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Acute Freshwater Aquatic Life Benchmarks for Eight Data-Limited PFAS: PFBA, PFHxA, PFNA, PFDA, PFBS, PFHxS, 8:2 FTUCA, and 7:3 FTCA

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Acronyms

AWQC	National Recommended Ambient Water Quality Criteria
CAS	Chemical Abstracts Service
CWA	Clean Water Act
DER	Data Evaluation Record
ECOTOX	ECOTOXicology database
EC_x	Effect concentration at x percent level
EPA	U.S. Environmental Protection Agency
FAV	Final Acute Value
7:3 FTCA	2H,2H,3H,3H-Pefluorodecanoic acid
8:2 FTUCA	2H-Perfluoro-2-decenoic acid
GMAV	genus mean acute value
hpf	hours post fertilization
ICE	Interspecies Correlation Estimation
LC_x	Lethal concentration at x percent level
LOECs	Lowest Observed Effect Concentrations
MDRs	minimum data requirements
MSE	Mean square error
NAMS	New Approach Methods
NOECs	No Observed Effect Concentrations
OCSPP	Office of Chemical Safety and Pollution Prevention
ORD	Office of Research and Development
OW	Office of Water
PFAS	per- and polyfluorinated substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFDA	Perfluorodecanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid, pentadecafluoro-1-octanoic acid, pentadecafluoro-n-octanoic acid, octanoic acid, pentadecafluoro-, perfluorocaprylic acid, pentadecafluorooctanoic acid, perfluoroalkyl carboxylic acid or perfluoroheptanecarboxylic acid
PFOS	Perfluorooctane sulfonic acid or perfluorooctane sulfonate
SMAV	Species Mean Acute Value
SOP	standard operating procedure
SSD	species sensitivity distribution
U.S.	United States
web-ICE	Web-based Interspecies Correlation Estimation
WWTPs	wastewater treatment plants

Notices

This document provides information that states and authorized tribes may consider in their water quality protection programs under the Clean Water Act (CWA) to protect freshwater aquatic life from the acute effects of eight individual per- and polyfluoroalkyl substances (PFAS) substances: Perfluorobutanoic acid (PFBA), Perfluorohexanoic acid (PFHxA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), Hexadecafluoro-2-decenoic acid (8:2 FTUCA), and Pentadecafluorodecanoic acid (7:3 FTCA). Toxicity data for these eight PFAS are limited relative to recommended data requirements traditionally used to develop ambient water quality criteria (AWQC) for the protection of aquatic life. Therefore, the United States Environmental Protection Agency (EPA) derived acute PFAS aquatic life freshwater benchmark values under Section 304(a)(2) of the CWA using the best available data, as informational values that states and Tribes may consider in their water quality protection programs, including establishing their water quality criteria. While this document contains the EPA's scientific analyses regarding benchmark values for ambient freshwater concentrations of these eight PFAS protective of aquatic life, this document does not substitute for the CWA or the EPA's regulations; nor is this document or the benchmarks values for the PFAS it presents a regulation itself. Thus, this document does not establish or affect legal rights or obligations, or impose legally binding requirements on the EPA, states, Tribes, or the regulated community. It cannot be finally determinative of the issues addressed. This document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency.

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

https://www.epa.gov/system/files/documents/2024-08/pfas-report-2024.pdf

Foreword

This document presents acute freshwater benchmark values for aquatic life in ambient water based upon consideration of all available toxicity information relating to the acute effects of eight individual per- and polyfluoroalkyl substances (PFAS) substances on freshwater aquatic organisms: Perfluorobutanoic acid (PFBA), Perfluorohexanoic acid (PFHxA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), Hexadecafluoro-2-decenoic acid (8:2 FTUCA), and Pentadecafluorodecanoic acid (7:3 FTCA). The EPA developed this document to provide information that states and authorized Tribes may consider in their water quality protection programs.

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Executive Summary

Per- and polyfluoroalkyl substances (PFASs) are organic, human-made chemical compounds composed of an alkyl chain bound to multiple fluorine atoms. There are estimated to be over 3,000 PFAS present in the global marketplace, that are used in a variety of specialized applications, including surface coatings and a variety of other industrial and commercial products (Wang et al. 2017). In addition to Perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonic acid (PFOS), for which the U.S. Environmental Protection Agency (EPA) has recently developed Aquatic Life Ambient Water Quality Criteria (U.S. EPA 2024b,c), the EPA recognizes that there may be thousands of other PFAS present in the environment. The EPA (2024b,c) provides a detailed discussion of the sources, occurrence, fate and transport of PFOA, PFOS and other PFAS in the environment.

The EPA has developed acute freshwater aquatic life acute ambient water quality benchmarks for eight selected polyfluorinated substances. The eight chemicals for which acute benchmarks were developed are: Perfluorobutanoic acid (PFBA), Perfluorohexanoic acid (PFHxA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), Hexadecafluoro-2-decenoic acid (8:2 FTUCA), and Pentadecafluorodecanoic acid (7:3 FTCA). These eight chemicals were selected based on interest by stakeholders and aquatic toxicity data availability.

The Clean Water Act (CWA) supports the protection of U.S. waters by helping to ensure that aquatic species have clean water to live in. States, Tribes, or the EPA may establish concentration limits of pollutants that will ensure that fish and other aquatic species can live, grow, and reproduce. CWA Section 304(a) directs the EPA to develop and publish water quality criteria that reflect the latest scientific knowledge.

National recommended ambient water quality criteria for the protection of aquatic life establish chemical concentrations (magnitude) that are averaged over a given time period (duration) and should not be exceeded more than once during a specified time period (frequency). The EPA's 304(a)(1) criteria recommendations generally follow the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (hereafter "Aquatic Life Criteria Guidelines" (U.S. EPA 1985). The Aquatic Life Criteria Guidelines recommend toxicity data for a minimum of eight families of aquatic animals be used in developing aquatic life criteria to protect aquatic ecosystems as a whole.

Aquatic life benchmarks, developed under 304(a)(2) of the CWA, are informational values that the EPA generates when there are limited high quality toxicity data available and data gaps exist for several families of aquatic organisms. Aquatic life benchmarks provide information that states and Tribes may consider in their water quality protection programs. In developing aquatic life benchmarks, data gaps may be filled using new approach methods (NAMs), such as computer-based toxicity estimation tools (e.g., the EPA's Web-ICE; Version 4.0) or other new approach methods intended to reduce reliance on animal-based testing (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-vertebrate-animals-chemical), including the use of read-across estimates based on other chemicals with similar structures. The EPA's aquatic life benchmark values are not regulatory, nor do they automatically become part of a state's water quality standards.

Because direct empirical data are more limited for the eight PFAS evaluated within this document and do not fulfill MDRs as described by the Aquatic Life Criteria Guidelines (U.S. EPA 1985), the EPA developed acute protective benchmarks for these eight PFAS using available freshwater species empirical test data in conjunction with the application of the EPA's

peer-reviewed web-based Interspecies Correlation Estimate (ICE) tool (Web-ICE; Version 4.0; https://www.epa.gov/webice/) (Raimondo et al. 2010). ICE models support the derivation of acute values, and do not currently support the derivation of chronic values. This document accordingly focuses on the derivation of acute aquatic life benchmarks. These acute aquatic life benchmarks, developed using the direct empirical and ICE (estimated) data for these chemicals, were calculated by applying methods described in the EPA Aquatic Life Criteria Guidelines (U.S. EPA 1985).

In addition to addressing data limitations through NAMs, the EPA also developed application factors to account for species that are known to show markedly greater sensitivity to PFOA and PFOS. Specifically, Soucek et al. (2023) and Razak et al. (2023) indicated the mayfly, *Neocloeon triangulifer,* and the cladoceran, *Moina micrura*, are markedly more sensitive to PFOA and PFOS relative to other species tested. The EPA accordingly derived the adjustment factors from the available PFOA and PFOS data and applied them to the eight PFAS benchmarks to account for greater sensitivity of these species. The inclusion of the application factors protect the many untested species in the environment, some of which may be similarly sensitive to these PFAS as the mayfly and cladoceran species noted above. Development of these PFAS benchmarks reflects goals in the EPA's PFAS Strategic Roadmap and aligns with the Agency's intention to reduce the use of additional animal testing through application of NAMs. They provide information to states and Tribes regarding protective values for aquatic life for these eight data-limited PFAS.

This document describes the process used and results of a systematic review of available direct empirical toxicity data for aquatic organisms identified via the EPA's literature search for the eight selected PFAS substances, inclusive of their anionic, acid, and ammonium salt forms.

The derived acute benchmark values are summarized for each of the eight evaluated PFAS in **Table Ex-1**. These concentrations are expected to be protective of 95% of freshwater genera exposed to the listed PFAS under short term conditions of one-hour of duration, if the one-hour average magnitude is not exceeded more than once in three years. However, because only limited toxicity test data were available for these eight chemicals and aquatic toxicity was estimated for some taxa to develop these benchmarks, the values are somewhat less certain than national recommended Aquatic Life AWQC developed using more robust empirical data sets (i.e., those that meet the MDRs according to the EPA's Aquatic Life Criteria Guidelines). Further, while the eight MDRs were fulfilled for six of the PFAS chemicals when direct empirical and ICE data were combined, only seven of the eight MDRs were fulfilled for PFBS and only six of the eight MDRs were fulfilled for PFHxS when data were combined, and there is considered to be greater uncertainty associated with these benchmark values.

Table Ex-1. Acute Freshwater Aquatic Life Benchmarks for Eight PFAS.

Chemical	PFBA	PFHxA	PFNA	PFDA	PFBS	PFHxS	8:2 FTUCA	7:3 FTCA
Magnitude[1]	5.3	4.8	0.65	0.50	5.0	0.21	0.037	0.012
Duration	One hour average							
Frequency	Not to be exceeded more than once in three years on average							

[1] Values expressed as mg/L, or ppm.

The EPA additionally explored the application of a data "binning" approach, used by Giddings et al. (2019) for pyrethroids, as an exploratory approach for deriving protective acute aquatic life values for grouped perfluoroalkyl carboxylic acids and grouped perfluoroalkyl sulfonic acids. This approach is based on combining chemicals with similar modes of action to provide more robust datasets to support value derivation in data-limited situations. The EPA followed this methodology to explore the calculation of protective benchmark values for six of

the PFAS, based on the combined perfluoroalkyl carboxylic acid (PFBA, PFHxA, PFNA, PFDA) and perfluoroalkyl sulfonic acid (PFBS, PFHxS) groupings, that were used to support value derivation.

1 BACKGROUND

This document describes the development of acute water quality benchmarks to protect freshwater aquatic life from acute toxic effects of eight PFAS substances and their related forms:

- Perfluorobutanoic acid (PFBA)(CAS# 375224, 45048622, 2218544)
- Perfluorohexanoic acid (PFHxA)(CAS# 307244, 92612527)
- Perfluorononanoic acid (PFNA)(CAS# 375951)
- Perfluorodecanoic acid (PFDA)(CAS# 335762)
- Perfluorobutanesulfonic acid (PFBS)(CAS# 375735, 108427527, 29420493)
- Perfluorohexanesulfonic acid (PFHxS)(CAS# 355464, 108427538, 3871996, 82382125)
- 2H-Perfluoro-2-decenoic acid (8:2 FTUCA)(CAS# 70887842)
- 2H,2H,3H,3H-Pefluorodecanoic acid (7:3 FTCA)(CAS# 812704)

These eight chemicals were selected based on interest by stakeholders and aquatic toxicity test data availability. Notably, the U.S. Department of Defense (DOD) published a set of draft Ecological Screening Values (Grippo et al. 2021) for six of these substances (PFBA, PFHxA, PFNA, PFDA, and PFHxS) based on stated concerns about the historic release of these substances at DOD facilities. The ECOTOXicology Knowledgebase (ECOTOX; https://cfpub.epa.gov/ecotox/), a curated source of high-quality toxicity data for aquatic life, terrestrial plants, and wildlife, was also searched to identify PFAS, besides PFOA and PFOS, that have some of the greatest numbers of empirical studies available to support benchmark development.

In addition to PFOA and PFOS, for which the EPA has recently developed final Aquatic Life AWQC, the EPA is aware of thousands of other PFAS chemicals that may be present in the environment. The above eight chemicals were selected to represent a range of PFAS that are present in aquatic ecosystems and of concern to stakeholders (e.g., states, Tribes, DOD. Another important consideration for selection was the availability of both acute direct empirical and ICE model toxicity data for these chemicals.

The EPA typically follows the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (U.S. EPA 1985), which requires toxicity data for a minimum of eight families be available to fulfill taxonomic MDRs to calculate freshwater Aquatic Life AWQC. The MDR groups represent taxa with different ecological, trophic, taxonomic and functional characteristics in aquatic ecosystems, and are intended to collectively represent the diversity and range of sensitivity of species within an aquatic community. The MDRs, as defined by the Aquatic Life Criteria Guidelines, consist of the following representative taxa:

a) Family Salmonidae in the class Osteichthyes
b) Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
c) Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
d) Planktonic crustacean (e.g., cladoceran, copepod, etc.)
e) Benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
f) Insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
g) Family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
h) Family in any order of insect or any phylum not already represented

For some contaminants, particularly those with newer or novel chemistries and emerging contaminants, these MDRs often cannot be met, and an alternative approach is necessary to provide information to states and Tribes under CWA Section 304(a). In this case, empirical acute data for these eight PFAS fulfill some but not all of these MDRs. Accordingly, the EPA applied the peer reviewed Interspecies Correlation Estimation (ICE) models (Raimondo et al. 2010, 2024) to estimate acute toxicity data to fulfill MDRs for which direct empirical toxicity data are not available. ICE models support the derivation of acute values, and do not currently support the derivation of chronic values and/or the values associated with potential bioaccumulative effects**.**

This document accordingly focuses on the derivation of acute aquatic life benchmarks. The EPA has previously used this same approach to derive final acute estuarine/marine benchmarks for PFOA and PFOS (U.S. EPA 2024b,c).

As noted above, the EPA used the acute empirical test data for these chemicals supplemented with the ICE values derived for the missing MDRs to derive acute freshwater benchmark recommendations for aquatic life using calculation procedures provided in the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The EPA also developed application factors to account for species that are known to show markedly greater sensitivity to PFOA and PFOS. Specifically, Soucek et al. (2023) and Razak et al. (2023) indicated the mayfly, *Neocloeon triangulifer,* and the cladoceran, *Moina micrura,* are markedly more sensitive to PFOA and PFOS relative to other species tested. The EPA accordingly derived the adjustment factors from the available PFOA and PFOS data and applied them to the eight PFAS benchmarks to account for greater sensitivity of these species. The inclusion of the application factors protect the many untested species in the environment, some of which may be similarly sensitive to these PFAS as the mayfly and cladoceran species noted above. The above approaches are consistent with the Aquatic Life Criteria Guidelines "good science" clause, the EPA's interest in providing useful information to states and Tribes regarding protective benchmark values for aquatic life, and also the EPA's intention to reduce the use of animal testing via application of NAMS in toxicity assessment (https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing).

The EPA additionally explored the application of a data "binning" approach, used by Giddings et al. (2019), to derive protective aquatic life values for grouped carboxylic acid PFASs and grouped sulfonic acid PFAS. This approach, which is detailed in **Appendix G**, is based on

combining chemicals with similar modes of action to provide more robust datasets to support value derivation in data-limited situations.

2 INTRODUCTION TO ICE

Interspecies Correlation Estimation (ICE) models are log-linear regressions of the acute toxicity (EC_{50}/LC_{50}) of two species across a range of chemicals that represent the relationship of inherent sensitivity between those species (**Figure 2-1**). ICE models are 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 (Raimondo et al. 2010, 2024). ICE models are used to predict the sensitivity of an untested taxon (predicted taxa are represented by the y-axis) from the known, measured sensitivity of a surrogate species (represented by the x-axis).

ICE models have been developed from a broad range of chemicals (e.g., metals and other inorganics, pesticides, solvents, and reactive chemicals) and across a wide range of toxicity values and have been validated as accurate predictors of acute toxicity when model criteria parameters are followed (Raimondo et al. 2024). The Web ICE application (v4.0, https://www3.epa.gov/webice/) contains approximately 4,800 statistically-significant ICE models for aquatic animal and plant species.

Validation and uncertainty analyses of ICE models are founded in both leave-one-out cross validation and direct comparison of measured and predicted acute values (Raimondo et al. 2010, 2024; Willming et al. 2016). Acute toxicity predictions have high accuracy when values are derived from models with robust parameters (e.g., mean square error and R^2 of regression between the tested and untested species), and variability of prediction accuracy is consistent with that of empirical test data (Raimondo et al. 2024). **Box 1** below outlines the parameter measures used to define acceptable ICE models for evaluation. Models identified as acceptable based on

the parameter measures identified in **Box 1** were further evaluated for use as discussed in **Sections 4.2** and **5.1**.

ICE models have undergone extensive peer review and their use has been supported through external peer review for development of aquatic life benchmark values for saltwater and estuarine species exposed to PFOA and PFOS (U.S. EPA 2024b,c). The application of ICE-predicted values to develop protective aquatic life values by multiple independent, international groups confirms that values developed from ICE-generated species sensitivity distributions (SSDs) provide a level of protection that is consistent with using measured laboratory data (Dyer et al. 2008; Feng et al. 2013; Fojut et al. 2012a,b; Palumbo et al. 2012; Wu et al. 2015, 2016; Wang et al. 2020; Zhang et al. 2017). A recent 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).

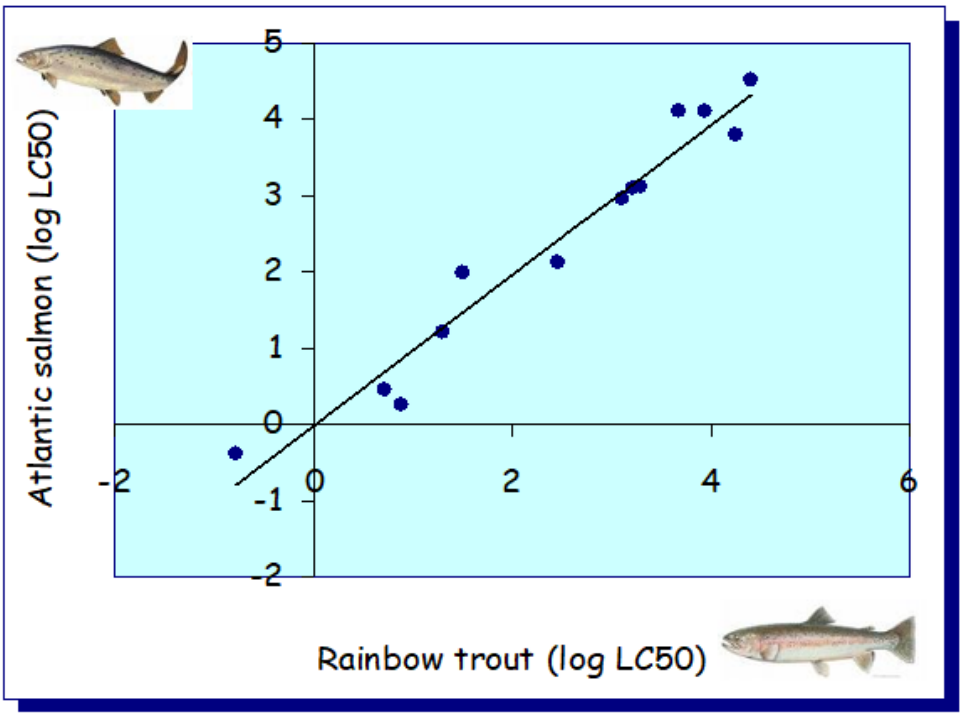

Figure 2-1. Example of ICE Model for Rainbow Trout (surrogate) and Atlantic Salmon (predicted).
Each model datapoint is a common chemical that was tested with both species to develop a log-linear model.

Box 1. ICE Model User Parameter Criteria for Robust Predictions (Willming et al. 2016, Raimondo et al. 2024):

- Low mean squared error (MSE) (<~ 0.95)
- High R^2 value (>~0.6)
- High slope (>~0.6; 0.66 – 1.33 when µg/L input is beyond model domain)
- Confidence interval range of 2 orders of magnitude between lower and upper limit
- 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

3 APPLICATION OF ICE WITH SELECTED PFAS

ICE models for freshwater species were used to predict acute toxicity of the eight PFAS from measured sensitivity of surrogate species listed in **Table 3-1**. For the application of ICE models, the type and number of predicted species available to fulfill missing MDRs in the selected PFAS acute criteria dataset was based on the number of models available within ICE for the surrogate species (**Table 3-1**). It is important to note, not all available ICE models met the prediction robustness criteria outlined in Box 1 for quantitative use. Accordingly, an evaluation of the predictions was conducted following the acquisition of all available predicted values from all available surrogate species for each compound.

Table 3-1. Measured Surrogate Species Sensitivity Values and Number of Available ICE Models for Eight PFAS.
The number of freshwater ICE models for each surrogate excludes models that predict values for species for which measured acute toxicity data are already available. Available ICE models are presented in Appendix E.

Chemical	Taxa	Surrogate Species	Acute Toxicity (mg/L)	Number of Freshwater ICE Models
PFBA	Vertebrate	*Danio rerio*	13,779	18 (5 Juveniles, 13 Embryo)
	Invertebrate	*Daphnia magna*	5,251	69
PFHxA	Invertebrate	*Daphnia magna*	1,048	69
	Vertebrate	*Lithobates catesbeianus*	1,105	8
PFNA	Invertebrate	*Chydorus sphaericus*	28	2
	Invertebrate	*Daphnia magna*	85	69
	Vertebrate	*Xenopus laevis*	336	4
PFDA	Invertebrate	*Chydorus sphaericus*	41	2
	Invertebrate	*Daphnia magna*	120	69
	Invertebrate	*Daphnia pulicaria*	150	2
	Vertebrate	*Oncorhynchus mykiss*	32	78
	Vertebrate	*Xenopus laevis*	77	4
	Invertebrate	*Daphnia magna*	2,183	69
	Vertebrate	*Lepomis macrochirus*	6,452	76
	Vertebrate	*Pimephales promelas*	1,938	74

Chemical	Taxa	Surrogate Species	Acute Toxicity (mg/L)	Number of Freshwater ICE Models
PFHxS	Vertebrate	*Danio rerio*	23	18 (5 Juveniles, 13 Embryo)
	Vertebrate	*Lithobates catesbeianus*	1,105	8
8:2 FTUCA	Invertebrate	*Daphnia magna*	3	69
	Vertebrate	*Oncorhynchus mykiss*	81	78
7:3 FTCA	Invertebrate	*Daphnia magna*	1	69
	Vertebrate	*Oncorhynchus mykiss*	32	78

PFAS acute values (typically reported as mg/L) can be beyond the range of values used to calibrate the ICE model (ICE database toxicity range $1x10^{-4}$ and $1x10^{8}$ μg/L) such that the input PFAS value of the surrogate would be outside the model domain. In situations where the input PFAS value is beyond the model range, a user can either:

- Enter the measured toxicity value (LC_{50}/ EC_{50}) into the ICE model as μg/L and allow the regression to **extrapolate** beyond the model range; or
- "**Scale**" the toxicity value by entering the measured LC_{50} value as mg/L, bringing the input value within the model range.

Because it is recognized that extrapolating beyond the model range can result in large confidence intervals around the prediction and can result in the acceptance of fewer ICE models based on acceptability parameters (see **Box 1**), a "scaled" approach was considered by Raimondo et al. (2024) as an alternative approach to predicting species sensitivity to place the input value within the model domain. The scaled approach underwent detailed evaluation by Raimondo et al. (2024), who compared the two approaches and reported that ICE models predict toxicity with the same level of accuracy using scaled and extrapolated input values, with smaller confidence intervals for the scaled approach (allowing acceptance of more ICE models). The first ("extrapolated") approach was selected as the primary approach for deriving the benchmark

values. The "scaled" approach was applied as an alternative approach and serves as an additional analysis to compare SSDs and HC5s with those based on extrapolated values and are summarized in **Section 5.10** and presented in detail in **Appendix F**.

Figure 3-1 provides an example of the approach taken to calculate a toxicity value when an LC_{50} or EC_{50} value falls beyond the ICE model range, through the use of either an "extrapolation" or "scaled" approach. The data plots and calculations below provide an illustrative example using an EC_{50} value for *Daphnia magna* for PFBS.

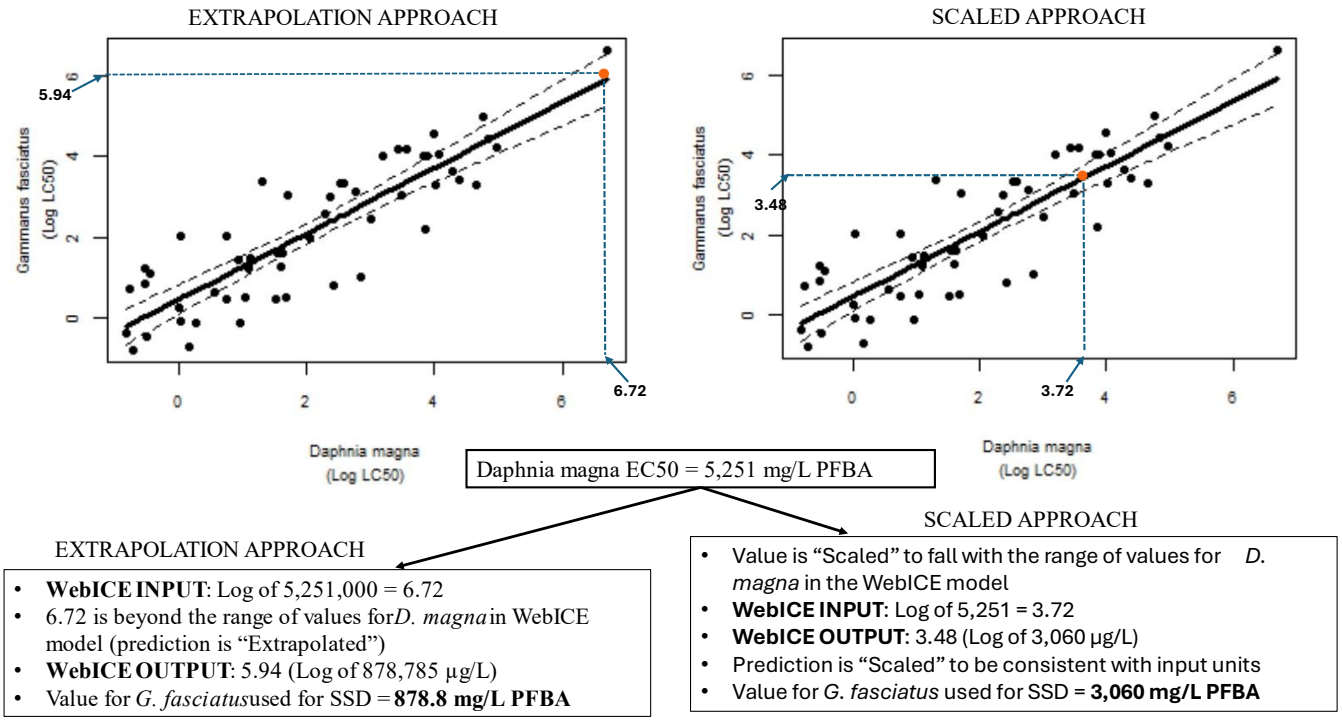

Figure 3-1. Example Calculation for *Daphnia magna* PFBA Toxicity Data Based on Extrapolation and Scaled Approaches.

4 EFFECTS ANALYSIS FOR AQUATIC LIFE

4.1 Summary and Evaluation of Available Empirical Toxicity Test Data

The EPA reviewed and considered all relevant empirical toxicity test data for the eight PFAS. Information available for all relevant species and genera were reviewed to identify data from acceptable tests that meet data quality standards. All available empirical studies relating to the acute toxicological effects of each PFAS on aquatic life were considered in the derivation of these freshwater acute benchmarks. Empirical data for possible inclusion in the derivation of the benchmarks were obtained from published literature reporting acute exposures of the selected PFAS that were associated primarily with mortality/survival. The development of benchmarks included the review of a range of forms of each PFAS substance for which data were available, as summarized in **Table 4-1**. **Figure 4-1** presents representative chemical structures for these PFAS. With the exception of 8:2 FTUCA, all of these PFAS can be analyzed using the EPA Method 1633 (U.S. EPA 2024a).

Table 4-1. Summary Table of PFAS Forms and Associated CAS Numbers Identified for the Evaluation of Available Empirical Data.

PFAS	Form	Chemical Formula	CAS Number
Perfluorobutanoic acid (PFBA)	PFBA	$C_4HF_7O_2$	375224
	PFBA Ion	$C_4F_7O_2^-$	45048622
	PFBA-Na	$C_4F_7NaO_2^-$	2218544
Perfluorohexanoic Acid (PFHxA)	PFHxA	$C_6HF_{11}O_2$	307244
	PFHxA Ion	$C_6F_{11}O_2^-$	92612527
Perfluorononanoic acid (PFNA)	PFNA	$C_9HF_{17}O_2$	375951
Perfluorodecanoic Acid (PFDA)	PFDA	$C_{10}HF_{19}O_2$	335762
Perfluorobutanesulfonic acid (PFBS)	PFBS	$C_4HF_9O_3S$	375735
	Tetrabutylammonium perfluorobutanesulfonate	$C_{20}H_{36}F_9NO_3S$	108427527
	PFBS.K	$C_4F_9KO_3S$	29420493

PFAS	Form	Chemical Formula	CAS Number
Perfluorohexanesulfonic acid (PFHxS)	PFHxS	$C_6HF_{13}O_3S$	355464
	Perfluorohexanesulfonate	$C_6F_{13}O_3S^-$	108427538
	PFHxS.K	$C_6F_{13}KO_3S$	3871996
	PFHxS.Na	$C_6F_{13}NaO_3S$	82382125
2H-Perfluoro-2-decenoic acid (8:2 FTUCA)	8:2 FTUCA	$C_{10}H_2F_{16}O_2$	70887842
2H,2H,3H,3H-Pefluorodecanoic acid (7:3 FTCA)	7:3 FTCA	$C_{10}H_5F_{15}O_2$	812704

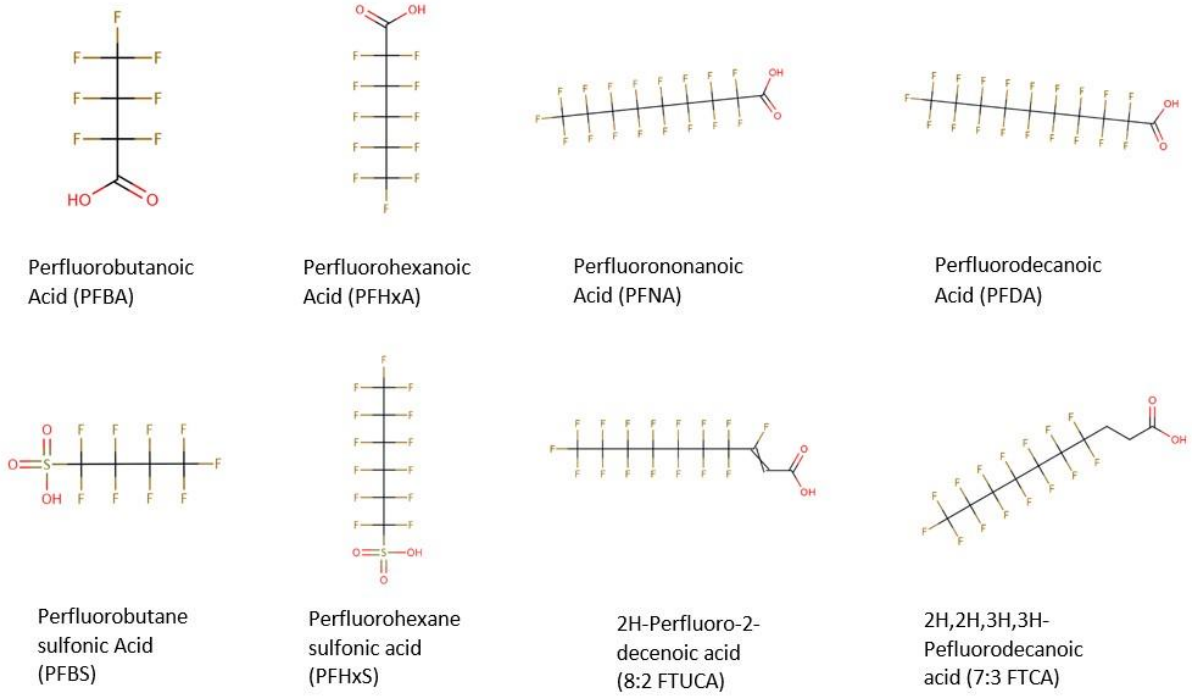

Figure 4-1. Chemical Structures of Selected PFAS.

Empirical studies available for the eight PFAS were identified using ECOTOX. Toxicity studies accessed from the ECOTOX database were further evaluated. Studies were evaluated for data quality as described by the EPA OW's data quality standard operating procedure (SOP), and

consistent with OW's data quality review approach (U.S. EPA 1985), and the EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S. EPA 2016). The EPA completed a Data Evaluation Record (DER) for each species empirical toxicity test from the studies identified by ECOTOX for the eight PFAS undergoing evaluation. This in-depth review ensured the studies used for derivation would result in robust, scientifically-defensible benchmarks. Studies that did not fully meet the data quality objectives outlined in the EPA SOP were not considered for inclusion in the benchmark derivation, including some studies with other PFAS exposures. Further, only single chemical toxicity tests (i.e., not tests with chemical mixtures) were considered for possible inclusion in benchmark derivation. When available, both controlled laboratory experiments and field observations/studies were evaluated for inclusion.

Due to the EPA's interest in using all available quality data, particularly for these data-limited chemicals, toxicity studies were considered for possible inclusion regardless of the test species residential status in North America. Use of non-North American residential species is also consistent with other published aquatic life criteria (U.S. EPA 2018). Non-North American resident species also help to serve as taxonomically-related surrogate organisms for untested resident species.

Toxicity data from tests reporting either measured or nominal concentrations were considered for quantitative use in benchmark derivation. Toxicity tests used in many of the EPA's previous aquatic life criteria documents are typically based only on measured chemical concentrations. For PFOA and PFOS, the EPA examined the issue of whether nominal (unmeasured) and measured concentrations are in close agreement with each other (U.S. EPA 2024b,c). For both PFOA and PFOS, pairs of nominal and corresponding measured

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. For PFOA 83% of measured freshwater concentrations and 82% of the measured concentrations for PFOS fell within 20% of paired nominal concentrations, which represent the test acceptability threshold identified by the EPA's OCSPP's Ecological Effects Test Guidelines. Based on the results for PFOA and PFOS (U.S. EPA 2024b,c), the EPA concluded that nominal test concentrations adequately represent actual PFAS exposures in standard acute laboratory-based toxicity tests and PFAS toxicity tests were not excluded from quantitative use in benchmark derivation on the basis of unmeasured test concentrations alone. Resultingly, renewal, and flow-through experiments were considered for inclusion for species mean acute values regardless of whether the PFAS concentrations were measured.

Available empirical acute data meeting the quality objectives and test requirements were utilized quantitatively in conjunction with ICE values in deriving benchmarks for aquatic life. The number of acute genus and species level mean values and MDRs fulfilled by the quantitatively acceptable empirical acute toxicity data available for each of the eight PFAS is summarized in **Table 4-2** and detailed in **Appendix A: Quantitatively Acceptable Freshwater Acute Toxicity Studies**. Studies that were determined to be qualitatively acceptable as supporting information, but not acceptable for quantitative use, are summarized in **Appendix C: Qualitative Freshwater Acute Toxicity Studies**. Studies that were not considered acceptable for quantitative or qualitative use are summarized in **Appendix D: Unused Freshwater Acute Toxicity Studies**.

Acute values were presented as reported by the study authors for each individual study. Author-reported toxicity values were included in each study summary included in corresponding

appendices, as applicable. The results of all toxicity values (e.g., individual toxicity test outcomes 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 benchmarks is stated for each study at the end of the summaries in **Appendix A**.

Table 4-2. Number of Acute Genus and Species Level Mean Values in the Empirical Toxicity Datasets and Freshwater Minimum Data Requirements Fulfilled per the Aquatic Life Criteria Guidelines for the Eight PFAS.

MDR	PFBA	PFHxA	PPFNA	PFDA	PFBS	PFHxS	8:2 FTUCA	7:3 FTCA
Family Salmonidae in the class Osteichthyes	-	-	-	1	-	-	1	1
Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species	1	-	-	-	3	1	-	-
Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	-	2	1	1	-	2	-	-
Planktonic Crustacean	3	1	2	3	1	-	1	1
Benthic Crustacean	-	-	-	-	-	-	-	-
Insect	-	-	-	-	-	-	-	-
Family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, or Mollusca)	-	-	-	-	-	-	-	-
Family in any order of insect or any phylum not already represented	1	1	-	-	-	-	-	-
Total number of empirical studies	**5**	**4**	**3**	**5**	**4**	**3**	**2**	**2**
MDRs fulfilled with empirical data	**3**	**3**	**2**	**3**	**2**	**2**	**2**	**2**

4.2 Prediction of Species Sensitivity Using ICE Models

Values for the selected PFAS were first predicted with Web-ICE v4.0 for all possible species. The resulting ICE models are summarized in **Appendix E.** These ICE models were then evaluated using the acceptance parameters (e.g., MSE, R^2, slope) summarized in **Box 1**, provided

above under **Section 2**. Only ICE models based on freshwater species were considered for use. Models that met these acceptance parameters were used to derive species toxicity data, which were used in conjunction with empirical toxicity data to support the derivation of the benchmark values. The ICE models selected for use are summarized in each of the chemical-specific discussions presented in **Section 5**.

5 DERIVATION OF ACUTE WATER QUALITY BENCHMARKS FOR SELECTED PFAS

5.1 Approach

5.1.1 Use of Empirical and ICE Data

The following section describes the process used to derive the water quality benchmarks for each of the eight PFAS. The first step encompassed development of the acute toxicity dataset used to support the derivation of each benchmark, which involved combining available empirical test data with ICE-derived toxicity data in order to both meet the eight MDRs, as described in the Aquatic Life Criteria Guidelines (U.S. EPA 1985), and provide as robust of a dataset as possible for benchmark derivation. The following approach was used to develop the dataset for the derivation of each acute PFAS benchmark:

- All acceptable empirical data (evaluated as described in **Section 4.1** and summarized in **Appendix A** and **B**) were used and prioritized for benchmark derivation. Accordingly, if both acceptable empirical and ICE-estimated LC_{50}/ EC_{50} values were available for a species, then only the empirical data were averaged to derive that SMAV, based on the greater certainty assumed to be associated with quantitatively-acceptable empirical test data.
- ICE-derived toxicity data were derived from acceptable ICE models (as described in **Section 4.2** and summarized in **Appendix E**), and used to derive toxicity data for each PFAS, with the objective of fulfilling gaps in the MDRs and creating an overall more robust acute dataset for benchmark derivation. As noted, ICE-estimated LC_{50}/EC_{50} values were used to derive SMAVs, except when empirical data were available. If multiple acceptable ICE models were available for a given species (based on prediction by

multiple surrogates) then only the model with the closest taxonomic relationship was used to support deriving the SMAV.

Following development of the toxicity database, values were then calculated using the same basic procedure as described in the Aquatic Life Criteria Guidelines (U.S. EPA 1985) for the derivation of acute criteria. Please refer to the Aquatic Life Criteria Guidelines for a detailed description of this approach. The primary difference between the derivation of aquatic life criteria versus the derivation of protective acute benchmarks in this document is in the composition of the constituent dataset, which consists of both empirical test and ICE-derived acute toxicity data, since the eight MDRs could not be fulfilled for these compounds using the available empirical test data.

Consistent with the Aquatic Life Criteria Guidelines (U.S. EPA 1985), acceptable empirical data were then used in conjunction with ICE estimated LC_{50} values to derive a "preliminary" value as follows:

- Species mean acute values (SMAVs) were derived based on the availability of multiple acceptable empirical or the selected ICE-estimated values by combining the accepted data into species-level groupings and calculating the means for each grouping.
- Genus Mean Acute Values (GMAVs) were then calculated by combining the SMAVs (comprised of both empirical data and ICE-estimated values) into genus-level groupings and calculating the means for each grouping.
- Sensitivity Distributions (SD) were developed from the GMAVs
- The four GMAVs closest to the 5^{th} percentile of the distribution were used to calculate the Final Acute Value (FAV).

- The FAV is then divided by two, which is intended to estimate a minimal effect level and provide an acute value that is protective of nearly all individuals in the distribution (U.S. EPA 1985).

5.1.2 Application Factor to Adjust for Additional Unrepresented Sensitive Species Data

It was noted that no acceptable empirical data and limited ICE-estimated toxicity data were available for some taxa. This is particularly notable as recent investigations with both PFOA and PFOS indicate the mayfly, *Neocloeon triangulifer* (Soucek et al. 2023) and the cladoceran, *Moina micrura* (Razak et al. 2023) are among the most acutely sensitive of any species tested to these substances amongst the acceptable data, as determined by the EPA:

- For PFOA, the author-reported acute EC_{50} (i.e., 0.4747 mg/L) for *M. micrura* from a new study (Razak et al. 2023) was determined to be acceptable for quantitative use and was averaged as a geometric mean with other quantitatively acceptable acute *Moina* data, yielding the most sensitive genus mean acute value (8.885 mg/L). The EPA-calculated acute LC_{50} (i.e., 13.045 mg/L) for *N. triangulifer* from Soucek et al. (2023) was acceptable for quantitative use and was the second most sensitive genus mean acute value in the final PFOA acute freshwater criterion.
- For PFOS, the EPA-calculated acute LC_{50} (i.e., 0.07617 mg/L) for *N. triangulifer* was acceptable for quantitative use and was the most sensitive genus mean acute value in the final PFOS acute freshwater criterion. The new author-reported acute LC_{50} (i.e., 0.5496 mg/L) for *M. micrura* (Razak et al. 2023) was acceptable for quantitative use and was averaged as a geometric mean with other *Moina* data to yield the second most sensitive genus mean acute value (3.075 mg/L) in the final acute PFOS criterion.

These data were not available when the draft freshwater PFOA and PFOS acute aquatic life criteria were released (U.S. EPA 2022a,b), but were incorporated into the final freshwater acute

criteria released for these compounds (U.S. EPA 2024b,c), significantly lowering the derived freshwater acute values for these substances:

- For PFOA, the final acute freshwater criterion is 3.1 mg/L, compared to the draft acute criterion of 49 mg/L, and the addition of the acute value for *N. triangulifer* along with the addition of the sensitive *M. micrura* data decreased the criterion by a factor of 15.8.
- For PFOS, the final acute freshwater criterion is 0.071 mg/L, compared to the draft acute criterion of 3.0 mg/L, and the addition decreased the criterion by a factor of 42.3.

Based on the limited available data and to account for this marked difference in sensitivity, the EPA applied an application factor to the calculation of each PFAS benchmark value, to account for the absence of empirical insect, and other sensitive invertebrate, data and the markedly greater sensitivity of the mayfly *N. triangulifer*, and *M. micrura.* The following application factors were used:

- For PFAS with carboxylate groups (PFBA, PFHxA, PFNA, PFDA, 8:2 FTUCA, and 7:3 FTCA), an application factor of 15.8 was used based on the difference in draft and final PFOA acute criterion values when the additional sensitive species data were added.
- For PFAS with sulfonate groups (PFBS and PFHxS), an application factor of 42.3 was similarly used.

These application factors were used to convert the "preliminary" benchmark value to a final benchmark value. **Table 5-1** provides a summary of the application factors developed to adjust for insect sensitivity.

Table 5-1. Application Factors Applied to Adjust for Additional Unrepresented Sensitive Species Data.

Chemical	Draft Acute Freshwater Criterion (mg/L)	Final Acute Freshwater Criterion (mg/L)	Application Factor	PFAS Applied To
PFOA	49	3.1	15.8	PFBA, PFHxA, PFNA, PFDA, 8:2 FTUCA, 7:3 FTCA
PFOS	3.0	0.071	42.3	PFBS, PFHxS

Final benchmark values were calculated by dividing the value derived from the sensitivity distribution using procedures presented in the Aquatic Life Criteria Guidelines (U.S. EPA 1985) by the relevant application factors, to derive benchmark values protective of the aquatic community, including sensitive aquatic insects. The following sections detail the derivation of the acute benchmark values for each of the eight PFAS.

5.2 Derivation of Acute Water Benchmark for Perfluorobutanoic Acid (PFBA)

Quantitatively acceptable empirical data for PFBA were available for five species comprising four genera and fulfilling three MDR groups (**Tables 4-2** and **5-3**). Because only three of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining five MDRs. A total of 22 ICE models were acceptable for use. Of these, three ICE models were not selected for use because empirical data were available for the three genera (i.e., models for *Daphnia pulex*, *Chydorus sphaericus* and *Danio rerio*). One other model was not used because there was an acceptable model for a more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Pimephales promelas*). A total of 18 ICE models were selected for use, resulting in ICE models predicting 18 SMAVs, representing 18 genera and seven MDRs (**Tables 5-2** and **5-3**).

The combined empirical and ICE data resulted in 22 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-3**. GMAVs for the four most sensitive genera were within a factor of eight of each other (**Table 5-4**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFBA is 166.1 mg/L (**Table 5-4**), which is lower than all of the GMAVs except for one tested species (the rotifer, *Brachionus calyciflorus*, GMAV=110 mg/L). The FAV was divided by two to obtain a preliminary value of 83.03 mg/L PFBA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 5.3 mg/L PFBA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFBA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-1**).

Table 5-2. Acceptable Models for ICE-estimated Species Sensitivity to PFBA.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	5,251**	0.0002	5,000	**878.8** (199.6-3,869.5)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	5,251	0.0003	8,694.5	**2,248.7** (1,108.2-4,562.9)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	5,251	0.0001	46,278.3	**959.0** (508.0-1,810.5)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	5,251	0.0003	46,278.3	**4,100.3** (2,125.4-7,909.9)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	5,251**	0.0002	4,894.7	4,297.8 (2,058.5-8,973.1)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	5,251	0.0002	46,500	1,174.4[b] (655.0-2,105.8)
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	13,779	0.023	54,579	**11,531.9** (6,189.6-21,485.3)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**2,013.5** (809.4-5,008.9)
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	13,779**	7.797	649.3	**85,148.8** (12,990.4-558,127.6)
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	0.145	304.5	**19,384.5** (4,544.6-82,682.6)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	5,251	0.37	14,500	**12,920.8** (3,394.0-49,189.4)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	5,251**	0.011	281.6	**7,457.2** (1,357.9-40,952.1)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**1,748.6** (862.3-3,545.6)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	5,251	0.0001	14,500	**677.7** (396.2-1,159.4)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**3,490.7** (670.8-18,164.6)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**3,740.1** (1,061.7-13,176.1)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	5,251**	0.014	4,894.7	**881.4** (169.6-4,582.0)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**2,852.6** (949.6-8,569.8)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**1,759.2** (742.4-4,168.8)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	5,251**	0.009	977.6	1,522.7 (748.6-3,097.1)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	5,251	0.014	8,694.5	**1,528.3** (497.6-4,694.2)
Zebrafish-embryo (Danio rerio-embryo)*	Daphnid (Daphnia magna)	5,251	0.0001	46,500	1,391.4 (413.4-4,683.2)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-3. Ranked PFBA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFBA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	**H**	**Rotifer**	***Brachionus calyciflorus***	**110.0**	**110.0**	**0.04**
2	A	Rainbow trout	*Oncorhynchus mykiss*	677.7	677.7	0.09
3	E	Amphipod	*Gammarus fasciatus*	878.8**	878.8	0.13
4	G	Threeridge	*Amblema plicata*	881.4**	881.4	0.17
5	B	Bluegill	*Lepomis macrochirus*	959.0	959.0	0.22
6	G	Western pearlshell	*Margaritifera falcata*	1,528	1,528	0.26
7	G	Paper pondshell	*Utterbackia imbecillis*	1,749	1,749	0.30
8	G	Washboard	*Megalonaias nervosa*	1,759	1,759	0.35
9	G	Fatmucket	*Lampsilis siliquoidea*	2,013	2,013	0.39
10	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	2,249	2,249	0.43
11	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	2,853	2,853	0.48
12	G	Swamp lymnaea	*Lymnaea stagnalis*	3,491	3,491	0.52
13	G	Tadpole physa	*Physella gyrina*	3,740	3,740	0.57
14	D	Daphnid	*Ceriodaphnia dubia*	4,100	4,100	0.61
15	**D**	**Daphnid**	***Chydorus sphaericus***	**>4,281**	**>4,281**	**0.65**
16	**D**	**Daphnid**	***Daphnia magna***	**4,741**	**4,741**	**0.70**
	D	**Daphnid**	***Daphnia pulicaria***	**>1,006[a]**		
17	E	Oriental river shrimp	*Macrobrachium nipponense*	7,457**	7,457	0.74
18	B	Fathead minnow	*Pimephales promelas*	11,532	11,532	0.78
19	F	Midge	*Paratanytarsus parthenogeneticus*	12,921	12,921	0.83
20	**B**	**Zebrafish**	***Danio rerio***	**13,779**	**13,779**	**0.87**
21	B	Goldfish	*Carassius auratus*	19,385**	19,385	0.91
22	C	Flagfish	*Jordanella floridae*	85,149**	85,149	0.96

a Not used in GMAV calculation, because values represents a greater than low value

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR 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, 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.

