EPA RESPONSE TO EXTERNAL PEER REVIEW COMMENTS

on the

EXTERNAL PEER REVIEW DRAFT AQUATIC LIFE AMBIENT WATER QUALITY CRITERION FOR SELENIUM - FRESHWATER 2014

September 25, 2015

Office of Water
U.S. Environmental Protection Agency
Washington, DC

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I. Background on External Peer Review Process and this Response to Peer Review Comments Document

EPA submitted its External Peer Review Draft Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2014 for contractor-led independent, external peer review from August 2014 to September 2014. Prior to this peer review, EPA had released the document for public comment for over 60 days, May 14 to July 28, 2014. The external peer reviewers provided their independent responses to EPA's charge questions. External peer reviewers also considered public comments on the 2014 draft document, which EPA shared with them to obtain external peer reviewers' feedback on the public comments.

The 2014 draft criterion update of selenium provided a chronic criterion that is composed of four elements. The recommended elements are: (1) a fish egg-ovary element; (2) a fish whole-body and/or muscle element; (3) a water column - element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent element to account for potential chronic effects from repeated, short-term exposures (one value for lentic and one value for lotic aquatic systems).

The following text lists the charge questions submitted to the peer reviewers, the external peer reviewers' comments regarding those questions, and EPA's responses to the peer reviewer's comments. EPA revised the 2014 draft document considering the peer review and public comments, and is releasing the revised 2015 draft selenium criterion document for a second period of public comment, for 75 days, from July 28 to October 10, 2015.

II. Peer Review Charge Questions

PART I: OVERARCHING CHARGE QUESTIONS FOR THE EXTERNAL PEER REVIEWERS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.
2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available
   - 2.a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:
     - 2.a.i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

2.a.ii. Is the primacy of the whole-body/fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

2.a.iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

2.a.iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10^{th} centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.
2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).
   - 2.a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.
   - 2.b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).
   - 2.c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared

to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al., 1990)?

2.d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).
- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).
- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).
- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).
- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).
- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times,

water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

2. Regarding the trophic transfer factor (*TTF)* values, did EPA use a scientifically defensible method to derive the *TTF* values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in *TTF* values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.
3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.
4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.
5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

III. Responses Sorted by Question

PART I: OVERARCHING CHARGE QUESTIONS FOR THE EXTERNAL PEER REVIEWERS

1. Please comment on the overall clarity of the document and construction of the criterion statement with its multiple elements.

External Peer Reviewers' Comments	**EPA Response**
1. I found the overall clarity of the document to be good, although there are several specific areas that require clarification (detailed in comments to specific charge questions). I also found the construction of the criterion statement to be quite clear and logical.	Thank you for your comment.
2. In general the document is clearly written, but there are numerous typographical errors, missing references (e.g., EPRI, 2006 cited first on p. 16), and incorrect citations (e.g., Table 12 cites Appendix L, but the Appendices only go to K). Some key words are poorly chosen (the freshwater criterion parts are called "elements"), especially considering that this document concerns an aquatic trace ELEMENT, and other elements such as mercury are also discussed; I recommend selecting another key word for this. The use of acronyms and abbreviations are unavoidable in a document like this, and while they provided a table listing them all (which should be numbered Table 1 on page xi), it would make the document more readable to those only looking for some specific details to periodically redefine these in the text, for example the first time it is used extensively in a new section. The criterion statement (largely in Section 3.8) is clearly written and presented, although I have serious scientific problems with parts of it to be elaborated below. While this was not directly requested in our charge, but has direct bearing on the problems	Regarding use of the word "element," EPA maintains that the word was used appropriately and specifically referenced the components of the criteria statement, and will retain this terminology. Regarding the reviewer's recommendation to periodically redefine acronyms in the document, EPA has adopted that practice for those terms that are that are heavily used in the document. Regarding the review of the biogeochemistry of selenium, the section has been rewritten to reflect the current scientific understanding of selenium chemistry in the environment. Regarding the role of speciation, the data available in the peer-

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in this document, the review section on the aquatic biogeochemistry of selenium (pp. 9-17) has factual errors that may reflect on the authors understanding of the selenium or on some biases. First, in Section 3.2 the statement that "…the effects are integrated across forms of selenium; thus water column values are based on total selenium exposure." is an oversimplification that leads to conceptual errors later. The amount of dissolved selenium that enters the food web through the first trophic level is strongly linked to the speciation of dissolved selenium (e.g., Reidel et al., 1991; Baines and Fisher, 2001; Baines et al., 2001; Baines et al., 2004), which for freshwater and marine/brackish species is: selenite=organic selenide>>selenate. So for a lotic or lentic water body that is dominated by selenate, the incorporation of selenate into the phytoplankton biomass is much lower than that if the selenium was in the +4 oxidation state. In the next section 3.2.1, it starts off with serious errors, in particular "organo-selenide" being selenomethionine. Data on the speciation of dissolved organic selenide show it to be in soluble peptides and proteins, not free amino acids (e.g., Cutter, 1982; Cutter and Cutter, 1995), so phytoplankton uptake studies using free selenomethionine are not using the actual dissolved forms and likely overestimating uptake. A following sentence says that selenite tends to dominate in "slow moving waters", presumably lentic environments. However, there are no data in the literature to support this statement (e.g., see compilations in Cutter, 1989a); selenite is only dominant when there is a large, fossil fuel-derived input, regardless of water residence time (e.g., Cutter, 1989a, 1989b). In this respect, on p. 14, 2nd complete sentence, they state that geologic AND anthropogenic sources often release mostly	reviewed literature to EPA for modeling are typically non-speciated and expressed as "total" selenium. Therefore, EPA has derived selenium criteria for fish tissue based on the relationship between total selenium and the effect observed in fish tissue, which integrates the effect of all selenium species to which the organism has been exposed over time. Regarding the bioaccumulation section, EPA has corrected the language in Section 3.2 (now Section 2.2) to more closely reflect our current understanding of speciation of selenium and incorporation into the food web by algae. Regarding the statement that selenite tends to dominate in "slow moving waters," EPA has corrected the text to reflect the appropriate geologic source and selenium speciation state as available from the current literature.

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selenate, but most anthropogenic sources produce selenite (e.g., Cutter, 1989a, 1989b; Cutter and Church, 1986), only geological sources (weathered or irrigated) yield selenate; the presence of selenite in surface waters can in fact be used as a fossil fuel-combustion source indicator (e.g., Cutter, 1989a, 1989b). Interestingly, the last paragraph on p. 14 is largely correct in stating that the concentration of particulate selenium in the first trophic level (algae) is highly dependent on the dissolved speciation; this begs the question of why the authors later ignore speciation and calculate EF on total (presumably dissolved) selenium in the water column and particles; see later comments. In the Bioaccumulation section (3.2.2), the major error, and this is significant in terms of bioavailability, is that dissolved selenium uptake results in elemental selenium and organoselenium (2nd to last sentence on p. 15). Elemental selenium is only produced by dissimilatory (heterotrophic) reduction under low oxygen conditions (many works of Oremland, but they correctly cite Oremland et al., 1989); autotrophs perform assimilatory reduction to selenide that is then coupled with acetyl CoA, serine, etc to produce seleno amino acids. Also, the use of the term "absorbed" is poorly chosen in that it implies simple exchange with no chemical reactions; dissolved selenium is assimilated (or incorporated) into autotrophic organic matter, which in the case of selenite uptake/assimilation/incorporation involves a change in oxidation state and chemical form (i.e., selenite is reduced to selenide and bonded with carbon to produce seleno amino acids like selenocysteine).	
3. There is a lot of information to digest and it may be difficult for non-technical readers to follow, but I feel that the document	Thank you for your comment.

External Peer Reviewers' Comments	EPA Response
was organized in a logical manner and that the approaches were adequately described. Although I have technical comments relative to the criterion statement, I feel that format for presenting the selenium criteria based on multiple elements is clearly presented and easily digestible to the reader. I have included here a few miscellaneous typos and editorial suggestions that I noted during my review: p. 59, Table 7a: Correct spelling of "Onchyrhynchus" to " Oncorhynchus " p. 60, paragraph below Table 7b: Correct spelling of "Leopmis" to "Lepomis" p. 62, 1st paragraph: Correct spelling of "Oncorhyncus" to " Oncorhynchus " p. 89, footnote a in Table 12: Appendix L should be Appendix K p. 114, 1st paragraph, last sentence: Correct spelling of "criteirion" to "criterion"	EPA has noted your suggested edits and corrected the typographical errors.
4. Reasonably clear, although some phrases and terms need further clarification.	Thank you for your comment. EPA has clarified phrases and terms to enhance readability.
5. The document is generally well-written and is based on a comprehensive evaluation of the extensive body of freshwater Se literature. This said, I found many typographical and other errors throughout the document, which I will address in a marked-up copy (Adobe would not let me use the edit text functions so I simply highlighted the text in yellow and provided a comment if necessary). There were also several areas that I believe require significant clarification, which I will address in	Regarding spelling and grammar, EPA has made many grammatical and typographical corrections during the most recent revisions. Regarding the tiered criterion: EPA has presented a tiered criterion, and is recommending that States and Tribes adopt all of the recommended tiers; tiering enhances both the usability

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my subsequent review comments found below. I agree with the concept of the tiered criterion approach, particularly that tissue (i.e., ovary, egg, muscle, or whole-body)-based Se concentrations ([Se]) are key to accurately assess the toxicological risk posed to fishes, and that egg/ovary [Se] overrides/supersedes whole-body or muscle [Se]. However, I do not fully agree with the approach, in the absence of tissue [Se] data, that a water-column criterion will be protective of aquatic species. There are many examples of aquatic systems, due to their specific biogeochemistry, ecology, and physiology, where very low dissolved [Se] (i.e., less than the proposed criteria for lentic or lotic systems) results in toxicologically significant bioaccumulation in fishes and their prey, and elevated frequencies of larval abnormalities. I suggest that dissolved [Se] be used as a "trigger" to initiate further monitoring (i.e., collection of fishes to determine tissue [Se]). I also do not agree with the intermittent exposure criterion; it is unclear why it was developed, how it could be implemented consistently and reliably, and in general I think it just adds too much complexity to an already complex (indeed perhaps the most complex) water quality criterion. These are my general comments, and more specific details can be found in my subsequent review comments.	and the scientific strength of the criterion. Regarding system variability with respect to the amount of tissue bioaccumulation relative to selenium in water; the water criterion element has an additional level of conservatism added, so that it is calculated to be protective of the expected tissue concentration 80% of the time for the most bioaccumulative fish in each system (Section 3.2.5). Regarding those aquatic systems where fish and their prey species can experience significant bioaccumulation of selenium despite low water concentrations, EPA recommends fish tissue be collected in waters where available data (e.g., unusually large EFs) suggest the possibility of "toxicologically significant bioaccumulation" despite low selenium water concentrations. Furthermore, the national criterion can easily be adapted to site-specific situations using site-specific data using the modeling information provided in the document Regarding the intermittent exposure criterion, EPA recognizes that not all exposures are continuous and developed the intermittent criterion element due to concern that intermittent discharge sources may not be accounted for with the national 30-day average chronic criteria, yet intermittent discharges of sufficient magnitude and frequency could accumulate through the food web and ultimately result in chronic impacts on aquatic life. EPA believes it is unnecessary to have an additional acute criterion element which addresses acute, water column-only

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	events, because selenium is bioaccumulative and toxicity primarily occurs through dietary exposure. Although selenium may cause acute toxicity at high concentrations, the most deleterious effect on aquatic organisms is due to its bioaccumulative properties; these effects are found at lower concentrations than acute effects. Chapman et al. (2009) noted that selenium acute toxicity has rarely been reported in the aquatic environment and that traditional methods for predicting effects based on direct exposure to dissolved concentrations do not work well for selenium.
6. On an overall basis, the 2014 Selenium Criterion is well-organized and well-written. The major sections of the document serve to critically analyze the scientific and regulatory background of the issue, and to develop and rigorously justify a tiered criterion. Overall, the writing is clear and communicative, with key details, data and background information appropriately appended to the main document. The included tables and figures act to support the analysis of cause for a substantially different approach to risk management and furthermore this information serves to validate this criterion approach by critically evaluating decades worth of peer-reviewed laboratory and field observations in a fair and scientifically valid manner. The concordance observed in many tables exploring and ground-truthing modeled approaches, available data, and a broad array of published study results yields exceptional weight and justification for this new approach developed for the protection of aquatic life. Importantly, the criterion statement on p. 96 does indicate dry weight basis for tissue analyses, and this is discussed in the text, however Table 15 and the tabular Summary on p. 4 do not carry	Regarding the comment regarding the overall evaluation of the document, EPA thanks you. Regarding the missing "dw," it has been added. Regarding those areas of the document that may require additional guidance, EPA recognizes that there are numerous aspects of the criteria that will benefit from technical support documents to enhance its application, and is planning to develop such documents. The intermittent element was developed to address a specific concern regarding intermittent discharge sources.

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the dry weight basis notation and this should be included. With the advantage of subsequent key published selenium research targeting trophic transfer and reproductive endpoints in fish, as well as the expert panel contributions published in Chapman et al., 2009, this current document is a significant improvement over the 2004 AWQC draft. In its presentation and treatment of a broad and diverse study and data set, the draft criterion document can be characterized as exhaustive in its attempt to quantitatively and qualitatively address the myriad issues related to this task under the CWA. Furthermore the draft criterion document addresses that task in a manner that synthesizes a new tiered criterion approach well-grounded in our current understanding of selenium risks in aquatic ecosystem and best available peer-reviewed knowledge. The draft approach balances knowns and unknowns, data and data gaps, simplicity and complexity in an overall sound attempt to address the time-value requirement of regulatory science. Although additional implementation guidance for this new tiered approach may be necessary, and observing that the discussion of background science, data and methods used in the intermittent exposure tier of the present criterion needs significant improvement, the draft document is overall remarkable for its clarity and completeness, in a scientifically driven and defendable analysis of a complex risk management challenge.	

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7. The *Draft Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater* is generally clearly written and logically organized. While there are some issues which require clarification in the document, these generally arise as technical issues (identified in subsequent sections of this review) rather than writing clarity within the document. In contrast to some of the public comments, this reviewer believes that the document clearly states the order of preference for criterion (e.g., egg/ovary over muscle and whole body over water column concentrations) and the ultimate primacy of the egg/ovary criterion. The lone issue of clarity in the document concerns the water column values of selenium. Table 15 (page 97) specifies that water column selenium concentrations are based on "dissolved total selenium in water" however, elsewhere in the document the criterion is described as including "all oxidation states (e.g., selenite, selenate, organic selenium, and any other form)". While clarity regarding the species and analytical methods for assessing water column selenium are provided in Appendix J (Analytical Methods for measuring Selenium), a more precise definition of water column Se is warranted within the body of the document.	Regarding the comment on the overall evaluation of the document, EPA thanks you. Regarding the basis of the water column concentrations as "dissolved total selenium in water," EPA recognizes that clarification of the definition of selenium was needed and has revised the draft with a better definition and discussion of selenium speciation in the water column in the document. Selenium data are collected and analyzed using various methods. The data available to EPA for modeling is typically non-speciated and expressed as "total" selenium. EPA has derived selenium criteria for fish tissue based on the relationship between total selenium and the effect observed in fish tissue, which integrates the effect of all selenium species to which the organism has been exposed over time. Because of the uncertainty as to the form and potential for bioaccumulation, the criterion is expressed as total dissolved selenium in water (e.g., the total of all oxidation states; selenite, selenate, organic selenium, and any other forms), realizing that multiple forms of inorganic and organic selenium species may be present in any one sample.

2. EPA has developed a tiered selenium criterion with four elements, with the fish tissue elements having primacy over the water-column elements, and the egg-ovary element having primacy over any other element. Inclusion of the fish whole-body or fish muscle element into the selenium criterion ensures the protection of aquatic life when fish egg or ovary tissue measurements are not available, and inclusion of the water column elements ensures protection when fish tissue measurements are not available.

2.a. Please comment on the tiered construction of the selenium chronic criterion; is it logical, and scientifically defensible as it applies to protection of freshwater aquatic life:

2.a.i. That is, is the primacy of the egg-ovary element over the other elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

External Peer Reviewers' Comments	EPA Response
1. Yes, primacy of the egg-ovary element is sound and well supported by the scientific literature. EPA has cited all of the key references for support of this approach.	Thank you for your comment.
2. Given the known, well documented, and published in the peer-reviewed literature information, choosing the egg-ovary compartment/vector/whatever (not element) is very well justified. The accuracy of then selecting a suitable value for various fish species depends on a critical evaluation of the literature, or new experiments.	Thank you for your comment.
3. Yes, in my opinion the tiered construction of the chronic selenium criterion is logical and scientifically defensible. First, the critical exposure route for fish is dietary organic selenium (Janz et al. 2010), which is the basis for all of the studies in which egg or ovary selenium concentrations are linked to toxicity in offspring. Dietary organic selenium exposures are implicit in those studies in which adult females were exposed in the field and explicit in those studies in which adult females were exposed in the laboratory (primarily through the use of	Regarding your support of the tiered construction of the chronic criterion, EPA thanks you. Regarding the relative sensitivity of reproductive and non-reproductive (e.g., larval growth) effects, EPA completed a new analysis and found that the reproductive endpoint is protective of juvenile survival and growth (see pages 134 and 135 in the revised 2015 document). EPA however has noted in the current

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diets enriched with organic selenium, such as selenomethionine). Second, the critical toxicity endpoint for fish exposed to selenium is larval mortality, deformities, and/or edema following exposure to selenium during absorption of the yolk-sac. The selenium concentration in the egg or ovaries is the most relevant exposure metric for this exposure route and toxicity endpoint. Third, and related to the second point, is that fish species partition varying amounts of their total selenium burden to the ovaries and eggs (deBruyn et al., 2008). Direct measurement of the selenium concentration in the eggs or ovaries addresses this between-species variability in selenium partitioning within tissues. Fourth, fish egg- or ovary-based selenium toxicity values (e.g., EC10s) are not highly variable among fish species, regardless of whether adult females were exposed to dietary organic selenium in the field or in the laboratory or whether species may be considered "warm-water" or "cold-water" species. Some studies have also shown that juvenile fish survival and growth can be relatively sensitive to dietary organic selenium. For this toxicity endpoint, of course, an egg or ovary selenium criterion would not be applicable (but a whole-body selenium criterion would be). An important question, therefore, is whether compliance with an egg or ovary selenium criterion would be protective of juvenile fish. DeForest (2008) evaluated this question by comparing dietary Se toxicity data for juvenile growth and effects on larvae via maternal transfer. Although data were limited to bluegill sunfish (*Lepomis macrochirus*) for that evaluation, it was concluded that juvenile bluegill are not more sensitive than bluegill larvae exposed to selenium via maternal transfer. This would indicate that an egg or ovary selenium criterion should be protective of effects on juvenile	criterion document (see pages 127 and 128 in the revised 2015 document) that the whole body tissue is the sample of choice to assess selenium exposure in freshwater environments in juvenile anadromous salmonids (i.e., Pacific Northwest species) from the larval swim up to the smolt stage prior to outmigration to the marine environment. Regarding the purpose of the water criterion elements, one of the purposes of the water-based criterion element(s) was to facilitate having an easily implemented value in permits. If a waterbody meets the fish tissue limits yet not a surface water criterion, for an existing input source, it is likely that the site specific dynamics of selenium bioaccumulation may be different than the basis of the national criterion, and this waterbody may be a candidate for a site-specific water value. Sufficient samples should be collected to evaluate the frequency of exceedance of the water value, and the concentration difference between the fish tissue sample(s) and the appropriate criterion element. An analysis of the available data may indicate that the fish tissue concentration may be increasing over time and may soon exceed the allowable tissue criterion element threshold. For example, if the selenium entering the environment is from a recent new or increased input, the fish tissue concentrations may not yet reflect the ultimate "steady state" accumulation of selenium in fish tissue, because of a lag time of accumulating selenium moving through the food web.

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survival and growth (if the observations for bluegill are translatable across fish species). Although I agree that the primacy of each criterion element is logical, it is not clearly stated whether a water Se criterion could be adopted into a permit limit. For example, if compliance with the lotic or lentic Se criterion is demonstrated, is measurement of fish tissue Se concentrations necessary? If a water body meets a fish tissue-based Se criterion, but not a surface water criterion, would the water body be considered in compliance? I believe the answer to the latter is "yes", but this does not seem to be clearly stated in the draft AWQC document. Literature cited: deBruyn A, Hodaly A, Chapman P. 2008. Tissue selection criteria: Selection of tissue types for the development of a meaningful selenium tissue threshold in fish. Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field Prepared for the North American Metals Council - Selenium Working Group, Washington, DC. DeForest D. 2008. Threshold development endpoints: Review of selenium tissue thresholds for fish: Evaluation of the appropriate endpoint, life stage, and effect level and recommendation for a tissue-based criterion. Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field Prepared for the North American Metals Council - Selenium Working Group, Washington, DC. Janz DM, DeForest DK, Brooks ML, Chapman PM, Gilron G, Hoff D, Hopkins WA, McIntyre DO, Mebane CA, Palace VP,	

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Skorupa JP, Wayland M. 2010. Selenium toxicity to aquatic organisms. 141-231 in Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, eds. Ecological assessment of selenium in the aquatic environment. SETAC Press, Pensacola, FL, USA.	
4. The tiered construction makes sense for most natural conditions, but not when acutely high Se levels are present (e.g., Kesterson reservoir). But for most sublethal concentrations this approach makes sense as a general approach for the EPA to adopt.	In waterbodies where no fish are present for tissue collection as a result of acutely toxic selenium concentrations, the water concentration criterion element would be applied.
5. Yes, it has been clearly shown in the scientific literature that egg/ovary [Se] provides the greatest certainty in predicting the toxicological risk associated with Se exposure in fishes. This is because (a) embryo-larval abnormalities are the most sensitive toxicological response, and (b) maternal transfer of Se to the eggs by adult female fishes provides the ultimate dose received by their offspring (i.e., during yolk resorption prior to swim-up). In addition, the frequency and severity of early life stage abnormalities caused by Se has clear ramifications for population dynamics; impaired recruitment of individuals into fish populations can alter demographics and ultimately result in extirpation. This is Ecotoxicology 101. Indeed, documented Se poisoning events (e.g., Belews Lake) provide some of the most convincing evidence of a cause-effect relationship between exposure to a toxic substance and resulting negative impacts on fish populations and communities. This is the goal of aquatic ecotoxicology: to protect populations and communities of organisms, not individuals.	Thank you for your comment.

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6. The primacy of the egg-ovary element over other elements of the selenium chronic criterion is logical and broadly scientifically defensible. As identified in the document, numerous published studies outline the major aquatic ecosystem impact of selenium, beyond its nutritional requirement, as a reproductive toxicant. While the specific bio-molecular mechanisms of reproductive toxicity and teratogenesis still require further work, it is well-established from controlled laboratory studies and field studies that the best indicator of the potential for reproductive end effects from selenium is in tissue concentrations, and specifically in egg-ovary concentrations. While the relationships of tissue and water concentrations can be studied, quantified, modeled, and tasked to risk assessment, the now well-established relationship of egg-ovary Se levels to toxicity endpoints fully justifies this primacy of this indicator.	Thank you for your comment.
7. The tiered construction of the chronic criterion is logical and scientifically defensible, and the primacy of the egg/ovary element over all other elements is also defensible. In fact, the primacy of the egg/ovary criterion was also recognized by a multidisciplinary and international group of selenium experts convened at a workshop in 2009. Proceedings from that workshop were published (Chapman et al. 2009) and in the executive summary it was noted "Selenium concentrations in eggs are the best predictors of effects in sensitive egg-laying vertebrates". Additional sections of that volume further supports the USEPA's Draft Document approach by recommending that measurement endpoints for risk assessment should be as closely associated with reproductive endpoints in egg laying vertebrates as possible and that measurements in eggs or ovaries, or in the absence of these measures, selenium concentrations in muscle or	Regarding your supportive comment on the tiered construction of the chronic criterion, EPA thanks you for your comment. Regarding the interpretation of the "never to be exceeded" criteria, this is an issue that will be addressed in the technical support documents.

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whole body are required. The scientific evidence supporting these conclusions has not changed substantively since the time of that volume's publication and the approach remains the most valid scientifically. In fact, this general approach was also recently adopted by the British Columbia Ministry of the Environment (BC MoE 2014) after an extensive, and peer reviewed, analysis of the literature relevant to the ecotoxicology of selenium. It is unclear however, how the USEPA will interpret the "never to be exceeded" criteria. Biological variability, coupled with uncertainty regarding the residence of mobile fish species, will make it likely that some fish in a given collection may exceed the guidelines. It is unclear if a result from one fish (i.e. a single exceedance) will render a given management area in non-compliance, or if some average value is intended as the trigger.	

2.a.ii. Is the primacy of the whole-body/fish muscle element over the water column elements scientifically sound, given the prevailing toxicological knowledge and the data and supporting scientific information currently available to the Agency? Please provide detailed comments.

External Peer Reviewers' Comments	**EPA Response**
1. Yes, in general a tissue-based criterion should have primacy over a water-based criterion for Se due to the complex site-specific nature for Se bioaccumulation. This is well documented in the literature. As discussed by EPA, an egg-ovary based criterion is highly desirable but may not always be achievable due to logistical constraints or the potentially significant impacts on populations of terminal sampling of ovaries for some threatened or endangered species. In such cases, whole body or muscle plugs provide a reasonable surrogate for the egg-ovary element. One item lacking from the WQC is guidance on when use of whole body or muscle elements is acceptable. Some questions that come to mind: 1.) Can WB or muscle elements be used instead of EO even when collection of EO samples is considered logistically and environmentally feasible? 2.) Are there seasonal considerations to use of WB and muscle samples? For example, is it acceptable to use WB or muscle samples collected in the Fall for a species that spawns in the Spring?	Regarding your support of the primacy of the tissue-based criterion, EPA thanks you for your comment. Regarding questions 1 and 2 posed by the reviewer. The design of the tiered criterion is such that the hierarchy allows for the assessment of samples that are available (i.e., muscle, egg ovary, or whole body). There are practical and scientific considerations for the collection and assessment of specific types of samples which EPA plans to address in a detailed technical support document. For example, whole body or muscle tissue from fish may provide the best estimate of critical body burdens of selenium in fish (male or female) that are not in the process of spawning. This and other issues will be discussed in the technical support document on fish tissue monitoring which is under development by EPA at this time.
2. Again, this is well documented and the only proviso would be the choice/selection of the CF value	Regarding your support of the primacy of the tissue-based criterion, EPA thanks you for your comment. Regarding the methodology used to derive a CF value for conversion of egg ovary to the whole body or muscle criterion

External Peer Reviewers' Comments	EPA Response
	elements; it was based on the most phylogenetically similar species for which there were data. There were more individual species data available for muscle than for whole body conversions. The choice or selection of a different CF value may play a role in the calculation of a site-specific criterion.
3. Yes, in my opinion the primacy of the whole-body or muscle selenium criterion over the water column criterion is scientifically sound. Selenium bioaccumulation potential from water to fish is highly site-specific (Brix et al., 2005; Presser and Luoma 2010; Stewart et al., 2010), so it is appropriate that a whole-body or muscle selenium criterion is given a priority over a water column selenium criterion. Consideration of only a water column selenium criterion (or a water column selenium criterion that is given priority over a fish tissue-based selenium criterion) would necessarily have to be very low to ensure protection of the sites with the greatest selenium bioaccumulation potential. However, this would potentially be problematic because it would trigger concerns (i.e., selenium criterion exceedances) at locations where selenium bioaccumulation potential is lower and not of ecological concern. Literature cited: Brix KV, Toll JE, Tear LM, DeForest DK, Adams WJ. 2005. Setting site-specific water-quality standards by using tissue residue thresholds and bioaccumulation data. Part 2. Calculating site-specific selenium water-quality standards for protecting fish and birds. Environ Toxicol Chem 24:231-237. Presser TS, Luoma SN. 2010. A methodology for ecosystem-	Regarding your support of the primacy of the tissue-based criterion, EPA thanks you for your comment. Regarding your comment on the potential problems associated with the adoption of only a water criterion element, EPA recommends adoption of all four elements of the selenium criterion. The hierarchy of the criteria is such that, for an existing input, a tissue sample will always supersede the result of the water element, because the tissue sample(s) are most closely associated with the endpoint of concern: reproductive impacts on aquatic species. In the situation the peer reviewer presented, tissue sample(s) could be collected to confirm or refute the result of the water sample(s). It may be necessary to collect multiple samples over a period of time to ensure that selenium is not building up in the system leading to increased bioaccumulation in the biota. For example, if the selenium entering the environment is from a recent new or increased input, the fish tissue concentrations may not yet reflect the ultimate "steady state" accumulation of selenium in fish tissue, because of a lag time of accumulating selenium moving through the food web.

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scale modeling of selenium. Integr Environ Assess Manag 6:685-710. Stewart R, Grosell M, Buchwalter D, Fisher N, Luoma S, Mathews T, Orr P, Wang W-X. 2010. Bioaccumulation and trophic transfer of selenium. 93-139 in Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, eds. Ecological assessment of selenium in the aquatic environment. SETAC Press, Pensacola, FL, USA.	
4. This approach is wholly justifiable because Se is accumulated by animals almost exclusively through diet rather than directly from the dissolved phase in ambient water. In fact, Se and perhaps methylmercury would be extreme examples in which this approach is appropriate.	Thank you for your comment.
5. Yes, in the absence of egg/ovary [Se], the next best thing is whole-body or muscle [Se]. Practically, whole-body or muscle samples are more reliably collected throughout the year since most adult female fishes do not have appreciable ovarian tissue mass during non-reproductive periods. This is especially true in small-bodied fishes. In addition, muscle tissue can be collected non-lethally in larger fishes, which may be particularly relevant to threatened species. It is important to note that [Se] in ovarian tissue containing only primary oocytes or pre-vitellogenic ovarian follicles (i.e., during the non-reproductive period spanning most of the year in many fishes) will likely provide similar information on Se risk as whole-body or muscle [Se]. This is because the ultimate Se dose is maternally delivered to eggs during the period of	Regarding your support of the primacy of the tissue-based criterion, EPA thanks you for your comment. EPA also thanks you for the important information on seasonal considerations of collection of egg-ovary versus whole body or muscle tissue and will consider this information in the technical support document on fish tissue sampling for selenium. EPA also appreciates the peer reviewer's clarification of the type of selenium incorporated into vitellogenin. Most of the toxicity studies evaluated by EPA for criteria derivation involving selenium contaminated food use selenomethionine. This likely results in a conservative estimate of the exposure dose, since selenium in the environment is in multiple forms, some of which are not as toxic as selenomethionine.

