United States Environmental Protection Agency
Office of Water
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April 2022

EPA Response to the

External Peer Review of U.S. EPA's

"*Draft Aquatic Life Ambient Water Quality Criteria for*

Perfluorooctanoic Acid (PFOA)"

(April 2022)

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, D.C.

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1.0 INTRODUCTION

The U.S. Environmental Protective Agency (EPA) Office of Water (OW) is charged with protecting ecological integrity and human health under the purview of the Clean Water Act (CWA). In support of this mission, EPA has developed draft water quality criteria to protect aquatic life and aquatic-dependent wildlife from the presence of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) in freshwater. Because there were only limited data for estuarine/marine species for PFOA and PFOS, EPA developed benchmarks for PFOA and PFOS in saltwater. The derivation of these criteria is described in two draft documents: *Aquatic Life Ambient Water Quality Criteria for Perfluorooctanoic Acid (PFOA)* and *Aquatic Life Ambient Water Quality Criteria for Perfluorooctane Sulfonate (PFOS)*.

An independent letter peer review of the EPA's draft *Aquatic Life Ambient Water Quality Criteria for Perfluorooctanoic Acid (PFOA)* was conducted by Eastern Research Group, Inc. (ERG), a contractor for EPA OW and developed an external peer review report (https://www.epa.gov/wqc/aquatic-life-criteria-perfluorooctanoic-acid-pfoa). Independent peer review of the draft *Aquatic Life Ambient Water Quality Criteria* for *Perfluorooctane Sulfonate (PFOS)* document is covered in a separate set of external peer review and EPA response documents.

This document provides EPA's responses to external peer review comments on the draft PFOA criteria document. Section 2.0 of this report presents the individual reviewer comments and EPA's responses organized by charge question.

1.1 Development of the Draft Documents

Toxicity studies used to derive the PFOA and PFOS criteria were carefully evaluated and thoroughly reviewed to ensure studies were of sufficient data quality to use in criteria derivation. Scientists from EPA OW and Office of Research and Development (ORD) conducted an extensive internal review of the PFOA and PFOS toxicity studies, primarily based on studies in EPA's ECOTOXicology database through September 2019. Additionally, EPA obtained replicate-level (or treatment-level, when replicates were unavailable) concentration-response (C-R) data from publications, supplemental materials, or via contacting authors so that EPA could independently fit C-R models to estimate acute LC_{50} and chronic EC_{10} values that were used to derive the criteria to ensure endpoints used were statistically sound. Individual C-R models and resultant point estimates were also reviewed and discussed between OW and ORD to ensure the most statistically robust models informed the derivation of the PFOA and PFOS criteria. In addition to contacting study authors for C-R data (when not reported in the open literature), EPA also consulted primary authors for methods clarifications in many instances during the data quality review phase to ensure that the studies used to derive criteria were of high quality.

Overall, due to the paucity of measured freshwater toxicity data, EPA included a number of tests with unmeasured treatments to derive criteria to ensure the dataset was representative of a range of taxa and there were sufficient data to develop criteria. EPA also conducted meta-analyses to evaluate the relationship between nominal and measured test concentrations using tests with measured treatment concentrations. These meta-analyses (described in detail as Appendix L of the PFOA criteria document and Appendix O of the PFOS criteria document) suggested measured concentrations were similar to nominal concentrations and that the use of unmeasured tests, in light of data limitations, was appropriate. Additionally, estuarine/marine toxicity data limitations did not allow for the direct derivation of acute or chronic estuarine/marine criteria for PFOA or PFOS. Therefore, to develop recommendations that states and tribes could use in adopting protective values for estuarine/marine waters, EPA developed acute PFOA and PFOS protective benchmarks using a New Approach Methodology (detailed in Appendix K of the PFOA criteria document and Appendix L of the PFOS criteria document).

Addressing data limitations to derive robust criteria/benchmarks, extensively reviewing studies, and calculating point estimates meant that the derivation of the PFOA and PFOS aquatic life criteria were developed via comprehensive, rigorous process that included collaborations across EPA scientists in OW and ORD. Beyond

detailed discussions between OW and ORD, the PFOA and PFOS drafts also underwent two rounds of review with the EPA Scoping Workgroup (consisting of additional scientists from both OW and ORD) and one round of review with a group of internal EPA reviewers that included representatives from the OW, ORD, other EPA Program Offices, and EPA Regions.

Subsequently, EPA contracted with ERG to organize an independent external peer review of both draft documents. External peer reviewer comments on the PFOA criteria document and EPA's responses to those comments are described in this report. Results of the PFOS review are documented in a separate report.

1.2 Peer Reviewers

ERG identified, screened, and selected the following five experts who met technical selection criteria provided by EPA and were determined by ERG to have no conflict of interest in performing this review:

- **Jason Conder, Ph.D**.; Principal, Geosyntec Consultants
- **Anu Kumar, Ph.D.**; Principal Research Scientist, Environment Protection and Technologies, Commonwealth Scientific and Industrial Research Organization (CSIRO)
- **Ryan Prosser, Ph.D.**; Associate Professor, University of Guelph
- **Christopher J. Salice, Ph.D.**; Director, Environmental Science and Studies Program, Towson University
- **Jamie G. Suski, Ph.D.**; Senior Scientist, EA Engineering, Science, and Technology, Inc.

ERG provided reviewers with instructions, the draft *Aquatic Life Ambient Water Quality Criteria for Perfluorooctanoic Acid (PFOA)*, and the charge to reviewers prepared by EPA. Reviewers worked individually to develop written comments in response to the charge questions. After receiving reviewer comments, ERG compiled responses by charge question (see Section 2.0) and included the responses organized by reviewer.

2.0 PEER REVIEWER COMMENTS AND EPA RESPONSES ORGANIZED BY CHARGE QUESTION

This section organizes reviewer comments by charge question.

2.1 Please comment on the overall clarity of the document as it relates to the derivation of each criterion.

2.1. Clarity of Document as it Relates to the Derivation of Each Criterion		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	Overall, the document is clear and the reader can follow the logic of criteria derivation, and track the values used back to the cited research articles or values calculated by EPA.	Thank you for your comment.
Reviewer 2	I thought that the document was well written and laid out. I thought that the document clearly laid out the approach that the EPA used to derive each criterion. I thought it clearly outlined the approach that the EPA chose in deciding which data to use in their derivation and how these data would be used in derivation. The appendices are very useful in providing added detail and the data that were used in the derivation of the criteria. The appendices allow for a high level of transparency around how the criteria were generated. In Table 3-1, the acronym "GMAV" was used in the caption, but I could not locate where this acronym was defined earlier in the document. The captions of figures and tables are not sufficiently detailed. Figures and tables should be able to stand on their own. Also, the use of acronyms in the caption of tables and figures decreases clarity, e.g., Fig. 3-5. The use of acronyms in the figure or table is fine, as long as they are defined in the caption of the figure or table.	Thank you for commenting on the clarity and transparency of the document, the criteria derivation process, and supporting appendices. Genus Mean Acute Value (i.e., GMAV) is now specified in the first in-text use. A list of all acronyms used is now included in the revised draft PFOA Aquatic Life Criteria document to aid readers in understanding of the text and figure/table captions.

2.1. Clarity of Document as it Relates to the Derivation of Each Criterion		
Reviewer	**Comments**	**EPA Response**
Reviewer 3	I have confidence is the PFOA draft criteria, these are more in-line with thresholds put forth by other agencies. Importantly, a discussion on the difference in derivation of the thresholds would be welcome; for instance, others are derived using Species Sensitivity Distributions of the complete dataset. In comparison, EPA uses a subset of data on the genus identified as most sensitive. Table 1 – does not list units of thresholds. Define CMC at first use Table 3-6 reverses order of sensitive taxa compared to the previous tables.	Thank you for describing your overall confidence in the PFOA criteria given the relative similarities between the draft PFOA criteria magnitudes and thresholds put forth by other agencies/jurisdictions. While other jurisdictions may use species sensitivity distributions (SSD) to identify hazard concentrations or protective thresholds, EPA used a genus sensitivity distribution (GSD) approach to derive draft PFOA criteria, which is consistent with EPA's 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*. Reviewer 3 incorrectly stated a subset of data were used to derive the draft criteria. The draft acute criterion is based on the final acute value (FAV), which is the 5th centile of the GSD. The chronic criterion was also based on the FAV transformed by the final acute to chronic ration (FACR). Consequently, both draft PFOA freshwater column-based criteria and were based on the entire distribution of available toxicity data. The acute and chronic freshwater column-based PFOA criteria were derived following EPA's 1985 Guidelines. Addition of new chronic toxicity data in the updated draft PFOA criteria document allowed for the calculation of a draft chronic freshwater criterion magnitude directly from the chronic GSD rather than basing the magnitude on the FAV transformed by the FACR, as was done in the draft PFOA criteria document that underwent peer review. Table 1-1 has been updated to include a column that describes the criteria or benchmark and calculation approach in response to Reviewer 3 suggesting "*discussion on the difference in derivation of the thresholds would be welcome*." Table 1-1 has also been updated to include the units of thresholds as mg/L. Criterion Maximum Concentration (CMC) is now fully described in the first in-text use and as a caption in the first table that contains "CMC." Additionally, a list of all acronyms used is now included in the revised draft PFOA Aquatic Life Criteria document to aid readers in understanding of the text and figure/table captions.

2.1. Clarity of Document as it Relates to the Derivation of Each Criterion		
Reviewer	**Comments**	**EPA Response**
		All tables describing relative species sensitivities are now consistent with one another where more sensitive species are at the top of the table and tolerant species are at the bottom of the tables.
Reviewer 4	EPA has drafted the PFOA aquatic life criteria to be consistent with methods described in EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (U.S. EPA 1985). I congratulate the EPA Team for a very thorough, comprehensive analysis of toxicological data to derive each criterion. • The report is technically sound and is very clearly written. • The criteria have been derived using strong science-based evidence. • Sub-sections on overview of PFAS, PFAS nomenclature, problem formulation, exposure pathways, transformation and degradation of PFOA precursors in the aquatic environment sources, concentration reported in environment and existing criteria (both nationally and internationally) help to set the scene before toxicological data is presented and assessed for developing various criterion. • The freshwater acute water column-based criterion, the chronic water column-based chronic criterion, the chronic fish whole-body tissue criterion, the chronic fish muscle tissue criterion and the chronic invertebrate whole-body tissue criterion have been developed and reported in this report. • Acute and chronic MDRs for PFOA estuarine/marine criteria derivation were not met due to fewer empirical PFOA toxicity data. EPA Team developed an acute aquatic life benchmark for	Thank you for your comment and thank you for describing specific sections of the draft PFOA criteria document that you felt were comprehensive.

2.1. Clarity of Document as it Relates to the Derivation of Each Criterion		
Reviewer	**Comments**	**EPA Response**
	estuarine/marine environments based on Interspecies Correlation Estimation (ICE) model. • The relative sensitivity of freshwater plants to PFOA exposures indicated plants are less sensitive than aquatic vertebrates and invertebrates so plant criteria were not considered. • EPA Team has provided extensive background information on toxicity data assessment and collated this information in various appendices such as Appendix A-Summary Table of Acceptable Quantitative Freshwater Acute PFOA Toxicity Studies; Appendix B-Detailed PFOA Acute Toxicity Study Summaries and Corresponding Concentration-Response Curves (when calculated; Appendix C: Acceptable Freshwater Chronic PFOA Toxicity Studies); Appendix D- Acceptable Estuarine/Marine Chronic PFOA Toxicity Studies; Appendix E-Acceptable Freshwater Plant PFOA Toxicity Studies; Appendix F- Acceptable Estuarine/Marine Plant PFOA Toxicity Studies; Appendix G-Summary Table of Qualitative Freshwater PFOA Toxicity Studies; Appendix H-Other Estuarine/Marine PFOA Toxicity Studies Unused PFOA Toxicity Studies; Appendix I-Unused PFOA Toxicity Studies; Appendix J- EPA Methodology for Fitting Concentration-Response Data and Calculating Effect Concentration-Fitting Concentration Response Data in R; Appendix K-Derivation of Acute Protective PFOA Benchmarks for Estuarine/Marine Waters through a New Approach Method (NAM); and Appendix L Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations.	

2.1. Clarity of Document as it Relates to the Derivation of Each Criterion		
Reviewer	**Comments**	**EPA Response**
Reviewer 5	Overall, similar to PFOS, the document for PFOA is very well written, generally free of grammatical errors and clear. It is long but not as long as PFOS and, therefore, easier to digest. I think the background material for both chemicals is especially good and provides an excellent overview and summary for readers less familiar with PFAS. In particular, the summary of PFOA concentrations in water bodies and other environmental media is wonderfully useful. To me, the derivation of the criteria for PFOA is easier to follow than PFOS, mostly because there are fewer data and, hence, fewer assumptions, calculations, and evaluations that need to be made. Unlike for PFOS, the criteria developed by EPA for PFOA are more similar to values derived by other jurisdictions for both acute and chronic values. To me, this lends strength to EPA's criteria values and is also consistent with the scientific method, in general. If we are all following the same threads of logic, using similar analyses, and looking at the same data, it makes sense that criteria values would be similar. So, again, in the case of PFOA the congruence with other published criteria values is a strength. I will use this as an argument for EPA to reconsider the criteria (and the data supporting it) for PFOS as, for that chemical, EPA's numbers are higher than other jurisdictions.	Thank you for your comment. Thank you for describing your overall confidence in the PFOA criteria given the relative similarities between the draft PFOA criteria and thresholds put forth by other jurisdictions. PFOS-specific comments described by Reviewer 5 and corresponding responses are further discussed in detail in the *PFOS Draft Criteria Document Peer Review Response to Comments*.

2.2 Please comment on the approach used to derive the draft criterion for PFOA. Please provide detailed comments.

- **Is the technical approach used to derive the criterion logical?**
- **Does the science support the conclusions?**
- **Is it consistent with the protection of freshwater aquatic life from acute, chronic, and bioaccumulative effects?**

2.2. The Technical Approach Used to Derive the Draft Criterion for PFOA		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	• **Is the technical approach used to derive the criterion logical?** Yes, the technical approach used to derive the criteria elements is generally logical. I disagree with some of the elements of the analyses, as noted in my detailed comments (see below, responses to charge question 8) • **Does the science support the conclusions?** In general, the science is supportive of the general conclusions. As noted in my below detailed responses to other charge questions, I believe the science is not supportive of the work in a few key instances including: 1. I believe the Criterion Continuous Concentration (CCC) should be potentially re-calculated considering my comments provided in response to charge question 5a. 2. The science does not support the assumption of a 10-year recovery time for PFOA in aquatic systems. 3. The generation of tissue criteria is weakly supported, and the uncertainty associated with these criteria should be emphasized. 4. The NAM-generated marine Final Acute Value (FAV) and FAV/2 values (Appendix K) are highly uncertain.	Thank you for your comment, stating the technical approach was generally logical. Specific elements referenced by Reviewer 1 are further described and responded to in section 2.8. Responses to key instances where Reviewer 1 does not believe the science is supportive of the draft PFOA Aquatic Life Criteria document are described below in corresponding numerical order: 1. Reviewer 1 believes the FACR should be 90 (calculated as the geometric mean of the *B. calyciflorus*, *M. macrocopa*, and *D. magna* Species Mean Acute to Chronic Rations [SMACR]) rather than 299 as it is in the draft criteria (based directly on the B. calyciflorus SMACR). Addition of new chronic toxicity data in the updated draft PFOA criteria document allowed for the calculation of a draft chronic freshwater criterion magnitude directly from the chronic GSD rather than basing the magnitude on the FAV transformed by the FACR, as was done in the draft PFOA criteria document that underwent peer review. 2. EPA responded to this comment from Reviewer 1 in greater detail in Section 2.7. In short, EPA considered the bioaccumulative nature and persistence of PFOA in aquatic systems, in combination with the documented recovery times of pollutants with similar chemical attributes (Lemly 1997; Gergs et al. 2016), to set a reasonable and protective exceedance frequency for tissue-based PFOA criteria. Furthermore, three of the remaining Expert Peer Reviewers were supportive of the 10-year exceedance frequency for the

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Reviewer	**Comments**	**EPA Response**
	5. It is unclear if the EPA-calculated Effective Concentration 10% (EC_{10}) values are supported; additional details on the modeling and the variability and fit of each EC_{10} model need to be provided. • **Is it consistent with the protection of freshwater aquatic life from acute, chronic, and bioaccumulative effects?** The criteria derived are aimed at protecting aquatic life (e.g., fish, invertebrates) from the direct acute and chronic toxicity of PFOA in water. Generally, the values applied are protective and are generally similar to protective values derived by other regulatory organizations and independent (i.e., academic, private sector) scientists. Although, as based on my comments, I believe there is room for improvement. The criteria derived for tissues attempt to provide criteria that take into account bioaccumulation so that measurements in tissue can be interpreted with respect to the potential for potential effects; however, the uncertainty with the tissue criteria is high. The water and tissue criteria are not intended protective of bioaccumulative effects that may affect higher trophic levels, such as wildlife that may consume aquatic life.	tissue-based PFOA criteria, with the remaining Reviewer (i.e., Reviewer 3) stating it was difficult to comment on the tissue-based criteria frequencies. 3. Please see EPA's response to Reviewer 1's comments below to Charge Question 2.6 regarding the generation of tissue-based criteria. EPA acknowledges the inherent uncertainties that are present with the use of bioaccumulation factors (BAF) to derive tissue criteria. EPA screened the BAF literature in a manner consistent with the evaluation criteria outlined in Burkhard (2021). Additionally, the use of BAFs to derive tissue criteria is consistent with previously derived criteria for both aquatic life (i.e., 2016 Selenium Aquatic Life Criterion for Freshwaters; U.S. EPA 2016a) and human health (U.S. EPA 2000). Given the potential bioaccumulation of PFOA through the aquatic food web, tissue-based criteria were needed to ensure the protection of aquatic life to PFOA exposures. 4. Please see EPA's response to Reviewer 1's comments below to Charge Question 2.3 regarding the New Approach Method (NAM)-generated acute saltwater benchmark derived in Appendix K of the draft PFOA Aquatic Life Criteria document. EPA added text to ensure uncertainty surrounding the acute saltwater benchmark is clearly stated. 5. Concentration-response (C-R) model type and figures of the C-R data with the fitted model are displayed in Appendices A.2 and C.2 for those tests that were used to quantitatively derive the PFOA criteria and were among the four most sensitive acute and chronic genera, respectively. Figures of the fitted C-R models in Appendices A.2 and C.2 are displayed with 95% confidence bands (relative to the Y-axis) allowing for a visual display of variability in organisms responses. Reviewer 1 further notes their review focused on "*key portions of the Draft of the Aquatic Life Water Quality Criterion*" and may have missed the C-R modeling results

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Reviewer	**Comments**	**EPA Response**
		presented in appendices A.2 and C.2 as well as the modeling methods presented in Appendix J. Reviewer 5, noted "*EPA's approach to fitting C-R data using the drc package in R is, in my opinion, state of the art.*" EPA thanks Reviewer 1 for describing the relative similarity between the draft PFOA criteria and protective thresholds from regulatory organizations and independent scientists. As noted in EPA's response to this comment above (item # 3 from the previous list), the draft PFOA tissue criteria are intended to protect aquatic life from PFOA exposures. EPA derived the tissue criteria by translating the chronic water column criterion into tissue concentrations through application of BAFs. There were insufficient data to derive tissue criteria directly from empirical toxicity tests with tissue-based exposure concentrations. However, the draft PFOA Aquatic Life Criteria document contained an evaluation of the tissue-based criteria relative to the limited tissue-based toxicity, concluding, "*these studies do not provide any evidence that the PFOA tissue-based criteria are not protective of aquatic species*." Furthermore, aquatic life tissue criteria are intended to be protective of aquatic life; aquatic -dependent wildlife taxa fall outside the scope of the current draft PFOA Aquatic Life Criteria document. EPA intends to review PFOA data focused on aquatic-dependent wildlife in the future and to potentially derive separate aquatic-dependent wildlife criteria for PFOA, if the data support the derivation of such criteria.
Reviewer 2	Yes, the technical approach used by the EPA to derive the criterion is logical and defensible. The approach is also clearly laid out in the document. Dividing the 5th centile of the acute GSD by 2 is sufficiently conservative to ensure the protection of 95% of species, based on the data	Thank you for the comment noting the approach used in the draft PFOA criteria document was "*logical and defensible*." EPA agrees with Reviewer 2 that use of the *Brachionus* Acute to Chronic Ratio (ACR) to derive the Final ACR (FACR) was a valid approach that resulted in a protective chronic freshwater column criterion in the draft that underwent peer review. For clarity, EPA notes the FACR