Table 5-4. PFBA Protective Aquatic Life Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=22 GMAVs total

Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	***Brachionus***	**110.0**	4.70	22.09	0.043	0.209
2	*Oncorhynchus*	677.8	6.52	42.49	0.087	0.295
3	*Gammarus*	878.8	6.78	45.95	0.130	0.361
4	*Amblema*	881.4	6.78	45.99	0.174	0.417
		Σ (Sum):	**24.78**	**156.53**	**0.43**	**1.28**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 125.08 S = slope
L = 2.612 L = X-axis intercept
A = 5.112 A = lnFAV
FAV = 166.1
FAV/2 = 83.03 mg/L (Preliminary Value)
Adjustment = 83.03 / 15.8 = 5.255 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **5.3 mg/L PFBA** (rounded to two significant figures)

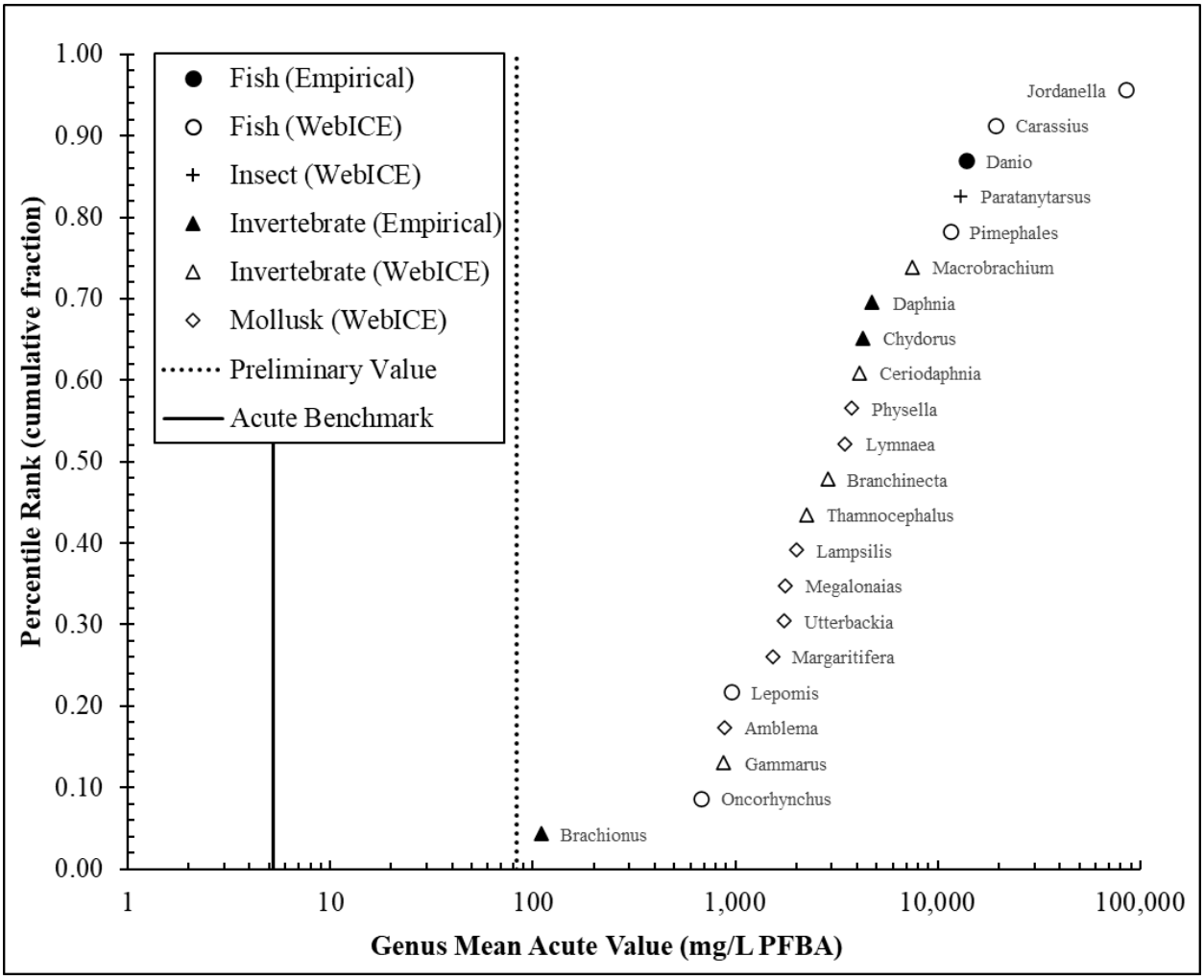

Figure 5-1. Ranked Acute PFBA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.3 Derivation of Acute Water Benchmark for Perfluorohexanoic Acid (PFHxA)

Quantitatively acceptable empirical data for PFHxA were available for four species comprising three genera and fulfilling three MDR groups (**Tables 4-2** and **5-6**). Because only three of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining five MDRs. A total of 28 ICE models were accepted for use. Of these, two ICE models were not selected for use because empirical data were available for the genus (i.e., models for *Daphnia magna* and *Daphnia pulex*). Three other ICE models were not used because

there was an acceptable model for a more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Lepomis macrochirus; Daphnia magna* predicting for *Pimephales promelas*; and *Daphnia magna* predicting for *Oncorhynchus mykiss*). A total of 23 ICE models were selected for use, resulting in ICE models predicting 23 SMAVs representing, 21 genera, and 6 MDRs (**Tables 5-5** and **5-6**).

The combined empirical and ICE data resulted in 24 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-6**. GMAVs for the four most sensitive genera were within a factor of 2.4 of each other (**Table 5-7**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFHxA is 150.4 mg/L (**Table 5-7**), which is lower than all of the GMAVs except for one tested species, the rotifer, *Brachionus calyciflorus*, GMAV=140 mg/L). The FAV was divided by two to obtain a preliminary value of 75.21 mg/L PFHxA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 4.8 mg/L PFHxA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFHxA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-2**).

Table 5-5. Acceptable Models for ICE-estimated Species Sensitivity of PFHxA.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	1,048	0.0002	5,000	**234.7** (64.5-853.7)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	1,048	0.0003	8,694.5	**517.3** (280.8-953.1)
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1,105**	0.003	233	**1,576.9** (395.3-6,289.9)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	1,048	0.0001	46,278	303.5[b] (176.0-523.2)
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**719.8** (362.4-1,429.7)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	1,048	0.0003	46,278	**785.1** (446.2-1,381.6)
Daphnid (Daphnia magna)*	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	424.1 (68.5-2,627.9)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	1,048	0.0002	4,894.7	841.4 (444.8-1,591.6)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	1,048	0.0002	46,500	384.7[b] (235.8-627.7)
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**1,083.6** (468.5-2,506.4)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**470.8** (220.2-1,006.8)
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**1,027.7** (405.9-2,602.1)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	1,048	0.37	14,500	**2,676.6** (917.7-7,806.3)
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	1,105	18.6	20,900	**805.1** (394.5-1,643.4)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	1,048**	0.042	166.8	**404.3** (69.6-2,349.3)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	1,048**	0.011	281.6	**1,172.2** (290.4-4,732.5)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**418.5** (231.7-755.9)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	1,048	0.0001	14,500	214.2[b] (135.8-337.8)
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**713.5** (200.3-2,541.4)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**762.0** (188.8-3,075.8)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**794.5** (277.3-2,276.3)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	1,048	0.014	4,894.7	**191.3** (48.0-761.8)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**623.8** (247.0-1,575.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**372.3** (181.1-765.2)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	1,048**	0.009	977.6	**329.4** (183.8-590.5)
Wavyrayed lampmussel (Lampsilis fasciola)	Daphnid (Daphnia magna)	1,048	166.8	4,894.7	**686.2** (127.8-3,684.7)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	1,048	0.014	8,694.5	**370.2** (145.8-940.1)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	1,048	0.0001	46,500	**481.7** (172.4-1,346.1)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-6. Ranked PFHxA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFHxA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	**H**	**Rotifer**	***Brachionus calyciflorus***	**140.0**	**140.0**	**0.04**
2	G	Threeridge	*Amblema plicata*	191.3	191.3	0.08
3	E	Amphipod	*Gammarus fasciatus*	234.7	234.7	0.12
4	D	Water flea	*Chydorus sphaericus*	329.4**	329.4	0.16
5	G	Western pearlshell	*Margaritifera falcata*	370.2	370.2	0.20
6	G	Washboard	*Megalonaias nervosa*	372.3	372.3	0.24
7	G	Paper pondshell	*Utterbackia imbecillis*	418.5	418.5	0.28
8	B	Zebrafish	*Danio rerio-embryo*	481.7	481.7	0.32
9	G	Wavyrayed lampmussel	*Lampsilis fasciola*	686.2	507.4	0.36
	G	Neosho mucket	*Lampsilis rafinesqueana*	404.3**		
	G	Fatmucket	*Lampsilis siliquoidea*	470.8		
10	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	517.3	517.3	0.40
11	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	623.8	623.8	0.44
12	A	Rainbow trout	*Oncorhynchus mykiss*	713.5	713.5	0.48
13	B	Channel catfish	*Ictalurus punctatus*	719.8	719.8	0.52
14	G	Swamp lymnaea	*Lymnaea stagnalis*	762.0	762.0	0.56
15	D	Daphnid	*Ceriodaphnia dubia*	785.1	785.1	0.60
16	G	Tadpole physa	*Physella gyrina*	794.5	794.5	0.64
17	B	Mosquitofish	*Gambusia affinis*	805.1	805.1	0.68
18	**C**	**Bullfrog**	***Lithobates catesbeianus***	**1,105**	**915.2**	**0.72**
	C	**Green frog**	***Lithobates clamitans***	**758.0**		
19	B	Goldfish	*Carassius auratus*	1,028	1,028	0.76
20	**D**	**Daphnia**	***Daphnia magna***	**1,048**	**1,048**	**0.80**
21	B	Fathead minnow	*Pimephales promelas*	1,084	1,084	0.84
22	E	Oriental river shrimp	*Macrobrachium nipponense*	1,172**	1,172	0.88
23	B	Bluegill	*Lepomis macrochirus*	1,577**	1,577	0.92
24	F	Midge	*Paratanytarsus parthenogeneticus*	2,677	2,677	0.96

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR 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, 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.

Table 5-7. PFHxA Final Acute Value and Protective Aquatic Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=24 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	***Brachionus***	**140.0**	4.94	24.42	0.040	0.200
2	*Amblema*	191.3	5.25	27.60	0.080	0.283
3	*Gammarus*	234.7	5.46	29.79	0.120	0.346
4	*Chydorus*	329.4	5.80	33.61	0.160	0.400
		Σ (Sum):	**21.45**	**115.42**	**0.40**	**1.23**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 17.41 S = slope
L = 4.080 L = X-axis intercept
A = 5.013 A = lnFAV
FAV = 150.4
FAV/2 = 75.21 mg/L (Preliminary Value)
Adjustment = 75.21 / 15.8 = 4.760 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **4.8 mg/L PFHxA** (rounded to two significant figures)

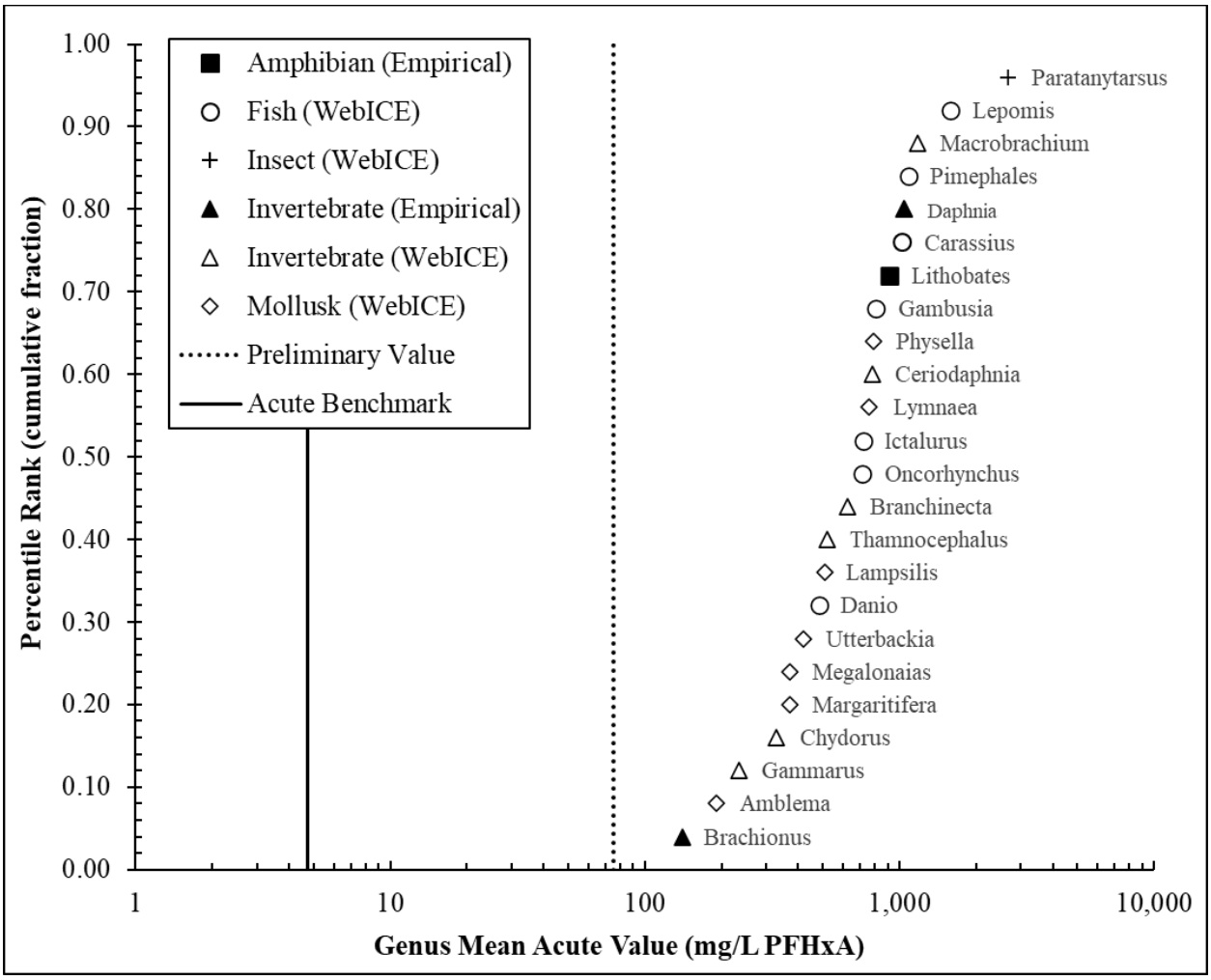

Figure 5-2. Ranked Acute PFHxA GMAVs Used for the Aquatic Life Acute Benchmark Calculations.

5.4 Derivation of Acute Water Benchmark for Perfluorononanoic Acid (PFNA)

Quantitatively acceptable empirical data for PFNA were available for three species comprising three genera and fulfilling two MDR groups (**Tables 4-2** and **5-9**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 29 ICE models were accepted for use. Of these, four ICE models were not selected for use because empirical data were available for two genera (i.e., models for *Daphnia magna*, *Daphnia pulex*, *Daphnia pulicaria* and *Chydorus sphaericus*). One other ICE model was

not used because there was an acceptable model for a more closely taxonomically-related model where the measured surrogate EC_{50} value fell within the ICE model range (i.e., *Daphnia magna* predicting for *Danio rerio*). A total of 24 ICE models were selected for use resulting in ICE models predicting 24 SMAVs representing 22 genera and 7 MDRs (**Tables 5-8** and **5-9**).

The combined empirical and ICE data resulted in 25 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-9**. GMAVs for the four most sensitive genera were within a factor of 2.0 of each other (**Table 5-10**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFNA is 20.58 mg/L (**Table 5-10**), which is lower than all of the GMAVs except for the one ICE-derived species value for the threeridge mussel, *Amblema plicata* (GMAV=17.58 mg/L). The FAV was divided by two to obtain a preliminary value of 10.29 mg/L PFNA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.65 mg/L PFNA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFNA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-3**).

Table 5-8. Acceptable Models for ICE-estimated Species Sensitivity to PFNA.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	84.51	0.0002	5,000	**29.8** (10.9-81.6)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	84.51**	0.0001	68.3	**57.2** (12.4-263.1)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	84.51	0.0003	8,694.5	**52.1** (32.2-84.2)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	84.51	0.0001	46,278	**50.3** (33.3-75.9)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	84.51	0.0003	46,278	**59.4** (38.2-92.1)
Daphnid (Daphnia magna)*	Water flea (Chydorus sphaericus)	27.84	0.007	462.0	76.4 (48.0-121.5)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	84.51	0.0002	4,894.7	65.8 (40.3-107.5)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	84.51	0.014	281.6	84.2 (18.0-392.8)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	84.51**	0.0002	7.2	**39.7** (8.4-187.6)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	84.51	0.0002	46,500	**67.3** (47.1-96.2)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**48.6** (27.6-85.6)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	84.51	0.0003	166.8	**1,261.8** (217.7-7314.9)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	84.51	0.37	14,500	**228.8** (101.9-513.5)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	84.51	0.042	166.8	**42.9** (12.1-152.7)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	84.51	0.003	281.6	**181.9** (43.1-768.2)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	84.51	0.011	281.6	**65.1** (24.3-174.3)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**44.8** (28.6-70.2)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	84.51	0.042	545.9	**54.4** (13.3-222.4)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	84.51	0.0001	14,500	**35.4** (25.2-49.7)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**70.7** (24.0-207.9)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**70.6** (31.3-159.2)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	84.51	0.014	4,894.7	**17.6** (6.1-50.8)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**58.0** (28.6-117.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**32.9** (18.5-58.5)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	84.51	0.009	977.5	30.1 (19.3-47.1)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	84.51	0.009	281.6	**44.3** (8.8-223.1)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	84.51	0.014	8,694.5	**40.4** (19.8-82.5)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	84.51**	0.0001	50.0	104.2[c] (15.4-703.8)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	84.51	0.0001	46,500	**91.8** (42.1-200.2)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-9. Ranked PFNA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFNA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	G	Threeridge	*Amblema plicata*	17.58	17.58	0.04
2	**D**	**Daphnid**	***Chydorus sphaericus***	**27.84**	**27.84**	**0.08**
3	G	Washboard	*Megalonaias nervosa*	32.90	32.90	0.12
4	A	Rainbow trout	*Oncorhynchus mykiss*	35.40	35.40	0.15
5	D	Daphnid	*Simocephalus serrulatus*	39.74**	39.74	0.19
6	G	Western pearlshell	*Margaritifera falcata*	40.41	40.41	0.23
7	E	Amphipod	*Gammarus fasciatus*	29.82	41.28	0.27
	E	Amphipod	*Gammarus pseudolimnaeus*	57.16**		
8	D	Water flea	*Moina macrocopa*	44.31	44.31	0.31
9	G	Paper pondshell	*Utterbackia imbecillis*	44.82	44.82	0.35
10	G	Neosho mucket	*Lampsilis rafinesqueana*	42.93	45.69	0.38
	G	Fatmucket	*Lampsilis siliquoidea*	48.63		
11	B	Bluegill	*Lepomis macrochirus*	50.29	50.29	0.42
12	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	52.08	52.08	0.46
13	G	Pheasantshell	*Ortmanniana pectorosa*	54.41	54.41	0.50
14	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	58.02	58.02	0.54
15	D	Daphnid	*Ceriodaphnia dubia*	59.35	59.35	0.58
16	E	Oriental river shrimp	*Macrobrachium nipponense*	65.10	65.10	0.62
17	B	Fathead minnow	*Pimephales promelas*	67.29	67.29	0.65
18	G	Tadpole physa	*Physella gyrina*	70.62	70.62	0.69
19	G	Swamp lymnaea	*Lymnaea stagnalis*	70.68	70.68	0.73
20	**D**	**Daphnid**	***Daphnia magna***	**84.51**	**84.51**	**0.77**
21	B	Zebrafish	*Danio rerio*	91.85	91.85	0.81
22	H	Oligochaete	*Limnodrilus hoffmeisteri*	181.9	181.9	0.85
23	F	Midge	*Paratanytarsus parthenogeneticus*	228.8	228.8	0.88
24	**C**	**Clawed frog**	***Xenopus sp.***	**335.8**	**335.8**	**0.92**
25	E	Isopod	*Asellus aquaticus*	1,262	1,262	0.96

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR 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, 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.

Table 5-10. PFNA Final Acute Value and Protective Aquatic Acute Benchmark.
Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=25 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Amblema*	17.58	2.87	8.22	0.038	0.196
2	***Chydorus***	**27.84**	3.33	11.07	0.077	0.277
3	*Megalonaias*	32.90	3.49	12.20	0.115	0.340
4	*Oncorhynchus*	35.40	3.57	12.72	0.154	0.392
		Σ (Sum):	**13.25**	**44.21**	**0.38**	**1.21**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 13.85 S = slope
L = 2.192 L = X-axis intercept
A = 3.024 A = lnFAV
FAV = 20.58
FAV/2 = 10.29 mg/L (Preliminary Value)
Adjustment = 10.29 / 15.8 = 0.6511 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.65 mg/L PFNA** (rounded to two significant figures)

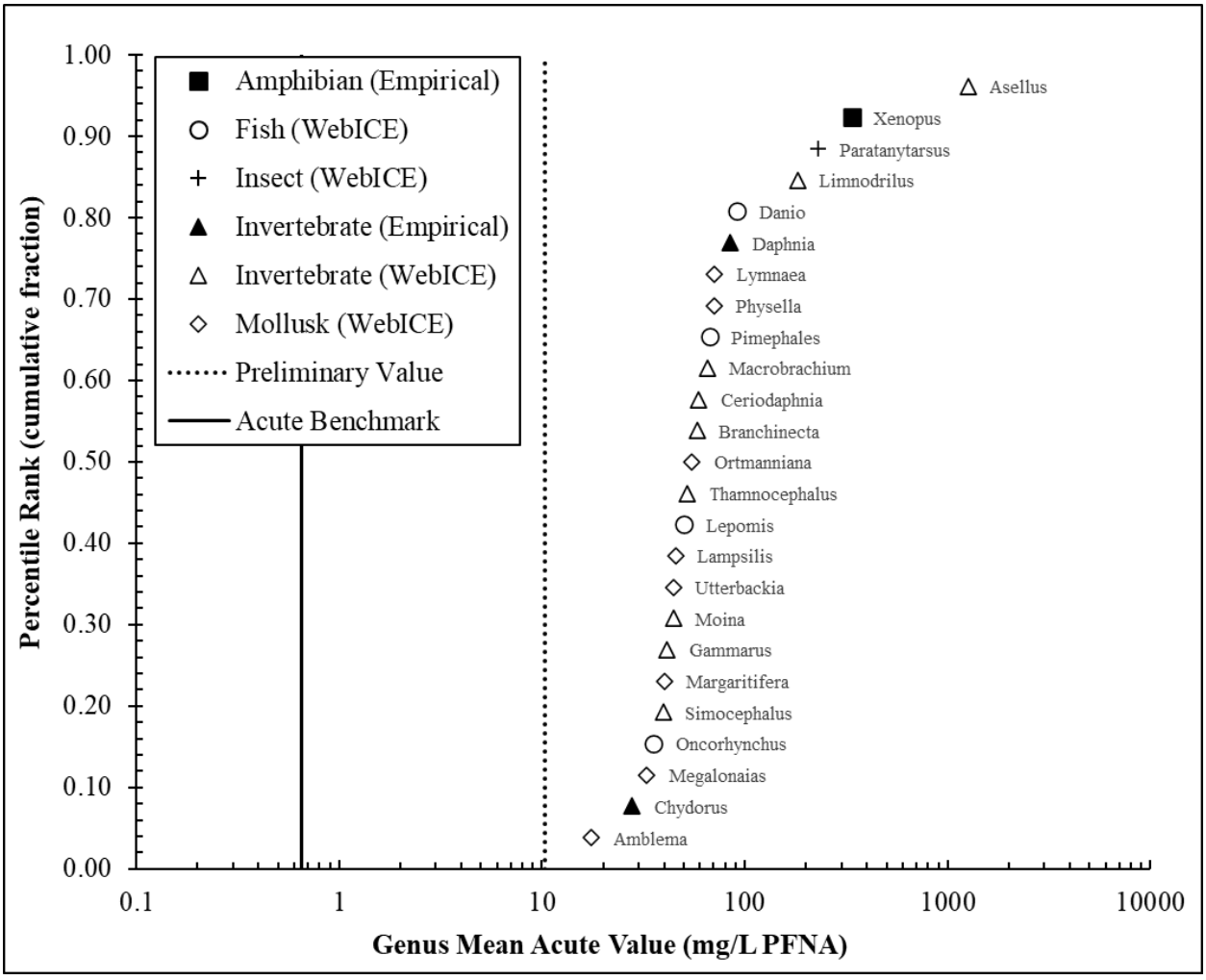

Figure 5-3. Ranked Acute PFNA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.5 Derivation of Acute Water Benchmark for Perfluorodecanoic Acid (PFDA)

Quantitatively acceptable empirical data for PFDA were available for five species comprising four genera and fulfilling three MDR groups (**Tables 4-2** and **5-12**). Because only three of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining five MDRs. A total of 61 ICE models were accepted for use. Of these, 11 ICE models were not selected for use because empirical data were available for the two genera (e.g., models for *Oncorhynchus gilae* and *Daphnia magna*). Three other ICE models were not used because

there was an acceptable model for a more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Lepomis macrochirus*; *Daphnia magna* predicting for *Pimephales promelas*; and *Daphnia magna* predicting for *Danio rerio*). A total of 46 ICE models were selected for use resulting in ICE models predicting 46 SMAVs representing 38 genera and all eight MDRs (**Tables 5-11** and **5-12**).

The combined empirical and ICE data resulted in 42 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-12**. GMAVs for the four most sensitive genera were within a factor of 2.9 of each other (**Table 5-13**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFDA is 15.71 mg/L (**Table 5-13**), which is lower than all of the GMAVs except for the ICE-derived species values for the isopod, *Caecidotea brevicauda*, GMAV= 8.17 mg/L. The FAV was divided by two to obtain a benchmark value of 7.856 mg/L PFDA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.50 mg/L PFDA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFDA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-4**).

Table 5-11. Acceptable Models for ICE-estimated Species Sensitivity to PFDA.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	119.7	0.0002	5,000	**39.7** (14.0-112.7)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	119.7**	0.0001	68.3	**78.6** (16.1-384.0)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	32**	0.0041	1.625	39.4 (9.0-173.4)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	95.86	**31.0** (15.6-61.3)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	119.7	0.0003	8,694.5	**71.5** (43.6-117.5)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	32	0.0001	8,341.5	**33.1** (27.6-39.6)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	119.7	0.0001	46,278	64.5[b] (42.0-99.0)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	32	0.003	43.1	**72.7** (12.6-420.2)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	96.7	**31.4** (18.8-52.5)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	95.9	**33.1** (19.8-55.3)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	13,400	**58.8** (23.7-145.4)
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	32**	0.004	1.625	**114.0** (33.0-394.0)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	13,400	**31.6** (19.9-50.1)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	32	0.003	724.4	40.0 (24.0-66.9)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	43.1	49.4 (38.4-63.6)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	198.4	**35.6** (16.3-77.7)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	198.4	27.1 (18.7-39.3)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	32	0.71	155.5	**51.8** (30.8-87.1)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	119.7	0.0003	46,278	**84.8** (53.8-133.7)
Daphnid (Daphnia magna)*	Water flea (Chydorus sphaericus)	41.13	0.007	462.0	114.5 (70.9-184.8)
Daphnid (Daphnia magna)*	Daphnid (Daphnia pulicaria)	149.6	0.009	237.4	122.1 (27.4-544.5)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	119.7	0.0002	4,894.7	93.7 (56.2-156.0)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	119.7	0.014	281.6	122.0 (24.2-614.6)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	119.7**	0.0002	7.2	**55.1** (11.0-275.1)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	13,400	**46.7** (36.0-60.7)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	119.7	0.0002	46,500	85.6[b] (58.9-124.4)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**66.6** (37.0-119.8)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	13,400	**62.2** (31.6-122.5)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	32**	<0.0001	19.9	**45.8** (19.9-105.3)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	198.4	**28.3** (7.6-105.3)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	119.7	0.0003	166.8	**1,699.9** (273.7-10,558.5)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	82.0	**8.2** (1.9-35.7)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	198.4	**18.0** (11.4-28.3)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	95.9	**19.5** (9.5-40.3)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	32	0.007	54.4	**52.1** (10.9-248.7)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	1,330	**105.8** (35.6-314.4)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	119.7	0.37	14,500	**321.4** (140.5-735.3)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	119.7	0.042	166.8	**58.5** (15.4-222.1)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	32	0.002	198.4	**64.7** (33.7-124.2)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	119.7	0.003	281.6	**232.8** (51.5-1,052.2)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	119.7	0.011	281.6	**97.1** (34.6-272.8)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**61.0** (38.3-97.2)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	119.7	0.042	545.9	**76.0** (17.4-332.2)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	119.7	0.0001	14,500	45.4 (31.9-64.7)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	32	0.003	43.1	**26.6** (5.3-134.0)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	32	0.03	95.9	**45.7** (11.2-185.6)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	32**	0.0014	7.075	**73.0** (29.7-179.7)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	32**	0.03	7.5	25.7 (4.0-164.6)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	32**	0.03	9.7	**312.8** (88.4-1,106.8)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**98.2** (32.2-299.3)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**98.7** (42.7-227.9)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	119.7	0.014	4,894.7	**24.5** (8.2-73.1)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**80.6** (38.8-167.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**46.0** (25.6-82.8)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	119.7	0.009	977.6	41.9 (26.5-66.4)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	119.7	0.009	281.6	**56.9** (10.6-305.9)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	119.7	0.014	8,694.5	**54.9** (26.3-114.5)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	32**	<0.0001	16.2	**22.2** (5.1-95.7)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	229.1	**17.6** (6.4-48.5)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	8,341.5	**34.4** (16.8-70.4)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	119.7	0.0001	46,500	115.5[b] (51.4-259.4)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-12. Ranked PFDA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFDA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	E	Isopod	*Caecidotea brevicauda*	8.173	8.173	0.02
2	B	Largemouth bass	*Micropterus salmoides*	19.52	19.52	0.05
3	B	Yellow perch	*Perca flavescens*	22.20**	22.20	0.07
4	A	Brook trout	*Salvelinus fontinalis*	31.44	23.80	0.09
	A	Lake trout	*Salvelinus namaycush*	18.01		
5	G	Threeridge	*Amblema plicata*	24.46	24.46	0.12
6	B	Zebrafish	*Danio rerio*	24.56	24.56	0.14
7	B	Razorback sucker	*Xyrauchen texanus*	26.57	26.57	0.16
8	B	Guppy	*Poecilia reticulata*	28.31	28.31	0.19
9	B	Channel catfish	*Ictalurus punctatus*	31.55	31.55	0.21
10	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**32.00**	**32.00**	**0.23**
11	A	Atlantic salmon	*Salmo salar*	30.96	32.02	0.26
	A	Brown trout	*Salmo trutta*	33.12		
12	B	Common carp	*Cyprinus carpio*	35.58	35.58	0.28
13	B	Green sunfish	*Lepomis cyanellus*	45.79**	38.92	0.30
	B	Bluegill	*Lepomis macrochirus*	33.08		
14	**D**	**Daphnid**	***Chydorus sphaericus***	**41.13**	**41.13**	**0.33**
15	B	Shortnose sturgeon	*Acipenser brevirostrum*	45.67	45.67	0.35
16	G	Washboard	*Megalonaias nervosa*	46.01	46.01	0.37
17	B	Fathead minnow	*Pimephales promelas*	46.72	46.72	0.40
18	C	Cuvier's foam froglet	*Physalaemus cuvieri*	51.77	51.77	0.42
19	B	Medaka	*Oryzias latipes*	52.12	52.12	0.44
20	G	Western pearlshell	*Margaritifera falcata*	54.89	54.89	0.47
21	D	Daphnid	*Simocephalus serrulatus*	55.07**	55.07	0.49
22	E	Amphipod	*Gammarus fasciatus*	39.66	55.84	0.51
	E	Amphipod	*Gammarus pseudolimnaeus*	78.62**		
23	D	Water flea	*Moina macrocopa*	56.93	56.93	0.53
24	G	Paper pondshell	*Utterbackia imbecillis*	61.04	61.04	0.56
25	B	Goldfish	*Carassius auratus*	62.18	62.18	0.58
26	G	Neosho mucket	*Lampsilis rafinesqueana*	58.54	62.42	0.60
	G	Fatmucket	*Lampsilis siliquoidea*	66.56		
27	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	71.54	71.54	0.63
28	B	Bonytail	*Gila elegans*	72.68	72.68	0.65
29	B	Silver perch	*Bidyanus bidyanus*	73.03**	73.03	0.67
30	G	Pheasantshell	*Ortmanniana pectorosa*	76.02	76.02	0.70
31	**C**	**Clawed frog**	***Xenopus sp.***	**76.50**	**76.50**	**0.72**

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
32	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	80.58	80.58	0.74
33	D	Daphnid	*Ceriodaphnia dubia*	84.82	84.82	0.77
34	E	Oriental river shrimp	*Macrobrachium nipponense*	97.09	97.09	0.79
35	G	Swamp lymnaea	*Lymnaea stagnalis*	98.19	98.19	0.81
36	G	Tadpole physa	*Physella gyrina*	98.69	98.69	0.84
37	C	Bullfrog	*Lithobates catesbeianus*	58.75	106.0	0.86
	C	Northern leopard frog	*Lithobates pipiens*	64.72		
	C	Southern leopard frog	*Lithobates sphenocephalus*	312.8**		
38	B	Cape Fear shiner	*Notropis mekistocholas*	114.0**	114.0	0.88
39	**D**	**Daphnid**	***Daphnia magna***	**145.5**	**147.5**	**0.91**
	D	**Daphnid**	***Daphnia pulicaria***	**149.6**		
40	F	Midge	*Paratanytarsus dissimilis*	105.8	184.4	0.93
	F	Midge	*Paratanytarsus parthenogeneticus*	321.4		
41	H	Oligochaete	*Limnodrilus hoffmeisteri*	232.8	232.8	0.95
42	E	Isopod	*Asellus aquaticus*	1,700	1,700	0.98

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR 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, 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.

Table 5-13. PFDA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=42 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Caecidotea*	8.17	2.10	4.41	0.023	0.152
2	*Micropterus*	19.52	2.97	8.83	0.047	0.216
3	*Perca*	22.20	3.10	9.61	0.070	0.264
4	*Salvelinus*	23.80	3.17	10.05	0.093	0.305
		Σ (Sum):	**11.34**	**32.90**	**0.23**	**0.94**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 57.22 S = slope
L = 1.063 L = X-axis intercept
A = 2.754 A = lnFAV
FAV = 15.71
FAV/2 = 7.856 mg/L (Preliminary Value)
Adjustment = 7.856 / 15.8 = 0.4972 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.50 mg/L PFDA** (rounded to two significant figures)

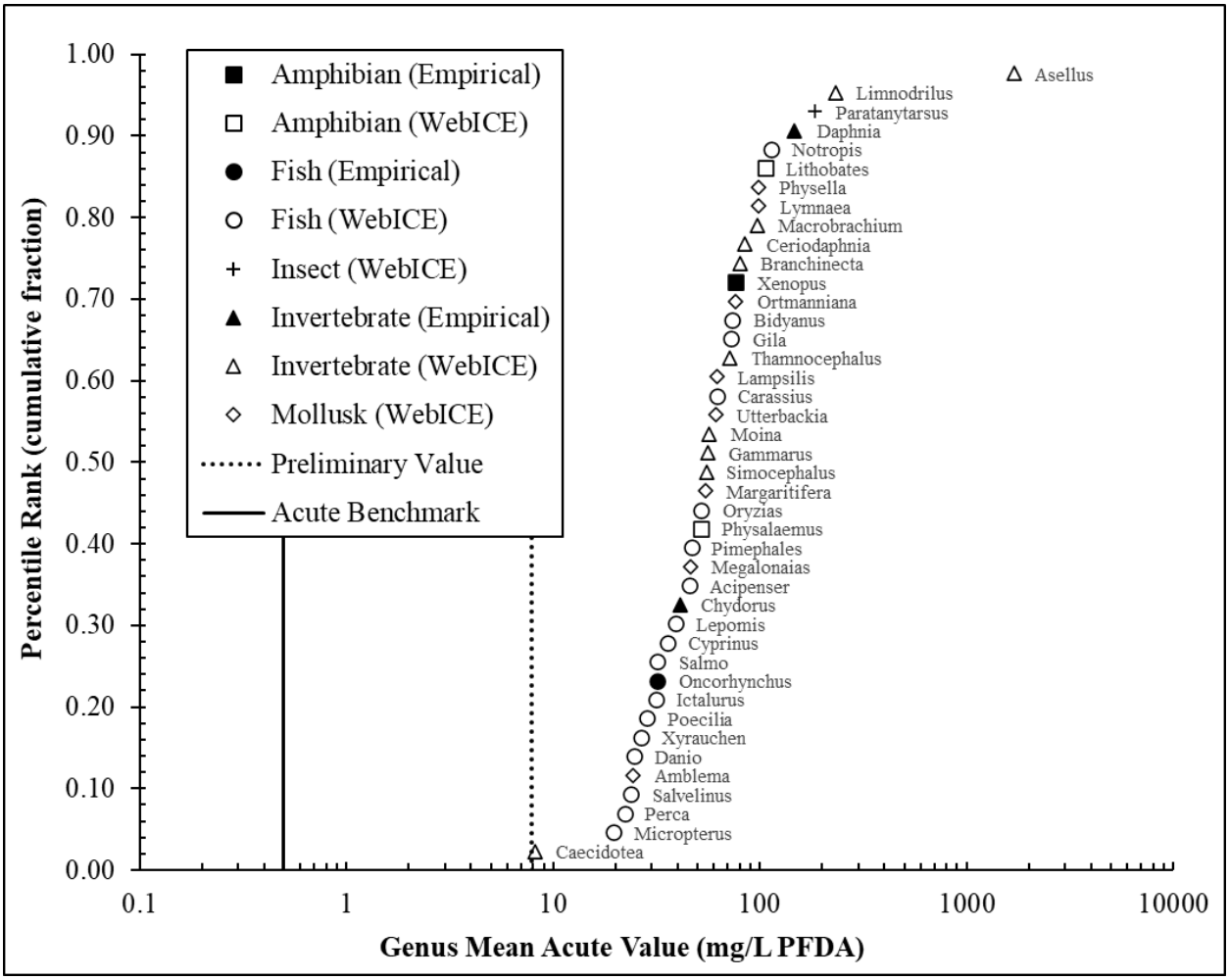

Figure 5-4. Ranked Acute PFDA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.6 Derivation of Acute Water Benchmark for Perfluorobutanesulfonic Acid (PFBS)

Quantitatively acceptable empirical data for PFBS were available for four species comprising four genera and fulfilling two MDR groups (**Tables 4-2** and **5-15**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 55 ICE models were acceptable for use. Of these, thirteen ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Lepomis macrochirus* and *Daphnia magna*). Three ICE models were not selected for use because

they were derived based on saltwater species surrogates (i.e., model for an amphipod, *Hyalella azteca*; daphnid, *Ceriodaphnia dubia*; and rainbow trout, *Oncorhynchus mykiss*). Four other models were not used because there were acceptable models for more closely-related surrogate species (i.e., *Lepomis macrochirus* predicting for *Cyprinus carpio*; *Lepomis macrochirus* predicting for *Carassius auratus*; *Daphnia magna* predicting for *Oncorhynchus mykiss*; and *Pimephales promelas* predicting for *Amblema plicata*). Two additional models (models for the bullfrog, *Lithobates catesbeianus* and channel catfish, *Ictalurus punctatus*) were not used because the measured EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related species with measured EC_{50} values within the ICE model range were used. A total of 33 ICE models were selected for use, resulting in ICE models predicting 30 SMAVs representing 26 genera and seven MDRs (**Tables 5-14** and **5-15**). An ICE model was not available to fulfill the MDR for a family in any order of insect or any phylum not already represented (**Tables 5-14** and **5-15**).

The combined empirical and ICE data resulted in 30 GMAVs that collectively fulfill seven MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-15**. Surrogate species EC_{50} values fell outside the ICE model range for 14 of the predicted SMAVs and the resultant GMAVs are less certain than GMAVs based on ICE models where surrogate EC_{50} values were in the range of the model. GMAVs for the four most sensitive genera were within a factor of 1.7 of each other (**Table 5-16**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFBS is 422.1 mg/L (**Table 5-16**), which is lower than all of the GMAVs except for the ICE-derived species value for the threeridge mussel, *Amblema plicata* (GMAV=383.6 mg/L). The FAV was

divided by two to obtain a preliminary value of 211.0 mg/L PFBS and then adjusted by the sulfonic acid application factor (42.3) to obtain the freshwater acute water column benchmark magnitude of 5.0 mg/L PFBS (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFBS under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-5**).

Table 5-14. Acceptable Models for ICE-estimated Species Sensitivity to PFBS.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	2,183	0.0002	5,000	**428.1** (107.9-1,697.9)
Amphipod (Hyalella azteca)	Mysid (Americamysis bahia)	372**	<0.0001	193.4	449.4[a] (93.4-2,162.8)
Atlantic salmon (Salmo salar)	Bluegill (Lepomis macrochirus)	6,452**	0.004	123.3	**14,523.6** (2,825.4-74,655)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	2,183	0.0003	8,694.5	**1,010** (525.1-1,942.9)
Bluegill (Lepomis macrochirus)*	Daphnid (Daphnia magna)	2,183	0.0001	46,278	512.5 (285.3-920.4)
Bluegill (Lepomis macrochirus)*	Fathead minnow (Pimephales promelas)	1,938	0.0002	50,154	751.0 (390.0-1,446)
Bluegill (Lepomis macrochirus)*	Mysid (Americamysis bahia)	372**	<0.0001	79.0	207.7 (107.0-403.2)
Brook trout (Salvelinus fontinalis)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	123.3	**3,505.7** (612.1-20,077)
Bullfrog (Lithobates catesbeianus)	Fathead minnow (Pimephales promelas)	1,938	0.001	26,500	**1,791.4** (756.1-4,244.6)
Bullfrog (Lithobates catesbeianus)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	201.0	3,420.6[c] (841.4-13,905)
Channel catfish (Ictalurus punctatus)	Fathead minnow (Pimephales promelas)	1,938	0.001	26,500	**1,617.3** (755.0-3,464.4)
Channel catfish (Ictalurus punctatus)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	517.8	1,863.7[c] (713.6-4,867.2)
Coho salmon (Oncorhynchus kisutch)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	150.8	**1,609.5** (248.6-10,421)
Common carp (Cyprinus carpio)	Fathead minnow (Pimephales promelas)	1,938**	0.005	133	**1,952.5** (498.7-7,644.4)
Common carp (Cyprinus carpio)	Bluegill (Lepomis macrochirus)	6,452**	0.0009	180	2,962.4[b] (569.2-15,418.0)

Predicted Species	Surrogate Species	Surrogate Measured EC_{50} (mg/L)	ICE Model Value Range (mg/L) Minimum	ICE Model Value Range (mg/L) Maximum	Predicted EC_{50} (Confidence Limits) (mg/L)
Cutthroat trout (Oncorhynchus clarkii)	Fathead minnow (Pimephales promelas)	1,938**	0.001	140.2	**602.6** (113.4-3,203.2)
Cutthroat trout (Oncorhynchus clarkii)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	180	**2,561.1** (548.4-11,961)
Daphnid (Ceriodaphnia dubia)	Mysid (Americamysis bahia)	372	<0.0001	4,100	171.7 [a] (36.6-804.9)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	2,183	0.0003	46,278	**1,666.6** (909.0-3,055.6)
Daphnid (Daphnia magna)*	Mysid (Americamysis bahia)	372	<0.0001	11,000	243.9 (143.9-413.4)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	2,183	0.0002	4,894.7	1,768.1 (893.9-3,497.1)
Fathead minnow (Pimephales promelas)*	Mysid (Americamysis bahia)	372	<0.0001	11,000	158.6 (74.5-337.5)
Fathead minnow (Pimephales promelas)*	Daphnid (Daphnia magna)	2,183	0.0002	46,500	639.5 (375.7-1,088.5)
Fathead minnow (Pimephales promelas)*	Bluegill (Lepomis macrochirus)	6,452	0.0001	27,540	3,399.2 (1,631.9-7080.3)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**912.5** (399.0-2,086.8)
Goldfish (Carassius auratus)	Fathead minnow (Pimephales promelas)	1,938	0.001	26,500	**2,152.4** (1,075.2-4,309.0)
Goldfish (Carassius auratus)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	201	1,922.7 [b] (312.9-11,813.0)
Green sunfish (Lepomis cyanellus)*	Bluegill (Lepomis macrochirus)	6,452**	0.004	113	2,817.7 (563.5-14,089.5)
Guppy (Poecilia reticulata)	Fathead minnow (Pimephales promelas)	1,938**	0.001	140.2	**499.3** (131.8-1,892.4)
Guppy (Poecilia reticulata)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	180	**687.3** (105.9-4,461.3)
Lake trout (Salvelinus namaycush)	Bluegill (Lepomis macrochirus)	6,452**	0.003	180	**320.8** (49.0-2,102.1)
Largemouth bass (Micropterus salmoides)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	123.3	**8,225.3** (2,938.4-23,024.8)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Medaka (Oryzias latipes)	Fathead minnow (Pimephales promelas)	1,938**	0.003	1,420	**1,013.6** (496.2-2,070.7)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	2,183	0.37	14,500	**5,481.6** (1,674.6-17,943.7)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	2,183**	0.042	166.8	**777.2** (115.1-5,248.1)
Nile tilapia (Oreochromis niloticus)	Bluegill (Lepomis macrochirus)	6,452**	0.002	6.969	**11,873.0** (8,879.5-15,875.6)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	2,183**	0.011	281.6	**2,722.2** (587.7-12,609.0)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**802.5** (422.2-1,525.5)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	2,183	0.0001	14,500	361.9[b] (221.2-592.1)
Rainbow trout (Oncorhynchus mykiss)	Fathead minnow (Pimephales promelas)	1,938	0.0002	26,500	**788.9** (505.7-1,230.8)
Rainbow trout (Oncorhynchus mykiss)	Bluegill (Lepomis macrochirus)	6,452	0.0001	7,100	**3,208.6** (2,275.7-4,524.0)
Rainbow trout (Oncorhynchus mykiss)	Mysid (Americamysis bahia)	372**	<0.0001	113	110.0 [a] (58.8-205.9)
Redear sunfish (Lepomis microlophus)*	Bluegill (Lepomis macrochirus)	6,452**	0.017	4.5	9,763.6 (7,469.9-12,761.7)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**1,523.7** (337.4-6,881.2)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**1,608.6** (512.8-5,046.1)
Threeridge (Amblema plicata)	Fathead minnow (Pimephales promelas)	1,938	0.814	10,831	250.3[b] (39.4-1,592.2)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	2,183	0.014	4,894.7	**383.6** (85.6-1,717.6)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**1,246.4** (457.0-3,399.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**755.1** (345.4-1,650.5)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	2,183**	0.009	977.6	**661.4** (349.2-1,252.8)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	2,183	0.014	8,694.5	**706.1** (255.8-1,948.9)
Yellow perch (Perca flavescens)	Bluegill (Lepomis macrochirus)	6,452**	0.0004	9.41	**3,426.2** (710.8-16,515)
Zebrafish (Danio rerio)*	Fathead minnow (Pimephales promelas)	1,938**	0.013	26.69	1,069.6 (242.1-4,726.4)
Zebrafish-embryo (Danio rerio-embryo)*	Daphnid (Daphnia magna)	2,183	0.0001	46,500	780.8 (257.2-2,370.6)
Zebrafish-embryo (Danio rerio-embryo)*	Fathead minnow (Pimephales promelas)	1,938	0.004	70,200	1,707.9 (1,061.5-2,748)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-15. Ranked PFBS Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFBS toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	G	Threeridge	*Amblema plicata*	383.6	383.6	0.03
2	E	Amphipod	*Gammarus fasciatus*	428.1	428.1	0.06
3	B	Guppy	*Poecilia reticulata*	585.8**	585.8	0.10
4	D	Water flea	*Chydorus sphaericus*	661.4**	661.4	0.13
5	G	Western pearlshell	*Margaritifera falcata*	706.1	706.1	0.16
6	G	Washboard	*Megalonaias nervosa*	755.1	755.1	0.19
7	G	Paper pondshell	*Utterbackia imbecillis*	802.5	802.5	0.23
8	G	Neosho mucket	*Lampsilis rafinesqueana*	777.2**	842.1	0.26
	G	Fatmucket	*Lampsilis siliquoidea*	912.5		
9	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	1,010	1,010	0.29
10	B	Medaka	*Oryzias latipes*	1,014**	1,014	0.32
11	A	Brook trout	*Salvelinus fontinalis*	3,506**	1,060	0.35
	A	Lake trout	*Salvelinus namaycush*	320.8**		
12	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	1,246	1,246	0.39
13	A	Cutthroat trout	*Oncorhynchus clarkii*	1,242**	1,471	0.42
	A	Coho salmon	*Oncorhynchus kisutch*	1,610**		
	A	Rainbow trout	*Oncorhynchus mykiss*	1,591		
14	G	Swamp lymnaea	*Lymnaea stagnalis*	1,524	1,524	0.45
15	G	Tadpole physa	*Physella gyrina*	1,609	1,609	0.48
16	B	Channel catfish	*Ictalurus punctatus*	1,617	1,617	0.52
17	D	Daphnid	*Ceriodaphnia dubia*	1,667	1,667	0.55
18	C	Bullfrog	*Lithobates catesbeianus*	1,791	1,791	0.58
19	**B**	**Fathead minnow**	***Pimephales promelas***	**1,938**	**1,938**	**0.61**
20	B	Common carp	*Cyprinus carpio*	1,952**	1,952	0.65
21	B	Goldfish	*Carassius auratus*	2,152	2,152	0.68
22	**D**	**Daphnid**	***Daphnia magna***	**2,183**	**2,183**	**0.71**
23	E	Oriental river shrimp	*Macrobrachium nipponense*	2,722**	2,722	0.74
24	**B**	**Zebrafish**	***Danio rerio***	**>3,000**	**>3,000**	**0.77**
25	B	Yellow perch	*Perca flavescens*	3,426**	3,426	0.81
26	F	Midge	*Paratanytarsus parthenogeneticus*	5,482	5,482	0.84
27	**B**	**Bluegill**	***Lepomis macrochirus***	**6,452**	**6,452**	**0.87**
28	B	Largemouth bass	*Micropterus salmoides*	8,225**	8,225	0.90
29	B	Nile tilapia	*Oreochromis niloticus*	11,873**	11,873	0.94
30	A	Atlantic salmon	*Salmo salar*	14,524**	14,524	0.97

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR 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, 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.