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vitellogenesis in fishes. Eggs will not be present in the ovary of most fish species for much of the year. During vitellogenesis (the period of egg "growth"), adult females synthesize the yolk precursor protein, vitellogenin, in their liver, where it is transported via the bloodstream to the ovary and taken up by growing (vitellogenic) ovarian follicles (eggs). Thus, the [Se] in the liver of adult female fishes may provide a better predictor of Se risk than whole-body or muscle [Se]. To be even more scientifically correct, it is the concentration of the seleno-amino acid, selenomethionine, in the liver of adult female fishes that is incorporated into vitellogenin in a non-specific, dose-dependent manner (replacing the amino acid methionine) that defines the ultimate dose of Se received by their offspring. For more details see the following paper, which was not cited in the EPA document: Janz, D.M. 2012. Selenium. Pp. 327-374 In: C.W. Wood, A.P. Farrell and C.J. Brauner (Eds.) *Fish Physiology Vol 31A, Homeostasis and Toxicology of Essential Metals*. Elsevier, San Diego, CA. Thus, I do not agree with the statement on page 27 (line 4) that "concentrations of Se in ovaries are considered equivalent to concentrations of Se in eggs…" because fish ovarian tissue during the non-reproductive phase contains somatic cells responsible for ovarian maturation processes (i.e., steroidogenic cells), and gametes (primary oocytes and pre-vitellogenic follicles), and the [Se] in these cells do not necessarily reflect the dose of Se that will be received by the eggs (i.e., in the yolk) during vitellogenesis. Further studies are needed to examine the relationship between [Se] in ovarian tissue vs. eggs. It is strongly suggested that the EPA inspect the ovary and egg data	Regarding the comment on egg-ovary equivalence, EPA notes that the text in the criterion document actually states: "In this document, concentrations of selenium in ovaries are considered equivalent to concentrations of selenium in eggs because most studies measured selenium in the ovaries prior to spawning." The last part of the sentence is the relevant part as to why EPA considers theses tissues equivalent in the document. The selenium egg values for the studies used in the criteria derivation were based on eggs just spawned or ripe for spawning (stripped and used for spawning), thus they have selenium levels representative of the embryo/larvae exposure. The issue of temporal considerations on collection of a specific tissue type (egg ripeness) for selected species is relevant and will be discussed (to the extent information is available) in the information developed for states to aid in the implementation of the criterion.

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carefully and attempt to derive the potential relationship between [Se] in ovarian tissue vs. eggs.	
6. With regards to many chemical exposures in aquatic ecosystems, tissue levels in resident or migratory aquatic animals often help to assess toxic risk by integrating the exposure and revealing the storage, distribution, metabolism, and excretion of the toxicant, regardless of the geography, hydrograph or acute-to-chronic exposure dynamic of the chemical. The bimodal nutrient-toxicant behavior of selenium adds to the complexity of evaluating approaches to risk management. Metabolic and environmental conditions can also add complexity and uncertainty to a full understanding of risk in selenium impacts aquatic ecosystems. It is clear from many studies that the physiological homeostasis (uptake/efflux) of Se is not well controlled and the biochemical metabolic co-relationship of Se and S pathways *in vivo* allows for chronic Se exposure to advance to toxic endpoints. A recurring issue in aquatic ecosystem Se management has been co-exposure to high levels of sulfur, typically as sulfate. While high sulfate co-exposure may impact Se toxicosis, tissue Se levels yield a high quality, aquatic Se toxic impact potential metric regardless of sulfate co-exposure or other co-factors in Se reproductive toxicity (e.g., synergists or antagonists), known or unknown. This Se fish tissue approach, including eggs, ovary, muscle or whole body, is robust with respect to the findings of several decades of peer-reviewed studies. Selenium levels in fish tissue are broadly accepted in the scientific community as a high quality indicator suitable for risk management of aquatic life. The document supports the tissue approach and key toxicological endpoints with a critical review of the peer-	Thank you for your comment.

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reviewed literature and inclusion or rejection of specific studies and the data or findings therein, in an overall transparent, logical and defendable manner. The tier placement of whole-body/fish muscle is appropriate since egg/ovary assessment may have practical challenges with some ecosystems, with some species, the size of the target fish, and with some aspects of the life-cycle of the target fish. The inclusion and tier level of fish tissue selenium gives flexibility in aquatic ecosystem risk assessment. Inclusion of water column Se levels in the tiered criteria will no doubt help screen for potential Se impacts in aquatic ecosystems that have not had a history or occurrence of selenium contamination, and in the prevention of discharges or other anthropogenic activities that present an unacceptable risk to water quality.	
7. Affording primacy to the measurement of selenium in tissues over measurements in the water column is scientifically sound. While egg/ovary are recognized as the best predictors of potential impacts of selenium in oviparous vertebrates, there may be situations where these tissues are not available or where technical expertise is not sufficient to allow collection. In this instance, muscle or whole body measures are the next best alternative to egg/ovary as a risk assessment tool. The use of water column concentrations of selenium as environmental assessment tools or as triggers for additional assessment is fraught with uncertainty from several sources, which are discussed in subsequent sections of this review. However, it is important to recognize that the use of these tissues for monitoring purposes introduces a layer of uncertainty	Regarding your support of the primacy of the tissue-based criterion, EPA thanks you for your comment. Regarding uncertainty associated with the water column criterion elements, EPA recognizes this uncertainty, but notes that the water column element is useful for implementing in permits, and in certain situations water will be the preferred sample in the case of water bodies with new discharges of selenium or where adequate fish samples are not available. In situations where both fish tissue and water samples are available, the water column values are backstopped by the fish tissue criteria elements in the hierarchal construction of the criterion and thus provide a means for reducing the uncertainty.

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<tr><th>External Peer Reviewers’ Comments</th><th>EPA Response</th></tr>
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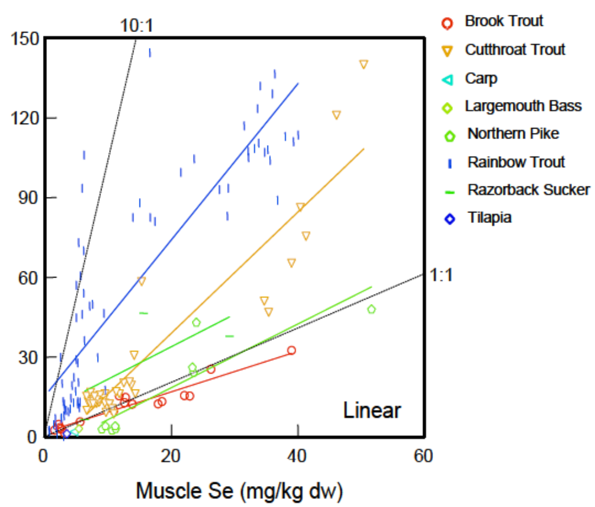
with regard to potential reproductive toxicity assessments. This uncertainty arises because selenium partitions between egg/ovary and muscle/whole body differently in different species. For example, regression plots of selenium concentrations in eggs versus those in muscle of 8 fish species revealed vastly different slopes and strengths of regression between species (see figure below reproduced directly from North America Metal Council ([NAMC] 2008), y axis scale is Egg Se (mg/kg dry weight (dw)). Due to this divergence, in order for muscle to be used as an effective surrogate for concentrations of selenium in egg/ovary, the specific regression for the fish species in question will have to be documented.



With regard to whole body as a criterion, it is unclear whether the USEPA intended to include visceral tissues (e.g., liver, kidney, gonads, gastrointestinal tract) with the carcass for whole
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Regarding variability in egg-ovary to whole body conversion factors (CFs), in the EPA dataset, variability in egg-ovary to whole body CFs was relatively low for the majority of species, ranging from 1.38-2.44 for 16 of 17 species. The inter-species range of CFs for egg ovary to muscle was comparable. EPA will address these and other issues in the technical support document on fish tissues monitoring for selenium.

Regarding the definition of a whole body tissue sample, the entire fish (carcass and visceral tissue) is homogenized, and then a sample of the homogenized tissue is collected and analyzed for selenium. The whole body criterion element is ranked as a lower tier than the egg-ovary criterion element because of the additional uncertainty associated with variable selenium concentrations across tissue types.
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body measurements of selenium. Because these tissues can account for a significant amount of the whole body pool of selenium, when Se concentrations in liver and gonads are elevated (especially during oogenesis [i.e. egg formation]), this requires clarification.	

2.a.iii. Please comment on the scientific uncertainty that may be associated with this tiered approach? Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

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1. There are of course a number of uncertainties in the tiered approach proposed by EPA. I provide specific comments on these uncertainties throughout this review. Overall though, I do not believe there are any currently available data sources, models or alternative approaches that EPA has not considered that would significantly reduce the uncertainty.	Thank you for your comment.
2. While the approach is scientifically justifiable, the propagation of errors that combine to make the total uncertainty is a bit daunting. Indeed, their frequent use of r or r^2 values for log/log plots completely masks the overall uncertainty; what are the correlations for direct concentration comparisons? I suspect they are much less than 0.4 and the p values would make them far less significant. Having said this, the trophic level transfers between higher levels (1 and above) are well described and parameterized in the literature, so the authors really should do a complete error/sensitivity analysis to quantify the overall error/uncertainty.	Thank you for your comment. EPA has considered the variability and uncertainty inherent with this derivation and has made some modifications to the document, including incorporation of new data, and modification of the Conversion Factor (CF) methodology to better reflect taxonomic similarities as described immediately below. Regarding development of CF and Trophic Transfer Factor (TTF) values that better reflected taxonomic similarities among species when species-specific data were not available, to estimate TTF or CF values, EPA sequentially considered higher taxonomic classifications (i.e., species, genus, family, order, then class) until one or more taxa for which a calculated TTF or CF value was available matched the taxon being considered. If the lowest matching taxon was common to more than one species with a TTF or CF value available, EPA used the median

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	TTF or CF from the matching species. This criterion was designed to allow for easy application to site-specific conditions; the variability and uncertainty will likely be lower with a dataset from a specific site.
3. Overall, I believe that the tiered approach is scientifically appropriate. I do have specific comments on the actual selenium criteria at each tier, which are provided under specific charge questions below.	Thank you for your comment.
4. The EPA can provide further levels of uncertainty with regard to toxicity associated with fish egg/ovary contamination. How many studies is this approach ultimately reliant upon? The report is based on a limited number of studies, but more studies are warranted before we can be assured that this approach is rock-solid.	Regarding the number of studies upon which this approach is ultimately reliant, EPA evaluated 80 studies on selenium toxicity to aquatic organisms, identified in appendices C, D, and E; and in section 3.1.3. The 13 GMCVs (not including the 2 "placeholder" GMCVs used to fulfill missing taxonomic minimum data requirements [MDRs] included in the sensitivity distribution [SD]) were calculated from 15 SMCVs, which were calculated from 19 chronic values obtained from 24 studies. An additional 21 non-reproductive toxicity values were obtained from 20 studies for 10 species, including 5 species that were not used in the SD. Fish reproductive and non-reproductive summaries are included in Appendix C and D, respectively, and were used to demonstrate that the egg-ovary based criterion protects against both reproductive and non-reproductive effects in aquatic organisms. An additional 21 toxicity values from 22 studies encompassing 18 species, seven of which were not included among the reproductive or non-reproductive studies listed above, were evaluated and are included in Appendix E (other data). Three field studies with multiple species were also evaluated qualitatively to assess the relative sensitivity of Cyprinidae to selenium, and are included in Appendix E.

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	Finally, 11 studies encompassing 11 species were qualitatively evaluated to assess selenium nutritional requirements and are included in Appendix E. While EPA agrees that additional studies are always desirable, the selenium criterion is based upon a substantial dataset.
5. One major source of uncertainty is the translation of whole-body or muscle [Se] to egg/ovary [Se]. This relationship has been documented for 10 fish taxa in the document (Tables 7a and 8a). These ratios vary about two-fold among taxa (1.21-2.44 for EO:WB and 0.95-1.92 for EO:M). Not all fish taxa have been studied, and more work is needed in this area. Importantly, in a given fish species these ratios may vary considerably among aquatic ecosystems due to differences in the food web, biogeochemistry of Se, and other factors. These ratios may also vary across seasons. Nonetheless, the data sources, models and approaches used by the EPA to derive these ratios are valid; we simply need more data to more accurately define these conversion factors. The major source of uncertainty in the tiered approach is the conversion of tissue (egg, ovary, muscle or whole-body) [Se] to water column [Se]. The approach used by the EPA is appropriate and uses, for the most part, the recent biodynamic modeling approach to derive water column [Se] from tissue [Se]. However, to use water column [Se] as a criterion in of itself in the absence of tissue [Se] data is a recipe for inappropriate conclusions, which may penalize industry (i.e., false positives) or cause harm to certain fish populations (i.e., false negatives). I strongly believe that water column [Se] should be used more as a "trigger" to initiate further monitoring that includes collection of fish for tissue [Se] determinations. I also think that a safety	Regarding inter-species variability in conversion factors, while not all fish taxa have been studied, major freshwater fish families that are phylogenetically distinct and diverse are represented, and yet there is only roughly a two-fold variability between them. Given the inherent variability (2-3) fold in sensitivity observed even among species with repeated toxicity tests, 2-fold variability is a small level of uncertainty compared to uncertainty associated with other pollutants. EPA agrees that more data for the tissue conversion factors are always desirable. EPA is moving forward with the current database to ensure protection of aquatic life based on the current state-of-the-science. Regarding the tiered approach, the water column criteria is backstopped with the hierarchal construction of the tissue criteria; exceedance of a water column value, can be confirmed via sampling of fish tissue. Regarding the lack of clarity in the definitions of lotic and lentic water bodies. Based on comments received, EPA examined the potential for residence time to classify the water bodies in the database, but available data were extremely limited. EPA also examined each waterbody to ensure that the available

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factor should be applied to the proposed 1.3 ug/L and 4.8 ug/L criteria for lentic and lotic systems, respectively, which would reduce these values as triggers for further ecosystem monitoring. There are many examples of lentic systems with < 1 ug/L dissolved [Se] where negative effects of Se on early life stage development of fishes have been demonstrated. This is an appropriate place to discuss the problems with a crude classification of systems as lentic vs lotic. Many rivers in the USA are impounded, essentially creating lentic systems for a significant portion of their river-miles, although they would still be classified as lotic. I think the EPA needs to more clearly define these terms. One suggestion is to use water residence time and/or mean annual flow velocity as more quantitative descriptors. Many of the studies that have shown lower Se bioaccumulation in lotic systems have been conducted in fast-flowing mountain streams, creeks and rivers. To classify a river in the southern USA that has numerous dams as a lotic system does not make sense.	characteristics and sample location indicated that it was either a lentic or lotic site. EPA's follow-up analyses ensured that sites such as run-of-the-river-reservoirs, an example discussed in the reviewers comment, were not misclassified as lotic.
6. The tiered approach presented in the criterion embodies the best available scientific knowledge of selenium in aquatic ecosystems actively studied by a broad range of investigators, disciplines and institutions, across a diverse range of water environments and potentially impacted organisms, over more than three decades of focused effort. While all science has uncertainty, the magnitude and diversity of the research effort in the environmental toxicology and regulatory science community to understand the complex risk dynamic of selenium in aquatic ecosystems is unprecedented in the history of U.S. environmental law. The 2014 Aquatic Life Ambient Water Quality Criterion for Selenium balances the available data,	Thank you for your comment.

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models, and approaches to risk management. The document and the tiered criteria within represent a balanced approach where assumptions and data uncertainties are clearly laid out and discussed. Published data or results that were not included in criterion determination were adequately and satisfyingly discussed and defended for exclusion. The data and peer-reviewed studies used in the quantitative and qualitative development of the criterion are sufficiently robust, sufficiently concordant in their conclusions, and sufficiently broad in their scope and number to result in a criterion that can protect aquatic organisms under the requirements of the Clean Water Act.	
7. Uncertainty associated with the US-EPA's tiered approach arises from the species specific disposition of selenium into egg/ovary versus muscle and whole body (noted above) and in the sampling methods used to obtain these tissues. In terms of sampling methods, timing may contribute to variability. A recent study showed that some fish may partition selenium to the eggs/ovaries more immediately from the diet than from their tissue stores (Conley 2014). In these species of fish, muscle/whole body might be less reflective of egg/ovary selenium concentrations than concentrations in the diet. However, the authors noted that spawning strategy may play a role in determining the importance of tissue reserves versus dietary sources accounting for selenium partitioned to egg/ovary. Specifically, for species with longer periods of oogenesis and which spawn only once annually, tissue stores may be better predictors of egg/ovary selenium concentrations than dietary sources. However, for multiple spawners, the diet may be a more important determinant. This has relevance to the both the egg/ovary and muscle/whole body criteria recommended by the	Regarding the effect of timing on the variability of selenium between tissues, the Conley (2014) reference was not yet available when the draft criterion was developed. However, the agency is aware that spawning strategy likely plays a role in accumulation, and maternal transfer and deposition of selenium into the eggs. This issue will be addressed in the technical support materials under development regarding the timing and type of sampling based on species spawning strategy (single batch versus multiple smaller batches) and seasonality (spring or fall [some salmonids] versus sporadic spring/summer [e.g., cyprinids]), while also providing flexibility to states to adapt their monitoring programs to address the additional requirements for fish tissue monitoring for selenium.

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US-EPA and the variability inherent in each. If muscle/whole body were used as a measure of compliance the timing of sampling within the fishes’ reproductive cycle could have an influence on the concentration of selenium in the tissue, especially among single spawners with extended oogenesis periods. Therefore, if muscle/whole body were sampled immediately following the spawning period lower concentrations of selenium might be expected than if the tissues were sampled prior to oogenesis.	

2.a.iv. Are the draft recommended magnitude, duration, and frequency for each criterion element scientifically sound and appropriate? Please provide detailed comments.

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1. I have provided specific comments on these issues in response to the questions below.	Thank you for your comment.
2. I found the time and frequency evaluations of the factors (not elements) well justified, with the exception of the EF, to be explained in later comments.	Thank you for your comment. The EPA response to the comment on EF is given below, adjacent to the peer reviewer's detailed comments.
3. The comments below are organized first by magnitude, duration, and frequency, and then by criterion element (i.e., fish egg or ovary, fish whole-body or muscle, and water column) within each of these categories. Magnitude *Fish Egg/Ovary Se Criterion* <u>Brown Trout</u> The draft fish egg/ovary selenium criterion is 15.2 mg/kg dw. This draft criterion is driven by brown trout (*Salmo trutta*), which had an EC10 of 15.91 mg/kg dw in the EPA's draft AWQC document. This study, conducted by Formation Environmental (2011a), has received tremendous scrutiny in how to best interpret the results and derive a defensible EC10. In my earlier review of that study on behalf of the Eastern Research Group (ERG) and EPA, I had concluded that the most relevant egg selenium EC10s that could be derived from that study ranged from 20.70-21.60 mg/kg dw. In that same review, however, I concluded that an egg selenium EC10 of 16.76 mg/kg dw was on the lower end of the range of possible EC10s	Regarding the brown trout study, EPA re-analyzed the available data on the Formation 2011 study after issuing the External Peer Review Draft Selenium Criterion document in 2014. Several analytical approaches were considered based on peer review and public comments. Because of uncertainty associated with how to best interpret the health and mortality of larval fish lost to an overflow event caused by a clogged drain, EPA re-evaluated the EC10 for mortality during the hatch through swim up portion of the test, and did not include the 15 day post swim up portion of the test. The hatch through swim up test is the more conventional test duration for these kinds of maternal transfer tests, and the resulting EC10 was determined to be 18.09 mg/kg dw. The description and supplementary analyses are included in the Effects Characterization section of the main text and in Appendix C. Regarding the comment on bluegill, following the peer review, EPA reanalyzed the Hermanutz bluegill study using a combined endpoint of larval survival and edema. Including larval survival in the endpoint eliminated the more variable nest data since it

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that could be derived from the study. Accordingly, in my opinion, the EC10 of 15.2 mg/kg dw used by the EPA is an overly conservative interpretation of the brown trout Se toxicity study. <u>Bluegill</u> The second lowest species mean chronic value (SMCV) was 18.41 mg/kg dw for bluegill sunfish (*Lepomis macrochirus*). This SMCV was based on the geometric of EC10s from three studies: (1) an EC10 of 20.05 mg/kg dw from Doroshov et al. (1992); (2) an EC10 of 24.55 mg/kg dw from Coyle et al. (1993); (3) an EC10 of 12.68 mg/kg dw from Hermanutz et al. (1992, 1996). The latter EC10 is much less than the other two EC10s for bluegill and less than even a very conservative interpretation of the EC10 for brown trout. I agree with the interpretations of the Doroshov et al. (1992) and Coyle et al. (1993) studies, but disagree with the interpretation of the Hermanutz et al. (1992, 1996) study. The EC10 of 12.68 mg/kg dw from Hermanutz et al. (1992, 1996) is driven by two treatments from Study 1: these were Streams 3 and 8 which had an ovary Se concentration of 17.71 mg/kg dw and 80% edema was observed and Steam 4 which had an ovary Se concentration of 15.46 mg/kg dw and 50.3% edema was observed. At first glance, there are three issues that stand out: • First, the water Se treatment concentration that resulted in an ovary Se concentration of 17.71 mg/kg dw in Study I was 10 µg/L—in the 10 µg Se/L treatment in Study II the ovary Se concentrations averaged 36.39 mg/kg dw and the average rate of edema was 83%. Thus, the rates	was not possible to measure survival in the nest. The combined endpoint fit the TRAP model well with an EC10 of 11.36 mg/kg. This EC10 was included in the bluegill SMCV in the accompanying 2015 draft criterion document. EPA recognizes the discrepancy between the bluegill tissue between Study I and II is 10 µg/L in streams. However, the 10 µg/L treatment from Study I was only one data point in the analysis and it did fit the curve well, so it was included. The 30 µg/L fish all died by the end of the test and Se accumulation was apparently affected by its toxicity. Overall, EPA recognizes the uncertainties in the Hermanutz bluegill study, but believes that the current scientific analysis is scientifically defensible and reasonable to include in criterion development. Regarding the use of the *Esox* data, EPA exhaustively examined the data. EPA does not believe that the authors' EC10 calculation is scientifically defensible. The spacing between exposures is too large to estimate the EC10 either by the authors' linear regression approach, or by EPA's nonlinear regression approach. But based on its cluster of three values with concentrations near 34 mg/kg dw, and having a 24% effect, EPA estimates that *Esox* is somewhat sensitive, but not among the four most sensitive species. Regarding the comment on the logic for setting the number of GMCVs to 14, the goal of aquatic life criteria is to ensure protection of populations of species representing the entire aquatic community, and not just fish, as described in the methodology for criteria development (Stephan et al. 1985).

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of edema were consistent between the 10 µg Se/L treatments in Study I and II, on average, but the ovary Se concentrations were widely different. The mean macroinvertebrate Se concentrations in the 10 µg Se/L treatments in Study I and II were similar (grand means among all invertebrate taxa were 21.6 and 22.8 mg/kg dw for Study I and Study II, respectively [Hermanutz et al., 1996]). The relatively large difference in the bluegill ovary Se concentrations in Study I compared to Study II, therefore, is unexpected. • Second, in Study I, the ovary Se concentration of 17.71 mg/kg dw in the 10 µg Se/L treatment was greater than the ovary Se concentration of 15.46 mg/kg dw in the 30 µg Se/L treatment. This is also unexpected because the grand mean Se concentration in invertebrate taxa collected from the 10 and 30 µg Se/L streams were 21.6 and 44.7 mg/kg dw, respectively. Thus, a higher ovary Se concentration in the 30 µg Se/L stream would be expected. This basis for this discrepancy is not clear, although the ovary Se concentration measured in the 30 µg Se/L stream was based on a single fish, which may have randomly had a lower ovary Se concentration. • Third, a potentially more important source of uncertainty is that the ovary Se concentrations in the Hermanutz et al. (1992, 1996) study were reported on a wet weight basis. Dry weight ovary Se concentrations were estimated assuming a moisture content of 76%, which was based on the average from Gillespie and Baumann (1986), 85%, and Nakamoto and Hassler (1992), 67%. If the true moisture content was 85%, the bluegill Se EC10	Estimated egg-ovary concentrations were originally calculated for invertebrates as a point of reference to show that they were typically less sensitive then the fish represented in the SSD, and would therefore be protected by a tissue criterion based on fish. In the current draft, fish and invertebrate SMCVs and GMCVs are listed in separate tables, and whole body and estimated egg-ovary values for invertebrates have been removed from the two tables showing the distribution of GMCVs used in the SSD for egg-ovary and whole body criteria, respectively. A separate Table (5b) was added showing the measured whole body SMCVs and GMCVs for the three invertebrate taxa with relevant test data, as well as what the predicted whole body fish tissue concentrations would be after multiplying the measured invertebrate SMCV&GMCV by the median tropic level 2 to trophic level 3 trophic transfer function of 1.27. The results described in sections 3.1.3-3.1.4 and shown in Table 5b demonstrate that invertebrates are less sensitive to selenium in fish, and while they are used implicitly in the SSD to fulfill taxonomic minimum data requirements, they are not included with fish values. These studies are taken into account as part of the total "N" in the criterion calculations, in accordance with the standard methodology for criteria development (EPA 2008). Regarding the comment on the inclusion of additional genera, the *Catostomus* and *Xyrauchen* studies, presented in Appendix D of the 2014 draft. EPA determined these studies are not of sufficient quality for quantitative use, therefore they have not been included in setting N. During development of the document, EPA considered increasing the N used in the criterion calculation to reflect inclusion of *Catostomus*, based on the

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from Hermanutz et al. (1992, 1996) would be 20.3 mg/kg dw (almost identical to the EC10 derived from Doroshov et al. [1992]). In contrast, if the true moisture content was 67%, the bluegill Se EC10 from Hermanutz et al. (1992, 1996) would be 9.2 mg/kg dw. In my opinion, the uncertainty in the moisture content of the bluegill ovaries in the Hermanutz et al. (1992, 1996), along with uncertainties in the ovary Se concentrations in Study I, are sufficiently great that this study should not be included in the SMCV for bluegill, as there are two other studies (Doroshov et al. [1992] and Coyle et al. [1993]) for which dry weight ovary Se concentrations were reported and the EC10s from those two studies were very comparable. The SMCV for bluegill based on those two studies would be 22.2 mg/kg dw. Alternatively, if data from Study I of Hermanutz et al. (1992, 1996) are pooled with data from Doroshov et al. (1992) and Coyle et al. (1993), the consistency in the concentration-response data is apparent and an EC10 of 21.4 mg/kg dw can be derived (Fig.1). Fig. 1. Concentration-response relationship for bluegill based on data pooled from Study I of Hermanutz et al. (1992, 1996), Doroshov et al. (1992), and Coyle et al. (1993). EC10 = 21.4 mg/kg dw based on logistic regression analysis in TRAP.	suggestive evidence from the de Rosemond et al. study, but decided that it would be more prudent not to, given that Crutchfield (2000) states that "By the early 1980s, the fish community had collapsed in the Hyco Reservoir and species such as [names of several taxa]… and suckers (Catostomidae) became much reduced throughout the reservoir." Regarding the Hamilton et al. study, EPA does not believe it can come to any reliable conclusion about the relative sensitivity of razorback suckers. Regarding the comment on adding new studies, the authors of the mountain whitefish study were contacted by EPA; however, the authors declined to provide these data to EPA at this time. The Formation Environmental Yellowstone cutthroat trout study has been analyzed and added to the database. Regarding the suggestion for EPA to consider using empirically measured whole-body Se (or muscle Se) data for those species where they are available, rather than applying CFs to egg/ovary Se data (EPA's current approach) using conversion factors, EPA determined that the approach used was consistent and linked criteria for other tissues to egg-ovary concentrations. Regarding the comment that notes that there are a few reproductive studies for which EC10s can be directly calculated as muscle or whole-body concentrations measured during the study, EPA opted to use the egg-ovary EC10 from such studies and then convert the SMCV or GMCV from EO to WB or M

