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*

Perfluorooctane Sulfonate (PFOS)"

(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 Perfluorooctane Sulfonate (PFOS)* was conducted by Eastern Research Group, Inc. (ERG), a contractor to EPA for EPA OW and developed an external peer review report (https://www.epa.gov/wqc/aquatic-life-criteria-perfluorooctane-sulfonate-pfos). Independent peer review of the draft *Aquatic Life Ambient Water Quality Criteria for Perfluorooctanoic Acid (PFOA)* 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 PFOS 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 review comments on the PFOS criteria document and EPA's responses to those comments are described in this report. Results of the PFOA external peer 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 Perfluorooctane Sulfonate (PFOS)*, 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	**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 as a heading in the table, 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 and headings of tables and figures decreases clarity, e.g., Figs 3-1, Tables 3-1, 3-2, 3-3, 3-4, 3-5. The use of acronyms in the figure or table is valid to save space, as long as they are defined in the caption of the figure or table.	Thank you for your comment. EPA added a list of acronyms to the draft PFOS Aquatic Life Criteria document. This list of acronyms can be found on page xii of the revised draft PFOS Aquatic Life Criteria Document.

2.1. Clarity of Document as it Relates to the Derivation of Each Criterion		
Reviewer	**Reviewer Comments**	**EPA Response**
Reviewer 3	Main Question: Perhaps this was missed in the draft document, but, is there guidance when one criteria is exceeded and the other is not? For example, if the tissue based criteria are exceeded yet, the CCC is not; perhaps this is an unlikely scenario as those receptors have been accumulating PFOS for a duration likely under higher water concentrations (> 0.014mg/L). Although if sediment concentrations remain elevated (but not water column concentrations) this may also be a likely route of PFOS exposure to fish with sediment dwelling prey. There are additional domestic criteria missing from the previously published criteria section; please review those for Texas, Florida and California. Are there two Sharpe et al.'s, I believe this is only one publication but flipping between Sharpe et al. 2010, Sharpe et al. 2010a and Sharpe et al. 2010b throughout the document. If the goal is to distinguish between supplemental vs the manuscript proper I suggest just clarifying in the text instead of the reader looking for two pubs by Sharpe et al. 2010. Page 238 – error: The study authors reported a 96-hour LC_{50} of 58.47 mg/L PFOS, based on the results of the range finding test. The independently-calculated toxicity value was x.xx mg/L. Page 296 – error: The independently-calculated toxicity value was x.xx mg/L. Table 3-6 is not referenced/described in the text. Additionally, the title reads "Six" most sensitive and lists "Seven". Overall comment: ranking of sensitive genera flips back and forth between most and least sensitive among tables, consistency would help the reader.	Thank you for raising the question regarding guidance for potential scenarios resulting in one criterion being exceeded while the other criteria are not. All of these PFOS (those for water column and tissue) are intended to be independently applicable and no one criterion takes primacy. As such all of the recommended criteria (acute and chronic water column and tissue criteria) are intended to be protective of aquatic life. As for the example provided in the comment, the chronic PFOS criteria consist of both the water-column based criterion and the tissue-based criteria (as fish and invertebrate whole-body tissue criteria and fish muscle tissue criterion). Therefore, given the independent applicability of the PFOS criteria, if one criterion is exceeded the PFOS criteria as a whole would be considered to be exceeded. Thank you for suggesting that EPA review domestic criteria for Texas, Florida, and California. EPA will review these criteria and add relevant details, as appropriate, to the section entitled: "Previously Derived PFOS Toxicity Values and Thresholds." Lastly, the editorial items regarding the in text citations and references for Sharpe et al, the independently-calculated values, and the text for Table 3-6 will be corrected. In particular, the following edits were made: (1) there should only be one citation for Sharpe et al. (2010) and any others will be removed, (2) the x.xx mg/L placeholders for the independently-calculated values on pages 238 and 296 will be removed or replaced as appropriate, (3) the text for Table 3-6 will be updated to reflect the most sensitive species listed are consistent with the text and (4) the tables ranking the sensitivity of genera were edited to ensure that all are consistent with listing genera based on sensitivity and reordered so that the species are listed as most sensitive to least sensitive.

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Reviewer	**Reviewer Comments**	**EPA Response**
Reviewer 4	EPA has drafted the PFOS aquatic life criteria to be consistent with methods described in EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (U.S. EPA 1985). I congratulate the EPA Team for a very thorough, comprehensive analysis of the 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 PFOS precursors in the aquatic environment • sources, concentration reported in environment and existing criteria (both national and international) 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 documented in this report are based on comprehensive assessment of the toxicological data and consistent with the *Guidelines*.	Thank you for your comment describing the specific sections of the draft PFOS Aquatic Life Criteria Document that you found to be comprehensive.

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Reviewer	**Reviewer Comments**	**EPA Response**
	• Acute and chronic MDRs for PFOS estuarine/marine criteria derivation were not met due to fewer empirical PFOS toxicity data. To address this gap, the EPA Team developed an acute aquatic life benchmark for estuarine/marine environments based on Interspecies Correlation Estimation (ICE) model. Such predictive models should be used when there is limited toxicity data. • EPA Team has provided extensive background information on toxicity data assessment and collated this information in various Appendices as additional line of evidence. • Tables and Figures are very well laid out throughout the document and provide additional information of the toxicity data used in developing Water Quality Criteria for PFOS.	
Reviewer 5	Overall, the document is clearly written and generally free of grammatical errors. I applaud the scientists and EPA for compiling an impressive amount of work and communicating it in a reasonably clean and coherent way. That said, there are a few instances of redundancy – literally, the same sentences repeated. I have made note of these in the actual report and will include that along with this document, if requested. Although these are easy enough to see with careful review. The document is VERY LONG and very detailed so any efforts to shorten or make more concise would be welcomed. As for the clarity of technical elements of the document, I feel that many of the decisions to use or not use data or endpoints could be more consistent and/or communicated better. For example, in some cases the geometric mean of endpoints for a certain taxa is used for the chronic value (pimephales) but for	Thank you for your comment regarding the general support for the level of detail and the overall structure of the draft PFOS criteria for aquatic life. EPA will remove the redundancies to make the draft PFOS Aquatic Life Criteria Document more concise and clearer. EPA made edits to ensure that the technical elements and decisions in the criteria document are consistent throughout and are clearly communicated in the draft. Regarding Reviewer 5's comment that a model was fit to the four most sensitive endpoints (i.e., four most sensitive GMAVs and GMCVs) to derive the criteria is not entirely accurate. 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

2.1. Clarity of Document as it Relates to the Derivation of Each Criterion		
Reviewer	**Reviewer Comments**	**EPA Response**
	other taxa, this is not the case (e.g. daphnia; zebrafish). In other cases, the decision to not use certain data seems as if it could be communicated more clearly. So while the language of the document is pretty clear, the actual technical aspects are less so. One major concern I have is with the overall approach of using the 4 most sensitive toxicity values to then derive the final acute and final chronic values. Using this approach it would seem the AWQC are then very sensitive to changes in any 1 of the 4 toxicity values. For example, when EPA explored the impact of different toxicity values on the chronic freshwater water column criteria, using higher toxicity values (e.g., pimephales in place of fatmucket Table 4-3) resulted in a lower chronic criteria. This is nonintuitive and suggests a possible flaw in the approach. It's possible this is not the case and it makes sense both mathematically (steeper slope) and perhaps even from a protection standpoint. Either way, EPA should explain why this happens and what it means for the overall approach. I suspect the EPA is somewhat constrained by the 1985 guidelines in developing the AWQC but I also see that New Approach Methods (WEB-ICE) were used to derive criteria with limited data. I wonder if using a species sensitivity distribution approach in which all the chronic or acute (freshwater)data are used would result in more defensible criteria that are less impacted by changes in any one toxicity value? At the very least, I think including a full SSD would be useful for comparison as part of the characterization piece. In the PFOA document I mention revisiting and publishing and updated 1985 guidelines…this is warranted when EPA has the bandwidth to do so. Having said all this, I am aware that EPA likely has justification for their approach of using the 4 most sensitive tox values but it would perhaps be good to mention this again as I suspect a lot of people that may read the AWQC will not also read the 1985 guidelines.	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 PFOS Aquatic Life Criteria document. Please see EPA's response to Reviewer 5's comments to Charge Question 2.2 below for greater details on how the approach used to derive the draft PFOS criteria follow the 1985 Guidelines. Specifically, 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. Further, the reviewer is correct in assuming that when EPA explored the impact of different toxicity values on the chronic freshwater criterion (see Section 4.2.2 of the draft PFOS Aquatic Life Criteria Document that underwent external peer review) the use of some higher toxicity values resulted in a lower chronic water column value for freshwater because the change in the chronic toxicity values used in these additional analyses resulted in a steeper slope. EPA made edits to the draft PFOS Aquatic Life Criteria Document to clarify this observation in Section 4.2.2. Lastly, many of the documented decisions and data requested by Reviewer 5 to be in a single table can be found in the summary tables of Appendices A through D. EPA made further edits to these tables to include additional information requested by the reviewer.

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	Lastly, I had a hard time keeping track of all the decisions to use or not use data for each of the tox values that supported the criteria. I think a more detailed table with all the tox values considered (data shown in Figure 3-3) and including whether the data used were author-reported, re-calculated by EPA, along with the lowest reported/calculated value that wasn't used and why. This may be asking a lot and this information is throughout the document but not in a single, easy to locate and read location.	

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

- **Is the technical approach used to derive the criterion elements 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 PFOS		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	• **Is the technical approach used to derive the criterion elements 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: I believe the Criterion Continuous Concentration (CCC) should be potentially re-calculated considering my comments provided in response to charge question 5a. The science does not support the assumption of a 10-year recovery time for PFOS in aquatic systems. The generation of tissue criteria is weakly supported, and the uncertainty associated with these criteria should be emphasized. The NAM-generated marine Final Acute Value (FAV) and FAV/2 values (Appendix L) are highly uncertain. It is unclear if the EPA-calculated Effective Concentration 10% (EC10) values are supported; additional details on the modeling	Thank you comments that state the technical approach used to derive the PFOS criteria were generally logical and for noting specific areas were the science is not supportive of the criteria in a few instances. In addition to EPA's response to this comment, please see EPA's responses to subsequent Reviewer 1 comments for specific Charge Questions that follow. Responses to key instances where Reviewer 1 does not believe the science is supportive of the draft PFOS Aquatic Life Criteria Document are described below in corresponding order mentioned in Reviewer 1's comment: 1. See EPA's response to Reviewer 1's comments under Charge Question 2.5.a below 2. See EPA's response to Reviewer 5's comments under Charge Question 2.7 below. EPA considered the bioaccumulative nature and persistence of PFOS 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 PFOS criteria. Three of the remaining Expert Peer Reviewers were supportive of the 10-year exceedance frequency for the tissue-based PFOS criteria, with the remaining Reviewer (i.e., Reviewer 3) stating it was difficult to comment on the tissue-based criteria frequencies.

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Reviewer	**Comments**	**EPA Response**
	and the variability and fit of each EC10 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 PFOS 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.	3. Please see EPA's response to Reviewer 1's comments below to Charge Question 2.6 regarding the generation of the tissue criteria. As such EPA acknowledges the inherent uncertainties that are present with the use of BAFs to derive tissue criteria. For these reasons, 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 (the 2016 Selenium Aquatic Life Criterion for Freshwaters; U.S. 2016) and human health (U.S. 2000). Thus, given the potential bioaccumulation of PFOS through the aquatic food web EPA concluded that tissue based criteria were needed to ensure the protection of aquatic life to exposures of PFOS. 4. Please see EPA's response to Reviewer 1's comments below to Charge Question 2.3 regarding the NAM-generated acute saltwater benchmark derived in Appendix L of the draft PFOS criteria. EPA made edits to ensure that any uncertainty surrounding the acute saltwater benchmark is clearly stated. 5. Concentration-response model type and graphs of the concentration-response data with the fitted model were provided in draft criteria Appendices A.2 and C.2 for those tests that were used to quantitatively to derive the PFOS criteria and were among the four most sensitive acute and chronic genera, respectively. Moreover, the graphs of the fitted concentration-response model in provided in the draft's appendices A.2 and C.2 displayed 95% confidence bands allowing for a visual display of variability. Reviewer 1 further notes their reviewed focused on "key portions" of the "Draft of the Aquatic Life Water Quality Criterion" and may have missed the

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Reviewer	**Comments**	**EPA Response**
		concentration-response modeling results presented in appendices A.2 and C.2 as well as the modeling methods presented in Appendix K. Lastly, EPA thanks Reviewer 1 for describing the relative similarity between the draft PFOS 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 tissue criteria derived in this draft PFOS criteria are intended to be protective of aquatic life from the bioaccumulation of PFOS. Currently, there are insufficient data to derive tissue criteria empirically from the toxicity literature. Instead, EPA derived the tissue criteria by translating the empirically derived chronic water column criterion into tissue concentrations with the use of bioaccumulation factors (BAFs). While this approach adds some uncertainty to the tissue criteria that were derived, EPA summarized the limited empirical tissue-based data for aquatic life to understand this potential uncertainty. In general, the comparison of the empirical tissue-based data demonstrates that the tissue criteria, which are based on fish and invertebrate whole-body and fish muscle, are protective of aquatic life with some specific studies indicating that the tissue criteria may not be protective of certain species, exposures, or endpoints. EPA will take a closer look at the overall approach to derive the tissue criteria, including the potential uncertainties, and make the needed adjustments to ensure that the tissue criteria are protective of all aquatic life. Furthermore, aquatic life tissue criteria are intended to be protective of aquatic life; aquatic-dependent wildlife fall outside the scope of the current draft PFOS criteria. EPA intends to review PFOS data focused on aquatic-dependent wildlife in the future and to potentially derive separate aquatic-dependent wildlife criteria for PFOS if the data support the derivation of such criteria.

2.2. The Technical Approach Used to Derive the Draft Criterion for PFOS		
Reviewer	**Comments**	**EPA Response**
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 currently available. 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.	Thank for your comment noting the approach used in the draft PFOS Aquatic Life Criteria Document was "logical and defensible" and that "the science supports the EPA's conclusions." EPA noted the studies provided by Reviewer 2 (and all other reviewers) that were not included in the draft PFOS criteria were considered for possible inclusion in the derivation of the criteria, as appropriate, based on the data quality, using the data quality review approach described in the draft document. To summarize, the review of these recommended studies was conducted to ensure they met the data quality objectives outlined by the 1985 Guidelines (U.S.EPA 1985) and the EPA 850 test guidelines (U.S.EPA 2016b). This further allowed EPA to determine if each individual provided study should be used quantitively in the derivation of the criteria, qualitatively as supporting information to the criteria, or not used in the criteria due to concerns with data quality or test methodology. Additionally, EPA reviewed all relevant PFOS toxicity studies currently included in EPA's Office of Research and Development's ECOTOX database through the September 2021 quarterly update. Between both the recommended and ECOTOX papers, the number of additional PFOS references that were reviewed total 41, which resulted in 51 individual new studies (as two of the references were large industry reports that contained many individual studies) The review and inclusion of these additional toxicity studies resulted in an update of the draft PFOS criteria, in which the acute, freshwater criterion changed very little (from 3.1 mg/L to 3.0 mg/L) and the chronic, freshwater criterion decreased slightly (from 0.014 mg/L to 0.0084 mg/L). Additionally, with the decrease in the chronic, freshwater criterion the tissue criteria decreased as well (resulting in an invertebrate whole-body criterion of 7.4 mg/kg

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Reviewer	**Comments**	**EPA Response**
		ww, a fish whole-body criterion of 25.8 mg/kg ww, and a fish muscle criterion of 15.2 mg/kg ww). Thank you for your comments on the evaluation of the influence of non-North American species on the derivation of the criteria. 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 PFOS criteria.
Reviewer 3	• **Is the technical approach used to derive the criterion elements logical?** This is logical and follows the established GLRI guidance; however, both Canada and Australia utilize a species sensitivity distributions to determine the 95th and 99th percentile of species protection. Is there a defensible reason why EPA did not employ this approach or at the very least present these distributions and analysis that would support the currently drafted criteria? Additionally, thresholds from those SSDs (and others published) are lower than the draft guidance here, this should be addressed: Australia – 0.13 µg/L Canada – 6.8 µg/L Salice et al. 2018 – 1.12 µg/L Conder et al. 2020 – 5.85 µg/L *Giesy et al. 2010 – 5.1 µg/L (using CCC based on GLRI guidance) • **Does the science support the conclusions?**	Thank you for your comments that state the technical approach used to derive the PFOS criteria were generally logical. However, it should be noted that the derivation of the PFOS criteria followed the 1985 Guidelines. As such the approach is generally consistent with the approaches used by both Canada and Australia, since all utilized species sensitivity distributions and similar percentiles of species protective (95 percentile for EPA's and Canada's PFOS criteria and 99th percentile for Australia's criteria). The observed differences between EPA's draft PFOS criteria and those derived for both Canada and Australia are largely due to differences in the toxicity studies and therefore data that were used. Based on the literature included in the draft PFOS criteria (those included in EPA's ORD ECOTOX database as of September 2019), the GMCV for zebrafish of 0.0165 mg/L was the most sensitive chronic value. However, EPA is aware that more recent toxicity studies that indicate that there are some more sensitive toxicity values for some aquatic life species. As noted in EPA's comment to Reviewer 2's comment to Charge Question 2.2 above, between ECOTOX and recommendations made by external peer reviewers, the number of additional PFOS studies

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Reviewer	**Comments**	**EPA Response**
	The GMCV for Zebrafish is 0.0165 mg/L, thus there are studies that result in chronic toxicity at concentrations lower than this mean; however, this is very close to the CCC of 0.014mg/L. This seems borderline protective when considering potential exposures to this species (and those more sensitive). • **Is it consistent with the protection of freshwater aquatic life from acute, chronic, and bioaccumulative effects?** I have confusion over Tables 3-9 and 4-6 calculations. How is it that the inclusion of *Lampsilis* with a higher GMAV results in a lower overall CMC (3.3 mg PFOS/L) compared to the CMC in table 3-9 (3.6 mg PFOS/L)? Actually, looking more closely at this, the ln(GMAV)^2 are inconsistent among the tables for *Xenopus*, this is likely are result of using table 3-6 as a template for 4-6. It is great to see the inclusion of the Burkhard et al. 2021 as this synthesis has been peer-reviewed and published and is an exceptional overview of PFOS bioaccumulation; unfortunately, there are not more current literature used within the draft document.	that were reviewed totals 51. The review and inclusion of these additional toxicity studies resulted in an update of the draft PFOS criteria, in which the acute, freshwater criterion changed very little (from 3.1 mg/L to 3.0 mg/L) and the chronic, freshwater criterion decreased slightly (from 0.014 mg/L to 0.0084 mg/L). Specifically, the addition of the new data shifted the relative sensitivity of genera in both the acute and chronic criteria derivation. The GMAVs shifted slightly with the addition of fathead minnow, which is now the most sensitive genus in the acute PFOS dataset. All of the other GMAVs among the 5 most sensitive genera did not change from the previous draft. In the chronic PFOS dataset, the midge and the zebrafish are the only species among the 4 most sensitive genera with new data. And all of the other GMCVs for the 4 most sensitive genera did not change from the previous draft with the exception of the fatmucket GMCV, which changed from the estimated EC_{10} of 0.0571 mg/L (calculated using acute mussel data), to the MATC of 0.0177 mg/L following comments from external peer reviewers indicating that the MATC and/or data from a chronic mussel study should be used to estimate an EC_{10} for this species (see EPA's response to Review 1's comment to Charge Question 2.5.a.ii below for more details). EPA thanks Reviewer 3 for pointing out the inconsistencies between Tables 3-9 and 4-6 in the draft PFOS Aquatic Life Criteria Document and has corrected the values for *Xenopus*. Thank you for your comments on the inclusion of Burkhard (2021). EPA has updated the literature cited throughout the draft PFOS Aquatic Life Criteria Document to be inclusive of more recently published studies, though September 2021. Updates in the literature includes both toxicity literature used to derive the criteria)and background literature related to topics discussed in

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Reviewer	**Comments**	**EPA Response**
		the Problem Formulation of the draft PFOS Aquatic Life Criteria Document.
Reviewer 4	This EPA report provides a critical review of toxicity data identified in EPA's literature search for PFOS, including the anionic form (CAS No. 45298-90-6), the acid form (CAS No. 1763-23-1), potassium salt (CAS No. 2795-39-3), an ammonium salt (CAS No. 56773-42-3), sodium salt (CAS No. 4021-47-0), and a lithium salt (CAS No. 29457-72-5). It quantifies the toxicity of PFOS to aquatic life, and provides criteria intended to protect aquatic life from the acute and chronic toxic effects of PFOS. 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 PFOS is very logical and consistent with the protection offered by acute and chronic aquatic life 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 were included for criteria development. For example, inclusion of non-resident species such as Planaria, *Dugesia japonica* and Japanese swamp shrimp, *Neocaridina denticulata* for calculating acute water quality criteria and zebra fish, *Danio rerio* for chronic criteria. The EPA team did not find any influence of excluding non-North American resident species in	Thank you for your comment describing specific sections of the draft PFOS Aquatic Life Criteria Document in detail. Please see responses to the comments pertaining to the acute and chronic MDRs for the acute estuarine/marine benchmark that Reviewer 4 mentions are described in response to Charge Question 2.3. Specifically, EPA noted the studies provided by Reviewer 4 (and all other reviewers) that were not included in the draft PFOS Aquatic Life Criteria document and all studies provided in the comments of this external peer review were considered for possible inclusion in the derivation of the criteria and acute estuarine/marine benchmark to determine if additional estuarine/marine MDRs were met.