Table 5-16. PFBS Protective Aquatic Life Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=30 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the sulfonic acid application factor (42.3)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	383.6	5.95	35.40	0.032	0.180
2	*Gammarus*	428.1	6.06	36.72	0.065	0.254
3	*Hyalella*	585.8	6.37	40.61	0.097	0.311
4	*Ceriodaphnia*	661.4	6.49	42.18	0.129	0.359
		Σ (Sum):	**24.88**	**154.90**	**0.32**	**1.10**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 11.02 S = slope
L = 5.303 L = X-axis intercept
A = 6.045 A = lnFAV
FAV = 422.1
FAV/2 = 211.0 mg/L (Preliminary Value)
Adjustment = 211.0 / 42.3 = 4.989 (Preliminary Value / Sulfonic Acid Application Factor)
Benchmark = **5.0 mg/L PFBS** (rounded to two significant figures)

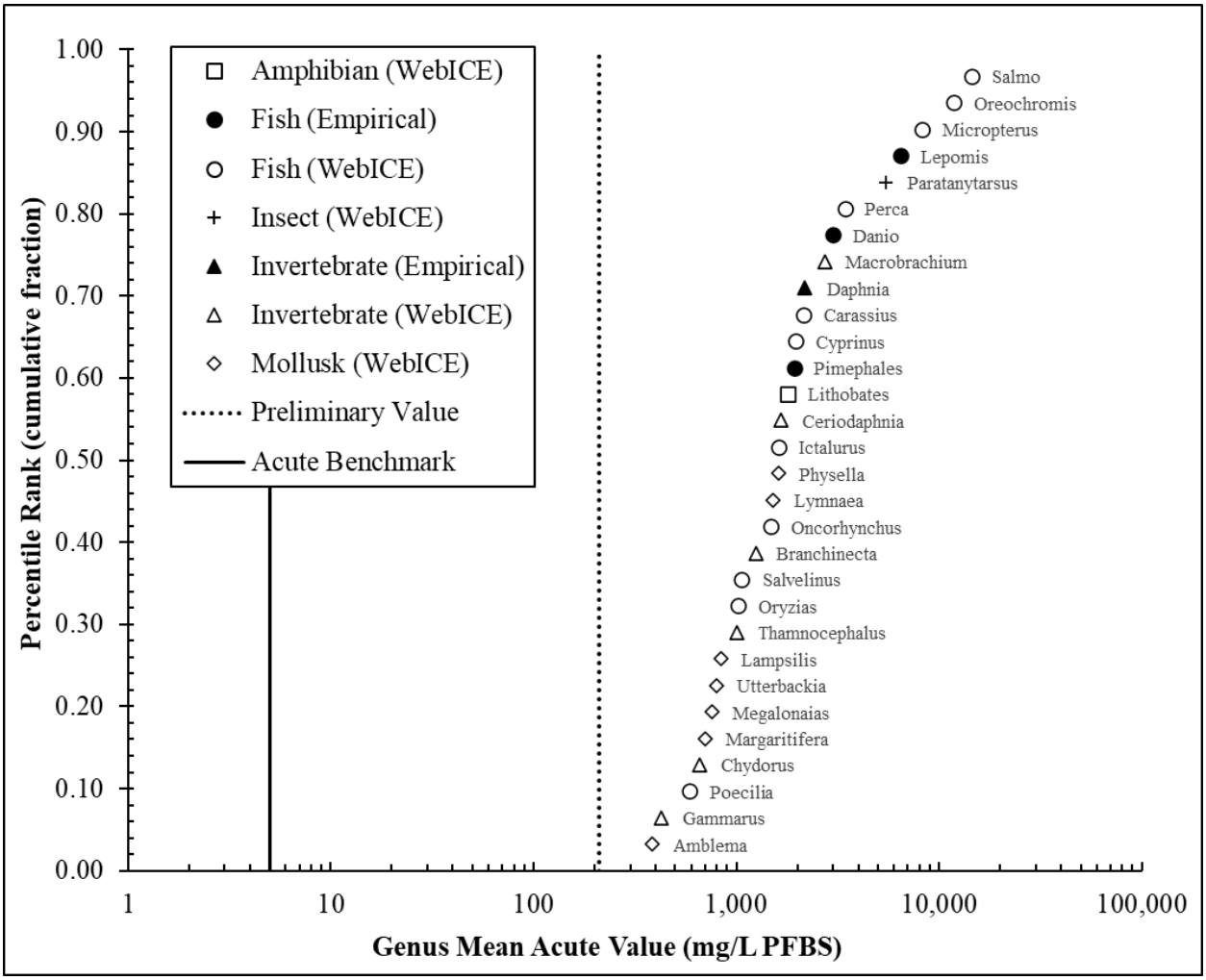

Figure 5-5. Ranked Acute PFBS GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.7 Derivation of Acute Water Benchmark for Perfluorohexanesulfonic Acid (PFHxS)

Quantitatively acceptable empirical data for PFHxS were available for three species comprising two genera and fulfilling two MDR groups (**Tables 4-2** and **5-18**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 17 ICE models were acceptable for use. Of these, four models were not used because there were acceptable models for more closely-related surrogate species (i.e., *Lithobates catesbeianus* predicting for *Ictalurus punctatus*; *Lithobates catesbeianus* predicting

for *Pimephales promelas*; *Lithobates catesbeianus* predicting for *Carassius auratus*; and *Lithobates catesbeianus* predicting for *Oncorhynchus mykiss*). A total of 13 ICE models were selected for use, resulting in ICE models predicting 10 SMAVs representing 10 genera and five MDRs (**Tables 5-17** and **5-18**). ICE models were not available to fulfill two MDRs: an insect, and a family in a phylum other than Arthropoda or Chordata.

The combined empirical and ICE data resulted in 12 GMAVs that collectively fulfill six of the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-18**. GMAVs for the four most sensitive genera were within a factor of 1.2 of each other (**Table 5-19**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFHxS is 18.07 mg/L (**Table 5-19**), which is lower than all of the GMAVs. The FAV was divided by two to obtain a preliminary value of 9.035 mg/L PFHxS and then adjusted by the sulfonic acid application factor (42.3) to obtain the freshwater acute water column benchmark magnitude of 0.21 mg/L PFHxS (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFHxS under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-6**). However, because only six of the eight MDRs were fulfilled for PFHxS (when both empirical test and ICE data were considered), there is considered to be greater uncertainty associated with this benchmark value compared with other PFAS benchmarks developed.

Table 5-17. Acceptable Models for ICE-estimated Species Sensitivity to PFHxS.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1,105**	0.003	233.0	**1,576.9** (395.3-6,289.9)
Channel catfish (Ictalurus punctatus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.145	304.5	**19.5** (9.2-41.0)
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	719.8[b] (362.4-1,429.7)
Daphnid (Daphnia magna)	Zebrafish (Danio rerio)	22.5	0.003	36.91	**5.2** (1.2-22.7)
Daphnid (Daphnia magna)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	**424.1** (68.5-2,627.9)
Fairy shrimp (Streptocephalus rubricaudatus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	4.622	5,100.7	**114.2** (33.4-390.2)
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.023	54,579	**18.3** (13.3-25.2)
Fathead minnow (Pimephales promelas)	Zebrafish (Danio rerio)	22.5	0.018	25.08	**26.0** (11.6-58.2)
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	1,083.6[b] (468.5-2,506.4)
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	22.5	7.797	649.3	**33.9** (19.7-58.4)
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.145	304.5	**37.3** (23.2-59.9)
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	1,027.7[b] (405.9-2,602.1)
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	1,105	18.6	20,900	**805.1** (394.5-1,643.4)
Oligochaete (Limnodrilus hoffmeisteri)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.145	239.3	**63.2** (9.9-403.1)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish-embryo (Danio rerio-embryo)	22.5	0.023	8,843.9	**9.6** (4.2-22.0)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Rainbow trout (Oncorhynchus mykiss)	Zebrafish (Danio rerio)	22.5	0.003	26.39	**38.3** (9.5-155.1)
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1,105	0.003	20,900	713.5[b] (200.3-2,541.4)

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-18. Ranked PFHxS Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFHxS toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	A	Rainbow trout	*Oncorhynchus mykiss*	19.19	19.19	0.08
2	B	Channel catfish	*Ictalurus punctatus*	19.46	19.46	0.15
3	B	Fathead minnow	*Pimephales promelas*	21.79	21.79	0.23
4	**B**	**Zebrafish**	***Danio rerio***	**22.50**	**22.50**	**0.31**
5	B	Flagfish	*Jordanella floridae*	33.90	33.90	0.38
6	B	Goldfish	*Carassius auratus*	37.28	37.28	0.46
7	D	Daphnid	*Daphnia magna*	46.87	46.87	0.54
8	H	Oligochaete	*Limnodrilus hoffmeisteri*	63.23	63.23	0.62
9	E	Fairy shrimp	*Streptocephalus rubricaudatus*	114.2	114.2	0.69
10	B	Mosquitofish	*Gambusia affinis*	805.1	805.1	0.77
11	**C**	**Bullfrog**	***Lithobates catesbeiana***	**1,105**	**915.2**	**0.85**
	C	**Green frog**	***Lithobates clamitans***	**758.0**		
12	B	Bluegill	*Lepomis macrochirus*	1,577**	1,577	0.92

****Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR 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, 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.

Table 5-19. PFHxS Final Acute Value and Protective Aquatic Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=12 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the sulfonic acid application factor (42.3)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)²	P=R/(N+1)	sqrt(P)
1	*Oncorhynchus*	19.19	2.95	8.73	0.077	0.277
2	*Ictalurus*	19.46	2.97	8.81	0.154	0.392
3	*Pimephales*	21.79	3.08	9.50	0.231	0.480
4	***Danio***	**22.50**	3.11	9.69	0.308	0.555
		Σ (Sum):	12.12	36.73	0.77	1.70

P = cumulative probability
R = rank
N = number of GMAVs
S = slope
L = X-axis intercept
A = lnFAV

S^2 = 0.45
L = 2.745
A = 2.894
FAV = 18.07
FAV/2 = 9.035 mg/L (Preliminary Value)
Adjustment = 9.035 / 42.3 = 0.2136 (Preliminary Value / Sulfonic Acid Application Factor)
Benchmark = **0.21 mg/L PFHxS** (rounded to two significant figures)

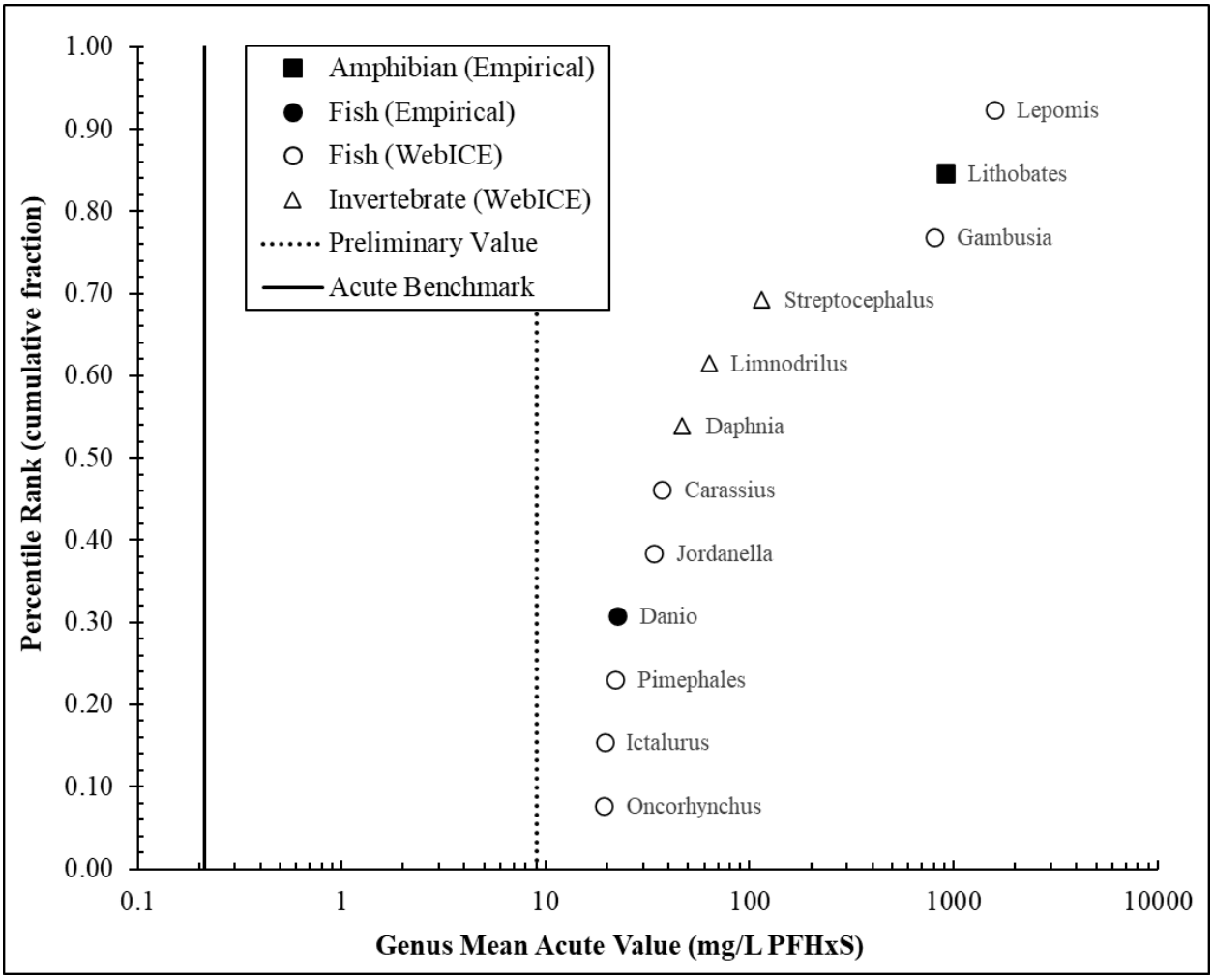

Figure 5-6. Ranked Acute PFHxS GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.8 Derivation of Acute Water Benchmark for Hexadecafluoro-2-decenoic Acid (8:2 FTUCA)

Quantitatively acceptable empirical data for 8:2 FTUCA were available for two species comprising two genera and fulfilling two MDR groups (**Tables 4-2** and **5-21**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 66 ICE models were accepted for use. Of these, nine ICE models were not selected for use because empirical data were available for the two genera (e.g., models for *Oncorhynchus gilae* and *Daphnia pulex*). Four other models were not used because there were

acceptable models for more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Lepomis macrochirus*; *Daphnia magna* predicting for *Pimephales promelas*; *Daphnia magna* predicting for *Danio rerio*). A total of 53 ICE models were selected for use, resulting in ICE models predicting 52 SMAVs representing 43 genera and all eight MDRs (**Tables 5-20** and **5-21**).

The combined empirical and ICE data resulted in 45 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV is summarized in **Table 5-21**. GMAVs for the four most sensitive genera were within a factor of 1.8 of each other (**Table 5-22**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for 8:2 FTUCA is 1.171 mg/L (**Table 5-22**), which is lower than all of the GMAVs except for the ICE-derived species value for the threeridge mussel, *Amblema plicata* (GMAV=0.79 mg/L). The FAV was divided by two to obtain a preliminary value of 0.5853 mg/L 8:2 FTUCA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.037 mg/L 8:2 FTUCA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to 8:2 FTUCA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-7**).

There is a visible gap in the data between the water flea (*Miona macrocopa*, SMAV = 4.2 mg/L) and bryozoan (*Pectinatella magnifica*, SMAV = 15.2 mg/L) for these ICE-generated values (see **Figure 5-7**). Although the exact reason is unclear for this large gap in values, it is noted that the lower portion of the curve is primarily composed of the phyla Mollusca and Arthropoda, while the upper portion of the curve is primarily composed of the phylum Chordata.

The distinct difference in sensitivity indicated by the gap in these data is thus likely to result from a greater estimated sensitivity of most invertebrates than vertebrates to 8:2 FTUCA. Additionally, the ICE estimates for the lower portion of the curve are based primarily on the surrogate *Daphnia magna*, while the ICE estimates for the upper portion of the curve are based primarily on the surrogate *Oncorhynchus mykiss*. However, these differences fall within the range of differences in acute response frequently observed between species. For example, within the PFOA and PFOS acute criteria datasets, there was single species sensitivity that spanned over two orders of magnitude, and the variation in acute toxicity values is frequently an order of magnitude and can be as high as three orders of magnitude (Hrovat et al. 2009, Schür et al. 2023, Raimondo et al. 2024). It is, therefore, not unexpected to see the difference in sensitivity observed between these species.

Table 5-20. Acceptable Models for ICE-estimated Species Sensitivity to 8:2 FTUCA.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	3.2	0.0008	166.8	**28.6** (7.0-116.1)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	3.2	0.0002	5,000	**2.0** (1.0-4.1)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	3.2	0.0001	68.3	**2.9** (1.0-7.8)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	81**	0.004	1.625	109.4 (20.3-589.1)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	95.86	**79.3** (36.6-171.6)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	3.2	0.0003	8,694.5	**2.6** (1.8-3.9)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	3.2	0.0001	46,278	4.9[b] (3.7-6.4)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	81	0.0001	8,341.5	**79.0** (64.3-97.1)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	96.69	**79.4** (44.1-142.9)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	95.86	**83.8** (47.0-149.4)
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	3.2**	0.014	0.232	**15.2** (3.5-66.6)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	13,400	**132.5** (51.0-344.2)
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	81**	0.004	1.625	**339.1** (82.8-1,388.7)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	81	<0.0001	13,400	**67.4** (39.8-114.0)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	81	0.003	724.4	102.9 (57.1-185.4)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	81**	<0.0001	43.07	128.3 (96.6-170.5)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	198.4	**75.2** (31.0-182.2)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	81	<0.0001	198.4	65.5 (42.9-100.0)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	81	0.7099	155.5	**91.1** (48.4-171.5)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	3.2	0.0003	46,278	**2.1** (1.5-2.9)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	3.2**	0.0001	0.646	3.1 (0.8-12.3)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	3.2	0.009	10.36	4.9 (1.0-23.7)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	3.2	0.0002	4,894.7	2.4 (1.7-3.3)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	3.2	0.014	281.6	2.6 (0.7-9.5)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	3.2	0.0002	7.2	**1.8** (0.7-5.2)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	3.2	0.0001	166.8	**1.7** (0.2-12.3)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	3.2	0.0002	46,500	7.0[b] (5.4-9.0)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	13,400	**107.7** (79.8-145.3)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**2.5** (1.6-4.1)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	81	<0.0001	13,400	**132.7** (62.2-283.3)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	3.2	0.014	166.8	**1.9** (0.4-9.4)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	81**	<0.0001	19.93	**105.8** (41.7-269)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	198.4	**54.9** (12.3-243.7)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	3.2	0.0003	166.8	**76.6** (23.0-255.1)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	82	**16.8** (3.2-89.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	198.4	**41.3** (24.5-69.7)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	81	<0.0001	95.86	**46.8** (20.6-106.2)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	81**	0.007	54.4	**121.6** (20.2-733.2)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	81	0.0006	1,330	**241.0** (72.2-804.7)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	3.2	0.37	14,500	**9.3** (3.5-24.7)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	3.2	0.0003	58	**1.3** (0.3-6.2)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	3.2	0.042	166.8	**2.3** (1.1-5.1)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	81	0.002	198.4	**125.5** (62.3-252.8)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	3.2	0.003	281.6	**17.9** (6.9-46.2)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	3.2	0.0001	4,894.7	**31.6** (7.4-134.7)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	3.2	0.011	281.6	**1.5** (0.7-3.4)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**2.5** (1.6-3.7)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	3.2	0.042	545.9	**2.3** (0.8-7.0)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	3.2	0.0001	14,500	3.4 (2.7-4.3)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	81**	0.003	43.07	**54.8** (8.5-352.2)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	81	0.028	95.86	**134.5** (26.7-677.1)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	81**	0.0014	7.075	**200.3** (73.6-544.8)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	81**	0.028	9.7	**1,023.9** (231.9-4,520.4)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**3.2** (1.2-8.4)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**3.0** (1.4-6.7)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	3.2	0.014	4,894.7	**0.8** (0.3-2.1)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**2.6** (1.4-4.9)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**1.4** (0.8-2.6)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	3.2	0.009	977.6	**1.3** (0.8-2.2)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	3.2	0.009	281.6	**4.2** (1.3-13.2)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	3.2	0.014	8,694.5	**2.3** (1.1-4.6)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	81**	<0.0001	16.24	**51.2** (10.0-262.6)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	3.2	0.0001	50	8.3[b] (2.5-28.0)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	229.1	**34.6** (11.3-106.0)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	3.2	0.0001	46,500	10.6[b] (5.7-19.9)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	81	0.0002	8,341.5	**65.2** (29.0-146.3)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-21. Ranked 8:2 FTUCA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical 8:2 FTUCA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	G	Threeridge	*Amblema plicata*	0.7894	0.7894	0.02
2	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	1.327	1.327	0.04
3	D	Water flea	*Chydorus sphaericus*	1.344	1.344	0.07
4	G	Washboard	*Megalonaias nervosa*	1.403	1.403	0.09
5	E	Oriental river shrimp	*Macrobrachium nipponense*	1.518	1.518	0.11
6	D	Daphnid	*Simocephalus serrulatus*	1.848	1.795	0.13
	D	Daphnid	*Simocephalus vetulus*	1.743		
7	G	Green floater	*Lasmigona subviridis*	1.900	1.900	0.15
8	D	Daphnid	*Ceriodaphnia dubia*	2.066	2.066	0.17
9	G	Western pearlshell	*Margaritifera falcata*	2.268	2.268	0.20
10	G	Pheasantshell	*Ortmanniana pectorosa*	2.347	2.347	0.22
11	E	Amphipod	*Gammarus fasciatus*	2.040	2.412	0.24
	E	Amphipod	*Gammarus pseudolimnaeus*	2.853		
12	G	Neosho mucket	*Lampsilis rafinesqueana*	2.325	2.430	0.26
	G	Fatmucket	*Lampsilis siliquoidea*	2.540		
13	G	Paper pondshell	*Utterbackia imbecillis*	2.454	2.454	0.28
14	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	2.632	2.632	0.30
15	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	2.645	2.645	0.33
16	G	Tadpole physa	*Physella gyrina*	3.035	3.035	0.35
17	**D**	**Daphnid**	***Daphnia magna***	**3.200**	**3.200**	**0.37**
18	G	Swamp lymnaea	*Lymnaea stagnalis*	3.210	3.210	0.39
19	D	Water flea	*Moina macrocopa*	4.192	4.192	0.41
20	H	Bryozoan	*Pectinatella magnifica*	15.21**	15.21	0.43
21	E	Isopod	*Caecidotea brevicauda*	16.82	16.82	0.46
22	H	Oligochaete	*Limnodrilus hoffmeisteri*	17.89	17.89	0.48
23	E	Amphipod	*Crangonyx pseudogracilis*	28.57	28.57	0.50
24	H	Oligochaete	*Tubifex tubifex*	31.57	31.57	0.52
25	B	Largemouth bass	*Micropterus salmoides*	46.83	46.83	0.54
26	B	Zebrafish	*Danio rerio*	47.47	47.47	0.57
27	F	Midge	*Paratanytarsus dissimilis*	241.0	47.45	0.59
	F	Midge	*Paratanytarsus parthenogeneticus*	9.343		
28	B	Yellow perch	*Perca flavescens*	51.18**	51.18	0.61
29	B	Razorback sucker	*Xyrauchen texanus*	54.83**	54.83	0.63

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
30	B	Guppy	*Poecilia reticulata*	54.85	54.85	0.65
31	A	Brook trout	*Salvelinus fontinalis*	79.36	57.25	0.67
	A	Lake trout	*Salvelinus namaycush*	41.30		
32	B	Channel catfish	*Ictalurus punctatus*	67.38	67.38	0.70
33	B	Common carp	*Cyprinus carpio*	75.18	75.18	0.72
34	E	Isopod	*Asellus aquaticus*	76.56	76.56	0.74
35	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**81.00**	**81.00**	**0.76**
36	A	Atlantic salmon	*Salmo salar*	79.29	81.49	0.78
	A	Brown trout	*Salmo trutta*	83.76		
37	C	Cuvier's foam froglet	*Physalaemus cuvieri*	91.14	91.14	0.80
38	B	Green sunfish	*Lepomis cyanellus*	105.8**	91.43	0.83
	B	Bluegill	*Lepomis macrochirus*	78.99		
39	B	Fathead minnow	*Pimephales promelas*	107.7	107.7	0.85
40	B	Medaka	*Oryzias latipes*	121.6**	121.6	0.87
41	B	Goldfish	*Carassius auratus*	132.7	132.7	0.89
42	B	Shortnose sturgeon	*Acipenser brevirostrum*	134.5	134.5	0.91
43	B	Silver perch	*Bidyanus bidyanus*	200.3**	200.3	0.93
44	C	Bullfrog	*Lithobates catesbeianus*	132.5	257.2	0.96
	C	Northern leopard frog	*Lithobates pipiens*	125.5		
	C	Southern leopard frog	*Lithobates sphenocephalus*	1,024**		
45	B	Cape Fear shiner	*Notropis mekistocholas*	339.1**	339.1	0.98

**Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR 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, 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.

Table 5-22. 8:2 FTUCA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=45 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	0.7894	-0.24	0.06	0.022	0.147
2	*Palaemonetes*	1.327	0.28	0.08	0.043	0.209
3	*Chydorus*	1.344	0.30	0.09	0.065	0.255
4	*Megalonaias*	1.403	0.34	0.11	0.087	0.295
		Σ (Sum):	**0.68**	**0.34**	**0.22**	**0.91**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 18.39 S = slope
L = -0.801 L = X-axis intercept
A = 0.158 A = lnFAV
FAV = 1.171
FAV/2 = 0.5853 mg/L (Preliminary Value)
Adjustment = 0.5853 / 15.8 = 0.03705 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.037 mg/L 8:2 FTUCA** (rounded to two significant figures)

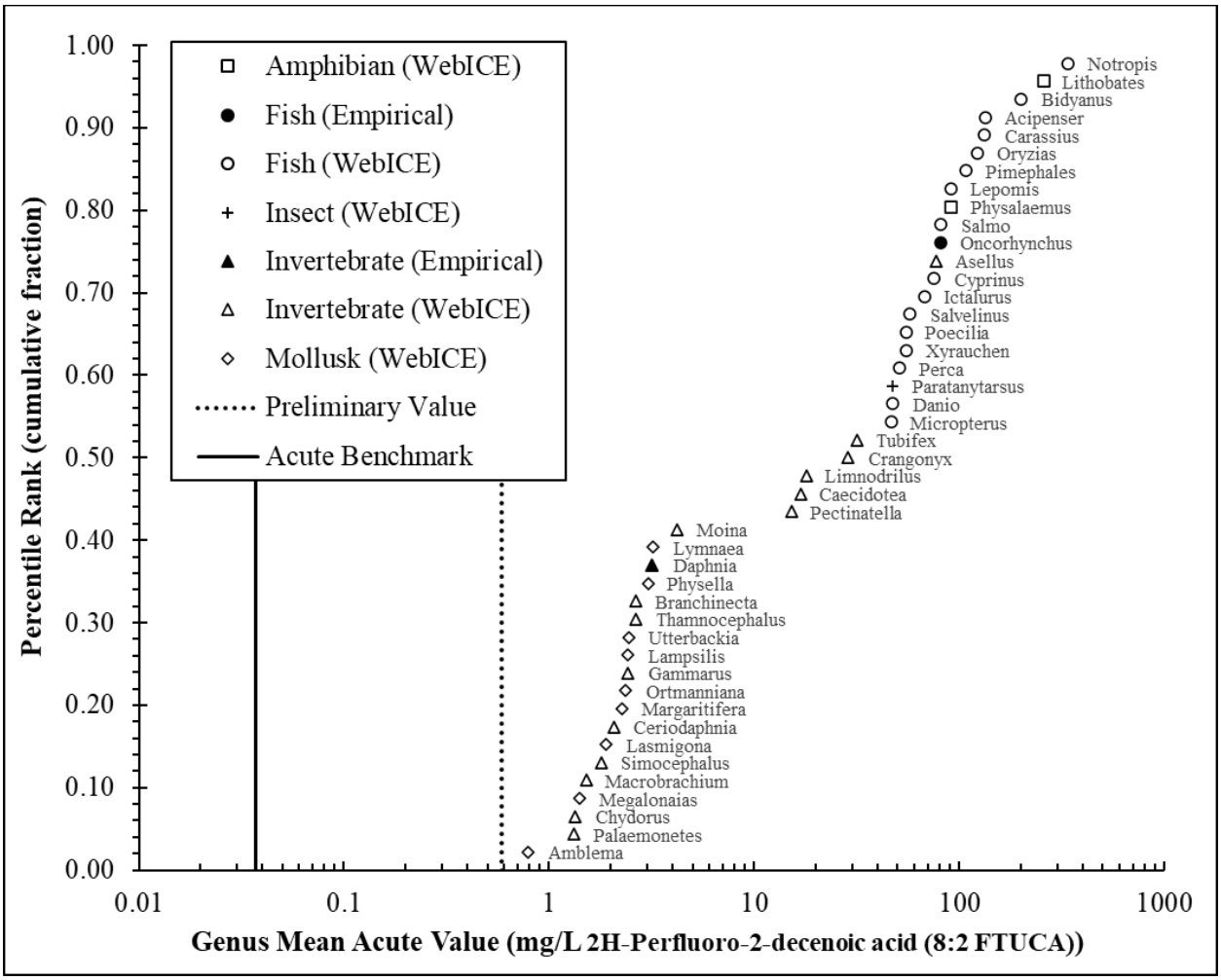

Figure 5-7. Ranked Acute 8:2 FTUCA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.9 Derivation of Acute Water Benchmark for Pentadecafluorodecanoic Acid (7:3 FTCA)

Quantitatively acceptable empirical data for 7:3 FTCA were available for two species comprising two genera and fulfilling two MDR groups (**Tables 4-2** and **5-24**). Because only two of the MDRs were met with empirical data, ICE-derived values were used to fulfill the remaining six MDRs. A total of 70 ICE models were accepted for use. Of these, ten ICE models were not selected for use because empirical data were available for the two genera (e.g., models for *Oncorhynchus gilae* and *Daphnia pulex*). Four other models were not used because there were

acceptable models for more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Lepomis macrochirus*; *Daphnia magna* predicting for *Pimephales promelas*; *Daphnia magna* predicting for *Danio rerio*). A total of 56 ICE models were selected for use, resulting in ICE models predicting 55 SMAVs representing 46 genera and all eight MDRs (**Tables 5-23** and **5-24**).

The combined empirical and ICE data resulted in 48 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table 5-24**. GMAVs for the four most sensitive genera were within a factor of 1.7 of each other (**Table 5-25**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for 7:3 FTCA is 0.3727 mg/L (**Table 5-25**), which is lower than all of the GMAVs except for the ICE-derived species value for the threeridge mussel, *Amblema plicata* (GMAV=0.2519 mg/L). The FAV was divided by two to obtain a preliminary value of 0.1864 mg/L 7:3 FTCA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.012 mg/L 7:3 FTCA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to 7:3 FTCA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure 5-8**).

There is a gap in the data between the water flea (*Moina macrocopa*, SMAV = 1.76 mg/L) and midge (*Chironomus tentans*, SMAV = 4.63 mg/L) for these ICE-generated values (see **Figure 5-8**). This trend was also noted for 8:2 FTUCA, the other fluorotelomer evaluated. Although it is unclear the exact reason for this gap in values, it is noted that the lower portion of the curve is primarily composed of the phyla Mollusca and Arthropoda, while the upper portion

of the curve is primarily composed of the phylum Chordata. The distinct difference in sensitivity indicated by the gap in these data is thus likely to result from a greater estimated sensitivity of most invertebrates than vertebrates to 7:3 FTCA. Additionally, and as for 8:2 FTUCA, the ICE estimates for the lower portion of the curve are based primarily on the surrogate *Daphnia magna*, while the ICE estimates for the upper portion of the curve are based primarily on the surrogate *Oncorhynchus mykiss*. However, these differences fall within the range of differences in acute response frequently observed between species. For example, within the PFOA and PFOS acute criteria datasets, there was single species sensitivity that spanned over two orders of magnitude, and the variation in acute toxicity values is frequently an order of magnitude and can be as high as three orders of magnitude (Hrovat et al. 2009, Schür et al. 2023). It is, therefore, not unexpected to see the difference in sensitivity observed between these species.

Table 5-23. Acceptable Models for ICE-estimated Species Sensitivity to 7:3 FTCA.

Includes values that were extrapolated as indicated. Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	0.9592	0.0008	166.8	**9.5** (2.8-32.4)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	0.9592	0.0002	5,000	**0.8** (0.4-1.4)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	0.9592	0.0001	68.3	**0.9** (0.4-2.2)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	32**	0.004	1.625	39.4 (9.0-173.4)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	95.9	**31.0** (15.6-61.3)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	0.9592	0.0003	8,694.5	**0.9** (0.6-1.3)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	0.9592	0.0001	46,278	2.1[b] (1.6-2.7)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	32	0.0001	8,341.5	**33.1** (27.6-39.6)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	32	0.003	43.07	**72.7** (12.6-420.2)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	96.69	**31.4** (18.8-52.5)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	95.86	**33.1** (19.8-55.3)
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	0.9592**	0.014	0.232	**5.3** (1.9-15.4)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	13,400	**58.8** (23.7-145.4)
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	32**	0.004	1.625	**114.0** (33.0-394.0)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	13,400	**31.6** (19.9-50.1)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	32	0.003	724.4	40.0 (24.0-66.9)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	43.07	49.4 (38.4-63.6)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	198.4	**35.6** (16.3-77.7)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	198.4	27.1 (18.7-39.3)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	32	0.7099	155.5	**51.8** (30.8-87.1)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	0.9592	0.0003	46,278	**0.6** (0.4-0.8)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	0.9592**	0.0001	0.646	1.0 (0.3-3.2)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	0.9592	0.0089	10.36	1.1 (0.3-4.3)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	0.9592	0.0002	4,894.7	0.7 (0.5-0.9)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	0.9592	0.014	281.6	0.7 (0.2-3.1)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	0.9592	0.0002	7.2	**0.6** (0.3-1.4)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	0.9592	0.0001	166.8	**0.7** (0.1-3.8)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	0.9592	0.0002	46,500	3.0[b] (2.3-3.9)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	13,400	**46.7** (36.0-60.7)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.9** (0.5-1.5)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	13,400	**62.2** (31.6-122.5)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	0.9592	0.014	166.8	**0.8** (0.2-3.5)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	32**	<0.0001	19.93	**45.8** (19.9-105.3)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	198.4	**28.3** (7.6-105.3)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	0.9592	0.0003	166.8	**27.3** (9.3-80.1)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	82.0	**8.2** (1.9-35.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	198.4	**18.0** (11.4-28.3)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	32	<0.0001	95.86	**19.5** (9.5-40.3)
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	0.9592	0.0008	4.369	**131.2** (18.7-920.2)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	32	0.007	54.4	**52.1** (10.9-248.7)
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	0.9592	0.0003	472	**4.6** (0.7-32.5)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	32	0.0006	1,330	**105.8** (35.6-314.4)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	0.9592	0.37	14,500	**2.9** (0.9-9.1)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	0.9592	0.0003	58	**0.5** (0.1-1.9)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	0.9592	0.042	166.8	**0.8** (0.4-1.6)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	32	0.002	198.4	**64.7** (33.7-124.2)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	0.9592	0.003	281.6	**7.6** (3.1-18.5)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	0.9592	0.0001	4,894.7	**10.8** (3.0-38.4)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	0.9592	0.011	281.6	**0.4** (0.2-0.9)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.8** (0.5-1.3)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	0.9592	0.042	545.9	**0.7** (0.2-2.4)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	0.9592	0.0001	14,500	1.4 (1.2-1.8)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	32	0.003	43.07	**26.6** (5.3-134.0)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	32	0.028	95.86	**45.7** (11.2-185.6)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	32**	0.001	7.075	**73.0** (29.7-179.7)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	32**	0.028	7.5	25.7 (4.0-164.6)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	32**	0.028	9.7	**312.8** (88.4-1,106.8)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**1.0** (0.4-2.9)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**1.0** (0.4-2.3)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	0.9592	0.014	4,894.7	**0.3** (0.1-0.7)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.8** (0.4-1.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.4** (0.2-0.9)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	0.9592	0.009	977.6	**0.4** (0.2-0.7)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	0.9592	0.009	281.6	**1.8** (0.6-5.2)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	0.9592	0.014	8,694.5	**0.8** (0.4-1.7)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	32**	<0.0001	16.24	**22.2** (5.1-95.7)

Predicted Species	Surrogate		ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Minimum	Maximum	
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	0.9592	0.0001	50	3.3[b] (1.1-10.0)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	229.1	**17.6** (6.4-48.5)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	0.9592	0.0001	46,500	4.8[b] (2.5-9.2)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	32	0.0002	8,341.5	**34.4** (16.8-70.4)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table 5-24. Ranked 7:3 FTCA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical 7:3 FTCA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
1	G	Threeridge	*Amblema plicata*	0.2519	0.2519	0.02
2	E	Oriental river shrimp	*Macrobrachium nipponense*	0.3806	0.3806	0.04
3	D	Water flea	*Chydorus sphaericus*	0.4278	0.4278	0.06
4	G	Washboard	*Megalonaias nervosa*	0.4393	0.4393	0.08
5	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	0.5324	0.5324	0.10
6	D	Daphnid	*Ceriodaphnia dubia*	0.6004	0.6004	0.12
7	D	Daphnid	*Simocephalus serrulatus*	0.5972	0.6489	0.14
	D	Daphnid	*Simocephalus vetulus*	0.7051		
8	G	Pheasantshell	*Ortmanniana pectorosa*	0.7379	0.7379	0.16
9	G	Western pearlshell	*Margaritifera falcata*	0.7857	0.7857	0.18
10	G	Neosho mucket	*Lampsilis rafinesqueana*	0.7949	0.8254	0.20
	G	Fatmucket	*Lampsilis siliquoidea*	0.8571		
11	G	Paper pondshell	*Utterbackia imbecillis*	0.8426	0.8426	0.22
12	E	Amphipod	*Gammarus fasciatus*	0.7600	0.8483	0.24
	E	Amphipod	*Gammarus pseudolimnaeus*	0.9468		
13	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	0.8488	0.8488	0.27
14	G	Green floater	*Lasmigona subviridis*	0.8497	0.8497	0.29
15	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	0.8773	0.8773	0.31
16	G	Tadpole physa	*Physella gyrina*	0.9531	0.9531	0.33
17	**D**	**Daphnid**	***Daphnia magna***	**0.9592**	**0.9592**	**0.35**
18	G	Swamp lymnaea	*Lymnaea stagnalis*	1.029	1.029	0.37
19	D	Water flea	*Moina macrocopa*	1.760	1.760	0.39
20	F	Midge	*Chironomus tentans*	4.630	4.630	0.41
21	H	Bryozoan	*Pectinatella magnifica*	5.340**	5.340	0.43
22	H	Oligochaete	*Limnodrilus hoffmeisteri*	7.621	7.621	0.45
23	E	Isopod	*Caecidotea brevicauda*	8.173	8.173	0.47
24	E	Amphipod	*Crangonyx pseudogracilis*	9.512	9.512	0.49
25	H	Oligochaete	*Tubifex tubifex*	10.75	10.75	0.51
26	F	Midge	*Paratanytarsus dissimilis*	105.75	17.45	0.53
	F	Midge	*Paratanytarsus parthenogeneticus*	2.880		
27	B	Largemouth bass	*Micropterus salmoides*	19.52	19.52	0.55
28	B	Yellow perch	*Perca flavescens*	22.20**	22.20	0.57
29	A	Brook trout	*Salvelinus fontinalis*	31.44	23.80	0.59

Rank	MDR Group[1]	Name	Species	SMAV (mg/L)	GMAV (mg/L)	Percentile Rank
	A	Lake trout	*Salvelinus namaycush*	18.01		
30	B	Zebrafish	*Danio rerio*	24.56	24.56	0.61
31	B	Razorback sucker	*Xyrauchen texanus*	26.57	26.57	0.63
32	E	Isopod	*Asellus aquaticus*	27.30	27.30	0.65
33	B	Guppy	*Poecilia reticulata*	28.31	28.31	0.67
34	B	Channel catfish	*Ictalurus punctatus*	31.55	31.55	0.69
35	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**32.00**	**32.00**	**0.71**
36	A	Atlantic salmon	*Salmo salar*	30.96	32.02	0.73
	A	Brown trout	*Salmo trutta*	33.12		
37	B	Common carp	*Cyprinus carpio*	35.58	35.58	0.76
38	B	Green sunfish	*Lepomis cyanellus*	45.79**	38.92	0.78
	B	Bluegill	*Lepomis macrochirus*	33.08		
39	B	Shortnose sturgeon	*Acipenser brevirostrum*	45.67	45.67	0.80
40	B	Fathead minnow	*Pimephales promelas*	46.72	46.72	0.82
41	C	Cuvier's foam froglet	*Physalaemus cuvieri*	51.77	51.77	0.84
42	B	Medaka	*Oryzias latipes*	52.12	52.12	0.86
43	B	Goldfish	*Carassius auratus*	62.18	62.18	0.88
44	B	Bonytail	*Gila elegans*	72.68	72.68	0.90
45	B	Silver perch	*Bidyanus bidyanus*	73.03**	73.03	0.92
46	C	Bullfrog	*Lithobates catesbeianus*	58.75	106.0	0.94
	C	Northern leopard frog	*Lithobates pipiens*	64.72		
	C	Southern leopard frog	*Lithobates sphenocephalus*	312.8**		
47	B	Cape Fear shiner	*Notropis mekistocholas*	114.0**	114.0	0.96
48	H	Leech	*Nephelopsis obscura*	131.2	131.2	0.98

******Measured EC_{50} falls outside range of ICE model and predicted SMAV is based on extrapolation beyond model bounds.

1: Freshwater MDR 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, 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.