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Reviewer	**Comments**	**EPA Response**
	currently available. The use of the ACR from Brachionus calyciflorus to construct a chronic GSD is a valid approach, along with being the most conservative option. Yes, I think the science supports the EPA's conclusions. However, there appears to be several studies that were not considered by the EPA. I have listed these studies below. Yes, I think the approach taken by the EPA is sufficiently conservative to be protective of freshwater aquatic life from acute, chronic, and bioaccumulative effects based on the data that was available at the time. It was a good idea to evaluate the influence on non-North American species on the derivation of the criteria.	did not construct the chronic GSD (as suggested by Reviewer 2) but was used to transform the Final Acute Value (FAV) to the Final Chronic Value (FCV), which is a valid approach that is consistent with the 1985 Guidelines and results in a protective chronic criterion. Addition of new chronic toxicity data in the updated draft PFOA criteria document allowed for the calculation of a draft chronic freshwater criterion magnitude directly from the chronic GSD rather than basing the magnitude on the FAV transformed by the FACR, as was done in the draft PFOA criteria document that underwent peer review. Thank you for reiterating that the science supports EPA's conclusion. All studies provided by Reviewer 2 (and all other reviewers) were reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc. and were included in the PFOA criteria document accordingly. EPA agrees that "*it was a good idea to evaluate the influence on non-North American species on the derivation of the criteria.*" Including non-North American species in the acute and chronic criteria derivation did not markedly affect the draft criteria magnitudes and ensures the fullest, high-quality dataset available is used to represent the thousands of untested aquatic taxa present in U.S. ecosystems when deriving the PFOA criteria.
Reviewer 3	• **Is the technical approach used to derive the criterion logical?** Why did EPA derive the LC_{50} for Chydorus when an EC_{50} was provided by the authors; however, accepted the EC50s for the two mussel species? This is an inconsistency. • **Does the science support the conclusions?** See response immediately below	*Chydorus* was the most sensitive acute genus. EPA calculated a C-R model because C-R data were available (provided by authors to EPA on 7/16/2020). Per the draft PFOA Aquatic Life Criteria document: *"Toxicity values, including LC_{50} and EC_{10} values, were independently calculated from the data presented in the toxicity studies meeting the inclusion criteria described above when adequate concentrations-response data were published in the study or could be obtained from authors. When concentration-response data were not presented in toxicity studies, concentration-response data were requested from study authors to independently calculate toxicity values. In cases where study authors did not respond to EPA's request for data or were unable to locate concentration-*

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Reviewer	**Comments**	**EPA Response**
	• **Is it consistent with the protection of freshwater aquatic life from acute, chronic, and bioaccumulative effects?** Overall, the draft criteria are in agreement with other thresholds generated using the species/genus sensitivity distributions; which seems like a more robust approach given data are not restricted to a subset of studies (albeit most sensitive). Although new data should be evaluated and potentially incorporated into these criteria calculations it is unclear if those data would alter these currently drafted thresholds.	*response data, the toxicity values were not independently calculated by EPA, and the reported toxicity values were retained for criteria deviation."* EC_{50} values for the two mussel species (i.e., *L. recta and L. siliquoidea*) were not calculable because C-R data were not available in the publication and could not be provided by authors. For both species, Appendix A.2 of the draft PFOA criteria document that underwent peer review stated, the EPA-Calculated LC_{50}s were "*Not calculable, concentration-response data not available*." Thank you for describing your overall confidence in the PFOA criteria given the relative similarities between the draft PFOA criteria and thresholds put forth by other jurisdictions. All studies provided by Reviewer 3 (and all other reviewers) were reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc., and were included in the PFOA criteria document, as appropriate.
Reviewer 4	This EPA report provides a critical review of toxicity data identified in EPA's literature search for PFOA, including the anionic form (CAS No. 45285-51-6), the acid form (CAS No. 335-67-1), and the ammonium salt (CAS No. 3825-26-1). It quantifies the toxicity of PFOA to aquatic life, and provides criteria intended to protect aquatic life from the acute and chronic toxic effects of PFOA. The detailed assessment is as follows: • These criteria have been derived using robust methods and the best available toxicity data on aquatic life. • The approach used to derive the draft criterion for PFOA is very logical and consistent with the protection offered by acute and chronic aquatic life	Thank you for your comment and thank you for describing specific sections of the draft PFOA criteria document in detail. Responses to the comments pertaining to the tissue-based criteria made by Reviewer 4 are described in response to charge question 2.5.

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Reviewer	**Comments**	**EPA Response**
	criteria derived using empirical data, as prescribed in the 1985 *Guidelines.* • Exclusion and inclusion criteria are appropriately discussed in the context of the toxicological data reported in the literature and provide additional evidence on the selection of toxicity data criteria development. • With limited toxicity datasets to North American resident species, non-North American resident species served as taxonomically-related surrogate test organisms. For example, *Oryzias latipes* is a common ecotoxicity test species that served as a surrogate for untested fish species residing in North America. • The acute measures of effect on aquatic organisms selected included the lethal concentration (LC_{50}), effect concentration (EC_{50}), or inhibitory concentration (IC_{50}) estimated to produce a specific effect in 50 percent of the test organisms • The endpoint for chronic exposures incorporated the effect concentration estimated to produce a chronic effect on survival, growth, or reproduction in 10 percent of the test organisms (EC_{10}). This approach has been also consistent with the harmonized guidelines from OECD and the generally preferred effect level for countries such as Canada, Australia, and New Zealand. • Reported (No Observed Effect Concentrations) (NOECs) and (Lowest Observed Effect Concentrations) (LOECs) were only used for the derivation of a chronic criterion when a robust EC_{10} could not be calculated for the genus. • Furthermore, EPA independently calculated these toxicity values if sufficient raw data were available	

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Reviewer	**Comments**	**EPA Response**
	for EPA to conduct statistical analyses. EPA's independently-calculated toxicity values were used preferentially, where available. • EPA developed protective tissue-based criteria through a bioaccumulation factor approach. This was based on the application of evaluation criteria for screening bioaccumulation factors (BAFs). • The freshwater Final Acute Value (FAV) for PFOA was calculated as 91.34 mg/L and freshwater acute criterion water column magnitude (criterion maximum concentration, CMC), as 46 mg/L PFOA, using the procedures described in the 1985 Guidelines. This values is expected to be protective of 95% of freshwater genera potentially exposed to PFOA under short-term conditions of one-hour of duration, if the one-hour average magnitude is not exceeded more than once in three years. • Toxicity data were available for only two families, an estuarine/marine FAV could not be calculated to derive an estuarine/marine acute criterion. Further benchmark was developed using predictive approach and discussed later in this document. • Tissue-based criteria were also developed using comprehensive methods and assessment is provided as response to charge Question 5.	
Reviewer 5	The overall approach to derive criteria for PFOA is logical…except for the use of only the 4 most sensitive endpoints and then a model was fitted (unspecified, I believe) to obtain the 5% most sensitive species (in general). I am not familiar with this as an approach as I have not seen other scientists use this and, instead, I have more commonly seen the application of a species	Thank you for your comment stating the "*overall approach to derive the criteria for PFOA is logical*." Reviewer 5 commented that a model was fit to the four most sensitive endpoints (i.e., four most sensitive GMAVs and GMCVs) to derive the criteria, which was not the case. Instead, derivation of the acute and chronic criteria followed long-established methods outlined in the 1985 Guidelines. The established criteria calculation outlined in the 1985 Guideline uses a log-

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Reviewer	**Comments**	**EPA Response**
	sensitivity distribution based on more data and usually following an s-shape. Indeed, many well-cited papers on toxicity thresholds and criteria have used this approach. I suspect EPA has justification for the approach used in the document and that it is well-supported. I would suggest adding any details, beyond just citing the 1985 guidelines, that supports a focus on just the 4 most sensitive toxicity endpoints for the criteria development. My apologies if this information is in the document and I missed it. . One point of clarification is needed in the explanation of the regression analysis (p. 64). The document states: "When LOECs and NOECs were used, a Max Aceept. Tox Conc. (MATC) was calculated, with is the geometric mean of the NOEC and LOEC. For the calculation of chronic criterion, **point estimates** were selected for use as the measur of effect in favor of the MATCs…" – WHAT IS MEANT BYT "POINT ESTIMATES"? Isn't the LOEC or NOEC a point estimate? This needs to be clarified here (and for PFOS). On p. 66 – I also recall that in some cases EPA obtained data from plots using web plot digitizer software. If I am correct, this should be explained on this page. One point worth mentioning with regard to the technical approach is that I think EPA was correct to consider non-North American resident species in developing the criteria. While I can understand why some scientists feel strongly about focusing on native species, I also cannot think of a clear example of widely different chemical tolerances among species from different countries. Sure, organisms from contaminated environments are likely to differ compared to the same organisms (species) from uncontaminated areas but barring this, it seem sensitivity to	triangular fit to determine the 5th centile of a GSD. Acute and chronic GSDs (which included all quantitatively acceptable toxicity data) were presented in the Effect Analysis section of the draft PFOA Aquatic Life Criteria document. When there are less than 59 genera in a GSD, the 5th centile is inherently based on the four most sensitive genera, with the remaining tests only influencing the FAV through the "*n*" in the calculation. Please see the excerpt from the 1985 Guidelines below for further explanation. "*Order the GMAVs from high to low.* *L. Assign ranks, R, to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.* *M. Calculate the cumulative probability, P, for each GMAV as R/(N+1).* *N. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are less than 59 GMAVs, these will always be the four lowest GMAVs).*" Additionally, research conducted since the 1985 Guidelines were published has continued to suggest use of a log-triangular distribution to estimate an HC_5 from sensitivity distributions is appropriate. USEPA (2011) concluded: "*Judging by bias at small sample sizes, distributions on log-transformed data (normal, logistic, triangular, Gumbel) generally outperformed distributions on untransformed data (Pareto, Weibull, and Burr$_{III}$) and of the former, the log-normal, log-logistic, and log-triangular showed very similar performance*." Lowest Observed Effect Concentrations (LOEC) and No Observed Effect Concentrations (NOEC) are determined through hypothesis-based testing. A LOEC is the lowest test concentration where test organism responses were statistically different from responses in the control organisms. A NOEC is the highest test concentration where

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	chemicals is not geographically determinated (again, barring extremes). Additionally, I think using the EC10 makes sense for PFAS chronic criteria and perhaps other chemicals as well. That said, I can't say that using a 50% effect level for acute toxicity/exposures makes sense. To me, using an EC20 or LC20 for acute would be more reasonable. As I understand it, the current approach divides the calculated FAVs by 2.0 to further ensure protection. Here again, it would seem easier and more straight-forward to use the EC20 or LC20. Perhaps dividing the FAV by 2.0, however, commonly results in a low threshold (like an LC20, for example). If this is the case, it would be worth EPA mentioning to give some sense of magnitude to what could be considered an arbitrary "safety factor" of 2.0. What highlighted this issue for me was the estuarine mussel species where there was a 27% effect (malformations) at 0.0001 mg PFOA/L but because an EC50 could not be determined, EPA chose to use the highest concentration of 1 mg/L. If I were in charge of managing resources in an estuary, I can't say I'd have much confidence in that 1 mg/L value. Or at least, I would be very uncomfortable. Having said all this, I understand that the frequency and duration of environmental exposures would make is such that the acute and chronic criteria would be protective but please see my comments below with regard to frequency and duration of exposure.	test organism responses were not statistically different from responses in the control organisms. A point estimate is an LC_x or EC_x value determined through regression analysis. The PFOA Aquatic Life Criteria document now includes a parenthetical indicating what a point estimate is, stating "*Regression analysis was used preferentially to characterize a concentration-effect relationship and to estimate concentrations at which chronic effects are expected to occur (i.e., Point Estimates*)." Additionally, footnote has been added to the first mention of webplotdigitizer in the draft PFOA criteria document describing its general use and providing a link to the application. EPA agrees that "*it was correct to consider non-North American resident species in developing the criteria*." Including non-North American species in the acute and chronic criteria derivation did not markedly affect the draft criteria magnitudes and ensures the fullest, high-quality dataset available is used to represent the thousands of untested aquatic taxa present in U.S. ecosystems when deriving the PFOA criteria. Thank you for your comment "*the EC10 makes sense for PFAS chronic criteria and perhaps other chemicals as well.*" EPA notes that use of a 10% effect concentration for deriving chronic criteria magnitudes is also consistent with the harmonized guidelines from OECD and the generally preferred effect level for countries such as Canada, Australia, and New Zealand (CCMC 2007; Warne et al. 2018). EPA's PFOA FAV was derived following a long-established approach described in the 1985 Guidelines. Briefly, the PFOA FAV is based on the 5th centile of a GSD, which was comprised of GMAVs calculated from LC_{50} values. Consistent with the 1985 Guidelines, the FAV was then divided by 2.0 to calculate the criterion maximum concentration (CMC). Dividing the FAV by 2.0 ensures the CMC represents a concentration that will not affect a large portion of sensitive organisms. This is based on the established analyses demonstrating that the ratio between LC50 values and corresponding LC_{low} values

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		(e.g., $LC_0 - LC_{10}$) is typically close to 2.0. Please see the excerpt from the 1985 Guidelines below for further explanation, "*the Criterion Maximum Concentration is now equal to one-half the Final Acute Value. The Criterion Maximum Concentration is intended to protect 95 percent of a group of diverse genera, unless a commercially or recreationally important species is very sensitive. However, a concentration that would severely harm 50 percent of the fifth percentile or 50 percent of a sensitive important species cannot be considered to be protective of that percentile or that species. Dividing the Final Acute Value by 2 is intended to result in a concentration that will not severely adversely affect too many of the organisms."* Additionally, EPA thanks Reviewer 5 for comments regarding the study on the, "*Adaptation of the bivalve embryotoxicity assay for the high throughput screening of emerging contaminants in Mytilus galloprovincialis*" by Fabbri et al. (2014). Fabbri et al. (2014) did not report an acute EC_{50} for *M. galloprovincialis* from exposure to PFOA (or PFOS) because it could not be calculated. The percent adverse effect at the highest concentration of PFOA tested (1 mg/L) did not exceed a 50% reduction in % Normal D-larvae relative to the negative control treatment, only producing about a 37% reduction (calculated from 83% Normal D-larvae in negative control and 52% Normal D-larvae in the 1 mg/L treatment; determined from visual inspection of Figure 4 of Fabbri et al. 2014). EPA's decision to use the acute value of > 1 mg PFOA/L for this study in the draft criteria document that underwent peer review was consistent with the 1985 Guidelines. Specifically, Under Section IV.E.2. - *"The result of a [acute] test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones, should be the 96-hr EC_{50} based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an EC_{50} is not available from a test, the lower of the 96-hr EC_{50} based on the percentage of organisms with incompletely*

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Reviewer	**Comments**	**EPA Response**
		developed shells and the 96-hr LC_{50} should be used in place of the desired 96-hr EC_{50}. If the duration of the test was between 48 and 96 hr, the EC_{50} or LC_{50} at the end of the test should be used." And, under Section IV.E.5.- "*If the tests were conducted properly, acute values reported as "greater than" values and those which are above the solubility of the test material should be used, because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species*." Thus, the appropriate acute value for entry into Table B.1 of the draft criteria document that underwent peer review for Fabbri et al. (2014) was the 48-h EC_{50} of > 1 mg/L PFOA. While the study clearly demonstrates an effect of PFOA on embryo development, the fact that a 50% reduction in % Normal D-larvae was not reached in the test resulted in a "greater than" EC_{50} value for the acute effect concentration; which is consistent with the authors being unable to determine an EC_{50}. Furthermore, the authors note PFOS and PFOA did not cause an increase in the percentage of malformations, but rather a reduction in number of fully developed D-larvae, suggesting delayed development effects rather than viability. NOECs, LOECs, and Maximum Acceptable Toxicant Concentrations (MATC) from acute tests are not used in the acute GSD for the derivation of acute criteria. Furthermore, the short 48-h duration of the test excludes its consideration in the development of a chronic criterion estuarine/marine criterion or benchmark. The *Mytilus galloprovincialis* SMAV was updated to 17.58 mg/L in the revised PFOA criteria document following incorporation of new data published by Hayman et al. (2021). Briefly, Hayman et al. (2021) exposed *M. galloprovinvialis* embryos to PFOA for 48-hours and determined an EC_{50} of 9.98 mg/L (endpoint = normal and surviving). EPA fit a model to the concentration-response data and determined an EPA-calculated EC_{50} of 17.58 mg/L from this test. The acute value from Hayman et al. (2021) was used preferentially over the acute value determined by Fabbri et al. (2014) to calculate the *M.*

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Reviewer	**Comments**	**EPA Response**
		galloprovinvialis SMAV in the revised PFOA draft criteria. Results from Fabbri et al. (2014) were not used in the *M. galloprovinvialis* SMAV calculation because the EC_{50} was a relatively low "greater than" value, while Hayman et al. (2021) provided a definitive EC_{50} value. Finally, thank you for reiterating how "*the frequency and duration of environmental exposures would make is such that the acute and chronic criteria would be protective*." EPA has provided further responses to Reviewer 5's comments on the frequency and duration components of the PFOA criteria below. EPA also notes *M. galloprovincialis* occurs in estuarine/marine habitats and the draft PFOA document did not contain acute or chronic estuarine/marine criteria but did include an estuarine/marine acute benchmark.

2.3 Please comment on the approach used to derive the draft acute estuarine/marine benchmark for PFOA. Given the limited estuarine/marine test data available, a new approach method was used to support the derivation of an acute estuarine/marine benchmark to provide states and tribes with a protective value. Please provide detailed comments.

- **Is the technical approach used to derive the benchmark logical?**
- **Does the science support the conclusions?**
- **Is it consistent with the protection offered by acute estuarine/marine aquatic life criteria derived using empirical data, as prescribed in the 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*?**

2.3. The Technical Approach used to Derive the Draft Acute Estuarine/Marine Benchmark for PFOA		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	• **Is the technical approach used to derive the benchmark logical?** The derivation of the acute marine benchmarks (FAV and Criterion Maximum Concentration (CMC)) using the New Approach Method (NAM) is highly uncertain, and I would recommend this analysis not be included as in this document. I do not feel that the analysis and subsequent criteria have high confidence for use in a regulatory application. I understand that similar analyses with other chemicals have about a 90% probability of the predicted effect value being within a factor of 5 of the actual value (Raimondo et al., 2010 – cited in document). Given the calculated CMC (3.4 mg/L), this implies the CMC has about a 90% probability of being within 0.68 to 17 mg/L. If the NAM approach stays in the document, this uncertainty and range of values should be acknowledged in the discussion. I would rather see tentative or provisional acute criterion developed from the limited empirical marine acute data highlighted in Appendix B and other recently published marine acute data. I place higher confidence in empirical data (even if limited to a few studies) and would suggest EPA emphasize it in addition to or in place of the values calculated by the NAM.	Thank you for your comment regarding uncertainties associated with the derivation of the acute estuarine/marine benchmark using a NAM. As discussed in Appendix K, ICE models have undergone extensive peer review and their use has been recommended for multiple similar applications, including direct toxicity estimation for endangered species (NRC 2013, Willming et al. 2016) and the development of SSDs (Awkerman et al. 2014, Bejarano et al. 2017, Dyer et al. 2006, Dyer et al. 2008, Raimondo et al. 2010, Raimondo et al. 2020). EPA has noted and quantified the epistemic uncertainty associated with the use of Web ICE-derived data to the extent possible in Appendix K, and further, has characterized the value as a "benchmark" to differentiate it from criteria values that have been derived solely with empirical test data from the chemical for which the criteria is being developed. Additional text has been added to compare the derived benchmarks to the available empirical test data with estuarine/marine species. Further, additional empirical estuarine/marine toxicity test data have become available since the benchmark values were first derived. The benchmarks have been revised to incorporate the additional acceptable empirical data. EPA continued to integrate new acceptable empirical data as they

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Reviewer	**Comments**	**EPA Response**
	I am hopeful that as new toxicity information on marine species are developed, these values can be supplanted with a proper and robust criteria calculation. If such a future analysis is possible, it should be noted. • **Does the science support the conclusions?** See above comment. • **Is it consistent with the protection offered by acute estuarine/marine aquatic life criteria derived using empirical data, as prescribed in the 1985 Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses?** The approach seems to be consistent with the approach in the 1985 guidelines. As noted above, the uncertainty with regards to the predictive capability of the interspecies correlations should be acknowledged quantitatively.	became available until the benchmark values were derived as final values. Thank you for your comment noting that the approach used to derive the acute estuarine/marine PFOA benchmark is consistent with the 1985 Guidelines. As noted above, additional text has been added to further summarize and clarify uncertainties associated with derivation of the benchmark value.
Reviewer 2	The technical approach using Web-ICE to determine an acute benchmark for estuarine/marine species is logical. The science has shown that Web-ICE can effectively be used to derive effect measures for additional species using species for which data is available. I think the approach taken by EPA has included sufficient conservatism to address the relatively large amount of uncertainty around the acute toxicity of PFOA to estuarine and marine species. The proposed acute benchmark for estuarine and marine species is an order of magnitude lower than the acute benchmark for freshwater species, which I think underscores the conservatism used by EPA in determining an acute benchmark for estuarine and marine species. That said, the benchmark should be used cautiously due to the relatively large amount of uncertainty and effort should	Thank you for your comment noting that the approach used to derive the acute estuarine/marine PFOA benchmark is logical and sufficiently conservative to account for uncertainties associated with data limitations. EPA agrees that additional estuarine/marine test data focused on PFOA would provide support for the derived benchmark value, or enable development of a criterion.