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	criteria derivations and decided to retain the full acute and chronic toxicity dataset. This was very rational decision and non-Northern American species served as surrogate species for the broad range of the thousands of untested species present in the freshwater environment in the U.S. • The acute measures of effect on aquatic organisms selected included the lethal concentration (LC50), effect concentration (EC50), or inhibitory concentration (IC50) estimated to produce a specific effect in 50 percent of the test organisms as per the *Guidelines*. • 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 for EPA to conduct statistical analyses. EPA's independently-calculated toxicity values were used preferentially, where available. • I agree with the authors' decision on not developing plant criteria based on their lesser sensitivity to PFOS than in comparison to aquatic vertebrates and	

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	invertebrates. The EPA team evaluated the toxicity data to plants as an additional line of evidence and confirmed that the proposed PFOS freshwater acute and chronic criteria are expected to be protective of freshwater plants. • 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). • Based on comprehensive toxicity data assessment, the EPA team has developed the following criteria using the procedures described in the 1985 *Guidelines*. The freshwater acute water column-based criterion magnitude is 3.6 mg/L and the chronic water column-based criterion magnitude is 0.014 mg/L. The chronic freshwater criterion also contains tissue-based criteria expressed as 43.0 mg/kg wet weight (ww) for fish whole-body, 25.3 mg/L ww for fish muscle tissue, and 12.3 mg/kg ww for invertebrate whole-body tissue. • Acute and chronic MDRs for PFOS estuarine/marine criteria derivation were not met and an estuarine/marine FAV could not be calculated to derive an estuarine/marine acute criterion. Further benchmark was developed using predictive approach and discussed in the follow-up question 3.	
Reviewer 5	• **Is the technical approach used to derive the criterion elements logical?** Overall, I think the technical approach is relatively sound although there are instances where it was difficult to keep track of all the decisions with regard to data and whether these were	Thank you for your comments that the technical approach used to derive the PFOS criteria were generally relatively sound and for noting that there are instances where it is difficult to follow technical decisions with data. And for stating that "EPA did an

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	consistent and logical. Admittedly, I think this is a tough chemical and a tough dataset and EPA did an excellent job with the background material and highlighted and used key studies (but more have been published since and will, I'm sure be included). Unfortunately, the technical approach to derive criterion elements is not universally logical. Moreover, as mentioned, using only the 4 most sensitive toxicity endpoints followed by a regression (what type? Was this specified?) seems less robust than using a full species sensitivity distribution with a more "natural" distribution of sensitivity (s-shaped, for example). This last statement may not be true so a reasonable compromise might be to include a full SSD as part of the characterization piece related to "considering other toxicity values impact on the FCV, etc.". What really confused me was that when EPA did what amounts to a sensitivity analysis of the FCV by replacing toxicity values, the FCV DECREASED when higher toxicity values were used. While I suspect this happened because switching to higher toxicity values steepened the slope (or something), it does not make intuitive sense to me and should be further explained. Alternatively, an explanation and justification, even brief, would be helpful in supporting the 4 most sensitive toxicity value approach. I am aware that the 1985 guidelines may include this but I suspect most users of the AWQC may not be familiar with the details of the guidelines. With regard to the tissue-based criteria, EPA mentions using "only PFOS studies in which organisms were exposed in the diet" (or similar; p. 88) but then go on to say the BAF approach was used. I would edit this section to start with mentioning that a BAF approach was used because there were not enough tissue data from laboratory studies. I mention this because it was confusing – there was a lot of explanation of using only dietary exposures and then one sentence (basically) stating…EPA explored a BAF approach.	excellent job with the background material and highlighted and used key studies." Responses to Reviewer 5 comments on question 2.2 described below in corresponding order of Reviewer 5's comment: 1. EPA thanks for your review for stating the "overall approach to derive the criteria for PFOS is relatively sound." Regarding Reviewer 5's comment that a model was fit to the four most sensitive endpoints (i.e., four most sensitive GMAVs and GMCVs) to derive the criteria The derivation of the acute and chronic criteria followed long established methods outlined in the 1985 Guidelines (U.S.EPA 1985). The established criteria calculation outlined in the 1985 Guideline uses a log-triangular fit to determine the 5th centile of a genus sensitivity distribution. Acute and chronic genus sensitivity distributions (which included all quantitatively acceptable toxicity data) were presented in the Effect Analysis section of the draft PFOS Aquatic Life Criteria Document. When there are less than 59 genera in a genus sensitivity distribution, the 5th centile magnitude is inherently based on the four most sensitive genera, with the remaining tests only influencing the final acute value (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.*

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	• **Does the science support the conclusions?** Well, offhand, I think the final chronic value for freshwater organisms should likely be lower. Importantly, several studies have been published in 2021 that should likely be included as toxicity values and they may result in lower toxicity estimates. The fact that EPA's criteria are higher than all other published criteria is worrisome. We are all using the same data and many in the field are quite capable scientists. These two papers come immediately to mind but I am sure there are others. Sensitivity and Accumulation of Perfluorooctanesulfonate and Perfluorohexanesulfonic Acid in Fathead Minnows (*Pimephales promelas*) Exposed over Critical Life Stages of Reproduction and Development J.G. Suski, C.J. Salice, M.K. Chanov, J. Ayers, J. Rewerts, J. Field Environmental Toxicology and Chemistry, 2021, pp. 811-819. Toxicological Response of *Chironomus dilutus* in Single-Chemical and Binary Mixture Exposure Experiments with 6 Perfluoralkyl Substances 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 Environmental Toxicology and Chemistry, 2021, pp. 2319-2333. In my view, it is essential that EPA incorporate newly published toxicity data for PFOS (and PFOA). Furthermore, in several cases, EPA's decisions to use what look like higher estimates of toxicity seem somewhat arbitrary and not internally consistent. I also noted above and mention here	*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).* 2. EPA also thanks you for your suggestions regarding the approach and data used to derive the PFOS tissue criteria. Edits were made to this section entitled "Translation Chronic Water Column Criterion to Tissue Criteria" in the draft PFOS document to clarify the approach and the data that were used to derive the tissue criteria. 3. EPA took note of the studies provided by the external peer reviewers that were not included in the draft PFOS criteria and all these studies were considered for possible inclusion in the draft criteria, as appropriate, based on the quality of the data. 4. EPA ensured to clearly justify each specific toxicity value used to derive the acute and chronic criteria in the draft PFOS Aquatic Life Criteria Document. The toxicity values used in the derivation of the PFOS criteria were the most defensible values based on careful consideration of the test methods, author-reported results, and EPA's independent analysis of the current toxicity literature. The toxicity values and the justifications for each value that was used are detailed in study summaries in the Effects Analysis section and in the corresponding appendices (A through D). 5. Your comment noting that the criteria are "nearly protective" of aquatic life was unclear and

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	again the sensitivity of the criteria development approach to changes in one of the 4 most sensitive taxa/toxicity values. • **Is it consistent with the protection of freshwater aquatic life from acute, chronic, and bioaccumulative effects?** I believe the criteria are "NEARLY" protective of freshwater aquatic life for acute, chronic and bioaccumulative effects of PFOS. I say "nearly" because it seems to me that the FCV, in particular, could and maybe should likely be lower. Also, below I comment on the appropriateness and utility of the frequency and duration elements of the criteria. Briefly, in my opinion the frequency and criteria elements of the criteria certainly help the criteria concentrations to be protective; it is unlikely that a 4-day exposure to the FCV would result in adverse effects to any taxa for which there are data; however, these data are not commonly reported (hourly or 4-day running average concentrations have never been reported to my knowledge).	unsubstantiated. EPA assumed based on the comments provided by Reviewer 5 to this questions that the criteria appeared to be nearly protective based largely on the more recent toxicity data that the peer review recommended that EPA review. Based on ORD's ECOTOX updates of the PFAS literature since the time of this draft development and September 2021, and recommended toxicity studies provided by the external peer reviewers, the number of additional PFOS studies that were reviewed totals 51 The additional studies were included in the subsequent draft, as appropriate, based on their data quality. 6. See EPA's response to Reviewer 5's comments under Charge Question 2.7 below. In summary, such an event that a 4-day exposure to the FCV would result in adverse effects is conceivable but unlikely considering the implementation of criteria duration and frequency and NPDES Permit limits because the four-day duration is represents the time period over which the chronic criterion is averaged.

2.3 Please comment on the approach used to derive the draft acute estuarine/marine benchmark for PFOS. 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?**

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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 (0.43 mg/L), this implies the CMC has about a 90% probability of being within 0.086 to 2.2 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. This suggests a reasonable interim FAV of approximately 1 mg/L, which is similar to that calculated using the NAM approach. I place higher confidence in the limited	Thank you for your comments regarding uncertainties associated with the derivation of the acute estuarine/marine benchmark using a New Approach Method (NAM). As discussed in Appendix L of the draft PFOS Aquatic Life Criteria document, ICE models have undergone extensive peer review and their use has been supported for multiple similar applications, including direct toxicity estimation for endangered species (NRC 2013; Willming et al. 2016) and the development of Species Sensitivity Distributions (Awkerman et al. 2014; Bejarano et al. 2017; Dyer et al. 2006; Raimondo and Barron 2020; Raimondo et al. 2010). EPA has noted and quantified uncertainties associated with the use of Web ICE data to the extent possible in Appendix L. Further, EPA 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 are being developed. However, additional text has been added to further clarify these uncertainties and compare the derived benchmarks to the available empirical test data. Further, additional empirical estuarine/marine toxicity test data have become available since the benchmark value was first derived. The benchmark has been revised to incorporate the additional acceptable empirical data.

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	empirical data and would suggest EPA emphasize it in addition to or in place of the values calculated by the NAM. 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.	EPA thanks you for your comments noting that the approach used to derive the acute estuarine/marine benchmark for PFOS seems 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 PFOS 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	Thank you for your comments that the derivation of the acute estuarine/marine benchmark using Web-ICE to determine an acute estuarine/marine benchmark is logical and includes sufficient conservatism to address uncertainties in the PFOS toxicity data for estuarine/marine species. EPA agrees that additional estuarine/marine test data focused on PFOS would provide support for the derived benchmark value. As such, EPA reviewed additional toxicity studies focused on the effects of PFOS on estuarine/marine species and updated the data used to derive the acute estuarine/marine benchmark. These updated data can be found in Appendix B of the revised draft PFOS

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	for estuarine and marine species. That said, the benchmark should be used cautiously due to the relatively large amount of uncertainty and effort should be made to generate acute and chronic toxicity data for PFOS on estuarine and marine species.	Aquatic Life Criteria Document. As a great deal of toxicity testing is underway, EPA will continue to integrate new acceptable empirical data as they become available until the benchmark value is derived as a final value.
Reviewer 3	• **Is the technical approach used to derive the benchmark logical?** After potential inclusion of the data mentioned below this approach may be appropriate. In the current form with the limited data it may be misleading. Can this guidance be updated? I am aware of other researchers investigating PFAS on marine species (Ed Wirth, NOAA) and maybe others that will be coming out soon. • **Does the science support the conclusions?** I believe the data are incorrect for Fabbri et al. 2014. In table B.1 the reported effect concentration is recorded as >1 mg/L. However, looking at the paper, I read, "*The PFCs PFOA and PFOS induced a dose-dependent effect, with significant decreases in normal larval development from 0.1 µg/L (17% and 27%, respectively; P 0.01). Maximal effects were observed at 100 µg/L (about 40% and 50%, respectively; P 0.001) with no further decreases at higher concentrations*". There is a monotonic concentration-response curve. The associated figure also supports an effect at 0.1µg PFOS/L, see below. Furthermore, if the EC50 of the test organisms is a needed endpoint (as noted in the PFOA justification, for which is lacking support in the current form) looking at the figure below % of normal D-larvae for PFOS (although incorrectly referred to in the legend as PFOAS) could be inferred at 0.1 mg/L. Furthermore, has EPA considered calculating the MATC from this study?	Thank you for your comments indicating that after including addition data from publications noted in the later sections of the comment the approach used to derive the acute estuarine/marine benchmark for PFOS "follows the spirit of the 1985 Guidelines." Additionally, EPA thanks Reviewer 3 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). In this work, the acute (48 h) bivalve embryo toxicity test was applied for screening the developmental effects of different emerging contaminants in *M. galloprovincialis*. The assay was adapted to 96-microwell plates, and standardized in order to obtain normal D-shaped larvae with acceptability of test results based on negative control and positive control (copper) comparable with those reported in literature for *Mytilus* spp. The assay was adapted from *International Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Mollusks* - ASTM E724-98 (ASTM 2004). The recorded test endpoint was the percentage of normal D-larvae in each well in respect to the total, including malformed larvae and pre-D stages. Larvae were considered normal when the shell was D-shaped (straight hinge) and the mantle did not protrude out of the shell, and malformed if had not reached the stage typical for 48-h (trochophore or earlier stages) or when some developmental defects were observed (concave, malformed or damaged shell, protruding mantle). The acceptability of test results was based on controls for a percentage of normal D-shell stage larvae > 75% (ASTM, 2004).

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	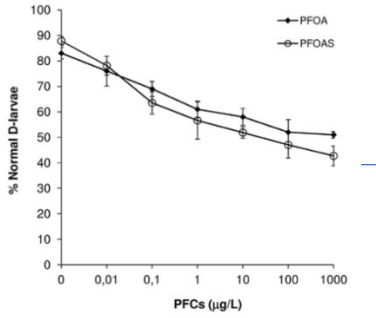 **Fig. 4.** Effects of PFOA and PFOS (0.01–0.1–1–10–100–1000 µg/L) on *M. galloprovincialis* normal larval development in 96-multiwell plates. Data are reported as in Fig. 3a. I did not see data included or the study evaluated for: Robertson JC (1986) Potential for environmental impact of AFA-6 surfactant. Beak Consultants Ltd. Missassauga, Ontario, Canada. EPA Docket AR226-1030a043. There are data for saltwater spp in the ITRC from this citation. • **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.	Fabbri et al. (2014) did not report an acute EC_{50} for *M. galloprovincialis* from exposure to PFOS (or PFOA) because it could not be calculated. The percent adverse effect at the highest concentration of PFOS tested (1,000 µg/L or 1 mg/L) did not exceed a 50% reduction in % Normal D-larvae relative to the negative control treatment. Using the raw data shown in Figure 4 of the publication, % Normal D-larvae in the control treatment of the acute toxicity test with PFOS was approximately 88%; at the highest concentration tested (1,000 µg PFOS/L, nominal) % Normal D-larvae was approximately 45% (a decrease of only 48.86% compared to the control). Based on this information, EPA recorded the acute effect concentration for the test (or 48-h EC_{50}) as > 1 mg/L in Table B.1 of the document (Summary Table of Acceptable Quantitative Estuarine/Marine Acute PFOS Toxicity Studies). EPA's decision to use the acute value of > 1 mg PFOS/L for this study is 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"*

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		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 for Fabbri et al. (2014) was the 48-h EC_{50} of > 1 mg PFOS/L. While the study clearly demonstrates an effect of PFOS on embryo development (with an author reported LOEC of 0.1 µg/L PFOS), 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 the chemicals (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 consideration of a calculated MATC for the study for development of a chronic criterion. The reported NOEC and LOEC are provided and the test results discussed in detail in Section 3.1.1.2 of the draft PFOS Aquatic Life Criteria Document. EPA noted the studies provided by Reviewer 3 (and all other reviewers) that were not included in the draft PFOS Aquatic Life Criteria document and all studies provided in the comments of this external peer review were considered for possible inclusion in the derivation of the criteria and acute estuarine/marine benchmark, as appropriate, based on the data quality review.
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	Thank you for your comment describing specific derivation of the draft acute estuarine/marine benchmark for PFOS in detail. And for noting that draft acute benchmark for estuarine/marine

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	benchmark recommendations for aquatic life using procedures consistent with those in the 1985 *Guidelines*. A total of 3104 datapoints from 398 models were evaluated. • ICE model has been recommended to predict the sensitivity of an untested taxon (predicted taxa are represented by the y-axis) from the known, measured sensitivity of a surrogate species (represented by the x-axis). The ICE model approach used is very reasonable to predict toxicity of untested taxa. • As documented in Section L.1, ICE-predicted models have been used by multiple independent, international groups and further confirms that values developed from ICE-generated SSDs will provide a level of protection that is consistent with using measured laboratory data. • In addition, prediction accuracy and robustness of the model is evaluated using robust parameters (e.g., mean square error, R2), that fall within a defined range of acceptability, and with close prediction confidence intervals that facilitate evaluating the fit of the underlying data. This confirms the robustness of the model. • ICE models predicted with acceptable accuracy for PFOS when invertebrates were used to predict to invertebrate species and vertebrates were used to predict to vertebrate species in these comparisons. • The draft acute benchmark for estuarine/marine aquatic life developed using this approach is 0.43 mg/L PFOS, it is lower than the recommended acute freshwater criterion(3.6 mg/L), suggesting that estuarine/marine species may be more acutely sensitive to PFOS. This is	aquatic indicates that estuarine/marine species may be more acutely sensitive to PFOS, which is in line with the findings from Hayman et al. (2021). EPA noted the studies provided by Reviewer 4 (and all other reviewers) that were not included in the draft PFOS Aquatic Life Criteria document and all studies provided in the comments of this external peer review were considered for possible inclusion in the derivation of the criteria and acute estuarine/marine benchmark.