Table 5-25. 7:3 FTCA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=48 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Amblema*	0.2519	-1.38	1.90	0.020	0.143
2	*Macrobrachium*	0.3806	-0.97	0.93	0.041	0.202
3	*Chydorus*	0.4278	-0.85	0.72	0.061	0.247
4	*Megalonaias*	0.4393	-0.82	0.68	0.082	0.286
		Σ (Sum):	**-4.02**	**4.23**	**0.20**	**0.88**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 17.53 S = slope
L = -1.923 L = X-axis intercept
A = -0.987 A = lnFAV
FAV = 0.3727
FAV/2 = 0.1864 mg/L (Preliminary Value)
Adjustment = 0.1864 / 15.8 = 0.01179 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.012 mg/L 7:3 FTCA** (rounded to two significant figures)

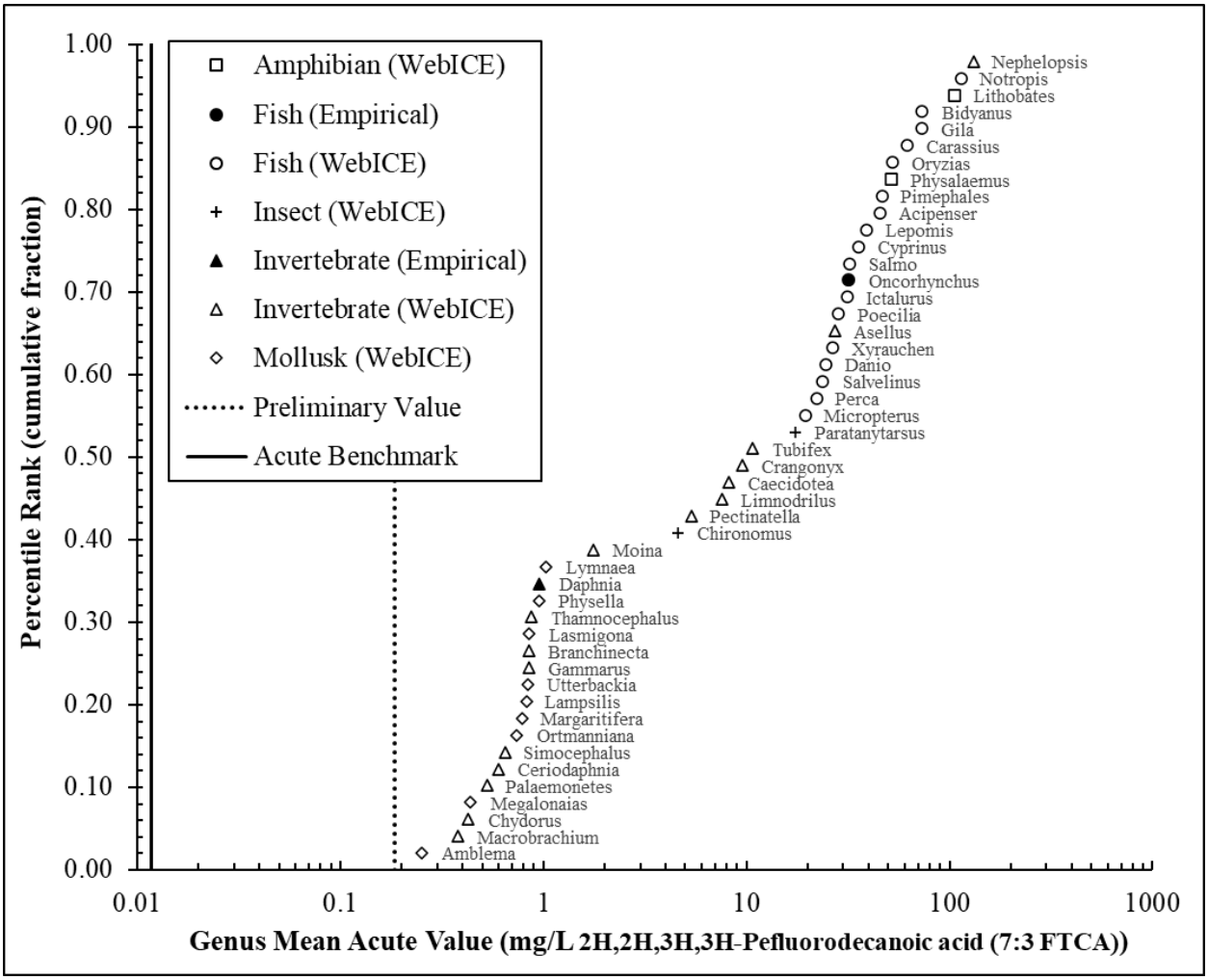

Figure 5-8. Ranked Acute 7:3 FTCA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

5.10 Summary of Benchmarks for Evaluated PFAS Substances and Effects Characterization

5.10.1 Summary and Evaluation of Benchmark Values

Freshwater acute benchmarks for the eight selected PFAS are summarized in **Table 5-26**. These concentrations are expected to be protective of 95% of freshwater genera exposed to these PFAS under short-term conditions of one-hour of duration, if the one-hour average magnitude is not exceeded more than once in three years. However, because only limited empirical toxicity test data were available for the eight PFAS and the use of ICE models were required to fill in

data gaps to derive the benchmarks, the recommended aquatic life acute ambient water quality benchmarks are less certain than the standard Aquatic Life AWQC typically developed.

Quantitatively acceptable empirical toxicity data were coupled with ICE-predicted values to try to fulfill the eight MDRs for deriving acute freshwater criteria per the Aquatic Life Criteria Guidelines (U.S. EPA 1985). Using this approach, the eight MDRs were fulfilled for six of the evaluated compounds (PFBA, PFHxA, PFNA, PFDA, 8:2 FTUCA, and 7:3 FTCA). Seven of the eight MDRs were fulfilled for PFBS while six of the eight MDRs were fulfilled for PFHxS; thus, there is somewhat greater uncertainty associated with these latter benchmarks. It is also noted that ICE models for some chemicals were highly dependent on one or a couple of surrogate species. For example, *Daphnia magna* comprised the majority of surrogate species for PFNA. This heavy reliance on a surrogate potentially increases the uncertainty associated with these ICE models. Conversely, there is somewhat less uncertainty associated with those PFAS benchmarks with empirical test endpoints amongst the most sensitive test species. Notably, empirical test data comprised the mean acute value for the most sensitive test species for PFBA (*Brachionus calyciflorus*), PFHxA (*Brachionus calyciflorus*), and PFHxS (*Danio rerio*), and the second most sensitive species for PFNA (*Chydorus sphaericus*). Mean acute values comprised of empirical test data did not fall amongst the most sensitive test species for the remaining PFAS for which acute benchmark values were derived. The resulting acute water column-based benchmark magnitudes range from 0.012 mg/L for 7:3 FTCA to 5.3 mg/L for PFBA.

Table 5-26. Acute Freshwater Benchmarks for Eight PFAS (mg/L, ppm).

Chemical	PFBA	PFHxA	PFNA	PFDA	PFBS	PFHxS	8:2 FTUCA	7:3 FTCA
Magnitude	5.3	4.8	0.65	0.50	5.0	0.21	0.037	0.012
Duration	One hour average							
Frequency	Not to be exceeded more than once in three years on average							

Table 5-27 compares the model inputs and freshwater acute benchmarks where the ICE input PFAS value (surrogate value) was beyond the model range and the model prediction used for deriving the EPA's acute PFAS benchmark values was based on the extrapolation approach or using an alternative scaled approach, as discussed in **Section 3** (see also **Appendix F)**. The alternative scaled approach adjusts the toxicity values to bring them within the ICE model range, when necessary, by changing the measured toxicity units from mg/L to µg/L so the values fall within the range of ICE toxicity values, rather than allowing regressions to extrapolate beyond the model range. This alternative approach underwent detailed evaluation by Raimondo et al. (2024) and values were calculated using the scaled approach to compare with values calculated using the extrapolation approach.

As shown in **Table 5-27**, the scaled approach allowed for the acceptance of up to two times more models than the extrapolation approach, based primarily on the greater number of scaled inputs and associated output values meeting the confidence interval acceptability parameters presented in **Box 1** (**Section 2**). Although there was a meaningful increase in the number of models accepted with the scaled approach, there was only a slight increase in the number of MDRs fulfilled. Using the scaled approach, MDRs were met for PFBS (an increase of one MDR) and one additional MDR was met for PFHxS (seven versus six MDRs met). The differences between the resulting acute benchmarks, although consistently higher using the scaled approach, were also small, with each of the benchmarks falling within a factor of $\leq$ 2.1 of

one another, indicating close agreement between benchmark values calculated using either approach. Raimondo et al. (2024) compared the extrapolated and scaled approaches and found no significant differences in prediction accuracy between the two approaches. It is likely the small but generally higher benchmark values (values were the same for PFHxS) resulting from the scaled approach result from the increase in the number of GMAVs available using the approach (see in **Table 5-27**), which will tend to mathematically increase the resulting values because of the effect of increasing the number of genera (n) in the Aquatic Life Criteria Guidelines calculation method.

Several investigations have indicated a trend of increasing toxicity with increasing carbon chain length of PFAS (Cousins et al. 2020, Chambers et al. 2021). This pattern was observed for both carboxylic acid and sulfonic acid PFAS in this document, as demonstrated by the decreasing benchmark values with an increasing number of carbons (i.e., increasing chain length) based on both the extrapolation and scaled approaches, as shown in **Table 5-28**. Further, the final acute aquatic life criterion recently derived by the EPA (U.S. EPA 2024b) for the eight carbon carboxylic acid PFOA is 3.1 mg/L, which falls between the benchmark values for PFHxA with six carbons (4.8 mg/L with the extrapolated approach; 6.0 mg/L with the scaled approach) and PFNA with nine carbons (0.65 mg/L with the extrapolated approach; 0.73 mg/L with the scaled approach) (see **Table 5-28**). Similarly, the acute aquatic life criterion recently derived by the EPA (U.S. EPA 2024c) for the eight carbon sulfonic acid PFOS is 0.071 mg/L, which falls below the benchmark values for PFHxS with six carbons (0.21 mg/L for both the extrapolated and scaled approaches). The trend of increasing toxicity with increasing chain length for carboxylic acid and sulfonic acid PFAS thus holds for both the calculated acute benchmark values and with the final acute criteria for PFOA and PFOS.

Table 5-27. Summary of Data Inputs, MDRs Fulfilled and Benchmark Outcomes Using the Extrapolation and Scaled Approach when Web-ICE Input PFAS Values Exceed Model Range.

Chemical	MDRs Met with Empirical Data	Number of Accepted ICE Predictions		MDRs Filled with ICE Models		Total MDRs Met (Number of GMAVs)		Acute Benchmark (mg/L)	
		Extrapolation Approach	Scaled Approach	Extrapolation Approach	Scaled Approach	Extrapolation Approach	Scaled Approach	Extrapolation Approach	Scaled Approach
Carboxylic Acids									
PFBA	3	22	43	5	5	8(22)	8(33)	5.3	11
PFHxA	3	28	43	5	5	8(24)	8(33)	4.8	6.0
PFNA	2	29	42	6	6	8(25)	8(33)	0.65	0.73
PFDA	3	61	85	5	5	8(42)	8(60)	0.50	0.65
Sulfonic Acids									
PFBS	2	55	119	5	6	7(30)	8(58)	5.0	7.6
PFHxS	2	17	18	4	5	6(12)	7(13)	0.21	0.21
Fluorotelomers									
8:2 FTUCA	2	66	80	6	6	8(45)	8(55)	0.037	0.041
7:3 FTCA	2	70	81	6	6	8(48)	8(59)	0.012	0.015

Table 5-28. Comparison of PFAS Chain Length to Perfluorocarboxylic and Perfluorosulfonic Acid Benchmarks and PFOA and PFOS Acute Criteria Values.

Chemical	Chain Length: Number of Carbons	Acute Benchmark (mg/L)		PFOA and PFOS Aquatic Life Criteria (mg/L) [1]
		Extrapolation Approach	Scaled Approach	
Carboxylic Acids				
PFBA	4	5.3	11	NA
PFHxA	6	4.8	6.0	NA
PFOA	8	NA	NA	3.1
PFNA	9	0.65	0.73	NA
PFDA	10	0.50	0.65	NA
Sulfonic Acids				
PFBS	4	5.0	7.6	NA
PFHxS	6	0.21	0.21	NA
PFOS	8	NA	NA	0.071

NA = Not Applicable

[1] Final aquatic life criteria for PFOA and PFOS are presented in U.S. EPA (2024b) and U.S. EPA (2024c), respectively.

5.10.2 Comparison of Benchmarks to Other Calculation Methods for Data-Limited PFAS

5.10.2.1 Comparison with Giddings et al. (2019) Approach of Combining Data Across Chemical Class

In addition to calculating PFAS benchmarks using a combination of empirical and predicted (ICE model) values for each individual PFAS, the EPA explored the application of an approach used by Giddings et al. (2019) to derive protective values for data-limited synthetic pyrethroid insecticides (detailed in **Appendix G**). In brief, Giddings et al. (2019) combined acute toxicity data across several synthetic pyrethroid insecticide active ingredients (e.g., bifenthrin, cypermethrin, permethrin, etc.) into a single pyrethroid species sensitivity distribution (SSD) to provide a more robust dataset for the derivation of protective aquatic life values. The Giddings et al. (2019) approach thus involved combining or "binning" data within a general chemical class, and expected similar mode of action, to develop a larger data set for their analyses. The EPA followed the same binning approach to estimate protective aquatic life values for PFAS, using quantitatively acceptable empirical data (**Appendix A**) for combined carboxylic acid PFAS (PFBA, PFHxA, PFNA, and PFDA) and combined sulfonic acid PFAS (PFBS and PFHxS) datasets. The EPA additionally incorporated quantitatively acceptable empirical data used for derivation of the acute PFOA and PFOS criteria (U.S. EPA 2024b,c) in the carboxylic acid and sulfonic acid data groupings, respectively, to further support derivation of these benchmark values. Data for carboxylic acid and sulfonic acid groupings were each then normalized by a common species and pooled according to PFAS grouping to derive group-specific SSDs. Group-specific SSDs were then generated using both the EPA's SSD Generator (as was done by Giddings et al. 2019) and following procedures described in the Aquatic Life Criteria Guidelines (U.S. EPA 1985). A more detailed description of the process used to derive these benchmark values is presented in **Appendix G**.

Exploratory acute benchmarks derived using the Giddings binning approach and SSD Generator-based values are summarized in **Table 5-29** and compared to those calculated using the Aquatic Life Criteria Guidelines-based values. Exploratory benchmark values for both carboxylic acids (5.6 times) and sulfonic acids (5.0 times) were consistently higher when calculated using the SSD Generator-based values versus the Aquatic Life Criteria Guidelines-based values. However, these benchmarks did not show a consistent pattern in magnitude of differences when compared to the EPA's recommended acute benchmark values (see **Table 5-29**). For carboxylic acid PFAS, for example, exploratory benchmark values calculated using the Aquatic Life Criteria Guidelines-based approach were between 0.5 and 3.7 times different (both higher and lower) compared to the EPA's recommended acute benchmarks. For sulfonic acid PFAS, exploratory benchmark values calculated using the Aquatic Life Criteria Guidelines-based values were between 0.5 and 3.0 times different (both higher and lower) compared to recommended benchmarks. Although the differences between these outcomes cannot be definitively determined, outcomes are likely to reflect inherent differences in the derivation processes being used. For example, use of the EPA's Aquatic Life Criteria Guidelines procedure to derive an SSD and calculate a protective value relies on a single distribution based on averaging the data for the four most sensitive genera around the fifth centile of the distribution, whereas the EPA's SSD Generator (used by Giddings et al. 2019) supports the calculation of four different distributions (normal, logistic, triangular, and Gumbel), with the protective value selected from the best-fit distribution. The limited size of the constituent empirical datasets available for the binning approach used to generate SSDs based on Aquatic Life Criteria Guidelines-based and SSD Generator-based values for the specific PFAS (24 studies total for PFBA, PFNA, PFDA, and PFHxA datasets and seven studies total for PFBS and PFHxS

datasets) and the limited number of datapoints available for the species used for normalization (one to three datapoints for PFBA, PFHxA, PFNA, and PFDA datasets and seven studies total for PFBS and PFHxS datasets) are also likely to influence the individual benchmark outcomes.

Table 5-29. Comparison of Benchmark Outcomes with Acute Benchmarks Calculated Based on Approach by Giddings et al. (2019) (Aquatic Life Criteria Guidelines and SSD Generator-Based Values).

Chemical	EPA Recommended Acute Benchmark (mg/L)	Acute Benchmarks Calculated Using Binning Approaches[1]		Factor Difference Between EPA Acute Benchmark and Binning-based Benchmark Values	
		Aquatic Life Criteria Guidelines-Based Values (mg/L)	SSD Generator-Based Values (mg/L)	Aquatic Life Criteria Guidelines-Based Values	SSD Generator-Based Values
Carboxylic Acids					
PFBA	5.3	19	110	3.6	21
PFHxA	4.8	4.3	24	0.9	5.0
PFNA	0.65	0.35	2.0	0.5	3.0
PFDA	0.50	0.49	2.8	1.0	5.6
Sulfonic Acids					
PFBS	5.0	15	75	3.0	15
PFHxS	0.21	0.11	0.56	0.5	2.7

[1] Acute benchmarks-binning approach is presented in Appendix G.

5.10.2.2 Comparison with Grippo et al. (2021) Approach of Deriving Water Quality-Based Ecological Screening Values

Grippo et al. (2021) calculated aquatic life surface water ecological screening values for each of the carboxylic acid and sulfonic acid PFAS for which the EPA developed benchmarks. Grippo et al. (2021) employed the Great Lakes Initiative (GLI) Tier II water quality value derivation approach (U.S. EPA 1995a,b,c), which uses secondary acute factors to account for the increased uncertainty associated with the reduced number of tested genera, when they fall below the eight MDRs. A comparison of the resulting screening values to the EPA's recommended

benchmark values is presented in **Table 5-30**. The Tier II GLI values calculated by Grippo et al. (2021) using the Tier II water quality value derivation approach were generally in agreement with the EPA benchmarks for carboxylic acid PFAS (within a factor of ≤ 8 of one another), whereas the Tier II GLI value calculated by Grippo et al. (2021) for sulfonic acid PFAS trended towards a greater difference (factor of 8 for PFHxS and 130 for PFBS). This trend towards a larger difference in Tier II GLI value versus the EPA benchmark for sulfonic acid PFAS is likely primarily due to differences between the application factor used by the EPA for calculation of the acute benchmark (42.3) and the application factor used by Grippo et al. (2021) for calculation of the Tier II GLI values (13), and some differences in the data used for the calculation of the Tier II values. If, for example, the Tier II GLI application factor of 13, used by Grippo et al. (2021) for PFBS and PFHxS, were applied to the EPA calculated benchmarks for these compounds (instead of the application factor used by the EPA) the difference between the two values decreases to a factor difference of 9.2 for PFBS and 2.4 for PFHxS). Overall, the EPA's acute benchmark values are lower than the Tier GLI values. This is primarily because the EPA used application factors to account for the high sensitivity of some invertebrate species to PFAS, as represented by the mayfly *Neocloeon triangulifer* (Soucek et al. 2023) and the cladoceran *Moina micrura* (Razak et al. 2023) to PFOA and PFOS. These sensitive species were not represented in the derived Tier II GLI values.

Table 5-30. Comparison of Benchmark Outcomes with Water Quality-Based Ecological Screening Values Calculated by Grippo et al. (2021) Using the Great Lakes Initiative (GLI) Approach.

Chemical	EPA Recommended Acute Benchmark (mg/L)	Grippo et al. (2021) Calculated Tier II GLI Values (mg/L)	Factor Difference Between EPA Acute Benchmark and Calculated Tier II GLI Values
Carboxylic Acids			
PFBA	5.3	13.75	2.6
PFHxA	4.8	17.5	3.6
PFNA	0.65	2.14	3.3
PFDA	0.50	4	8
Sulfonic Acids			
PFBS	5.0	149.1	30
PFHxS	0.21	1.7	8

5.10.3 Summary and Conclusions

The EPA developed recommended aquatic life acute ambient water quality benchmarks for eight PFAS with limited empirical test data in accordance with the provisions of section 304(a) of the CWA. Benchmarks were developed for the following eight chemicals: Perfluorobutanoic acid (PFBA), Perfluorohexanoic acid (PFHxA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulfonic acid (PFHxS), 2H-Perfluoro-2-decenoic acid (8:2 FTUCA), and 2H,2H,3H,3H-Pefluorodecanoic acid (7:3 FTCA).

Because of limitations in the available direct empirical test data, the EPA developed acute protective benchmarks for these PFAS using available freshwater species empirical test data in conjunction with the application of the EPA, Office of Research and Development's (ORD's) peer-reviewed Web-based Interspecies Correlation Estimate tool (Web-ICE; Version 4.0; https://www.epa.gov/webice/) (Raimondo et al. 2024). Two approaches were used for

benchmark development because PFAS acute values can extend beyond the range of values used to calibrate the ICE model. One approach allowed extrapolation of the data beyond the calibrated range of the model, while the other (presented in **Appendix F**) "scaled" the toxicity values to bring them within the ICE model range, when necessary.

Table 5-27 summarizes and compares the model inputs and aquatic life ambient water quality benchmark values between the extrapolation approach and alternative scaled approach. Benchmark values calculated using the scaled approach were consistently higher than those calculated using the extrapolation approach, though the difference was small (within a factor of $\leq$ 2.1 of one another), indicating close agreement between the two approaches. It is likely this small but consistent difference results from an increase in the number of GMAVs available when using the scaled approach, which tends to mathematically increase the resulting values.

Several investigations have indicated a trend of increasing toxicity with increasing carbon chain length of PFAS compounds (Cousins et al. 2020, Chambers et al. 2021). This pattern was also observed for carboxylic acid and sulfonic acid PFAS, as demonstrated by the decreasing benchmark values with an increasing number of carbons (i.e., increasing chain length) for both the extrapolation and scaled approaches (**Table 5-28**).

Table 5-31 compares the EPA benchmark values with those calculated using alternative approaches. The EPA explored the application of an alternative data "binning" approach, developed by Giddings et al. (2019) (see **Table 5-31**), which combines data for multiple related compounds to provide a more robust dataset. This approach supported derivation of protective aquatic life values for grouped perfluoroalkyl carboxylic acids and grouped perfluoroalkyl sulfonic acids. SSDs were derived using both Aquatic Life Criteria Guidelines -based procedures, which derive a single distribution and determine the benchmark value based on

averaging the data for the four most sensitive genera around the fifth centile, and by using the EPA's SSD Generator, which was used by Giddings et al. (2019) and supports the calculation of four different distributions and selects a protective value from the best-fit distribution using all of the genera. Exploratory benchmark values for both carboxylic acids and sulfonic acids were consistently higher (5.6 to 5.8 times and 5.0 times, respectively) when derived using the SSD Generator versus the Aquatic Life Criteria Guidelines procedures but did not show a consistent pattern in magnitude when compared to the EPA's recommended benchmark values (**Tables 5-31** and **5-29**). For carboxylic acid PFAS, for example, exploratory benchmark values calculated using Aquatic Life Criteria Guidelines-based values were between 1.02 and 3.6 times different (both higher and lower) compared to EPA's recommended acute benchmarks. For sulfonic acid PFAS, exploratory benchmark values calculated using Aquatic Life Criteria Guidelines-based values were between 1.9 and 3.0 times different (both higher and lower) compared to recommended benchmarks. These mostly small differences likely reflect inherent differences in the derivation processes being used and the constituent datasets.

Grippo et al. (2021) employed the Great Lakes Initiative (GLI) Tier II water quality value derivation approach (U.S. EPA 1995a,b,c) to derive draft ecological screening values for the same carboxylic acid and sulfonic acid PFAS evaluated in this document. This GLI approach uses secondary acute factors to account for the increased uncertainty associated with the reduced number of tested genera, when the number of genera for which data is available falls below the Aquatic Life Criteria Guidelines eight MDRs. The Grippo et al. (2021) calculated draft Tier II GLI values were in generally close agreement with the EPA's acute benchmarks for both carboxylic acid PFAS (within a factor of ≤ 8 of one another) and sulfonic acid PFAS (30 for PFBS and 8 for PFHxS) (**Table 5-31**). These differences likely reflect the use of different

application factors and some differences in the data used for the calculation of the draft Grippo et al. (2021) Tier II values, which did not include the sensitive mayfly (*N. triangulifer*) data as the Grippo et al. (2021) publication preceded the publication of the mayfly data.

These acute aquatic life benchmarks, developed under Section 304(a)(2) of the CWA, are informational values that the EPA generated because there are limited high quality toxicity data available and data gaps exist for several aquatic organism families, such that 304(a)(1) criteria could not be developed. The EPA developed these aquatic life benchmarks to provide information that states and Tribes may consider in their water quality protection programs, including establishing their water quality criteria. However, the EPA's aquatic life benchmark values are not regulatory, nor do they automatically become part of a state's or Tribe's water quality standards.

Table 5-31. Summary of the EPA Acute Benchmark Values and Acute Values Calculated Using Alternative Methods

Chemical	EPA Acute Benchmarks – Extrapolation Approach (mg/L)	Acute Benchmarks Calculated Using Binning Approaches		Grippo et al (2021) Calculated Draft Tier II GLI Values (mg/L)
		Aquatic Life Criteria Guidelines-Based Values (mg/L)	SSD Generator-Based Values (mg/L)	
PFBA	5.3	19	110	13.75
PFHxA	4.8	4.3	24	17.5
PFNA	0.65	0.35	2.0	2.14
PFDA	0.50	0.49	2.8	4
PFBS	5.0	15	75	149.1
PFHxS	0.21	0.11	0.56	1.7
8:2 FTUCA	0.037	-	-	-
7:3 FTCA	0.012	-	-	-

6 REFERENCES

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Appendices

Appendix A Quantitatively Acceptable Freshwater Acute Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	Species Mean Acute Value (mg/L)	Reference
Perfluorobutanoic acid (PFBA)									
Rotifer (neonate, <2 h), *Brachionus calyciflorus*	S, U	24 hr	PFBA 98%	7.0	20	LC_{50}	110	110	Wang et al. 2017
Cladoceran (neonate, <24 hours), *Chydorus sphaericus*	S, M	48 hr	PFBA 98%	-	20	EC_{50} (immobilization)	>4,280.8	>4,280.8	Ding et al. 2012
Zebrafish (embryo, 60 mpf), *Danio rerio*	S, U	96 hr	PFBA >97%	7.2-7.5	26	LC_{50}	>3,000[b]	-	Hagenaars et al. 2011
Zebrafish (gastrula, 4.5 hpf), *Danio rerio*	R, U	96 hr	PFBA NR	7.0 - 7.5	28	LC_{50}	13,779	13,779	Godfrey et al. 2017
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, U	48 hr	PFBA >97%	NR	21	EC_{50} (immobilization)	>1,006[b]	-	Boudreau 2002
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, M	48 hr	PFBA 98%	8.0	20	EC_{50} (immobilization)	>4,280.8	-	Ding et al. 2012
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, M	48 hr	PFBA >98%	7.0 – 7.82	20	EC_{50} (immobilization)	5,251	4,741	Barmentlo et al. 2015
Cladoceran (neonate, <24 hr), *Daphnia pulicaria*	S, U	48 hr	PFBA >97%	-	21	EC_{50} (immobilization)	>1,006	>1,006	Boudreau 2002
Perfluorobutanesulfonic acid (PFBS)									
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, M	48 hr	PFBS 97.3-97.9%	8.1 – 8.5	19.8 – 20.1	EC_{50} (immobilization)	2,183	2,183	Wildlife International Ltd. 2001
Fathead minnow (juvenile), *Pimephales promelas*	S, M	96 hr	PFBS 97.3 – 97.9%	8.4 – 8.5	20.7 – 22.8	LC_{50}	1,938	1,938	Wildlife International Ltd. 2001
Bluegill (juvenile), *Lepomis macrochirus*	S, M	96 hr	PFBS 97.3 – 97.9%	8.1 – 8.4	22.1 – 23.9	LC_{50}	6,452	6,452	Drottar and Krueger 2001

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	Species Mean Acute Value (mg/L)	Reference
Zebrafish (embryo, 60 mpf), *Danio rerio*	S, U	96 hr	PFBS >97%	7.2 – 7.5	26	LC_{50}	>3,000	>3,000	Hagenaars et al. 2011
Perfluorononanoic acid (PFNA)									
Cladoceran (<24 hours), *Chydorus sphaericus*	S, M	48 hr	PFNA 97%	-	20	EC_{50} (immobilization)	27.84	27.84	Ding et al. 2012
Cladoceran (neonate, 24 hours), *Daphnia magna*	S, U	48 hr	PFNA 98%	7.7 – 8.4	20	EC_{50} (immobilization)	43.42	-	Lu et al. 2016
Cladoceran (neonate, 24 hours), *Daphnia magna*	S, U	48 hr	PFNA >97%	-	21	EC_{50} (immobilization)	91.89	-	Boudreau 2002
Cladoceran (neonate, 24 hours), *Daphnia magna*	S, M	48 hr	PFNA 97%	-	20	EC_{50} (immobilization)	151.29	84.51	Ding et al. 2012
Clawed frog (blastula), *Xenopus sp.*	R, U	96 hr	PFNA NR	-	23	LC_{50}	335.8	335.8	Kim et al. 2013
Perfluorodecanoic acid (PFDA)									
Cladoceran (neonate, <24 hours), *Chydorus sphaericus*	S, U	48 hr	PFDA 98%	-	20	EC_{50} (immobilization)	41.13	41.13	Ding et al. 2012
Cladoceran (neonate, <24 hours), *Daphnia magna*	S, U	48 hr	PFDA 99.6%	-	20	EC_{50}	>100[b]	-	Hoke et al. 2012
Cladoceran (neonate, <24 hours), *Daphnia magna*	S, U	48 hr	PFDA >97%	-	21	EC_{50} (immobilization)	129.54	-	Boudreau 2002
Cladoceran (neonate, <24 hours), *Daphnia magna*	S, U	48 hr	PFDA	-	20	EC_{50} (immobilization)	163.48	145.5	Ding et al. 2012
Cladoceran (neonate, <24 hours), *Daphnia pulicaria*	S, U	48 hr	PFDA, >97%	-	21	EC_{50} (immobilization)	149.59	149.59	Boudreau 2002
Clawed frog (blastula), *Xenopus sp.*	R, U	96 hr	PFDA NR	-	23	LC_{50}	76.5	76.5	Kim et al. 2013

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	Species Mean Acute Value (mg/L)	Reference
Rainbow trout (not reported, 28-61 dph), *Oncorhynchus mykiss*	S, U	96 hr	PFDA 99.6%	-	12	LC_{50}	32	32	Hoke et al. 2012
Perfluorohexanoic acid (PFHxA)									
Rotifer (neonate, <2 h), *Brachionus calyciflorus*	S, U	24 hr	PFHxA 98%	7.0	20	LC_{50}	140	140	Wang et al. 2017
Cladoceran (neonate, <24 hours), *Daphnia magna*	S, M	48 hr	PFHxA >97%	7.0 – 7.82	20	EC_{50} (immobilization)	1,048	1,048	Barmentlo et al. 2015
American bullfrog (larva, 26 go), *Lithobates catesbeianus* (formerly, *Rana catesbeiana*)	S, U	96 hr	PFHxA >98%	-	21	LC_{50}	1,105	1,105	Tornabene et al. 2021
Green frog (larva, 26 gosner stage), *Lithobates clamitans* (formerly, *Rana clamitans*)	S, U	96 hr	PFHxA NR	-	21	LC_{50}	758	758	Tornabene et al. 2021
Perfluorohexanesulfonic acid (PFHxS)									
Zebrafish (embryo, 3 hpf), *Danio rerio*	R, U	96 hr	PFHxS NR	7.2	28	NOEC (growth, length)	22.5	22.5	Annunziato et al. 2020
American bullfrog (larva, 26 gosner stage), *Lithobates catesbeianus* (formerly, *Rana catesbeiana*)	S, U	96 hr	PFHxS NR	-	21	LC_{50}	1,105	1,105	Tornabene et al. 2021
Green frog (larva, 26 gosner stage), *Lithobates clamitans* (formerly, *Rana clamitans*)	S, U	96 hr	PFHxS NR	-	21	LC_{50}	758	758	Tornabene et al. 2021
H-Perfluoro-2-decenoic acid (8:2 FTUCA)									
Cladoceran (neonate, 24 hr), *Daphnia magna*	S, U	48 hr	8:2 FTUCA 99.8%	-	20	EC_{50} (immobilization)	3.2	3.2	Hoke et al. 2012

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Conc. (mg/L)	Species Mean Acute Value (mg/L)	Reference
Rainbow trout (not reported, 28-61 dph), *Oncorhynchus mykiss*	S, U	96 hr	8:2 FTUCA 99.8%	-	12	LC_{50}	81	81	Hoke et al. 2012
2H,2H,3H,3H-Pefluorodecanoic acid (7:3 FTCA)									
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, M	48 hr	7:3 FTCA >99.%	-	20	EC_{50} (immobilization)	0.4	-	Hoke et al. 2012
Cladoceran (neonate, <24 hr), *Daphnia magna*	S, U	48 hr	7:3 FTCA >99%	-		EC_{50} (immobilization)	2.3	0.9592	Hoke et al. 2012
Rainbow trout (not reported, 28-61 dph), *Oncorhynchus mykiss*	S, U	96 hr	7:3 FTCA >99%	-	12	LC_{50}	32	32	Hoke et al. 2012

a S=static, R=renewal, F=flow-through, U=unmeasured, M=measured.
b Not used in species mean calculation because the value is greater than low value.

A.1 References

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Appendix B Summary of Lowest Quantitatively Acceptable Freshwater Acute Toxicity Studies

B.1 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies Used to Derive Aquatic Life Benchmarks

Quantitatively acceptable empirical acute toxicity data were available for 27 freshwater species across the 8 PFAS: PFBA (5 species), PFBS (4 species), PFNA (3 species), PFDA (4 species), PFHxA (4 species), PFHxS (3 species), 8:2 FTUCA (2 species), and 7:3 FTCA (2 species) (**Table 4-2**; **Appendix A**). The following presents summaries of the freshwater toxicity studies with empirical effect values that were used quantitatively to derive acute benchmarks, which consist of toxicity values falling amongst the four most sensitive values. These values are used quantitatively to derive the benchmarks consistent with procedures presented in the Aquatic Life Criteria Guidelines (U.S. EPA 1985) designed to protect 95% of aquatic taxa. These "most sensitive" empirical studies consist of one study each for PFBA, PFNA, PFHxA, and PFHxS. Study summaries for the most sensitive taxa are presented below for each of these substances. Acute values are presented as reported by the study authors, unless indicated otherwise.

B.2 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies for Perfluorobutanoic acid (PFBA)

Wang et al. (2014) exposed the rotifer, *Brachionus calyciflorus*, to perfluorobutanoic acid (PFBA, CAS # 375-22-4, 98% purity) in a 24-hour acute toxicity test. The test was performed under static, non-renewal conditions beginning with <2-hour old neonates. Exposures consisted of 10 test organisms per 15 mL well in 6-well cell culture plates containing 10 mL of test medium. The control and each of nine PFBA test concentrations were replicated three times for a total of 30 neonates per treatment. Nominal test concentrations were 0.0 mg/L for the control and treatments ranged from 40-140 mg/L PFBA. Neonates were exposed at pH 7.0 and 20 ± 1°C under darkness. After 24 h of exposure, the number of dead and live rotifers was

enumerated under a microscope at 10× magnification. Rotifers were deemed inactive if they did not move in a period of 30s. The author-reported acute LC_{50} was 110 mg/L. This value was acceptable for quantitative use in deriving the recommended acute freshwater PFBA benchmark.

B.3 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies for Perfluorohexanoic acid (PFHxA)

Wang et al. (2014) exposed the rotifer, *Brachionus calyciflorus*, to perfluorohexanoic acid (PFHxA, CAS # 307-24-4, 98% purity) in a 24-hour acute toxicity test. The test was performed under static, non-renewal conditions beginning with <2-hour old neonates. Exposures consisted of 10 test organisms per 15 mL well in 6-well cell culture plates containing 10 mL of test medium. The control and each of nine PFHxA test concentrations were replicated three times for a total of 30 neonates per treatment. Nominal test concentrations were 0.0 mg/L for the control and treatments ranged from 40-140 mg/L PFHxA. Neonates were exposed at pH 7.0 and 20 ± 1°C under darkness. After 24 h of exposure, the number of dead and live rotifers was enumerated under a microscope at 10× magnification. Rotifers were deemed inactive if they did not move in a period of 30s. The author-reported acute LC_{50} was 140 mg/L. This value was acceptable for quantitative use in deriving the recommended acute freshwater PFHxA benchmark.

B.4 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies for Perfluorononanoic acid (PFNA)

Ding et al. (2012) exposed the cladoceran, *Chydorus sphaericus*, to perfluorononanoic acid (PFNA, CAS # 375-95-1, 97% purity) in a 48-hour acute toxicity test. The test was performed under static, non-renewal conditions beginning with <24-hour old neonates. Exposures consisted of five test organisms per 2 ml vial to which 250 µl test solution was added. Because the water solubility of PFNA was low, dimethylsulfoxide (DMSO) was used for preparing the stock solution. The concentration of DMSO in test solutions did not exceed 0.2%.

A solvent control was tested simultaneously with a negative (dilution water) control. The two controls and each of six PFNA test concentrations were replicated four times for a total of 20 neonates per treatment. Nominal test concentrations were 0.0 (controls), 0.05, 0.07, 0.1, 0.15, 0.2 and 0.25 mM PFNA, or 0, 23.20, 32.49, 69.61, 92.82 and 116.0 mg/L after conversion using a molecular weight for PFNA of 464.08 mg/mmol. Samples containing the highest and the lowest test concentrations were analyzed to confirm the nominal concentrations. Although partial chemical analysis was conducted, only nominal concentrations were reported. To assess the test concentration variability, all samples at a selected nominal concentration were analyzed, with a relative standard deviation of 6.5%. The measured concentrations were between 82 and 91% of the nominal concentration. Neonates were exposed at 20 ± 1°C under a 16:8-hour light:dark cycle. The animals were not fed during the test period and were checked under a reverse dissecting microscope at 24 and 48 h. Immobilization was determined based on inactivation of the animals after slightly tapping with a finger to the vial and monitoring them for 30s. The author-reported acute immobilization LC_{50} was 0.060 mM (or 27.84 mg/L). This value was acceptable for quantitative use in deriving the recommended acute freshwater PFNA benchmark.

B.5 Summary of Lowest Quantitatively Acceptable Empirical Toxicity Studies for Perfluorohexanesulfonic acid (PFHxS)

Annunziato et al. (2020) exposed the wildtype AB strain of zebrafish, *Danio rerio*, to perfluorohexanesulfonic acid (PFHxS, CAS # 355-46-4, purity not reported) in a 93-hour acute toxicity test following OECD Test No. 236 (OECD 2013). The test was performed under static-renewal conditions beginning with 3-hour post fertilization (hpf) embryos. Fifteen embryos were assigned at random to each treatment or control group, with each embryo placed into an individual glass vial containing 1 mL of solution. Test solutions were prepared and refreshed daily. Because of the low water solubility of PFHxS, dimethylsulfoxide (DMSO) was used for

preparing the stock solution. The concentration of DMSO in test solutions did not exceed 0.01%. A solvent control and each of seven PFHxS test concentrations were replicated three times for a total of 45 organisms per treatment. Nominal test concentrations were 0.0 (control), 0.07, 1.41, 2.81, 5.63, 11.25, 22.50, and 45.00 mg/L PFHxS. Embryos were exposed at pH 7.2, 28 ± 1°C under a 14:10-hour light:dark cycle and monitored daily through 96 hpf. There was >90% health and survival in the control group. Test concentrations of PFHxS were too low to derive an LC_{50} value. Larval growth (length) was assessed at test termination. The author-reported a growth (weight) NOEC of 22.5 mg/L. This value was acceptable for quantitative use in deriving the recommended acute freshwater PFHxS benchmark.

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Appendix C Qualitative Freshwater Acute Toxicity Studies

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Perfluorobutanoic acid (PFBA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	4.5 hr	Perfluorobutanoic acid / 98%	NR	NR	EC_{50} (population photosynthesis)	262.2[b]	Test duration too short	Ding et al. 2012
Duckweed, *Lemna gibba*	S, U	7 d	Perfluorobutyric acid / ≥97%	NR	25	IC_{50} (population/ weight)	>1,006[b]	Test duration too long	Boudreau 2002
Japanese medaka (embryo, <6 hpf), *Oryzias latipes*	R, U	48 hr post hatch	Perfluorobutyric acid / NR	7.0-7.5	25	LOEC (velocity)	137	Only one concentration tested; atypical endpoint	Godfrey 2017
Japanese medaka, *Oryzias latipes*	S, U	96 hr	Perfluorobutyric acid / NR	7.0-7.5	25	NOEC (mortality)	>13,795	Only one concentration tested	Godfrey et al. 2019
Japanese medaka (embryo, <6 hpf), *Oryzias latipes*	R, U	10 d	Perfluorobutyric acid / NR	7.0-7.5	25	NOEC (growth - length)	137	Only one concentration tested; duration too short for a chronic test	Godfrey et al. 2019
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	144 hr post fert.	Perfluorobutyric acid / NR	7.2-7.6	26	EC_{50} (heart rate, hatching time)	2,200	Duration too long for an acute test and too short for a chronic test	Ulhaq et al. 2013
Zebrafish (embryo, 5-6 hpf), *Danio rerio*	R, U	90-91 hr	Perfluorobutyric acid / >99%	7	28	LC_{50}	9,703	Test duration too short	Wasel et al. 2020

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Perfluoro-n-butanoic acid / >98%	NR	28	NOEC (mortality)	0.2504[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Perfluorobutanesulfonic acid (PFBS)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, M	96 hr	Perfluorobutane sulfonate potassium salt / 97.3%	6.9-8.7	23.8-24.5	EC_{50} (population abundance)	2,146		Wildlife International 2001
Green alga, *Raphidocelis subcapitata*	S, U	48 hr	1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-Nonafluoro-, Potassium salt / ≥95%	7.2	20	EC_{10} (population growth rate)	299	Test duration too short	Kusk et al. 2018
Zebrafish (embryo), *Danio rerio*	S, U	144 hr post fert.	Perfluorobutane sulfonic acid / NR	7.2-7.6	26	LC_{50}	1,500	Duration too long for an acute test and too short for a chronic test	Ulhaq et al. 2013
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Potassium nonafluoro-1-butanesulfonate / NR	NR	28	NOEC (growth stunting)	20.84[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo), *Danio rerio*	R, U	168 hr post fert.	Nonafluorobutane-1-sulfonic acid / NR	7.5	28-28.5	LC_{50}	921.9	Duration too long for an acute test and too short for a chronic test	Stinckens et al. 2018
Zebrafish (embryo, 3 hpf), *Danio rerio*	R, U	5 d post fert.	Nonafluorobutane-1-sulfonic acid / 97%	NR	NR	LC_{50}	406.2[b]	Test duration too long for an acute test and too short for a chronic test	Sant et al. 2019

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Potassium nonafluoro-1-butanesulfonate / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	27.75[b]	Test duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 5-6 hpf), *Danio rerio*	R, U	90-91 hr	Potassium perfluorobutane-sulfonate / >99%	NR	28	LC_{50}	1,394	Test duration too short	Wasel et al. 2020
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Potassium perfluoro-1-butanesulfonate / >98%	NR	28	NOEC (mortality)	0.2295[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Zebrafish (3-yr old adults), *Danio rerio*	R, U	14 d	Perfluorobutanesulfonate / >98%	NR	NR	LOEC (multiple genetic and biochemistry endpoints)	0.100	Only non-apical endpoints evaluated	Liu et al. 2022
Perfluorohexanoic acid (PFHxA)									
Rainbow trout (oocyte, ova), *Oncorhynchus mykiss*	S, M	3 hr	Perfluorohexanoic acid / >97%	8.5	6	NOEC (accumulation residue)	10.83	Test duration too short	Raine et al. 2021
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorohexanoic acid / NR	NR	28	NOEC (growth stunting)	20.10[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorohexanoic acid / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	22.18[b]	Duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 5-6 hpf), *Danio rerio*	R, U	90-91 hr	Perfluorohexanoic acid / >99%	7	28	LC_{50}	6,486	Test duration too short	Wasel et al. 2020
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	120 hr	Perfluoro-n-hexanoic acid / >98%	NR	28	MATC (mortality)	5.367	Duration too long for an acute test and too short for a chronic test	Guo et al. 2021

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Perfluoro-n-hexanoic acid / >98%	NR	28	NOEC (mortality)	0.2512[b]	Duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Zebrafish (embryo, 0.5-2 hpf), *Danio rerio*	R, U	94-95.5 hr	Perfluorohexanoic acid / >99%	NR	28	MATC (mortality)	5.367	Test duration too short	Zhang et al. 2022
Perfluorohexanesulfonic acid (PFHxS)									
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Potassium perfluorohexanesulfonate / NR	NR	28	MATC (mortality)	8.889[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Potassium perfluorohexanesulfonate / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	1.968[b]	Duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Sodium perfluoro-1-hexanesulfonate / >98%	NR	28	NOEC (mortality)	0.2503[b]	Duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Perfluorononanoic acid (PFNA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	4.5 hr	Perfluorononanoic acid / 97%	NR	NR	EC_{50} (population photosynthesis)	481.7[b]	Test duration too short	Ding et al. 2012
Duckweed, *Lemna gibba*	S, U	7 d	Perfluorononanoic acid / ≥97%	NR	25	IC_{50} (population/ weight)	89.10[b]	Test duration too long	Boudreau 2002
Cladoceran (<24 h old), *Daphnia pulicaria*	R, U	21 d	Perfluorononanoic acid / ≥97%	NR	21	MATC (reproduction: young/brood)	8.694[b]	Lack of procedural details cannot judge against data quality objectives	Boudreau 2002
Zebrafish (embryo, 0 hpf), *Danio rerio*	S, U	72 hr post fert.	Perfluorononanoic acid / 97%	8.3	28.5	LC_{50}	84	Test duration too short	Zheng et al. 2012

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Zebrafish (embryo), *Danio rerio*	S, U	144 hr post fert.	Perfluorononanoic acid / NR	7.2-7.6	26	LC_{50}	>10	Duration too long for an acute test and too short for a chronic test	Ulhaq et al. 2013
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorononanoic acid / NR	NR	28	MATC (mortality)	0.9392[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo, 1 hpf), *Danio rerio*	S, U	23 hr	Perfluorononanoic acid / 97%	7.2-8.0	28	LC_{50}	140.2[b]	Test duration too short	Liu et al. 2015
Zebrafish (embryo, 3 hpf), *Danio rerio*	S, U	117 hr	Perfluorononanoic acid / NR	7.2-7.7	26-28	MATC (growth - length)	0.2935[b]	Duration too long for an acute test and too short for a chronic test	Jantzen et al. 2016
Zebrafish (embryo, 72 hpf), *Danio rerio*	S, M	48 hr	Perfluorononanoic acid / NR	NR	27	LC_{50}	108.6	Test duration too short	Rainieri et al. 2017
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorononanoic acid / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	17.10[b]	Duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Perfluoro-n-nonanoic acid / 97%	NR	28	LOEC (development deformation)	45.02[b]	Duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
African clawed frog (embryo, 8-11 stage), *Xenopus laevis*	R, U	96 hr	Perfluorononanoic acid / NR	6.5-7.5	24	NOEC (mortality, growth)	3.5	Only one concentration evaluated	Mitchell 2010
Perfluorodecanoic acid (PFDA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	4.5 hr	Perfluorodecanoic acid / 98%	NR	NR	EC_{50} (population photosynthesis)	437.5[b]	Test duration too short	Ding et al. 2012
Green alga, *Raphidocelis subcapitata*	S, U	72 hr	n-Perfluorodecanoic acid / 99.6%	~7.5	24	EC_{50} (population biomass)	10.6	Test duration too short	Hoke et al. 2012

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Duckweed, *Lemna gibba*	S, U	7 d	Perfluorodecanoic acid / ≥97%	NR	25	IC_{50} (population/ weight)	99.22[b]	Test duration too long	Boudreau 2002
Zebrafish (embryo), *Danio rerio*	S, U	144 hr post fert.	Perfluorodecanoic acid / NR	7.2-7.6	26	LC_{50}	8.4	Duration too long for an acute test and too short for a chronic test	Ulhaq et al. 2013
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorodecanoic acid / NR	NR	28	NOEC (growth stunting)	32.90[b]	Duration too long for an acute test and too short for a chronic test	Truong et al. 2014
Zebrafish (embryo, 6 hpf), *Danio rerio*	S, U	114 hr	Perfluorodecanoic acid / NR	NR	NR	BMD_{10} (mortality) (benchmark dose)	5,141[b]	Duration too long for an acute test and too short for a chronic test	Thomas et al. 2019
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	Perfluoro-n-decanoic acid / 98%	NR	28	NOEC (mortality)	50.38[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
African clawed frog (embryo, 8-11 stage), *Xenopus laevis*	R, U	96 hr	Perfluorodecanoic acid / NR	6.5-7.5	24	NOEC (mortality, growth)	3.5	Only one concentration evaluated	Mitchell 2010
Hexadecafluoro-2-decenoic acid (8:2 FTUCA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, M	72 hr	8:2 Fluorotelomer unsaturated carboxylate / >98%	6.5-7.5	24	EC_{10} (population abundance)	2.3	Test duration too short	Mitchell et al. 2011
Green alga, *Raphidocelis subcapitata*	S, U	72 hr	8:2 FTUCA / 99.8%	~7.5	24	EC_{50} (population biomass)	7.5	Test duration too short	Hoke et al. 2012

Species (lifestage)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Effect	Author Reported Effect Concentration (mg/L)	Deficiencies	Reference
Green alga (exponential growth phase (log), 3-7 d), *Chlorella vulgaris*	S, M	72 hr	8:2 Fluorotelomer unsaturated carboxylate / >98%	6.5-7.5	24	EC_{10} (population abundance)	2.2	Test duration too short	Mitchell et al. 2011
Amphipod, *Hyalella azteca*	R, M	10 d	8:2 Fluorotelomer unsaturated carboxylate / >98%	6.5-7.5	23	NOEC (mortality and growth)	6.1	Duration too long for an acute test and too short for a chronic test	Mitchell et al. 2011
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	2H-Perfluoro-2-decenoic acid / >98%	NR	28	NOEC (mortality)	0.2520[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021
Pentadecafluorodecanoic acid (7:3 FTCA)									
Green alga, *Raphidocelis subcapitata* (formerly, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*)	S, U	72 hr	4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Pentadecafluorodecanoic acid / >99%	~7.5	24	EC_{50} (population biomass)	2.1	Test duration too short	Hoke et al. 2012
Zebrafish (embryo, 6-8 hpf), *Danio rerio*	S, U	112-114 hr	3-Perfluoroheptyl propanoic acid / >98%	NR	28	NOEC (mortality)	0.2520[b]	Only one concentration tested; duration too long for an acute test and too short for a chronic test	Rericha et al. 2021

[a] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured.