2.3. The Technical Approach used to Derive the Draft Acute Estuarine/Marine Benchmark for PFOA		
Reviewer	**Comments**	**EPA Response**
	be made to generate acute and chronic toxicity data for PFOA on estuarine and marine species, particularly fish.	
Reviewer 3	• **Is the technical approach used to derive the benchmark logical?** See comment immediately below • **Does the science support the conclusions?** The PFOA - LOEC reported for *Mytilus* in Fabbri et al. 2014 is 0.1µg/L; in the draft criteria this is listed as >1 mg/L. The justification provided for dismissing this effect given 50% of the test organisms did not experience is not compelling. • **Is it consistent with the protection offered by acute estuarine/marine aquatic life criteria derived using empirical data, as prescribed in the 1985** *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses***?** No, this is a new approach; however, it follows the spirit of the 1985 guidelines.	Thank you for your comment regarding the study on the, "*Adaptation of the bivalve embryotoxicity assay for the high throughput screening of emerging contaminants in Mytilus galloprovincialis*" by Fabbri et al. (2014). Fabbri et al. (2014) did not report an acute EC_{50} for *M. galloprovincialis* from exposure to PFOA (or PFOS) because it could not be calculated. The percent adverse effect at the highest concentration of PFOA tested (1 mg/L) did not exceed a 50% reduction in % Normal D-larvae relative to the negative control treatment, only producing about a 37% reduction (calculated from 83% Normal D-larvae in negative control and 52% Normal D- larvae in the 1 mg/L treatment; determined from visual inspection of Figure 4 of Fabbri et al. 2014). EPA's decision to use the acute value of > 1 mg PFOA/L for this study in the draft PFOA criteria document that underwent peer review was consistent with the 1985 Guidelines. Specifically, Under Section IV.E.2. - *"The result of a [acute] test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones, should be the 96-hr EC_{50} based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an EC_{50} is not available from a test, the lower of the 96-hr EC_{50} based on the percentage of organisms with incompletely developed shells and the 96-hr LC_{50} should be used in place of the desired 96-hr EC_{50}. If the duration of the test was between 48 and 96 hr, the EC_{50} or LC_{50} at the end of the test should be used."* And, under Section IV.E.5.- "*If the tests were conducted properly, acute values reported as "greater than" values*

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Reviewer	**Comments**	**EPA Response**
		and those which are above the solubility of the test material should be used, because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species." Thus, the appropriate acute value for entry into Table B.1 of the draft criteria document that underwent peer review for Fabbri et al. (2014) was the 48-h EC_{50} of > 1 mg/L PFOA. While the study clearly demonstrates an effect of PFOA on embryo development, the fact that a 50% reduction in % Normal D-larvae was not reached in the test resulted in a "greater than" EC_{50} value for the acute effect concentration; this is consistent with the authors being unable to determine an EC_{50}. Furthermore, the authors note that PFOS and PFOA did not cause an increase in the percentage of malformations, but rather a reduction in number of fully developed D-larvae, suggesting delayed development effects rather than viability. NOECs, LOECs, and MATCs from acute tests are not used in the acute GSD for the derivation of acute criterion. Furthermore, the short 48-h duration of the test excludes its consideration in the development of a chronic criterion estuarine/marine criterion or benchmark. In response to Reviewer 3 noting the apparent sensitivity of *Mytilus galloprovincialis* (with a reported LOEC of 0.1 µg/L), EPA notes The *Mytilus galloprovincialis* SMAV was updated to 17.58 mg/L in the revised PFOA criteria document following incorporation of new data published by Hayman et al. (2021). Briefly, Hayman et al. (2021) exposed *M. galloprovinvialis* embryos to PFOA for 48-hours and determined an EC_{50} of 9.98 mg/L (endpoint = normal and surviving). EPA fit a model to the concentration-response data and determined an EPA-calculated EC_{50} of 17.58 mg/L from this test. The acute value from Hayman et al. (2021) was used preferentially over the acute value determined by Fabbri

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Reviewer	**Comments**	**EPA Response**
		et al. (2014) to calculate the *M. galloprovinvialis* SMAV in the revised PFOA draft criteria. Results from Fabbri et al. (2014) were not used in the *M. galloprovinvialis* SMAV calculation because the EC_{50} was a relatively low "greater than" value, while Hayman et al. (2021) provided a definitive EC_{50} value.
Reviewer 4	• EPA applied The ICE model predictions to supplement the available test dataset to help fill missing MDRs and allow the derivation of acute estuarine/marine benchmark recommendations for aquatic life using procedures consistent with those in the 1985 Guidelines**.** Total of 3104 datapoints from 398 models were evaluated. • The draft acute benchmark for estuarine/marine aquatic life is lower than the recommended acute freshwater criterion (46 mg/L), suggesting that estuarine/marine species may be more acutely sensitive to PFOA. According to Hayman et al., 2021, marine species, compared to freshwater, may have a higher sensitivity to PFOA. • There are two more studies published, the toxicity values for marine/estuarine species. o Stuart L. Simpson, Yawen Liu, David A. Spadaro, Xinhong Wang; Rai S. Kookana and Graeme E. Batley Chronic effects and thresholds for estuarine and marine benthic organism exposure to perfluorooctane sulfonic acid (PFOS)-contaminated sediments: Influence of organic carbon and exposure routes https://doi.org/10.1016/j.scitotenv.2021.146008 o Nicholas T Hayman , Gunther Rosen , Marienne A Colvin , Jason Conder , Jennifer A Arblaster Aquatic toxicity evaluations of PFOS and PFOA for five standard marine endpoints. https://doi.org/10.1016/j.chemosphere.2021.129699	Thank you for your comment and for summarizing the derivation of the draft acute estuarine/marine benchmark for PFOA in detail. Furthermore, Reviewer 4 reiterated the draft PFOA estuarine/marine benchmark is lower than the draft acute freshwater PFOA criterion, while noting how this is consistent with results reported by Hayman et al. (2021). Thank you for providing additional references. All studies provided by Reviewer 4 (and all other reviewers) were reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc., and were included in the PFOA criteria document, as appropriate. This includes recalculation of the acute estuarine/marine benchmark, as suggested by Reviewer 4, to include suggested estuarine/marine studies.

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Reviewer	**Comments**	**EPA Response**
	It is recommended to assess the quality of the toxicity data on marine/estuarine species and recalculate estuarine criteria based on this recently available information.	
Reviewer 5	The most sensitive estuarine/marine species was Mytilus g. in the study by Fabbri et al. (2014). EPA chose to use 1 mg/L PFOA because there was not a 50% effect level even at the highest tested concentration (1 mg/L). HOWEVER, there was 27% effect at the LOEC (0.0001 mg/L). Is this not problematic? If I were concerned about mussels or other bivalves in an estuary, I don't think I would hang my hat on a 1 mg/L PFOA concentration given there was a 27% decrease in normal D-larvae at a concentration several orders of magnitude below that. This raises the issue of why EPA is using a 50% effect level for acute criteria – this seems excessively high, doesn't it? EPA justified using the EC_{10} for chronic criteria so it seems reasonable to use the EC/LC20 for acute. A 50% effect, if it occurs, is likely to manifest as ecologically relevant for any species…20% may not be protective depending on the species and endpoint. As mentioned before, I understand that there is a frequency/duration element to all the criteria but from a functional standpoint, I don't see how the ferequency and duration elements are helpful because nobody collects or reports environmental data on a relevant temporal scale (every hour; 4-day running average??). See below for more on frequency and duration. Given the data, I believe the new approach methods based on WEB-ICE are appropriate. The estimation tool has been in development and used for a considerable length of time and several publications have supported it's use. Of course it would be better to have more data but, again, given the lack of data for estuarine/marine species, the WEB-ICE approach is likely the best available.	Thank you for your comment regarding the study on the, "*Adaptation of the bivalve embryotoxicity assay for the high throughput screening of emerging contaminants in Mytilus galloprovincialis*" by Fabbri et al. (2014). Fabbri et al. (2014) did not report an acute EC_{50} for *M. galloprovincialis* from exposure to PFOA (or PFOS) because it could not be calculated. The percent adverse effect at the highest concentration of PFOA tested (1 mg/L) did not exceed a 50% reduction in % Normal D-larvae relative to the negative control treatment, only producing about a 37% reduction (calculated from 83% Normal D-larvae in negative control and 52% Normal D- larvae in the 1 mg/L treatment; determined from visual inspection of Figure 4 of Fabbri et al. 2014). EPA's decision to use the acute value of > 1 mg PFOA/L for this study in the draft PFOA criteria document that underwent peer review was consistent with the 1985 Guidelines. Specifically, Under Section IV.E.2. - *"The result of a [acute] test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones, should be the 96-hr EC_{50} based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an EC_{50} is not available from a test, the lower of the 96-hr EC_{50} based on the percentage of organisms with incompletely developed shells and the 96-hr LC_{50} should be used in place of the desired 96-hr EC_{50}. If the duration of the test was between 48 and 96 hr, the EC_{50} or LC_{50} at the end of the test should be used."*

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Reviewer	**Comments**	**EPA Response**
		And, under Section IV.E.5.- "*If the tests were conducted properly, acute values reported as "greater than" values and those which are above the solubility of the test material should be used, because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species*." Thus, the appropriate acute value for entry into Table B.1 of the draft PFOA criteria document that underwent peer review for Fabbri et al. (2014) was the 48-h EC_{50} of > 1 mg/L PFOA. While the study clearly demonstrates an effect of PFOA on embryo development, the fact that a 50% reduction in % Normal D-larvae was not reached in the test resulted in a "greater than" EC_{50} value for the acute effect concentration; this is consistent with the authors being unable to determine an EC_{50}. Furthermore, the authors note PFOS and PFOA did not cause an increase in the percentage of malformations, but rather a reduction in number of fully developed D-larvae, suggesting delayed development effects rather than viability. NOECs, LOECs, and MATCs from acute tests are not used in the acute GSD for the derivation of acute criterion. Furthermore, the short 48-h duration of the test excludes its consideration in the development of a chronic criterion estuarine/marine criterion or benchmark. The *Mytilus galloprovincialis* SMAV was updated to 17.58 mg/L in the revised PFOA criteria document following incorporation of new data published by Hayman et al. (2021). Briefly, Hayman et al. (2021) exposed *M. galloprovinvialis* embryos to PFOA for 48-hours and determined an EC_{50} of 9.98 mg/L (endpoint = normal and surviving). EPA fit a model to the concentration-response data and determined an EPA-calculated EC_{50} of 17.58 mg/L from this test. The acute value from Hayman et al. (2021) was used preferentially over the acute value determined by Fabbri et al. (2014) to calculate the *M. galloprovinvialis* SMAV in the revised PFOA draft

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Reviewer	**Comments**	**EPA Response**
		criteria. Results from Fabbri et al. (2014) were not used in the *M. galloprovinvialis* SMAV calculation because the EC_{50} was a relatively low "greater than" value, while Hayman et al. (2021) provided a definitive EC_{50} value. EPA's acute estuarine/marine GSD consisted GMAVs calculated from empirical acute LC_{50} values supplemented with estimated LC_{50} values determined through ICE. The 5th percentile of the GSD served as the estuarine/marine FAV. Consistent with the 1985 Guidelines, the estuarine/marine FAV was then divided by 2.0 to calculate the draft estuarine/marine benchmark. Dividing the FAV by 2.0 ensures the estuarine/marine benchmark represents a concentration that will not affect a large portion of sensitive organisms. This is based on the established premise that the ratio between LC_{50} values and corresponding LC_{low} values (e.g., LC_0 – LC_{10}) is typically close to 2.0. Please see the excerpt from the 1985 Guidelines below for further explanation, "*the Criterion Maximum Concentration is now equal to one-half the Final Acute Value. The Criterion Maximum Concentration is intended to protect 95 percent of a group of diverse genera, unless a commercially or recreationally important species is very sensitive. However, a concentration that would severely harm 50 percent of the fifth percentile or 50 percent of a sensitive important species cannot be considered to be protective of that percentile or that species. Dividing the Final Acute Value by 2 is intended to result in a concentration that will not severely adversely affect too many of the organisms."* In addition to criteria magnitudes themselves, the frequency and duration components of criteria ensure species are adequately protected. For example, the 1985 Guidelines state:

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Reviewer	**Comments**	**EPA Response**
		"Duration components (or averaging periods) "*in national criteria have been made short enough to restrict allowable fluctuations in the concentration of the pollutant in the receiving water and to restrict the length of time that the concentration in the receiving water can be continuously above a criterion concentrations*." In addition to monitoring, the magnitude, duration, and frequency components of criteria are particularly important in the design of wastewater treatment plants. For example, the 1985 Guidelines state: *"one of the most important uses of criteria is for designing waste treatment facilities. Such facilities are designed based on probabilities and it is not possible to design for a zero probability. Thus, one of the important design parameters is the probability that the four-day average or the one-hour-average will be exceeded, or, in other words, the frequency with which exceedences will be allowed."* Overall, EPA agrees with Reviewer 5's assertion that, "*given the data,.....the new approach methods based on WEB-ICE are appropriate*" for deriving an acute estuarine/marine benchmark. In the absence of a large acute estuarine/marine toxicity dataset, the acute estuarine/marine benchmark provides information regarding a protective value that States and Tribes may consider for use.

2.4 Please comment on the use of measured and unmeasured toxicity tests to derive the respective criterion. In particular please comment on the supporting justification for using unmeasured toxicity tests in Appendix L.

2.4. The Use of Measured and Unmeasured Toxicity Tests to Derive Respective Criterion		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	The consideration of toxicity data from experiments in which PFOA measurements were not made seems appropriate. The Appendix L analysis is supportive of the general observation that actual concentrations in the toxicity test waters approximated nominal values for the freshwater studies.	Thank you for your comment indicating the appropriateness of using acute and chronic toxicity data from studies that did not measure the PFOA treatment concentrations. EPA thanks Reviewer 1 for indicating "*the Appendix L analysis is supportive of the general observation that actual concentrations in the toxicity test waters approximated nominal values for the freshwater studies*."
Reviewer 2	I am concerned with the approach of using the agreement of measured and nominal concentrations from studies that measured the concentration of PFOA in their tests to determine whether to use toxicity data from studies that did not measure the concentration PFOA in their tests. My concern stems from this approach having to assume that studies that did not measure the concentration of PFOA in their experiments performed the dosing of PFOA with the same care and skill as those studies that did measure the concentration of PFOA in their experiments and measured concentrations within 20% of nominal. My concern is compound by 79.5% and 60% of the acute and chronic tests, respectively, only reporting nominal test concentrations. The EPA's approach uses the agreement of measured and nominal concentration in a minority of studies to determine whether to include the majority of studies on their assessment. I am assuming that there wouldn't be sufficient data to determine a criterion without using data from studies that did not measure the concentrations of PFOA in their experiment?	Thank you for your comment noting that by using both measured and unmeasured toxicity studies in the derivation of the draft PFOA Aquatic Life Criteria, the assumption was made that unmeasured toxicity studies conducted dosing with the same accuracy and care as measured toxicity tests. EPA acknowledges that the *Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations* in Appendix L of the draft PFOA criteria document does not eliminate this concern. However, unmeasured studies that were used quantitatively to derive the PFOA criteria all otherwise met EPA's test quality guidelines (EPA's 1985 Guidelines and 850 Test Guidelines; U.S. EPA 1985 and U.S. EPA 2016b). Given the relative high occurrence of unmeasured PFOA toxicity tests, typically attributed to the relatively high stability of PFOA and/or difficulty in measuring test concentrations by individual study authors, there would be insufficient data to derive PFOA criteria for aquatic life without the inclusion of both measured and unmeasured tests. Therefore, EPA chose to use the best available science to develop criteria recommendations to support states and stakeholders in protecting aquatic life. Thank you for your comment noting the approach EPA used to determine the level of agreement between nominal and measured concentrations was logical and valid. With the meta-analysis in

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Reviewer	**Comments**	**EPA Response**
	I think the approach that the EPA has used to determine the level of agreement between the nominal and measured concentration of PFOA in the studies that measured the concentration is logical and valid. It is encouraging that the agreement on average is high. Again, my largest concern is assuming this agreement in a minority of studies is present in all studies.	Appendix L of the draft PFOA criteria document, EPA evaluated any potential differences between nominal and measured test concentrations that may be due to water type (salt or freshwater) or experimental conditions such as (1) acute and chronic test duration; (2) whether test organisms were fed or unfed; (3) test vessel material (e.g., glass or plastic); (4) use of solvent or no solvent; and (5) the presence of a substrate. Because experimental conditions did systematically produce differences between nominal and measured concentrations of PFOA, EPA used both measured and unmeasured toxicity studies that otherwise meet EPA's test quality guidelines to derive the PFOA criteria for aquatic life.
Reviewer 3	*Reviewer 3's response to this question was provided in this person's review of the draft PFOS document, with a statement that the comment also pertained to PFOA; therefore, it is copied here.* This seems acceptable for the time being. Having worked in the laboratory with PFOS, I can make a first-hand testament that mixing PFOS into exposures solutions does not guarantee a homogenous mixture despite working at solutions well below the solubility limit. There are nuances associated with achieving homogeneity of the exposure solution, we have developed a PFAS mixing protocol to reduce chemical clumping and this increases uniformity of the solutions. Furthermore, there is approximately 30% variability of PFOS quantitatively (see…Rewerts et al. 2020); so, the best measurement still has significant variability.	A statement has been included in Appendix L of the updated PFOA criteria indicating recent PFAS literature indicates standard variability between nominal and measured concentrations may be as high as 30% (citing Coats et al. 2017 and Rewerts et al. 2021, as suggested by Reviewer 3).
Reviewer 4	PFOA is a highly stable compound, resistant to hydrolysis, photolysis, volatilization, and biodegradation (as described in Section 1.1.1 of the Report) and, therefore, expected to vary only minimally in the course of a toxicity test. To	Thank you for your comment and summarization of the *Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations* in Appendix L of the draft PFOA Aquatic Life Criteria document. Thank you for describing your

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Reviewer	**Comments**	**EPA Response**
	determine if nominal and measured PFOA concentrations were typically in close agreement, pairs of nominal and corresponding measured PFOA concentrations were compared to one another through (1) linear correlation analysis and (2) an assessment of measured concentrations as a percent of its paired nominal concentration. The analysis conducted by EPA Team showed strong correlation (correlation = 0.9995) of the 79 pairs of nominal and measured concentrations from freshwater studies, and similar strong correlation (correlation = 0.9999) of the 11 pairs of nominal and measured concentrations from saltwater studies (Figure L1 in the Report). In addition, the experimental conditions did not influence the correlation between nominal and measured concentrations. ***This confirms inclusion of unmeasured PFOA toxicity tests for quantitative use in criteria derivation.*** Personal experience on analyzing PFOA in ecotoxicological studies using freshwater and saltwater species have also exhinited strong correlation between nominal and measured concentrations. **Additional information for L.I.4 summary section** could include additional information based on the two additional published papers and the key points from these studies are listed below. *Despite the concerns and avoidance of glass, few studies have presented data describing the sorption of perfluorooctanoate (PFOA) to glass and other container materials (**Lath et al. 2019**). Lath et al. (2019), who had reported that sorptive losses of PFOA for lower concentration (~20 µg/L) aqueous solutions*	experience analyzing PFOA in ecotoxicological studies for freshwater species, which have exhibited strong correlation between nominal and measured concentrations. EPA notes the suggestion of Reviewer 4 to consider Rewerts et al. (2021). A statement has been included in Appendix L of the updated PFOA criteria indicating recent PFAS literature indicates standard variability between nominal and measured concentrations may be as high as 30% (citing Coats et al. 2017 and Rewerts et al. 2021). At the suggestion of Reviewer 4, a statement has been included in Appendix L of the updated PFOA Aquatic Life Criteria document that discusses the results of Lath et al. (2019) in relation to the three tests across two publications (Oakes et el. 2004 and Colombo et al. 2008) that exhibited a relatively high number of measured treatment concentrations that were not within 20% of nominal concentrations. In short, Colombo et al. (2008) did not report the test vessel material, while Oaks et al. (2004) reported test vessels were lined with food-grade PVC. PFOA in treatments described by Oakes et al. (2004) may have rapidly sorbed to the test vessel (in addition to added substrate and/or macrophytes). This is consistent with results of Lath et al. (2019), who determined PFOA was more likely to sorb to plastic test vessels than glass, stating: "*Contrary to suggestions in the literature, our results indicated that the greatest sorption losses for PFOA occurred on PP* [polypropylene]*, whereas losses on glass tubes were much lower*."

