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DRAFT

AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA

for

PERFLUOROOCTANOIC ACID

(PFOA)

April 2022

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

Washington, D.C.

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ACRONYMS

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

HIS	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 draft document provides information to states and tribes authorized to establish water quality standards under the Clean Water Act (CWA), to protect aquatic life from toxic effects of Perfluorooctanoic acid (PFOA). Under the CWA, states and tribes are to establish water quality criteria to protect designated uses. State and tribal decision makers retain the discretion to adopt approaches that are scientifically defensible that differ from these criteria to reflect site-specific conditions. While this document contains the Environmental Protection Agency's (EPA) draft scientific recommendations regarding ambient concentrations of PFOA that protect aquatic life, the draft PFOA Criteria Document does not substitute for the Clean Water Act or the EPA's regulations; nor is it a regulation itself. Thus, the document when final would not impose legally binding requirements on the EPA, states, tribes, or the regulated community, and might not apply to a particular situation based upon the circumstances. The EPA intends to finalize this document in the future. This draft document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency.

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

FOREWORD

The Clean Water Act Section 304(a)(l) (P.L. 95-217) directs the Administrator of the EPA to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including groundwater. This document is a draft ambient water quality criteria (AWQC) document for the protection of aquatic life based upon consideration of all available information relating to effects of perfluorooctanoic acid on aquatic organisms.

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

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

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EXECUTIVE SUMMARY

U.S. Environmental Protection Agency (EPA) developed the draft recommended perfluorooctanoic acid (PFOA) aquatic life ambient water quality criteria in accordance with the provisions of section304(a) of the Clean Water Act. This document provides EPA's basis for and derivation of the draft national PFOA ambient water quality criteria recommendations for fresh and saltwater environments to protect aquatic life. EPA has drafted the PFOA aquatic life criteria to be consistent with methods described in EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (U.S. EPA 1985).

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

The draft Aquatic Life Ambient Water Quality Criteria for the draft 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 none of the eight MDRs for deriving a chronic estuarine/marine criterion per the 1985 Guidelines. EPA did, however, include an acute aquatic life benchmark for estuarine/marine environments in Appendix L, using available estuarine/marine species toxicity

data and the New Approach Methods (NAMS) application of 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/). Both the freshwater criteria and the acute estuarine/marine benchmark are draft recommendations for states/authorized tribes to consider as protective values in their state water quality protection programs. However, the acute estuarine/marine benchmark magnitude is less certain than the freshwater criteria since it was based on both empirical and estimated acute toxicity data (Appendix L).

The draft freshwater acute water column-based criterion magnitude is 49 mg/L, and the draft chronic water column-based chronic criterion magnitude is 0.094 mg/L. The draft chronic freshwater criterion also contains tissue-based criteria with magnitudes of 6.10 mg/kg wet weight (ww) for fish whole-body, 0.125 mg/kg ww for fish muscle tissue, and 1.11 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 are expected to protect the freshwater aquatic community.

Table Ex-1. Draft Recommended Freshwater Perfluorooctanoic acid (PFOA) Aquatic Life Ambient Water Quality Criteria

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	49 mg/L	0.094 mg/L	1.11 mg/kg ww	6.10 mg/kg ww	0.125 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 more than once in ten years on average		

[1] All five of these water column and tissue criteria are intended to be independently applicable and no one criterion takes primacy. All of the above recommended criteria (acute and chronic water column and tissue criteria) are intended to be protective of aquatic life. These criteria are applicable throughout the year.
[2] Tissue criteria derived from the chronic water column concentration (CCC) with the use of bioaccumulation factors and are expressed as wet weight (ww) concentrations.
[3] Tissue data provide instantaneous point measurements that reflect integrative accumulation of PFOS over time and space in aquatic life population(s) at a given site.
[4] Criterion Maximum Concentration; applicable throughout the water column.
[5] Criterion Continuous Concentration; applicable throughout the water column.

Table Ex-2. Draft Recommended 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 tribes by defining ambient water concentrations that will protect against unacceptable adverse ecological effects to aquatic life resulting from exposure to pollutants found in water. Once EPA publishes final CWA section 304(a) recommended water quality criteria, states and authorized tribes may 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 are to submit new and revised WQS to EPA for review and approval or disapproval. When approved by EPA, the state's/tribe's WQS become the applicable WQS for CWA purposes. Such purposes include 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. EPA would recommend 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. The draft estuarine/marine benchmarks are provided in Appendix L as additional protective values that states and tribes may consider in their water quality programs.

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

EPA derived the draft recommended criteria using the best available data to reflect the latest scientific knowledge on the toxicological effects of PFOA on aquatic life. EPA developed the criteria following the general approach outlined in the EPA's "*Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses"* (U.S. EPA 1985). The draft PFOA criteria are expected to be protective of most aquatic organisms in the community (i.e., approximately 95 percent of tested aquatic organisms representing the aquatic community) and are derived to be protective of aquatic life designated uses established by states and authorized tribes for freshwaters. The draft 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 draft criteria presented herein are EPA's best estimate of the maximum 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, two states have acute and chronic protective values that were developed to be numerical translations of CWA Section 303(c) narrative water quality criteria (e.g., Michigan

and Minnesota) and other states have published draft/interim acute and chronic ecological screening level values/benchmarks for the protection of aquatic life (e.g., 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). No acute estuarine/marine criteria, benchmarks, or protective values have been established for PFOA. 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)

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 US EPA Great Lakes Initiative [GLI; US 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 US EPA Great Lakes Initiative [GLI; US 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.	STS/MPCA 2007
Florida	20	Secondary Acute Value (SAV) calculated using US EPA Great Lakes Initiative (GLI; USEPA 1995) 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)	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. (2017) and Batley et al. (2014)	CRC CARE 2017, EPAV 2016, HEPA 2020
	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)		
California	0.54 (99% species protection)	HC1 calculated from an acute and chronic NOEC-based SSD as reported in SERDP Project ER18-1614 (SERDP 2019). Acute NOEC values were converted to chronic values using mean acute-to-chronic ratios derived from Giesy et al. (2010). This value represents an "Interim Final Environmental Screening Level" and does not represent a CWA Section 303(c) approved water quality Standard for PFOA.	San Francisco Bay RWQCB 2020; SERDP 2019
Michigan	0.88	Final Chronic Value (FCV) was calculated as the FAV ÷ Final Acute: Chronic ratio (ACR) (following the GLI; US 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 US EPA Great Lakes Initiative (GLI; USEPA 1995) 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 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.	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 SERDP Project ER18-1614 (SERDP 2019). Acute NOEC values were converted to chronic values using mean acute-to-chronic ratios derived from Giesy et al. (2010). This value represents an "Interim Final Environmental Screening Level" and does not represent a CWA Section 303(c) approved water quality Standard for PFOA.	San Francisco Bay RWQCB 2020; SERDP 2019

State/Country of Applicability	Aquatic Life Protective Value (mg/L)	Criteria or Benchmark and Calculation Approach	Source
Australia, New Zealand	0.019 (99% species protection – high conservation value 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
	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)		

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. EPA's Office of Pollution Prevention and Toxics (OPPT) defines a PFAS as: any chemical substance or mixture that structurally contains the unit R-(CF2)-C(F)(R′)R″. Both the CF2 and CF moieties are saturated carbons. None of the R groups (R, R′ or R″) can be hydrogen. The carbon-fluorine (C-F) bond is strong and stable due to the strong electronegativity and intermediate atomic size of fluorine. The chemical structure of the perfluoroalkyl moiety make PFAS water and oil repellent, chemically and thermally stable, and exhibit surfactant properties. Due to these properties, PFAS have been used in a wide range of industrial and consumer products since the 1940s with common uses including wetting agents, lubricants, corrosion inhibitors, firefighting foams, and stain-resistant treatments to leather, paper, and clothing.

There are many families of PFAS, and each contain many individual homologues and isomers (Buck et al. 2011). These PFAS families can be divided into two primary categories: nonpolymers and polymers. The nonpolymer PFAS include perfluoroalkyl and polyfluoroalkyl substances. Polymer PFAS include fluoropolymers, perfluoropolyethers, and side-chain fluorinated polymers (Table 1-2).

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

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

[1] Modified from 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)	$-SO_3H$	Perfluorooctane sulfonic acid (PFOS)
	$-SO_3^-$	Perfluorooctane sulfonate (PFOS)
Perfluoroalkyl sulfinic acids (PFSIAs)	$-SO_2H$	Perfluorooctane sulfinic acid (PFOSI)
Perfluoroalkyl phosphonic acids (PFPAs)	$-P(=O)(OH)_2$	Perfluorooctyl phosphonic acid (C8-PFPA)
Perfluoroalkyl phosphinic acids (PFPIAs)	$-P(=O)(OH)(C_mF_{2m+1})$	Bis(perfluorooctyl) phosphinic acid (C8/C8-PFPIA)
Perfluoroalkylether carboxylic acids (PFECAs)	$CF_3(OCF_2)_nCOO^-$	perfluoro (3,5,7-trioxaoctanoic) acid
Perfluoroalkylether sulfonic acids (PFESAs)	$CF_3(OCF_2)_nSO_3H$	6:2 Cl-PFESA
Perfluoroalkyl dicarboxylic acids (PFdiCAs)	$HOOC\text{-}C_nF_{2n}\text{-}COOH$	9:3 Fluorotelomer betaine
Perfluoroalkane disulfonic acids (PFdiSAs)	$HO_3S\text{-}C_nF_{2n}\text{-}SO_3H$	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 $C_nF_{2n+1}COOH$. 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).

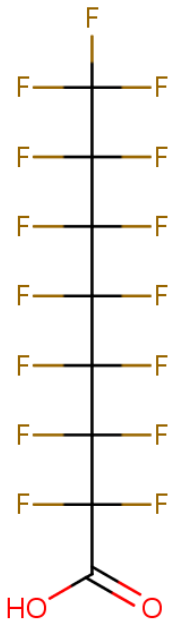

Figure 1-1. Chemical Structure of the Linear Isomer of Perfluorooctanoic acid (PFOA). (Source: United States EPA Chemistry Dashboard; https://comptox.epa.gov/dashboard).

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 (ammonia 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 (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 ammonia salt (CASRN 2795-39-3). 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). 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 (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 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.

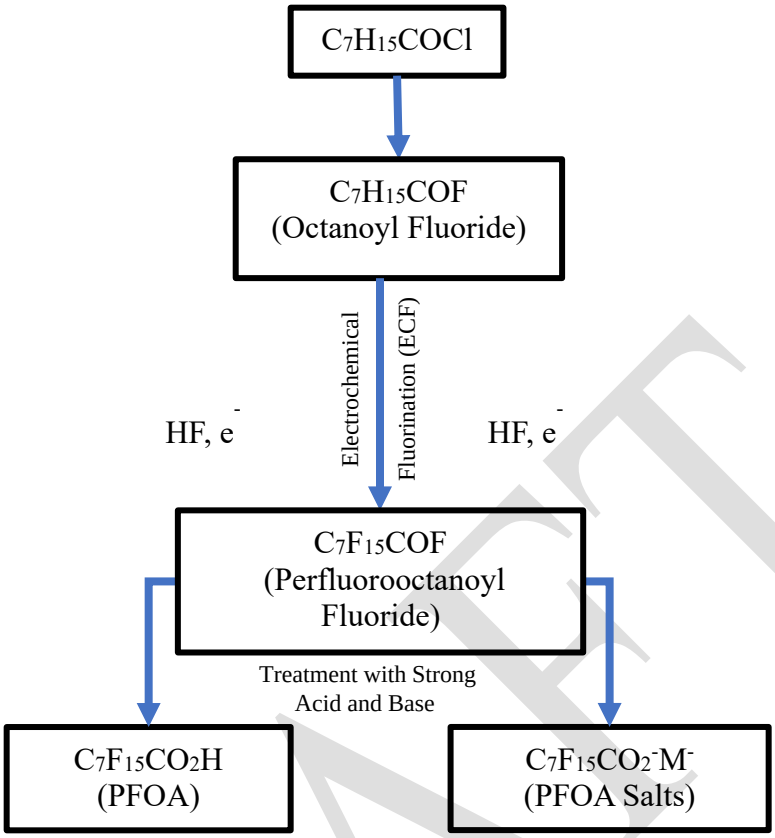

Figure 2-1. Synthesis of Perfluorooctanoic acid (PFOA) by Electrochemical Fluorination (ECF).
Modified from Buck et al. (2011).

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 3200 tons to 7300 tons (Prevedouros et al. 2006). In 2006, 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 2000) 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. 2011). 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 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 (Russel 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 (Environment 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; Schoeib 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, the kinetic modeling approach, estimated half-lives of PFOA 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, Russel 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). See Occurrence of PFOA in the Aquatic Environment (Section 2.4.1) below. 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).

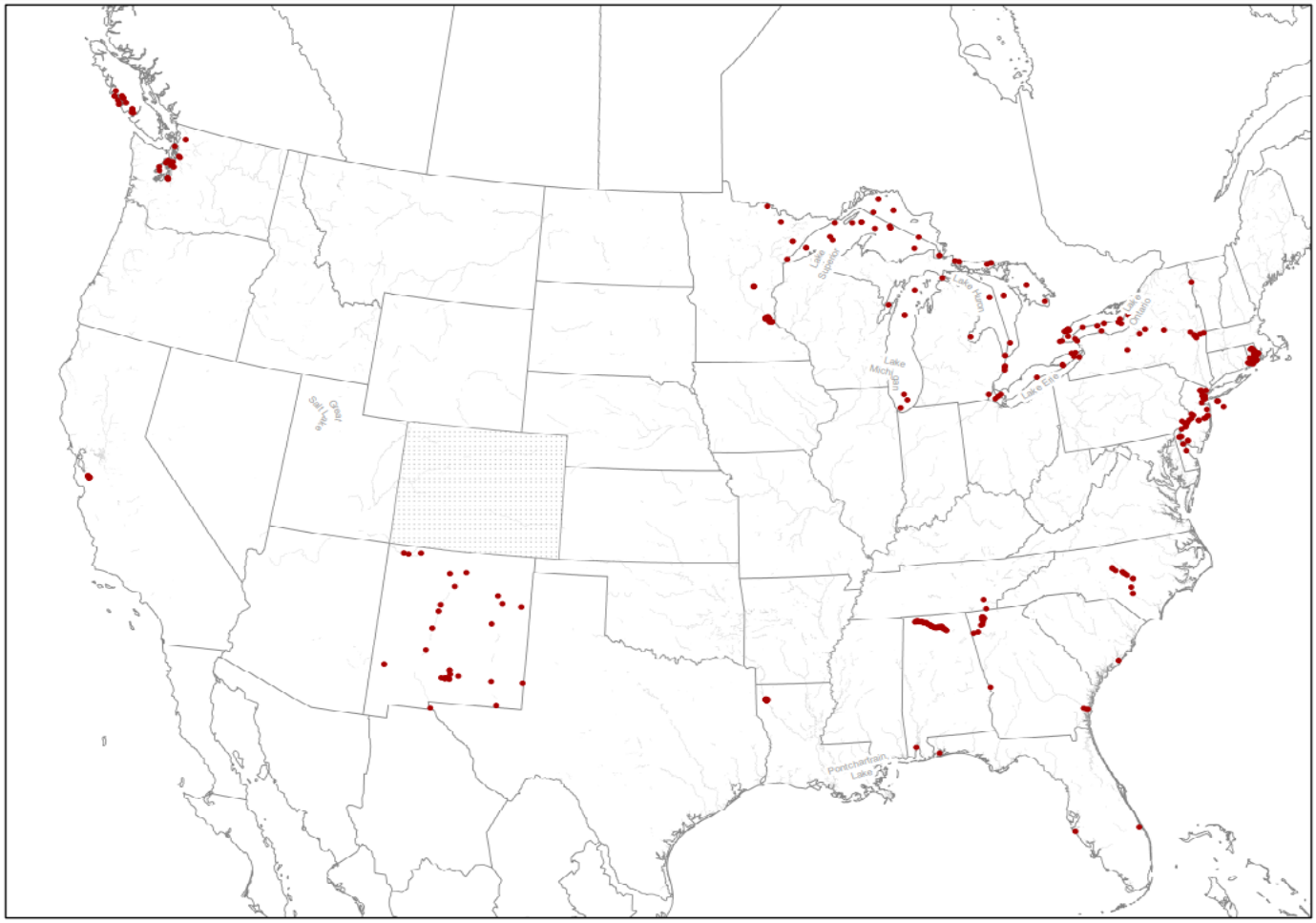

Figure 2-2. Map Indicating Sampling Locations for Perfluorooctanoic acid (PFOA) Measured in Surface Waters Across the United States (U.S.) Based on Data Reported in the Publicly Available Literature.
Colorado sampling coordinates were not available, these data are represented by the dash marks to indicate 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.

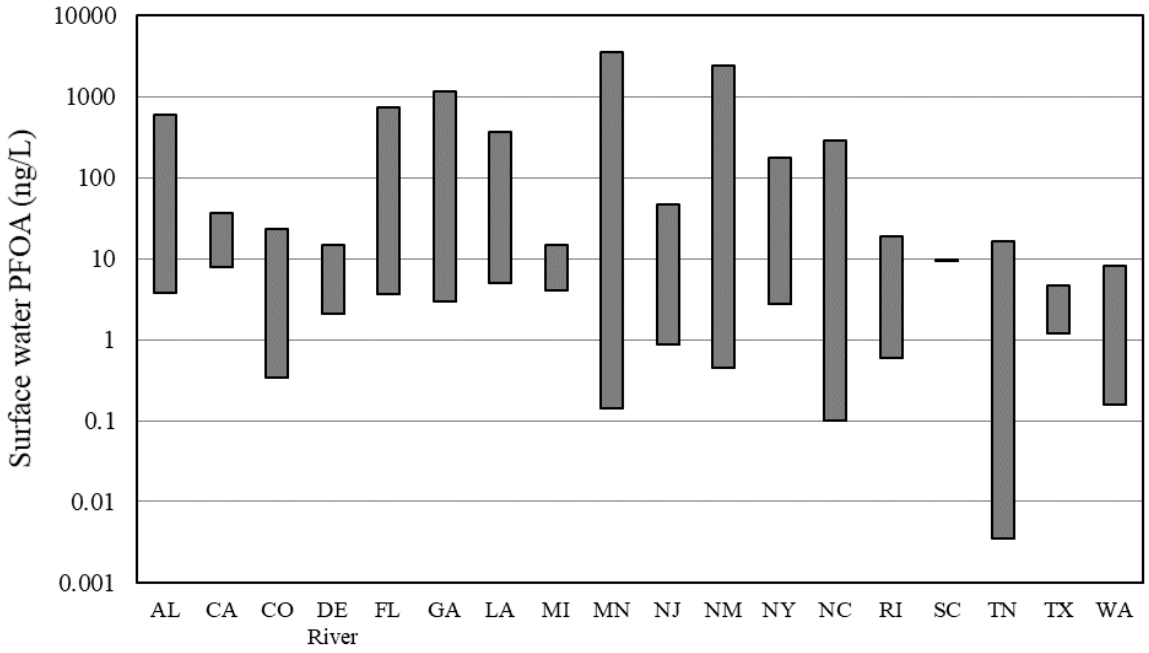

Figure 2-3. Distribution of the minimum and maximum concentrations (ng/L) of Perfluorooctanoic acid (PFOA) measured in surface waters for each state or waterbody (excluding the Great Lakes) with reported data in the publicly available literature.
The distribution is arranged alphabetically by state and 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 N for further discussion of PFOA occurrence in surface waters and other abiotic media such as aquatic sediments, groundwater, air, and ice.

2.5 Bioaccumulation and Biomagnification of 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. 2006). 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. 2003a). 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 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. 2003b).

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. (2018) 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. 2003b). 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. 2006b; 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. 2006a). Since

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. 2006a).

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 (5^{th} 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. (2006b) 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. 2006b).

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, and Appendix A through Appendix H summarize the current 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 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. 2006; Keiter et al. 2012; Wang et al. 2017; see Section 2.4.1). Although 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. Findings of the 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 and can have unpredictable, exposure and species-specific effects. For example, in a single *in vivo* exposure of zebrafish (*D. rerio*) embryos, synergism, additivity and antagonism were all reported for different combinations/ratios of PFOA and PFOS and endpoints (Ding et al. 2013), thereby illustrating the complexity and uncertainty associated with mixture studies. Importantly, neither the concentration addition model nor the independent-action model could predict the combined effects when strong interactive effects existed. More

recently, Yang et al. (2019) exposed the water flea, *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. Finally, U.S. EPA (2021, unpublished) observed PFOA and PFOS interacting in an additive manner to reduce pup body weight, pup liver weight, and maternal liver weight in the Sprague-Dawley rat.

2.8 Conceptual Model of 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.

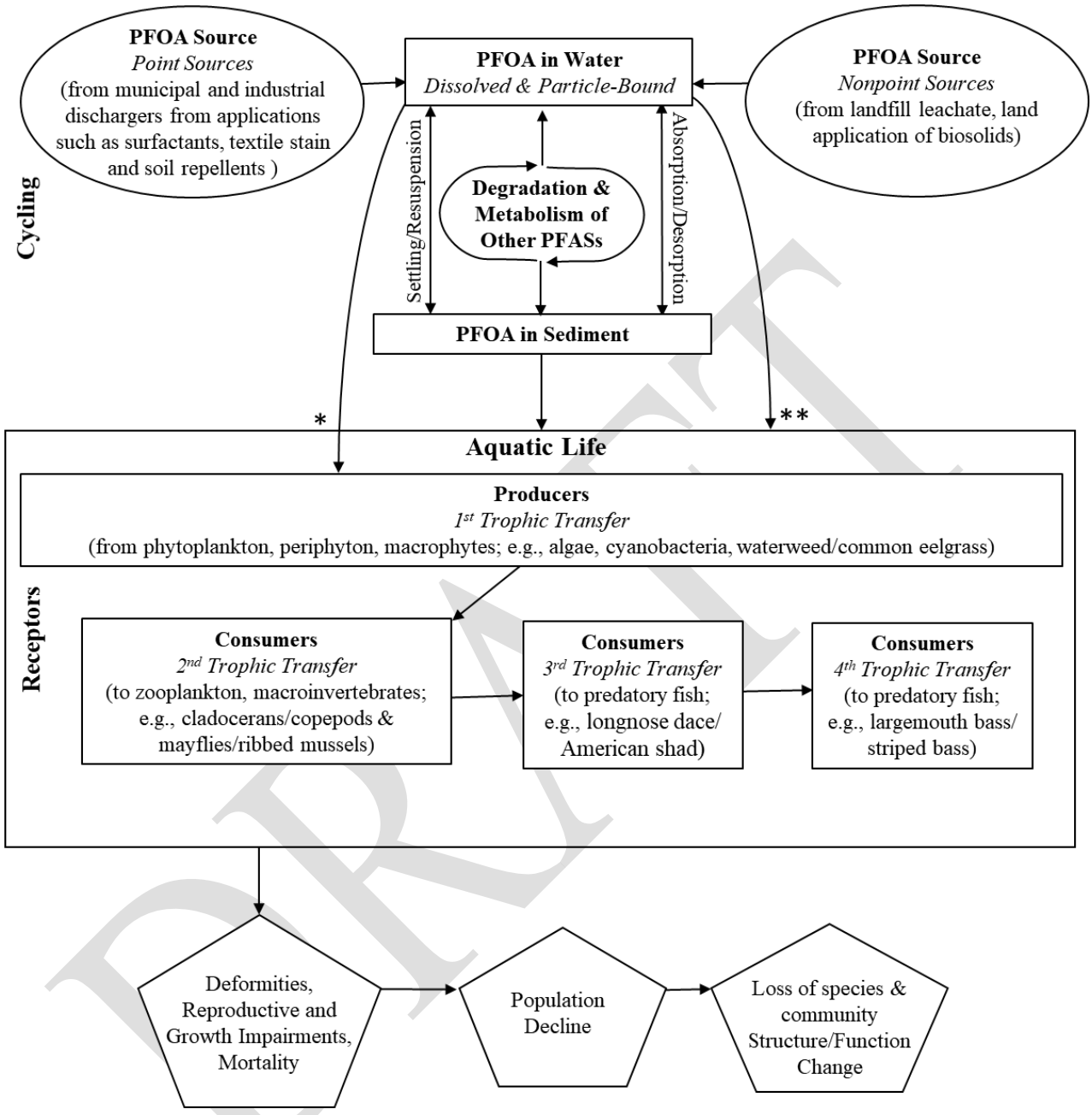

Figure 2-4. Conceptual Model Diagram of Sources, Compartmental Partitioning, and Trophic Transfer Pathways of Perfluorooctanoic acid (PFOA) in the Aquatic Environment and its Bioaccumulation and Effects in Aquatic Life and Aquatic-dependent Wildlife.

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: bioconcentration by producers (*) and direct exposure to all trophic levels within box (**). 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 5^{th} 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, 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-

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 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).

Based on EPA's data review, PFOA toxicity studies typically used the linear PFOA isomer for dosing with fewer studies using the branched isomer. 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 Office of Water. All studies were evaluated for data quality as described by U.S. EPA (1985), EPA's Office of Chemical Safety and Pollution Prevention (OPP)'s Ecological

Effects Test Guidelines (U.S. EPA 2016c), and EPA OW's internal data quality standard operating procedure (SOP), which is consistent with OPP's data quality review approach (U.S. EPA 2016c). 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 Q 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 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 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 2018b). 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 EPA aquatic life criteria documents are typically based on measured chemical concentrations only. For PFOA, EPA has examined the issue of whether nominal (unmeasured) and measured concentrations are in close agreement with each other (see Appendix M). Briefly, approximately 24.3% of the 152 freshwater acute and chronic toxicity tests determined to be quantitatively or qualitatively acceptable reported measured PFOA concentrations in at least one treatment. Approximately 57.1% of the 14 saltwater acute and chronic toxicity tests determined to be quantitatively or qualitatively acceptable reported measured PFOA concentrations in at least one treatment. Pairs of nominal and corresponding measured PFOA concentrations were compared to one another through: (1) linear correlation analysis and; (2) an assessment of measured concentrations as a percent of its paired nominal concentration. Linear correlation between measured and corresponding nominal concentrations suggests a high degree of precision between paired observations across all test conditions and 83% of measured freshwater concentrations and all of measured saltwater concentrations fell within 20% of paired nominal concentrations, which represent the test acceptability threshold identified by EPA's OCSPP's Ecological Effects Test Guidelines. Instances where measured concentrations were not within 20% of nominal were isolated to a few studies. In these isolated cases, suspected dosing errors, unexplained phenomena, and/or presence of substrate (e.g., sediment) may have contributed to observed differences. Overall, 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 et al. 2011). Because suspected dosing errors were a relatively rare occurrence and toxicity tests with substrate and nominal concentrations only were not used quantitatively in PFOA criteria derivation, 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., EPA's 1985 Guidelines and 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}). 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 (CCMC 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 2. NOEC and LOEC concentrations in water For effects from chronic exposure: 1. EC_{10} concentrations in water 2. NOEC and LOEC concentrations in water; Only used when an EC_{10} could not be calculated for a genus.

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 EPA's request for data or were unable to locate concentration-response data, the toxicity values were not independently calculated by EPA, and the reported toxicity values were retained for criteria deviation. 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, 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 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 criterion were met for seven of the eight MDRs. Because nearly all MDRs were met for deriving both acute and chronic criteria, EPA derived the acute and chronic freshwater column criteria based on the seven MDRs. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide information to evaluate the sensitivity of insects to acute and chronic PFOA exposures before the PFOA criteria document is finalized.

Acute and chronic MDRs for PFOA estuarine/marine criteria derivation were not met and, consequently, acute and chronic estuarine/marine criteria were not derived. 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). 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. 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, EPA derived PFOA chronic tissue-based criteria by translating the chronic freshwater column criterion (see Section 3.2.1.3) into tissue-based criteria magnitudes using bioaccumulation factors and the following equation:

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

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:

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

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

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 organisms, 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 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 P). For further details on BAFs compilation and ranking, see Burkhard (2021).

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

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).

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. 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 25 freshwater species, representing 18 genera and 16 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 11 freshwater species, representing 10 genera and nine families in three phyla. There were no quantitatively acceptable chronic studies with estuarine/marine organisms. 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. EPA independently calculated these toxicity values if sufficient raw data were available for EPA to conduct statistical analyses. EPA's independently-calculated toxicity values were used preferentially, where available. Author-reported toxicity values and 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.

	Freshwater			
MDR[a, b]	**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	5	2	3
Benthic Crustacean	1	1	1	1
Insect	0[a]	0[a]	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	0[b]	0[b]
Total	**18**	**25**	**10**	**11**
	Saltwater[c]			
MDR	**GMAV**	**SMAV**	**GMCV**	**SMCV**
Family in the phylum Chordata	0	0	0	0
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	0	0
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	0	0

a One acute MDR, for aquatic insects, was not fulfilled. Of the available qualitatively-acceptable insect data, only Yang et al. (2014) conducted a test for the standard 96 acute exposure. Other qualitatively acceptable insect toxicity data were based on either chronic or sub-chronic exposure durations Yang et al. (2014) specifically conducted a 96-hour renewal test with measured PFOA concentrations on the midge, *Chironomus plumosus*. This study was not acceptable for quantitative use due to the potential problematic source of the organisms but was retained for qualitative use. The reported LC_{50} was 402.24 mg/L PFOA. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide additional information to fully evaluate on the sensitivity of insects to acute PFOA exposures before the PFOA criteria document is finalized.

b One chronic MDR, for any order of aquatic insects or any phylum not already represented, was not fulfilled with quantitatively acceptable chronic data. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide information to evaluate the sensitivity of insects to chronic PFOA exposures before the PFOA criteria document is finalized.

c The 1985 Guidelines require that data from a minimum of eight families are needed to calculate an estuarine/marine criterion. Insufficient data exist to fulfill all eight of the taxonomic MDR groups. Consequently, EPA cannot derive an estuarine/marine acute criterion, based on the 1985 Guidelines. However, EPA has developed draft estuarine/marine benchmarks through use of surrogate data to fill in missing MDRs using EPA's 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 18 genera representing seven of the eight taxonomic MDR groups. 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 11 freshwater invertebrate species, representing nine genera and eight families, and fulfilled four of the eight MDRs. Data for acute PFOA toxicity were available for 10 freshwater amphibian species, representing five genera and five families fulfilling one of the MDRs. The missing MDR is a representative from an insect family. Therefore, qualitatively acceptable data in Appendix G were examined to determine if any "Qualitative Data" provided information on the relative sensitivity of aquatic insects. Yang et al. (2014) conducted a test for the standard 96 hour acute exposure. Other qualitatively acceptable insect toxicity data were based on either chronic or sub-chronic exposure durations. Yang et al. (2014) specifically conducted a 96-hour renewal test with measured PFOA concentrations on the midge, *Chironomus plumosus*. This study was not acceptable for quantitative use due to the potential problematic source of the organisms but was retained for qualitative use. The reported LC_{50} was 402.24 mg/L PFOA. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide additional information to fully evaluate the sensitivity of insects to acute PFOA exposures before the PFOA criteria document is finalized. Summaries of studies for the most sensitive acute genera are describe 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	Comment
1	*Chydorus*	93.17	Cladoceran (*Chydorus sphaericus*)	North American resident species
2	*Daphnia*	144.1	Cladoceran (*Daphnia carinata*)	Non-North American resident species
			Cladoceran (*Daphnia magna*)	North American resident species
			Cladoceran (*Daphnia pulicaria*)	North American resident species
3	*Brachionus*	150.0	Rotifer (*Brachionus calyciflorus)*	North American resident species
4	*Ligumia*	161.0	Black sandshell mussel (*Ligumia recta*)	North American resident species

3.1.1.1.1 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). 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* Genus Mean Acute Value (GMAV).

3.1.1.1.2 Second 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 (2000a) 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 replicates. 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 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). 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. EPA performed C-R analysis for each individual test. All three tests had acceptable curves with 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). 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%) 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). 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 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). 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 a 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.

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 et al. (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), and Yang et al. (2019; LC_{50} = 117.2 mg/L) were taken together as a geometric mean value to calculate the *D. magna* SMAV of 220.0 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., 220.0 mg/L) were used to determine the *Daphnia* GMAV of 144.1 mg/L.

3.1.1.1.3 Third most acutely sensitive genus: Brachionus (rotifer)

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. PFOA concentrations were not measured in the rotifer exposures, but rather, in a side experiment that showed that the concentration of PFOA measured every eight hours over a 24-hour period in rotifer medium with green algae incurred minimal change. C-R data were not included in the publication and could not be obtained for independent calculation of the test-specific LC_{50}. Therefore, the study reported 24-hour LC_{50} of 150.0 mg/L was considered acceptable for quantitative use. No other quantitatively acceptable acute toxicity data were available for *Brachionus calyciflorus* or other members of the genus *Brachionus*. Therefore, the LC_{50} (i.e., 150.0 mg/L) from this test served directly as the *Brachionus calyciflorus* SMAV and the *Brachionus* GMAV.

3.1.1.1.4 Fourth most acutely sensitive genera: Ligumia (mussel)

Hazelton et al. (2012, 2013) evaluated the acute effects of PFOA (96% purity) on the freshwater mussel, *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). Authors stated the tests followed ASTM E2455-06 (2006). Measured test concentrations of PFOA were within 10% of target in water from acute tests. Mortality of mussels in the negative control was <10% in all exposures. C-R data were not reported in the publication and could not be obtained for independent calculation of EC_{50} values from these tests. The 24-hour EC_{50} reported for *L. recta* glochidia. was 161.0 mg/L (95% C.I. 135.0-192.7 mg/L). The 96-hour LC_{50} value for the juvenile *L. recta* was greater than the highest test concentration (500 mg/L). The juvenile life stage was determined to be relatively tolerant to

acute PFOA exposures and, therefore, the LC_{50} from the acute juvenile mussel test was not used to derive the acute PFOA criterion. No other quantitatively acceptable acute toxicity data were available for *Ligumia recta* or other members of the genus *Ligumia*. Therefore, the EC_{50} (i.e., 161.0 mg/L) from this test served directly as the *Ligumia recta* SMAV and the *Ligumia* GMAV (the fourth most sensitive GMAV).

3.1.1.1.5 Missing Insect MDR

The acute data set for PFOA contained 18 genera (Table 3-3) representing seven of the eight taxonomic MDR groups. The missing MDR was a representative from an insect family. The EPA examined data in Appendix G to determine if any qualitatively acceptable freshwater PFOA toxicity studies could be used to evaluate the missing MDR group. Yang et al. (2014) conducted a 96-hour renewal, measured PFOA acute test with the midge, *Chironomus plumosus*, which is described in greater detail in Appendix G.2.1.5. The source of the test organisms was the Beijing City Big Forest Flower Market, which potentially was a problematic source given uncertainties associated with previous exposures to PFOA. Consequently, this study was not considered acceptable for quantitative use but was considered qualitatively by providing relative species sensitivity information. The reported LC_{50} was 402.24 mg/L PFOA indicating this insect species may not be one of the more sensitive taxonomic groups (Figure 3-1). EPA will continue to seek additional acute PFOA insect data to further evaluate the sensitivity of insects. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide additional information to fully evaluate on the sensitivity of insects to acute PFOA exposures before the PFOA criteria document is finalized.

EPA calculated the PFOA Criterion Maximum Concentration (CMC) using all acceptable quantitative studies from Appendix A, but did not include the insect data in the criterion

calculation (i.e., the relatively tolerant insect LC_{50} value was not included in the total count ("n") of Genus Mean Acute Values in the criterion calculation).

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

Rank[a]	GMAV (mg/L PFOA)	MDR Group[c, d]	Genus	Species	SMAV[b] (mg/L PFOA)
1	93.17	D	*Chydorus*	Cladoceran, *Chydorus sphaericus*	93.17
2	144.1	D	*Daphnia*	Cladoceran, *Daphnia carinata*	66.80
				Cladoceran, *Daphnia magna*	220.0
				Cladoceran, *Daphnia pulicaria*	203.7
3	150.0	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	150.0
4	161.0	G	*Ligumia*	Black sandshell, *Ligumia recta*	161.0
5	164.4	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	164.4
6	166.3	D	*Moina*	Cladoceran, *Moina macrocopa*	166.3
7	377.0	C	*Xenopus*	Frog, *Xenopus sp.*	377.0
8	383.6	H	*Dugesia*	Planaria, *Dugesia japonica*	383.6
9	413.2	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	413.2
10	431.5	E	*Neocaridina*	Green neon shrimp, *Neocaridina denticulata*	431.5
11	572.4	B	*Danio*	Zebrafish, *Danio rerio*	572.4
12	646.2	C	*Hyla*	Gray treefrog, *Hyla versicolor*	646.2
13	664.0	B	*Lepomis*	Bluegill, *Lepomis macrochirus*	664.0
14	681.1	G	*Physella*	Bladder snail, *Physella acuta*	681.1
15	689.4	C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	1,070

Rank[a]	GMAV (mg/L PFOA)	MDR Group[c, d]	Genus	Species	SMAV[b] (mg/L PFOA)
				Small-mouthed salamander, *Ambystoma texanum*	407.3
				Eastern tiger salamander, *Ambystoma tigrinum*	752.0
16	793.9	C	*Anaxyrus*	American toad, *Anaxyrus americanus*	793.9
17	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
18	1,682	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	1,682

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.

d Of the available qualitatively-acceptable insect data, only Yang et al. (2014) conducted a test for the standard 96 hour acute exposure. Other qualitatively acceptable insect toxicity data were based on either chronic or sub-chronic exposure durations Yang et al. (2014) specifically conducted a 96-hour renewal test with measured PFOA concentrations on the midge, *Chironomus plumosus*. This study was not acceptable for quantitative use due to the potential problematic source of the organisms but was retained for qualitative use. The reported LC_{50} was 402.24 mg/L PFOA. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide additional information to fully evaluate on the sensitivity of insects to acute PFOA exposures before the PFOA criteria document is finalized.

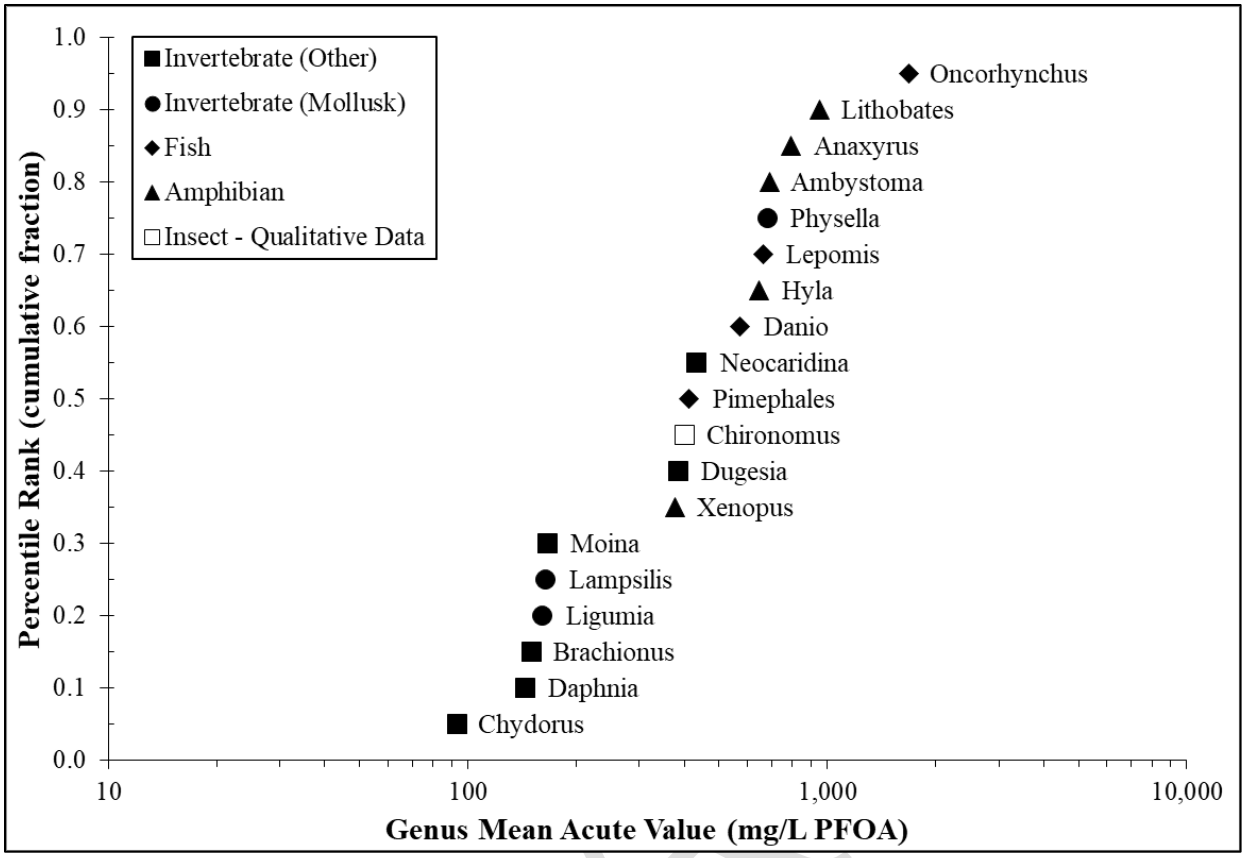

Figure 3-1. Ranked Freshwater Acute PFOA GMAVs Fulfilling the Acute Family MDR.
Note: *Chironomus* is only displayed as a visual representation to show relative rank of the species based on the qualitative data. The *Chironomus* GMAV is not used in the Final Acute Value calculation.

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 recommendations to states/authorized tribes on protective values, EPA developed an estuarine/marine acute benchmark using the available empirical data supplemented with toxicity values generated through the use of new approach methods, specifically through the use of the EPA Office of Research and Development's peer-reviewed publicly-available Web-based

Interspecies Correlation Estimation (WebICE) tool (Raimondo et al. 2010). These benchmarks are provided in Appendix L.

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	Comment
1	*Siriella*	15.5	Mysid (*Siriella armata*)	Not a North American resident species, but a member of the Mysidae Family and serves as a surrogate for untested mysid species residing in North America.
2	*Mytilus*	17.58	Mediterranean mussel (*Mytilus galloprovincialis*)	North American resident species
3	*Strongylocentrotus*	20.63	Purple sea urchin (*Strongylocentrotus purpuratus*)	North American resident species
4	*Americamysis*	24	Mysid (*Americamysis bahia*)	North American resident species

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 residing in North America.

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 survival larvae 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 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. 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, EPA used the available acute toxicity data and ORD's peer-reviewed webICE 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.

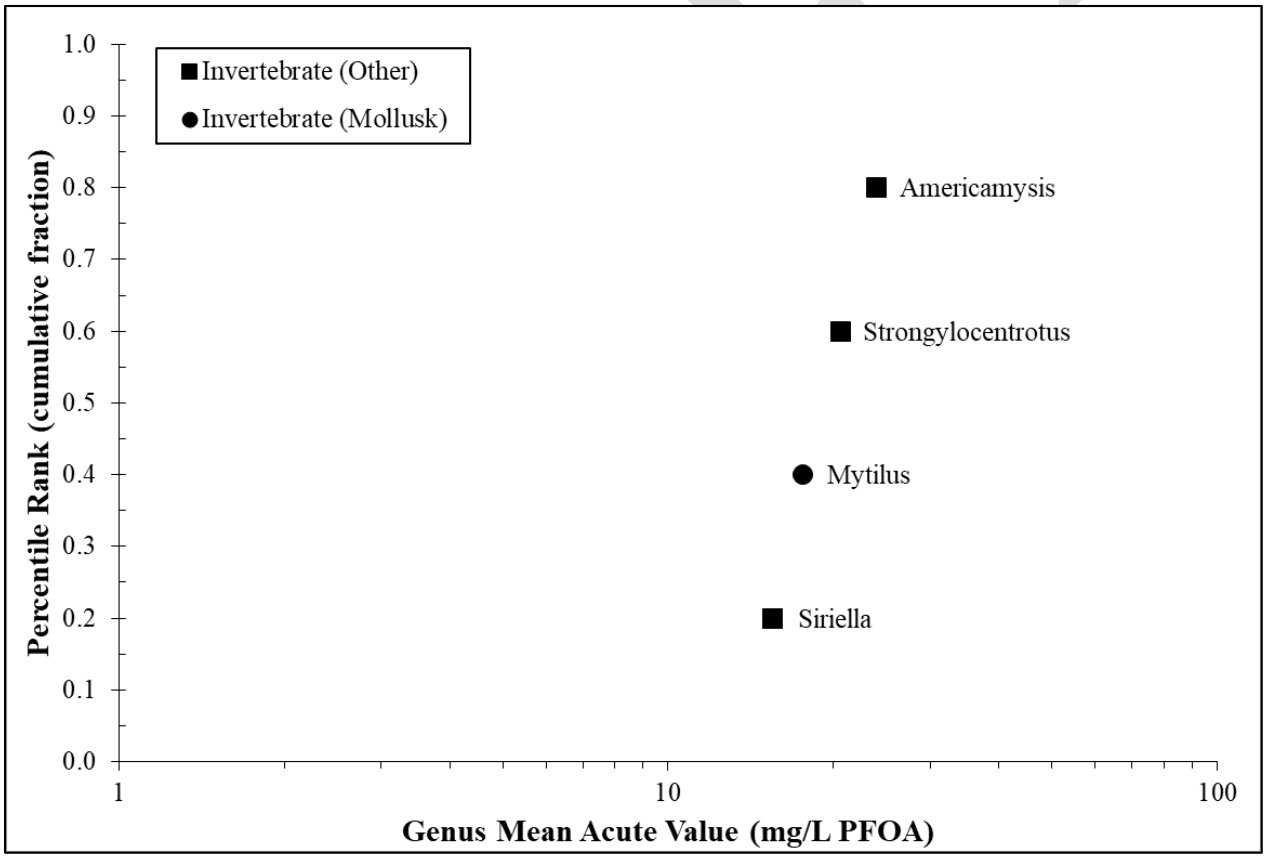

Figure 3-2. Acceptable Estuarine/Marine GMAVs.

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 11 species representing 10 genera and nine families in three phyla. A total of seven of the eight required MDRs were fulfilled. Data for one MDR group (any other phylum not already represented or a second insect order not already represented) remains unfulfilled. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide information to evaluate the sensitivity of insects to chronic PFOA exposures before the PFOA criteria document is finalized. 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). Study summaries for the four most sensitive genera are provided below to describe all sensitive genera up to the point where four established North American species are represented.

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

Rank	Genus	GMCV (mg/L)	Species	Comment
1	*Hyalella*	0.147	Amphipod (*Hyalella azteca*)	North American resident species
2	*Lithobates*	0.288	American bullfrog (*Lithobates catesbeiana*)	North American resident species
3	*Daphnia*	0.3700	Cladoceran (*Daphnia carinata*)	Not a resident species
			Cladoceran (*Daphnia magna*)	North American resident species
4	*Brachionus*	0.7647	Rotifer (*Brachionus calyciflorus*)	North American 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. 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. 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* SMCV and the *Hyalella* 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. EPA could not independently calculate an EC_{10} value because there were minimal effects observed across the limited number of treatment concentrations tested. Consequently, 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 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. 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. 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.

Colombo et al. (2008) conducted a 21-day renewal measured chronic test on PFOA with the daphnid, *Daphnia magna*. Authors stated the toxicity test was 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. 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. EPA performed C-R analysis for each reported endpoint. The most sensitive endpoint with an acceptable C-R curve was average number of live young, with an EPA-calculated EC_{10} of 20.61 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 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). EPA performed C-R analysis for each reported endpoint. 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. 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 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. 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) 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 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 0.0716 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. 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. 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 draft chronic water column criterion.

The chronic values from Colombo et al. (2008; EC_{10} = 20.61 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., 4.330 mg/L). The *D. carinata* SMCV (i.e., 0.03162 mg/L) and the *D. magna* SMCV (i.e., 4.330 mg/L) were used to calculate the *Daphnia* GMCV of 0.3700 mg/L.

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. EPA calculated EC_{10}s 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. (2014) 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 1 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. (2014), 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.

3.1.1.3.5 Missing Minimum Data Requirements

The chronic data set for PFOA based on quantitatively acceptable data contains 10 genera representing seven of the eight taxonomic MDR groups (Table 3-7). The MDR group missing is the any other phylum not already represented, or a second insect order not already represented. The EPA examined data in Appendix G and did not identify any qualitatively acceptable studies with species that could be used to inform the relative sensitivity of the missing MDR group. Two qualitatively acceptable chronic/sub-chronic tests with *Dugesia japonica*, a species that falls into the missing MDR category, were available to inform the potential sensitivity of the missing MDR. Overall, these two tests (Yuan et al. 2016b, Yuan et al. 2017) did not measure apical effects associated with growth, survival and reproduction. Therefore, the qualitatively acceptable data provided by these studies did not provide information about the relative sensitivity of this species. Because nearly all MDRs were met, EPA derived the chronic freshwater column criterion based on the genus sensitivity distribution (GSD) of the 10 genera representing seven MDRs. EPA will continue to seek additional chronic PFOA insect data to further evaluate the

sensitivity of insects. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide information to evaluate the sensitivity of insects to chronic PFOA exposures before the PFOA criteria document is finalized.

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	0.3700	D	*Daphnia*	Cladoceran, *Daphnia carinata*	0.03162
				Cladoceran, *Daphnia magna*	4.330
4	0.7647	G	*Brachionus*	Rotifer, *Brachionus calyciflorus*	0.7647
5	2.194	D	*Moina*	Cladoceran, *Moina macrocopa*	2.194
6	9.487	C	*Oryzias*	Medaka, *Oryzias latipes*	9.487
7	>30	B	*Gobiocypris*	Rare minnow, *Gobiocypris rarus*	>30
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.

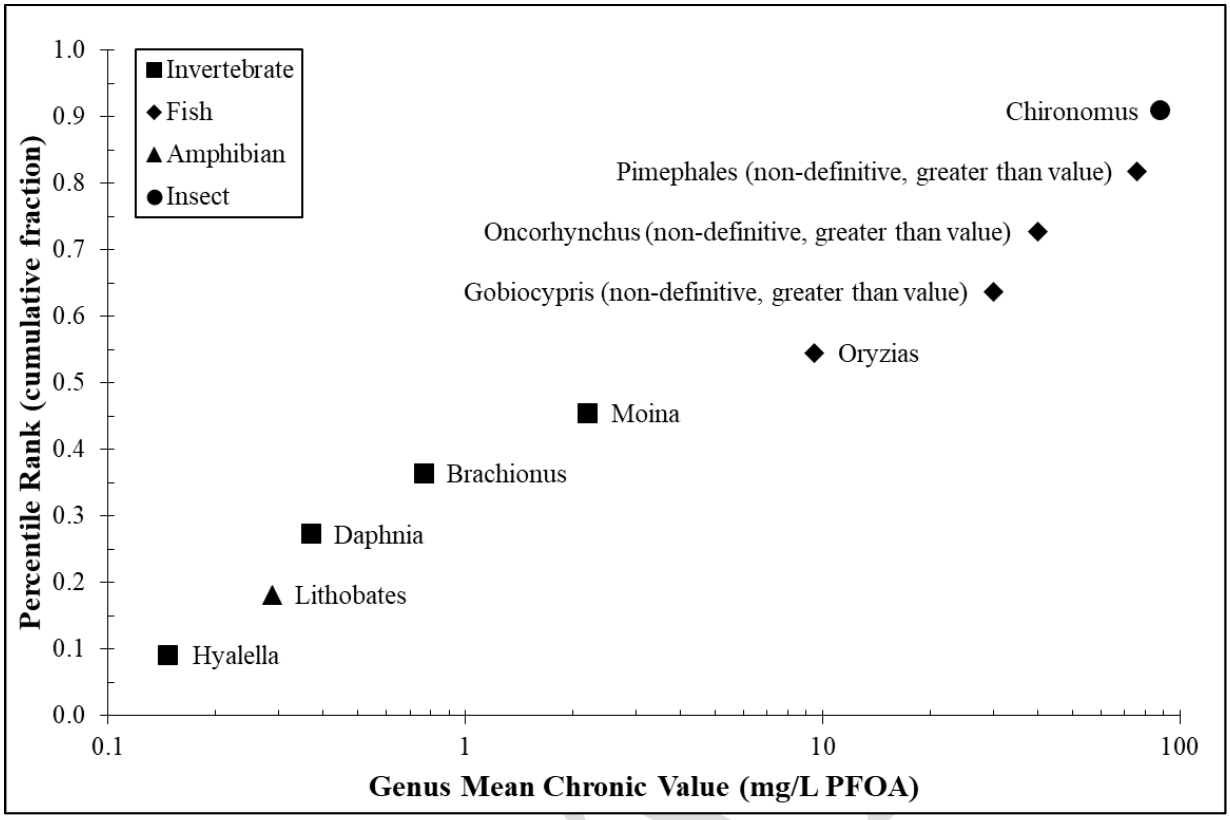

Figure 3-3. Freshwater Genus Mean Chronic Values for PFOA.

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

There are no quantitatively acceptable chronic estuarine/marine PFOA toxicity studies at this time.

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 18 genera representing seven of the eight taxonomic MDR groups. The missing MDR is a representative from an insect family. GMAVs for the four most sensitive genera were within a factor of 1.7 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 97.14 mg/L, calculated using the procedures

described in the 1985 Guidelines (Table 3-8). The FAV is lower than all the GMAVs for the tested species, except the *Chydorus* GMAV (i.e., 93.17 mg/L). The FAV was then divided by two to obtain a concentration yielding a minimal effects acute criterion value. Based on the above, the FAV/2, which is the freshwater acute criterion water column magnitude (criterion maximum concentration, CMC), is 49 mg/L PFOA (rounded to two significant figures) and is expected to be protective of 95% of freshwater 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 3-4).

Table 3-8. Freshwater Final Acute Value and Criterion Maximum Concentration.

Calculated Freshwater FAV based on 4 lowest values: Total Number of GMAVs in Data Set = 18						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Chydorus*	93.17	4.53	20.56	0.053	0.229
2	*Daphnia*	144.1	4.97	24.71	0.105	0.324
3	*Brachionus*	150.0	5.01	25.11	0.158	0.397
4	*Ligumia*	161.0	5.08	25.82	0.211	0.459
		Σ (Sum):	**19.60**	**96.20**	**0.53**	**1.41**

S^2 =	6.28	S = slope	
L =	4.016	L = X-axis intercept	
A =	4.576	A = lnFAV	
FAV =	97.14	P = cumulative probability	
CMC =	**49 mg/L PFOA** (rounded to two significant figures)		

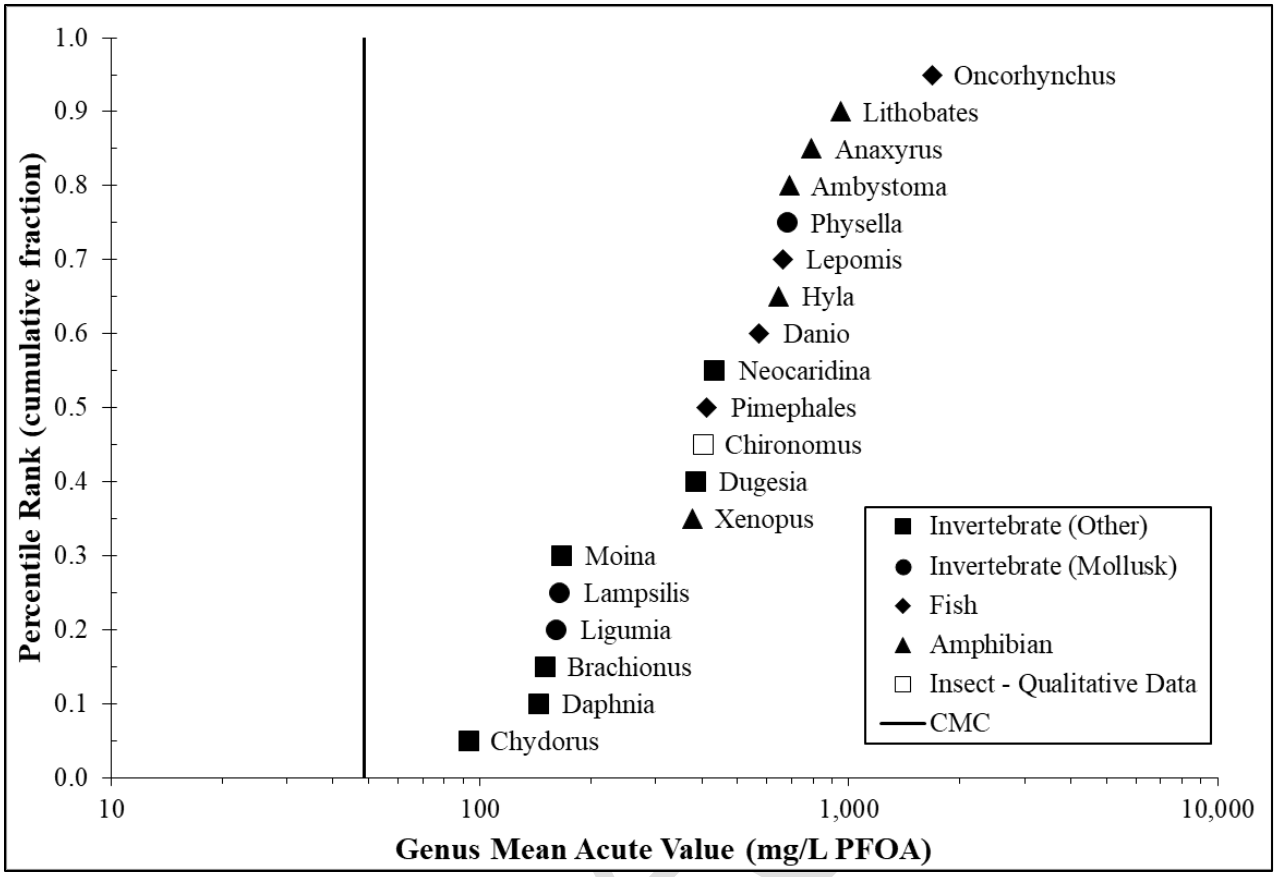

Figure 3-4. Ranked Freshwater Acute PFOA GMAVs used for the Criterion Calculation and the Qualitative Value for the Insect MDR Group.

Note: *Chironomus* is only displayed as a visual representation to show relative rank of the species based on the qualitative data. The *Chironomus* GMAV is not used in the Final Acute Value calculation.

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). EPA has, however, developed an acute benchmark value using available empirical data and 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 0.09404 mg/L, calculated using the procedures described in the 1985 Guidelines (Table 3-9). The freshwater chronic criterion water column magnitude (CCC) is the FCV rounded to two significant figures, or 0.094 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 3-9. Freshwater Final Chronic Value and Criterion Continuous Concentration.

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	*Daphnia*	0.3700	-0.994	0.988	0.273	0.522
4	*Brachionus*	0.7647	-0.268	0.072	0.364	0.603
		Σ (Sum):	**-4.42**	**6.29**	**0.91**	**1.85**

S^2 =	27.54	S = slope	
L =	-3.538	L = X-axis intercept	
A =	-2.364	A = lnFCV	
FCV =	0.09404	P = cumulative probability	
CCC =	**0.094 mg/L PFOA** (rounded to two significant figures)		

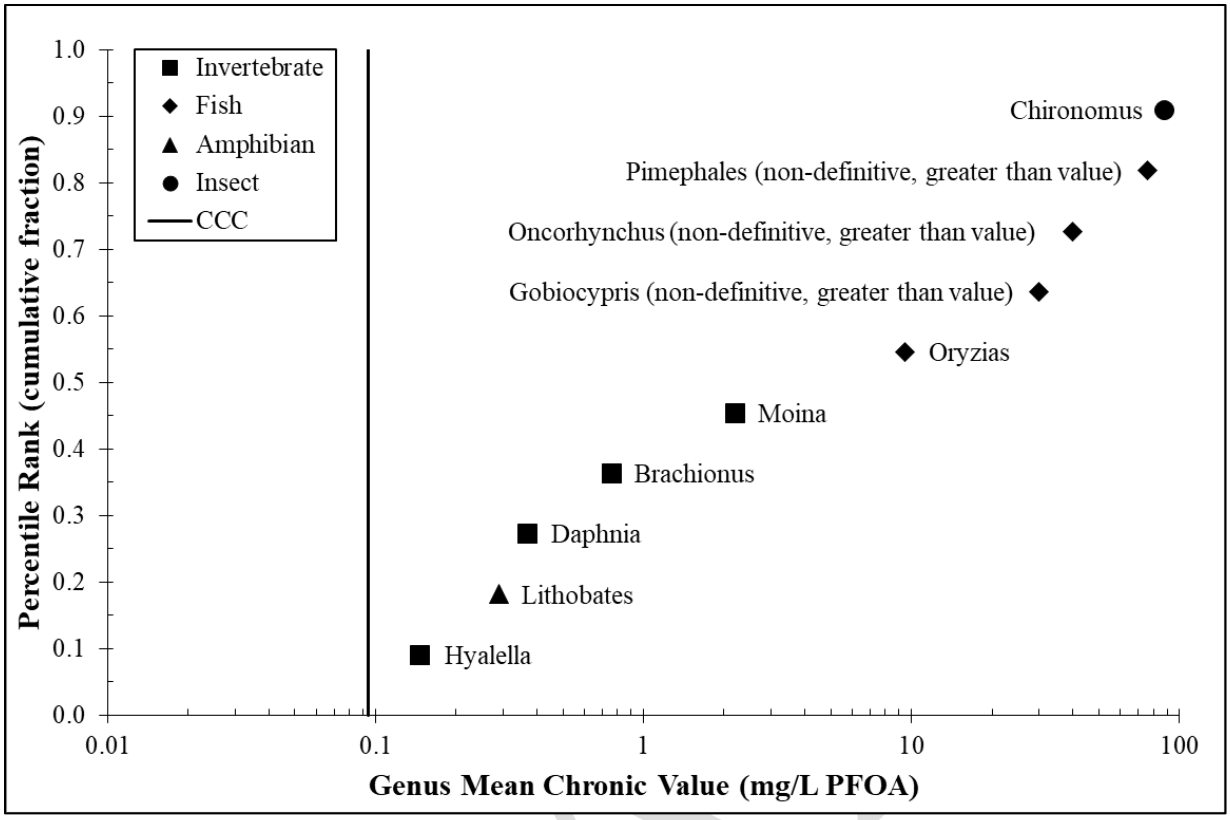

Figure 3-5. Freshwater Quantitative GMCVs used for the Criterion Calculation.

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 deceased 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, 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

There are no quantitatively acceptable GMCVs for estuarine/marine genera. Consequently, the EPA could not derive an estuarine/marine chronic criterion.

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., 0.094 mg/L; see equation *X-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 3-10 and individual BAFs are provided in Appendix P.

Table 3-10. 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).

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 *X-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 0.094 mg/L, which resulted in a chronic invertebrate whole-body tissue criterion of 1.11 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 0.094 mg/L, which resulted in a chronic fish muscle-based chronic criterion of 0.125 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 0.094 mg/L, which resulted in a chronic fish whole-body tissue criterion of 6.10 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 more than once in ten years.

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 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

Ecological recovery times following chemical disturbances are situational-specific, being largely dependent on: (1) biological variables such as the presence of nearby source populations or generational time of taxa affected; (2) physical variables such as lentic and lotic habitat considerations where recovery rates in lentic systems may be slower than lotic systems where the pollutant may be quickly flushed downstream, and; (3) chemical variables such as the persistence of a chemical and potential for residual effects. Given the large variation in possible biological and physical variable influencing ecological recovery, EPA focused on the known chemical

attributes of PFOA to inform the recommended ten-year exceedance frequencies for the chronic tissue-based criteria.

Metals and other chemical pollutants may be retained in the sediment and biota, where they can result in residual effects over time that further delay recovery. Few studies are available concerning 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 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 has 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 in North Carolina (a freshwater reservoir) had recovered significantly in the decade since selenium discharges were halted in 1985, the threat to fish had not been eliminated. The 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; 75^{th} centile ~ three years; n = 20).