[b] Converted to mg/L based on molecular weight.

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Hexadecafluoro-2-decenoic acid (8:2 FTUCA)

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Pentadecafluorodecanoic acid (7:3 FTCA)

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Appendix D Unused Freshwater Acute Toxicity Studies

Author	Citation	Reason Unused
Perfluorobutanoic acid (PFBA)		
Blanc, M., J. Ruegg, N. Scherbak and S.H. Keiter	2019. Environmental chemicals differentially affect epigenetic-related mechanisms in the zebrafish liver (ZF-L) cell line and in zebrafish embryos. Aquat. Toxicol. 215: 105272-9999.	No control information
Perfluorononanoic acid (PFNA)		
Dale, K., F. Yadetie, T. Horvli, X. Zhang, H.G. Froysa, O.A. Karlsen and A. Goksoyr	2022. Single PFAS and PFAS mixtures affect nuclear receptor- and oxidative stress-related pathways in precision-cut liver slices of Atlantic cod (*Gadus morhua*). Sci. Total Environ. 814: 1-12.	*In vitro* exposure
Garoche, C., A. Boulahtouf, M. Grimaldi, B. Chiavarina, L. Toporova, M.J. Den Broeder, J. Legler, W. Bourguet and P. Ba	2021. Interspecies differences in activation of peroxisome proliferator-activated receptor gamma by pharmaceutical and environmental chemicals. Environ. Sci. Technol. 55(24): 16489-16501.	*In vitro* exposure

Appendix E ICE Models Available in Web-ICE v4.0 for Freshwater Predicted Species Based on Surrogates with Measured Toxicities

Table E-1. All ICE Models Selected for the Derivation of PFAS Benchmarks.

Model parameters are used to evaluate prediction robustness. Cross-validation success is the percentage of all model data that were predicted within five-fold of the measured value through leave-one-out cross-validation (Raimondo et al. 2024). 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	PFAS Compound
African clawed frog (Xenopus laevis)	Fathead minnow (Pimephales promelas)	0.76	0.77	4	0.91	0.00	0.14	18.0	140,225.3	100	5	PFBS
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	0.91	1.26	12	0.75	0.00	0.76	0.8	166,819.4	50	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	0.82	0.44	51	0.75	0.00	0.76	0.2	5,000,000.0	49	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Amphipod (Gammarus fasciatus)	Mysid (Americamysis bahia)	0.91	0.58	26	0.70	0.00	0.81	0.0	4,400.0	57	4	PFBS
Amphipod (Gammarus minus)	Fathead minnow (Pimephales promelas)	0.73	1.50	2	0.96	0.02	0.05	239.0	107,584.8	75	6	PFBS
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	0.92	0.25	30	0.75	0.00	0.75	0.1	68,300.0	63	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Amphipod (Gammarus pseudolimnaeus)	Mysid (Americamysis bahia)	0.82	0.22	18	0.80	0.00	0.56	0.0	7,170.0	55	4	PFBS
Amphipod (Hyalella azteca)	Mysid (Americamysis bahia)	1.03	-0.10	30	0.88	0.00	0.68	0.0	193,390.8	63	4	PFBS

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	PFAS Compound
Apache trout (Oncorhynchus gilae)	Rainbow trout (Oncorhynchus mykiss)	1.10	-0.35	3	0.99	0.00	0.02	4.1	1,624.7	100	1	PFDA, 7:3 FTCA, 8:2 FTUCA
Arctic grayling (Thymallus arcticus)	Bluegill (Lepomis macrochirus)	1.14	-0.59	1	1.00	0.02	0.01	37.9	150,750.0	N/A	4	PFBS
Atlantic salmon (Salmo salar)	Bluegill (Lepomis macrochirus)	1.15	-0.64	11	0.95	0.00	0.13	4.3	123,267.3	92	4	PFBS
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	1.01	-0.07	12	0.96	0.00	0.11	0.2	95,857.7	86	2	PFDA, 7:3 FTCA, 8:2 FTUCA
Atlantic salmon (Salmo salar)	Fathead minnow (Pimephales promelas)	1.28	-1.51	6	0.68	0.01	0.84	44.4	140,225.3	63	4	PFBS
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	0.91	0.22	18	0.97	0.00	0.12	0.3	8,694,505.9	95	4	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Black bullhead (Ameiurus melas)	Rainbow trout (Oncorhynchus mykiss)	0.74	1.21	12	0.60	0.00	0.95	0.0	9,809.6	43	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Black bullhead (Ameiurus melas)	Fathead minnow (Pimephales promelas)	1.07	-0.10	3	0.97	0.00	0.17	1.2	20,922.0	80	4	PFBS
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	0.71	1.18	270	0.65	0.00	0.84	0.1	46,278,306.5	57	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Bluegill (Lepomis macrochirus)	Mysid (Americamysis bahia)	0.71	1.38	150	0.71	0.00	0.55	0.0	79,000.0	72	6	PFBS
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	0.94	0.30	314	0.89	0.00	0.22	0.1	8,341,462.7	90	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Bluegill (Lepomis macrochirus)	Fathead minnow (Pimephales promelas)	0.94	-0.02	108	0.81	0.00	0.43	0.2	50,154,461.4	82	4	PFBS

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	PFAS Compound
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1.10	-0.48	5	0.98	0.00	0.10	2.5	233,000.0	100	5	PFHxS, PFHxA
Bonytail (Gila elegans)	Bluegill (Lepomis macrochirus)	0.96	-0.37	6	0.69	0.01	0.62	53.9	150,750.0	50	4	PFBS
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	0.92	0.70	6	0.89	0.00	0.22	3.3	43,073.0	75	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Bonytail (Gila elegans)	Fathead minnow (Pimephales promelas)	1.01	-0.39	5	0.62	0.04	0.50	58.3	8,320.3	43	2	PFBS
Brook trout (Salvelinus fontinalis)	Bluegill (Lepomis macrochirus)	0.97	-0.06	21	0.86	0.00	0.27	0.4	123,267.3	83	4	PFBS
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	1.00	0.01	25	0.95	0.00	0.11	0.6	96,692.7	93	2	PFDA, 7:3 FTCA, 8:2 FTUCA
Brook trout (Salvelinus fontinalis)	Fathead minnow (Pimephales promelas)	0.95	-0.43	17	0.70	0.00	0.75	1.2	140,225.3	68	4	PFBS
Brown trout (Salmo trutta)	Bluegill (Lepomis macrochirus)	0.91	-0.10	16	0.73	0.00	0.68	2.3	123,267.3	78	4	PFBS
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	1.00	0.02	20	0.97	0.00	0.07	0.2	95,857.7	100	2	PFDA, 7:3 FTCA, 8:2 FTUCA
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	0.87	1.14	2	0.98	0.01	0.01	14.1	231.7	100	6	PFNA, PFDA, 7:3 FTCA, 8:2 FTUCA
Bullfrog (Lithobates catesbeianus)	Bluegill (Lepomis macrochirus)	0.89	0.48	5	0.98	0.00	0.08	0.4	201,000.0	100	5	PFBS
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	0.88	0.82	9	0.95	0.00	0.31	0.6	13,400,000.0	73	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Bullfrog (Lithobates catesbeianus)	Fathead minnow (Pimephales promelas)	0.97	0.15	7	0.98	0.00	0.10	1.2	26,500,000.0	100	5	PFBS

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	PFAS Compound
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	1.17	-0.23	3	0.99	0.00	0.01	4.1	1,624.7	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Catla (Gibelion catla)	Fathead minnow (Pimephales promelas)	1.09	-0.36	2	0.97	0.02	0.02	333.0	8,320.3	100	2	PFBS
Channel catfish (Ictalurus punctatus)	Zebrafish-embryo (Danio rerio-embryo)	0.77	0.95	5	0.91	0.00	0.07	144.8	304,541.0	86	4	PFHxS, PFBA
Channel catfish (Ictalurus punctatus)	Bluegill (Lepomis macrochirus)	0.81	0.74	77	0.77	0.00	0.43	0.4	517,824.6	82	4	PFBS
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	0.82	0.82	98	0.76	0.00	0.50	0.0	13,400,000.0	76	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Channel catfish (Ictalurus punctatus)	Fathead minnow (Pimephales promelas)	0.98	0.06	52	0.87	0.00	0.30	1.2	26,500,000.0	85	4	PFBS
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1.02	-0.28	7	0.99	0.00	0.08	2.5	20,900,000.0	100	5	PFHxS, PFHxA
Channel catfish (Ictalurus punctatus)	African clawed frog (Xenopus laevis)	1.00	0.47	3	0.93	0.01	0.20	18.4	122,473.4	100	5	PFNA
Chinook salmon (Oncorhynchus tshawytscha)	Bluegill (Lepomis macrochirus)	0.91	-0.10	13	0.65	0.00	0.95	4.1	150,750.0	67	4	PFBS
Chinook salmon (Oncorhynchus tshawytscha)	Rainbow trout (Oncorhynchus mykiss)	1.02	0.02	18	0.96	0.00	0.11	3.3	724,430.8	95	1	PFDA, 7:3 FTCA, 8:2 FTUCA
Chinook salmon (Oncorhynchus tshawytscha)	Fathead minnow (Pimephales promelas)	1.16	-1.22	9	0.71	0.00	0.82	10.5	140,225.3	45	4	PFBS
Coho salmon (Oncorhynchus kisutch)	Bluegill (Lepomis macrochirus)	0.91	0.01	25	0.79	0.00	0.47	0.4	150,750.0	78	4	PFBS
Coho salmon (Oncorhynchus kisutch)	Rainbow trout (Oncorhynchus mykiss)	1.03	0.06	36	0.99	0.00	0.04	0.0	43,073.0	100	1	PFDA, 7:3 FTCA, 8:2 FTUCA

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	PFAS Compound
Coho salmon (Oncorhynchus kisutch)	Fathead minnow (Pimephales promelas)	0.91	-0.23	19	0.65	0.00	0.69	1.2	20,922.0	67	4	PFBS
Colorado squawfish (Ptychocheilus lucius)	Rainbow trout (Oncorhynchus mykiss)	0.91	0.92	7	0.73	0.00	0.56	3.3	43,073.0	67	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Colorado squawfish (Ptychocheilus lucius)	Fathead minnow (Pimephales promelas)	1.07	-0.58	6	0.68	0.01	0.49	58.3	20,922.0	63	2	PFBS
Common carp (Cyprinus carpio)	Zebrafish-embryo (Danio rerio-embryo)	0.83	0.45	5	0.66	0.03	0.37	86.0	34,190.0	71	2	PFBA
Common carp (Cyprinus carpio)	Bluegill (Lepomis macrochirus)	0.86	0.60	30	0.79	0.00	0.47	0.9	180,000.0	81	4	PFBS
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	0.81	0.92	47	0.74	0.00	0.52	0.2	198,443.9	76	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Common carp (Cyprinus carpio)	Fathead minnow (Pimephales promelas)	0.99	0.06	25	0.84	0.00	0.21	4.7	133,000.0	89	2	PFBS
Cutthroat trout (Oncorhynchus clarkii)	Bluegill (Lepomis macrochirus)	0.88	0.42	32	0.79	0.00	0.36	0.4	180,000.0	71	4	PFBS
Cutthroat trout (Oncorhynchus clarkii)	Rainbow trout (Oncorhynchus mykiss)	0.95	0.15	36	0.96	0.00	0.08	0.0	198,443.9	97	1	PFDA, 7:3 FTCA, 8:2 FTUCA
Cutthroat trout (Oncorhynchus clarkii)	Fathead minnow (Pimephales promelas)	0.99	-0.45	22	0.82	0.00	0.35	1.2	140,225.3	79	4	PFBS
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	0.61	1.97	2	0.99	0.01	0.01	709.9	155,541.7	100	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	1.03	-0.28	40	0.95	0.00	0.24	0.3	46,278,306.5	83	2	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA

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	PFAS Compound
Daphnid (Ceriodaphnia dubia)	Mysid (Americamysis bahia)	0.98	-0.21	25	0.84	0.00	0.77	0.0	4,100,000.0	52	5	PFBS
Daphnid (Ceriodaphnia reticulata)	Daphnid (Daphnia magna)	0.87	-0.03	7	0.70	0.01	0.24	0.8	231.7	89	2	PFNA, PFDA, 8:2 FTUCA
Daphnid (Daphnia galeata)	Daphnid (Daphnia magna)	0.92	0.27	4	0.96	0.00	0.08	0.1	646.2	83	1	PFNA, PFDA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Daphnia longispina)	Daphnid (Daphnia magna)	1.22	-0.57	2	0.99	0.01	0.06	8.9	10,359.2	100	1	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Daphnia magna)	Water flea (Chydorus sphaericus)	1.04	0.27	7	0.98	0.00	0.06	7.1	462,000.0	100	3	PFNA, PFDA
Daphnid (Daphnia magna)	Zebrafish (Danio rerio)	0.92	-0.31	11	0.71	0.00	0.74	3.1	36,910.0	54	6	PFHxS, PFBA
Daphnid (Daphnia magna)	Daphnid (Daphnia pulicaria)	0.88	0.51	4	0.94	0.00	0.19	9.3	237,367.4	83	1	PFDA
Daphnid (Daphnia magna)	Mysid (Americamysis bahia)	0.82	0.83	246	0.73	0.00	0.76	0.0	11,000,000.0	70	5	PFBS
Daphnid (Daphnia magna)	Bullfrog (Lithobates catesbeianus)	0.82	0.68	9	0.84	0.00	0.79	2.5	20,900,000.0	73	6	PFHxS, PFHxA
Daphnid (Daphnia pulex)	Daphnid (Daphnia magna)	1.01	-0.17	40	0.96	0.00	0.15	0.2	4,894,739.3	95	1	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Daphnia pulicaria)	Daphnid (Daphnia magna)	1.07	-0.32	4	0.94	0.00	0.23	14.1	281,641.4	83	1	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA

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	PFAS Compound
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	0.94	-0.02	21	0.86	0.00	0.28	0.2	7,200.0	87	2	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	0.75	0.61	5	0.88	0.00	0.35	0.1	166,819.4	57	2	PFNA, PFHxA, PFDA, PFBS, 7:3 FTCA, 8:2 FTUCA
Fairy shrimp (Streptocephalus rubricaudatus)	Zebrafish-embryo (Danio rerio-embryo)	0.60	2.45	2	0.95	0.03	0.06	4622.4	5,100,709.2	75	6	PFHxS
Fathead minnow (Pimephales promelas)	Zebrafish (Danio rerio)	1.07	-0.25	4	0.97	0.00	0.05	18.0	25,076.0	100	2	PFHxS, PFBA
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	1.00	-0.11	41	0.93	0.00	0.22	23.0	54,578,562.0	84	2	PFHxS, PFBA
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	0.69	1.42	236	0.64	0.00	0.76	0.2	46,500,000.0	66	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Fathead minnow (Pimephales promelas)	Bluegill (Lepomis macrochirus)	0.87	0.62	108	0.81	0.00	0.40	0.1	27,540,000.0	81	4	PFBS
Fathead minnow (Pimephales promelas)	Mysid (Americamysis bahia)	0.62	1.74	104	0.62	0.00	0.75	0.0	11,000,000.0	60	6	PFBS
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	0.90	0.62	146	0.87	0.00	0.27	0.2	13,400,000.0	86	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1.01	-0.10	7	0.98	0.00	0.10	2.5	20,900,000.0	100	5	PFHxS, PFHxA
Fathead minnow (Pimephales promelas)	African clawed frog (Xenopus laevis)	1.20	-0.62	4	0.91	0.00	0.22	44.7	122,473.4	67	5	PFNA

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	PFAS Compound
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	0.90	0.24	17	0.94	0.00	0.19	14.1	8,694,505.9	79	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Fatmucket (Lampsilis siliquoidea)	Fathead minnow (Pimephales promelas)	0.94	-0.40	13	0.65	0.00	0.93	13.4	10,830,770.1	47	6	PFBS
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	1.22	-0.78	2	0.99	0.00	0.01	7797.4	649,307.3	100	4	PFHxS, PFBA
Flagfish (Jordanella floridae)	Bluegill (Lepomis macrochirus)	0.86	0.47	7	0.89	0.00	0.30	0.4	113,000.0	78	4	PFBS
Flagfish (Jordanella floridae)	Fathead minnow (Pimephales promelas)	0.91	0.11	8	0.84	0.00	0.46	1.2	32,537.5	80	4	PFBS
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	0.97	0.33	7	0.96	0.00	0.06	144.8	304,541.0	100	2	PFHxS, PFBA
Goldfish (Carassius auratus)	Bluegill (Lepomis macrochirus)	0.78	0.98	27	0.71	0.00	0.51	0.4	201,000.0	72	4	PFBS
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	0.82	1.12	35	0.83	0.00	0.49	0.0	13,400,000.0	81	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Goldfish (Carassius auratus)	Fathead minnow (Pimephales promelas)	0.98	0.19	26	0.94	0.00	0.14	1.2	26,500,000.0	93	2	PFBS
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1.04	-0.30	7	0.98	0.00	0.14	2.5	20,900,000.0	89	5	PFHxS, PFHxA
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	0.67	0.94	2	0.97	0.02	0.08	14.1	166,819.4	75	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Green floater (Lasmigona subviridis)	Rainbow trout (Oncorhynchus mykiss)	0.60	1.44	4	0.73	0.03	0.36	3.3	95,857.7	67	6	PFDA
Green sunfish (Lepomis cyanellus)	Bluegill (Lepomis macrochirus)	0.85	0.63	13	0.92	0.00	0.14	4.1	113,000.0	93	1	PFBS

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	PFAS Compound
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	0.90	0.60	13	0.95	0.00	0.13	0.0	19,926.0	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Green sunfish (Lepomis cyanellus)	Fathead minnow (Pimephales promelas)	0.90	-0.09	6	0.64	0.02	0.89	10.5	26,685.4	50	4	PFBS
Greenthroat darter (Etheostoma lepidum)	Rainbow trout (Oncorhynchus mykiss)	1.05	-0.04	3	0.93	0.01	0.11	4.1	1,624.7	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Greenthroat darter (Etheostoma lepidum)	Fathead minnow (Pimephales promelas)	0.85	-0.09	3	0.92	0.01	0.13	5.7	8,320.3	100	4	PFBS
Guppy (Poecilia reticulata)	Bluegill (Lepomis macrochirus)	0.70	1.04	22	0.75	0.00	0.39	0.4	180,000.0	67	4	PFBS
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	0.71	1.24	24	0.63	0.00	0.68	0.6	198,443.9	69	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Guppy (Poecilia reticulata)	Fathead minnow (Pimephales promelas)	0.85	0.35	27	0.78	0.00	0.39	1.2	140,225.3	66	4	PFBS
Indian bullfrog (Euphlyctis hexadactylus)	Bluegill (Lepomis macrochirus)	0.88	0.02	4	0.86	0.01	0.29	37.9	113,000.0	67	5	PFBS
Indian bullfrog (Euphlyctis hexadactylus)	Rainbow trout (Oncorhynchus mykiss)	1.25	-0.12	5	0.78	0.01	0.59	28.3	19,926.0	57	5	8:2 FTUCA
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	0.86	1.88	7	0.91	0.00	0.31	0.3	166,819.4	67	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Isopod (Asellus aquaticus)	Bluegill (Lepomis macrochirus)	1.09	-0.19	5	0.91	0.00	0.37	2.8	123,267.3	43	6	PFBS
Isopod (Asellus aquaticus)	Mysid (Americamysis bahia)	0.79	1.69	4	0.89	0.00	0.31	0.0	6,982.6	83	4	PFBS
Isopod (Caecidotea brevicauda)	Bluegill (Lepomis macrochirus)	0.86	0.28	17	0.74	0.00	0.48	0.4	26,700.0	68	6	PFBS

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	PFAS Compound
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	0.78	0.41	19	0.70	0.00	0.52	0.6	82,000.0	57	6	PFDA, 7:3 FTCA, 8:2 FTUCA
Isopod (Caecidotea brevicauda)	Fathead minnow (Pimephales promelas)	0.76	-0.05	8	0.73	0.00	0.49	1.2	20,922.0	60	6	PFBS
Lake trout (Salvelinus namaycush)	Bluegill (Lepomis macrochirus)	0.69	0.80	20	0.72	0.00	0.27	2.8	180,000.0	77	4	PFBS
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	0.89	0.23	26	0.94	0.00	0.07	0.2	198,443.9	96	2	PFDA, 7:3 FTCA, 8:2 FTUCA
Lake trout (Salvelinus namaycush)	Fathead minnow (Pimephales promelas)	0.77	0.04	12	0.66	0.00	0.34	44.4	133,000.0	79	4	PFBS
Largemouth bass (Micropterus salmoides)	Bluegill (Lepomis macrochirus)	1.03	-0.10	33	0.93	0.00	0.12	0.4	123,267.3	94	2	PFBS
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	0.94	0.04	32	0.89	0.00	0.21	0.0	95,857.7	91	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	1.16	1.67	2	0.98	0.01	0.13	0.8	4,369.3	75	6	PFNA, PFDA, 7:3 FTCA
Mayfly (Drunella grandis)	Rainbow trout (Oncorhynchus mykiss)	1.01	0.64	3	0.98	0.00	0.13	0.6	95,857.7	100	6	PFDA, 7:3 FTCA, 8:2 FTUCA
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	0.91	0.61	3	0.95	0.01	0.16	6.6	54,399.6	80	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Medaka (Oryzias latipes)	Fathead minnow (Pimephales promelas)	0.86	0.60	6	0.98	0.00	0.05	2.7	1,420,000.0	88	4	PFBS
Midge (Chironomus plumosus)	Mysid (Americamysis bahia)	0.74	0.93	13	0.78	0.00	0.66	0.0	87,600.0	67	5	PFBS

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	PFAS Compound
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	0.97	0.76	6	0.89	0.00	0.82	0.3	472,000.0	50	5	PFNA, PFDA, 7:3 FTCA
Midge (Chironomus tentans)	Bluegill (Lepomis macrochirus)	1.03	0.11	5	0.94	0.00	0.39	0.1	517,824.6	71	6	PFBS
Midge (Paratanytarsus dissimilis)	Bluegill (Lepomis macrochirus)	0.85	1.02	5	0.89	0.00	0.49	0.4	201,000.0	57	6	PFBS
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	0.89	1.03	8	0.90	0.00	0.33	0.6	1,330,000.0	80	6	PFDA, 7:3 FTCA, 8:2 FTUCA
Midge (Paratanytarsus dissimilis)	Fathead minnow (Pimephales promelas)	0.86	0.97	8	0.85	0.00	0.52	1.2	1,430,000.0	80	6	PFBS
Midge (Paratanytarsus dissimilis)	Bullfrog (Lithobates catesbeianus)	1.09	-0.27	3	0.97	0.00	0.25	2.5	3,019,983.4	40	6	PFHxS, PFHxA
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	0.98	0.55	3	0.99	0.00	0.06	370.0	14,500,000.0	100	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Midge (Paratanytarsus parthenogeneticus)	Fathead minnow (Pimephales promelas)	0.99	0.57	2	0.98	0.01	0.09	280.0	10,600,000.0	100	6	PFBS
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	0.76	0.47	20	0.63	0.00	0.72	0.3	58,000.0	64	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Mosquitofish (Gambusia affinis)	Fathead minnow (Pimephales promelas)	0.95	0.01	2	0.99	0.01	0.12	5.7	26,500,000.0	100	4	PFBS
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	0.97	0.07	1	1.00	0.01	0.00	18600.0	20,900,000.0	N/A	5	PFHxS, PFHxA

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	PFAS Compound
Mozambique tilapia (Oreochromis mossambicus)	Fathead minnow (Pimephales promelas)	0.86	0.36	9	0.73	0.00	0.33	44.4	140,225.3	64	4	PFBS
Mrigal carp (Cirrhinus mrigala)	Rainbow trout (Oncorhynchus mykiss)	0.76	1.03	4	0.71	0.04	0.43	0.6	1,624.7	67	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Mrigal carp (Cirrhinus mrigala)	Fathead minnow (Pimephales promelas)	1.11	-0.59	3	0.98	0.00	0.01	196.9	8,320.3	100	2	PFBS
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	0.89	0.24	2	0.99	0.00	0.02	42.0	166,819.4	100	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Nile tilapia (Oreochromis niloticus)	Bluegill (Lepomis macrochirus)	1.04	-0.04	1	1.00	0.00	0.00	2.3	6,969.1	N/A	3	PFBS
Northern leopard frog (Lithobates pipiens)	Bluegill (Lepomis macrochirus)	0.80	1.13	1	1.00	0.02	0.01	7.9	180,000.0	N/A	5	PFBS
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	0.71	1.60	1	1.00	0.01	0.00	2.3	198,443.9	N/A	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Northern pike (Esox lucius)	Rainbow trout (Oncorhynchus mykiss)	1.04	-0.30	6	0.80	0.00	0.27	4.7	1,822.3	88	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Oligochaete (Limnodrilus hoffmeisteri)	Zebrafish-embryo (Danio rerio-embryo)	1.17	-0.31	3	0.92	0.01	0.24	144.8	239,250.6	60	6	PFHxS, PFBA
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	0.71	1.77	10	0.81	0.00	0.36	3.2	281,641.4	67	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Oligochaete (Limnodrilus hoffmeisteri)	Rainbow trout (Oncorhynchus mykiss)	0.71	1.60	6	0.67	0.01	0.76	3.3	707,000.0	50	6	8:2 FTUCA

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	PFAS Compound
Oligochaete (Lumbriculus variegatus)	Fathead minnow (Pimephales promelas)	0.94	0.28	14	0.82	0.00	0.43	1.2	140,225.3	75	6	PFBS
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	0.89	1.37	13	0.80	0.00	0.78	0.1	4,894,739.3	47	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Oligochaete (Tubifex tubifex)	Bluegill (Lepomis macrochirus)	0.72	0.67	7	0.72	0.00	0.84	0.1	113,000.0	56	6	PFBS
Oligochaete (Tubifex tubifex)	Fathead minnow (Pimephales promelas)	1.04	-0.10	8	0.87	0.00	0.53	15.3	10,830,770.1	50	6	PFBS
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	1.15	-0.84	3	0.99	0.00	0.06	11.0	281,641.4	100	5	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	0.89	0.28	11	0.98	0.00	0.08	14.1	8,694,505.9	100	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Paper pondshell (Utterbackia imbecillis)	Fathead minnow (Pimephales promelas)	0.86	-0.16	9	0.65	0.00	0.85	134.2	10,830,770.1	18	6	PFBS
Peppered loach (Lepidocephalichthys guntea)	Fathead minnow (Pimephales promelas)	0.78	0.72	1	1.00	0.03	0.00	196.9	43,785.6	N/A	3	PFBS
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	0.96	0.00	3	0.97	0.00	0.11	42.0	545,927.5	100	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Rainbow trout (Oncorhynchus mykiss)	Zebrafish (Danio rerio)	1.31	-1.12	5	0.96	0.00	0.24	3.1	26,390.0	71	4	PFHxS, PFBA

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	PFAS Compound
Rainbow trout (Oncorhynchus mykiss)	Zebrafish-embryo (Danio rerio-embryo)	1.16	-1.06	27	0.80	0.00	0.61	23.0	8,843,890.0	66	4	PFHxS, PFBA
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	0.71	1.03	377	0.63	0.00	0.84	0.1	14,500,000.0	60	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Rainbow trout (Oncorhynchus mykiss)	Bluegill (Lepomis macrochirus)	0.95	0.03	314	0.89	0.00	0.22	0.1	7,100,000.0	91	4	PFBS
Rainbow trout (Oncorhynchus mykiss)	Mysid (Americamysis bahia)	0.66	1.35	201	0.62	0.00	0.72	0.0	113,000.0	68	6	PFBS
Rainbow trout (Oncorhynchus mykiss)	Fathead minnow (Pimephales promelas)	0.97	-0.20	146	0.87	0.00	0.29	0.2	26,500,000.0	87	4	PFBS
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1.09	-0.71	9	0.95	0.00	0.38	2.5	20,900,000.0	73	5	PFHxS, PFHxA
Razorback sucker (Xyrauchen texanus)	Bluegill (Lepomis macrochirus)	0.81	0.01	6	0.67	0.01	0.48	53.9	150,750.0	75	4	PFBS
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	0.78	0.91	6	0.87	0.00	0.19	3.3	43,073.0	88	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Razorback sucker (Xyrauchen texanus)	Fathead minnow (Pimephales promelas)	0.98	-0.36	5	0.66	0.03	0.40	58.3	8,320.3	71	3	PFBS
Redear sunfish (Lepomis microlophus)	Bluegill (Lepomis macrochirus)	1.03	-0.03	2	1.00	0.00	0.00	17.0	4,500.0	100	1	PFBS
Riceland prawn (Macrobrachium lanchesteri)	Rainbow trout (Oncorhynchus mykiss)	0.85	0.97	2	0.95	0.03	0.09	3.3	2,040.4	100	6	PFDA, 7:3 FTCA, 8:2 FTUCA
Rohu (Labeo rohita)	Bluegill (Lepomis macrochirus)	0.94	0.77	4	0.94	0.00	0.21	0.8	7,326.2	83	4	PFBS
Rohu (Labeo rohita)	Rainbow trout (Oncorhynchus mykiss)	1.07	0.86	4	0.89	0.00	0.37	0.6	1,624.7	67	4	PFDA, 7:3 FTCA, 8:2 FTUCA

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	PFAS Compound
Rohu (Labeo rohita)	Fathead minnow (Pimephales promelas)	0.98	0.32	4	0.87	0.01	0.30	2.3	8,320.3	83	2	PFBS
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	1.16	-0.58	3	0.98	0.00	0.06	28.3	95,857.7	100	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	1.09	-0.03	1	1.00	0.01	0.00	1.4	7,075.2	N/A	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Snipefly (Atherix variegata)	Rainbow trout (Oncorhynchus mykiss)	0.94	0.88	1	1.00	0.02	0.00	0.6	68.4	N/A	6	PFDA, 7:3 FTCA
Sockeye salmon (Oncorhynchus nerka)	Rainbow trout (Oncorhynchus mykiss)	0.85	0.60	5	0.85	0.00	0.14	28.3	7,500.0	71	1	PFDA, 7:3 FTCA, 8:2 FTUCA
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	1.28	-0.26	4	0.98	0.00	0.04	28.3	9,700.0	100	5	PFDA, 7:3 FTCA, 8:2 FTUCA
Spotfin chub (Erimonax monachus)	Rainbow trout (Oncorhynchus mykiss)	1.20	-0.41	3	0.97	0.00	0.06	4.1	1,624.7	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	0.94	0.20	6	0.96	0.00	0.23	14.1	8,694,505.9	88	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	0.96	0.11	8	0.96	0.00	0.20	14.1	8,694,505.9	80	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Tadpole physa (Physella gyrina)	Fathead minnow (Pimephales promelas)	1.22	-1.60	6	0.84	0.00	0.58	813.9	10,830,770.1	25	6	PFBS
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	0.95	-0.43	4	0.97	0.00	0.13	14.1	4,894,739.3	100	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA

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	PFAS Compound
Threeridge (Amblema plicata)	Fathead minnow (Pimephales promelas)	1.30	-2.81	3	0.98	0.00	0.13	813.9	10,830,770.1	100	6	PFBS
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	0.94	0.12	5	0.98	0.00	0.07	14.1	8,694,505.9	100	4	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Walleye (Sander vitreus)	Bluegill (Lepomis macrochirus)	0.74	0.54	7	0.78	0.00	0.22	4.3	6,722.1	67	3	PFBS
Walleye (Sander vitreus)	Rainbow trout (Oncorhynchus mykiss)	0.68	0.60	7	0.69	0.01	0.28	4.7	16,235.6	56	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	0.96	-0.23	9	0.97	0.00	0.13	14.1	8,694,505.9	91	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	0.95	-0.20	7	0.98	0.00	0.06	8.9	977,589.9	100	3	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	0.72	1.10	3	0.97	0.00	0.11	8.9	281,641.4	100	3	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Wavyrayed lampmussel (Lampsilis fasciola)	Daphnid (Daphnia magna)	1.18	-1.28	1	0.99	0.05	0.01	166819.4	4,894,739.3	N/A	6	PFHxA
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	0.88	0.27	8	0.96	0.00	0.16	14.1	8,694,505.9	80	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA

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	PFAS Compound
Western toad (Anaxyrus boreas)	Rainbow trout (Oncorhynchus mykiss)	1.33	-0.51	3	0.90	0.01	0.15	28.3	1,624.7	80	5	PFDA, 7:3 FTCA, 8:2 FTUCA
White sucker (Catostomus commersonii)	Bluegill (Lepomis macrochirus)	1.05	-0.32	7	0.92	0.00	0.18	35.0	123,267.3	89	4	PFBS
White sucker (Catostomus commersonii)	Fathead minnow (Pimephales promelas)	1.15	-0.37	5	0.93	0.00	0.21	43.9	140,225.3	86	3	PFBS
Yellow perch (Perca flavescens)	Bluegill (Lepomis macrochirus)	0.98	-0.11	17	0.92	0.00	0.15	0.4	9,409.7	89	3	PFBS
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	0.90	0.29	23	0.77	0.00	0.58	0.0	16,235.6	84	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Yellow perch (Perca flavescens)	Fathead minnow (Pimephales promelas)	0.94	-0.38	12	0.74	0.00	0.54	1.2	20,922.0	64	4	PFBS
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	0.77	1.22	11	0.71	0.00	0.62	0.1	50,000.0	62	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Zebrafish (Danio rerio)	Mysid (Americamysis bahia)	0.73	1.91	7	0.68	0.01	0.83	0.0	5,666.0	33	6	PFBS
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	0.73	0.96	5	0.96	0.00	0.14	0.2	229,100.0	100	4	PFDA, 7:3 FTCA, 8:2 FTUCA
Zebrafish (Danio rerio)	Fathead minnow (Pimephales promelas)	0.91	0.31	4	0.97	0.00	0.04	13.4	26,685.4	100	2	PFBS
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	0.66	1.72	43	0.67	0.00	0.87	0.1	46,500,000.0	56	6	PFNA, PFHxA, PFDA, PFBS, PFBA, 7:3 FTCA, 8:2 FTUCA
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	0.69	1.43	27	0.80	0.00	0.37	0.2	8,341,462.7	76	4	PFDA, 7:3 FTCA, 8:2 FTUCA

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	PFAS Compound
Zebrafish-embryo (Danio rerio-embryo)	Fathead minnow (Pimephales promelas)	0.93	0.38	41	0.93	0.00	0.20	4.0	70,200,000.0	81	2	PFBS

Appendix F Derivation of PFAS Benchmarks using Scaled Data

As discussed in **Section 3.0**, PFAS acute values (typically reported as mg/L) can be greater than those used to develop an ICE model (ICE database toxicity range $1x10^{-4}$ and $1x10^{8}$ µg/L) such that the input PFAS value of the surrogate would be outside the model domain. In these situations, a user can either enter the measured toxicity value (LC_{50}/EC_{50}) into the ICE model as µg/L and allow the regression to extrapolate beyond the range of the model or enter a "scaled" toxicity value (i.e., enter the measured LC_{50} value as mg/L) (Raimondo et al. 2024). **Figure 3-1** provides an example data plots and calculation of an EC_{50} value for *Daphnia magna* for PFBS for a value that falls beyond the model range, when calculated using an "extrapolation" and "scaled" approach. Calculations presented in **Section 5** of this document allowed extrapolation beyond the range of the regression model, recognizing that such an extrapolation has inherent uncertainties and can result in larger confidence intervals around the prediction than would typically occur using the scaled approach. Within this section, we apply the alternative scaled approach to explore calculating benchmark values for the eight PFAS using this alternative method (Raimondo et al. 2024).

For the scaled approach, values are directly entered as µg/L in situations when the surrogate species measured PFAS value falls within the range of the ICE model. In cases where the surrogate species measured value is beyond the range of the ICE model, the value is then entered as mg/L, so that it falls within the range of the model data. For example, if a hypothetical concentration of 15 µg/L is beyond the range of the ICE model data for a chemical, it is then entered as 15 mg/L to bring it within range of the model data. Values were also scaled in some situations where the measured value falls within the model range but where the confidence interval was large (>50 fold), and use of scaling resulted in acceptable confidence intervals.

The use of scaled values has been validated through a comprehensive analysis of ICE data, where it has been demonstrated that 3,104 values predicted in this manner had the same level of accuracy as values entered into ICE models as µg/L within and beyond the model domain for models with slopes ranging from 0.66 - 1.33 (Raimondo et al. 2024). Raimondo et al. (2024) also demonstrated that using ICE models in this manner does not violate any of the model assumptions, which 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, toxicity unit). Additionally, there are no statistical assumptions of linear regressions (linear relationship, normality, homoscedasticity, no auto-correlation, no or little multicollinearity; Dowdy et al. 2011) violated by this application. The following sections present the calculations and resulting alternative benchmark values for each of eight selected PFAS using the scaled approach. ICE models selected for use for the derivation of recommended PFAS benchmarks are presented in **Table E-1** and the empirical data used for this evaluation are presented in **Appendix A**.

F.1 Derivation of Acute Water Benchmark for Perfluorobutanoic Acid (PFBA)

Quantitatively acceptable empirical data for PFBA were available for five species comprising four genera and fulfilling three MDR groups (**Table F-2**). A total of 43 ICE models were accepted for use. Of these, seven ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Chydorus sphaericus* and *Daphnia magna*). Three other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Pimephales promelas*). One additional model (*Danio rerio* predicting for *Pimephales promelas*) was not used because the measured

EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related species with measured EC_{50} values within the ICE model range were used. A total of 32 ICE models were selected for use, resulting in ICE models predicting 31 SMAVs, representing 29 genera (**Tables F-1** and **F-2**).

The combined empirical and ICE data resulted in 33 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-2.** GMAVs for the four most sensitive genera were within a factor of 13.9 of each other (**Table F-3**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFBA is 347.1 mg/L (**Table F-3**), which is lower than all of the GMAVs except for one tested species (the rotifer, *Brachionus calyciflorus,* GMAV=110 mg/L). The FAV was divided by two to obtain a preliminary value of 173.5 mg/L PFBA and then adjusted by the carboxylic acid application factor (15.8) to obtain the alternative freshwater acute water column benchmark magnitude of 11 mg/L PFBA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFBA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-1**).

Table F-1. Acceptable models for ICE-estimated Species Sensitivity to PFBA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC50 (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0008	166.8	**44,908.2** (10,018-201,313)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0002	5,000	**3,060.4** (1,474.4–6,352.5)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0001	68.3	**4,490.4** (1,523.0–13,240)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0003	8,694.5	**2,248.7** (1,108-4,563)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0001	46,278.3	**959.02** (507.98-1,810.6)
Channel catfish (Ictalurus punctatus)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.145	304.5	**13,361.7** (6,856.1-26,037)
Common carp (Cyprinus carpio)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.086	34.19	**7,908.4** (1,225.4–51,039)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0003	46,278.3	**4,100.3** (2,125.4-7,909.9)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.009	10.36	8,995.3 (1,641.2-49,301)
Daphnid (Daphnia magna)*	Zebrafish (Danio rerio)	13,779**	13,779 (mg/L)	0.003	36.91	3,291.4 (831.19-13,034)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0002	4,894.7	3,957.7 (2,788.6-5,616.9)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.014	281.6	4,361.5 (1,223.6-15,546)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0002	7.2	**2,939.0** (964.77–8,953.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC50 (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0002	46,500	1,174.4[b] (655.01–2,105.8)
Fathead minnow (Pimephales promelas)	Zebrafish (Danio rerio)	13,779**	13,779 (mg/L)	0.018	25.08	15,343.0[c] (7,383.2-31,884)
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	13,779	13,779,000 (µg/L)	0.023	54,579	**11,532.0** (6,189.6-21,485)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**2,013.5** (809.37-5,008.9)
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	13,779**	13,779 (mg/L)	7.797	649.3	**18,640.2** (10,316-33,681)
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.145	304.5	**23,117.1** (14,833-36,028)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.014	166.8	**2,645.9** (481.11-14,552)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0003	166.8	**116,979.2** (32,795-417,264)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.37	14,500	**12,921.0** (3,394.0-49,189)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0003	58	**1,932.2** (372.75-10,016)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.042	166.8	**3,613.7** (1,572.1-8,306.4)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.003	281.6	**25,412.0** (9,374.1-68,889)
Oligochaete (Limnodrilus hoffmeisteri)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.145	239.3	35,536.4[b] (6,336.9-199,284)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0001	4,894.7	**49,159.1** (10,555-228,948)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.011	281.6	**2,680.1** (1,224.4-5,866.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC50 (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**1,748.6** (862.33-3,545.6)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.042	545.9	**3,775.7** (1,269.6-11,229)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0001	14,500	677.8[b] (396.18–1,159.4)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish (Danio rerio)	13,779**	13,779 (mg/L)	0.003	26.39	**20,148.4** (5,451.2-74,471)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish-embryo (Danio rerio-embryo)	13,779**	13,779 (mg/L)	0.023	8,843.9	**5,447.6** (2,518.2- 11,785)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**3,490.7** (670.81-18,165)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**3,740.1** (1,061.7-13,176)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.014	4,894.7	**1,262.4** (480.17-3,318.7)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**2,852.6** (949.6-8,569.8)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**1,759.2** (742.37-4,168.8)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.009	977.6	2,151.3 (1,364.7- 3391.2)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.009	281.6	**5,989.5** (1,810.5-19,815)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.014	8,694.5	**1,528.3** (497.59-4,694.2)
Zebrafish (Danio rerio)*	Daphnid (Daphnia magna)	5,251**	5,251 (mg/L)	0.0001	50	12,209.2 (3,373.7-44,185)
Zebrafish-embryo (Danio rerio-embryo)*	Daphnid (Daphnia magna)	5,251	5,251,000 (µg/L)	0.0001	46,500	1,391.4 (413.39-4,683.2)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.
[a] Saltwater surrogate species were not used.
[b] Not used because other more closely taxonomically-related models were available.
[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-2. Ranked PFBA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFBA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	**H**	**Rotifer**	***Brachionus calyciflorus***	**110**	110	0.03
2	B	Bluegill	*Lepomis macrochirus*	959	959	0.06
3	G	Threeridge	*Amblema plicata*	1,262**	1,262	0.09
4	G	Western pearlshell	*Margaritifera falcata*	1,528	1,528	0.12
5	G	Paper pondshell	*Utterbackia imbecillis*	1,749	1,749	0.15
6	G	Washboard	*Megalonaias nervosa*	1,759	1,759	0.18
7	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	1,932**	1,932	0.21
8	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	2,249	2,249	0.24
9	G	Green floater	*Lasmigona subviridis*	2,646**	2,646	0.26
10	E	Oriental river shrimp	*Macrobrachium nipponense*	2,680**	2,680	0.29
11	G	Neosho mucket	*Lampsilis rafinesqueana*	3,614**	2,697	0.32
	G	Fatmucket	*Lampsilis siliquoidea*	2,013		
12	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	2,853	2,853	0.35
13	D	Daphnid	*Simocephalus serrulatus*	2,939**	2,939	0.38
14	G	Swamp lymnaea	*Lymnaea stagnalis*	3,491	3,491	0.41
15	E	Amphipod	*Gammarus fasciatus*	3,060**	3,707	0.44
	E	Amphipod	*Gammarus pseudolimnaeus*	4,490**		
16	G	Tadpole physa	*Physella gyrina*	3,740	3,740	0.47
17	G	Pheasantshell	*Ortmanniana pectorosa*	3,776**	3,776	0.50
18	D	Daphnid	*Ceriodaphnia dubia*	4,100	4,100	0.53
19	**D**	**Daphnid**	***Chydorus sphaericus***	**>4,280.8**	>4,280.8	0.56
20	**D**	**Daphnid**	***Daphnia magna***	**4,741**	4,741	0.59
	D	**Daphnid**	***Daphnia pulicaria***	**>1,006[a]**		
21	D	Water flea	*Moina macrocopa*	5,989**	5,989	0.62
22	B	Common carp	*Cyprinus carpio*	7,908**	7,908	0.65
23	A	Rainbow trout	*Oncorhynchus mykiss*	10,477**	10,477	0.68
24	B	Fathead minnow	*Pimephales promelas*	11,532	11,532	0.71
25	F	Midge	*Paratanytarsus parthenogeneticus*	12,921	12,921	0.74
26	C	Channel catfish	*Ictalurus punctatus*	13,361**	13,361	0.76
27	**B**	**Zebrafish**	***Danio rerio***	**13,779**	13,779	0.79
28	C	Flagfish	*Jordanella floridae*	18,640**	18,640	0.82
29	B	Goldfish	*Carassius auratus*	23,117**	23,117	0.85
30	H	Oligochaete	*Limnodrilus hoffmeisteri*	25,412**	25,412	0.88
31	E	Amphipod	*Crangonyx pseudogracilis*	44,908**	44,908	0.91
32	H	Oligochaete	*Tubifex tubifex*	49,159**	49,159	0.94
33	E	Isopod	*Asellus aquaticus*	116,979**	116,979	0.97

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

a Not used in GMAV calculation, because value represents a greater than low value

1: Freshwater MDR 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, 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.