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	were highest in polypropylene centrifuge tubes. However, the container type did not influence the measured concentration as reported in section – of this report. According to ***Rewerts et al., 2021*** *additional handling steps, which are not typically reported for ecotoxicological studies but may contribute to variability, include solution homogenization, subsampling procedures, and the container materials selected for storage*. https://doi.org/10.1002/etc.4667 Lath S, Knight ER, Navarro DA, Kookana RS, McLaughlin MJ. 2019. Sorption of PFOA onto different laboratory materials: Filter membranes and centrifuge tubes. Chemosphere 222: 671– 678. DOI: 10.1016/j.chemosphere.2019.01.096	
Reviewer 5	Similar to PFOS, the approach EPA used for PFOA was to consider studies in which the chemical was not measured. This was justified based on an analysis EPA did comparing nominal and measured concentrations and finding close agreement. This generally seems reasonable to me especially considering the stability of the chemical. However, EPA used a criteria of 20% (p. 61) which is not consistent with the analytical precision of most methods used to analyze PFAS. According to several very prolific environmental chemists that have made a career of measuring PFAS, they have communicated to me that the methods are accurate to within 30%. That means that if the measured were within 30% of nominal, we basically have concluded these were nominal. I would encourage EPA to explore their 20% acceptability threshold and perhaps offer an explanation as to why this is appropriate.	EPA acknowledges participants of the Society of Environmental Toxicology and Chemistry (SETAC) North America Focused Topic Meeting on Environmental Risk Assessment of PFAS (held August 2019, Durham, NC) commented that analytical confirmation of test concentrations is needed. Additionally, previous aquatic life ambient water quality criteria for other chemicals have preferentially relied on measured toxicity tests, particularly those tests with relatively sensitive taxa. Given the relative rarity of measured PFOA toxicity tests in the current literature there would be insufficient data to derive PFOA criteria for aquatic life without the inclusion of both measured and unmeasured tests. Considering that the results of the meta-analysis (described in Appendix L of the draft PFOA Aquatic Life Criteria document) that strongly indicated nominal concentrations were relatively similar to measured concentrations regardless of experimental condition, EPA used both measured and unmeasured toxicity studies that otherwise meet EPA's test quality guidelines to derive the draft PFOA aquatic life criteria.

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Reviewer	**Comments**	**EPA Response**
		Thank you for your comment noting that several analytical chemists have recommended a 30% threshold for determining if measured and nominal concentrations are different. EPA used the 20% threshold (as opposed to the 30% threshold) to be consistent with EPA's 850 Test Guidelines (U.S. EPA 2016b). Moreover, in freshwater 89.9% of measured concentration were within 20% of the paired nominal concentration, indicating a high degree of correlation between nominal and measured concentrations. Adjusting to a 30% threshold (as opposed ot the 20% threshold difference in Appendix L of the draft PFOA criteria Document) would not meaningfully alter conclusions.

2.5 Please comment on the toxicity data used to derive the draft criteria.

- **Were the data selected and/or excluded from the derivation of the criteria derivation appropriately utilized?**
- **Are there relevant data that you are aware of that should be added to the analyses (note that EPA is working on updating the toxicity data to reflect the data in ECOTOX between Sept. 2019 through the latest update)? If so, please provide references for consideration.**

In particular, please comment on:

2.5.a. The toxicity values used to derive the PFOA criteria, with a particular emphasis on:

2.5.a.i. the selection and the Acute to Chronic Ratio (ACR) to serve as the Final Acute to Chronic Ratio (FACR) and its application to derive the Final Chronic Value (FCV).

2.5.a.ii. the use of the qualitatively acceptable acute midge (*Chironomus plumosus*) data from Yang et al. (2014) to suggest aquatic insects are relatively tolerant to acute PFOA exposures. Specifically, Yang et al. (2014) conducted a 96-hour renewal, measured PFOA acute test with the midge, *Chironomus plumosus*. This study was not acceptable for quantitative use due to the potentially problematic source of the organisms. The reported LC_{50} was 402.24 mg/L PFOA indicating that these insects may not be one of the more sensitive taxonomic groups. Therefore, this test was excluded from the criterion calculation, but used to waive the missing insect MDR.

2.5.b. EPA's approach for fitting concentration-response (C-R) data (described in Appendix J) as well as the specific acute LC_{50} values (Appendix A.2) and chronic EC_{10} values (Appendix C.2) that were estimated (for sensitive genera when C-R data were available) and used to derive criteria.

2.5. The Toxicity Data to Derive the Draft Criterion		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	• **Were the data selected and/or excluded from the derivation of the criteria derivation appropriately utilized?** In most cases, yes. Please see detailed comments on particular studies and interpretations in response to other charge questions.	Thank you for your comment noting data were appropriately utilized in the draft PFOA Aquatic Life Criteria document in most cases, Instances where Reviewer 1 believes data may have been used inappropriately have been addressed in responses to other charge questions. All studies provided by Reviewer 1 (and all other reviewers), including Hayman et al. (2021) were all reviewed to ensure they met

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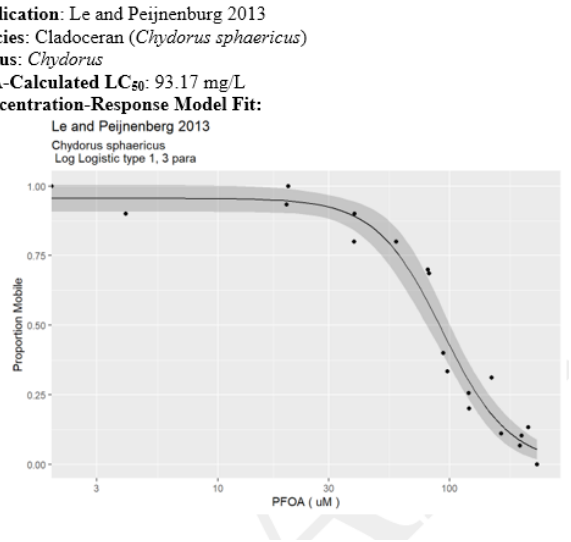
	• **Are there relevant data that you are aware of that should be added to the analyses (note that EPA is working on updating the toxicity data to reflect the data in ECOTOX between Sept. 2019 through the latest update)? If so, please provide references for consideration.** Hayman, N.T., Rosen, G., Colvin, M.A., Conder, J., Arblaster, J.A. 2021. Aquatic toxicity evaluations of PFOS and PFOA for five standard marine endpoints. Chemosphere 273:129699. **2.5.a** 2.5.a.i As stated in the 1985 guidelines, "the Final Acute-Chronic Ratio should be calculated as the geometric mean of the acute-chronic ratios for species whose SMAVs are close to the Final Acute Value." The guidance does not quantify "close", but it also does not specify that the Acute to Chronic Ratio (ACR) should only be derived <u>from a single ACR</u> from the study with the acute value that is closest to the Final Acute Value (FAV). In the case of the PFOA document, EPA is using only one ACR value, which is derived from the Zhang et al. (2013) rotifer study in which the LC_{50} is 150 mg/L. I would agree 150 mg/L relatively close to the FAV of 94 mg/L. However, I would also argue that the LC_{50} of 166 mg/L from the study with *Moina macrocopa* is also "close" to 94 mg/L (it is only 11% higher than 150 mg/L). The Species Mean Acute Value (SMAV) for *D. magna* is 253.7 mg/L is well within a factor of 3 of 94 mg/L and could also be considered "close". These three data points tend to cluster, as shown in	data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc., and were included in the PFOA criteria document as appropriate based on their data quality. Responses to key instances where Reviewer 1 does not believe the data were appropriately utilized to derive the draft PFOA Aquatic Life Criteria are described below in the corresponding order to those comments by Reviewer 1. Specific to comments to charge question 2.5.a: 2.5.a.i Addition of new chronic toxicity data in the updated draft PFOA criteria document allowed for the calculation of a draft chronic freshwater criterion magnitude directly from the chronic GSD rather than basing the magnitude on the FAV transformed by the FACR, as was done in the draft PFOA criteria document that underwent peer review. 2.5.a.ii Based on further consideration and peer reviewer comments, tests with organisms from Yang et al. (2014) that were collected from the Beijing City Big Forest Flower Market and tests from Yuan et al. (2015) where organisms were collected from a fountain in Quanhetou, Boshan, China are now both considered qualitatively acceptable for criteria derivation. Specific to comments to charge question 2.5.b: The draft PFOA criteria document reviewed by peer reviewers contained model type and figures of the C-R data with the fitted model and corresponding 95% confidence bands for tests that were among the four most sensitive acute or chronic genera (and C-R data were reported in the publication or could be obtained from authors). Appendix A.2 contained test-specific C-R modeling for relatively sensitive acute tests, while test-specific C-R models for relatively

2.5. The Toxicity Data to Derive the Draft Criterion		
Reviewer	**Comments**	**EPA Response**
	Figure 3-5. Taking the geometric mean of the three ACRs from these three studies, which are studies that have acute values all within a factor of 2-3 of the 94 mg/L FAV, seems more consistent with the 1985 guidance to select several ACRs that are "close" to the FAV, rather than simply selecting a single ACR. The resulting recalculated ACR would be approximately 90, which provides a good, but conservative measure of central tendency and is higher than all but the highest ACR (299) shown in Table 3-8. Applying an ACR of 90 to the FAV would result in a FCV of approximately 1 mg/L, which is protective of chronic values shown in Figure 3-6 (note the Zhang et al.-derived rotifer SMCV is 0.8 mg/L, which is only slightly lower 1 mg/L). Such as recalculation would better incorporate variability in the ACRs and avoid both water-based criteria being completely driven by the results from a single organism (rotifers) as reported in a single study (Zhang et al., 2013).	sensitive chronic tests were included in Appendix C.2. Please see below for an example of test-specific C-R modeling results that was reported in Appendix A.2 of the draft PFOA Criteria Document. **Publication**: Le and Peijnenburg 2013 **Species**: Cladoceran (*Chydorus sphaericus*) **Genus**: *Chydorus* **EPA-Calculated LC_{50}**: 93.17 mg/L **Concentration-Response Model Fit:**  Additional details on the functions and model specifications within the R.drc package are publicly available by following the link here: https://cran.r-project.org/web/packages/drc/drc.pdf Standard errors for both the model parameters and ECx estimates were calculated during evaluation, but this level of statistical detail was not included in the Peer Review draft of the PFOA Aquatic Life Criteria document. In response to Reviewer 1, Appendix A.1, A.2, C.1, and C.2 of the updated PFOA criteria document now include 95% Confidence Intervals (CI) as parentheticals following discussion of all point estimates (i.e., LC_{50} and EC_x values) calculated by EPA (irrespective of whether or not the point estimate was used in calculation of the four most sensitive GMAVs and/or GMCVs).
	2.5.a.ii I disagree with excluding this data point from the acute criteria calculations. I assume this data has been removed under the assumption that these animals may have been pre-exposed to PFOA and may have been more tolerant of PFOA exposures, which would result in biased-high median lethal concentration (LC_{50}) values. If so, this should be explicitly stated. Assuming these *Chironomus* can develop tolerance to PFOA, it seems that they would have to be exposed to rather high mg/L ranges of PFOA in water given the reported 96-hour LC_{50} of 402 mg/L. Based on published	

2.5. The Toxicity Data to Derive the Draft Criterion		
Reviewer	**Comments**	**EPA Response**
	literature, I am unaware of natural ecosystems in China (where the animals may have been originally harvested) with concentrations of PFOA that approach this order of magnitude range (in which they could build up a tolerance). The animals were obtained from a local market, so it is also possible that they were cultured for several generations, presumably using uncontaminated water (which would further reduce the chance that multiple generations were exposed at these levels). Overall, I think it is more reasonable to assume that the animals used in the experiment have not built up an acute lethal tolerance to PFOA, and the that LC_{50} result is unbiased. It does seem clearly show that insects may be less sensitive to acute lethality effects of PFOA. As such, I think it should be included as a quantitative endpoint. Additionally, it seems inconsistent to exclude this Yang et al (2014) study, when acute data from a study by Yuan et al. (2015) were included for quantitative consideration. As noted on page A-10, the animals in the Yuan et al. (2015) study were "collected from a fountain in Quanhetou, Boshan, China, and acclimated in the laboratory for an unspecified time period before use". The source of the animals is just as uncertain as the Yang et al (2014) animals, and it is unclear (if PFOA tolerance at lethal levels is possible) how many generations would be needed to shed adaptive tolerance and how this time period would compare to an "unspecified time period." Simply put, if data from experiments like Yuan et al. (2015) are quantitatively included, those from Yang et al.	

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Reviewer	**Comments**	**EPA Response**
	(2014) should also be quantitatively included (with some notes on the uncertainty of the animal sources). **2.5.b.** More details need to be provided on the dose response modeling using R. Appendix J is helpful for providing the reader with details on the general approach, but where EC_{10}s are modeled by EPA, the model being used (out of the 22 available in the R software package) needs to be specified. Providing some indication of variability (such as a 95% confidence interval) for the model-generated EC_{10}s is standard practice for dose response modeling, and this information should be provided somewhere in the document. Showing the R package output of the goodness of fit statistics (or equivalent) for the modeling in an Appendix would be helpful; since this was used to select the model used in each instance of an EC_{10} calculation, it must be available, so I would recommend including it for full transparency and to aid future efforts in understanding the aquatic toxicology of this chemical. Additionally, it would be helpful to show the selected model fits for all calculated EC_{10}s (as shown for the most sensitive EC_{10}s estimated). These steps would be helpful to ensure and demonstrate quality of the model fits and reproducibility of the modeling work.	
Reviewer 2	• **Were the data selected and/or excluded from the derivation of the criteria appropriately utilized?** I think the data used in the derivation of the criteria were appropriate. As mentioned above, I am a little concerned about the use of toxicity data from studies that did not	Given the high occurrence of unmeasured PFOA toxicity tests (typically attributed to the relatively high stability of PFOA and/or difficulty in measuring test concentrations by individual study authors) there would be insufficient data to derive PFOA criteria for aquatic life without the inclusion of both measured and unmeasured

2.5. The Toxicity Data to Derive the Draft Criterion		
Reviewer	**Comments**	**EPA Response**
	measure the concentration of PFOA in their experiments, especially considering the proportion of studies that did not measure the concentrations. The confirmation of exposure concentrations is an important principle of sound ecotoxicology. • **Are there relevant data that you are aware of that should be added to the analyses (note that EPA is working on updating the toxicity data to reflect the data in ECOTOX between Sept. 2019 through the latest update)? If so, please provide references for consideration.** Hayman, N.T., Rosen, G., Colvin, M.A., Conder, J., Arblaster, J.A., 2021. Aquatic toxicity evaluations of PFOS and PFOA for five standard marine endpoints. Chemosphere 273, 129699.. doi:10.1016/j.chemosphere.2021.129699 Logeshwaran, P., Sivaram, A.K., Surapaneni, A., Kannan, K., Naidu, R., Megharaj, M., 2021. Exposure to perfluorooctanesulfonate (PFOS) but not perflurorooctanoic acid (PFOA) at ppb concentration induces chronic toxicity in Daphnia carinata. Science of The Total Environment 769, 144577.. doi:10.1016/j.scitotenv.2020.144577 Bartlett, A.J., De Silva, A.O., Schissler, D.M., Hedges, A.M., Brown, L.R., Shires, K., Miller, J., Sullivan, C., Spencer, C., Parrott, J.L., 2021. Lethal and sublethal toxicity of perfluorooctanoic acid (PFOA) in chronic tests with Hyalella azteca (amphipod) and early-life stage tests with Pimephales promelas (fathead minnow). Ecotoxicology and	tests. EPA appreciates Reviewer 2 previously noting the approach EPA used to determine the level of agreement between nominal and measured concentrations was logical and valid. Appendix L of the draft PFOA Aquatic Life Criteria document contained EPA evaluation of potential differences between nominal and measured test concentrations that may be due to water type (salt or freshwater) and/or experimental conditions such as (1) acute and chronic test duration; (2) whether test organisms were fed or unfed; (3) test vessel material (glass or plastic); (4) use of solvent or no solvent; and (5) the presence of a substrate. Because experimental conditions did systematically produce influence discrepancies between nominal and measured concentrations of PFOA, EPA use both measured and unmeasured toxicity studies that otherwise meet EPA's test quality guidelines to derive the PFOA criteria for aquatic life. All toxicity studies provided by Reviewer 2 (and all other reviewers) were reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc., and were included in the PFOA criteria document as appropriate based on study data quality. BAFs used to calculate the tissue criteria were obtained from the BAF database created Lawrence Burkhard (U.S. EPA, Office of Research and Development) in support of his publication: Burkhard, L.P. (2021) Evaluation of Published Bioconcentration Factor (BCF) and Bioaccumulation Factor (BAF) Data for Per- and Polyfluoroalkyl Substances Across Aquatic Species. ET&C 40: 1530-1543. BAFs reported in Prosser et al. 2016 were not in the Burkhard (2021) database and were therefore not used to calculate BAFs. Upon review of Prosser et al. (2016), it appears this study was not included in the BAF database developed by Burkhard (2021) because this addressed study biota sediment accumulation factors (BSAFs) instead of BAFs. It is for this same reason EPA did not include Prosser et al. (2016) among the BAFs that were used to derive the tissue criteria for PFOA.

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Reviewer	**Comments**	**EPA Response**
	Environmental Safety 207, 111250.. doi:10.1016/j.ecoenv.2020.111250 Tornabene, B.J., Chislock, M.F., Gannon, M.E., Sepúlveda, M.S., Hoverman, J.T., 2021. Relative acute toxicity of three per- and polyfluoroalkyl substances on nine species of larval amphibians. Integrated Environmental Assessment and Management 17, 684–690.. doi:10.1002/ieam.4391 Flynn, R.W., Iacchetta, M., Perre, C., Lee, L., Sepúlveda, M.S., Hoverman, J.T., 2021. Chronic Per-/Polyfluoroalkyl Substance Exposure Under Environmentally Relevant Conditions Delays Development in Northern Leopard Frog (Rana pipiens) Larvae. Environmental Toxicology and Chemistry 40, 711–716.. doi:10.1002/etc.4690 BAF data provided in the supplementary information of Prosser et al. 2016 study for three freshwater species does not appear to have been considered. Prosser, R.S., Mahon, K., Sibley, P.K., Poirier, D., Watson-Leung, T., 2016. Bioaccumulation of perfluorinated carboxylates and sulfonates and polychlorinated biphenyls in laboratory-cultured Hexagenia spp., Lumbriculus variegatus and Pimephales promelas from field-collected sediments. Science of The Total Environment 543, 715–726. doi:10.1016/j.scitotenv.2015.11.062 **2.5.a.** 2.5.a.i I think the approach that led to the selection of the ACR for Brachionus calyciflorus was appropriate. It is also the most conservative approach with the	Specific to comments to Charge Question 2.5.a: 2.5.a.i Thank you for your comment. 2.5.a.ii Thank you for your comment. The *Chironomus plumosus* test by Yang et al. (2014) has been retained as qualitatively acceptable because of the atypical source (i.e., Beijing City Big Forest Flower Market) of the test organisms. Specific to comments to Charge Question 2.5.b: Thank you for your comment noting the inclusion of C-R models for tests with species among the four most sensitive acute and/or chronic genera in Appendices A.2 and C.2 "*generates a high level of transparency in the derivation of the criterion*." Additional details on the functions and model specifications within the R.drc package are publicly available via the link here: https://cran.r-project.org/web/packages/drc/drc.pdf

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	ACR for this species being the largest of the four species with ACRs. 2.5.a.ii I think the EPA's decision that the data from Yang et al. (2014) was not acceptable for quantitative use was appropriate. The source of the larvae is problematic. The conclusion that insects may not be one of the most sensitive taxa is valid. The NOEC for Chironomus tentans of 100 mg/L reported by MacDonald et al. (2004) also supports this conclusion. **2.5.b.** I think the approach that the EPA used to determine effect measure from concentration-response data was appropriate. The use of the drc package in R to fit 22 different models to the empirical data and then using several criteria (e.g., AIC, residual standard errors, confidence intervals) to evaluate the fit of the different models is robust. It would have been useful if the EPA reported the 22 different models in Appendix J. I think the LC_{50} and EC_{10} values determined by the EPA using the approach mentioned in the previous paragraph was appropriate. It is valid for these effect measures to be determined when the concentration-response data has been provided by the authors of the study. The EPA has also made is clear in Appendix A.2 and C.2 how they determined these effect measures using the concentration-response data provide in the studies. This generates a high level of transparency in the derivation of the criterion.	