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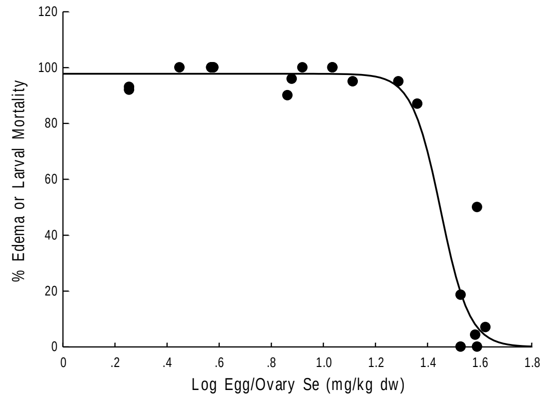 <u>Other Fish Species in the SSD</u> The draft fish egg/ovary Se criterion derived following EPA guidelines is based on the four lowest GMCVs and the total number of GMCVs. The two lowest GMCVs in the EPA's draft document are for *Salmo* (represented by brown trout) and *Lepomis* (represented by bluegill), which were both discussed above. The 3rd and 4th lowest GMCVs are for *Micropterus* (represented by largemouth bass) and *Oncorhynchus* (represented by cutthroat trout and rainbow trout). I do not disagree with EPA's interpretation of the studies for those genera. The *Esox* GMCV of <34 mg/kg dw, represented by northern pike, is an EC24 because the data were not amenable to derivation of an EC10 using TRAP. The EPA compared this EC24 to the EC24 that could be derived for rainbow trout and noted that the two species appear to be similar in sensitivity, with northern pike perhaps slightly less tolerant. In contrast, the original study authors for the northern pike study, Muscatello et	using all available WB/EO or M/EO ratios. Although EPA recognizes the merits of the commenter's suggestion, EPA used the current approach because it is also scientifically defensible. Regarding the fish whole-body and muscle selenium criteria, the primary driver for the low translated water numbers at two lentic sites were large EFs, which were site specific. The application of generic TTFs to site-species combinations was done for purposes of consistency, as site-specific TTFs could not be calculated in many cases. As noted above in response to the comment of Peer Reviewer 2 regarding Part I, Question 2.a.iii., EPA modified the TTF methodology in the 2015 draft to better reflect taxonomic similarities when data were lacking. Also, compared to EFs, TTFs are relatively small and less variable, and have less influence on a translated water value. Regarding the surface water selenium criteria monthly average, EPA reanalyzed the data after considering this peer review comment and recalculated the lentic and lotic water column elements of the criterion to reflect appropriate consideration of both high and low exposure sites. In the 2014 External Peer Review Draft, translated lentic and lotic water criteria were calculated from 44 and 88 site-species combinations, respectively. A single site could have as many as 8 sampled fish species. For example, of the 44 site-species used for the lentic derivation via the 20th percentile value, 12 of the lowest 13 values are for Badin and High Rock. These lakes each have one EF, but each of its EFs is used six times, once for each of six fish species. The particulate concentrations measured in both of

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al. (2006), reported an EC10 of 20.38 mg/kg dw based on linear regression. The EC10 of 20.38 mg/kg dw would make the *Esox* GMCV the 4th lowest in the EPA's dataset. This change alone, however, would have a negligible influence on the draft fish egg/ovary Se criterion—it would raise it slightly from 15.2 mg/kg dw to 15.6 mg/kg dw (lowering the 4th lowest GMCV steepens the slope of the line through the four lowest GMCVs, which increases the 5th percentile). <u>Number of GMCVs Assumed in Fish Egg/Ovary Se Criterion Calculation</u> The logic for setting the number of GMCVs to 14 is flawed in my opinion. This number is based on 9 fish genera, 3 invertebrate genera with tissue-based toxicity data available, and 2 crustacean genera that were waived. In my opinion, a genus sensitivity distribution based on Se toxicity values for fish eggs/ovaries, and for which the resulting criterion will be a Se concentration in fish eggs/ovaries, and for which compliance will be determined by measuring Se concentrations in fish eggs/ovaries, cannot include data for non-fish taxa. It must be remembered that a criterion based on an internal tissue concentration is not the same as a criterion based on an external concentration to which the entire aquatic community may be exposed. One will not be able to measure Se concentrations in invertebrates in order to determine compliance with the fish tissue-based Se criterion, so they should not be included in the SSD. Further, if I understand correctly, the three whole body Se EC10s for invertebrates (37.84 mg/kg dw for *B. calyciflorus*, >140 mg/kg dw for *L. variegatus*, and 24.2 mg/kg dw for *C. triangulifer*) were multiplied by a (1) diet-to-whole body fish TTF and (2) a whole body-to-egg/ovary conversion factor in	these lakes are near the median observed in EPA's lentic database, but their water concentrations are among the lowest. Conversely, several lotic sites (e.g., McElmo Cr., Spring Creek at LaBoca, etc.) had very low EFs (and by extension, high translated water concentrations), but each EF was used several times, once for each fish species. As a result of the peer reviewer's comments, EPA completed a reanalysis of the data to remove any overweighting of a few key high and low end sites in the calculations. To account for overweighting, EPA used one fish species per site – the species most sensitive to selenium bioaccumulation, to yield an appropriately protective water column criterion element for both lentic and lotic values. In addition to adjustments to correct for overweighting due to the influence of multiple species at high and low EF sites, changes to several TTFs and CFs, reflecting incorporation of new information, were made yielding site-species lentic and lotic values of 0.9 µg/L and 4.2 µg/L, respectively. Subsequent adjustments to correct for overweighting due to the influence of multiple species at high and low EF sites resulted in an increase in the translated lentic water concentration criterion from 0.9 µg/L to 1.2 µg/L, and a decrease in the translated lotic water concentration criterion from 4.2 µg/L to 3.1 µg/L. Regarding the intermittent criteria, this criterion element fills a need to account for intermittent discharges that may be elevated but infrequent, and to account for associated potential loadings and chronic risk to downstream waters. Modifications of the criterion can be made on a site-specific basis, with data from the site. As mentioned above, biokinetic modeling, if used should be adapted to a site using appropriate data – the values EPA

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order to estimate the Se concentrations in fish eggs/ovaries that may result from the toxicity thresholds for invertebrates. These values were then used as "SMCV & GMCV as estimated EO concentration in an accompanying fish assemblage (mg Se/kg dw EO)" in Table 6b of the draft AWQC document. However, these are simply predicted concentrations in fish eggs/ovary and are not effect concentrations for fish. I believe that n should equal the number of fish genera, which is 9 based on the draft AWQC document. <u>Additional Genera that Could be Added to the Total N</u> Although the EPA did not include the egg/ovary Se toxicity data for white suckers (*Catostomus commersonii*; de Rosemond et al. 2005) and razorback suckers (*Xyrauchen texanus*; Hamilton et al. 2005a,b) because reliable toxicity thresholds (EC10s or other) could not be derived, there does appear to be sufficient evidence that they would be among the four most sensitive genera. Thus, the number of GMCVs used in the criterion calculation could be increased from 9 fish genera to 11 fish genera. <u>Toxicity Data for an Additional Fish Species</u> Nautilus Environmental in Burnaby, British Columbia has conducted a Se maternal transfer toxicity study with mountain whitefish (*Prosopium williamsoni*). This species does not appear to be especially sensitive (i.e., it would not be among the four lowest GMCVs), but it would added another genus to the sensitivity distribution. I recommend that the EPA investigate	presented represented default values that will be generally protective in these situations. Regarding the suggested EC10 values for brown trout and bluegill, EPA revisited the analyses of these relevant studies and made appropriate revisions in the 2015 document. Regarding the criterion frequency, selenium is persistent, however, it may be sequestered in an environmental compartment that precludes it from uptake in biota (e.g., burial in sediment). EPA's AWQC are traditionally based on aqueous concentrations since toxicity for the chemicals for which criteria have been developed is generally due to aqueous exposure. These concentrations can vary significantly both short-term and long term, allowing organisms in the aquatic community to recover. In contrast, selenium is a bioaccumulative pollutant, and fish tissue concentrations have been directly correlated with the adverse effect. Also reductions in fish tissue concentrations occur slowly, even after removal of a selenium source. Since exceedance of these concentrations is correlated with adverse effect occurs EPA determined that these levels cannot be exceeded in order to be protective of the aquatic community. Technical support information regarding fish tissue sampling issue is being developed by EPA.

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whether this study is publically available and, if so, whether it meets the EPA guideline for test acceptability and inclusion in the sensitivity distribution. The Se toxicity study with Yellowstone cutthroat trout (Formation Environmental 2011b) should also be considered. Influence of Potential Changes to GMCVs and N As summarized above, in my opinion, the most conservative and reasonable EC10 that can be derived for brown trout is 16.76 mg/kg dw (although the weight-of-evidence suggest to me that the EC10 falls between about 20.7-21.6 mg/kg dw) and that the bluegill SMCV should be 22.2 mg/kg dw. If the four lowest GMCVs were 16.76 mg/kg dw for *Salmo*, 20.35 mg/kg dw for *Micropterus*, 22.2 mg/kg dw for *Lepomis*, and 22.53 mg/kg dw for *Oncorhynchus*, and the total number of fish genera was set equal to 11 (with inclusion of the two sucker genera), the resulting criterion would be 16.0 mg/kg dw. Alternatively, if the *Esox* (northern pike) GMCV was adjusted from <34 mg/kg dw to 20.4 mg/kg dw, the resulting criterion would change slightly to 16.1 mg/kg dw. *Fish Whole-body and Muscle Se Criteria* The draft fish whole-body and muscle selenium criteria are 8.1 and 11.8 mg/kg dw, respectively. In general, I believe that the approach for deriving these draft criteria is reasonable and that the magnitudes of these criteria are consistent with the toxicological literature. My only suggestion is that the EPA consider using empirically measured whole-body Se (or muscle Se) data for those species where it is available, rather than applying CFs to egg/ovary Se data. It would be interesting to see	

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whether that has a significant influence on the draft whole-body or muscle Se criteria. And of course if any modifications are made to the egg/ovary Se GMCVs, this would influence the draft whole-body and muscle Se criteria, as would a change to the number of genera, if my suggestions above are considered. *Surface Water Se Criteria - Monthly Average* The draft water column selenium criteria are 4.8 and 1.3 µg/L for lotic and lentic waters, respectively. In general, I do not agree with the approach used by the EPA in deriving these water column criteria. Although I do not agree with the approach, I do believe that the draft criterion of 4.8 µg/L for lotic waters is reasonable and consistent with our understanding of the range of Se bioaccumulation potential into fish across a wide range of lotic sites. However, for the draft lentic Se criterion of 1.3 µg/L, the approach used by the EPA results in this criterion being almost exclusively driven by data for two reference locations. This in turn is mostly due to what I perceive as a flaw in the approach, where site-specific Se data in invertebrates and fish are ignored and instead non-site-specific TTFs and CFs are applied that are inconsistent with the site-specific data. This resulted in cases where erroneously high modeled Se concentrations in fish tissue are linked with low water Se concentrations (i.e., reference site concentrations), and then these become the "drivers" for the draft lentic criterion of 1.3 µg/L. Please see my detailed comments on this issue in Part III. *Surface Water Se Criteria - Intermittent Exposure*	

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The draft intermittent exposure Se criteria represent a mathematical manipulation of the monthly average criteria in order to derive values that would still result in 30-day average concentrations of 4.8 and 1.3 µg/L for lotic and lentic waters, even if those were exceeded for *x* number of days. A limitation of this approach is that it does not consider the uptake and elimination kinetics of Se in aquatic food chains and the influence of exposure duration and magnitude on these biokinetic parameters. In my opinion, a biokinetic modeling-based approach would be more appropriate for deriving intermittent, or acute, criteria that are protective against exceeding fish tissue-based Se criteria. More details are provided in my comments in Part III below. Duration *Fish Egg/Ovary, Whole-body, and Muscle Se Criteria* The draft fish tissue-based selenium criteria (eggs, ovaries, whole-body, muscle) are "instantaneous measurements" as "Fish tissue data provide point measurements that reflect integrative accumulation of selenium over time and space in the fish at a given site" and "Selenium concentrations in fish tissue are expected to change only gradually over time in response to environmental fluctuations." I agree with the EPA's decision that the duration for fish tissue Se measurements should be an instantaneous measurement since, for most scenarios and fish species, the Se concentrations in fish tissue will be reflective of a longer term exposure. *Surface Water Se Criteria - Monthly Average and Intermittent Exposures* In my opinion, 30 days for an average exposure duration is	

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reasonable, especially since an intermittent criterion is being considered (although, as noted, I believe the intermittent criterion would best be derived using a biokinetic modeling approach). Biokinetic data for algae and several freshwater invertebrates indicate that steady-state Se concentrations in the food chain may be achieved within this time frame. Frequency *Fish Egg/Ovary, Whole-body, and Muscle Se Criteria* Although the EPA's AWQC, including the draft water Se criteria, are not to be exceeded more than once in three years, the fish tissue-based Se criteria are "never to be exceeded." To my knowledge, the "frequency" component of AWQC is rarely incorporated into permit limitations, so the implications of fish tissue-based Se criteria "never to be exceeded" are not entirely clear to me. The "frequency" component was initially incorporated into AWQC based on the premise that ecosystems will not be harmed if the number of criterion excursions is limited and/or there are compensating periods of time below the criterion over which the ecosystem can recover. As far as I can tell, the draft AWQC document for Se does not explain the basis for the "never to be exceeded" frequency decision for fish tissue. It seems that there should be some level of consistency between the allowable "frequency" for fish tissue-based and water-based Se criteria. *Surface Water Se Criteria - Monthly Average and Intermittent Exposures*	

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The "frequencies" of "not more than once in three years on average" are consistent with the EPA guidelines and AWQC for other chemicals. As noted above, however, I am not aware of the "frequency" component of AWQC being incorporated into most effluent limitation so am unsure of the significance of this component. The fixed monitoring benchmark (FMB) approach, which has initially been developed for copper and biotic ligand model (BLM)-based criteria, represents a method that does explicitly account for exceedance frequency (USEPA 2012). However, this approach is for use under a site-specific context and would not apply to the national (non-site-specific) Se criteria. A reasonable excursion frequency for Se in water should be determined carefully, however, as Se is bioaccumulative and has variable persistence depending on receiving water conditions. For example, more frequent excursion frequencies may not be consequential in lotic systems with low biological productivity and short resident times, while an excursion frequency greater than once every three years may be warranted for lentic systems with high biological productivity and long residence times. In summary, I think the "frequency" decisions should be evaluated and explained in more detail. Literature cited: Coyle JJ, Buckler DR, Ingersoll CG, Fairchild JF, May TW. 1993. Effect of dietary selenium on the reproductive success of bluegills (Lepomis macrochirus). Environ Toxicol Chem 12:551-565. de Rosemond SC, Liber K, Rosaasen A. 2005. Relationship between embryo selenium concentration and early life stage	

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Toxicol Chem 11:217-224. Hermanutz RO, Allen KN, Detenbeck NE, Stephan CE. 1996. Exposure of bluegills (*Lepomis macrochirus*) to selenium in outdoor experimental streams. U.S. Environmental Protection Agency, Duluth, MN, USA. Nakamoto RJH, T.J. 1992. Selenium and other trace elements in bluegills from agricultural return flows in the San Joaquin Valley, California. Arch Environ Contam Toxicol 22:88-98. USEPA. 2012. Calculation of BLM fixed monitoring benchmarks for copper at selected monitoring sites in Colorado. Office of Water, USEPA. 820R12009.	
4. I do not see obvious errors in their approach.	Thank you for your comment.
5. The egg/ovary criterion of 15.2 mg/kg relies strongly on the reassessment of brown trout data, in particular the Formation study. It seems that much of the issue is related to the lab accident where larval trout were removed from an aquarium due to a faulty standpipe. The EPA has chosen to assume the worst-case, that 100% of the fish that escaped were dead and/or deformed, resulting in an EC10 of 15.91 mg/kg egg. However it is plausible that certain of these fish were not dead or deformed, as discussed in certain public comment documents. The EPA has reanalyzed these data to account for different scenarios, and shown that the EC10 varies from 15.91 to 21.16 mg/kg egg. It seems to me that the 15.91 mg/kg EC10 may be overly conservative. Due to the lack of knowledge regarding the status of these escaped fish (dead, deformed, or healthy), perhaps the assumption could be made that 50% of the escaped fish were dead/deformed, and 50% were normal. This would only slightly	The brown trout egg ovary EC10 has been re-evaluated by EPA based on recognition of confounding occurring due to the lab accident that caused aquaria to overflow. The new EC10 is based on larval mortality from hatch to swim up, the normal duration for these tests, and no longer includes data from the post swim up portion of the test after the lab accident This yielded an EC10 of 18.09 mg/kg dw. Regarding collecting fish tissue samples, EPA agrees that technical support information is needed on sampling approaches for each element of the tissue criterion, since there are various situations where one tissue type may be favored over another, or the sample logistics (e.g., fish size) may limit the sample to a certain type of tissue sample. EPA is developing information for states, tribes and stakeholders to consider when sampling fish

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increase the EC10 value from which the 15.2 mg/kg egg/ovary criterion is being largely driven. This is only a suggestion of a reasonable compromise given the diverse opinions on this lab occurrence. For the egg/ovary criterion, the timing of fish sampling is absolutely critical, and the EPA provides no guidance on sampling design for determining egg/ovary [Se] in the document. As discussed above in 2a(ii), it is the [Se] in eggs that drives early life stage toxicity, so adult female fish absolutely must be collected during the late vitellogenic or preovulatory periods of oogenesis for this criterion to be scientifically and toxicologically meaningful. Measuring [Se] in ovarian tissue during other periods of oogenesis will be much less informative (i.e., about as informative as muscle or whole-body [Se]). The EPA must provide guidance for specific times of the year to collect adult female fish for egg [Se] determinations. For synchronous spawning species (e.g., salmonids, esocids, catostomids, ictalurids), this will be a defined period of 1-2 months on average (usually spring). For asynchronous (batch) spawning species (e.g., cyprinids), this period will be less defined and will usually be 3-6 months (usually spring to late summer or early fall). For the whole-body and muscle criteria, the EPA has used best available knowledge and approaches to derive these values, and they are of appropriate magnitude, duration and frequency. Collecting fish at any time of the year and determining whole-body or muscle [Se] will provide sufficient information on Se bioaccumulation. Although there will likely be some variation across seasons, due to prey availability, temperature and other factors, this approach should work.	tissue for the purposes of implementing the freshwater selenium criterion. In addition EPA has derived and is recommending that states adopt all of the fish tissue elements to provide maximum flexibility for a states monitoring and assessment program. Adopting all elements covers all potential logistical, spatial, temporal, and species- and life history-specific considerations.

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6. The recommended magnitude, duration, and frequency for each criterion element are scientifically sound and appropriate. The derivation of the tissue based criteria are well-supported by including the major published works in the related fields and by rejecting with transparent cause and inclusion/rejection standards those studies that do not attain the stated benchmark for quality and reproducibility (e.g., NOECs). The criterion development satisfyingly addresses a diverse range of major fish types indicative of aquatic ecosystem health in geographically diverse lentic and lotic systems. With chronic exposure, fish egg-ovaries are now recognized as the best indicator of toxic selenium risk, however practical monitoring may require whole body-muscle tissue analysis. Water column selenium values fill the need for screening and analysis of potential for risk, abatement of new contamination pathways, and managing discharge, as well as other activities that may impact water quality.	Thank you for your comment.
7. As noted in our response above, there is some confusion regarding how "never to be exceeded" concentrations of selenium in the tissue based criterion will be applied (i.e., is this applied to analysis of single fish or to arithmetic or geometric means from sampled populations?). Clarification on this question is required before the scientific defensibility of the duration and frequency can be assessed for the two tissue based criterion. With regard to the magnitude of the tissue based elements, it would appear that at least two issues may challenge the scientific defensibility of these criteria. First, it is our understanding that the egg/ovary criterion was developed from	Selenium is persistent. However, it may be sequestered in an environmental compartment that precludes it from uptake in biota (e.g., burial in sediment). EPA's AWQC are traditionally based on aqueous concentrations since toxicity, for the chemicals for which criteria have been developed, is generally due to aqueous exposure. These concentrations can vary significantly both short-term and long term, allowing organisms in the aquatic community to recover. In contrast, selenium is a bioaccumulative pollutant, and fish tissue concentrations have been directly correlated with the adverse effect. Also reductions in fish tissue concentrations take a long time, even after removal of a selenium source. Since exceedance of these concentrations is correlated with adverse effect occurs EPA determined that

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EC10 values derived from the literature. Where multiple results of acceptable quality for a given species were available, a geometric mean was calculated. In the case of the EC10 for bluegill (*Lepomis macrochirus*), the mean EC10 resulted from 4 studies, published by three authors: Hermanutz et al., 1992 and 1996, Doroshov et al. (1992) and Coyle et al. (1993). However, the EC10 value calculated from the Hermanutz et al. studies (=12.7mg/kg) is quite different from the values rom the other two studies (20 and 24.6 mg/kg respectively), indicating cause for investigation of the reasons for the difference, especially in light of their importance for determining the egg/ovary tissue based criterion. One of the supplemental comments provided as additional information with this package (Docket ID EPA-HQ-OW-2004-0019-0331) indicates that the TRAP model plot of the Hermanutz et al. data provide a poor fit. While we were not afforded access to figure 1, which cited in that docket submission, if the data are indeed poorly fit, it is appropriate to consider them questionable and eliminate them from the geometric mean calculation for this species. A second, and potentially more serious issue with regard to the magnitude of the egg/ovary tissue based criterion, is the reliance on the reanalysis of data from the brown trout (*Salmo trutta)* study (Formation 2011). Uncertainty in this study arises because some fry escaped from their respective incubation chambers and could not be assigned to a given treatment. As a result, several scenarios were calculated based on whether the escaped fry had similar deformity rates relative to the retained fry, were all deformed, or were all normal. While this cannot be resolved, the criterion was calculated based upon the most conservative	these levels cannot be exceeded in order to be protective of the aquatic community. Technical support information regarding the specifics of fish tissue sampling for selenium is being developed by EPA. Regarding the Hermanutz study, EPA re-analyzed this study and removed the nest data, which was introducing substantial variability, resulting in the poor fit in the EC10 TRAP model in the previous draft. When only egg-cup data were used, EPA determined the most appropriate endpoint to be a combined deformity and mortality endpoint. Figure 1 (now Figure 2) was in the Hermanutz et al. chronic summary for bluegill in Appendix C of the previous draft, although it was not labeled. EPA has labeled all Figures in the Hermanutz et al. bluegill summary in the current draft. Regarding the brown trout data from the Formation 2011 study, EPA agrees with the reviewer and has re-analyzed the data and excluded effects observations of the 15 day post swim-up test when the lab accident occurred and caused the confounding issues. The brown trout egg ovary EC10 has been re-evaluated by EPA based on recognition of confounding occurring due to the lab accident that caused aquaria to overflow. The new EC10 is based on larval mortality from hatch to swim up, the normal duration for these tests, and no longer includes data from the post swim up portion of the test after the lab accident This yielded an EC10 of 18.09 mg/kg dw.

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approach: that all fry were dead or deformed. This conservative approach to calculating an EC10 value for brown trout result in it being the most sensitive species, thereby affecting the overall egg/ovary criterion. Subsequently, because other criterion (i.e., muscle/whole body and the water based criterion) are back calculated based on the egg/ovary value, conservatism is compounded in the values for these criterion as well. For the water column based criterion, two separate elements are prescribed in the Draft Document: a monthly average and a separate element for intermittent (discontinuous) exposures. Each of these is further delineated to apply to either lentic or lotic systems. Presumably the definitions for lotic and lentic systems would be based on residence time of water or some related criteria, but the Draft Document does not contain an explicit definition of either type of system. Back calculating from egg/ovary to muscle/whole body and then down through trophic levels to derive allowable water column criterion for each of these types of aquatic systems is not scientifically valid, because of the use of generic conversion factors and broadly based trophic transfer factors. These generic terms do not incorporate site specific information, including concentration dependent uptake kinetics and consideration for important influencing factors (e.g., sulfate). The water based criterion is therefore, conservative and variable. As evidence for this, the monthly average exposure value for lentic systems is 1.3 ug/L. This value is at the upper end of background values for freshwater and may be exceeded even in the absence of industrial inputs in areas receiving runoff from seleniferous soils. The value is also lower than recently recommended lentic	Regarding the classification of lentic and lotic waterbodies, there were few residence time data available for the water bodies evaluated, therefore EPA could not use this parameter for classification. Based on the comments of peer reviewers as well as stakeholders in the public process, the EPA went through each waterbody and determined whether it was lentic or lotic, being careful to note specific issues like run of the river reservoirs which could cause misclassification. Regarding the commenter's concern of back-calculating (translating) the tissue criterion to water, EPA re-evaluated conversion factors used and refined the approach to reflect the most phylogenetically proximate data in its criterion calculations, strengthening its previous draft analyses, as noted above EPA also re-evaluated the lentic and lotic water column values, and developed values that are based on the best available, scientifically defensible science to yield water column values that are protective of aquatic life based on available data.

External Peer Reviewers' Comments	EPA Response
values based on similar analysis (2 and 2.1 ug/L respectively (Deforest et al., 2104, BC MoE 2014).	

PART II: FISH TISSUE CRITERION ELEMENTS DERIVATION: DERIVATION OF FISH EGG-OVARY, WHOLE BODY AND MUSCLE CRITERION ELEMENT(S)

EPA is requesting a technical review of the methods and procedures used to derive a chronic selenium criterion based on an egg-ovary concentration, as well as its translation to a criterion element applicable to whole-body and muscle tissue. Please address the following questions:

1. Please comment on EPA's use of the effects concentration 10th centile (EC_{10}) as the measurement endpoint for the fish reproductive toxicity studies used to derive the egg-ovary element.

External Peer Reviewers' Comments	EPA Response
1. It is unclear to me why EPA has selected the EC10 as the measurement endpoint for these studies. EPA argues because it is a tissue-based criterion, the measure of exposure is less variable than might occur for a water-based criterion. I understand the point EPA is making and agree that a tissue-based criterion is more integrative of exposure than a water-based criterion. However, following this logic, EPA is then stating that for a chemical with a water-based criterion in a system where the exposure concentration is consistently above the EC10 (e.g., very stable at a concentration equivalent to the EC15) that it is not sufficiently protective. It seems to me that the ECx selected should be based on the level of protection EPA intends to provide and that this is independent of variability in exposure. Variability in exposure is more appropriately addressed via averaging periods as EPA has done with the intermittent exposure element of the criterion. In fact, by considering both an intermittent exposure element and using an EC10, EPA is addressing the same issue twice. Given the above, I do not believe EPA has provided a scientific rationale for use of the EC10 in a tissue-based criterion as	When considering the use of the EC10 versus the EC20, an EC10 was determined to be a more appropriate endpoint for tissue-based criteria given the nature of exposure and effects for this bioaccumulative chemical. EC20s have historically been used in the derivation of EPA criteria applicable to the water medium. While water concentrations may vary rapidly over time, tissue concentrations of bioaccumulative chemicals are expected to vary gradually. Thus, where concentrations of selenium in fish tissue are used as an effect threshold, there is potential for sustained impacts on aquatic systems, relative to chemicals that are not as bioaccumulative. This calls for use of a lower level of effect to attain sufficient protection. Also, detection in fish tissue above these levels can potentially have longer-term impacts due to the bioaccumulative nature of selenium. This is further demonstrated by field observations that reproductive effects in fish can take on the order of a decade for a selenium contaminated ecosystem to recover from (e.g., Belews Lake, NC). Further, the steepness of the dose-response curve for selenium argues for a more protective value, not less, because small increases in concentrations can have larger

External Peer Reviewers' Comments	EPA Response
providing an equivalent level of protection as an EC20 in a water-based criterion. I recommend EPA evaluate how use of the EC20 would affect the final criteria calculations. I suspect given the sharp dose-response relationships for Se, it will not dramatically change the final criteria calculations. Alternatively, if EPA now believes the EC10 is an appropriate level of protection for WQC, then this should be applied across chemicals.	impacts than for toxicants with shallower dose-response curves. The EC10 was also preferred for selenium over the NOEC or LOEC as these measures of effect are influenced by study design, specifically the concentrations tested, the number of concentrations tested, the number of replicates for each concentration, and the number of organisms in each replicate. As noted by Campbell (2011), EC10s and NOECs are generally of similar magnitude, but EC10s have the advantage of being more reproducible than NOECs (Van der Hoeven et al. 1997; Warne and van Dam 2008). The intermittent criterion was developed to address situations where a system is subject to elevated concentrations for short durations. The kinetic considerations used to set the averaging period for the chronic water criterion, from which the intermittent criterion is calculated, address a different (but not unrelated) facet of the time variability problem than do the considerations for using the EC10. The selection for use of the EC10 considered the appropriate level of effect for a criterion that fish tissue concentrations may approach for extended periods of time, having already contaminated all of the lower trophic levels with a bioaccumulative pollutant. The environmental concern is thus greater than that involving water criteria that typically are only infrequently approached by rapidly varying concentrations in the water column. It is for this reason that EPA has derived its selenium tissue criteria based on the EC10 while it continues to derive water criteria for other pollutants based on the EC20. Further, the steepness of the dose-response curve argues for a more protective value, not less, because small increases in concentrations can have larger

External Peer Reviewers' Comments	EPA Response
	impacts than for toxicants with shallower dose-response curves. The intermittent criterion is meant to protect receiving and downstream waters from bioaccumulative impacts by limiting the amount of selenium that is available to be taken up by biota and bioaccumulated to levels of concern in sensitive species. Because (a) the derivation of the 30-day averaging period, and the subsequent derivation of the intermittent criterion, and (b) the considerations upon which EPA based its decision to use the EC10 both involve the kinetics of bioaccumulation, EPA understands the connection the comment is making between the two. However, EPA does not agree that it is addressing the same issue twice. Rather EPA is addressing two distinct issues that are both affected by bioaccumulation and kinetics. For these reasons EPA finds it is reasonable and protective to select the EC10 as the measurement endpoint for this tissue-based criterion.
2. This seems like a statistically-valid approach to setting the threshold, but toxicology is not my field of expertise.	Thank you for your comment.
3. The draft AWQC document notes that "an EC10 was determined to be a more appropriate endpoint for tissue-based criteria given the nature of exposure and effects for this bioaccumulative chemical. EC20s have historically been used in the derivation of EPA criteria applicable to the water medium. While water concentrations may vary rapidly over time, tissue concentrations of bioaccumulative chemicals are expected to vary gradually. Thus, where concentrations of selenium in fish tissue approach an effect threshold, there is potential for sustained impacts on aquatic systems, relative to chemicals that	Thank you for your comment.