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	in line with Hayman et al., (2021), confirming marine species have a higher sensitivity to PFOS than compared to the freshwater organisms. • In this report, *Mytilus galloprovincialis* was not used in the FAV calculation because the value was not definitive, and true sensitivity of this species is unknown. There are two more studies published reporting the toxicity values for marine/estuarine species, including *Mytilus galloprovincialis*. 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 **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	• **Is the technical approach used to derive the benchmark logical?**	Thank you for your comment indicating that given the limited availability of PFOS toxicity data for estuarine/marine species the application of Web-ICE to derive an acute benchmark is a reasonable approach. And for further indicating that overall, the

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	Yes, given the lack of PFOS toxicity data for acute estuarine/marine species, I think applying WEB-ICE is a REASONABLE APPROACH…perhaps the only approach that is defensible. Clearly, more (or some) data would be a wonderful contribution. WEB-ICE, as mentioned, has been reviewed and published quite a bit so I think, as an approach, it has merit and support of the scientific community. EPA also did a good job presenting the approach and being clear about the criteria being a draft. Overall, when data have been lacking, EPA has used state-of-the-art approaches to developing criteria (my concerns are mostly when sufficient data are available). • **Does the science support the conclusions?** Yes, the science supports the conclusions. Interestingly, the acute criteria for estuarine/marine species (o.43 mgPFOS/L) is almost an order of magnitude lower than the acute criteria for freshwater organisms (3.6 mg/L). Whether estuarine/marine species are truly more sensitive remains to be seen but, to me, it is more reasonable, given the lack of data, that the criteria draft is lower. • **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*?** Yes, and EPA justified this in the explanation of WEB-ICE that occurs in Appendix L and, overall, the approach and resulting criteria are consistent with the protection of estuarine and marine species.	approach and resulting acute estuarine/marine benchmark for PFOS is consistent with the protection of estuarine and marine species. Additionally, EPA thanks Reviewer 5 for noting that the acute estuarine/marine benchmark for PFOS is almost an order of magnitude lower than the acute freshwater criterion. EPA reviewed Hayman et al (2021), cited by Reviewer 4, and other studies recommended by the peer reviewers for inclusion of recommended PFOS toxicity studies, and studies were included as appropriate, based on their data quality.

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 O.

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 PFOS measurements were not made seems appropriate. The Appendix O analysis is supportive of the general observation that actual concentrations in the toxicity test waters approximated nominal values for freshwater. I agree that actual concentrations in the toxicity test waters for the marine test may be lower than nominal values, thus, effect data originating from marine studies that only report nominal concentrations may be biased high in some cases. Given the tentative/temporary nature of the marine criteria developed in this study, this bias is manageable until additional empirical data from experiment with measured concentrations in water can be provided.	Thank you for your comment indicating the appropriateness of using data from toxicity studies that did not measure the PFOS concentrations and the Appendix O of the draft PFOS Aquatic Life Criteria Document supported this use. EPA intends to continue the consideration and use of toxicity data with unmeasured test concentrations so long as all other test quality guidelines are met in the study. Also, EPA thanks Reviewer 1 for noting that although the effect data from estuarine/marine studies that only reported nominal concentrations may biased high (and therefore the draft acute estuarine/marine benchmark may be slightly under protective) As the results from the measured meta-analysis (presented in Appendix O of the draft PFOS Aquatic Life Criteria) indicate that measured and nominal concentrations from saltwater tests were not in close agreement, with most measured concentrations being lower than nominal. However, it should be noted that the saltwater data were limited compared to the freshwater data. And therefore, it was difficult to discern if these observed differences between measured and nominal concentrations was a result of experimental conditions in saltwater. EPA has reviewed additional toxicity data, both from studies recommended by the peer reviewers and identified in recent ECOTOX quarterly updates and has updated both the freshwater criteria and the acute estuarine/marine benchmark.
Reviewer 2	I am concerned with the approach of using the agreement of measured and nominal concentrations from studies that measured the concentration of PFOS in their tests to determine whether to use toxicity data from studies that did not measure the concentration PFOS in their tests. My concern stems from	EPA appreciates the comment noting that by using both measured and unmeasured toxicity studies in the derivation of the PFOS criteria the assumption was made that unmeasured toxicity studies conducted dosing with the same accuracy and care as those that did measure PFOS test concentrations in their

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	this approach having to assume that studies that did not measure the concentration of PFOS in their experiments performed the dosing of PFOS with the same care and skill as those studies that did measure the concentration of PFOS in their experiments and measured concentrations within 20% of nominal. My concern is compound by 58% and 65% of the freshwater and saltwater 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 PFOS in their experiment? I think the approach that the EPA has used to determine the level of agreement between the nominal and measured concentration of PFOS 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.	experiments. And EPA acknowledges that the Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations in Appendix O of the draft PFOS Aquatic Life Criteria Document does not eliminate this concern. However, unmeasured studies that were used quantitatively to derive the PFOS 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 PFOS and/or difficulty in measuring test concentrations by individual study authors, there would be insufficient data to derive PFOS 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 Appendix O of the draft PFOS 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 and/or experimental conditions that were previously suggested in the PFOS literature (Boudreau et al. 2003a; Boudreau et al. 2003b; Hansen et al. 2001; Martin et al. 2004), 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.

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Reviewer 3	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.	Thank you for your comment that this analysis and the use of both measured and unmeasured toxicity tests in the derivation of the draft PFOS criteria seems acceptable given the data currently available. EPA appreciates Reviewer 3's comment regarding the difficulty of working with PFOS when mixing the chemical into solutions and the variability in PFOS measurements. EPA will include these difficulties in the discussions of PFOS dosing and analytics in Appendix O and the draft PFOS Aquatic Life Criteria Document. Additionally, EPA noted and reviewed the recommended publication by Rewerts et al. (2021). Based on this review, EPA added a statement to Appendix O of the draft PFOS Aquatic Life Criteria Document indicating recent PFAS literature demonstrates the standard variability between nominal and measured concentrations may be as high as 30% (Rewerts et al. 2021; which was referred to Rewerts et al. 2020 in the comment based on the first online publication of this article) as suggested by Reviewer 3.
Reviewer 4	PFOS 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 determine if nominal and measured PFOS concentrations were typically in close agreement, pairs of nominal and corresponding measured PFOS 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 authors reported, 22 freshwater studies with PFOS measured concentrations, yielding 373 pairs of measured and nominal concentrations. In addition, there were 7 estuarine/marine studies with measured concentrations, yielding 142 pairs of measured and nominal concentrations. The data were grouped by	Thank you for your comment summarizing the analysis and resulting conclusions of the Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations in Appendix O and for providing input that the conclusions of the analysis support the inclusion of unmeasured toxicity tests in the derivation of the draft PFOS criteria for aquatic life. Thank you for your comment regarding your experience analyzing PFOS in ecotoxicological studies for freshwater species, which have exhibited strong correlation between nominal and measured concentrations.

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	classifications including water type (salt/fresh) and experimental conditions (acute/chronic; solvent/no solvent; fed/unfed, etc.). Data displayed a high degree of linear correlation and measured, and nominal concentrations were in close agreement The analysis conducted by EPA Team showed strong correlation (correlation = 0.9998) of the 326 pairs of nominal and measured concentrations from freshwater studies. In addition, the experimental conditions did not influence the correlation between nominal and measured concentrations. The detailed analyses of the data in Appendix O and the relevant Tables and Figures provide very comprehensive analyses – this is very useful information and will assist ecotoxioclogist in designing future experiments. ***This confirms inclusion of unmeasured PFOS toxicity tests for quantitative use in criteria derivation.*** Personal experience on analyzing PFOS in ecotoxicological studies using freshwater species have also exhibited strong correlation between nominal and measured concentrations. The authors reported the strong correlation (0.8993) of the 142 pairs of nominal and measured concentrations, the ratio of measured to nominal concentrations from the saltwater dataset showed bias with a geometric mean value of 0.6178. Additionally, the median percent difference between measured and nominal concentration was 30.82%. Furthermore, the saltwater comparison of nominal and measured concentrations indicated that these experimental conditions (acute/chronic and unfed/fed) could influence the observed differences between measured and nominal concentrations. These results suggest that measured and nominal concentrations from saltwater tests were not in close agreement, but this analysis was based on limited set of data.	EPA noted the recommended papers, both the two toxicity papers with measured test concentrations and Rewerts et al. (2021) with details pertaining to potential sources of PFOS variability. All studies suggested for inclusion by reviewers were reviewed to ensure they met data quality objectives outlined by the 1985 Guidelines and EPA's 850 test guidelines (U.S.EPA 1985; U.S.EPA 2016b)., and were included in the draft PFOS Aquatic Life Criteria document, as appropriate, based on their data quality.. A statement has been included in Appendix O of the updated PFOS criteria document indicating recent PFAS literature designates standard variability between nominal and measured concentrations may be as high as 30% (citing Coats et al. 2017 and Rewerts et al. 2021). And the following statement was added to the conclusion of the Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations in Appendix O of the draft PFOS Aquatic Life Criteria Document: *Recent PFAS literature has indicated standard variability between nominal and corresponding measured concentrations may even be as high as 30%. For example, Rewerts et al. (2021) states,* "To agree with nominal concentration, measured concentrations for both stock and exposure solutions were required to fall within the margin of $100 \pm 30\%$, as specified by the guidelines in the consolidated Quality Systems Manual for Environmental Laboratories set by the US Department of Defense and the US Department of Energy (Coats et al. 2017)." *Further, Rewerts et al. (2021) conclude the variability between measured and nominal concentrations may be influenced by solution homogenization and subsampling*

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	The measured concentrations in the recently published paper on marine/estuarine toxicity of PFOS should also be included in this assessment: 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 The second paper is on benthic organisms and PFOS is measured in overlying water, porewater and sediment. This may provide further guidance on difference between PFOS measured and nominal concentrations. 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 **Additional information for Appendix O based on a recently published paper:** *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	*procedures. And noted that for PFOS, it would appear that storage container type may influence agreement between measured and nominal concentrations based on the concerns stated in previous literature. However, it should be noted that container type (as glass or plastic) did not appear to influence the observed differences between measured and nominal PFOS concentrations in EPA's Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations.*
Reviewer 5	I think the comparison of measured and nominal concentrations was an interesting read and a useful contribution. That said, many toxicologists focused on PFAS have commented that	EPA acknowledges that many toxicologists working with PFASs, particularly those at the SETAC North America Focused Topic Meeting on Environmental Risk Assessment of PFAS in

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	analytical confirmation is necessary for a high quality study – this was echoed (loudly) at the SETAC Workshop on Risk of PFAS that occurred in summer, 2019. As well, in my own experience there have been challenges in sometimes matching nominal and measured concentrations for aquatic exposures. The paper by Rewerts et al. 2020 highlights some of the challenges and provides recommendations for accurate solutions of PFAS. As a general rule, we have erred on the side of reporting measured concentrations. Two important thoughts. First, several very prominent analytical chemists that have made a career of measuring PFAS have indicated to me that the analytical method is only about 30% accurate – meaning that if the analytical measure was +/- 30% of nominal, they would be considered "the same". EPA used 20% as a threshold (for deciding nominal and measured were the same) and I'm not sure why this is. As far as I can tell, 30% is a more reasonable threshold. Second, in the review and derivation of toxicity values for the MacDonald et al. 2014 paper, EPA elected not to use the 20-day emergence rate endpoint, in part, because the nominal and measured did not agree. This makes no sense to me. As long as the solutions were confirmed analytically and reported, that should be good enough and, in fact, preferred over nominal alone. Paper worth including in the section on nominal vs. measured PFOS concentrations: Key Considerations for Accurate Exposures in Ecotoxicological Assessments of Perfluorinated Carboxylates and Sulfonates. Justin N. Rewerts, Emerson C. Christie, Alix E. Robel, Todd A. Anderson,	the August 2019 in Durham, North Carolina, have 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 PFOS toxicity tests in the current literature there would be insufficient data to derive PFOS criteria for aquatic life without the inclusion of both measured and unmeasured tests. Considering that the results of the meta-analysis (described in Appendix O of the draft PFOS 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 PFOS aquatic life criteria. Responses to key instances where Reviewer 5 provides particular comments on the Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations in Appendix O of the draft PFOS Aquatic Life Criteria Document are described below in corresponding order mentioned in Reviewer 5's comment: 1. 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 2016). Adjusting to a 30% threshold (as opposed ot the 20% threshold difference in Appendix O of the draft PFOS Aquatic Life Criteria Document) would not meaningfully alter the conclusions.

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	Christopher McCarthy, Christopher J. Salice, Jennifer A. Field Environmental Toxicology and Chemistry, 2020	2. EPA agrees with Reviewer 5's comment that the observed differences between the measured and nominal concentrations in the 20-day emergence endpoint of MacDonald et al. (2004) should not be the reason this test endpoint was not considered for use in the derivation of the chronic water column criterion since the test was measured and those concentrations could be used. However, this was not the reason the 20-day emergence endpoint from this study was not used to derive the chronic water column criterion for PFOS. Instead, this endpoint was not used because it is not considered to be a reliable endpoint at this time given the disparities in the calculated EC_{10}s and the level of data that was presented in the paper, which made independent calculation of the toxicity values less accurate. This particular detail is noted on page C-19 of the appendices in the draft PFOS Aquatic Life Criteria document. Further, EPA clarifies that in the Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations, the 20-day test from MacDonald et al. (2004) was determined to have systematic discrepancies between the measured and nominal test concentrations. Data suggest that the 20-day test had a dosing issue. Given the apparent systematic dosing issue in the 20-day test, all five treatments from this test were removed from the measured meta-analysis alone. 3. EPA noted and reviewed the recommended publication by Rewerts et al. (2021), which was referred to as Rewerts et al. (2020) in the comment based on the first online publication of this article, for possible inclusion in the discussions in the draft PFOS Aquatic Life Criteria document. Please see EPA's response to Review 4's comment to the same Charge Question above

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		regarding the edits that were made to Appendix O of the draft PFOS Aquatic Life Criteria Document for details on how Rewerts et al. (2021) was incorporated.

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 PFOS criteria, with a particular emphasis on:

2.5.a.i. 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 PFOS exposures. Specifically, Yang et al. (2014) conducted a 96-hour renewal, measured PFOS acute test with the midge. This study *Chironomus plumosus* was not acceptable for quantitative use due to the potential problematic source of the organisms. The reported LC50 was 182 mg/L for PFOS indicating that insects may not be one of the more sensitive taxonomic groups. Therefore, this test was excluded from the acute criterion calculation, but used to waive the missing insect MDR.

2.5.a.ii. the use of the quantitatively acceptable chronic toxicity value for mussel (*Lampsilis siliquoidea*) from Hazelton et al. (2012). Specifically, Hazelton et al. (2012) conducted a 36-day renewal, measured PFOS chronic test with fatmucket, *Lampsilis siliquoidea*. The estimated EC10 was 0.05713 mg/L, which was extrapolated from the author-reported data and the exposure response slope from another PFOS toxicity study focused on another mussel species (*Ellipto complamata*) as explained in Section 3.1.1.3.3. Therefore, this test was used in the chronic criterion calculation.

2.5.a.iii. the use of the quantitatively acceptable chronic toxicity value for damselfly (*Enallagma cyathigerum*) from Bots et al. (2010). Bots et al. (2010) conducted a 320-day renewal, unmeasured PFOS chronic test with blue damselfly nymphs, *Enallagma cyathigerum*. The MATC was 0.03162 mg/L, which was calculated from the author-reported value for nymph survival as explained in Section 3.1.1.3.2. Therefore, this test was used in the chronic criterion calculation.

2.5.a.iv. the use of the quantitatively acceptable chronic toxicity value for midge (*Chironomus dilutus*) from MacDonald et al. (2004). MacDonald et al. (2004) conducted a 20-day renewal, measured PFOS chronic test with midge lava, *Chironomus dilutus*. The EC10 was 0.05963 mg/L, which was an EPA-calculated value for 10-day growth as explained in Section 3.1.1.3.4. Therefore, this test was used in the chronic criterion calculation.

2.5.b. EPA's approach for fitting concentration-response (C-R) data (described in Appendix K) as well as the specific acute LC50 values (Appendix A.2) and chronic EC10 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 Criteria		
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. • **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 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 PFOS and may have been more tolerant of PFOS exposures, which would result in biased-high median lethal concentration (LC50) values. If so, this should be explicitly stated. Assuming these *Chironomus* can develop tolerance to PFOS, it seems that they would have to be exposed to rather high mg/L ranges of	Thank you for your comment noting that in most cases the data selected and/or excluded from the derivation were appropriately utilized. And all studies provided in the comments of this external peer review were considered for possible inclusion in the derivation of the criteria, following the approach described in the draft document. To summarize, the review of these recommended studies was conducted to ensure they met the data quality objectives outlined by the 1985 Guidelines and the EPA 850 test guidelines (U.S. EPA 1985 and U.S. EPA 2016, respectively). This further allowed EPA to determine if each individual study should be used quantitively in the derivation of the criteria, qualitatively as supporting information to the criteria, or not used in the criteria due to concerns with data quality or test methodology. Additionally, EPA reviewed all relevant PFOS toxicity studies currently included in EPA's Office of Research and Development's ECOTOX database through the September 2021 quarterly update. Between both the recommended and ECOTOX papers, the number of additional PFOS studies that were reviewed total 51 The review and inclusion of these additional toxicity studies resulted in an update of the draft PFOS criteria, in which the acute, freshwater criterion changed very little (from 3.1 mg/L to 3.0 mg/L) and the chronic, freshwater criterion decreased slightly (from 0.014 mg/L to 0.0084 mg/L).. Responses to key instances where Reviewer 1 does not believe the data were appropriately utilized to derive the draft PFOS criteria are described below in corresponding order mentioned in Reviewer 1's comment:

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	PFOS in water given the reported 96-hour LC50 of 182 mg/L. Based on published literature, I am unaware of natural ecosystems in China (where the animals may have been originally harvested) with concentrations of PFOS 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 PFOS, and the that LC50 result is unbiased. It does seem clearly show that insects may be less sensitive to acute lethality effects of PFOS. 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 chronic data from an unpublished study by Funkhouser (2015) were included for quantitative consideration. As noted on page C-25, the animals in the Funkhouser (2015) study were "purchased from a private collector" and then kept for "several" generations prior to testing. The source of the animals is just as uncertain as the Yang et al (2014) animals, and it is unclear (if PFOS tolerance at lethal levels is possible) how many generations would be needed to shed adaptive tolerance and how it would compare to "several." Simply put, if data from experiments like Funkhouser (2015) are quantitatively included, those from Yang et al. (2014) should also be	Specific to comments to Charge Question 2.5.a: 2.5.a.i Test organisms were obtained from Yang et al. (2014) were collected from Beijing City Big Forest Flower Market and therefore, both the potential exposure to PFOS and other contaminants was unknown. Additionally, the test organisms were held 7 days prior to testing, thus the age of the test organisms was unknown despite the recommended use of 2nd or 3rd instar (ASTM 1994). For these reasons, EPA considered the chironomid toxicity data from Yang et al. (2014) for qualitative use in the criterion derivation. Further, EPA also included additional analyses of the acute criterion which included the LC_{50} of 182.12 mg/L and concluded the chironomid toxicity data had very little influence on the derivation (with a CMC of 3.6 mg/L, which is the same as the draft acute freshwater criterion). Further, additional toxicity data for the genus have become publicly available following the initiation of this external peer review. These additional data were reviewed by EPA and included in the draft PFOS Aquatic Life Criteria Document However, these new data did not include acute toxicity data for this genus as the exposure duration was a minimum of 10 days as opposed to the recommended 4-day exposure duration stated in chironomid test guidelines (OECD 2004a; OECD 2004b). Therefore, EPA retained the acute insect toxicity data discussed previously as qualitative studies and waived the aquatic insect MDR in the derivation of the acute freshwater criterion for PFOS. Regarding Reviewer 1's comments related to the use of the data from Funkhouser (2014), incorrectly