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

3.3 Summary of PFOA Aquatic Life Criteria

This Aquatic Life Ambient Water Quality Criteria for PFOA document includes water column based acute and chronic criteria and tissue-based criteria for freshwaters. 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 49 mg/L, and the chronic water column-based chronic criterion magnitude is 0.094 mg/L. The fish whole-body tissue criterion magnitude is 6.10 mg/kg wet weight, the fish muscle tissue criterion magnitude is 0.125 mg/kg wet weight and the invertebrate whole-body tissue criterion magnitude is 1.11 mg/kg wet weight (Table 3-11). 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 3-11. Recommended Freshwater Perfluorooctanoic acid (PFOA) Aquatic Life Ambient Water Quality Criteria.

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	49 mg/L	0.094 mg/L	1.11 mg/kg ww	6.10 mg/kg ww	0.125 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 more than once in ten years on average		

[1] All five of these water column and tissue criteria are intended to be independently applicable and no one criterion takes primacy. All of the above recommended criteria (acute and chronic water column and tissue criteria) are intended to be protective of aquatic life. These criteria are applicable throughout the year.
[2] Tissue criteria derived from the chronic water column concentration (CCC) with the use of bioaccumulation factors and are expressed as wet weight (ww) concentrations.
[3] Tissue data provide instantaneous point measurements that reflect integrative accumulation of PFOS over time and space in aquatic life population(s) at a given site.
[4] Criterion Maximum Concentration; applicable throughout the water column.
[5] Criterion Continuous Concentration; applicable throughout the water column.

4 EFFECTS CHARACTERIZATION FOR AQUATIC LIFE

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) evaluation of the acute insect MDR (Section 4.3); (4) describes the available PFOA ACRs (Section 4.4); (5) compares the tissue-based criteria magnitudes to the empirical tissue-based effect concentrations available (Section 4.5); and (5) evaluates aquatic plant tolerance to PFOA exposures (Section 4.6).

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

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 (USFWS 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 22 species representing 16 genera (Table 4-1). The freshwater acute dataset truncated to North American resident species only was missing two MDR groups (a benthic crustacean and an insect). While the SMAV for *Daphnia carinata* was the most sensitive genus, its removal and 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 47 mg/L PFOA, which was slightly lower than the CMC (49 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, the number of genera in the dataset would have decreased by one, and the resultant exploratory FAV and acute water column concentration would have been 92.97 mg/L PFOA and 46 mg/L PFOA, respectively. 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	93.17	D	*Chydorus*	Cladoceran, *Chydorus sphaericus*	93.17
2	150.0	H	*Brachionus*	Rotifer, *Brachionus calyciflorus*	150.0
3	161.0	G	*Ligumia*	Black sandshell, *Ligumia recta*	161.0
4	164.4	G	*Lampsilis*	Fatmucket, *Lampsilis siliquoidea*	164.4
5	166.3	D	*Moina*	Cladoceran, *Moina macrocopa*	166.3
6	211.7	D	*Daphnia*	Cladoceran, *Daphnia magna*	220.0
				Cladoceran, *Daphnia pulicaria*	203.7
7	377.0	C	*Xenopus*	Frog, *Xenopus sp.*	377.0
8	413.2	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	413.2
9	572.4	B	*Danio*	Zebrafish, *Danio rerio*	572.4
10	646.2	C	*Hyla*	Gray treefrog, *Hyla versicolor*	646.2
11	664.0	B	*Lepomis*	Bluegill, *Lepomis macrochirus*	664.0
12	681.1	G	*Physella*	Bladder snail, *Physella acuta*	681.1
13	689.4	C	*Ambystoma*	Jefferson salamander, *Ambystoma jeffersonianum*	1,070
				Small-mouthed salamander, *Ambystoma texanum*	407.3
				Eastern tiger salamander, *Ambystoma tigrinum*	752.0
14	793.9	C	*Anaxyrus*	American toad, *Anaxyrus americanus*	793.9
15	951.5	C	*Lithobates*	American bullfrog, *Lithobates catesbeiana*	1,020

Rank[a]	GMAV (mg/L PFOA)	MDR Group[c]	Genus	Species	SMAV[b] (mg/L PFOA)
				Green frog, *Lithobates clamitans*	1,070
				Northern leopard frog, *Lithobates pipiens*	751.7
				Wood frog, *Lithobates sylvatica*	999
16	1,682	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	1,682

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 = 16						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Chydorus*	93.17	4.53	20.56	0.059	0.243
2	*Brachionus*	150.0	5.01	25.11	0.118	0.343
3	*Ligumia*	161.0	5.08	25.82	0.176	0.420
4	*Lampsilis*	164.4	5.10	26.03	0.235	0.485
		Σ (Sum):	**19.73**	**97.52**	**0.59**	**1.49**

S^2 =	6.59	S = slope
L =	3.975	L = X-axis intercept
A =	4.549	A = lnFAV
FAV =	94.58	P = cumulative probability
CMC =	**47 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 eight species representing eight genera (Table 4-3). The freshwater chronic dataset truncated to North American resident species was missing one MDR group (another phylum or a second insect order not already represented). Calculating the FCV based on the chronic GSD comprised of

North American resident species only resulted in an exploratory FCV of 0.04825 mg/L and a chronic water column criterion of 0.048 mg/L (rounded to two significant figures; Table 4-4). The exploratory chronic water column concentration CCC (0.048 mg/L PFOA) based on North American species only was about half of the CCC based on North American and non-North American species (0.094 mg/L PFOA) and is lower than the 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 0.094 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	4.330	D	*Daphnia*	Cladoceran, *Daphnia magna*	4.330
6	>40	A	*Oncorhynchus*	Rainbow trout, *Oncorhynchus mykiss*	>40
7	>76	B	*Pimephales*	Fathead minnow, *Pimephales promelas*	>76

Rank[a]	GMCV[b] (mg/L PFOA)	MDR Group[c,d]	Genus	Species	SMCV[b] (mg/L PFOA)
8	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 = 8						
Rank	**Genus**	**GMCV (mg/L)**	**ln(GMCV)**	**ln(GMCV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Hyalella*	0.147	-1.917	3.676	0.111	0.333
2	*Lithobates*	0.288	-1.245	1.550	0.222	0.471
3	*Brachionus*	0.7647	-0.268	0.072	0.333	0.577
4	*Moina*	2.194	0.786	0.617	0.444	0.667
		Σ (Sum):	**-2.64**	**5.91**	**1.11**	**2.05**

S^2 =	67.46	S = slope	
L =	-4.868	L = X-axis intercept	
A =	-3.031	A = lnFCV	
FCV =	0.04825	P = cumulative probability	
CCC =	**0.048 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 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 reported 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 qualitatively 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. Additionally, qualitatively-acceptable data for species within the one unfulfilled chronic MDR (i.e., another phylum or a second insect order not already represented) are evaluated relative to the chronic water column criterion. 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, Chydorus

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

4.2.1.1.2 Second most acutely sensitive genus, Daphnia

3M Co. (2000) 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 but was retained for qualitative use.

3M Co. (2000) 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 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 did not make these tests acceptable for quantitative use and they were retained for qualitative use.

3M Co. (2000) 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 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 did not make this test acceptable for quantitative use. This test was retained for qualitative use only.

3M Co. (2000) 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 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. (2000) 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 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 and other perfluoro analogue compounds the test was not acceptable for quantitative use but was retained for qualitative use.

3M Co. (2000) 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 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., 97.12 mg/L) and the acute criterion magnitude (i.e., 49 mg/L). These additional data suggest the *Daphnia* GMAV (i.e., 144.1 mg/L) used to derive the acute criterion was sufficiently protective.

4.2.1.1.3 Third most acutely sensitive genus, Brachionus

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

4.2.1.1.4 Fourth most acutely sensitive genus, Ligumia

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

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 underproductive and to provide additional supporting evidence of the potential toxicity of PFOA to aquatic organisms.

4.2.1.2.1 Genus: Dugesia (planarian)

Yuan et al. (2015) conducted a 96-hour, unmeasured renewal acute test on PFOA (96% purity) with *Dugesia japonica*. The study reported 96-hour LC_{50} was 39.35 mg/L (95% C.I. = 32.38 - 46.32 mg/L). The test was not acceptable for quantitative use because the test organisms were collected from a fountain in Quanhetou, Boshan, China, where there may have been potential exposures to PFAS from the source of the test organisms. Overall, three additional tests were available for this species (Li 2008, 2009), which resulted in a SMAV of 383.6, suggesting

this species is tolerant to acute PFOA exposures. The apparent sensitivity of the LC_{50} reported by Yuan et al. (2015) may have been the result of compounding chemical stressors originating from the source of the test organisms (i.e., fountain in Quanhetou, Boshan, China)

4.2.1.2.2 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 hpf (114-hour test 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.

Dasgupta et al. (2020) evaluated the acute effects of PFOA (CAS # 335-67-1, 96% purity, purchased from Acros Organics) on zebrafish (*Danio rerio*) via a 66-hour unmeasured, static study. At test termination there were no significant effects on survival or development of zebrafish embryos. The 66-hour NOEC of 20.70 mg/L PFOA, based on survival, was acceptable for qualitative use only due to the short exposure period (i.e., 66-hour exposure instead of the established 96-hour acute exposure for this species).

Wasel et al. (2020) reported the results of an unmeasured, renewal acute toxicity test with larval *D. rerio* and PFOA (>99% purity). The 90-91 hour LC_{50} reported by the authors was 57.6 mg/L for the unbuffered test solution. Based on the starting age of the organisms (embryo, five to six hpf), the acute test was too short to be used quantitatively, so values were acceptable

for qualitative use only, especially since other acute quantitatively acceptable tests for this species were available.

Quantitatively acceptable acute tests used to calculate the SMAV (Hagenaars et al. 2011; Godfrey et al. 2017a; Stengel et al. 2017) suggested *D. rerio* is relatively tolerant to acute PFOA exposures with a *D. rerio* SMAV of 572.4 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 five quantitatively-acceptable values available indicated the LC_{50} was an outlier, falling out more than an order of magnitude lower than the other five LC_{50} values. It is expected that *D. rerio* will be tolerant to acute PFOA exposures.

4.2.1.2.3 Genus: Pimephales (fathead minnow)

3M Co. (2000) reported the results of a 96-hour static, unmeasured acute toxicity test with the fathead minnow, *Pimephales promelas* and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot number 83 and was characterized as 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). No specific test protocol was identified. The authors extrapolated the concentration-response data graphically to estimate a 96-hour LC_{50} of 70 mg/L APFO. This test was not acceptable for quantitative use because of the lack of replicates and lack of observed effects in the test, as well as the possible mixture effects of other perfluoro analogue compounds. Although the LC_{50} reported in this test is sensitive relative to the FAV (i.e., 97.14 mg/L), quantitatively acceptable acute data with this species (Corrales et al. 2017) suggest *P. promelas* is tolerant with a SMAV of 413.2 mg/L

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

There were no qualitatively acceptable chronic tests for species within the genus, *Hyalella*.

4.2.2.1.2 Second most 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 test toxicity 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 as 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.

Overall, these two 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.

4.2.2.1.3 Third most sensitive genus, Daphnia

3M Co. (2000) 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 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 but does not suggest *D. magna* will be chronically sensitive relative to the chronic freshwater criterion.

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. 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 underproductive 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; further described in Appendix G.2.2.3) 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. 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: Danio (zebrafish)

Jantzen et al. (2017b) 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 three-hours post fertilization (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., five-day) exposure duration compared to other acceptable acute exposures that indicated *D. rerio* was tolerant to brief (i.e., 96-hours) PFOA exposures.

4.2.2.2.3 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, 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 be rejected because of pseudoreplication resulting from a lack of replicates in the hatching stage. Since this test is a static-renewal unmeasured test, 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).

4.2.2.3 Consideration of Qualitatively Acceptable Data from Missing Chronic MDRs

4.2.2.3.1 Another Phylum or a Second Insect Order not Already Represented

Yuan et al. (2016b) conducted a 10-day renewal, unmeasured test on PFOA with the planarian, *Dugesia japonica* (a non-North American species). No apical endpoints were measured as the study focused on neural genes expression and neuronal morphology in the planarian. The lowest test concentration, 0.5 mg/L, decreased the mRNA expression levels of neural genes DjFoxD, DjotxA and DjotxB. Due to a lack of apical endpoints and insufficient test

duration, the LOEC was not used quantitatively; however, it was considered acceptable for qualitative use by providing relevant toxicity information to inform relative species sensitivity as well as potential sublethal affects to inform mode of action and adverse outcome pathway (AOP) considerations.

Yuan et al. (2017) conducted another 10-day static, unmeasured test on with the planarian, *Dugesia japonica*. No apical endpoints were measured as the study focused on stress responses. The lowest test concentration, 0.5 mg/L, exhibited elevated lipid peroxidation, increased mRNA expression levels of *HSP40* and *HSP70*, two stress response genes. Due to a lack of apical endpoints and test duration, the LOEC was not used quantitatively but considered acceptable for qualitative use by providing relevant toxicity information as well as potential sublethal affects to inform mode of action and AOP considerations.

Chronic toxicity values for *Dugesia japonica* reported by Yuan et al. (2016b) and Yuan et al. (2017) were not based on apical effects associated with growth, survival or reproduction. Therefore, the qualitative data provided by these studies did not provide useful information about the relative sensitivity of this species. As a result, the MDR for another phylum or a second insect order not already represented remains unfulfilled. However, authors did not report noticeable effects to apical endpoints at the test concentration of 0.5 mg/L, suggesting the chronic water column criterion (i.e., 0.094 mg/L) is protective of *Dugesia japonica* and potentially other members of the unfulfilled MDR.

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

The acute data set for PFOA contained 18 genera (Table 3-3) representing seven of the eight taxonomic MDR groups. The missing MDR was a representative from an insect family. Evaluation of qualitatively acceptable insect data (i.e., Yang et al. 2014) relative to the acute

criterion magnitude was the primary line of evidence used to inform insect sensitivity to acute PFOA exposures (see section 3.1.1.1.5). Acute insect LC_{50} data were estimated using Web-ICE and compared to the acute criterion as a secondary line of evidence to evaluate insect sensitivity to acute PFOA exposures.

EPA's web-ICE tool is described in detail in Appendix L.1.1. Briefly, ICE models are log-linear regressions of the acute toxicity (EC_{50}/LC_{50}) of two species across a range of chemicals, thus representing the relationship of inherent sensitivity between those species (Raimondo et al. 2010). ICE models can be used predict the sensitivity of an untested taxon (predicted taxa are represented by the y-axis) from the known, measured sensitivity of a surrogate species (represented by the x-axis). This analysis focused on all possible ICE models that used insects as a predictor species (i.e., y-axis) and a corresponding surrogate input species (i.e., x-axis) for which a SMAV (see Table 3-3) was available. These models are shown in Table 4-5 along with use classifications for each individual model based on a host of statistical metrics described by Willming et al. (2016; see box one of Appendix L.1.1 for additional discussion on model use criteria).

Table 4-5. All ICE models available in Web-ICE v3.3 for predicted insect 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)	R2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µg/L)	Surrogate model maximum value (µg/L)	Cross-validation Success (%)	Taxonomic Distance	Use Classification
Atherix variegata	*Lepomis macrochirus*	0.85	0.9	2	0.91	0.0428	0.08	0.36	59.53	100	6	Accepted qualitatively
Atherix variegata	*Oncorhynchus mykiss*	0.94	0.73	2	0.91	0.0439	0.08	0.61	59.27	100	6	Accepted qualitatively
Chironomus plumosus	*Americamysis bahia*	0.64	1.1	9	0.65	0.0026	0.97	0.01	5083	45	5	Rejected
Chironomus plumosus	*Daphnia magna*	0.63	1.05	19	0.5	0.0002	1.14	0.13	39000	29	5	Rejected
Chironomus plumosus	*Lepomis macrochirus*	0.74	0.37	21	0.43	0.0006	1.1	0.77	45166	26	6	Rejected
Chironomus plumosus	*Oncorhynchus mykiss*	0.78	0.3	21	0.5	0.0001	1.04	0.82	140000	35	6	Rejected
Chironomus plumosus	*Pimephales promelas*	1.03	-0.46	15	0.64	0.0001	0.99	2.27	97000	35	6	Rejected
Chironomus tentans	*Daphnia magna*	0.83	0.94	7	0.79	0.0011	1.03	0.32	472000	33	5	Rejected
Chironomus tentans	*Lepomis macrochirus*	0.95	0.05	6	0.8	0.0027	0.88	2.85	517825	25	6	Accepted
Chironomus tentans	*Oncorhynchus mykiss*	1.11	-0.64	5	0.81	0.005	0.95	11.24	905704	29	6	Accepted qualitatively
Chironomus tentans	*Pimephales promelas*	1.21	-1.04	5	0.8	0.006	1.34	19.63	766452	57	6	Rejected
Claassenia sabulosa	*Americamysis bahia*	0.34	0.4	3	0.77	0.049	0.04	0.04	8.85	100	5	Rejected
Claassenia sabulosa	*Lepomis macrochirus*	0.4	-0.34	7	0.63	0.0102	0.19	0.36	7326	78	6	Rejected
Claassenia sabulosa	*Oncorhynchus mykiss*	0.42	-0.43	7	0.55	0.0213	0.23	0.61	1638	67	6	Rejected
Claassenia sabulosa	*Pimephales promelas*	0.33	-0.62	6	0.63	0.0182	0.22	1.24	110000	75	6	Rejected
Paratanytarsus dissimilis	*Daphnia magna*	0.57	2.17	8	0.41	0.0441	1.96	0.66	1190000	50	5	Rejected

Predicted Species	Surrogate Species	Slope	Intercept	Degrees of Freedom (N-2)	R2	p-value	Mean Square Error (MSE)	Surrogate model minimum value (µg/L)	Surrogate model maximum value (µg/L)	Cross-validation Success (%)	Taxonomic Distance	Use Classification
Paratanytarsus dissimilis	*Lepomis macrochirus*	0.88	0.84	4	0.92	0.0024	0.41	0.36	201000	50	6	Accepted
Paratanytarsus dissimilis	*Lithobates catesbeianus*	0.92	0.67	4	0.84	0.0093	0.99	2.50	3019983	67	6	Rejected
Paratanytarsus dissimilis	*Oncorhynchus mykiss*	0.8	1.45	8	0.83	0.0002	0.54	0.61	1330000	70	6	Accepted
Paratanytarsus dissimilis	*Pimephales promelas*	0.8	1.27	10	0.8	0	0.52	1.24	1430000	75	6	Accepted
Paratanytarsus parthenogeneticus	*Daphnia magna*	0.93	0.74	5	0.98	0	0.04	9.91	14500000	100	5	Accepted
Paratanytarsus parthenogeneticus	*Lepomis macrochirus*	1	0.63	5	0.83	0.0037	0.66	103.94	7100000	57	6	Accepted qualitatively
Paratanytarsus parthenogeneticus	*Oncorhynchus mykiss*	0.86	1.45	4	0.78	0.0193	1.1	32	9800000	50	6	Rejected
Paratanytarsus parthenogeneticus	*Pimephales promelas*	1.05	0.22	4	0.97	0.0002	0.13	92	10600000	83	6	Accepted
Pteronarcella badia	*Americamysis bahia*	0.72	0.83	4	0.83	0.0112	0.4	0.12	7300	50	5	Accepted
Pteronarcella badia	*Lepomis macrochirus*	0.45	0	12	0.32	0.0343	0.76	0.36	7326	71	6	Rejected
Pteronarcella badia	*Oncorhynchus mykiss*	0.59	-0.21	15	0.48	0.0018	0.88	0.61	1100000	47	6	Rejected
Pteronarcella badia	*Pimephales promelas*	0.28	-0.06	8	0.7	0.0023	0.09	1.24	110000	100	6	Rejected
Pteronarcys californica	*Daphnia magna*	0.63	0.72	24	0.54	0	0.94	0.15	68300	42	5	Rejected
Pteronarcys californica	*Lepomis macrochirus*	0.64	0	43	0.28	0.0001	1.72	0.36	95000	31	6	Rejected
Pteronarcys californica	*Oncorhynchus mykiss*	0.63	0.05	44	0.25	0.0003	1.7	0.61	70500	35	6	Rejected

Table 4-6 shows model outputs from all the rejected, qualitatively acceptable, and acceptable ICE models listed in Table 4-5. PFOA acute values are typically reported as mg/L and are, therefore, often greater than the toxicity values used to develop an ICE model, meaning the input PFOA LC_{50} value of the surrogate was typically outside the model domain. In these cases, the input toxicity value could be entered as µg/L and model would be allowed to extrapolate beyond its range or the input toxicity value could be a "scaled" mg/L value (i.e., estimate the value as mg/L). Table 4-6 includes a column to denote whether the input toxicity data were µg/L or a "scaled" mg/L value for individual models. Please see Appendix L for further discussion on the selection process for identifying whether a µg/L or a "scaled" mg/L value was used as the input toxicity value for individual models.

Within Table 4-6, bolded and underlined values in the "Estimated Toxicity" column represent the estimated LC_{50} values from the acceptable models. Only estimated toxicity values from acceptable models were used to develop the estimated insect SMAVs reported in Table 4-6. When more than one acceptable ICE model was available for an individual predicted insect species, all the acceptable estimated toxicity values (i.e., LC_{50} values) were taken together as a geometric mean to represent the estimated SMAV.

Table 4-6. ICE-estimated Insect Species Sensitivity to PFOA.
Values in bold and underlined are used for estimated insect SMAVs.

Common Name	Predicted Species	Surrogate Species	µg/L or mg/L input	Estimated Toxicity (mg/L)	95% Confidence Intervals (mg/L)	SMAV
Snipefly	*Atherix variegata*	*Lepomis macrochirus*	mg/L	2027.47[ab]	35.46 - 115910.82	NA
		Oncorhynchus mykiss	mg/L	6271.94[ab]	38.26 - 1028076.54	
Midge	*Chironomus plumosus*	*Americamysis bahia*	µg/L	8.33[bc]	0.44 - 159.47	NA
		Daphnia magna	µg/L	28.83[bc]	2.61 - 318.13	
		Lepomis macrochirus	µg/L	51.17[bc]	2.95 - 888.56	
		Oncorhynchus mykiss	µg/L	155.22[bc]	8.12 - 2966.13	
		Pimephales promelas	µg/L	210.74[bc]	12.78 - 3492.24	
Midge	*Chironomus tentans*	*Daphnia magna*	µg/L	251.45[c]	15.1 - 4186.53	575.87
		Lepomis macrochirus	mg/L	**575.87**	86.87 - 3817.34	
		Oncorhynchus mykiss	mg/L	868.79[a]	97.49 - 7742.26	
		Pimephales promelas	µg/L	621.10[ac]	12.55 - 30731.5	
Stonefly	*Claassenia sabulosa*	*Americamysis bahia*	µg/L	0.083[bc]	0.002 - 3.32	NA
		Lepomis macrochirus	µg/L	0.10[bc]	0.006 - 1.8	
		Oncorhynchus mykiss	µg/L	0.16[bc]	0.004 - 6.07	
		Pimephales promelas	µg/L	0.017[bc]	0.002 - 0.13	
Midge	*Paratanytarsus dissimilis*	*Daphnia magna*	µg/L	168.49[ac]	5.34 - 5318.67	1557.92
		Lepomis macrochirus	mg/L	**575.87**	388.32 - 12682.13	
		Lithobates catesbeianus	µg/L	1627.49[ac]	30.27 - 87514.09	
		Oncorhynchus mykiss	mg/L	**11063.24**	3183.00 - 38452.77	
		Pimephales promelas	µg/L	**593.51**	110.45 - 3189.21	
Midge	*Paratanytarsus parthenogeneticus*	*Daphnia magna*	µg/L	**568.96**	313.37 - 1033.01	890.25
		Lepomis macrochirus	µg/L	3147.45[a]	152.44 - 64987.96	
		Oncorhynchus mykiss	µg/L	6405.37[ac]	55.24 - 742788.68	
		Pimephales promelas	µg/L	**1392.97**	351.36 - 5522.46	
Stonefly	*Pteronarcella badia*	*Americamysis bahia*	mg/L	**67.06**	10.47 - 429.35	67.06
		Lepomis macrochirus	µg/L	0.44[bc]	0.01 - 16.72	
		Oncorhynchus mykiss	µg/L	3.27[bc]	0.15 - 69.67	
		Pimephales promelas	µg/L	0.033[bc]	0.01 - 0.1	
Stonefly	*Pteronarcys californica*	*Daphnia magna*	µg/L	13.96[bc]	1.19 - 163.96	NA
		Lepomis macrochirus	µg/L	5.55[bc]	0.45 - 68.82	
		Oncorhynchus mykiss	µg/L	10.6[bc]	0.52 - 215.73	

[a] Both confidence intervals >1.5 order magnitude.
[b] Input data outside model range.
[c] Guidance for model mean square error, R^2, and/or slope not met.

Overall, acceptable ICE models and empirical acute PFOA LC_{50} values as model input data were available to support the estimation of SMAVs for four individual insect species (Table 4-6). Estimated insect SMAVs ranged from 67.06 mg/L for the stonefly, *Pteronarcella badia*, to 1,557.92 mg/L for the midge, *Paratanytarsus dissimilis*. Beyond the *P. badia* estimated SMAV, the remaining three estimated SMAVs were greater than the FAV (i.e., 97.14 mg/L; see section 3.2.1.1) by more than a factor of five. The FAV was greater than the *P. badia* estimated SMAV by nearly a factor of 1.5, suggesting *P. badia* may be relatively sensitive to acute PFOA exposures. However, the *P. badia* estimated SMAV was based on a single estimated LC_{50} value of 67.06 mg/L with corresponding 95% confidence intervals that ranged from 10.47 - 429.35 mg/L.

Three of the four estimated insect SMAVs were greater than the FAV. Further, qualitatively acceptable empirical insect toxicity data (i.e., Yang et al. 2014; see section 3.1.1.1.5) suggested insects may not be among the most sensitive genera to acute PFOA exposures. PFOA insect toxicity testing is an active and ongoing area of research within the ecotoxicological scientific community that will likely provide information to evaluate the sensitivity of insects to acute and chronic PFOA exposures before the PFOA criteria document is finalized. EPA will continue to seek additional acute PFOA insect data to further evaluate the sensitivity of insects.

4.4 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. For descriptive purposes, 11 individual ACRs for four

invertebrate species, one fish, 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 14.5 to 3,493 across all species and SMACRs ranged from <17.68 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.

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. There was an apparent relationship between SMACRs and SMAVs, but only after excluding the *L. catesbeiana* ACR. The 1985 Guidelines do not provide for calculation of a FACR under these circumstances. However, if one were calculated as the geometric mean of the six SMACRs reported in Appendix I, it would be 207.5.

4.5 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 (4.932 mg/L, measured), which corresponded to liver, gill, and muscle PFOA concentrations of 17.11, 35.13, and 6.07 mg/kg wet 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 2 mg/L for 56 days. Corresponding tissue PFOA concentrations in blood, liver, and muscle 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 2 mg/L for 56 days. PFOA liver concentrations in fish exposed to 2 mg/L for 56 days were 0.0284 mg/kg

wet weight, similar to Giari et al. (2016). 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 (10.5, 10.92, and 1,110 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 3.87 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 1.08 mg/kg wet weight. No effects of PFOA on mortality, growth, or development time were reported.

Tissue PFOA concentrations reported in these qualitative studies were lower than the tissue-based criteria calculated from BAFs, with the exceptions of the 96-hour liver- and muscle-based NOECs of 17.11 mg/kg and 6.07 mg/L, respectively, reported by Feng et al. (2015), which were greater than the corresponding liver tissue value of 0.221 mg/kg and muscle tissue criterion magnitude of 0.125 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.

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.

4.6 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., 0.094 mg/L PFOA), with the exception of a green alga (*Chlorella pyrenoidosa*) with a 96-hour growth-based NOEC of >0.1 mg/L (Li et al. 2021a). Excluding the low NOEC reported by Li et al. (2021; NOEC > 0.1 mg/L), effect concentrations

for freshwater plants and algae ranged from 5.7 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.

4.7 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 Perfluorooctanoic acid (PFOA) document includes water column based acute and chronic criteria and tissue-based criteria for freshwaters. 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 49 mg/L, and the chronic water column-based chronic criterion magnitude is 0.094 mg/L. The fish whole-body tissue criterion magnitude is 6.10 mg/kg wet weight, the fish muscle tissue criterion magnitude is 0.125 mg/kg wet weight and the invertebrate whole-body tissue criterion magnitude is 1.11 mg/kg wet weight (Table 3-11). Although empirical PFOA toxicity data for estuarine/marine species were not available to fulfill the eight MDRs directly, EPA included an acute aquatic life benchmark for estuarine/marine environments in Appendix L, using available estuarine/marine species toxicity data and a NAM application of ORD's peer-reviewed webICE tool. The estuarine/marine acute water column-based benchmark magnitude is 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.

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, 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, 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

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	EPA Calculated Effect Conc. (mg/L)	Final Effect Conc. (mg/L)[e]	Species Mean Acute Value (mg/L)	Reference
Cladoceran (<24 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
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]**	220.0	Yang et al. 2019
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

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
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**	-	DuPont Haskell Laboratory 2000
Rainbow trout (juvenile, 40-50 mm), *Oncorhynchus mykiss*	S, U	96 hours	APFO 99.7%	6.0-8.5	13-17	LC_{50}	707	-	**707**[d]	1,682	Colombo et al. 2008
Zebrafish (embryo), *Danio rerio*	S, U	96 hours	PFOA ≥97%	7.2-7.5	26	LC_{50}	>500	-	**>500**	-	Hagenaars et al. 2011
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**	572.4	Stengel et al. 2017
Fathead minnow (larva), *Pimephales promelas*	S, U	96 hours	PFOA Unreported	7.5	25	LC_{50}	413.2	-	**413.2**	413.2	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
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

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 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
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

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
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	-	1,070[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 determined as 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 lifestages.

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 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 EPA request. In such cases, EPA did not independently calculate toxicity values and the author-reported effect concentrations were used to derive the criterion.

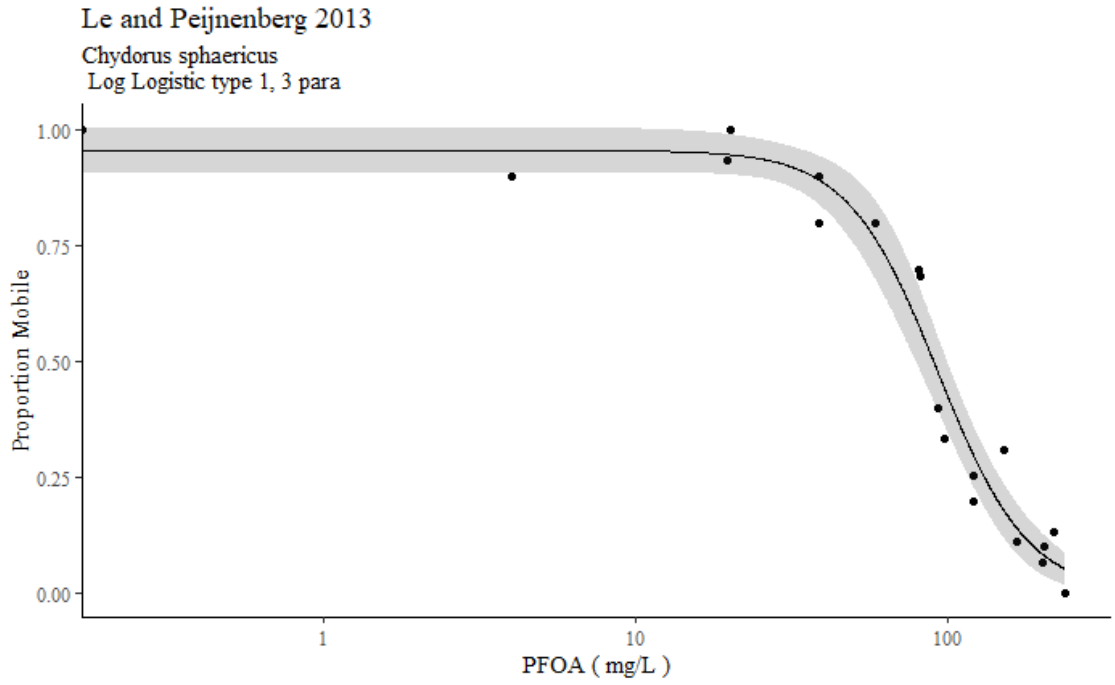
A.2.1 Most acutely sensitive genus – *Chydorus*

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 µL 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 ± 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). 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.2.2 Second most acutely sensitive genus – *Daphnia*

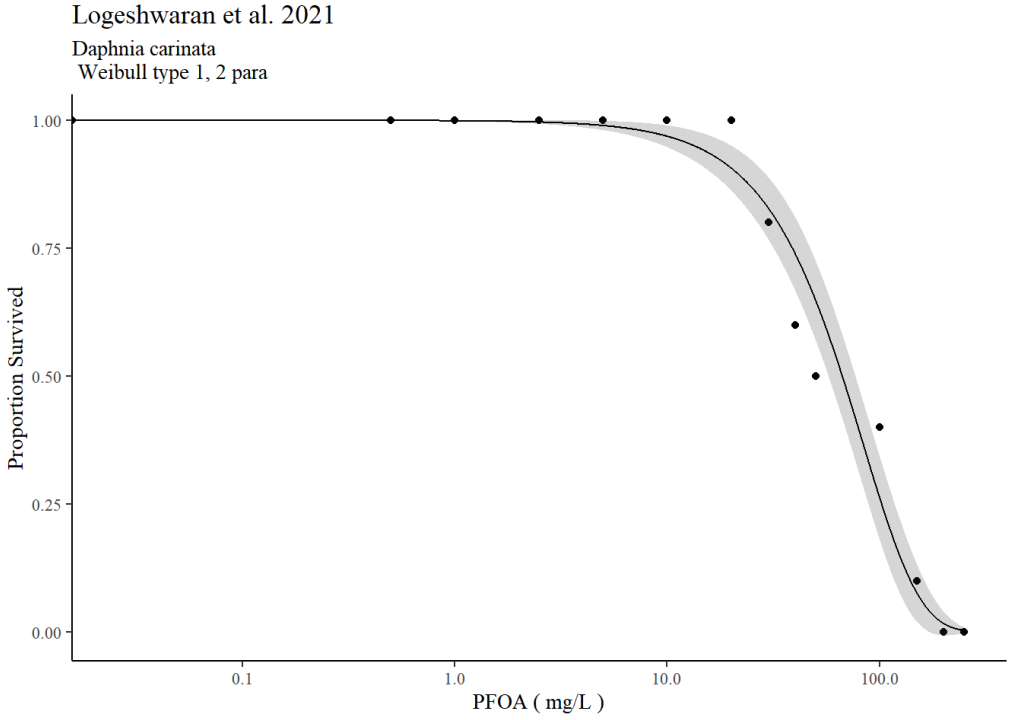
Logeshwaran et al. (2021) conducted acute and chronic toxicity tests with the cladoceran, *Daphnia carinata*, and PFOA (95% purity, purchased from Sigma-Aldrich Australia). In-house cultures of daphnids were maintained in 2 L glass bottles with 30% natural spring water in deionized water, 21°C and a 16-hour:8-hour light:dark photoperiod. The acute test protocol followed OECD guidelines (2000a) 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:



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. 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 ± 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 PFOA (ammonium salt, 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 was 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, 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.

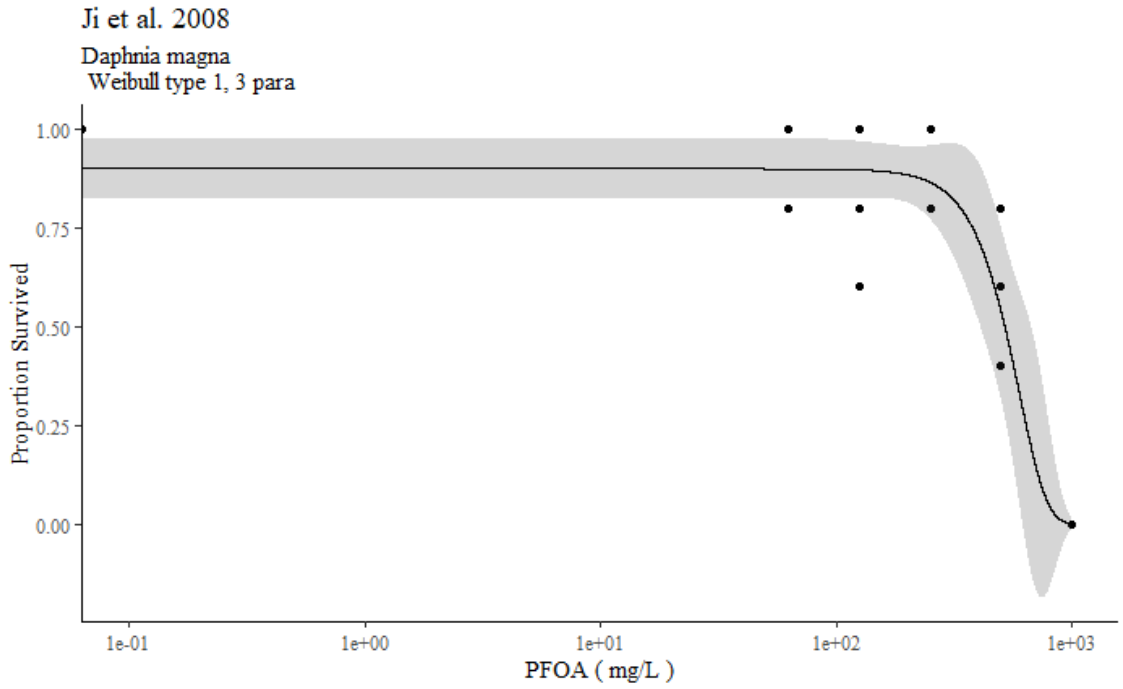
Publication: Colombo et al. (2008)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated LC_{50}: Not calculable, unable to fit a model with significant parameters
Concentration-Response Model Fit: Not Applicable

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 (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 ± 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. 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). 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:



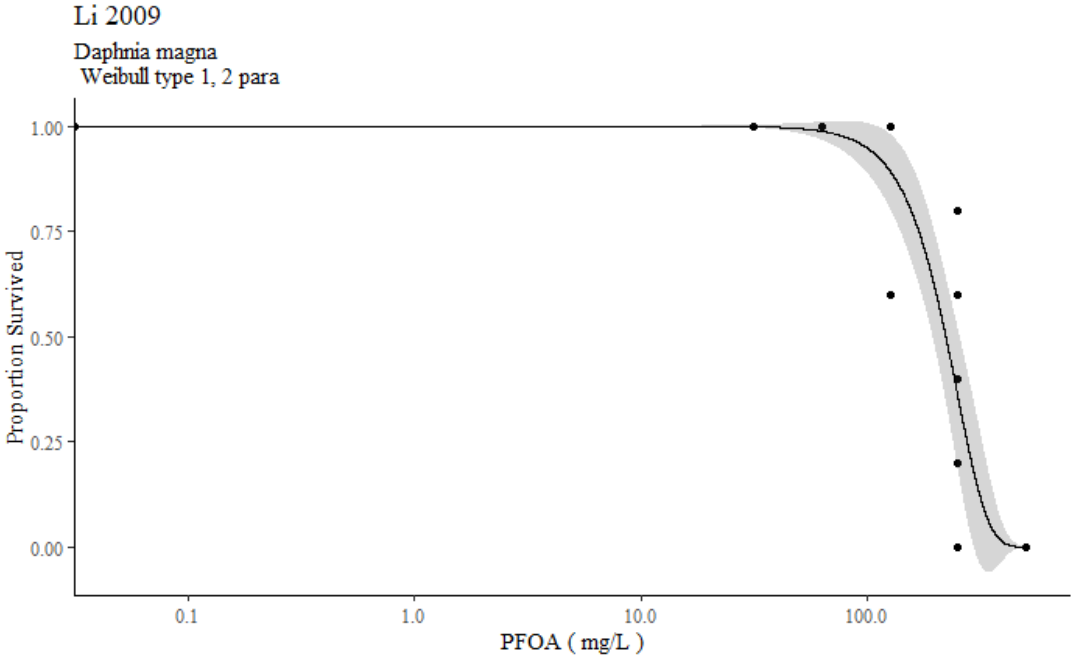
Li (2009) conducted a 48-hour static unmeasured acute test on PFOA (ammonium salt, >98% purity) with *Daphnia magna*. The authors stated that the test followed OECD 202 (1984) with slight modifications. *D. magna* used for the test were less than 24-hours old at test initiation. Dilution water was dechlorinated 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 ± 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 ± 6.8% saturation and 101.8 ± 6.8 µS/cm. After the 48-hour testing period, dissolved oxygen and specific conductivity were 55.6 ± 1.26% saturation (implying 4.56 mg/L) and 109.1 ± 3.5 µS/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. EPA performed C-R analysis for each individual test. All three tests had acceptable curves with EPA-calculated EC_{50}s 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	$< 2.2\ e^{-16}$

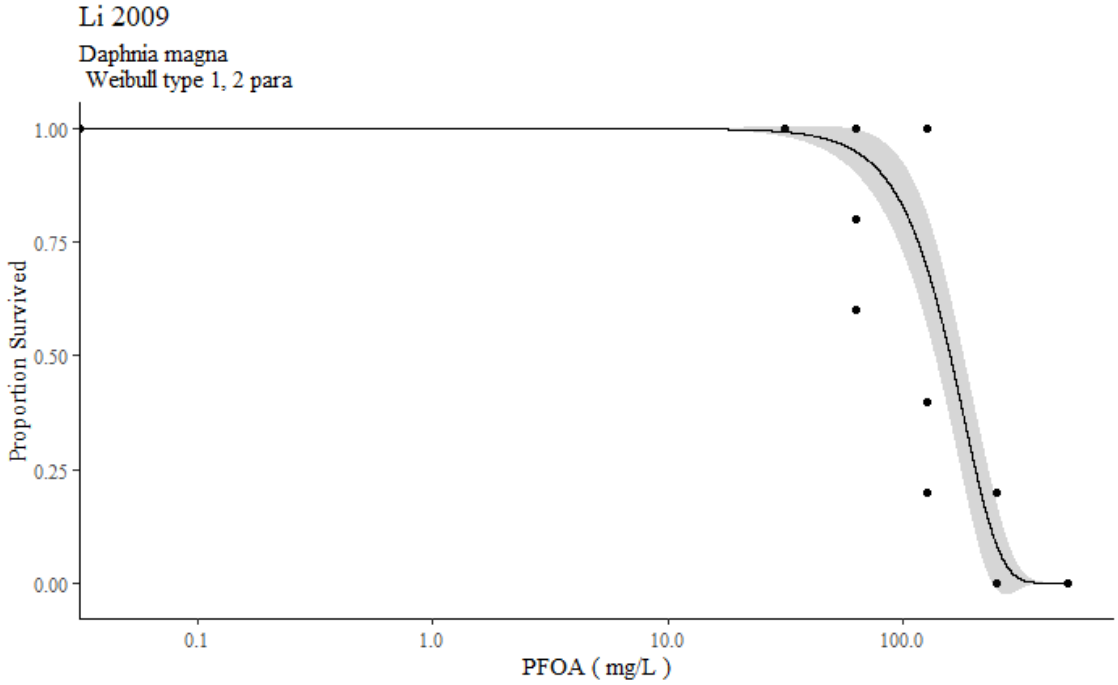
Concentration-Response Model Fit:



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	$< 2.2\ e^{-16}$

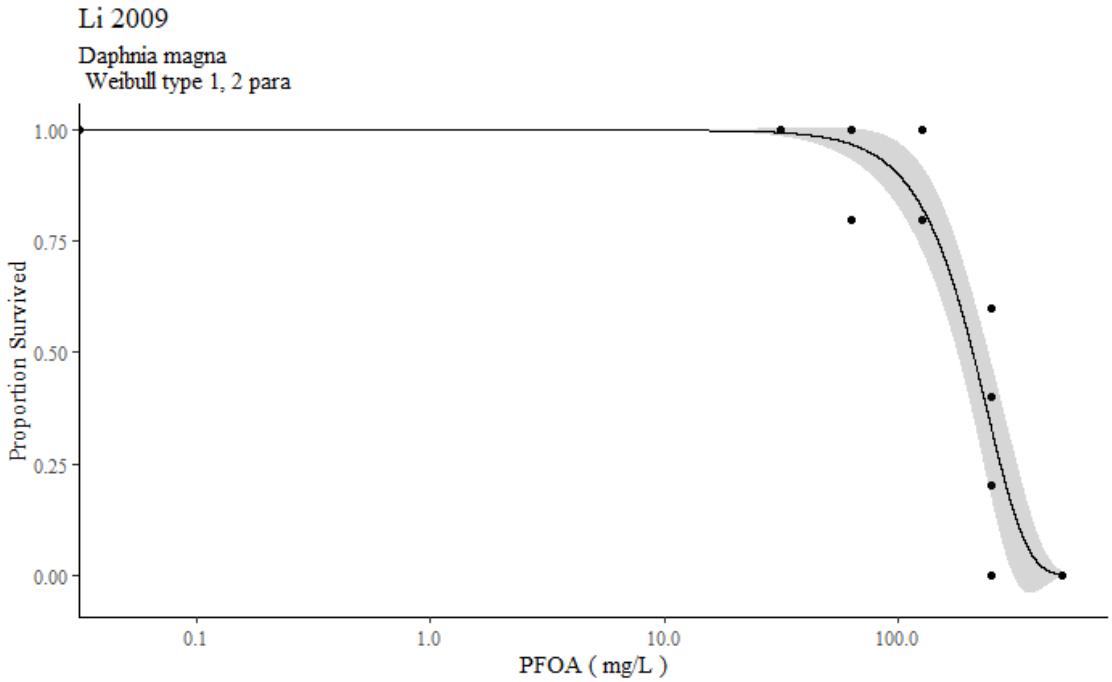
Concentration-Response Model Fit:



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:



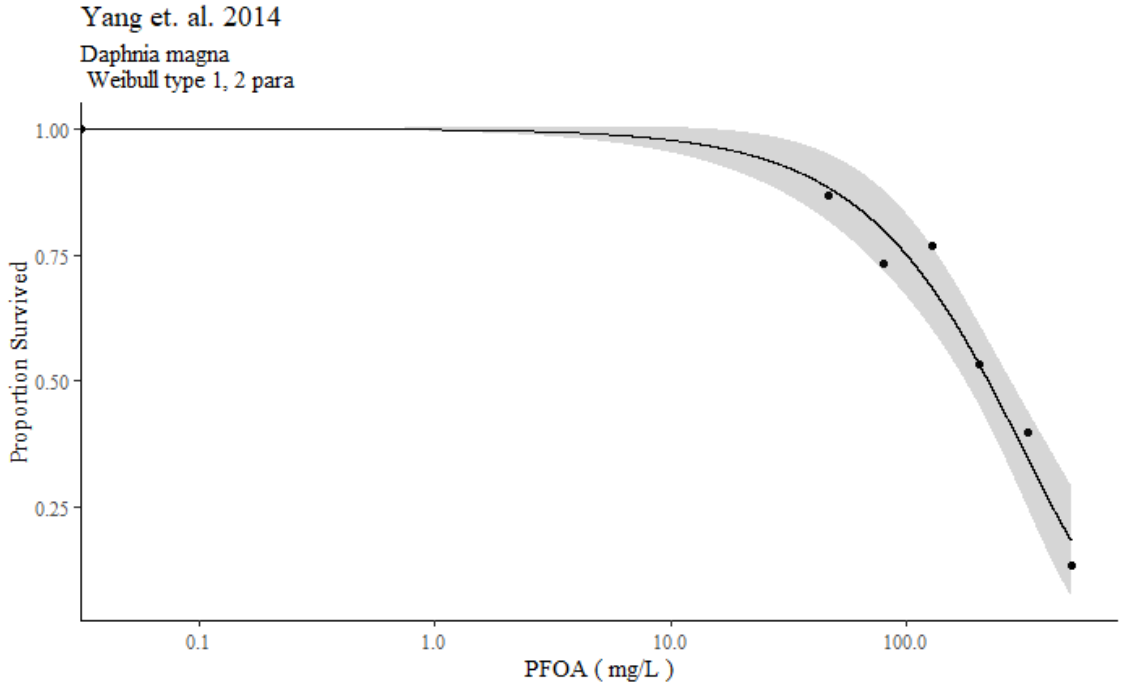
Yang et al. (2014) conducted a 48-hour measured acute test of PFOA (CAS # 335-67-1, 99% purity) with *Daphnia magna*, following ASTM E729 (1993). Although the authors termed the test conditions "static", they also mentioned PFOA measurements before and after renewal; based on this distinction the test 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). 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:



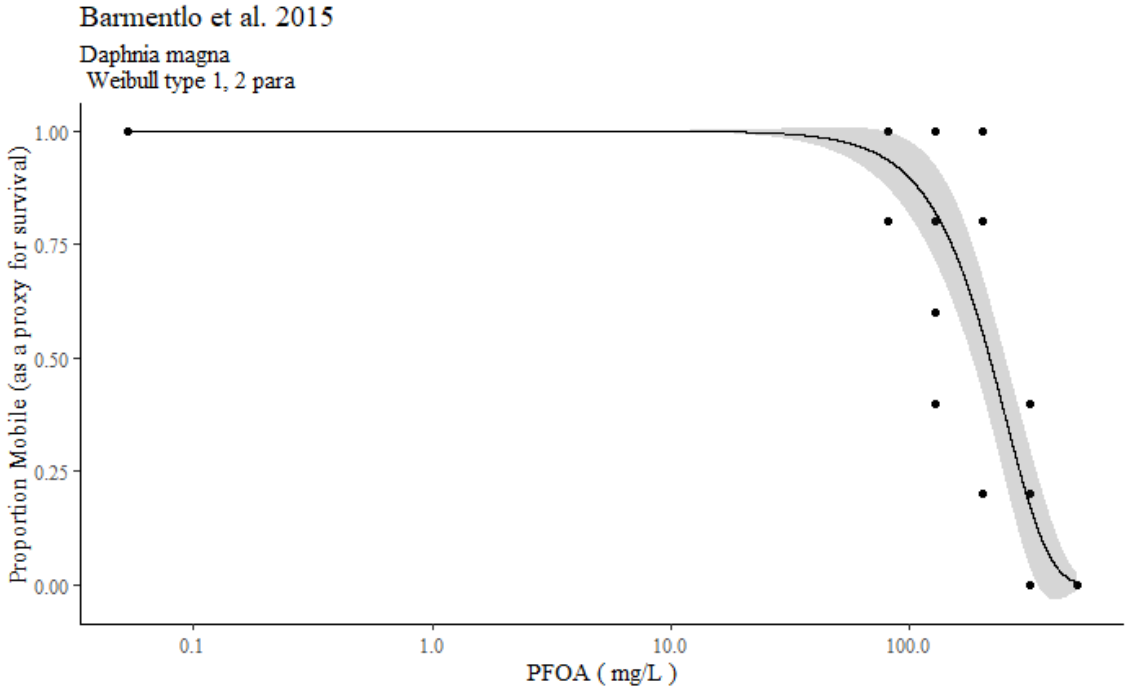
Barmentlo et al. (2015) performed a 48-hour static, measured acute test of PFOA (CAS # 335-67-1, >96%) with *Daphnia magna*. Authors stated the test followed OECD 202 (2004) guidelines for testing. *D. magna* used for testing were obtained from Grontmij, Amsterdam, and cultured in M4 media according to OECD 211 (2008). 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 ± 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). 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:



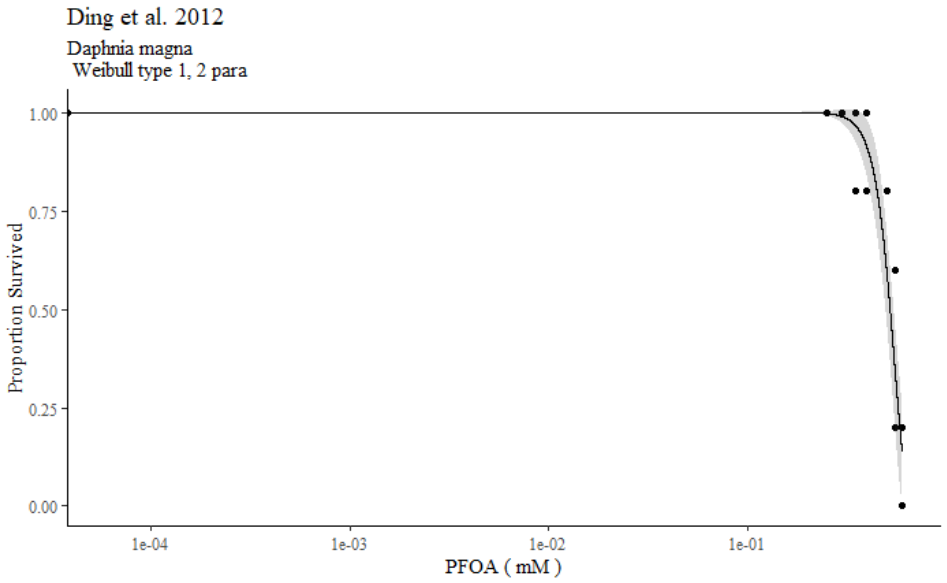
Ding et al. (2012a) conducted a 48-hour static, partially measured acute test on PFOA (CAS # 335-67-1; 96% purity from Sigma Aldrich) with *D. magna*. The test was performed following OECD test guideline 202 (2004) with slight modifications. *D. magna* used for testing were purchased from local suppliers 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 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. 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.

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:



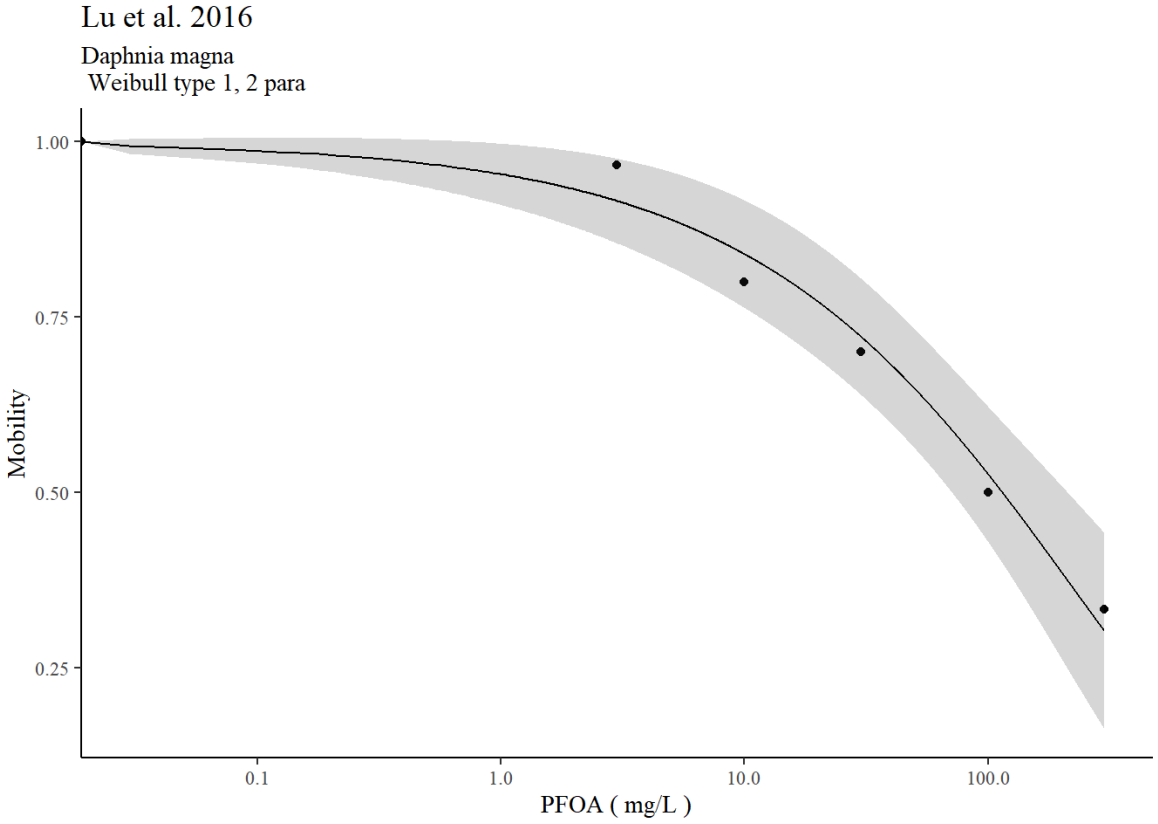
Lu et al. (2016) evaluated the acute toxicity of PFOA (CAS# 335-67-1, 98% purity, purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) on *Daphnia magna* immobilization. Reconstituted daphnia culture media was used for both culturing and test solution preparation as described in OECD Test 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 h/8 h 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). 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:



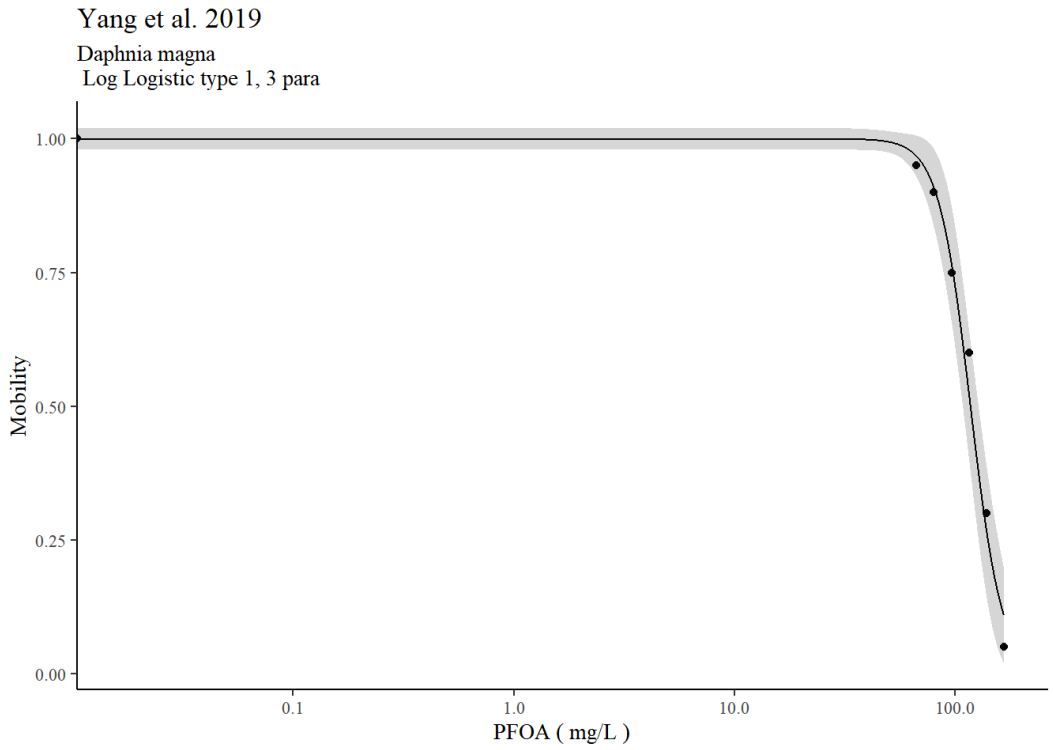
Yang et al. (2019) evaluated the acute effects of PFOA (CAS# 335-67-1, purchased from Sigma-Aldrich in St. Louis, MO) on *Daphnia magna* via a 48-hour unmeasured static exposure. *D. magna* cultures were originally obtained from the Institute of Hydrobiology of Chinese Academy of Science in Wuhan, China. 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 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:



A.2.3 Third 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.2.4 Fourth and fifth most acutely sensitive genera – *Ligumia* and *Lampsilis* (mussels)

Hazelton et al. (2012) and Hazelton (2013) 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). Authors stated 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.

Text pertaining to C-R modeling below is only described for *L. recta* because this species was among the four most sensitive acute genera; *L. siliquoidea* was among the fifth most sensitive genus and C-R curves were only displayed for those species within the four most sensitive acute genera.

Publication: Hazelton et al. (2012, 2013)
Species: Black sandshell, (*Ligumia recta*)
Genus: *Ligumia*
EPA-Calculated LC_{50}: Not calculable, concentration-response data not available
Concentration-Response Model Fit: Not Applicable

A.2.5 Sixth 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 ± 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). 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.

A.2.6 Seventh 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 ± 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 ± 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). 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 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.2.7 Eighth most acutely sensitive genus – *Dugesia*

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 ± 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 ± 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 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. EPA performed C-R analysis for each individual test. Two of the tests had acceptable curves with 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.2.8 Ninth most acutely sensitive genus – *Pimephales*

Corrales et al. (2017) 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 h:8 h 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.2.9 Tenth most acutely sensitive genus – *Neocaridina*

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 ± 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. EPA performed C-R analysis for each individual test. All three tests had acceptable curves with 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 Eleventh most acutely sensitive genus – *Danio*

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

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 ± 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, 4.5- 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. EPA performed C-R analysis for each individual test. Three tests had acceptable curves with 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) 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. 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. EPA performed C-R analysis for this test with an EPA-calculated LC_{50} of 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 five quantitatively-acceptable zebrafish LC_{50} values available (Godfrey et al. 2017a; Hagenaars et al. 2011; Stengel et al. 2017), indicated the LC_{50} reported by **Corrales et al. (2017)** was an outlier, falling out more than an order of magnitude lower than the other four LC_{50} values.

A.2.11 Twelfth 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 2008, 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 to10 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 Appendix G.

A.2.12 Thirteenth 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-h 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. 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.2.13 Fourteenth 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 ± 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. EPA performed C-R analysis for each individual test. All three tests had acceptable curves with 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.2.14 Fifteenth most acutely sensitive genus – *Ambystoma*

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 2008, 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℃ 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, lifestage 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. 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. 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 EPA was unable to fit a C-R model with significant parameters and relied on the author reported value as quantitatively acceptable.

A.2.15 Sixteenth most acutely sensitive genus – *Anaxyrus*

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 2008, 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 all treatments 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 26 Gosner stage test and was 806.6 (95% C.I. = 760.6 – 852.6 mg/L) mg/L for the 41 Gosner stage test, both of which were quantitatively acceptable for use.

A.2.16 Seventeenth 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. 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 2008, 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. 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. EPA was unable to fit a C-R model with significant parameters and relied on the author reported value as quantitatively acceptable.

A.2.17 Eighteenth most acutely sensitive genus – *Oncorhynchus*

The acute effects of ammonium perfluorooctanoate (APFO, 99.4% purity) to *Oncorhynchus mykiss* was 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. 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. This study was classified as quantitatively acceptable for use in criterion derivation.

Colombo et al. (2008) also evaluated the acute toxicity of ammonium perfluorooctanoate (APFO, CAS # 3825-26-1, 99.7% purity) to *O. mykiss*. Authors stated that the unmeasured static 96-hour GLP test followed OECD test guideline 203 and EU Commission Directive 92/69/EEC. APFO stock solutions were prepared by dissolving the test substance directly in the dilution water and then diluting the stock solution to provide a geometric series of test concentrations (nominal concentrations of 31.3, 62.5, 125, 250, 500 and 1,000 mg/L APFO). Analyses to confirm the APFO test concentrations were not performed during the acute tests based on the known stability of the test substance in water, but tests concentrations were measured in the chronic test. One replicate test chamber containing seven fish was used for the control and each test solution concentration. Test organisms were randomly assigned to the test solutions after a pre-test acclimation period of 12 days. Test organisms loading was 0.76 g/L during the study

with juvenile fish length ranging from 40 to 50 mm. Dilution water was filtered, dechlorinated tap water that was treated by a softening system to obtain the desired total hardness of 150 ± 20 mg/l as $CaCO_3$, and pH of 6.0-8.5. A light:dark cycle of 16-hours:8-hours, a temperature of 13-17 ± 1°C, and dissolved oxygen greater than 60% saturation were used for acclimation and testing. Fish were fed trout chow twice daily during the acclimation period, but were not fed during the 24-hour pre-test period or during acute testing. Fish were observed for mortality and visible abnormalities at 0, 2, 4, 24, 48, 72 and 96-hours. The study reported a 96-hour LC_{50} of 707 mg/L as PFO^-. 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. EPA does not believe ammonia was a significant contributor of toxicity (see section C.2.8). Furthermore, Table 7 of Colombo et al. (2008) reports unionized ammonia at *the calculated APFO* [chronic] *NOEC of 40 mg/L* to be 0.013 mg/L un-ionized ammonia, nearly half the unionized ammonia concentration described in the supportive text (i.e., 0.021 mg/L). Therefore, this acute rainbow trout toxicity test is classified as quantitatively acceptable.