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Reviewer	**Comments**	**EPA Response**
Reviewer 3	• **Were the data selected and/or excluded from the derivation of the criteria derivation appropriately utilized?** With the exception of the Fabbri et al. 2014, data currently evaluated, and the associated decision matrix seem appropriate. However, as noted below, there are new data available following this draft. • **Are there relevant data that you are aware of that should be added to the analyses (note that EPA is working on updating the toxicity data to reflect the data in ECOTOX between Sept. 2019 through the latest update)? If so, please provide references for consideration.** o McCarthy et al. 2021 - freshwater o Hayman et al. 2021 – marine o Logeshwaran et al. 2021 – freshwater o Li et al. 2021 – freshwater/plant o Etc. **2.5.a.** 2.5.a.i The use of ACR is appropriate given data limitations. However, either I am missing it or it is unclear as to why Chydorus is not included in the chronic data set when it is the most sensitive in the acute. 2.5.a.ii McCarthy et al. 2021 reported midge data for PFOA with an EC50 of 192 mg PFOA/L following a 20-day exposure. Following EPA review of this publication, this may fulfill the	Please see EPA's response to Reviewer 3's comments on the use of Fabbri et al. (2014) under charge question 2.3. EPA thanks Reviewer 3 for noting remaining data were used appropriately. Specific to comments to Charge Question 2.5.a: 2.5.a.i Thank you for your comment on the appropriateness of the selection and use of the FACR to derive the draft chronic criterion. There were no ACRs for species within the genus, *Chydorus* (most sensitive acute genus in the draft criteria document), because chronic toxicity data were not available precluding the calculation of an ACR for any species within the genus, *Chydorus*. Addition of new chronic toxicity data in the updated draft PFOA criteria document allowed for the calculation of a draft chronic freshwater criterion magnitude directly from the chronic GSD rather than basing the magnitude on the FAV transformed by the FACR, as was done in the draft PFOA criteria document that underwent peer review. 2.5.a.ii All toxicity studies provided by Reviewer 3 (and all other reviewers), including McCarthy et al. (2021), were all reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc., and were included in the PFOA criteria document as appropriate, based on their data quality. Specific to comments to Charge Question 2.5.b: Thank you for your comment on EPA's independent calculation of acute LC_{50} and chronic EC_{10} values, nothing this "*is a sound scientific approach*." Section 2.10.3.3 (*Summary of Independent Calculation of Toxicity Values*) of the draft PFOA Aquatic Life Criteria document describes that EPA independently calculated acute LC_{50} and chronic EC_{10} values when C-R data could be obtained from the publication, supplemental material, and/or through direct contact with study

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	missing insect MDR; however, still supports the conclusion of likely not a sensitive taxa. **2.5.b.** This is a sound scientific approach, what is unclear is when EPA employs this vs other times when it is not used. i.e. chydorus vs two mussle spp in the acute studies	authors. Acute concentration-response data for *L. siliquoidea* and *L. recta* were not reported in Hazelton et al. (2012) and could not be obtained from authors to inform C-R modeling. Section 2.10.3.3 of the draft PFOA criteria document states: "*Toxicity values, including LC_{50} and EC_{10} values, were independently calculated from the data presented in the toxicity studies meeting the inclusion criteria described above when adequate concentrations-response data were published in the study or could be obtained from authors. When concentration-response data were not presented in toxicity studies, concentration-response data were requested from study authors to independently calculate toxicity values. In cases where study authors did not respond to EPA's request for data or were unable to locate concentration-response data, the toxicity values were not independently calculated by EPA, and the reported toxicity values were retained for criteria deviation.*"
Reviewer 4	• **Were the data selected and/or excluded from the derivation of the criteria derivation appropriately utilized?** The data selected to derive PFOA criteria are appropriate. Studies that did not fully meet the data quality objectives outlined in the 1985 Guidelines were not considered for inclusion in the criteria derivation, including some studies with other PFAS exposures, but were considered qualitatively as supporting information. A brief summary of each study describing the experimental conditions and summary tables providing all the relevant information such as strengths and limitations of each study, end points selected for deriving criteria are well documented by the EPA team.	Thank you for commenting "*information such as strengths and limitations of each study, end points selected for deriving criteria are well documented by the EPA team*" and for summarizing how data were selected and/or excluded from the derivation of the PFOA criteria. All toxicity studies provided by Reviewer 4 (and all other reviewers) were all reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc., and were included in the PFOA criteria document as appropriate, based on their data quality. Specific to comments to Charge Question 2.5.a: 2.5.a.i Thank you for summarizing the selection and application of the FACR to derive the chronic PFOA criteria. Thank you for commenting, "*The selection and the Acute to Chronic Ratio (ACR) to serve as the Final Acute to Chronic Ratio (FACR) and its application to derive the Final Chronic Value (FCV) for PFOA is acceptable.*"

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	The key acceptable exclusion/inclusion criteria used to derive draft criteria are listed below: • Only single chemical toxicity tests with PFOA were considered for possible inclusion in criteria derivation, studies that tested chemical mixtures, including mixtures with PFAS compounds were excluded from criteria derivation. • Both controlled laboratory experiments and field observations/studies were included. • PFOA toxicity tests were not excluded from quantitative use in criteria derivation on the basis of unmeasured test concentrations alone. • Only single chemical toxicity tests with PFOA were considered for possible inclusion in criteria derivation, studies that tested chemical mixtures, including mixtures with PFAS compounds were excluded from criteria derivation. • Due to lower sensitivity, insect MDR was excluded from the criterion calculation, but were used to waive the missing insect MDR. • Further supporting information on acceptable and unused studies for acute and chronic endpoints and for freshwater and marine studies are documented and summarized as appendices in this report. • **Are there relevant data that you are aware of that should be added to the analyses (note that EPA is working on updating the toxicity data to reflect the data in ECOTOX between Sept. 2019**	Addition of new chronic toxicity data in the updated draft PFOA criteria document allowed for the calculation of a draft chronic freshwater criterion magnitude directly from the chronic GSD rather than basing the magnitude on the FAV transformed by the FACR, as was done in the draft PFOA criteria document that underwent peer review. 2.5.a.ii Thank you for summarizing the data used to derive the criteria and for summarizing the relative tolerance of aquatic insects to acute PFOA exposures based on data available in the draft PFOA Aquatic Life Criteria document. Specific to comments to Charge Question 2.5.b: Thank you for summarizing EPA's independent calculation of acute LC_{50} and chronic EC_{20} values and for stating, "*This is an excellent approach utilized by the EPA Team. EPA's approach for fitting concentration-response (C-R) data resulted in consistent approach across various ecotoxicological studies*."

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	through the latest update)? If so, please provide references for consideration. Additional toxicity data published over the last six months is listed below: **Marine/estuarine** Stuart L. Simpson, Yawen Liu, David A. Spadaro, Xinhong Wang; Rai S. Kookana and Graeme E. Batley Chronic effects and thresholds for estuarine and marine benthic organism exposure to perfluorooctane sulfonic acid (PFOS)-contaminated sediments: Influence of organic carbon and exposure routes https://doi.org/10.1016/j.scitotenv.2021.146008 Nicholas T Hayman , Gunther Rosen , Marienne A Colvin , Jason Conder , Jennifer A Arblaster Aquatic toxicity evaluations of PFOS and PFOA for five standard marine endpoints. https://doi.org/10.1016/j.chemosphere.2021.129699 **Fresh water** Christopher J. McCarthy, Shaun A. Roark, Demitria Wright, Kelly O'Neal, Brett Muckey, Mike Stanaway, Justin N. Rewerts, Jennifer A. Field, Todd A. Anderson, Christopher J. Salice, Toxicological Response of Chironomus dilutus in Single-Chemical and Binary Mixture Exposure Experiments with 6 Perfluoralkyl Substances, Environmental Toxicology and Chemistry, 10.1002/etc.5066, **40**, 8, (2319-2333), (2021). **2.5.a.**	

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	2.5.a.i The selection and the Acute to Chronic Ratio (ACR) to serve as the Final Acute to Chronic Ratio (FACR) and its application to derive the Final Chronic Value (FCV) for PFOA is acceptable. The 1985 Guidelines allow the use of a Final Acute-Chronic Ratio (FACR) to convert the FAV to the FCV (i.e., FAV/FACR=FCV), which is equivalent to the chronic criterion (Criterion Continuous Concentration, CCC), intended to protect 95 percent of the taxa in aquatic ecosystems. For PFOA, the 8-family MDR requirement was not met for the chronic dataset, as acceptable chronic studies for species representing three MDR groups were not available (benthic crustacean and third phylum or second insect order not already represented). Therefore, the Final Chronic Value (FCV) was calculated with the use of an ACR (acute-chronic ratio). When more than a single ACR was calculated for the same species, the Species Mean Acute-Chronic Ratio (SMACR) was calculated as the geometric mean value of all ACRs for that species. . The specifications for derivation of a FACR for aquatic animals was met for PFOA based on 1985 Guidelines: ACRs for at least three different families provided that at least one was a fish, at least one was an invertebrate, and at least one was an acutely sensitive freshwater species. The 1985 Guidelines provides recommendations to calculate the FACR when SMACRs are dissimilar. The 1985 Guidelines states that if SMACRs tend to increase or decrease as the SMAV increases, the FACR should be calculated as the geometric mean of the ACRs for species whose SMAVs are close to the FAV. For PFOA, relationship between SMAV	

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	and SMACR showed that SMACRs decreased as the SMAVs increased. SMAV of the rotifer *B. calyciflorus* was closest to the FAV. The CCC was calculated by dividing the FAV by the FACR to determine the FCV (91.34/299.1=0.3054). *The PFOA FCV derived by this method is lower than the all of the quantitatively-acceptable chronic values (ranges between 0.76- 40 mg/L) and listed in listed in Table C.1*.	
	2.5.a.ii The acute data set for PFOA contained 14 genera representing seven of the eight taxonomic MDR groups. The missing MDR was a representative from an insect family. There was no evidence to suggest aquatic insect are among the four most sensitive genera. EPA calculated the PFOA CMC using all acceptable quantitative studies, but did not include the insect data in the criterion calculation (i.e., the relatively tolerant insect LC_{50} value was not included in the total count ("n") of Genus Mean Acute Values in the criterion calculation). In addition, waiving an unfulfilled MDR when available data suggest it is not among the four most sensitive genera is consistent with previous EPA criteria documents, including U.S. EPA (2016). In addition, Stefani et al. (2014), Macdonald et al. (2004), and Marziali et al. (2019) conducted chronic toxicity tests with *Chironomus* spp. and reported apical endpoints. *Results of these studies, taken together, also suggest that insects may not be among the most sensitive taxa to chronic PFOA exposures. Therefore, these tests were excluded*	

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	from the criterion calculation, but were used to waive the missing insect MDR. **2.5.b.** • This is an excellent approach utilized by the EPA Team. EPA's approach for fitting concentration-response (C-R) data resulted in consistent approach across various ecotoxicological studies. The R drc package was used to fit 22 different models to each individual C-R dataset. A single model was then selected from the 22 models to serve as the representative C-R model. The selected model represented the most statistically-robust model available. In certain cases, this approach even improved and helped to select most sensitive toxicological endpoint, for example, o Page 85- Ji et al. (2008) conducted a chronic life-cycle test on the effects of PFOA (with *Moina macrocopa*). The *M. macrocopa* 7-day NOEC (reproduction: number of young per adult) reported by authors was 3.125 mg/L, the LOEC was 6.25 mg/L, and the MATC is 4.419 mg/L. EPA performed C-R analysis for this study and determined the number of young per starting female as the most sensitive endpoint with an acceptable C-R curve. The EPA-calculated EC_{10} was 2.194 mg/L PFOA for *M. macrocopa* and used it directly as the *Moina* GMCV. o Page 88 Yang et al., 2014 Chronic survival using EPA's method was more sensitive	

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	than reproduction endpoint related EC_{10} values reported by the authors. • In depth analyses and associated dose-response graphs in Appendix A.2 and Appendix C.2 provides further in-depth information on the EPA's approach for fitting concentration-response (C-R) data.	
Reviewer 5	In general, the data selected or excluded for criteria development were appropriately used. A study published by McCarthy et al. in 2021 reports toxicity of PFOA to Chironomus dilutus – if I recall correctly, I believe these data (on an insect) indicate that PFOA is not very toxic to C. dilutus…even for exposures greater than 96 hours. This agrees with EPA's assessment of available acute toxicity data for freshwater insects; that PFOA is generally not toxic to insects. I would, however, still urge more studies on aquatic insects as this is an obviously diverse taxa with many sensitive species. It is very possible that the available test species of insects are not sensitive but other insects such as mayflies or damselflies, etc. may, in fact, be quite sensitive. The write up of the Zhang et al. (2013 and 2014) papers has a few grammatical errors and could benefit from additional editing for clarity. I agree that using the intrinsic rate of increase (or similar) is a very relevant endpoint but it reads as though this was based on a 4 day observation period for the 2014 paper. This does not quite make sense to me…offhand, I would hypothesize that the reported/calculated effect level of 1.166 mg/L would decrease with a longer observation period. I think I may be misunderstanding the experimental design – some editing would help clarify. This is an important series of studies	All toxicity studies provided by Reviewer 5 (and all other reviewers), including McCarthy et al. (2021), were all reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc., and were included in the PFOA Aquatic Life Criteria document as appropriate, based on their data quality. Thank you for your comment, agreeing with EPA's assessment of the relative tolerance of *C. dilutus* to acute PFOA exposures based on data available at the time of the draft PFOA Aquatic Life Criteria document. EPA agrees further insect toxicity testing would be beneficial. The Zhang et al. (2013, 2014) summaries in the updated PFOA Aquatic Life Criteria draft have been edited for clarity. Regarding the differences in the intrinsic rate of increase (r) endpoint observation periods between the two studies, it is correct that the calculation of "r" was based on full lifecycle observations (5.5-6.0 days for all treatments except for the second highest treatment level, which was 8.3 days) in Zhang et al. (2013), and following a four-day experiment in Zhang et al. (2014). In Section 3.2 (p. 117) of Zhang et al. (2014), the authors argue that four days is of sufficient duration to calculate "r" for the following reasons: "*The tested rotifer B. calyciflorus can produce multiple broods and the F1 generation also produces neonates in 4 d as we have observed during a long observation period. This meets the*

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	(see below regatding resting egg production) so clarity is critical. NOTE: the EC_{10} of 0.076 mg/L for resting egg production observed by Zhang et al. (2014b) is potentially a big deal. EPA appears justified in not using this because it was only one replicate, etc. but these data clearly point to a potentially relevant effect at a relatively low concentration. **2.5.a.** **2.5.a.i** The EPA followed the 1985 guidelines allowing them to calculate the FCV using the ACR approach as outlined around p. 101. Given the lack of data, this seems like a reasonable approach. The actual calculation of the FCV (based on the data from B. calyciflorus) is appropriate and the use of this FACR to determine the FCV of 0.3054 is appropriate. Morevoer, as stated above, this value is also generally in line with other criteria for PFOA published by other jurisdictions. **2.5.a.ii** I commented on this above but will mention again. Overall, I think EPA is correct that the available data on chironimids (Yange et al. 2014 and McCarthy et al. 2021) indicate that chironomids are not sensitive to PFOA. That said, it is probably the case that other insects such as mayflies or damselflies (or other species?) are more sensitive than chironomids. In contrast to this statement, however, is that chironomids are among the most sensitive to PFOS. Bottom line: for PFOA and available insect toxicity data, it appears PFOA is not toxic to insect but, clearly,	*requirements of reproductive bioassays (Snell and Moffat, 1992). In addition, a period of four days is sufficiently long for compounds to achieve steady-state body burdens (Jones and de Voogt, 1999) making a 4 d test in favor of predicting effects of chronic exposure*." Because the chronic exposure durations used to calculate "r" in Zhang et al. (2014) and Zhang et al. (2013) were relatively similar, in combination with the stated appropriateness of the 4-day exposure duration, EPA has retained use of the chronic EC_{10} (endpoint of intrinsic rate of natural increase) from Zhang et al. (2013) and Zhang et al. (2014) to calculate the *B. calyciflorus* SMCV. Regarding the resting egg production endpoint, EPA acknowledges the potential importance of the resting egg production endpoint and thanks Reviewer 5 for noting, "*EPA appears justified in not using this because it was only one replicate*." Specific to comments to Charge Question 2.5.a: 2.5.a.i Thank you for noting the reasonableness of the FACR used to derive the FCV and reiterating the relative similarities in magnitude between EPA's draft chronic water column PFOA criterion and "*other criteria for PFOA published by other jurisdictions*." Addition of new chronic toxicity data in the updated draft PFOA criteria document allowed for the calculation of a draft chronic freshwater criterion magnitude directly from the chronic GSD rather than basing the magnitude on the FAV transformed by the FACR, as was done in the draft PFOA criteria document that underwent peer review. 2.5.a.ii All toxicity studies provided by Reviewer 5 (and all other reviewers), including McCarthy et al. (2021), were all reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines, EPA 850 test guidelines, etc., and were included in the PFOA Aquatic Life Criteria

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	more data are needed to improve confidence in this estimate. **2.5.b.** EPA's approach to fitting C-R data using the drc package in R is, in my opinion, state of the art. The method can easily test a variety of curves and the fit criteria can be used to select the best fitting curves. Comments related to specific studies and LC_{50} and EC_{10} estimates are elsewhere in these comments. In general, my opinion is that the PFOA criteria are slightly more defensible than the PFOS criteria; this is explained more in the review of PFOS.	document as appropriate, based on their data quality. EPA thanks Reviewer 5 for their comment noting that they agree with EPA's assessment of the relative tolerance of *C. dilutus* to acute PFOA exposures based on data available at the time of the draft PFOA Aquatic Life Criteria document. EPA agrees further insect toxicity testing would be beneficial. Specific to comments to Charge Question 2.5.b: Thank you for your comment and for denoting the scientific defensibility of the PFOA criteria. Responses to comments related to specific studies and LC_{50} and EC_{10} estimates that are referenced by Reviewer 5 are provided elsewhere in this document.

2.6 Please comment on the translation of the chronic water column criterion elements for aquatic life to derive the tissue-based criterion elements, considering the bioaccumulation of PFOA and PFOS. In particular, please comment on:

2.6.a. Uncertainty surrounding the bioaccumulation factors (BAFs) used to translate of the chronic water column criterion elements into tissue-based criterion elements.

2.6.b. EPA's determination of appropriate BAFs and the tissue types that the tissue criterion elements were based.

