PEER REVIEW OF THE DRAFT AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA FOR CADMIUM – 2015

PEER REVIEW SUMMARY REPORT

September 30, 2015

Submitted to:
U.S. Environmental Protection Agency
Office of Water, Office of Science and Technology
Health and Ecological Criteria Division
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1.0 INTRODUCTION

This report documents the results of an independent peer review of the *Draft Aquatic Life Ambient Water Quality Criteria (AWQC) for Cadmium – 2015,* developed by the U.S. Environmental Protection Agency (EPA). Eastern Research Group, Inc. (ERG, a contractor to EPA) organized this review and developed this report. Sections 2.1 to 2.4 of this report present, for each charge question, the individual reviewer comments and a summary of those comments; Section 2.5 presents additional minor comments provided by one reviewer. New information (e.g., references) provided by reviewers is presented in Section 3. Appendices A and B provide, respectively, the charge to reviewers and the complete set of comments submitted by each reviewer.

1.1 Background

EPA's Office of Water is charged with protecting ecological integrity and human health from adverse anthropogenic, water-mediated effects, under the purview of the Clean Water Act (CWA) Section 304(a)(l). The Agency has been working to update water quality criteria to protect aquatic life and aquatic-dependent wildlife from the presence of cadmium in freshwater and estuarine/marine environments in order to reflect the latest scientific knowledge.

EPA's AWQC for cadmium presents draft acute and chronic criteria expressed as concentrations of cadmium in fresh and estuarine/marine waters (dissolved). The document represents an update to the draft cadmium water quality criteria that was last published in 2001, and incorporates additional toxicological data, while using the same criteria derivation process that was used in 2001.

1.2 Peer Reviewers

ERG searched for, identified, and selected five reviewers who met the technical selection criteria provided by EPA and who had no conflict of interest in performing this review:

- Kevin V. Brix, Ph.D.; Principal Scientist, EcoTox, and Visiting Scientist, University of Miami
- David Buchwalter, Ph.D.; Associate Professor, Department of Biological Sciences, North Carolina State University
- Nicholas S. Fisher, Ph.D.; Distinguished Professor, School of Marine and Atmospheric Sciences, State University of New York
- Christopher Mebane, B.Sc.; Water Quality Specialist, U.S. Geological Survey
- Daniel Schlenk, Ph.D.; Professor of Environmental Toxicology, Department of Environmental Sciences, University of California, Riverside

ERG provided reviewers with instructions, the review document (including appendices), the charge to reviewers (Appendix A of this report) prepared by EPA, and supporting reference materials as described in the charge. Reviewers worked individually to develop written comments in response to the charge questions. After receiving reviewer comments, ERG summarized reviewers' responses to the charge questions, noting areas of agreement and disagreement, where relevant (see Section 2).

2.0 SUMMARIES ORGANIZED BY CHARGE QUESTION

This section presents summaries of reviewer comments organized by charge question. Each summary is followed by a table presenting the individual reviewer comments by charge question. Individual comments are copied directly from written comments as submitted by each reviewer and presented in Appendix B.

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

All reviewers found the document to be relatively clear. Reviewer 4 noted that it was helpful that the document was organized in a risk assessment format, and also found that the comparisons to previous cadmium criteria documents made the changes in the updated criteria more transparent.

All reviewers noted areas where the document could be improved. Reviewer 4 suggested that EPA consolidate and reduce the many redundancies in the document. Reviewer 5 noted that the Problem Formation section seemed to be a forced fit in the document.

Reviewer 1 commented that the document does not present new ideas or insights. Reviewer 2 mentioned a minor concern – that the document did not include sources of cadmium described in emerging materials – and noted that the document did not discuss sub-lethal effects in the freshwater acute and chronic sections or in the estuarine/marine chronic section. Reviewer 2 also commented that, overall, the uncertainty analysis section should be extended to include aspects of uncertainty associated with the data used for criteria derivation (e.g., uncertainty associated with flow-through versus static exposure values). He also suggested that the document clarify the lack of a standard salinity value to compare toxicity values, since it appears that the most sensitive toxicity value is being used regardless of the salinity.

Reviewer 3 pointed out that there were several places in the document where decisions were made without proper justification and thus appear to be arbitrary. He also questioned EPA's claim that Mebane (2014) concluded that cadmium is unlikely to accumulate in tissue to levels that would result in adverse effects to aquatic invertebrates or fish. Species vary in their abilities to store and sequester cadmium in physiologically inert forms; however, this does not mean that bioaccumulated metals are non-toxic, as is implied by the language in this document.