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	LC_{50}	24	-	**24**	24	Hayman et al. 2021
Myside (neonate, <24 hours), *Siriella armata*	S, U	96 hours	PFOA 96%	-	20	-	LC_{50}	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, 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's 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 Second most acutely sensitive estuarine/marine genus - *Mytilus* (mussel)

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 ± 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, 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*. 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 Third most acutely sensitive estuarine/marine genus - *Strongylocentrotus* (sea urchin)

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*. Authors noted 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℃. 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℃ 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*. Authors noted 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℃ 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. 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 (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%	-	18-22	EC_{10} (average # of live young)	29.73	20.61	**20.61**[d]	-	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
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]**	4.330	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

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Chronic Value Endpoint	Author Reported Chronic Value (mg/L)	EPA Calculated Chronic Value (mg/L)	Final Chronic Value (mg/L)[c]	Species Mean Chronic Value (mg/L)	Reference
Midge (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
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	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 determined as 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 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 EPA request. In such cases, EPA did not independently calculate toxicity values and the author-reported effect concentrations were used to derive the criterion.

C.2.1 Most chronically sensitive genus – *Hyalella*

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. 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. 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.

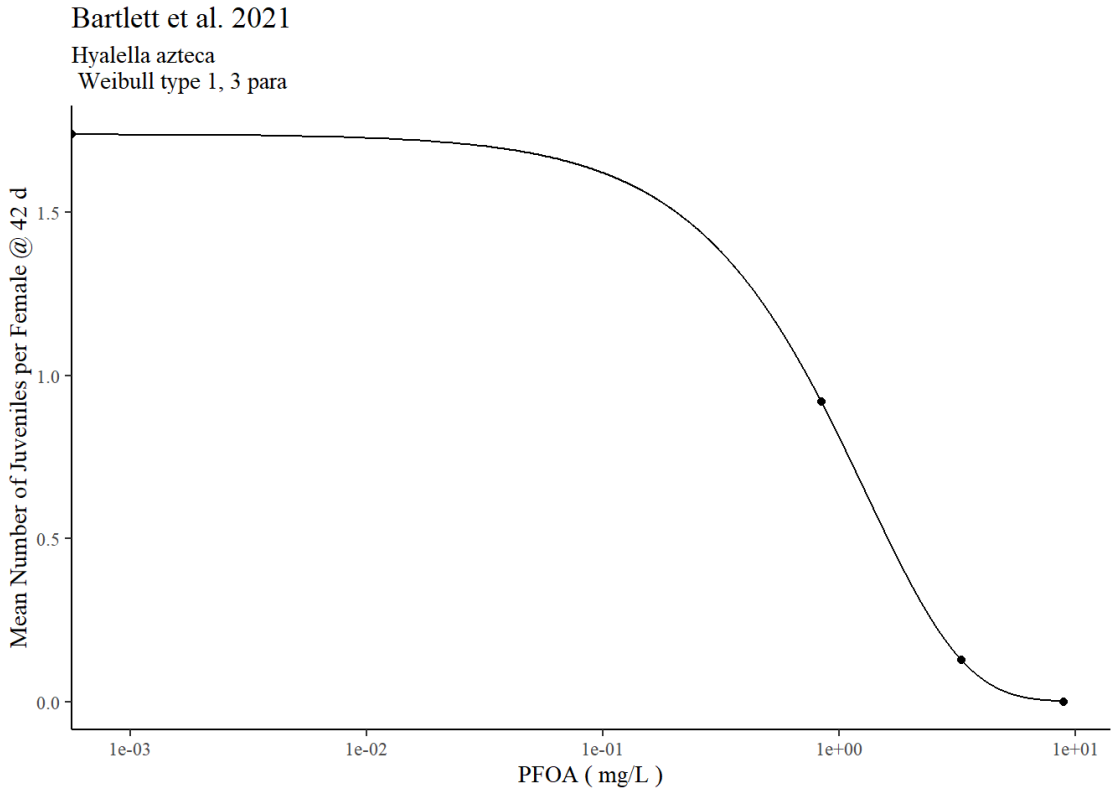
Citation	Species	Endpoint	EPA-Calculated EC_{50} (mg/L)	EPA-Calculated EC_{10} (mg/L)	EC_{50}:EC_{10} Ratio
Ji et al. 2008	*Daphnia magna*	(# young/starting female)	61.67	7.853	7.853
Li et al. 2008	*Daphnia magna*	(# young/starting female)	40.75	12.89	3.161
Ji et al. 2008	*Moina macrocopa*	(# young/starting 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:



C.2.2 Second most chronically sensitive genus – *Lithobates*

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. EPA could not independently calculate an EC_{10} value because there were minimal effects observed across the limited number of treatment concentrations tested. Consequently, 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 tests 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℃ 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. 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

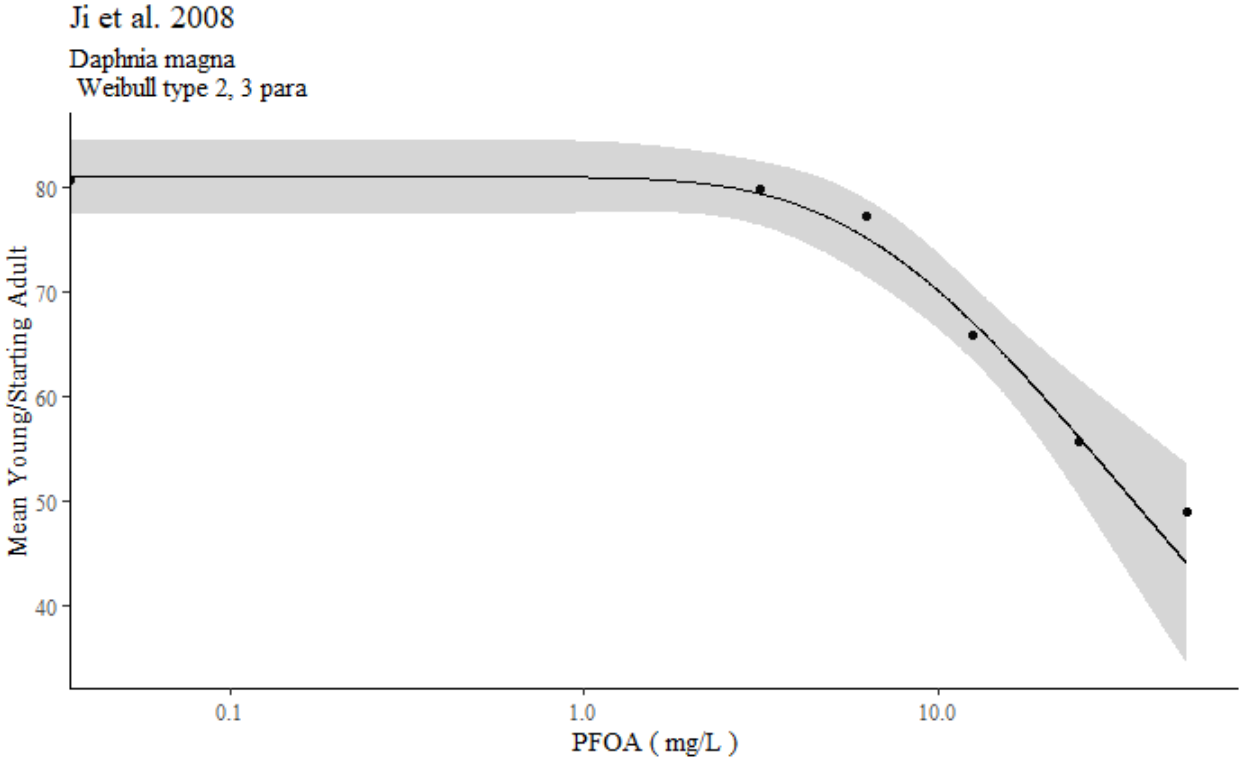
Ji et al. (2008) conducted a chronic life-cycle test on the effects of PFOA (CAS # 335-67-1, purity was not reported; obtained from Sigma Aldrich, St. Louis, MO, USA) with *Daphnia magna*. The test was done under renewal conditions over a 21-day period and test solutions were not analytically confirmed. 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 ± 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 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:



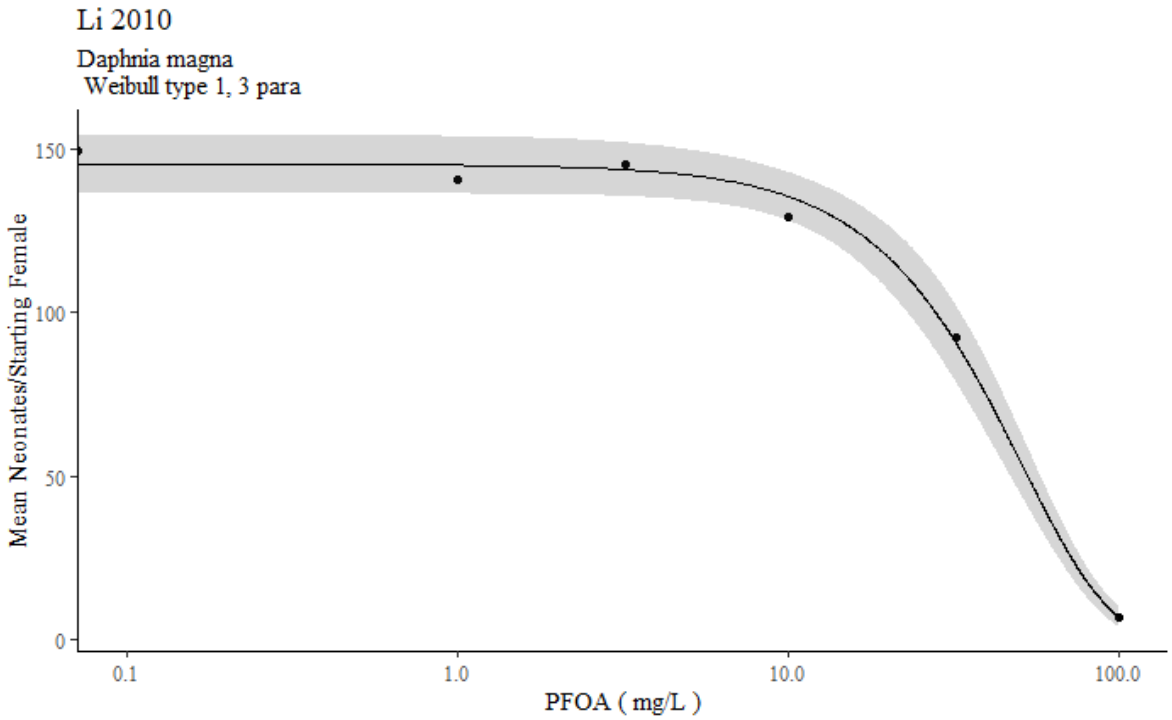
Li (2010) conducted an unmeasured chronic life cycle 21-day test on the effects of PFOA (ammonium salt, >98% purity) on *Daphnia magna*. Authors stated that the test followed OECD 211 (1998). *D. magna* used for the test were maintained in the laboratory for more than one year and were less than 24-hours 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 ± 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). EPA performed C-R analysis for each reported endpoint. 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:



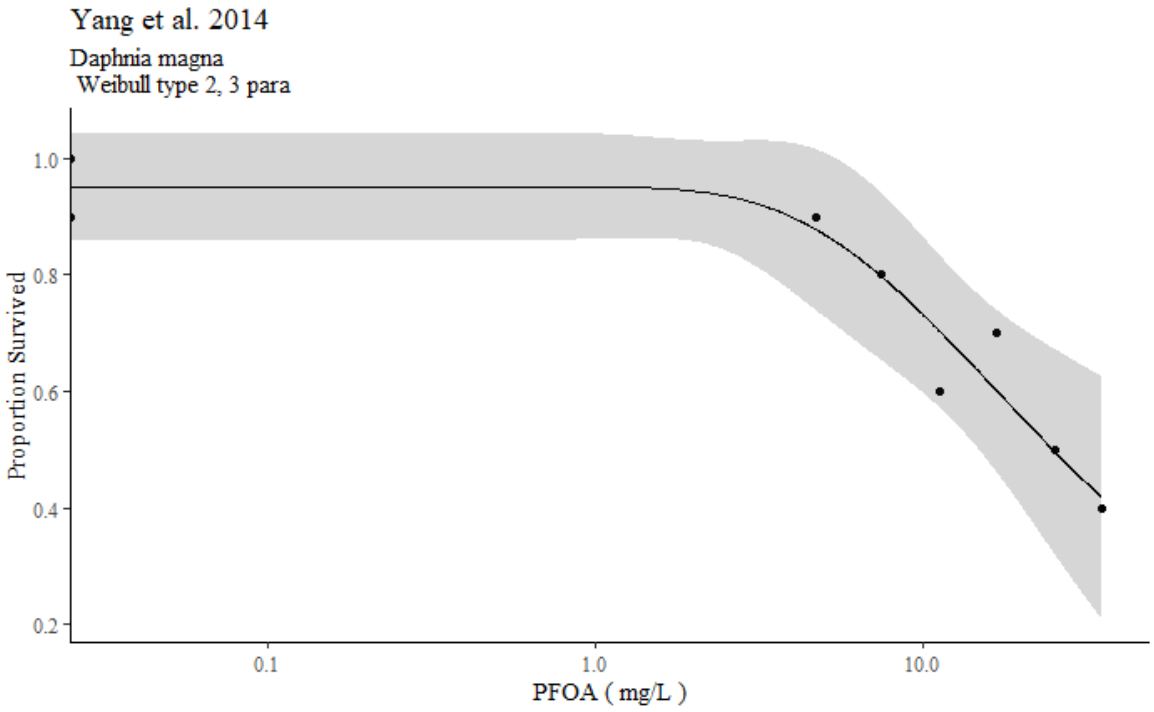
Yang et al. (2014) evaluated the chronic 21-day renewal, measured test of PFOA (CAS # 335-67-1, 99% purity) with *Daphnia magna*, following ASTM E729 (1993). Daphnids used for the test were donated by the Chinese Research Academy of Environmental Sciences. The daphnids were less than 24-hours old at 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. 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 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:



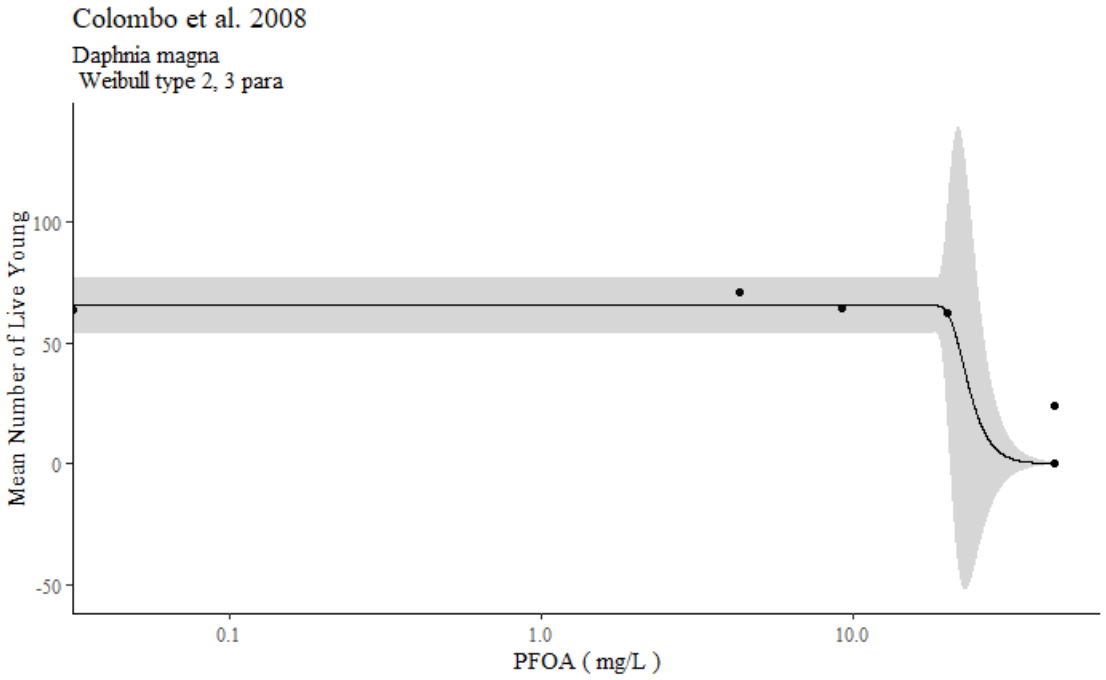
Colombo et al. (2008) conducted a 21-day renewal measured chronic test on PFOA (ammonium salt, CAS # 3825-26-1, 99.7% purity) with the daphnid, *Daphnia magna*. Authors stated that the toxicity test was conducted followed OECD test guideline 211. There were 10 replicates for each test treatment containing 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 was 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. EPA performed C-R analysis for each reported endpoint. The most sensitive endpoint with an acceptable C-R curve was average number of live young, with an EPA-calculated EC_{10} of 20.61 mg/L PFOA (95% C.I. = 11.29 – 29.93 mg/L), which was acceptable for quantitative use. Although the *D. magna* C-R curve from Colombo et al. (2008) displayed a 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.

Publication: Colombo et al. (2008)
Species: Cladoceran (*Daphnia magna*)
Genus: *Daphnia*
EPA-Calculated EC_{10}: 20.61 mg/L (95% C.I. = 11.29 – 29.93 mg/L)
Concentration-Response Model Estimates:

Parameter	Estimate	Std. Error	t-stat	p-value
b	3.3397	0.7219	4.6265	0.0190
d	66.9304	2.0761	32.2383	$6.559\ e^{-5}$
e	43.8667	0.8789	49.9126	$1.771\ e^{-5}$

Concentration-Response Model Fit:



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. 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) 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 of these 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

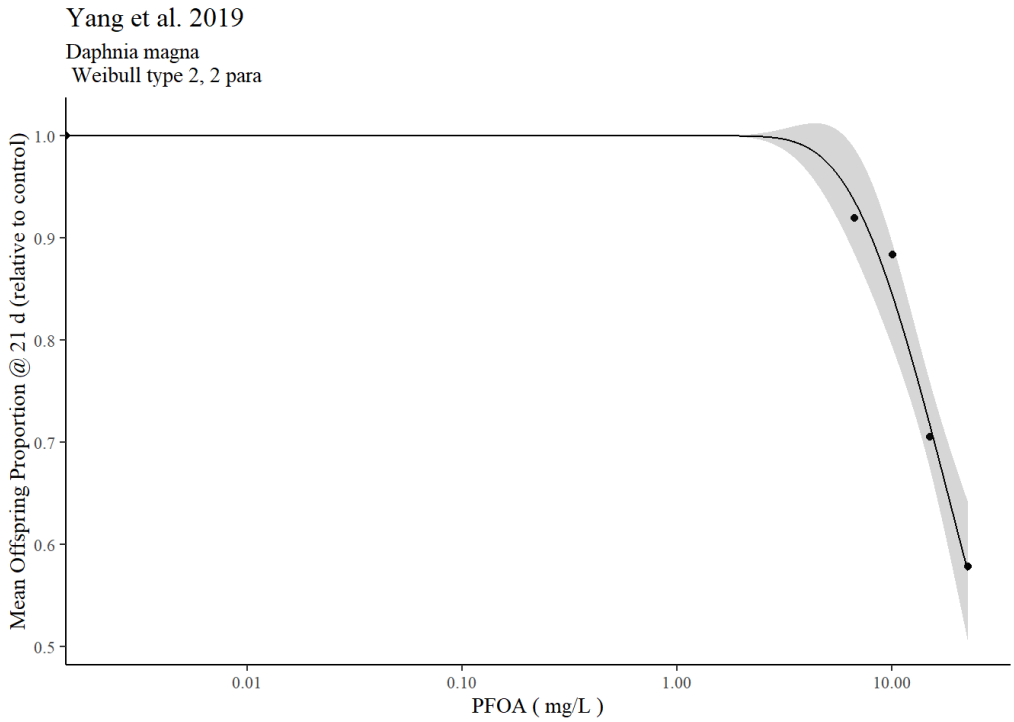
Yang et al. (2019) evaluated the chronic effects of PFOA (CAS# 335-67-1, purchased from Sigma-Aldrich in St. Louis, MO) on *Daphnia magna* via a 21-day unmeasured, static-renewal test that assessed reproductive effects. *D. magna* cultures were originally obtained from the Institute of Hydrobiology of 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 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 ± 1ºC 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. 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 draft 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 Fourth most chronically sensitive genus - *Brachionus*

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)

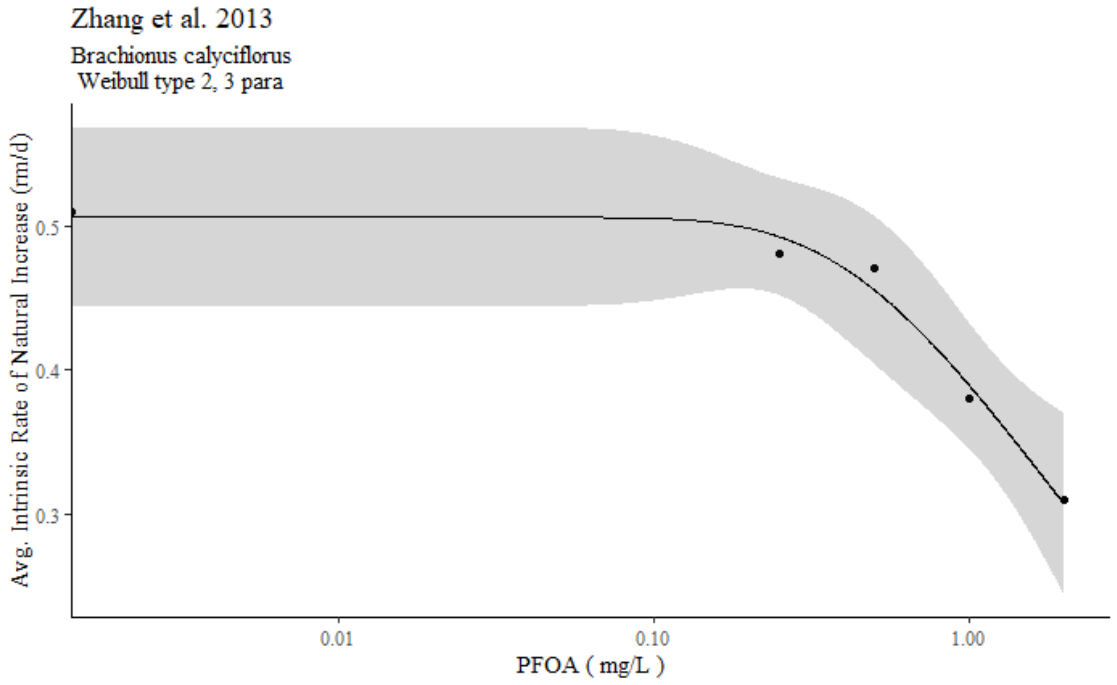
for more than six months before toxicity testing to acclimate to the experimental conditions. Culture medium was an artificial inorganic medium and all toxicity tests were carried out in the same culture medium and under the same conditions as during culture (i.e., pH, temperature, illumination). Solvent-free 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. 100% 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. 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; 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. (2014) 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. (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

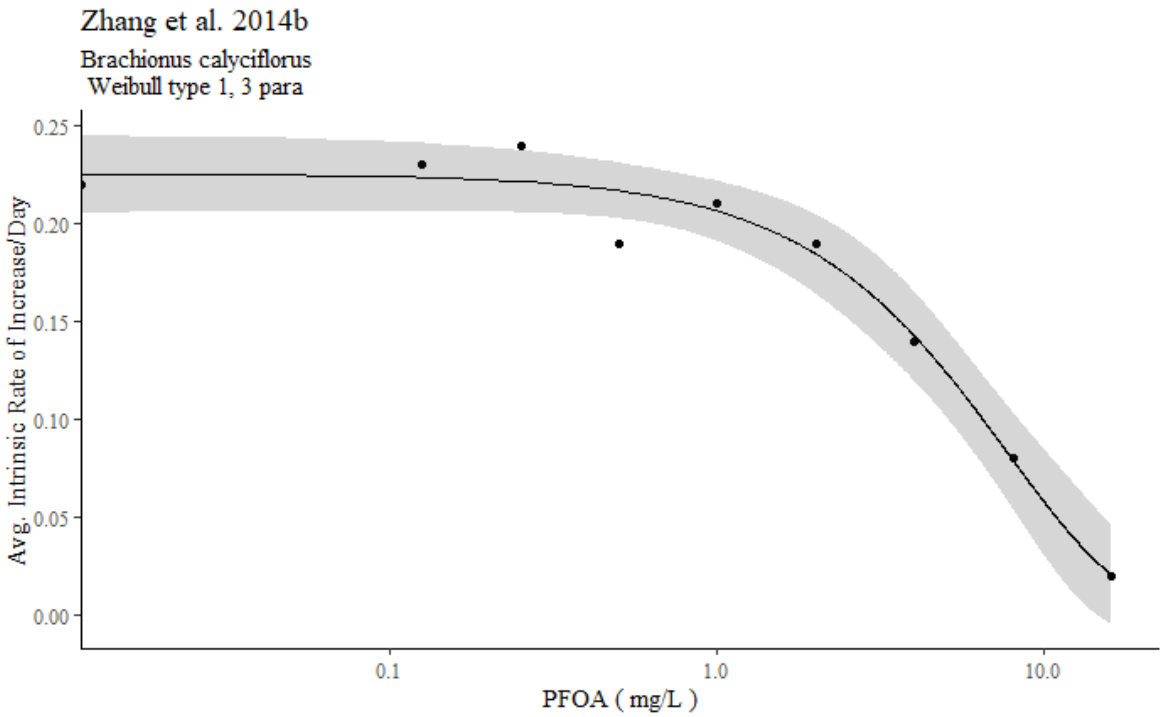
[1] WebPlotDigitizer is an online application used to convert values shown in figures to numerical values. This application was used to obtain numerical concentration-response data when they were only reported in figures. The application is free and available online (WebPlotDigitizer - Extract data from 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 ± 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. 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 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 - *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 each 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), 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.2.7 Seventh most chronically sensitive genus – *Gobiocypris*

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 ± 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.2.8 Eighth most chronically sensitive genus - *Oncorhynchus*

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 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 EPA's chronic value for rainbow trout re-expressed as an un-ionized ammonia concentration for the test condition. Therefore, 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.2.9 Ninth 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: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. 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.2.10 Tenth most chronically sensitive genus - *Chironomus*

McCarthy et al. (2021) conducted a 19-day chronic 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), which is further described in Appendix G. 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

No data at this time.

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
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 (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 (9×10^5 cells/mL), *Chlorella pyrenoidosa*	S, U	96 hours	PFOA ≥95%	-	25	NOEC (growth)	0.1	Li et al. 2021b
Green alga (1.5×10^4 cells/mL), *Chlorella vulgaris*	S, U	96 hours	PFOA 95%	-	23	IC_{50} (cell density)	115.5	Boudreau 2002
Green alga (1.5×10^4 cells/mL), *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	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 (7.0×10^5 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
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

Species	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Reported Effect Concentration (mg/L)	Reference
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
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 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 each 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.2.2 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 ($7.0x10^5$ 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.

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℃ under a 12-hour:12-hour light:dark photoperiod (2000 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 $9x10^5$ 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.2.3 Green alga, *Chlorella vulgaris*

Boudreau (2002) performed a 96-hour static algal growth inhibition test on PFOA (acid form, CAS # 335-67-1, ≥97% purity) with *Chlorella vulgaris* as part of a Master's thesis at the University of Guelph, Ontario, Canada. Authors 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 $1.5x10^4$ 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 0.014 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 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.2.4 Green alga, *Raphidocelis subcapitata*
(formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*)

Boudreau (2002) also performed a 96-hour static algal growth inhibition test on PFOA (acid form, CAS # 335-67-1, ≥97% purity) with *Raphidocelis subcapitata* as part of the Master's thesis at the University of Guelph, Ontario, Canada. Authors 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 $1.5x10^4$ 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, 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 ($7.0x10^5$ 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 $1x10^4$ 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.2.5 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 $5x10^3$ 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 each 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 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.2.6 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%) 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 $2.0x10^4$ 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 ± 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.2.7 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.2.8 Lettuce, *Lactuca sativa*

Ding et al. 2012b conducted a microcosm study where water lettuce, *Lactuca sativa*, was exposed to PFOA (CAS# 335-67-1) for 5 days. Authors stated the test protocols followed U.S. EPA (1996b). 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

No data at this time.

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
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 2000
Green alga, *Raphidocelis subcapitata*	S, U	96 hours	APFO 96.5-100%	-	23	EC_{10} (cell count)	-	5.3	Possible mixture effects of other perfluoro homologue compounds	3M Company 2000
Green alga, *Raphidocelis subcapitata*	S, U	7 days	APFO 96.5-100%	-	23	EC_{10} (cell count)	-	3.3	Possible mixture effects of other perfluoro homologue compounds	3M Company 2000
Green alga, *Raphidocelis subcapitata*	S, U	10 days	APFO 96.5-100%	-	23	EC_{10} (cell count)	-	2.9	Possible mixture effects of other perfluoro homologue compounds	3M Company 2000
Green alga, *Raphidocelis subcapitata*	S, U	14 days	APFO 96.5-100%	-	23	EC_{10} (cell count)	-	5	Possible mixture effects of other perfluoro homologue compounds	3M Company. 2000

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Green alga, *Raphidocelis subcapitata*	S, U	96 hours	APFO Unknown	-	-	EC_{50} (cell count)	-	1,980	Test substance is considered a mixture of APFO and other impurities	3M Company. 2000
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
Tubificid worm (0.03g, 0.8cm), *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

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Planarian, *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
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	3M Company 2000
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	3M Company 2000
Cladoceran, *Daphnia magna*	S, U	48 hours	APFO 96.5-100%	-	-	EC_{50} (death/immobility)	-	126	Possible mixture effects of other perfluoro analogue compounds	3M Company 2000
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	3M Company 2000
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	3M Company 2000

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Cladoceran (<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	3M Company 2000
Cladoceran, *Daphnia magna*	R, U	48 hours	APFO 96.5-100%	-	-	EC_{50} (death/immobility)	-	266	Missing test details, possible mixture effects of other perfluoro analogue compounds	3M Company 2000
Cladoceran, *Daphnia magna*	R, U	21 days	APFO 96.5-100%	-	-	MATC (survival and reproduction)	22-36	28.14	Missing test details, possible mixture effects of other perfluoro analogue compounds	3M Company 2000
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
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	Range-finding experiment; duration too long for an acute test and too short for a chronic test	MacDonald et al. 2004

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
Midge (larva, 10 days old), *Chironomus dilutus*	S, M	10 days	PFOA 97%	-	-	MATC (mortality)	26-272	84.10	Range-finding experiment; duration too long for an acute test and too short for a chronic test	McCarthy et al. 2021
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
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

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
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
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
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
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
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

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 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
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

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 (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. 2017b
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. 2017b
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, 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), *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, 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, 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

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, 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, *Danio rerio*	R, U	21 days	PFOA Unreported	-	-	LOEC (mRNA gene expression in kidneys)	0.05-0.1	0.1	Atypical endpoint	Zhang et al. 2021
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. 2008a
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. 2008b
Rare minnow (adult, 9 months old), *Gobiocypris rarus*	F, U	28 days	PFOA 98%	-	-	LOEC (protein spots identified by MALDl-TOF/TOF)	<3-3	3	Non-apical endpoint	Wei et al. 2008b
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

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 (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 considered a mixture of APFO and other impurities	3M Company 2000
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	3M Company 2000
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 2000
Fathead minnow, *Pimephales promelas*	S, U	96 hours	APFO 96.5-100%	-	-	LC_{50}	-	70	Possible mixture effects of other perfluoro analogue compounds, lack of replication	3M Company 2000
Fathead minnow, *Pimephales promelas*	S, U	96 hours	APFO 96.5-100%	7.9-8.0	19	LC_{50}	-	776	Possible mixture effects of other perfluoro homologue compounds, lack of replication	3M Company 2000
Fathead minnow, *Pimephales promelas*	S, U	96 hours	APFO 96.5-100%	7.9-8.0	19	LC_{50}	-	754	Possible mixture effects of other perfluoro homologue compounds, lack of replication	3M Company 2000

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Chronic Limits (NOEC-LOEC) (mg/L)	Reported Effect Conc. (mg/L)	Deficiencies	Reference
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	3M Company 2000
Fathead minnow, *Pimephales promelas*	S, U	96 hours	PFOA 95-98%	7.5-7.7	19-20	LC_{50}	-	843	Possible mixture effects of other perfluoro impurities	3M Company 2000
Fathead minnow (juvenile), *Pimephales promelas*	S, U	96 hours	APFO Unreported	7.4-8.4	21-22	LC_{50}	-	>1,000	Possible mixture effects of the inert perfluorinated compounds and other perfluoro analogue compounds	3M Company 2000
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	Possible mixture effects of other perfluoro analogue compounds, lack of replication	3M Company 2000
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	3M Company 2000
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

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
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, possible mixture effects of other perfluoro analogue compounds	3M Co. 2000
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

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	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
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 (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
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
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
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
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.

G.2 Qualitatively Acceptable PFOA Toxicity Study Summaries for Tests Not Described in the Effects Characterization

G.2.1 Summary of Acute PFOA Toxicity Studies Used Qualitatively in the Freshwater Aquatic Life Criterion Derivation

G.2.1.1 Worms

Yang et al. (2014) conducted a 96-hour measured acute test of PFOA (CAS # 335-67-1, 99% purity) with the annelid worm, *Limnodrilus hoffmeisteri*. 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. Authors stated that the test followed ASTM E729 (1993). *L. hoffmeisteri* (0.03 g, 0.8 cm) used for the test were obtained from Beijing City Big Forest Flower Market and allowed to acclimate for at least seven days before testing. Dilution water was dechlorinated tap water (pH, 7.0 ± 0.5; dissolved oxygen, 7.0 ± 0.5 mg/L; total organic carbon, 0.02 mg/L; and 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 90 cm Petri dishes containing 10 mL of test solution. The test employed three replicates of 10 worms each in six test concentrations plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 300, 390, 507, 659.1, 856.83 and 1,113.88 mg/L. Test concentrations were measured in low and high treatments only. The authors provided mean measured concentrations before and after renewal: 295.3 and 259.31 mg/L (lowest concentration) and 1,098.05 and 987.37 mg/L (highest concentration). Analyses of test solutions were performed using high performance liquid chromatography with mass spectrometric detection (HPLC/MS) and negative electrospray ionization. The concentration of 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 are not reported. Negative control and solvent control mortality was 0% and 3%, respectively. The 96-hour LC_{50} was 568.2 mg/L (95% C.I.: 476.3-677.8 mg/L). The acute value was not acceptable for quantitative use because of the uncertainties and potential exposures to PFAS from the source of the test organisms but was retained for qualitative use by providing relevant toxicity information.

Li (2009) conducted a second 96-hour static, unmeasured acute test of PFOA (ammonium salt, >98% purity) with *Dugesia japonica*, which is described in greater detail in Appendix A.2.7: Eighth most acutely sensitive genus – *Dugesia*. 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. EPA performed C-R analysis for each individual test. Two of the tests had acceptable curves while the third curve had a poor concentration response and the LC_{50} (427.7 mg/L) was, therefore, not acceptable for quantitative use but was retained for qualitative use only.

Yuan et al. (2015) also conducted a 96-hour, unmeasured acute test on PFOA (96% purity) with *Dugesia japonica*, with daily solution changes. Planarians used for testing were originally collected from a fountain in Quanhetou, Boshan, China, and acclimated in the laboratory for an unspecified time period before use. The planarians had a body length of 10-12 mm at test initiation. Dilution water was aerated tap water. No details were provided regarding photoperiod or light intensity. A primary stock solution was prepared by dissolving the salt in dimethyl sulfoxide (DMSO). The control and exposed planarians received 0.005% DMSO (v/v). Exposure vessels' material, dimensions and fill volume were not reported. The test employed

three replicates of 10 planarians each in seven test concentrations plus a solvent control. Nominal test concentrations were 0 (solvent control), 10, 30, 35, 40, 45, 50 and 55 mg/L PFOA. The test temperature was reported as 20°C. No other water quality parameters were reported. Mortality of solvent control animals was also not reported. The study reported 96-hour LC_{50} was 39.35 mg/L (95% C.I. = 32.38 - 46.32 mg/L). The test was not acceptable for quantitative use because of the uncertainties and potential exposures to PFAS from the source of the test organisms.

G.2.1.2 Mollusks

Yang et al. (2014) conducted a 96-hour measured acute test of PFOA (CAS # 335-67-1, 99% purity) with a non-North American snail species, *Cipangopaludina cathayensis*. 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. Authors stated that the test followed ASTM E729 (1993). The test organisms (4.0 g, 2.0 cm) were purchased from the Beijing Dahongmen Jingshen Seafood Market and allowed to acclimate for at least seven days before testing. Dilution water was dechlorinated tap water (pH, 7.0 ± 0.5; dissolved oxygen, 7.0 ± 0.5 mg/L; total organic carbon, 0.02 mg/L; and 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 snails each in six test concentrations plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 300, 420, 588, 823.2, 1,152.48 and 1,613.47 mg/L. Test concentrations were measured in low and high treatments only. The authors provided mean measured concentrations before and after

renewal: 293.55 and 261.77 mg/L (lowest concentration) and 1,596.62 and 1,468.08 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 96-hour LC_{50} was 740.07 mg/L (95% C.I.: 597.7-916.4 mg/L). The acute value was not acceptable for quantitative use because of the uncertainties and potential exposures to PFAS from the source of the test organisms but was retained to for used in a qualitative manner.

G.2.1.3 Planktonic crustaceans

3M Company (2000) exposed *D. magna* to PFOA (CAS # 335-67-1) in a 48-hour static, unmeasured acute toxicity test. 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 substance was dissolved in a 50:50 water:isopropanol solution to make a primary solution of 1,000 mg/L test substance and isopropanol. Another toxicity test conducted by the authors showed no mortality or sublethal effects at 390 mg/L isopropanol on the same species. It was then diluted with reconstituted water to make five nominal test concentrations (130, 200, 360, 600 and 1,000 mg/L test substance or 65, 110, 180, 300 and 500 mg/L PFOA) plus a control (reconstituted water only). The test followed USEPA-TSCA Guideline 797.1300. Exposures were conducted in 300 mL glass beakers with 250 mL of test solution with 10 daphnids per beaker. There were two replicates for

each treatment. Test conditions throughout the experiment varied little (D.O.: 8.7-8.8 mg/L; pH: 7.5-8.4; 19.4-20.2°C). No mortality occurred in the control treatment and PFOA treatments ≤180 mg/L. The 48-hour reported EC_{50}, based on death/immobility, was 720 mg/L test substance and isopropanol or 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, but was retained for qualitative use.

3M Company (2000) 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 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). No specific test protocol was identified. Solutions of APFO were made in carbon filtered well water. Exposures were conducted in 250 mL glass beakers with 200 mL of test solution and 10-20 daphnids per beaker. There were two replicates for each treatment and test. The authors noted that the results of the four various tests were inconsistent and that the inconsistencies in the effect concentrations may be due to diet (specifics not provided). The 48-hour EC_{50}s determined from tests conducted in May 1982, based on death/immobility, were >1,000 mg/L APFO, while the EC_{50} for a subsequent test in June 1982 was reported to be 126 mg/L APFO. The results of the acute tests were reported by the study authors to have been superseded by a more recent study reported in 3M Co. (2002). These inconsistencies and the possible mixture effects of other perfluoro analogue compounds rendered these tests unacceptable for quantitative use and they were retained for qualitative use.

3M Company (2000) 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 mixture of APFO (78-93% of the compound) and C_5, C_6 and C_7 perfluoro analogue compounds (7-22% of the compound). No specific test protocol was identified. Solutions of APFO were made in carbon filtered well water and included five nominal test concentrations (100, 180, 320, 560, and 1000 mg/L test substance) plus a control (well water only). Exposures were conducted in 250 mL glass beakers with 200 mL of test solution and 10 daphnids (<24 hours old) per beaker. There were two replicates for each treatment. Test conditions throughout the experiment varied minimally (D.O.: 8.8-9.0 mg/L; pH: 8.0-8.1; 21°C). No mortality occurred in the control and 100 mg/L APFO treatments. 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 precluded this test for quantitative use, and it was therefore retained for qualitative use only.

3M Company (2000) 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 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 acute test followed USEPA-TSCA Guideline 797.1300 protocol. Solutions of the test substance were made in reconstituted water and included five nominal test concentrations plus a control. Exposures were conducted in 300 mL glass beakers with 250 mL of test solution and 10 daphnids (<24 hours old) per beaker. There were two replicates for each treatment. Test conditions throughout the experiment varied minimally (D.O.: 8.1-9.1 mg/L; pH: 8.1-8.3; 20.3-20.8°C). No mortality occurred in the control treatment and in 730 mg/L test substance treatment. 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 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 Company (2000) 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 2327 and was characterized as 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 acute test followed test guidance from OECD 202. Solutions of APFO were made in well water and included five nominal test concentrations (150, 250, 400, 600, and 1,000 mg/L test substance) plus a control (well water only). Exposures were conducted in 250 mL glass beakers with 200 mL of test solution and 5 daphnids (<24 hours old) per beaker. There were four replicates for each treatment. Test conditions throughout the experiment varied little (D.O.: 9.0-9.5 mg/L; pH: 8.1-8.4; 19.5-20.1°C). No mortality occurred in the control and treatments ≤400 mg/L. 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 and other perfluoro analogue compounds the test was not acceptable for quantitative use but was retained for qualitative use.

3M Company (2000) 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 little 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 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.

G.2.1.4 Benthic crustaceans

Yang et al. (2014) conducted a 96-hour measured acute test of PFOA (CAS # 335-67-1, 99% purity) with the freshwater prawn species, *Macrobrachium nipponense* (a non-North American species). 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. Authors stated that the test followed ASTM E729 (1993). *M. nipponense* (0.30 g, 4.0 cm) used for the test were purchased from the Beijing Dahongmen Jingshen Seafood Market and allowed to acclimate for at least seven days before testing. Dilution water was dechlorinated tap water (pH, 7.0 ± 0.5; dissolved oxygen, 7.0 ± 0.5 mg/L; total organic carbon, 0.02 mg/L; and 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 2 L beakers of unreported material type containing 1.5 L of test solution. The test employed three replicates of 10 prawns each in six test concentrations (measured in low and high treatments) plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 200.00, 300.00, 450.00, 675.00, 1,012.50 and 1,518.75 mg/L. Test concentrations were measured in low and high treatments only. The authors provided mean measured concentrations before and after renewal: 179.46 and 196.25 mg/L (lowest concentration) and 1,344.28 and 1,492.75 mg/L (highest concentration). Analyses of test

solutions were performed using high performance liquid chromatography with mass spectrometric detection (HPLC/MS) and negative electrospray ionization. The concentration of 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% and 3%, respectively. The 96-hour LC_{50} was 366.66 mg/L (95% C.I.: 253.09-531.18 mg/L). The acute value was not considered for quantitative use because of the uncertainties and potential exposures to PFAS from the source of the test organisms but was retained for qualitative use by providing relevant toxicity information.

G.2.1.5 Insects

Yang et al. (2014) performed a 96-hour measured acute test of PFOA (CAS # 335-67-1, 99% purity) with the midge, *Chironomus plumosus*. Authors stated that the test followed 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. *C. plumosus* (0.05 g, 1.2 cm) used for the test were purchased from the Beijing City Big Forest Flower Market and allowed to acclimate for at least seven days before testing. Dilution water was dechlorinated tap water (pH, 7.0 ± 0.5; dissolved oxygen, 7.0 ± 0.5 mg/L; total organic carbon, 0.02 mg/L; and 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 90 cm Petri dishes containing 10 mL of test solution. The test employed three replicates of 10 midges each in six test concentrations (measured in low and high treatments) plus a negative and solvent control. Nominal concentrations were 0 (negative and solvent controls), 200, 300, 450, 675, 1,012.5 and 1,518.75 mg/L. Test concentrations were measured in low and high treatments only. The authors provided mean measured concentrations before and after renewal: 196.25 and 178.48 mg/L (lowest concentration) and 1,488.4 and 1,364.97 mg/L (highest concentration). Analyses of test solutions were performed using high performance liquid chromatography with mass spectrometric detection (HPLC/MS) and negative electrospray ionization. The concentration of 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% and 3%, respectively. The 96-hour LC_{50} was 402.24 mg/L (95% C.I.: 323.8-499.6 mg/L). The acute value was not acceptable for quantitative use because of the uncertain source and unreported previous exposure to PFAS of the test organisms but was retained for qualitative use.

G.2.1.6 Cyprinid fishes

Yang et al. (2014) evaluated the toxicity of the acidic form of perfluorooctanoic acid (PFOA, CAS #335-67-1, 99% purity) with *Carassius auratus* for 96-hours using measured conditions (the authors note that the experiments followed ASTM standards and U.S. EPA procedures for deriving water quality criteria). 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. The goldfish (6.0 g, 7.0 cm) were purchased from the Beijing Chaoyang Spring Flower Market, which is considered an atypical source. The organisms were allowed to acclimate for at least seven days before testing, and the test was conducted at 22 ± 2°C with 12-hours of illumination, 10 fish per replicate, and three replicates per concentration. Beakers used for exposure are assumed to be made of glass but was not specified by study authors. PFOA was dissolved in deionized water and the carrier solvent dimethylsulfoxide (DMSO) to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 200, 320, 512.0, 819.2, 1,311 and 2,097 mg/L PFOA. Water quality parameters reported were pH = 7.0 ± 0.5, dissolved oxygen = 7.0 ± 0.5 mg/L, total organic carbon = 0.02 mg/L and total hardness = 190.0 ± 0.1 mg/L as $CaCO_3$. The supplemental data provided for the study included a comparison of measured PFOA concentrations before and after solution renewal in the low and high test concentrations. PFOA concentrations in the test water did not fluctuate by more than 15% during experiments. The 96-hour LC_{50} reported for the study, 606.6 mg/L PFOA, was not acceptable for quantitative use because the test organisms were obtained from an atypical source and previous exposure to contaminants was unknown but was retained for qualitative use.

The effect of PFOA (CAS #335-67-1, >98% purity) on oxidative stress enzyme responses of *C. auratus* juveniles (27.85 g) was evaluated by **Feng et al. (2015)**. The 96-hour static measured exposure was conducted at a temperature of 23°C, pH of 7.25, dissolved oxygen of 6.5 mg/L and total hardness of 174.3 mg/L as $CaCO_3$. The fish were purchased from a local aquatic breeding base and acclimated in dechlorinated tap water for at least for 10 days, with the total mortality near zero. After acclimatization, five fish were randomly selected and placed in each glass tank (two replicates for treatments and five control replicates) containing 20 L of test

solution (nominal concentrations of 1.21 or 12.10 µM PFOA, or approximately 0.5 and 5.0 mg/L PFOA) or 20 L of dechlorinated tap water. PFOS was dissolved in DMSO to prepare a 120.77 mmol/L stock solution. The tanks were continuously aerated, and water was refreshed to minimize the contamination from metabolic wastes. Antioxidant enzyme activity (CAT, SOD and GPx) and lipid peroxidation were not adversely impacted at 4.931 mg/L PFOA at test termination, but these data were not considered quantitatively because of the atypical endpoints reported and only two exposure concentrations were evaluated. This study was retained for qualitative use by providing non- apical endpoint which may inform mode of action and AOP considerations.

Kim et al. (2010) evaluated the effects of PFOA (99.8% purity) to biomarker responses exhibited by *Cyprinus carpio* exposed for 96-hours under flow-through, measured conditions. PFOA stock solutions were prepared in N,Ndimethylformamide (<100 mg/L) and diluted with carbon-filtered and dechlorinated tap water to give nominal concentrations of 0.050, 0.500, 5.000 and 50.00 mg/L. Dechlorinated tap water was used as a control. The measured exposure concentrations of PFOA ranged from 81 to 138% of the nominal concentrations (0, 0.041, 0.483, 6.582, 55.57 mg/L), and where appropriate, the average of the PFOA measured concentrations was used to calculate endpoints when not within ± 20% of the nominal concentrations. The carp were obtained from the Chungcheongnam-do Experimental Station for Inland Waters Development (Republic of Korea) and held in 2,000 L tanks with flowing dechlorinated tap water at 23 ± 2°C, which was also used in the study (pH, 6.9; alkalinity, 28.0 mg/L as $CaCO_3$; total hardness, 47.8 mg/L as $CaCO_3$). Ten juvenile fish (~12 cm; ~20 g) were held in each 100 L glass exposure tank (assume one replicate per concentration) under 16-hours of illumination, and water temperature maintained at 23 ± 1°C. At test termination, the fish were removed from the

tanks and evaluated for biochemical and genetic responses. Vitellogenin activity was determined to be the most sensitive endpoint, with a 96-hour LOEC of 6.582 mg/L. This study was not used quantitatively due to the non-apical endpoint reported. This study was retained for qualitative use by providing non- apical endpoint which may inform mode of action and AOP considerations.

Ding et al. (2012c, 2013) evaluated the acute effects of APFO (CAS # 3825-26-1, 98% purity, purchased from Sigma-Aldrich) to *Danio rerio* embryos via a 96-hour static-renewal, unmeasured exposure. Adult AB strain zebrafish were cultured in aerated and biologically-filtered reconstituted freshwater at 26 ± 1°C. The day before a test, male and female zebrafish, at a ratio of 1:1, were placed in spawning tanks before the onset of darkness. Mating, spawning, and fertilization take place within 30 minutes after light onset in the morning. Eggs were collected from spawn traps and washed with clean OECD water. Unfertilized or abnormal eggs were removed under a stereomicroscope. APFO was dissolved in reconstituted water to achieve the desired test concentrations; no solvents were used. For the toxicity test, the authors stated that six exposure concentrations were performed with three replicates each. Graphically, seven PFOA concentrations are shown as $\log_{10}$(mol/L) concentrations. These were converted to mg/L (105.1, 204.0, 381.6, 681.7, 812.0, 1238, and 1,314 mg/L, respectively) given the molecular weight of the form of PFOA used in the study (CAS # 3825-26-1; molecular weight of 431.1 g/mol). Response data for the control treatment were not presented graphically or reported in the text. Twenty fertilized eggs per exposure concentration were divided into a 24-well plate with one embryo per well, containing 2 mL test solution. The remaining four wells were filled with control water and a single embryo. An embryo was considered dead when one of four end points (i.e., coagulation of the embryo, non-detachment of the tail, non-formation of somites and non-detection of the heartbeat) was observed. The survival rates were monitored and documented at

72- and 96-hour post fertilization (hpf). The test solutions were half renewed every 24 hours. The author-reported 96-hour LC_{50} was 0.896 mM, or 386.3 mg/L APFO. The graphical presentation of concentration-response data in the publication without including the control response was considered problematic. EPA attempted to contact the study authors on July 13, 2021, to request the data from the paper, but did not hear back as of October 26, 2021. Therefore, the limited level of data presented in the paper and the apparent lack of data from the control treatment precluded this test from being used quantitatively. The reported LC_{50} from the study was considered acceptable for qualitative use only.

A 72-hour exposure of PFOA (CAS # 335-67-1, 95% purity) to *D. rerio* embryos was conducted by **Zheng et al. (2012)** following OECD (1996) methodology. No solvent was used for PFOA because of its high water solubility (9,500 mg/L); therefore, exposure solutions were diluted from the stock solutions with embryonic water. Adult wild-type zebrafish were obtained from the Model Animal Research Center of Nanjing University and kept in a semiautomatic rearing system (tap water), with five females and ten males in each 10 L tank at 28 ± 1°C. Water was exchanged at a rate of 1/3 daily and the lighting was 14-hours of illumination at 1,000 lux. Spawning and fertilization took place within 30 minutes after the lights were turned on in the morning. Embryos were transferred to exposure solutions (reconstituted embryo water) immediately after fertilization and examined under a stereomicroscope. Damaged or unfertilized embryos were discarded. Zebrafish embryos were exposed in 24-well cell culture plates (material not identified) with 2 mL solution per well (pH of 8.3 ± 0.2, dissolved oxygen concentration of 6.07 ± 0.24 mg/L at the beginning and end of experiments). Twenty normally shaped fertilized embryos were assigned to each treatment (nominal concentrations of 0, 150, 200, 212, 225, 240, 255, 270 mg/L). All concentrations were repeated in triplicate at different days with different

batches of eggs. Embryos were cultured in an incubator at 28.5°C after exposure. Control performance was reported as >80% proportion of normal embryos. The reported 72-hour LC_{50} and 96-hour EC_{50} (malformations) were 262 and 198 mg/L PFOA, respectively. However, the data were not considered in a quantitative manner because the duration was too short for an acute exposure and the data were instead considered qualitatively acceptable.

Du et al. (2013) investigated the effect of PFOA (CAS # 335-67-1, unreported purity) on the survival, malformation and suppression of steroidogenic enzyme synthesis of *D. rerio* embryos exposed via renewal unmeasured conditions for 120-hours. PFOA stock solutions were prepared in DMSO at a concentration of 1 M and stored at -20°C. They were diluted to desired concentrations in culture medium immediately before use, and the final concentration of DMSO in the culture medium did not exceed 0.1% (v/v). Wild-type adult male and female zebrafish, obtained from the Model Animal Center of Nanjing University, were maintained on a 14-hour:10-hour light:dark cycle at 28°C under semi-static conditions with charcoal-filtered water. Spawning was induced in the morning when the lights were turned on. Fertilized eggs were collected 30 minutes later and examined under the microscope. Only those that had developed normally were selected. Embryos were incubated with reconstituted embryo medium in Petri dishes for subsequent experiments. Zebrafish embryos at four hours post-fertilization were exposed (three replicates, 40 fish per replicate) to 0.100, 0.200 and 0.500 mg/L PFOA and 0.001% DMSO (control) at 28°C, with daily renewal of the embryo medium. Embryo survival and stage of embryonic development were recorded daily until test termination (120 hours post-fertilization). The five-day NOEC and LOEC for the increase in relative mRNA expression of hhex and pax eight were reported as 0.100 and 0.200 mg/L, respectively. Since only non-apical

endpoints were reported, and the five-day exposure period, these data were not classified as acceptable for quantitative use but retained for qualitative use.

A six-day static unmeasured test was utilized by **Ulhaq et al. (2013)** to determine the toxicity of PFOA (CAS # 335-67-1, purity not reported) to *D. rerio*. PFOA stock solutions were freshly prepared in reconstituted water in concentrations below the limit for water solubility. Adult zebrafish (AB strain) were held in charcoal-filtered tap water. Breeding groups including three males and two females were placed in 10 L glass aquaria equipped with spawning nets separating the parental fish from the eggs. A half an-hour after the onset of lights the eggs were collected, rinsed for removal of debris, and then only normally developed fertilized eggs at least in the four-cell stage were selected. The zebrafish embryos were then (within 15 minutes after collection) exposed to a series of concentrations of the test substance dissolved in reconstituted water (exposure medium). Fertilized eggs (four-cell stage) were randomly distributed individually into flat bottom, 48-well polystyrene plates along with 750 µL of the exposure medium. PFOA was tested at six consecutive nominal concentrations differing by a factor of 3.3 based on logarithmic scale fitting (3-1,000 mg/L). For each test, four 48-well plates were used, with a total of 24 embryos per concentration as well as 24 in the water control group. Each treatment group was equally distributed to each of the four well plates (i.e., six embryos/concentration/plate), giving a total of 168 embryos. The plates were covered with parafilm, and the embryos were exposed to the chemical until 144-hour post fertilization (hpf). Fish laboratory conditions throughout the study were kept at pH 7.2-7.6, a water temperature of 26 ± 1°C and a light cycle of 14-hours. Observations of mortality and sublethal endpoints were made after 24, 48, 120 and 144 hours post-fertilization using a stereomicroscope. Sublethal endpoints such as presence of edema, malformations, not-hatched eggs, lack of circulation and

reduced pigmentation were also observed. Heart rate was recorded at 48 hours post-fertilization and hatching time was determined using time-lapse photography. The 144-hour LC_{50} was 430 mg/L PFOA, and the EC_{50} (lethal and sublethal effects) was 350 mg/L PFOA. Neither value was used in a quantitative manner because of test duration, but both were considered in a qualitative manner.

Bouwmeester et al. (2016) tested various chemicals, including PFOA (CAS 335-67-1) in single-chemical exposures to evaluate the potential of *D. rerio* embryos as a screening tool to examine DNA methylation modifications after xenobiotic exposure. Embryos were exposed from 0 to 72 hours post fertilization. In the single PFOA chemical tested (i.e., 320 µM, which converts to 132 mg/L based on the molecular weight of PFOA (414.07 µg/µM) authors reported no embryonic effects. The reported NOEC of 132 mg/L was not acceptable for quantitative use because the test did not measure acute apical effects and the test duration was too short. This study was retained for qualitative use.

PFOA (CAS # 335-67-1, 96% purity) acute exposure to *D. rerio* embryos was evaluated by **Kalasekar et al. (2015)** via a 72-hour static unmeasured exposure. PFOA was dissolved and diluted in DMSO to make a 1,000x stock solution. Adult wild type (TAB 14) zebrafish were maintained in 3.5 L tanks in a Tecniplast system supplied continuously with circulating filtered water at 28.5°C with 14-hours of illumination. Embryos were harvested after spawning and allowed to develop in a Petri dish at 28.5°C containing reconstituted E3 media. At two days post fertilization (dpf), a clutch of 20 embryos were transferred into each well of a six-well plate containing 4 mL of E3. The embryos were exposed to PFOA dissolved in dimethylsulfoxide (DMSO) or to DMSO alone (vehicle-control). At five dpf, the larvae were transferred to 96-well plates (one embryo/well in approximately 100 µL E3), manually imaged on an Olympus IX51

inverted fluorescence microscope using a 4x objective, and images captured using an Olympus XM10 camera with CellSens Dimension v1.9 software. Exposures were not performed directly in 96 well plates because the small volume could result in motility restriction, thereby causing decreased yolk absorption. The larvae were not anaesthetized, and thus swam in an upright position, facilitating image capture of the ventral side of the larvae. Chemical exposure experiments for all concentrations were repeated two to four times, with one to two sets of 20 embryos per experiment. To determine LC_{50} values and morphological malformations, a clutch of 20 embryos were exposed at two dpf, and lethality and morphology were scored at five dpf. The 72-hour LC_{50} was reported as 3.8×10^{-4} M PFOA (157.3 mg/L PFOA) and was not classified as quantitatively acceptable due to the short test duration but was retained for qualitative use.

Rainieri et al. (2017) evaluated the acute effects of PFOA (purchased from Acros, Geel, Belgium) on zebrafish (*Danio rerio*) in a 48-hour static measured study. A 2 mg/ml stock solution was prepared by dissolving PFOA in methanol and storing the stock solution in darkness at <4°C until use. Wild type fish were obtained from AZTI Zebrafish Facility and maintained in 60 L tanks at 27°C and a 12-hour light/12-hour dark cycle, and were fed twice daily with commercial feed. Embryos were held in embryo water for 72 hours before testing. Twenty-five hatched embryos (72 hpf) were exposed to 10 mL of test solution in glass petri dishes 6 cm in diameter at 27°C under a 12-hour:12-hour light-dark photoperiod for 48 hours. Triplicate exposures ranged from 10 to 500 mg/L with a maximum of 0.45% DMSO in any exposure. Samples of each exposure solution were taken at the beginning and at the end of the test to determine PFOA concentrations. The reported 48-hour LC_{50} value was >500 mg/L PFOA, but

the value was acceptable for qualitative use only because of the short test duration (i.e., only 48 hours rather than the established 96-hour acute exposure period for this species).

Vogs et al. (2019) evaluated the acute effects of PFOA (>96% purity, CAS # 335-67-1, 0.002 mg/L solubility at 25°C) on zebrafish (*Danio rerio*) embryos in a 118-hour measured, static-renewal study. AB strain fish used in this study were provided by the Zebrafish Core Facility at Comparative Medicine, Karolinska Institute. Three male and three female adults were grouped, and embryos were collected in E3 medium directly after spawning. Study authors reported following OECD TG 236 for fish embryo toxicity testing. A stock solution was prepared by dissolving PFOA into dimethyl sulfoxide to achieve initial measured concentrations of 21, 41 and 340 µM PFOA. Thirty embryos (two hpf) were placed in 30 mL of E3 exposure medium in 50 mL glass petri dishes maintained at 28 ± 1°C under dark conditions throughout the exposure. A 118-hour EC_{50} value of 210.8 mg/L (509 µM) PFOA was reported for mortality, and the EC_{20} for non-inflated swim bladders, pericardial and yolk sac edemas, and scoliosis was 147.2 mg/L (355.6 µM). The 118-hour cumulative EC_{50} was qualitatively acceptable because 118-hour exposure duration was longer than standard 96-hour exposure duration prescribed by OECD Test No. 236: Fish Embryo Acute Toxicity (FET) Test. Additionally, true replication was not used because authors conducted replicates at different times (confirmed by through personal communication with the corresponding author [C. Vogs] on 9/3/2021).

Dasgupta et al. (2020) evaluated the acute effects of PFOA (CAS # 335-67-1, 96% purity, purchased from Acros Organics) on zebrafish (*Danio rerio*) via a 66-hour unmeasured, static study. A stock solution was prepared with either DMSO or NaOH and stored in 5 mL glass vials and kept at room temperature. The working solution (50 mM PFOA) was freshly prepared by spiking stock solutions into water derived from the recirculating water system. Adult,

wildtype (5D) zebrafish were maintained and bred in the same water system. Eight embryos (six hpf) were incubated and exposed to 10 mL of either a solvent control or 50 µM PFOA until 72 hpf at 28°C under a 14-hour:10-hour light-dark photoperiod. At test termination there was no significant effect of survival or development on zebrafish embryos. The 66-hour NOEC of 20.70 mg/L (50 µM) PFOA, based on survival, was acceptable for qualitative use only due to the short exposure period (i.e., 66-hour exposure instead of the established 96-hour acute exposure for this species).

Pecquet et al. (2020) conducted acute exposures with zebrafish embryos to determine the LC_{50} of PFOA, and to examine sublethal exposures of PFOA on neutrophil migration in response to wounding. A stock solution of PFOA (1 mg/L) was prepared in DMSO and diluted with fish water containing 60 mg/L Instant Ocean salts. In the acute LC_{50} tests, 20 embryos (one hpf) were exposed to one of seven PFOA concentrations (0-1,000 mg/L PFOA) with three replicates for each test concentration. Each test was replicated five times and results were pooled. Tests followed the OECD Fish Embryo Toxicity Test Guideline 236, and the U.S. EPA Fish Early-life Stage Toxicity Test Guideline 210. Exposures were static and lasted for 48 hours at 28°C. In a separate experiment, embryos (one hpf) were exposed for 24 hours to 0, 0.5 or 5.0 mg/L PFOA for 24 hours and checked for mortality and deformity. Measured PFOA concentrations were 0.685 and 6.16 mg/L PFOA for the test treatments and 0.089 mg/L PFOA was detected in the DMSO control. Afterwards PTU (1-phenyl-2-thiourea) (0.003%) was added to the remaining viable embryos to inhibit melanin formation (pigmentation), and the embryos were incubated for an additional 24 hours in their respective PFOA or control treatments. Fish were subsequently wounded and allowed to recover for three hours to facilitate neutrophil recruitment. The reported 48-hour LC_{50} was 300 mg/L PFOA, but the test duration was too short (i.e., only 48-hours rather

than the established 96-hour acute exposure period for this species) to be used quantitatively. In the separate experiment, neutrophil migration was significantly increased in the 0.685 mg/L PFOA exposure compared to the unwounded control fish. Results of this publication were acceptable for qualitative use.