External Peer Reviewers' Comments	EPA Response
are not as bioaccumulative." I agree with this logic for using the EC10 as the measurement endpoint for tissue-based toxicity values, where this effects statistic can be derived. I also agree with the use of an EC10 rather than a no-observed-effect concentration (NOEC), lowest-observed-effect concentration (LOEC), or geometric mean of the two, for the reasons discussed in the draft AWQC document.	
4. Strikes me as rather arbitrary.	Regarding the use of the EC_{10}, see response to Peer Reviewer 1, Part II, Question 1, above.
5. The EC10 is absolutely the appropriate endpoint for early life stage toxicity in fish to be used to derive the egg/ovary criterion. This is due to the very steep dose-response relationships observed for larval abnormalities/mortality as a function of egg [Se]. Thus, EC10 provides a toxicologically relevant threshold for appearance of such toxicities, that is, only a marginal increase in egg [Se] will result in a much greater frequency of toxicity. In addition, the main alternative endpoint (EC20) will not differ greatly from EC10 for a given species due to this steep dose-response relationship. Something the EPA should consider when developing the genus sensitivity distribution is the nature of the experiment for each taxa (lab- vs. field-based). In lab studies, adult female fish are most commonly exposed to selenomethionine (SeMet), which is valid because it is the dominant Se species (60-80% of total Se) found in organisms throughout food webs, particularly at higher trophic levels. In field studies, fish are exposed to SeMet and several other selenium species that likely vary in their toxicity,	Thank you for your comments. EPA recognizes the issue noted by the peer reviewer regarding differences in laboratory and field exposures. EPA used all of the available reliable data for the selenium fish effects analyses. Laboratory and field experiments did not yield apparent wide differences in predictions of effect level, suggesting that effects were more related to the concentrations of selenium in tissues rather than the proportions of the forms of selenium in diets. Thus, EPA considers the current criterion to be protective and scientifically defensible.

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and in fact are likely less toxic than SeMet. Thus, lab exposures using pure SeMet may overestimate toxicity (i.e., generate lower EC10 values) compared to real-world exposures.	
6. The EC10 is an appropriate endpoint to use in the development of the egg-ovary element of the tiered criterion. Egg-ovary Se concentration is well recognized in the peer-reviewed scientific literature as a high quality indicator of reproductive toxic risk in fish. Because selenium is a reproductive toxicant, special considerations in risk management are warranted. For precedent, the Food Quality Protection Act of 1996 which manages risk of chemical exposure in the human food system, uses an extra ten-fold safety factor for chemicals used in food production that have reproductive toxicology or neurotoxic endpoints. This extra safety factor results from our common understanding in toxicology that those chemicals with repro- or neuro-toxic activity represent an exceptional risk and thus require exceptional safeguards. Reproductive toxicity is a significant threat to the population of the impacted aquatic organisms and thus to the aquatic food-web. There are valid questions whether the EC10 is sufficiently protective of endangered aquatic species and the criterion document should address these concerns more thoroughly. Overall the EC10 egg-ovary endpoint is scientifically consistent and defendable with the intent and required actions of the CWA.	Thank you for your comment. Aquatic life criteria are designed to be protective of approximately 95% of aquatic genera present in ecosystems. Risk assessment practices that are specifically tailored to the particular objectives and requirements of the Food Quality Protection Act of 1996 to protect human health are not necessarily appropriate models for the implementation of the Clean Water Act protection of aquatic life. The extra ten-fold safety factor is to ensure protection of children, where data are not available and does not establish a precedent for the protection of the aquatic community. Regarding consideration of endangered species, EPA has provided a summary of available data on the adverse effects of selenium to endangered species, and taxonomically related surrogates. EPA identified data for white sturgeon (a species with one population listed as endangered, and a closely related taxonomic surrogate for other endangered sturgeon), several species in the family Salmonidae, Cyprinidae as well as a pupfish in the Cyprinodontidae, and several less closely related species with tests in the order Perciformes (sunfish and bass) as surrogates for endangered darters. Regarding the comment that it is possible that the EC10 might not be sufficiently protective of a particular endangered aquatic

External Peer Reviewers' Comments	EPA Response
	species, the commenter did not recommend any specific studies, regarding any particular endangered species, to be addressed more thoroughly. Further, published data indicates that endangered fish species have not been found to be more sensitive to toxicants than common species (Sappington et al 2001, and other). EPA acknowledges that there may be locations where a lower criterion could be applicable on a site specific basis due to the site-specific presence of a particularly sensitive species.
7. The slope of the response curve for selenium rates of deformities plotted against selenium concentrations in eggs/ovaries rises rapidly above the EC10 value. Therefore, use of the 10^{th} percentile as the measurement endpoint is scientifically defensible, appropriate and consistent with USEPA's assessment of toxicity of other compounds as well as the assessment of reproductive toxicity in other jurisdictions (BC MoE 2014).	Thank you for your comment.

2. Data used to derive the final chronic egg-ovary criterion element were differentiated based on the type of effect (reproductive vs. non-reproductive effects). Acceptable chronic toxicity data on fish reproductive effects are available for a total of nine fish genera. The genus Sensitivity Distribution (SD) is predominantly populated with data on fish genera because field evidence demonstrates that fish communities can be affected by selenium even when there is no observable change in the invertebrate community diversity and abundance. As a result, decades of aquatic toxicity research have focused primarily on fish. Available field and laboratory studies indicate that invertebrates are more tolerant to selenium than most of the tested fish species (Criteria document, Table 6c, Section 4.1.2). The data set used to derive the selenium criterion marks a change from the traditional method used to derive water quality criteria that requires toxicity tests with aquatic organisms from 8 phylogenetically distinct taxa (including three vertebrate and five invertebrate genera) in order to derive aquatic life criteria (Stephan et al., 1985).

2.a. Given selenium's more taxon-specific and life stage-specific toxicity, please comment on EPA's use of the available data to derive the egg-ovary tissue element.

External Peer Reviewers' Comments	EPA Response
1. Overall, I found EPA's use of the available data to derive the egg-ovary element to be scientifically sound. However, see caveats in b and c below. I did find EPA's use of the data for *Gambusia* to be questionable. Given the variability in the EO:WB ratio across species and the complete lack of data on this ratio for ovovivaprous fish, the EO-based threshold for this genus is highly questionable. Given this uncertainty and that these are the only data used in the WQC calculation in which EO Se was not directly measured, in my opinion, data for this genus should not be used in the WQC calculation.	EPA did not use *Gambusia* data directly to derive the selenium egg ovary criterion, given they are a viviparous fish. EPA did use these data to calculate the whole body criterion, as well as counted in the "N" for derivation of the egg-ovary criterion. The available data indicates that *Gambusia* is insensitive compared to other species in the database.
2. In as much as fish are the most vulnerable to Se toxicity, and it is manifested primarily at reproduction, the egg-ovary focus is justified. The availability of data that passed the EPA criteria is somewhat limited, but statistically valid. Having said this, I am not well-versed in fish toxicity literature, so I rely on the other reviewers to point out data sets that may have been overlooked	Thank you for your comment.

External Peer Reviewers' Comments	EPA Response
(e.g., I know they missed many water column data).	
3. I agree with the EPA's approach of only considering fish data in the genus sensitivity distribution as fish are the most sensitive aquatic taxa (although the sensitivity of amphibians relative to fish is still uncertain). There is a fundamental difference in a criterion that is based on an internal organism concentration versus an external environmental concentration (such as a water concentration). If fish are accepted to be the most sensitive taxa, and if selenium criteria are to be based on the selenium concentration in fish tissue (either eggs/ovaries or whole body), then the toxicity data and genus sensitivity distribution need to necessarily be based only on selenium concentrations in fish tissue. Development of a tissue-based genus sensitivity distribution that includes toxicity data for other taxa would not be relevant to the application of any criterion that could be derived using such an approach.	EPA has considered this comment as well as similar comments from other reviewers and the public. EPA agrees with the reviewer that the SD using fish egg-ovary data should only use the fish egg-ovary data. However, criteria are developed to protect the entire aquatic community, and the available data should reflect the sensitivity range for various components (e.g., fish, invertebrates) of the aquatic system. The invertebrate whole body data will be portrayed in the whole body SD in that it shows the relative sensitivity of invertebrates versus fish. EPA also identified limited data on amphibians and characterized effects in comparison to fish. Data indicate that the selenium fish tissue criterion is protective of the aquatic community including invertebrates and amphibians.
4. I have no particular insight on this issue.	Thank you for your comment.
5. This certainly makes the regulator's job easier due to the exquisite sensitivity of oviparous fish species to Se, and the well-established, characteristic and diagnostic response pattern in fishes (larval deformities and edema) that have clear links to population-level impacts. So yes, the egg/ovary tissue element is appropriate. However, it is important to note that we have limited data for all species, whether vertebrate or invertebrate. Recent work in David Buchwalter's lab at NC State U has observed a certain invertebrate taxon (Ephemeroptera I think) to be very sensitive to Se, and should be considered by EPA in the future criterion document. Nevertheless, in my opinion	Thank you for your comment. In responding to peer review and public comments, EPA examined Buchwalter's (Conley et al. 2013) recent study. There were 2 studies with the same exposures but different food rations (1x and 2x). There were major differences in the effects levels observed, which seemed to be a diet effect, rather than a selenium toxicity effect. These data are discussed in the revised draft selenium criterion document.

External Peer Reviewers' Comments	EPA Response
protecting fish based of an egg/ovary criterion will be protective of aquatic ecosystem sustainability. To my knowledge, the EPA has used a scientifically sound procedure to use available data on 9 fish species to derive the egg/ovary criterion.	
6. The use of fish data to drive the tiered criteria, and specifically the egg-ovary tissue element is fully justified and well-supported in the criterion document and the relevant scientific literature. While the sources, pathways, receptors and controls of chemicals impacting water quality have inherent diversity, selenium demonstrates significant trophic transfer potential and potential for fish reproductive effects in aquatic ecosystems. The reproductive endpoints observed in peer-reviewed, published controlled and field studies strongly suggest the potential for accumulation, magnification, and trophic transfer, and thus population level effects in a higher tropic level organism such as fish. The concomitant food-web impacts and observed impacts to aquatic birds support the criterion approach. The guidelines of Stephan et al., 1985 pre-date much of the knowledge base of Se in aquatic ecosystems, and the somewhat unique behavior and impact potential of this toxicant across trophic levels did not come into a more complete understanding for nearly two decades since that work. Hence, deviation from prior risk assessment approaches that pre-date our current knowledge base and the evolution of understanding of Se behavior in aquatic ecosystems is broadly justified in the risk management of selenium.	Thank you for your comment. While the Guidelines EPA uses to derive aquatic life criteria do not specifically address bioaccumulative pollutants like selenium, there are several aspects of criteria derivation which are similar to or an adaptation of the Guidelines. EPA followed the Guidelines and used the conceptual approach from EPA's Ecological Risk Assessment Paradigm in deriving the selenium criterion, 1. First EPA gathered all data on the toxic effects of selenium on aquatic organisms, as criteria are meant to protect the entire aquatic community. 2. Second, EPA recognized that the mode of action of selenium and its duality as a nutrient and a toxicant would require special consideration in the criteria derivation process. Given the available data, and deliberations from the 2009 Pellston on selenium, EPA was able to focus on toxicity tests that used maternal transfer of selenium via diet, and subsequent larval toxicity. 3. Using the SD methodology of the Guidelines, we assembled a fish egg-ovary SD based on EC10s for all available reliable studies. We are also including the invertebrate tests implicitly and available data on amphibians, although they were not represented in the SD.

<table>
<tr><th>External Peer Reviewers' Comments</th><th>EPA Response</th></tr>
<tr><td></td><td>4. We also used principles developed in an EPA White Paper reviewed by the SAB that provided for considering MOA and other characteristics of pollutants like Contaminants of Emerging Concern (CECs) (and to some extent selenium). (U.S. EPA 2008). We were able to apply these principles because we have specific information about the action of selenium in aquatic ecosystems established at a SETAC Pellston workshop (Chapman et al. 2009), specifically, that egg-laying vertebrates are the most sensitive to selenium, and that reproductive effects are the most sensitive measurement endpoint.</td></tr>
<tr><td>7. The use of reproductive effects in fish to derive the sensitivity distribution is appropriate because non-reproductive effects may arise from mechanisms that are not central to the primary ecological effects of selenium; reproductive toxicity in oviparous vertebrates manifested by maternal transfer of selenium to eggs. Additionally, as noted in the Draft Document, non-reproductive effects thresholds are highly variable and provide less confidence for deriving threshold values for selenium. The use of data from fish as the most sensitive organisms is appropriate and likely to be protective of invertebrates. However, it should be noted that sensitivity among invertebrates is highly variable and that some invertebrate taxa do exhibit sensitivity at low µg/L concentrations (see BC MoE 2014 for a review of this data).

While we agree that the Draft Document <u>predominantly uses</u> data from fish generally sensitivity, the approach in the Draft Document is not a complete departure from the principles surrounding the use of eight phylogenetically distinct taxa. The</td><td>Thank you for your comment.

EPA has considered this comment as well as similar comments from other reviewers and the public regarding the fish and invertebrate values. In the current draft document, fish and invertebrate SMCVs and GMCVs are no longer presented on the same tables, in agreement with the peer reviewer's comment, and invertebrate SMCVs and GMCVs are presented as whole body concentrations. However, criteria are developed to protect the entire aquatic community, such that the available data should reflect the sensitivity range for various components (e.g., fish, invertebrates) of the aquatic system. Towards this end, invertebrate sensitivity to selenium was evaluated in terms of both measured whole body concentrations as well as in terms of what the whole body tissue concentration of a representative fish would be were it to consume each invertebrate with a whole body concentration at the SMCV & GMCV, by multiplying each invertebrate GMCV by 1.27, the median TTF for all fish species. When evaluated with or without the trophic level</td></tr>
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External Peer Reviewers' Comments	EPA Response
US-EPA has attempted to increase taxonomic coverage of the sensitivity distribution by converting results from studies of three invertebrate taxa into fish reproductive endpoints. Specifically, threshold concentrations of selenium in the invertebrates were converted to predicted fish concentrations of selenium in egg/ovary based on consumption of the invertebrates by fish. These values were then included in the fish distribution (Figure 5, page 58). The variability inherent in this calculation is large because a generic trophic transfer factor of 1.27 was applied to convert invertebrate concentrations to fish whole body concentrations and then a generic conversion factor of 1.71 was applied to convert whole-body concentrations to egg/ovary. The result is a highly variable, and scientifically questionable, series of three additional data points that were added to the distribution of reproductive effects for fish.	biomagnification, the available data demonstrate that compared to fish, invertebrates are not sensitive to selenium, and do not comprise any of the four lowest GMCVs. Consistent with the 1985 Guidelines, they are used to fulfill the taxonomic minimum data requirements, and are counted in the total number of genera ("N") in the calculations. We note the numeric impact of increasing "N" though inclusion of the invertebrate data in the "N" for the criterion calculation is minimal, reflecting an approximately 3% change in the egg-ovary criterion element value.

2.b. Given the greater general sensitivity of oviparous fish to selenium compared to aquatic invertebrates, please comment on the appropriateness of EPA's fish tissue-based criterion for affording protection to the aquatic community as a whole (e.g., including invertebrates).

External Peer Reviewers' Comments	EPA Response
1. I agree with EPA that currently available data indicates oviparous fish are more sensitive than aquatic invertebrates to Se. However, it is important to note that there is a paucity of data for invertebrates. I agree with EPA's approach to translate available invertebrate data to an EO threshold for purposes of developing a species sensitivity distribution (SSD). However, I strongly disagree with the addition of 2 hypothetical crustaceans to the SSD. This is scientifically indefensible (just making up data) and the WQC calculation should be based only on taxa for which there are actually data available. By this logic, why add only 2 crustacean taxa, why not 3 or 5?	EPA agrees that the estimated egg-ovary concentrations for invertebrates should be deleted. The data available do indicate that the invertebrates are somewhat less sensitive to fish on a whole-body basis and thus invertebrates are included in the "N" for the criterion derivation. Invertebrates are portrayed explicitly in the whole body SD with the converted whole-body fish tissue concentrations for comparative purposes.
Note, EPA needs to include the data from Conley et al. (2011, 2013, and 2014) in its assessment of Se toxicity and trophic transfer to mayflies.	EPA examined Buchwalter's (Conley et al. 2013) recent study. There were 2 studies with the same exposures but different food rations (1x and 2x). There were major differences in the effects levels observed, which seemed to be a diet effect, rather than a selenium toxicity effect. These data are discussed in the revised draft selenium criterion document. EPA has also examined the Conley data and have included the data that has met the data quality requirements for quantitative consideration in the criteria derivation process.
Overall, given the limited data, I think EPA has overstated the certainty with which we can conclude fish are more sensitive than invertebrates. All we can really say is that based on a relatively small data set, available data suggests the tissue based WQC will be protective of invertebrates.	EPA agrees that, based on available reliable data, the draft selenium fish tissue criterion will be protective of invertebrates. EPA further agrees that additional data on invertebrate sensitivity to selenium would be useful to further support this conclusion.

External Peer Reviewers' Comments	EPA Response
2. In the aquatic systems in which I have worked with selenium, we have never encountered Se problems with invertebrates, and the literature seems to bear this out. So it seems to me that setting the criteria for the most at risk population is the best approach.	Thank you for your comment.
3. Although it has perhaps not been rigorously evaluated at all levels of food chain structure and function, field data indicates that adverse Se-related effects on fish can occur when there is no evidence of effects to food chain organism communities, including invertebrates. Selenium trophic transfer factors (TTFs) for invertebrates-to-fish typically average about 1 for whole body Se concentrations in fish and ≥2 for egg/ovary Se concentrations in fish (with the latter being more variable). Thus, a whole body Se criterion of 8.1 mg/kg dw and an egg/ovary Se criterion of 15.2 mg/kg dw may, on average, both be associated with an invertebrate Se concentration of about 8 mg/kg dw. Based on a review of Se toxicity to invertebrate taxa, deBruyn and Chapman (2007) identified two studies in which whole body invertebrate Se concentrations of <8 mg/kg dw were associated with adverse effects. Both of these studies were based on growth effects in larval midges (*Chironomus decorus*). deBruyn and Chapman (2007) reported an EC40 of 1.0 mg/kg dw from Alaimo et al. (1994) and an EC15 and EC46 of 2.6 and 4.1 mg/kg dw, respectively, from Malchow et al. (1995). However, in Alaimo et al. (1994), Se was below the detection limit in the treatment with a 40% reduction in growth relative to the control, which suggests the growth reduction was due to other factors. In Malchow et al. (1995), whole-body Se LOECs of 2.6 and 4.1	Thank you for your analysis and your comments.

External Peer Reviewers' Comments	EPA Response
mg/kg dw in midges were observed after 96-hr exposures. It is unclear whether growth effects would be related to tissue concentrations under such a short exposure period, but perhaps the water concentrations themselves (10 µg/L of either selenate or selenite) were directly responsible for the reduced growth. More recent data for a mayfly (*C. triangulifer*) suggest that the whole-body Se toxicity threshold for this species is also >8 mg/kg dw (Conley et al. 2009, 2011, 2013). Overall, in my opinion, the above provides support that a fish tissue-based Se criterion should ensure protection of the aquatic community as a whole, including invertebrates. Literature cited: Alaimo J, Ogle RS, Knight AW. 1994. Selenium uptake by larval *Chironomus decorus* from a *Ruppia maritima*-based benthic/detrital substrate. Arch Environ Contam Toxicol 27:441-448. Conley JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. Environ Sci Technol 43:7952-7957. Conley JM, Funk DH, Cariello NJ, Buchwalter DB. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. Ecotoxicology 20:1840-1851. Conley JM, Funk DH, Hesterberg DH, Hsu L-C, Kan J, Liu Y-T, Buchwalter DB. 2013. Bioconcentration and biotransformation of selenite versus selenate exposed periphyton and subsequent toxicity to the mayfly	

External Peer Reviewers' Comments	EPA Response
Centroptilum triangulifer. Environ Sci Technol 47:7965-7973. deBruyn AMH, Chapman PM. 2007. Selenium toxicity to invertebrates: Will proposed thresholds for toxicity to fish and birds also protect their prey? Environ Sci Technol 41:1766-1770. Malchow DE, Knight AW, Maier KJ. 1995. Bioaccumulation and toxicity of selenium in *Chironomus decorus* larvae fed a diet of seleniferous *Selenastrum capricornutum*. Arch Environ Contam Toxicol 29:104-109.	
4. Until we find more Se-sensitive groups of freshwater animals than fish, the fish tissue-burden approach seems warranted.	Thank you for your comment.
5. See previous comment regarding aquatic insects. In my opinion the tissue-based criteria in fish will protect freshwater aquatic communities.	Thank you for your comment.
6. The fish tissue-based criterion affords protection to the aquatic community as a whole and is appropriately placed in the tiered criterion. Since tissue Se integrates chronic and intermittent acute aquatic Se exposure, it provides a good quality indicator of impacts and potential impacts to the broader aquatic community. The complex interactions of predator-prey relationships in these environments rely on nominal stability in each tropic level and the food-web as a whole. In field practice and in published controlled studies, fish tissue Se has been shown to provide a valuable assessment and management tool for Se impacted aquatic ecosystems. Except where fish populations are absent, very low, endangered or otherwise	Thank you for your comment.

External Peer Reviewers' Comments	EPA Response
insufficient, tissue monitoring is a high quality indicator of water quality with regards to selenium.	
7. As noted above, the use of data from oviparous fish as the most sensitive aquatic organisms to derive criterion is appropriate and likely to be protective of invertebrates. However, the USEPA may wish to consider sensitivity data for some invertebrate taxa that do exhibit sensitivity at low µg/L concentrations (see BC MoE 2014 for a review of this data).	Thank you for your comment. EPA is aware of the available invertebrate data that reported selenium impacts at low concentrations, however our review of these data uncovered data quality flaws that limit the quantitative use of these studies for criteria derivation. This discussion is located in Section 3.1.3.

2.c. With respect to the tests that quantified non-reproductive effects, did the EPA use that data to the best extent possible given its limitations (e.g., relevance compared to reproductive tests, and data quality concerns which increased uncertainty (e.g., Hamilton et al., 1990)?

External Peer Reviewers' Comments	EPA Response
1. I agree with EPA, that generally, the reproductive endpoint is more sensitive than other endpoints such as juvenile growth. However, in the case of salmonids, there is at least some evidence (e.g., Hamilton et al., 1990) that juvenile growth is comparable in sensitivity to reproduction. It is also worth pointing out that these studies did not include pre-exposure of the parents and subsequent maternal transfer, so it is possible that exposure and subsequent effects on juvenile growth have been underestimated. Further, juvenile salmonids have a much more limited home range and potentially higher intensity of exposure if they rear in Se contaminated areas compared to adult salmonids (particularly migratory species). Given this, it is unclear to me that placing primacy on the egg-ovary element will necessarily be protective of these species. EPA should consider the potential that juvenile whole body Se concentrations for migratory salmonids may need primacy or at least concurrent compliance monitoring to ensure the protection of these important species.	EPA had significant concerns with the 90-day endpoint in Hamilton et.al. 1990, most significantly that the 90-day control survival (67%) was below toxicity test acceptability thresholds, such that we could not use those effects data. We did consider and use the 60-day time point for this study, and concluded that the egg-ovary transformed whole body criteria of 8 mg/kg dw would protect against growth effects in juvenile salmonids. EPA does agree that salmonids, particularly anadromous Pacific salmonid species are not represented by the dietary maternal transfer model upon which the egg-ovary criterion element is based for several reasons: Salmonids migrate as smolts to the marine environment mature and then migrate back to reproduce, however, they cease feeding before entering the freshwater environment resulting in a lack of exposure to these contaminants in freshwater. Also, these species are semelparous, meaning they die after spawning, so there is no opportunity for selenium to be stored. This is different than other salmonid species that are freshwater species (e.g., brown trout) and do not die after spawning. Since the maturing juvenile to the smolt stage is the critical lifestage of interest for these species, monitoring of whole body concentrations in smolts using the whole body criterion element threshold of 8 mg/kg dw is expected to insure protection of this critical life stage from impacts associated with dietary selenium. EPA also completed further analyses of this issue in response to this peer

External Peer Reviewers' Comments	EPA Response
	review comment. These analyses are presented in Section 6 of the 2015 draft criterion document and conclude that the whole body criterion element should be used in combination with smolt-stage fish from species of anadromous Pacific salmon to ensure their protection from selenium exposure in freshwater. Thus, EPA's selenium criterion is protective of these species if applied as recommended.
2. Again, fish toxicity is not my expertise, so I cannot adequately respond to this question.	Thank you for your comment.
3. Overall, I generally agree with the EPA's interpretation of the non-reproductive effects data and the draft whole-body Se criterion appears to be protective of the toxicity endpoints evaluated in those studies (at least the GMCVs reported in Table 17 of the draft AWQC document certainly are). The one study that could be interpreted somewhat differently is the juvenile Chinook salmon study conducted by Hamilton et al. (1990). The EPA derived whole-body Se EC10s of 7.355 and 11.14 mg/kg dw for juvenile growth based on a seleno-DL-methionine spiked diet and San Luis Drain (SLD)-spiked diet. For comparison, DeForest and Adams (2011) had derived a whole-body Se EC10 of 6.4 mg/kg dw based on the seleno-DL-methionine spiked diet, using a different concentration-response model (they excluded the SLD-spiked diet due to concerns associated with other contaminants). Overall, the model fit by the EPA to the data using TRAP appears to be quite good and the greater EC10 that they derived based on SLD-diet provides support that other contaminants did not adversely affect growth in the juvenile Chinook. Accordingly, I do not disagree with the SMCV (and GMCV) of 9.052 mg/kg dw that the EPA derived from juvenile	Thank you for your comment and analysis. See comment to reviewer number 1 above.

External Peer Reviewers' Comments	EPA Response
Chinook salmon. This would also support that the draft whole-body Se criterion of 8.1 mg/kg dw based on reproductive effects would be protective against growth effects in juvenile Chinook. Literature cited: DeForest DK, Adams WJ. 2011. Selenium accumulation and toxicity in freshwater fishes. 193-229 in Beyer WN, Meador JP, eds. Environmental contaminants in biota: Interpreting tissue concentrations Second edition. CRC Press, Boca Raton, FL, USA. Hamilton SJ, Buhl KJ, Faerber NL, Wiedmeyer RH, Bullard FA. 1990. Toxicity of organic selenium in the diet to chinook salmon. Environ Toxicol Chem 9:347-358.	
4. I'm not sure.	Thank you for your comment.
5. Since the non-reproductive effects occur at tissue [Se] equal to or more commonly greater than reproductive effects, and since reproductive effects have clearer links to population-level impacts than non-reproductive effects such as reduced growth or altered behavior, the EPA has appropriately chosen not to use non-reproductive effects in their derivation of tissue-based criteria.	Thank you for your comment.
6. The non-reproductive fish data, limited in scope and diversity, were adequately explored and treated in the development of the tiered criterion. The increased concerns over reproductive effects from a risk management perspective, study diversity (e.g., species, geography, lentic/lotic), in addition to the quality and quantity of reproductive toxicity endpoint data and studies reproductive toxic risk the superior driver of selenium	Thank you for your comment. We have added a figure to Section 6 where the reproductive effects and non-reproductive effects are compared, demonstrating that the whole body criterion element translated from egg ovary criterion threshold is expected to be protective against non-reproductive effects.

External Peer Reviewers' Comments	EPA Response
risk management in aquatic ecosystems. The summary statement that the non-reproductive data were less reproducible (p. 57) suggests that including them would have added uncertainty to the final criterion values. It is reasonable, acceptable, and scientifically defensible to have reproductive toxicity as the driving endpoint for criterion development, as these criteria appear to afford protection from non-reproductive toxic effects.	
7. Because non reproductive tests do not evaluate the most sensitive measure of selenium ecotoxicology, their use as regulatory criteria are questionable. However, the USEPA has provided summaries of non-reproductive tests and compared the results from these studies with the criterion derived using reproductive data. In most cases, the studies have evaluated growth or survival of fish. The species mean chronic values (SMCV) and genus mean chronic values (GMCV) from the non-reproductive tests are generally greater than the egg/ovary criterion and, therefore, it is expected that the criteria derived from the reproductive studies (e.g., Egg/ovary) will be protective of non-reproductive endpoints as well.	Thank you for your comment. EPA has provided additional description in the 2015 draft criterion document by developing and displaying sensitivity distributions with both the egg-ovary (reproductive) translated whole body values, as well as the whole body values from non-reproductive tests to show the relationship between and demonstrate the protectiveness of the whole body translated criterion element.

2.d. EPA also rejected studies that used the injection route of exposure for selenium due to uncertainty related to uptake, distribution and metabolism/transformation kinetics when compared with the dietary and/or maternal transfer routes of exposure. Was this reasonable? Does the panel envision an appropriate and scientifically defensible use for this type of data? Please provide detailed comments.

External Peer Reviewers' Comments	EPA Response
1. Yes, it was reasonable to reject these studies for the reasons stated by EPA. In my opinion, there is currently insufficient information to have confidence that injection studies replicate realistic environmental exposures with respect to Se homeostasis. Indeed, the fact that the catfish study resulted in such an unusually low effect level suggests there may be different processes occurring in these types of studies. EPA has adequately documented that catfish do not appear to be uniquely sensitive based on available field abundance data in Se-impacted systems, counter to the lab-based injection study.	Thank you for your comment.
2. I cannot recommend using any artificial means of introducing selenium to tissues; exposure must be through food and the assimilation pathways it follows for a given species. In this respect, chemical speciation is very important, so the exact form of organic selenide (peptide vs free amino acid, seleno methionine vs seleno cysteine; cytosol vs proteins) is critical to its uptake and eventual assimilation (e.g., Reinfelder and Fisher, 1994; Luoma et al., 1992).	Thank you for your comment.
3. In my opinion it was reasonable to exclude microinjection studies because there are sufficient questions as the environmental relevance of the exposure. For example, Linville (2006) exposed white sturgeon larvae to selenium using two different approaches: (1) by microinjection of L-selenomethionine into larval yolk sacs immediately after	Thank you for your comment.