2.5. The Toxicity Data to Derive the Draft Criteria			
Reviewer	**Comments**	**EPA Response**	
	quantitatively included (with some notes on the uncertainty of the animal sources).		referred to as Funkhouser (2015), EPA acknowledges that the source of these test organisms should be considered carefully. However, EPA considered the source and potential previous exposure to be less of a concern for Funkhouser (2014) despite the original source being from a private collector as the test organisms ultimately used in the PFOS experiment were from an established laboratory line housed at Texas Tech University and were reared for several generations before initiation of the PFOS experiment.
	2.5.a.ii There were only three exposure levels in this experiment, including the control. One PFOS dose (4.5 µg/L) indicated an absence of detectable effects on metamorphosis, the other (69.5 µg/L) indicated an approximate 35% reduction relative to controls. This is not a definitive test; there is little dose response information to fully confirm the effects/absence of effects and predict an effective concentration (EC) value with a dose response model. Application of another study's dose response curve to generate EC10 values for this study does not address this fundamental shortcoming, and simply carries too much uncertainty. Although there are only two PFOS doses, which is highly uncertain, use of a Maximum Acceptable Toxicant Concentration (MATC) value may be a less uncertain path to including this study in quantitative calculations. This would result in a more conservative chronic value for this study (0.018 mg/L instead of 0.057 mg/L). Given the high uncertainty of using this result (due to only 2 PFOS doses), I believe this value should be caveated in some way and re-evaluated for use or excluded in future criteria derivation. For example, on page C-22, the Spachmo and Arukwe (2012) value (which also featured a limited PFOS dose design), the document notes that the limited doses "may limit its future use in the criteria derivation pending independent verification of the toxicity values by EPA."	2.5.a.ii Thank you for your comments noting the shortcomings related to the number of exposure levels in the chronic experiment in Hazelton et al. (2012b). EPA noted that this was not a definitive test and that both the study design (which only included two treatment groups) and level of data presented (which are only presented graphically in Figure 2 of the paper) in the publication lack the details needed to fully understand the effects of chronic PFOS exposures to the glochidia and juvenile life stages of *Lampsilis siliquoidea.* EPA made edits to the draft PFOS Aquatic Life Criteria document to ensure that these concerns are clearly discussed (see edits in Section 3.1.1.3.3 in revised draft). 2.5.a.iii Reviewer 1 expressed concerns with using another study's dose response curve to generate an EC_{10} for the chronic exposures to PFOS from Hazelton et al. (2012) as this application carries uncertainty and does not address the shortcomings noted above. The reviewer instead suggests that EPA use a MATC for this study. Following the recalculation of the estimated EC_{10} for Hazelton et al. (2012) using a chronic study by Liu et al. (2013), which was	

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Reviewer	**Comments**		**EPA Response**	
	2.5.a.iii	I agree with the interpretation of the Bots et al. (2010) study and selection of the MATC.		reviewed following the external peer review, the estimated EC_{10} was updated to 0.123 mg/L. Given the similarity between this EC_{10} and the author-reported MATC for Hazelton et al. (2012), the MATC of 0.0177 mg/L was used instead of the estimated EC_{10} to derive the chronic criterion for PFOS. Additionally, as stated in the draft PFOS Aquatic Life Criteria Document, EPA will further refine the toxicity value used in the derivation of the chronic freshwater criterion if EPA is able to obtain the treatment level data from the study authors. Also, additional chronic PFOS data for mussels would be useful to better inform the general use of Hazelton et al. (2012) and the chronic effects of PFOS to mussels in general. However, given the data currently available, EPA included an additional analysis to Section 4.2.2 of the draft PFOS Aquatic Life Criteria document that used the recalculated EC_{10} for Hazelton et al. (2012) using a chronic study by {Liu, 2013 #1137@@author-year instead of the MATC as part of a line-of-evidence discussion to consider the effect the toxicity value has on the chronic freshwater criterion. This additional analysis yielded a chronic water column concentration of 0.0071 mg/L, which resulted in a modest influence when compared the recommended CCC value of 0.0084 mg/L and therefore, the author-reported MATC was used instead. See Section 4.2.2 of the revised draft PFOS Aquatic Life Document for more details Thank you for your comment regarding the interpretation of Bots et al. (2010) and the use of the MATC to derive the chronic freshwater criterion.
	2.5.a.iv	First, on page 104, the document mentioned "an EC10 of 0.0586 mg/L for growth following 10-days of exposure", but on page 115, the document noted "10-day growth with an EC10 of 0.05963 mg/L". In Appendix C (page C-19), the document states "the independently-calculated 10-day EC10 for growth was 0.0586 mg/L." There's some inconsistencies in the value being obtained from EPA's EC10 modeling using this reference; please correct or clarify. One justification for using the 10-day EC10 growth result rather than other results is a lack of being able to calculate EC10s. I do think the emergence results should not be discounted, however. EPA notes "as for the emergence endpoint, there was a lack of a concentration-response relationship and there were very similar levels of observed effects (which ranged between 42.6 and 50.1%) despite the more than nine-fold increase in the mid-range treatment concentrations (0.0023, 0.0144, 0.0217 mg/L, respectively)." The magnitude of the effect (relative to controls), and the fact that there were statistically-detectable differences from controls in some of these doses (0.0144, 0.0217 mg/L) seems to indicate an ecologically meaningful adverse effect is occurring due to PFOS. This range of concentrations just might be a portion of dose response curve that is relatively flat. There is a very clear adverse effect at 0.0949 mg/L. I think it would be reasonable to select the MATC for emergence (0.0071 mg/L reported on page C-19) and treat it a second study point since it	2.5.a.iv	The inconsistencies in the EC_{10} for chironomid growth following 10-days of PFOS exposure were corrected. The correct value of 0.0596 mg/L was used to derive

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	was a completely different experiment from the 10-day experiment used to provide the EC10 of 0.05963 (or 0.0586) mg/L value. A Species Mean Chronic Value using the 0.0071 and 0.0586 mg/L results would be 0.020 mg/L. This 0.020 mg/L value would seem to be protective while including the growth and emergence data from these two experiments. **2.5.b.** More details need to be provided on the dose response modeling using R. Appendix K is helpful for providing the reader with details on the general approach, but where EC10s 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 EC10s 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 EC10 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 EC10s (as shown for the most sensitive EC10s estimated). These steps would be helpful to ensure and demonstrate quality of the model fits and reproducibility of the modeling work. Additionally, somewhere in the document (Appendix K),the 22 dose response model equations should be provided to the reader. Alternately, a reference could be made to a document that	the chronic freshwater criterion and the corresponding text was updated. With regards to using the author-reported MATC of 0.0071 mg/L for emergence in addition to the EPA-calculated EC_{10} of 0.05963 mg/L for growth to calculate a Species Mean Chronic Value that is utilized in the derivation of the draft chronic freshwater criterion, EPA disagrees and continues to use the EPA-calculated EC_{10} of 0.05963 mg/L for growth alone, because the concerns EPA provided in the draft PFOS Aquatic Life Criteria document remained. Consistent with other aquatic life criteria for other chemicals, EPA utilized the most sensitive scientifically-defensible endpoint for this particular study. EPA determined emergence not to be the most scientifically-defensible endpoint despite appearing to be the most sensitive. Specific to comments to Charge Question 2.5.b: In instances where EPA independently fit a model to derive a LC_{50} or EC_{10} estimates for most sensitive genera to acute and chronic exposures to PFOS, the model types are displayed in appendices A and C along with graphs of the data and fitted model with 95% confidence bands for the fit. Details on the functions and model specifications within the R.drc package are available via an internet search or by following the link here: https://cran.r-project.org/web/packages/drc/drc.pdf Standard errors for both the model parameters and LC_{50} or EC_{10} estimates are calculated during evaluation, but this level of statistical detail was not included in version of the draft document that underwent peer review. In response to Reviewer 1, Appendix A.2 and C.2 of the updated PFOS criteria document now include 95% Confidence Intervals (C.I.) as parentheticals

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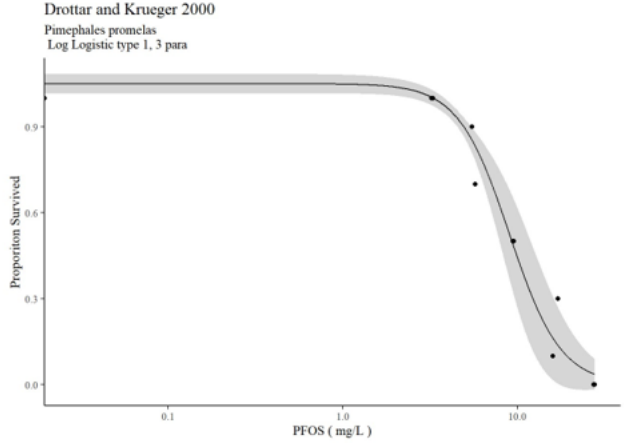
	clearly provides this information (ideally a peer-reviewed or EPA document) containing all 22 models.	following discussion of all point estimates (i.e., LC_{50} and EC_x values) calculated by EPA (irrespective of whether or not the point estimate was used in calculation of the four most sensitive GMAVs and/or GMCVs). Please see below for an example of test-specific C-R modeling results that were reported in Appendix A.2 and C.2 of the draft PFOS Criteria Document. *A.2.1.1 Drottar and Krueger 2000 Concentration Response Curve – Pimephales (fathead minnow)* **Publication**: **Species**: Fathead minnow, *Pimephales promelas* **Genus**: Pimephales **EPA-Calculated LC_{50}**: 9.012 (95% C.I. 7.146315 - 10.892956) mg/L  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
Reviewer 2	• **Were the data selected and/or excluded from the derivation of the criteria appropriately utilized?**	Thank you for your comment. Given the high occurrence of unmeasured PFOS toxicity tests (typically attributed to the relatively high stability of PFOS and/or difficulty in measuring

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	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 measure the concentration of PFOS 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.** I have listed a number of papers below that were published in 2020 and 2021 that the EPA may want to consider in their assessment. 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 perfluorooctanoic 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 Simpson, S.L., Liu, Y., Spadaro, D.A., Wang, X., Kookana, R.S., Batley, G.E., 2021. Chronic effects and thresholds for estuarine and marine benthic organism exposure to perfluorooctane sulfonic acid (PFOS)-contaminated	test concentrations by individual study authors) there would be insufficient data to derive PFOS criteria for aquatic life without the inclusion of both measured and unmeasured 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 (see Reviewer comment to Charge Question 2.4 above). Appendix O of the draft PFOS 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 not systematically indicate discrepancies between nominal and measured concentrations of PFOS, EPA used both measured and unmeasured toxicity studies that otherwise meet EPA's test quality guidelines to derive the PFOS criteria for aquatic life. Additionally, EPA noted the studies provided by Reviewer 2 (and all other reviewers) that were not included in the draft PFOS criteria and all studies provided in the comments of this external peer review were considered for possible inclusion in the derivation of the criteria,as appropriate, based on study data quality.. Please see EPA's response to Reviewer 1's comments to the same Charge Question above for a summary of EPA's review of these studies. Responses to specific comments related to key studies used to derive the draft PFOS criteria are summarized below in corresponding order mentioned in Reviewer 2's comment: Specific to comments to Charge Question 2.5a:

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	sediments: Influence of organic carbon and exposure routes. Science of The Total Environment 776, 146008.. doi:10.1016/j.scitotenv.2021.146008 Li, R., Tang, T., Qiao, W., Huang, J., 2020. Toxic effect of perfluorooctane sulfonate on plants in vertical-flow constructed wetlands. Journal of Environmental Sciences 92, 176–186.. doi:10.1016/j.jes.2020.02.018 Aquilina-Beck, A.A., Reiner, J.L., Chung, K.W., Delise, M.J., Key, P.B., Delorenzo, M.E., 2020. Uptake and Biological Effects of Perfluorooctane Sulfonate Exposure in the Adult Eastern Oyster Crassostrea virginica. Archives of Environmental Contamination and Toxicology 79, 333–342.. doi:10.1007/s00244-020-00765-4 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 Suski, J.G., Salice, C.J., Chanov, M.K., Ayers, J., Rewerts, J., Field, J., 2021. Sensitivity and Accumulation of Perfluorooctanesulfonate and Perfluorohexanesulfonic Acid in Fathead Minnows (Pimephales promelas) Exposed over Critical Life Stages of Reproduction and Development. Environmental Toxicology and Chemistry 40, 811–819.. doi:10.1002/etc.4936 Mccarthy, C.J., Roark, S.A., Wright, D., O'Neal, K., Muckey, B., Stanaway, M., Rewerts, J.N., Field, J.A., Anderson, T.A., Salice, C.J., 2021. Toxicological Response of Chironomus dilutus in Single-Chemical and Binary Mixture Exposure Experiments with 6 Perfluoralkyl	2.5.a.i	EPA noted Reviewer 2's support for not considering the chironomid data from Yang et al. (2014) for quantitative use in the derivation of the acute freshwater criterion for PFOS. And the reviewer raises a good point that aquatic insects appear to be sensitive to chronic exposures of PFOS. However, given the toxicity data that were available during the development of the draft PFOS Aquatic Life Criteria Document that was peer reviewed and in the literature review that was conducted following the external peer review, EPA concluded that aquatic insects appear not to be sensitive to acute exposure of PFOS. EPA will continue to review additional PFOS toxicity literature as it becomes available to better inform the effects of acute exposures of PFOS to aquatic insects.
		2.5.a.ii	EPA thanks Reviewer 2 for providing editorial comments for the summary of Hazelton et al. (2012). Edits were made to the draft PFOS Aquatic Life Criteria Document to correct these editorial issues. Drottar and Krueger (2000) was an acute toxicity study on *Elliptio complanata (*formerly, *Unio complamatus*). A summary for this study can be found in Appendix A (specifically Section A.2.1.1) of the draft PFOS Aquatic Life Criteria Document. Therefore, the observed endpoints between Drottar et al. (2000) and Hazelton et al. (2012) were not the same. Following the external peer review EPA recalculated the estimated EC_{10} for Hazelton et al. (2012) using a chronic study by Liu et al. (2013), which not previously included in the PFOS draft that underwent review. And the estimated EC_{10} was updated to 0.0123 mg/L. Given

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	Substances. Environmental Toxicology and Chemistry 40, 2319–2333.. doi:10.1002/etc.5066 **2.5.a.** 2.5.a.i 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. However, I don't agree with the conclusion that insects may not be one of the most sensitive taxa. Chironomus tentans is a relatively sensitive taxa to chronic exposure to PFOS (MacDonald et al. 2004). In Table C.1, the EC10 for C. tentans is reported as 0.05963 mg/L. Chironomus tentans was also the fourth most sensitive species used in calculating the chronic freshwater criterion (Table 3-6). Also, another insect, Enallagma cyathigerum, another insect species, was the second most sensitive species used in calculating the chronic freshwater criterion (Table 3-6). 2.5.a.ii EPA used an EC10:EC35.4 from Drottar et al. (2000) for Elliptio complanata and applied this ratio to derive an EC10 from the data reported in Hazelton et al. (2012) for Lampsilis siliquoidea. The problem is that EPA have not clearly outlined in section 3.1.1.3.3 what endpoint that Drottar et al. (2000) was measuring in Elliptio complanata (also note that the genus and species are not spelled correctly in section 3.1.1.3.3). Is the endpoint measured in E. complanata the same as the endpoint measure in L. siliquoidea? I tried to look up the endpoint measure in Drottar et al. (2000) but I could not find the study and there was no reference provided in the reference section for Drottar et al. (2000). This missing information makes it difficult to	the similarity between the recalculated EC_{10} and the author-reported MATC for Hazelton et al. (2012), the MATC of 0.0177 mg/L was used instead of the estimated EC_{10} to derive the chronic criterion for PFOS. In addition to these changes in the toxicity value used, EPA updated the text related to the updated estimated EC_{10} for Hazelton et al. (2012) to clarify the specific exposure duration and test endpoints between the two studies included in the estimated EC_{10}. 2.5.a.iii Thank you for your comment regarding the inclusion and interpretation of Bots et al. (2010) in the derivation of the chronic freshwater criterion for PFOS. 2.5.a.iv EPA thanks Reviewer 2 for clarifying the species tested in MacDonald et al. (2004). The authors used the species name *Chironomus tentans*, however, this particular species name has changed in recent years to the species name that EPA used in the draft PFOS Aquatic Life Criteria document of *Chironomus dilutus.* EPA edited the text for MacDonald et al. (2004) in the draft PFOS Aquatic Life Criteria Document to "*Chironomus dilutus,* formerly *Chironomus tentans*" to clarify the issue of the species name. EPA has not been able to obtain additional treatment level data or clarification regarding study design details from the study authors for MacDonald et al. (2004). Instead, EPA independently calculated toxicity values for these experiments using the data that was provided in the publication. As such details for some of the

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	comment on the validity of the approach that EPA has taken to derive an EC10 for L. siliquoidea.	endpoints (i.e., survival) were lacking in critical information relating to sample size and EPA was not able to reliably fit a model to independently calculate toxicity values. However, these details were provided in the paper for other endpoints (i.e., growth) and thus EPA was able to reliably fit a model to independently calculate toxicity values. EPA ensured that these details were clearly provided in the study summary for MacDonald et al. 2004 (see Section 3.1.1.3.4 and Appendix C.2.4).
	2.5.a.iii I think the EPA's justification for the use of the survival data from Bots et al. (2010) is valid. While control mortality reached 40% in the control, the plateau in control mortality after 60 days, the total duration of the test being 200 days, and 82.57% survival in the control from day 60 to 200, justifies the inclusion of the MATC derived from Bots et al. (2010) for Enallagma cyathigerum in the derivation of a chronic criterion.	
	2.5.a.iv First, the wrong species is referenced in relation to the MacDonald et al. (2004) study. MacDonald et al. (2004) reported the toxicity of PFOS to Chironomus tentans. The EPA's derivation of a 10-d EC10 for Chironomus tentans using the data from MacDonald et al. (2004) is not clear. In Appendix C, section C.2.4, the EPA writes, "EPA could not fit a curve to independently verify the 10-day survival (due to a lack of a specific sample size for this endpoint as the number of replicates was not stated in the paper; however, the number of replicates was between 2 and 4 and EPA sought to obtain clarification and treatment level data from the study authors)" It is not clear how EPA got the information necessary, e.g., number of replicates, to fit a curve. It is also not clear what EPA means by "…and treatment level data from the study authors."? Did EPA acquire the raw data for growth from the 10-day toxicity test with C. tentans? If that is the case, they have not made that clear. If that is the case, it would also strengthen their independently derived EC10 for growth in C. tentans. I think the EPA needs to more clearly explain where they got the	Following the external peer review of the draft PFOS Aquatic Life Document, EPA reviewed recently published PFOS toxicity literature and updated the PFOS criteria to include new data that met test quality guidelines. This update includes a recently published chironomid study by McCarthy et al. (2021). The inclusion of this additional study changed the chironomid GMCV from 0.05963 mg/L to 0.009731 mg/L and resulted in this genus being the most sensitive in the chronic PFOS dataset as opposed to the fourth most sensitive. Specific to comments to Charge Question 2.5.b: Thank you for your comment indicating that EPA's independently-calculated toxicity values from concentration-response data was appropriate. Details pertaining to model specifications can be found here: https://cran.r-project.org/web/packages/drc/drc.pdf (Ritz and Streibig 2016) In instances where EPA independently fit a model to derive an ECx estimate, the model types are displayed in appendices A and C of the draft PFOS Aquatic Life Criteria Document along with graphs of the data and fitted model with 95% confidence bands for the fit.