Wasel et al. (2020) reported the results of toxicity tests with the zebrafish, *Danio rerio*, and PFOA in either whole larvae (*in vivo*) or as zebrafish embryonic cell line (*in vitro*). The results of the *in vivo* tests are summarized from this study since they represent apical endpoints with whole animals. PFOA (CAS # 335-67-1, >99% purity, purchased from Sigma-Aldrich, St. Louis, MO) stock solution was prepared in reverse osmosis water and diluted with embryo medium to make test concentrations ranging from 10-9,000 mg/L PFOA. Two acute toxicity tests were run, one where pH in test solutions was not buffered and another where test solutions were buffered with $NaHCO_3$ to pH 7. Wild-type zebrafish from in-house cultures were used to supply embryos (five to six hours post fertilization, hpf) for testing. Exposures were conducted in culture plates with one embryo per well and 20 embryos per plate. For each experiment there were at least three plates for each treatment. Embryos were observed for development until 96 hpf under test conditions (solutions renewed daily, 28±1°C, and a 14-hour:10-hour light:dark photoperiod). The 90-91 hour LC_{50}s reported by the authors were 57.6 and 487.4 mg/L PFOA, for the unbuffered and buffered test solutions, respectively. Based on the starting age of the organisms, the acute test was too short to be used quantitatively, so values were acceptable for qualitative use only, especially since other acute quantitatively acceptable tests for this species were available.

3M Company (2000) summarized a 96-hour static, unmeasured acute toxicity test with the fathead minnow, *Pimephales promelas*, and APFO (CAS # 3825-26-1). 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 acute test followed USEPA-TSCA Guideline 797.1400 protocol. Solutions of the test substance were made in reconstituted water and included five nominal test concentrations (530, 830, 1330, 2100, and 3330 mg/L test substance) plus a control. Exposures were conducted in 20 L glass tanks with 15 L of test solution and 20 juvenile fish per tank. There were two replicates for each treatment. Test conditions throughout the experiment ranged as follows: D.O.: 5.7-9.2 mg/L; pH: 7.2-7.9; and temperature: 21.8-22.5°C. No mortality occurred in the control and treatments with ≤830 mg/L test substance. The author-reported 96-hour LC_{50} was 2,470 mg/L test substance. The authors reported that the test substance was considered a mixture of APFO and other impurities, so the LC_{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 Company (2000) reported the results of two 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 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 first test lacks important test details including the dilution water, stock preparation, source of organisms, number of organisms per replicate and exposure vessels. Six nominal test concentrations (control, 50, 125, 250, 375 and 500 mg/L PFOA) were used, with each test treatment only replicated one time. The measured dissolved oxygen in the control and 375 mg/L test concentration was also low; ranging from 3.8-5.7 over the test period. Therefore, the authors questioned the health of the test fish, even though there was 100% survival at the end of the test period except the highest concentration with zero survivors. The

author-reported 96-hour LC_{50} for the test was 440 mg/L, but the lack of test details and the possible mixture effects of other perfluoro homologue compounds did not make the value acceptable for quantitative use. In the second acute test, with the same PFOA compound, authors dissolved the test substance in a 50:50 water:isopropanol solution to make a primary solution of 1,000 mg/L test substance and isopropanol. Another toxicity test conducted by the authors showed no mortality or sublethal effects at 500 mg/L isopropanol on the same species. The stock solution was then diluted with reconstituted water to make five nominal test concentrations (130, 220, 360, 600 and 1,000 mg/L test substance or 65, 110, 180, 300 and 500 mg/L PFOA) plus a control (reconstituted water only). The test followed USEPA-TSCA Guideline 797.1400. Exposures were conducted in 20 L glass aquaria with 15 L of test solution and 10 fish per tank (0.23 g/L loading). There were two replicates for each treatment. The pH of the three highest test solutions was very low at test initiation (3.0-4.3) which would adversely affect fish survival. No mortality occurred in the control and treatments with ≤110 mg/L PFOA. The 96-hour reported LC_{50} was 280 mg/L test substance and isopropanol or 140 mg/L PFOA. The test was not acceptable for quantitative use because of the possible mixture effects of other perfluoro homologue compounds and the low pH of test solutions. Results of both tests described here were retained for qualitative use.

3M Company (2000) reported the results of a 96-hour static, unmeasured acute toxicity test with the fathead minnow, *Pimephales promelas* and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot number 83 and was characterized as 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). No specific test protocol was identified. The test lacks important test details including the stock preparation, source of organisms, number of organisms per replicate and

exposure vessels. Six nominal test concentrations (control, 10, 20, 30, 40 and 50 mg/L APFO) were made in carbon-filtered well water. Each test treatment only replicated one time. The measured dissolved oxygen in the control and 50 mg/L test concentration was low; ranged from 4.0-5.9 over the test period. There was 90% survival in the control at test termination, 100% survival at ≤40 mg/L and 80% survival at the highest test concentration (50 mg/L). The authors extrapolated graphically to estimate a 96-hour LC_{50} of 70 mg/L APFO. This test was not acceptable for quantitative use because of the lack of replicates and lack of observed effects in the test, as well as the possible mixture effects of other perfluoro analogue compounds. This test was retained for qualitative use only.

3M Company (2000) reported the results of a 96-hour static, unmeasured acute toxicity test with the fathead minnow, *Pimephales promelas* and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot number 37 and was characterized as 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 USEPA 660/3-75-009 (1975) test protocol. Preparation of the stock was not provided, but the dilution water was noted as carbon filtered well water. The acute test included six nominal test concentrations (0, 560, 650, 750, 870 and 1,000 mg/L APFO). Exposures were conducted in glass aquaria with 16 L of test solution and 12 fish per tank (0.5 g/L loading). There were two replicates for each treatment. Test conditions throughout the experiment ranged by the following: D.O.: 4.0-5.0 mg/L; pH: 7.9-8.0; temperature: 19°C. The authors report a 96-hour LC_{50} for each replicate (776 and 754 mg/L), with an average LC_{50} of 766 mg/L APFO. Results of this test were not acceptable for quantitative use because of possible mixture effects of other perfluoro homologue compounds and the lack of true replicates and exposure details. Results of this test were retained for qualitative use only.

3M Company (2000) summarized a 96-hour static, unmeasured acute toxicity test with the fathead minnow, *Pimephales promelas*, and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot number 390 and was characterized as mixture of APFO (78-93% of the compound) and C_5, C_6 and C_7 perfluoro analogue compounds (7-22% of the compound). No specific test protocol was identified. Solutions of APFO were made in carbon filtered well water and included five nominal test concentrations (100, 180, 320, 560, and 1000 mg/L test substance) plus a control (well water only). Exposures were conducted in 4 L glass beakers with 3 L of test solution and six fish per beaker (0.6 g/L fish loading). There were two replicates for each treatment. Test conditions throughout the experiment varied little (D.O.: 5.6-7.4 mg/L; pH: 7.7-8.0; 20°C). No mortality occurred in the control and APFO treatments ≤180 mg/L. The author-reported 96-hour LC_{50} was 301 mg/L APFO. Because of possible mixture effects of other perfluoro analogue compounds, this test was not acceptable for quantitative use but was retained for qualitative use.

3M Company (2000) summarized 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 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 ≤750 mg/L. The

author-reported 96-hour LC_{50} was 843 mg/L PFOA. Because of possible mixture effects of other perfluoro analogue compounds, this test was not acceptable for quantitative use but was retained for qualitative use.

3M Company (2000) summarized a 96-hour static, unmeasured acute toxicity test with the fathead minnow, *Pimephales promelas*, and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot number 2327 and was characterized as 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 acute test followed test guidance from OECD 203. Solutions of APFO were made in well water and included five nominal test concentrations (150, 250, 400, 600, and 1,000 mg/L test substance) plus a control (well water only). Exposures were conducted in 19.6 L glass aquaria with 15 L of test solution and 10 fish (juveniles) per aquaria (0.30 g/L fish loading). There were two replicates for each treatment. Test conditions throughout the experiment varied little (D.O.: 6.1-9.2 mg/L; pH: 7.4-8.4; 21.0-22.0°C). No mortality occurred in the control and any treatment involving the test substance. The author-reported 96-hour LC_{50} was >1,000 mg/L test substance. Because of possible mixture effects of other perfluoro analogue compounds, this test was not acceptable for quantitative use but was retained for qualitative use.

Topmouth gudgeons, *Pseudorasbora parva*, were exposed to PFOA (99% purity) for 96-hours by **Yang et al. (2014)** via a static measured exposure (the authors note that the experiments followed ASTM standards and USEPA procedures for deriving water quality criteria). The topmouth gudgeon (4.0 g, 4.0 cm) were purchased from the Beijing Chaoyang Spring Flower Market, which was considered an atypical source. The organisms were allowed to acclimate for seven days before testing, and the test was conducted at 22 ± 2°C with a light:dark

cycle of 12-hour:12h-hour, with 10 fish per replicate and three replicates per concentration. Beakers used for exposure were assumed glass but was not specified by the study authors. PFOA was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 100, 140, 196, 274.4, 384.2 and 537.8 mg/L PFOA. Water quality parameters reported were pH = 7.0 ± 0.5, dissolved oxygen = 7.0 ± 0.5 mg/L, total organic carbon = 0.02 mg/L and total hardness = 190.0 ± 0.1 mg/L as $CaCO_3$. The supplemental data provided for the study includes a comparison of measured PFOA concentrations before and after solution renewal in the low and high acute and chronic test concentrations. PFOA concentrations in the test water did not fluctuate by more than 15% during experiments. The 96-hour LC_{50} reported for the study was 365.0 mg/L PFOA, which was not used quantitatively because of the uncertainties and potential exposures to PFAS from the source of the test organisms. The test was instead acceptable for qualitative use.

G.2.1.7 Adrianichthyidae fishes

The effects of PFOA (CAS # 335-67-1, purity not provided) on swim bladder development of *Oryzias latipes* was investigated by **Godfrey (2017)**. The stock solution was prepared by dissolving PFOA in 1 L of reverse osmosis water containing 12.5 mL Replenish, with the pH adjusted to neutral (7-7.5). The stock solution was then diluted to obtain 4.7 mg/L PFOA (only one concentration evaluated) based on data from a previous study. Adult, see-through Japanese medaka SK2MC strain were maintained in a controlled recirculating system, with a 14-hour:10-hour light:dark photoperiod and a temperature of 25 ± 1ºC. Adult fish were fed *ad libitum* twice daily with a combination of hatched *Artemia* nauplii and commercial food (Otohime). Adult fish were bred by placing them in breeding tanks (1:1 male to female ratio) in an environmental chamber with the same conditions described earlier. Fertilized eggs (<6-hours

post-fertilization) were collected from the bottom of the tank or gently brushed off from females. Eggs were then immediately disinfected in a 0.005% bleach solution and moved to six-well plastic plates containing 10 mL of the designated exposure or control media and no more than five embryos per well. Test solutions were fully changed every other day. The total length of the exposure lasted until 48-hours post-hatch. Fish were sexed with aid of leucophores along the body axis of males only prior to hatching. The LOEC for swim bladder development was 4.7 mg/L PFOA, which was not used quantitatively because only one concentration was tested; however, the tests results were retained for qualitative use.

G.2.1.8 Centrarchidae fishes

3M Company (2000) summarized two 96-hour static, unmeasured acute toxicity tests with the bluegill sunfish, *Lepomis macrochirus*, and APFO (CAS # 3825-26-1). The toxicant was part of the 3M production lot number 83 and was characterized as 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). No specific test protocol was identified in either acute test. In the first test, solutions of APFO were made in carbon filtered well water and included five nominal test concentrations (135, 180, 240, 320, and 420 mg/L test substance) plus a control (well water only). Exposures were conducted in 16 L tanks with 20 fish per tank. There were no replicates for each treatment. Test conditions throughout the experiment varied little (D.O.: 5.1-6.9 mg/L; pH: 7.8-8.0; 18-19°C). No mortality occurred in the control and treatments with ≤240 mg/L of test substance. The author-reported 96-hour LC_{50} was >420 mg/L. Since only one fish died in the highest test treatment, the experiment was re-run with higher test treatments (0, 420, 560, 750, 1000, and 1350 mg/L test substance). The 96-hour LC_{50} for the second test was 569 mg/L test substance.

Both effect concentrations were not acceptable for quantitative use because of possible mixture effects of other perfluoro analogue compounds, but they were retained for qualitative use.

G.2.1.9 Amphibians

Tornabene et al. (2021) conducted an acute PFOA (purchased from Sigma Aldrich, Catalog # 171468-25G; purity not provided) toxicity tests with the gray treefrog, *Hyla versicolor*. The acute test followed standard 96-hour acute toxicity test guidance (U.S. EPA 2002; ASTM 2008, 2017). Frog egg masses 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. The experiments began when frogs reached Gosner stage 40. 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 six to seven replicates for each treatment and two of the seven frogs died in the controls (i.e., 28% control mortality). The author-reported 96-hour LC_{50} was 191 mg/L PFOA, which was not acceptable for quantitative use because of high control mortality but was retained for qualitative use. Note, the authors also reported a quantitatively acceptable test for the same species (Gosner stage 26) that is described in A.2.11.

Yang et al. (2014) evaluated the acute toxicity of acidic form of perfluorooctanoic acid (PFOA, CAS #335-67-1, 99% purity) to the Asiatic toad, *Bufo gargarizans* via 96-hour renewal measured exposures (the authors note that the experiments followed ASTM standards and U.S.

EPA procedures for deriving water quality criteria). The tadpoles (0.048 g, 1.8 cm) were purchased from the Beijing Olympic Park, which is considered an atypical source. The organisms were allowed to acclimate for seven days before testing, and the test was conducted at 22 ± 2°C with a light:dark cycle of 12-hours:12-hours, with 10 tadpoles per replicate and three replicates per concentration. Beaker material used for exposure was not specified by study authors. PFOA was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 35, 56, 89.60, 143.36, 229.38 and 367 mg/L PFOA. Water quality parameters reported were pH = 7.0 ± 0.5, dissolved oxygen = 7.0 ± 0.5 mg/L, total organic carbon = 0.02 mg/L and total hardness = 190.0 ± 0.1 mg/L as $CaCO_3$. The supplemental data provided for the study includes a comparison of measured PFOA concentrations before and after solution renewal in the low and high acute and chronic test concentrations. PFOA concentrations in the test water did not fluctuate by more than 15% during experiments. The 96-hour LC_{50} of 114.74 mg/L PFOA was not used quantitatively due to the atypical test organism source but was retained for qualitative use.

G.2.2 Summary of Chronic PFOA Toxicity Studies Used Qualitatively in the Freshwater Aquatic Life Criterion Derivation

G.2.2.1 Worms

Yuan et al. (2016b) conducted a 10-day renewal, unmeasured test on PFOA (96% purity) with the planarian, *Dugesia japonica* (a non-North American species). The test organisms were originally collected from a fountain in Quanhetou Boshan, China, and cultivated in the laboratory for an unspecified time period before use. Dilution water was aerated tap water. No details were provided regarding photoperiod or light intensity. A primary stock solution was prepared by dissolving the salt in dimethyl sulfoxide (DMSO). The control and exposed

planarians received 0.005% DMSO (v/v). Exposure vessels were beakers of unreported material type and dimensions and a 50 mL fill volume. The test employed three replicates of 10 planarians each in five test concentrations: 0 (solvent control), 0.5, 5, 10, and 20 mg/L PFOA. The test temperature was reported as 20°C. No other water quality parameters were reported as having been measured in test solutions. Survival of solvent control animals was not reported. No apical endpoints were measured as the study focused on neural genes expression and neuronal morphology in the planarian. The lowest test concentration, 0.5 mg/L, decreased the mRNA expression levels of neural genes DjFoxD, DjotxA and DjotxB. Due to a lack of apical endpoints, insufficient test duration, and uncertainties associated with the source of the test organism, the LOEC was not acceptable for quantitative use but was retained for qualitative use.

Yuan et al. (2017) conducted another 10-day static, unmeasured test on PFOA (purity not provided) with the planarian, *Dugesia japonica*. Many of the exposure details were similar to the previous experiment. The test organisms were collected from a Quanhetou stream (Zibo, China), and cultivated in the laboratory for two weeks before use. Intact *D. japonica* (>1 cm) were starved for seven days before exposures. Dilution water was aerated tap water. No details were provided regarding photoperiod or light intensity. A primary stock solution was prepared by dissolving the salt in dimethyl sulfoxide (DMSO). The control and exposed planarians received 0.005% DMSO (v/v). Exposure vessels were glass tanks of unreported material, dimension, and fill volume. The test employed three replicates of 100 planarians each in five nominal test concentrations: 0 (solvent control), 0.5, 5, 10, and 20 mg/L PFOA. The test temperature was reported as 20°C. No other water quality parameters were reported as having been measured in test solutions. Survival of solvent control animals was not reported. No apical endpoints were measured as the study focused on stress responses. The lowest test concentration,

0.5 mg/L, exhibited elevated lipid peroxidation and increased mRNA expression levels of *HSP40* and *HSP70*, two stress response genes. Due to a lack of apical endpoints and insufficient test duration, the LOEC was not acceptable for quantitative use but was retained for qualitative use.

G.2.2.2 Planktonic crustaceans

3M Company (2000) summarized a 21-day static-renewal, unmeasured chronic toxicity tests 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 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 USPEA (1982) and OECD (1997) test protocols. Solutions of APFO were made in carbon filtered well water and include six test treatments (5.0, 8.0, 13, 22, 36, and 60 mg/L APFO) plus a control. Exposures were conducted in 250 mL glass beakers with 200 mL of test solution and five daphnids per beaker. There were four replicates for each treatment and each test solution was renewed every two days. Over the 21 day exposure period none of the daphnids died in the controls, but 80% of daphnids died in the highest test treatment. Reproduction was also affected by APFO with a significant decrease in the mean live young per adult at ≥36 mg/L APFO at test termination. 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. Note: as part of this publication the authors reported a 48-hour EC_{50} of 266 mg/L APFO but provide very little details about test methodology. Because of possible mixture effects of other perfluoro analogue compounds, this test was not acceptable for quantitative use but was retained for qualitative use.

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. *D. magna* ephippia were purchased from MicroBioTests Inc. (Belgium) and were activated by rinsing in tap water. Ephippia were hatched by incubating at 20-22ºC for 72 to 90 hours in standard freshwater under a continuous light intensity (6,000 lux). Newly hatched neonates (<24-hours old) were fed a suspension of *Spirulina* micro-algae two hours before testing. Nominal concentrations of 0 (control), 1, 10 and 25 µM (or 0 (control), 0.4141, 4.141, and 10.35 mg/L given the molecular weight of the form of PFOA used in the study, CAS # 335-67-1, of 414.07 g/mol) were prepared by mixing the respective amounts of PFOA in dimethyl sulfoxide (DMSO). Ten <24-hour old neonates, exposed in triplicate, were placed into 250 mL crystallization dishes with 100 mL of test solution. A mean temperature of 23 ± 1ºC, dissolved oxygen of 8 to 9 mg/L, total hardness of 250 mg/L as $CaCO_3$, pH of 7.5 ± 0.25 and salinity of 0.02% were reported in the exposure water. *D. magna* were fed a mixture of *Spirulina* microalgae and yeast (*Saccharomyces cerevisiae*) daily during the test, and 50% of the test solution was changed every other day. Neonates were counted daily and removed. The 21-day reproductive (fecundity) LOEC of 1 µM, or 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. 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*.

G.2.2.3 Aquatic insects

MacDonald et al. (2004) conducted a 10-day renewal, unmeasured range-finding test of PFOA (≥97% purity) with the midge, *Chironomus dilutus*. PFOA data were limited since no effects were observed at the highest treatment concentration (100,000 µg/L). Limited details were provided about the range-finding test. Authors stated that the test followed the general guidance given by EPA-600-R99-064 (USEPA 2002) and ASTM E 1706-00 (ASTM 2000). These were methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates and have different exposure durations than those typically considered for aqueous exposures, as well as different control survival requirements and recommendations. *C. dilutus* used for the test were 10-day old larvae from in-house cultures. Dilution water was reconstituted hard water according to ASTM (2002), but specifics were not provided. Temperature and D.O. concentrations were remained within acceptable ranges (21.0-23.0°C; D.O.>5.0 mg/L). The photoperiod consisted of 16-hours of illumination, at an unreported intensity. A primary stock solution was proportionally diluted with dilution water to prepare the test concentrations. Exposure vessels were 250 mL polypropylene beakers containing 240 mL of test solution and 50 mL of clean cultured sand. Assuming the range-finding test followed that later definitive test with PFOS; the PFOA test employed at least two replicates of 10 midges each in unmeasured PFOA concentrations that ranged from 1-100 mg/L PFOA. No effect on survival or growth were observed at the highest test concentration (100 mg/L PFOA) so the authors did not follow up with a definitive test. The 10-day NOEC of 100 mg/L, based on

survival and growth, was not acceptable for quantitative use but was retained for qualitative use by providing toxicity information on the relative tolerance of insects to PFOA exposure.

McCarthy et al. (2021) conducted a 10-day sub-chronic PFOA (97% purity, purchased from Sigma-Aldrich) test on the midge, *Chironomus dilutus*. The PFOA stock solution was dissolved in reconstituted moderately hard water without the use of a solvent stored in polyethylene at room temperature until use. The 10-day exposure was considered a range finding test for a follow up 19-day duration test (which is further described in C.2.10) and included concentrations spaced by ~100x with only mortality measured. Exposure vessels were 1 L high-density polyethylene beakers containing natural-field collected sediment with 60 mL of sediment and 105 mL of test solution. PFOA in test solutions was added via pipette to the beakers with the tip just above the sediment substrate. Nominal test concentrations were 0, 0.05, 26, 2,600, 26,000 µg/L. Egg cases were obtained from Aquatic Biosystems or USGS Columbia Environmental Research Center and held for 10 days. Each test beaker held 12 organisms with five replicates per exposure treatment. Solutions were renewed every 48-72 hours in the 10-day exposure. Water samples of test concentrations were measured on day one and day 10 and measured test concentrations ranged from 0-98% of nominal. Based on nominal concentrations, the author-reported NOEC and LOEC (endpoint = mortality) values were 26 and 272 mg/L PFOA, respectively. Results of this test were not used quantitatively because the exposure duration was too short for a chronic test and too long for an acute test; however, results were retained as qualitatively acceptable for use.

Stefani et al. (2014) conducted a chronic (10 generation) test of PFOA (form and purity not reported) with a midge, *Chironomus riparius*. The 10 generations (each approximately 20 to 28 days) were tested under static conditions. Authors stated that the test followed OECD 218

(2004c), OECD 219 (2004b), OECD 233 (2010), and a specific protocol for multigenerational assays using *C. riparius* developed and published by Nowak et al. (2006, 2007a, 2008, 2009) and Vogt et al. (2007b, 2007c, 2010). The specific protocol for multigenerational assays was designed to highlight neutral evolutionary responses caused by exposure to contaminants. A native population collected in the Lambro River (Milan, Lombardy, Italy) was used as a starting population for the test. *C. riparius* used to initiate the test were L1 (first instar) larvae. Dilution water was reconstituted water according to U.S. EPA (2000); test hardness was not specified, pH 7.8-8.2. Photoperiod was 16-hours:8-hours, light:dark with an intensity 500-1,000 lux. Treatments with two replicates each (i.e., two cages with five vessels each per treatment and 60 larvae per vessel, or approximately 300 larvae per treatment) were tested, by spiking 15 L of test water with 150 µL of methanolic solution at 1 g/L of PFOA to achieve a nominal concentration of 0.010 mg/L. Exposure vessels were glass tanks (19 cm x 19 cm x 18 cm) containing an unspecified amount of test solution and 1 cm thick layer of formulated sediment (75% of the volume constituted by 250-300 µm grain size aquarium quartz sand and 25% of the volume by 63-250 µm grain size natural sediment collected in an unimpacted river, sieved and sterilized). The measured exposure concentration diminished significantly over the course of the exposure (mean concentration at beginning of experiment: 0.0114 ± 0.0011 mg/L and concentration at end of experiment: 0.0064 ± 0.0011 mg/L), meaning later generations were exposed to less PFOA than earlier generations. The reported time-weighted measured concentration was 0.0089 mg/L. Mass-balance evaluations at the end of a generation showed that most of the PFOA was detected in the sediment (35% of the added amount). Test temperature was controlled at 20 ± 1°C, and dissolved oxygen remained above 66% saturation. No other water quality parameters were reported as having been measured in the test solutions. Controls were considered acceptable

because they fulfilled the validity criteria for survival according to OECD guideline 218 (OECD, 2004a). In the control group, most vessels in all generations reached the emergence of at least 70% individuals. There were no significant effects on mutation rate, emergence, reproduction, or sex ratio. The NOEC for the study was 0.0089 mg/L PFOA. The results from this study were not acceptable for quantitative use because of the lack of details pertaining to the characteristics of the sediment used in the exposure.

In a companion study to Stefani et al. (2014), **Marziali et al. (2019)** similarly conducted a chronic (10 generation) test of PFOA (form and purity not reported) with the midge, *Chironomus riparius*. The test was done under static conditions for 10 generations, each approximately 36 days (or 1/10 of this year-long, 10 generation test). The test followed OECD 218 and 233 (OECD 2004, 2010), with slight variations. *C. riparius* used for testing were from in-house cultures originating from a native population collected in the Lambro River (Milan, Lombardy, Italy). *C. riparius* used to initiate the test were first instar larvae. Dilution water was reconstituted water according to U.S. EPA/600/R-711 99/064 (U.S. EPA 2000); the hardness was not specified, pH 7.8-8.2. The photoperiod included 16-hours of unspecified illumination. Authors tested a single treatment (0.010 mg/L nominal) and solvent control. Authors stated there were two replicates per treatment. PFOA was dissolved in pure methanol (>99%) in order to achieve stock solutions at 1 g/L of PFOA. Each stock solution was then diluted in reconstituted water in order to achieve a nominal concentration of 0.01 mg/L. Exposure vessels were glass tanks (19 cm x 19 cm x 18 cm) containing 1 L of test solution and 1 cm of formulated sediment (75% of the volume aquarium quartz sand and 25% of sterilized natural sediment). The reported time-weighted measured concentration was 0.0098 mg/L and PFOA was found primarily in the water column. Water temperature, dissolved oxygen and pH were measured every three to five

days in two to three replicates per treatment. Test temperature was controlled at 20.1 ± 0.7°C, dissolved oxygen remained ≥66% saturation, and pH stayed within the range of 7.8-8.2. Each generation test was considered valid if emergence in the control was >70% in at least six replicates (i.e., vessels) of the 10 included. Emergence in the control groups by generation was as follows: 88 (primary emphasis for criteria development), 71, 53, 61.6, 78.6, 91.9, 62, 53.5, 79.1, 75.5. Generations one, two, five, six, nine, and 10 met control survival acceptability. The LOEC based on developmental time, adult weight, was 0.0098 mg/L (time-weighted average; NOEC and MATC <0.0098 mg/L). 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 this study were not acceptable for quantitative use because of limited test concentrations assessed, and uncertainty pertaining to sediment characteristics, and poor control survival in four of the 10 generations.

G.2.2.4 Salmonid fishes

The chronic toxicity of perfluorooctanoic acid (PFOA, purity not reported) to *Oncorhynchus mykiss* via a dietary exposure was evaluated by **Tilton et al. (2008)**. Two separate experiments were performed: 1) A tumor experiment involved feeding 10-week post-hatch fry 200 or 1,800 ppm PFOA for six months (30 weeks); and 2) A microarray experiment involved exposing 12-18-month-old juvenile fish trout diets containing 500 or 1,800 ppm PFOA for 14 days. PFOA in diets was not measured in either experiment, and tissues were not measured to ensure body burdens reflected doses used. Mt. Shasta strain rainbow trout were hatched and reared at the Oregon State University Sinnhuber Aquatic Research Laboratory in 14°C flowing

well water on a 12-hour:12-hour light:dark cycle. In the tumor experiment, approximately 1,000 fry were initiated at 10 weeks post-hatch with an aqueous exposure to 0.01 ppm aflatoxin B1 (AFB1) for 30 min. Sham-exposed trout were exposed to vehicle alone (0.01% ethanol) and served as noninitiated controls for each treatment. After initiation, fry were fed Oregon Test Diet (OTD), a semi-purified casein-based diet, for three months (Lee et al. 1991). Trout were then randomly (within initiator group) divided into experimental treatment groups (140 animals/treatment) and fed experimental diets containing 200 or 1,800 ppm PFOA, 1,800 ppm clofibrate (CLOF), or 1,800 ppm dehydroepiandrosterone (DHEA) *ad libitum* (2.8-5.6% body weight) five days/week for six months, a protocol similar to that previously described for DHEA (Orner et al. 1995). The PFOA concentrations in the diet for 200 and 1,800 ppm were equivalent to 5 and 50 mg/kg/day, respectively. Diets were prepared monthly and stored frozen at -20°C until 2-4 days prior to feeding, when diets were allowed to thaw at 4°C. At nine months post-initiation, juvenile fish were euthanized by deep anesthesia with 250 ppm tricaine methane sulfonate and sampled for liver tumors over a two-day period. Livers were fixed in Bouin's solution for two to seven days for histologic identification and examination of tumors with hematoxylin and eosin. In the microarray experiment, juvenile trout, 12-18 months of age, were maintained in separate 375 L tanks (three tanks) for each treatment, with five fish per tank. Animals were fed a maintenance ration (2.8% wet weight) of OTD. Administration of experimental diets containing 500 or 1,800 ppm PFOA, 1,800 ppm CLOF, 750 ppm DHEA, 5 ppm E2, or 0.1% dimethyl sulfoxide (DMSO) vehicle control was carried out for 14 days. The concentrations of 17β-estradiol (E2) and DHEA were chosen based on their ability to maximally induce vitellogenin (VTG) and/or act as hepatic tumor promoters in trout (Nunez et al. 1989). On day 15, fish were euthanized by deep anesthesia with 250 ppm tricaine methane sulfonate.

Approximately 100 mg liver tissue from individual fish was minced, stored in TRIzol Reagent and quick-frozen in liquid nitrogen for gene expression analysis. The rest of the liver was quick-frozen in liquid nitrogen for enzyme assays. The 10-week MATC (liver somatic index) and 6-month MATC (palmitoyl CoA β-oxidation-liver enzyme) were both 600 mg/kg PFOA diet (NOEC = 200; LOEC = 1,800 mg/kg). The test was not acceptable for quantitative use based on the non-apical test endpoints. This study was retained for qualitative use by providing non-apical endpoints which may inform mode of action and AOP considerations.

Benninghoff et al. (2011) evaluated the chronic effects of PFOA (CAS # 335-67-1, purchased from Sigma-Aldrich in St. Louis, MO) on rainbow trout (*Oncorhynchus mykiss*) juveniles in a 15-day static, unmeasured study. Mount Shasta strain rainbow trout were hatched and reared in the Sinnhuber Aquatic Research Laboratory at Oregon State University in Corvallis, OR. Fish were maintained at 12°C and a 12-hour:12-hour light:dark cycle in a 375 L tank filled with carbon filtered tap water. Two weeks before testing, fish were fed a semipurified casein-based diet with menhaden oil at a rate of 2% body weight. A stock solution was prepared by dissolving PFOA in dimethyl sulfoxide (DMSO) that was then added to oil in the fish diet to prepare a nominal concentration of 250 ppm PFOA wet weight. Eleven-month-old fish weighing approximately 70 g were placed in treatment groups, six fish per group, and were fed either 0.1, 1 or 5 mg/kg body weight/day PFOA laced food five times per week for 15 days: correlating to a diet concentration of 5, 50 or 250 ppm PFOA per day. Four replicates were included for each dietary treatment, along with a negative control group, and a vehicle control group (treated dietarily with 0.5 ppm DMSO). A positive control group (treated dietarily with 5 ppm estradiol) was also included with an additional twelve fish. Fish were sacrificed and weighed on day 15. The 50 and 250 ppm PFOA test diets caused a significant increase in plasma vitellogenin (Vtg)

levels. The lack of description of the dilution water and the test methodology (dietary exposure) rendered this study unacceptable for quantitative use and was retained for qualitative use only.

Benninghoff et al. (2012) also evaluated the chronic effects of PFOA (CAS No. 335-67-1, analytical grade purchased from Sigma Aldrich in St. Louis, MO) on rainbow trout (*Oncorhynchus mykiss*) in a ≈9-month unmeasured study. Mount Shasta strain rainbow trout were hatched and reared in the Sinnhuber Aquatic Research Laboratory at Oregon State University in Corvallis, OR. Fish were raised in a 375 L tank filled with carbon filtered tap water and maintained at 12°C and a 12-hour:12-hour light:dark cycle. Fry (10-15 weeks post spawn) were exposed to a cancer-causing agent for 30 minutes, then fed a semi purified casein-based diet for one month. Fish were fed experimental diets containing 2,000 ppm PFOA (approximately 50 mg/kg body weight/day) five-days per week for a period of six months. Fish were sacrificed at test termination (12.5 months post spawn) and examined for tumor presence. The dietary PFOA treatment significant increased tumor multiplicity and size. The lack of description of the dilution water and the test methodology (dietary exposure) deemed this study unacceptable for quantitative use and was retained for qualitative use only.

Atlantic salmon (*Salmo salar*) embryos were evaluated by **Spachmo and Arukwe (2012)** via a 52-day flow-through unmeasured exposure to PFOA (95% purity). Eggs were obtained from Lundamo Hatcheries, Norway (Aquagen) and transported to the Norwegian University of Science and Technology Centre of Fisheries and Aquaculture in Trondheim, Norway. The eggs were kept in plastic tanks (25 L) at 5-7°C with filtered, re-circulating and aerated water. Approximately one-third of the water volume was changed once per week. At age 404-679 day degrees (dd: number of days multiplied by degree Celsius), the eggs and larvae were exposed to PFOA (0.10 mg/L). PFOA was dissolved in methanol (carrier solvent: 0.01%) and the control

group was exposed to the carrier solvent only. Hatching occurred at 20 calendar days after start of exposure, at an effective developmental age of 504 dd, after which a riverbed environment was simulated by tank bed gravel and continuous water flow. Fish sampling was performed at 21, 35, 49 and 56 calendar days after exposure, or at respective developmental age of 549, 597, 679 and 721 dd. The exposure was terminated at 679 dd, and 712 dd represents the end of a one-week exposure-free recovery period. Thus, day 49 sampling was performed 24-hours after terminating the exposure and no exposure related differences in hatching rate were observed. The 52-day growth NOEC and LOEC were 0.10 and >0.10 mg/L PFOA, respectively. These data were deemed 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.

G.2.2.5 Cyprinid fishes

Giari et al. (2016) evaluated the effects of PFOA (96% purity) on tissue accumulation and histological alterations of specific organs of adult *Cyprinus carpio*. Thirty-one two-year-old common carp were purchased from a local fish farm and acclimated according to OECD 305 test guidelines (1996). Four weeks prior to the start of the experiment, fish were transferred into the test tanks to adapt to the exposure environmental conditions. The fish were fed pelleted feed three times per week with feeding stopped two days before fish were killed. Feeding was carried out manually to ensure rapid and complete consumption. Uneaten feed and feces were removed

from the tank to minimize PFOA sequestration by these organic substances. Fish were placed into each of three 120 L glass aquaria filled with tap water; with a continuous supply of fresh water provided at a flow-through rate of 500 mL/min. PFOA stock solutions were continuously dispensed and diluted to deliver the tested concentrations to the tanks over a period of 56 days. Exposures were conducted at 0.0002 mg/L (n = 10) and 2 mg/L (n = 11) levels based on environmental reports (Loos et al. 2008, 2009) and on reported experimental data, respectively (Oakes et al. 2004; Wei et al. 2007). A control group of 10 carp were held in tap water only. The stock solutions were prepared by dissolving PFOA in distilled water and delivered into the treatments tanks by a peristaltic pump at a flow rate of 0.42 mL/min. At time zero of exposure, an initial volume of the stock solution was added to the treatments tanks to immediately achieve the desired exposure concentration. Water parameters were monitored and recorded three times weekly for temperature (10-15°C), pH (6.70-8.00), and oxygen saturation (>80 %). PFOA concentration in the test water from each test tank was measured by liquid chromatography-mass spectrometry three times during the exposure period. The results of these analyses indicate that PFOA concentration was maintained at >80 % of each nominal concentration. At the end of the 56-day exposure, the fish were anesthetized with MS-222, killed by a deep cut through the neck, and dissected. Sex was recorded, and animals were measured for total length, body wet weight, and liver and gonad weight. Condition factor, hepato-somatic index, and gonado-somatic index were also calculated. No mortality was observed in the controls or either treatment level. The 56-day LOEC (PCNA-positive hepatocyte abundance) was reported as 2.0 mg/L PFOA, which was not used quantitatively because only two exposure concentrations were tested, lack of replication, and the test initiated with insensitive two-year-old adults. Results were retained for qualitative use.

The chronic toxicity of PFOA to *Cyprinus carpio* was also evaluated by **Manera et al. (2017)** under the same conditions as Giari et al. (2016). Stock solutions were prepared by dissolving PFOA (96% purity) in distilled water. Thirty two-year-old carp (19.3 ± 2.5 cm; 104.8 ± 27.8 g) were obtained from a local fish farm, randomly divided into three groups/tank (two PFOA treated tanks and one unexposed tank) of 10 fish each. Fish were acclimated for four weeks before starting the experiment and treated according to OECD (2012) test guidelines. The carp were fed with a commercial pellet food (Tetra Pond Pellets Mini) three times per week at 2% of their total body weight. Waste and uneaten food were removed regularly. A flow-through exposure test was conducted for 56 days by a system that continuously delivered PFOA to the test tanks to maintain concentrations of 0.0002 mg/L or 2 mg/L. Test tanks were 120 L glass aquaria filled with a continuous supply of tap water at a flow-through rate of 500 mL/min. The tested concentrations were selected, respectively, on the basis of environmental reports (Loos et al. 2008, 2009) and experimental data from the literature (Oakes et al. 2004; Road et al. 2007; Wei et al. 2007). The stock solution was delivered into the treatments tanks by a peristaltic pump at a flow rate of 0.42 mL/min. At time zero of exposure, an initial volume of the stock solution was added to the treatments tanks to immediately achieve the desired exposure concentration. Water parameters were monitored and recorded three times weekly for temperature (10-15°C), pH (6.70-8.00), and oxygen saturation (> 80%). At the end of the 56-day exposure, the fish were anesthetized with MS-222, pithed, dissected and sexed. In each group of carp, the sex ratio was approximately 1:1. The 56-day LOEC (liver biomarkers) was 2 mg/L PFOA, which was not used quantitatively because only two exposure concentrations were tested, lack of replication, and the test initiated with insensitive two-year-old adults. Results were retained for qualitative use.

The chronic effects of PFOA on the zebrafish, *Danio rerio*, have been reported by numerous researchers. However, all data available for this species were classified as qualitatively acceptable. **Hagenaars et al. (2013)** exposed *D. rerio* adults to PFOA (purity 96%) under renewal unmeasured conditions for 28 days. Adult zebrafish were obtained from a commercial supplier (Aqua hobby, Heist-op-den-berg, Belgium) and acclimated for four weeks prior to treatment in aerated medium-hard reconstituted freshwater (OECD 203) at 26 ± 0.5°C. Fish were subjected to a 14-hour light cycle and fed daily with Sera flakes at a rate of 2% of their mean body weight. After acclimation, fish were exposed to nominal concentrations of 0.1, 0.5 and 1 mg/L PFOA for four and for 28 days while the control fish were kept in clean water. Separate experiments with an identical setup were performed for four and 28 days. Every 48-hours, the water was totally renewed by water with the correct nominal PFOA concentrations. For both experiments, three different 25 L aquaria per exposure concentration were used with each aquarium containing eight male and eight female zebrafish. After respectively four and 28 days, fish were decapitated and dissected. The livers of six male fish and six female fish were pooled separately per aquarium and snap frozen in liquid nitrogen. The livers of fish exposed for 28 days to 0, 0.1 and 1 mg/L PFOA were used for transcriptomic and proteomic analyses. Whole bodies of all exposure concentrations were used for biochemical analyses (28 day) as well as for the determination of PFOA concentrations (four and 28 days). Based on the results of the first experiment, a second experiment was conducted to study the mitochondrial dysfunction caused by PFOA in more detail. The activity of the mitochondrial electron transport chain was measured. Male zebrafish were exposed to 1 mg/L PFOA for 14 days using the same exposure conditions as in the previous experiments. Three biological replicates were used for both the control and the PFOA exposed fish, with each aquarium containing 18 males. Males were chosen

in this experiment as they accumulated more PFOA than females. The liver was dissected to assess the electron transport chain (ETC) activity. ETC activity was also measured at the beginning of the experiment (time point zero) in three biological replicates consisting of 18 males each. The 28-day LOEC (reproduction-based endpoints, including fecundity, fertility, and hatching) was >1 mg/L PFOA, which was not used quantitatively because the test was initiated with a relatively tolerant adult life stage. A 28-day LOEC (non-apical alterations of gene transcripts) of 0.1 mg/L PFOA was also reported but was not used quantitatively due to the non-apical endpoint. Results of this study were, therefore, only considered qualitatively acceptable.

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 test design and results of the toxicity test with APFO and PFOA are highlighted here. A stock solution of APFO and PFOA were made with 100% DMSO at a concentration of 20 mM (final DMSO concentrations was 0.64% vol/vol) and diluted with standard embryo medium to make one of six test concentrations (0, 0.0064, 0.064, 0.64, 6.4 and 64 µM) for each chemical of interest. One zebrafish embryo (six hpf) from in-house cultures was placed individually in a well along with 90 µL of test solution in a 96-well culture plate. Thirty-two replicates were used for each test treatment. The effects of APFO and PFOA on mortality, growth, behavior morphology, histology and physiology were observed until 120 hpf (114-hour test duration) with the water quality conditions not reported. The most sensitive endpoint was mortality with a reported LOEC of 0.064 µM APFO, or 0.02759 mg/L APFO, based on a molecular weight of 431.1 g/mol for APFO. There were no effects of PFOA on mortality for zebrafish embryos with a reported NOEC of 64 µM PFOA or 26.50 mg/L PFOA based on a molecular weight of 414.07 g/mol for 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.

Zhang et al. (2014a) exposed adult *D. rerio* to PFOA (CAS # 335-67-1, 96% purity) for 21 days via renewal unmeasured exposure conditions. PFOA was dissolved directly in dilution water to obtain nominal concentrations (likely prepared a stock solution first). Adult wild-type zebrafish were obtained from a local fish dealer and acclimatized for three weeks prior to treatment in well-aerated tap water at 27 ± 0.5°C. Fish were subjected to a 12-hour light/dark cycle and fed live bloodworms and fish flakes (Tetramin) twice a day. After acclimation, fish were exposed to nominal concentrations of 0.05, 0.1, 0.5 and 1 mg/L PFOA. Separate experiments with an identical setup were performed. Every 24-hours, aquarium water was completely replenished with a water solution containing the correct nominal PFOA concentrations. For three experiments, five different 6 L aquaria per exposure concentration were used with each aquarium containing 15 male and 15 female zebrafish. After 21 days, fish were decapitated and dissected. The spleens from 15 male and 15 female fish were pooled and frozen in liquid nitrogen. The 21-day MATC (decrease in inflammatory cytokines, IL-1ß and IL-21, in spleen) was 0.0707 mg/L PFOA (NOEC = 0.05, LOEC = 0.1 mg/L). The chronic value was not acceptable for quantitative use because exposure was initiated with of adult fish and only non-apical endpoints were reported. Consequently, results of this study were only retained for qualitative use.

The effects of PFOA (CAS # 335-67-1, unreported purity) on *Danio rerio* thyroid disruption and subsequent swim bladder development was investigated by **Godfrey et al. (2017b)**. Stock solutions were prepared by dissolving PFOA in 1 L of reverse osmosis water containing 12.5 µL Replenish (Seachem Laboratories Inc.) and then adjusted to neutral pH (7-

7.5). The stock solution was then diluted to 1% of the respective LC_{50} based on data from a previous acute test (96-hour LC_{50} = 473 mg/L PFOA) (Godfrey et al. 2017a). Thus, the tested exposure concentration was 4.7 ppm PFOA. Adult zebrafish, AB wild-type, were maintained at a water temperature of 28 ±1°C and a photoperiod of 14-hours L:10-hours 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 two males to one female. Embryos were collected (gastrula stage, 4.5-hpf) and randomly placed into Petri dishes containing 25 mL of test solution which was renewed daily throughout the duration of the exposure. Each Petri dish contained 20 embryos and each test consisted of a minimum of three replicates per dose with experiments repeated three times. In order to cover the complete period of swim bladder development, zebrafish embryos were exposed starting immediately after fertilization either subchronically for six days (zero to six days post fertilization, dpf) or chronically for 28 days (zero to 28 dpf). For the subchronic exposures, embryos were maintained in Petri dishes for six days, after which they were imaged, and flash frozen for qPCR analysis. For the chronic exposures, larvae were moved after six days to a 500 mL glass mason jar containing 200 mL solution. Fish were not fed during the sub-chronic exposures since they rely on their yolk sac until swim-up. From six to 14 dpf larvae were fed *ad libitum* paramecia once a day, and from 15 to 28 dpf larvae were fed *Artemia* nauplii in the morning and Tetramin in the afternoon. Embryos and larvae were maintained in an environmental chamber at a temperature of 28 ± 1°C and a photoperiod of 14-hour:10-hour light:dark. The 28-day LOEC (swim bladder development) was 4.7 mg/L PFOA, which was not used quantitative since there was only one concentration tested; however, this value was retained for qualitative use.

Jantzen et al. (2017b) evaluated the effects of PFOA (purity not provided) on the morphometric, behavioral and gene expression in *D. rerio* exposed via five-day static unmeasured exposures (OECD Method 212, 2011). The AB strain of zebrafish (Zebrafish International Resource Center, Eugene, OR) were used for all experiments. Breeding stocks were bred and housed in recirculating systems under a 14-hour light cycle. System water was obtained by carbon/sand filtration of municipal tap water and water quality was maintained at a pH between 7.2 and 7.7, and water temperature between 26 and 28°C. Zebrafish embryos were exposed at 3-hpf to PFOA at concentrations of 0, 0.02, 0.2 or 2.0 μM (or 0, 0.02, 0.20, and 2.0 mg/L as reported by the authors) for 120-hours (four replicates, 30-38 fish per replicate). All compounds were dissolved in water. After this time, fish were transferred to non-treated system water and fed two times daily with Zeigler Larval AP50. Therefore, the only exposure was through the water from 3-hpf to 120-hpf (~five days), which corresponds to an embryonic to yolk sac larval exposure. At 120-hpf, morphometric measurements were recorded, and gene expression analyzed. Morphometric measurements were also taken at 7-dpf and 14-dpf. At 14-dpf, gene expression data and swim activity endpoints were collected. Each treatment compound and corresponding control group was set up as individual experiments, and the sample size was dependent on number of embryos produced from the stock breeding sets. No experiment had mortality greater than 20% of the starting sample size. The five-day (plus nine-days for observation) chronic value for growth based apical endpoints, including body length, was a MATC of 0.6325 mg/L (NOEC = 0.2 mg/L; LOEC = 2.0 mg/L). A MATC for swimming activity, a non-apical endpoint, was also 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., five-day)

exposure duration compared to other acceptable acute exposures that indicated *D. rerio* was tolerant to brief (i.e., 96-hours) PFOA exposures.

Annunziato (2018) investigated the cellular and behavioral alterations of zebrafish exposed to PFOA (purity not provided, purchased from Sigma Aldrich) for 333 hours. Zebrafish from in-house cultures were maintained at a pH of 7.2-7.7, temperature of 27±1°C and a 14-hour:10-hour light:dark cycle, and were fed twice daily a diet of *Artemia* in the mornings and aquatox/tetramin flake mix in the evenings. A stock solution of PFOA (2,000 µM) was prepared in egg water. Twenty-five embryos (3-hpf) were exposed under static conditions to one of four nominal treatments (0, 0.02, 0.2 and 2.0 µM PFOA) for five days and then transferred to control water for morphometric analysis of stained cartilage and yolk membranes of hatched larvae at 14 dpf. Control survival was >85%. At 14 dpf the stained neural area in larvae was less than the control fish and the normalized neural strain intensity was greater at the two highest test concentrations. The 333-hour (117 hour plus nine-day observation) NOEC and LOEC based on morphology were 0.02 and 0.2 µM PFOA, respectively; or 0.008281 and 0.08281 mg/L PFOA based on a molecular weight of 414.07 g/mol. The study was acceptable for qualitative use only because of the relatively short test duration for a chronic test.

Stinckens et al. (2018) report the results of 12 chemicals using an adverse outcome pathway testing strategy on zebrafish, *Danio rerio*. Twenty-four viable embryos (wild type), from in-house cultures, were transferred to polystyrene 24-well plates (one embryo/well; two mL/well). The first column of each plate consisted of a negative control (reconstituted water), resulting in 20 exposed embryos per plate and four control embryos per plate. Four plates were used for the median effect estimation (e.g., LC_{50}). Seven nominal test concentrations (0, 10, 50, 100, 250, 500 and 1000 mg/L PFOA) were used to determine the LC_{50} of zebrafish held under

static conditions for 168 hours. No solvent was used in stock preparation. The 168-hour LC_{50} was 362.5 mg/L PFOA, which was not acceptable for quantitative use because of the atypical exposure duration (too long for an acute test and too short for a chronic test). The test was retained for qualitative use only.

Zhang et al. (2021) summarized the immunotoxicity of PFOA via the NF-κβ pathway in zebrafish, *Danio rerio*, kidney. PFOA (CAS # 335-67-1, 95% purity) purchased from Sigma-Aldrich (St. Louis, MO) was mixed with dechlorinated tap water to create five nominal test concentrations (0, 0.05, 0.1, 0.5 and 1 mg/L PFOA). Zebrafish from a commercial supplier (specifics not provided) were acclimated for seven days in glass aquaria. Over the course of the 21-day exposure, fish were fed daily in a 12-hour:12-hour light:dark photoperiod and test solutions were renewed daily. At test termination 40 fish were sampled from each treatment and kidneys were excised. The specific number of replicates and number of organisms per replicate was not provided. PFOA kidney concentrations increased with increasing exposure durations. Additionally, as PFOA concentrations increased the kidneys became enlarged and the color faded. The mRNA expression levels of IFN and IL-1β peaked when zebrafish were exposed to 0.1 mg/L PFOA, increasing by 150% and 170%, respectively, compared to the control. The mRNA expression level was reduced when the PFOA concentration was higher than 0.1 mg/L. The mRNA expression level of IL-1β was lower than the control group at 1 mg/L PFOA. The 21-day LOEC of 0.1 mg/L (endpoint = mRNA gene expression levels in the kidney) was not acceptable for quantitative use because it was a non-apical endpoint, but it was retained for qualitative use.

3M Company (2000) summarized a flow-through, unmeasured chronic toxicity tests with the fathead minnow, *Pimephales promelas*, and APFO (CAS # 3825-26-1). The toxicant

was part of the 3M production lot number 83 and was characterized as 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 USEPA (1972) proposed chronic test protocols. Solutions of APFO were made in aerated well water and included six test treatments (0, 6.2, 12.5, 25, 50, and 100 mg/L APFO). Exposures started with 60 eggs (48 hours post fertilization) sourced from U.S. EPA (Duluth, MN) in oscillating eggs cups. Each test treatment had two test replicates (aquaria). After hatch, 40 fry from each cup were placed in their respective aquarium and exposures continued for an additional 30 days under flow-through conditions. Fry were fed two to three times per day. Test conditions throughout the test were D.O. >95% saturation, pH 7.0-7.3 and temperatures of 25 ± 1°C. At test termination there were no significant effects on egg hatchability, and fry survival and growth. The 30-day post hatch NOEC of 100 mg/L APFO, based on all test endpoints, was not acceptable for quantitative use because of the possible mixture effects of other perfluoro analogue compounds and lack of observed chronic effects. Results of this test were retained for qualitative use only.

3M Company (2000) summarized the uptake/depuration of APFO (CAS # 3825-26-1) by the fathead minnow, *Pimephales promelas*, in a 13-day sub-chronic exposure. The toxicant 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 exposure included one test concentration of APFO (25 mg/L) dissolved in carbon-filtered well water plus a control. Exposures were static and included a 13-day uptake phase followed by a 15-day depuration phase containing no PFOA. Thirty fathead minnows (64 days old) were obtained from Aquatic Biosystems (Fort Collins, CO) and held in five gallon high density polyethylene tanks containing 15 L of test solution (0.1246 g/L fish loading). No treatments were replicated. Measured

concentrations of APFO ranged from 25.0-25.9 mg/L in test solutions and <1 mg/L in control solutions. Fish were fed daily and five fish were sampled on day eight and day 13 in the uptake phase and days one, four, seven, and 10 in the depuration phase for whole body APFO concentrations. Average APFO measured in whole bodies of fathead minnows was 46.7 µg/g wet weight at test day 13, with a calculated BCF of 1.8 L/Kg. Authors reported no effects of survival and growth in the single treatment concentration evaluated, resulting in a NOEC of >25 mg/L. Results of this study were not acceptable for quantitative use because of possible mixture effects of other perfluoro analogue compounds, the lack of replicates, and because of the relatively short exposure duration. Results of this test were retained for qualitative use only.

Oakes et al. (2004) exposed fathead minnows to PFOA in an outdoor microcosm experiment. The University of Guelph Microcosm Facility is located at the Guelph Turfgrass Institute (ON, Canada) and consists of 30 artificial ponds of approximately 12,000 L. The microcosms were constructed below grade to a depth of 1.2 meters using galvanized steel panels lined with food-grade polyvinylchloride. Each microcosm had a diameter of 3.9 meters, was filled with water to a depth of approximately 1 meter and was flush with ground level. The water supply for the microcosms was an irrigation pond supplied by a well located on site. Sediment trays containing a 1:1:1 (v/v/v) mixture of sand, loam, and organic matter, as well as potted macrophytes (*Myriophyllum spicatum*) were added to each microcosm. Prior to being treated with PFOA, water was circulated among all microcosms for two weeks at a flow rate of approximately 12 m^3/d, ensuring homogeneous water chemistry, zooplankton, and algae assemblages. PFOA (19.4% wet-weight aqueous solution from the 3M Company; however, purity of PFOA that constituted the 19.4 wet weight was not reported) was added to the microcosms once by subsurface injection. Water samples from each microcosm were obtained

using a metal depth-integrating water-column sampler at one-hour and one, two, four, seven, 14, 21, 28 and 35 days after PFOA addition to calculate time-weighted mean PFOA concentrations. Microcosms were treated in triplicate at nominal concentrations of 0.3, 1.0, 30 and 100 mg/L PFOA (mean time weighted average concentrations of 0.27, 0.65, 23.9 and 74.1 mg/L PFOA). Three additional microcosms served as controls and did not receive any PFOA. Fathead minnows were purchased from Silhanek Baitfish Farms (Bobcageon, ON, Canada) and acclimated in the adjacent irrigation pond for 10 days prior to PFOA exposure (under a natural photoperiod). Breeding pairs were held in two wooden frames with 5 mm aperture polyvinylchloride mesh cages. Each microcosm held two cages, with each PFOA concentration replicated in three microcosms. Cages were divided into four quadrants, and each quadrant contained a single breeding pair for a total of 16 fish per microcosm. Fish were initially sexed prior to exposure based on size and presence of secondary sex characteristics. Sexes were subsequently confirmed at the conclusion of the exposure after the fish were killed. A 15 cm piece of 10 cm round polyvinyl chloride pipe cut in half lengthwise served as a breeding substrate within each quadrant and was examined for egg deposition daily. Both egg production and oviposition (spawning) frequency were recorded and used for the subsequent calculation of egg and oviposition frequency per female, per microcosm, and cumulatively per dose. At the conclusion of the 39-day exposure, measurements of total length, total weight, gonad weight, and liver weight were taken, and gonadosomatic indices (GSI), liver-somatic indices (LSI), and condition factor (K) were calculated. Mean water-quality parameters (collected mid-depth) sampled over the course of the exposure include dissolved oxygen (7.6 mg/L), temperature (21.7°C), pH (8.5) and alkalinity (112.9 mg/L as $CaCO_3$). The 39-day NOEC was 74.1 mg/L PFOA based on mean total egg production over the course of the experiment. The large outdoor

microcosm experiment contained sediment, algae, macrophyte and zooplankton and therefore the chronic value was not used quantitatively but was considered in a qualitative manner.

Wei et al. (2008a) later evaluated the hepatic protein profiles in *Gobiocypris rarus* exposed to PFOA (98% purity) for 28 days. Adult male and female rare minnows (~nine months old) with an average body weight of 1.4 ± 0.4 g and an average total length of 47.7 ± 3.6 mm were obtained from a laboratory hatchery and held in 20 L glass tanks (~2 g body weight/L). Fish were acclimated and treated as previously described (Wei et al. 2007). Briefly, fish were supplied with dechlorinated tap water under continuous flow-through conditions at 25 ± 2°C with a photoperiod of 16-hours:8-hours (light/dark). Commercial granular food (Tetra) was supplied at a daily rate of 0.1% body weight. Waste and uneaten food were removed daily. Gender determination was based on the shape of the abdomen and the distance between the abdominal fin and the tail fin. After a one-week acclimation period, equal numbers of randomly selected male and female rare minnows were assigned to one of four treatment groups: PFOA exposure at 0, 3, 10 or 30 mg/L. These concentrations were selected based on previous studies (Oakes et al. 2004); however, the actual PFOA concentrations in the tanks were not verified by chemical analysis. Each treatment group contained ten male and ten female minnows in duplicate tanks. The flow rate of the test solution was 8 L/hour. At the end of the 28-day exposure period, all fish were anesthetized on ice. Gonadal tissues from all fish and hepatic tissues from four male and four female fish per treatment group were quickly dissected and fixed in 10% formalin for histological analysis. The livers from the remaining six males and six females per treatment group were removed and immediately frozen in liquid nitrogen and stored at -80 °C until analysis. The 28-day LOEC (PCR alterations of genes in liver) was 3 mg/L PFOA, which was not used quantitatively because it was a non-apical endpoint, but was qualitatively acceptable to

potentially inform mode of action and AOP considerations. Following the same methodology described above, **Wei et al. (2008b)** reported a 28-day MATC (change in m-RNA muscle-heart-fatty acid binding protein, a hepatic protein) of 5.477 mg/L PFPA, and a 28-day LOEC (protein spots identified by matrix-assisted laser desorption/ionization (MALDI) tandem time-of-flight mass spectrometry (TOF/TOF) analysis) of 3 mg/L PFOA. These data were also not used quantitatively but were retained for qualitative use.

Liu et al. (2008b) evaluated the effects of PFOA (98%) on the mRNA levels in the rare minnow, *Gobiocypris rarus*. The flow-through unmeasured test was conducted for 28 days. All rare minnows were obtained from a laboratory hatchery. Two hundred and forty mature males and females (about nine months old, 1.4 ± 0.4 g, 47.7 ± 3.6 mm) were randomly assigned to eight 20 L glass tanks (30 individuals per tank) and acclimated for one week. Fish were supplied with dechlorinated tap water under continuous flow-through conditions at 25 ± 2°C and subjected to a photoperiod of 16-hours:8-hours light:dark. 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, 15 randomly selected male and 15 randomly selected 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 each of the four groups (0, 3, 10 or 30 mg/L PFOA). Each treatment was carried out in duplicate tanks (15 male and 15 female fish in each tank). The flow rate of the test solution was 8 L/hour. After a 28-day exposure period, fish were anesthetized on ice. Gills from four male and four female fish from each treatment group were quickly dissected and fixed in 10% formalin for histological analysis. The gills from six male and six female fish in each group were removed and immediately frozen in liquid nitrogen and stored at -80°C until further real-time PCR analysis. Various tissues,

including muscle, liver, brain, gonad, gill, and intestine from two male and two female fish in the control group were used for semi-quantitative PCR analysis. The remaining samples were used for proteomic analysis. The 28-day MATC (increase relative mRNA expression of AhR in gills) was 5.477 mg/L PFOA for males, and the MATC (decrease relative mRNA expression of CYP1a and increase relative mRNA expression of PXR in gills) for females was 17.32 mg/L PFOA. Both toxicity values were not used quantitatively because they were based on non-apical endpoints but were considered qualitatively acceptable.

Liu et al. (2009) again investigated the effects of PFOA (98%) on the mRNA levels in the gill and liver of the rare minnow, *Gobiocypris rarus*, but for males and females separately. Minnows (about nine months old), with a body mass of 1.4 ± 0.4 g and total length of 47.7 ± 3.6 mm, were obtained from a laboratory hatchery. Briefly, male and female rare minnows were randomly allocated into four treatment groups (0, 3, 10 or 30 mg/L PFOA). Six males and six females were included in each treatment group. After a 28-day exposure in dechlorinated tap water under flow-through conditions at 25 ± 2°C with a 16-hour light, 8-hour dark photoperiod, fish were anesthetized on ice and then sampled. The gills and livers from fish in the PFOA treatment groups and the muscle, livers, brains, gonads, gills, and intestines from fish in the control group were removed, immediately frozen in liquid nitrogen, and stored at -80°C until analysis. The 28-day MATC (increase relative mRNA expression of PPARγ in gills) was 5.477 mg/L PFOA for females, and the MATC (increase relative mRNA expression of PPARγ and PPARα in gills and CYP4T11 in liver) for males was 17.32 mg/L PFOA. Both toxicity values were not used quantitatively because they were based on non-apical endpoints but were considered qualitatively acceptable.

The effects of PFOA (98% purity) on the gene expression of *Gobiocypris rarus* was also evaluated by **Fang et al. (2010)**. Nine-month-old male rare minnows with an average body mass of 1.3 ± 0.3 g were obtained from a laboratory hatchery. Fish were kept in an indoor aquaria system with flowing dechlorinated water at 25 ± 2°C and a photoperiod of 16-hours:8-hours (light:dark). After acclimation for one week to ensure the absence of disease, fish were randomly assigned to 20 L glass tanks (10 individuals per tank) and exposed under flow-through conditions to various concentrations (0, 3, 10 or 30 mg/L) of PFOA for 14 days. Each treatment was in duplicate tanks. The flow rate of the test solution (8 L/hour), dissolved oxygen (>80%), water temperature (25 ± 2°C) and the functioning of the delivery system were monitored throughout the study. Commercial granule food (Tetra) was supplied at a rate of 0.1% body weight per day during the experiment. Waste and uneaten food were removed daily. No decrease in food consumption or other adverse effects was observed during the experiment. At the end of the exposure, ten fish per group were anesthetized on ice. The livers were removed, and six livers from each group were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Hepatic tissues of the other four individuals per group were quickly dissected and fixed in 10% formalin for histological examination. The 14-day LOEC (apolipoprotein gene expression) was 3 mg/L PFOA, which was not used quantitatively because of the test duration and non-apical endpoint; however, the results were retained for qualitative use.

Yang et al. (2014) evaluated the toxicity of PFOA (CAS # 335-67-1, 99% purity) to *Pseudorasbora parva* via a 30-day renewal measured exposure (the authors note that the experiments followed ASTM standards and USEPA procedures for deriving water quality criteria). The topmouth gudgeon (4.0 g, 4.0 cm) were purchased from the Beijing Chaoyang Spring Flower Market, which was considered an atypical source. The organisms were allowed to

acclimate for at least seven days before testing, and the test was conducted at 22 ± 2°C with a light cycle of 12-hours, 10 fish per replicate and three replicates per concentration. Beakers used for exposure were assumed glass but was not specified by study authors. PFOA was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 10, 15, 22.5, 33.75, 50.63 and 75.94 mg/L PFOA. Blank and solvent controls were also included. Water quality parameters reported were pH = 7.0 ± 0.5, dissolved oxygen = 7.0 ± 0.5 mg/L, total organic carbon = 0.02 mg/L and total hardness = 190.0 ± 0.1 mg/L $CaCO_3$. The supplemental data provided for the study included a comparison of measured PFOA concentrations before and after solution renewal in the low and high acute and chronic test concentrations. PFOA concentrations in the test water did not fluctuate by more than 15% during experiments. The 30-day survival EC_{10} of 11.78 mg/L PFOA was not used quantitatively due to the atypical fish source and the older/unspecified life stage of the test organisms at test initiation. Results of this test were, instead, considered qualitatively acceptable.