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Reviewer	**Comments**	**EPA Response**
Reviewer 1	The derivation of the tissue criteria in this manner is highly uncertain. To my knowledge this is the first time EPA has applied ambient water quality criteria protective of aquatic life direct toxicity with uptake factors (bioaccumulation factors (BAFs), bioconcentration factors (BCFs)) in this manner to calculate tissue criteria. References are made to the selenium tissue criteria, but those are used in the reverse (i.e., criteria based on measured concentrations in tissue used to calculate water criteria). The use of criteria for water with a assumed uptake factor carries a large amount of uncertainty, and in general, the use of measured concentrations in tissue linked to adverse effects is a more straightforward approach since it does not involve uptake model predictions. This needs to be noted in the text. Also, are the predicted tissue criteria meant to be a temporary stop-gap until tissue effect data become available? This should be discussed and clarified. 2.6.a. The use of BAFs derived from field studies is inherently uncertain due to the wide variety of techniques used in the compiled studies, their analytical data quality, the differences in species and ecosystems, experimental designs, spatial uncertainties for mobile animals like fish, etc. That being said, the use of a BAF value (or BCF) in	Thank you for your comment indicating that the derivation of the tissue criteria by translating the chronic freshwater column criterion to tissue concentrations with the use of BAFs is highly uncertain. Reviewer 1 is correct that the derivation of these tissue criteria was the reverse process of the previously derived criterion for selenium, which instead translated a fish tissue criterion into corresponding lentic and lotic water column criteria. The derivation of the PFOA tissue criteria were translated in the manner presented in the draft criteria document because measured effect concentrations in tissue were limited, with no quantitatively acceptable and only 5 qualitatively-acceptable toxicity studies (across four publications) evaluating tissue-based effects. Therefore, there were insufficient data to derive a chronic tissue criterion using a GSD approach from empirical tissue data. These details were provided in Section 3.2.2 of the draft PFOA Aquatic Life Criteria document. EPA also included a revised comparative discussion between the tissue-based criteria magnitudes and the limited empirical tissue data in Section 4.5 of the draft PFOA Aquatic Life Criteria document. Section 4.5 of the draft PFOA Aquatic Life Criteria document states: "*Tissue-based PFOA toxicity data were reported for four species (three fish and one frog species) across five publications, all of which were classified as qualitatively acceptable…Tissue PFOA concentrations reported in these qualitative studies were lower than*

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	criteria derivation is consistent with other criteria developed by EPA. As noted above, the use of the tissue criteria needs to be considered carefully, and I think empirical tissue data from toxicity experiments should form the basis of a next iteration of a tissue criteria. 2.6.b. The development of BAFs for invertebrates, fish (whole body), and fish (muscle) seems reasonable for the application in estimating a draft or interim tissue criteria until empirical tissue data can be used to calculate tissue criteria directly	*the tissue-based criteria calculated from BAFs. However, no statistically significant effects of apical endpoints were observed in any of these studies. Results of these studies do not provide any evidence that the aquatic community will experience unacceptable chronic effects at tissue-based criteria magnitudes."* Finally, text has been added to the revised PFOA Aquatic Life Criteria document to highlight the uncertainty of the tissue-based criteria relative to the water column-based criterion. The revised draft PFOA Aquatic Life Criteria document now states: *"The freshwater chronic water column criterion is more strongly supported than the chronic tissue-based criteria because the water column-based chronic criterion was derived directly from the results of empirical toxicity tests. The chronic tissue-based criteria are relatively less certain because they were derived by transforming the chronic water column criterion into tissue concentrations through BAFs, with any uncertainty and variability in the underlying BAFs then propagating into the resultant tissue-based criteria magnitudes."* Specific to comments to Charge Question 2.6.a: EPA acknowledges the inherent uncertainties resulting from the use of BAFs to derive tissue criteria. These uncertainties are present given the differences in analytical methods used, the specific species and habitats with paired tissue and water column measurements, and experimental designs utilized across studies. For these reasons, EPA screened the BAF literature in a manner consistent with the evaluation criteria outlined in Burkhard (2021) and focused on factors relating to: 1) number of water samples collected, 2) number of organism samples collected, 3) water and organism temporal coordination in sample collection, and 4) water and organism spatial coordination in sample collection. Additionally, the general experimental design was evaluated. Further, these screening criteria

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		were consistent with those used for paired concentrations (both tissue and water and tissue and diet concentrations) in the 2016 Selenium Aquatic Life Criterion for Freshwaters (U.S.EPA 2016a). These screening details are provided in Table 2-3 of the draft PFOA criteria document that underwent external peer review. These screening criteria for the BAF data reduce the impacts of the inherent uncertainties that are present with the use of BAFs to derive tissue criteria. Section 4.5 of the draft PFOA Criteria Document described a comparative analysis between empirical tissue-based toxicity data and the draft PFOA tissue-based criteria, which did not provide any evidence that the aquatic community will experience unacceptable chronic effects at tissue-based criteria magnitudes. Additionally, EPA recognizes differences between field-derived and experimentally-derived (or those linked to adverse effects) BAFs. Despite the uncertainties noted in Reviewer 1's comments, EPA only used field-derived BAFs to derive the tissue criteria for PFOA. Use of field-derived BAFs is consistent with previously derived criteria for both aquatic life (the 2016 Selenium Aquatic Life Criterion for Freshwaters; U.S.EPA 2016a) and human health (U.S.EPA 2000). Although field-derived BAFs have inherent uncertainties discussed above, field-derived BAFs better represent real-world bioaccumulation of contaminants, including PFOA, through the aquatic food web. Specific to comments to Charge Question 2.6.b: Thank you for your comment regarding the reasonableness of the development and use of fish and invertebrate BAFs. Currently there are insufficient data to derive chronic tissue criteria using a GSD approach from empirical tissue data from toxicity studies. However, as described in EPA's response to this comment above, the limited empirical tissue concentrations were compared to the translated tissue criteria magnitudes and there was no evidence that the aquatic

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		community will experience unacceptable chronic effects at tissue-based criteria magnitudes. EPA agrees additional empirical tissue data linked to adverse effects would be helpful to better understand the translated tissue criteria and/or to develop chronic tissue criterion using a GSD approach from empirical tissue data from toxicity studies.
Reviewer 2	2.6.a. There appears to be an error in sub-section 2.11.3; "The resulting tissue-based criteria magnitudes correspond to the tissue type from the geometric mean BAF used in the equation (see Section 2.12.3.1)." I cannot locate sub-section 2.12.3.1. I assume the authors meant sub-section 2.11.3.1? Sub-section 2.12.3 and sub-section 2.12.3.1 are also referenced on pages 105 & 106, respectively. I think the EPA has sufficiently addressed the uncertainty around the use of BAFs and the chronic water column criterion in the derivation of tissue-based criterion. They have indicated that tissue-based criterion should only be observed once in 10 years. The use of the geometric mean of the reported BAFs incorporates the range of BAFs that may be present for different invertebrate and fish species. The use of the chronic water column criterion also builds in added conservatism to the tissue-based criterion. Prosser et al. (2016) reported BAFs for PFOA in three freshwater species (two invertebrates and one fish) (See Tables S29-31 in Supplementary Information), but it was not considered in this	Thank you for your comment regarding the translation of the chronic freshwater criterion into tissue-based criteria with the use of BAFs. Specific to comments to Charge Question 2.6.a: Section 2.12.3.1 should have read Section 2.11.3.1. The section cross-references have been updated. The BAFs used to calculate the tissue criteria were obtained from the BAF database created Lawrence Burkhard in support of his publication: Burkhard, L.P. (2021) Evaluation of Published Bioconcentration Factor (BCF) and Bioaccumulation Factor (BAF) Data for Per- and Polyfluoroalkyl Substances Across Aquatic Species. ET&C 40: 1530-1543. BAFs reported in Prosser et al. (2016) were not in the Burkhard (2021) database and were, therefore, not used to calculate BAFs. Upon review of Prosser et al. (2016), this study was not included in the BAF database developed by Burkhard (2021) because this study reported biota sediment accumulation factors (BSAFs) instead of BAFs. It is for this same reason EPA did not include Prosser et al. (2016) among the BAFs that were used to derive the tissue criteria for PFOA. Specific to comments to Charge Question 2.6.b:

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	assessment. It is not clear why it was not considered. Prosser, R.S., Mahon, K., Sibley, P.K., Poirier, D., Watson-Leung, T., 2016. Bioaccumulation of perfluorinated carboxylates and sulfonates and polychlorinated biphenyls in laboratory-cultured Hexagenia spp., Lumbriculus variegatus and Pimephales promelas from field-collected sediments. Science of The Total Environment 543, 715–726. doi:10.1016/j.scitotenv.2015.11.062 2.6.b. The evaluation criteria for BAFs outline in Table 2-3 are appropriate and the decision to only use high and medium quality BAFs is justified based on the criteria that would make a BAF low quality. It was a good idea to use fish BAFs based on the concentration in muscle and whole body. Muscle tissue is usually exclusively sampled in large fish, especially as part of fish consumption guidelines. The whole body is more appropriate for small fish and invertebrate species, e.g., minnows, benthic macroinvertebrates.	Thank you for your comment regarding the evaluation criteria that were used for the BAFs and the use of fish BAFs for whole-body and muscle.
Reviewer 3	This sentence is very confusing: "EPA examined the potential for criteria using only those studies in which test organisms were exposed to PFOA in their diet, because such studies would most closely replicate real-world exposures (diet and/or diet plus water)." The tissue criteria are based on water exposures, the relevance of this statement and evaluation is lost on me.	EPA agrees with Reviewer 3 that the original sentence was unclear. The sentence has since been revised to state: "*EPA considered deriving tissue-based criteria using empirical toxicity tests with studies that exposed organisms to PFOA in water and/or diet and reported exposure concentrations based on measured tissue concentrations*." EPA used the PFOA BAFs that were compiled by and can be found in Burkhard (2021). As such, tables summarizing the information requested in Reviewer 3's comment can also be found in the

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	A table summarizing the species used to derive the BAFs would be helpful to evaluate the comprehensiveness, i.e., pelagic vs sediment feeders.	supporting information of the paper (see: https://doi.org/10.1002/etc.5010). EPA also added an appendix to the draft PFOA Aquatic Life Criteria document that described the BAFs used in development of the tissue-based criteria magnitudes.
Reviewer 4	2.6.a. Tissue criteria derived from the chronic water column concentration (CCC) with the use of bioaccumulation factors were developed by EPA. The chronic fish whole-body tissue criterion is 54.1 mg/kg wet weight, the chronic fish muscle tissue criterion is 9.37 mg/kg wet weight and the chronic invertebrate whole-body tissue criterion is 23.9 mg/kg wet weight. The freshwater chronic PFOA toxicity data with measured tissue concentrations was limited, with no quantitatively acceptable tissue-based tests. Qualitatively acceptable tissue-based tests were reported for four species (three fish species and one amphibian) across five publications. Therefore, there were insufficient data to derive tissue-based criteria using a GSD approach from empirical tissue data from toxicity studies. EPA thus developed protective tissue-based criteria through a bioaccumulation factor approach (Burkhard 2021). Only BAFs of high and medium quality were used to derive the tissue criteria. BAFs used in the derivation of the PFOA tissue-based criteria consisted of > 2 water and organism samples each and were collected within one year and 2 km distance of one another. Criteria for protection of aquatic life and wildlife will need to use whole-organism BAFs because the criteria are based on	Thank you for your comment summarizing the translation of the chronic freshwater criterion to tissue criteria using PFOA BAFs. Specific to comments to Charge Question 2.6.a: The draft PFOA aquatic life criteria were derived to be protective of aquatic life and did not explicitly consider aquatic-dependent wildlife. If a potential aquatic-dependent wildlife criterion were derived in the future it would be preferentially based on empirical tissue-based toxicity data to evaluate effects. Evaluation of exposure potential for these species, however, would likely consider PFOA exposures in diet through the consumption of whole animals, in which case, use of whole-body BAFs would be appropriate (among other possible options for evaluating exposure via diet such as modeling approaches). The freshwater tissue-based criteria are all equally protective of the aquatic community whether they are expressed as fish whole-body, fish muscle, or invertebrate tissues because these tissue-based criteria were all translated from the same chronic effects-based water column concentration (i.e., chronic water column criterion). Specific to comments to Charge Question 2.6.b: EPA did not derive PFOA aquatic life criteria in tissues because these are the tissues consumed by humans; the draft PFOA Aquatic Life Criteria were derived to protect aquatic communities from acute and chronic PFOA exposures and are not intended to be protective of human health endpoints. Criteria were derived for fish whole-body, fish muscle, and invertebrate tissues because these are the tissue types

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	whole-body toxicology for aquatic life (e.g., fish) and for wildlife (e.g., birds), both of which consume the whole fish (Stephan et al. 1985). 2.6.b. Within the body, PFOA tends to bioaccumulate within protein-rich tissues, such as the blood serum proteins and liver. BAFs are different for muscle/fillet and whole-body tissues. Humans consume muscle/fillets from fish and soft tissues from bivalves, therefore the water quality criteria recommended by EPA used BAFs based on these tissues. EPA calculated additional tissue values for liver, blood, and reproductive tissues by transforming the freshwater chronic water column criterion (i.e., 0.31 µg/L) into representative tissue concentrations using tissue-specific bioaccumulation factors (BAFs). Furthermore, EPA team justified to use female reproductive tissues due to its relevance for potential maternal transfer to offspring. There additional tissue-based values were calculated for comparative purposes and were not proposed as recommended criteria.	most commonly monitored by States and Tribes. Additional tissue-based values were calculated and were presented in Appendix M of the draft PFOA Aquatic Life Criteria document for informational purposes.
Reviewer 5	2.6.a. Overall, this seems like a reasonable approach – to estimate tissue-based criteria using the water column criteria multiplied by the bioaccumulation factors. The difficulty arises when we consider the accuracy or robustness of the BAFs. I agree with the criteria in Table 2-3 and especially emphasize the importance of concurrent collections in space AND time for tissues and environmental media.	Specific to comments to Charge Question 2.6.a: Thank you for your comment regarding the translation of the chronic freshwater criterion into tissue-based criteria with the use of BAFs and your agreement with the BAF selection criteria in table 2-3 of the draft PFOA Aquatic Life Criteria document. Specific to comments to Charge Question 2.6.b:

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	2.6.b. I am very familiar with the Burkhard (2021) paper which the PFOA document follows closely in terms of BAFs. The BAFs used by EPA are appropriate given the data. I also agree that the most useful/appropriate tissues for BAFs are invertebrates, fish muscle and fish whole body – these are the most commonly analyzed and most abundant in the literature. For what it's worth, in my own research in which we collected and analyzed fish tissues and co-located water samples, our calculated BAFs for PFOA (and PFOS) were close to the central tendency BAFs reported by Burkhard (2021). I also agree that co-located and sampled at the same time yield the most defensible BAFs as PFAS concentrations can vary considerably in space and time (not often shown in the literature).	Thank you for your comment noting the appropriateness of the tissue types included in the draft PFOA criteria document. Thank you for noting your research in calculating PFOA BAFs yielded similar results to the central tendency BAFs reported by Burkhard (2021), which served as the BAF dataset for the draft PFOA aquatic life criteria.

2.7 Please comment on the frequency and duration of the criterion elements, in particular please comment on the frequency and duration components of the tissue-based criterion elements.

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Reviewer 1	The 4-day duration seems to be supported by the time scale of toxicity discussed for the limited chronic studies selected by EPA. This assumption should be revisited as more data become available (please note in the document). For the tissue-based criterion (page 107), there is no clear support for assuming a 10-year exceedance frequency. Given the uncertainty with the BAF-predicted tissue criteria, and how little is known regarding the recalcitrance of PFOA in aquatic ecosystems and recovery time if PFOA inputs in water were halted, the assignment of a 10-year exceedance frequency at this stage seems completely arbitrary. We simply do not yet know the time frame over which aquatic ecosystems recover from PFOA. It is not technically supported to cite recovery times for selenium to support a 10-year recovery time for PFOA, these are completely different toxicants that have their own unique fate and behavior. USEPA (1985) guidance suggests assuming a 3-year frequency as a default, and the discussion on page 17-108 is not scientifically convincing enough to modify it to 10 years. Additionally, it should be noted that the exceedance frequency for another organic chemical, Tributyltin (TBT) was set at 3 years by EPA in derivation of that criteria. TBT exhibits uptake factors similar to PFOA (i.e., BCF of approximately 2,000 L/kg, wet weight for goldfish, as noted in the EPA TBT criteria document, which is higher than the PFOA BAFs of 30-175 L/kg, wet weight being used to calculate the fish tissue criteria). TBT is also persistent in aquatic ecosystems, as noted by EPA. Given	Thank you for your comment stating the 4-day duration for the chronic water column criterion is appropriate based on available data. EPA agrees with reviewer 1 that "*We simply do not yet know the time frame over which aquatic ecosystems recover from PFOA*." However, we do know that PFOA is stable in water and air (UNEP 2015), and thus, unless the source is eliminated, PFOA is likely to remain in aquatic systems over time. Therefore, EPA considered the bioaccumulative nature and persistence of PFOA in aquatic systems, in combination with the documented recovery times of pollutants with somewhat similar chemical attributes (Lemly 1997; Gergs et al. 2016) set a reasonable and protective exceedance frequency for tissue-based PFOA criteria. As described by Reviewer 1, USEPA (1985) suggests a three-year exceedance frequency; however, the suggestion of three years in USEPA (1885) was intended to be for water column-based criteria. Tissue-based exposures exceeding criteria magnitudes cannot diminish at a rate of water column-based exposures and initiation of subsequent recovery is delayed. Therefore, it is logical that the exceedance frequency for tissue-based criteria for bioaccumulative pollutants (such as selenium; USEPA 2016) be longer than three years. Unlike the draft PFOA Aquatic Life Criteria, the Tributyltin (TBT) Aquatic Life Criteria (U.S. EPA 2004) does not include tissue-based criteria. Only the draft tissue-based PFOA criteria specified exceedance frequencies of 10 years. The 10-year frequency for tissue-based criteria for PFOA was set to provide time for tissue concentrations that accumulate through food webs to diminish, if possible, in source reservoirs lower in the food web before being

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	TBT is at least an organic chemical, it is a closer analog than selenium, which is an element. As such, the exceedance frequency for the PFOA tissue criterion should be set at the default of 3 years unless EPA can provide convincing technical information specific to recovery times for PFOA. Additionally, on page 108, the paragraph that begins with "Metals and other chemical pollutants such as PFOA..." is not convincing as any quantitative support for EPA's 10-year exceedance frequency for the chronic tissue-based criteria. The text as written may give the reader the conclusion that PFOA recovery may be "on the order of decades", as EPA notes for selenium. There is no support for the conjecture that PFOA recovery may be "relatively slow" or require decades, as noted in my above comment.	eliminated in higher trophic level species and allowing for subsequent potential ecological recovery. Unless data suggest otherwise, a three-year recovery interval remains appropriate for water column criteria where ecological recovery can begin when chemical concentrations no longer exceed the criteria magnitudes and durations. Consequently, the draft chronic water column-based criterion for PFOA and the chronic TBT criterion both specified a three-year exceedance frequency, as recommended by the 1985 Guidelines. Based on ecological recovery times for other bioaccumulative and persistent chemicals, ecological recovery times following elevated PFOA concentrations in the tissues of aquatic organisms is expected to be relatively long to allow for the dissipation of PFOA throughout the food web and subsequent recovery. The draft PFOA Aquatic Life Criteria document was revised in response to the comment from Reviewer 1. The full text reference by Reviewer 1 (with added strike throughs to represent deletions in response to Reviewer 1) is provided below for informational purposes: *"Metals and other chemical pollutants* ***~~such as PFOA,~~*** *may be retained in the sediment and biota, where they can result in residual effects over time that further delay recovery.* ***Long-term uptake and subsequent excretion rates of PFOA has been extensively studies in humans relative to aquatic life. Li et al. (2017) reported a median PFOA half-life of 2.7 years in human serum following exposure to PFOA in drinking water, which authors stated was in the range of previously published estimates. Due to chemical retention in tissues, ~~As a result,~~*** *ecosystems impacted by discharges of bioaccumulative pollutants (such as* ***~~PFOA or~~*** *selenium) recover from chemical disturbances at relatively slow rates. For example, Lemly (1997) concluded that although water quality in Belews Lake in North Carolina (a freshwater reservoir) had recovered significantly in the decade since selenium discharges were halted in 1985, the threat to fish had not been eliminated. The selenium dischargers that led to severe