External Peer Reviewers' Comments	EPA Response
hatching and (2) by exposing parent females to dietary selenium (as selenized yeast) for up to six months before they deposited eggs (i.e., maternal transfer exposure). In larvae that received L-selenomethionine microinjections, mortality was a more sensitive endpoint than developmental- related effects. In contrast, in the maternal transfer test, larval developmental effects was a more sensitive endpoint than larval mortality. Further the egg Se EC10 for white sturgeon was 15.8 mg/kg dw in the maternal transfer study versus 6.77 mg/kg dw in the microinjection study (as derived by Beckon [2012]). The microinjection methodology has not been validated in other studies and the results from Linville (2006) suggest that it is not an appropriate substitute for maternal transfer. Further, to my knowledge, studies on injection of Se into muscle tissues and subsequent maternal transfer of Se to the ovaries and eggs, and comparison to maternal transfer data following dietary Se exposures, have not been conducted. (Although the data from Linville [2006] are sufficient to make some comparisons between maternal transfer and microinjection studies, the concentration-response data are too limited to derive an EC10 that would be considered reliable in a sensitivity distribution for criteria development. Further, the egg Se EC10 from the maternal transfer test was estimated from the larval Se EC10 using a regression relationship between egg and larval Se concentrations from a microinjection test.) Literature cited: Beckon WN. 2012. Evaluation of the toxicity of selenium to white and green sturgeon. U.S. Fish and Wildlife Service, Sacramento, CA. Linville RG. 2006. Effects of excess selenium on the health and	EPA concluded that the white sturgeon EC10 of 16.3 mg/kg for the combined edema + skeletal deformity endpoint from the Linville 2006 maternal transfer study were of sufficient quality to include in the criterion document. The test fulfilled Guidelines requirements, larval control survival was acceptable, and the highest test concentration indicated evidence of reproductive effects. While an EC_{10} based on one partial response would not ordinarily be included in the chronic data set, special consideration was given to these results for the following reasons: 1) White sturgeon is listed as endangered in specific regions, such as the Kootenai River white sturgeon in Idaho and Montana; 2) White sturgeon serves as a surrogate for other sturgeon listed as threatened or endangered (e.g., pallid and shovelnose sturgeon); 3) Linville 2006 reported a statistically significant relationship between selenium concentrations in larvae and occurrence of skeletal + edema effect incidence, and calculated a similar EC10 using a logit model; and 4) there are supporting data (De Riu et al. 2014, Appendix E) that suggest the federally threatened green sturgeon is also sensitive to selenium.

External Peer Reviewers' Comments	EPA Response
reproduction of white sturgeon (*Acipenser transmontanus*): Implications for San Francisco Bay-delta. Ph.D. Thesis, University of California, Davis. 232 pp.	
4. It is hard to argue on behalf of egg injection studies in favor of dietary uptake (the obviously more natural process) studies. This is particularly the case if the Se contents of the tissues and eggs are measured during the dietary exposure.	Thank you for your comment.
5. I think the EPA should use studies that use maternal injection of Se as the route of exposure (e.g., the Doroshov et al. (1992) study in catfish). Whether Se is absorbed from the gut or injected into adult female fish, it will reach the systemic circulation and become part of the Se pool, some of which will be incorporated into vitellogenin in the liver and transported/deposited into eggs. Including the Doroshov et al. (1992) study is thus scientifically sound, and will add an additional fish taxon (ictalurids) into the species sensitivity distribution.	EPA has not previously accepted injection studies as a valid exposure method for aquatic life criteria development, and this exposure route is considered particularly critically regarding selenium for several reasons. 1. A 100% selenomethionine exposure does not reflect natural dietary sources. 2. Microinjection does not include the natural metabolic detoxification and storage processes occurring in the female over time in the diet, as opposed to a bolus dose of a single form of selenium which likely overwhelms the body's metabolic processes. We note that most other reviewers agreed with the exclusion of the injection route of exposure, and several provided additional lines of reasoning for excluding injection studies from consideration. In addition, in the effects characterization EPA describes field evidence from Hyco Reservoir that found catfish representing multiple year classes present even after most other fish species were reproductively extirpated from the lake. This indicates that

External Peer Reviewers' Comments	EPA Response
	at a minimum, catfish are no more sensitive than other species for which we have reliable egg-ovary data for (i.e., centrarchids like bass and bluegill), and that they are likely less sensitive, due to their presence in these studies after other species disappeared. Thus, EPA concluded that the egg-ovary criterion is expected to be protective for ictalurids, despite the absence of valid egg-ovary test data.
6. The rejection of injection exposure route studies is reasonable. Injection based toxicology studies have their place in understanding the interface of chemistry and biology. They are of significant value when metabolism of the toxicant is of interest or when digestive and absorption processes (i.e., bioavailability) confound or complicate study goals. Since controlled feed/water laboratory exposure trials, and field observation data and published studies are available in overall sufficient quantity, diversity, and quality for establishment of the criterion, the rejection of injection-based trials results yield a data set more amenable to generalization of aquatic ecosystem exposure and dose, as well as the subsequent analysis of trophic transfer and potential for toxic end effects. Although injection route studies have scientific value, they are not necessary or required for a qualitative and quantitative understanding of Se aquatic ecosystem risk potential given the other peer-reviewed resources presently available.	Thank you for your comment.
7. The US-EPA rejected the Doroshov et al. (1992) study in which female catfish were injected intramuscularly with seleno-methionine and effects were determined in their offspring. The chemical form of selenium was appropriate for injection into these fish, but it could be argued that injection circumvents	EPA has reexamined the study data and determined that it will not accept injection studies as a valid exposure method for selenium aquatic life criterion development. The majority of the other peer reviewers' comments support this decision. Please see EPA comments to reviewer 5 above for more detail.

External Peer Reviewers' Comments	EPA Response
dietary uptake, tissue partitioning and timing of muscular uptake with respect to reproductive cycle of the fish. Some may therefore consider this injection study to be invalid. However, relating selenium concentrations in egg/ovary to reproductive effects was the primary focus of the USEPA's assessment. While several compromises have been established to allow data to be included in the development of the criterion (see discussion of the bluegill and brown trout data from earlier comments), the exclusion of the data from the Doroshov et al. (1992) study appears arbitrary. Moreover, citing abundance of Ictalurids in the Hyco Reservoir (Crutchfield (2000) and at Belews Lake (Young et al. 2010) at selenium concentrations that may have affected abundance of other fish species is not sufficient evidence to dismiss the data from the Doroshov et al. (1992) study. A reexamination of the data and consideration to include them in the egg/ovary criterion is warranted.	

3. Was the method (Section 4.1.5, 7.1.7) used to translate the fish egg-ovary criterion element into muscle and whole body criterions elements understandable, transparent and scientifically defensible? Was there sufficient data for making the translations for each element?

External Peer Reviewers' Comments	**EPA Response**
1. Yes, I found the egg-ovary to muscle and whole body translations to be understandable and scientifically defensible.	Thank you for your comment.
2. The methodology is well described and documented, but as above I would like to see a more thorough error analysis for the resulting CFs.	Thank you for your comment. We have expanded the discussion and clarified the derivation of the CFs and modified the methodology to leverage more of the taxonomic relationships available in the dataset. See response to Peer Reviewer 2, in Part I, Question 2.a.iii.
3. In general, I am hesitant about considering tissue-to-tissue Se relationships in order to estimate toxicity thresholds for one tissue based on measured concentrations in another tissue. However, the "EO/WB" ratios shown in Table 7a appear bracket the ratios typically observed, while still reflecting the variability observed between different species and families. The resulting draft whole-body Se criterion of 8.1 mg/kg dw is not inconsistent with other whole-body fish Se guidelines that have been recommended based on direct whole-body Se measurements. DeForest and Adams (2011), for example, recommended a whole-body fish Se guideline of 8.1 mg/kg dw following a different approach. However, per my above comment, I believe that the number of GMCVs should be 11 rather than 14 (or 12 if a recently conducted study for mountain whitefish were added to the sensitivity distribution. In addition, for those species with measured Se concentrations in whole-body tissue or muscle, why not use the empirical	Regarding the comment on the number of GMCVs, EPA agrees that the invertebrate and the *Gambusia* data should not be represented in the egg-ovary sensitivity distribution, and they have been removed from that distribution. EPA 304(a) criteria are developed to protect the entire aquatic community, and so the "N" should consider the other less sensitive taxa data implicitly; even though they are not represented in the SD, they are still protected by the criterion. We also evaluated new studies, and so the "N" reflects the number of studies that are used quantitatively, and represented explicitly in the SD; as well as those less sensitive taxa that cannot be represented in the SD, but are part of the total number of taxa where data are available that show that the criterion is protective.

measurements? For example, for Dolly Varden, McDonald et al. (2010) reported a whole body Se EC10 of 44 mg/kg dw based on the site-specific relationship between egg and WB Se in their study (this would not influence the draft whole-body Se criterion because *Salvelinus* is not among the four most sensitive genera, but it would be more accurate). Likewise, Coyle et al. (1993) and Hermanutz et al. (1992, 1996) report whole body Se concentrations in bluegills. This could be checked for other species as well. Finally, perhaps it should be noted that, if possible or desired, site- and species-specific relationships between egg/ovary Se and whole-body or muscle Se could be derived and used in place of the draft criteria of 8.1 and 11.8 mg/kg dw. Literature cited: Coyle JJ, Buckler DR, Ingersoll CG, Fairchild JF, May TW. 1993. Effect of dietary selenium on the reproductive success of bluegills (*Lepomis macrochirus*). Environ Toxicol Chem 12:551-565. DeForest DK, Adams WJ. 2011. Selenium accumulation and toxicity in freshwater fishes. 193-229 in Beyer WN, Meador JP, eds Environmental contaminants in biota: Interpreting tissue concentrations Second edition. CRC Press, Boca Raton, FL, USA. Hermanutz RO, Allen KN, Roush TH, Hedtke SF. 1992. Effects of elevated selenium concentrations on bluegills (*Lepomis macrochirus*) in outdoor experimental streams. Environ Toxicol Chem 11:217-224. Hermanutz RO, Allen KN, Detenbeck NE, Stephan CE. 1996. Exposure of bluegills (*Lepomis macrochirus*) to selenium in outdoor experimental streams. U.S. Environmental	(See responses to questions 2.a.6. and 2.b.1 above for further discussion.) Section 6.1.11 includes a figure showing the distribution of egg-ovary GMCVs converted to whole body concentrations overlaid with the distribution of GMCVs using empirical whole body measurements, plus those converted from muscle to whole body. Both overlays include invertebrate GMCVs. EPA supports the development of site-specific criteria, where data are available and has specifically designed this criterion so that its modification from a national criterion to a site-specific value, using site-specific data, could be easily facilitated. The discussion on how to develop site specific criteria is presented in Appendix K

Protection Agency, Duluth, MN, USA. McDonald BG, deBruyn AMH, Elphick JRF, Davies M, Bustard D, Chapman PM. 2010. Developmental toxicity of selenium to Dolly Varden char (*Salvelinus malma*). Environ Toxicol Chem 29:2800-2805.	
4. It seemed reasonably clear to me.	Thank you for your comment.
5. The EPA used an appropriate approach to translate the egg/ovary element to whole-body and muscle elements. Unfortunately, data are limited to few fish species. As discussed above in 2a(iii), conversion ratios vary by about two-fold for both EO:WB and EO:M. In addition, within-species ratios may vary throughout the year. These aspects all create uncertainty, but these are the data we have and this is the best approach. It is suggested that as more studies measure [Se] in egg/ovary, whole-body and muscle, that these data be used to update criteria through time. One thing that was not clear. In certain cases it appears that [Se] in egg/ovary and whole-body were determined in the same fish. If eggs were removed for [Se] determination prior to determination of whole-body [Se], then how did the removal of eggs influence the whole-body [Se]? Was the absolute quantity of Se removed by subsampling eggs added back into the whole-body quantity, and was the mass of eggs removed added back to the whole-body?	Thank you for your comment. The majority of the data for the E/O to whole body [Se] relationship analysis came from Osmundson et al (2007) who did have egg data and whole body [Se] data from the same fish. The whole body [Se] was calculated by adding back the egg Se that was removed for analysis. Osmundson et al (2007) had 9 of the 10 species in EPA's data set for this analysis. Coyle et al (1993) also added back egg Se for the whole body same fish comparison. Formation (2011) and Doroshov et al. did not specify how the whole body [Se] was determined. Hermanutz (1996) and Hardy (2005) apparently measured whole body and egg Se in different fish with the same exposure. EPA has added clarifying discussion to the section discussing fish tissue relationships.
6. The approach and method of translating the fish egg-ovary criterion into muscle/whole body is transparent and broadly scientifically defensible, and there appears to be sufficient data to make the translation. Although there is some variability in the	Thank you for your comment. The Figure 5 (now Figure 3.1) that the comment refers to has been simplified to eliminate the problem of needing to refer to

calculated results of whole body and muscle calculations, the relative consistency across taxon gives significant support to the modeling approach and in the data used to derive the values. The Figure 5 references to Table 10 and 11 should be introduced and explained in the body text prior to using them in a Figure caption since the reader has not seen that data. Some editing in this regard would improve clarity and help the reader understand and follow the approach. The body text of paragraph 1 of page 59 needs to be rewritten for clarity; statements of "it can be seen" assume much and explain little. Because the paragraph references a subsequent Section 4.2, editing page 59 to introduce and summarize the detail of 4.2 would be an improvement in clarity for the reader. Table 7a and 8a would be improved with units (mg Se/kg DW) for tissue concentrations. Footnotes on these important tables cross-referencing the specific source, table or appendix where the data originated would be helpful and aid in reader understanding and transparency.	material described later in the document.
7. The methods used to translate egg/ovary to muscle and whole body criteria are understandable and transparent, but as we noted in our earlier comments, there are scientific issues with some of the transformations. The USEPA attempts to use matched pairs of muscle and whole body concentrations of selenium for each species, but only a few fish species provided data for directly assessing the conversion (Page 78). As a result, US-EPA used the median conversion value for all species (i.e., 1.27) to convert muscle selenium to whole body concentrations where species specific data were not available. Continued use of this generic ratio would be expected to introduce additional variability and uncertainty, particularly for the conversion from egg/ovary to whole body because in many cases this requires a two step conversion (i.e., from egg/ovary to muscle and then from muscle	The EPA agrees with the reviewer comment and has revised its approach in the 2015 draft criterion document accordingly and now uses species-specific muscle-to-whole body conversion factors where data are available. Where species-specific data are not available, the EPA uses the same taxonomic classification procedure for the CF as used to estimate TTF values for species without data. (See response to Peer Reviewer 2 above, in Part I, Question 2.a.iii.) Section 3.2.2 of the 2015 draft criterion document provides a complete description of this procedure.

to whole body). More specifically, almost half (i.e., 7 of 16) of the Conversion Factor (CF) values for egg/ovary to whole body were derived by including the generic muscle to whole body conversion ratio. The issue is less important for conversion of egg/ovary to the muscle criteria because for most species (other than desert pupfish) there were data available to calculate the conversion directly.	

PART III: EVALUATION OF THE TRANSLATION PROCEDURE TO DERIVE THE WATER COLUMN ELEMENT(S)

EPA is also requesting a technical review of the methods and procedures used to translate the egg-ovary element of the chronic selenium criterion to water-column elements. Relevant sections of the document include:

- A description of the method used to derive an equation to translate the egg-ovary element to a monthly water-column element in perennial (lentic and lotic) waters and an equation that can be used to convert the monthly water-column element to an intermittent water column element (Sections 3.8.3, 3.8.4, 4.2.1, 4.3, and Appendix G).
- An analysis of the translation equation precision using data obtained from published literature (Sections 7.2.1, 7.2.2, and Appendix H).
- A description of the method and data sources used to derive the translation equation parameters (Sections 4.2.2, 4.2.3, and Appendix B).
- A description of the method and data sources used to categorize waterbody types where a single water-column chronic criterion concentration value would be adequately protective in most circumstances (Section 4.2.4).
- A description of the method and data sources used to derive water-column chronic criterion concentration values for established categories of waters (Section 4.2.5).
- A description of the method and data sources used to derive water-column chronic criterion concentration values for intermittent discharges that may occur in lentic and lotic waterbodies (Section 4.3).

Please address the following questions:

1. Please comment on the scientific defensibility of EPA's translation equation method for translating the concentration of selenium in fish tissue to a concentration of selenium in the water-column. Please comment on major sources of uncertainty in applying the translation equation to different types of waterbodies (e.g., with differing retention times, water chemistries, and/or species present). Are there other data sources, models, or approaches that EPA should consider that would reduce uncertainty? Please provide detailed comments.

External Peer Reviewers' Comments	EPA Response
1. I appreciate that EPA is dealing with a very difficult issue in terms of translating a tissue-based criteria to water for routine monitoring and screening purposes. I agree with the general conceptual model EPA has developed for making this	EPA has used all available data in developing a series of site specific food chain models in order to derive the national criteria. Although there is some uncertainty in the TTFs and CFs due to a paucity of species specific data, the EPA believes

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translation. Having said that, the details of how EPA has implemented this conceptual model I think are very problematic. My concerns center on two major themes – compounding multiple uncertain values in the food chain transfer models and lack of transparency on what level of protection the proposed water elements provide. I am very concerned that EPA is placing too much value on extrapolated and modeled values. The translation approach involves building food chain models for 69 sites that in many cases have significant data gaps (e.g., dietary composition, extrapolated TTFs, extrapolated CFs, etc.). To address these uncertainties, EPA developed a series of protocols for filling in the data gaps (e.g., using TTFs for species in the same order).While I appreciate the logic and largely agree with these protocols, ultimately, information derived in this manner is not measured data. This approximated information is then used in a very quantitative manner for setting the water-based WQC. Figure 11 in particular I find very misleading. How many of the data points in those two distributions (lotic and lentic) are based on sites where all parameters in the food chain models were actually measured? I did not take the time to calculate this, but EPA must explicitly provide this information. I suspect the percentage will be quite low. What do these distributions in Figure 11 look like if based on only studies where all parameters were directly measured? In my view, use of such data provides a potentially very inaccurate picture of what we actually know about the distribution of waterborne Se concentrations associated with the tissue-based WQC. This seems to be a significant departure from previous WQC criteria derivation processes where if data for a particular study were insufficient, the study was simply excluded and the resulting uncertainty	using taxonomically related surrogates provides the most scientifically defensible approach since the range of variability across these parameters is typically small compared to sources of variability in other aspects of criteria development (e.g., range in sensitivity across species in a given genus for a particular toxicant). See response to Peer Reviewer 2 above, in Part I, Question 2.a.iii. The reviewer would like a better accounting of the uncertainty associated with each step in the translation process. Unfortunately, most of the studies that provide the data used in the translation do not provide enough information to quantitatively estimate the associated uncertainty. The approach EPA uses to translate the egg-ovary criterion element into water-column measurements represents the best available science, data and information on selenium bioaccumulation at the present time. EPA has used a peer reviewed approach shown in Presser and Luoma (2010). It allows for use of the available data in the most scientifically defensible manner possible. The kinetic analyses recommended in the comment would be impossible to complete nationally because sufficient data do not exist to allow for of a rate-based dynamic model using speciated selenium data. As discussed in response to comment #2 below, EPA believes that confining its national assessment to a very small number of well-studied sites and to laboratory studies where such measurements are available would reduce rather than increase confidence in the appropriateness of the national criterion.

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from having relatively few complete data sets was reflected in a lower WQC (e.g., a WQC less than the most sensitive taxa tested if n<20). An important element of previous WQC was transparency in the level of protection being provided (e.g., 95 % of taxa) and the assumptions underlying that protection (e.g., that tested taxa were representative of aquatic communities in the US). It is entirely unclear to me what level of protection is being provided by the water element of proposed WQC. The proposed water-based WQC is based on the 20th percentile for lotic and lentic sites that were modeled (see concerns about this in the previous paragraph). But even this is not correct, because for some sites, multiple fish species were modeled per site. This raises numerous questions regarding independence of values in the distribution, whether the sites evaluated are biased towards those with known Se issues, etc. EPA has also not made it clear why protection of 80% of sites is a desirable regulatory objective. Why not 70%, 90%, or 95%? I appreciate that EPA has undertaken a ground truthing exercise to evaluate the proposed water element WQC. However, it is unclear exactly how EPA undertook this analysis. Were there truly over 3,000 independent sites that EPA evaluated? If this exercise concluded that <10% of sites would result in false negatives, then what does this say about the representativeness of the 69 sites and what is the real level of protection being provided? The overall approach of considering selenium's pathway from the water column, dissolved state, through trophic levels, and into tissues such as reproductive organs is well justified, particularly the trophic transfer model that is dynamic and rate/kinetically based (uptake rate * assimilation efficiency/elimination rate); the trophic transfer approach largely developed by Nick Fisher and collaborators. However, the water to first trophic level approach	Nevertheless, where sufficient site-specific data are available to support rate-based estimates of uptake of separate selenium species, interested parties could use those data to develop a site-specific criterion. Regarding the reviewer's comment about the data points in Figure 11 being based on estimated values, that figure is clearly labeled as model results. There are not sufficient data at any single site to derive all the necessary input values to translate through all trophic levels to the water value only using measurements taken at a particular site. Nevertheless, EPA did require that fish be measured at the site and that the EF value be measured at the site. Nationally derived TTFs and CFs were then applied to the site translation. Because Chapman et al. (2010) contains numerous examples of (a) EFs and TTFs are not derived from rate data, (b) water concentrations expressed as total dissolved selenium, EPA does not agree that the Pellston Workshop calls upon EPA to restrict itself to the commenter's recommendation to use only kinetically based EFs and TTFs and to use only separately measured concentrations of selenite and selenate. EPA believes that the tiered structure of its selenium criterion, with the range of site-specific options that it offers, is fully consistent with the recommendations of the Workshop. EPA has addressed the relationship between its approach and the kinetic approach with selenite and selenate in the response to the

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is completely unacceptable in that it is not dynamic or rate-based (actually assumes equilibrium) and completely ignores the effects of speciation. The latter is curious in that they seem to be relying on the Chapman et al (2009 and 2010) recommendations from the SETAC Pellston workshop which specifically states, "Understanding Se speciation is critical to understanding its mobility, transformation, partitioning in the environment, and potential risk to aquatic ecosystems." and "The single largest step in the bioaccumulation of Se occurs at the base of food webs, characterized by an "enrichment function"; thermodynamic or equilibrium-based principles are not appropriate for predicting Se bioaccumulation at the base of food webs." The choice of the Presser and Luoma model used in this EPA document is completely contrary to these recommendations since the water/particle ratio called the Enrichment Factor (EF) is only a renamed equilibrium distribution coefficient (K_d) that was used long ago for metal cations. Dissolved and particulate selenium speciation cannot be modeled with equilibrium approaches, it must consider the kinetics of the transfers/transformations (e.g., Cutter, 1992). Since the transfer of dissolved selenium in any of its chemical forms to the particulate state (largely assimilation by phytoplankton and conversion to organic selenide – seleno amino acids in proteins) changes the chemical forms, how does one calculate a distribution coefficient (EF)? For selenium, dissolved selenite or selenate are not what are in the particulate state (organic selenides), so which dissolved species and which particulate species do you use to calculate EF? And, they are certainly not reversible (selenite uptake followed by regeneration does not return selenite, but rather organic selenide…which may later oxidize back to selenite and selenate;	comment immediately below. It can be seen that under steady-state conditions, the equations of EPA's approach with dissolved selenium can be derived from the equations of the kinetic approach with separate selenite and selenate. The first advantage of EPA's approach is that it allows far more site data to be brought to bear in the derivation. EPA believes that the variability in EF values across sites far exceeds any uptake differences attributable to the ratio of selenite to selenate across sites. That is, the selenite/selenate ratio is only one factor that should not be focused on in a way causes the assessment to lose sight of the larger body of site data. Furthermore, site EFs represent the composite uptake of all TL1 species at the site, and are not reliably captured by measurements of a single TL1 species in the lab. The second advantage of EPA's approach is that it produces a criterion that applicable to the selenium water measurements commonly available across the U.S. Regarding the comment's question of whether there were "truly over 3,000 independent sites:" There were over 3000 whole-body fish tissue measurements that could be coupled with water concentrations measured. The 3000 measurements include same-site values of (a) different species measured at the same time, (b) the same species measured at different times, and (c) different species measured at different times.

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Cutter, 1982; Cutter and Bruland, 1984). In this EPA document, they "solve" this issue by only considering total dissolved selenium, in contradiction to the recommendations at the Pellston workshop.	With regard to the relationship between EPA's approach and other facets of more complex approaches, in addition to the response to Reviewer 2 immediately below, please see also the response to the comment in Part III, Question 2, Reviewer 3, which addresses the issue of the model's linearity. Regarding the comment's issue with use of the 20th percentile translated water value, the level of protection is determined by the egg-ovary criterion element. The water-column element is a translation of the egg-ovary element and thus is intended to provide the same level of protection as the egg-ovary element. However, some level of uncertainty in translating the egg-ovary element to the water-column element is unavoidable. To address this uncertainty, the EPA chose the 20th percentile of translated water-column values using the most bioaccumulative food web present at each site to select a protective national water column criterion element. Limitations of available data only allowed translation of the egg-ovary criterion element at 69 unique aquatic sites. The 20th %tile was selected because it results in a low probability of false negatives, (i.e., low probability of failure to indicate exceedance of the egg-ovary criterion element via the water column element.) In response to the selenium chemistry comments, EPA has modified the selenium chemistry section to reflect the uptake and transformation of selenium in the aquatic food web. Those changes can be found in Section 2.