G.2.2.6 Melanotaeniidae fishes

Miranda et al. (2020) evaluated the sub-chronic effects of PFOA (CAS# 335-67-1, ≥96% purity, purchased from Sigma-Aldrich) on the Murray River rainbowfish (*Melanotaenia fluviatilis*). One-year-old male adult fish were purchased from a commercial supplier and held in a flow-through system in 50 L tanks with carbon filtered aerated water to acclimate to test conditions. During the exposure period, the test temperature was 23°C, pH was 7.1-7.4 s.u. and dissolved oxygen was >80% saturation. Stock solutions were made with Milli-Q water and diluted to one of four test treatments (0.01, 0.1, 1 and 10 mg/L). Four fish were assigned to 12 L tanks, with three tanks per treatment, for a total of 12 fish per test concentration or control. Water

was changed and fish were fed frozen brine shrimp daily during the 14-day exposure period. Measured test concentrations at test initiation and after the first 24-hour renewal averaged <0.01, 0.01, 0.1, 0.91 and 9.0 mg/L PFOA, respectively. There was no effect on mortality, condition factor or gonadosomatic index (GSI) between any treatment or the control at test termination. The hepatosomatic index was significantly reduced at the three highest test concentrations as compared to the control fish, suggesting increasing energy demands of the exposed fish. The NOEC of 9.0 mg/L PFOA, based on growth and mortality, was acceptable for qualitative use only because of the short test duration for a chronic exposure.

G.2.2.7 Adrianichthyidae fishes

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. Solvent-free stock solutions of PFOA (2,000 mg/L) were prepared by dissolving the solid in MilliQ® water via sonication. Chemical measurements were not made, and nominal concentrations were used throughout the study. Medaka were maintained in the laboratory for several years at 25 ± 1°C, a 16-hour:8-hour light:dark photoperiod and fed with *Artemia* nauplii (<24-hours after hatching) twice daily. For the F0 fish exposure study, breeding medaka pairs (~2.5 cm) were maintained at 25 ± 1°C for at least seven days in 1 L beakers filled with dechlorinated tap water, which was prepared by serial filtration through a sediment and two granular activated carbon filters. Thirty-six mating pairs that spawned more than eight eggs per breeding and bred more than five times per week were selected and randomly separated into four groups. Nine mating pairs were assigned to each treatment group and the control. Definitive test PFOA concentrations were 0.1, 1 and 10 mg/L, based on the preliminary range-finding results using adult medaka. The exposure duration for F0 fish was limited to 14 days, during which the fish were fed *Artemia*

nauplii (<24-hours after hatching) *ad libitum* twice daily. The exposure medium was renewed at least three times per week. Dead fish were removed as soon as possible. Eggs were counted every day, and the eggs spawned on the seventh day were saved for the F1 generation exposure study. On day 14, all surviving fish were euthanized, and body length and weight were measured, from which the condition factor (K) was calculated. The gonads and livers were also measured, and the gonadosomatic index (GSI) and the hepatosomatic index (HSI) were calculated. For the F1 fish exposure study, fertilized eggs collected from F0 fish exposed to each concentration of PFOA and the control were randomly separated into groups of 25 eggs each and then assigned to varying concentrations of PFOA (0, 0.1, 1 or 10 mg/L), with only one replicate per treatment. Because eggs were compiled into a single replicate for the hatching stage, results reported beyond hatching (even when larvae/juveniles were separated into replicates) are based on pseudoreplication. During the egg stage for the F1 generation, investigators maintained all possible combinations of F0 x F1 exposure concentrations for a given compound. Exposure was initiated in 50 mL beakers less than 12 hours after fertilization. The developing embryos were observed daily under a stereoscopic microscope, and dead embryos were removed. This procedure was repeated until all living embryos had hatched. Hatching was defined as the disruption of the chorion. Newly hatched larvae were then randomly transferred to 100 mL beakers and observed daily for swim-up success and survival for an additional two weeks. Larvae were fed *Artemia* nauplii *ad libitum* twice daily. After 14 days, replicates with five fry each were randomly selected from each treatment group and transferred to 1 L beakers for the 100-day post hatch observation. All survivors were sacrificed 100 days after hatching, and body length and weight were measured. The gonads and livers were weighed to determine GSI and HSI. The F0 (parental generation) adult survival, condition factor and adult male and female GSI

and HSI 14-day LOECs were all >10 mg/L PFOA. For the F1 (progeny generation), the percent hatchability, swim-up success and larval growth NOECs were all 10 mg/L PFOA, while the NOEC for the time to hatch endpoint was 1.0 mg/L, suggesting these were tolerant endpoints relative to the criterion magnitude of 0.094 mg/L. 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, 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 be rejected because of pseudoreplication resulting from a lack of replicates in the hatching stage. Since this test is a static unmeasured test, 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).

Kang et al. (2019) evaluated the chronic effects of PFOA (96% purity, CAS # 335-67-1 purchased from Sigma Aldrich, St. Louis, MO) on Japanese medaka (*Oryzias latipes*) in a 21-day unmeasured, static-renewal study. A stock solution was prepared by dissolving PFOA into dimethyl sulfoxide and stored at 4°C. The 10 mg/L working solution was prepared by diluting the stock solution in fish culture water (carbon-filtered dechlorinated tap water). Adult fish (16 ± 2 weeks, 0.38 ± 0.06 g) were obtained from the fish culture facility at the Korea Institute of Technology in Jinju, Gyeongnam, South Korea. Fish were acclimated for 7 days in carbon-filtered dechlorinated tap water at 25°C with a 14-hour:10-hour light-dark photoperiod. Eight male and eight female fish were introduced to a 20 L glass tank filled with 15 L of working

solution at concentrations of 0 (control), 0 (solvent control) and 10 mg/L PFOA. Fish were fed brine shrimp and Tetramin daily, and the working solution was renewed twice weekly. Authors reported following OECD 229 exposure guidance, with conditions maintained the same as during the acclimation period. Eggs were harvested and counted twice daily at seven, 14 and 21 days. A significant reduction in fecundity was shown for all time periods, but there was no abnormal behavior or mortality observed. The 21-day fecundity LOEC was 10 mg/L PFOA and only considered acceptable for qualitative use because it only reported a relatively tolerant LOEC value.

G.2.2.8 Amphibians

Hoover et al. (2017) tested chronic PFOA (96% purity) toxicity on the northern leopard frog, *Lithobates pipiens* (formerly, *Rana pipiens*) in a chronic renewal test using measured PFOA treatment concentrations. Stock solutions consisted of 1 g of chemical dissolved in 1 L of Milli-Q water, followed by pH adjustment to 6.95-7.05, and lastly vacuum-filtration before storage in polycarbonate bottles. Eight northern leopard frog egg masses were collected during early spring from a temporary pond at the Purdue Wildlife Area in West Lafayette, IN, and randomly assigned to outdoor ~100 L wading pools. After hatching, larvae were checked daily for mortality and fed Purina Rabbit Chow *ad libitum*. Treatments consisting of control and exposure to PFOA at three concentrations (nominally 0.01, 0.1, and 1.0 mg/L) were placed in two replicates on adjacent shelves within an environmental chamber. Experimental units consisted of 15 L plastic aquaria filled with 7.5 L of filtered, UV-irradiated well water. Tadpoles (n=35 per aquarium) were randomly assigned to the experimental units. Prior to addition to aquaria, a subset of animals was examined to confirm development at Gosner stage 26, when hind limb buds start to develop. Tadpoles with visible irregularities in morphology, coloration, or

behavior were excluded. Animals were maintained at 20 ± 2°C with a 12-hour:12-hour light:dark photoperiod for 10 days to acclimate to indoor conditions and were fed a Tetramin slurry *ad libitum*. Water changes (100%) were conducted every four days. Tadpoles were exposed for 40 days and were monitored daily for survival and abnormalities. A water sample (~5 mL) was taken immediately prior to and after each water change to monitor concentration of test chemicals. Every 10 days, six animals were randomly collected from each aquarium. The animals were euthanized, measured (total length at 10 days, snout-vent length otherwise), and staged (Gosner) prior to storage at -20°C for chemical analyses. After 40 days, the depuration phase was initiated by removing animals, cleaning each aquarium with a methanol-soaked sponge, and rinsing to remove adsorbed compound. Aquaria were refilled with clean water; animals were returned to the same aquarium and monitored as described above. Water changes were carried out every four days with fresh water, and a water sample was taken prior to each water change. Two tadpoles were sampled every 10 days for an additional 30 days. Survival was >90% for all treatments and no significant sublethal effects were observed. 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 as 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. Frog egg masses were collected from an ephemeral pond at the Purdue Wildlife area in West Lafayette, Indiana. Egg masses were held in covered 190-L outdoor tubs containing 80 L of well water. Once hatched, the larvae were fed *ad libitum* with Purina rabbit chow. A control treatment (replicated four times) and three nominal sediment exposure concentrations of 10, 100 and 1,000 ppb PFOA (each replicated five times) were set up in 180-L plastic wading pools filled with 75 L well water. The stock solution was made by dissolving 2.0 g PFOA into 1 L of reverse osmosis Milli-Q water in polycarbonate bottles. Sediment was collected from the upper 5 to 8 cm of a permanent pond in the same wildlife area. The sediment was air dried for eight days, with 10.1 kg of the dried homogenized sediment placed in each experimental unit. Sediment was spiked with the assigned PFOA dose by adding the appropriate volume of stock solution to 6 L of water, stirred for five minutes, and then allowed to equilibrate for seven days. Once equilibrated, 75 L of water was added to the experimental chamber and allowed to sit for an additional three days. The water was then inoculated with algae and zooplankton from local pond water and allowed to establish for five days, after which Gosner stage 25 frog larvae were added to each tank.

Reported average water quality conditions include a pH of 7.8 and temperature of 26.2°C. Overlying water PFOA measured water concentrations were 2.9, 7.3 and 66 µg/L for the nominal sediment concentrations of 10, 100 and 1,000 ppb, respectively. At test termination (30 days) there was no effect on survival and growth (snout-vent length and weight). The 30-day NOEC, based on survival and growth, was 66 µg/L (or 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 acceptable for qualitative use only because the test design was an outdoor spiked sediment mesocosm exposure with algal and zooplankton communities present.

Yang et al. (2014) evaluated the chronic toxicity of PFOA (CAS #335-67-1, 99% purity) to the Asiatic toad, *Bufo gargarizans* via a 30-day renewal measured exposure (the authors note that the experiments followed ASTM standards and USEPA procedures for deriving water quality criteria). The tadpoles (0.048 g, 1.8 cm) were purchased from the Beijing Olympic Park, which was considered an atypical source. The organisms were allowed to acclimate for seven days before testing, and the test was conducted at 22 ± 2°C with a light:dark cycle of 12-hours:12-hours. There were 10 tadpoles per replicate and three replicates per concentration. Beakers used for exposure were assumed glass but was not specified by study authors. PFOA was dissolved in deionized water and carrier solvent DMSO to obtain a 7 mg/mL stock solution, and then diluted with dechlorinated tap water to yield nominal exposure concentrations of 5, 7.5, 11.25, 16.88, 25.31 and 37.97 mg/L PFOA. Water quality parameters reported were pH = 7.0 ± 0.5, dissolved oxygen = 7.0 ± 0.5 mg/L, total organic carbon = 0.02 mg/L and total hardness = 190.0 ± 0.1 mg/L as $CaCO_3$. The supplemental data provided for the study included a comparison of measured PFOA concentrations before and after solution renewal in the low and

high acute and chronic test concentrations. PFOA concentrations in the test water did not fluctuate by more than 15% during experiments. The 30-day EC_{10} (survival) reported for the study, 5.89 mg/L PFOA, was not used quantitatively due to the atypical test organism source (obtained from Beijing Olympic Park) and limited details pertaining to the source of the test organisms and any potential previous exposure to PFOA or any other contaminant. Consequently, this study was considered qualitatively acceptable.

G.2.3 Summary of Qualitatively Acceptable Plant PFOA Toxicity Studies

G.2.3.1 Cyanobacteria, Anabaena sp.

Rodea-Palomares et al. (2012) examined the toxicity of PFOA (acid form, CAS # 335-67-1, 96% purity) with the bioluminescent cyanobacterium, *Anabaena sp*. (CPB4337 strain) following OECD Guidelines No. 23 (OECD 2000b) and Rodea-Palomares et al. (2009). The inhibition of constitutive luminescence was examined over a 24-hour test period. Very little information was provided about the exposure details (i.e., test media, test vessel, cell density per replicate, water quality parameters). PFOA was dissolved in the exposure media with no solvent and was measured in the highest test concentration and one concentration near the reported EC_{50}. The cyanobacteria were exposed to five to seven test concentrations with replicate samples. Each test was repeated three times. The reported EC_{50} was 19.81 mg/L based on bioluminescence inhibition and was not acceptable for quantitative e use, based on the short test duration and lack of exposure details. This test was, therefore, considered qualitatively acceptable for use in criteria derivation.

Rodea-Palomares et al. (2015) conducted a similar 24-hour static, unmeasured test on PFOA (acid form, 96% purity) with the bioluminescent cyanobacterium, *Anabaena sp*. (CPB4337 strain). The test was performed with 1.5 mL of cyanobacterial growth media (AA/8+N, Allen and Arnon 1955) in transparent 24-well microtiter plates. The pH of the growth

media was 7.8. The plates were incubated at 28°C under continuous illumination on a rotary shaker. The cyanobacteria in the log-growth phase (initial optical density at 750 nm = 0.1) were exposed to seven nominal concentrations ranging from 0-200 mg/L. A description of test solution preparation was lacking, but it does not appear that a solvent was used. Each test was repeated three times. The reported EC_{50} was 78.88 mg/L based on bioluminescence inhibition was not acceptable for quantitative use given the short test duration. This test was considered qualitatively acceptable.

G.2.3.2 Green alga, Raphidocelis subcapitata

(Formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*)

3M Company (2000) exposed the green alga, *Raphidocelis subcapitata* (formerly, *Selenastrum capricornutum*) to PFOA (CAS # 335-67-1) in a 96-hour static, unmeasured acute toxicity test. The toxicant was part of the 3M production lot number 269 and was characterized as a 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 substance was dissolved in a 50:50 water:isopropanol solution to make a primary solution of 1,000 mg/L test substance and isopropanol. A separate test conducted by the authors showed no growth effects at 1,000 mg/L isopropanol on the same species. The primary solution was diluted with algal medium to make five nominal test concentrations (63, 125, 250, 500 and 1,000 mg/L test substance or 32, 63, 130, 250 and 500 mg/L PFOA) plus a control (algal medium). The report stated the test followed USEPA-TSCA Guideline 797.1050. Exposures were conducted in 250 mL glass beakers with 50 mL of test solution and an initial cell loading of 10,000 cells/mL. There were three replicates for each treatment. The pH of the highest test concentration was low (2.3-3.0) over the course of the experiment as compared to the control (7.4-10.3). The mean number of cells increased in the

control and ≤125 mg/L test substance treatments but decreased at all other treatments. The 96-hour reported EC_{50}, based on cell density and growth rate, was 180 mg/L test substance and isopropanol or 90 mg/L PFOA. Because of the possible mixture effects of other perfluoro homologue compounds, the toxicity value from the study was not acceptable for quantitative use but was retained for qualitative use.

3M Company (2000) provides 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 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 USEPA-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. 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 $1x10^4$ cells/mL were placed in 250 mL flasks with 50 mL of test solution. There were three replicates for each of the six nominal test concentrations (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 5.3, 3.3, 2.9, and 5 mg/L, for the four, seven, 10 and 14 day exposures, respectively. Note: the authors did not specify if the EC_{10}s were determined after the exposure period or the post observation period. Because of the

possible mixture effects of other perfluoro analogue compounds in the tests these toxicity values were not acceptable for quantitative use but were retained for qualitative use.

3M Company (2000) exposed *Raphidocelis subcapitata* (formerly, *Selenastrum capricornutum*) to APFO (CAS # 3825-26-1) in a 96-hour static, unmeasured acute toxicity test. The toxicant was part of the 3M production lot number HOGE205 and was not sufficiently characterized but was considered a mixture of APFO (30% of the compound), other impurities, and water. A stock solution (3330 mg/L) was made without the use of a solvent, which was then. then diluted with algal medium (USEPA 1978) to make five nominal test concentrations (210, 430, 830, 1670 and 3330 mg/L APFO) plus a control (algal medium). The test followed USEPA-TSCA Guideline 797.1050 and OECD 201. Exposures were conducted in 250 mL glass beakers with 100 mL of test solution and an initial cell loading of 10,000 cells/mL. There were three replicates for each treatment. The 96-hour reported EC_{50}, based on cell count, was 1,980 mg/L APFO. The authors reported that the test substance is considered a mixture of APFO and other impurities and stated the EC_{50} may not accurately reflect the toxicity of APFO. Therefore, the value was not acceptable for quantitative use and was retained for qualitative use.

Rosal et al. (2010) performed a 72-hour static, measured growth inhibition test with perfluorooctanoic acid (96% purity) on the green alga, *Raphidocelis subcapitata* following OECD TG 201 Protocol. While limited details were provided about the exposure, the authors state they were following the OECD protocol. The algae were cultured in 96-well microplates with a total volume of 200 µL. No solvents were used to make test solutions. Specific test concentrations were not provided, but the authors noted that nominal and measured concentrations did not have significant deviations. The 72-hour growth inhibition (based on

biomass) EC_{50} of 96.2 mg/L PFOA was not acceptable for quantitative use because of the short test duration but was considered qualitatively acceptable.

Ding et al. (2012b) performed a rapid, 4.5-hour static algal growth inhibition test on PFOA (acid form, CAS # 335-67-1, 96% purity) with *Raphidocelis subcapitata*. The test follows the protocol of PAM Test: Acute effects on photosynthesis in algae developed in the Dutch National Institute of Public Health, RIVM (Verweij et al. 2009). Dilution medium consisted of Dutch standard water. *R. subcapitata* used for testing were from an in-house culture. Algae at a cell density of $3x10^6$ cells/mL were inoculated in medium containing PFOA at nominal concentrations of 0 (negative control), 1, 1.5, 1.8, 2, 2.2, 2.5, 3 mM PFOA, or 414.1, 621.1, 745.3, 828.1, 911.0, 1,035, and 1,242 mg/L PFOA when converted by multiplying the reported mM concentration by a molecular weight of 414.07 g/mol. There were two replicates for each exposure concentration. No details were provided for lighting, temperature, or other dilution or test solution parameters. The reported 4.5-hour EC_{50} based on photosynthetic efficiency was 1.807 mM (748 mg/L). The EC_{50} was not quantitatively acceptable due to the short duration but was retained for qualitative use.

G.2.3.3 Green alga, Scenedesmus obliquus

Liu et al. (2008a) conducted a 72-hour unmeasured exposure with *Scenedesmus obliquus* to evaluate the effects of PFOA (acid form, CAS #335-67-1, purity not reported) at the cellular level, measured by flow cytometry. Authors stated that the test followed OECD (2002) methodology with *S. obliquus* that were obtained from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences (Beijing). The algal test medium was prepared according to OECD (2002) using deionized water and analytically pure chemicals, adjusted to pH 7.5 ± 0.2. The authors did not provide details regarding how the PFOA treatments

were prepared. Algae in exponential growth phase and a cell density of $1x10^4$ cells/mL were inoculated in medium containing PFOA at nominal concentrations of 0 (negative control), 500, 1,000, 1,500, and 2,000 µM PFOA, or 0.0, 207.0, 414.1, 621.1, and 828.1 mg/L when converted based on molecular weight of PFOA, 414.07 g/mol. Experiments were initiated in replicated 50 mL flasks (n = 3 per treatment) containing a total volume of 20 mL of algal cell suspension. Algal cells were incubated at 22 ± 1°C under cool-white lights (6,000 lux) with a 14-hour:10-hour light:dark cycle. The 72-hour NOEC (growth rate reduction, based on optical density) of PFOA was 828.1 mg/L; the LOEC was >828.1 mg/L. The plant value from the study was not used quantitatively because of the short exposure duration (less than 96-hours) and the missing exposure details. The test was instead considered qualitatively acceptable.

G.2.3.4 Duckweed, Lemna gibba

Boudreau et al. (2003) performed a 7-day static acute algal growth inhibition test on PFOA (acid form, CAS # 335-67-1, ≥97% purity) with duckweed, *Lemna gibba*. The study was part of a Master's thesis at the University of Guelph, Ontario, Canada. Authors stated that the test followed protocols found in ASTM E1415-91 (ASTM 1991), Greenberg et al. (1992) and Marwood et al. (2001). Duckweed was obtained from laboratory culture maintained according to Marwood et al. (2001), and originally acquired from University of Waterloo. All treatment concentrations were prepared in laboratory-grade distilled water. Toxicity testing consisted of six test treatments plus a negative control (0, 10, 30, 50, 100, 300, and 500 mg/L) in 10 mL of Hunter's growing media in 60 x 15 mm polyethylene disposable petri dishes. There were three to four replicates per treatment, but the number of plants and fronds per plant were not reported. Tests were continuously illuminated with cool-white, fluorescent light between 5,800 and 6,200 lux and incubated at 25 ± 1°C. Endpoints used to determine inhibition of growth were mean

frond number and biomass, measured as wet weight. The reported IC_{10}, IC_{25} and IC_{50}, based on wet weight, were 0.052 M (95% C.I.: 0.042-0.065), 0.127 M (95% C.I.: 0.117-0.146) and 0.193 M (95% C.I.: 0.142-0.210), respectively. Note that although ICx for PFOA were reported in molar (M) units, 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, ICx now considered as mM, were converted to mg/L by molecular weight of 414.07 g/mol PFOA. The calculated 7-day IC_{10}, IC_{25} and IC_{50} expressed as mg/L from the study were 21.53, 52.59, and 79.92, respectively and were not acceptable for quantitative use given the lack of exposure details and uncertainties with the reported units but were retained for qualitative use.

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 d	PFOA Unknown	-	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
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
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
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.

H.2 Estuarine/Marine Qualitatively Acceptable PFOA Toxicity Study Summaries

H.2.1 Summary of Acute PFOA Toxicity Studies Used Qualitatively

H.2.1.1 Invertebrates

Mhadhbi et al. (2012) conducted a 48-hour static, unmeasured acute test with PFOA (96% purity) on the sea urchin, *Paracentrotus lividus* (a non-North American species). 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 (at a final maximum DMSO concentration of 0.01% (v/v) in the test medium). However, authors did not indicate what was considered a high test concentration. If a DMSO was used, a solvent control was also included with the test. Sea urchin embryos were exposed to one of ten nominal PFOA treatments (1, 2, 5, 10, 20, 50, 100, 200, 500 and 750 mg/L). Four hundred fertilized eggs (within 30 minutes of fertilization) were transferred to glass vials containing 10 mL of test solutions with four replicates per PFOA treatment and five replicates per control. Vials were incubated at 20°C in the dark for 48-hours. At test termination samples were fixed in formalin and 35 larvae per vial was measured for growth (length). The 48-hour EC_{50} (growth inhibition) was 110.0 mg/L and was not acceptable for quantitative use due to the atypical acute endpoint and short test duration but was retained for qualitative use

H.2.1.2 Fish

Mhadhbi et al. (2012) conducted a six-day renewal, unmeasured acute test with PFOA (96% purity) on the turbot, *Scopthalmus maximus* (formerly, *Psetta maxima*; a non-North American species). 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, authors did not indicate what was considered a high test concentration. If a DMSO was used a solvent control was also included. Fish were exposed to one of eight nominal PFOA treatments (1.5, 3, 5, 10, 12, 24, 100 and 200 mg/L). Turbot eyed eggs from a single stock of adults were supplied by a nearby fish hatchery (PESCANOVA Insuina) and acclimated to laboratory conditions before use. At 72-hpf, the floating fertilized eggs were collected and the non-fertilized eggs at the bottom discarded. Embryos that had reached the blastula stage were used for testing. Fifty normal embryos were added to glass beakers containing 500 mL of test solution. Each treatment had four replicates and were incubated in the dark for six days at 18°C with no food or aeration provided. Dead embryos and larvae were removed daily. Endpoints included dead embryos, malformation, hatch success at 48-hours and larvae survival (missing heartbeat and a non-detached tail) at six days. The 6-day LC_{50} of 11.9 mg/L PFOA was not used quantitatively because of the atypical acute test duration but was considered qualitatively acceptable.

H.2.2 Summary of Chronic PFOA Toxicity Studies Used Qualitatively

H.2.2.1 Mollusks

Li et al. (2021a) examined the physiological, transcriptomic, and metabolomic responses to PFOA in the blue mussel, *Mytilus edulis*. Mussels were collected from the Jinhuang Gulf (Yellow Sea, China) and acclimated in flow-through tanks at 16-19℃ and a 12-hour light:dark photoperiod for seven days. PFOA was dissolved in DMSO and diluted with natural seawater to create three test treatments (20, 200 and 2000 µg/L PFOA) plus a control. Each treatment was replicated three times with sixty mussels per each replicate. Water in exposure tanks was replaced daily and ten mussels were subsampled on test day 0, 1, 3, 7. 21. At test termination enzyme activity was measured and CAT and SOD activity decreased in the 200 and 2000 µg/L PFOA treatments groups as compared to the controls. The 21-day LOEC, based on catalase

activity, was 0.2 mg/L PFOA. The lack of apical effect assessed precluded this study from being acceptable for quantitative use, but it was retained for qualitative use.

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. The mussels were obtained from a local farm in Singapore, and subsequently acclimated to laboratory conditions for seven days before testing. Mussels were kept at a salinity of 25 ppt (artificial seawater) and a temperature of 25ºC. Forty mussels (60-65 mm length) per 50-L polypropylene tank, each duplicated, were exposed to measured PFOA concentrations of 0 (control), 0.08, 1.2, 11.4, 99 and 1,120 µg/L. Mussels were fed a commercial marine micro-algae purchased from Reed Mariculture on renewal days, which occurred every two days, two hours before the solution renewal. PFOA concentrations were verified through water and muscle tissue samples via liquid chromatography-tandem mass spectrometry. Weights and lengths were determined on days zero and seven. A NOEC of 11.4 µg/L and a LOEC of 99 µg/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., 99 ug/L) and the highest test concentration, which contained a PFOA concentration that was more than 10X greater (i.e., 1120 ug/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.

Lui et al. (2014a,b) and Liu and Gin (2018) conducted a series of seven-day renewal, measured experiments with perfluorooctanoic acid (PFOA, 96% purity) on the green mussel, *Perna viridis* (a non-North American species). All of these studies utilized a similar test design, but with each publication providing a different level of test details. In **Liu et al. (2014a)**, green mussels were obtained from a local fish farm and acclimated to laboratory conditions prior to PFOA exposure. Adult organisms were exposed in 70 L polypropylene tanks in artificial seawater at a temperature of 25°C and at salinity of 30 ppt. Mussels were exposed to one of five nominal PFOA concentrations (0.0001, 0.001, 0.010, 0.1 and 1.0 mg/L) or a control. Each tank contained 60-65 mussels, with two tanks per exposure concentration or control. During exposures, mussels were fed with microalgae and each tank was cleaned and refilled every two days. After seven days of exposure and seven days of depuration, various biomarkers were measured. The EC_{50} (integrative genotoxicity) was reported as 0.594 mg/L PFOA and was based on three genotoxic endpoints (DNA fragmentation and single strand breaks (comet assay), chromosomal breaks (micronucleus test) and apoptosis (DNA diffusion assay). Results of this study were not used quantitatively due to the short exposure duration and endpoint but was considered qualitatively acceptable.

In **Lui et al. (2014b)**, the oxidative damage of PFOA (96% purity) to green mussels was assessed after seven days under similar conditions as Lui et al. (2014a). Green mussels were obtained from a local fish farm in Singapore and acclimated to laboratory conditions for one week prior to exposures. Organisms (60-65 mm) were exposed in polypropylene tanks containing artificial seawater at a temperature of 25°C and at salinity of 25 ppt. Mussels were exposed to one of six nominal PFOA concentrations (0.0001, 0.001, 0.010, 0.1, 1.0 and 10.0 mg/L) or a control. Nominal concentrations were similar to measured concentration (0.00008,

0.0012, 0.0114, 0.099, 1.120 and 9.630 mg/L, respectively) and no PFOA was detected in the controls. Each treatment was replicated with an unreported number of mussels per replicate. Again, during the exposure mussels were fed with microalgae and each tank was cleaned and refilled every two days. The most sensitive parameters to PFOA were activation of antioxidant enzymes (catalase [CAT] and superoxide dismutase [SOD]), which is an adaptive response to the excessive reactive oxygen species. Significant effects were observed at 0.099 mg/L PFOA, but not at 1.12 mg/L. The seven-day MATC (CAT and SOD activity) was 0.3330 mg/L, which was not used quantitatively because of the atypical endpoint and duration but was considered qualitatively acceptable for use.

Liu and Gin (2018) employed the same test design and nominal PFOA concentrations as Lui et al. (2014a). The most sensitive biomarker endpoint reported was hemocyte cell viability with a NOEC and LOEC of 0.0114 and 0.099 mg/L, respectively. Again, the MATC of 0.03359 mg/L PFOA was not used quantitatively due to the atypical test duration but was considered qualitatively acceptable.

Bernardini et al. (2021) reported the results of a 21-day chronic study with the Manila clam, *Ruditapes philippinarum*, and PFOA (CAS # 335-67-1). Clams were collected from the field (Venice Lagoon, Italy) and acclimated to laboratory conditions (aerated natural seawater, salinity 35 ± 1 ppt and 12 ± 0.5°C) for one week. Ninety healthy individuals (3.64 cm shell length) were evenly divided amongst two aquaria exposed to either PFOA at a nominal 1 µg/L or control seawater for 21 days. Clams were fed and test solutions were renewed every other day. Subsamples of clams (n=20) were collected on test days seven and 21 for soft tissue PFOA concentrations and haemolymph analysis. No significant effects of mortality were observed in the single treatment group throughout the exposure. The measured concentration of PFOA was

0.93 µg/L. At the end of the experiment over 113 genes were upregulated and 362 genes were downregulated in exposed compared to control clams. The 21-day NOEC, based on mortality, was 0.00093 mg/L PFOA, which was not acceptable for quantitative use because apical endpoints were the focus of the study and only one exposure concentration was employed which did not result in apical effects at a relatively low concentration. Results of this study were retained for qualitative use only.

H.2.2.2 Fish

Yang (2010) performed a seven-day, renewal unmeasured toxicity test with the ammonium salt of perfluorooctanoic acid (98% purity) using the medaka, *Oryzias latipes* (a non-North American species). Male medaka were gradually acclimated to a high saline condition (specific salinity not reported) in the laboratory over 15 days. Freshwater was mixed with equal parts seawater, with half of the volume replaced with seawater every day. No mortality was observed during this acclimation period. The F3 generation from these parental fish were used in the experiments. Twelve adult male medaka were added to unreplicated 2 L glass tanks and exposed to one of three nominal PFOA concentrations (10, 50, 100 mg/L) or controls for seven days. Solutions were renewed daily and fish were maintained at 25 ±1°C under a constant photoperiod of 16-hours:8-hours (light:dark). After seven days, fish were euthanized and various endpoints were measured: condition factor (K), gonadosomatic index (GSI), liver somatic index (LSI), and other enzymatic responses. Across all treatments none of the fish died, and there were no significant differences between PFOA treatments and controls for K, GSI and LSI. The survival-based seven-day NOEC of 100 mg/L PFOA was not acceptable for quantitative use because of the atypical exposure duration and lack of replication in the experiment but was used qualitatively.

H.2.3 Summary of Qualitatively Acceptable Data for Saltwater Plants

H.2.3.1 Bacterium, Vibrio fischeri

Rosal et al. (2010) conducted a 15-minute static, measured bioluminescence inhibition test with perfluorooctanoic acid (PFOA, 96% purity) on the bacterium, *Vibrio fischeri* following ISO 11348-3 standard protocol. While limited details were provided about the exposure, the authors stated they followed the standard protocol. The experiment used a commercially available Biofix Lumi test (Macherey-Nagel, Germany), where the bacterium is supplied freeze-dried. It was reconstituted and incubated at 3°C for five min before use. The experiment employed a 0.34 M NaCl (2% w/v) test medium conducted at 18°C. No solvents were used to make test solutions. Specific test concentrations were not provided, but the authors noted that nominal and measured concentrations did not have significant deviations. The 15-minute EC_{50}, based on bioluminescence inhibition, was 524 mg/L. The test was not used quantitively because of the short test duration and lack of exposure details but was considered qualitatively acceptable.

H.2.3.2 Cyanobacterium, Anabaena sp.

Rosal et al. (2010) also conducted a 24-hour static, measured bioluminescence inhibition test with PFOA (96% purity) on the cyanobacterium, *Anabaena sp*. Limited details were provided about the exposure, but the authors stated they were following the test design in Rodea-Palomares et al. (2009). The cyanobacterium *Anabaena*, CPB4337 strain, was grown at 28°C on a rotary shaker in 50 mL AA/8 media supplemented with nitrate (5 mM) in 125 ml Erlenmeyer flasks and 10 mg/mL of neomycin sulphate. No solvents were used to make test solutions. Specific test concentrations were not provided, but the authors noted that nominal and measured concentrations did not have significant deviations. The 24-hour EC_{50}, based on bioluminescence

inhibition was 72.3 mg/L and was not used quantitatively because of the short test duration and lack of exposure details but was retained for qualitative use.

H.2.3.3 Cyanobacterium, Geitlerinema amphibium

Latala et al. (2009) performed a 72-hour static algal growth inhibition test on PFOA (acid form, CAS # 335-67-1) with *Geitlerinema amphibium*. The purity of PFOA was not reported, but the authors stated they used the highest grade commercially available. Authors stated the algal growth inhibition tests followed protocols found in ISO 10253 (ISO 1995) and ISO 8692 (ISO 1993), but used f/2 medium, different test species and a different photoperiod than the test protocols. The blue-green alga *G. amphibium* BA-13 strain was isolated from Baltic Sea coastal waters and maintained as monoalgal cultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdansk. Algae were batch-cultured in f/2 medium prepared in distilled water and brought to a salinity of 8 PSU was using Tropic Marin® sea salt. Cultures were acclimated for ten days at 20ºC and a 16-hour photoperiod (25 µmol photons m^{-2} s^{-1}). The pH was stabilized at 7.6–7.8 with NaOH. These conditions were maintained throughout the test. Aliquots (9.5 cm^3) of cell cultures in the log growth phase were added to conical glass flasks to which different concentrations of PFOA or distilled water was added. Nominal test concentrations ranged from 0.000005-50 mM, or 0.0 to 20,703.5 mg/L PFOA. After 72-hours the number of cells was determined by measuring optical density spectrophotometrically. The 72-hour EC_{50} for growth inhibition was reported as 0.60 mM, or 248.4 mg/L PFOA. The EC_{50} of 248.4 mg/L PFOA was not acceptable for quantitative use, due to the short test duration but was retained for qualitative use.

H.2.3.4 Dinoflagellate, Pyrocystis lunula

Hayman et al. (2021) conducted a short-term, sublethal 24-hour exposure to examine the effects of PFOA on the bioluminescent dinoflagellate (*Pyrocystis lunula*) following ASTM E1924-97 (ASTM 2004). Test solution water was 0.45 µm filtered seawater collected from North San Diego Bay, CA spiked with PFOA. Spiking consisted of the addition of stock solutions of PFOA (CAS # 335-67-1; 95% purity) dissolved in methanol; highest methanol concentration of 0.02% (v/v). Concentrations of PFOA for the toxicity tests were determined from a range finding study. Measured concentrations for PFOA were 0 (control and solvent control), 1.5, 4.7, 11, 16, 29, and 52 mg/L. Approximately 3,000 cells of *P. lunula* were added to 2.5 mL of test solution in acrylic test cuvettes, with six replicates per treatment concentration. *P. lunula* were exposed for 24 hours in a 19°C incubator with a reversed (e.g., dark during the typical "day" period) 12-hour:12-hour light:dark cycle. Test cuvettes were removed from the incubator after 24 hours and after being in the dark period for approximately three hours, inserted and analyzed in a specialized spectrometer (QwikLite 200 Biosensor System, Assure Controls, Carlsbad, CA) and the light output was recorded. Less light output relative to concurrently evaluated controls is indicative of an adverse effect. The 24-hour bioluminescence EC_{50} for *P. lunula* was determined to be 18 mg/L PFOA. The chronic value was used qualitatively and was not acceptable for quantitative use because of the short exposure duration and lack of apical endpoints.

H.2.3.5 Golden brown alga, Isochrysis galbana

A 72-hour static, unmeasured algal growth inhibition test on PFOA (96% purity) with *Isochrysis galbana* was performed by **Mhadhbi et al. (2012)** following OECD (2006) test methodology. Golden brown algae were provided by Estacion de Ciencias Marinas de Toralla (ECIMAT). The cultures were maintained in 250 mL glass Erlenmeyer flasks with autoclaved filtered sea water and EDTA-free f/2 culture medium. PFOA stock solutions were prepared in

DMSO and added to the dilution water with a maximum DMSO concentration of 0.01% (v/v). Nominal test concentrations were solvent control, 25, 50, 100, 200 and 400 mg/L PFOA. Each flask was inoculated at a density of 10,000 cells/mL, with the algae in the exponential growth phase. Each treatment was replicated three times. Flasks were kept at 20ºC with a 24-hour light period, and manually shaken daily. Cell counts were carried out every 24-hours, with a reported 72-hour EC_{50} based on growth inhibition of 163.6 mg/L PFOA. Results of this test were not acceptable for quantitative use, due to the short test duration but was retained for qualitative use.

H.2.3.6 Green alga, Chlorella vulgaris

Latala et al. (2009) conducted a 72-hour static algal growth inhibition test on PFOA (acid form, CAS # 335-67-1) with *Chlorella vulgaris*. The purity of PFOA was not reported, but the authors stated they used the highest grade commercially available. Authors stated that the algal growth inhibition test followed protocols found in ISO 10253 (ISO 1995) and ISO 8692 (ISO 1993), but used f/2 medium, different test species and a different photoperiod than the test protocols. The green alga *C. vulgaris* BA-02 strain was isolated from Baltic Sea coastal waters and maintained as monoalgal cultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdansk. Algae were batch-cultured in f/2 medium prepared in distilled water and brought to a salinity of 8 PSU was using Tropic Marin® sea salt. Cultures were acclimated for ten days at 20ºC and a 16-hour photoperiod (25 µmol photons m^{-2} s^{-1}). The pH was stabilized at 7.6–7.8 with NaOH. These conditions were maintained throughout the test. Aliquots (9.5 cm^3) of cell cultures in the log growth phase were added to conical glass flasks to which different concentrations of PFOA or distilled water was added. Nominal test concentrations ranged from 0.000005-50 mM, or 0.0 to 20,703.5 mg/L PFOA. After 72-hours the number of cells was determined by measuring optical density spectrophotometrically. The 72-

hour EC_{50} for growth inhibition was reported as 2.36 mM, or 977.2 mg/L PFOA. The EC_{50} of 977.2 mg/L PFOA was not acceptable for quantitative use, due to the short test duration but was instead classified at qualitatively acceptable.

H.2.3.7 Diatom, Skeletonema marinoi

Latala et al. (2009) conducted a 72-hour static growth inhibition test on PFOA (acid form, CAS # 335-67-1) with the diatom, *Skeletonema marinoi*. The purity of PFOA was not reported, but the authors stated they used the highest grade commercially available. Authors stated that the algal growth inhibition tests followed protocols found in ISO 10253 (ISO 1995) and ISO 8692 (ISO 1993), but used f/2 medium, different test species and a different photoperiod than the test protocols. The diatom *S. marinoi* BA-98 strain was isolated from Baltic Sea coastal waters and maintained as monoalgal cultures in the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdansk. Diatoms were batch-cultured in f/2 medium prepared in distilled water and brought to a salinity of 8 PSU was using Tropic Marin® sea salt. Cultures were acclimated for ten days at 20ºC and a 16-hour photoperiod (25 µmol photons m^{-2} s^{-1}). The pH was stabilized at 7.6–7.8 with NaOH. These conditions were maintained throughout the test. Aliquots (9.5 cm^3) of cell cultures in the log growth phase were added to conical glass flasks to which different concentrations of PFOA or distilled water was added. Nominal test concentrations ranged from 0.000005-50 mM, or 0.0 to 20,703.5 mg/L PFOA. After 72-hours the number of cells was determined by measuring optical density spectrophotometrically. The 72-hour EC_{50} for growth inhibition was reported as 0.89 mM, or 368.5 mg/L. The EC_{50} of 368.5 mg/L PFOA was not used quantitatively due to the short test duration but was retained for qualitative use.

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	20.61	23.29	-	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	26.96	Yang et al. 2019
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

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
Rainbow trout, *Oncorhynchus mykiss*	APFO 99.7%	96 hours	85 days (ELS)	LC_{50}	NOEC (growth and mortality)	707	40	<17.68	<17.68	Colombo et al. 2008
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
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
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
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	2017a. 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

Author	Citation	Reason Unused
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. Journal of Applied Toxicology 37: 699-708.	In vitro exposure, zebrafish liver cell cultures
Martin, J.W., S.A. Mabury, K.R. Solomon and D.C.G. Muir	2003b. 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	2013a. 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
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)

Author	Citation	Reason Unused
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)
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
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
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 alleviate 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

Author	Citation	Reason Unused
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 perflurooctanoic 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 EPA's request for data or were unable to locate concentration-response data, the toxicity values were not independently calculated by EPA, and the reported toxicity values were retained for criteria deviation. EPA also retained author-reported effect concentrations when data availability did not support effect concentration calculation by EPA. This retention was done to be consistent with use of author-reported toxicity values in previous criteria documents and retain informative toxicity values (that would have otherwise not been used only on the basis of lacking the underlying C-R data). Where concentration-response data were available, they were analyzed using the statistical software program R (version 3.6.2) and the associated dose-response curve (drc) package.

In some cases, the author-reported toxicity values were different than the corresponding effect concentrations calculated by EPA. Overall, the magnitude of such discrepancies 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 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, 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, EPA was able to develop a draft 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 estuarine/marine benchmark recommendations for aquatic life using procedures consistent with those in the 1985 Guidelines. This is important as it provides an approach by which values that are protective of aquatic life communities can be developed, even when MDRs are not fulfilled by direct PFOA test data. This approach is consistent with both the 1985 Guidelines "good science" clause, EPA's interest in providing useful information to states and tribes regarding

protective values for aquatic life, and EPA's intention to reduce the use of animal testing via application of NAMS (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing).

L.1.1 Introduction to Web-ICE

ICE models, developed by EPA's Office of Research and Development, are log-linear regressions of the acute toxicity (EC_{50}/LC_{50}) of two species across a range of chemicals, thus representing the relationship of inherent sensitivity between those species (Raimondo et al. 2010). Each model is derived from an extensive, standardized database of acute toxicity values by pairing each species with every other species for which acceptable toxicity data are available. Once developed, ICE models can be used predict the sensitivity of an untested taxon (predicted taxa are represented by the y-axis) from the known, measured sensitivity of a surrogate species (represented by the x-axis; Figure L-1).

ICE models have been developed for a broad range of different 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).

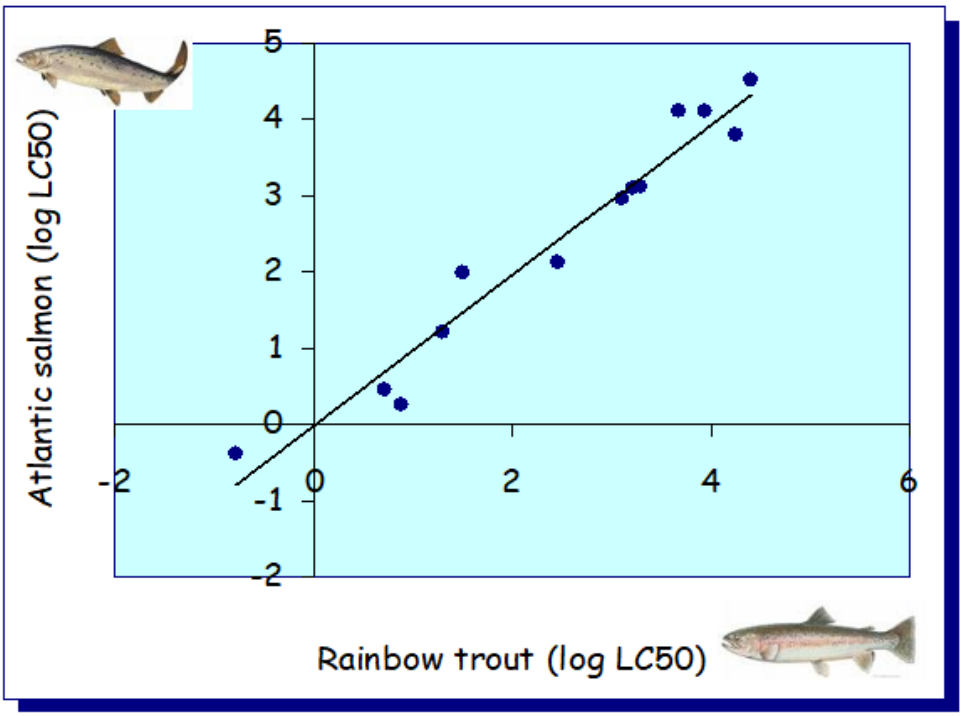


Figure L-1. Example ICE Model for Rainbow Trout (surrogate) and Atlantic Salmon (predicted).
Each model datapoint is a common chemical that was tested in both species to develop a log-linear regression.

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. in prep).
- For models between vertebrates and invertebrates, using those with lower MSE or MOA-specific models (not available for PFAS) has been recommended for listed species predictions (Willming et al. 2016).

L.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 (v3.3) and is described in detail in Raimondo et al. (in prep). Briefly, ICE models containing a minimum of 10 datapoints and spanning at least five orders of magnitude were separated into two subsets: 1) a lower subset that contained all paired chemical data corresponding to values below the 75^{th} 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. in prep). Comparison of scaled values with those predicted from µg/L values beyond the model domain showed that predicted values varied by a factor of 10 for models with slopes ranging from 0.66 – 1.33. Toxicity values predicted from models with slopes within this range had a median fold difference of 2.4 using mg/L values

and 2.8 using µg/L values (Wilcoxon paired rank sum test, V = 1334749, p-value 0.77). These results and a detailed review of ICE model assumptions are provided in Raimondo et al. (in prep).

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 draft 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.

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]	1020	9
Amphibian	Clawed frog	*Xenopus sp.* [b]	377	2
Crustacean	Mysid	*Americamysis bahia*	24	28
Crustacean	Water flea	*Daphnia magna*	220	53
Fish	Zebrafish	*Danio rerio*	572.4	2 (juvenile models) 6 (embryo models)
Fish	Bluegill	*Lepomis macrochirus*	664	68
Fish	Rainbow trout	*Oncorhynchus mykiss*	1682	77
Fish	Fathead minnow	*Pimephales promelas*	413.2	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

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. in prep). For accuracy assessments, the fold difference provides a simplified metric to easily see how close predictions are to measured values at a glance. A 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) (Raimondo et al. in prep).

Measured SMAVs are for the predicted species as listed in Appendix A.1, Appendix B.1 and Table L-1. Footnotes indicate where predictions or models do not meet one or more of the user guidance criteria.

		Toxicity Values Potentially Beyond Model Domain				Scaled Toxicity Values			
Predicted Species	**Surrogate Species**	**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*)	1,020,000	351962.71	47028.33 - 2634108.91	2.9[ab]	1,020	1085.27	358.55 - 3284.91	1.1
	Daphnid (*Daphnia magna*)		250407.71	42580.36 - 1472604.12	4.1		265.51	41.71 - 1689.91	3.8
	Fathead minnow (*Pimephales promelas*)		378090.72	131569.51 - 1086517.65	2.7		600.01	258.97 - 1390.14	1.7
	Rainbow trout (*Oncorhynchus mykiss*)		1802334.46	759319.17 - 4278055.44	1.8		4035.49	2152.23 - 7566.66	4.0
African clawed frog (*Xenopus laevis*)	Fathead minnow (*Pimephales promelas*)	377,000	232893.16	4680.66 - 11587923.00	1.6[ab]	377	626.72	64.49 - 6090.47	1.7[a]
Mysid (*Americamysis bahia*)	Bluegill (*Lepomis macrochirus*)	24,000	101324.58	39595.31 - 259290.08	4.2[b]	24	88.82	61.09 - 129.12	3.7
	Daphnid (*Daphnia magna*)		30882.91	15730.51 - 60630.86	1.3		95.62	67.73 - 134.98	4.0
	Fathead minnow (*Pimephales promelas*)		18018.04	3248.57 - 99936.19	1.3[c]		24.09	9.84 - 58.98	1.0[c]
	Rainbow trout (*Oncorhynchus mykiss*)		171630.73	59300.92 - 496739.45	7.2[bc]		295.1	195.02 - 446.52	12.3[c]
Daphnid (*Daphnia magna*)	Bluegill (*Lepomis macrochirus*)	220,000	159433.9	88808.47 - 286224.59	1.4[c]	220	256.05	191.88 - 341.67	1.2[c]
	Bullfrog (*Lithobates catesbeianus*)		482481.09	76477.24 - 3043886.00	2.2		1174.46	256.08 - 5386.36	5.3
	Fathead minnow (*Pimephales promelas*)		68202.89	35104.94 - 132506.54	3.2[c]		132.34	81.18 - 215.76	1.7[c]
	Fatmucket (*Lampsilis siliquoidea*)		331623.04	78520.34 - 1400577.72	1.5		111	31.65 - 389.26	2.0
	Mysid (*Americamysis bahia*)		22344.14	12674.03 - 39392.40	9.8		78.04	54.00 - 112.78	2.8
	Rainbow trout (*Oncorhynchus mykiss*)		250884.54	128137.85 - 491213.58	1.1[c]		798.29	599.09 - 1063.73	3.6[c]
	Zebrafish embryo (*Danio rerio* - embryo)		113414.26	16337.79 - 787303.02	1.9[c]		71.66	10.27 - 499.77	3.1[c]

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
Bluegill (*Lepomis macrochirus*)	Bullfrog (*Lithobates catesbeianus*)	664,000	1501135.53	114395.51 - 19698393.38	2.3[ab]	664	601.13	166.16 - 2174.76	1.1
	Daphnid (*Daphnia magna*)		81671.31	52906.69 - 126074.85	8.1		801.26	627.74 - 1022.74	1.2
	Fathead minnow (*Pimephales promelas*)		155867.21	82472.16 - 294579.23	4.3		255.38	171.26 - 380.83	2.6
	Fatmucket (*Lampsilis siliquoidea*)		408413.42	32355.12 - 5155335.38	1.6[ac]		295.92	21.91 - 3996.50	2.2[ac]
	Mysid (*Americamysis bahia*)		23663.56	13808.02 - 40553.53	28.1		266.08	195.07 - 362.94	2.5
	Rainbow trout (*Oncorhynchus mykiss*)		1388883.84	1036631.76 - 1860832.75	2.1		2138.11	1903.46 - 2401.68	3.2
	Zebrafish embryo (*Danio rerio* - embryo)		347447.26	41366.56 - 2918288.94	1.9[a]		274.69	48.73 - 1548.44	2.4
Rainbow trout (*Oncorhynchus mykiss*)	Bluegill (*Lepomis macrochirus*)	1,682,000	353682.57	274243.78 - 456131.98	4.8	1,682	524.72	466.57 - 590.13	3.2
	Bullfrog (*Lithobates catesbeianus*)		781571.06	313951.07 - 1945695.88	2.2		376.93	177.31 - 801.30	4.5
	Daphnid (*Daphnia magna*)		56931.33	35836.37 - 90443.76	29.5[c]		630.92	485.50 - 819.91	2.7[c]
	Fathead minnow (*Pimephales promelas*)		145155.57	92010.78 - 228996.44	11.6		179.81	133.78 - 241.68	9.4
	Fatmucket (*Lampsilis siliquoidea*)		736514.01	14043.59 - 38626348.23	2.3[abc]		201.6	39.91 - 1018.32	8.3[c]
	Mysid (*Americamysis bahia*)		17330.67	9740.08 - 30836.72	97.1		192.8	137.35 - 270.65	8.7
	Zebrafish embryo (*Danio rerio* – embryo)		410453.8	77544.98 - 2172575.38	4.1		121.34	42.07 - 349.98	13.9
Fathead minnow (*Pimephales promelas*)	African clawed frog (*Xenopus laevis*)	413,200	524824.76	4876.97 - 56477848.18	1.3[ab]	413.2	257.81	16.14 - 4116.88	1.6[a]
	Bluegill (*Lepomis macrochirus*)		387148.61	201381.26 - 744279.98	1.1		1427.45	1024.71 - 1988.48	3.5
	Bullfrog (*Lithobates catesbeianus*)		1012520.93	299871.50 - 3418793.08	2.5		758.77	320.24 - 1797.81	1.8
	Daphnid (*Daphnia magna*)		111877.81	66019.39 - 189590.43	3.7[c]		1709.52	1209.11 - 2417.01	4.1[c]
	Fatmucket (*Lampsilis siliquoidea*)		675590	54952.40 - 8305767.53	1.6[ac]		3450.51	576.51 - 20651.62	8.4[c]
	Mysid (*Americamysis bahia*)		34114.93	11148.28 - 104395.31	12.1[c]		635.95	321.17 - 1259.27	1.5[c]
	Rainbow trout (*Oncorhynchus mykiss*)		1557418.83	896689.52 - 2705009.20	3.8		3950.04	3110.36 - 5016.41	9.6

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
	Zebrafish embryo (*Danio rerio* - embryo)		498531.23	249260.29 - 997083.75	1.2		901.61	385.51 - 2108.61	2.2
Fatmucket (*Lampsilis siliquoidea*)	Black sandshell (*Ligumia recta*)	164,400	153582.08	28669.47 - 822737.61	1.1	164.4	109.67	9.40 - 1278.86	1.5[a]
	Bluegill (*Lepomis macrochirus*)		97837.12	12334.24 - 776059.00	1.7[ac]		733.53	111.00 - 4847.23	4.5[c]
	Daphnid (*Daphnia magna*)		69944.45	23967.21 - 204121.63	2.4		406.14	160.97 - 1024.70	2.5
	Fathead minnow (*Pimephales promelas*)		17586.46	2323.63 - 133103.22	9.3[ac]		78.68	7.86 - 787.28	2.1[ac]
	Rainbow trout (*Oncorhynchus mykiss*)		26218.81	2516.16 - 273203.88	6.3[abc]		729.52	264.03 - 2015.66	4.4[c]
Black sandshell (*Ligumia recta*)	Fatmucket (*Lampsilis siliquoidea*)	161,000	171536.98	34311.02 - 857594.23	1.1	161	237.72	25.46 - 2219.07	1.5[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.

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 and described more thoroughly in Raimondo et al. (in prep) 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. in prep). Cases in which both units were outside the model domain were not included quantitatively, but the value with the narrowest confidence intervals was included for qualitative considerations. Values (using either µg/L or mg/L input value) were excluded quantitatively from the SMAVs but retained for qualitative consideration if an evaluation of confidence intervals, model parameters, and the model domain indicated the relationship between surrogate and predicted species was not informed by robust underlying data. At this stage, specific predictions should be based on holistic evaluation of all available information provided by the model, confidence interval, and data used to develop the model. Decisions to exclude a prediction from the SMAV are clarified in footnotes. Because the sensitivity of a single species can be predicted by multiple surrogates, we calculated the SMAV where multiple robust models were available for a predicted species. Each predicted species was then assigned to the appropriate saltwater MDRs as defined in the 1985 Guidelines:

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	38514.70	50	5	Rejected
Allorchestes compressa	*Daphnia magna*	0.83	1.59	3	0.80	0.0390	0.12	5.00	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	<0.001	0.93	0.07	840000.00	64	5	Accepted
Americamysis bahia	*Lepomis macrochirus*	1.01	-0.92	138	0.66	<0.001	0.95	0.13	290000.00	59	6	Accepted
Americamysis bahia	*Oncorhynchus mykiss*	0.92	-0.50	150	0.60	<0.0001	1.08	0.06	1100000.00	57	6	Rejected
Americamysis bahia	*Pimephales promelas*	0.95	-1.12	46	0.55	<0.001	1.75	2.27	70200000.00	35	6	Rejected
Chelon labrosus	*Lampsilis siliquoidea*	1.27	1.50	1	0.99	0.0403	0.00	19.01	281.00	na	6	Accepted
Chelon macrolepis	*Pimephales promelas*	1.51	-1.04	2	0.97	0.0114	0.05	26.00	2533.38	100	4	Accepted qualitatively
Crassostrea virginica	*Americamysis bahia*	0.44	1.76	114	0.34	<0.001	0.88	0.003	117648.20	55	6	Rejected
Crassostrea virginica	*Daphnia magna*	0.44	1.54	116	0.28	<0.001	1.08	0.08	137171.43	58	6	Rejected
Crassostrea virginica	*Lampsilis siliquoidea*	0.82	-0.28	3	0.95	0.0041	0.06	30.00	22000.00	100	4	Accepted
Crassostrea virginica	*Lepomis macrochirus*	0.66	0.71	112	0.51	<0.001	0.64	0.36	290000.00	69	6	Rejected
Crassostrea virginica	*Oncorhynchus mykiss*	0.59	0.97	120	0.50	<0.001	0.68	0.02	570000.00	68	6	Rejected
Crassostrea virginica	*Pimephales promelas*	0.75	0.44	24	0.61	<0.001	0.68	1.24	206300.75	69	6	Accepted
Cyprinodon bovinus	*Lepomis macrochirus*	0.66	0.70	1	0.99	0.0326	0.00	7.43	7326.20	na	4	Accepted
Cyprinodon bovinus	*Oncorhynchus mykiss*	0.72	0.80	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	0.00	10.49	7847.42	100	4	Accepted
Cyprinodon variegatus	*Americamysis bahia*	0.57	1.88	88	0.56	<0.001	0.67	0.003	182000.00	64	6	Rejected
Cyprinodon variegatus	*Daphnia magna*	0.53	1.79	84	0.49	<0.001	0.72	0.08	304000.00	64	6	Rejected
Cyprinodon variegatus	*Lampsilis siliquoidea*	0.72	0.76	1	0.99	0.0392	0.00	30.00	22000.00	na	6	Accepted
Cyprinodon variegatus	*Lepomis macrochirus*	0.74	0.87	82	0.65	<0.001	0.47	0.77	157000.00	82	4	Accepted
Cyprinodon variegatus	*Oncorhynchus mykiss*	0.75	0.90	87	0.65	<0.001	0.56	0.82	12700000.00	75	4	Accepted
Cyprinodon variegatus	*Pimephales promelas*	0.69	0.98	24	0.74	<0.0001	0.43	2.27	16500000.00	77	4	Accepted
Farfantepenaeus duorarum	*Americamysis bahia*	1.03	0.06	6	0.81	0.0022	0.55	0.01	720.00	50	4	Accepted

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	*Daphnia magna*	1.08	0.14	16	0.76	<0.0001	1.32	0.04	65686.02	44	5	Rejected
Farfantepenaeus duorarum	*Lepomis macrochirus*	1.16	-1.21	15	0.67	<0.0001	1.88	2.32	130000.00	35	6	Rejected
Farfantepenaeus duorarum	*Oncorhynchus mykiss*	1.20	-1.36	15	0.72	<0.0001	1.54	0.57	221000.00	47	6	Rejected
Fenneropenaeus merguiensis	*Daphnia magna*	0.82	1.43	4	0.66	0.0473	0.40	5.00	1251.41	67	5	Accepted
Gasterosteus aculeatus	*Lepomis macrochirus*	1.15	0.00	3	0.95	0.0039	0.09	0.36	340.00	80	4	Accepted qualitatively
Gasterosteus aculeatus	*Oncorhynchus mykiss*	1.05	0.29	4	0.90	0.0038	0.18	0.61	890.00	83	4	Accepted qualitatively
Hydroides elegans	*Daphnia magna*	0.49	1.59	2	0.96	0.0182	0.01	5.00	1251.41	100	6	Rejected
Hydroides elegans	*Oncorhynchus mykiss*	0.20	2.30	1	0.99	0.0179	0.00	1.84	13390.93	na	6	Rejected
Lagodon rhomboides	*Lepomis macrochirus*	1.61	-2.02	1	0.99	0.0301	0.00	110.00	760.00	na	3	Accepted qualitatively
Litopenaeus stylirostris	*Americamysis bahia*	1.04	0.01	5	0.60	0.0401	0.29	0.58	24.09	57	4	Accepted qualitatively
Menidia beryllina	*Lepomis macrochirus*	0.79	0.90	5	0.89	0.0012	0.19	12.30	93800.00	86	4	Accepted
Menidia menidia	*Lepomis macrochirus*	1.05	-0.35	4	0.96	0.0005	0.14	2.85	97000.00	83	4	Accepted
Menidia	*Oncorhynchus mykiss*	1.28	-1.40	3	0.94	0.0050	0.23	11.24	91000.00	60	4	Accepted
Menidia peninsulae	*Americamysis bahia*	0.63	0.91	3	0.88	0.0162	0.32	0.01	1160.00	80	6	Accepted qualitatively
Menidia peninsulae	*Lepomis macrochirus*	0.90	-0.10	3	0.97	0.0012	0.06	0.77	2480.00	100	4	Accepted
Menidia peninsulae	*Oncorhynchus mykiss*	1.01	-0.36	2	0.91	0.0421	0.35	0.82	1600.00	50	4	Accepted qualitatively
Metamysidopsis insularis	*Daphnia magna*	0.86	0.93	3	0.94	0.0057	0.18	6.97	317472.74	80	5	Accepted
Metamysidopsis insularis	*Lampsilis siliquoidea*	1.03	0.62	2	0.99	0.0027	0.02	19.01	87705.88	75	6	Accepted
Mugil cephalus	*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*	0.60	1.73	3	0.92	0.0095	0.10	1.20	11202.42	80	6	Accepted qualitatively
Tigriopus japonicus	*Pimephales promelas*	0.81	1.12	5	0.76	0.0103	0.11	195.14	27000.00	86	6	Accepted
Tisbe battagliai	*Daphnia magna*	0.86	1.25	2	0.94	0.0289	0.08	0.61	184.54	100	5	Accepted 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	225.75
		Pimephales promelas	mg/L	**225.75**	115.43 - 441.47	
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	171.63[bc]	59.3 - 496.74	
		Pimephales promelas	µg/L	18.02[c]	3.25 - 99.94	
Thicklip mullet	*Chelon labrosus*	*Lampsilis siliquoidea*	mg/L	**21448.80**	5726.99 - 80330.23	21448.80
Bigscale mullet	*Chelon macrolepis*	*Pimephales promelas*	mg/L	851.39[d]	248.17 - 2920.75	NA
Eastern oyster	*Crassostrea virginica*	*Americamysis bahia*	µg/L	5.08[c]	2.55 - 10.1	96.96
		Daphnia magna	µg/L	8.16[bc]	3.27 - 20.37	
		Lampsilis siliquoidea	mg/L	**34.96**	13.16 - 92.90	
		Lepomis macrochirus	µg/L	37.78[bc]	15.7 - 90.87	
		Oncorhynchus mykiss	µg/L	51.55[bc]	20.64 - 128.77	
		Pimephales promelas	mg/L	**268.94**	124.11 - 582.78	
Leon springs pupfish	*Cyprinodon bovinus*	*Lepomis macrochirus*	mg/L	**385.84**	100.03 - 1488.19	321.5
		Oncorhynchus mykiss	mg/L	1405.12[ab]	117.55 - 16795.20	
		Pimephales promelas	mg/L	**267.81**	163.81 - 437.84	
Sheepshead minnow	*Cyprinodon variegatus*	*Americamysis bahia*	µg/L	24.42[c]	12.11 - 49.24	300.4
		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	**975.20**	695.11 - 1368.14	
		Oncorhynchus mykiss	µg/L	**432.19**	164.38 - 1136.32	
		Pimephales promelas	µg/L	**81.82**	24.12 - 277.55	
Pink shrimp	*Farfantepenaeus duorarum*	*Americamysis bahia*	mg/L	**31.16**	5.31 - 182.77	31.16
		Daphnia magna	µg/L	825.20[bc]	47.76 - 14258.5	
		Lepomis macrochirus	µg/L	350.34[bc]	11.8 - 10398.14	
		Oncorhynchus mykiss	µg/L	1468.52[bc]	47.52 - 45386.61	

Common Name	Predicted Species	Surrogate Species	Input Unit	Estimated Toxicity (mg/L)	95% Confidence Intervals (mg/L)	SMAV
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	4934.49[ab]	357.05 - 68194.76	
Polychaete	*Hydroides elegans*	*Daphnia magna*	µg/L	17.26[bc]	1.79 - 166.56	NA
		Oncorhynchus mykiss	µg/L	3.90[bc]	1.96 - 7.76	
Pinfish	*Lagodon rhomboides*	*Lepomis macrochirus*	mg/L	342.29[d]	121.48 - 964.44	NA
Blue shrimp	*Litopenaeus stylirostris*	*Americamysis bahia*	mg/L	28.40[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	491.5
		Oncorhynchus mykiss	mg/L	**576.41**	110.75 - 2999.99	
Tidewater silverside	*Menidia peninsulae*	*Americamysis bahia*	mg/L	61.53[a]	8.51 - 444.50	279.81
		Lepomis macrochirus	mg/L	**279.81**	97.91 - 799.61	
		Oncorhynchus mykiss	mg/L	818.72[ab]	11.88 - 56382.63	
Mysid	*Metamysidopsis insularis*	*Daphnia magna*	mg/L	**894.87**	209.96 - 3814.04	853.2
		Lampsilis siliquoidea	mg/L	**813.42**	361.18 - 1831.90	
Striped mullet	*Mugil cephalus*	*Lepomis macrochirus*	mg/L	730.34[ab]	36.69 - 14537.49	NA
		Oncorhynchus mykiss	mg/L	18685.50[ab]	81.57 - 4280001.49	
Harpacticoid copepod	*Tigriopus japonicus*	*Lepomis macrochirus*	mg/L	2812.24[d]	976.85 - 8096.09	1810.69
		Pimephales promelas	mg/L	**1810.69**	533.03 - 6150.86	
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 5^{th} 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). This draft acute benchmark for estuarine/marine aquatic life is lower than the recommended acute freshwater criterion (49 mg/L), suggesting that estuarine/marine species may be more acutely sensitive to PFOA and emphasizing the importance of having a separate benchmark value for the protection of estuarine/marine aquatic life.