Table F-3. PFBA Protective Aquatic Life Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=33 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	***Brachionus***	**110.0**	4.70	22.09	0.029	0.171
2	*Lepomis*	959.0	6.87	47.14	0.059	0.243
3	*Amblema*	1262.4	7.14	50.99	0.088	0.297
4	*Margaritifera*	1528.3	7.33	53.76	0.118	0.343
		Σ (Sum):	**26.04**	**173.98**	**0.29**	**1.05**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 273.69 S = slope
L = 2.150 L = X-axis intercept
A = 5.849 A = lnFAV
FAV = 347.1
FAV/2 = 173.5 mg/L (Preliminary Value)
Adjustment = 173.5 / 15.8 = 10.98 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **11 mg/L PFBA** (rounded to two significant figures)

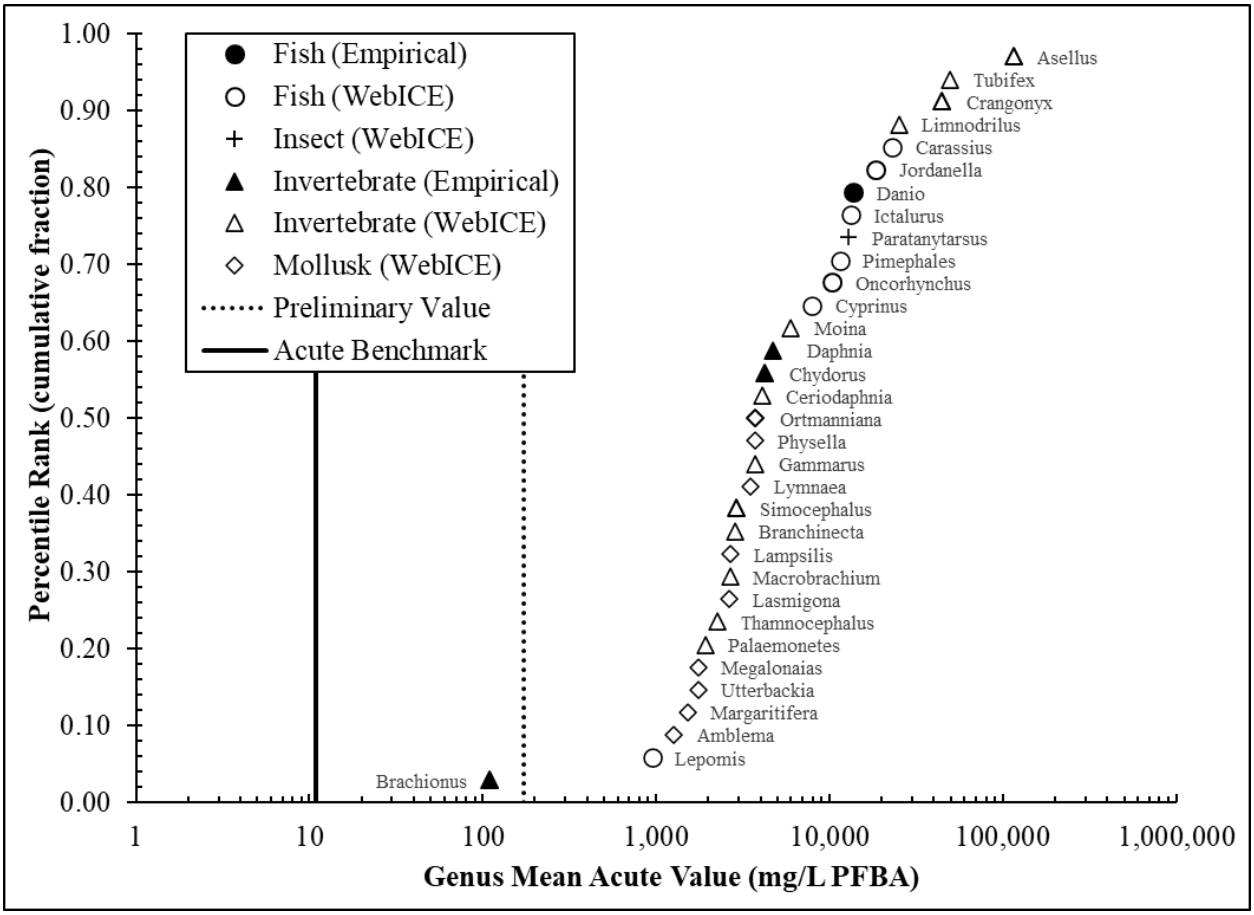

Figure F-1. Ranked Acute PFBA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.2 Derivation of Acute Water Benchmark for Perfluorohexanoic Acid (PFHxA)

Quantitatively acceptable empirical data for PFHxA were available for four species comprising three genera and fulfilling three MDR groups (**Table F-5**). A total of 43 ICE models were accepted for use. Of these, four ICE models were not selected for use because empirical data were available for the genus (e.g., models for *Daphnia longispina* and *Daphnia magna*). Three other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Lepomis macrochirus*). One additional model (*Daphnia magna* predicting for *Danio rerio*) was not used because the measured EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related

species with measured EC_{50} values within the ICE model range were used. A total of 35 ICE models were selected for use, resulting in ICE models predicting 35 SMAVs representing 30 genera (**Tables F-4** and **F-5**).

The combined empirical and ICE data resulted in 33 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-5**. GMAVs for the four most sensitive genera were within a factor of 2.7 of each other (**Table F-6**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFHxA is 189.1 mg/L (**Table F-6**), which is lower than all of the GMAVs except for one tested species (the rotifer, *Brachionus calyciflorus*, GMAV=140 mg/L). The FAV was divided by two to obtain a preliminary value of 94.57 mg/L PFHxA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 6.0 mg/L PFHxA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFHxA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-2**).

Table F-4. Acceptable models for ICE-estimated Species Sensitivity to PFHxA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0008	166.8	**10,313.0** (3,000.6-35,445)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0002	5,000	**234.7** (64.5-853.7)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0001	68.3	**1,026.8** (431.3-2,444.8)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0003	8,694.5	**517.3** (280.8-953.1)
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1,105**	1,105 (mg/L)	0.003	233	**766.4** (378-1,553.6)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0001	46,278	303.5[b] (176-523.2)
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**719.8** (362.4-1,429.7)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0003	46,278	**785.2** (446.2-1,381.6)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.009	10.36	1,267.8 (334.3-4,808.1)
Daphnid (Daphnia magna)*	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	424.1 (68.5-2,627.9)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0002	4,894.7	841.4 (444.8-1,591.6)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.014	281.6	783.4 (181.5-3,381.5)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0002	7.2	**648.9** (269.3-1,563.9)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0001	166.8	**753.7** (136-4,175.6)
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**1,083.6** (468.5-2,506.4)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0002	46,500	384.7[b] (235.8-627.7)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**470.8** (220.2-1,006.8)
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**1,027.7** (405.9-2,602.1)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.014	166.8	**901.5** (216-3,761.6)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0003	166.8	**29,446.1** (9,967-86,994)
Midge (Paratanytarsus dissimilis)	Bullfrog (Lithobates catesbeianus)	1,105^	1,105 (mg/L)	0.003	3,020.0	**1,069.7** (176.6-6,480.9)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.37	14,500	**2,676.6** (917.7-7,806.3)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0003	58	**569.4** (155.9-2,080.1)
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	18.6	20,900	**805.1** (394.5-1,643.4)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.042	166.8	**860.1** (421.3-1,755.9)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.003	281.6	**8,114.2** (3,337.6-19,727)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	1,048^	1,048 (mg/L)	0.0001	4,894.7	**11,637.0** (3,223.3-42,013)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.011	281.6	**421.3** (176.7-1004.7)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**418.5** (231.7-755.9)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.042	545.9	**803.4** (252.6-2,555.1)
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**713.5** (200.3-2,541.4)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0001	14,500	214.2[b] (135.8-337.8)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**762.0** (188.8-3,075.8)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**794.5** (277.3-2,276.3)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	4,894.7	**191.3** (48-761.8)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**623.8** (247-1,575.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**372.3** (181.1-765.2)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.009	977.6	**465.4** (271.8-796.9)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.009	281.6	**1,876.3** (633.2-5,560.2)
Wavyrayed lampmussel (Lampsilis fasciola)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	166.8	4,894.7	**686.2** (127.8-3,684.7)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.014	8,694.5	**370.2** (145.8-940.1)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	1,048**	1,048 (mg/L)	0.0001	50	3,520.9[c] (1,155.2-10,731)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	1,048	1,048,000 (µg/L)	0.0001	46,500	**481.7** (172.4-1,346.1)

*****Acceptable models that were not used because genus level empirical data were available.

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.
^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.
[a] Saltwater surrogate species were not used.
[b] Not used because other more closely taxonomically-related models were available.
[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-5. Ranked PFHxA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFHxA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	**H**	**Rotifer**	***Brachionus calyciflorus***	**140.0**	140.0	0.03
2	G	Threeridge	*Amblema plicata*	191.3	191.3	0.06
3	G	Western pearlshell	*Margaritifera falcata*	370.2	370.2	0.09
4	G	Washboard	*Megalonaias nervosa*	372.3	372.3	0.12
5	G	Paper pondshell	*Utterbackia imbecillis*	418.5	418.5	0.15
6	E	Oriental river shrimp	*Macrobrachium nipponense*	421.3**	421.3	0.18
7	D	Water flea	*Chydorus sphaericus*	465.4**	465.4	0.21
8	B	Zebrafish	*Danio rerio*	481.7	481.7	0.24
9	E	Amphipod	*Gammarus fasciatus*	234.7	490.9	0.26
	E	Amphipod	*Gammarus pseudolimnaeus*	1,027**		
10	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	517.3	517.3	0.29
11	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	569.4**	569.4	0.32
12	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	623.8	623.8	0.35
13	G	Wavyrayed lampmussel	*Lampsilis fasciola*	686.2	652.6	0.38
	G	Neosho mucket	*Lampsilis rafinesqueana*	860.1**		
	G	Fatmucket	*Lampsilis siliquoidea*	470.8		
14	D	Daphnid	*Simocephalus serrulatus*	648.9**	699.3	0.41
	D	Daphnid	*Simocephalus vetulus*	753.7**		
15	A	Rainbow trout	*Oncorhynchus mykiss*	713.5	713.5	0.44
16	B	Channel catfish	*Ictalurus punctatus*	719.8	719.8	0.47
17	G	Swamp lymnaea	*Lymnaea stagnalis*	762.0	762.0	0.50
18	B	Bluegill	*Lepomis macrochirus*	766.4**	766.4	0.53
19	D	Daphnid	*Ceriodaphnia dubia*	785.2	785.2	0.56
20	G	Tadpole physa	*Physella gyrina*	794.5	794.5	0.59
21	G	Pheasantshell	*Ortmanniana pectorosa*	803.4**	803.4	0.62
22	B	Mosquitofish	*Gambusia affinis*	805.1	805.1	0.65
23	G	Green floater	*Lasmigona subviridis*	901.5**	901.5	0.68
24	**C**	**Bullfrog**	***Lithobates catesbeianus***	**1,105**	915.2	0.71
	C	**Green frog**	***Lithobates clamitans***	**758.0**		
25	B	Goldfish	*Carassius auratus*	1,028	1,028	0.74
26	**D**	**Daphnia**	***Daphnia magna***	**1,048**	1,048	0.76
27	B	Fathead minnow	*Pimephales promelas*	1,084	1,084	0.79
28	F	Midge	*Paratanytarsus dissimilis*	1,070	1,692	0.82
	F	Midge	*Paratanytarsus parthenogeneticus*	2,677		
29	D	Water flea	*Moina macrocopa*	1,876**	1,876	0.85
30	H	Oligochaete	*Limnodrilus hoffmeisteri*	8,114**	8,114	0.88
31	E	Amphipod	*Crangonyx pseudogracilis*	10,313**	10,313	0.91
32	H	Oligochaete	*Tubifex tubifex*	11,637	11,637	0.94
33	E	Isopod	*Asellus aquaticus*	29,446**	29,446	0.97

**Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR 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, 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.

Table F-6. PFHxA Final Acute Value and Protective Aquatic Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=33 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	***Brachionus***	**140.0**	4.94	24.42	0.029	0.171
2	*Amblema*	191.3	5.25	27.60	0.059	0.243
3	*Margaritifera*	370.2	5.91	34.98	0.088	0.297
4	*Megalonaias*	372.3	5.92	35.04	0.118	0.343
		Σ (Sum):	**22.03**	**122.04**	**0.29**	**1.05**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 44.02 S = slope
L = 3.759 L = X-axis intercept
A = 5.242 A = lnFAV
FAV = 189.1
FAV/2 = 94.57 mg/L (Preliminary Value)
Adjustment = 94.57 / 15.8 = 5.985 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **6.0 mg/L PFHxA** (rounded to two significant figures)

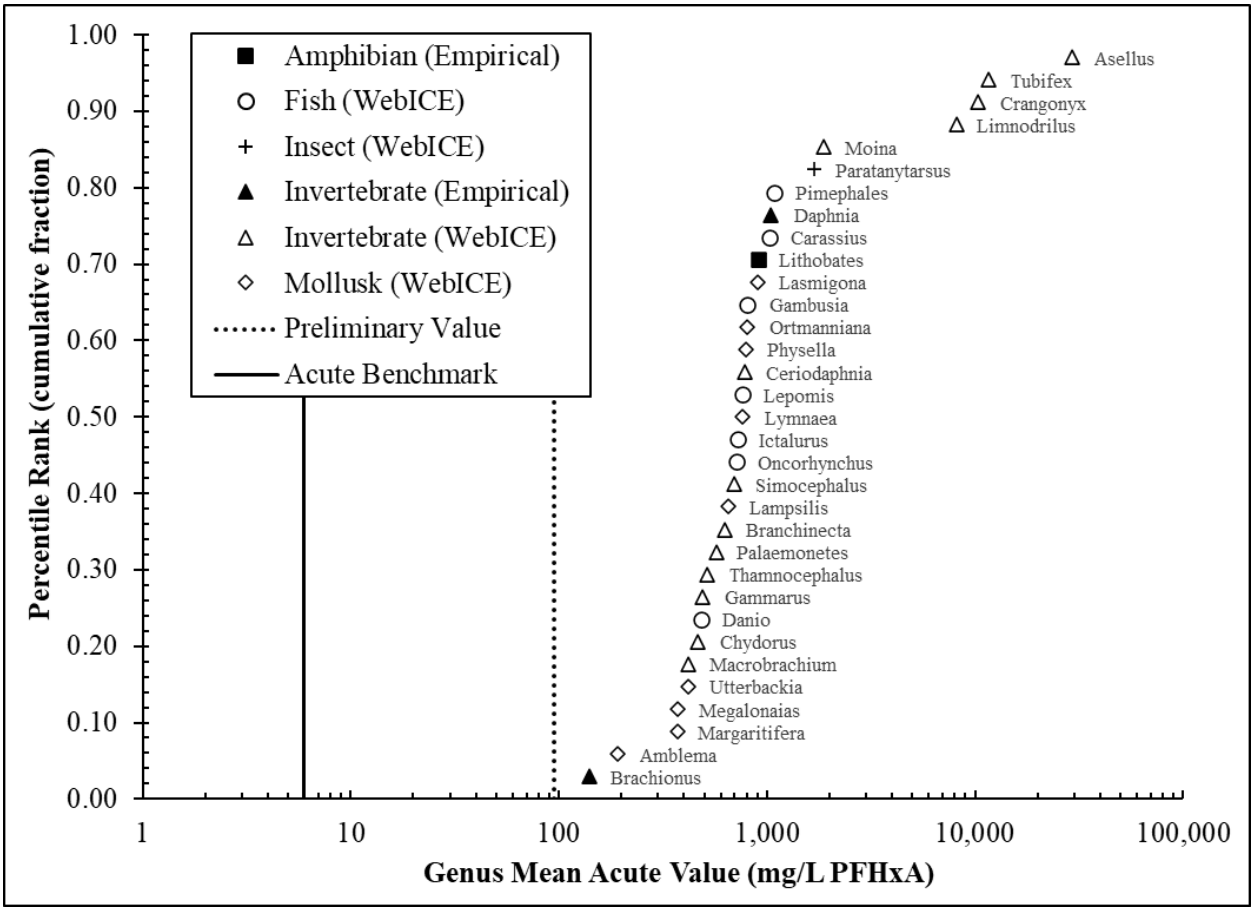

Figure F-2. Ranked Acute PFHxA GMAVs Used for the Aquatic Life Acute Benchmark Calculations.

F.3 Derivation of Acute Water Benchmark for Perfluorononanoic Acid (PFNA)

Quantitatively acceptable empirical data for PFNA were available for three species comprising three genera and fulfilling two MDR groups (**Table F-8**). A total of 42 ICE models were accepted for use. Of these, six ICE models were not selected for use because empirical data were available for the genus (e.g., models for *Daphnia longispina* and *Daphnia magna*). One other model was not used because there was an acceptable model for more closely-related surrogate species (i.e., *Daphnia magna* predicting for *Pimephales promelas*). One additional model (i.e., *Daphnia magna* predicting for *Danio rerio*) was not used because the measured EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related

species with measured EC_{50} values within the ICE model range were used. A total of 34 ICE models were selected for use, resulting in ICE models predicting 34 SMAVs representing 30 genera (**Tables F-7** and **F-8**).

The combined empirical and ICE data resulted in 33 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-8**. GMAVs for the four most sensitive genera were within a factor of 2.0 of each other (**Table F-9**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFNA is 23.18 mg/L (**Table F-9**), which is lower than all of the GMAVs except for the one ICE-derived species value (the threeridge mussel, *Amblema plicata*, GMAV=17.58 mg/L). The FAV was divided by two to obtain a preliminary value of 11.59 mg/L PFNA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.73 mg/L PFNA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFNA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-3**).

Table F-7. Acceptable models for ICE-estimated Species Sensitivity to PFNA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.0008	166.8	**1,035.5** (296.9-3,611.9)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0002	5,000	**29.8** (10.9-81.6)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0001	68.3	**102.4** (51.2-204.7)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0003	8,694.5	**52.1** (32.2-84.2)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0001	46,278	**50.3** (33.3-75.9)
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.014	0.232	**648.8** (426-988.3)
Channel catfish (Ictalurus punctatus)	African clawed frog (Xenopus laevis)	335.8**	335.8 (mg/L)	0.018	122.5	**977.6** (162.9-5,867.9)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0003	46,278	**59.4** (38.2-92.1)
Daphnid (Ceriodaphnia reticulata)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0008	0.232	**44.5** (16.1-123.4)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0001	0.646	109.2 (49.2-242.4)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.009	10.36	59.4 (16.2-217.7)
Daphnid (Daphnia magna)*	Water flea (Chydorus sphaericus)	27.84	27,840 (µg/L)	0.007	462	76.4 (48-121.5)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0002	4,894.7	65.8 (40.3-107.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	281.6	84.2 (18-392.8)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0002	7.2	**61.3** (33.8-111)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.0001	166.8	**113.7** (29.4-439.6)
Fathead minnow (Pimephales promelas)	African clawed frog (Xenopus laevis)	335.8**	335.8 (mg/L)	0.045	122.5	**260.9** (48.9-1,393.3)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0002	46,500	67.3[b] (47.1-96.2)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**48.6** (27.6-85.6)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.014	166.8	**167.6** (38.9-721.5)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0003	166.8	**1,261.8** (217.7-7,314.9)
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0008	4.369	**7,904.0** (1,279-48,846)
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.0003	472	**435.6** (71.7-2,647.8)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.37	14,500	**228.8** (101.9-513.5)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0003	58	**84.4** (33.9-210)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.042	166.8	**42.9** (12.1-152.7)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.003	281.6	**181.9** (43.1-768.2)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	84.51^	84.51 (mg/L)	0.0001	4,894.7	**1,225.2** (393.2-3,817.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.011	281.6	**65.1** (24.3-174.3)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**44.8** (28.6-70.3)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.042	545.9	**54.4** (13.3-222.4)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0001	14,500	**35.4** (25.2-49.7)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**70.7** (24-208)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**70.6** (31.3-159.2)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	4,894.7	**17.6** (6.1-50.8)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**58.0** (28.6-117.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**32.9** (18.5-58.5)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.009	977.6	30.1 (19.3-47.1)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.009	281.6	**44.3** (8.8-223.1)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.014	8,694.5	**40.4** (19.8-82.5)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	84.51**	84.51 (mg/L)	0.0001	50	504.6[c] (137.4-1,853.4)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	84.51	84,510 (µg/L)	0.0001	46,500	**91.9** (42.1-200.2)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

[a] Saltwater surrogate species were not used.
[b] Not used because other more closely taxonomically-related models were available.
[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-8. Ranked PFNA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFNA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	G	Threeridge	*Amblema plicata*	17.58	17.58	0.03
2	**D**	**Daphnid**	***Chydorus sphaericus***	**27.84**	27.84	0.06
3	G	Washboard	*Megalonaias nervosa*	32.90	32.90	0.09
4	A	Rainbow trout	*Oncorhynchus mykiss*	35.40	35.40	0.12
5	G	Western pearlshell	*Margaritifera falcata*	40.41	40.41	0.15
6	D	Water flea	*Moina macrocopa*	44.31	44.31	0.18
7	G	Paper pondshell	*Utterbackia imbecillis*	44.82	44.82	0.21
8	G	Neosho mucket	*Lampsilis rafinesqueana*	42.93	45.69	0.24
	G	Fatmucket	*Lampsilis siliquoidea*	48.63		
9	B	Bluegill	*Lepomis macrochirus*	50.29	50.29	0.26
10	D	Daphnid	*Ceriodaphnia dubia*	59.35	51.41	0.29
	D	Daphnid	*Ceriodaphnia reticulata*	44.54**		
11	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	52.08	52.08	0.32
12	G	Pheasantshell	*Ortmanniana pectorosa*	54.41	54.41	0.35
13	E	Amphipod	*Gammarus fasciatus*	29.82	55.26	0.38
	E	Amphipod	*Gammarus pseudolimnaeus*	102.4**		
14	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	58.02	58.02	0.41
15	E	Oriental river shrimp	*Macrobrachium nipponense*	65.1	65.10	0.44
16	G	Tadpole physa	*Physella gyrina*	70.62	70.62	0.47
17	G	Swamp lymnaea	*Lymnaea stagnalis*	70.68	70.68	0.50
18	D	Daphnid	*Simocephalus serrulatus*	61.28**	83.48	0.53
	D	Daphnid	*Simocephalus vetulus*	113.7		
19	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	84.41**	84.41	0.56
20	**D**	**Daphnid**	***Daphnia magna***	**84.51**	84.51	0.59
21	B	Zebrafish	*Danio rerio*	91.85	91.85	0.62
22	G	Green floater	*Lasmigona subviridis*	167.6	167.6	0.65
23	H	Oligochaete	*Limnodrilus hoffmeisteri*	181.9	181.9	0.68
24	F	Midge	*Paratanytarsus parthenogeneticus*	228.8	228.8	0.71
25	B	Fathead minnow	*Pimephales promelas*	260.9**	260.9	0.74
26	**C**	**Clawed frog**	***Xenopus sp.***	**335.8**	335.8	0.76
27	F	Midge	*Chironomus tentans*	435.6	435.6	0.79
28	H	Bryozoan	*Pectinatella magnifica*	648.8**	648.8	0.82
29	B	Channel catfish	*Ictalurus punctatus*	977.6**	977.6	0.85
30	E	Amphipod	*Crangonyx pseudogracilis*	1,035	1,035	0.88
31	H	Oligochaete	*Tubifex tubifex*	1,225	1,225	0.91
32	E	Isopod	*Asellus aquaticus*	1,262	1,262	0.94
33	H	Leech	*Nephelopsis obscura*	7,904**	7,904	0.97

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR 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, 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.

Table F-9. PFNA Final Acute Value and Protective Aquatic Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Calculated Freshwater FAV based on 4 lowest values; N=33 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	17.58	2.87	8.22	0.029	0.171
2	***Chydorus***	**27.84**	3.33	11.07	0.059	0.243
3	*Megalonaias*	32.90	3.49	12.20	0.088	0.297
4	*Oncorhynchus*	35.40	3.57	12.72	0.118	0.343
		Σ (Sum):	**13.25**	**44.21**	**0.29**	**1.05**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 18.12 S = slope
L = 2.192 L = X-axis intercept
A = 3.143 A = lnFAV
FAV = 23.18
FAV/2 = 11.59 mg/L (Preliminary Value)
Adjustment = 11.59 / 15.8 = 0.7337 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.73 mg/L PFNA** (rounded to two significant figures)

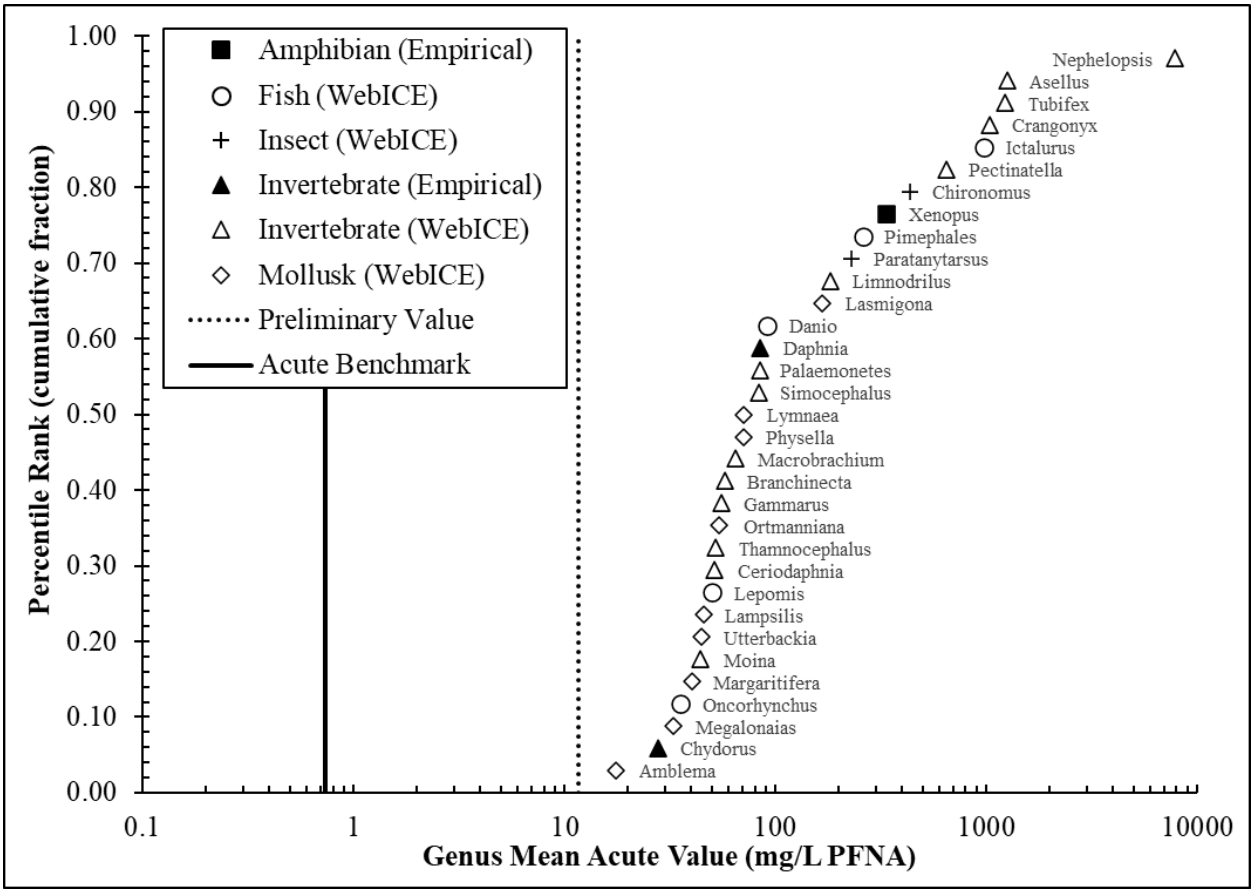

Figure F-3. Ranked Acute PFNA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.4 Derivation of Acute Water Benchmark for Perfluorodecanoic Acid (PFDA)

Quantitatively acceptable empirical data for PFDA were available for five species comprising four genera and fulfilling three MDR groups (**Table F-11**). A total of 85 ICE models were accepted for use. Of these, thirteen ICE models were not selected for use because empirical data were available for the genus (e.g., models for *Chydorus sphaericus* and *Daphnia magna*). Four other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Pimephales promelas*). A total of 68 ICE models were selected for use, resulting in ICE models predicting 67 SMAVs representing 56 genera (**Table F-10** and **F-11**).

The combined empirical and ICE data resulted in 60 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-11**. GMAVs for the four genera used in the FAV calculation were within a factor of 1.3 of each other (**Table F-12**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for PFDA is 20.53 mg/L (**Table F-12**), which is lower than all of the GMAVs except for three of the ICE-derived species values (the isopod, *Caecidotea brevicauda*, GMAV= 8.170 mg/L the northern pike, *Esox lucius*, GMAV=18.36 mg/L, and the largemouth bass, *Micropterus salmoides*, GMAV=19.52 mg/L). The FAV was divided by two to obtain a preliminary value of 10.27 mg/L PFDA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.65 mg/L PFDA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFDA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-4**).

Table F-10. Acceptable models for ICE-estimated Species Sensitivity to PFDA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.0008	166.8	**1,422.9** (423.1-4,784.7)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0002	5,000	**39.7** (14-112.7)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0001	68.3	**140.9** (70-283.6)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.004	1.625	20.0 (12.3-32.6)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	95.86	**31.0** (15.7-61.3)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0003	8,694.5	**71.5** (43.6-117.5)
Black bullhead (Ameiurus melas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	9.810	**207.8** (56.1-770)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0001	46,278	64.5[b] (42-99)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0001	8,341.5	**33.1** (27.6-39.6)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	43.07	**72.7** (12.6-420.2)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	96.69	**31.4** (18.8-52.5)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	95.86	**33.1** (19.8-55.3)
Bryozoan (Pectinatella magnifica)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.014	0.232	**877.8** (553.4-1,392.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	13,400	**58.8** (23.7-145.4)
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0041	1.625	**34.4** (22.8-51.9)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	13,400	**31.6** (19.9-50.1)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	724.4	40.0 (24-66.9)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	43.07	49.4 (38.4-63.6)
Colorado squawfish (Ptychocheilus lucius)	Rainbow trout (Oncorhynchus mykiss)	32^	32 (mg/L)	0.0033	43.07	**195.1** (34.5-1,103.6)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	198.4	**35.6** (16.3-77.7)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	198.4	27.1 (18.7-39.3)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.7099	155.5	**51.8** (30.8-87.1)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0003	46,278	**84.8** (53.8-133.7)
Daphnid (Ceriodaphnia reticulata)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0008	0.232	**60.4** (19.8-184.4)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0001	0.646	150.2 (65.6-344.1)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.009	10.36	90.7 (25.8-318.7)
Daphnid (Daphnia magna)*	Daphnid (Daphnia pulicaria)	149.6	149,600 (µg/L)	0.009	237.4	122.1 (27.4-544.5)
Daphnid (Daphnia magna)*	Water flea (Chydorus sphaericus)	41.13	41,130 (µg/L)	0.007	462	114.5 (70.9-184.8)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0002	4,894.7	93.7 (56.2-156)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	281.6	122.0 (24.2-614.7)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0002	7.2	**84.9** (45.5-158.4)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.0001	166.8	**147.7** (37.1-587.8)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0002	46,500	85.6[b] (58.9-124.4)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	13,400	**46.7** (36-60.7)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**66.6** (37-119.8)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	13,400	**62.2** (31.6-122.5)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.014	166.8	**211.5** (51.2-874.2)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	19.93	**90** (54.2-149.3)
Greenthroat darter (Etheostoma lepidum)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0041	1.625	**34.9** (11-110.9)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	198.4	**28.3** (7.6-105.3)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0003	166.8	**1,699.9** (273.7-10,559)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	82	**8.2** (1.9-35.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	198.4	**18.0** (11.4-28.4)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	95.86	**19.5** (9.5-40.3)
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0008	4.369	**11,822** (1,982-70,511)
Mayfly (Drunella grandis)	Rainbow trout (Oncorhynchus mykiss)	32^	32 (mg/L)	0.0006	95.86	**143.5** (43.1-477.3)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.007	54.4	**52.1** (10.9-248.7)
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.0003	472	**611.2** (100.7-3,709.3)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	1,330	**105.8** (35.6-314.4)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.37	14,500	**321.5** (140.5-735.3)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	119.7**	119.7 (mg/L)	0.0003	58	**109.9** (42.7-282.7)
Mrigal carp (Cirrhinus mrigala)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	1.625	**147.7** (24.4-896.1)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.042	166.8	**58.5** (15.4-222.1)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.002	198.4	**64.7** (33.7-124.2)
Northern pike (Esox lucius)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.005	1.822	**18.4** (6.5-52)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.003	281.6	**232.8** (51.5-1,052.2)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	119.7^	119.7 (mg/L)	0.0001	4,894.7	**1,672.6** (538.3-5,196.9)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.011	281.6	**97.1** (34.6-272.8)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**61.0** (38.4-97.2)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.042	545.9	**76.0** (17.4-332.2)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0001	14,500	45.4 (31.9-64.7)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0033	43.07	**26.6** (5.3-134)
Riceland prawn (Macrobrachium lanchesteri)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.003	2.04	**177.6** (34.2-922.7)
Rohu (Labeo rohita)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	1.625	**302** (61.5-1,484.6)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.028	95.86	**45.7** (11.2-185.7)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0014	7.075	**40.3** (25.8-62.8)
Snipefly (Atherix variegata)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	0.068	**194.9** (89.6-424)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	7.5	74.6 (17.1-325.1)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	9.7	**46.3** (19.9-107.9)
Spotfin chub (Erimonax monachus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0041	1.625	**24.5** (10.4-57.6)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**98.2** (32.2-299.3)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**98.7** (42.7-227.9)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	4,894.7	**24.5** (8.2-73.1)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.506	**80.6** (38.8-167.4)
Walleye (Sander vitreus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.005	16.24	**41.8** (12.1-144.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**46.0** (25.6-82.8)
Water flea (Chydorus sphaericus)*	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.009	977.6	41.9 (26.5-66.4)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.009	281.6	**56.9** (10.6-305.9)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.014	8,694.5	**54.9** (26.3-114.5)
Western toad (Anaxyrus boreas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	1.625	**31.3** (4.6-211.6)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	16.24	**44.5** (21-94.5)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	119.7**	1,19.7(mg/L)	0.0001	50	660.1[b] (190-2,293.1)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	229.1	**17.6** (6.4-48.5)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	119.7	119,700 (µg/L)	0.0001	46,500	115.5[b] (51.4-259.4)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	8,341.5	**34.4** (16.8-70.4)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

Table F-11. Ranked PFDA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFDA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	E	Isopod	*Caecidotea brevicauda*	8.170	8.170	0.02
2	B	Northern pike	*Esox lucius*	18.36**	18.36	0.03
3	B	Largemouth bass	*Micropterus salmoides*	19.52	19.52	0.05
4	A	Brook trout	*Salvelinus fontinalis*	31.44	23.80	0.07
	A	Lake trout	*Salvelinus namaycush*	18.01		
5	G	Threeridge	*Amblema plicata*	24.46	24.46	0.08
6	B	Spotfin chub	*Erimonax monachus*	24.47**	24.47	0.10
7	B	Zebrafish	*Danio rerio*	24.57	24.57	0.11
8	B	Razorback sucker	*Xyrauchen texanus*	26.57	26.57	0.13
9	B	Guppy	*Poecilia reticulata*	28.31	28.31	0.15
10	C	Western toad	*Anaxyrus boreas*	31.29**	31.29	0.16
11	B	Channel catfish	*Ictalurus punctatus*	31.55	31.55	0.18
12	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**32.00**	32.00	0.20
13	A	Atlantic salmon	*Salmo salar*	30.96	32.02	0.21
	A	Brown trout	*Salmo trutta*	33.12		
14	B	Cape Fear shiner	*Notropis mekistocholas*	34.42**	34.42	0.23
15	B	Greenthroat darter	*Etheostoma lepidum*	34.87**	34.87	0.25
16	B	Common carp	*Cyprinus carpio*	35.58	35.58	0.26
17	B	Silver perch	*Bidyanus bidyanus*	40.28**	40.28	0.28
18	**D**	**Daphnid**	***Chydorus sphaericus***	**41.13**	41.13	0.30
19	B	Walleye	*Sander vitreus*	41.80**	41.80	0.31
20	B	Yellow perch	*Perca flavescens*	44.48**	44.48	0.33
21	B	Shortnose sturgeon	*Acipenser brevirostrum*	45.67	45.67	0.34
22	G	Washboard	*Megalonaias nervosa*	46.01	46.01	0.36
23	B	Fathead minnow	*Pimephales promelas*	46.72	46.72	0.38
24	C	Cuvier's foam froglet	*Physalaemus cuvieri*	51.77	51.77	0.39
25	B	Medaka	*Oryzias latipes*	52.12	52.12	0.41
26	B	Green sunfish	*Lepomis cyanellus*	89.98**	54.56	0.43
	B	Bluegill	*Lepomis macrochirus*	33.08		
27	G	Western pearlshell	*Margaritifera falcata*	54.89	54.89	0.44
28	C	Bullfrog	*Lithobates catesbeianus*	58.75	56.04	0.46
	C	Northern leopard frog	*Lithobates pipiens*	64.72		
	C	Southern leopard frog	*Lithobates sphenocephalus*	46.28**		
29	D	Water flea	*Moina macrocopa*	56.93	56.93	0.48
30	G	Paper pondshell	*Utterbackia imbecillis*	61.04	61.04	0.49
31	B	Goldfish	*Carassius auratus*	62.18	62.18	0.51
32	G	Neosho mucket	*Lampsilis rafinesqueana*	58.54	62.42	0.52
	G	Fatmucket	*Lampsilis siliquoidea*	66.56		
33	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	71.54	71.54	0.54
34	D	Daphnid	*Ceriodaphnia dubia*	84.82	71.55	0.56
	D	Daphnid	*Ceriodaphnia reticulata*	60.35**		
35	B	Bonytail	*Gila elegans*	72.68	72.68	0.57
36	E	Amphipod	*Gammarus fasciatus*	39.66	74.75	0.59
	E	Amphipod	*Gammarus pseudolimnaeus*	140.9**		

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
37	G	Pheasantshell	*Ortmanniana pectorosa*	76.02	76.02	0.61
38	**C**	**Clawed frog**	***Xenopus sp.***	**76.50**	76.50	0.62
39	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	80.58	80.58	0.64
40	G	Swamp lymnaea	*Lymnaea stagnalis*	98.19	98.19	0.66
41	G	Tadpole physa	*Physella gyrina*	98.69	98.69	0.67
42	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	109.9**	109.9	0.69
43	D	Daphnid	*Simocephalus serrulatus*	84.92**	112.0	0.70
	D	Daphnid	*Simocephalus vetulus*	147.7		
44	E	Riceland prawn	*Macrobrachium lanchesteri*	177.6**	131.3	0.72
	E	Oriental river shrimp	*Macrobrachium nipponense*	97.09		
45	F	Mayfly	*Drunella grandis*	143.5	143.5	0.74
46	**D**	**Daphnid**	***Daphnia magna***	**145.5**	147.5	0.75
	D	**Daphnid**	***Daphnia pulicaria***	**149.6**		
47	B	Mrigal carp	*Cirrhinus mrigala*	147.7**	147.7	0.77
48	F	Midge	*Paratanytarsus dissimilis*	105.8	184.4	0.79
	F	Midge	*Paratanytarsus parthenogeneticus*	321.5		
49	F	Snipefly	*Atherix variegata*	194.9**	194.9	0.80
50	B	Colorado squawfish	*Ptychocheilus lucius*	195.1	195.1	0.82
51	B	Black bullhead	*Ameiurus melas*	207.8**	207.8	0.84
52	G	Green floater	*Lasmigona subviridis*	211.5	211.5	0.85
53	H	Oligochaete	*Limnodrilus hoffmeisteri*	232.8	232.8	0.87
54	B	Rohu	*Labeo rohita*	302.0**	302.0	0.89
55	F	Midge	*Chironomus tentans*	611.2	611.2	0.90
56	H	Bryozoan	*Pectinatella magnifica*	877.8**	877.8	0.92
57	E	Amphipod	*Crangonyx pseudogracilis*	1,423	1,423	0.93
58	H	Oligochaete	*Tubifex tubifex*	1,673	1,673	0.95
59	E	Isopod	*Asellus aquaticus*	1,700	1,700	0.97
60	H	Leech	*Nephelopsis obscura*	11,822**	11,822	0.98

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR 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, 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.

Table F-12. PFDA Final Acute Value and Protective Aquatic Acute Benchmark

Calculated Freshwater FAV based on 4 values closest to 0.05 percentile; N=60 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
2	*Esox*	18.36	2.91	8.47	0.033	0.181
3	*Micropterus*	19.52	2.97	8.83	0.049	0.222
4	*Salvelinus*	23.80	3.17	10.05	0.066	0.256
5	*Amblema*	24.46	3.20	10.22	0.082	0.286
		Σ (Sum):	**12.25**	**37.57**	**0.23**	**0.95**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 =	9.92	S = slope
L =	2.318	L = X-axis intercept
A =	3.022	A = lnFAV
FAV =	20.53	
FAV/2 =	10.27 mg/L (Preliminary Value)	
Adjustment =	10.27 / 15.8 = 0.6498 (Preliminary Value / Carboxylic Acid Application Factor)	
Benchmark =	**0.65 mg/L PFDA** (rounded to two significant figures)	

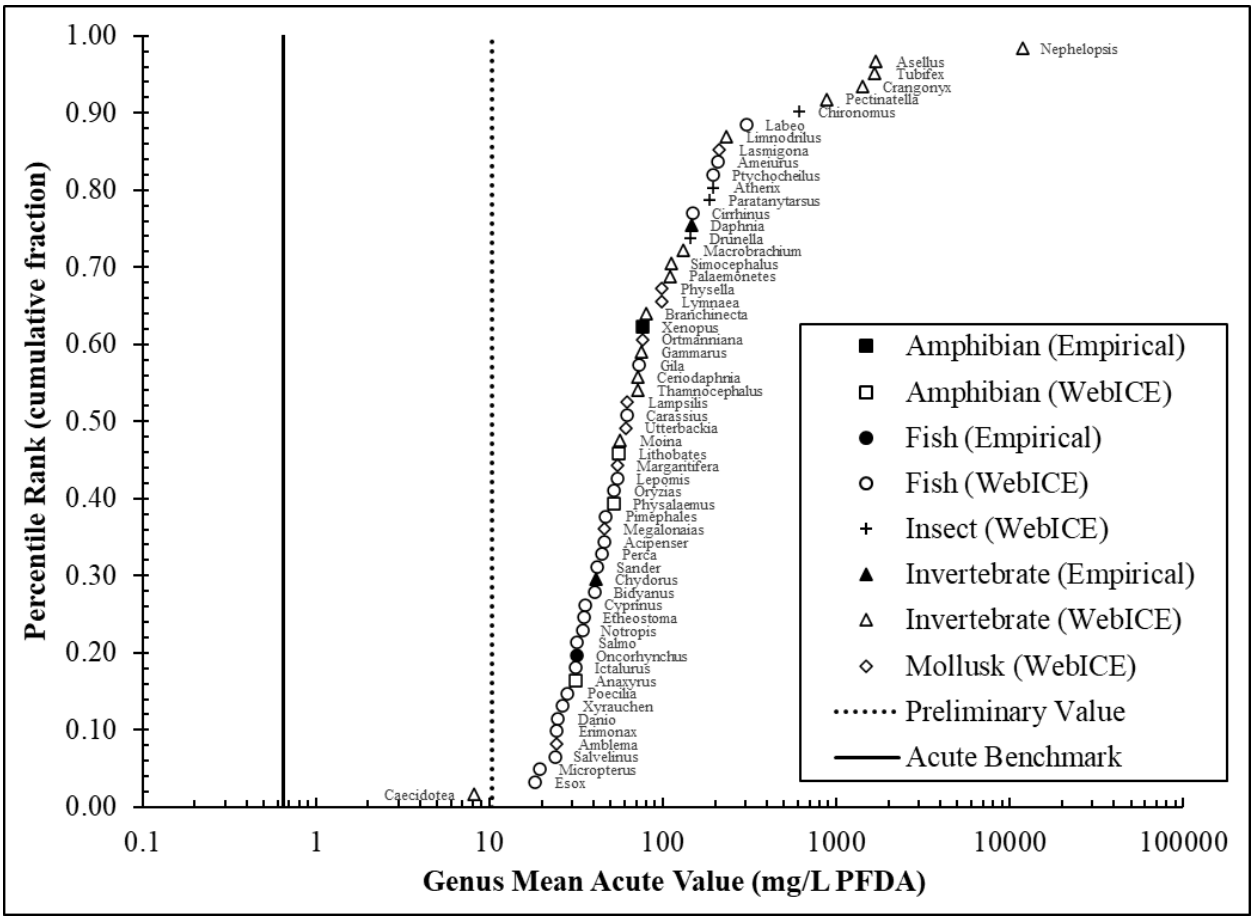

Figure F-4. Ranked Acute PFDA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.5 Derivation of Acute Water Benchmark for Perfluorobutanesulfonic Acid (PFBS)

Quantitatively acceptable empirical data for PFBS were available for four species comprising four genera and fulfilling two MDR groups (**Table F-14**). A total of 119 ICE models were accepted for use. Of these, seventeen ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Lepomis macrochirus* and *Daphnia magna*). Seven ICE models were not selected for use because they were derived based on saltwater species surrogates (e.g., *Americamysis bahia* predicting for *Gammarus fasciatus*). Sixteen other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Lepomis macrochirus* predicting for *Cyprinus carpio*). Two

additional models (e.g., *Ictalurus punctatus* predicting for *Lithobates catesbeianus*) were not used because the measured EC_{50} values fell outside of the ICE model range and other acceptable models for taxonomically-related species with measured EC_{50} values within the ICE model range were used. A total of 77 ICE models were selected for use, resulting in ICE models predicting 66 SMAVs, representing 54 genera (**Tables F-13** and **F-14**).

The combined empirical and ICE data resulted in 58 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-14**. GMAVs for the four most sensitive genera were within a factor of 1.9 of each other (**Table F-15**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFBS is 646.3 mg/L (**Table F-15**), which is lower than all of the GMAVs except for two of the ICE-derived species values (the threeridge mussel, *Amblema plicata*, GMAV=383.6 mg/L and the greenthroat darter, *Etheostoma lepidum*, GMAV=518.0 mg/L). The FAV was divided by two to obtain a preliminary benchmark value of 323.1 mg/L PFBS and then adjusted by the sulfonic acid application factor (42.3) to obtain the freshwater acute water column benchmark magnitude of 7.6 mg/L PFBS (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFBS under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-5**).