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2. The use of the Presser and Luoma (2006, 2010) model for any aquatic ecosystem to predict dissolved or particulate concentrations is questionable for the simple reason that while it acknowledges the importance of chemical speciation, and the rates of processes (kinetics as opposed to equilibrium thermodynamics), it largely ignores them in application. It is a totally empirical model designed for the San Francisco Bay-Delta system, so its application to other systems may not work. To reiterate the preceding paragraph in detail, the primary problem with this model is the exchange between the dissolved and particulate phases, in this case the first trophic level (autotrophs/primary producers). While there is some adsorption of dissolved selenite and selenate to suspended particles (e.g., Doblin et al., 2006), most particulate selenium in organic matter is organic selenide in the form of seleno-amino acids in proteins (Wrench, 1978). In other words, the uptake of dissolved selenite and selenate from the water column by phytoplankton changes their chemical forms, it is reductively incorporated (Cutter, 1982; Cutter and Bruland, 1984). Biological uptake of dissolved nutrients such as nitrogen, and metals, is best (most accurately) modeled using Michaelis-Menten kinetics, or at least pseudo-first order rate expressions. The release of this particulate organic selenide back into the water column as dissolved organic selenide is coupled to oxic (or anoxic) respiration (Cutter, 1982; Cutter and Bruland, 1984), which is also modeled using an appropriate rate expression (e.g., first order; see discussion in Meseck and Cutter, 2006). The critical point here is that the speciation of particulate selenium has no relation to that in the water column – reductive incorporation and subsequent regeneration obliterates this	Please see response to reviewer 1 above. In short, EPA has used an approach shown in Presser and Luoma (2010). The specific applications of the model cited in Presser and Luoma (2006, 2010) were parameterized using site specific empirical data from the SF Bay. However, the model described in those studies is a basic food web model that can be applied to any system. The kinetic analyses recommended in the comment would be impossible to complete nationally because sufficient data do not exist to allow for of a rate-based dynamic model using speciated selenium data. Thus, EPA uses total dissolved selenium to reflect the selenium concentration in water that is available for uptake and assimilation at TL1. As mentioned above in the response to Reviewer 1, the document's narrative on the uptake and transformation of selenium has been rewritten to reflect the actual processes in nature. EPA has followed up on the reviewer's recommendation that: "all that is needed is a model that covers dissolved to first trophic level interactions, and from there the existing biodynamic part of the Presser and Luoma (2006; 2010) could be employed. In this case, using Equations 4-6, and 7, in the Meseck and Cutter (2006) paper (and related equations in the Appendices) could suffice." EPA coupled the above with consideration of the reviewer's recommendation to: "Use … simple first order rate equations (and values)

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relationship and only a rate-based (kinetic) approach can accurately quantify it. However, the Presser and Luoma (2006, 2010) model uses equilibrium distribution coefficients (K_d or in this EPA document EF) to quantify how particulate selenium in the first trophic level reflects the dissolved concentration in the water column. The distribution coefficient approach works well for divalent metal cations where no oxidation state change occurs. For a given K_d value, if the dissolved concentration goes up, more adsorbs to the particles (to maintain equilibrium), and when the dissolved concentration drops, the particulate-bound metal desorbs. But, when there is a redox change between dissolved and particulate conversions, the equilibrium concept is violated. For example, if the concentration of selenite goes up, the rate of uptake increases, and the concentration of particulate organic selenide increases; in a crude fashion, the use of a K_d could mimic this biochemical process. But, when the concentration of dissolved selenite goes down, particulate organic selenide doesn't desorb to balance it; they are different chemical species. Particulate organic selenide is only released through respiration/regeneration, not adsorption/desorption (for which the K_d concept was created). So in this scenario, the Presser and Luoma (2006) cannot accurately predict the response to a change in dissolved concentration, and more importantly cannot predict the speciation of selenium. Interestingly, Presser and Luoma (2006) note that as more recycling (i.e., the regeneration part of the selenium cycle depicted in Cutter and Bruland, 1984) occurs, organic selenide concentrations increase. Indeed, they do, but their model cannot reproduce this, a problem if you "reverse" their model to predict water column dissolved concentrations of selenium for a given particulate concentration in the food web (e.g., 11.8 ppm Se in	described in the literature." The reviewer's recommended Equation 7 relates selenium water concentrations to sediment flux rates, and although it is applicable to reviewer's San Francisco Bay site, it cannot be applied to the national criterion derivation for lack of information on the direction and magnitude of sediment flux rates in the wide range of sites EPA considered. However, the implications of the reviewer's recommended equations 4-6 are of interest. Those equations are as follows, where D signifies dissolved and P particulate, and Se(-II) is organic selenium: $\partial DSe(IV)/\partial t = k3\ [DSe(IV)] - k5\ [DSe(VI)]$ $\partial DSe(IV)/\partial t = k2\ [DSe(-II)] - k3\ [DSe\ (IV)] - k4\ [DSe\ (IV)]$ $\partial DSe(-II)/\partial t = k1\ [PSe(-II)] - k2\ [DSe\ (-II)] - k6\ [DSe\ (-II)]$ The above rate coefficients are for the following reactions: k1: PSe(-II) → DSe(-II) k2: DSe(-II) → DSe(IV) k3: DSe(IV) → DSe(VI) k4: Phytoplankton uptake rate for DSe(IV) k5: Phytoplankton uptake rate for DSe(VI) k6: Phytoplankton uptake rate for DSe(-II) Lacking information on rates of concentration change, the approach is unworkable unless a steady-state assumption is

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fish muscle; this document). This latter (highlighted) point is exactly what Section 4.2 is doing. On a related matter, the Presser and Luoma model suggests that it handles selenium speciation, but only in the dissolved phase, and then rather than using separate K_ds for each species, and presumably summing the contributions from each from to derive the particulate selenium concentration, they simply average the K_ds to one value and omit speciation. To put this modeling approach into another perspective, it has been observed (Cutter, 2005) that the aquatic selenium and nitrogen cycles are very similar/parallel. Adding N cycling to the Se cycle depicted in Cutter and Bruland (1984) gives:	imposed, as commonly done in such problems. At steady state, concentrations do not change over time. Consequently, the left side of each equation is zero, and the problem becomes workable. The equations then reduce to: k3 [DSe(IV)] = k5 [DSe(VI)] Hence, with first order kinetics, [DSe(IV)] $\propto$ [DSe(VI)] k2 [DSe(-II)] = k3 [DSe (IV)] + k4 [DSe (IV)] Hence, [DSe(-II)] $\propto$ [DSe (IV)] k1 [PSe(-II)] = k2 [DSe (-II)] + k6 [DSe (-II)] Hence, [PSe(-II)] $\propto$ [DSe (-II)] With first-order kinetics and steady-state assumptions, particulate organic selenium, PSe(-II), in the primary producers is proportional to dissolved organic selenium, DSe(-II), which in turn is proportional to dissolved Se(IV), which in turn is proportional to dissolved Se(VI).Consequently, under these model assumptions, selenium in the primary producers is proportional to total dissolved selenium in the water column. The reviewer's recommended approach thus appears to be a more detailed articulation of the approach EPA applied, provided that the first-order kinetic assumption is taken to be one of the acceptable approaches, as the reviewer seems to suggest, and if the commonly used steady-state modeling assumption is applied, as was done for all other trophic levels. Reiterating a point made in response comment #1 immediately above, this comment is technically incorrect in calling EFs an equilibrium concept. EFs, like TTFs and BAFs, are better characterized as steady-state concepts. Steady state does not

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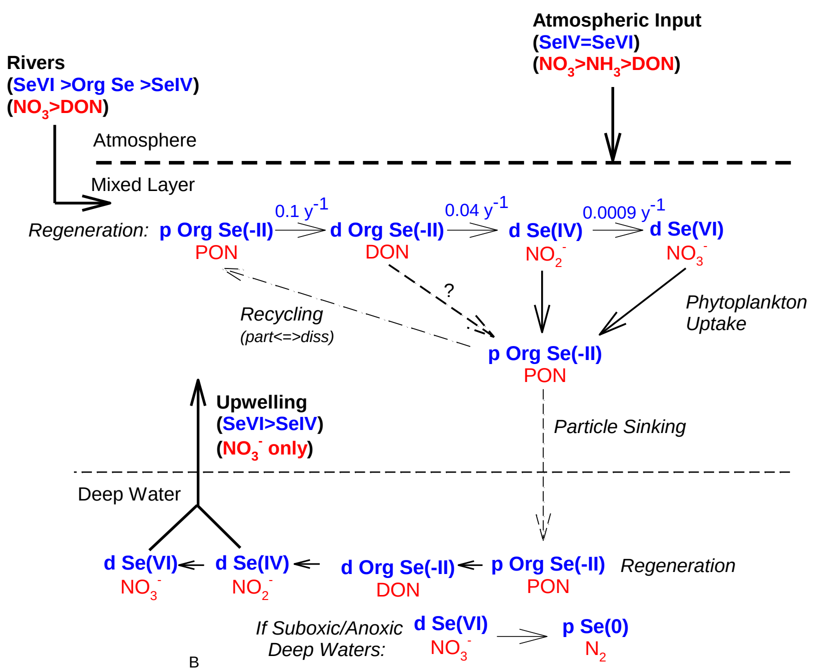
Biogeochemical Cycle of Selenium in a stratified lake (with nitrogen analogs)  Thus, I ask those who wrote this document if they would use the Presser and Luoma (2006, 2010) approach to model nitrogen cycling and therefore set N discharge, etc limits? I suspect the answer would be no, and my response then would be, why use it for selenium? To be constructive, what modeling approach should be used? In Cutter (1992) it was argued that a kinetic/rate approach, and not	require equilibrium or process reversibility. For example, irreversible uptake coupled with growth dilution would still yield a steady-state EF. In site-specific situations where ample measurements are available for all forms of Se, EPA recognizes the value of the reviewer's detailed articulation of the organic-inorganic Se processes. However, because such data are rarely available, the reviewer's detailed approach is not feasible to capture the wide diversity of sites represented via the derivation of the national criterion. EPA believes that confining its assessment to a very small number of well-studied sites where such measurements are available would reduce rather than increase confidence in the appropriateness of the national criterion. Likewise, an analysis based on a few site- or lab-specific measurements of k1 through K6 would also decrease rather than increase confidence, when compared to an approach that homogenizes the rate coefficients but uses the end-resulting ratios of concentrations observed in water and primary producers at a wide diversity of sites.

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an equilibrium thermodynamic one (EFs are an equilibrium concept) is the only way to quantify the selenium cycle. There are at least two existing kinetic models for the selenium cycle: for lakes there is the one described in Porcella et al. (1991) and Bowie et al. (1996), and one for estuaries, Meseck and Cutter (2006). The Meseck and Cutter model focuses on the dissolved to first trophic level dynamics and includes the full speciation of selenium in the dissolved and particulate states in an estuary (San Francisco Bay/Delta). The Bowie et al. (1996) model uses a kinetic approach to modeling selenium speciation and dynamics from the dissolved state to all trophic levels in freshwaters, and was designed to assist in mitigation/restoration efforts. The Meseck and Cutter (2006) model also has direct applications to mitigation via scenario modeling (what if…). However, this model includes components to simulate sediment resuspension, mixing and dispersion, and primary production (light-limited in this case), so it may be too complicated for the application needed here. Indeed, all that is needed is a model that covers dissolved to first trophic level interactions, and from there the existing biodynamic part of the Presser and Luoma (2006; 2010) could be employed. In this case, using Equations 4-6, and 7, in the Meseck and Cutter (2006) paper (and related equations in the Appendices) could suffice. Or, use simple Michealis-Menten equations and values in the literature (e.g., Riedel et al., 1991), and simple first order rate equations (and values) described in the literature (e.g., Cutter, 1982; Cutter and Bruland, 1984; Reinfelder et al., 1993).	
3. I believe that the EPA's translation method is not unreasonable, but I have three primary concerns: (1) TTFs and CFs derived for taxa from other studies are applied to sites	The model prediction itself is correct with respect to the translation calculations. In applying the translation model, the egg-ovary criterion element concentration is used to translate

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regardless of whether those TTFs and CFs are reflective of site-specific trophic transfer data; (2) the EFs and TTFs are treated as constants regardless of exposure concentrations; and (3) the level of protection associated with the draft criteria is unclear. These are discussed further below (in response to questions 1 and 2).	that value to a water concentration by dividing that egg-ovary criterion element by the product of a species-specific composite TTF, a species specific egg-ovary to whole body to CF, and a site specific EF). The model values in Table 12 are correct calculations.
Model for translating fish egg/ovary Se criterion to lentic and lotic water Se criteria is not always consistent with site-specific information: The EPA identified sites where Se EFs could be calculated based on reported co-located Se concentrations in surface water and particulates (algae, detritus, sediment). Information on the fish species present at those sites was then used to develop food web models, which determined the CFs and TTFs that were then applied in translating from the draft fish egg/ovary Se criterion back to corresponding water Se concentrations. Site-specific food web information was used where reported, but the EPA mostly relied on the NatureServe database (http://www.natureserve.org) for information on the typical diet and/or eating habits of the fish at each site.	To maximize the applicability of the available information, EPA treated EF as site-specific and TTFs as taxon-specific. Site-specific food-web observations (prey species consumed by predator species) were always used when available. When not available from the study, the NatureServe database was used. EPA believes it has used the available data in a comprehensive and scientifically defensible way. Regarding the reviewer's point about EFs and TTFs not varying with concentration, see responses in the TTF section (Question 2) below.
A limitation of this modeling approach is that it ignored site-specific information on Se bioaccumulation in fish and their diets. The EFs used were site-specific, but Se modeling up the rest of the food chain and into fish was based on assumed model parameters. This becomes particularly important when considering the data "drivers" for the draft lentic Se criterion of 1.3 µg/L. This value is driven almost exclusively by data for two reference lakes (Badin Lake and High Rock Lake, NC, USA). Badin Lake was reported to have a water Se concentration of 0.32 µg/L and High Rock Lake a water Se concentration of 0.67	Regarding the influence of Badin and High Rock Lakes, EPA agrees that they have a strong influence on the derived lentic value. Of the 44 site-species used for the lentic derivation via the 20th percentile value, 12 of the lowest 13 values are for Badin and High Rock. These lakes each have one EF, but each of its EFs is used six times, once for each of six fish species. The particulate concentrations measured in both of these lakes are near the median observed in EPA's lentic database, but their water concentrations are among the lowest. As a result of the peer reviewer's comments, EPA completed a reanalysis of the

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µg/L (Lemly 1985). For comparison, the mean water Se concentrations translated from a fish egg/ovary Se criterion of 15.2 mg/kg dw were 0.54 µg/L for Badin Lake and 1.2 µg/L for High Rock Lake. The former falls between the water Se concentrations reported for these two reference lakes and the latter almost equals the draft lentic criterion of 1.3 µg/L. Since six fish species were assumed to represent each of these two sites, these two reference sites are the drivers for the draft lentic Se criterion of 1.3 µg/L. In addition to two reference sites being the drivers for the draft lentic Se criterion of 1.3 µg/L, the model for translating a fish egg/ovary Se criterion of 15.2 µg/L to a water Se concentration does not appear to be correct for these two sites. Although fish egg/ovary Se concentrations were not reported for Badin Lake and High Rock Lake, muscle Se concentrations were. Those muscle Se concentrations were reported on a wet weight basis and converted to a dry weight basis by assuming a moisture content of 75%. The muscle-to-egg CFs reported in Table 12 of the draft AWQC document were then used to estimate fish egg Se concentrations. These estimated fish egg Se concentrations for the two reference sites were, on average, less than one-half of the draft fish egg/ovary Se criterion of 15.2 mg/kg dw. Further, the muscle Se concentrations at the references sites ranged from 2.3 to 5.8 mg/kg dw, which are well below the draft muscle Se criterion of 11.8 mg/kg dw. The above demonstrates that the food web model for these two reference sites does not accurately reflect Se bioaccumulation potential at these two sites and in fact greatly overestimates Se bioaccumulation potential. Overall opinion on method for translating from a fish tissue criterion to water Se criteria:	data to remove any overweighting of a few key high and low end sites in the calculations. To account for overweighting, EPA used one fish species per site – the species most sensitive to selenium bioaccumulation, to yield an appropriately protective water column criterion element.

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In my opinion, the approach should rely more on empirical data in order to eliminate cases where the food web models do not reflect the site-specific data. One alternative approach is that described in DeForest et al. (2014). That approach was also based on multi-step Se partitioning, but rather than using EFs and TTFs, the empirical relationships between (1) water and particulate Se; (2) particulate and invertebrate Se; and (3) invertebrate and fish egg/ovary Se were used. Quantile regression was used to work backward from an egg/ovary Se threshold to conservative Se concentrations in lentic and lotic water bodies. This regression-based approach accounts for the breadth of data on Se enrichment and trophic transfer potential, which can essentially represent the bounds of Se bioaccumulation potential from water to fish eggs/ovaries. The regression-based approach also accounts for the slopes of the relationships between water and particulate Se, particulate and invertebrate Se, and invertebrate and fish Se. This would be one example of an alternative model that could be considered. Level of protection associated with draft water selenium criteria unclear: The draft lentic and lotic criteria are based on the 20th percentiles of the data points plotted in Fig. 11 of the draft AWQC document. Those data points in Fig. 11 are for individual fish species at a given site. For example, 18 of the 51 data points for lentic systems (35%) are for just three water bodies (six fish species per water body). It is unclear what the 20th percentiles of those lentic and lotic distributions are protective of, as they do not represent 20% protection of sites or 20% protection of fish species. The latter was presumably not the intent, as those levels of protection would not be acceptable	

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for national AWQC recommendations. Literature cited: DeForest DK, Brix KV, Gilron G, Hughes SA, Tear LM, Elphick JR, Rickwood CJ, DeBruyn AMH, Adams WJ. 2014. Selenium partitioning between water and fish tissue in freshwater systems: Development of water-based selenium screening guidelines. http://www.namc.org/docs/Selenium%20 Integrated%20Report%20-%20Final%20(2014-05-20).pdf Lemly AD. 1985. Toxicology of selenium in a freshwater reservoir: Implications for environmental hazard evaluation and safety. Ecotoxicol Environ Saf 10:314-338.	
4. The EPA is justified in simplifying the bioaccumulation equations by eliminating the growth rate constant (g) because it is negligible compared to the loss rate constant of Se from aquatic animals. This is generally the case for most metals and metalloids, with some notable exceptions where the loss rate constants are very low (e.g., methylmercury). Their equations 2 and 3 (pages 64-65) have already been published, and the reference for this should be cited. (Reinfelder, J.R., N.S. Fisher, S.N. Luoma, J.W. Nichols, and W.-X. Wang. 1998. Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Science of the Total Environment 219: 117-135.) The authors should note that the loss rate constant of some contaminants can differ following uptake from the aqueous phase and uptake from diet---this is because the contaminant may deposit in different tissues from these two uptake routes. As such, the term ke should be converted to kef and kew (following uptake from food and uptake from water). For Se, fortunately,	Thank you for your comment. EPA added the reference you noted. EPA recognizes that ke consists of kef and kew, but has decided to retain the mathematical expression as is for simplicity. As the reviewer noted, uptake from the aqueous phase is negligible.

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this correction is unlikely to be an important one because uptake from the aqueous phase (water) is negligible compared to dietary uptake. But strictly speaking, the mathematical expression (Eq. 2) should reflect two different loss rate constants. By using tissue concentrations of Se in fish to calculate dissolved Se concentrations in ambient water, one must ultimately calculate the Se concentration in organisms at the base of the food chain, namely phytoplankton. This is because none of the animals in the food chain appreciably take up Se from the aqueous phase. The problem of inferring Se concentrations in water from phytoplankton Se concentrations is that the enrichment factors (or bioconcentration factors) of Se in phytoplankton can vary by up 2 or 3 orders of magnitude, depending on the type of phytoplankton that happen to be dominant in the water. Chlorophyceae (green algae), for example, bioconcentrate Se far less than diatoms, and so the variability in these calculations would depend heavily on which types of phytoplankton happen to be dominating the community, and this can change temporally and geographically.	
5. The EPA has used the modern and scientifically valid biodynamic model approach to derive water quality elements from tissue-based elements. I am not aware of other data sources, models or approaches that would reduce the inherent uncertainty. However, based on comments provided above (in 1 and especially 2a(iii)), relying on water column dissolved [Se] has a high likelihood of generating both false positive and false negative results with respect to regulatory action. I think the proposed water column criteria (a) should be used as triggers to initiate further monitoring of fish tissue [Se], (b) should be made more conservative (reduced) by application of a safety factor to	Thank you for your comment. The water column criterion element values were derived using the egg-ovary criterion element as the basis of the calculation. EPA developed water column criterion values because routine water concentration monitoring of selenium is more practically feasible than routine tissue monitoring, and used a hierarchal (tiered) approach to insure that water column values indicating a selenium problem could be vetted using a fish tissue sample, because the fish tissue selenium concentrations are indicative of potential effects.. Water column values are necessary because they may be the only media data available – i.e. streams where fish are absent, or

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avoid false negatives, and (c) that the simple classification of a water body as lentic or lotic should be modified to include more quantitative measures of flow such as water residence time and/or mean annual water velocity. Given that many impounded riverine systems in the USA are essentially lentic systems for much of their river-miles, perhaps a water column trigger [Se] could be set at 1 ug/L (same as the current Canadian [CCME] water quality guideline for Se). If exceeded, this trigger value would result in further action in terms of fish collections for tissue [Se].	temporally necessary due to a new discharge where selenium has not accumulated in the system, and so there is uncertainty in the relationship between the discharge and the potential for bioaccumulation in fish at the site.
6. The translation equation approach used to convert toxicologically relevant fish tissue concentrations to water-column concentrations is broadly scientifically sound and defensible, and represents our best available understanding of these relationships across trophic levels in an aquatic ecosystem food web. This may be especially true because the approach is based on a straightforward model, and alternative approaches that introduce complexity can also introduce uncertainty from the requirements of additional data beyond that currently available. Risk estimation rarely has perfection due to situational variability and uncertainty involving the integration of exposure, uptake, and biokinetics. The draft criterion approach uses qualified data and reasonable analysis to reduce complexity and increase the transparency of criterion. Modeling dynamic relationships in complex multi-level systems with innate variability is a significant environmental management challenge, however the effort can yield a valuable management tool. Figure 8 (p. 73) graphically demonstrates "hysteresis" with regards to aquatic food chain selenium levels and potential for toxic impact as well as the temporal relationship to periodic sampling. Any	Thank you for your comment.

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challenges in application of this approach across diverse aquatic ecosystem types with variable water chemistries and annual variability (e.g., flow and flux), are equally met by the challenges of sufficiently devising specific criteria to address every subset of variables with less or equal uncertainty in the protection of aquatic life. The duration and frequency requirements of the water column selenium criterion address the potential for system variability (e.g., year to year weather/hydrograph changes) and propagation of system uncertainty (e.g., non-selenium related chemical or biological changes) in this risk management.	
7. The scientific method for translating concentrations of selenium in fish tissues to allowable concentrations in the water column is clearly written and understandable. However, while we understand the regulatory need for triggers to initiate site investigation where selenium is suspected of being an issue, the derivation of allowable water column concentrations from eggs or ovaries is oversimplified and likely to need site specific inputs for refinement. Back calculating from egg/ovary to muscle/whole body and then down through trophic levels to derive allowable water column criterion for each of these types of aquatic systems is not scientifically valid, because of the use of generic CF, assumptions regarding proportions of prey items consumed by resident fish and broadly applied trophic transfer factors. These generic terms do not incorporate site specific information, including concentration dependent uptake kinetics and consideration for important influencing factors (e.g., sulfate, organic carbon, temperature, etc.). The water based criterion developed in the Draft Document are therefore, necessarily conservative. As evidence for this, the monthly average	EPA has developed the national criterion based on the best-available science. EPA also agrees on the utility of site-specific criteria developed using site-specific data to estimate risk in the most refined manner possible. EPA has included a discussion of the development of site-specific criteria in the criterion document main text body, and describes approaches to developing site-specific criteria in detail in Appendix K. In each site-species translation equation an egg-ovary selenium concentration is divided by the product of: a species (or closest taxonomical surrogate) specific CF, a species-specific component TTF, and a site specific EF. At many sites, site-specific data are not available to calculate site specific CFs and TTFs for each species, so the application of CFs and TTFs represents the best available information. Also, see response to Peer Reviewer 2, in Part I, Question 2.a.iii above.

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exposure value for lentic systems is 1.3 µg/L. This value is at the upper end of background values for freshwater and may be exceeded even in the absence of industrial inputs in areas receiving runoff from seleniferous soils. The value is also lower than recently recommended lentic values based on similar analysis (Deforest et al. 2104, BC MoE 2014).	In effect, EPA is positing that although partitioning from water to particulate matter is highly variable and can best be evaluated using data gathered from the specific site under consideration (if available), selenium partitioning among tissues (CF), and from particulate matter to fish through the food chain (TTF) is relatively similar for a given species regardless of site. Although this introduces some uncertainty to the translated values, CFs and TTFs are less variable across sites than EFs based on available data. (See response to Peer Reviewer 2, in Part I, on modification of CF and TTF derivation method to incorporate taxonomic proximity.)

2. Regarding the trophic transfer factor (TTF) values, did EPA use a scientifically defensible method to derive the TTF values (p. 71-77 of the criteria document)? Were the exclusion criteria, (pp. 71-77 of the criteria document) developed by EPA to screen the available data applied in a consistent and scientifically defensible manner? In particular, EPA noticed that application of the exclusion criteria resulted in TTF values for aquatic insect larvae that differ from other published values. Given this, are you aware of any other methods of screening data that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included, if appropriate? Please provide detailed comments.

External Peer Reviewers' Comments	EPA Response
1. In general, EPA has used a scientifically defensible method to derived TTFs. However, I am concerned that the TTFs derived from field data by EPA are biased low and potentially not protective. I note that the data in Figure 16 appear to show a rather significant bias towards under-prediction of EO selenium concentrations, consistent with this concern. As recognized by EPA, there is typically an inverse relationship between the exposure concentration and the TTF such that low dietary Se will result in relatively high TTFs for a given predator-prey species pair. Many of the field data sets used by EPA are from sites with high levels of Se contamination (10's to 100's μg l^{-1} waterborne Se). Conversely, a number of the data sets are from extremely low Se environments (e.g., mayfly). Perhaps, for TTF derivation purposes, EPA should constrain calculation of the median TTF to conditions that approximate the range of WQC (e.g., 0.5-10 μg l^{-1} in water) that EPA might consider on a site-specific basis, or the range of concentrations typically associated with the EC10 for sensitive fish species. Otherwise, individual TTFs have the potential to be biased either low or high depending on the site(s) from which they were collected. EPA should carefully review the biokinetic data using similar criteria.	EPA used TTFs derived from both lab and field data. TTFs derived from field data are expected to be sufficiently protective of aquatic life in the field since they are estimates based on real-world conditions, and not controlled experiments in the lab. Field data were considered superior to lab data for several reasons, such as having representative diets, steady-state conditions and an absence of artifacts that can be observed in laboratory exposures (e.g., poor nutrition, selenium speciation issues). Although there is a strong correlation between predicted and observed egg-ovary concentration values, Figure 16 does show more data points above the y = x (observed egg-ovary concentration vs predicted egg- ovary concentration) line at low selenium concentrations. This result suggests the model underestimates bioaccumulation at low selenium concentrations. However, within the range of concentrations near the egg-ovary criterion element value, the relationship between predicted and observed selenium concentrations are evenly dispersed around the y = x line. Thus the model is unlikely to result in biased estimates near the egg-ovary criterion concentration, the focus

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	of the criterion development.
2. After the dissolved to first trophic level particulate selenium part of the model that I am criticizing above, the rest of the Presser and Luoma (2006) model (including the derivation of TTFs) is excellent and accurately predicts bioaccumulation through the various parts of the food web (and earlier documented in the Luoma and Rainbow (2005) peer-reviewed paper). The reason here is that once into the first trophic level, the primary speciation of particulate selenium is organic selenide, and the concepts of assimilation efficiency, trophic transfer factors, ingestion and depuration (egestion) work well for selenium (and any other metal or nutrient). The screening of data followed well-set protocols and are quite defensible. I am not aware of additional data to be included, but I'm sure there must be some in the grey literature.	Thank you for your comment.
3. Overall, I generally agree with how the EPA derived TTFs from either physiological coefficients or from field data. Following are specific comments. TTFs from empirical measurements in laboratory studies: Laboratory-based TTFs were calculated from physiological coefficients (AE, IR, ke), but it does not appear that TTFs were calculated from laboratory data in which Se concentrations were empirically measured in invertebrates or fish and their diets. This approach is analogous to the field-based TTFs calculated by the EPA, but there is less uncertainty in the dietary Se concentration because the dietary Se concentration is known in laboratory studies. Is there a reason why these studies were not considered?	When EPA collected data for calculating TTF values, the search was restricted to field data. Field data were considered superior to lab data for several reasons, such as: having representative diets, steady-state conditions and an absence of artifacts that can be observed in laboratory exposures (e.g., poor nutrition, selenium speciation issues). The dynamic nature of selenium transfer from one trophic level to another is better represented in field data than in laboratory or mesocosm data. EPA did include TTFs from the multiple Conley et al. publications based on the life-cycle exposure of selenium to mayflies via a complex periphyton diet. There are concerns regarding the Conley mayfly TTFs when insufficient

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TTFs are not constants across exposure concentrations: As previously noted, one potential limitation of the modeling approach is that TTFs tend to be inversely related to exposure concentration (i.e., TTFs are inversely related to the corresponding dietary Se concentration). However, the TTFs in the model used by the EPA are constants that are specific to the exposure concentration in the test from which they were derived. The EPA did note, on p. 74, that the "distribution of ratios could be biased high toward larger values if the data are obtained from aquatic systems with low selenium concentrations" and on p. 75 a regression-based approach was considered. EPA ultimately used what was described as a hybrid approach, in which ordinary least squares (OLS) linear regression was used to confirm that a significant ($p \leq 0.05$) and positive relationship was observed, and then the median of individual ratios was used to estimate central tendency and avoid bias from systems with very low or very high selenium concentrations. This helps to partially address the issue, but a regression-based approach may still be more appropriate (see previous comment).	food resulted in different TTF (and toxicity) measurements, which illustrate the potential bias with laboratory studies. The decision to include the Conley studies was based on the weight of evidence of similar TTFs for most of the exposures and the need to fill a data gap for a TTF of an important fish prey item. Regarding the commenters question on regression versus median approaches, ultimately, a hybrid approach (median ratios with regression statistics used to screen data quality) was selected by EPA because the use of ratios was less sensitive to outliers or from regressions where the y-intercept was notably different from zero, where the slope of a constrained regression (following the approach used by Presser and Luoma) had a poor fit. Every approach (median ratios, constrained regression, and conventional OLS regression) has inherent strengths and weaknesses, and ultimately, the hybrid approach was determined to be the most robust.
TTFs for insect larvae: The draft AWQC document includes Se TTFs of 1.97 for a dragonfly (Anisoptera), 2.88 for a damselfly (Coenagrionidae), 1.28 for a mayfly (*Centroptilum triangulifer*), 1.90 for a midge (Chironomidae), and 1.48 for a corixid (Corixidae). • Dragonflies and damselflies: The dragonfly and damselfly TTFs do not always appear to be calculated as described. On p. B-63 it is noted that the Se concentration in dragonfly and damselfly food is the median selenium concentration in all invertebrate tissues	EPA has carefully considered its assumption that EFs and TTFs do not vary with concentration. The following graphs show the water-TL1, TL1-TL2, and TL2-TL3 relationships, from the standpoint of total least squares (error in x and y), and ordinary least squares (error in y only). One advantage of total least squares (TLS) is that the regression relationship is unaffected by which variable is assigned to the x and y axes. Another advantage is that introducing sampling noise into an underlying relationship does not consistently flatten the TLS slope (in contrast to its flattening of the OLS slope). Because concentrations in both media have essentially equal uncertainty,

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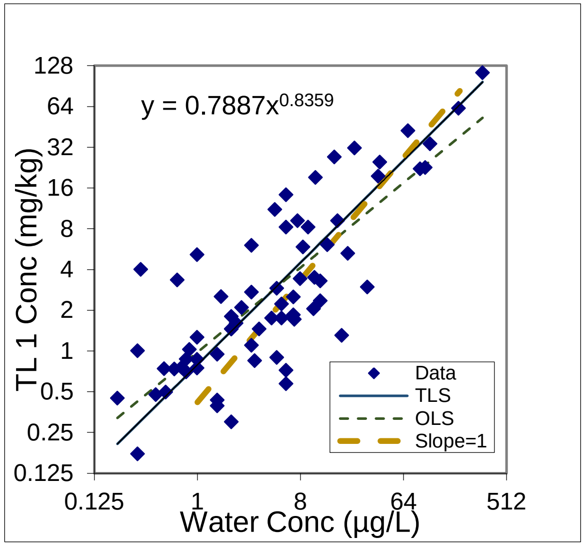
that co-occur with an Odonate species. For Site 29 in Birkner (1978), however, only corixids are considered in the damselfly diet, even though data for chironomids are available. The damselfly Se concentration at this site was 55.0 mg/kg dw and the corixid Se concentration was 29.4 mg/kg dw, which resulted in a TTF of 1.87. However, if chironomids were also considered part of the diet, which had a Se concentration of 58.2 mg/kg dw, the median Se concentration in the damselfly diet would be 43.8 mg/kg dw and the TTF would be 1.26. I recommend that the EPA double-check the dietary data used to calculate the TTFs for these taxa. • Mayfly (*C. triangulifer*): The Se TTF of 1.28 for this species may be too low. This value was based on biokinetic data from Riedel and Cole (2001). However, empirical laboratory data from Conley et al. (2009, 2011, 2013) indicate that the Se TTF may range from about 1-3, with a mean of about 2 depending on exposure and test conditions. I recommend that the EPA consider these studies, which may result in a higher Se TTF for *C. triangulifer*. • Midges (Chironomidae): The Se TTF of 1.90 for this taxa may be high when considering laboratory-based TTFs, for which the dietary Se concentration is known. Based data for chironomids from Malchow et al. (1995) and Rickwood and Jatar (2013), mean and maximum Se TTFs are 0.3 and 1.4. The chironomid Se TTFs derived from field data by the EPA include dietary Se assumptions that may underestimate the dietary Se concentration and result in relatively high Se TTFs. For	total least squares might be the preferred approach. In the graphs below show, (a) the data, (b) the TLS line and its formula, (c) the OLS line, and (d) a slope=1 line going through the median of x and y. 