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

Values in bold 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*	96.96	96.96	6	0.43
E	Amphipod	*Allorchestes compressa*	225.8	225.8	7	0.50
A	Leon springs pupfish	*Cyprinodon bovinus*	321.5	310.8	8	0.57
	Sheepshead minnow	*Cyprinodon variegatus*	300.4			
B	Inland silverside	*Menidia beryllina*	1,373	573.8	9	0.64
	Atlantic silverside	*Menidia menidia*	491.5			
	Tidewater silverside	*Menidia peninsulae*	279.8			
C	Mysid	*Metamysidopsis insularis*	853.2	853.2	10	0.71
G	Harpacticoid copepod	*Tigriopus japonicus*	1,811	1,811	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.

Calculated Estuarine/Marine FAV based on 4 lowest values; n=13						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
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)	

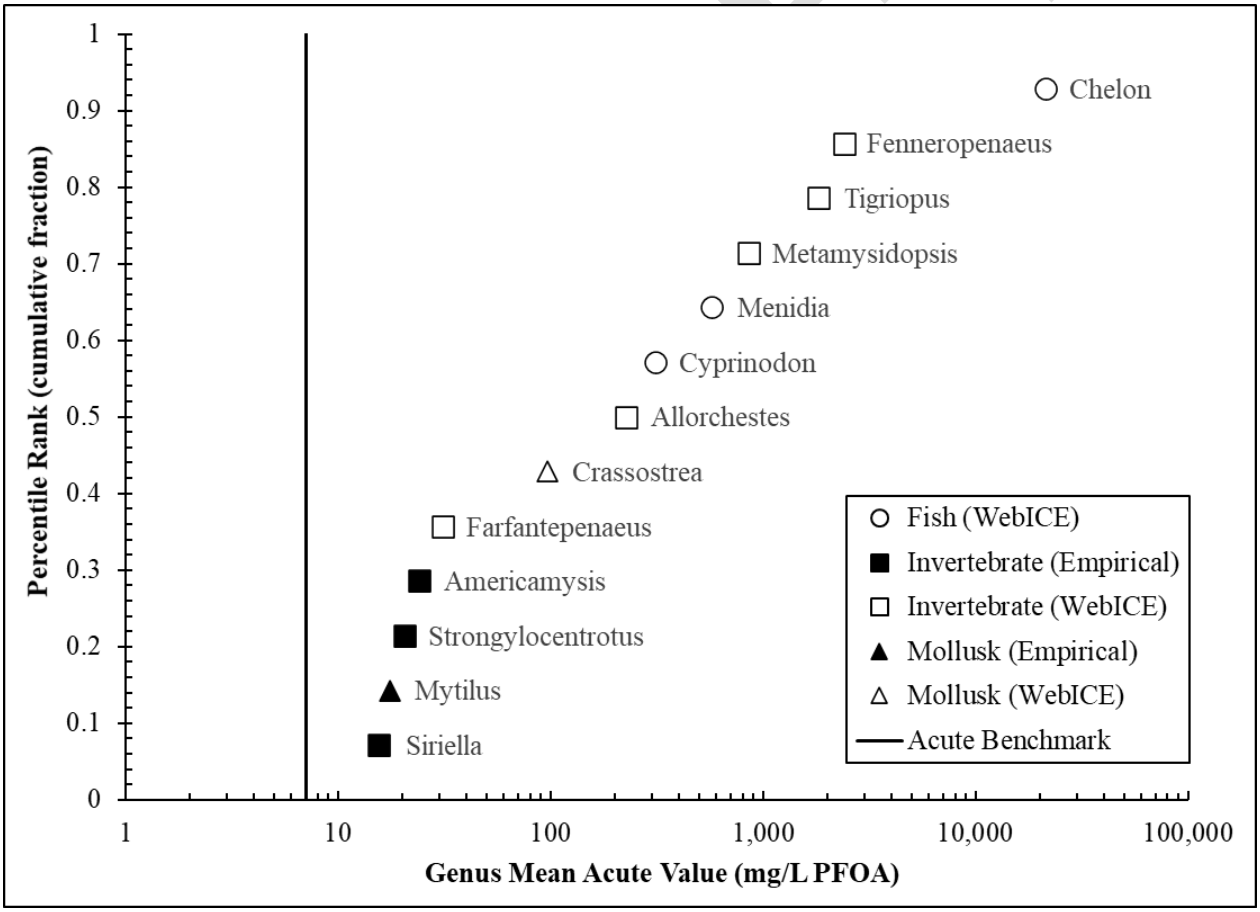


Figure L-2. Ranked Estuarine/Marine Acute PFOA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

L.5.1 Estuarine Marine/Benchmark Uncertainty

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. Interestingly, 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. It is unclear if ICE-estimated data were greater than empirical data because of a simple coincidence or a systematic mechanistic reason. A systematic mechanistic reason why ICE-estimated acute values were greater than empirical acute values

could be attributed to the use of freshwater species to predict to estuarine/marine species in the ICE regressions. For example, estuarine/marine LC_{50} values from quantitatively acceptable studies (Appendix B.1) were typically smaller than acute LC_{50} values for freshwater species (Appendix A.1). The apparent increase in PFOA toxicity in estuarine/marine environments relative to freshwaters may represent a unique toxicological consideration of PFOA (and possibly other PFAS) that was not a toxicological attribute of the other chemicals used to build the supporting ICE models, which would result in artificially high PFOA LC_{50} estimates for estuarine/marine species.

The estuarine/marine benchmark still appears adequately protective based on the available high quality empirical data (Appendix B.1). The acute 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*. EPA further evaluated the appropriateness of the estuarine/marine benchmark by comparing it to empirical, but qualitatively acceptable, data for estuarine/marine species. EPA specifically focused on qualitatively-acceptable estuarine/marine tests reported in Table H.1 that (1) tested an animal species, (2) exposed test organisms to a 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). 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 at 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 of the test duration was longer than the

standard 96 hour acute exposure. Nevertheless, this six-day tests suggests early life stages of *S. maximus* may be sensitive to acute PFOA exposures. 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).

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

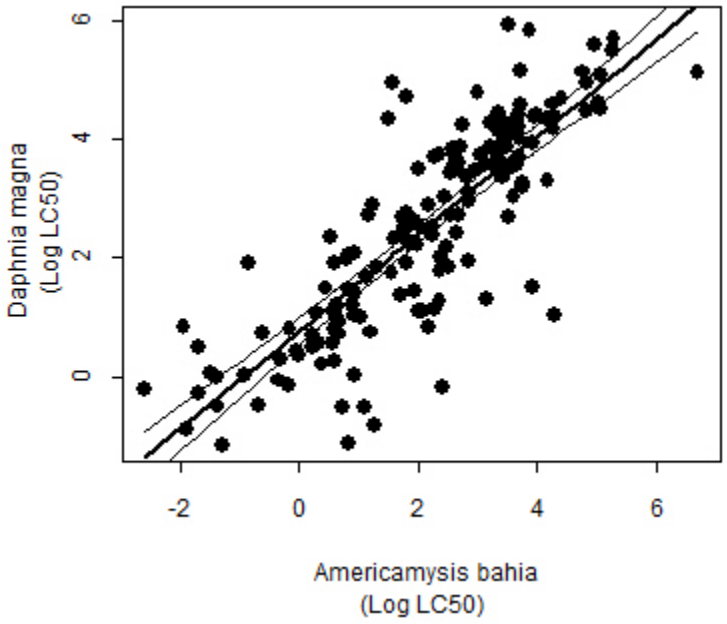


Figure L-3. *Americamysis bahia* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

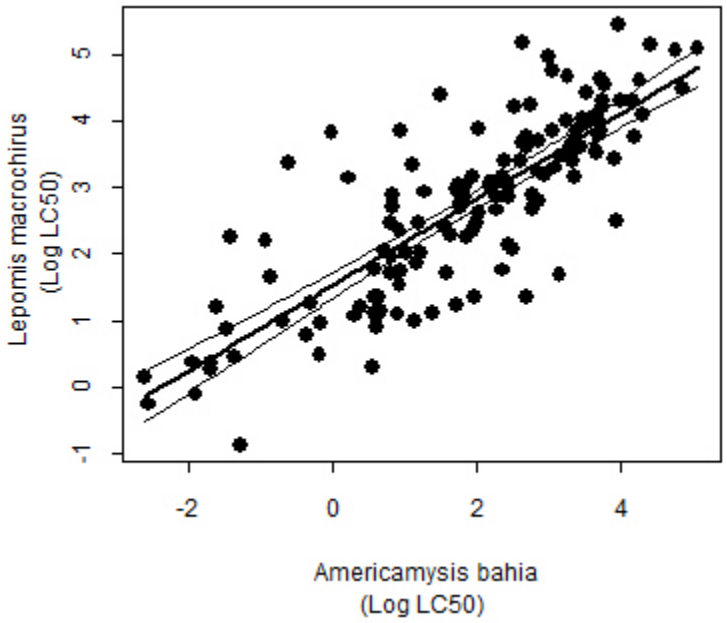


Figure L-4. *Americamysis bahia* (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.

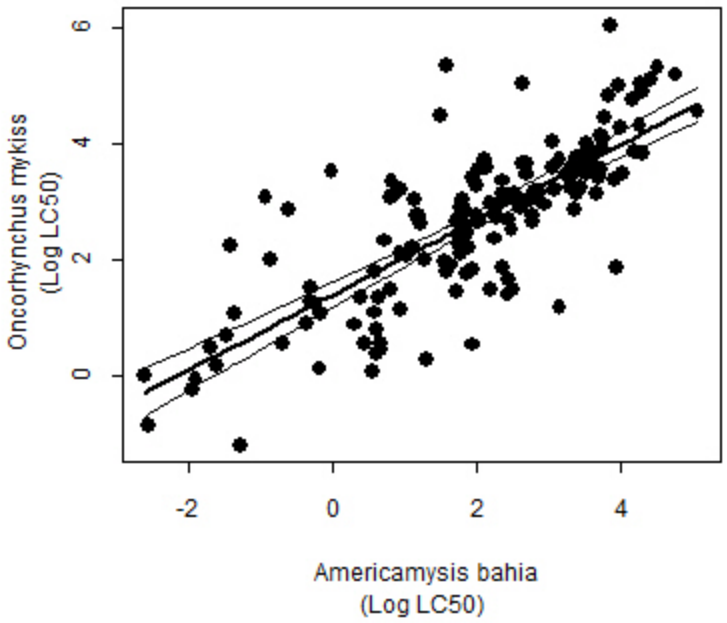


Figure L-5. *Americamysis bahia* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

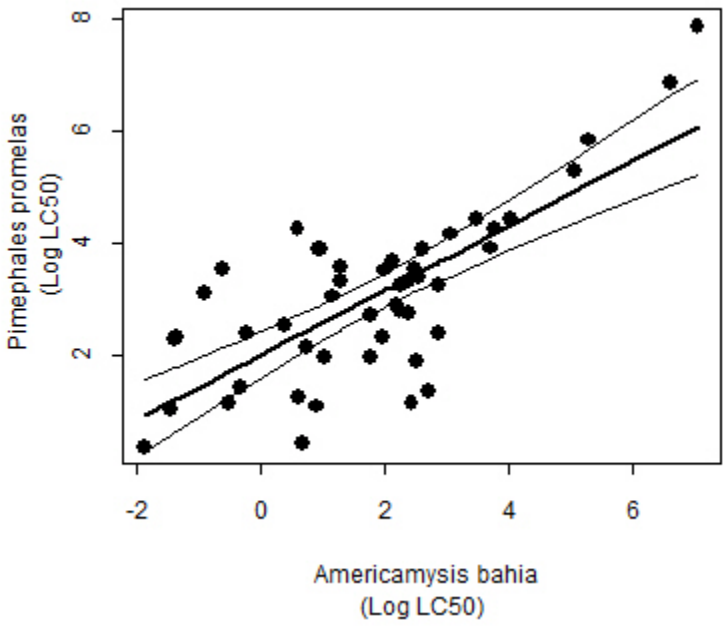


Figure L-6. *Americamysis bahia* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

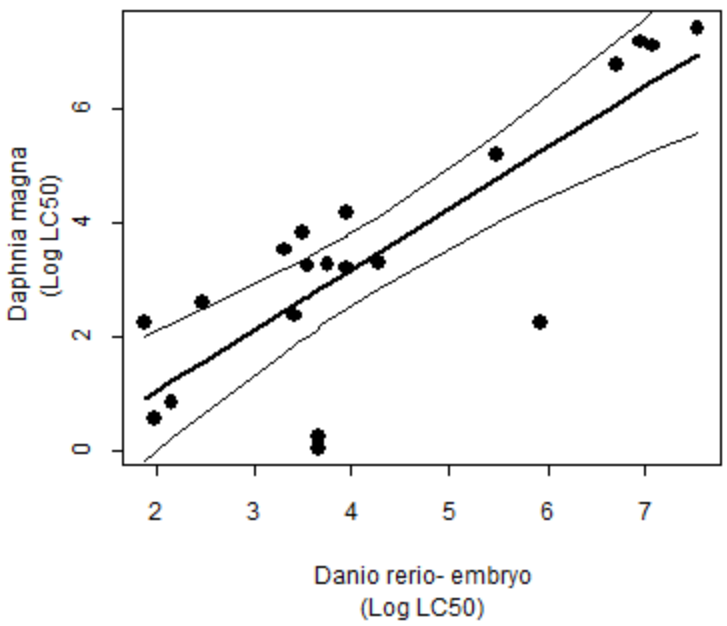


Figure L-7. *Danio rerio* - embryo (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

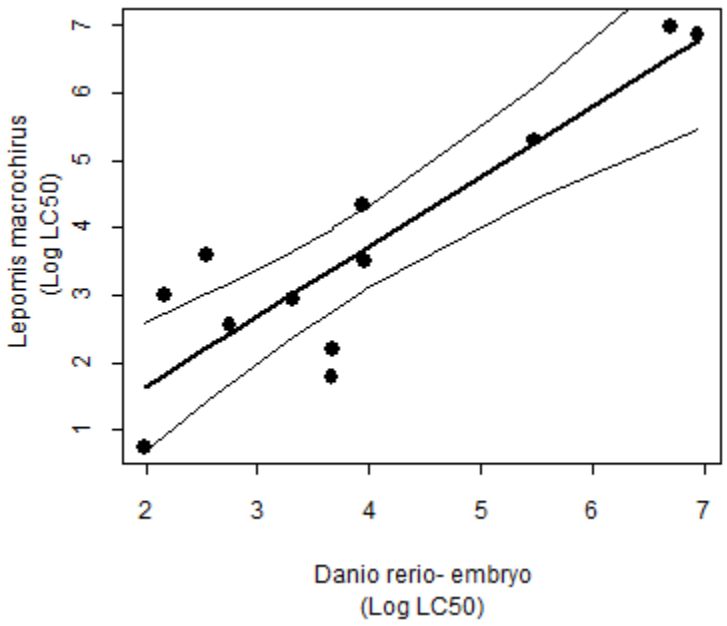


Figure L-8. *Danio rerio* - embryo (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.

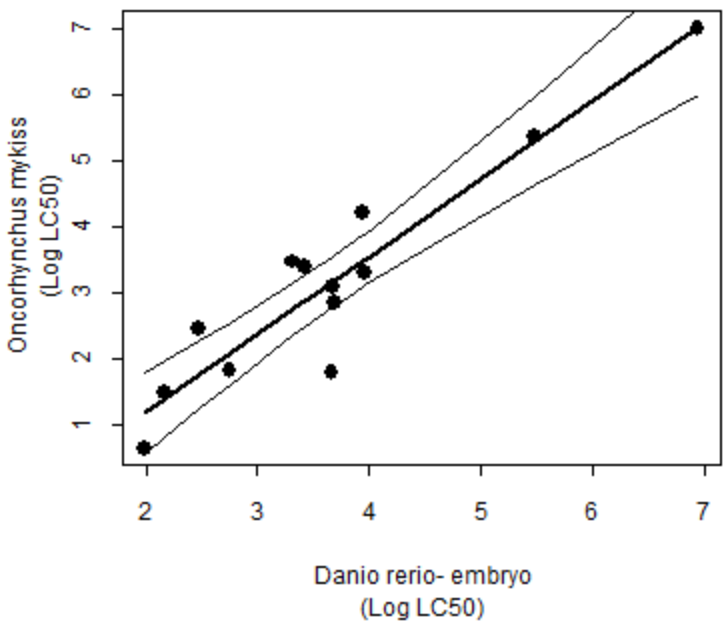


Figure L-9. *Danio rerio* - embryo (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

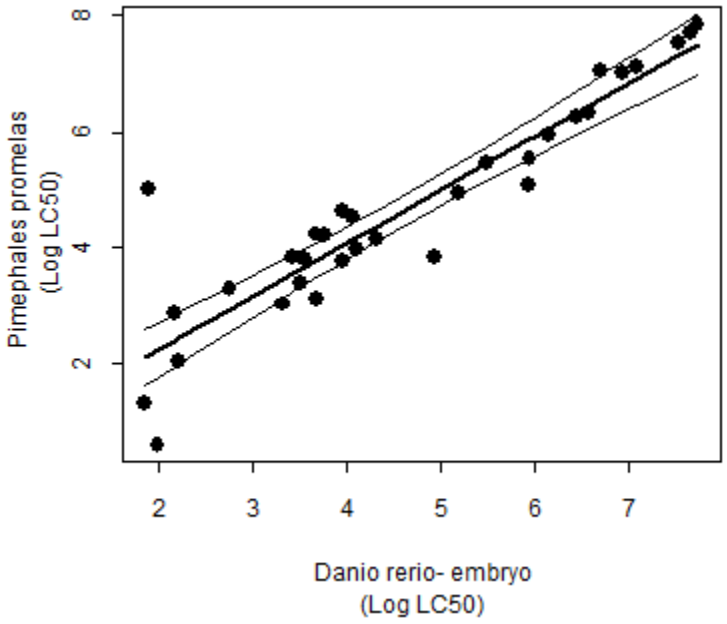


Figure L-10. *Danio rerio* - embryo (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

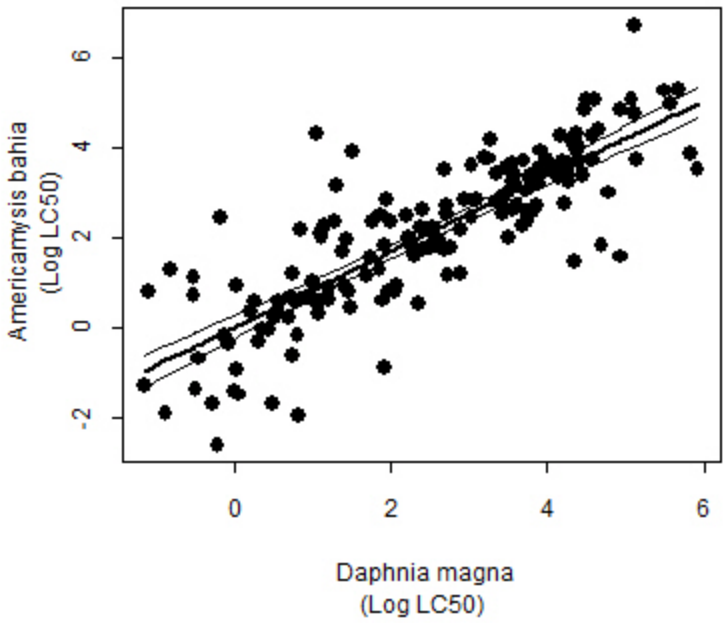


Figure L-11. *Daphnia magna* embryo (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

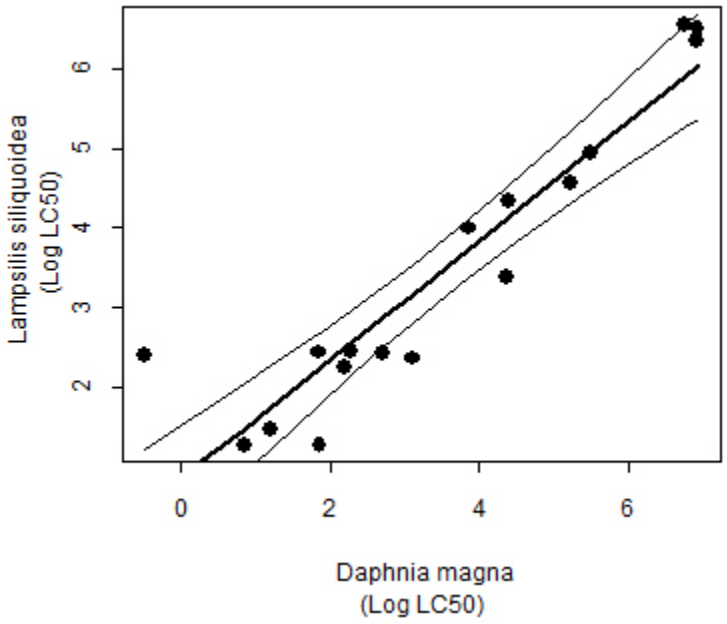


Figure L-12. *Daphnia magna* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

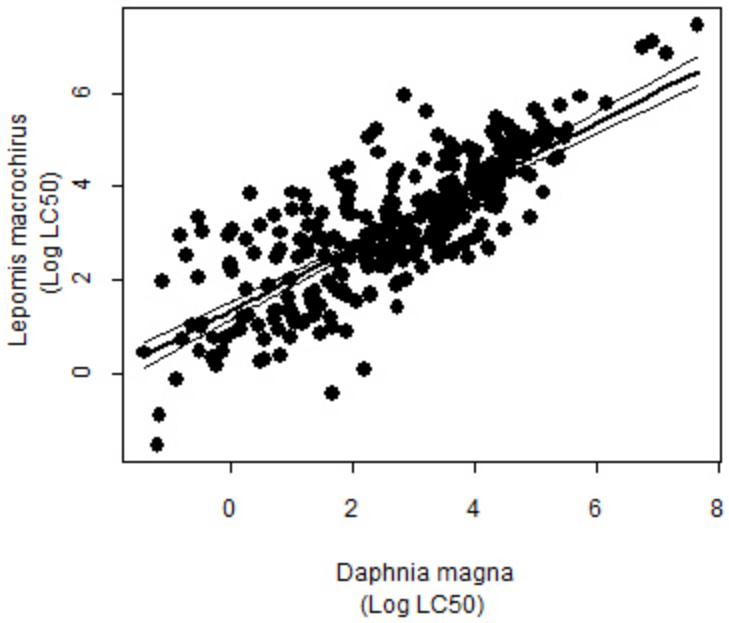


Figure L-13. *Daphnia magna* (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.

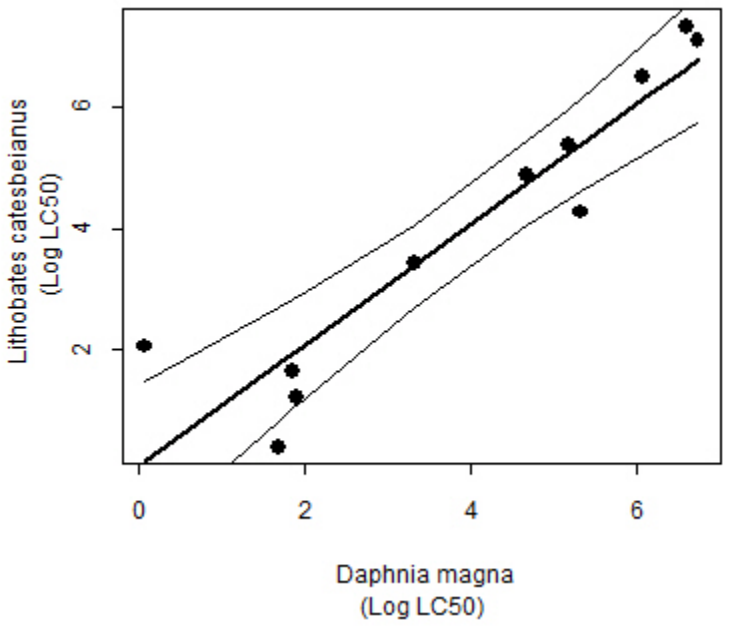


Figure L-14. *Daphnia magna* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

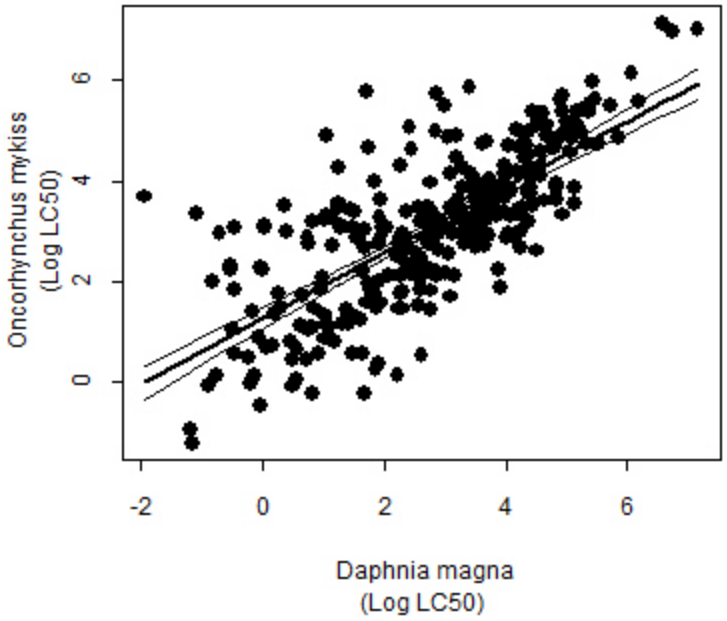


Figure L-15. *Daphnia magna* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

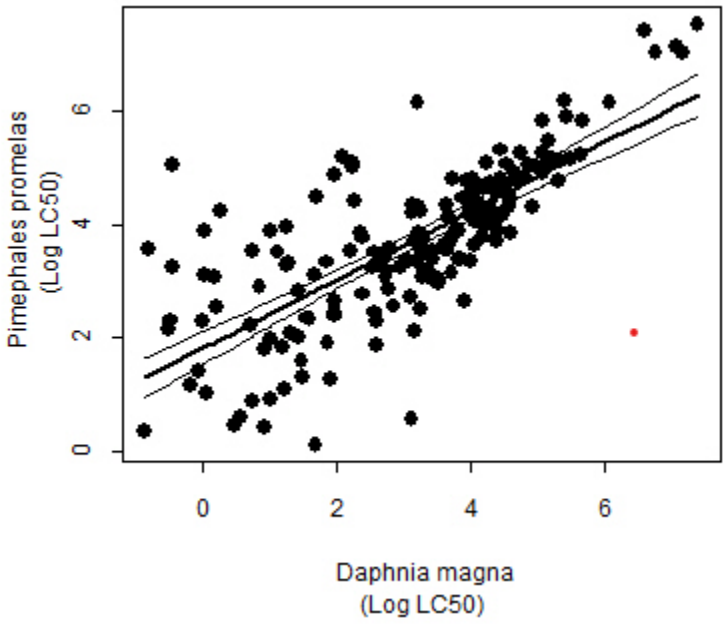


Figure L-16. *Daphnia magna* embryo (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

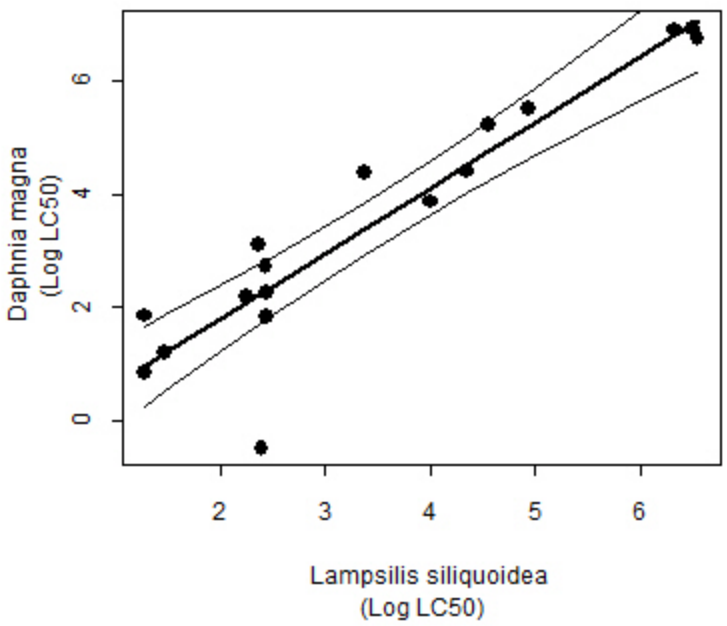


Figure L-17. *Lampsilis siliquoidea* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

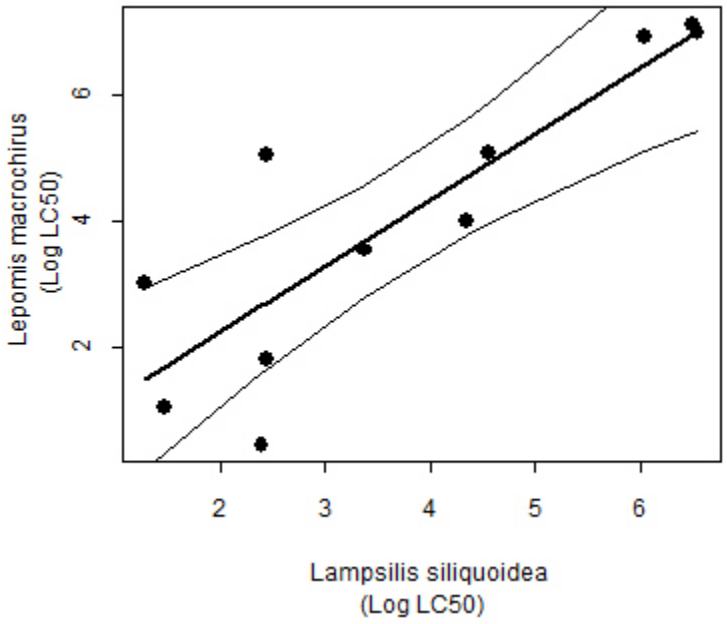


Figure L-18. *Lampsilis siliquoidea* (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.

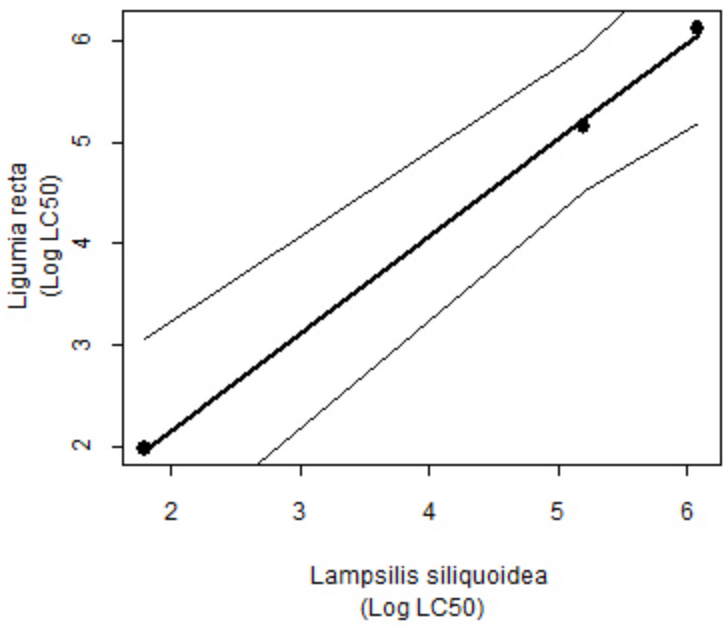


Figure L-19. *Lampsilis siliquoidea* (X-axis) and *Ligumia recta* (Y-axis) regression model used for ICE predicted values.

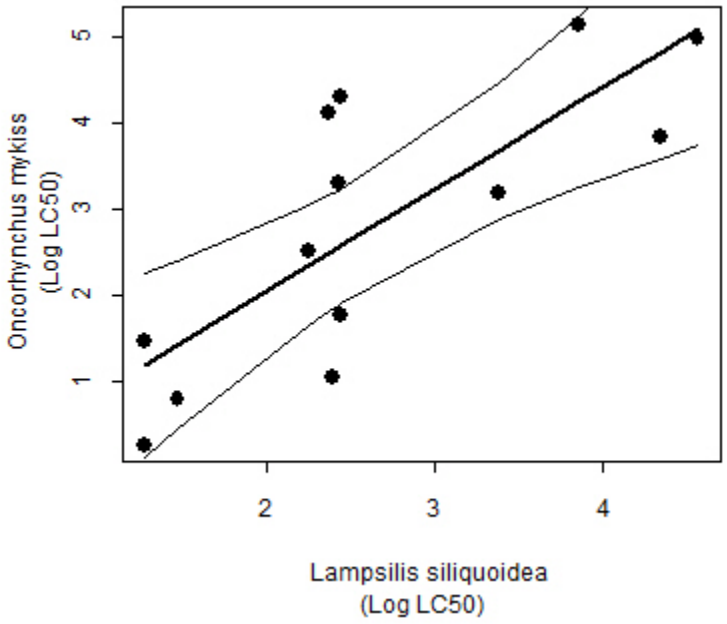


Figure L-20. *Lampsilis siliquoidea* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

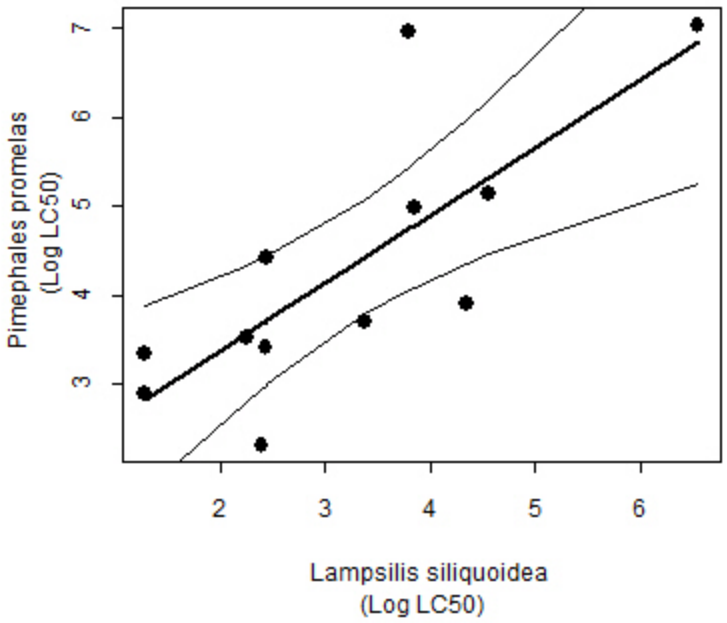


Figure L-21. *Lampsilis siliquoidea* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

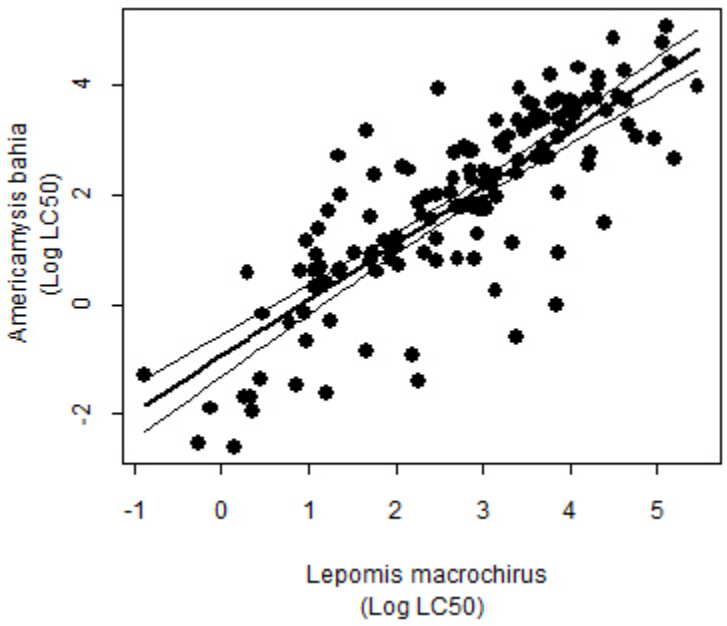


Figure L-22. *Lepomis macrochirus* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

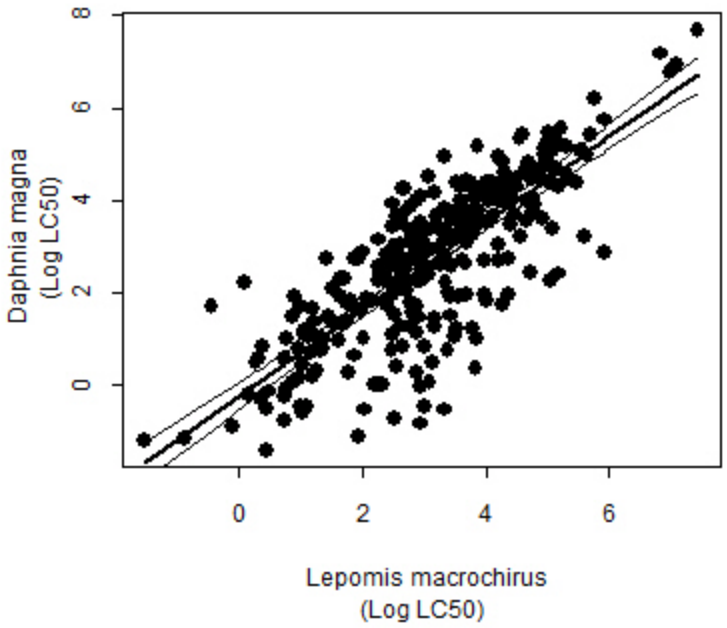


Figure L-23. *Lepomis macrochirus* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

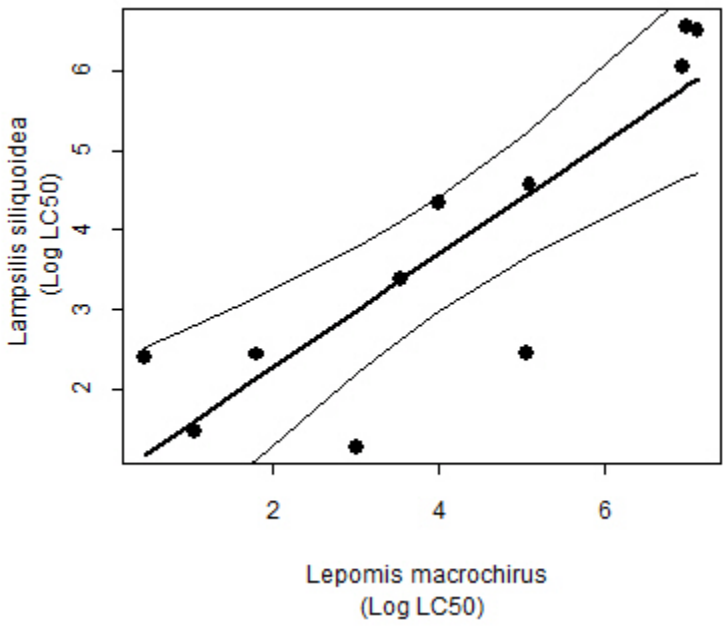


Figure L-24. *Lepomis macrochirus* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

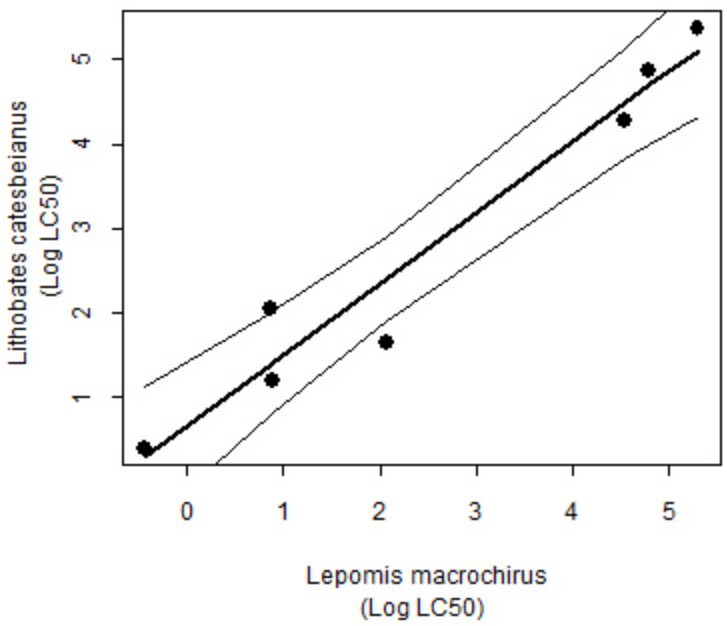


Figure L-25. *Lepomis macrochirus* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

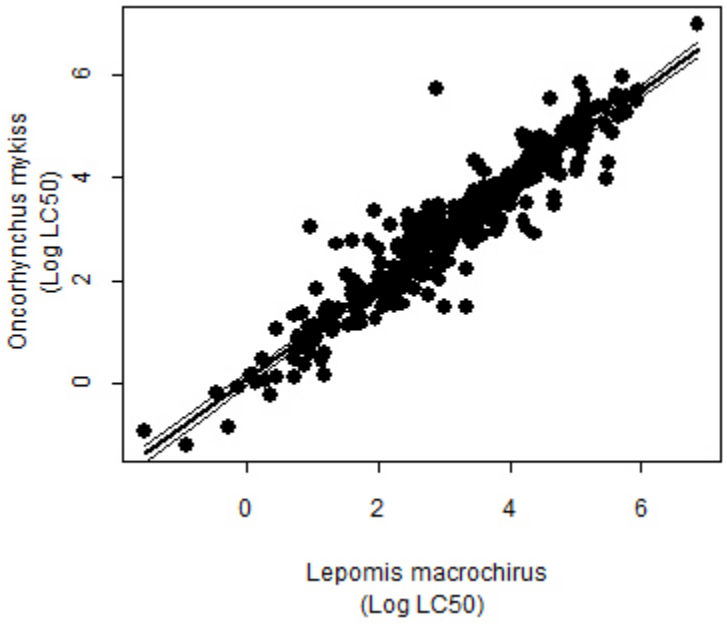


Figure L-26. *Lepomis macrochirus* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

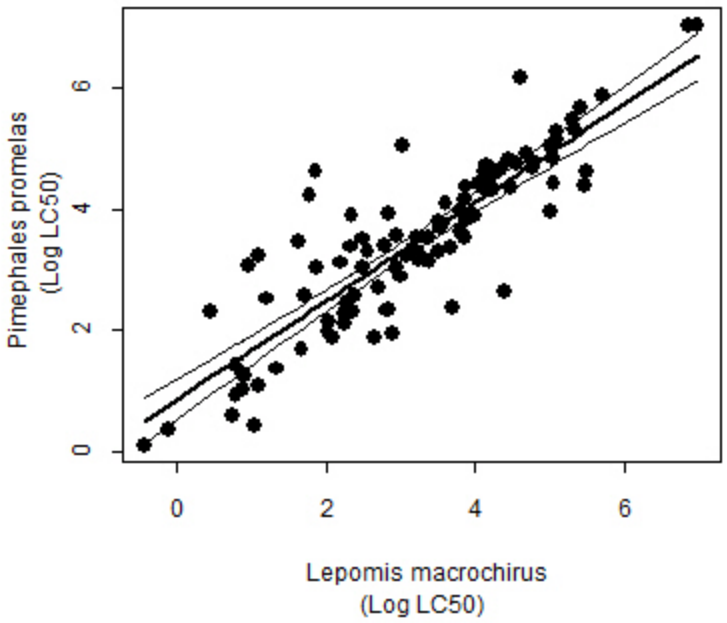


Figure L-27. *Lepomis macrochirus* embryo (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

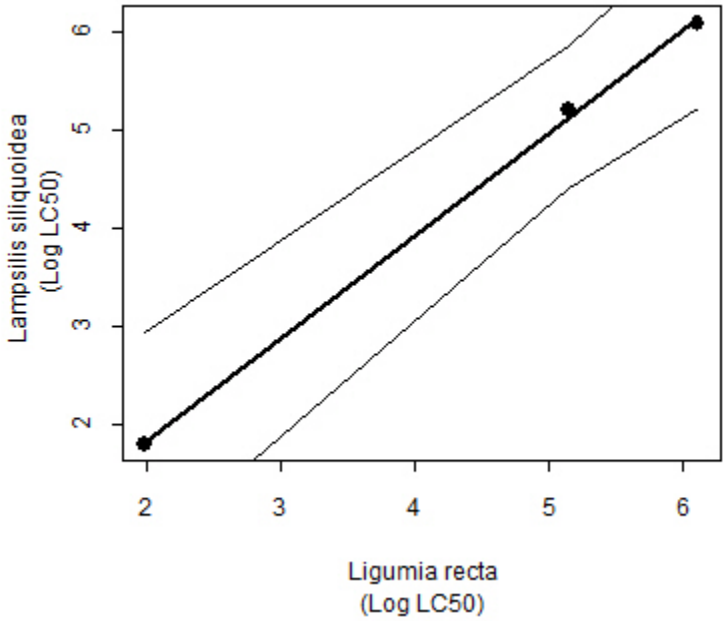


Figure L-28. *Ligumia recta* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

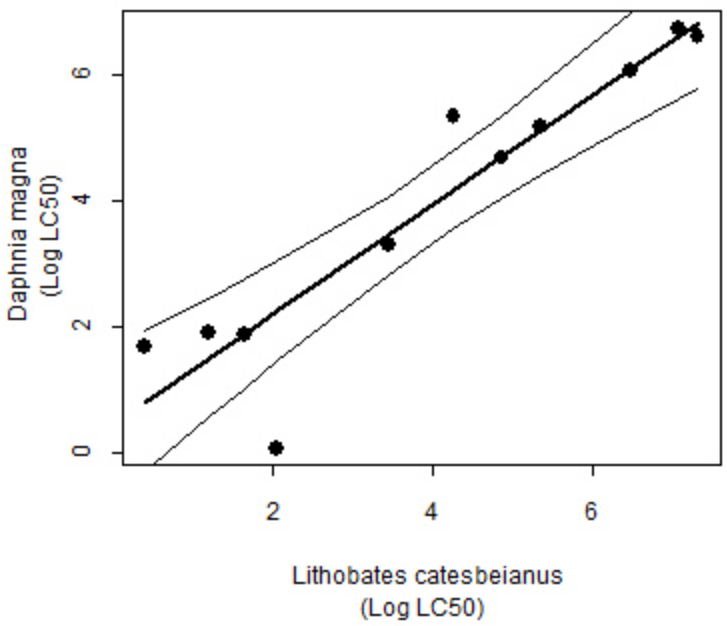


Figure L-29. *Lithobates catesbeianus* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

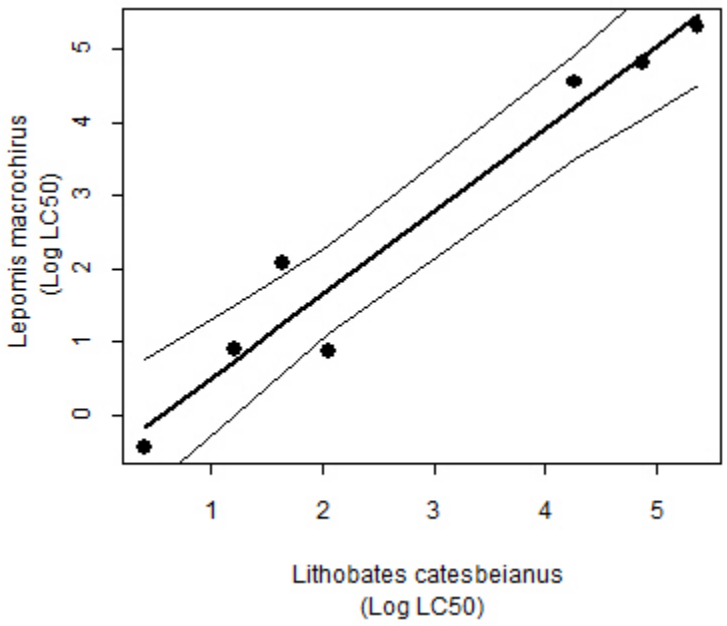


Figure L-30. *Lithobates catesbeianus* (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.

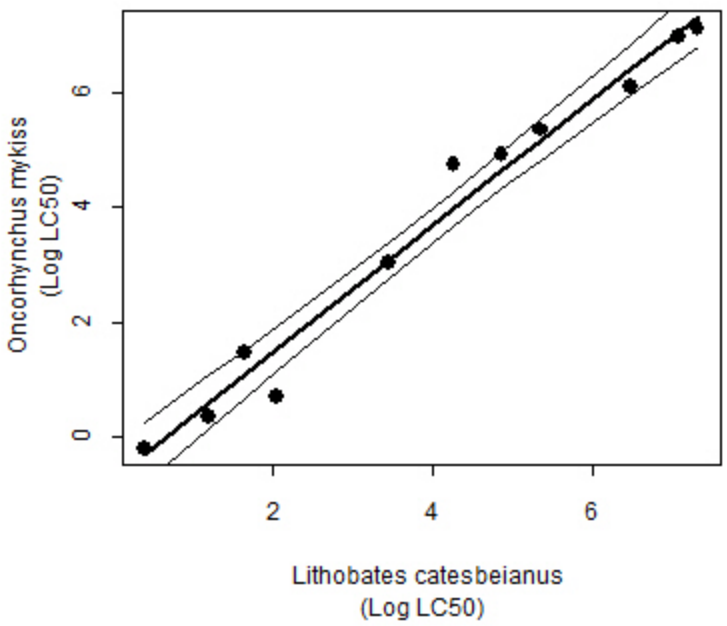


Figure L-31. *Lithobates catesbeianus* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

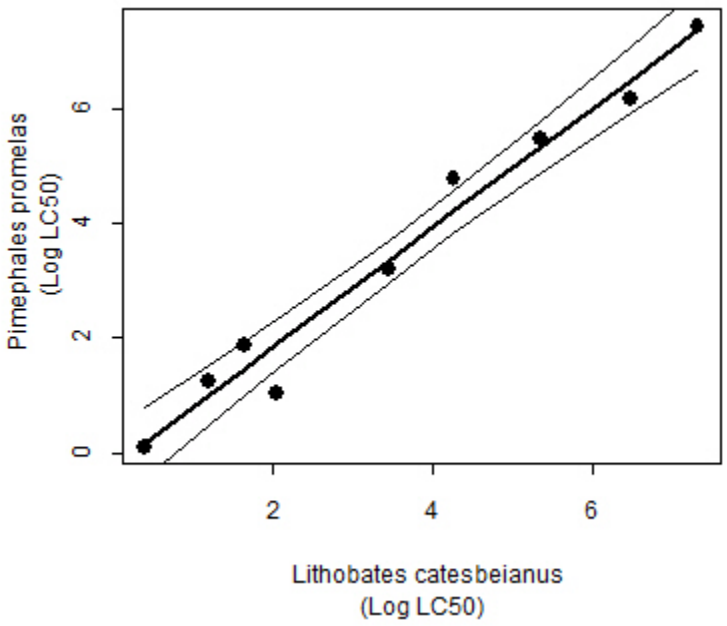


Figure L-32. *Lithobates catesbeianus* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

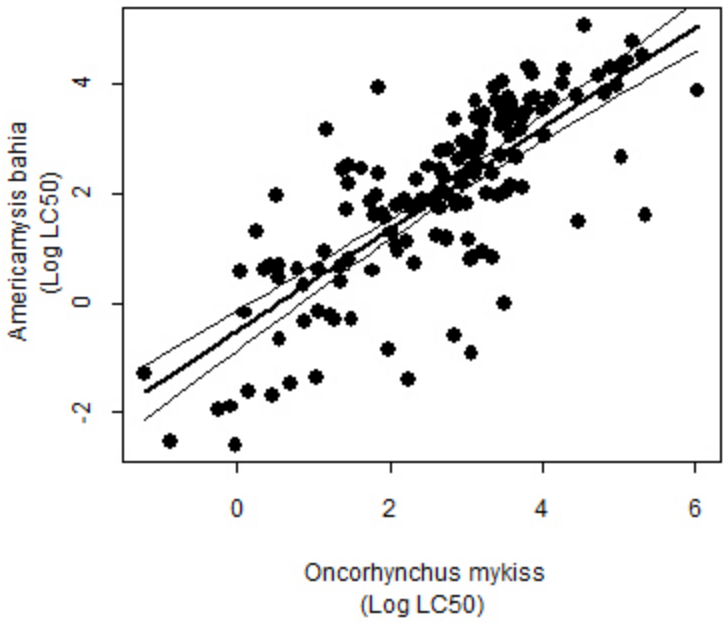


Figure L-33. *Oncorhynchus mykiss* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

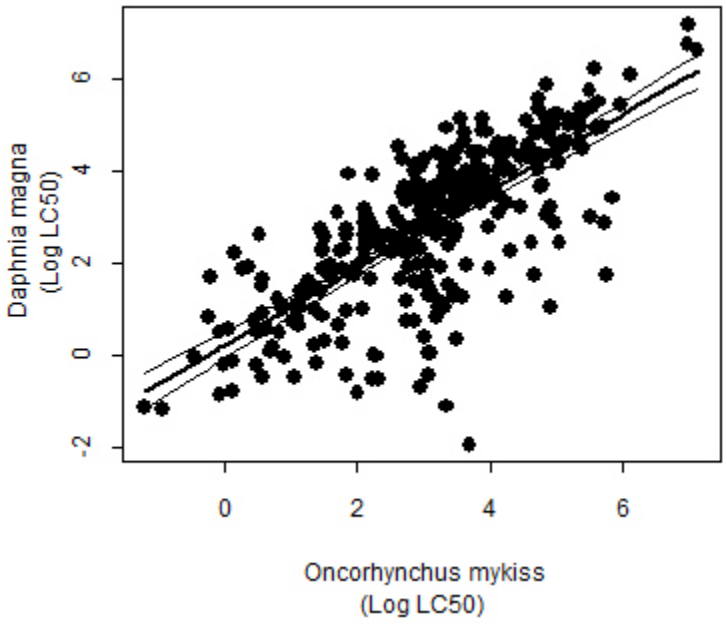


Figure L-34. *Oncorhynchus mykiss* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

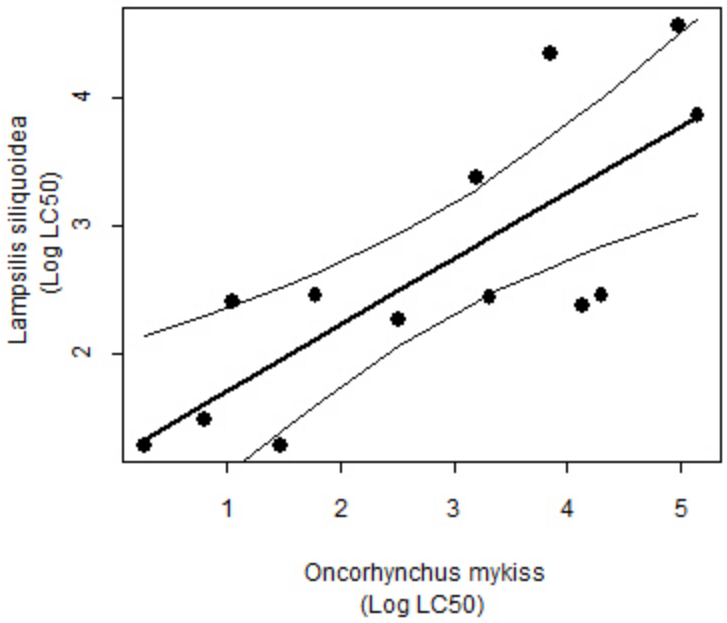


Figure L-35. *Oncorhynchus mykiss* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

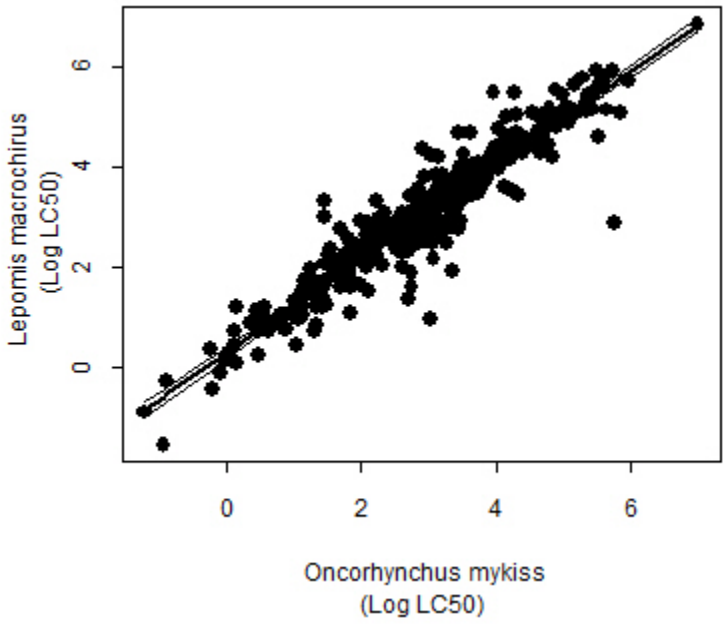


Figure L-36. *Oncorhynchus mykiss* (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.

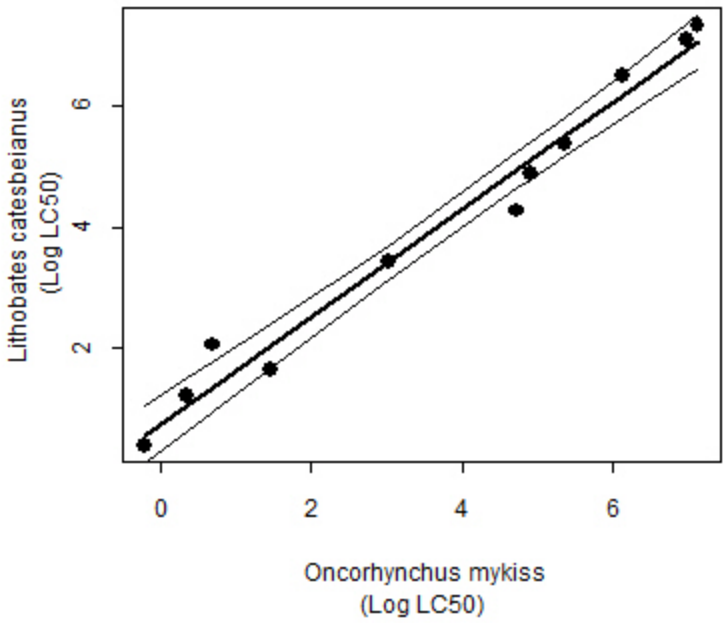


Figure L-37. *Oncorhynchus mykiss* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

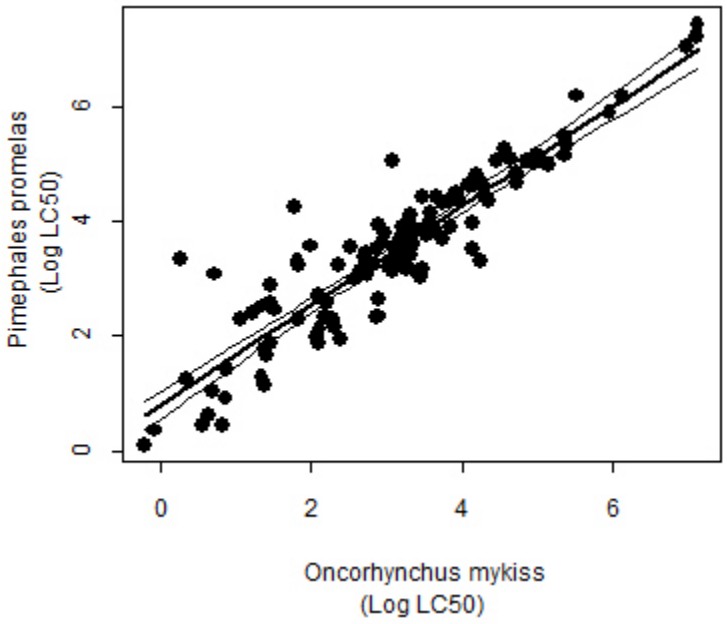


Figure L-38. *Oncorhynchus mykiss* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

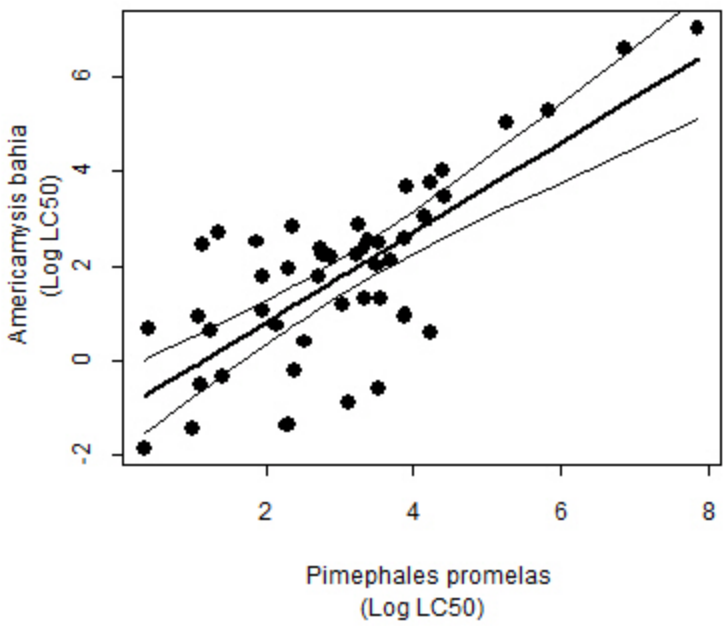


Figure L-39. *Pimephales promelas* (X-axis) and *Americamysis bahia* (Y-axis) regression model used for ICE predicted values.