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		reproductive failure and deformities in fish, was still measurable (fish deformities) in 1992 (seven years later) and in 1996 (ten years later). Lemly (1997, pg. 280) estimated based on these data that "the timeframe necessary for complete recovery from selenium contamination from freshwater reservoirs can be on the order of decades." *Beyond bioaccumulation, chemical-specific considerations such as degradation vs. persistence may also provide a mechanism influencing ecological recovery rates. The persistence of PFOA has been attributed to the strong C-F bond, with no known biodegradation or abiotic degradation processes for PFOA. Similarly, metals do not degrade and may persist in aquatic systems following elevated discharge. The persistence of metals may explain why metals had the second longest median recovery time of any disturbance described in a systematic review of aquatic ecosystem recovery (Gergs et al. 2016). Gergs et al. (2016) showed recovery times following metal disturbances ranged from roughly six months to eight years (median recovery time = 1 year; 75th centile ~ 3 years; n = 20)."*
Reviewer 2	As per Table 0-1, I think the chosen durations and frequencies for the acute and chronic criteria are appropriate. They will ensure protection of aquatic life. The duration of the tissue-based criterion is appropriate as the concentration will be measured when biota is collected. The 10 year frequency is appropriate considering that for biota to reach the tissue-based criteria, they would likely to have be exposed to concentrations at or above the chronic criteria for an extended period of time.	Thank you for your comment.
Reviewer 3	This is a not an easy statement to comment on, as it may be unlikely that the aquatic receptors will exceed or reach these tissue concentrations prior to exceedances from the CCC. Importantly, PFOA is not particularly	Thank you for your comment. Even if it is unlikely that aquatic receptors will exceed or reach these tissue concentrations prior to exceedances from the CCC (as suggested by Reviewer 3), EPA notes the draft PFOA Aquatic Life Criteria document stated:

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	bioaccumulative compared to PFOS and this likely a less sensitive threshold.	*"All of these water column and tissue criteria are intended to be independently applicable and no one criterion takes primacy. All of the above recommended criteria (acute and chronic water column and chronic tissue criteria) are intended to be protective of aquatic life."* Because the chronic freshwater criteria are independently applicable, they are protective of the scenario described by Reviewer 3. Although PFOA is less bioaccumulative than PFOS (as noted by Reviewer 3), EPA reiterates the PFOA tissue-based criteria were derived using PFOA-specific BAFs.
Reviewer 4	PFOA concentrations in tissues are generally expected to change only gradually over time in response to environmental fluctuations. The chronic tissue-based criteria averaging periods, or duration components, were therefore specified as instantaneous, because tissue data provide point, or instantaneous, measurements that reflect integrative accumulation of PFOA over time and space in population(s) at a given site. It was appropriate for EPA to inform the recommended ten-year exceedance frequencies for the chronic tissue-based criteria given the large variation in possible biological and physical variable influencing ecological recovery.	Thank you for your comment noting that "*It was appropriate for EPA to inform the recommended ten-year exceedance frequencies for the chronic tissue-based criteria*."
Reviewer 5	Conceptually, the frequency and duration of the criterion elements seem reasonable – the acute water column criterion can't be exceeded for more than one-hour of duration which is then not to be exceeded more than once in three years. Even though many of the effect levels are 50%, this is likely protective given the duration of most acute toxicity studies is certainly and convincingly more than one hour. This applies to the chronic water column	Thank you for your comment. EPA agrees with Reviewer 5 that the one-hour acute duration is protective since "*the duration of most acute toxicity studies is certainly and convincingly more than one hour*." EPA also agrees with Reviewer 5 that the chronic water column criterion duration is also protective because 4 days is shorter than typical chronic toxicity tests. The acute PFOA criterion is not based directly on 50% effect levels. EPA's PFOA FAV was derived following a long-established approach

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	criteria as well which is based on not exceeding a 4-day duration; most chronic studies are much longer than 4 days. The only real issue with this is that, for all intents and purposes, these frequency and duration elements are not measured in practice. In other words, I am unaware of hourly measurements of PFAS in water or 4-day running averages. So, conceptually, I believe the frequency and duration elements are protective. However, in practice it is not clear to me how these would be useful or would help with regulation because the data related to frequency and magnitude of PFAS in water is not at a fine enough temporal resolution. The frequency and duration for tissue-based criteria is a little different, however. Tissue concentrations (as mentioned in the document) represent an integration through time and so a measure of fish tissues, for example, provides some insight to exposures that have occurred over longer than an hour or 4 days. In this case, not exceeding the tissue-based criteria more than once in 10 years is likely protective. It is also more likely that monitoring programs would sample fish at least yearly which means this criteria is likely the most useful from a monitoring and clean-up perspective. It is still possible that high concentrations in tissues will be "missed" with only sampling once a year but this is far better than the frequency and duration elements for the water column criteria.	described in the 1985 Guidelines. Briefly, the PFOA FAV is based on the 5th centile of a GSD, which was comprised of GMAVs calculated from LC_{50} values. Consistent with the 1985 Guidelines, the FAV was then divided by 2.0 to calculate the CMC. Dividing the FAV by 2.0 ensures the CMC represents a concentration that will not affect a large portion of sensitive organisms. This is based on the established premise that the ratio between LC_{50} values and corresponding LC_{low} values (e.g., $LC_0 – LC_{10}$) is typically close to 2.0. Please see the excerpt from the 1985 Guidelines below for further explanation: "*the Criterion Maximum Concentration is now equal to one-half the Final Acute Value. The Criterion Maximum Concentration is intended to protect 95 percent of a group of diverse genera, unless a commercially or recreationally important species is very sensitive. However, a concentration that would severely harm 50 percent of the fifth percentile or 50 percent of a sensitive important species cannot be considered to be protective of that percentile or that species. Dividing the Final Acute Value by 2 is intended to result in a concentration that will not severely adversely affect too many of the organisms."* Similar to magnitudes, the duration and frequency components of criteria are based on exposure-response relationships and toxicological principles, irrespective of monitoring considerations. Absent of continuous monitoring data, EPA agrees that it may be difficult to assess PFAS concentrations in water bodies with enough temporal resolution to continually assess average acute concentrations over the course of a one-hour duration or average chronic concentrations over the course of four days. Nevertheless, States and Tribes have adopted and implemented water column-based water quality standards containing the standard acute 1-hour and chronic 4-day durations, as well as the 3-year frequency, dating back to the 1985 Guidelines. In addition to monitoring, duration and frequency components of criteria are particularly important for setting National Pollution Discharge

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		Elimination System (NPDES) permit limits (U.S. EPA 1991). For example, the 1985 Guidelines state: *"one of the most important uses of criteria is for designing waste treatment facilities. Such facilities are designed based on probabilities and it is not possible to design for a zero probability. Thus, one of the important design parameters is the probability that the four-day average or the one-hour-average will be exceeded, or, in other words, the frequency with which exceedences will be allowed*." Thank you for your comment indicating both the protectiveness and utility of the magnitude, duration, and frequency components associated with the tissue-based criteria.

2.8 Please provide any additional technical comments that you believe should be considered.

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Reviewer 1	I have the additional detailed comments: a) Please note that the comments provided in this file reflect a focus on of key portions of the "Draft of the Aquatic Life Water Quality Criterion…" document as directed by the above charge questions provided to me. Given time and resource constraints and the scope of my review, it was not feasible to provide a detailed review of the entire document and all of the supporting references and their associated results and conclusions. As such, I reserve my right to supplement or amend my comments in future, pending additional review or new information. Thank you for the opportunity to assist EPA in its work on this very important matter, and I was honored to be selected as a reviewer. b) Page 7: Please note in Figure 1-1 that this is the linear isomer of PFOA. It would be helpful to note that the PFOA data in this study are likely from experiments with water spiked with the linear PFOA isomer. It is hypothesized that toxicity and bioaccumulation may differ between branched and linear forms of PFCAs and PFASs. Linear PFOA is thought to be more accumulative (as noted on Page 61) and potentially more toxic to aquatic life when the dose is expressed as an external water concentration. At some sites, a portion of the concentrations of PFOA in water (which are reported as the sum of branched and linear PFOA) can be branched PFOA, so criteria derived from linear PFOA could be overly protective. Please include this uncertainty in the discussion in the document. c) Page 47: With regard to the discussion of biomagnification factors (BMFs), please acknowledge the Martin et al. 2003 paper where the BMF for fish was noted to be less than one. This paper is very helpful for understanding biomagnification from the diet in fish, as it was a controlled PFOA-spiked food study. d) Page 51: Starting here on this page and in the rest of this section, most of the units need to be specified for dry weight or wet weight for concentrations of	Thank you for your comments. Responses to corresponding alphabetical comments are provided below. a) Thank you for your comment. EPA has received the final peer review report from the contractor, and there is no ability to supplement or amend your comments in the final report. Thank you for your review. b) The caption to Figure 1-1 has been updated to specify that it is the linear isomer of PFOA. As stated in the draft PFOA Aquatic Life Criteria document, the criteria document provides a critical review of all aquatic toxicity data identified in EPA's literature search for PFOA, including the anionic form (CAS No. 45285-51-6), the acid form (CAS No. 335-67-1), and the ammonium salt (CAS No. 3825-26-1). Further, EPA added the requested text to the Measurement Endpoints section of draft PFOA Aquatic Life Criteria document to note that PFOA toxicity studies typically used the linear PFOA isomer for dosing with fewer studies using the branched isomer. c) Reviewer 1 is assumed to be referencing the publication; *Martin, J.*

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	PFOA in tissue. There were other instances of this error in the document as well. For units of every concentration of PFOA in tissue, please be sure to specify dry weight or wet weight. e) Page 61: Regarding "nearly 90% of measured concentrations fell within 20% of paired nominal concentrations", I believe that applies to the freshwater studies reviewed (please note). f) Page 64: The use of EC_{10} values instead of effective concentration 20% (EC20) values for chronic values is inconsistent with EPA's general practice for developing aquatic life values. The selection of EC_{10}s for the selenium criteria (EPA, 2016) was associated with the derivation of tissue guidelines. In the EPA (2016) document, EPA noted "EC20s have historically been used in the derivation of EPA criteria applicable to the water medium". As noted in the EPA (2016) selenium guidance EC_{10}s were selected over EC20s "given the nature of exposure and effects for this bioaccumulative chemical." Additionally EPA (2016) selected EC_{10} for selenium because "it was found that the dose-response curves for selenium across a broad range of fish genera are very steep, such that a small change in selenium tissue concentration yielded a large increase in observed adverse effect." g) First, all the derivation of aquatic life criteria for "bioaccumulative chemicals" have not followed the process used for selenium, and there is no quantitative discussion in the current document that compares the bioaccumulation values for selenium to those of PFOA in a manner than justifies the use of EC_{10}s. For example, EPA in its 2016 aquatic life criteria for cadmium noted that cadmium "can bioaccumulate in aquatic organisms", but EC20s (not EC_{10}s) were used as chronic values in the derivation of aquatic life criteria in that document. Fundamentally, there is a logical disconnect between adding additional conservativism (i.e., using EC_{10}s instead of EC_{20}s) simply because a chemical has a higher bioaccumulative potential than another chemical or exceeds a BCF or BAF criteria used to determine a chemical has "bioaccumulative" status by typical chemical registration guidelines. The use of chronic exposure toxicology data generally assumes that concentrations in the organisms have reached steady	*W., S. A. Mabury, K. R. Solomon and D. C. Muir. 2003a. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry: An International Journal. 22(1): 189-195.* Following page 47 of the draft PFOA criteria document (referenced by Reviewer 1), page 48 of the draft PFOA criteria had cited Martin et al. 2003a. Please see text below from the draft PFOA Aquatic Life Criteria document that referenced Martin et al. (2003a) when referring to the lack of PFOA biomagnification in aquatic food webs: "*PFOA is nearly always shown not to biomagnify (Loi et al. 2011; Martin et al. 2004; Tomy et al. 2004; Xu et al. 2014; Zhou et al. 2012), unless aquatic-dependent species, such as aquatic-dependent birds, are included in the food web model (Houde et al. 2006b; Kelly et al. 2009; Tomy et al. 2009). The overall lack of biomagnification in PFOA relative to PFOS is attributed to its physical-chemical properties, including a shorter perfluorinated chain length and the carboxylate head group, both of which are*

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	state and, and thus, any bioaccumulation that has occurred is accounted for and manifests in toxic action. Coincidentally, the general assumption is that toxic responses have plateaued as well and that effective doses (measured via external concentrations in water or concentrations in the organism) will not change significantly with additional exposure time. The bioaccumulative nature of the toxicant at that point is a moot point with regards to toxic effects in an aquatic organism, so there seems no need to add additional conservatism in the estimation of a threshold for potential ecologically-significant effects on aquatic life. Adding additional conservatism to the aquatic life criteria to protect other trophic levels (i.e. wildlife that consume aquatic life) or human consumers of aquatic life, which does involve bioaccumulation of chemicals in aquatic organisms, is not justified. Criteria to protect wildlife and humans exposed via exposure pathways involving bioaccumulation of chemicals in aquatic life are handled via separate approaches, and are completely disconnected from the acute and chronic toxicity data developed to evaluate the risks to aquatic invertebrates and lower trophic level vertebrates like fish and amphibians. h) Second, EPA has not provided any analysis of the dose response curves that demonstrates the need for EC_{10}s versus EC20s (as was mentioned for selenium). Additionally, justification of the use of EC_{10}s by simply referencing the regulatory policies of other countries seems to be insufficient as the basis for a US policy, and is unsatisfying from a scientific perspective. i) More discussion is needed to support the poorly-supported move from EC20s to EC_{10}s, or alternately, EC20s need to be used in throughout the document, as consistent with past EPA practice in aquatic life criteria derivation. EC_{10}s are more conservative than EC20s, but there is often greater variability and uncertainty associated with EC_{10} values given the typical 50% effect ranges that are generally targeted in the experimental designs of typical toxicological studies. Additionally, as noted in EPA's 2016 aquatic life criteria document for cadmium, EC_{10}s are "rarely statistically significantly different from the control treatment." A 20% effect has often been discussed as a point of departure of ecologically-significant population- and community-level effects (e.g., Suter, 2000: Suter, G.W.,	*associated with less efficient assimilation into tissues and faster excretion rates (e.g., Martin et al. 2003a, 2003b).*" d) EPA ensured all tissue concentrations reported in the draft PFOA Aquatic Life Criteria document were reported in wet or dry weight (ww or dw, respectively). EPA specified that the information was not provided instances where authors did not specify whether or not tissue concentrations were reported in ww or dw. e) Reviewer 1 is correct. Text on page 61 has been updated to include the underlined text (see below) and more clearly state the paired nominal and measured concentrations were from freshwater tests: "*Linear correlation between measured and corresponding nominal concentrations in freshwater suggests a high degree of precision between paired observations across all test conditions and nearly 90% of measured concentrations fell within 20% of paired nominal concentrations in <u>freshwater toxicity tests</u>, which represent the test acceptability threshold identified by EPA's OCSPP's*

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	Efroymson, R.A., Sample, B.E., & Jones, D.S. (2000). Ecological Risk Assessment for Contaminated Sites. CRC Press. April). j) Overall, the adoption of a more conservative 10% effect level (i.e., EC_{10}) for chronic values used in criteria calculation carries large environmental management and policy implications. As noted above, clarification and careful justification is needed. EPA needs to clearly articulate (ideally with ample scientific support) why the additional conservatism is needed. This important potential policy matter deserves an open and earnest discourse among the scientific, stakeholder, and regulated communities. k) Page 67: It appears that only studies in which organisms exposed via diet were included for evaluation of tissue criteria. Is this correct? It is questionable to exclude effect concentrations in tissue from experiments in which exposure of PFAS was only via water. EPA (2016) took the "dietary exposure only" approach with selenium because the primary exposure route for selenium has been shown to be via the diet in natural ecosystems. In contrast, for many aquatic animals (especially lower trophic level fish and invertebrates), a significant portion of the exposure to PFOA is via non-dietary pathways. Part of this is due to the fact that controlled studies (e.g., Martin et al., 2003 studies cited in the document) have found that water-to-organism BCFs for aquatic life such as fish are generally larger than diet-to-organism biomagnification factors (BMFs). Additionally, there is no reason to expect dietary or non-dietary exposure pathways would affect toxic responses given the relatively rapid internal kinetics of PFAS in aquatic life (i.e., half-life of hours or days), especially for small invertebrates and fish that are in relative equilibrium with their surrounding exposure water. l) Page 82: The intrinsic rate of increase (basis of the chronic value from the Zhang et al. (2013) study is not a typical endpoint used in aquatic life criteria derivation. This chronic value has a large influence over the criteria calculated in this document, as noted below. More detail is needed on the calculation of the endpoints used in the two Zhang et al. papers, and some additional information should be provided on what this endpoint means ecologically in comparison to typical reproductive endpoints used in criteria	*Ecological Effects Test Guidelines.*" f - j) Response applies to comments "f" through "j." EPA has retained use of chronic EC_{10} values to ensure species protection, considering the long-term persistence of PFOA in environmental media. Further, use of a 10% effect concentration for deriving chronic criteria magnitudes is also consistent with the harmonized guidelines from OECD and the generally preferred effect level for countries such as Canada, Australia, and New Zealand (CCMC 2007; Warne et al. 2018). EPA also retained use of EC10 values to further afford protection of aquatic life from this bioaccumulative "forever" chemical. Additionally, steady state is not an assumption of chronic tests, particularly for chemicals that take longer to reach equilibrium. Chronic tests simply measure the effects of longer-term, lower exposures, that don't generally outright produce lethality. k) EPA agrees the statement referenced by Reviewer 5 was unclear. The text within the revised PFOA criteria document has been updated to state: "*EPA considered deriving tissue-based criteria using empirical toxicity tests with*

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
	derivation. Note that for stable populations, the intrinsic rate is 0, so achieving a maximal rate of increase is not always ecologically sustainable. Some additional explanation would be helpful to the reader. m) Page 98: Both the Criterion Maximum Concentration (CMC) and Criterion Continuous Concentration (CCC) are heavily influenced by toxicity tests on a single organism (rotifers, Brachionus calyciflorus) conducted by Zhang et al. (2013) and Zhang et al. (2014). For example, the rotifer acute value is the second lowest value of the four values used to calculate the FAV and CMC. The ACR used to calculate the CCC (using the with the FAV) is derived entirely from the Zhang et al. (2013) study (Page 101). I would recommend the ACR be recalculated to reflect more of a central tendency estimate that incorporates other ACRs, rather than relying solely on the Zhang et al. (2013) result (see above 5a comment). Regardless, I think a few sentences should be added to note the strong influence of these rotifer studies on PFOA criteria. Given this, additional experimentation and verification of the Zhang et al. results seems would seem to be useful and this is worth mentioning in the document as well. n) Page G-1: Please explain the acceptable duration acceptable for tests for which "Duration too short" is noted. It would be good to provide the acceptable durations that would be considered acceptable for these species. Perhaps a summary table for acceptable durations for particular endpoints could be provided in this document. o) Appendix G: The endpoints such as "decrease mRNA expression levels of neural genes DjFoxD, DjotxA and DjotxB" and "decrease in inflammatory cytokines (IL-1ß and IL-21) in spleen" are atypical. These should be noted under "Deficiencies". p) Page H-2: First use of "atypical duration" in the table. This entry is inconsistent with other entries (e.g., "duration too short") and does not clearly describe why the experiment is not considered. Please explain this table entry.	*studies that exposed organisms to PFOA in water and/or diet and reported exposure concentrations based on measured tissue concentrations*." l) The intrinsic rate of increase ("r"), while not a typical endpoint in criteria derivation for many species, has been used as a reproductive endpoint for rotifer taxa, which have rapid generation rates, in previous criteria. While a conventional reproductive endpoint measures some aspect of population growth (e.g., egg production, young per adult), the intrinsic rate of natural increase is actually more comprehensive, accounting for all births and deaths over a given time interval, as it is expressed as the net change in a given population per day. In toxicity studies, it is commonly applied to rotifers, as their generation time is at a similar time scale as many toxicity studies. Although "r" is typically near or at zero for a stable population for a long-lived species, in an experimental setting with a short-lived, rapidly reproducing species, intrinsic rate of increase is an appropriate measure of reproduction, as test animals are not

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
		limited by food or physical space during the test duration. m) EPA responded to the selection of the FACR in detail in Section 2.5.a.1. Addition of new chronic toxicity data in the updated draft PFOA criteria document allowed for the calculation of a draft chronic freshwater criterion magnitude directly from the chronic GSD rather than basing the magnitude on the FAV transformed by the FACR, as was done in the draft PFOA criteria document that underwent peer review. n) Table G.1 of the draft PFOA Aquatic Life Criteria document provided summary information (including test durations) for those tests that were considered qualitatively acceptable. Appendix G.2 subsequently provided detailed summaries of all studies referenced in Table G.1. Acceptable acute and chronic study durations (including taxa-specific test protocols) can be found established test protocols/methods, that were referenced in EPA's draft PFOA Aquatic Life Criteria document. The

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
		draft PFOA Aquatic Life Criteria document specifically stated: "*Studies were then further reviewed by EPA, Office of Water (OW) to determine test acceptability for use in criteria derivation. Studies that did not fully meet the data quality objectives outlined in the 1985 Guidelines, EPA's Office of Chemical Safety and Pollution Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S. EPA 2016b), and EPA OW's internal data quality standard operating procedure (SOP; which are consistent with OCSPP's data quality review approach; U.S. EPA 2018) were not considered for inclusion in the criteria derivation, including some studies with other PFAS exposures, but were considered qualitatively as supporting information and are characterized in the Effects Characterization.*" o) Within table G.1 and H.1 (qualitatively acceptable freshwater and estuarine/marine toxicity data), the deficiencies column now includes "non-apical endpoint" for studies that did not report apical endpoint,

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
		including the examples described by Reviewer 1. p) Use of "*atypical duration*" occurred five times within Table H.1 and once in Appendix H.2 (i.e., summaries of studies in Table H.1) and did not occur elsewhere in to draft PFOA Aquatic Life Criteria document. Instances where "*atypical duration*" was used in Table H.1 described tests with exposure durations of either 6 or 7 days. In these instances, Table H.1 has been revised to state: *"Exposure duration too short for chronic test and too long for acute test."*
Reviewer 2	I think the EPA's criteria for PFOA are very defensible based on the science and data available. I think they did a great job clearly laying out how they derived the criteria and providing all of the data that was used in the derivation.	Thank you for your comments. All previously mentioned comments have been responded to and addressed accordingly in the updated PFOA draft.
Reviewer 3	All technical comments have been previously mentioned	Thank you for your comments.
Reviewer 4	Some additional edits/suggestions are listed below: **1-** Appendix list in Table of Contents in missing **2-** List of Tables not matching with Tables listed in the text Table 2-4. is Table 2.1 Measured Perfluorooctanoic acid (PFOA) Concentrations in Surface Waters Across the United States. . **Error! Bookmark**	Thank you for your comments. Responses to corresponding numerical comments are provided below.