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	data necessary to derive the EC10 for C. tentans used in the chronic criterion. **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 K. I think the LC50 and EC10 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.	
Reviewer 3	See collective responses below • **Were the data selected and/or excluded from the derivation of the criteria derivation appropriately utilized?** Data selection and waiving of the MDR for insect family in the FAV seem reasonable. • **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**	Thank you for your comment indicating that the screening and ultimately waiving the aquatic insect MDR was reasonable for the derivation of the acute freshwater criterion for PFOS. EPA noted the studies provided by Reviewer 3 (and all other reviewers) that were not included in the draft PFOS criteria and all studies provided in the comments of this external peer review were considered for possible inclusion in the derivation of the criteria, as appropriate, based on data quality, following the approach described in the draft document. Further, EPA intends to continue reviewing PFOS toxicity literature as it becomes available. Particularly, as Reviewer 3 noted in the comments,

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	latest update)? If so, please provide references for consideration. The data selection for the derivation of the draft criteria are limited to published and/or available studies from 2018 and prior. This significantly reduces the studies used in the derivation as a number of publications have become available in recent years. For example: A newly published study is available for fathead minnows exposed to PFOS for chronic duration and over the course of reproduction and development. Although, this study was static-renewal, PFOS concentrations are measured ; importantly, this study resulted in a NOEC of 88µg/L based on reduced biomass seen in the second generation (Suski et al. 2020). Importantly, follow-on work (in prep) indicates that this may be a maternal transfer impact as PFOS exposures to juvenile fish alone do not share results. Also, from the authors noted above is an ongoing full life-cycle fathead PFOS and PFAS mixture exposure. This study is being conducted under flow through conditions and is expected to reach termination in December 2021. McCarthy et al. 2021 published data on chironomids (EC20 = 1.7µg/L), these are also not included here. Bryan Brooks (Baylor) and Matt Simcik (UMN) also have acute data on the fathead minnow with measured concentrations, these are not published just yet. David Moore (Army Corps) is near completion of a full life-cycle fish study	since many relevant PFOS toxicity studies are currently underway and/or in preparation for publication. Responses to specific comments related to key studies used to derive the draft PFOS criteria are summarized below in corresponding order mentioned in Reviewer 3's comment: Specific to comments to Charge Question 2.5.a: 2.5.a.i EPA noted Reviewer 3's support for not considering the chironomid data from Yang et al. (2014) based on the source of the test organisms being problematic. EPA continued to not consider the chironomid data from Yang et al. (2014) for quantitative use in the derivation of the acute freshwater criterion for PFOS and instead used the data qualitatively as supporting information. 2.5.a.ii EPA thanks Reviewer 3 for the editorial comment regarding the summary of the chronic exposures to fatmucket in Hazelton et al. (2012) and edits were made to the text to clarify. The text in the draft PFOS Aquatic Life Criteria Document is now: *"The in marsupia exposure was followed by a 24-hour free glochidia exposure consisting of a factorial design. As such the free glochidia from the control group of the marsupia exposure were divided between a control and the two PFOS treatments and the PFOS treatments were split into control and the same PFOS treatment group as the marsupia exposure. This factorial design allowed for the comparison of PFOS effects in two different life-stages."*

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	In particular, SERDP has been funding this research for years and those data are published, recently published or near final. EPA should reach out to SERDP PIs for data inquiries and potential inclusion in these draft criteria. **2.5.a.** 2.5.a.i This seems appropriate, the flower market is most definitely an odd place to purchase research organisms. 2.5.a.ii From Section 3.1.1.3.3: "*The in marsupia exposure was followed by a 24-hour free glochidia exposure consisting of a factorial design, such that free glochidia from the control group of the marsupia exposure were divided between a control and the two PFOS treatments and the PFOS treatments were split into control and the same PFOS treatment group as the marsupia exposure."* - Comment: This is an exceptionally long and confusing sentence please revise to help the reader understand this complex study and overall approach that EPA took. The approach seems ok given the limited data availability at this time. 2.5.a.iii Given the duration of the study the researchers likely hovered around the nominal concentrations of PFOS. Inclusion seems appropriate. 2.5.a.iv I am uncomfortable with this conclusion presented here, it may be more appropriate to use MacDonald et al. data from the 20-day endpoint considering recent publication from McCarthy et al. 2020 as noted above. **2.5.b.**	2.5.a.iii EPA also noted and thanks Reviewer 3 for indicating that the approach used to estimate an EC_{10} fatmucket in Hazelton et al. (2012) seem reasonable given the limited data available. Thank you for your comment regarding the inclusion of Bots et al. (2010) in the derivation of the chronic freshwater criterion for PFOS. 2.5.a.iv Following the external peer review of the draft PFOS Aquatic Life Document, EPA reviewed recently published PFOS toxicity literature, including the study by McCarthy et al. (2021) and updated the PFOS criteria to include new data that met test quality guidelines. This update includes chronic data from McCarthy et al. (2021). The inclusion of this additional study changed the chironomid GMCV from 0.05963 mg/L to 0.009731 mg/L and resulted in this genus being the most sensitive in the chronic PFOS dataset as opposed to the fourth most sensitive in the previous draft that underwent external peer review. Specific to comments to Charge Question 2.5.b: Thank you for your comment indicating that EPA's independently-calculated toxicity values from concentration-response data was appropriate and defensible.

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	This seems like a reasonable and defensible approach if it is applied consistently across genera.	
Reviewer 4	The data selected to derive PFOS 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 and provides further confidence in data selection process. The key acceptable exclusion/inclusion criteria used to derive draft criteria are listed below: • Only single chemical toxicity tests with PFOS 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. • PFOS toxicity tests were not excluded from quantitative use in criteria derivation on the basis of unmeasured test concentrations alone. • Due to lower sensitivity, insect MDR was excluded from the criterion calculation, but were used to waive the missing insect MDR.	Thank you for your comment summarizing the test quality guidelines that were considered in the review of PFOS toxicity data for providing three additional papers that EPA should review for possible inclusion in the derivation of the draft PFOS criteria. There is one point in Reviewer 4's comments that EPA would clarify related to the acute toxicity data for aquatic insects. In the process of deriving an acute freshwater criterion for PFOS, it was determined that there were no quantitative acute, PFOS toxicity data focused on aquatic insects and that one MDR would not be met given the data available at the time. As the derivation of a PFOS acute freshwater criterion is important for the protection of aquatic life exposed to PFOS, EPA considered qualitative data (see Appendix G of the draft PFOS Aquatic Life Criteria Document) to determine if the relative sensitivity of aquatic insects could be ascertained and if the requirement of the missing MDR group could be waived if there was no evidence to suggest aquatic insect are among the four most sensitive genera. As such, there were qualitative data from two acute studies focused on aquatic insects (Yang et al. 2014 and Olson 2017). And the relative sensitivity of aquatic insects following acute exposures to PFOS could not be ascertained as these two qualitative studies indicated contrasting relative sensitivity of aquatic insects. However, the data on midge from Yang et al. (2014) were considered to be more robust as the test was based on measured exposure concentrations, the author reported LC_{50} could be assessed by EPA on a statistical basis since model parameters were provided, and all other study design elements beyond the source of the test organism met EPA's test quality recommendations. Therefore, EPA utilized the data on midge from Yang et al. (2014) to conclude that aquatic insects are likely

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	• 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. Additional toxicity data published over the last six months is listed below: **Marine/estuarine** 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 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 **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). https://doi.org/10.1002/etc.5066	not among the four most sensitive genera and waived the unfulfilled MDR in the derivation of the acute freshwater criterion for PFOS that underwent external peer review. Additional toxicity data aquatic insects have become publicly available following the initiation of this external peer review. These additional data were reviewed by EPA and included in the draft PFOS Aquatic Life Criteria Document However, these new data did not include acute toxicity data any aquatic insects, including chironomid as the exposure duration in the one new test by McCarthy et al. (2021) was a minimum of 10 days as opposed to the recommended 4-day exposure duration stated in chironomid test guidelines (OECD 2004a; OECD 2004b). Therefore, EPA retained the acute insect toxicity data discussed previously as qualitative studies and waived the aquatic insect MDR in the derivation of the acute freshwater criterion for PFOS. Given the recent PFOS toxicity literature that EPA reviewed Also, EPA noted that additional insect toxicity data for PFOS would be very useful for further examining the relative sensitivity of insects to PFOS exposures. EPA noted the studies provided by Reviewer 4 (and all other reviewers) that were not included in the draft PFOS criteria and all studies provided in the comments of this external peer review were considered for possible inclusion in the derivation of the criteria, as appropriate, based on study data quality, following the approach described in the draft document. Responses to specific comments related to key studies used to derive the draft PFOS criteria are summarized below in corresponding order mentioned in Reviewer 4's comment: Specific to comments to Charge Question 2.5.a: 2.5.a.i EPA thanks Reviewer 4 for the comments pertaining to the waiving of the acute MDR for aquatic insects.

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	2.5.a. 2.5.a.i 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). At this stage, I do not fully agree with the statement that midge larvae are tolerant to acute exposures. The OECD protocol recommends 48h acute test for midge larvae and the 48h exposure period is acceptable duration for assessing acute toxicity. The study by Olson (2017) has limitations but this study can't be fully ruled out. The chronic toxicity data exhibits sensitivity of insects to PFOS and this statement is also supported by the authors. 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 are among sensitive taxa to chronic PFOS exposures (with adverse effects reports at low ug/L)* I support the recommendation '*Additional insect toxicity data for PFOS would be very useful for further examining the relative sensitivity of insects to PFOS exposures''*. Unpublished work from our lab shows acute toxicity to midge larva, *Chironomus tepperi* at 1 mg/L PFOS (48 h EC50 value). 2.5.a.ii The authors have provided detailed assessment of this study and explained the approach used for the calculation of chronic toxicity value (section C.2.3-Third Sensitive Freshwater Genus for Chronic Toxicity: *Lampsilis siliquoidea* (mussel). Hazelton et	EPA agrees that the PFOS toxicity date from Olson (2017) should be taken into consideration for the derivation of the acute freshwater criterion for PFOS. However, given the multiple concerns that EPA noted with this study (specifically that publication was missing important exposure details, the author reported LC_{50} and concentration-response curve could not be assessed by EPA on a statistical basis since model parameters were not provided, and there was insufficient treatment level data to independently calculate toxicity values) it was considered for qualitative use and was not used in the derivation of the criterion. Instead, EPA included this study in the additional analyses section of the Effects Characterization of the draft PFOS criteria draft (see Section 4.2.1). This additional analysis indicated that the inclusion of data for yellow fever mosquito (*Aedes aegypti*) from Olson (2017) affects the calculated value for the freshwater acute water column criterion for PFOS, decreasing the criterion magnitude by a factor of 6.9 below the value calculated when waiving the unfulfilled MDR. However, EPA decided not to use data from Olson (2017) in the acute freshwater criterion derivation and noted that additional toxicity data on aquatic insects are needed to fully understand the potential acute effects of PFOS on aquatic insects, especially considering the comparison between qualitative data for midge and mosquito, which indicated very different sensitivities among insects for which data are available. As for the results from MacDonald et al. (2004); Marziali et al. (2019); Stefani et al. (2014), the reviewer suggests that aquatic insects appear to be sensitive to chronic exposures of PFOS. However,

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		al. 2012 used robust study design in spite of including only two concentration of PFOS in this study. The PFOS exposure concentration was measured, and metamorphosis success was used as an endpoint for inclusion in the criteria development. While viability of free glochidia at 24 hours post removal from females was a less sensitive endpoint and did not meet the acceptability criteria. The reduction in metamorphosis success at the 0.0695 mg/L was estimated to be 35.4% but EC10 could not be calculated based on only two PFOS concentrations tested in this study. The EPA team has calculated EC10 (0.05713 mg/L) using the exposure response slope from PFOS toxicity study on another mussel species (*Ellipto complamata*). The explanation and logic provided is reasonable to include the calculated EC10 value to derive the freshwater chronic criterion and to better understand the effects of PFOS on aquatic insects.	given the toxicity data that were available during the development of the draft PFOS Aquatic Life Criteria Document that was peer reviewed, EPA concluded that aquatic insects appear not to be sensitive to acute exposure of PFOS and that the relative sensitivity of aquatic insects may differ between acute and chronic exposures. Thus, making it difficult to ascertain the relative sensitivity of aquatic insects given the available data. And while the recent PFOS toxicity literature that EPA reviewed following the completion of the external peer review includes additional data for aquatic insects these new data did not include acute toxicity data any aquatic insect species, including chironomid, In particular, the exposure duration in the one new test by McCarthy et al. (2021) was a minimum of 10 days as opposed to the recommended 4-day exposure duration stated in chironomid test guidelines (OECD 2004a; OECD 2004b). Therefore, EPA retained the acute insect toxicity data discussed previously as qualitative studies and waived the aquatic insect MDR in the derivation of the acute freshwater criterion for PFOS.
	2.5.a.iii	As a weight of evidence approach, EPA ran additional analyses with some of the other toxicity values for *E. cyathigerum* to understand the influence of this study on the overall chronic criterion. The 150-day MATC was more comparable to the other aquatic insect data and more representative of life cycle effects than the 10-day MATC or NOEC at 60 and 320 days of exposure (Table 4.3 of the report). EPA has concluded that the 150-day MATC should be used quantitatively to derive the chronic freshwater criterion toxicity. In addition, the control survival of test organisms was determined to be acceptable at this time point in the test. I am in agreement with this decision.	2.5.a.ii Thank you for your comment summarizing the chronic exposure of PFOS to fatmucket in Hazelton et al. (2012) and EPA's estimation of an EC_{10} given data limitations, and for indicating that approach used to estimate an EC_{10} for fatmucket from Hazelton et al. (2012) seems reasonable and aids to better understanding of the effects of PFOS. 2.5.a.iii Thank you for your comment regarding the inclusion of Bots et al. (2010) in the derivation of the chronic freshwater criterion for PFOS.

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	2.5.a.iv The observed effects of PFOS on *C. dilutus* reported in the paper by the study authors include survival and growth as weight (measured as mg of ash-free dry mass per individual) for both the 10-day and 20-day exposure durations and emergence and reproduction over the 20-day exposure duration. The author reported 10-day growth and survival EC10s for the study were 0.0492 and 0.1079 mg/L, respectively. The study authors also reported NOECs of 0.0491 mg/L, LOECs of 0.0962 mg/L, and MATCs of 0.0687 mg/L for both endpoints. The author reported 20-day EC_{10}s for growth, survival, and total emergence were 0.0882, 0.0864, and 0.0893 mg/L, respectively. And the study authors also reported NOECs of 0.0217 mg/L for growth and survival and < 0.0023 mg/L for emergence, LOECs of 0.0949 mg/L for growth and survival and 0.0217 mg/L for emergence, and MATCs of 0.0454 mg/L for growth and survival and 0.0071 mg/L for emergence. Independent statistical analyses were conducted by EPA Team for both the 10-day and 20-day exposure durations using data that were estimated The 20-day EC_{10}s for survival and emergence were not considered to be reliable endpoints given the disparities in the calculated EC_{10}s and the level of data that was presented in the paper, which made independent verification of the toxicity values less accurate. The dosing of the 20-day exposure was more of a concern than the 10-day exposure, which had measured concentrations that were much more in line with the expected nominal concentrations. The independently-calculated 10-day EC_{10} for growth was 0.0586 mg/L	2.5.a.iv Thank you for your comment summarizing the chronic toxicity values for chironomid. Following the external peer review of the draft PFOS Aquatic Life Document, EPA reviewed recently published PFOS toxicity literature, including a chironomid toxicity study by McCarthy et al. (2021) and updated the PFOS criteria to include new data that met test quality guidelines. This update includes chronic data for chironomid, which changed the chironomid GMCV from 0.05963 mg/L to 0.009731 mg/L and resulted in this genus being the most sensitive in the chronic PFOS dataset as opposed to the fourth most sensitive in the previous draft that underwent external peer review. Specific to comments to Charge Question 2.5.b: Thank you for your comment indicating that EPA's independently-calculated toxicity values from concentration-response data were appropriate. EPA noted and corrected the missing values Reviewer 4 highlighted in comments to Charge Question 2.8 below.

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	was used quantitatively to derive the chronic aquatic life criterion. The EPA team has reviewed publications by Stefani et al. (2014) and Marziali et al. (2019) as additional supporting information. These authors conducted chronic toxicity tests with *Chironomus* spp. and reported chronic apical endpoints (at low ug/l) but at only at one concentration. **Use of the chronic toxicity data for PFOS in a recent publication should also be considered to assess the reliability of 20-day endpoints (adverse effects reported at 2-3 µg/L) .** 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). https://doi.org/10.1002/etc.5066 **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	

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	available. In certain cases, this approach even improved and helped to select most sensitive toxicological endpoint. 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. As noted in Section 8 some of the values are missing.	
Reviewer 5	• **Were the data selected and/or excluded from the derivation of the criteria derivation appropriately utilized?** As mentioned, I feel that there are some inconsistencies with how some data were included or excluded. In the previous comment, for example, some data were excluded from the MacDonald et al. 2014 paper because there was some disagreement between nominal and measured. With regard to PFAS, I would say measured is almost always better than nominal and the fact that these sometimes don't agree should not be too big of a deal as long as they are not wildly different. EPA put substantial effort into sometimes justifying nominal – in all cases, excluding studies that had analytical confirmation is less defensible than including studies that only report nominal, in my opinion. This last statement is, of course, provided the analytical methods are robust. • **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.**	Please see EPA's response to Reviewer 5's comments to Charge Question 2.4 regarding the use of measured and unmeasured toxicity tests in the derivation of the draft PFOS criteria above. EPA agrees that the observed differences between the measured and nominal concentrations in the 20-day emergence endpoint of MacDonald et al. (2004) should not be the reason this test endpoint was not considered for use in the derivation of the chronic water column criterion. Instead, this endpoint was not used because it is not considered to be a reliable endpoint at this time given the disparities in the calculated EC_{10}s (with EPA's independently-calculated EC_{10} being 0.0102 mg/L and the author-reported EC_{10} equaling 0.0893 mg/L) and the level of data that was presented in the paper, which made independent calculation of the toxicity values by EPA less accurate. This particular detail is noted on page C-19 of the appendices in the draft PFOS Aquatic Life Criteria Document. Further, EPA would clarify that in the Meta-Analysis of Nominal Test Concentrations Compared to Corresponding Measured Test Concentrations, the 20-day test from MacDonald et al. (2004) was determined to have systematic discrepancies between the measured and nominal test concentrations. Given the apparent systematic dosing issue in the 20-day test, all five treatments from this test were identified as outliers and were removed from the measured meta-analysis alone.

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	Sensitivity and Accumulation of Perfluorooctanesulfonate and Perfluorohexanesulfonic Acid in Fathead Minnows (*Pimephales promelas*) Exposed over Critical Life Stages of Reproduction and Development. J.G. Suski, C.J. Salice, M.K. Chanov, J. Ayers, J. Rewerts, J. Field Environmental Toxicology and Chemistry, 2021, pp. 811-819. Toxicological Response of *Chironomus dilutus* in Single-Chemical and Binary Mixture Exposure Experiments with 6 Perfluoralkyl Substances. 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. Environmental Toxicology and Chemistry, 2021, pp. 2319-2333. **2.5.a.** 2.5.a.i Given that insects are among the most sensitive organisms for the chronic exposures to PFOS, it seems the Yang et al. 2014 paper is not very consistent with the prevailing data. Additionally, McCarthy et al. 2021 reports toxicity to chironomids similar to that of MacDonald et al. Additionally, while the Olson 2017 data for Aedes species was not acceptable (for valid reasons), nonetheless the study shows very high sensitivity of another insect species to acute exposures to PFOS. That said, given the EPA's stance and justification that "nominal generally equal measured PFOS concentrations", I'm inclined to put more confidence in Olson's study. Same for the 20-day data in the MacDonald et al. paper on chironomids. In that case, there was a "relatively large difference between measured and nominal concentrations (p. 278)" and so	EPA noted the studies provided by Reviewer 5 (and all other reviewers) that were not included in the draft PFOS criteria and all studies provided in the comments of this external peer review were considered for possible inclusion in the derivation of the criteria, as appropriate, based on study data quality, following the approach described in the draft document. Please see EPA's response to Reviewer 1's comments for a summary of EPA's review of these studies. Specific to comments to Charge Question 2.5.a: 2.5.a.i EPA thanks Reviewer 5 for the comments pertaining to the waiving of the acute MDR for aquatic insects based on the acute toxicity data from Yang et al. (2014). Please see EPA's response to Reviewer 4's comments to the same Charge Question (question 2.5a) above regarding the sensitivity of aquatic insects to PFOS exposure and EPA's previous comments regarding the use of the 20-day chironomid data from MacDonald et al. (2004) immediately above. As mentioned in the comment, additional toxicity data for the genus have become publicly available following the initiation of this external peer review. These additional data were reviewed by EPA and included in the revised draft PFOS Aquatic Life Criteria Document However, these new data did not include acute toxicity data for aquatic insects as the exposure duration was a minimum of 10 days as opposed to the recommended 4-day exposure duration stated in chironomid test guidelines (OECD 2004a; OECD 2004b). Therefore, EPA retained the acute insect toxicity data discussed previously as qualitative studies and waived the aquatic insect MDR in the derivation of the acute freshwater criterion for PFOS.