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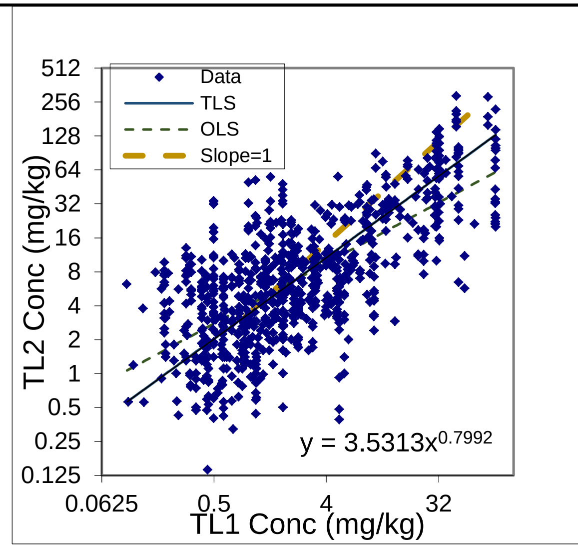
example, the TTFs from Saiki et al. (1993) average 1.0 when a detritus-based food chain is assumed, as suggested by the study authors. I recommend that the EPA consider the dietary assumptions in the field studies in light of the laboratory data. • Corixids (Corixidae): Additional Se TTF data for corixids are available from a laboratory study with *Trichorixa reticulata* (water boatman). In this study, the TTF was very high (32.6) in the control with a low dietary Se concentration of <0.1 mg/kg dw, but then TTFs were <1 at dietary Se concentrations of about 6 to 86 mg/kg dw. It is recommended that this laboratory study be included in deriving the corixid and be used to check the dietary assumptions in the field studies. Additional potentially relevant TTF data sources: *Laboratory data:* • Conley et al. (2009, 2011, 2013) - *Centroptilum triangulifer* (mayfly) • Malchow et al. (1995) - *Chironomus decorus* (chironomid) • Rickwood and Jatar (2013) - *Chironomus dilutus* (chironomid) • Besser et al. (1989) - *Daphnia magna* (cladoceran) • Besser et al. (1993) - *Daphnia magna* (cladoceran) • Guan and Wang (2004) - *Daphnia magna* (cladoceran) • Thomas et al. (1999) - *Trichorixa reticulata* (water	

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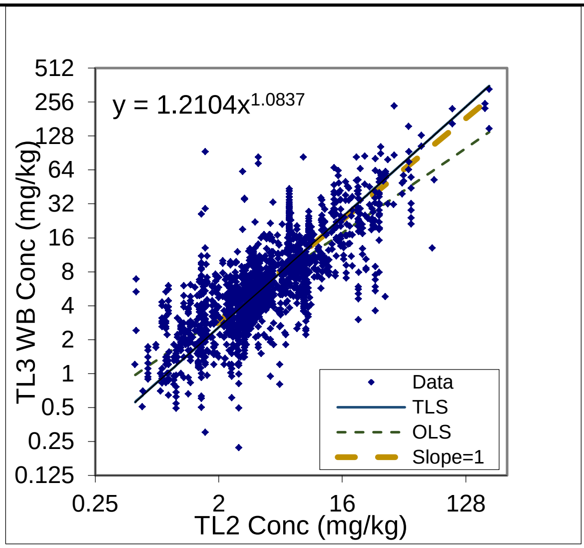
boatman) Literature cited: Besser JM, Huckins JN, Little EE, La Point TW. 1989. Distribution and bioaccumulation of selenium in aquatic microcosms. Environ Pollut 62:1-12. Besser JM, Canfield TJ, La Point TW. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. Environ Toxicol Chem 12:57-72. Conley JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly *Centroptilum triangulifer* in a life-cycle, periphyton-biofilm trophic assay. Environ Sci Technol 43:7952-7957. Conley JM, Funk DH, Cariello NJ, Buchwalter DB. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. Ecotoxicology 20:1840-1851. Conley JM, Funk DH, Hesterberg DH, Hsu L-C, Kan J, Liu Y-T, Buchwalter DB. 2013. Bioconcentration and biotransformation of selenite versus selenate exposed periphyton and subsequent toxicity to the mayfly *Centroptilum triangulifer*. Environ Sci Technol 47:7965-7973. Guan R, Wang W-X. 2004. Dietary assimilation and elimination of Cd, Se, and Zn by *Daphnia magna* at different metal concentrations. Environ Toxicol Chem 23:2689-2698. Malchow DE, Knight AW, Maier KJ. 1995. Bioaccumulation and toxicity of selenium in *Chironomus decorus* larvae fed a	 In the above plots, the linear assumption (log-log slope=1 or EF and TTF constant with concentration) serves well for water-TL1 (EF) and for TL2-TL3 (TTF into fish). In the TL1-TL2 relationship, TTF into invertebrates, the linear assumption at high values of x fits the corresponding upper bound values of y, indicating environmentally conservative results at high concentrations. Regarding midges, chironomids were considered. The modeled diet for site 29 of Birkner 1978 consists of 3 invertebrate taxa: a chironomid, a corixid, and an amphipod. The median value of 29.4 happens to be for the corixid. However, the chironomid and amphipod values were used in the determination of the median.

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diet of seleniferous *Selenastrum capricornutum*. Arch Environ Contam Toxicol 29:104-109. Rickwood CJ, Jatar M. 2013. Investigation into the fate and effects of selenium on the life-cycle of a benthic invertebrate (*Chironomus dilutus*). CanmetMINING, Project: 603994. Natural Resources Canada (NRCan), Ottawa, Canada. Riedel GF, Cole L. 2001. Selenium cycling and impact in aquatic ecosystems: Defining trophic transfer and water-borne exposure pathways. Chapter 3 in EPRI Report 2001. EPRI, Palo Alto, CA. Thomas BV, Knight AW, Maier KJ. 1999. Selenium bioaccumulation by the water boatman *Trichocorixa reticulata* (Guerin-Meneville). Arch Environ Contam Toxicol 36:295-300.	Regarding the new TTF data, EPA thanks you for providing additional sources of TTF data. EPA reviewed and used the additional data determined to pass data quality and relevance screening. EPA has included empirical laboratory data from the Conley et al. studies in the 2015 draft criterion to calculate the mayfly TTF, which is now 2.48, changed from the TTF 1.28 value used in the 2014 draft.
4. I am more familiar with the marine literature and am not well-versed in the freshwater literature regarding Se TTF values.	Thank you for your comment.
5. The method used to derive TTF values is scientifically sound by using the widely accepted biodynamic modeling approach, which is particularly appropriate for Se. The EPA also demonstrated that temporal changes in TTF are for the most part not a factor that may cause large data discrepancies. Since the EPA used a large dataset to derive TTF values for insects, any differences between the EPA-derived values and values reported from individual studies are not of concern to this reviewer. I am not aware of any other data, other than the recent work by Buchwalter mentioned in II2a above. It is suggested the EPA include an updated literature search for this and other supporting data prior to the next revision of the document.	Thank you for your comment. EPA is providing an updated bibliography in the 2015 draft selenium criterion document based on literature suggested by the public and the peer reviewers, particularly the references detailed by peer reviewer 3 above.

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6. The trophic transfer factor (TTF) values were developed as an application of a peer-reviewed, published approach that represents our best available scientific information. The method and data used are adequately described, and the approach is satisfyingly direct. The confounding dynamic to this approach could be the bi-modal essential-toxic behavior of selenium where low-level exposure has different metabolic and storage behavior that non-essential metals and therefore different toxicodynamics across a broad range of exposures. This dynamic is adequately discussed (p. 74). The screening criteria for data used in TTF calculations appear defensible and reasonable, and complete with regard to major published works.	Thank you for your comment.
7. The derivation of Trophic Transfer Factors (TTF) by the US-EPA in the Draft Document is clearly outlined and presented. However there are several issues which, again, result in the introduction of error and therefore an element of conservatism in the data that was derived. For example, the USEPA matched selenium concentrations in consumers and their likely prey items from a thorough investigation of the available data. However, where matched data from more than one prey item was identified from a site, the median of lower trophic organisms was used to calculate a TTF. While we understand the rationale for this practice from a data handling perspective, by not acknowledging that prey items may comprise different proportions of the diet ultimately introduces variability in the calculated TTF, with the potential for an influence in either direction. Additionally, while the US-EPA presents a statistical argument for the validity of matching pairs of samples taken from an aquatic site over a year, it is also acknowledged that some sites may present selenium loads or bioaccumulation	EPA used a median of concentrations of lower trophic organisms since most predators are opportunistic, preying on the organism that they encounter at random. Site-specific studies would reveal predator prey preference or prey abundance relationships that may warrant consideration of a different approach. EPA used a time limit threshold of 1 year to characterize samples as "matched" to maximize the available data that are likely to be temporally similar. EPA understands that there are uncertainties associated with this assumption, but this approach was deemed necessary in order to obtain sufficient data from sufficient sites in order to develop water column criteria. It may be more appropriate to collect site specific data for a known impacted site to insure that both temporal and spatial considerations at the site are accounted for. Uncertainties are "backstopped" by the egg-ovary criterion, the ultimate indicator

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kinetics that require different collection time criteria. Recognizing that the Draft Document will largely be applied to impacted receiving environments that are influenced by industrial activity and which present dynamic ranges in selenium loading, it appears likely that establishing a precedent to allow matching concentrations of selenium in aquatic compartments collected a year apart will, in most cases, not be appropriate. Finally, the USEPA designated single TTF based on the median value of only those regressions that were significant (Page 75). While this is a conservative approach, it does not fully incorporate consideration for differential uptake among lower trophic organisms at varying concentrations of selenium exposure.	of ecosystem protection in a waterbody.

3. Regarding the conversion factor (*CF*) values used, did EPA use an appropriate and scientifically defensible method to derive those values (p. 78-79 of the criteria document and Appendix B)? Are you aware of any other methods that EPA should consider? Also, are you aware of any data that was not considered in this effort and should be screened and included? Please provide detailed comments.

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1. EPA has used a scientifically defensible method for deriving CFs. I am not aware of any other data EPA should consider. It could be argued that a regression based approach be used instead of the ratio approach EPA has adopted. In some cases, it appears that residuals are structured, suggesting that assumptions of the CF approach may be violated. At least for the 4-5 most sensitive taxa, EPA should conduct a sensitivity analysis of the regression-based approach versus the ratio approach and particularly consider confidence in the CF at concentrations that approximate the EC10.	The median ratio approach was used because it was less subject to variability in slope imposed using the constrained regression (0 intercept) approach, which was problematic when the y-intercept was notably different from 0. Also, the median approach appeared less sensitive to issues encountered using unconstrained regression for those slopes where the y-intercept was notably different than zero. EPA considered all approaches, and determined the ratio-based approach to be less affected by issues related to outliers and y-intercept.
2. The calculation of the CF values was rather straightforward, with my only concern, as noted above, being a thorough quantification of the resulting errors in the CF values. As an overall statement, error propagation seems to have been largely ignored in this document.	Thank you for your comment. EPA has clarified the uncertainties in the document by providing more information on the variability behind the calculation of the factors in the 2015 draft document.
3. I think the EPA used a reasonable approach for deriving CFs. As a partial confirmation of those values, fish species for which diet-to-egg TTFs can be derived could be compared to the combined CFs and TTFs values.	Thank you for your comment.
4. See my response to question 2. RE - I am more familiar with the marine literature and am not well-versed in the freshwater literature regarding Se TTF values.	Thank you for your comment.

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5. EO:WB conversion factors ranged from 1.38 to 7.39 with a median value of 1.27. As mentioned in II3 above, it was unclear how determination of [Se] in both whole-body and egg were determined in the same fish, and this should be clarified in the document. Similarly when muscle and whole body were determined in the same fish. Overall, this is a simple method and I am not aware of any alternative methods, nor data sources for these analyses.	Thank you for your comment. For 16 of the 17 species for which data were available, EO/WB CFs ranged from 1.38-2.44, with the EO/WB ratio for mountain whitefish being 7.39. The E/O to WB ratio for all fish (based on a hierarchal taxonomic approach using available data) is 1.63. (See response to Peer Reviewer 2, in Part I, Question 2.a.iii.for additional information regarding the hierarchal taxonomic approach.) The mountain whitefish has a fairly small distribution in the US, when considering overlap with Se enriched areas. Thus, median values are considered more appropriate. As noted above (response to Question 3, part 2, peer reviewer response number 5), regarding determination of selenium concentration in both whole-body and egg were determined in the same fish. The vast majority of the data for the E/O to whole body [Se] relationship analysis came from Osmundson et al (2007) who did report egg data and whole body [Se] data from the same fish. The whole body [Se] was calculated by adding back the egg Se that was removed for analysis. Osmundson et al (2007) had 9 of the 10 species in EPA's data set for this analysis. Coyle et al (1993) also added back egg Se for the whole body same fish comparison. Formation (2011) and Doroshov et al. did not specify how the whole body [Se] was determined. Hermanutz (1996) and Hardy (2005) apparently measured whole body and egg Se in different fish with the same exposure. EPA has added a clarifying discussion to the section discussing fish tissue relationships. Please see response to peer review comment 7 below for details

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	on how the CF methodology was improved from the 2014 draft approach.
6. There is inherent uncertainty and variability in deriving conversion factors given the diversity of fish types, lifecycle stage, and environmental conditions. The single 1.27 conversion factor approach appears to be a straightforward and reasonable approach given the limitations of data and species data sets. This is especially true in practice where a criterion will be applied to fish types including those not subjected to controlled studies. While species specific CFs are desirable, this would require considerably more data that currently available especially in regards to life cycle of the target fish analyzed. The conversion factor (CF) method and input data appear to be a reasonable and defendable approach to addressing data limitations and practical application of the criterion. Other numerical approaches can also rise to developing CFs however it is unclear if the absence of data would bias those results or create similar uncertainties as well. The calculation approach in the current draft is straightforward and robust. Appendix B appears to have most freshwater fish data used in the CF analyses addressed in multiple published scientific papers or agency reports. Because of the critical nature of this calculation to criterion development, updating literature searches for new research data is important.	Thank you for your comment. EPA updated literature sources for the 2015 draft criterion document.
7. As noted in our response to Charge Question #2, almost half (i.e., 7 of 16) of the Conversion Factor (CF) values for egg/ovary to whole body were derived using a generic (i.e., not species specific) muscle to whole body conversion ratio that was calculated as the median value of the available data for all fish species. This practice will have likely contributed to the	Thank you for your comment. In the 2015 draft document, EPA improved its methodology from the approach used in the 2014 draft to calculate CF values when matched pairs of selenium measurements in eggs and/or ovaries and whole body tissue were not available by using species-specific or most-closely-taxonomically-related muscle to whole body and egg-ovary to

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variability in the dataset.	muscle conversion factors. See response to Peer Reviewer 2 above, in Part I, Question 2.a.iii. For those species of fish with neither sufficient data to directly calculate an egg-ovary to whole body CF, nor data to calculate a conversion factor for egg-ovary to muscle or whole body to muscle, EPA estimated CF following the approach described for the estimation of TTF values. In this approach, EPA sequentially considered higher taxonomic classifications until one or more taxa for which a calculated CF value was available matched the taxon being considered, and if the lowest matching taxon was common to more than one species with a CF value available, EPA used the median CF from the matching species. For fish species without sufficient data to directly calculate an egg-ovary to whole body CF but which had sufficient data to calculate a conversion factor for either egg-ovary to muscle or whole body to muscle, EPA followed a two stage approach based on taxonomic similarity similar to that described above. If a fish species had a species-specific egg-ovary to muscle conversion factor, but no whole-body data with which to calculate an egg to whole body CF, then available data for other species were used to estimate a muscle-to-whole-body conversion factor for that species based on taxonomic relatedness. The estimated muscle-to-whole-body factor would be multiplied by the directly measured egg-ovary-to-muscle factor to estimate an egg-ovary-to-whole-body CF for that species.

4. Regarding the derivation of enrichment factor (*EF*) values, was the method EPA used to screen data from the literature applied appropriately and consistently (see inclusion/exclusion criteria on p. 71-77 of the criteria document)? Was the method for deriving *EF* values applied to those data in a consistent manner so as to derive *EF* values for selected waters in a scientifically defensible manner? Is the method that EPA used to establish the lentic and lotic categories for *EF* values reasonable given the available data? Are you aware of other methods or relevant data the EPA should consider? Please provide detailed comments.

External Peer Reviewers' Comments	EPA Response
1. Yes, the method for deriving EFs was scientifically defensible and appears to have been applied in a consistent manner. However, similar to my comments regarding TTFs, there is frequently an inverse relationship between water Se and EF. EPA should carefully examine the distribution of EFs as a function of water Se and assess whether their data set is unduly biased by EFs measured in systems with unusually low or high waterborne Se. It would be helpful if Table 12 included the mean or median water Se concentration at the site. Note, in the section on calculation of EFs, there is no reference to where the EFs for the 69 individual sites can be found (i.e., Appendix L).	Thank you for your comment. A figure has been added to section 3.2.4 that examines the relationship between EF and water concentration. There is a statistically significant inverse relationship between EF and Se concentration in water. EPA carefully re-examined the distribution of EFs and corrected the data for overweighting by EFs measured in systems with unusually low or high waterborne selenium, as described above, in response to Peer Reviewer 3's comments to Question 2.a.iv, regarding magnitude, duration and frequency. A new appendix (G) has been created that lists all of the EF values, by site, and the values used to make the calculations.
2. See above comments; I feel the EF values are completely useless and in fact incapable of being calculated given that they really need to include the chemical speciation of dissolved selenium. They did however miss lots of dissolved and particulate data, many examples including: Cutter, 1989a; Cutter, G. A. 1991., Riedel and Cole, 2001 in their reference list, and river data in Cutter, 1989b and Cutter and San Diego-McGlone that are also in their reference list.	The consequences of the absence of the chemical speciation of dissolved selenium has been addressed in a previous response. Thank you for including a list of references including particulate and dissolved Se data. EPA has reviewed these sources to determine whether they can be included in our translation dataset and included those that were relevant and passed data quality review.

<table>
<tr><th>External Peer Reviewers’ Comments</th><th>EPA Response</th></tr>
<tr><td>3. Overall, I believe that the EPA used a reasonable approach in calculating EF values. However, I do not necessarily agree that Se concentrations should be available for at least two particulate types in order to derive an EF. Periphyton, for example, may be the dominant particulate in certain lotic systems and in my opinion such data should be included. I do agree that Se concentrations in sediment alone is insufficient for deriving EF values. I have greater reservations in how the EFs (and CFs and TTFs) were ultimately used to translate from the draft fish egg/ovary Se criterion to water Se criteria.

Potential sources of additional EF data may include:

Bowie GL, Sanders JG, Riedel GF, Gilmour CC, Breitburg DL, Cutter GA, Porcella DB. 1996. Assessing selenium cycling and accumulation in aquatic ecosystems. Water Air Soil Pollut 90:93-104.

Casey R. 2005. Results of aquatic studies in the McLeod and Upper Smoky River systems. Alberta Environment. 64 pp.

Fan TW-M, Swee JT, Hinton DE, Higashi RM. 2002. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Aquat Toxicol 57:65-84.

Greater Yellowstone Coalition. 2005. Technical Reports on selenium concentrations in water, macrophytes, macroinvertebrates, and fish.

Hamilton SJ, Buhl KJ. 2003a. Selenium and other trace elements in water, sediment, aquatic plants, aquatic invertebrates, and fish from streams in southeastern Idaho near phosphate mining operations: September 2000. US Geological Survey.</td><td>To reduce uncertainty in estimating site-specific EF values, EPA limited its analysis to those aquatic sites with at least two particulate selenium <u>measurements</u> with corresponding water column measurements, and only used sediment measurements if there was at least one other measurement from either algae or detritus. That is, EPA would calculate an EF from algae (or detritus) alone; however, in order to qualify, at least 2 algal (or detritus) samples from a site were required. In contrast, sediment data alone were insufficient to calculate an EF. In order for sediment to be included, additional algal or detrital data were also required.

Thank you for providing additional potential data for EF determination. EPA added the data from Bowie et al 1996, Casey 2005, Fan et al 2002, McDonald and Strosher 1998 and Zhang and Moore 1996 to the EF data set. EPA already had Minnow Environmental 2007 in the EF data set which includes the data contained in Orr et al 2006.</td></tr>
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64 pp. Hamilton SJ, Buhl KJ. 2003b. Selenium and other trace elements in water, sediment, aquatic plants, aquatic invertebrates, and fish from streams in southeastern Idaho near phosphate mining operations: May 2001. US Geological Survey. 61 pp. Hamilton SJ, Buhl KJ, Lamothe PJ. 2002. Selenium and other trace elements in water, sediment, aquatic plants, aquatic invertebrates, and fish from streams in southeastern Idaho near phosphate mining operations: June 2000. USGS, Yankton, SD and Denver, CO. 72 pp. McDonald LE, Strosher MM. 1998. Selenium mobilization from surface coal mining in the Elk River basin, British Columbia: A survey of water, sediment and biota. Ministry of Environment, Land and Parks, Cranbrook, BC. 46 pp. + appendices. Orr PL, Guiguer KP, Russel CK. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotoxicol Environ Saf 63:175-188. Orr PL, Wiramanaden CIE, Paine MD, Franklin W, Fraser C. 2012. Food chain model based on field data to predict westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) ovary selenium concentrations from water selenium concentrations in the Elk Valley, British Columbia. Environ Toxicol Chem 31:672-680. Presser TS, Luoma SN. 2009. Modeling of selenium for the San Diego Creek watershed and Newport Bay, California. US Geological Survey, Open-File Report 2009-1114. 48 pp. Zhang Y, Moore JN. 1996. Selenium fractionation and	

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speciation in a wetland system. Environ Sci Technol 30:2613-2619.	
4. See my response to question 2. RE - I am more familiar with the marine literature and am not well-versed in the freshwater literature regarding Se TTF values.	Thank you for your comment.
5. EF values were derived from all available data that I am aware of and used scientifically valid approaches, including inclusion/exclusion criteria. See comments above regarding the simple distinction used for lentic vs. lotic systems.	Thank you for your comment. EPA revised the lotic and lentic section to better identify the difference between lotic and lentic in Section 3.2.4. Unfortunately, a parameter like residence time was not available for most of the waters in the dataset.
6. The enrichment factor (EF) approach and method is scientifically defensible and represents our best understanding of selenium dynamics in aquatic ecosystems. While all modeling approaches have uncertainties and limits in application, the approach is reasonable, transparent, appropriately applied and representative of the present selenium knowledge base. The criterion document uses available data in a consistent manner, and extending the water system terminology used by study authors for data used in EF value determinations is a best practice. The evaluation of categories of aquatic systems is well treated in the analysis. The grouping of streams, drains, washes and creeks into a common category is reasonable. The results of Figure 9 and 10, and furthermore in Figure 11, help to validate the EF approach of the criterion document when measured against our cumulative knowledge base of selenium behavior in different aquatic systems. The use of a 20^{th} percentile approach for water column values accommodates system variability and system uncertainty that is inherent in all modeling approaches. Whereas tissue levels of Se can more reliably predict toxic risk,	Thank you for your comment.

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a 20th percentile affords adequate protection in many risk management situations such as water quality-based effluent limits, especially in light of the primacy of the tissue based components of the criterion.	
7. Derivation of Enrichment Factors (EF) based on paired concentrations of selenium determined in water and particulate would have been influenced by the practice of allowing data to be paired if they were collected up to a year apart. In terms of application of EF to categories for lentic and lotic systems it is difficult to judge because of the lack of specific criteria to distinguish between the two types of systems in the Draft Document. While the US-EPA acknowledges the importance of residence time for defining aquatic systems as either lentic or lotic, the criterion for their initial assignment to each category is not apparent (Page 82). Despite statistical comparisons that support their aggregation, it is very likely that lakes, reservoirs, ponds and marshes will have vastly different selenium kinetics, and yet they are all designated as lentic systems. Likewise, selenium uptake into aquatic food-webs of creeks, drains, washes, rivers and streams may differ markedly. The wide range of variability in the aggregated categories (Figure 10, page 84) is compelling evidence in support of this point. Additional specific guidance is required to distinguish between the two types of aquatic systems and the applicability of EFs for each.	Thank you for your comment. As discussed above, EPA has rewritten the description of lotic and lentic sites and has investigated each site individually since a common delineation parameter such as residence time was not available for most of the waters for which data were available in the study.

5. Please comment on the scientific defensibility of EPA's conversion of the selenium fish tissue – water translation equation into an equation that allows for calculation of a criterion for waters that may be subject to intermittent discharges of selenium. Please comment on major sources of uncertainty in this approach. Is this method appropriate, given the bioaccumulative nature of selenium? Please comment on the uncertainty associated with the application of this conversion equation to intermittent discharges that may occur in different types of waterbodies and/or in different locations, particularly with respect to loads transported to potentially more sensitive aquatic systems. Does the method employed result in criteria that are similarly protective to the 30-day chronic criterion? Are there any other models or approaches that EPA should consider that would reduce this uncertainty? Please provide detailed comments.

External Peer Reviewers' Comments	EPA Response
1. EPA's proposed method for addressing intermittent and time-varying discharges appears reasonable given available data. Ideally, intermittent criteria would be based on a biokinetic modeling approach and EPA's effort to evaluate their proposed approach using biokinetic modeling is encouraging. However, given the limited biokinetic data currently available, it is probably premature to implement such an approach for setting WQC. Further use of such an approach may be unnecessarily complicated if the simpler approach proposed by EPA continues to achieve the same objective as the biokinetic approach. A major uncertainty in the approach and subsequent biokinetic evaluation is the near complete lack of kinetic data for EF. If depuration kinetics are slower than EPA has assumed for primary producers, then this will have significant impacts on the validity of this approach. The issue of generating pulse loads of Se that may ultimately result in Se accumulation in sensitive downstream systems (e.g., pulse loads in a river that discharges to a wetland) is a legitimate concern. However, in my opinion, this is a site-specific issue and it is not reasonable to establish national WQC that ensure protection of these sites without dramatically increasing the false	Thank you for your comment. The intermittent criterion elements were developed to address intermittent elevated input scenarios that could result in chronic effects via bioaccumulation in an ecosystem, effects that may not be captured through assessing impacts considering only continuous 30-day exposure scenarios. See the response to comment #3 for the relationship between the intermittent criterion and the kinetic analysis of the 2014 Appendix G. EPA has since revised the 2014 Appendix G (now Appendix J) kinetic model to include a water-TL1 step. .

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positive rate for the WQC. However, it would be useful for EPA to provide specific language on the need to consider loading to downstream environments when regulating intermittent discharges or developing site-specific WQC.	
2. If a realistic concentration can be established using a more appropriate modeling approach (as above), then the calculation for intermittent discharges is fine. However, the propagation of errors must be carefully evaluated.	Thank you for your comment. EPA provided further discussion of uncertainty in Appendix J, section 3 of the 2015 draft criterion document.
3. I am not sure that the criterion equation for intermittent dischargers is meaningful, as it is basically a mathematical manipulation and does not in any way account for selenium uptake and elimination kinetics. An alternative approach that the EPA may want to consider is based on biokinetic modeling, such as that described in Brix and DeForest (2008). The method they described was based on modeling of a food chain comprised of periphyton, an invertebrate (mayfly), and a fish (fathead minnow). Inputs to the model include the background water Se concentration, the magnitude of an intermittent Se pulse, and the duration of the Se pulse. This provides a tool for evaluating whether a Se pulse of a given magnitude and duration could result in exceedance of a whole-body fish Se criterion, or short-term Se criteria could be derived for given short-term durations. For a comparison of the biokinetic-based approach to the intermittent criterion equation in the draft AWQC document, I assumed that the background water Se concentration is 1 µg/L, the lotic criterion is 4.8 µg/L, and the number of days elevated is 4. The intermittent criterion would be 29.5 µg/L. Just as an example, if a lotic food chain consisting of periphyton→mayflies→fathead minnows were assumed, a 4-d	Regarding the comment on the biokinetic approach, EPA's kinetic analysis was presented in Appendix G of the 2014 draft. It was similar to the work of Brix and DeForest (2008). It has since been revised to make it even more similar in structure to Brix and DeForest (2008). It continues to have a similar response time as Brix and DeForest because the limiting kinetic rate used in both Brix and DeForest and the 2014 Appendix G is based on the same fathead minnow study as referred to in the comment: Bertram and Brooks (1986). Regarding the biokinetic model and intermittent criteria, the kinetic analysis of Appendix G of the 2014 draft specifically addressed intermittent exposure. When applied to intermittent exposure, the analysis demonstrated the protectiveness of the 30-day averaging period that EPA is recommending. The intermittent criterion is a rearrangement of the water chronic criterion and its kinetic model-based 30-day averaging period. The 2014 intermittent criterion thus hinged on the kinetic model

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pulse of 29.5 µg Se/L would not be nearly sufficient to reach a whole body Se concentration of 8.1 mg/kg dw (Fig. 2). There is a rapid increase in predicted Se concentrations in periphyton and mayflies and then a rapid elimination, but uptake is slower in fathead minnows. In my opinion, a biokinetic-based modeling approach would be more appropriate for deriving acute or intermittent water Se criteria. Fig. 2. Example of modeled selenium uptake and elimination in a model food chain exposed to a 4-d pulse of 29.5 µg Se/L. Literature cited: Brix KV, DeForest DK. 2008. Selenium. Pages 123-172 in Gensemer RW, Meyerhoff RD, Ramage KJ, Curely EF, eds. Relevance of ambient water quality criteria in ephemeral and effluent-dependent watercourses of the arid western US. SETAC Press, Pensacola, FL, USA.	of the 2014 Appendix G. In the revised document Appendix G is now Appendix J.