<table>
<tr><th colspan="3">2.8. Additional Technical Comments to Consider</th></tr>
<tr><th>Reviewer</th><th>Comments</th><th>EPA Response</th></tr>
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not defined.

Table 2-5. is Table 2.2 Summary of Assessment Endpoints and Measures of Effect Used in the Criteria Derivation for PFOA. 74

Table 2-6 is Table 2.3 . Evaluation Criteria for Screening Bioaccumulation Factors (BAFs) in the Public Literature. Error! Bookmark not defined.

3- Page xiv Table 0-1. Recommended Freshwater Perfluorooctanoic acid (PFOA) Aquatic Life Ambient Water Quality Criteria-
Superscript 3 [3] listed as footnote in the Table but not referenced in the table

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

<table>
<tr><th>Assessment Endpoints for the Aquatic Community</th><th>Measures of Effect</th></tr>
<tr><td>Aquatic Life: Survival, growth, and reproduction of freshwater and estuarine/marine aquatic life (i.e., fish, amphibians, aquatic invertebrates)</td><td>For effects from acute exposure:
1. LC_{50} concentrations in water, diet, and/or tissue (e.g., muscle, blood, egg)
2. NOEC and LOEC concentrations in water, diet, and/or tissue (e.g., muscle, blood, egg)

For effects from chronic exposure:
1. EC_{10} concentrations in water, diet, and/or tissue (e.g., muscle, blood, egg)</td></tr>
</table>
</td><td>
1. A list of appendices is now included at the beginning of the document.

2. The list of tables has been corrected.

3. Table 0-1 of the draft PFOA Aquatic Life criteria document that underwent Peer Review contained a superscript "*3*" following "*Instantaneous*" within the table.

4. Table 2-2 has been revised to only include assessment endpoints and measures of effect as they pertain to water column concentrations, since only water-column exposures were used to derive the draft PFOA criteria.

5. Table 1.1 has been updated to include current information for Australia and New Zealand.

6. Section 1.2 of the PFOA Aquatic Life criteria document has been updated to refer to OECD (2021), specifically stating:

"EPA's Office of Pollution Prevention and Toxics (OPPT) defines a PFAS chemical as: "a structure that contains the unit R-CF2-CF(R')(R"), where R, R', and R" do not equal "H" and the carbon-carbon bond is saturated (note: branching, heteroatoms,
</td></tr>
</table>

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
	<table><tr><td></td><td>2. NOEC and LOEC concentrations in water, diet, and/or tissue (e.g., muscle, blood, egg); Only used when an EC_{10} could not be calculated for a genus.</td></tr></table> Please review if the highlighted muscle, blood and egg would be relevant to this section. **5- Section 1.1 Previously Derived PFOA Toxicity Values and Thresholds** Table 1.1 to be updated by current information for Australia and new Zealand. Freshwater values are to be used on an interim basis until final marine guideline values can be set using the nationally-agreed process under the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Marine guideline values developed by CRC CARE are under consideration through the nationally-agreed water quality guideline development process. The Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG, 2018; ANZWQG, 2000) and Australia's PFAS Environmental Management Plan (HEPA, 2020) both recommend that a 99% species protection level be used as a guideline for bioaccumulating substances such as PFOS, PFOA, and PFHxS (as given below) as a precautionary approach.	*and cyclic structures are included).*" No edits were made to Table 1-2 of the draft PFOA Aquatic Life Criteria document as this table reflects the terminology in OECD (2021), specifically the general terms used to simply categorize PFASs based on simple traits in Figure 11 of OECD (2021). 7. Table 1-3 was updated to reflect the latest PFAS nomenclature provided in OECD (2021), specifically the addition of PFAAs that were not considered to be PFASs previously by Buck et al. (2011) are now included in Table 1-3 based on those PFAAs identified in Figures 9 and 10 of OECD (2021). 8. The effects symbol in the conceptual model now states: "*Deformities, Reproductive and Growth Impairments, Mortality*." 9. The cross reference has been corrected.

<table>
<tr><th colspan="3">2.8. Additional Technical Comments to Consider</th></tr>
<tr><th>Reviewer</th><th>Comments</th><th>EPA Response</th></tr>
<tr><td></td><td>
<table>
<tr><th>Exposure scenario</th><th>PFOA</th><th>Exposure scenario</th><th>Comments and source</th></tr>
<tr><td rowspan="4">Freshwater</td><td>19 µg/L</td><td>99% species protection - high conservation value systems</td><td rowspan="4">Australian and New Zealand Guidelines for Fresh and Marine Water Quality - technical draft default guideline values for PFOA.

The draft guidelines do not account for effects which result from the biomagnification of toxicants in air-breathing animals or in animals which prey on aquatic organisms.

The WQGs advise P40FP40F[1]PP that the 99% level of protection be used for slightly to moderately disturbed systems. This approach is generally adopted for chemicals that bioaccumulate and biomagnify in wildlife. Regulators may specify or environmental legislation may prescribe</td></tr>
<tr><td>220 µg/L</td><td>95% species protection - slightly to moderately disturbed systems</td></tr>
<tr><td>632 µg/L</td><td>90% species protection - highly disturbed systems</td></tr>
<tr><td>1824 µg/L</td><td>80% species protection - highly disturbed systems</td></tr>
</table>
</td><td></td></tr>
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[1] https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/local-conditions#bioaccumulation.

2.8. Additional Technical Comments to Consider					
Reviewer	**Comments**				**EPA Response**
				the level of species protection. required, rather than allowing for case-by-case assessments.	
	Exposure scenario	**PFOA**	**Exposure scenario**	**Comments and source**	
	Interim marine	19 µg/L	99% species protection - high conservation value systems	As above. Freshwater values are to be used on an interim basis until final marine guideline values can be set using the nationally-agreed process under the Australian and New Zealand Guidelines for	
		220 µg/L	95% species protection - slightly to moderately disturbed systems		
		632 µg/L	90% species protection - highly disturbed systems		

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	1824 µg/L	80% species protection - highly disturbed systems	Fresh and Marine Water Quality. The WQG advise that in the case of estuaries, the most stringent of freshwater and marine criteria apply, taking account of any available salinity correction. Marine guideline values developed by CRC CARE are under consideration through the nationally-agreed water quality guideline development process.

6- Table 1 2. Two Primary Categories of PFAS
Please refer to OECD 2021 to be consistent with PFAS terminology/nomenclature

OECD (2021), Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance, OECD Series on Risk Management, No. 61, OECD Publishing, Paris

7- Table 1.3 page 5
Please review Figure 9 OECD 2021 (also attached as PDF)

8- Conceptual Model of PFOA in the Aquatic Environment and Effects
Figure 2.7 page 56- Growth as an endpoint missing in the endpoints – first pentagon

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
	9- Page 69 last paragraph- Table 2-6 outlines the screening criteria for study evaluation and ranking. Cross reference to be updated to Table 2.3	
Reviewer 5	Overall, I think the PFOA criteria document and the reported criteria are robust given the constraints imposed by data availability. I agree with most assumptions made by EPA and the actual criteria values reported. I would, however, suggest that EPA consider revising the 1985 Guidelines. Re-evaluating the guidelines and publishing and update, even if changes are minimal would be a valued effort. I am sure that EPA has more than enough to do but given the importance of AWQC, I think it would be better to base the science on a more recent effort. There has been a lot of solid science in the last 35 years with a focus on criteria, species sensitivity distributions, etc. Although not used heavily for PFOA the use of the 4 most sensitive taxa and then a linear regression to estimate the criteria seems less robust than other methods. I could be wrong but here is where an updated Guidelines would be helpful – that may be an effort worth exploring quantitatively.	Thank you for your comments. EPA uses the best available science in developing AWQC. EPA has initiated an effort to update the 1985 Guidelines. When a draft revision is completed it will be peer reviewed and made available for public comment. Reviewer 5 commented that a model was fit to the four most sensitive endpoints (i.e., four most sensitive GMAVs and GMCVs) to derive the criteria, which was not the case. Instead, derivation of the acute and chronic criteria followed long-established methods outlined in the 1985 Guidelines. The established criteria calculation outlined in the 1985 Guideline uses a log-triangular fit to determine the 5th centile of a GSD. Acute and chronic GSDs (which included all quantitatively acceptable toxicity data) were presented in the Effect Analysis section of the draft PFOA Aquatic Life Criteria document. When there are less than 59 genera in a GSD, the 5th centile is inherently based on the four most sensitive genera, with the remaining tests only influencing the FAV through the "*n*" in the calculation. Please see the excerpt from the 1985 Guidelines below for further explanation.

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
		"*Order the GMAVs from high to low.* *L. Assign ranks, R, to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.* *M. Calculate the cumulative probability, P, for each GMAV as R/(N+1).* *N. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are less than 59 GMAVs, these will always be the four lowest GMAVs).*" Additionally, research conducted since the 1985 Guidelines were published has continued to suggest use of a log-triangular distribution to estimate an HC_5 from sensitivity distributions is appropriate. USEPA (2011) concluded: "*Judging by bias at small sample sizes, distributions on log-transformed data (normal, logistic, triangular, Gumbel) generally outperformed distributions on untransformed data (Pareto, Weibull, and $Burr_{III}$) and of the former, the log-normal, log-logistic, and log-triangular showed very similar performance*."

3 REFERENCES CITED BY EPA IN RESPONSES

Awkerman, J. A., S. Raimondo, C. R. Jackson and M. G. Barron. 2014. Augmenting aquatic species sensitivity distributions with interspecies toxicity estimation models. Environmental toxicology and chemistry. 33(3): 688-695.

Bejarano, A. C., S. Raimondo and M. G. Barron. 2017. Framework for optimizing selection of interspecies correlation estimation models to address species diversity and toxicity gaps in an aquatic database. Environmental science & technology. 51(14): 8158-8165.

Buck, R. C., J. Franklin, U. Berger, J. M. Conder, I. T. Cousins, P. de Voogt, A. A. Jensen, K. Kannan, S. A. Mabury and S. P. van Leeuwen. 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess. Manag. 7(4): 513-541.

Burkhard, L. P. 2021. Evaluation of published bioconcentration factor (BCF) and bioaccumulation factor (BAF) data for per-and polyfluoroalkyl substances across aquatic species. Environmental Toxicology and Chemistry.

CCME (Canadian Council of Ministers of the Environment). 2007. Protocol for the derivation of water quality guidelines for the protection of aquatic life. Winnipeg, Manitoba: Canadian Council of Ministers of the Environment. Available online at: http://ceqg-rcqe.ccme.ca/download/en/220.

Coats, K., G. Long and J. Adelson. 2017. Quality Systems Manual (QSM) for Environmental Laboratories. Department of Defense, Washington, DC.

Dyer, S. D., D. J. Versteeg, S. E. Belanger, J. G. Chaney and F. L. Mayer. 2006. Interspecies correlation estimates predict protective environmental concentrations. Environmental science & technology. 40(9): 3102-3111.

S.D. Dyer, D.J. Versteeg, S.E. Belanger, J.G. Chaney, S. Raimondo, and M.G. Barron. 2008. Comparison of Species Sensitivity Distributions derived from interspecies correlation models to distributions used to derive water quality criteria, Environ. Sci. Technol. 42; 3076–3083.

Fabbri, R., M. Montagna, T. Balbi, E. Raffo, F. Palumbo and L. Canesi. 2014. Adaptation of the bivalve embryotoxicity assay for the high throughput screening of emerging contaminants in Mytilus galloprovincialis. Mar Environ Res. 99: 1-8.

Gergs, A., S. Classen, R. Strauss, R. Ottermanns, T. Brock, H. Ratte, U. Hommen and T. Preuss. 2016. Ecological Recovery Potential of Freshwater Organisms: Consequences for Environmental Risk Assessment of Chemicals. Rev. Environ. Contam. Toxicol. 236: 259-294.

Hayman, N. T., G. Rosen, M. A. Colvin, J. Conder and J. A. Arblaster. 2021. Aquatic toxicity evaluations of PFOS and PFOA for five standard marine endpoints. Chemosphere. 273: 129699.

Hazelton, P. D., W. G. Cope, T. J. Pandolfo, S. Mosher, M. J. Strynar, M. C. Barnhart and R. B. Bringolf. 2012. Partial Life-Cycle and Acute Toxicity of Perfluoroalkyl Acids to Freshwater Mussels.Environ. Toxicol. Chem., 31: 1611-1620.

Jones, K.C. and P. de Voogt. 1999. Persistent organic pollutants (POPs): state of the science. Environ. Pollut. 100: 209-221.

Lath S, Knight ER, Navarro DA, Kookana RS, McLaughlin MJ. 2019. Sorption of PFOA onto different laboratory materials: Filter membranes and centrifuge tubes. Chemosphere 222: 671– 678.

Lemly, A. D. 1997. Ecosystem recovery following selenium contamination in a freshwater reservoir. Ecotoxicology and Environmental Safety. 36(3): 275-281.

Li, M.H. 2008. Effects of nonionic and ionic surfactants on survival, oxidative stress, and cholinesterase activity of planarian. Chemosphere 70(10): 1796-1803.

Li, M.H. 2009. Toxicity of perfluorooctane sulfonate and perfluorooctanoic acid to plants and aquatic invertebrates. Environ. Toxicol. 24(1): 95-101.

Martin, J. W., S. A. Mabury, K. R. Solomon and D. C. Muir. 2003a. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry: An International Journal. 22(1): 189-195.

McCarthy, C.J., S. A. Roark, D. Wright, K. O'Neal, B. Muckey, M. Stanaway, J. N. Rewerts, J. A. Field, T. A. Anderson, C. J. Salice. 2021. Toxicological Response of Chironomus dilutus in Single-Chemical and Binary Mixture Exposure Experiments with 6 Perfluoralkyl Substances. Environ. Contam. Toxicol. 40(8): 2319-2333.

NRC. 2013. Assessing risks to endangered and threatened species from pesticides. National Academies Press Washington, DC. pp 142

OECD. (Organisation for Economic Co-operation and Development). 2021. Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance. Series on Risk Management No.61. Paris, France. ENV/CBC/MONO(2021)25. pp.34. https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/CBC/MONO(2021)25&docLanguage=en

Prosser, R. S., K. Mahon, P. K. Sibley, D. Poirier and T. Watson-Leung. 2016. Bioaccumulation of perfluorinated carboxylates and sulfonates and polychlorinated biphenyls in laboratory-cultured Hexagenia spp., Lumbriculus variegatus and Pimephales promelas from field-collected sediments. Sci Total Environ. 543(Pt A): 715-726.

Raimondo, S. and M. G. Barron. 2020. Application of Interspecies Correlation Estimation (ICE) models and QSAR in estimating species sensitivity to pesticides. SAR and QSAR in Environmental Research. 31(1): 1-18.

Raimondo, S., C. R. Jackson and M. G. Barron. 2010. Influence of taxonomic relatedness and chemical mode of action in acute interspecies estimation models for aquatic species. Environmental science & technology. 44(19): 7711-7716.

Rewerts, J. N., E. C. Christie, A. E. Robel, T. A. Anderson, C. McCarthy, C. J. Salice and J. A. Field. 2021. Key considerations for accurate exposures in ecotoxicological assessments of perfluorinated carboxylates and sulfonates. Environmental toxicology and chemistry. 40(3): 677-688.

Ritz, C. and J. C. Streibig. 2016. Analysis of dose-response curves.Package 'drc'. 3.0-1. https://cran.r-project.org/web/packages/drc/drc.pdf

Snell, T.W. and B.D. Moffat. 1992. A 2-d Life cycle test with the rotifer Brachionus calyciflorus Environ. Toxicol. Chem. 11: 1249-1257

U.S.EPA. (US Environmental Protection Agency). 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. Office of Research and Development. PB85-227049. https://www.epa.gov/sites/default/files/2016-02/documents/guidelines-water-quality-criteria.pdf

U.S. EPA (United States Environmental Protection Agency). 1991. Technical support document for water quality-based toxics control. U.S. EPA, Office of Water, Washington, DC. EPA/505/2-90-001, PB91-127415.

U.S.EPA. (U.S. Environmental Protection Agency). 2000. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health. Office of Water. EPA-822-B-00-004. https://www.epa.gov/sites/default/files/2018-10/documents/methodology-wqc-protection-hh-2000.pdf

U.S.EPA. (U.S. Environmental Protection Agency). 2003. Ambient Aquatic Life Ambient Water Quality Criterion for Tributyltin (TBT). Office of Water. Washington, DC. EPA 822-R-03-031. https://www.epa.gov/sites/default/files/2019-02/documents/ambient-wqc-tributyltin-final.pdf

U.S.EPA (United States Environmental Protection Agency). 2011. Review of methods for characterizing effects of pesticides and other chemical stressors to aquatic organisms. Office of Pesticide Programs. Washington, D.C. EPA-HQ-OPP-2011-0898-0005.

U.S.EPA. (U.S. Environmental Protection Agency). 2016a. Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater. Office of Water. Washington, DC. EPA 822-R-16-006. https://www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf

U.S.EPA (U.S. Environmental Protection Agency). 2016b. Series 850 - Ecological Effects Test Guidelines. Office of Chemical Safety and Pollution Prevention, Washington, DC. Accessed March 2021. https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines.

Willming, M. M., C. R. Lilavois, M. G. Barron and S. Raimondo. 2016. Acute toxicity prediction to threatened and endangered species using interspecies correlation estimation (ICE) models. Environmental science & technology. 50(19): 10700-10707.

Warne, M.St.J., G.E. Batley, R.A. van Dam, J.C. Chapman, D.R. Fox, C.W. Hickey and J.L. Stauber. 2018. Revised method for deriving Australian and New Zealand water quality guideline values for toxicants – update of 2015 version. Prepared for the revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Governments and Australian state and territory governments, Canberra, 48 pp.

Yang, S., F. Xu, F. Wu, S. Wang and B. Zheng. 2014. Development of PFOS and PFOA Criteria for the Protection of Freshwater Aquatic Life in China. Sci. Total Environ. 470/471: 677-683.

Yuan, Z., J. Zhang, Y. Zhang, H. Zhen and Y. Sun. 2015. The effect of perfluorooctanoic acid on the planarian *Dugesia japonica*. Pol. J. Environ. Stud. 24(2): 801-807.

Zhang, L., J. Niu, Y. Li, Y. Wang and D. Sun. 2013. Evaluating the sub-lethal toxicity of PFOS and PFOA using rotifer *Brachionus calyciflorus*. Environ. Pollut. 180: 34-40.

Zhang, L., J. Niu, Y. Wang, J. Shi and Q. Huang. 2014. Chronic effects of PFOA and PFOS on sexual reproduction of freshwater rotifer *Brachionus calyciflorus*. Chemosphere 114: 114-120.