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		the data were not used. This seems odd to me – as long as there are measured data, that's what I would suggest using. Regardless, the MacDonald et al. paper points to the sensitivity of insects so, collectively, I'd be disinclined to say that the Yang et al. paper shows insects are not sensitive and the data requirement can be waived. I wonder if it's possible to somehow estimate an acute toxicity value for aquatic insects based on chronic toxicity data? Basically, a reverse of the Acute/Chronic ratio approach.	2.5.a.ii Please see EPA's response to Reviewer 2's comments for the same Charge Question. Edits were made to the draft PFOS Aquatic Life Criteria Document to correct these editorial issues. Drottar et al. (2000) was an acute toxicity study on *Elliptio complanata (*formerly, *Unio complamatus*). A summary for this study can be found in Appendix A (specifically Section A.2.1.1) of the draft PFOS Aquatic Life Criteria Document. Thus, the observed endpoints between Drottar et al. (2000) and Hazelton et al. (2012) were not the same. Following the external peer review EPA recalculated the estimated EC_{10} for Hazelton et al. (2012) using a chronic study by Liu et al. (2013), which not previously included in the PFOS draft that underwent review; the estimated EC_{10} was updated to 0.123 mg/L. Given the similarity between the recalculated EC_{10} and the author-reported MATC for Hazelton et al. (2012), the MATC of 0.0177 mg/L was used instead of the estimated EC_{10} to derive the chronic criterion for PFOS. In addition to these changes in the toxicity value used, EPA updated the text related to the updated estimated EC_{10} for Hazelton et al. (2012) to clarify the specific exposure duration and test endpoints between the two studies included in the estimated EC_{10}.
	2.5.a.ii	Unfortunately, I cannot find the Drottar et al. (2000) paper which is the basis of estimating the EC10 from the EC35 generated in the Hazelton et al. (2012) study. And..I think the citation in the document is incorrect and this should be Drottar and Kreugar (2000g)… or check to make sure the citations in text and references match. Moroever, the Drottar paper appears to be an acute test which is VERY different than the Fatmucket study. This approach seems like a "stretch" and, again, somewhat inconsistent with the approaches and decision matrix EPA has used to utilize or discard other data and endpoints.	
	2.5.a.iii	Well, clearly it would have been better to be able to estimate an EC10 but this appears appropriate. I note that for this study the exposure concentrations were an order of magnitude apart; in other cases, EPA has used "too big of a difference between exposure concentrations" to discard a study or two. Somewhere, it would be good to know at what point there is too great a difference among exposure concentrations (10x, 20x, ?) for the study to be deemed acceptable for use quantitatively.	EPA updated the text related to the estimated EC_{10} for Hazelton et al. (2012) to clarify that the exposure duration and test endpoints were not the same. Additionally, EPA is hopeful that additional chronic toxicity data for mussels will become available to better inform the estimated EC_{10} for Hazelton et al. (2012) and the overall relative sensitivity of this taxon.

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	2.5.a.iv I do not agree with the toxicity value used by EPA as obtained from the MacDonald et al. study. The lowest toxicity value is the MATC for 20-day emergence of 0.0071 mg PFOS/L. It is not clear why EPA did not use this value? Emergence is clearly extremely ecologically relevant, and the value generated seems as defensible as most of the other endpoints EPA has chosen to include? Additionally, and as mentioned above, see the paper by McCarthy et al. (2021) that was just published in Environmental Toxicology and Chemistry. Those data appear robust and should meet acceptability criteria. **2.5.b.** In general, the approach for fitting C-R data that EPA used is basically state-of-the-art. The drc package is very powerful and provides a way to test many different curves to then select the best fit model. Although EPA described some of this in the several sections related to "fitting x data (K 1.2)", I think more details would be warranted. The description for the criteria to select best fit models is rather vague. Perhaps a table of specific fit criteria would be helpful? Perhaps this is not doable because every dataset is different. When I teach modules on Akaike Information Criteria (AIC) I emphasize that the metric "penalizes" fit for more parameters within a model. So, using AIC can yield the simplest, best model that fits the data. This is because models with more parameters tend to yield a better fit purely based on statistical properties and not the actual phenomena being studied. I am not aware that AIC is a measure of the model fit to "true outcomes" which are only theoretical constructs, I think. If we knew the "true outcomes" we would not really need the model. Anyway, I would encourage the authors to review the AIC section and	2.5.a.iii Thank you for your comment regarding the inclusion of Bots et al. (2010) in the derivation of the chronic freshwater criterion for PFOS. EPA notes that other studies that were not considered for quantitative use (and therefore not used in the derivation of the PFOS criteria) based on the wide range of the test concentrations, such as an order of magnitude difference between each treatment group, also had other concerns with either the study design or the level of data provided. These concerns are all noted in the study summaries of draft PFOS. 2.5.a.iv The mentioned endpoint from MacDonald et al. (2004) was not used quantitatively to derive the chronic freshwater criterion for PFOS given the disparities in the calculated EC_{10}s (those reported by the study authors and independently by EPA) and the author-reported MATC. The disparities in the EC_{10}s are likely due to the level of data that was presented in the paper, which made independent calculation of the toxicity values less certain (e.g., authors used concentration-response data at replicate level data while EPA only had treatment mean data available). As mentioned in the comment, additional toxicity data for this genus have become publicly available following the initiation of this external peer review. These additional data from McCarthy et al. (2021)were reviewed by EPA and included in the revised draft PFOS Aquatic Life Criteria Document. The inclusion of this additional study changed the chironomid GMCV from 0.05963 mg/L to 0.009731 mg/L and resulted in this genus being the most sensitive in the chronic PFOS dataset as opposed to

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	make edits if necessary and certainly cite the source of the explanation. For section K.2.2. are there actually any criteria (i.e., numbers) that are used to determine when a model fit is appropriate? As a simple example, maybe one would consider an r^2 of 0.8 or better to be a "good model" for linear regression? Some statements to this effect and any details regarding actual criteria used to select "good models" would be helpful. So, overall the curve fitting approach is appropriate but more, specific details would be helpful.	the fourth most sensitive in the previous draft that underwent external peer review. Specific to comments to Charge Question 2.5.b: Thank you for your comment indicating that EPA's independently-calculated toxicity values from concentration-response data was appropriate. Details pertaining to model specifications can be found here: https://cran.r-project.org/web/packages/drc/drc.pdf (Ritz and Streibig 2016). In instances where EPA independently fit a model to derive an ECx estimate, the model types are displayed in appendices A and C along with graphs of the data and fitted model with 95% confidence bands for the fit. The AIC provides an initial ranking of candidate models to yield a subset of possible models to fit. Models are then judged on a suite of statistical metrics (detailed in Appendix K) taken as a whole. There is no one number that would indicate that a model is appropriate. Further, the reviewer is correct concerning individual datasets: each dataset presents distinct challenges in assessing fit. Also, the reviewer is correct that the "true outcomes" phrasing should be revised. As such, edits were made by EPA to clarify the model fits and the selection of toxicity values from these model fits in the derivation of the PFOS criteria.

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 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 bioaccumulation factors (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 fish tissue criterion into water column criterion. The derivation of the PFOS tissue criteria were translated in the manner presented in the draft criteria document because measured effect concentrations in tissue were limited, with only 13 toxicity studies reporting tissue concentrations linked to adverse effects. From these studies, only three of the eight MDRs were met. Therefore, EPA concluded that there are currently insufficient data to derive a chronic tissue criterion using a GSD approach from empirical tissue data from toxicity studies. These details were provided in Section 3.2.2 of the draft PFOS Aquatic Life Criteria Document that underwent external peer review. EPA included a comparison of the translated tissue criteria to the empirical tissue data linked to adverse effects in Section 4.6 of the draft PFOS Aquatic Life Criteria Document that underwent external peer review. This comparison and these studies provided context to the translation of tissue criteria. EPA updated both the tissue criteria and the comparison of these criteria to the empirical tissue data. EPA notes the draft PFOS Aquatic Life Criteria document

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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.	continues to summarize the comparison between the empirically measured and translated tissue concentrations, stating: *"Measured PFOS tissue data were reported in 14 publications focused on freshwater species, six of which were quantitatively acceptable and eight of which were qualitatively acceptable (Table 4-9). The six quantitatively acceptable studies included data for one invertebrate, two fish, and one amphibian species, and the eight qualitatively acceptable studies included data for two invertebrate and four fish species. Results of these studies are summarized in Section 4.6.1 and Section 4.6.2.* *Tissue concentration data from these toxicity studies were compared to the translated tissue values for invertebrates and fish to better understand the protectiveness of the aquatic life tissue criteria. While tissue concentrations from toxicity literature were limited, overall, the comparison indicated that the translated tissue concentrations for invertebrate whole-body, fish whole-body and fish muscle were consistent with those from toxicity studies with direct aqueous exposure. However, tissue concentrations from toxicity studies focused on maternal transfer indicated that the tissue criteria may be under protective and that a reproductive tissue criterion may be needed to ensure protection from PFOS through this exposure pathway (Hazelton et al. 2012a; Wang et al. 2011). Nevertheless, BAF data for reproductive tissues are currently limited; and therefore, a reliable reproductive tissue criterion cannot be derived at this time (see Appendix Q).* *As for other tissue types and taxa with limited data, tissue concentrations from available toxicity studies suggest that the translated tissue concentrations for fish liver and reproductive tissues may be under protective. And while no*

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		amphibian tissue criteria are available, tissue concentrations from two amphibian toxicity tests indicate that the fish tissue criteria may not be protective of amphibians. However, tissue data for these tissues and taxa are limited and additional data are needed." Specific to comments to Charge Question 2.6.a: EPA acknowledges the inherent uncertainties that are present with 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 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). Only studies with greater than two water and tissue samples were collected for each media type, water samples collected within one year and within one to two km. These screening details are provided in Table 2-4 of the draft PFOS Aquatic Life Criteria Document that underwent external peer review. EPA determined that 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. EPA recognizes differences between field-derived and experimentally-derived (or those linked to adverse effects) BAFs. Despite the uncertainties that are noted in Reviewer 1's comments,

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		EPA only used field-derived BAFs to derive the tissue criteria for PFOS. This 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). Additionally, while field-derived BAFs have inherent uncertainties discussed above, EPA concluded that field-derived BAFs better represent real-world bioaccumulation of contaminants, including PFOS, 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 the data indicate that that the PFOS tissue-based criteria are generally expected to be protective of aquatic species. 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. 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	Thank you for your comment regarding the translation of the chronic freshwater criterion into tissue-based criteria with the use of bioaccumulation factors (BAFs). Specific to comments to Charge Question 2.6.a:

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Reviewer	**Comments**	**EPA Response**
	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 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 2-4 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 (Table 3-12). 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.	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. Further, 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 only biota sediment accumulation factors (BSAFs) were developed and BAFs were not. 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 PFOS. Specific to comments to Charge Question 2.6.b: 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.

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Reviewer	**Comments**	**EPA Response**
Reviewer 3	Please clarify, the following sentence: "BAFs used in the derivation of the PFOS tissue criteria consisted of > 2 water and organism samples each and were collected within one year and 2 km distance." It is unclear if the >2 samples refer to the tissue & water samples being mismatched temporally or if there where >2 sets of water and tissue samples that were collected in different years. If the latter then this approach seems appropriate; if the former, EPA should discuss differences in water chemistry between years to alleviate any concerns with matching tissue concentration data to water samples that may have significant environmental temporal variability. A table summarizing the animal tissues used in deriving the BAFs would be helpful to assess the range of fish species and their dietary preferences.	The sentence in question refers to the generalized ranking system that evaluates the number of water samples, the number of organism samples, and the water and organism temporal and spatial coordination that were used to classify BAFs of medium and high quality in Burkhard 2021, which was the source of BAFs used in the translation of the chronic freshwater criterion to tissue criteria for PFOS. Details relating to the factors considered in screening and the overall classification of BAFs were provided by EPA in Table 2-4 of the draft PFOS Aquatic Life Criteria Document that underwent peer review. Regarding the relationship between water quality information for all time periods when water and tissue samples were not collected simultaneously, EPA determined this was impossible to know, because in this scenario water samples were not collected on dates when only tissue samples were collected. Additionally, in the review of PFOA and PFOS BAFs by Burkhard (2021) there is no information regarding the systematic influence of water quality parameters on measured PFOS concentrations in water or organism tissue. However, Review 5 provided comments that "there are some datasets that show considerable temporal and spatial variability in PFAS water concentrations over the course of a few weeks and over a spatial distance of less than 0.5 km" indicating that water quality parameters may influence the concentrations of PFOS. EPA added a table summarizing the information related to the water column and/or tissue concentrations used in the calculation of PFOS BAFs. Further it should be noted that EPA used the PFOS BAFs that were compiled by and can be found in Burkhard (2021). As such tables detailing all the information related to the PFOS BAFs can be found in the supporting information of the paper (see https://doi.org/10.1002/etc.5010).

2.6. The Translation of the Chronic Water Column Criterion Elements for Aquatic Life to Derive the Tissue-Based Criterion Elements Considering Bioaccumulation		
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Reviewer 4	2.6.a. The freshwater chronic PFOS toxicity data with measured tissue concentrations was limited, with no quantitatively acceptable tissue-based tests. Therefore, there were insufficient data to derive tissue-based criteria using a GSD approach from empirical tissue data from toxicity studies. Tissue criteria derived from the chronic water column concentration (CCC) with the use of bioaccumulation factors were developed by EPA. The chronic freshwater criterion also contains tissue-based criteria expressed as 43.0 mg/kg wet weight (ww) for fish whole-body, 25.3 mg/kg ww for fish muscle tissue, and 12.3 mg/kg ww for invertebrate whole-body tissue. EPA developed protective tissue-based criteria through a bioaccumulation factor approach. The authors reviewed PFOS BAF literature based on four criteria 1) number of water samples, 2) number of organism samples, 3) water and organism temporal coordination in sample collection, and 4) water and organism spatial coordination in sample collection and developed a ranking system. BAFs used in the derivation of the PFOS tissue-based criteria consisted of > 2 water and organism samples each and were collected within one year and 2 km distance. This scheme assured selection of only BAFs of high and medium quality to derive the tissue criteria. 2.6.b. BAFs are different for muscle/fillet and whole-body tissues. Humans consume muscle/fillets from	Thank you for your comment summarizing the translation of the chronic freshwater criterion to tissue criteria using PFOS BAFs.

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	fish and soft tissues from bivalves, therefore the water quality criteria recommended by EPA used BAFs based on these tissues. In addition, muscle and whole-body are the most commonly sampled tissue types in monitoring programs. These criteria were developed based on the values reported for 50-60 samples (Table 3-12). Within the body, PFOS tends to bioaccumulate within protein-rich tissues, such as the blood serum proteins and liver. EPA Team calculated additional tissue values for liver, blood, and reproductive tissues by transforming the freshwater chronic water column criterion into representative tissue concentrations using tissue-specific bioaccumulation factors (BAFs). Author's decision uses agreement on the use of female reproductive tissues due to its relevance for potential maternal transfer to offspring. These additional tissue-based values were calculated for comparative purposes and were not proposed as recommended criteria.	
Reviewer 5	2.6.a. Using the BAF for PFOS to determine the tissue-based criterion elements is, I think, an interesting and useful approach given the lack of tissue-based metrics associated with toxicity data. The variability in observed bioaccumulation of PFOS is an active area of research but the work by Burkhard 2021 (also an author on the AWQC) provides an excellent synthesis and compendium of available BAFs for PFOS. That said, I noticed that the criteria for co-located tissue and water	Thank you for your comment regarding the translation of the chronic freshwater criterion into tissue based criteria with the use of bioaccumulation factors (BAFs). Specific to comments to Charge Question 2.6.a: In the review of PFOA and PFOS BAFs by Burkhard (2021) there is no information regarding the systematic influence of water quality parameters on measured PFOS concentrations in water or organism tissue. However, Review 5 provided comments that "there are some datasets that show considerable temporal and spatial variability in

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	samples for PFOS was that they were collected within a year of each other and within 2 km distance (p. 134) – this likely contributes to significant variability in the BAFs. Although there are few published datasets, there are some datasets that show considerable temporal and spatial variability in PFAS water concentrations over the course of a few weeks and over a spatial distance of less than 0.5 km. I wonder if the variability in BAFs would decrease if the criteria were narrowed to co-collected samples measured at the same time? Might be worth the exercise. Given the variability in PFOS BAFs, why not use something like the 25% percentile BAF instead of the geometric mean? When developing a protective criteria and there is a very noisy data set, it might be beneficial to err on the side of caution until better data (many co-located samples in space and time) were available. So, in summary, I think the approach of using BAFs to estimate tissue-based criteria is reasonable but given the variability in BAFs, I would encourage using a lower BAF instead of the geometric mean or reconsidering the data that went into the BAFs used for criteria development. 2.6.b. Invertebrate whole body, fish whole body, and fish muscle are appropriate tissues for the tissue-based criterion. These are the most commonly collected tissue types and are relevant to monitoring efforts and are even useful for considerations of fish advisories. That said, the only other tissue worth considering would be for liver in fish since this tissue accumulates considerably more PFOS than	PFAS water concentrations over the course of a few weeks and over a spatial distance of less than 0.5 km" indicating that water quality parameters may influence the concentrations of PFOS. Please see EPA's response to Reviewer 3's comment regarding the influence of water quality parameters on PFOS BAFs above. The geometric mean of the BAFs were used to be consistent with the BAF methodology (U.S. EPA 2000). Specific to comments to Charge Question 2.6.b:Thank you for your comment regarding the appropriateness of the tissue types included in the draft PFOS Aquatic Life Criteria Document.

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	muscle – these are included in the appendices so this is appropriate. Overall, tissues used for the tissue-based criteria are appropriate.	