Table F-13. Acceptable models for ICE-estimated Species Sensitivity to PFBS using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
African clawed frog (Xenopus laevis)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.018	140.2	**1,868.8** (685.1-5,097.5)
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0008	166.8	**20,152** (5,302.8-76,585)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0002	5,000	**428.1** (107.9-1,697.9)
Amphipod (Gammarus fasciatus)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	4.4	826.2[a] (280.5-2,433.7)
Amphipod (Gammarus minus)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.239	107.6	**7,872.6** (1,934.1-32,046)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0001	68.3	**2,010.4** (770.9-5,242.9)
Amphipod (Gammarus pseudolimnaeus)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	7.17	220.9[a] (87-560.8)
Amphipod (Hyalella azteca)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	193.4	357.7[a] (156.5-817.5)
Arctic grayling (Thymallus arcticus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.038	150.8	**5,722.4** (958.9-34,148)
Atlantic salmon (Salmo salar)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.004	123.3	**5,330.1** (2,929.8-9,696.8)
Atlantic salmon (Salmo salar)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.044	140.2	**493.4** (76.8-3,169.5)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0003	8,694.5	**1,010.1** (525.1-1,942.9)
Black bullhead (Ameiurus melas)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	20.92	**2,628.4** (591-11,689)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Bluegill (Lepomis macrochirus)*	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0001	46,278	512.5 (285.3-920.4)
Bluegill (Lepomis macrochirus)*	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.0002	50,154	751.0 (390-1,446)
Bluegill (Lepomis macrochirus)*	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	79	1,574.6 (1,174.5-2,111)
Bonytail (Gila elegans)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.054	150.8	1,895.4[b] (374.7-9,588.1)
Bonytail (Gila elegans)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.058	8.32	**880.6** (168.2-4,610.9)
Brook trout (Salvelinus fontinalis)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	123.3	**4,301.9** (2,141.8-8,640.5)
Brook trout (Salvelinus fontinalis)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	140.2	**506.7** (192.5-1,334)
Brown trout (Salmo trutta)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.002	123.3	**2,390.2** (732.9-7,795.5)
Bullfrog (Lithobates catesbeianus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	201	7,340[c] (3,632.2-14,833)
Bullfrog (Lithobates catesbeianus)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.001	26,500	**1,791.4** (756.1-4,244.6)
Catla (Gibelion catla)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.333	8.32	**1,718.3** (810.4-3,643.3)
Channel catfish (Ictalurus punctatus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	517.8	6,851.9[c] (4,607-10,191)
Channel catfish (Ictalurus punctatus)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.001	26,500	**1,617.3** (755.1-3,464.4)
Chinook salmon (Oncorhynchus tshawytscha)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.004	150.8	**2,417.4** (631.8-9,249.7)
Chinook salmon (Oncorhynchus tshawytscha)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.011	140.2	**397.2** (94.8-1,664.3)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Coho salmon (Oncorhynchus kisutch)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	150.8	**3,010.5** (1,399.5-6,475.7)
Coho salmon (Oncorhynchus kisutch)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	20.92	**589.1** (232.4-1,493.1)
Colorado squawfish (Ptychocheilus lucius)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.058	20.92	**868.0** (215.4-3,498.7)
Common carp (Cyprinus carpio)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0009	180	7,708.8[b] (3,816-15,573)
Common carp (Cyprinus carpio)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.005	133	**2,074.7** (1,359.3-3,166.5)
Cutthroat trout (Oncorhynchus clarkii)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	180	**5,874.5** (3,202.5-10,776)
Cutthroat trout (Oncorhynchus clarkii)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	140.2	**645.0** (354.7-1,172.7)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0003	46,278	**1,666.6** (909-3,055.6)
Daphnid (Ceriodaphnia dubia)	Mysid (Americamysis bahia)	372	372,000 (µg/L)	<0.0001	4,100	171.7[a] (36.6-804.9)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.009	10.36	3,094.2 (706.1-13,559)
Daphnid (Daphnia magna)*	Mysid (Americamysis bahia)	372	372,000 (µg/L)	<0.0001	11,000	243.9 (143.9-413.4)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0002	4,894.7	1,768.1 (893.9-3,497.1)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.014	281.6	1,712.1 (445.5-6,580.5)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0002	7.2	**1,291.0** (482.8-3,451.9)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0001	166.8	**1,307.9** (202.3-8,457.1)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Fathead minnow (Pimephales promelas)*	Bluegill (Lepomis macrochirus)	6,452	6,452,000 (µg/L)	0.0001	27,540	3,399.2 (1,631.9-7,080.3)
Fathead minnow (Pimephales promelas)*	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0002	46,500	639.5 (375.7-1,088.5)
Fathead minnow (Pimephales promelas)*	Mysid (Americamysis bahia)	372	372,000 (µg/L)	<0.0001	11,000	158.6 (74.6-337.5)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**912.5** (399-2,086.8)
Fatmucket (Lampsilis siliquoidea)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.013	10,831	487.4[b] (125.7-1,889.9)
Flagfish (Jordanella floridae)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	113	**5,648.7** (1,648.7-19,354)
Flagfish (Jordanella floridae)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	32.54	**1,232.7** (388.5-3,911.1)
Goldfish (Carassius auratus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	201	8,832.2[b] (4,286.5-18,199)
Goldfish (Carassius auratus)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.001	26,500	**2,152.4** (1,075.2-4,309)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.014	166.8	**1,471.9** (318.2-6,807.8)
Green sunfish (Lepomis cyanellus)*	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.004	113	7,710.8 (3,925.7-15,146)
Green sunfish (Lepomis cyanellus)*	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.011	26.69	720.9 (107.3-4,841.9)
Greenthroat darter (Etheostoma lepidum)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.006	8.32	**518.0** (105.9-2,533.1)
Guppy (Poecilia reticulata)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	180	**5,295.7** (2,358.2-11,892)
Guppy (Poecilia reticulata)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	140.2	**1,407.4** (816.4-2,426.3)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Indian bullfrog (Euphlyctis hexadactylus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.038	113	**2,330.8** (410.6-13,232)
Isopod (Asellus aquaticus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.003	123.3	9,389.6[b] (2,238.7-39,383)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0003	166.8	**55,185** (17,354-175,483)
Isopod (Asellus aquaticus)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	6.983	5,240.6[a] (964.7-28,468.1)
Isopod (Caecidotea brevicauda)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	26.7	**3,753.7** (1,068.9-13,183)
Isopod (Caecidotea brevicauda)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	20.92	**286.2** (83.9-976.8)
Lake trout (Salvelinus namaycush)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.003	180	**2,719.2** (1,374-5,381.5)
Lake trout (Salvelinus namaycush)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.044	133	**374.7** (171.7-817.4)
Largemouth bass (Micropterus salmoides)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	123.3	**6,684.3** (4,375-10,213)
Medaka (Oryzias latipes)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.003	1,420	**2,670.2** (1,679.9-4,244.3)
Midge (Chironomus plumosus)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	87.6	672.1[a] (228.5-1,977.3)
Midge (Chironomus tentans)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0001	517.8	**10,675** (1,696.1-67,189)
Midge (Paratanytarsus dissimilis)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	201	**18,234** (3,711.6-89,583)
Midge (Paratanytarsus dissimilis)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	1,430	**6,295.4** (1,795.6-22,072)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.37	14,500	**5,481.6** (1,674.6-17,944)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Midge (Paratanytarsus parthenogeneticus)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.28	10,600	6,562.8[b] (1,034.9-41,616)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0003	58	**993.2** (233.2-4,230.7)
Mosquitofish (Gambusia affinis)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.006	26,500	**1,349.6** (216.3-8,421.7)
Mozambique tilapia (Oreochromis mossambicus)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.044	140.2	**1,578** (636.6-3,911.6)
Mrigal carp (Cirrhinus mrigala)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.1969	8.32	**1,132.5** (742.8-1,726.6)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.042	166.8	**1,653.6** (781.8-3,497.4)
Nile tilapia (Oreochromis niloticus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.002	6.969	**8,705.2** (7,662.2-9,890.2)
Northern leopard frog (Lithobates pipiens)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.008	180	**14,976.0** (2,672.6-83,915)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.003	281.6	**13,646.0** (5,444.3-34,203)
Oligochaete (Lumbriculus variegatus)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	140.2	**2,368.1** (1,042.6-5,378.4)
Oligochaete (Tubifex tubifex)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0001	113	2,696.5[b] (423.5-17,169)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	2,183^	2,183 (mg/L)	0.0001	4,894.7	**22,428.0** (5,594.7-89,907)
Oligochaete (Tubifex tubifex)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.015	10,831	2,039.0[b] (591.9-7,024)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.011	281.6	**978.3** (434.4-2,203.5)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**802.5** (422.2-1,525.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Paper pondshell (Utterbackia imbecillis)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.134	10,831	478.6[b] (99.6-2,299.1)
Peppered loach (Lepidocephalichthys guntea)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.1969	43.79	**1,937.8** (699.6-5,367.4)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.042	545.9	**1,625.4** (540-4,892.6)
Rainbow trout (Oncorhynchus mykiss)	Bluegill (Lepomis macrochirus)	6,452**	6,452,000 (µg/L)	0.0001	7,100	**3,208.6** (2,275.7-4,524)
Rainbow trout (Oncorhynchus mykiss)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0001	14,500	361.9[b] (221.2-592.1)
Rainbow trout (Oncorhynchus mykiss)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.0002	26,500	**788.9** (505.7-1,230.8)
Rainbow trout (Oncorhynchus mykiss)	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	113	1,127.7[a] (854.1-1,489)
Razorback sucker (Xyrauchen texanus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.054	150.8	1,223[b] (295.2-5,067.6)
Razorback sucker (Xyrauchen texanus)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.058	8.32	**742.7** (170.7-3,231.1)
Rohu (Labeo rohita)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0008	7.326	22,073[b] (3,315.4-146,959)
Rohu (Labeo rohita)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.002	8.32	**3,518.1** (750.6-16,489)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**1,523.7** (337.4-6,881.2)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**1,608.6** (512.8-5,046.1)
Tadpole physa (Physella gyrina)	Fathead minnow (Pimephales promelas)	1,938^	1,938 (mg/L)	0.814	10,831	263.4[b] (41-1,694.5)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	4,894.7	**383.6** (85.7-1,717.6)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Threeridge (Amblema plicata)	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.814	10,831	250.3[b] (39.4-1,592.2)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**1,246.4** (457-3,399.4)
Walleye (Sander vitreus)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.004	6.722	**2,272.6** (535.8-9,639.4)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**755.1** (345.4-1,650.5)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.009	977.6	**934.5** (569.8-1,532.6)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.009	281.6	**3,183.1** (1,038.3-9,758.3)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.014	8,694.5	**706.1** (255.8-1,948.9)
White sucker (Catostomus commersonii)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.035	123.3	4,920.4[b] (2,000.4-12,103)
White sucker (Catostomus commersonii)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.044	140.2	**2,552.9** (896.1-7,273)
Yellow perch (Perca flavescens)	Bluegill (Lepomis macrochirus)	6,452**	6,452 (mg/L)	0.0004	9.41	**4,050.0** (2,122-7,729.6)
Yellow perch (Perca flavescens)	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.001	20.92	504.1[b] (168.3-1,509.3)
Zebrafish (Danio rerio)*	Daphnid (Daphnia magna)	2,183**	2,183 (mg/L)	0.0001	50	6,202.4 (1,930.8-19,924)
Zebrafish (Danio rerio)*	Fathead minnow (Pimephales promelas)	1,938**	1,938 (mg/L)	0.013	26.69	2,005.9 (1,169.6-3,440.3)
Zebrafish (Danio rerio)*	Mysid (Americamysis bahia)	372**	372 (mg/L)	<0.0001	5.666	6,109.6 (1,057.2-35,308)
Zebrafish-embryo (Danio rerio-embryo)*	Daphnid (Daphnia magna)	2,183	2,183,000 (µg/L)	0.0001	46,500	780.8 (257.2-2,370.6)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Zebrafish-embryo (Danio rerio-embryo)*	Fathead minnow (Pimephales promelas)	1,938	1,938,000 (µg/L)	0.004	70,200	1,707.9 (1,061.5-2,748)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-14. Ranked PFBS Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFBS toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	G	Threeridge	*Amblema plicata*	383.6	383.6	0.02
2	B	Greenthroat darter	*Etheostoma lepidum*	518.0**	518.0	0.03
3	G	Western pearlshell	*Margaritifera falcata*	706.1	706.1	0.05
4	B	Razorback sucker	*Xyrauchen texanus*	742.7**	742.7	0.07
5	G	Washboard	*Megalonaias nervosa*	755.1	755.1	0.08
6	G	Paper pondshell	*Utterbackia imbecillis*	802.5	802.5	0.10
7	B	Colorado squawfish	*Ptychocheilus lucius*	868.0**	868.0	0.12
8	B	Bonytail	*Gila elegans*	880.6**	880.6	0.14
9	D	Water flea	*Chydorus sphaericus*	934.5**	934.5	0.15
10	E	Oriental river shrimp	*Macrobrachium nipponense*	978.3**	978.3	0.17
11	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	993.2**	993.2	0.19
12	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	1,010	1,010	0.20
13	E	Isopod	*Caecidotea brevicauda*	1,036**	1,036	0.22
14	B	Mrigal carp	*Cirrhinus mrigala*	1,132**	1,132	0.24
15	A	Brook trout	*Salvelinus fontinalis*	1,476**	1,221	0.25
	A	Lake trout	*Salvelinus namaycush*	1,009**		
16	G	Neosho mucket	*Lampsilis rafinesqueana*	1,654**	1,228	0.27
	G	Fatmucket	*Lampsilis siliquoidea*	912.5		
17	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	1,246	1,246	0.29
18	D	Daphnid	*Simocephalus serrulatus*	1,291**	1,299	0.31
	D	Daphnid	*Simocephalus vetulus*	1,308**		
19	B	Mosquitofish	*Gambusia affinis*	1,350	1,350	0.32
20	A	Cutthroat trout	*Oncorhynchus clarkii*	1,946**	1,418	0.34
	A	Coho salmon	*Oncorhynchus kisutch*	1,332**		
	A	Rainbow trout	*Oncorhynchus mykiss*	1,591		
	A	Chinook salmon	*Oncorhynchus tshawytscha*	979.9**		
21	G	Green floater	*Lasmigona subviridis*	1,472**	1,472	0.36
22	G	Swamp lymnaea	*Lymnaea stagnalis*	1,524	1,524	0.37
23	G	Tadpole physa	*Physella gyrina*	1,609	1,609	0.39
24	B	Channel catfish	*Ictalurus punctatus*	1,617	1,617	0.41
25	G	Pheasantshell	*Ortmanniana pectorosa*	1,625**	1,625	0.42
26	D	Daphnid	*Ceriodaphnia dubia*	1,667	1,667	0.44
27	B	Catla	*Gibelion catla*	1,718**	1,718	0.46
28	C	African clawed frog	*Xenopus laevis*	1,869**	1,869	0.47
29	E	Amphipod	*Gammarus fasciatus*	428.1	1,892	0.49
	E	Amphipod	*Gammarus minus*	7,873**		
	E	Amphipod	*Gammarus pseudolimnaeus*	2,010**		
30	B	Peppered loach	*Lepidocephalichthys guntea*	1,938**	1,938	0.51
31	**B**	**Fathead minnow**	***Pimephales promelas***	**1,938**	1,938	0.53
32	A	Atlantic salmon	*Salmo salar*	1,622**	1,969	0.54
	A	Brown trout	*Salmo trutta*	2,390**		
33	B	Common carp	*Cyprinus carpio*	2,075**	2,075	0.56

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
34	B	Goldfish	*Carassius auratus*	2,152	2,152	0.58
35	**D**	**Daphnid**	***Daphnia magna***	**2,183**	2,183	0.59
36	B	Walleye	*Sander vitreus*	2,273**	2,273	0.61
37	C	Indian bullfrog	*Euphlyctis hexadactylus*	2,331**	2,331	0.63
38	H	Oligochaete	*Lumbriculus variegatus*	2,368**	2,368	0.64
39	B	White sucker	*Catostomus commersonii*	2,553**	2,553	0.66
40	B	Black bullhead	*Ameiurus melas*	2,628**	2,628	0.68
41	B	Flagfish	*Jordanella floridae*	2,639**	2,639	0.69
42	B	Medaka	*Oryzias latipes*	2,670**	2,670	0.71
43	B	Guppy	*Poecilia reticulata*	2,730**	2,730	0.73
44	**B**	**Zebrafish**	***Danio rerio***	**>3,000**	>3,000	0.75
45	D	Water flea	*Moina macrocopa*	3,183**	3,183	0.76
46	B	Rohu	*Labeo rohita*	3,518**	3,518	0.78
47	B	Mozambique tilapia	*Oreochromis mossambicus*	1,578**	3,706	0.80
	B	Nile tilapia	*Oreochromis niloticus*	8,705**		
48	B	Yellow perch	*Perca flavescens*	4,050**	4,050	0.81
49	C	Bullfrog	*Lithobates catesbeianus*	1,791	5,180	0.83
	C	Northern leopard frog	*Lithobates pipiens*	14,976**		
50	A	Arctic grayling	*Thymallus arcticus*	5,722**	5,722	0.85
51	**B**	**Bluegill**	***Lepomis macrochirus***	**6,452**	6,452	0.86
52	B	Largemouth bass	*Micropterus salmoides*	6,684**	6,684	0.88
53	F	Midge	*Paratanytarsus dissimilis*	10,714**	7,664	0.90
	F	Midge	*Paratanytarsus parthenogeneticus*	5,482		
54	F	Midge	*Chironomus tentans*	10,675**	10,675	0.92
55	H	Oligochaete	*Limnodrilus hoffmeisteri*	13,646**	13,646	0.93
56	E	Amphipod	*Crangonyx pseudogracilis*	20,152**	20,152	0.95
57	H	Oligochaete	*Tubifex tubifex*	22,428**	22,428	0.97
58	E	Isopod	*Asellus aquaticus*	55,185**	55,185	0.98

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR 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, 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.

Table F-15. PFBS Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=58 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the sulfonic acid application factor (42.3)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	383.6	5.95	35.40	0.017	0.130
2	*Etheostoma*	518.0	6.25	39.06	0.034	0.184
3	*Margaritifera*	706.1	6.56	43.03	0.051	0.225
4	*Xyrauchen*	742.7	6.61	43.70	0.068	0.260
		Σ (Sum):	**25.37**	**161.18**	**0.17**	**0.80**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 29.92 S = slope
L = 5.248 L = X-axis intercept
A = 6.471 A = lnFAV
FAV = 646.3
FAV/2 = 323.1 mg/L (Preliminary Value)
Adjustment = 323.1 / 42.3 = 7.639 (Preliminary Value / Sulfonic Acid Application Factor)
Benchmark = **7.6 mg/L PFBS** (rounded to two significant figures)

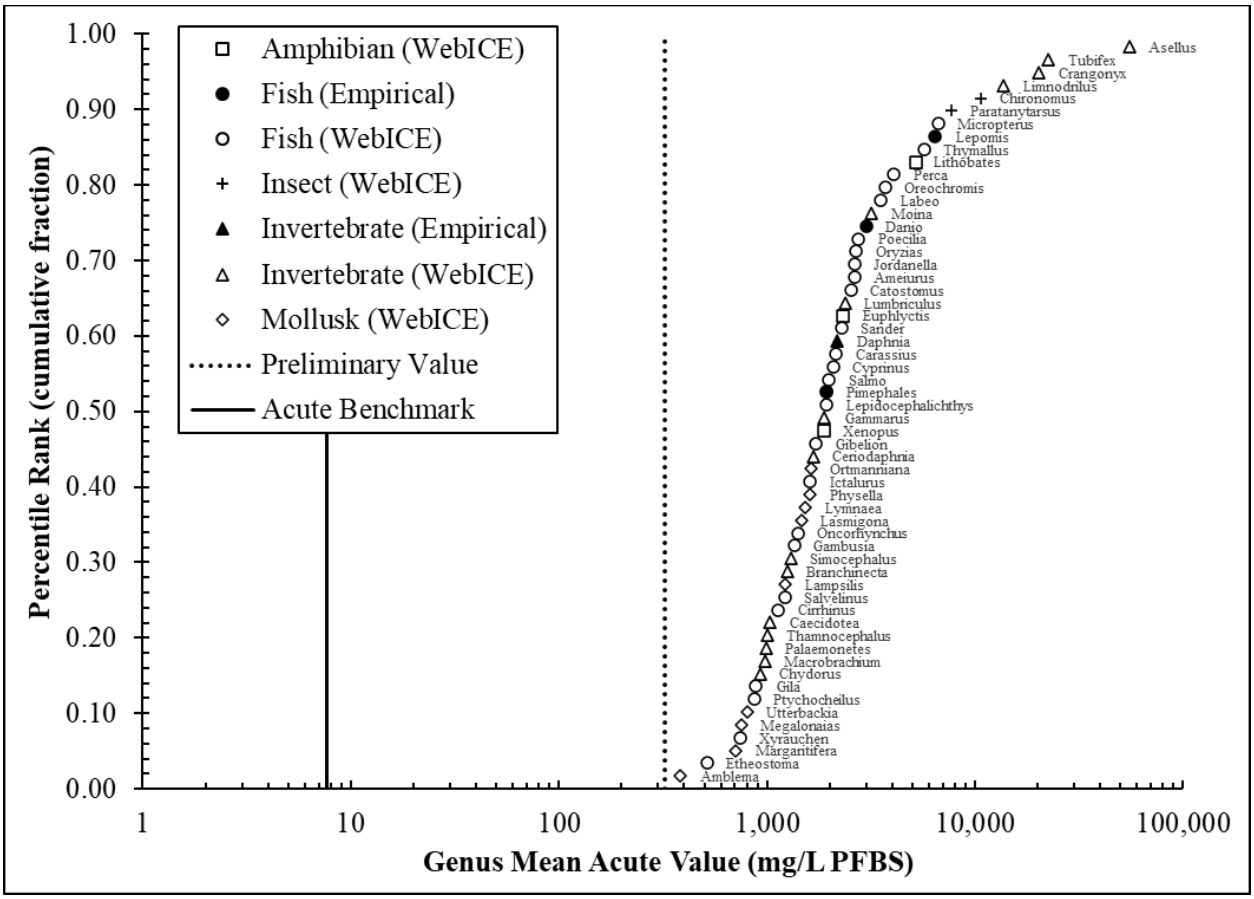

Figure F-5. Ranked Acute PFBS GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.6 Derivation of Acute Water Benchmark for Perfluorohexanesulfonic Acid (PFHxS)

Quantitatively acceptable empirical data PFHxS were available for three species comprising two genera and fulfilling two MDR groups (**Table F-17**). A total of 18 ICE models were accepted for use. Of these, four other models were not used because there were acceptable models for more closely-related surrogate species (i.e., *Lithobates catesbeianus* predicting for *Ictalurus punctatus*). A total of 14 ICE models were selected for use, resulting in ICE models predicting 11 SMAVs representing 11 genera (**Tables F-16** and **F-17**).

The combined empirical and ICE data resulted in 13 GMAVs that collectively fulfill seven of the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life

Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-17**. GMAVs for the four most sensitive genera were within a factor of 1.2 of each other (**Table F-18**). The freshwater FAV (the 5^{th} percentile of the genus sensitivity distribution) for PFHxS is 18.17 mg/L (**Table F-18**), which is lower than all of the GMAVs. The FAV was divided by two to obtain a preliminary value of 9.084 mg/L PFHxS and then adjusted by the sulfonic acid application factor (42.3) to obtain the freshwater acute water column benchmark magnitude of 0.21 mg/L PFHxS (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to PFHxS under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-6**).

Table F-16. Acceptable models for ICE-estimated Species Sensitivity to PFHxS using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Bluegill (Lepomis macrochirus)	Bullfrog (Lithobates catesbeianus)	1,105**	1,105 (mg/L)	0.003	233	**766.4** (378-1,553.6)
Channel catfish (Ictalurus punctatus)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	719.8[b] (362.4-1,429.7)
Channel catfish (Ictalurus punctatus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.145	304.5	**19.5** (9.3-41)
Daphnid (Daphnia magna)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	**424.1** (68.5-2,627.9)
Daphnid (Daphnia magna)	Zebrafish (Danio rerio)	22.5	22,500 (µg/L)	0.003	36.91	**5.2** (1.2-22.7)
Fairy shrimp (Streptocephalus rubricaudatus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	4.622	5,100.7	**114.2** (33.4-390.3)
Fathead minnow (Pimephales promelas)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	1,083.6[b] (468.5-2,506.4)
Fathead minnow (Pimephales promelas)	Zebrafish (Danio rerio)	22.5	22,500 (µg/L)	0.018	25.08	**26.0** (11.6-58.2)
Fathead minnow (Pimephales promelas)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.023	54,579	**18.3** (13.3-25.2)
Flagfish (Jordanella floridae)	Zebrafish (Danio rerio)	22.5	22,500 (µg/L)	7.797	649.3	**33.9** (19.7-58.4)
Goldfish (Carassius auratus)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	1,027.7[b] (405.9-2,602.1)
Goldfish (Carassius auratus)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.145	304.5	**37.3** (23.2-59.9)
Midge (Paratanytarsus dissimilis)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	3,020.0	**1,069.7** (176.6-6,480.9)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Mosquitofish (Gambusia affinis)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	18.6	20,900	**805.1** (394.5-1,643.4)
Oligochaete (Limnodrilus hoffmeisteri)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.145	239.3	**63.2** (9.9-403.1)
Rainbow trout (Oncorhynchus mykiss)	Bullfrog (Lithobates catesbeianus)	1,105	1,105,000 (µg/L)	0.003	20,900	713.5[b] (200.3-2,541.4)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish (Danio rerio)	22.5	22,500 (µg/L)	0.003	26.39	**38.3** (9.5-155.1)
Rainbow trout (Oncorhynchus mykiss)	Zebrafish-embryo (Danio rerio-embryo)	22.5	22,500 (µg/L)	0.023	8,843.9	**9.6** (4.2-22)

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-17. Ranked PFHxS Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical PFHxS toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	A	Rainbow trout	*Oncorhynchus mykiss*	19.19	19.19	0.07
2	B	Channel catfish	*Ictalurus punctatus*	19.46	19.46	0.14
3	B	Fathead minnow	*Pimephales promelas*	21.80	21.80	0.21
4	**B**	**Zebrafish**	***Danio rerio***	**22.50**	22.50	0.29
5	B	Flagfish	*Jordanella floridae*	33.90	33.90	0.36
6	B	Goldfish	*Carassius auratus*	37.28	37.28	0.43
7	D	Daphnid	*Daphnia magna*	46.87	46.87	0.50
8	H	Oligochaete	*Limnodrilus hoffmeisteri*	63.23	63.23	0.57
9	E	Fairy shrimp	*Streptocephalus proboscideus*	114.2	114.2	0.64
10	B	Bluegill	*Lepomis macrochirus*	766.4**	766.4	0.71
11	B	Mosquitofish	*Gambusia affinis*	805.1	805.1	0.79
12	**C**	**Bullfrog**	***Lithobates catesbeiana***	**1,105**	915.2	0.86
	C	**Green frog**	***Lithobates clamitans***	**758.0**		
13	F	Midge	*Paratanytarsus dissimilis*	1,070	1,070	0.93

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR 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, 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.

Table F-18. PFHxS Final Acute Value and Protective Aquatic Acute Benchmark.

Bold values represent genera for which empirical toxicity data were available.

Note: Missing MDR Group: G

Calculated Freshwater FAV based on 4 lowest values; N=13 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the sulfonic acid application factor (42.3)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Oncorhynchus*	19.19	2.95	8.73	0.071	0.267
2	*Ictalurus*	19.46	2.97	8.81	0.143	0.378
3	*Pimephales*	21.80	3.08	9.50	0.214	0.463
4	***Danio***	**22.50**	3.11	9.69	0.286	0.535
		Σ (Sum):	**12.12**	**36.73**	**0.71**	**1.64**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 0.48
S = slope

L = 2.744
L = X-axis intercept

A = 2.900
A = lnFAV

FAV = 18.17

FAV/2 = 9.084 mg/L (Preliminary Value)

Adjustment = 9.084 / 42.3 = 0.2147 (Preliminary Value / Sulfonic Acid Application Factor)

Benchmark = **0.21 mg/L PFHxS** (rounded to two significant figures)

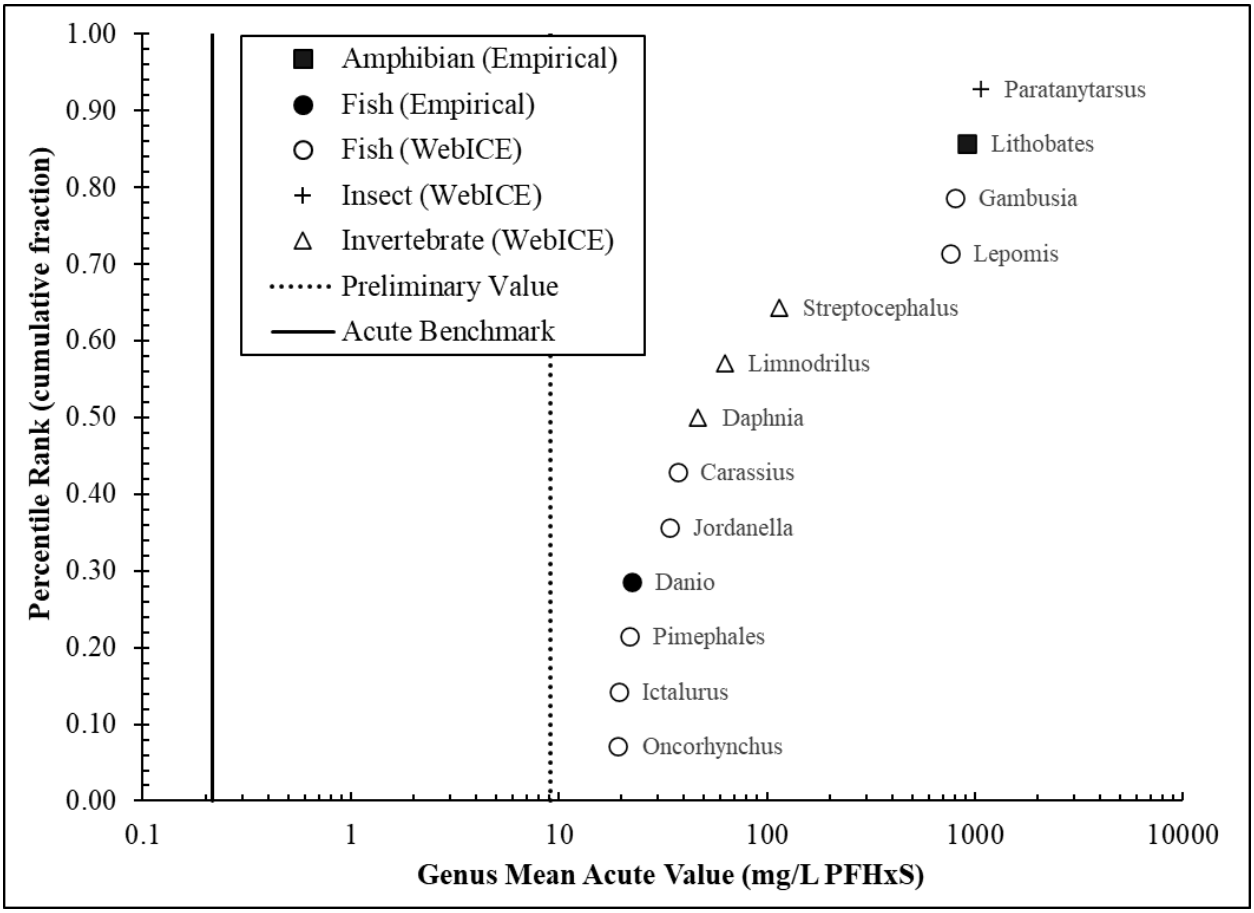

Figure F-6. Ranked Acute PFHxS GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.7 Derivation of Acute Water Benchmark for Hexadecafluoro-2-decenoic Acid (8:2 FTUCA)

Quantitatively acceptable empirical data for HEXA were available for two species comprising two genera and fulfilling two MDR groups (**Table F-20**). A total of 80 ICE models were accepted for use. Of these, ten ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Oncorhynchus tshawytscha* and *Daphnia magna*). Five other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Lepomis macrochirus* predicting for *Cyprinus carpio*). A total of 65 ICE models were selected for use, resulting in ICE models predicting 64 SMAVs representing 53 genera (**Tables F-19** and **F-20**).

The combined empirical and ICE data resulted in 55 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV is summarized in **Table F-20**. GMAVs for the four most sensitive genera were within a factor of 1.8 of each other (**Table F-21**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for 8:2 FTUCA is 1.292 mg/L (**Table F-21**), which is lower than all of the GMAVs except for the ICE-derived species value for the threeridge mussel, *Amblema plicata*, GMAV = 0.79 mg/L. The FAV was divided by two to obtain a preliminary value of 0.6458 mg/L 8:2 FTUCA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.041 mg/L 8:2 FTUCA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to 8:2 FTUCA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-7**).

Table F-19. Acceptable models for ICE-estimated Species Sensitivity to 8:2 FTUCA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0008	166.8	**28.6** (7-116.1)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0002	5,000	**2.0** (1-4.1)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	68.3	**2.9** (1-7.8)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.004	1.625	55.5 (35-87.9)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	95.86	**79.3** (36.6-171.6)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0003	8,694.5	**2.6** (1.8-3.9)
Black bullhead (Ameiurus melas)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	<0.0001	9.81	**411.3** (105.2-1,607.8)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	46,278	4.9[b] (3.7-6.4)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0001	8,341.5	**79.0** (64.3-97.1)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.003	43.07	**288.8** (102.7-811.8)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	96.69	**79.4** (44.1-142.9)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	95.86	**83.8** (47-149.4)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	13,400	**132.5** (51-344.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.004	1.625	**102.3** (69.6-150.5)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	<0.0001	13,400	**67.4** (39.8-114)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.003	724.4	102.9 (57.1-185.4)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	<0.0001	43.07	106.1 (91.6-122.9)
Colorado squawfish (Ptychocheilus lucius)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.003	43.07	**453.5** (101.9-2,018.3)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	198.4	**75.2** (31-182.2)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	<0.0001	198.4	65.5 (42.9-100)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.7099	155.5	**91.1** (48.4-171.5)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0003	46,278	**2.1** (1.5-2.9)
Daphnid (Ceriodaphnia reticulata)	Daphnid (Daphnia magna)	3.2**	3.2 (mg/L)	0.0008	0.232	**2.6** (0.6-11.3)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	3.2**	3.2 (mg/L)	0.0001	0.646	5.4 (2.1-14.2)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.009	10.36	4.9 (1-23.7)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0002	4,894.7	2.4 (1.7-3.3)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	281.6	2.6 (0.7-9.5)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0002	7.2	**1.9** (0.7-5.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	166.8	**1.7** (0.3-12.3)
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0002	46,500	7.0[b] (5.4-9)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	13,400	**107.7** (79.8-145.3)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**2.5** (1.6-4.1)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	<0.0001	13,400	**132.7** (62.2-283.3)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	166.8	**1.9** (0.4-9.4)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	<0.0001	19.93	**208** (129.4-334.4)
Greenthroat darter (Etheostoma lepidum)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.004	1.625	**92.6** (31.2-274.7)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	198.4	**54.9** (12.3-243.7)
Indian bullfrog (Euphlyctis hexadactylus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.028	19.93	**184.0** (26-1,301.7)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0003	166.8	**76.6** (23-255.1)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	82	**16.8** (3.2-89.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	198.4	**41.3** (24.5-69.8)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	<0.0001	95.86	**46.8** (20.7-106.2)
Mayfly (Drunella grandis)	Rainbow trout (Oncorhynchus mykiss)	81^	81 (mg/L)	0.0006	95.86	**365.0** (106.1-1,255.8)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0006	1,330	**241.0** (72.2-804.7)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.37	14,500	**9.3** (3.5-24.7)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0003	58	**1.3** (0.3-6.2)
Mrigal carp (Cirrhinus mrigala)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.0006	1.625	**298.8** (53.6-1,666.3)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.042	166.8	**2.3** (1.1-5.1)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.002	198.4	**125.5** (62.3-252.8)
Northern pike (Esox lucius)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.005	1.822	**48.1** (16.4-141.3)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.003	281.6	**17.9** (6.9-46.2)
Oligochaete (Limnodrilus hoffmeisteri)	Rainbow trout (Oncorhynchus mykiss)	81^	81 (mg/L)	0.003	707	889.5[b] (128.1-6,176.8)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	4,894.7	**31.6** (7.4-134.7)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.011	281.6	**1.5** (0.7-3.4)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**2.5** (1.6-3.7)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.042	545.9	**2.4** (0.8-7)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	3.2**	3200 (µg/L)	0.0001	14,500	3.4 (2.7-4.3)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.003	43.07	**250.4** (96.5-649.5)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Riceland prawn (Macrobrachium lanchesteri)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.003	2.04	**392.0** (87.1-1,764.3)
Rohu (Labeo rohita)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.0006	1.625	**818.9** (162.1-4,136.2)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.028	95.86	**134.5** (26.7-677.1)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.001	7.075	**110.5** (70.9-172.1)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.028	7.5	163.6 (50.5-529.6)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.028	9.7	**151.5** (76.9-298.6)
Spotfin chub (Erimonax monachus)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.004	1.625	**74.3** (33.2-166.2)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**3.2** (1.2-8.4)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**3.0** (1.4-6.7)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	4,894.7	**0.8** (0.3-2.1)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**2.6** (1.4-4.9)
Walleye (Sander vitreus)	Rainbow trout (Oncorhynchus mykiss)	81*	81 (mg/L)	0.005	16.24	**78.4** (27.6-222.5)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**1.4** (0.8-2.6)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.009	977.6	**1.3** (0.8-2.2)
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.009	281.6	**4.2** (1.3-13.2)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.014	8,694.5	**2.3** (1.1-4.6)
Western toad (Anaxyrus boreas)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	0.028	1.625	**107.9** (25.9-450.6)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	81**	81 (mg/L)	<0.0001	16.24	**102.6** (49.6-212)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	50	8.3[b] (2.5-28)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	229.1	**34.6** (11.3-106)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	3.2	3,200 (µg/L)	0.0001	46,500	10.7[b] (5.7-19.9)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	81	81,000 (µg/L)	0.0002	8,341.5	**65.2** (29-146.3)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC50 falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-20. Ranked 8:2 FTUCA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical 8:2 FTUCA toxicity tests with the species.

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
1	G	Threeridge	*Amblema plicata*	0.7900	0.7900	0.02
2	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	1.330	1.330	0.04
3	D	Daphnid	*Chydorus sphaericus*	1.340	1.340	0.05
4	G	Washboard	*Megalonaias nervosa*	1.400	1.400	0.07
5	D	Daphnid	*Simocephalus serrulatus*	1.850	1.794	0.09
	D	Daphnid	*Simocephalus vetulus*	1.740		
6	G	Green floater	*Lasmigona subviridis*	1.900	1.900	0.11
7	G	Western pearlshell	*Margaritifera falcata*	2.270	2.270	0.13
8	D	Daphnid	*Ceriodaphnia reticulata*	2.560**	2.302	0.14
	D	Daphnid	*Ceriodaphnia dubia*	2.070		
9	G	Pheasantshell	*Ortmanniana pectorosa*	2.350	2.350	0.16
10	E	Amphipod	*Gammarus fasciatus*	2.040	2.411	0.18
	E	Amphipod	*Gammarus pseudolimnaeus*	2.850		
11	G	Neosho mucket	*Lampsilis rafinesqueana*	2.320	2.428	0.20
	G	Fatmucket	*Lampsilis siliquoidea*	2.540		
12	G	Paper pondshell	*Utterbackia imbecillis*	2.450	2.450	0.21
13	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	2.630	2.630	0.23
14	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	2.640	2.640	0.25
15	G	Tadpole physa	*Physella gyrina*	3.030	3.030	0.27
16	**D**	**Daphnid**	***Daphnia magna***	**3.200**	3.200	0.29
17	G	Swamp lymnaea	*Lymnaea stagnalis*	3.210	3.210	0.30
18	D	Water flea	*Moina macrocopa*	4.190	4.190	0.32
19	E	Isopod	*Caecidotea brevicauda*	16.82	16.82	0.34
20	H	Oligochaete	*Limnodrilus hoffmeisteri*	17.89	17.89	0.36
21	E	Riceland prawn	*Macrobrachium lanchesteri*	392.0**	24.41	0.375
	E	Oriental river shrimp	*Macrobrachium nipponense*	1.520		
22	E	Amphipod	*Crangonyx pseudogracilis*	28.57	28.57	0.39
23	H	Oligochaete	*Tubifex tubifex*	31.57	31.57	0.41
24	B	Largemouth bass	*Micropterus salmoides*	46.83	46.83	0.43
25	F	Midge	*Paratanytarsus dissimilis*	241.0	47.44	0.45
	F	Midge	*Paratanytarsus parthenogeneticus*	9.340		
26	B	Zebrafish	*Danio rerio*	47.48	47.48	0.46
27	B	Northern pike	*Esox lucius*	48.13**	48.13	0.48
28	B	Guppy	*Poecilia reticulata*	54.85	54.85	0.50
29	A	Brook trout	*Salvelinus fontinalis*	79.36	57.25	0.52
	A	Lake trout	*Salvelinus namaycush*	41.30		
30	B	Channel catfish	*Ictalurus punctatus*	67.38	67.38	0.54
31	B	Spotfin chub	*Erimonax monachus*	74.30**	74.30	0.55
32	B	Common carp	*Cyprinus carpio*	75.18	75.18	0.57
33	E	Isopod	*Asellus aquaticus*	76.56	76.56	0.59
34	B	Walleye	*Sander vitreus*	78.36**	78.36	0.61

Rank	MDR Group[1]	Name	Species	SMAV	GMAV	Percentile Rank
35	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**81.00**	81.00	0.63
36	A	Atlantic salmon	*Salmo salar*	79.29	81.49	0.64
	A	Brown trout	*Salmo trutta*	83.76		
37	C	Cuvier's foam froglet	*Physalaemus cuvieri*	91.14	91.14	0.66
38	B	Greenthroat darter	*Etheostoma lepidum*	92.55**	92.55	0.68
39	B	Cape Fear shiner	*Notropis mekistocholas*	102.3**	102.3	0.70
40	B	Yellow perch	*Perca flavescens*	102.6**	102.6	0.71
41	B	Fathead minnow	*Pimephales promelas*	107.7	107.7	0.73
42	C	Western toad	*Anaxyrus boreas*	107.9**	107.9	0.75
43	B	Silver perch	*Bidyanus bidyanus*	110.5**	110.5	0.77
44	B	Green sunfish	*Lepomis cyanellus*	208.0**	128.2	0.79
	B	Bluegill	*Lepomis macrochirus*	78.99		
45	B	Goldfish	*Carassius auratus*	132.7	132.7	0.80
46	B	Shortnose sturgeon	*Acipenser brevirostrum*	134.5	134.5	0.82
47	C	Bullfrog	*Lithobates catesbeianus*	132.5	136.0	0.84
	C	Northern leopard frog	*Lithobates pipiens*	125.5		
	C	Southern leopard frog	*Lithobates sphenocephalus*	151.5**		
48	C	Indian bullfrog	*Euphlyctis hexadactylus*	184.0**	184.0	0.86
49	B	Razorback sucker	*Xyrauchen texanus*	250.4**	250.4	0.88
50	B	Bonytail	*Gila elegans*	288.8**	288.8	0.89
51	B	Mrigal carp	*Cirrhinus mrigala*	298.8**	298.8	0.91
52	F	Mayfly	*Drunella grandis*	365.0	365.0	0.93
53	B	Black bullhead	*Ameiurus melas*	411.3**	411.3	0.95
54	B	Colorado squawfish	*Ptychocheilus lucius*	453.5**	453.5	0.96
55	B	Rohu	*Labeo rohita*	818.9**	818.9	0.98

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR 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, 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.

Table F-21. 8:2 FTUCA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=55 GMAVs total
Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)

Rank	Genus	GMAV (mg/L)	ln(GMAV)	ln(GMAV)^2	P=R/(N+1)	sqrt(P)
1	*Amblema*	0.79	-0.24	0.06	0.018	0.134
2	*Palaemonetes*	1.33	0.29	0.08	0.036	0.189
3	*Chydorus*	1.34	0.29	0.09	0.054	0.231
4	*Megalonaias*	1.40	0.34	0.11	0.071	0.267
		Σ (Sum):	**0.68**	**0.34**	**0.18**	**0.82**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 22.23
S = slope
L = -0.798
L = X-axis intercept
A = 0.256
A = lnFAV
FAV = 1.292
FAV/2 = 0.6458 mg/L (Preliminary Value)
Adjustment = 0.6458 / 15.8 = 0.04087 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.041 mg/L 8:2 FTUCA** (rounded to two significant figures)

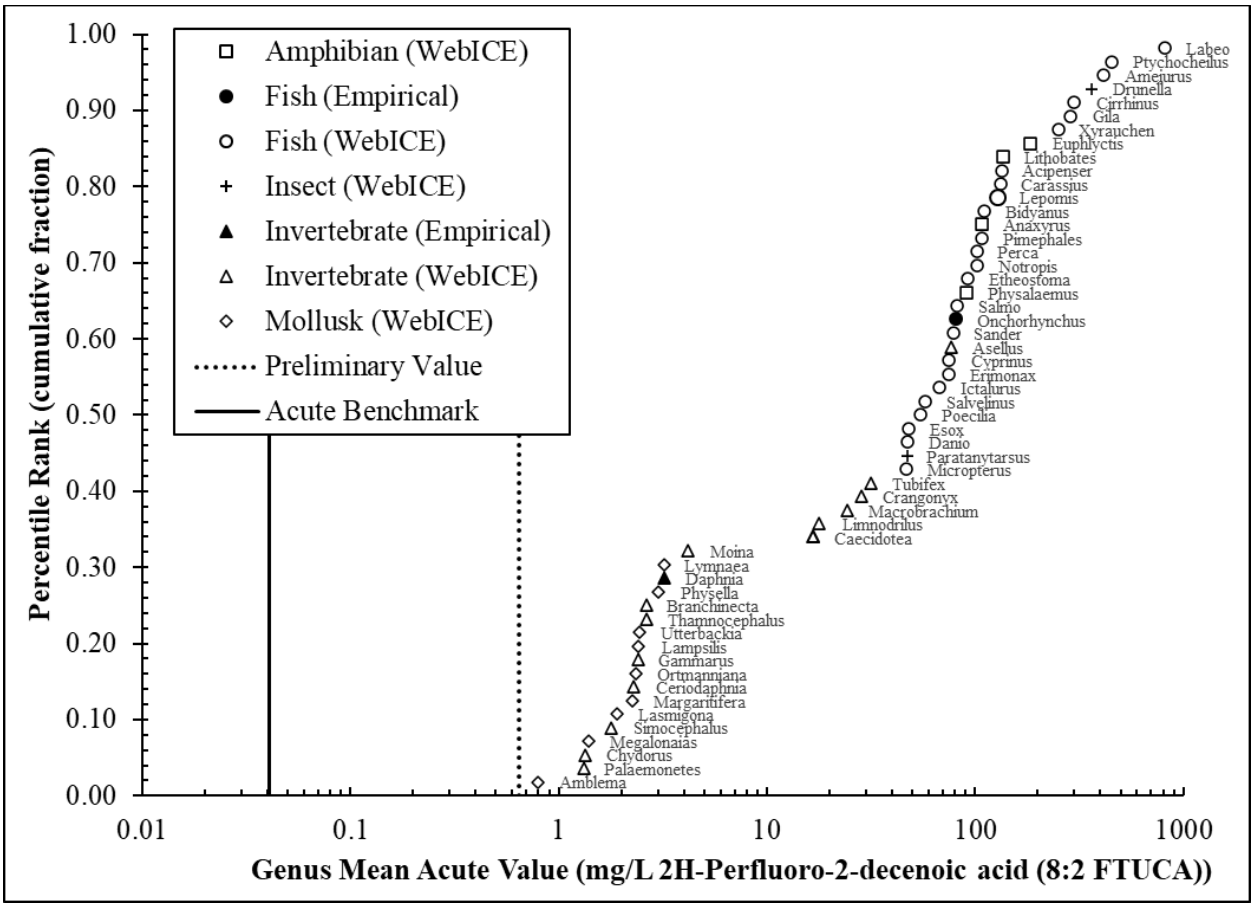

Figure F-7. Ranked Acute 8:2 FTUCA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.8 Derivation of Acute Water Benchmark for Pentadecafluorodecanoic Acid (7:3 FTCA)

Quantitatively acceptable empirical data for 7:3 FTCA were available for two species comprising two genera and fulfilling two MDR groups (**Table F-23**). A total of 81 ICE models were accepted for use. Of these, ten ICE models were not selected for use because empirical data were available for the genera (e.g., models for *Oncorhynchus tshawytscha* and *Daphnia pulex*). Four other models were not used because there were acceptable models for more closely-related surrogate species (e.g., *Daphnia magna* predicting for *Lepomis macrochirus* predicting for *Cyprinus carpio*). A total of 67 ICE models were selected for use, resulting in ICE models predicting 66 SMAVs representing 57 genera (**Tables F-22** and **F-23**).

The combined empirical and ICE data resulted in 59 GMAVs that collectively fulfill the eight MDR groups for deriving a freshwater criterion as defined by the Aquatic Life Criteria Guidelines (U.S. EPA 1985). The ranked GMAVs for these combined data along with the MDR met by each GMAV are summarized in **Table F-23**. GMAVs for the four most sensitive genera were within a factor of 2.1 of each other (**Table F-24**). The freshwater FAV (the 5th percentile of the genus sensitivity distribution) for 7:3 FTCA is 0.4609 mg/L (**Table F-24**), which is lower than all of the GMAVs except for three of the ICE-derived species values (the threeridge mussel, *Amblema plicata*, GMAV=0.25 mg/L, the water flea *Chydorus sphaericus*, GMAV=0.43 mg/L, and the washboard, *Megalonaias nervosa*, GMAV=0.44 mg/L). The FAV was divided by two to obtain a preliminary value of 0.2305 mg/L 7:3 FTCA and then adjusted by the carboxylic acid application factor (15.8) to obtain the freshwater acute water column benchmark magnitude of 0.015 mg/L 7:3 FTCA (rounded to two significant figures). This value is expected to be protective of 95% of freshwater genera exposed to 7:3 FTCA under short-term conditions of one-hour duration, if the one-hour average magnitude is not exceeded more than once in three years (**Figure F-8**).