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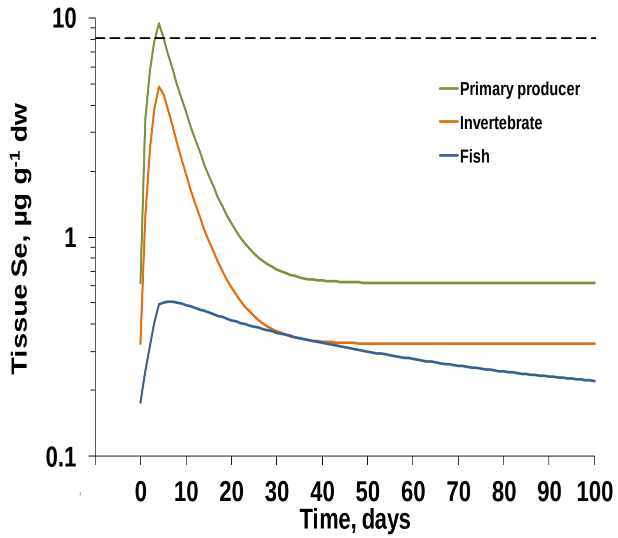	
4. See my response to question 1 of Part III.	See response provided above in Part III, Question 1, Reviewer 4.
5. EF values were derived from all available data that I am aware of and used scientifically valid approaches, including inclusion/exclusion criteria. See comments above regarding the simple distinction used for lentic vs. lotic systems.	Thank you for your comment.
6. While the need for a criterion tier that addresses intermittent discharges is clear, this part of the document is not well documented for scientific support as evidenced in the main document by no citations in this section beyond that of the general Chapman et al. 2009 reference. Appendix G Part 3.0 documents the modeling approach, however a list of references is missing. Since this is original work, further description of methods, key data inputs, and model run output may be useful	The document's 86-page list of references contains all citations used in the main text and the appendices. The bioaccumulation modeling approach is rather standard and is similar to Brix and DeForest (2008) referred to elsewhere in comments. The primary difference is that in EPA's application, all concentrations have been normalized to their criterion (in water and TL3) or corresponding benchmarks (in TL1 and TL2). Appendix J presents the model equations and input values.

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for potential replication of the results by others. A citation on page G-6 (EPA 1986; should be USEPA 1986) may be important to sourcing this modeling approach, but it is unclear in the writing whether this is so; without references to Appendix G, validation of scientific defensibility of the intermittent water-column criterion is not possible. Infrequently, some of the writing in Appendix G is informal or tech-speak and should be edited for clarity. Figure captions should contain a short description of all relevant model inputs to increase communication value and transparency. The modeling approach and the results of Appendix G appear to be a reasonable and defendable approach to developing a criterion for intermittent water column selenium values, although the polished execution of this important part of the tiered criterion is lacking in comparison to the other criterion elements. Thus, there appears to be sufficient support for the criterion approach in Appendix G and this information should be summarized and referenced in the main document body. This part of the tiered criterion is the most difficult to study in the field, although our practical and experiential knowledge of Se bioaccumulation in aquatic ecosystems suggests it has high importance in protecting aquatic life. The practical implementation of this tier of the criterion will require enhanced guidance and regulatory sensitivity to the cost of monitoring.	Appendix J uses a single set of kinetic parameters (explained in the text). Its different figures address different assumptions about the time series of water concentrations. In the 2015 draft, the Appendix J (replacing Appendix G in the 2014 draft document) has been modified, edited, and simplified..
7. It is not clear how the intermittent criterion outlined in the Draft Document will be applied. The mathematical expression of the criteria on page 93 is clear but the terms surrounding the application of the criterion are not. For example, the criterion is not intended to apply to "ordinary smoothly varying concentrations" (Page 94). However, what specifically will	The document's language about the intermittent criterion not being intended to apply to ordinary smoothly varying concentrations has been deleted. The intermittent criterion provides the same protection as the 30-day chronic criterion (from which it is derived). Whereas the 30-day chronic expresses the criterion in terms of one average of all

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constitute a discharge curve that is not "smooth" has not been defined. It is also not clear what magnitude of selenium concentration spikes would designate a discharge as having to be regulated as an intermittent discharge. Finally, designation of an intermittent criterion appears to contradict the data in Appendix G and the statement on page 94 that "kinetics of selenium accumulation and depuration are sufficiently slow that attainment of the water criterion concentration element by ambient 30 day averages will protect sensitive aquatic life species even where concentrations exhibit a high degree of variability. While outside the area of our expertise it is noted that several comments in the public registry suggest that a biokinetic model may be more appropriate than the application of an expansion of the 30-day average calculation for determining intermittent criterion.	concentrations occurring within a 30-day period, the intermittent criterion applies to two averages, occurring for two different durations within the 30-day period, one labeled the intermittent and one labeled background. The intermittent criterion will achieve its purpose no matter where the line is drawn between intermittent concentration and background concentration. The intermittent criterion elements were developed to address intermittent elevated input scenarios that could result in chronic effects via bioaccumulation in an ecosystem, effects that may not be captured when considering only continuous 30-day exposure scenarios. The kinetic model of Appendix G in the 2014 draft was used to derive the 30-day averaging period. It specifically evaluated intermittent exposure. The validity of the intermittent criterion thus hinges on the validity of the derivation of the chronic water criterion and the Appendix G (now Appendix J in the revised document) kinetic model derivation of the 30-day averaging period.

PART IV: SIGNIFICANCE OF SCIENTIFIC VIEWS FROM THE PUBLIC/STAKEHOLDERS

EPA will also be providing scientific views and other comments from stakeholders and the public received via the public docket to the peer review panel. Although EPA will be providing the full contents from the docket, EPA is only requesting a review of any scientific views/public comments that may be of technical significance to the selenium criterion.

1. Has the peer review panel identified any scientific views from the public or stakeholders as being technically significant to the draft of the selenium criterion going forward; that is, has information or data been introduced during the comment period that would change the scientific direction of the criterion? Is there any information or data that may refine or enhance the scientific defensibility of this criterion that EPA should consider further? Please provide detailed comments on specific issues of technical significance or refinement.

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1. After reviewing the public/stakeholder comments, I highlight the following comments which I would also make above and beyond responses to specific review questions EPA has asked: 1.) Because some states will continue to use an acute WQC for Se, I agree EPA needs to clarify its position on the scientific credibility of the existing acute WQC. 2.) There were a number of comments indicating that use of an instantaneous averaging period and "never to exceed" for the tissue element is inappropriate and inconsistent with the Guidelines.I disagree with these comments and support EPA's decision. 3.) I agree with several commenters that EPA must develop rigorous definitions of lentic and lotic as guidance for regulators. 4.) EPA needs to provide some guidance on how small first order and ephemeral streams that naturally do not support	Thank you for considering the public comments and providing your feedback. In response to your points 1-4, EPA provides the following: 1. EPA has indicated that acute toxicity is not included in the assessment (Section 2.6 and Section 2.7.3). Some reviewers have stated that the intermittent criterion may serve as a surrogate for the acute criterion. 2. Thank you for your support – additional information is being developed regarding frequency and duration as it relates to application of the criterion. 3. EPA has revised the lentic and lotic sections of the criterion document, and has investigated each waterbody/sample used in the criterion derivation.. 4. EPA is currently working on developing technical information that will help in the implementation of the Se criteria.

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fish populations should be regulated. There are a large number of these streams in the western US that have Se issues. Note, in these types of systems or in small wetland systems without fish, aquatic-dependent birds may be the most sensitive receptor. This leads to the obvious comment that if this WQC is intended to protect all US surface waters, EPA must develop guidance on the protection of aquatic-dependent wildlife.	
2. I examined the public comments AFTER I had reviewed the document and written the above comments, so as to not bias my own evaluation. The comments (by my count, 429) ranged from editorial ones, to simple criticisms, to detailed scientific evaluations and suggestions. Of the later, the most common concerned "implementation" (16% of total), followed by "translation" (to water column criteria; 14%), and site specific criteria (13%). If we combine all the "criteria" comments (site, tiered, tissue, intermittent), these received the most comments (30%). Of these, most dealt with the details of developing the criteria (justifying the calculation methods, literature missed, apparent oversights or conflicts with existing procedures). Thus, the peer-review community (it seems that most of these comments came from consulting companies, municipal and state agency scientists, and some from the academic sector) feels the document needs considerable attention to reformulating the criteria. The next most important topic was then implementing the criteria (16% by itself) and in this respect most comments (actually criticisms) were directed to the water column formulation. Related to this was the "translation" of the tissues (all)-based criteria to the water column (14% of comments), and most of these comments were directed to the inappropriate use	Thank you for considering the public comments and providing your feedback. EPA disagrees with the peer reviewer's view that the public comments indicate that the previously peer-reviewed and published methodology (Presser and Luoma, 2010) applied by EPA to derive the criteria needs to be reformulated. EPA worked with the scientists who published the methodology in applying their model in the context of a national criterion development to ensure appropriate application of their model. As acknowledged in the comment, most public comments dealt with specific issues with specific data. EPA has responded by correcting, modifying, or adding specific items that went into the criterion derivation. Further, none of the other six peer reviewers has recommended or indicated that EPA needs to reformulate the methodology used for setting water column criteria. EPA has made revisions to several sections of the document in order to further support the existing defensibility of the science

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of the Presser and Luoma model. Considering my review above and the community response, it would seem that the EPA needs to reformulate their methodology for setting water column criteria.	used, and also to help clarify and reduce some of the uncertainty related to the criterion and supporting data. EPA develops criteria that are national in scope, based on available scientifically-defensible data and approaches. EPA describes the appropriate use of the Presser and Luoma model in the criteria document. EPA realizes that the nature of selenium bioaccumulation may be effectively reflected through the development of site-specific criteria. Other public comments that reflect implementation issues are beyond the scope of the scientific criterion document and will be addressed in technical support documents under development by EPA. In response to comments in Part III, Question 1, Items 1 and 2, a description of preferability of the approach EPA used is provided, including, why the reviewer's recommendation to use a kinetic-based model in place of EPA's EF database would not strengthen the national applicability of the criterion because it would restrict analyses to a small a database. In Part III, Question 2, Item 3, EPA addressed another model issue, its linearity (implying first-order kinetics), and how the available data support such a formulation. Nevertheless, in conclusion, EPA encourages the development of site-specific criteria at sites with high quality, robust databases indicating the appropriateness of alternate model assumptions or formulations.
3. A substantial number of comments from stakeholders and the public were provided. These comments covered a large variety of topics and were often conflicting. I did not identify any comments that would lead me to think that the scientific direction of the criterion should be changed. The comments	Thank you for considering the public comments and providing your feedback. EPA, in a parallel process to the criterion development, is working to develop technical information that will assist in the implementation of the selenium criteria. EPA notes that several reviewers viewed the intermittent criterion

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relative to interpretation of toxicity studies and derivation of EC10 values should all be carefully reviewed by the EPA, as some suggested that certain EC10 values should be lowered and other suggested they should be raised (although I personally believe that the GMCVs values derived by the EPA were generally conservative, especially for *Salmo* and *Lepomis*). Aside from the technical comments and disagreements that are related to magnitudes of the various Se criterion elements, it appears that there is a desire (or need) for the EPA to more clearly define how the draft Se criteria should be implemented by the states. Perhaps case studies could be provided as examples? It is also apparent that the basis of the intermittent criterion, and its relationship to an acute criterion (if there is a relationship), needs to be more clearly explained. Although some comments seem to agree that an acute Se criteria is not necessary any longer, there does still appear to be a need for acute Se criteria from the perspectives of certain states. Finally, again related to implementation, is the question of whether the lotic and lentic water Se criteria can be replaced by a different metric, such as residence time. In my opinion, the latter would be worthy of further consideration by the EPA, although I wonder whether more reliable categories could be developed based on existing datasets.	element as a reasonable surrogate for an acute criterion for protecting aquatic ecosystems, particularly downstream lentic waterbodies, from the effects of intermittent discharges of selenium. EPA has also further examined the lentic/lotic classification issue, and has evaluated each site used in the criterion development individually to ensure it was not mischaracterized. Unfortunately, residence time was not a common metric available in the available studies.
4. Some of the comments made about acute toxicity are valid, but are unlikely to be relevant to most real-world situations. Note that acute toxicity can affect other than reproduction, but such effects are rarely seen (I think).	Thank you for considering the public comments and providing your feedback.
5. I have read through the entire package of views from public and stakeholders, not just the summarized Excel file but the	Thank you for considering the public comments and providing your feedback.

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actual documents, some of which are >100 pages. The EPA should pay close attention to these documents, since some excellent scientific issues are raised in many of them. It is good to see that there presently exists such good knowledge of the aquatic ecotoxicology of Se among stakeholders; 10 years ago this would not be true. The public/stakeholder views represent the classic range, from industry-based opinions that the proposed criteria are too conservative, to conservation group-based opinions that the proposed criteria will not protect all aquatic life. Both sides of the argument present many good points that should be considered carefully by the EPA. I will provide my views on each category of public/stakeholder comments at the end of this section.	The EPA uses the best available science in its development of Water Quality Criteria (WQC); the Agency is also bound to rigorous data quality standards under the Data Quality Act. The EPA has Guidelines for the use of toxicity data in its derivation of WQC for the protection of aquatic life and its uses, and has followed those guidelines in the derivation of the selenium criterion. The nature of selenium bioaccumulation may vary on a site-by-site basis in a manner sufficient to justify development of site-specific criteria. The reviewer identifies several important issues that will be addressed in the technical information to be developed to guide the implementation of the criteria.
The bottom line is that industry would prefer the egg/ovary criterion to be about 20 mg/kg egg (or greater), whereas conservation groups would prefer it to be about 10 mg/kg egg (or lower). Perhaps the 15.2 mg/kg criterion represents a workable compromise between these two extremes? I believe the EPA document for the most part has used current, scientifically sound approaches without significant bias in either direction (but see my comments regarding the Formation brown trout study). Since the proposed EPA criteria would still allow some aspect of site-specific assessment at the State level, then there could be modifications based on site specific issues such as relatively high background [Se] in certain areas, fish species not included in derivation of the egg/ovary criterion, lack of fish species ("fishless" waters), high aqueous sulfate, the presence of listed/threatened/endangered fish species, the presence of critical aquatic-dependent wildlife such as birds, or other	Regarding public concerns pertaining to the Formation brown trout, the study data were re-evaluated. Ultimately, EPA determined that the model instabilities resulting in uncertainty surrounding the EC10 value, such as multiple minima for the optimistic and worst-case deformity endpoint, were the result of high variability in deformities at low selenium concentrations, as well as the assumptions associated with how to interpret the health and survival of fry lost to overflow caused by a clogged drain during a 15-day post swim-up feeding trial. Most maternal transfer studies do not include a post swim-up test, and for this test, the uncertainty introduced by the laboratory accident was concluded to outweigh the additional information gained by extending the test. EPA performed an analysis, described in detail in both Section 6 and Appendix C, showing that there was no relationship between fish health and whether or not a given fish was lost to the overflow event. EPA recalculated the EC10

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biological/chemical/physical factors. Specific comments on public/stakeholder documents: An acute criterion is not needed and is not relevant. If you are releasing Se into the aquatic environment at levels that cause acute toxicity to fish, then you have a big problem! Lentic and lotic systems must be clearly defined and perhaps a more quantitative approach should be used as I have discussed above. The EPA should read the public/stakeholder input carefully and use these suggestions to come to a final decision on the Formation brown trout study. This is of critical importance since brown trout was found to be the most sensitive fish species and the egg/ovary criteria is driven largely by the brown trout EC10. Elevated sulfate ion in aquatic systems may reduce Se bioaccumulation in food webs by competing with selenate for uptake by primary producers, particularly algae. However, if regulatory limits are based on fish tissue [Se] then any modification of Se uptake by primary producers will be reflected in fish tissue [Se]. In my opinion sulfate is not really a regulatory issue when fish tissue [Se] is used. Ideally freshwater criteria for Se should include aquatic-dependent wildlife such as birds. However this makes the Se criteria more complicated than perhaps it needs to be. The issue of birds could be considered on a site-specific basis in certain ecosystems inhabited by ecologically significant avian populations and migrating water birds. The EPA must provide guidance on several aspects related to implementation of the tiered criteria approach, at the very least	(18.09 mg/kg dw) for larval survival for the hatch through swim up portion of the test, and this recalculation is reflected in the 2015 draft selenium criterion document. Regarding risk to aquatic dependent wildlife, EPA understands the potential for risk to birds from selenium exposure and has begun to investigate the potential for a national criteria that would protect aquatic-dependent wildlife. Regarding key issues pertaining to fish-tissue sampling, EPA is developing technical information related to fish-tissue sampling, as we realize this is a complex but important subject. Regarding the zebrafish studies, EPA has evaluated the available zebrafish data and identified several important issues with the quantitative use of those data. This information is included and fully discussed in the revised 2015 draft criterion document. This issue was also highlighted in the FR Notice in July 2015 with a request for additional data on zebrafish and other cyprinids. A major problem is that the concentration-response curve was so unusually shallow that zebrafish could be interpreted to be among the most or least sensitive species depending on the level of effect considered. Further, high control mortality (47%) at the end of the study raised concerns about the health of the fish at the time of testing. In addition, since the zebrafish is a non-native cyprinid species, EPA assessed the information available on zebrafish sensitivity to selenium compared to the sensitivity of native cyprinid

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including (a) when to sample fish so that females are in vitellogenic or pre-ovulatory stages of oogenesis, (b) what sample size of fish to collect for tissue [Se] determinations (I suggest a minimum of n=10 female fish per site), (c) recommended analytical procedures for quantification of Se, (d) guidelines for implementation of the 30-day average water column criterion element (how, when, where), and (e) guidelines for implementation of the intermittent water column criterion, if the EPA chooses to keep it in the tiered criterion. An interesting comment made in one of the public/stakeholder documents (US Fish and Wildlife Service, document 354-A2)) regards the use of recently published studies in zebrafish, a non-native cyprinid, in the species sensitivity distribution for larval deformities as a function of egg [Se]. They present a compelling argument to consider these data in the criterion development.	(minnow) species across the United States (Appendix D in the criteria document), including several studies where native cyprinids were investigated in selenium-impacted waters. Data from these studies suggest that native cyprinids are likely less sensitive to selenium than the currently available non-native zebrafish data suggest. The results of these analyses, particularly a comparison of the concentration response relationships of zebrafish versus all of the other fish species for which we have similar data, raises a concern. Given these concerns, EPA has not used the zebrafish data quantitatively in the derivation of the revised criterion. As noted above, EPA is seeks additional information on cyprinid taxa sensitivity to selenium, and particularly additional data on zebrafish, and solicited such information in the Federal Register Notice announcing the opening of the public comment period on the selenium draft criterion in July 2015. Such studies should be submitted to the docket in similar fashion as scientific views on the criterion document. EPA will then consider this information in finalizing the selenium criterion document.
6. Acute criterion: The comments largely support or request guidance concerning abandonment of an acute criterion. The intermittent water column tier of the draft selenium criterion	Thank you for your comments.

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does much to address potential ecosystem impact potential from discharge concentrations historically regarded as having "acute" toxic potential. Alternative more sensitive endpoint: Comment lacks clarity and method/approach publication or peer-review to fully consider the point being made. Aquatic dependent wildlife: Sound points are made concerning the potential for impact to aquatic birds. The author overstates that the criterion set a *de facto* limit for invertebrates. While the comments are broadly valid and demonstrate the complexity of the Se aquatic impact issue, equal concerns should be weighed on the relative balance of over- or under- protection of the draft criteria if deployed. The rigor of this present document to address aquatic life ambient water quality is significant, broadly inclusive and broadly defendable. The tier approach may be expected to have significant impact in overall water quality and aquatic dependent wildlife because of the integrative exposure nature of the tissue criterion. Averaging period: Comment reasonably addresses the need for clearer implementation guidance of the intermittent water column criterion. Bioaccumulation factors: The context of this question is addressed in the document, however additional clarification may be useful. Biphasic modeling: The comment author expresses an opinion regarding the modeling approach. The available peer-reviewed published studies supporting this approach for selenium in fish/aquatic ecosystems are limited and thus of less value in setting the criterion. The author may have a good point however	EPA agrees with your comment regarding the potential surrogacy of the intermittent criterion element for the acute criterion. EPA is currently investigating the potential for a national criteria for aquatic-dependent wildlife. EPA is developing information to assist in the implementation of this and other criterion aspects. Regarding comments on the Formation brown trout study, EPA has reviewed the comments from the public and other peer reviewers on the brown trout study. This resulted in a re-analysis of the available data and a revision of the EC10 from 15.9 to 18.1 based on larval mortality only from hatch to day 15 and excluded data beyond the lab overflow accident, which was a major confounder. Regarding the CF (and TTF) methodologies, EPA has revised its CF derivation methodology and uses taxonomic proximity to make it as species-specific as the data will allow. (See response to the comment of Peer Reviewer 2 to Part I, Question 2.a.iii for details.) EPA has decided to retain the use of the median rather than a higher percentile as the general range of CFs is relatively narrow with the exception of the mountain whitefish, so use of a higher percentile threshold would allow the CF to be unduly influenced by this species. (See 2015 draft, page 72.)

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the availability of published work limits its practical consideration. The Atlantic salmon graph referenced appears to be a Wikipedia selenium entry without attribution. Bluegill Hermanutz: The conclusion that the Hermanutz data are outliers is not supported in the comment by any numerical/statistical analysis and thus must be treated as opinion, unless otherwise verified. Data variability in biological systems can be tested to determine outliers however it is unlikely the data count would support exclusion, thus inclusion is more defendable. Brown trout study: The presentation and role of the brown trout study, related serial reviews, and re-reviews in the draft criterion document and supporting resources raises questions in the public comments. While some of the questions addressed in public comments are broadly addressed in the draft document, additional effort should be made by EPA to specifically address concerns outlined in these comments. The use of the study data is confounded by unfortunate experimental system failure encountered during the study. Clarification: The comment authors state reasonable requests for clarification that can be addressed in the main text body. Conversion factors: Several of the public comments regarding conversion factors represent valid concerns. Some of the issues are addressed in the draft document and thus additional explanation could be useful. The suggested approach of using species specific CFs and determining a 80 or 90th percentile cut is a solid suggestion for an alternative approach. Correction: These should be validated and corrected.	Regarding consideration of new data, EPA has acquired new data through the peer review and public process and has included these data, as appropriate, in the derivation of the criterion. Regarding endangered species, EPA recognizes the concern regarding protection of listed species. EPA has considered and described available data on impacts of selenium on endangered species in the criterion document as well as impacts on species that are close phylogenetic surrogates of listed species dataset. EPA notes that site-specific criteria can be developed if sensitive endangered species not represented by surrogates in the dataset used in the criterion are present at a specific site. Regarding the concerns over lentic/lotic, MDRs, and mayfly data, EPA has addressed the issues of lentic/lotic waters, mayfly, new information, Minimum Data Requirements (MDRS), particularly the invertebrates issue and several other concerns pointed out by the public and other reviewers in the revised 2015 draft criterion document and above. Regarding comments on the tissue derivation procedures, EPA has reviewed the approaches for both the derivation of the muscle as well as the whole body criteria elements and modified the criterion document, as appropriate

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Criteria are over-protective: these are speculative comments. Criteria are under-protective: There are valid concerns expressed, especially in the apparent disconnection between agencies working towards similar goals. Concerns over the water column tier of the criteria are adequately addressed by the primacy of the tissue tiers. The risk differentiation argued between 4-6 mg/kg and 8.1 whole body/muscle tissue selenium, in light of the egg-ovary tissue primacy in the draft criterion, is moot. Data analysis: This comment should be explored for its validity. Data paucity affecting criterion: This comment appears to somewhat understate the available data. An additional literature search may yield new studies that increase egg-ovary data counts. Define terms in document: Solid points are made to enhance clarity. Dietary requirements of Se in fish: The identified citations are of value. Document process: No comment. EC10 clarification: Editing error identified; requires correction. Endangered species protection: This process observation should be considered. Exclude invertebrates: Risk assessment using extrapolations from animal models is a keystone of toxicology. The approach in the document is a modeling effort based on a similar extrapolation of available data. While not perfect, the data have	Regarding concerns over specific studies, See previous responses starting on page 33 of this document regarding the Hermanutz bluegill study, Hardy cutthroat trout study, inclusion of Doroshov catfish study, and zebrafish study. Regarding the clarity of the criterion as a freshwater criterion, EPA provides clarification in the 2015 draft document, but the document is clearly labeled freshwater, and the data included in the criteria are all freshwater or anadromous/diadromous fish that have a significant life history in freshwater. Regarding implementation issues, EPA is addressing all of the issues mentioned (as well as others) in its development of technical support documents that will aid in the implementation of the selenium criterion. Regarding the translation procedure, EPA has addressed the public and reviewer comments on the translation of the fish tissue criterion to water, both in this document as responses to the charge question comments specific to translation. EPA has also addressed the water column value issues to the extent that the available data allow. Regarding criteria to protect birds, EPA is currently investigating the potential for a national criteria for aquatic-dependent wildlife. (See response to peer reviewer 5 regarding this issue.)

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value. General comments: Many opinions expressed. Sulfate impacts can be argued to be adequately incorporated into the primacy of the egg-ovary criterion. GMCV alternative: There are several useful comments, including apparently revised data that should be addressed. Human health: This comment contains information useful in addressing human health implications of the draft criterion. Implementation: The public comments express thoughtful concerns and practical implementation questions that can serve as prompts to draft additional guidance. Importance of Se speciation: The comment expresses academically valid concerns however the practicality and data quality issues of speciated Se analyses for routine sampling and monitoring discount this concern. There are additional confounding issues of analytical sensitivity and result uncertainty at the criterion levels. Total dissolved Se sampling will filter out selenite that is readily adsorbed to suspended sediment particles. Intermittent criterion: Several good points are raised in the public comments. Suggestions to abandon one model for another do not provide adequate support for the suggestion. Practical implementation concerns are valid and should be addressed. Lentic lotic clarification: The public comments express thoughtful concerns and practical questions that can serve as prompts to draft additional guidance and supporting information. Mayfly toxicity: This study should be reviewed for inclusion.	

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Mercury interaction: This observation is not unequivocal in the scientific literature and thus does not require significant consideration in criterion development. Misunderstanding of MDRs: Some points are valid, however the practice of extrapolating and translating data is commonplace in toxicology. Mode of action: The authors correctly identify an oversimplification of the wording in the draft criterion document. Natural background: The public comments correctly identify concerns of naturally occurring selenium contamination of waters and impacted aquatic life. The draft criterion should explicitly address these concerns in regards to implementation of the draft criterion. New information: Some of the submitted information has value and should be considered for inclusion. Sulfate modification to selenium impacts are addressed in the primacy of the egg-ovary criterion which reasonably characterizes endpoint risk regardless of modified uptake. Number of GMCVs in data set: Draft text should be modified to address clarification. Other comments: Most labs report 2 significant figures for water Se analysis at these levels. Rainbow trout study clarification: Clarifying language should be added to the draft text. Recommend other studies: These studies should be reviewed for inclusion in the data set.	

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Recommended modifications: This is a summary state of previous suggestions in the list. Data updates once validated are reasonable requests. Recommended muscle criterion: The approach should be critically reviewed. Recommended research: While interesting, the method is not used in all studies. Citable references are absent from the comment. Recommended whole body criterion: The approach should be critically reviewed. Recommends alternative analysis of Hardy cutthroat trout: The commenter's calculation lacks peer review and detail. Recommends alternative statistical analysis for Hermanutz bluegill: The commenter's calculation lacks peer review and detail. Recommends alternatives to Guidelines SSD: Several practical comments are contained in this collection that can assist in drafting clarifying language and guidance. Recommends including catfish study: The comments are well developed but not necessarily compelling for inclusion, especially in light of previous comments directed at lowering the outcome of the criterion development. Recommends including zebrafish in data set: A sound argument is forwarded to include this new dataset. Requests clarification of GEI fathead minnow analysis and its exclusion: This request can be reasonably addressed in the draft	

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document. Salinity freshwater distinction: Guidance should be included to address these concerns. Se speciation: The comments addressing plant Se speciation are correct in that the draft text is overly simplified and dated in its discussion of plant Se. Mesocosm studies will also adopt a test water that will influence Se speciation and thus similar Se species exposure concerns will be present as will transferability or differential sediment/particulate/container reactivity of Se species in the test system. Site-specific criteria: There are numerous public comments that should be addressed in guidance for implementation. Tiered criteria: There are numerous public comments that should be addressed in guidance for implementation. Tissue criterion: There are numerous public comments that should be addressed in guidance for implementation. Translation: There are numerous public comments that should be addressed in the draft document. Update data set: If practical and possible, this is always a consideration. Water column values: The concerns should be addressed in the draft document text. Wildlife criterion: It is apparent from FWS comments that there is significant concern with the draft criteria potential for protection of aquatic dependent wildlife and fish as well. The pathway for further consideration and development of protection proposed in the draft document appear reasonable to move CWA	

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requirements forward. Winter stress: Comments opine on winter stress exclusion. Ww to dw conversions: The comment should be addressed in the draft criterion text as best as possible. It is unlikely that the variability of WW-DW can be uniformly captured in a standardized approach.	
7. Relevant comments from the public or stakeholders have been acknowledged where they are relevant to the other charge questions above. No further specific issues arising from our review of the public comments are noted.	Thank you for your comment.
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