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

2.7. The Frequency and Duration of the Criterion Elements		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	The 4-day duration seems to be supported by the more sensitive chronic endpoints used to derive the CCC. For the tissue-based criterion (page 135), 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 PFOS in aquatic ecosystems and recovery time if PFOS 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 PFOS. It is not technically supported to cite recovery times for selenium to support a 10-year recovery time for PFOS, 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 135-136 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 PFOS (i.e., BCF of approximately 2,000 L/kg, wet weight for goldfish, as noted in the EPA TBT criteria document, which is similar to the PFOS BAFs of 1,800-3,100 L/kg, wet weight being used to calculate the fish tissue criteria). TBT is also persistent in aquatic ecosystems, as noted by EPA. Given 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 PFOS tissue criterion should	Thank you for your comment noting that 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 PFOS*." However, we do know that PFOS is stable in water and air (UNEP 2015), and thus, unless the source is eliminated, PFOS is likely to remain in aquatic systems over time. Therefore, EPA considered the bioaccumulative nature and persistence of PFOS in aquatic systems, in combination with the documented recovery times of pollutants with somewhat similar chemical attributes (Gergs et al. 2016; Lemly 1997) set a reasonable and protective exceedance frequency for tissue-based PFOS criteria. The selection of the 10-year exceedance frequency for tissue criteria was not arbitrary. Rather, it was based on disturbance to ecological recovery time relationships with chemicals of similar properties. 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 PFOS Aquatic Life Criteria, the Tributyltin (TBT) Aquatic Life Criteria does not include tissue-based criteria. Only the draft tissue-based PFOS criteria specified exceedance frequencies of 10 years. The 10 year frequency for PFOS tissue-based criteria was set with the intent to provide time for tissue concentrations that accumulate through food webs to diminish, if possible, in source

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	be set at the default of 3 years unless EPA can provide convincing technical information specific to recovery times for PFOS. Additionally, on page 136, the paragraph that begins with "Metals and other chemical pollutants such as PFOS..." 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 PFOS recovery may be "on the order of decades", as EPA notes for selenium. There is no support for the conjecture that PFOS recovery may be "relatively slow" or require decades, as noted in my above comment.	reservoirs lower in the food web before being eliminated in higher trophic level species and allowing for subsequent potential ecological recovery. 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 criteria for PFOS and the chronic TBT criteria both specified a three-year exceedance frequency, as recommended by the 1985 Guidelines (U.S.EPA 1985). Based on ecological recovery times for other bioaccumulative and persistent chemicals, ecological recovery times following elevated PFOS concentrations in the tissues of aquatic organisms is expected to be relatively long to allow for the dissipation of PFOS throughout the food web. The draft PFOS Aquatic Life Criteria document was revised in response to the comment from Reviewer 1. The full text referenced by Reviewer 1 (with added strike throughs to represent deletions and bold text to represent insertions to remove conjecture from the writing) is provided below for information purposes: *"Metals and other chemical pollutants ~~such as PFOS,~~ 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 PFOS has been extensively studies in humans relative to aquatic life. Li et al. (2018) reported a median PFOS half-life of 3.4 years in human serum following exposure to PFOS in drinking water, which authors stated was in the range of previously published estimates.*** *~~As a result,~~* ***Due to chemical retention in tissues,*** *ecosystems impacted by discharges of bioaccumulative pollutants (such as ~~PFOS 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 reproductive failure and deformities in fish, was still measurable (fish deformities) in 1992 (seven years later) and in 1996 (ten years*

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		later). Lemly (1997, pg. 280) estimated based on these data that "the timeframe necessary for complete recovery from selenium contamination from freshwater reservoirs can be on the order of decades." *Beyond bioaccumulation, chemical-specific considerations such as degradation vs. persistence may also provide a mechanism influencing ecological recovery rates. The persistence of PFOS has been attributed to the strong C-F bond, with no known biodegradation or abiotic degradation processes for PFOS. Somewhat similarly, as elements, metals do not degrade and may persist in aquatic systems following elevated discharge. The persistence of metals may explain why metals had the second longest median recovery time of any disturbance described in a systematic review of aquatic ecosystem recovery (Gergs et al. 2016). Gergs et al. (2016) showed recovery times following metal disturbances ranged from roughly six months to eight years (median recovery time = 1 year; 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 been 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.	Thank you for your comment regarding guidance for potential scenarios resulting in one criterion being exceeded while the other criteria are not. Even if it is unlikely that aquatic receptors will exceed or reach these tissue concentrations prior to exceedances from the

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	What I am not clear on is, if tissue concentrations exceed these proposed thresholds yet, PFOS water concentrations do not exceed the CCC, what would be the proposed guidance?	CCC (as suggested by Reviewer 3), EPA notes the draft PFOS Aquatic Life Criteria document stated: *"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."*
Reviewer 4	PFOS 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 PFOS 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 nothing that "*It was appropriate for EPA to inform the recommended ten-year exceedance frequencies for the chronic tissue-based criteria*."
Reviewer 5	In my opinion, the frequency and duration of criterion elements is among the most uncertain and potentially contentious elements of any type of protective criteria. The frequency and duration for tissue-based criteria is that the tissue-based criteria cannot be exceeded more than once in a 10 year period. This means that if the PFOS criterion for whole body in fish of 43 mg/kg bw is exceeded more than once in a 10 year period then the criteria is exceeded. This also means the fish was likely exposed to the 0.014 mg/l	Thank you for your comments. For clarity EPA has broken down and responded to various points in Reviewer 5's comment. **Reviewer 5 Comment A**: *In my opinion, the frequency and duration of criterion elements is among the most uncertain and potentially contentious elements of any type of protective criteria. The frequency and duration for tissue-based criteria is that the tissue-based criteria cannot be exceeded more than once in a 10 year period. This means that if the PFOS criterion for whole body in fish of 43 mg/kg bw is*

2.7. The Frequency and Duration of the Criterion Elements		
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	concentration for longer than an instantaneous exposure and likely longer than 4 days. So, to me, does this not mean that if 43 mg/kg bw was measured in a fish tissue, then the fish was likely exposed to 0.014 mg PFOS/L for longer than 4 days, doesn't it? And, this also means that if fish whole body concentrations were 42.5 mg/kg bw for 10 years, the criterion would not be exceeded. I would suspect that long term PFOS exposures that consistently lead to 42.5 mg/kg bw in fish would likely translate to adverse ecological impacts in some biota present in the same system. When I think of it this way, these criteria do not seem appropriately protective. In my view, the water column continuous exposure criteria should be adjusted downward which would then translate to a lower tissue-based criteria which might be more reasonable. Although, as mentioned, another protective approach would be to use something like the 25th percentile BAF or something other than the mean. That said, at least in many cases fish tissue monitoring occurs on a yearly basis so there is some potential for the criteria to be reasonably assessed against environmental data. It is still possible that fish tissue concentrations could be exceeded every year and this be missed by monitoring efforts. Nonetheless, because tissue concentrations are an intergrative measure and because many monitoring programs probably do measure fish every year, this is a better match than the water column criteria. When we consider the acute and chronic water column criteria, the frequency and duration elements are protective, in my opinion. The problem is that nobody knows if the criteria for acute toxicity are exceeded for more than 1 hour or whether the chronic criteria was exceeded for more than 4 days – this extent of temporal resolution (hourly concentrations or 4-day running averages) just does not exist. So while I agree that conceptually, the frequency and	*exceeded more than once in a 10 year period then the criteria is exceeded.* **EPA Response A**: Following a single instances of tissue values being greater than the corresponding criterion would mean that one excursion (i.e., the event where both criteria magnitude and duration are not met *in situ*) has occurred. If no other excursions occur within the ten-year frequency, then a criterion exceedance will not occur. If additional excursions reoccur within ten years, on average, then the criterion will be exceeded and Clean Water Act (CWA) Section 303(d) action may be taken. **Reviewer 5 Comment B**: *This also means the fish was likely exposed to the 0.014 mg/l concentration for longer than an instantaneous exposure and likely longer than 4 days.* **EPA Response B**: The four-day duration represents the time period over which the chronic criterion is averaged. Please see the except from the 1985 Guidelines below for further explanation: "*The Criterion Continuous Concentration (CCC) is intended to be a good estimate of this threshold of unacceptable effect. If maintained continuously, any concentration above the CCC is expected to cause an unacceptable effect. On the other hand, the concentration of a pollutant in a body of water can be above the CCC without causing an unacceptable effect if (a) the magnitudes and durations of the excursions above the CCC are appropriately limited and (b) there are compensating periods of time during which the concentration is below the CCC. The higher the concentration is above the CCC, the shorter the period of time it can be tolerated. But it is unimportant whether there is any upper limit on concentrations that can be tolerated instantaneously or even for one minute because concentrations outside mixing zones rarely change substantially in such short periods of time*."

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	duration elements definitely would add to the protection of aquatic life…I just don't see how these can be implemented or regulated. Perhaps EPA is aware that my concern is not warranted because in relevant circumstances, appropriately timed environmental data are obtained.	**Reviewer 5 Comment C**: *So, to me, does this not mean that if 43 mg/kg bw was measured in a fish tissue, then the fish was likely exposed to 0.014 mg PFOS/L for longer than 4 days, doesn't it?* **EPA Response C**: It's logical to assume a fish would have to be exposed to PFOS at the chronic criterion magnitude for longer than 4 days for tissue concentrations to reach tissue-criteria magnitudes; however, the four-day duration component of the chronic criterion does not specify actual exposure duration, only the time period in which the chronic criterion is averaged over (see EPA Response B above). **Reviewer 5 Comment D**: *And, this also means that if fish whole body concentrations were 42.5 mg/kg bw for 10 years, the criterion would not be exceeded. I would suspect that long term PFOS exposures that consistently lead to 42.5 mg/kg bw in fish would likely translate to adverse ecological impacts in some biota present in the same system. When I think of it this way, these criteria do not seem appropriately protective. In my view, the water column continuous exposure criteria should be adjusted downward which would then translate to a lower tissue-based criteria which might be more reasonable. Although, as mentioned, another protective approach would be to use something like the 25th percentile BAF or something other than the mean.* **EPA Response D**: The criteria magnitudes for tissues were based on the PFOS water column-criterion, which was the fifth centile of the chronic Genus Sensitivity Distribution (GSD; composed primarily of EC_{10} values). Consequently, the chronic criterion is protective aquatic life from unacceptable chronic PFOS exposures in water, including approximately 95% of taxa at a 10% effect level. Because the tissue criteria are based on the chronic water column-criterion itself, Reviewer 5 is incorrect to postulate the water column criterion may not be protective based on the magnitude, duration, and frequency components of the tissue-based criteria. The 10-year frequencies of the tissue criteria do not explicitly imply species will be exposed to

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		PFOS in the water column continually for 10 years. Instead, the 10-year frequency for tissue-based criteria was set to provide time for tissue concentrations that accumulate up through food webs to potentially diminish in source reservoirs lower in the food web before finally being eliminated in higher trophic level species and allowing for subsequent potential ecological recovery. Finally, EPA notes the draft PFOS water quality criteria document explicitly stated: *"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."* **Reviewer 5 Comment E**: *That said, at least in many cases fish tissue monitoring occurs on a yearly basis so there is some potential for the criteria to be reasonably assessed against environmental data. It is still possible that fish tissue concentrations could be exceeded every year and this be missed by monitoring efforts. Nonetheless, because tissue concentrations are an integrative measure and because many monitoring programs probably do measure fish every year, this is a better match than the water column criteria.* **EPA Response E:** EPA thanks Reviewer 5 for indicating both the protectiveness and utility of the magnitude, duration, and frequency components associated with the tissue-based criteria. **Reviewer 5 Comment F**: *When we consider the acute and chronic water column criteria, the frequency and duration elements are protective, in my opinion. The problem is that nobody knows if the criteria for acute toxicity are exceeded for more than 1 hour or whether the chronic criteria was exceeded for more than 4 days – this extent of temporal resolution (hourly concentrations or 4-day running averages) just does not exist. So while I agree that conceptually, the frequency and duration elements definitely would add to the*

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Reviewer	**Comments**	**EPA Response**
		protection of aquatic life…I just don't see how these can be implemented or regulated. Perhaps EPA is aware that my concern is not warranted because in relevant circumstances, appropriately timed environmental data are obtained. **EPA Response F**: 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 an hour duration or average chronic concentrations of 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 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 exceedances will be allowed*."

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

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
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) In general, the document needs some quality copy editing effort. I found many typographical errors, issues with formatting, reference/citation issues, and in some cases, poorly-worded text. I have noted a few of these instances below. c) Page xv: "25.3 mg/L ww" is not correct units for a concentration in tissue. d) Page 6: "The carbon chain can be fully fluorinated…". Please specify that this applies to PFAS in general, not to PFOS. e) Page 6: The reference to "Table 2-1"; should that be Table 1-2? f) Page 9: Please note in Figure 2-1 that this is the linear isomer of PFOS. It would be helpful to note that the PFOS data in this study are likely from experiments with water spiked with the linear PFOS isomer. It is hypothesized that toxicity and bioaccumulation may differ between branched and linear forms of PFCAs and PFASs. Linear PFOS is thought to be more accumulative (as noted on Page 45) and potentially more toxic to aquatic life when the dose is	Thank you for your comments. Responses to the corresponding alphabetical comments are provided below: a. Thank you for your comment. EPA has received the final peer review report from the contractor. This peer review has concluded and comments received are reflected in this final report. There is no ability to supplement or amend comments. Thank you for your review. b. - e. The grammatical errors and requested clarifications that were noted by the peer reviewers have been corrected in the revision to the draft PFOS Aquatic Life Criteria document. Thank you for your comments. f. EPA edited the caption for Figure 2-1 to note that this is the linear isomer of PFOS. And, as stated in the draft PFOS Aquatic Life Criteria document, the criteria provides a critical review of all aquatic toxicity data identified in EPA's literature search for PFOS, including the anionic form (CAS No. 45298-90-6), the acid form (CAS No. 1763-23-1), a potassium salt (CAS No. 2795-39-3), an ammonium salt (CAS No. 56773-42-3), a sodium salt (CAS No. 4021-47-0), and a lithium salt (CAS No. 29457-72-5). Further, EPA added the requested text to note that PFOS toxicity studies typically

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	expressed as an external water concentration. At some sites, a portion of the concentrations of PFOS in water (which are reported as the sum of branched and linear PFOS) is branched PFOS, so criteria derived from linear PFOS could be overly protective. Please include this uncertainty in the discussion in the document. g) Page 18: To my knowledge, FTSAs degrade to PFCAs, not PFSAs like PFOS. See Zhang et al. (2016): Zhang, S., Lu, X., Wang, N., & Buck, R. C. (2016). Biotransformation potential of 6:2 fluorotelomer sulfonate (6:2 FTSA) in aerobic and anaerobic sediment. Chemosphere, 154, 224–230. doi:10.1016/j.chemosphere.2016.03.062 h) Page 62: Regarding "The importance of the sediment pathway for PFOS bioaccumulation…" Larson et al. (2018) conducted some insightful food web modeling on benthic and pelagic sources of PFOS. See: Larson, E.S., Conder, J.M., Arblaster, J.A. 2018. Modeling avian exposures to perfluoroalkyl substances in aquatic habitats impacted by historical aqueous film forming foam releases. https://doi.org/10.1016/j.chemosphere.2018.03.004 Chemosphere 201:335-341. i) Page 66: 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 PFOS in tissue. There were other instances of this error in the document as well. For units of every concentration of PFOS in tissue, please be sure to specify dry weight or wet weight. j) Page 73-75: There are a few scientific names on these pages that are not italicized. Also may occur in other portions of the document. k) Page 78: USEPA (1998) is not cited in the references section; I fear there may be other similar omissions.	utilize the linear PFOS isomer for dosing with fewer studies using the branched isomer. g. EPA thanks Reviewer 1 noting that FTSAs do not appear to degrade to PFSAs like PFOS and agrees. The corresponding text was edited in the draft PFOS Aquatic Life Criteria document to correct the text. h. Since the draft PFOS Aquatic Life Criteria document is solely focused on exposures to aquatic life, Larson et al. (2018) was not included in the discussion of bioaccumulation of PFOS because this paper focused on food web modeling for aquatic birds. i. EPA will ensure that all tissue concentrations reported in the draft PFOS Aquatic Life Criteria Document are reported in either wet or dry weight (ww or dw, respectively). And in the instances where this information is not provided by study authors, EPA will specify that the information was not provided. j. EPA edited text throughout the draft PFOS Aquatic Life criteria Document to ensure that all species names are italicized. k. EPA ensured that all cited references are included in the references section.

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	l) Page 78: Where the 1985 guidelines are mentioned, please cite to USEPA (1985). m) Page 78: Replace the "Stephan et al. (1985)" citation with USEPA (1985). Also, I believe the surname of senior author of USEPA (1985) is Stephen, not Stephan. n) Page 80: At the start of Section 2.10.2, it would be good to discuss linear and branched PFOS. o) Page 86: The use of EC10 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 EC10s 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 EC10s were selected over EC20s "given the nature of exposure and effects for this bioaccumulative chemical." Additionally EPA (2016) selected EC10 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." p) 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 PFOS in a manner than justifies the use of EC10s. For example, EPA in its 2016 aquatic life criteria for cadmium noted that cadmium "can bioaccumulate in aquatic organisms", but EC20s (not EC10s) 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 EC10s instead of EC20s) simply because a chemical has a higher bioaccumulative	l. & m. The 1985 Guideline citation was changed to U.S. EPA 1985. n. Please see EPA's response to item f. above. o. – s. EPA retained the use of the chronic EC_{10} values to ensure species protection, considering the long-term persistence of PFOS in the aquatic environment. Further, the use of the EC_{10} to derive chronic criteria magnitudes is also consistent with the harmonized guidelines from OECD (OECD 2001) and the generally preferred effect level utilized in the derivation of protective values for contaminants, including PFOS, in other countries such as Canada, Australia, and New Zealand (CRCCare 2017; ECCC 2018; EPAV 2017). EPA also retained use of EC10 values to further afford protection of aquatic life from this bioaccumulative "forever" chemical. t. EPA agrees that the previous text stating that empirically derived tissue criteria would be derived from only studies in which test organisms were exposure via diet. This statement has been revised to state: *"EPA considered deriving tissue-based criteria using empirical toxicity tests with studies that exposure test organisms to PFOS via water, diet,*

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	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 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. q) Second, EPA has not provided any analysis of the dose response curves that demonstrates the need for EC10s versus EC20s (as was mentioned for selenium). Additionally, justification of the use of EC10s 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. r) More discussion is needed to support the poorly-supported move from EC20s to EC10s, or alternately, EC20s need to be used in throughout the document, as consistent with past EPA practice in	*and/or maternal transfer and reported exposure concentrations based on measured tissue concentrations."* u. Reviewer 1 is correct that there is only one citation for Bots et al. (2010). EPA corrected the citations to reflect this. v. EPA decided to intentionally leave the qualitative *Aedes* data point off of Figure 3-5 as the qualitative *Chironomus* data point was considered to be more robust and was used to conclude that aquatic insects are likely not among the four most sensitive genera and used to waive the unfulfilled MDR. This justification is described in greater detail in Section 3.1.1.1.8 of the draft PFOS Aquatic Life Criteria Document. EPA concluded that including the *Aedes* data point in Figure 3-5 would lead to confusion. w. Reviewer 1 is correct. The noted sentence should state for PFOS instead of PFOA. EPA corrected the text. x. The noted reference to Appendix Q was incorrect in the draft PFOS Aquatic Life Criteria Document that underwent external peer review. This reference was updated to be Appendix P. y. EPA ensured that all percent effects related to controls were added for studies where the LOEC was used to derive the criteria. EPA included such text in both

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	aquatic life criteria derivation. EC10s are more conservative than EC20s, but there is often greater variability and uncertainty associated with EC10 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, EC10s 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., Efroymson, R.A., Sample, B.E., & Jones, D.S. (2000). Ecological Risk Assessment for Contaminated Sites. CRC Press. April). s) Overall, the adoption of a more conservative 10% effect level (i.e., EC10) 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. t) Page 88: 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 PFOS 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).	the main body and the appendices of the draft PFOS Aquatic Life Criteria Document. z. EPA agrees that the noted endpoints are non-apical endpoints and therefore were not used in the derivation of the draft aquatic life criteria for PFOS. However, inclusion in Table 4-9 under the qualitative studies section was meant to be part of the line-of-evidence discussion comparing empirically measured tissue concentrations from toxicity studies to the tissue-based criteria derived for PFOS. Therefore, EPA retained these non-apical endpoints in this particular section and Table 4-9. aa. EPA updated the text relating the endpoint for this study to reflect the specific Gosner Stage the length was measured. bb. The endpoint referred to in the comment is time to a specific Gosner Stage, not just the Gosner Stage itself as stated in the comment. EPA edited the text to clarify the endpoint as *"this time (in days) to reach Gosner Stage 40."* This particular endpoint as the time to reach a specific Gosner Stage (in this case of 40) is a growth endpoint as it speaks to the development and overall growth of the test organism.