Table F-22. Acceptable models for ICE-estimated Species Sensitivity to 7:3 FTCA using the scaling approach for data outside of the model bounds as in cases indicated.

Bold predicted EC_{50}s used for SMAV calculations.

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Amphipod (Crangonyx pseudogracilis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0008	166.8	**9.5** (2.8-32.4)
Amphipod (Gammarus fasciatus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0002	5,000	**0.8** (0.4-1.4)
Amphipod (Gammarus pseudolimnaeus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	68.3	**1.0** (0.4-2.2)
Apache trout (Oncorhynchus gilae)*	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0041	1.625	20.0 (12.3-32.6)
Atlantic salmon (Salmo salar)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	95.86	**31.0** (15.7-61.3)
Beaver-tail fairy shrimp (Thamnocephalus platyurus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	8,694.5	**0.9** (0.6-1.3)
Black bullhead (Ameiurus melas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	9.81	**207.8** (56.1-770)
Bluegill (Lepomis macrochirus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	46,278	2.1[b] (1.6-2.7)
Bluegill (Lepomis macrochirus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0001	8,341.5	**33.1** (27.6-39.6)
Bonytail (Gila elegans)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	43.07	**72.7** (12.6-420.2)
Brook trout (Salvelinus fontinalis)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	96.69	**31.4** (18.8-52.5)
Brown trout (Salmo trutta)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	95.86	**33.1** (19.8-55.3)
Bullfrog (Lithobates catesbeianus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	13,400	**58.8** (23.7-145.4)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Cape Fear shiner (Notropis mekistocholas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.004	1.625	**34.4** (22.8-51.9)
Channel catfish (Ictalurus punctatus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	13,400	**31.6** (19.9-50.1)
Chinook salmon (Oncorhynchus tshawytscha)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	724.4	40.0 (24-66.9)
Coho salmon (Oncorhynchus kisutch)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	43.07	49.4 (38.4-63.6)
Colorado squawfish (Ptychocheilus lucius)	Rainbow trout (Oncorhynchus mykiss)	32^	32 (mg/L)	0.003	43.07	**195.1** (34.5-1,103.6)
Common carp (Cyprinus carpio)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	198.4	**35.6** (16.3-77.7)
Cutthroat trout (Oncorhynchus clarkii)*	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	198.4	27.1 (18.7-39.3)
Cuvier's foam froglet (Physalaemus cuvieri)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.7099	155.5	**51.8** (30.8-87.1)
Daphnid (Ceriodaphnia dubia)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	46,278	**0.6** (0.4-0.9)
Daphnid (Daphnia galeata)*	Daphnid (Daphnia magna)	0.9592**	0.9592 (mg/L)	0.0001	0.646	1.8 (0.6-5.8)
Daphnid (Daphnia longispina)*	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.009	10.36	1.1 (0.3-4.3)
Daphnid (Daphnia pulex)*	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0002	4,894.7	0.7 (0.5-1)
Daphnid (Daphnia pulicaria)*	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	281.6	0.7 (0.2-3.1)
Daphnid (Simocephalus serrulatus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0002	7.2	**0.6** (0.3-1.4)
Daphnid (Simocephalus vetulus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	166.8	**0.7** (0.1-3.8)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Fathead minnow (Pimephales promelas)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0002	46,500	3.0[b] (2.3-3.9)
Fathead minnow (Pimephales promelas)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	13,400	**46.7** (36-60.7)
Fatmucket (Lampsilis siliquoidea)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.9** (0.5-1.5)
Goldfish (Carassius auratus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	13,400	**62.2** (31.6-122.5)
Green floater (Lasmigona subviridis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	166.8	**0.9** (0.2-3.5)
Green sunfish (Lepomis cyanellus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	19.93	**90.0** (54.2-149.3)
Greenthroat darter (Etheostoma lepidum)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.004	1.625	**34.9** (11-110.9)
Guppy (Poecilia reticulata)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	198.444	**28.3** (7.6-105.3)
Isopod (Asellus aquaticus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	166.8	**27.3** (9.3-80.1)
Isopod (Caecidotea brevicauda)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	82	**8.2** (1.9-35.7)
Lake trout (Salvelinus namaycush)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	198.4	**18.0** (11.4-28.4)
Largemouth bass (Micropterus salmoides)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	<0.0001	95.86	**19.5** (9.5-40.3)
Leech (Nephelopsis obscura)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0008	4.369	**131.2** (18.7-920.2)
Mayfly (Drunella grandis)	Rainbow trout (Oncorhynchus mykiss)	32^	32 (mg/L)	0.0006	95.86	**143.5** (43.1-477.3)
Medaka (Oryzias latipes)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.007	54.400	**52.1** (10.9-248.7)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Midge (Chironomus tentans)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	472	**4.6** (0.7-32.5)
Midge (Paratanytarsus dissimilis)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0006	1,330	**105.8** (35.6-314.4)
Midge (Paratanytarsus parthenogeneticus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.37	14,500	**2.9** (0.9-9.1)
Mississippi grass shrimp (Palaemonetes kadiakensis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0003	58	**0.5** (0.2-1.9)
Mrigal carp (Cirrhinus mrigala)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	1.625	**147.7** (24.4-896.1)
Neosho mucket (Lampsilis rafinesqueana)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.042	166.8	**0.8** (0.4-1.6)
Northern leopard frog (Lithobates pipiens)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.002	198.4	**64.7** (33.7-124.2)
Northern pike (Esox lucius)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.005	1.822	**18.4** (6.5-52)
Oligochaete (Limnodrilus hoffmeisteri)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.003	281.6	**7.6** (3.1-18.5)
Oligochaete (Tubifex tubifex)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	4,894.7	**10.8** (3-38.4)
Oriental river shrimp (Macrobrachium nipponense)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.011	281.6	**0.4** (0.2-0.9)
Paper pondshell (Utterbackia imbecillis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.8** (0.5-1.3)
Pheasantshell (Ortmanniana pectorosa)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.042	545.9	**0.7** (0.2-2.4)
Rainbow trout (Oncorhynchus mykiss)*	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	14,500	1.4 (1.2-1.8)
Razorback sucker (Xyrauchen texanus)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.003	43.07	**26.6** (5.3-134)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Riceland prawn (Macrobrachium lanchesteri)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.003	2.04	**177.6** (34.2-922.7)
Rohu (Labeo rohita)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	1.625	**302.0** (61.5-1,484.6)
Shortnose sturgeon (Acipenser brevirostrum)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.028	95.86	**45.7** (11.2-185.7)
Silver perch (Bidyanus bidyanus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.001	7.075	**40.3** (25.8-62.8)
Snipefly (Atherix variegata)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.0006	0.068	**194.9** (89.6-424)
Sockeye salmon (Oncorhynchus nerka)*	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	7.5	74.6 (17.1-325.1)
Southern leopard frog (Lithobates sphenocephalus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	9.7	**46.3** (19.9-107.9)
Spotfin chub (Erimonax monachus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.004	1.625	**24.5** (10.4-57.6)
Swamp lymnaea (Lymnaea stagnalis)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**1.0** (0.4-2.9)
Tadpole physa (Physella gyrina)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**1.0** (0.4-2.3)
Threeridge (Amblema plicata)	Daphnid (Daphnia magna)	0.9592	0.9592 (mg/L)	0.014	4,894.7	**0.3** (0.1-0.7)
Vernal pool fairy shrimp (Branchinecta lynchi)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.9** (0.4-1.6)
Walleye (Sander vitreus)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.005	16.24	**41.8** (12.1-144.6)
Washboard (Megalonaias nervosa)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.4** (0.2-0.9)
Water flea (Chydorus sphaericus)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.009	977.6	**0.4** (0.3-0.7)

Predicted Species	Surrogate			ICE Model Value Range (mg/L)		Predicted EC_{50} (Confidence Limits) (mg/L)
	Species	Measured EC_{50} (mg/L)	Entered EC_{50} (units)	Minimum	Maximum	
Water flea (Moina macrocopa)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.009	281.6	**1.8** (0.6-5.2)
Western pearlshell (Margaritifera falcata)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.014	8,694.5	**0.8** (0.4-1.7)
Western toad (Anaxyrus boreas)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	0.028	1.625	**31.3** (4.6-211.6)
Yellow perch (Perca flavescens)	Rainbow trout (Oncorhynchus mykiss)	32**	32 (mg/L)	<0.0001	16.24	**44.5** (21-94.5)
Zebrafish (Danio rerio)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	50.000	3.3[b] (1.1-10)
Zebrafish (Danio rerio)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	229.1	**17.6** (6.4-48.5)
Zebrafish-embryo (Danio rerio-embryo)	Daphnid (Daphnia magna)	0.9592	959.2 (µg/L)	0.0001	46,500	4.8[b] (2.5-9.2)
Zebrafish-embryo (Danio rerio-embryo)	Rainbow trout (Oncorhynchus mykiss)	32	32,000 (µg/L)	0.0002	8,341.5	**34.4** (16.8-70.4)

*****Acceptable models that were not used because genus level empirical data were available.

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

^ µg/L CI greater than 50-fold. Used mg/L with acceptable CI.

[a] Saltwater surrogate species were not used.

[b] Not used because other more closely taxonomically-related models were available.

[c] Not used because other more closely taxonomically-related models where the measured surrogate EC_{50} values fell within the ICE model range were available.

Table F-23. Ranked 7:3 FTCA Genus Mean Acute Values.

Values in bold and highlighted are derived from empirical 7:3 FTCA toxicity tests with the species.

Rank	MDR Group[1]	Common Name	Species	SMAV (mg/L)	GMAV	Percentile Rank
1	G	Threeridge	*Amblema plicata*	0.2500	0.2500	0.02
2	D	Daphnid	*Chydorus sphaericus*	0.4300	0.4300	0.03
3	G	Washboard	*Megalonaias nervosa*	0.4400	0.4400	0.05
4	E	Mississippi grass shrimp	*Palaemonetes kadiakensis*	0.5300	0.5300	0.07
5	D	Daphnid	*Ceriodaphnia dubia*	0.6000	0.6000	0.08
6	D	Daphnid	*Simocephalus serrulatus*	0.6000	0.6527	0.10
	D	Daphnid	*Simocephalus vetulus*	0.7100		
7	G	Pheasantshell	*Ortmanniana pectorosa*	0.7400	0.7400	0.12
8	G	Western pearlshell	*Margaritifera falcata*	0.7900	0.7900	0.13
9	G	Neosho mucket	*Lampsilis rafinesqueana*	0.7900	0.8243	0.15
	G	Fatmucket	*Lampsilis siliquoidea*	0.8600		
10	G	Paper pondshell	*Utterbackia imbecillis*	0.8400	0.8400	0.17
11	E	Amphipod	*Gammarus fasciatus*	0.7600	0.8497	0.18
	E	Amphipod	*Gammarus pseudolimnaeus*	0.9500		
12	G	Green floater	*Lasmigona subviridis*	0.8500	0.8500	0.20
13	E	Vernal pool fairy shrimp	*Branchinecta lynchi*	0.8500	0.8500	0.22
14	E	Beaver-tail fairy shrimp	*Thamnocephalus platyurus*	0.8800	0.8800	0.23
15	G	Tadpole physa	*Physella gyrina*	0.9500	0.9500	0.25
16	**D**	**Daphnid**	***Daphnia magna***	**0.9592**	0.9592	0.27
17	G	Swamp lymnaea	*Lymnaea stagnalis*	1.030	1.030	0.28
18	D	Water flea	*Moina macrocopa*	1.760	1.760	0.30
19	F	Midge	*Paratanytarsus parthenogeneticus*	2.880	2.880	0.32
20	F	Midge	*Chironomus tentans*	4.630	4.630	0.33
21	H	Oligochaete	*Limnodrilus hoffmeisteri*	7.620	7.620	0.35
22	E	Isopod	*Caecidotea brevicauda*	8.170	8.170	0.37
23	E	Riceland prawn	*Macrobrachium lanchesteri*	177.6**	8.214	0.38
	E	Oriental river shrimp	*Macrobrachium nipponense*	0.3800		
24	E	Amphipod	*Crangonyx pseudogracilis*	9.510	9.510	0.40
25	H	Oligochaete	*Tubifex tubifex*	10.75	10.75	0.42
26	B	Northern pike	*Esox lucius*	18.36**	18.36	0.43
27	B	Largemouth bass	*Micropterus salmoides*	19.52	19.52	0.45
28	A	Brook trout	*Salvelinus fontinalis*	31.44	23.80	0.47
	A	Lake trout	*Salvelinus namaycush*	18.01		
29	B	Spotfin chub	*Erimonax monachus*	24.47**	24.47	0.48
30	B	Zebrafish	*Danio rerio*	24.57	24.57	0.50
31	B	Razorback sucker	*Xyrauchen texanus*	26.57	26.57	0.52
32	E	Isopod	*Asellus aquaticus*	27.30	27.30	0.53
33	B	Guppy	*Poecilia reticulata*	28.31	28.31	0.55
34	C	Western toad	*Anaxyrus boreas*	31.29**	31.29	0.57
35	B	Channel catfish	*Ictalurus punctatus*	31.55	31.55	0.58
36	**A**	**Rainbow trout**	***Oncorhynchus mykiss***	**32.00**	32.00	0.60

Rank	MDR Group[1]	Common Name	Species	SMAV (mg/L)	GMAV	Percentile Rank
37	A	Atlantic salmon	*Salmo salar*	30.96	32.02	0.62
	A	Brown trout	*Salmo trutta*	33.12		
38	B	Cape Fear shiner	*Notropis mekistocholas*	34.42**	34.42	0.63
39	B	Greenthroat darter	*Etheostoma lepidum*	34.87**	34.87	0.65
40	B	Common carp	*Cyprinus carpio*	35.58	35.58	0.67
41	B	Silver perch	*Bidyanus bidyanus*	40.28**	40.28	0.68
42	B	Walleye	*Sander vitreus*	41.80**	41.80	0.70
43	B	Yellow perch	*Perca flavescens*	44.48**	44.48	0.72
44	B	Shortnose sturgeon	*Acipenser brevirostrum*	45.67	45.67	0.73
45	B	Fathead minnow	*Pimephales promelas*	46.72	46.72	0.75
46	C	Cuvier's foam froglet	*Physalaemus cuvieri*	51.77	51.77	0.77
47	B	Medaka	*Oryzias latipes*	52.12	52.12	0.78
48	B	Green sunfish	*Lepomis cyanellus*	89.98**	54.56	0.80
	B	Bluegill	*Lepomis macrochirus*	33.08		
49	C	Bullfrog	*Lithobates catesbeianus*	58.75	56.04	0.82
	C	Northern leopard frog	*Lithobates pipiens*	64.72		
	C	Southern leopard frog	*Lithobates sphenocephalus*	46.28**		
50	B	Goldfish	*Carassius auratus*	62.18	62.18	0.83
51	B	Bonytail	*Gila elegans*	72.68	72.68	0.85
52	F	Midge	*Paratanytarsus dissimilis*	105.8	105.8	0.87
53	H	Leech	*Nephelopsis obscura*	131.2	131.2	0.88
54	F	Mayfly	*Drunella grandis*	143.5	143.5	0.90
55	B	Mrigal carp	*Cirrhinus mrigala*	147.7**	147.7	0.92
56	F	Snipefly	*Atherix variegata*	194.9**	194.9	0.93
57	B	Colorado squawfish	*Ptychocheilus lucius*	195.1	195.1	0.95
58	B	Black bullhead	*Ameiurus melas*	207.8**	207.8	0.97
59	B	Rohu	*Labeo rohita*	302.0**	302.0	0.98

******Measured EC_{50} falls outside range of ICE model and a "scaled" EC_{50} was therefore entered in ICE model.

1: Freshwater MDR 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, 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.

Table F-24. 7:3 FTCA Final Acute Value and Protective Aquatic Acute Benchmark.

Calculated Freshwater FAV based on 4 lowest values; N=59 GMAVs total Benchmark calculated by dividing the FAV by 2 and by the carboxylic acid application factor (15.8)						
Rank	**Genus**	**GMAV (mg/L)**	**ln(GMAV)**	**ln(GMAV)^2**	**P=R/(N+1)**	**sqrt(P)**
1	*Amblema*	0.25	-1.39	1.92	0.017	0.129
2	*Chydorus*	0.43	-0.84	0.71	0.033	0.183
3	*Megalonaias*	0.44	-0.82	0.67	0.050	0.224
4	*Palaemonetes*	0.53	-0.63	0.40	0.067	0.258
		Σ (Sum):	**-3.69**	**3.71**	**0.17**	**0.79**

P = cumulative probability
R = rank
N = number of GMAVs

S^2 = 33.93 S = slope
L = -2.077 L = X-axis intercept
A = -0.775 A = lnFAV
FAV = 0.4609
FAV/2 = 0.2305 mg/L (Preliminary Value)
Adjustment = 0.2305 / 15.8 = 0.01459 (Preliminary Value / Carboxylic Acid Application Factor)
Benchmark = **0.015 mg/L 7:3 FTCA** (rounded to two significant figures)

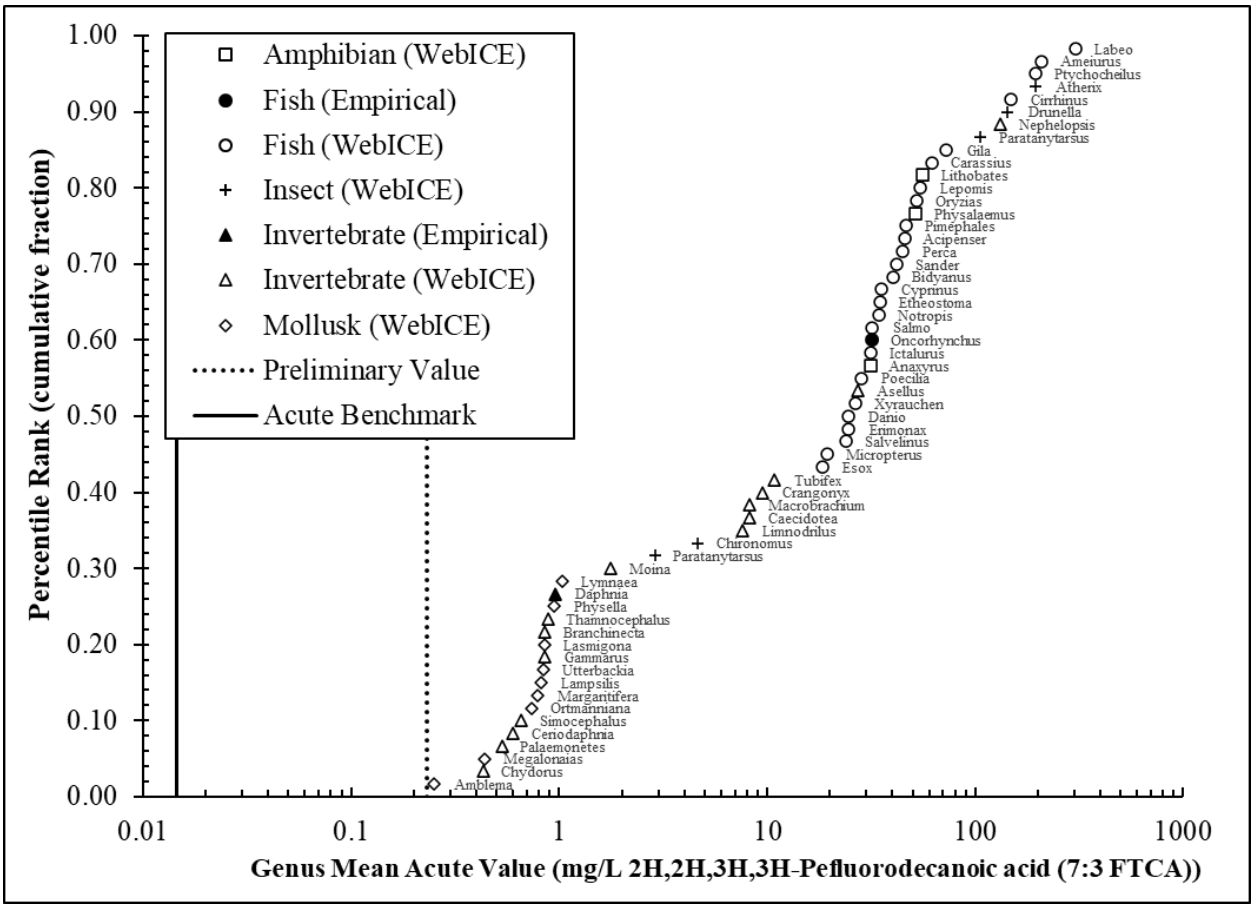

Figure F-8. Ranked Acute 7:3 FTCA GMAVs Used for the Aquatic Life Acute Benchmark Calculation.

F.9 Summary of Benchmarks for Evaluated PFAS Substances

Acute freshwater Aquatic Life Ambient Water Quality Benchmarks for the eight selected PFAS are summarized in **Table F-25**. These concentrations are expected to be protective of 95% of freshwater genera exposed to the listed PFAS under short term conditions of one-hour of duration, if the one-hour average magnitude is not exceeded more than once in three years. Quantitatively-acceptable empirical toxicity data were coupled with ICE predicted values to fulfill the eight MDRs for deriving acute freshwater criteria per the Aquatic Life Criteria Guidelines (U.S. EPA 1985). Using this approach, the eight MDRs were fulfilled for seven of the evaluated compounds (PFBA, PFHxA, PFNA, PFDA, PFBS, 8:2 FTUCA, and 7:3 FTCA).

Seven of the eight MDRs were fulfilled for PFHxS and there is considered to be greater uncertainty associated with this benchmark value compared to benchmarks developed with all MDRs met. The resulting acute water column-based benchmark magnitudes range from 0.015 mg/L for 7:3 FTCA to 11 mg/L for PFBA.

Table F-25. Acute Freshwater Benchmarks for Eight PFAS.

Chemical	PFBA	PFHxA	PFNA	PFDA	PFBS	PFHxS	8:2 FTUCA	7:3 FTCA
Magnitude[1]	11	6.0	0.73	0.65	7.6	0.21	0.041	0.015
Duration	One hour average							
Frequency	Not to be exceeded more than once in three years on average							

[1] Values expressed as mg/L, or ppm.

F.1 References

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U.S. EPA. (United States Environmental Protection Agency). 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. EPA Report 822/R-85-100 (NTIS Report PB85-227049).

Appendix G PFAS Acute Benchmark Calculations using a Data Binning Approach

The EPA investigated the utility of applying an approach described in Giddings et al. (2019) for synthetic pyrethroid insecticides as another alternative for deriving benchmark values for data-limited PFAS. For this approach, Giddings et al. (2019) combined acute toxicity data available for multiple data-limited synthetic pyrethroid insecticide active ingredients (e.g., bifenthrin, cypermethrin, permethrin, etc.) to create a more robust combined species sensitivity distribution (SSD) with the grouped pyrethroids. Before combining, the available toxicity data were normalized across the individual pyrethroid active ingredients. This was done by normalizing the data across the individual pyrethroid active ingredients based on the relative sensitivity of a single selected "key" species, that has data for each constituent active ingredient. Giddings et al. (2019) then combined the normalized species data (i.e., key species equivalents) for the individual pyrethroids and calculated an HC_5 (hazard concentration at the 5% level) based on the combined pyrethroid SSD composed of normalized acute toxicity values. Estimated HC_5s were then back-calculated for individual constituent pyrethroids based on the relative sensitivity of the species used for the normalization.

The EPA followed the same methodology to calculate predicted HC_5 values for selected PFAS combined into two groups: carboxylic acids and sulfonic acids. Constituent PFAS for these groups are as follows:

- Carboxylic acid PFAS substances for which acceptable acute toxicity data were available:
    - PFOA (perfluorooctanoic acid or perfluorooctanoate)
    - PFBA (perfluorobutanoic acid or perfluorobutanoate)
    - PFHxA (perfluorohexanoic acid or perfluorohexanoate)
    - PFNA (perfluorononanoic acid or perfluorononanoate)
    - PFDA (perfluorodecanoic acid or perfluorodecanoate)
- Sulfonic acid PFAS substances for which acceptable acute toxicity data were available:
    - PFOS (perfluorooctane sulfonate or perfluorooctane sulfonate acid)
    - PFBS (perfluorobutane sulfonic acid or perfluorobutane sulfonate)
    - PFHxS (perfluorohexane sulfonic acid or perfluorohexane sulfonate)

The EPA conducted the following steps to complete this evaluation:

1. High quality acute toxicity data for each PFAS were compiled. These toxicity data consist of the same quantitatively acceptable empirical data used for derivation of the ICE-based values (see **Appendix A**) for PFBA, PFBS, PFHxA, PFHxS, PFNA, PFDA, and the same quantitatively acceptable data used for derivation of the PFOA and PFOS criteria (U.S. EPA 2024a,b). All data were converted into mg/L.
2. Species mean acute values (SMAVs) were identified. If multiple acute toxicity values were available for a single species and PFAS substance, then the geometric mean of those values was calculated.
3. "Key" species to be used for normalizing the data across the constituent substances within each PFAS grouping (sulfonic acid PFAS and carboxylic acid PFAS grouping) were identified and species equivalent values were calculated. Zebrafish (*Danio rerio*) was identified as the key species for sulfonic acid PFAS, and the planktonic crustacean (*Daphnia magna*) was identified as the key species for carboxylic acid

PFAS. The SMAV for each constituent substance within each PFAS grouping was then divided by the corresponding normalization value for the key species to calculate species equivalent values. The following examples illustrate the procedure for calculating key species equivalents for daphnids for PFOA within the carboxylic acid PFAS group:

- Equivalent for *Daphnia magna*:
    - *D. magna* PFOA SMAV of 213.9 mg/L ÷ *D. magna* PFOA SMAV of 213.9 mg/L PFOA = 1.00 *D. magna* equivalent
- Equivalent for *Daphnia pulicaria*:
    - *D. pulicaria* PFOA SMAV of 203.7 mg/L PFOA ÷ *D. magna* PFOA SMAV of 213.9 mg/L PFOA = 0.9523 *D. magna* equivalent

The species equivalent values were then ranked. The key species equivalent acute toxicity values and their rankings are shown in **Table G-1** for sulfonic acids and **Table G-2** for carboxylic acids.

4. Species equivalent values for constituent substances were pooled for each PFAS grouping to derive SSDs for sulfonic acid PFAS and for carboxylic acid PFAS. If a key species equivalent value was available for a species having two or more constituent PFAS within a group, then the geometric mean of those values was calculated as the SMAV equivalent for that species. These SMAVs representing key species equivalent values were then ranked to create SSDs for their respective PFAS group (sulfonic acid and carboxylic acid PFAS). SSDs were derived for sulfonic acid PFAS and carboxylic acid PFAS using both the EPA SSD Generator[1], as was done by Giddings et al. (2019), and procedures described in the Aquatic Life Criteria

[1] Available online at: Download Software | US EPA. Use of this tool results in the estimation of an HC_5.

Guidelines (U.S. EPA 1985), as is typically done for the derivation of aquatic life criteria. The SSDs generated by the EPA's SSD Generator are shown in **Figure G-1a** for sulfonic acid PFAS and **Figure G-2a** for carboxylic acid PFAS. The SSDs generated using procedures described in the Aquatic Life Criteria Guidelines are shown in **Figure G-1b** for sulfonic acid PFAS and **Figure G-2b** for carboxylic acid PFAS.

5. HC_5 values were calculated for sulfonic acid PFAS and carboxylic acid PFAS from the SSDs using both the EPA SSD Generator[2] and procedures described in the Aquatic Life Criteria Guidelines (termed Final Acute Value [FAV] for the Aquatic Life Criteria Guidelines calculations [U.S. EPA 1985]). For any given species within the PFAS group-specific SSD, a key species equivalent greater than 1.0 indicated it was less sensitive than the key species, and a value less than 1.0 indicated it was more sensitive than the key species. Once the key species equivalent HC_5 (or FAV) was calculated, the HC_5 (or FAV) species equivalent value calculated from the SSD was multiplied by the key species toxicity value (i.e., SMAV) for the PFAS of interest within that group (sulfonic or carboxylic PFAS) to generate a substance-specific HC_5 (or FAV). For example:

   - FAV for PFHxS:
     - FAV for Sulfonic Acid PFAS of 0.01004 *x D. rerio* PFHxS SMAV of 22.5 mg/L = PFHxS FAV of 0.2260 mg/L
   - FAV for PFBA:
     - FAV for Carboxylic Acid PFAS of 0.008213 *x D. magna* PFBA SMAV of 4,741 mg/L = PFBA FAV of 38.94 mg/L

The calculations and values are summarized in **Tables G-3** through **G-6**.

[2] Available online at: Download Software | US EPA. Use of this tool results in the estimation of an HC_5.

Table G-1. Acute LC_{50}s, SMAVs, normalized *Danio rerio* equivalent values and rank of SMAV *D. rerio* equivalent values for the sulfonic acid PFAS.

Bolding indicates key species used for normalizing data across constituent substances.

PFAS	Species	Species Common Name	SMAV[a] (mg/L)	*Danio rerio* equivalent[b]	SMAV *D. rerio* equivalent[c]	Rank of SMAV *D. rerio* equivalent[d]
PFOS	*Ambystoma jeffersonianum*	Jefferson salamander	51.71	1.86	1.86	17
PFOS	*Ambystoma texanum*	Small-mouthed salamander	30.00	1.08	1.08	14
PFOS	*Ambystoma tigrinum*	Eastern tiger salamander	68.63	2.46	2.46	23
PFOS	*Anaxyrus americanus*	American toad	56.49	2.03	2.03	18
PFOS	*Brachionus calyciflorus*	Rotifer	61.80	2.22	2.22	21
PFBS	*Danio rerio*	**Zebrafish**	**>3,000**	**1.00**	**1.00**	**13**
PFHxS	*Danio rerio*	**Zebrafish**	**22.50**	**1.00**		
PFOS	*Danio rerio*	**Zebrafish**	**27.86**	**1.00**		
PFOS	*Daphnia carinata*	Daphnid	11.56	0.41	0.41	5
PFBS	*Daphnia magna*	Daphnid	2,183	0.73	1.16	15
PFOS	*Daphnia magna*	Daphnid	51.86	1.86		
PFOS	*Daphnia pulicaria*	Daphnid	134.0	4.81	4.81	27
PFOS	*Dugesia japonica*	Planaria	22.48	0.81	0.81	12
PFOS	*Elliptio complanata*	Eastern elliptio	64.35	2.31	2.31	22
PFOS	*Hyla versicolor*	Gray treefrog	19.88	0.71	0.71	11
PFOS	*Lampsilis siliquoidea*	Fatmucket	16.50	0.59	0.59	9
PFBS	*Lepomis macrochirus*	Bluegill	6,452	2.15	2.15	20
PFOS	*Ligumia recta*	Black sandshell	13.50	0.48	0.48	6
PFHxS	*Lithobates catesbeianus*	Bullfrog	1,105	49.11	15.33	30
PFOS	*Lithobates catesbeianus*	American bullfrog	133.3	4.78		
PFOS	*Lithobates clamitans*	Green frog	113.0	4.06	4.06	25
PFOS	*Lithobates pipiens*	Northern leopard frog	72.72	2.61	2.61	24
PFOS	*Lithobates sylvatica*	Wood frog	130.0	4.67	4.67	26

PFAS	Species	Species Common Name	SMAV[a] (mg/L)	*Danio rerio* equivalent[b]	SMAV *D. rerio* equivalent[c]	Rank of SMAV *D. rerio* equivalent[d]
PFOS	*Moina macrocopa*	Daphnid	17.20	0.62	0.62	10
PFOS	*Moina micrura*	Daphnid	0.5496	0.02	0.02	2
PFOS	*Neocaridina denticulata*	Japanese swamp shrimp	15.61	0.56	0.56	7
PFOS	*Neocleon triangulifer*	Mayfly	0.07617	0.003	0.003	1
PFOS	*Oncorhynchus mykiss*	Rainbow trout	7.515	0.27	0.27	3
PFOS	*Physella acuta*	Bladder snail	183.0	6.57	6.57	29
PFOS	*Physella heterostropha pomilia*	Snail	161.8	5.81	5.81	28
PFBS	*Pimephales promelas*	Fathead minnow	1,938	0.65	0.40	4
PFOS	*Pimephales promelas*	Fathead minnow	6.950	0.25		
PFOS	*Pontastacus leptodactylus*	Crayfish	48.81	1.75	1.75	16
PFOS	*Procambarus fallax f. virginalis*	Crayfish	59.87	2.15	2.15	19
PFOS	*Xenopus laevis*	African clawed frog	15.99	0.57	0.57	8

[a] Data used for PFBS and PFHxS are presented in **Appendix A**; data used for PFOS are the same as the quantitatively acceptable data used for derivation of the PFOS criteria (US EPA 2024b).

[b] *Danio rerio* equivalents determined according to the following equation: Species *x* SMAV for PFAS ÷ *D. rerio* SMAV for PFAS *x* = *D. rerio* equivalent for species.

[c] SMAV *Danio rerio* equivalents are the geometric means of the *D. rerio* equivalents for that species.

[d] Rank order of the *D. rerio* equivalents.

Table G-2. Acute LC_{50}s, SMAVs, normalized *Daphnia magna* equivalent values and rank of SMAV *D. magna* equivalent values for carboxylic acid PFAS.

Bolding indicates key species used for normalizing data across constituent substances.

PFAS	Species	Species Common Name	LC_{50} (mg/L)	SMAV[a] (mg/L)	*Daphnia magna* equivalent[b]	SMAV *D. magna* equivalent[c]	Rank of SMAV *D. magna* equivalent[d]
PFOA	*Ambystoma jeffersonianum*	Jefferson salamander		1,070	5.00	5.00	27
PFOA	*Ambystoma texanum*	Small-mouthed salamander		407.3	1.90	1.90	14
PFOA	*Ambystoma tigrinum*	Eastern tiger salamander		752.0	3.52	3.52	24
PFOA	*Anaxyrus americanus*	American toad		793.9	3.71	3.71	25
PFBA	*Brachionus calyciflorus*	Rotifer	110	110.0	0.02	0.13	3
PFHxA	*Brachionus calyciflorus*	Rotifer	140	140.0	0.13		
PFOA	*Brachionus calyciflorus*	Rotifer		150.0	0.70		
PFBA	*Chydorus sphaericus*	Daphnid	>4,280.8	>4,280.8	0.90	0.46	5
PFDA	*Chydorus sphaericus*	Daphnid	41.13	41.13	0.34		
PFNA	*Chydorus sphaericus*	Daphnid	27.84	27.84	0.33		
PFOA	*Chydorus sphaericus*	Daphnid		93.17	0.44		
PFBA	*Danio rerio*	Zebrafish	>3,000[e]	13,779	2.91	2.47	18
PFBA	*Danio rerio*	Zebrafish	13,779				
PFOA	*Danio rerio*	Zebrafish		450.4	2.11		
PFOA	*Daphnia carinata*	Daphnid		66.80	0.31	0.31	4
PFBA	***Daphnia magna***	**Daphnid**	**>1,006**	**4,741**	**1.00**	**1.00**	**10**
PFBA	***Daphnia magna***	**Daphnid**	**>4,280.8**				
PFBA	***Daphnia magna***	**Daphnid**	**5,251**				
PFDA	***Daphnia magna***	**Daphnid**	**81**	**119.7**	**1.00**		
PFDA	***Daphnia magna***	**Daphnid**	**129.54**				
PFDA	***Daphnia magna***	**Daphnid**	**163.48**				
PFHxA	***Daphnia magna***	**Daphnid**	**1048**	**1,048**	**1.00**		
PFNA	***Daphnia magna***	**Daphnid**	**43.42**	**84.51**	**1.00**		
PFNA	***Daphnia magna***	**Daphnid**	**91.89**				

PFAS	Species	Species Common Name	LC_{50} (mg/L)	SMAV[a] (mg/L)	*Daphnia magna* equivalent[b]	SMAV *D. magna* equivalent[c]	Rank of SMAV *D. magna* equivalent[d]
PFNA	***Daphnia magna***	**Daphnid**	**151.29**				
PFOA	***Daphnia magna***	**Daphnid**		**213.9**	**1.00**		
PFBA	*Daphnia pulicaria*	Daphnid	>1,006	>1,006	0.21	0.63	6
PFDA	*Daphnia pulicaria*	Daphnid	149.59	149.6	1.25		
PFOA	*Daphnia pulicaria*	Daphnid		203.7	0.95		
PFOA	*Dugesia japonica*	Planaria		383.6	1.79	1.79	12
PFOA	*Hyla versicolor*	Gray treefrog		646.2	3.02	3.02	20
PFOA	*Lampsilis siliquoidea*	Fatmucket		164.4	0.77	0.77	8
PFOA	*Lepomis macrochirus*	Bluegill		664.0	3.10	3.10	21
PFOA	*Ligumia recta*	Black sandshell		161.0	0.75	0.75	7
PFHxA	*Lithobates catesbeiana*	Bullfrog	1,105	1,105	1.05	2.24	17
PFOA	*Lithobates catesbeiana*	American bullfrog		1,020	4.77		
PFHxA	*Lithobates clamitans*	Green frog	758	758.0	0.72	1.90	13
PFOA	*Lithobates clamitans*	Green frog		1,070	5.00		
PFOA	*Lithobates pipiens*	Northern leopard frog		751.7	3.51	3.51	23
PFOA	*Lithobates sylvatica*	Wood frog		999.0	4.67	4.67	26
PFOA	*Moina macrocopa*	Daphnid		166.3	0.78	0.78	9
PFOA	*Moina micrura*	Daphnid		0.4747	0.0022	0.0022	1
PFOA	*Neocaridina denticulata*	Green neon shrimp		431.5	2.02	2.02	15
PFOA	*Neocloeon triangulifer*	Mayfly		13.05	0.061	0.061	2
PFDA	*Oncorhynchus mykiss*	Rainbow trout	32	32.00	0.27	2.24	16
PFOA	*Oncorhynchus mykiss*	Rainbow trout		4,001	18.71		
PFOA	*Physella acuta*	Bladder snail		681.1	3.18	3.18	22
PFOA	*Pimephales promelas*	Fathead minnow		593.6	2.78	2.78	19
PFDA	*Xenopus sp.*	Clawed frog	76.5	76.50	0.64	1.65	11
PFNA	*Xenopus sp.*	Clawed frog	335.8	335.8	3.97		

PFAS	Species	Species Common Name	LC_{50} (mg/L)	SMAV[a] (mg/L)	*Daphnia magna* equivalent[b]	SMAV *D. magna* equivalent[c]	Rank of SMAV *D. magna* equivalent[d]
PFOA	*Xenopus sp.*	Frog		377.0	1.76		

[a] Data used for PFBA, PFNA, PFDA, and PFHxA are presented in **Appendix A**; data used for PFOA are the same as the quantitatively acceptable data used for derivation of the PFOA criteria (US EPA 2024a).

[b] *Daphnia magna* equivalents determined according to the following equation: Species x SMAV for PFAS ÷ *D. magna* SMAV for PFAS x = *D. magna* equivalent for species.

[c] SMAV *Daphnia magna* equivalents are the geometric means of the *D. magna* equivalents for that species.

[d] Rank order of the *D. magna* equivalents.

[e] Non-definitive value, not used in SMAV calculation.

Table G-3. Results of lognormal regression analysis of combined PFAS group SSDs based on key species equivalents and using the EPA's SSD Generator.

HC5 value expressed as mg/L.

PFAS Group	N[a]	Intercept	Slope	R^2	HC_5 (95% prediction interval)
Sulfonic acid[b]	30	4.970	1.241	0.827	0.0500 (0.0120 – 0.2079)
Carboxylic acid[c]	27	4.946	1.193	0.732	0.04636 (0.00705 – 0.30478)

[a] Number of species in SSD.
[b] *Danio rerio* equivalents.
[c] *Daphnia magna* equivalents.

Table G-4. Calculated freshwater FAV based on four lowest SMAV key species equivalents for sulfonic acid PFAS.

Rank	Species	SMAV (mg/L)	ln(SMAV)	$ln(SMAV)^2$	P=R/(N+1)	sqrt(P)
1	*Neocloeon triangulifer*	0.003	-5.90	34.83	0.032	0.180
2	*Moina micrura*	0.02	-3.93	15.41	0.065	0.254
3	*Oncorhynchus mykiss*	0.27	-1.31	1.72	0.097	0.311
4	*Pimephales promelas*	0.40	-0.91	0.83	0.129	0.359
		Σ(Sum):	-12.05	52.79	0.32	1.10

N	30	
S^2 =	919.63	**S = slope**
L =	-11.382	**L = X-axis intercept**
A =	-4.601	**A = lnFAV**
FAV =	0.01004	**P = cumulative probability**

Table G-5. Calculated freshwater FAV based on four lowest SMAV key species equivalents for carboxylic acid PFAS.

Rank	Species	SMAV (mg/L)	ln(SMAV)	$ln(SMAV)^2$	P=R/(N+1)	sqrt(P)
1	*Moina micrura*	0.0022	-6.11	37.34	0.036	0.189
2	*Neocloeon triangulifer*	0.061	-2.80	7.82	0.071	0.267
3	*Brachionus calyciflorus*	0.13	-2.04	4.18	0.107	0.327
4	*Daphnia carinata*	0.31	-1.16	1.35	0.143	0.378
		Σ(Sum):	-12.11	50.69	0.36	1.16

N	27	
S^2 =	705.20	**S = slope**
L =	-10.740	**L = X-axis intercept**
A =	-4.802	**A = lnFAV**
FAV =	0.008213	**P = cumulative probability**

Table G-6. PFAS acute benchmarks for individual PFAS within sulfonic and carboxylic acid groups calculated using approach presented by Giddings et al. (2019): Summary of values determined from SSDs derived using Aquatic Life Criteria Guidelines-based and SSD Generator-based values.

PFAS Substance Group	Aquatic Life Criteria Guidelines-Based Values, mg/L		SSD Generator-Based Values, mg/L	
	FAV	Benchmark[a]	HC_5,	Benchmark[a]
PFBS FAV	30.13	15.07	150.0	74.98
PFHxS FAV	0.2260	0.1130	1.125	0.5623
PFOS FAV	0.2798	0.1399	1.393	0.6963
PFBA FAV	38.94	19.47	219.8	109.9
PFNA FAV	0.6941	0.3471	3.918	1.959
PFDA FAV	0.983	0.4916	5.550	2.775
PFHxA FAV	8.607	4.304	48.59	24.29
PFOA FAV	1.757	0.8784	9.917	4.959

[a] Benchmarks by dividing the FAV or HC_5 by two, consistent with approach described in the Aquatic Life Criteria Guidelines (U.S. EPA 1985) for derivation of acute aquatic life criteria.

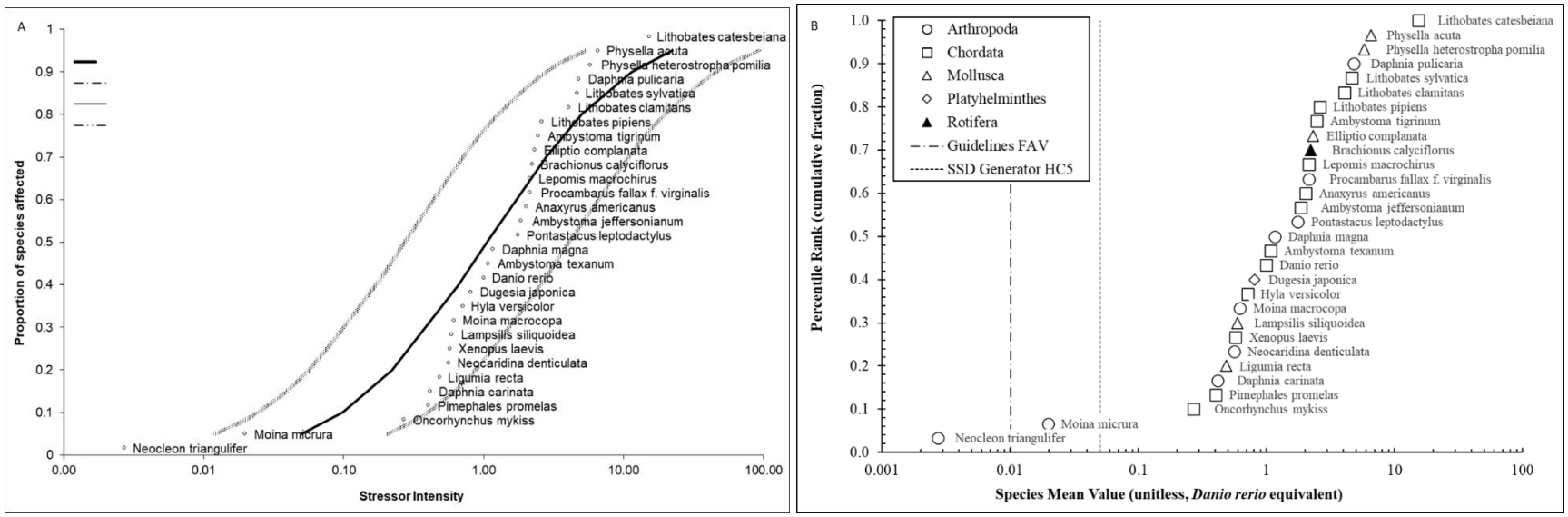

Figure G-1. Species sensitivity distributions for sulfonic acid PFAS based on *Danio rerio* equivalents, using the EPA's SSD Generator (left panel - A) or the Aquatic Life Criteria Guidelines procedure (right panel - B).

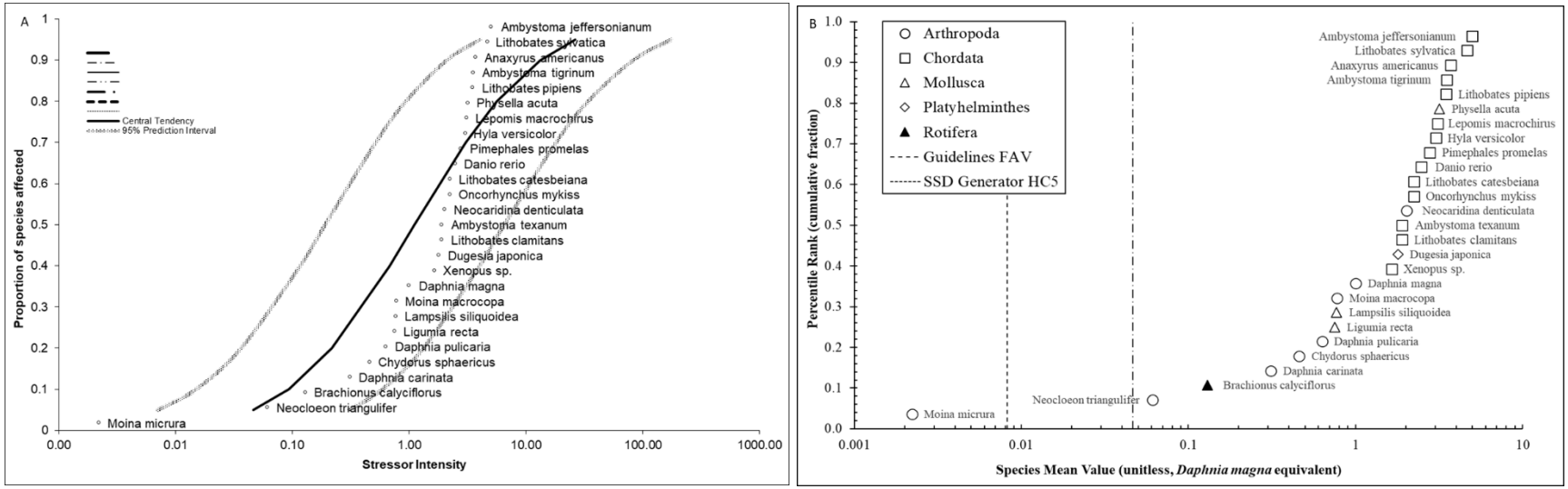

Figure G-2. Species sensitivity distributions for carboxylic acid PFAS based on *Daphnia magna* equivalents, using the EPA's SSD Generator (left panel - A) or the Aquatic Life Criteria Guidelines procedure (right panel - B)

G.1 References

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