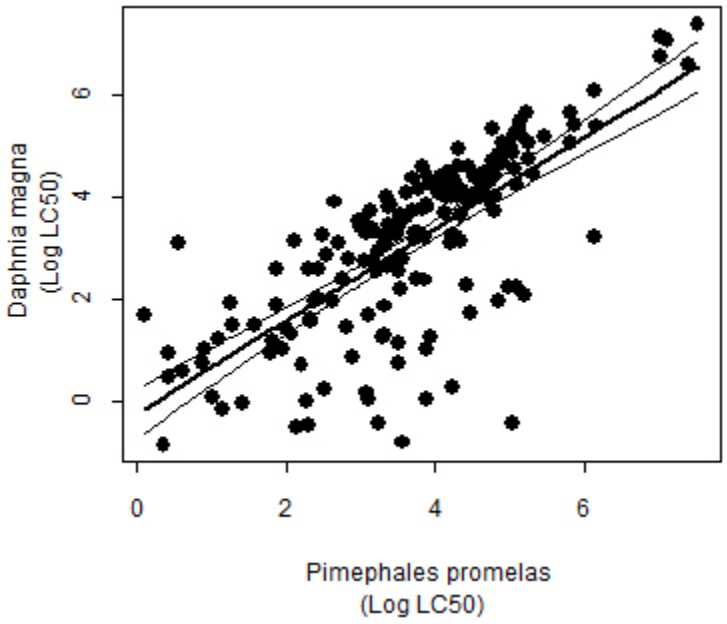


Figure L-40. *Pimephales promelas* (X-axis) and *Daphnia magna* (Y-axis) regression model used for ICE predicted values.

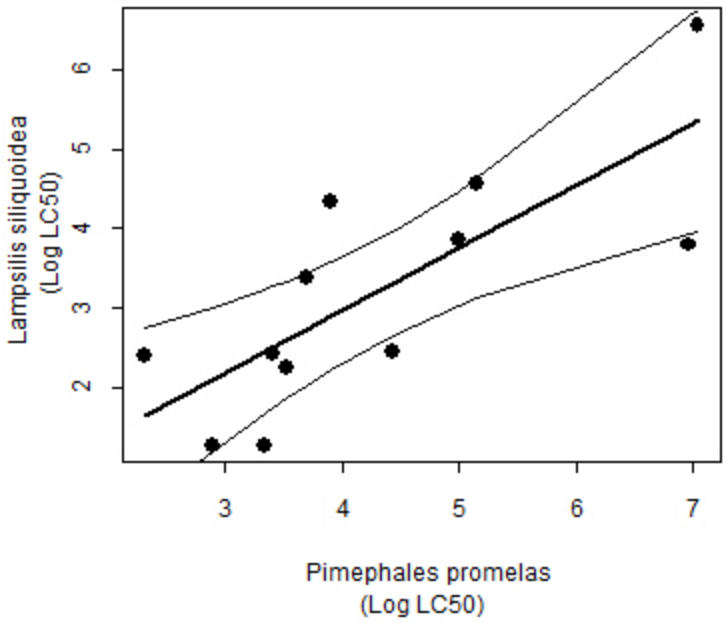


Figure L-41. *Pimephales promelas* (X-axis) and *Lampsilis siliquoidea* (Y-axis) regression model used for ICE predicted values.

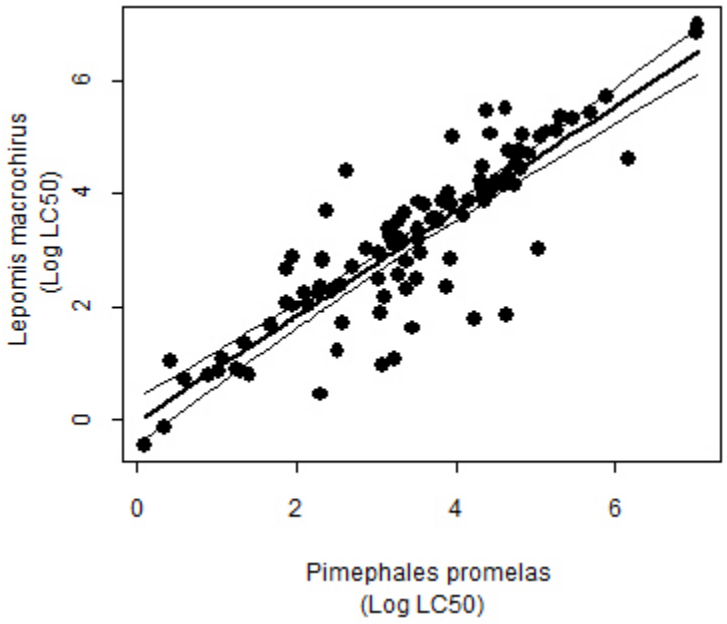


Figure L-42. *Pimephales promelas* (X-axis) and *Lepomis macrochirus* (Y-axis) regression model used for ICE predicted values.

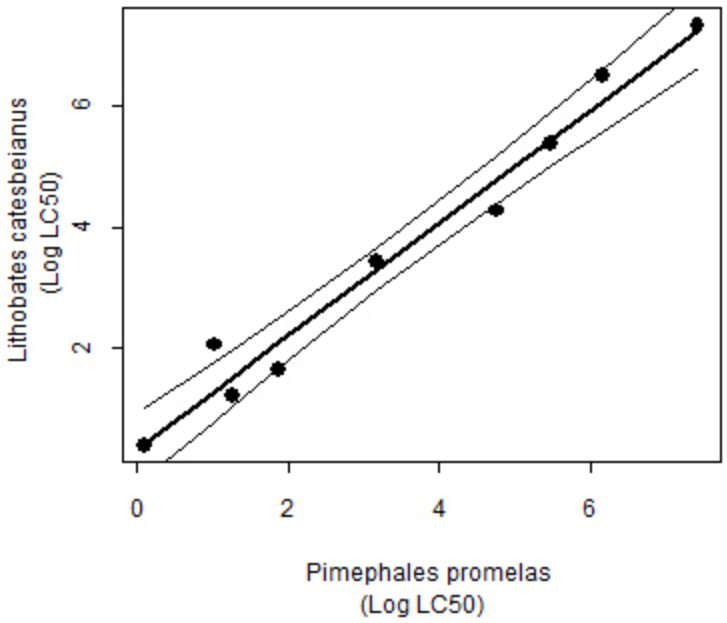


Figure L-43. *Pimephales promelas* (X-axis) and *Lithobates catesbeianus* (Y-axis) regression model used for ICE predicted values.

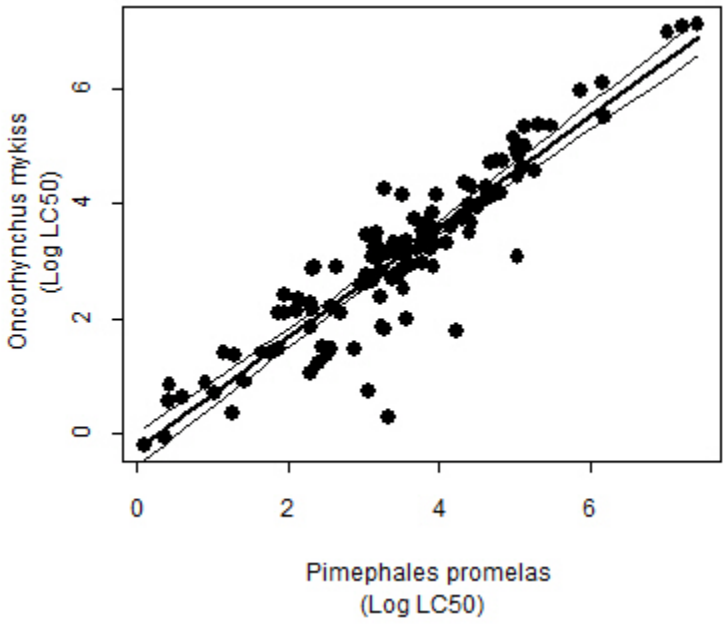


Figure L-44. *Pimephales promelas* (X-axis) and *Oncorhynchus mykiss* (Y-axis) regression model used for ICE predicted values.

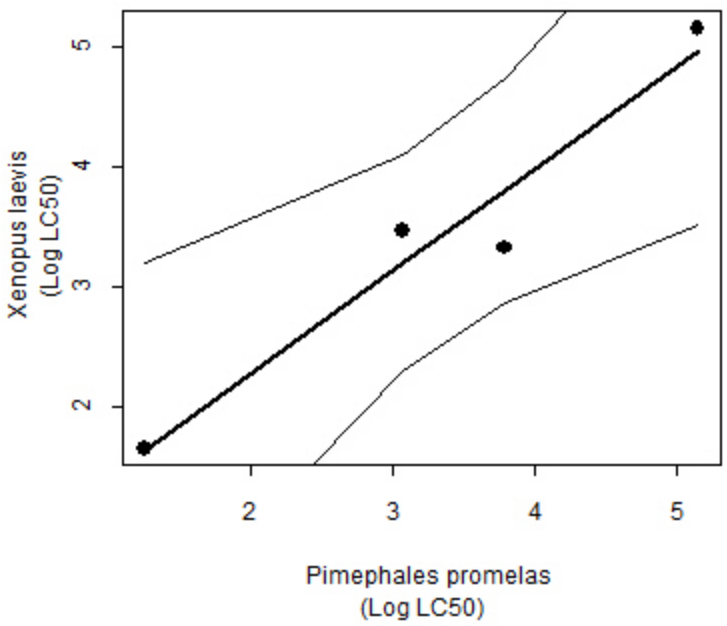


Figure L-45. *Pimephales promelas* (X-axis) and *Xenopus laevis* (Y-axis) regression model used for ICE predicted values.

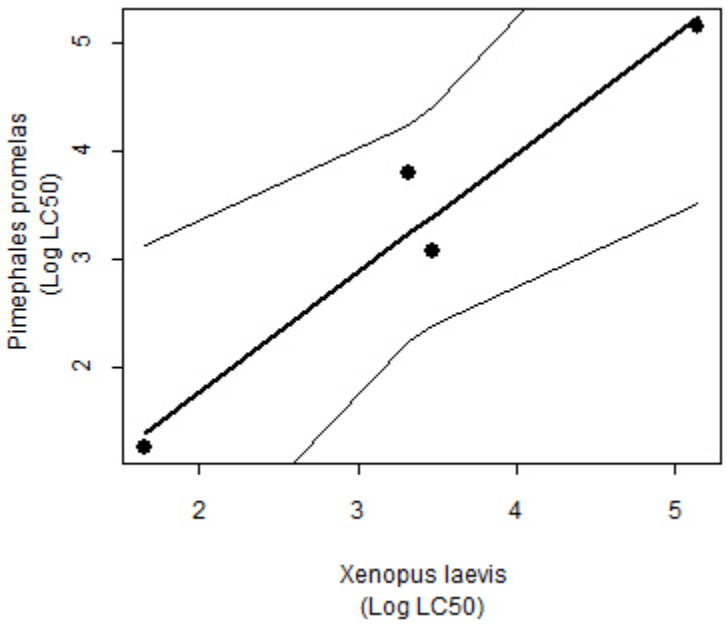


Figure L-46. *Xenopus laevis* (X-axis) and *Pimephales promelas* (Y-axis) regression model used for ICE predicted values.

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

M.1 Analysis

M.1.1 Introduction

Of the freshwater acute toxicity tests considered quantitatively acceptable for criteria derivation, 15.4% reported measured test concentrations while the remaining 84.6% only reported nominal test concentrations (see Appendix A). Of the freshwater chronic toxicity tests considered quantitatively acceptable for criteria derivation, 41.2% reported measured test concentrations while the remaining 58.8% only reported nominal test concentrations (see Appendix C). Therefore, EPA determined if nominal and measured PFOA concentrations were typically in close agreement (i.e., measured within 20% of nominal) to inform whether nominal concentrations from unmeasured tests provide reasonable approximations of actual PFOA exposures. If nominal and measured PFOA concentrations were systematically similar across tests, then EPA retained unmeasured PFOA toxicity tests for quantitative use in criteria derivation.

M.1.2 Meta-Analysis Methods

All freshwater and saltwater acute and chronic tests with animals that were determined to be quantitatively or qualitatively acceptable for criteria derivation (i.e., studies documented in Appendices A, B, C, D, G and H) were reviewed to identify those that reported both nominal and measured PFOA concentrations in at least one treatment concentration. Approximately 24% of the 152 freshwater toxicity tests and 57% of the 14 saltwater toxicity tests reported measured PFOA concentrations in at least one treatment. Pairs of nominal and measured concentrations were not considered when the nominal concentration was 0.0 (i.e., controls). Additionally, all nominal and measured pairs available from publications were used regardless of when the

measured concentration was collected or if the measured concentration was an average value because of broad inconsistencies in the timepoints concentrations were measured and reported in publications. Authors typically either reported measured concentrations at various time points or reported measured concentrations as an average value. The database of paired nominal and measured concentrations as well as the timepoint each measured concentration was determined are reported in Appendix M.2. Pairs of nominal and corresponding measured PFOA concentrations were compared to one another through (1) linear correlation analysis and (2) an assessment of measured concentrations as a percent of its paired nominal concentration.

M.1.2.1 Linear Correlation Analysis

The linear correlation analysis plotted nominal concentrations on the X-axis and corresponding measured concentrations on the Y-axis and assessed correlation between nominal and measured concentrations across all freshwater studies and again with all saltwater studies. Keeping freshwater and saltwater studies separate, the linear correlation analysis was then systematically repeated on subsets of studies based on specific experimental conditions to determine if specific experimental conditions may attribute to discrepancies between nominal and measured PFOA concentrations. Specific experimental conditions that were subsequently reassessed through linear correlation analysis included: (1) acute studies; (2) chronic studies; (3) unfed studies; (4) fed studies; (5) studies that used solvent vehicles; (6) studies that did not employ a solvent vehicle; (7) studies with substrate; (8) studies without substrate; (9) studies that used glass test vessels; (10) studies that used plastic test vessels, and; (11) studies with test vessels of an unspecified material.

M.1.2.2 Assessment of Measured Concentrations as a Percent of Nominal

The assessment of percent differences was used to identify the proportion of paired observations in which the measured concentration was not within 20% or 30% of the corresponding nominal concentration. Measured concentrations within 20% of corresponding nominal concentrations were considered in close agreement within one another based on EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines. For example, when describing test acceptability rules, U.S. EPA (2016b) states, "*measured concentration of test substance at each treatment level remains within plus or minus (±) 20% of the time-weighted average concentration for the duration of the test*." Similarly, U.S. EPA (1996a) states, "*In any case there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period*" when describing data quality. Finally, the Organization for Economic Cooperation and Development (OECD 2019) defines a stable exposure concentration as, "*A condition in which the exposure concentration remains within 80-120% of nominal or mean measured values over the entire exposure period*." Recently, in a study of key considerations for accurate exposures in ecotoxicological assessments of perfluorinated carboxylates and sulfonates, Rewerts et al. (2021) used a threshold of ± 30% to agree with nominal concentration for both stock and exposure solutions, as specified by the guidelines in the consolidated Quality Systems Manual for Environmental Laboratories set by the US Department of Defense and the US Department of Energy (Coats et al. 2017). The proportion of instances where measured concentrations differed from corresponding nominal concentrations by > either 20% or 30% was used to inform whether nominal and measured concentrations are expected to be systematically similar to one another.

Similar to the linear correlation analysis experimental conditions, the proportion of paired observations with measured concentration within 20% or 30% of nominal was also assessed across specific experimental conditions to determine if any test attributes consistently explained such discrepancies.

M.1.3 Results

M.1.3.1 Linear Correlation Analysis

Overall, nominal and measured concentrations from the freshwater PFOA toxicity literature indicated a high degree of correlation; however, measured concentrations were generally slightly lower than nominal concentrations. For example, the geometric mean measured/nominal ratios across freshwater and saltwater pairs were 0.8741 and 0.9665, respectively. Figure M-1 (Panel A) displays the strong correlation (correlation = 0.9995) of the 124 pairs of nominal and measured concentrations from freshwater studies, with the measured/nominal ratios mostly falling in a tight range (close to 1.0) with a geometric mean of 0.8741. Similarly, Figure M-1 (Panel B) displays the strong correlation (correlation = 0.9999) of the 12 pairs of nominal and measured concentrations from saltwater studies, with the measured/nominal ratios mostly falling in a tight range (close to 1.0) with a geometric mean of 0.9665.

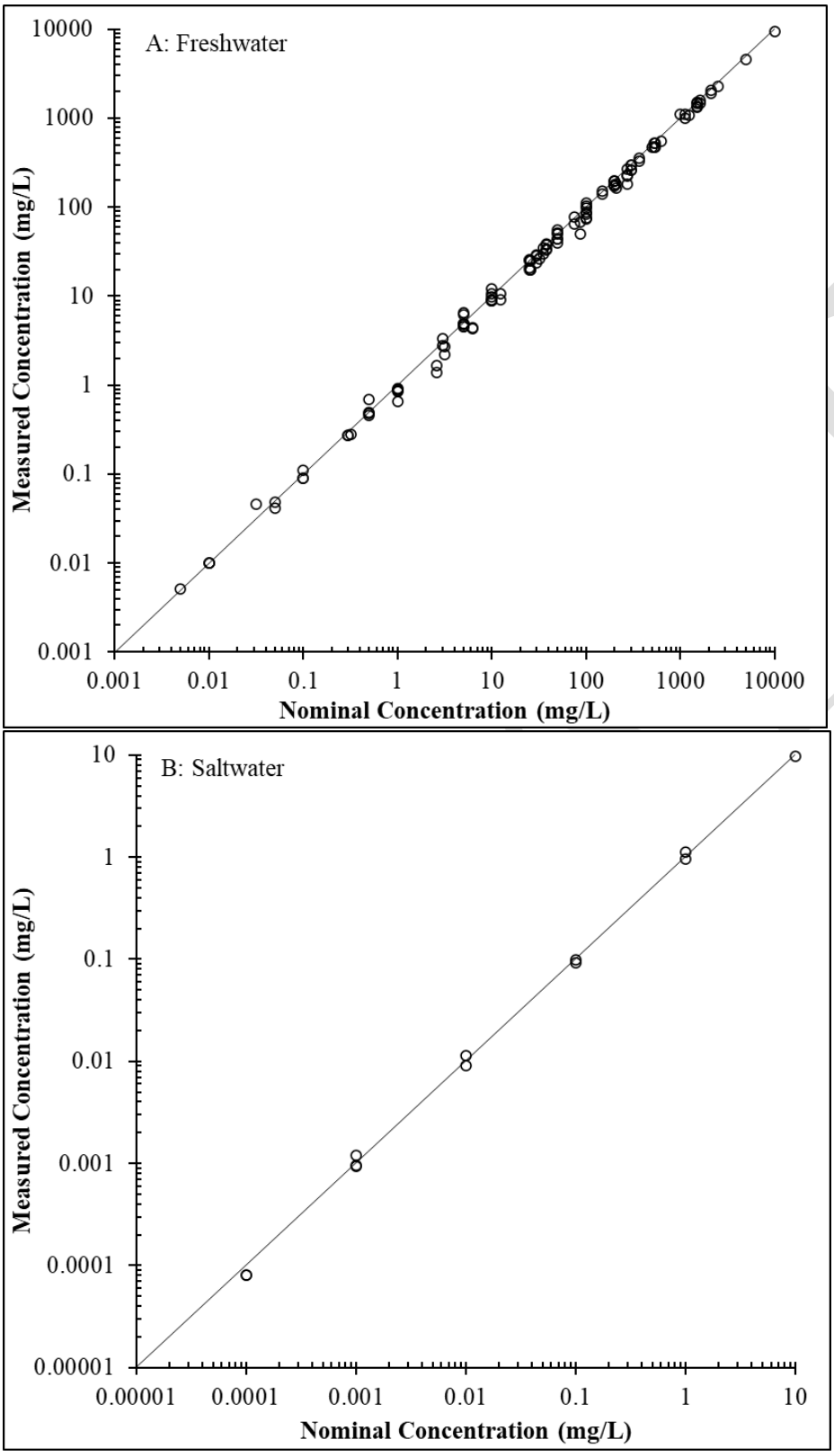


Figure M-1. PFOA measured vs. nominal concentrations for freshwater (Panel A, top) and saltwater (Panel B, bottom) data.

Nominal and measured concentration pairs in freshwater were further compared in terms of experimental conditions. In general, the nominal and measured concentrations displayed a high degree of correlation across experimental conditions. In particular, strong correlations were observed across all experimental conditions in freshwater tests (correlations > 0.98; Table M-1). Nominal and measured concentration pairs in saltwater were not further compared across various experimental conditions because of the three saltwater tests identified with measured PFOA treatments, two were performed by the same authors and followed the same methodologies. The only difference between the three exposures (saltwater tests) was the test vessel material; thus there were inadequate numbers of nominal and measured concentration pairs in saltwater to make any supportable conclusions.

Table M-1. Correlations of paired nominal and measured PFOA concentrations across various experimental conditions in freshwater toxicity tests.

Freshwater				
Experimental Condition	**# of Paired Observations**	**Correlation**	**Geometric Mean of Measured/Nominal**	**Median Percent Difference**
Acute	51	0.9996	0.9572	8.80
Chronic	73	0.9899	0.8204	11.00
Unfed	51	0.9996	0.9572	8.80
Fed	73	0.9899	0.8204	11.00
Solvent	52	0.9982	0.9561	9.07
No solvent	72	0.9997	0.8193	11.18
Substrate	25	0.9853	0.6560	17.65
No substrate	99	0.9995	0.9399	9.92
Glass	23	0.9989	0.9653	4.80
Plastic/Steel	45	0.9997	0.7798	12.50
Unspecified material	56	0.9984	0.9199	10.02

Correlations displayed in Table M-1 were high between paired observations in freshwaters across experimental conditions. Therefore, experimental conditions did not influence the correlation between nominal and measured concentrations. For example, Figure M-2 presents

log-scale plots of freshwater nominal and measured concentrations with data for acute tests (with 51 pairs; Panel A) and chronic tests (with 73 pairs; Panel B). Additionally, all acute tests displayed in Figure M-2 (Panel A) constituted all the unfed tests while all the chronic tests displayed in Figure M-2 (Panel B) constituted all the fed tests. Measured and nominal concentrations under acute (and unfed) and chronic (and fed) test conditions were highly correlated (0.9996 and 0.9899, respectively).

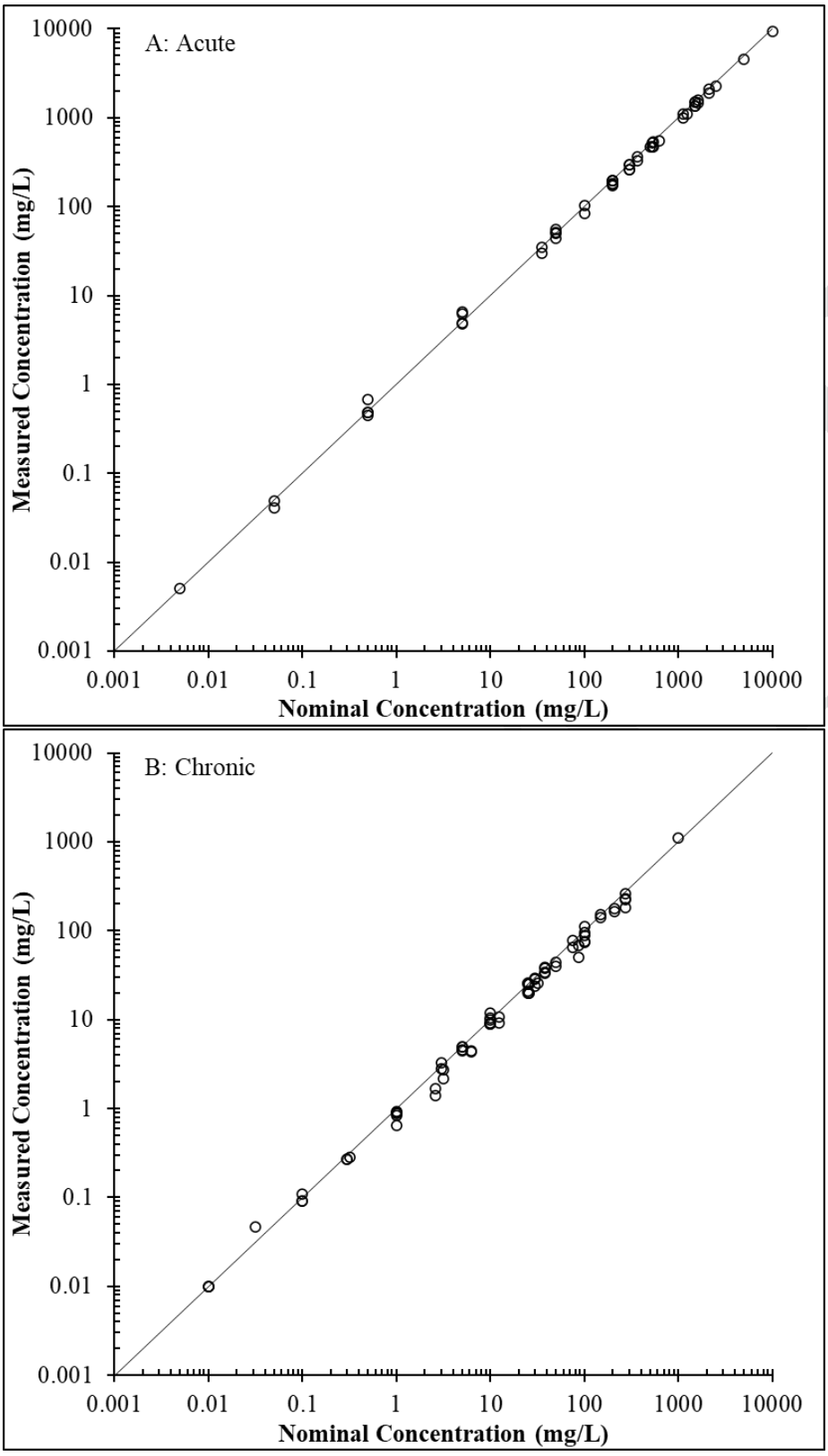


Figure M-2. PFOA measured vs. nominal concentrations for freshwater acute (Panel A, top) and freshwater chronic (Panel B, bottom) data.

As a secondary example, the nominal and measured concentrations from different test vessel types (e.g., plastic/steel or glass test vessels) are displayed in Figure M-3 below. Nominal and measured PFOA concentrations pairs were highly correlated in both the plastic/steel test vessels (45 pairs; correlation = 0.9997), glass test vessels (23 pairs; correlation = 0.9989) and unspecified test vessels (56 pairs; correlation = 0.9984).

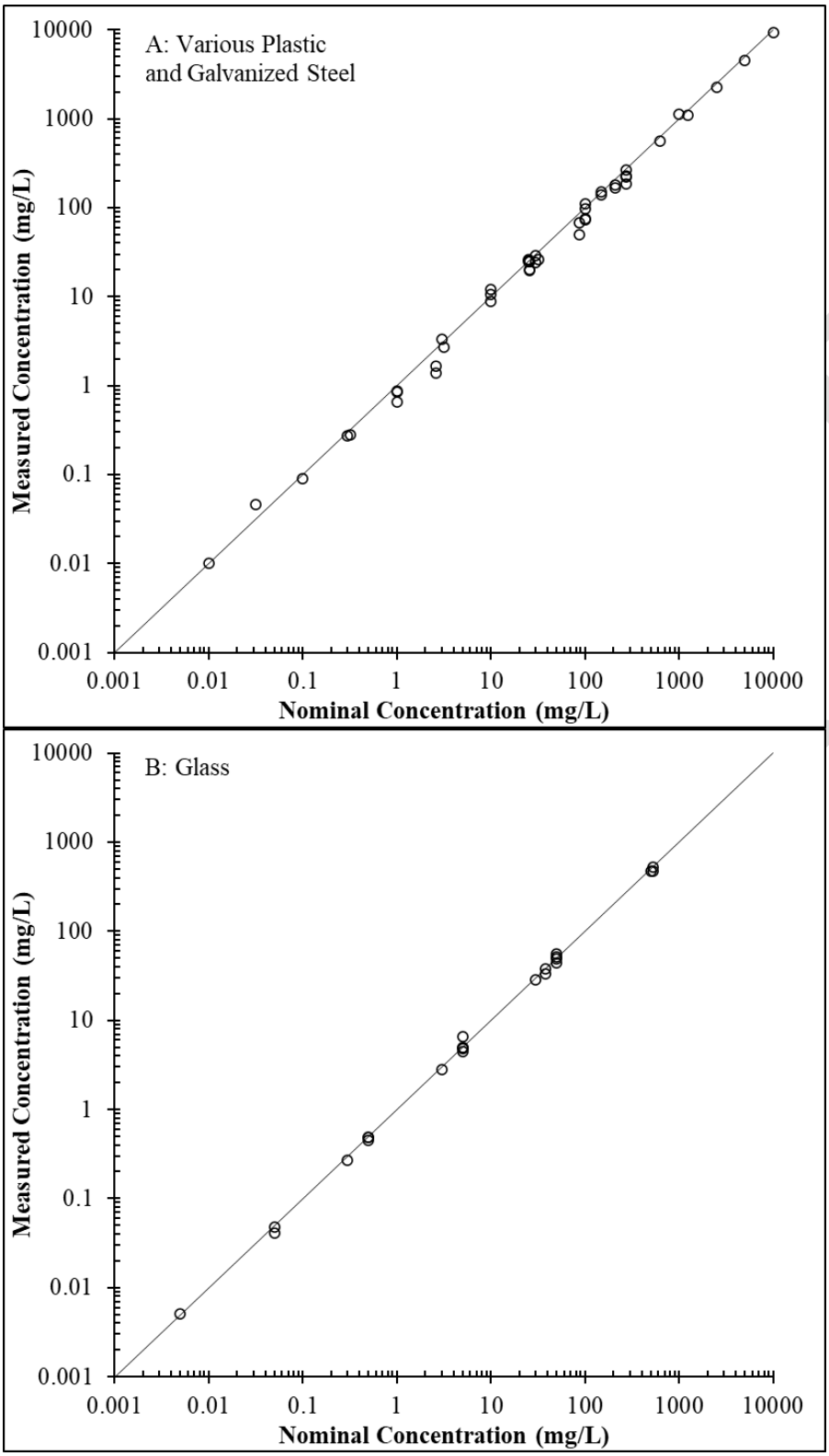


Figure M-3. PFOA measured vs. nominal concentrations for freshwater tests conducted in plastic/steel test vessels (Panel A, top) and freshwater tests conducted in glass test vessels (Panel B, bottom) data.

M.1.3.2 Assessment of Measured Concentrations as a Percent of Nominal

In freshwater, 21 (or 16.9%) of the 124 measured samples differed by corresponding nominal concentrations by more than 20%. Of these 21 pairs, 17 measured samples were <80% of the corresponding nominal concentration, while only 4 sample was >120% of the corresponding nominal concentration (Figure M-4). The trend of measured concentrations being less than nominal concentrations was also observed across the 124 paired observations in freshwaters, where measured concentrations were less than nominal concentrations in 80.6% of paired observations. Additionally, measured concentration as a percent of nominal concentrations was not influenced by the magnitude of the nominal concentrations, suggesting relatively low- or high-test concentrations did not systematically produce discrepancies between nominal and measured concentrations (Figure M-4). For comparison, only 11 of the 124 measured samples differed from corresponding nominal concentrations by more than 30% (data not shown).

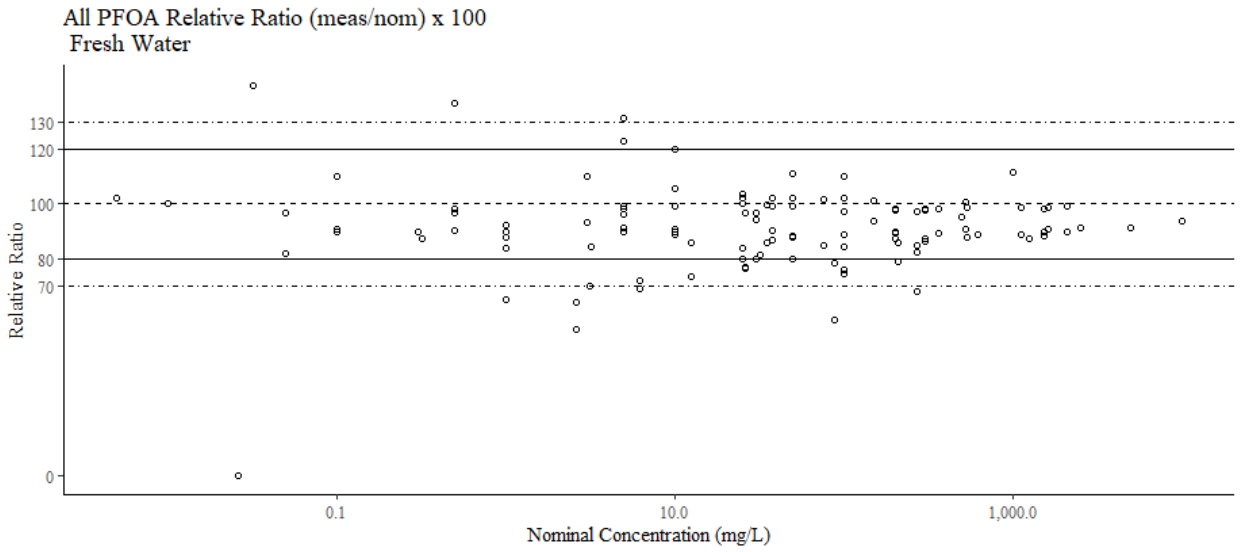


Figure M-4. Measured concentrations as a percent of corresponding nominal concentrations with horizontal lines to denote where the relative ratio (i.e., Y-axis) differs by more than 20% and 30%.
The horizontal line coding: 100% (dash line); +/- 20% (solid lines); +/- 30% (dash/dot lines).

In saltwater, none of the 12 measured samples differed from nominal concentrations by more than 20%. Similar to freshwater, 9 (or 75.0%) of the 12 measured concentrations were less than corresponding nominal concentrations, suggesting measured concentrations in both freshwater and saltwater may be systematically lower than nominal concentrations; however, the relative difference between measured and corresponding nominal concentrations was relatively minimal across all paired observations.

Measured as a percent of paired nominal concentrations from freshwater tests were further compared across experimental conditions, but similar comparisons were not performed for saltwater tests because of a lack of differences in experimental conditions across the limited number of saltwater tests available for such a comparison (Table M-4). Acute tests (which were the same as unfed tests), tests with solvent, tests without substrate, and tests conducted in glass test vessels or test vessels of an unspecified material had relatively low proportions of measured

concentrations that were not within 20% of nominal concentrations (range = 4.3% - 10.7%; Table M-2) and correspondingly lower proportion not within 30%, suggesting these test conditions may represent test scenarios where nominal concentrations accurately depict actual test concentrations in unmeasured tests.

Of the 21 measured PFOA concentrations that were not within 20% of nominal concentrations, 18 paired measured and nominal concentrations occurred across six different toxicity tests reported in four publications (Oakes et al. 2004, Colombo et al. 2008, McCarthy et al. 2021, Pecquet et al. 2020). These 18 pairs also all largely reoccurred across the remaining test condition categories with a high proportion of measured concentrations that were not within 20% of nominal concentrations (i.e., chronic/fed tests, tests without solvent, tests with substrate, and tests conducted in plastic test vessels; Table M-2). As a result, the six tests reported in Oakes et al. (2004), Colombo et al. (2008), McCarthy et al. (2021), and Pecquet et al. (2020) were further evaluated to determine if there were unique aspects of these individual tests that produced discrepancies between paired concentrations or if the discrepancies were explained by a systematic error that is expected to occur across PFOA toxicity tests in similar experimental conditions.

Table M-2. Percentage of Measured PFOA Concentrations Falling Outside of 20% of Corresponding Nominal Concentrations as well as the Minimum and Maximin of Measured as Percent of Nominal Concentrations (i.e., Measured/Nominal*100) across Range of Experimental Conditions.

Experimental Condition	Freshwater			
	Total # Nominal and Measured Pairs	Percent of Measured Concentrations outside of 20% of Paired Nominal Concentrations (%)[a]	Minimum of Measured Concentration as a Percent of Nominal (%)	Maximum of Measured Concentration as a Percent of Nominal (%)
All	124	16.9	0.38	144
Acute	51	5.9	82.0	137
Chronic	73	**24.7**	0.38	144
Unfed	51	5.9	82.0	137
Fed	73	**24.7**	0.38	144
Solvent	52	5.8	82.0	137
No Solvent	72	**25.0**	0.38	144
Substrate	25	**48.0**	0.38	110
No Substrate	99	9.1	76.0	144
Glass	23	4.3	82.0	132
Plastic/Steel	45	**31.1**	0.38	144
Unspecified	56	10.7	69.0	137

[a] Bold values represent test conditions with a high proportion of measured concentrations that were not within 20% of nominal concentrations.

McCarthy et al. (2021) conducted a 10-day test and a separate 20-day test on *C. dilutes* (note, organisms in the 20-day test were not exposed until 4 dph, so actual exposure was only 16-days in the 20-day test. For consistency with study authors, "20-day" test was used here). For both tests, PFOA stock solutions were dissolved in reconstituted moderately hard water without the use of a solvent and stored in polyethylene at room temperature until use. In both tests, the exposure vessels were 1 L high-density polyethylene beakers containing natural-field collected sediment with 60 mL of sediment and 105 mL of test solution. PFOA in test solutions of both the 10-day and 20-day tests was added via pipette to the beakers with the tip just above the sediment substrate.

- ***McCarthy at al. (2021) 10-Day test***: Solutions were renewed every 48-72 hours and test concentrations were measured on day 1 and day 10. Measured test concentrations ranged from 0-97% of nominal. At day one of the exposure, five of

the seven measured concentrations were lower than corresponding paired nominal concentrations by more than 20%. Of these five pairs, four pairs had measured concentrations that were lower than nominal by more than 30% (see data in Appendix M.2).

- ***McCarthy at al. (2021) 20-Day Test***: Solutions were renewed every 48 hours and PFOA treatment concentrations were measured on days 10, 15 and 20. At day 20, only one of the nine samples had a measured PFOA concentration that was larger than the corresponding nominal concentration (percent difference in this instance was 101.3%). Of the eight remaining measurements, four measured concentrations were less than corresponding nominal concentration by more than 20%, including one instance where the difference exceeded 30% (see data in Appendix M.2).

McCarthy et al. (2021) acknowledged the challenges associated with accounting for the differences between measured and nominal concentrations but could not credibly offer a reason why measured concentrations were consistently lower than nominal concentrations. It could represent a clear systematic dosing error, added substrate could have bound to PFOA thereby reducing the water column concentrations, and/or there could be other, unexplained, reasons why measured PFOA concentrations in McCarthy et al. (2021) were lower than paired nominal concentrations.

Oakes et al. (2004) exposed fathead minnows to PFOA in an outdoor microcosm experiment that contained sediment trays and potted macrophytes. With the exception of the lowest treatment concentration, all measured PFOA treatments were not within 20% of the nominal concentrations; however, Oakes et al. (2004) noted minimal variation in PFOA

concentrations throughout the course of the exposure period compared to those concentrations determined after one hour. The relatively minimal discrepancy between measured and nominal concentrations following one hour suggests there was possibly an error in the nominal test solutions themselves and/or the added PFOA rapidly sorbed to the sediment, organic matter, and macrophytes in the microcosms. Additionally, PFOA may have rapidly sorbed to the test vessel which was described as galvanized steel panels lined with food-grade PVC, rather than glass test vessels. For example, Lath et al. (2019) determined PFOA was more likely to sorb to plastic test vessels than glass, stating "*Contrary to suggestions in the literature, our results indicated that the greatest sorption losses for PFOA occurred on PP* [polypropylene]*, whereas losses on glass tubes were much lower.*"

Colombo et al. (2008) conducted acute and chronic freshwater aquatic toxicity studies with algae (*Pseudokirchneriella subcapitata*), *Daphnia magna*, and rainbow trout. Treatment waters were not measured in the acute tests because of "*the known stability of the test substance in water*" but treatment waters were measured in the chronic tests on the three species. Paired measured and nominal concentrations from the algae test were not specifically considered in this comparative analysis of measured and nominal concentrations because a Final Plant Value was not derived, and the intent was to focus on toxicity tests with animals.

In reference to the 21-day *Daphnia magna* test (test vessel material was unspecified), authors stated, "*In the Daphnia chronic test, some of the measured test concentrations were not within 20% of the nominal values but the maximum coefficient of variation of the measured concentrations was 8.5%.*" Specifically, the measured concentration in the two lowest treatments (excluding control) were 69.0% and 73.3% of nominal concentrations, respectively. All other treatments were within 20% of corresponding nominal concentrations. In reference to the 85-day

rainbow trout test, Colombo et al. (2008) stated, "*the measured test concentrations again were not within 20% of the nominal concentrations in some instances.*" Specifically, the measured concentration in the two lowest treatments (excluding control) were 69.6% and 71.7% of nominal concentrations, respectively. All other treatments were within 20% of corresponding nominal concentrations.

Interestingly, the two lowest treatment concentrations in both the rainbow trout and *Daphnia magna* tests had the greatest discrepancies between measured and nominal concentrations. Colombo et al. (2008) does not provide a reason for the discrepancies, but did note that the algal chronic test had all measured concentrations within 20% of the nominal concentrations, which suggests potential dosing errors or unknown phenomena did not occur across all tests conducted by Colombo et al. (2008). Unlike Oakes et al. (2004) which reported the use of PVC lined test vessels and McCarthy which reported HDPE test vessels, Colombo et al. (2008) did not report the test vessel material.

Finally, Pecquet et al. (2020) conducted 48-hour static exposures with zebrafish embryos and a separate 24-hr exposure to assess neutrophil migration. Pecquet et al. (2020) only measured exposure concentrations in the 24-hr test, which included a control and two treatments. Measured concentrations were 137% and 123.2% of corresponding nominal concentrations. Authors also note 0.089 mg/L PFOA was detected in the DMSO control. Consequently, it was assumed that relatively high measured concentrations were the result of a dosing error.

Beyond Pecquet et al. (2020), it is difficult to conclude discrepancies between nominal and measured concentrations observed by McCarthy et al. (2021), Oakes et al. (2004), and Colombo et al. (2008) were attributed directly to dosing errors. Nevertheless, the lack of explanatory variables, in combination with relative similarities (i.e., measured within 30% of

nominal) between concentrations across the remaining 89% of paired observations suggests dosing errors are relatively rare and no specific test condition(s) systematically produce discrepancies between measured and nominal concentrations.

M.1.4 Meta-Analysis Conclusions

Linear correlation between measured and corresponding nominal concentrations suggests a high degree of precision between paired observations across all test conditions. Nearly 83% of freshwater measured concentrations fell within 20% of paired nominal concentrations and 91% fell within 30% of paired nominal concentrations, which represent the test acceptability thresholds identified by EPA's OCSPP's Ecological Effects Test Guidelines and DoD's Quality Systems Manual for Environmental Laboratories, respectively. For example, Rewerts et al. (2021) states, "*To agree with nominal concentration, measured concentrations for both stock and exposure solutions were required to fall within the margin of 100 ± 30%, as specified by the guidelines in the consolidated Quality Systems Manual for Environmental Laboratories set by the US Department of Defense and the US Department of Energy (Coats et al.2017)*."

Instances where measured concentrations were not within 20% of nominal were isolated to a few studies. In these cases, suspected dosing errors, unexplained phenomena, and/or presence of substrate (i.e., McCarthy et al. 2021; Oakes et al. 2004) may have contributed to observed differences. Dosing errors were not considered to be a systemic issue with PFOA toxicity tests since large discrepancies from suspected dosing errors were only observed in a small subset of observed pairs in isolated toxicity tests. Use of substrate in tests may contribute to discrepancies between measured and nominal concentrations; however, no unmeasured tests with added substrate were used to derive the PFOA criteria. Therefore, PFOA toxicity tests were not excluded from quantitative use in criteria derivation on the basis of unmeasured test concentrations alone.

Although measured concentrations were relatively similar to nominal concentrations, measured concentrations were typically less than nominal concentrations, meaning PFOA toxicity tests with unmeasured treatments may overestimate exposure, thereby underestimating PFOA toxicity. As a result, it was particularly important to ensure tests with sensitive species were not excluded solely because treatment concentrations were not measured. For example, a relatively sensitive GMCV (i.e., *Brachionus*) was based on two unmeasured tests (Zhang et al. 2013a; Zhang et al. 2014b). Exclusion of these tests would have been inappropriate given the relative similarities between measured and nominal concentrations and would have also resulted in water column- and tissue-based chronic criterion magnitudes that were under productive.

M.2 Paired Nominal and Measured PFOA Concentrations Data Table Used to Inform the Meta-Analysis of Nominal and Measured Concentrations

Table M-3. Paired Nominal and Measured PFOA Concentrations from Quantitatively and Qualitatively Acceptable Freshwater Toxicity Tests that Reported Measured PFOA Concentrations.

Treatment concentrations where the nominal concentration was 0.0 mg/L (i.e., controls) were not included.

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOA / 96%	96 hours	Glass (assumed based on reference to glass beakers in the chronic PFOS test within this article)	None	High Performance Liquid Chromatography / Mass Spectrometry	0 hours	0.005	0.0051	Hazelton et al. 2012, 2013
					0 hours	0.05	0.0484	
					0 hours	0.5	0.49	
					0 hours	5	4.8	
					0 hours	50	51	
					0 hours	500	476	
PFOA / 99%	48 hours	Glass (assumed based on authors reference to beakers in acute test methods)	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	50	49.62	Yang et al. 2014
					Before Renewal	50	43.93	
					After Renewal	524.29	526.9	
					Before Renewal	524.29	476.41	
APFO / 99%	96 hours	Stainless steel	None	High Performance Liquid Chromatography / Tandem Mass Spectrometry.	Details about when samples are taken are not provided	625	554	DuPont Haskell Laboratory 2000
						1250	1090	
						2500	2280	
						5000	4560	
						10000	9360	
APFO / 99.7%	21 days	Not reported.	None	Ion Chromatography	Mean measured concentrations taken from 14 samples, details of when samples were taken are not provided.	6.25	4.31	Colombo et al. 2008
						12.5	9.16	
						25	20	
						50	44.2	
						100	88.6	
PFOA / 99%	21 days	Glass (assumed)[b]	DMSO	High Performance Liquid	After Renewal	5	4.96	Yang et al. 2014
					Before Renewal	5	4.49	
					After Renewal	37.97	37.66	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
				Chromatography / Mass Spectrometry	Before Renewal	37.97	32.88	
APFO / 99.7%	85 days	Not reported.	None	Ion Chromatography	Mean measured concentrations taken from 21 samples, details of when samples were taken are not provided.	3.13	2.18	Colombo et al. 2008
						6.25	4.48	
						12.5	10.7	
						25	20.9	
						50	40	
PFOA / 19.4% (wet weight aqueous solution)	39 days	Galvanized steel panels lined with food-grade PVC	None	Ion Chromatography	Mean measured concentrations taken from 14 samples, details of when samples were taken are not provided.	0.3	0.27	Oakes et al. 2004
						1	0.65	
						30	23.9	
						100	74.1	
PFOA / 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	300	295.3	Yang et al. 2014
					Before Renewal	300	259.31	
					After Renewal	1113.88	1098.05	
					Before Renewal	1113.88	987.37	
PFOA / 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	300	293.55	Yang et al. 2014
					Before Renewal	300	261.77	
					After Renewal	1613.47	1596.62	
					Before Renewal	1613.47	1468.08	
PFOA / 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After Renewal	200	196.25	Yang et al. 2014
					Before Renewal	200	179.46	
					After Renewal	1518.75	1492.75	
					Before Renewal	1518.75	1344.28	
PFOA / 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After renewal.	200	196.25	Yang et al. 2014
					Before renewal.	200	178.48	
					After renewal.	1518.75	1488.43	
					Before renewal.	1518.75	1364.97	
PFOA / 99%	96 hours	Not reported.	DMSO	High Performance Liquid	After renewal.	200	195.2	Yang et al. 2014
					Before renewal.	200	174.2	
					After renewal.	2097.15	2079.56	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
				Chromatography / Mass Spectrometry	Before renewal.	2097.15	1886.77	
PFOA / 99.8%	96 hours	Glass with Teflon steel components	N,N-dimethylformamide	Liquid Chromatography / Mass-Mass Spectrometry	Concentration values were averaged across all samples	0.05	0.041	Kim et al. 2010
						0.5	0.483	
						5	6.582	
						50	55.565	
PFOA / 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After renewal	100	102.3	Yang et al. 2014
					Before renewal	100	84.1	
					After renewal	537.82	529.82	
					Before renewal	537.82	473.06	
PFOA / 99%	30 days	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After renewal	10	9.91	Yang et al. 2014
					Before renewal	10	8.96	
					After renewal	75.94	77.06	
					Before renewal	75.94	64.55	
PFOA / 99%	96 hours	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After renewal	35	34.9	Yang et al. 2014
					Before renewal	35	30.05	
					After renewal	367	359.83	
					Before renewal	367	328.13	
PFOA / 99%	30 days	Not reported.	DMSO	High Performance Liquid Chromatography / Mass Spectrometry	After renewal	5	4.97	Yang et al. 2014
					Before renewal	5	4.57	
					After renewal	37.97	38.85	
					Before renewal	37.97	34.21	
PFOA / 96%	40 days	Plastic	None	LC-MS/MS	40 days	10	10.55	Hoover et al. 2017
						100	110.4	
						1000	1118	
PFOA / Unknown	259 days	Glass	None	LC-MS/MS	7 days	0.3	0.269	Lee et al. 2017
						3	2.792	
						30	28.215	
PFOA 96%	42 days	HDPE	None	LC-MS/MS	n/a	1	0.84	Bartlett et al. 2021
						3	3.3	
						10	8.9	
						30	29	
						100	97	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOA 96%	21 days	HDPE	None	LC-MS/MS	n/a	0.01	0.01	Bartlett et al. 2021
						0.032	0.046	
						0.1	0.091	
						0.32	0.28	
						1	0.88	
						3.2	2.7	
						10	12	
						32	26	
						100	76	
PFOA 97%	10 days	HDPE	None (assumed)	LC/MS	1 day	2.6	1.66	McCarthy et al. 2021
						2.6	1.4	
						0.026	0.0001	
						26	19.9	
						26	25.1	
						272	184	
						272	265	
PFOA 97%	16 days	HDPE	None (assumed)	LC/MS	0 day	26	19.7	McCarthy et al. 2021
					20 day	26	20	
					0 day	87.5	68.6	
					20 day	87.5	50.1	
					0 day	149	140	
					20 day	149	151	
					0 day	210	180	
					20 day	210	165	
					0 day	272	230	
					20 day	272	224	
PFOA >98%	96 hours	Glass	DMSO	High Performance Liquid	See Note	0.501	0.4513363	Feng et al. 2015
					See Note	5.010	4.9315737	

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
				Chromatography/Mass Spectrometry				
PFOA Not reported	24 hours	Not provided	DMSO	USEPA Method 537	Not reported	0.5	0.685	Pecquet et al. 2020
						5	6.16	
APFO 96.5-100%	13 days	Polypropylene	None	Ion-selective electrode (F-)	0 hr	25	25	3M Company 2000
					192 hr	25	25.5	
					312 hr	25	25.9	
PFOA ≥96%	14 days	Not provided	None	LC-MS-MS	0 hr	10	8.9	Miranda et al. 2020
					24 hr before renewal	10	9.1	
					0 hr	1	0.9	
					24 hr before renewal	1	0.92	
					0 hr	0.1	0.09	
					24 hr before renewal	0.1	0.11	
					0 hr	0.01	0.01	
					24 hr before renewal	0.01	0.01	

n/a = not applicable

[a] Assumed glass based on test outline for the chronic exposure.

[b] Specific details not provided.

Table M-4. Paired Nominal and Measured PFOA Concentrations from Quantitatively and Qualitatively Acceptable Saltwater Toxicity Tests that Reported Measured PFOA Concentrations.

Treatment concentrations where the nominal concentration was 0.0 mg/L (i.e., Controls) were not included.

Chemical / Purity	Test Duration	Test Vessel Material	Solvent Used	Measurement Method	Time Measured	Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Reference
PFOA / 96%	7 days	Polypropylene	None	Liquid Chromatography - tandem mass spectrometry	Every 24 hours	0.0001	0.00008	Liu et al. 2014a
						0.001	0.00095	
						0.01	0.0091	
						0.1	0.093	
						1	0.95	
PFOA / 96%	7 days	Polypropylene	None	Liquid Chromatography - tandem mass spectrometry	Samples were taken every 2 days. Twelve samples per concentration.	0.0001	0.00008	Liu et al. 2014b,c; Liu and Gin 2018
						0.001	0.0012	
						0.01	0.0114	
						0.1	0.099	
						1	1.120	
						10	9.630	
PFOA / Not reported	21 days	Glass	None	LC-MS	See note	0.001	0.00093	Bernardini et al. 2021

Appendix N Occurrence of PFOA in Abiotic Media

N.1 PFOA Occurrence in Surface Waters

Table N-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 et al. 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	
	Big Thompson River	2.90	2.90	2.90	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	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	
	Sand Creek	14.25	14.25	5.50-23.0	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	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. 2006
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	Simcik and Dorweiler 2005
	Lake Calhoun	19.44	19.44	19.44	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	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	
	Mountain Orchard MDWCA	<0.89	<0.89	<0.89	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	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	
	Pawtuxet River	7.546	7.546	7.546	

State	Waterbody[1]	Arithmetic Mean PFOA Concentration (ng/L)	Median PFOA Concentration (ng/L)	Range of PFOA Concentrations (ng/L)	Reference
	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. 2006
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
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.

N.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 N-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).

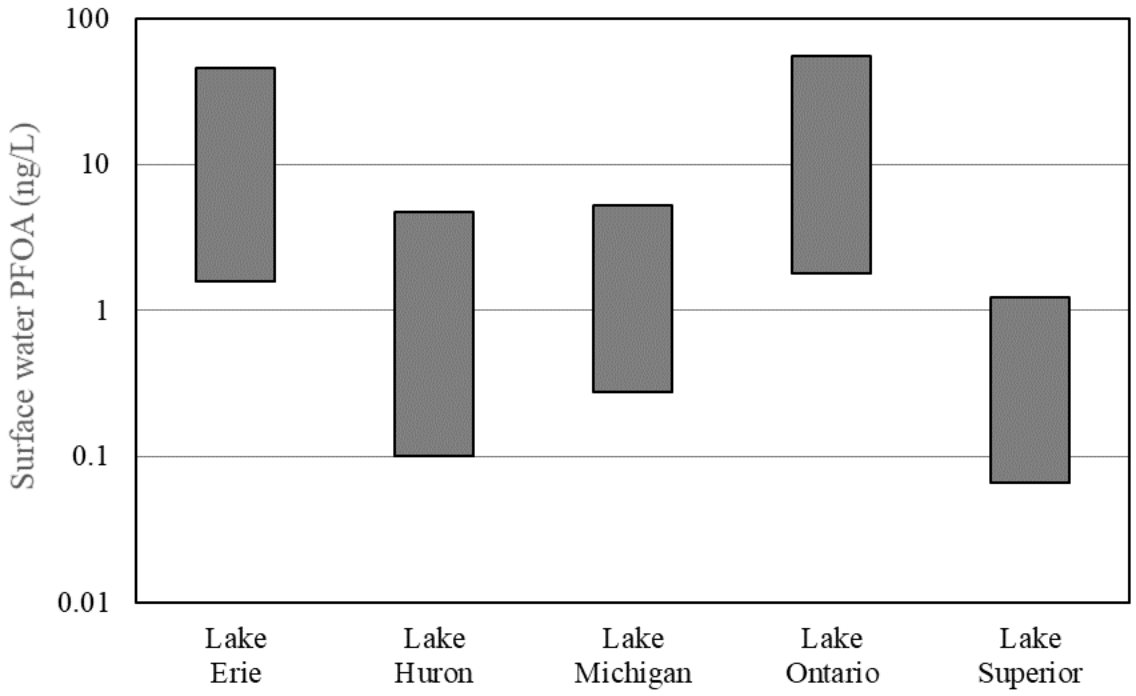


Figure N-1. Distribution of the minimum and maximum concentrations (ng/L) of Perfluorooctanoic acid (PFOA) measured in surface water samples collected from the Great Lakes as reported in the publicly available literature.
This distribution is arranged alphabetically by waterbody.

N.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.

N.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, 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.

N.1.4 PFOA Occurrence and Concentrations in the Southeastern U.S.

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 to the study area (Nakayama et al. 2007).

N.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 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).

N.1.6 Summary of PFOA Occurrence and Concentrations across the U.S.

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 N-2). Additional data, particularly in saltwater systems, are needed to better understand PFOA occurrence in aquatic ecosystems.

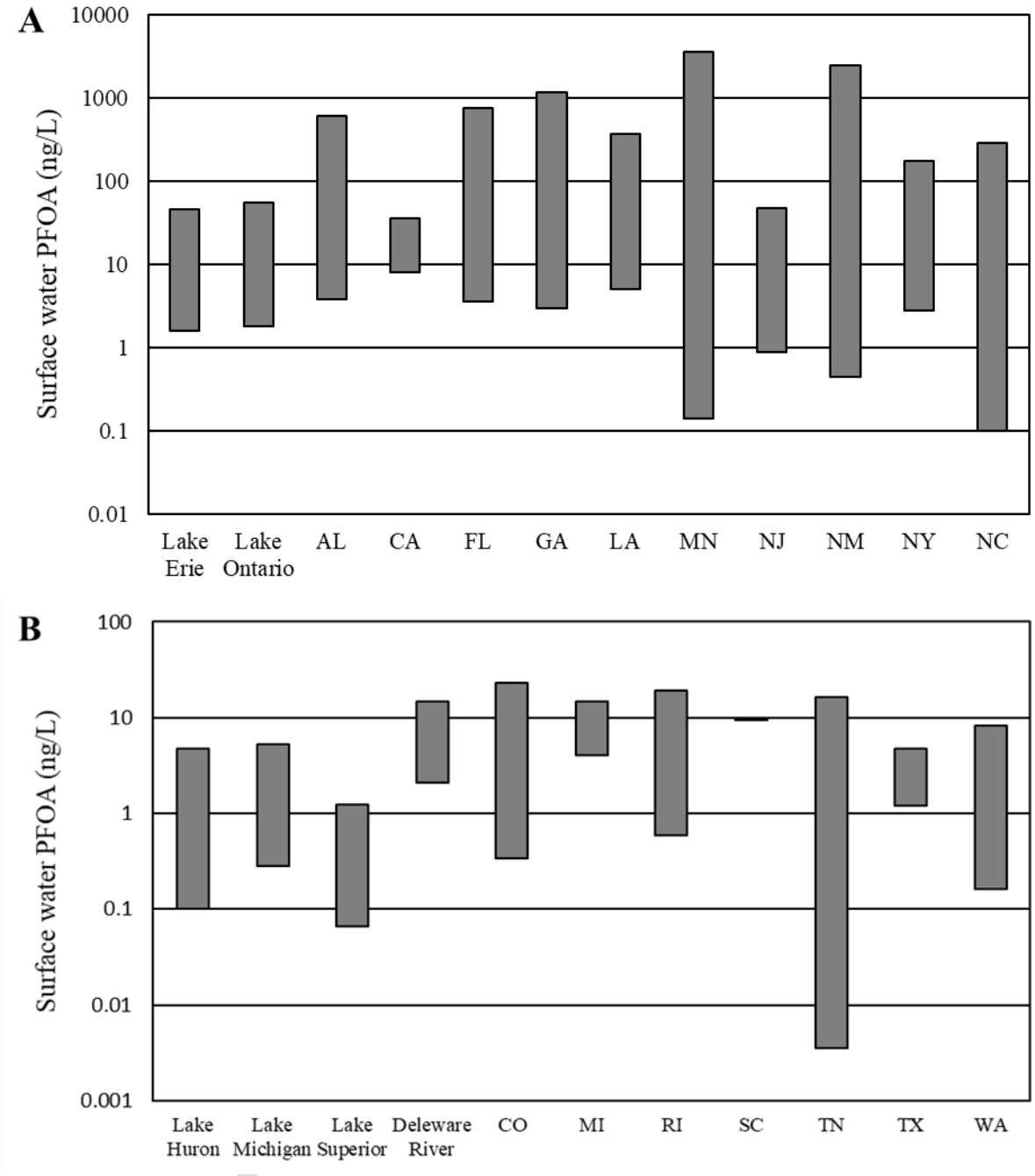


Figure N-2. Comparison of relatively high (A; greater than 30 ng/L) and low (B; less than 30 ng/L) maximum Perfluorooctanoic acid (PFOA) concentrations (ng/L) measured in surface water samples collected across the United States (U.S.) as reported in the publicly available literature.

N.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 (Gewurts 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; Pan et al. 2016).

PFOA concentrations observed in surface waters across eastern Asia were broadly similar to the US, 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; Gewurts et al. 2013; Loos et al. 2007; Loos et al. 2009; Scott et al. 2009).

N.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, CA; 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 (<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); (<0.05-0.2 µg/kg wet weight, Toronto, Ontario; Vedagiri et al. 2018).

N.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.

N.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 with 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).

N.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 O 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., 0.094 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:

$$BAF = \frac{C_{biota}}{C_{water}} \qquad (Eq.\ O\text{-}1)$$

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 O-1.

Table O-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 O-2) and individual BAFs are provided in Appendix P.

Table O-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 O-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 O-2:

$$Tissue\ Value = Chronic\ Water\ Column\ Criterion\ \times 20th\ Centile\ BAF \qquad (Eq.\ O\text{-}2)$$

The resulting tissue values that correspond to the 20th centile tissue-specific BAFs used in Equation *O-2* are reported in Table O-3. The values reported in Table O-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 O-3 are only presented for comparative purposes and are not recommended criteria.