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	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. u) Page 112: There's only one "Bots et al. 2010" in the references section. Multiple instances of "Bots et al 2010b" are cited in this document. I believe there is only one Bots et al. 2010 paper. Please clarify. v) Page 125: The Aedes data point is missing from Figure 3-5. If the qualitative Chironomus data point is included please include Aedes. w) Page 132: "expected to protect P. primulas from chronic time-variable PFOA exposures"… should that be "PFOS" instead? x) Page 135: A reference for "Appendix Q" is made. Please provide Appendix Q. y) The percentage effect for LOECs (relative to controls) needs to be clearly noted in the Appendices, for example, in Table C.1 and in the detailed summary text for the reviews of each paper. This should be provided when LOECs or MATCs are used as chronic values. z) Page 173: "Reduction in superoxide dismutase" and "Changes in protein expression" are atypical endpoints not well tied to ecologically significant effects. These should be removed from the table and subsequent discussion, or presented separately as qualitative analyses only. aa) Page 173: It is not appropriate to refer to the Gosner stage endpoint as "Length at metamorphosis" in the table. Refer to it as "Gosner stage" if it is to be included.	cc. Please see EPA's response to comment y above. dd. EPA provided additional details to clarify how the SMAV/SMCVs were calculated for this and all other species. ee. EPA provided details relating to the additional treatment-level data requested from individual study authors, but has not shared these data publicly as part of the draft PFOS Aquatic Life Criteria Document as these data are subject to copyright by the individual publishing journals and EPA was not granted permission to share data publicly beyond the scope of the request to the individual authors. ff. A model figure for the chronic tests from Hazelton et al. (2012) is not missing from Section C.2.3.1 as the EC_{10} was not estimated like the other studies in the draft PFOS Aquatic Life Criteria through model fitting, but was estimated by assuming the 0.0695 mg/L treatment represents an $EC_{35.4}$, and estimating the EC_{10} using the exposure response slope from another PFOS toxicity study focused on another mussel species (*Perna viridis*). Please see the summary under Charge Question 2.5.a.ii above and Section 3.1.1.3.3 of the draft PFOS Aquatic Life Criteria Document for additional details.

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	bb) Page 176: Gosner stages are not a typical endpoint, and the use of the growth data would be much more supportable. See comment below regarding Page C-42. cc) Page C-2: When the MATC is used in tables in the Appendices, it would be helpful to provide the percent effect level (relative to control) for the LOEC associated with the MATC. Also, in cases in which the LOEC is provided as the chronic value, please provide the percent effect level. dd) Page C-3: How was the Species Mean Chronic Value (SMCV) for leopard frog calculated? There is only one chronic value that is bolded in the data, and it does not equal the SMCV. Please add text to clearly discuss which values are included (and how the ">" values are used in subsequent calculations like geometric means). ee) Page C-7: For Wang et al., since this value is the lowest used in the criterion derivation, please share a table of the raw data graphed in the Figure on page C-7. ff) Page C-16: Appears to be a missing figure. gg) Page C-29: Typo "XX.XX mg/L". There are other typos like this in the document (search for "XX"). hh) Page C-42: The amount of detail for the review of the Hoover et al. (2017) experiment is insufficient. The selection of the Gosner stage as an endpoint requires additional detail. The relationship between Gosner stage and more typical endpoints clearly linked to ecological health (growth, reproduction, and survival is unclear). The effects on Gosner stage in this study are subtle; all dosed animals indicated they had reached tadpole stage (Gosner stages 25-41) at the 40-day endpoint noted. The maximum difference in Gosner stages noted in the study was approximately 2 (control Gosner stage result of ~30, 100 and 1000 µg/L Gosner stage results of ~28). A 7% difference in Gosner stages (especially when both 28 and 30 values fall within a tadpole Gosner stage development range) is difficult to translate to	gg. The noted typo of XX.XX mg/L was intended to be placeholder for a value. All instances of these placeholders have been updated throughout the draft PFOS Aquatic Life Criteria Document. hh. EPA thanks you for your comment on (Hoover et al. 2017). EPA reconsidered the study and the endpoints that were used to derive the draft PFOS aquatic life criteria. This re-evaluation of this study will also take other recently published studies on amphibians. ii. The noted repeated text was deleted. jj. EPA thanks you for your comment on Han et al. (2015). EPA reconsidered the study and the endpoints that were used to derive the draft PFOS aquatic life criteria. The revised criteria document now states: *The publication is unclear about the method used for the reproduction test endpoint and whether it was an independently conducted 10-day test or a subsample of reproducing adults were observed from the 20-day test. EPA sought but did not receive responses to clarifying questions posed to the authors. Additionally, the authors were asked if control survival for the test was above 80% and if the authors could provide the data. Based on the information presented in the paper without additional information and*

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	adverse ecological impact. As shown in the Gosner stage chart for anurans (Virginia Herpetological Society, http://www.virginiaherpetologicalsociety.com/amphibians/amphibian-development/amphibian-development.htm) the difference between stage 28 and stage 30 is the shape of the tail. It is unclear if this statistically detectable difference in the tail shape that distinguishes Gosner stage 28 and 30 would result in an ecologically significant decrease in the overall time period required to reach sexual maturity or ultimately translate to a developmental malformation that would result in an ecologically meaningful population-level effect (decrease in survival, decrease in reproductive output, etc.). The uncertainty with this atypical endpoint is high, and given the slight difference (~7%) between NOEC and LOEC exposures, I would recommend this datum be removed from the quantitative analysis. Notably, Figure S2 of this paper presents results for a measurement of growth via the Snout Vent Length (SVL) measurement endpoint. This endpoint provide more a continuous measurement of growth and is more typical of endpoints used in criteria derivation. ii) Page C-44: "In the later phases of the tests, (Bots et al. 2010a)" is repeated. jj) Page D-3: Regarding the Han et al (2015) study, I disagree with the selection of the less conservative growth endpoint. The reproductive effect does look to be valid and a reasonable endpoint to consider. EPA's reasoning to exclude it is not compelling and is unclear. The exposure duration was at least 10 days, which is likely sufficient for many marine invertebrates with relatively short life cycles (i.e., mysids). Perhaps EPA could reach out to the study authors to clarify the uncertainty around the exposure time (10 days or 20 days?). At any rate, I think the MATC should rely on the reproductive endpoint, and given the good dose-response for the reproductive data, a robust EC10 or EC20 value could likely be calculated. kk) Page G-3: Seems like the Olson (2017) snail experiment provides some useful chronic (21-day exposure?) data for a relevant sublethal	*data provided by the authors to clarify adherence to EPA data quality objectives and independent calculation and verification of point estimates, the developmental stage is considered for quantitative use and the reproductive endpoint for qualitative use. The use of the reproductive endpoint could be changed based on input on clarifying questions from the study authors. The 20-day MATC (based on time to reach development stage) was 0.7071 mg/L and currently recommended by EPA as acceptable for quantitative use.* kk. & ll. Table G.1 of the draft PFOS 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 in established test protocols/methods, that were referenced in EPA's draft PFOS Aquatic Life Criteria document. The draft PFOS Aquatic Life Criteria document specifically stated: *"All studies were evaluated for data quality generally as described by U.S.EPA (1985) in the 1985 Guidelines and in EPA's Office of Chemical Safety and Pollution*

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	growth endpoints. Please explain why this data was excluded from the chronic evaluation. Simply listing "Duration" in this table does not provide enough detail. ll) Page H-1: Please explain the acceptable duration acceptable for the urchin test and other tests. Simply listing "Duration too short" without noting the acceptable duration that would be considered is not helpful. Perhaps a summary table for acceptable durations for particular endpoints could be provided in this document. mm) 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. nn) Page P-12: The Hoover et al. (2017) paper is included twice. There may be more errors like this in the document, it needs to be reviewed closely by a technical editor. oo) Appendix L: The references cited in this section seem to be missing.	*Prevention (OCSPP)'s Ecological Effects Test Guidelines (U.S.EPA 2016b), and EPA OW's internal data quality SOP, which is consistent with OCSPP's data quality review approach (U.S.EPA 2018). These toxicity data were further screened to ensure that the observed effects could be primarily attributed to PFOS exposure*. mm. Use of "*atypical duration*" occurred 15 times within Table H.1 and twice in Appendix H.2 (i.e., summaries of studies in Table H.1) and did not occur elsewhere in to draft PFOS 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."* nn. The in-text citations and references have been updated to remove any duplicates as noted with Hoover et al. (2017). oo. EPA ensured that all cited references, including those in Appendix L, are included in the reference section of the

<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></td><td>draft PFOS Aquatic Life Criteria Document.</td></tr>
<tr><td>Reviewer 2</td><td>I think the EPA's criteria for PFOS 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.</td><td>Thank you for your comment.</td></tr>
<tr><td>Reviewer 3</td><td>All technical comments have been previously mentioned</td><td>Thank you for your comment.</td></tr>
<tr><td>Reviewer 4</td><td>Additional suggestions are listed below:

1. The species listed in the table is Mytilus galloprovincialis not M. edulis

Table 3-1. The Three Most Sensitive Acute Estuarine/Marine Genera.

Ranked Below from Most to Least Sensitive.
<table>
<tr><th>Rank</th><th>Genus</th><th>Species</th><th>GMAV (mg/L PFOS)</th><th>Comments</th></tr>
<tr><td></td><td>Mytilus[1]</td><td>Mediterranean mussel,
M. edulis
Mytilus galloprovincialis</td><td>> 1</td><td>Not a resident species in North America, but other species in this genus are resident, commercially, or ecologically important species</td></tr>
</table>
2. Page 115- second paragraph (values highlighted in red and underlined are not consistent)</td><td>Thank you for your comment. Responses to corresponding numerical order are provided below:

1. The species name for Mediterranean mussel was changed from M. edulis to M. galloprovincialis.

2. The noted values were changed to ensure that they are consistent. The first noted value of 0.0271 mg/L is the correct value.

3. The units were corrected in the noted text to state:
"The chronic freshwater criteria also contain tissue-based criteria expressed as 43.0 mg/kg wet weight (ww) for fish whole-body, 25.3. mg/kg ww for fish muscle tissue, and 12.3 mg/kg ww for invertebrate whole-body tissue."

4. The noted text was a placeholder for text and was replaced with the following text:</td></tr>
</table>

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	The author reported 10-day growth and survival EC_{10}s for the study were 0.0492 and 0.1079 mg/L, respectively. The study authors also reported NOECs of 0.0491 mg/L, LOECs of 0.0962 mg/L, and MATCs of 0.0687 mg/L for both endpoints. And the author reported 20-day EC_{10}s for growth, survival, and total emergence were 0.0882, 0.0864, and 0.0893 mg/L, respectively. And the study authors also reported NOECs of 0.0217 mg/L for growth and survival and < 0.0023 mg/L for emergence, LOECs of 0.0949 mg/L for growth and survival and 0.0217 mg/L for emergence, and MATCs of 0.0454 mg/L for growth and survival and 0.0071 mg/L for emergence. Also, it should be noted, the paper reported contrasting NOECs for 20-day survival. The text in the paper stated that the NOEC was 0.0271 mg/L and Table 2 of the paper stated 0.0949 mg/L. EPA assumed the NOEC in Table 2 of the paper was not correct and that 0.0217 mg/L was the correct NOEC based on the data presented in Figure 3A of the paper. This assumption was applied to the summary of the study results presented in this PFOS draft criteria. 3. Page 138-middle of the paragraph The chronic freshwater criteria also contain tissue-based criteria expressed as 43.0 mg/kg wet weight (ww) for fish whole-body, 25.3 mg/ ---ww for fish muscle tissue and 12.3 mg/kg ww for invertebrate whole-body tissue. 4. Page A-21 last paragraph The noted toxicity values provided in each study summary above (ADD NUMBERS), comprising of both author-reported and independently-calculated LC_{50} values, were used to calculate the GMAV value (as the geometric mean of the three LC_{50} values previously mentioned) of 22.48 mg/L, which was used to derive the freshwater aquatic life criterion. 5. Page A-24- Fourth line from bottom-The study author reported LC50 was 22.2 ± 4.6 mg/L for PFOS. The independently-calculated toxicity value was x.xx mg/L. The study author reported value was used quantitatively to derive the draft acute water column criterion.	*"The noted toxicity values provided in each study summary above (17, 22.68, and 29.46 mg/L), comprising of both author-reported and independently-calculated LC_{50} values, were used to calculate the GMAV value (as the geometric mean of the three LC_{50} values previously mentioned) of 22.48 mg/L, which was used to derive the freshwater aquatic life criterion."* 5 – 11. The noted text of x.xx mg/L were placeholders that were either replaced with relevant independently-calculated toxicity values or were removed if independently-calculated toxicity values could not be calculated by EPA. 12. Table 2-3 was 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 PFOS criteria at this time given the toxicity literature that is currently available, which focus largely on direct aqueous exposures. 13. The noted protective values for Australia and New Zealand were updated Section 1.1 of the draft PFOS Aquatic Life Criteria Document to reflect the updated values and information. 14. Section 1.2 of the draft PFOS Aquatic Life Criteria document was updated, specifically stating:

<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>6. Page A-25- Fourth line from bottom The study author reported 96-hour LC_{50} was 50.51 mg/L PFOS. The independently-calculated toxicity value was x.xx mg/L. The study author reported value was used quantitatively to derive the draft acute water column criterion.
7. Page A-27- Fourth line from bottom. The independently-calculated toxicity value was x.xx mg/L. The study author reported value was used quantitatively to derive the draft acute water column criterion.
8. Page A-29- First paragraph For comparison, the 7-day LC50 was 39.71 mg/L. The independently-calculated toxicity value was x.xx mg/L. The 96-hour study author reported value was used quantitatively to derive the draft acute water column criterion.
9. Page A-30- First paragraph The independently-calculated toxicity value was x.xx mg/L. The study author reported value was used quantitatively to derive the draft acute water column criterion.
10. Page A-36- 5th line from bottom in complete data x.xx mg/L.
11. Also at A-37 in complete data x.xx mg/L.
Table 2-3. Summary of Assessment Endpoints and Measures of Effect Used in the Criteria Derivation for PFOS
<table><tr><th>Assessment Endpoints for the Aquatic Community</th><th>Measures of Effect</th></tr></table></td><td>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, and cyclic structures are included).
Further, no additional edits were made to Table 1-2 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).
15. 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).
16. The effects symbol in the conceptual model (Figure 2-9) of the draft PFOS Aquatic Life Criteria Document now states:
" Deformities, Reproductive and Growth Impairments, and Mortality."</td></tr>
</table>

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	Aquatic Life: Survival, growth, and reproduction of freshwater and estuarine/marine aquatic life (i.e., fish, amphibians, aquatic invertebrates)	For effects from acute exposure: 1. LC_{50} 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) 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.* *Note: only chronic exposures were considered for derivation of the tissue-based criteria since PFOS is a bioaccumulative chemical. These chronic tissue-based criteria are expected to be protective of acute effects, because acute effects were observed at much greater concentrations than chronic effects.*
	Please review if the highlighted muscle, blood and egg would be relevant to this section in terms of LC50, EC10, LOEC and NOEC endpoints . **12. 1.1.2 and page 3- Previously Published Chronic Water Criteria for Direct Aqueous Exposure** The information on Australian guidelines to be updated based on NEMP2 published in 2020. I will attach it as a PDF.	

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	https://www.environment.gov.au/system/files/resources/2fadf1bc-b0b6-44cb-a192-78c522d5ec3f/files/pfas-nemp-2.pdf *"Previously published freshwater chronic values were available for two states (Minnesota and Michigan) and three countries or geographic regions (Australia/New Zealand, Canada, and Europe). These publicly available values for other jurisdictions were 0.019 mg/L and 0.14 mg/L for Minnesota (STS/MPCA 2007) and Michigan (EGLE 2010), respectively, and were 0.00013 mg/L in Australia/New Zealand (CRC CARE 2017; EPAV 2016), 0.00680 mg/L in Canada (ECCC 2018), and 0.000023 mg/L in Europe (RIVM 2010). Previously published estuarine/marine chronic values were available for two geographic regions (Australia/New Zealand and Europe). These publicly available values were 0.0000046 mg/L in Europe (RIVM 2010) and 0.0078 mg/L in Australia/New Zealand (CRC CARE 2017; EPAV 2016)"* The CRC marine guidelines are not valid as they are not based on the framework Freshwater values are to be used on an interim basis **13. Page 4- Table 1.1 to be updated accordingly**	

Exposure scenario	PFOS	Exposure scenario	Comments and source
Freshwater	0.00023 µg/L	99% species protection - high conservation value systems	Australian and New Zealand Guidelines for Fresh and Marine Water Quality - technical draft default guideline values for PFOS and PFOA. Note 1: The 99% species protection level for PFOS is close to the level of detection. Agencies may wish to apply a 'detect' threshold in such circumstances
	0.13 µg/L	95% species protection - slightly to moderately disturbed systems	

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
	2 µg/L | 90% species protection - highly disturbed systems 31 µg/L | 80% species protection - highly disturbed systems rather than a quantified measurement. Note 2: The draft guidelines do not account for effects which result from the biomagnification of toxicants in airbreathing animals or in animals which prey on aquatic organisms. Note 3: The WQGs advise[a] 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 the level of species protection required, rather than allowing for case by-case assessments. **Exposure scenario** | **PFOS** | **Exposure scenario** | **Comments and source**	

2.8. Additional Technical Comments to Consider					
Reviewer	**Comments**				**EPA Response**
	Interim marine	0.00023 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 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.	
		0.13 µg/L	95% species protection - slightly to moderately disturbed systems		
		2 µg/L	90% species protection - highly disturbed systems		
		31 µg/L	80% species protection - highly disturbed systems		
	[a]https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/local-conditions#bioaccumulation **14. Table 1 2. Two Primary Categories of PFAS** Please refer to OECD 2021 to be consistent with PFAS terminology/nomenclature				

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
	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 **15. Table 1.3 Page 8** Please review Figure 9 OECD 2021 (also attached as PDF) **16. Conceptual Model of PFOS in the Aquatic Environment and Effects** Figure 2.9 page 77- Growth as an endpoint missing in the endpoints – first pentagon	
Reviewer 5	Specific comments to the various elements of the PFOS AWQC are above. Here, I want to suggest that EPA revisit the 1985 Guidelines and publish either an updated version or an amendment. Basing critically important criteria on documents published in 1985 and then using this to justify decisions seems like it would not pass muster in the scientific community. I've had papers rejected because they did not include enough recent citations, for example. Moreover, I've mentioned my concerns with the 4- most sensitive taxa + linear regression for criteria derivation. No paper I've read on generating the 5th percentile most sensitive species has used this approach. Granted, I may have missed them but my sense is that it is more common to use a full SSD. It would be helpful, for example, if the revised Guidelines explored this further or other means of criteria development (including new approach methods) and published, used, and cited and updated guidelines document. I'd like to think we still generally lead the world (more or less) in environmental protection so having an updated document would be welcomed.	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. Thank you for your 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 PFOS Aquatic Life Criteria document. When there are less than 59 genera in a GSD, the

2.8. Additional Technical Comments to Consider		
Reviewer	**Comments**	**EPA Response**
		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 in EPA's response to Reviewer 5's comments to Charge Question 2.2. 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. (U.S.EPA 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*."

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