Table O-3. PFOA Concentrations for Additional Fish Tissue Values.[1, 2]

Category	PFOA Concentration (mg/kg ww)
Liver	0.2208
Blood	1.401
Reproductive Tissue	0.2914

[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 P Bioaccumulation Factors (BAFs) Used to Calculate PFOA Tissue Values

P.1 Summary Table of PFOA BAFs used to calculate tissue criteria and supplemental fish tissue values

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
goldfish	*Carassius auratus*	Blood	2.786	611.0	high	17 sites in six major rivers, Korea	Lam et al. 2014
mandarin	*Siniperca scherzeri*	Blood	2.869	739.0	high	17 sites in six major rivers, Korea	Lam et al. 2014
common carp	*Cyprinus carpio*	Blood	1.930	85.11	high	Xiaoqing River, China	Pan et al. 2017
Crucian carp	*Carassius carassius*	Blood	1.267	18.50	high	Tangxun Lake	Shi et al. 2018
Crucian carp	*Carassius carassius*	Blood	1.532	34.06	high	Xiaoqing River	Shi et al. 2018
Crucian carp	*Carassius carassius*	Blood	2.803	635.6	high	Beijing Airport, China	Wang et al. 2016
European perch	*Perca fluviatilis*	Blood	1.146	14.00	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
Crucian carp	*Carassius carassius*	Gonad	0.667	4.641	high	Tangxun Lake	Shi et al. 2018
Crucian carp	*Carassius carassius*	Gonad	0.819	6.594	high	Xiaoqing River	Shi et al. 2018
Crucian carp	*Carassius carassius*	Gonad	1.932	85.41	high	Beijing Airport, China	Wang et al. 2016
European perch	*Perca fluviatilis*	Gonad	0.491	3.100	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
common shiner	*Notropis cornutus*	Liver	1.390	24.55	high	Spring/Etobicoke Creek, Toronto, Canada	Awad et al. 2011
European chub	*Leuciscus cephalus*	Liver	1.000	10.00	high	Orge River, near Paris, France	Labadie and Chevreuil 2011
common carp	*Carassius auratus*	Liver	2.127	134.0	high	17 sites in six major rivers, Korea	Lam et al. 2014
mandarin	*Siniperca scherzeri*	Liver	2.779	601.0	high	17 sites in six major rivers, Korea	Lam et al. 2014
common carp	*Cyprinus carpio*	Liver	1.240	17.38	high	Xiaoqing River, China	Pan et al. 2017
Bream	*Parabramis pekinensis*	Liver	2.900	794.3	high	Pearl River Delta, China	Pan et al. 2014
goldfish	*Carassius auratus*	Liver	3.000	1000	high	Pearl River Delta, China	Pan et al. 2014
Common carp	*Cyprinus carpio*	Liver	3.400	2512	high	Pearl River Delta, China	Pan et al. 2014
Chub	*Hypophthalmichthys molitrix*	Liver	2.600	398.1	high	Pearl River Delta, China	Pan et al. 2014
Tilapia	*Tilapia aurea*	Liver	2.300	199.5	high	Pearl River Delta, China	Pan et al. 2014
Snakehead	*Ophicephalus argus*	Liver	2.300	199.5	high	Pearl River Delta, China	Pan et al. 2014

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Leather catfish	*Clarias fuscus*	Liver	2.200	158.5	high	Pearl River Delta, China	Pan et al. 2014
grass carp	*Ctenopharyngodon idellus*	Liver	3.600	3981	high	Pearl River Delta, China	Pan et al. 2014
Crucian carp	*Carassius carassius*	Liver	0.800	6.316	high	Tangxun Lake	Shi et al. 2018
Crucian carp	*Carassius carassius*	Liver	0.937	8.654	high	Xiaoqing River	Shi et al. 2018
Silver perch	*Bidyanus bidyanus*	Liver	1.146	14.00	high	Shoalhaven region, Australia	Terechovs et al. 2019
Crucian carp	*Carassius carassius*	Liver	3.045	1109	high	Beijing Airport, China	Wang et al. 2016
European perch	*Perca fluviatilis*	Liver	0.851	7.100	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
Mozambique tilapia	*Oreochromis mossambicus*	Liver	-0.051	0.888	medium	uMvoti, Estuary Mouth	Fauconier et al. 2020
Mozambique tilapia	*Oreochromis mossambicus*	Liver	-0.136	0.732	medium	uMvoti, Gledhow	Fauconier et al. 2020
Mozambique tilapia	*Oreochromis mossambicus*	Liver	0.846	7.017	medium	aMatikulu, N2 Bridge	Fauconier et al. 2020
Cape stumpnose	*Rhabdosargus holubi*	Liver	0.434	2.714	medium	aMatikulu, N2 Bridge	Fauconier et al. 2020
tilapia	tilapia	Liver	1.826	67.00	medium	Key River, Taiwan	Lin et al. 2014
tilapia	tilapia	Liver	1.724	53.00	medium	Key River, Taiwan	Lin et al. 2014
tilapia	tilapia	Liver	1.740	55.00	medium	Key River, Taiwan	Lin et al. 2014
Mud carp	*Cirrhinus molitorella*	Liver	3.900	7943	medium	Pearl River Delta, China	Pan et al. 2014
European perch	*Perca fluviatilis*	Muscle	1.568	37.00	high	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
minnow	*Hemiculter leucisculus*	Muscle	2.130	135.0	high	Taihu Lake, China	Fang et al. 2014
silver carp	*Hypophthalmichthys molitrix*	Muscle	1.153	14.21	high	Taihu Lake, China	Fang et al. 2014
white bait	*Reganisalanx brachyrostralis*	Muscle	2.245	175.6	high	Taihu Lake, China	Fang et al. 2014
Japanese crucian carp	*Carassius cuvieri*	Muscle	1.988	97.24	high	Taihu Lake, China	Fang et al. 2014
Lake Saury	*Coilia mystus*	Muscle	2.496	313.0	high	Taihu Lake, China	Fang et al. 2014
common carp	*Cyprinus carpio*	Muscle	2.328	213.0	high	Taihu Lake, China	Fang et al. 2014
Mongolian culter	*Culter mongolicus*	Muscle	2.252	178.7	high	Taihu Lake, China	Fang et al. 2014

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
mudfish	*Misgurnus anguillicaudatus*	Muscle	2.292	196.1	high	Taihu Lake, China	Fang et al. 2014
Chinese bitterling	*Rhodeus sinensis Gunther*	Muscle	2.023	105.5	high	Taihu Lake, China	Fang et al. 2014
gobies	*Ctenogobius giurinus*	Muscle	1.656	45.28	high	Taihu Lake, China	Fang et al. 2014
eel	*Anguilla anguilla*	Muscle	1.120	13.18	high	Netherlands	Kwadijk et al. 2010
Crucian carp	*Carassius cuvieri*	Muscle	0.159	1.442	high	Asan Lake, South Korea	Lee et al. 2020
Adult char	*Salvelinus alpinus*	Muscle	0.770	5.882	high	Meretta Lake, Canadian High Arctic	Lescord et al. 2015
Adult char	*Salvelinus alpinus*	Muscle	1.571	37.23	high	Resolute Lake, Canadian High Arctic	Lescord et al. 2015
Anchovy	*Engraulis encrasicolus*	Muscle	1.929	85.00	high	Gironde estuary, SW France	Munoz et al. 2017
Common seabass	*Dicentrarchus labrax*	Muscle	1.995	98.89	high	Gironde estuary, SW France	Munoz et al. 2017
Spotted seabass	*Dicentrarchus punctatus*	Muscle	2.280	190.6	high	Gironde estuary, SW France	Munoz et al. 2017
common carp	*Cyprinus carpio*	Muscle	0.460	2.884	high	Xiaoqing River, China	Pan et al. 2017
Crucian carp	*Carassius carassius*	Muscle	0.192	1.557	high	Tangxun Lake	Shi et al. 2018
Crucian carp	*Carassius carassius*	Muscle	0.344	2.207	high	Xiaoqing River	Shi et al. 2018
Silver perch	*Bidyanus bidyanus*	Muscle	0.954	9.000	high	Shoalhaven region, Australia	Terechovs et al. 2019
goby	*Gobio gobio*	Muscle	2.817	655.6	medium	Roter Main, Upper Franconia, Germany	Becker et al. 2010
Black Crappie	*Pomoxis nigromaculatus*	Muscle	1.400	25.12	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Brown Bullhead	*Ameiurus nebulosus*	Muscle	1.000	10.00	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Channel Catfish	*Ictalurus punctatus*	Muscle	1.000	10.00	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Common Carp	*Cyprinus carpio*	Muscle	1.100	12.59	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Largemouth Bass	*Micropterus salmoides*	Muscle	0.900	7.943	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Northern Pike	*Esox lucius*	Muscle	0.900	7.943	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Pumpkinseed	*Lepomis gibbosus*	Muscle	1.000	10.00	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Smallmouth Bass	*Micropterus dolomieu*	Muscle	0.900	7.943	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
White Crappie	*Pomoxis annularis*	Muscle	1.100	12.59	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Yellow Perch	*Perca flavescens*	Muscle	0.900	7.943	medium	Lake Niapenco, Ontario, Canada.	Bhavsar et al. 2016
Mozambique tilapia	*Oreochromis mossambicus*	Muscle	-0.535	0.292	medium	uMvoti, Estuary Mouth	Fauconier et al. 2020
Mozambique tilapia	*Oreochromis mossambicus*	Muscle	-0.420	0.380	medium	uMvoti, Gledhow	Fauconier et al. 2020
Slender glassy	*Ambassis natalensis*	Muscle	0.257	1.809	medium	aMatikulu, Estuary Mouth	Fauconier et al. 2020
Mozambique tilapia	*Oreochromis mossambicus*	Muscle	0.157	1.434	medium	aMatikulu, N2 Bridge	Fauconier et al. 2020
Cape stumpnose	*Rhabdosargus holubi*	Muscle	0.066	1.163	medium	aMatikulu, N2 Bridge	Fauconier et al. 2020
tilapia	tilapia	Muscle	1.708	51.00	medium	Key River, Taiwan	Lin et al. 2014
tilapia	tilapia	Muscle	1.653	45.00	medium	Key River, Taiwan	Lin et al. 2014
tilapia	tilapia	Muscle	1.681	48.00	medium	Key River, Taiwan	Lin et al. 2014
common shiner	*Notropis cornutus*	WB[a]	0.880	7.586	high	Spring/Etobicoke Creek, Toronto, Canada	Awad et al. 2011
medaka	*Oryzias latipes*	WB	2.519	330.0	high	Seven locations across Japan	Iwabuchi et al. 2015
Juvenile char	*Salvelinus alpinus*	WB	1.887	77.06	high	Meretta Lake, Canadian High Arctic	Lescord et al. 2015
Juvenile char	*Salvelinus alpinus*	WB	3.731	5387	high	Resolute Lake, Canadian High Arctic	Lescord et al. 2015
Juvenile char	*Salvelinus alpinus*	WB	2.638	434.8	high	9-Mile Lake, Canadian High Arctic	Lescord et al. 2015
Spotted seabass	*Dicentrarchus punctatus*	WB	2.535	343.0	high	Gironde estuary, SW France	Munoz et al. 2017
Crucian carp	*Carassius carassius*	WB	0.570	3.713	high	Xiaoqing River	Shi et al. 2018
Crucian carp	*Carassius carassius*	WB	0.443	2.775	high	Tangxun Lake	Shi et al. 2018
lake trout	*Salvelinus namaycush*	WB	1.740	55.00	high	Lake Superior	De Silva et al. 2011

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
lake trout	*Salvelinus namaycush*	WB	1.491	31.00	high	Lake Erie	De Silva et al. 2011
walleye	*Sander vitreus*	WB	2.425	266.0	high	Lake Erie	De Silva et al. 2011
lake trout	*Salvelinus namaycush*	WB	2.604	402.0	high	Lake Ontario	De Silva et al. 2011
Crucian carp	*Carassius carassius*	WB	2.807	641.9	high	Beijing Airport, China	Wang et al. 2016
Common carp	*Cyprinus carpio*	WB	2.260	182.0	high	Baiyangdian Lake, China	Zhou et al. 2012
European perch	*Perca fluviatilis*	WB	0.000	1.000	medium	Lake Halmsjön, near Stockholm, Sweden	Ahrens et al. 2015
Grass carp	*Ctenopharyngodon idellus*	WB	3.912	8,160	medium	Bantou Reservoir, Xiamen Sea, China	Dai and Zeng 2019
Chameleon goby	*Tridentiger trigonocephalus*	WB	4.211	16,273	medium	Gulf Park, Xiamen Sea, China	Dai and Zeng 2019
Lake Trout	*Salvelinus namaycush*	WB	3.300	1995	medium	Lake Superior	Furdui et al. 2007
Lake Trout	*Salvelinus namaycush*	WB	3.600	3981	medium	Lake Huron	Furdui et al. 2007
Lake Trout	*Salvelinus namaycush*	WB	2.900	794.3	medium	Lake Erie	Furdui et al. 2007
Lake Trout	*Salvelinus namaycush*	WB	2.600	398.1	medium	Lake Ontario	Furdui et al. 2007
Lake Trout	*Salvelinus namaycush*	WB	3.400	2512	medium	Lake Michigan	Furdui et al. 2007
herring	*Clupea harengus membras*	WB	2.340	218.8	medium	Baltic Sea	Gebbink et al. 2016
spat	*Sprattus*	WB	2.520	331.1	medium	Baltic Sea	Gebbink et al. 2016
Sea Bass	*Lateolabrax*	WB	2.469	294.1	medium	Omuta River mouth and estuary, Japan	Kobayashi et al 2018
Grey mullet	*Mugil cephalus*	WB	2.582	382.4	medium	Omuta River mouth and estuary, Japan	Kobayashi et al 2018
Yellowfin goby	*Acanthogobius flavimanus*	WB	3.388	2441	medium	Omuta River mouth and estuary, Japan	Kobayashi et al 2018
Chinese icefish	*Neosalanx tangkahkeii taihuensis*	WB	1.792	61.90	medium	Lake Chaohu, China	Pan et al. 2019
Common carp	*Cyprinus carpio*	WB	2.200	158.5	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Mullet	*Liza*	WB	2.000	100.0	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Roach	*Rutilus rutilus*	WB	2.100	125.9	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Rudd	*Scardinius erythrophtalmus*	WB	1.900	79.43	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
European catfish	*Silurus glanis*	WB	2.100	125.9	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Ebro chub	*Squalius laietanus*	WB	2.000	100.0	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
Bleak	*Alburnus alburnus*	WB	2.300	199.5	medium	Xerta, Ebro Delta, Spain	Pignotti et al. 2017
grass goby	*Zosterisessor ophiocephalus*	WB	2.125	133.4	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. 2013
grass goby	*Zosterisessor ophiocephalus*	WB	1.987	97.07	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. 2013
grass goby	*Zosterisessor ophiocephalus*	WB	2.123	132.7	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. 2013
zooplankton	zooplankton	Invert[b]	1.747	55.91	high	Taihu Lake, China	Fang et al. 2014
zooplankton	zooplankton	Invert	1.940	87.10	high	Taihu Lake, China	Xu et al. 2014
zooplankton	zooplankton	Invert	2.300	199.5	medium	Baltic Sea	Gebbink et al. 2016
zooplankton	zooplankton	Invert	1.068	11.70	medium	Mai Po Marshes, Hong Kong	Loi et al. 2011
amphipod	*Gammarus, Hyalella*	Invert	3.413	2591	high	Welland River, Hamilton, Ontario, Canada	De Solla et al. 2012
freshwater mussel	Unionidae	Invert	1.177	15.04	high	Taihu Lake, China	Fang et al. 2014
pearl mussel	Unionidae	Invert	1.678	47.64	high	Taihu Lake, China	Fang et al. 2014
Copepods	Copepoda	Invert	0.398	2.500	high	Gironde estuary, SW France	Munoz et al. 2019
mysids	Mysidacea	Invert	0.398	2.500	high	Gironde estuary, SW France	Munoz et al. 2019
white shrimp	*Palaemon longirostris*	Invert	0.362	2.300	high	Gironde estuary, SW France	Munoz et al. 2019
brown shrimp	*Crangon crangon*	Invert	0.398	2.500	high	Gironde estuary, SW France	Munoz et al. 2019
Oyster	*Crassostrea gigas*	Invert	1.301	20.00	high	Gironde estuary, SW France	Munoz et al. 2017
snails	*Bithynia tentaculata*	Invert	1.672	47.01	high	Hogsmill, Chertsey Bourne, Blackwater Rivers	Wilkinson et al. 2018
amphipod	*Gammarus pulex*	Invert	1.048	11.16	high	Hogsmill, Chertsey Bourne, Blackwater Rivers	Wilkinson et al. 2018

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Pacific Oyster	*Crassostrea gigas*	Invert	3.986	9680.00	medium	Gulf Park, Xiamen Sea, China	Dai and Zeng 2019
Pacific Oyster	*Crassostrea gigas*	Invert	3.807	6410.00	medium	Jimei Bridge, Xiamen Sea, China	Dai and Zeng 2019
Ghost Crab	*Ocypode stimpsoni*	Invert	3.872	7440.00	medium	Fenglin, Xiamen Sea, China	Dai and Zeng 2019
Ghost Crab	*Ocypode stimpsoni*	Invert	3.812	6490.00	medium	Jimei Bridge, Xiamen Sea, China	Dai and Zeng 2019
Orange-striped hermit crab	*Clibanarius infraspinatus*	Invert	3.797	6263.26	medium	Jimei Bridge, Xiamen Sea, China	Dai and Zeng 2019
Shrimp	*Caridea*	Invert	-0.007	0.98	medium	uMvoti, Gledhow	Fauconier et al. 2020
Snail	*Gastropoda*	Invert	0.584	3.84	medium	uMvoti, Gledhow	Fauconier et al. 2020
Snail	*Gastropoda*	Invert	1.025	10.58	medium	aMatikulu, N2 Bridge	Fauconier et al. 2020
Snail	*Cerithidea rhizophorarum*	Invert	2.428	267.6	medium	Omuta River mouth and estuary, Japan	Kobayashi et al 2018
waterlouse, water boatmen, amphipods, roundworm	Isopoda, Hemiptera, amphipoda, nematoda	Invert	2.233	171.0	medium	site A Stockholm Arlanda Airport	Koch et al. 2019
Fresh water amphipods	Amphipoda	Invert	2.716	520.0	medium	site R Ronneby Airport	Koch et al. 2019
Mayflies, Caddisflies, Dragonflies, Water boatmen, Waterlouse, Fresh water amphipods	Ephemeroptera, Trichoptera, Odonata, Hemiptera, Isopoda, Amphipoda	Invert	1.699	50.0	medium	site K the Kvarntorp area	Koch et al. 2019
worms	Sabellidae	Invert	1.646	44.21	medium	Mai Po Marshes, Hong Kong	Loi et al. 2011
Copepods	Copepoda	Invert	2.554	358.0	medium	Gironde estuary, SW France	Munoz et al. 2017
Mysids	Mysidacea	Invert	2.033	108.0	medium	Gironde estuary, SW France	Munoz et al. 2017
Gammarids	*Gammarus*	Invert	3.097	1250	medium	Gironde estuary, SW France	Munoz et al. 2017
White shrimp	*Palaemon longirostris*	Invert	2.576	377.0	medium	Gironde estuary, SW France	Munoz et al. 2017

Common Name	Scientific Name	Tissue	Log BAF (L/kg-ww)	BAF (L/kg-ww)	Ranking	Location	Reference
Brown shrimp	*Crangon crangon*	Invert	2.661	458.0	medium	Gironde estuary, SW France	Munoz et al. 2017
bivalve	*Mytilus galloprovincialis*	Invert	1.074	11.86	medium	PC Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Mytilus galloprovincialis*	Invert	2.108	128.3	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Mytilus galloprovincialis*	Invert	2.516	327.9	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Mytilus galloprovincialis*	Invert	2.383	241.8	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	2.335	216.4	medium	PC Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	2.145	139.8	medium	LC Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	2.008	102.0	medium	M Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	1.928	84.78	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	1.794	62.24	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. 2013
bivalve	*Ruditapes decussatus*	Invert	1.586	38.56	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	2.323	210.2	medium	PC Site, Orbetell lagoon, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	2.370	234.2	medium	LC Site, Orbetell lagoon, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	2.024	105.6	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	1.760	57.55	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. 2013
crab	*Carcinus aestuarii*	Invert	1.675	47.34	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	2.270	186.4	medium	PC Site, Orbetell lagoon, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	2.313	205.6	medium	LC Site, Orbetell lagoon, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	2.056	113.8	medium	AC Site, Orbetell lagoon, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	1.817	65.62	medium	NC Site, Orbetell lagoon, Italy	Renzi et al. 2013
prawn	*Palaemon serratus*	Invert	1.723	52.81	medium	FC Site, Orbetell lagoon, Italy	Renzi et al. 2013

a – Fish whole body
b – Invertebrate whole body

P.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 P.1. Summary statistics for the BAFs from this table used to derive tissue criteria and additional tissue values (i.e., lowest species-level BAF from each site) are reported in Table 3-10 and Table O-2, respectively. Rankings for individual BAFs were determined by Lawrence (2021), who devised a ranking system based on five characteristics: 1) number of water samples; 2) number of tissue samples; 3) spatial coordination of water and tissue samples; 4) temporal coordination of water and tissue samples; and 5) general experimental design. For the first four characteristics, a score of one to three was assigned, based on number of samples or how closely the water and tissue measurements were paired. For the experimental design characteristic, a default value of zero was assigned; unless the measured tissues were composites of mixed species, in which case it was assigned a three (Lawrence 2021). These sub-scores were then summed and assigned a rank based on the final score. Studies with high quality rankings had scores of four or five, studies with medium quality rankings had scores of five or six, and studies with low quality rankings had scores of seven or higher (Lawrence 2021). Parameters for the scores assigned to the five characteristics are listed in Table 2-2, and additional details can be found in Burkhard (2021). Only BAFs from studies with high or medium quality rankings were included for the final BAF geometric mean calculations used to derive tissue criteria (Table 3-11) and supplemental tissue values (Table O-3).

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Appendix Q 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 EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S. EPA 2016c), and EPA OW's internal data quality SOP, which is consistent with OCSPP's data quality review approach (U.S. EPA 2018). These toxicity data were further screened to ensure that the observed effects could be primarily attributed to PFOS exposure. Office of Water completed a DER for each species by chemical combination from the PFOS studies identified by ECOTOX. Example DERs are presented here to convey the meticulous level of evaluation, review, and documentation each PFOS study identified by ECOTOX was subject to. Appendix Q.1 shows an example fish DER and Appendix Q.2 shows an example aquatic invertebrate DER.

Q.1 Example Fish DER

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

____ **Controlled Experiment** (*manipulated*) ____ **Field Study/Observation** (*not manipulated*) (*Place X by One*)

Primary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)

Secondary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)
(*At least one reviewer should be from EPA for sensitive taxa*)

Citation: *Indicate: author(s), year, study title, journal, volume, and pages*.
(e.g., Slonim, A.R. 1973. Acute toxicity of beryllium sulfate to the common guppy. J. Wat. Pollut. Contr. Fed. 45(10): 2110-2122)

Companion Papers: *Identify any companion papers associated with this paper using the citation format above.*

-

Were other DERs completed for Companion Papers? ____ **Yes** ____ **No** (*If yes, list file names of DERs below*)

Study Classification for Aquatic Life Criteria Development: *Place X by One Based on Highest Use*

_____ Acceptable for Quantitative Use
_____ Acceptable for Qualitative Use
_____ Not Acceptable for Use/Unused

General Notes: *Provide any necessary details regarding the study's use classification for all pertinent endpoints, including non-apical endpoints within the study (e.g., note all study classifications for each endpoint if the use varies)*

Major Deficiencies (note any stated exclusions): *Check all that apply. Checking any of these items make the study "**Not Acceptable for Use**"*

_____ Mixture (for controlled experiments only)
_____ No Controls (for controlled experiments only)
_____ Excessive Control Mortality (> 10% for acute and > 20% for chronic)
_____ Dilution water not adequately characterized
_____ Bioaccumulation: steady state not reached
_____ Dermal or Injection Exposure Pathway
_____ Review paper or previously published without modification

_____ Other: *(if any, list here*)

POTENTIAL CHEMICAL MIXTURES: *Describe any potential chemicals mixtures as characterized by study authors (including any confirmation of chemical mixtures).*

DESCRIPTION OF DILUTION WATER: *Describe concerns with characterization of and/or major deficiencies with dilution water.*

General Notes:

Minor Deficiencies: *List and describe any minor deficiencies or other concerns with test. These items may make the study "**Acceptable for Qualitative Use**"* **(exceptions may apply as noted)**

***For Field Studies/Observations**: A field study/observation may be considered "**Acceptable for Quantitative Use**" if it consisted of a range of exposure concentrations and the observed effects are justifiably contributed to a single chemical exposure*

_____ Mixture (observed effects not justifiably contributed to single chemical exposure)
_____ Uncharacterized Reference Sites/Conditions

POTENTIAL CHEMICAL MIXTURES PRESENT AT SITE: *Describe any potential chemicals mixtures present at the site as characterized by study authors (including any confirmation of chemicals present at study site).*

EXPOSURE VARIABILITY ACROSS STUDY SITE(S): *Describe any exposure variability across study site(s) as characterized by study authors (i.e., description of study design with reference and contaminated sites).*

General Notes:

Reviewer's Comments: *Provide additional comments that do not appear under other sections of the DER.*

ABSTRACT: *Copy and paste abstract from publication*.

SUMMARY*: Fill out and modify as needed.*

Acute:

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration (mg/L)	Classification
											Quantitative / Qualitative / Unused

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

Chronic:

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L or µg/g)	Verified Chronic Value (mg/L or µg/g)	Chronic Value Endpoint	Classification
												Quantitative / Qualitative / Unused

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

II. Results *Provide results as reported in the publication (including supplemental materials). Include screen shots of tables and/or figures reporting results from the article <u>following</u> tabulated data table in each associated results section for <u>all studies</u>. Complete tabulated data tables for all studies for studies marked "**Acceptable for Quantitative Use"** and **"Acceptable for Qualitative Use**".*

Water Quality Parameters: *If only general summary data of water quality parameters is provided by study authors (i.e., no specific details of water quality parameters on a treatment level is provided), summarize any information regarding water quality parameters under General Notes below and indicate data not provided in* Table A.II.1.

General Notes: *For aquatic life criteria development, measured water quality parameters in the treatments nearest the toxicity test endpoint(s), e.g., LC_{50}, EC_{20}, etc., are most relevant.*

-

Table A.II.1. Measured Water Quality Parameters in Test Solutions.

Dissolved oxygen, temperature, pH and [other parameters (hardness, salinity, DOC)] in test solutions during the *[X]*-day exposure of *[test organism]* to *[concentration of treatment(s)]* of *[test substance]* under *[static renewal/flow-through]* conditions.

Parameter	Treatment	Mean	Range
Dissolved Oxygen (% saturation or mg/L)	*[1]*		
	[2]		
	j		
	j		
Temperature (°C)	*[1]*		
	[2]		
	j		
	j		
pH	*[1]*		
	[2]		
	j		
	j		
Other (e.g., hardness, salinity, DOC)	*[1]*		
	[2]		
	j		
	j		

Chemical Concentrations: *Summarize the concentration verification data from test solutions/media. Expand table to include measured concentration data for each media type (i.e., water, diet, muscle, liver, blood, etc.).*

General Notes: *Provide any necessary detail regarding the measured concentrations, including any identified cause for substantial differences between nominal and measured concentrations, if samples were collected on separate days (and if so provide details), and any potential cross contamination.*

Table A.II.2. Measured (and Nominal) Chemical Concentrations in Test Solutions/Media.
[Analytical Method] verification of test and control concentrations during an [X]-day exposure of [test organism] to [test substance] under [static renewal/flow-through] conditions.

Treatment	Nominal Concentration (units)	[Mean] Measured Concentration (units)	Number of Samples	Non-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
j							

[a]Non-Detect: 0 = measured and detected; 1= measured and not detected; if not measured or reported enter as such

Mortality: *Briefly summarize mortality results (if any).*

General Notes: *Comment on concentrations response relationship and slope of response if provided. Compare mortality in treatments with control group and/or the reference chemical.*

Table A.II.3. Mean Percent [Mortality or Survival].

Mean percent mortality [or number of immobilized, survival] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment	[Mean % Mortality]	[Standard Deviation or Standard Error]
Control		
[1]		
[2]		
[3]		
[4]		
[5]		
[6]		
[LCx]		
NOEC		
LOEC		

[a] Use superscript to identify the values reported to be significantly different from control.

Growth: *Briefly summarize growth results (if any).*

General Notes: *Comment on concentrations response relationship and slope of response if provided. Compare growth endpoints in treatments with control group and/or the reference chemical.*

Table A.II.4. Mean [Growth].

Mean growth [length and/or weight] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment	Mean Growth [Length/Weight] (units)	[Standard Deviation or Standard Error]	Mean Percent Change in [Length/ Biomass]	[Standard Deviation or Standard Error]
Control				
[1]				
[2]				
[3]				
[4]				
[5]				
[6]				
j				
[ECx]				
NOEC				
LOEC				

[a] Use superscript to identify the values reported to be significantly different from control.

Reproductive: *Briefly summarize reproduction endpoint results (if any). For multi-generational studies, copy and paste* Table A.II.5 *below for each generation with reproductive effects data.*

General Notes: *Comment on concentrations response relationship and slope of response if provided. Compare reproductive endpoints in treatments with control group and/or the reference chemical.*

Table A.II.5. Mean [Reproductive] Effect.

Mean [reproductive] effects for [generation] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment (units)	[Mean Number of Spawns]	[Standard Deviation or Standard Error]	[Mean Number of Eggs]	[Standard Deviation or Standard Error]	[Mean Percent Hatch]	[Standard Deviation or Standard Error]	[Mean Hatch Percent Survival Post]	[Standard Deviation or Standard Error]
Control								
[1]								
[2]								
[3]								
[4]								
[5]								
[6]								
j								
[ECx]								
NOEC								
LOEC								

[a] Use superscript to identify the values reported to be significantly different from control.

Sublethal Toxicity Endpoints: *Include other sublethal effect(s), including behavioral abnormalities or other signs of toxicity, if any. Copy* Table A.II.6 *as needed to provide details for each sublethal effect observed.*

General Notes: *Briefly summarize observed sublethal effects otherwise not captured in the results table(s) below.*

Table A.II.6. Mean [Sublethal] Effect.

*Mean [*Sublethal effect*, (e.g., behavioral abnormalities, etc.)]* in *[test organism]* during [test duration (*acute/chronic*)] exposure to *[test substance]* under *[static/renewal/flow-through]* conditions.

Treatment	[Mean Sublethal Response] (units)	[Standard Deviation or Standard Error]
Control		
[1]		
[2]		
[3]		
[4]		
[5]		
[6]		
j		
[ECx]		
NOEC		
LOEC		

[a] Use superscript to identify the values reported to be significantly different from control

Reported Statistics: *Copy and paste statistical section from publication*

Part B: Detailed Review
I. Materials and Methods

Protocol/Guidance Followed: *Indicate if provided by authors.*

Deviations from Protocol: *If authors report any deviations from the protocol noted above indicate here.*

Study Design and Methods: *Copy and paste methods section from publication.*

TEST ORGANISM: *Provide information under Details and any relevant or related information or clarifications in Remarks.*

Parameter	Details	Remarks
Species:	Common Name: Scientific Name:	North American species? ____ Surrogate for North American Taxon? ____ *(Place X if applicable)*

Strain/Source:
• Wild caught from unpolluted areas [1]
  o Quarantine for at least 14 days or until they are disease free, before acclimation [1]
• Must originate from same source and population [1]
• Should not be used:
  o If appeared stressed, such as discoloration or unusual behavior [1]
  o If more than 5% die during the 48 hours before test initiation [1]
  o If they were used in previous test treatments or controls [2]
• No treatments of diseases may be administered:
  o Within 16 hour of field collection [1]
  o Within 10 days or testing or during testing [1]		

Age at Study Initiation:
Acute:
• Juvenile stages preferred [1]
Chronic:
• Life-cycle test:
  o Embryos or newly hatched young < 48 hours old [2]
• Partial life-cycle test:
  o Immature juveniles at least 2 months prior to active gonad development [2]
• Early life-stage test:
  o Shortly after fertilization [2]		

Was body weight or length recorded at test initiation?	____ Yes ____ No	
Was body weight or length recorded at regular intervals?	____ Yes ____ No *If yes, describe regular intervals:*	

STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for <u>both Controlled Experiments and Field Studies/Observations.</u>*

	Parameter	Details	Remarks
For Both Controlled Experiments and Field Observations	**Number of Replicates per Treatment Group:** • At least 2 replicates/treatment recommended for acute tests [1] • At least 2 replicates/treatment recommended for chronic tests [3]	Control(s): Treatment(s):	
	Number of Organisms per Replicate/ Treatment Group: • At least 10 organisms/treatment recommended [3] • At least 7 organisms/treatment acceptable [4]	Control(s): Treatment(s):	
	Exposure Pathway: *(i.e., water, sediment, gavage, or diet).* *Note: all other pathways (e.g., dermal, single dose via gavage, and injection) are unacceptable.*		
	Exposure Duration: **Acute** • Should be 96 hours [2] **Chronic** • Life-cycle tests: o Ensure that all life stages and life processes are exposed [2] o Begin with embryos (or newly hatched young), continue through maturation and reproduction, and should end not less than 24 days (90 days for salmonids) after the hatching of the next generation [2] • Partial life-cycle tests: o Allowed with species that require >1 year to reach sexual maturity, so that all major life stages can be exposed to the test material in <15 months [2] o Begin with immature juveniles at least 2 months prior to active gonad development, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation [2] • Early life-cycle tests: o 28 to 32 day (60 day post hatch for salmonids) exposures from shortly after fertilization through embryonic, larval, and early juvenile development [2]	____ Acute ____ Partial Life Cycle ____ Early Life Stage ____ Full Life Cycle ____ Other *(please remark):*	
	Test Concentrations (remember units): *Recommended test concentrations include at least three concentrations other than the control; four or more will provide a better statistical analysis* [3]	Nominal: Measured: Media measured in:	
	Observation Intervals: • Should be an appropriate number of observations over the study to ensure water quality is being properly maintained [4]		

CONTROLLED EXPERIMENT STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for Controlled Experiments only.*

For Controlled Experiments Only

Parameter	Details	Remarks
Acclimation/Holding: • Should be placed in a tank along with the water in which they were transported o Water should be changed gradually to 100% dilution water (usually 2 or more days) [1] o For wild-caught animals, test water temperature should be within 5°C of collection water temperature [1] o Temperature change rate should not exceed 3°C within 72 hours [1] • To avoid unnecessary stress and promote good health: o Organisms should not be crowded [1] o Water temperature variation should be limited [1] o Dissolved oxygen: ▪ Maintain between 60 - 100% saturation [1] ▪ Continuous gentle aeration if needed [1] o Unionized ammonia concentration in holding and acclimation waters should be < 35 µg/L [1]	Duration: Feeding: Water type: Temperature (°C): Dissolved Oxygen (mg/L): Health (*any mortality observed?*):	*Identify number of individuals excluded from testing and/or analysis (if any):*
Acclimation followed published guidance? *Describe, if any*	____ Yes ____ No *If yes, indicate which guidance:*	
Test Vessel: • Test chambers should be loosely covered [1] • Test chamber material: o Should minimize sorption of test chemical from water [1] o Should not contain substances that can be leached or dissolved in solution and are free of substances that could react with exposure chemical [1] o Glass, No. 316 stainless steel, nylon screen and perfluorocarbon (e.g. Teflon) are acceptable [1] o Rubber, copper, brass, galvanized metal, epoxy glues, lead and flexible tubing should not come into contact with test solution, dil. water, or stock [1] • Size/volume should maintain acceptable biomass loading rates (see Biomass Loading Rate below) [1]	Material: Size: Fill Volume:	*Briefly describe the test vessel:*
Test Solution Delivery System/Method: • Flow-through preferred for some highly volatile, hydrolysable or degradable materials [2] o Concentrations should be measured often enough using acceptable analytical methods [2] • Chronic exposures: o Flow-through, measured tests required [2]	Test Concentrations Measured ____ Yes ____ No Test Solution Delivery System: ____ Static ____ Renewal *Indicate Interval:* ____ Flow-through *Indicate Type of Diluter:*	
Source of Dilution Water: • Freshwater hardness range should be < 5 mg/L or < 10% of the average (whichever is greater) [1] • Saltwater salinity range should be < 2 g/kg or < 20% of the average (whichever is greater) [1] • Dilution water must be characterized (natural surface water, well water, etc.) [3] o Distilled/deionized water without the addition of appropriate salts should not be used [2] • Dilution water in which total organic carbon or particulate matter >5 mg/L should not be used [2] o Unless data show that organic carbon or particulate matter do not affect toxicity [2]		
Dilution Series (*e.g., 0.5x, 0.6x, etc.*):		

	Parameter	Details	Remarks
For Controlled Experiments Only	**Dilution Water Parameters:** *Measured at the beginning of the experiment or averaged over the duration of the experiment (details of water quality parameters measured in test solutions should be included under the results section)*	Dissolved Oxygen (mg/L): pH: Temperature (°C): Hardness (mg/L as $CaCO_3$): Salinity (ppt): Total Organic Carbon (mg/L): Dissolved Organic Carbon (mg/L):	
	Aeration: • Acceptable to maintain dissolved oxygen at 60 - 100% saturation at all times [1] • Avoid aeration when testing highly oxidizable, reducible and volatile materials [1] • Turbulence should be minimized to prevent stress on test organisms and/or re-suspend fecal matter [1] • Aeration should be the same in all test chambers at all times [1]	____ Yes ____ No	
	Describe Preparation of Test Concentrations (e.g., water exposure, diet):		
	Test Chemical Solubility in Water: *List units and conditions (e.g., 0.01% at 20ºC)*		
	Were concentrations in water or diet verified by chemical analysis? *Measured test concentrations should be reported in* Table A.II.2 *above.*	__Yes __No *Indicate media:*	
	Were test concentrations verified by chemical analysis in tissue? *Measured test concentrations can be verified in test organism tissue (e.g., blood, liver, muscle) alone if a dose-response relationship is observed.* *Measured test concentrations should be reported in* Table A.II.2 *above.*	__Yes __No *Indicate tissue type:*	*If test concentrations were verified in test organism tissue, was a dose-response relationship observed?*
	Were stability and homogeneity of test material in water/diet determined?	__Yes __No	
	Was test material regurgitated/avoided?	__Yes __No	
	Solvent/Vehicle Type (Water or Dietary): • When used, a carrier solvent should be kept to a minimum concentration [1] • Should not affect either survival or growth of test organisms [1] • Should be reagent grade or better [1] • Should not exceed 0.5 ml/L (static) or 0.1 ml/L (flow through) unless it was shown that higher concentrations do not affect toxicity [3]		
	Negative Control:	____ Yes ____ No	
	Reference Toxicant Testing:	____ Yes ____ No	*If Yes, identify substance:*
	Other Control: *If any (e.g. solvent control)*		

	Biomass Loading Rate: • Loading should be limited so as not to affect test results. Loading will vary depending on temperature, type of test (static vs. flow-through), species, food/feeding regime, chamber size, test solution volume, etc. [1] • This maximum number would have to be determined for the species, test duration, temperature, flow rate, test solution volume, chamber size, food, feeding regime, etc. • Loading should be sufficiently low to ensure: o Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [1,5] o Unionized ammonia does not exceed 35 µg/L [1] o Uptake by test organisms does not lower test material concentration by > 20% [1] o Growth of organisms is not reduced by crowding • Generally, at the end of the test, the loading (grams of organisms; wet weight; blotted dry) in each test chamber should not exceed the following: o Static tests: > 0.8 g/L (lower temperatures); > 0.5 g/L (higher temperatures) [1] o Flow through tests: > 1 g/L/day or > 10 g/L at any time (lower temperatures); > 0.5 g/L/day or > 5 g/L at any time (higher temperatures) [1] • Lower temperatures are defined as the lower of 17˚C or the optimal test temperature for that species [1]		

	Parameter	Details	Remarks

For Controlled Experiments Only	**Feeding:**
	• Unacceptable for acute tests [2]
	  o Exceptions:
	  ▪ Data indicate that the food did not affect the toxicity of the test material [2]
	  ▪ Test organisms will be severely stressed if they are unfed for 96 hours [2]
	  ▪ Test material is very soluble and does not sorb or complex readily (e.g., ammonia) [2]
		____ Yes ____ No	

	Lighting:
	• Depends on the type of test (acute or chronic) and endpoint (e.g., reproduction) of interest.
	  o Embryos should be incubated under dim incandescent lighting (≤ 20 fc) or total darkness during early life-stage toxicity testing
	  o Embryos must not be subjected to prolonged exposure to direct sunlight, fluorescent lighting, or high intensity incandescent lighting
	• Generally, ambient laboratory levels (50-100 fc) or natural lighting should be acceptable, as well as a diurnal cycle consisting of 50% daylight or other natural seasonal diurnal cycle.
	• Artificial light cycles should have a 15 – 30-minute transition period to avoid stress due to rapid increases in light intensity [1]		

Study Design/Methods Classification: *(Place X by One Based on Overall Study Design/Methods Classification)*
Provide details of Major or Minor Deficiencies/Concerns with Study Design in Associated Sections of Part A: Overview
This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A.

_____ Study Design Acceptable for Quantitative Use
_____ Study Design Acceptable for Qualitative Use
_____ Study Design Not Acceptable for Use

Additional Notes: *Provide additional considerations for the classification of study use based on the study design.*

OBSERVATIONS: *Provide information under Details and any relevant information in Remarks. This information should be consistent with the Results Section in Part A.*

Parameter	Details	Remarks

Parameters measured including sublethal effects/toxicity symptoms:
Common Apical Parameters Include:
Acute
• EC_{50} based on percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus percentage of organisms killed [2]
  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.

Q.2 Example Aquatic Invertebrate DER

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

____ **Controlled Experiment** (*manipulated*) ____ **Field Study/Observation** (*not manipulated*) (*Place X by One*)

Primary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)

Secondary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)
(*At least one reviewer should be from EPA for sensitive taxa*)

Citation: *Indicate: author(s), year, study title, journal, volume, and pages.*
(e.g., Keller, A.E and S.G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. Environ. Toxicol. Chem. 10(4): 539-546.)

Companion Papers: *Identify any companion papers associated with this paper using the citation format above.*

Were other DERs completed for Companion Papers? ____ **Yes** ____ **No** (*If yes, list file names of DERs below*)

Study Classification for Aquatic Life Criteria Development:

_____ Acceptable for Quantitative Use
_____ Acceptable for Qualitative Use
_____ Not Acceptable for Use/Unused

General Notes: *Provide any necessary details regarding the study's use classification for all pertinent endpoints, including non-apical endpoints within the study (e.g., note all study classifications for each endpoint if the use varies)*

Major Deficiencies (note any stated exclusions): *Check all that apply. Checking any of these items make the study "**Not Acceptable for Use**"*

_____ Mixture (for controlled experiments only)
_____ No Controls (for controlled experiments only)
_____ Excessive Control Mortality (> 10% for acute and > 20% for chronic)
_____ Dilution water not adequately characterized
_____ Bioaccumulation: steady state not reached
_____ Dermal or Injection Exposure Pathway
_____ Review paper or previously published without modification

_____ Other: *(if any, list here)*

POTENTIAL CHEMICAL MIXTURES: *Describe any potential chemicals mixtures as characterized by study authors (including any confirmation of chemical mixtures).*

DESCRIPTION OF DILUTION WATER: *Describe concerns with characterization of and/or major deficiencies with dilution water.*

General Notes:

Minor Deficiencies: *List and describe any minor deficiencies or other concerns with test. These items may make the study "**Acceptable for Qualitative Use**"* **(exceptions may apply as noted)**

***For Field Studies/Observations**: A field study/observation may be considered "**Acceptable for Quantitative Use**" if it consisted of a range of exposure concentrations and the observed effects are justifiably contributed to a single chemical exposure*

_____ Mixture (observed effects not justifiably contributed to single chemical exposure)
_____ Uncharacterized Reference Sites/Conditions

POTENTIAL CHEMICAL MIXTURES PRESENT AT SITE: *Describe any potential chemicals mixtures present at the site as characterized by study authors (including any confirmation of chemicals present at study site).*

EXPOSURE VARIABILITY ACROSS STUDY SITE(S): *Describe any exposure variability across study site(s) as characterized by study authors (i.e., description of study design with reference and contaminated sites).*

General Notes:

Reviewer's Comments: *Provide additional comments that do not appear under other sections of the template*.

ABSTRACT: *Copy and paste abstract from publication*.

SUMMARY*: Fill out and modify as needed.*

Acute:

Species (lifestage)	Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration (mg/L)	Classification
											Quantitative / Qualitative / Unused

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

Chronic:

Species (lifestage)	Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L or µg/g)	Verified Chronic Value (mg/L or µg/g)	Chronic Value Endpoint	Classification
												Quantitative / Qualitative / Unused

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

II. Results *Provide results as reported in the publication (including supplemental materials). Include screen shots of tables and/or figures reporting results from the article following tabulated data table in each associated results section for all studies. Complete tabulated data tables for all studies for studies marked "**Acceptable for Quantitative Use"** and **"Acceptable for Qualitative Use**".*

Water Quality Parameters: *If only general summary data of water quality parameters is provided by study authors (i.e., no specific details of water quality parameters on a treatment level is provided), summarize any information regarding water quality parameters under General Notes below and include data not provided in* Table A.II.1.

General Notes: *For aquatic life criteria development, measured water quality parameters in the treatments nearest the toxicity test endpoint(s), e.g., LC_{50}, EC_{20}, etc., are most relevant.*

Table A.II.1. Measured Water Quality Parameters in Test Solutions.
Dissolved oxygen, temperature, pH and [other parameters (hardness, salinity, DOC)] in test solutions during the *[X]*-day exposure of *[test organism]* to *[concentration of treatment(s)]* of *[test substance]* under *[static renewal/flow-through]* conditions.

Parameter	Treatment	Mean	Range
Dissolved oxygen (% saturation or mg/L)	*[1]*		
	[2]		
	j		
	j		
Temperature (℃)	*[1]*		
	[2]		
	j		
	j		
pH	*[1]*		
	[2]		
	j		
	j		
Other (e.g., hardness, salinity, DOC)	*[1]*		
	[2]		
	j		
	j		

Chemical Concentrations: *Summarize the concentration verification data from test solutions/media. Expand table to include each measured concentration data for each media type (i.e., muscle, liver, blood, etc.).*

General Notes: *Provide any necessary detail regarding the measured concentrations, including any identified cause for substantial differences between nominal and measured concentrations, if samples were collected on separate days (and if so provide details), and any potential cross contamination.*

Table A.II.2. Measured (and Nominal) Chemical Concentrations in Test Solutions/Media.

[Analytical Method] verification of test and control concentrations during an [X]-day exposure of [test organism] to [test substance] under [static renewal/flow-through] conditions.

Treatment	Nominal Concentration (units)	[Mean] Measured Concentration (units)	Number of Samples	Non-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
j							

[a]Non-Detect: 0 = measured and detected; 1=measured and not detected; if not measured or reported enter as such

Mortality: *Briefly summarize mortality results (if any).*

General Notes: *Comment on concentrations response relations and slope of response if provided. Compare mortality with control treatment and/or the reference chemical.*

Table A.II.3. Mean Percent [Mortality or Survival].

Mean percent mortality [or number of immobilized] or survival of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment	[Mean % Mortality]	[Standard Deviation or Standard Error]
Control		
[1]		
[2]		
[3]		
[4]		
[5]		
[6]		
[LC_x]		
NOEC		
LOEC		

[a] Use superscript to identify the values reported to be significantly different from control.

Growth: *Briefly summarize growth results (if any).*

General Notes: *Comment on concentrations response relations and slope of response if provided. Compare growth endpoints with control treatment and/or the reference chemical.*

Table A.II.4. Mean [Growth].

Mean growth [length and/or weight] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment	Mean Growth [Length/Weight] (units)	[Standard Deviation or Standard Error]	Mean Percent Change in [Length/ Biomass]	[Standard Deviation or Standard Error]
Control				
[1]				
[2]				
[3]				
[4]				
[5]				
[6]				
j				
[EC_x]				
NOEC				
LOEC				

[a] Use superscript to identify the values reported to be significantly different from control.

Reproductive: *Briefly summarize reproduction endpoint results (if any). For multi-generational studies, copy and paste* Table A.II.5 *below for each generation with reproductive effects data.*

General Notes: *Comment on concentrations response relations and slope of response if provided. Compare reproduction endpoints with control treatment and/or the reference chemical.*

Table A.II.5. Mean [Reproductive] Effect.

Mean [reproductive] effects for [generation] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

Treatment (units)	[Mean Number of Spawns]	[Standard Deviation or Standard Error]	[Mean Number of Eggs]	[Standard Deviation or Standard Error]	[Mean Number of Offspring]	[Standard Deviation or Standard Error]
Control						
[1]						
[2]						
[3]						
[4]						
[5]						
[6]						
j						
[ECx]						
NOEC						
LOEC						

[a] Use superscript to identify the values reported to be significantly different from control.

Sublethal Toxicity Endpoints: *Include other sublethal effect(s), including behavioral abnormalities or other signs of toxicity, if any. Copy Table A.II.6 as needed to provide details for each sublethal effect observed.*

General Notes: *Briefly summarize observed sublethal effects otherwise not captured in the results table(s) below.*

Table A.II.6. Mean [Sublethal] Effect.

*Mean [*Sublethal effect*, (e.g., behavioral abnormalities, etc.)]* in *[test organism]* during [test duration (*acute/chronic*)] exposure to *[test substance]* under *[static/renewal/flow-through]* conditions.

Treatment	[Mean Sublethal Response] (units)	[Standard Deviation or Standard Error]
Control		
[1]		
[2]		
[3]		
[4]		
[5]		
[6]		
j		
[ECx]		
NOEC		
LOEC		

[a] Use superscript to identify the values reported to be significantly different from control

Reported Statistics: *Copy and paste statistical section from publication.*

Part B: Detailed Review

I. Materials and Methods

PROTOCOL/GUIDANCE FOLLOWED: *Indicate if provided by authors.*

DEVIATIONS FROM PROTOCOL: *If authors report any deviations from the protocol noted above indicate here.*

Study Design and Methods: *Copy and paste methods section from publication.*

TEST ORGANISM: *Provide information under Details and any relevant or related information or clarifications in Remarks.*

Parameter	Details	Remarks
Species:	Common Name: Scientific Name:	North American species? ____ Surrogate for North American Taxon? ____ *(Place X if applicable)*

Strain/Source:
• Wild caught from unpolluted areas [1]
  o Quarantine for at least 7 days or until they are disease free, before acclimation [1]
• Must originate from same source and population [1]
• Should not be used:
  o If appeared stressed, such as discoloration or unusual behavior [1]
  o If more than 5% die during the 48 hours before test initiation [1]
  o If they were used in previous test treatments or controls [2]
• No treatments of diseases may be administered:
  o Within 16 hours of field collection [1]
  o Within 10 days of testing or during testing [1]		

Age at Study Initiation:
Acute:
• Larval stages preferred [1]
• Mayflies and Stoneflies
  o Early instar [1]
• Daphnids/cladocerans:
  o < 24-hr old [1]
• Midges:
  o 2nd or 3rd instar larva [1]
• *Hyalella azteca* (chronic exposure)
  o Generally, 7 - 8 days old [3]
• Freshwater mussels (chronic exposure)
  o Generally, 2 month old juveniles [4]
• Mysids (chronic exposure)
  o < 24-hr old [1]		

Was body weight or length recorded at test initiation and/or at regular intervals?	____ Yes ____ No	
Was body weight or length recorded at regular intervals?	____ Yes ____ No *If yes, describe regular intervals:*	

STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for both Controlled Experiments and Field Studies/Observations.*

For Both Controlled Experiments and Field Observations

Parameter	Details	Remarks
Number of Replicates per Treatment Group: • At least 2 replicates/treatment recommended for acute tests [1] • At least 2 replicates/treatment recommended for chronic tests [5]	Control(s):	
	Treatment(s):	
Number of Organisms per Replicate/ Treatment Group: • At least 10 organisms/treatment recommended.	Control(s):	
	Treatment(s):	
Exposure Pathway: *(i.e., water, sediment, or diet). Note: all other pathways (e.g., dermal, injection) are unacceptable.*		
Exposure Duration: **Acute** • Cladocerans and midges should be 48 hours [2] o Longer durations acceptable if test species not fed and had acceptable controls [2] • Freshwater mussel glochidia should be a maximum of 24 hours [4] o Shorter durations (6, 12, 18 hours) acceptable so long as 90% survival of control animals achieved (see below) [4] • Embryo/larva (bivalve mollusks, sea urchins, lobsters, crabs, shrimp and abalones) should be 96 hours, but at least 48 hours [2] • Other invertebrate species should be 96 hours **Chronic** • Daphnids/cladocerans should be 21 days (3-brood test) [2] o Exception 7 days acceptable for *Ceriodaphnia dubia* [2] • Freshwater juvenile mussels should be at least 28 days [4] • *Hyalella azteca* should be at least 42 days o Beginning with 7 - 8 day old animals [3] • Mysids should continue until 7 days past the median time of first brood release in the controls [4]	____ Acute ____ Chronic ____ Other *(please remark):*	
Test Concentrations (remember units): *Recommended test concentrations include at least three concentrations other than the control; four or more will provide a better statistical analysis.*	Nominal:	
	Measured:	
	Media measured in:	
Observation Intervals: • Should be an appropriate number of observations over the study to ensure water quality is being properly maintained [1]		

CONTROLLED EXPERIMENT STUDY PARAMETERS: *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for Controlled Experiments only.*

For Controlled Experiments Only

Parameter	Details	Remarks
Acclimation/Holding: • Should be placed in a tank along with the water in which they were transported [1] o Water should be changed gradually to 100% dilution water (usually 2 or more days) [1] o For wild-caught animals, test water temperature should be within 5°C of collection water temperature [1] o Temperature change rate should not exceed 3°C within 72 hours [1] • To avoid unnecessary stress and promote good health: o Organisms should not be crowded [1] o Water temperature variation should be limited o Dissolved oxygen: ▪ Maintain between 60 - 100% saturation [1] ▪ Continuous gentle aeration if needed [1] o Unionized ammonia concentration in holding and acclimation waters should be < 35 µg/L [1]	Duration: Feeding: Water: Temperature (°C): Dissolved Oxygen (mg/L): Health (*any mortality observed?*):	*Identify number of individuals excluded from testing and/or analysis (if any):*
Acclimation followed published guidance? *Describe, if any*	____ Yes ____ No *If yes, indicate which guidance:*	
Test Vessel: • Test chambers should be loosely covered [1] • Test chamber material: o Should minimize sorption of test chemical from water [1] o Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical [1] o Glass, No. 316 stainless steel, nylon screen and perfluorocarbon (e.g. Teflon) are acceptable [1] o Rubber, copper, brass, galvanized metal, epoxy glues, lead and flexible tubing should not come into contact with test solution, dilution water or stock [1] • Size/volume should maintain acceptable biomass loading rates (see below) [1] • Substrate: o Required for some species (e.g., *Hyalella azteca*) [3] o Common types: stainless steel screen, nylon screen, quartz sand, cotton gauze and maple leaves [3] o More inert substances preferred over plant material, since plants may break down during testing and promote bacterial growth [3] o Consideration should be given between substrate and toxicant [3] ▪ Hydrophobic organic compounds in particular can bind strongly to Nitex® screen, reducing exposure concentrations, especially for studies using static or intermittent renewal exposure methods [3]	Material: Size: Fill Volume: Substrate Used *(if applicable)*:	*Briefly describe the test vessel here*

	Parameter	Details	Remarks

For Controlled Experiments Only	**Test Solution Delivery System/Method:**
	• Flow-through preferred for some highly volatile, hydrolyzable or degradable materials [2]
	  o Concentrations should be measured often enough using acceptable analytical methods [2]
	• Chronic exposures:
	  o Flow-through, measured tests required [2]
	  o Exception: renewal is acceptable for daphnids [2]
		Test Concentrations Measured
		____ Yes ____ No
		
		Test Solution Delivery System:
		____ Static
		____ Renewal
		Indicate Interval:
		
		____ Flow-through
		Indicate Type of Diluter:	

	Source of Dilution Water:
	• Freshwater hardness range should be < 5 mg/L or < 10% of the average (whichever is greater) [1]
	• Saltwater salinity range should be < 2 g/kg or < 20% of the average (whichever is greater) [1]
	• Dilution water must be characterized (natural surface water, well water, etc.) [2]
	  o Distilled/deionized water without the addition of appropriate salts should not be used [2]
	• Dilution water in which total organic carbon or particulate matter exceed 5 mg/L should not be used
	  o Unless data show that organic carbon or particulate matter do not affect toxicity [2]
	• Dilution water for tests with *Hyalella azteca*
	  o Reconstituted waters should have at least 0.02 mg bromide/L; natural ground or surface water presumed to have sufficient bromide [3]
	  o Recommended that control/dilution waters have chloride concentrations at or above 15 mg/L [3]		

	Dilution Series (*e.g., 0.5x, 0.6x, etc.*):		
	Dilution Water Parameters: *Measured at the beginning of the experiment or averaged over the duration of the experiment (details of water quality parameters measured in test solutions should be included under the results section)*	Dissolved Oxygen (mg/L): pH: Temperature (°C): Hardness (mg/L as $CaCO_3$): Salinity (ppt): Total Organic Carbon (mg/L): Dissolved Organic Carbon (mg/L):	
	Aeration: • Acceptable to maintain dissolved oxygen at 60 - 100% saturation at all times [1] • Avoid aeration when testing highly oxidizable, reducible and volatile materials • Turbulence should be minimized to prevent stress on test organisms and/or re-suspend fecal matter [1] • Aeration should be the same in all test chambers at all times [1]	____ Yes ____ No	
	Describe Preparation of Test Concentrations (e.g., water exposure, diet):		

	Parameter	Details	Remarks
For Controlled Experiments Only	**Test Chemical Solubility in Water:** • List units and conditions (e.g., 0.01% at 20ºC)		
	Were concentrations in water or diet verified by chemical analysis? *Measured test concentrations should be reported in* Table A.II.2 *above.*	____ Yes ____ No *Indicate media:*	
	Were test concentrations verified by chemical analysis in tissue? *Measured test concentrations can be verified in test organism tissue (e.g., blood, liver, muscle) alone if a dose-response relationship is observed.* *Measured test concentrations should be reported in* Table A.II.2 *above.*	____ Yes ____ No *Indicate tissue type:*	*If test concentrations were verified in test organism tissue, was a dose-response relationship observed?*
	Were stability and homogeneity of test material in water/diet determined?	____ Yes ____ No	
	Was test material regurgitated/avoided?	____ Yes ____ No	
	Solvent/Vehicle Type: • When used, a carrier solvent should be kept to a minimum concentration [1] • Should not affect either survival or growth of test organisms [1] • Should be reagent grade or better [1] • Should not exceed 0.5 ml/L (static), or 0.1 ml/L (flow through) unless it was shown that higher concentrations do not affect toxicity [5]		
	Negative Control:	____ Yes ____ No	
	Reference Toxicant Testing:	____ Yes ____ No *If yes, identify substance:*	
	Other Control: *If any (e.g. solvent control)*		
	Biomass Loading Rate: • Loading should be limited so as not to affect test results. Loading will vary depending on temperature, type of test (static vs. flow-through), species, food/feeding regime, chamber size, test solution volume, etc. [1] • This maximum number would have to be determined for the species, test duration, temperature, flow rate, test solution volume, chamber size, food, feeding regime, etc. • Loading should be sufficiently low to ensure: o Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [1,6] o Unionized ammonia does not exceed 35 µg/L [1] o Uptake by test organisms does not lower test material concentration by > 20% [1] o Growth of organisms is not reduced by crowding • Generally, at the end of the test, the loading (grams of organisms; wet weight; blotted dry) in each test chamber should not exceed the following: o Static tests: > 0.8 g/L (lower temperatures); > 0.5 g/L (higher temperatures) [1] o Flow through tests: > 1 g/L/day or > 10 g/L at any time (lower temperatures); > 0.5 g/L/day or > 5 g/L at any time (higher temperatures) [1] o Lower temperatures are defined as the lower of 17˚C or the optimal test temperature for that species. [1]		

For Controlled Experiments Only	**Feeding:**
	• Unacceptable for acute tests [2]
	  ○ Exceptions:
	  ▪ Data indicate that the food did not affect the toxicity of the test material [2]
	  ▪ Test organisms will be severely stressed if they are unfed for 96 hours [2]
	  ▪ Test material is very soluble and does not sorb or complex readily (e.g., ammonia) [2]
		____ Yes ____ No	

	Lighting: • No specific requirements for lighting • Generally, ambient laboratory levels (50 - 100 fc) or natural lighting should be acceptable, as well as a diurnal cycle consisting of 50% daylight or other natural seasonal diurnal cycle • Artificial light cycles should have a 15 - 30 minute transition period to avoid stress due to rapid increases in light intensity [1] • Depends on the type of test (acute or chronic) and endpoint (e.g., reproduction) of interest.		

Study Design/Methods Classification: *(Place X by One Based on Overall Study Design/Methods Classification)*
Provide details of Major or Minor Deficiencies/Concerns with Study Design in Associated Sections of Part A: Overview
This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A.

_____ Study Design Acceptable for Quantitative Use
_____ Study Design Acceptable for Qualitative Use
_____ Study Design Not Acceptable for Use

Additional Notes: *Provide additional considerations for the classification of study use based on the study design.*

OBSERVATIONS: *Provide information under Details and any relevant information in Remarks. This information should be consistent with the Results Section in Part A.*

Parameter	Details	Remarks
Parameters measured including sublethal effects/toxicity symptoms: **Common Apical Parameters Include:** **Acute** • Daphnids/cladocerans: o EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed [2] • Embryo/larva (bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones): o EC_{50} based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed [2] ▪ If not available, the lower of the 96 hour EC_{50} based on the percentage of organisms with incompletely developed shells and the 96-hr LC_{50} should be used [2] • Freshwater mussel (glochidia and juveniles): o Glochidia: EC_{50} based on 100 x number closed glochidia after adding NaCl solution - number closed glochidia before adding NaCl solution) / Total number open and closed glochidia after adding NaCl solution [4] o Juvenile: EC_{50} based on percentage exhibiting foot movement within a 5-min observation period [4] • All other species and older life stages: o EC_{50} based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed [2] ▪ If not available, the 96 hour LC_{50} should be used [2] **Chronic** • Daphnid: o Survival and young per female [2] • Mysids: o Survival, growth and young per female [2]	*List parameters:*	
Was control survival acceptable? **Acute** • > 90% control survival at test termination [2] o Glochidia 90% after 24 hours, or, the next longest duration less than 24 hours that had at least 90% survival [4] **Chronic** • > 80% control survival at test termination [2] o 80% in 42 day test with *Hyalella azteca*, slightly lower in tests substantially longer than 42 days [3]	____ Yes ____ No Control survival (%):	

Parameter	Details	Remarks
Were individuals excluded from the analysis?	____ Yes ____ No *If yes, describe justification provided:*	
Was water quality in test chambers acceptable? • If appropriate, describe any water quality issues (e.g., dissolved oxygen level below 60% of saturation)	____ Yes ____ No	
Availability of concentration-response data: • Were treatment level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • • If treatment and/or replicate level concentration-response data were included, how was data presented? *(check all that apply)* • Were concentration-response data estimated from graphs study publication or supplemental materials? Should additional concentration-response data be requested from study authors? *If concentration-response data are available, complete **Verification of Statistical Results (Part C)** for sensitive species.*	____ Yes ____ No ____ Yes ____ No ____ Tables ____ Graphs ____ Supplemental Files ____ Yes ____ No *If yes, indicate software used:* ____ Yes ____ No Requested by: Request date: Date additional data received:	

Part C: Statistical Verification of Results

I. Statistical Verification Information: *Report the statistical methods (e.g., EPA TRAP, BMDS, R, other) used to verify the reported study or test results for the five (5) most sensitive genera and sensitive apical endpoints (including for tests where such estimates were not provided). If values for the LC_{50}, LT_{50} and NOEC are greater than the highest test concentration, use the ">" symbol.*

Primary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)

Secondary Reviewer: _____ **Date:** _____ ____ **EPA** ____ **Contractor** (*Place X by One*)
(*At least one reviewer should be from EPA for sensitive taxa*)

Endpoint(s) Verified:

Additional Calculated Endpoint(s):

Statistical Method (e.g., TRAP, BMDS, R, other):

II. Toxicity Values: *Include confidence intervals if applicable*

NOEC:
LOEC:
MATC:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

Dose-Response Curve Classification: *(Place X by One)*
This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A

_____ Dose-Response Curve Acceptable for Quantitative Use
_____ Dose-Response Curve Acceptable for Qualitative Use
_____ Dose-Response Curve Not Acceptable for Use

Summary of Statistical Verification: *Provide summary of methods used in statistical verification.*

Additional Notes:

-

Attachments:

1. *Provide attachments to ensure all data used in Part C is captured, whether from study results reported in the publication and/or from additional data requested from study authors*
    - *Data from study results of the publication should be reported in Results section of Part A*
    - *Additional data provided upon request from study authors should be reported in Table C.II.1 below and original correspondence with study authors should be included as attachments*
2. *Model assessment output (including all model figures, tables, and fit metrics)*
3. *Statistical code used for curve fitting*

III. Attachments: *Include all attachments listed above after the table below.*

Additional Data Used in Response-Curve: *Provide all data used to fit dose-response curve not captured in Results section of DER above in Part A, rows as needed. First row in italicized text is an example.*

Table C.II.1 Additional Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[a] N = number of individuals per treatment; k = number of replicates per treatment level; n = number of individuals per replicate

Part D: References to Test Guidance

6. ASTM Standard E 739, 1980. 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken, PA.
7. Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses. PB85-227049. National Technical Information Service, Springfield, VA.
8. Mount, D.R. and J.R. Hockett. 2015. Issue summary regarding test conditions and methods for water only toxicity testing with *Hyalella azteca*. Memorandum to Kathryn Gallagher, U.S. EPA Office of Water. U.S. EPA Office of Research and Development. MED. Duluth, MN. 9 pp.
9. Bringolf, R.B., M.C. Barnhart, and W.G. Cope. 2013. Determining the appropriate duration of toxicity tests with glochidia of native freshwater mussels. Submitted to Edward Hammer. U.S. EPA. Chicago, IL, May 8, 2013. 39 pp.
10. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.
11. American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. Part 8000 - Toxicity. APHA. Washington, DC.