Reviewer	Comments	EPA Response to Comments
Reviewer 1	This report makes for very dull reading, but it is well-written and it is usually clear what the author is trying to say. There are no insightful comments or new ideas presented in this report, but the report is laid out in a clear, logical fashion.	
Reviewer 2	Overall the document is relatively clear with formatting in a risk assessment format which allows the reader to evaluate each criteria. Of minor concern was the lack of inclusion of emerging materials as sources of cadmium such as quantum dots which do make up photovoltaic substances (mentioned). However, the increased use of these materials as "inorganic" Cd sources and the uncertainties surrounding the potential absorption and effects of these materials to aquatic organisms needs some discussion. In addition, some inconsistencies were noted with regard to sub-lethal effects mentioned in the Estuarine/Marine	

	Acute section. While present in this section, discussions of sublethal effects were largely omitted in the Freshwater sections and chronic sections of both water types. There was also inconsistencies with regard to the use of flow-through vs. static exposures and whether more or less uncertainty is involved in utilization of the values. For example, flow-through methods were stated for *Salmo trutta*, but methods for *Morone* were static or static-renewal. One would clearly suggest the flow through values should be given greater weight with regard to uncertainty assessments. As it reads right now, it appears there are no differences between using static or flow-through exposures. The inability to determine salinity relationships to toxicity is also a concern but it is likely due to varied salinity regimes confounded with temperature and solute constituents in experimental designs (see comments below). It is noteworthy that a 1ppt value is considered "estuarine" for the *Morone* value, when there are "freshwater" systems that likelyhave higher conductance than this value. There should also be some statement or better clarity documenting the lack of a standard salinity value being utilized to compare toxicity values. It appears that the most sensitive toxicity value is being used regardless of the salinity. Overall, the uncertainty analysis section should be extended to include aspects of uncertainty with the data used for the derivation of the criteria. As it stands presently, the emphasis seems to be more on justification of data not utilized for the derivations.	
Reviewer 3	In general, the document language is reasonably clear. However, throughout the document, there are several instances where certain decisions are made that appear to be rather arbitrary without sufficient justification as to how or why these decisions were made (see details below). **Minor comments:** p. 8 and elsewhere: use mass units rather than ppm, ppb etc. p. 9: quantify concentrations found in impaired water ("several micrograms per liter" is vague)	

	p. 10: is the suggestion that precipitated/particulate forms of Cd that ultimately end up in sediments are not bioavailable? p. 19: do data exist for any other salts of Cd that has been excluded? P.63: Please be explicit about how the constants in the equations are derived for both the CMC and CCC. P. 67: Define the values listed under the two tables: (S^2, L, A) **Major comments:** p. 12: "Mebane (2014) conclude that, although there were not adequate data to establish acceptable tissue effects concentrations for aquatic life, <u>cadmium is unlikely to accumulate in tissue to levels that would result in adverse effects to aquatic invertebrates or fish</u>. The evaluation of direct exposure effects is therefore considered to be more applicable to the development of criteria for aquatic life." This line of reasoning is questionable on many levels. Establishing critical tissue effects thresholds that work across species is problematic, especially in invertebrates, because species vary in their abilities to store/sequester Cd in physiologically inert forms. However, this does not mean that bioaccumulated metals are non-toxic as is implied by the language in this document. I think Mebane is being grossly misquoted here (aside from the fact that there is no 2014 reference). Here are some quotes from his 2010 document that directly refute the underlined text above: • "Thus the consequences of elevated tissue residues or effects of dietary exposures may be important when estimating protective thresholds for cadmium and other pollutants (McCarty and Mackay, 1993; Meyer and others, 2005)." P. 32 • "A diet of cadmium-contaminated green algae *Chlorella* sp caused reduced growth in the amphipod *Hyalella azteca* in a recent study (Ball and others, 2006)." P. 38 • "Dietary cadmium exposures appear to be an important risk for at least some invertebrates. The data reviewed on dietary effects of cadmium to invertebrates indicated that adverse effects could	

	occur at concentrations realistic in cadmium-polluted waters". P. 38 • "Toxicity to mayflies from feeding on cadmium-contaminated algal mats at environmentally realistic concentrations was observed (Irving and others, 2003). P. 38 I understand that dealing with dietary exposures is incredibly inconvenient in the context of the 1985 Guidelines, but pretending that they are not important in 2015 is irresponsible because we know better. The Irving et al., 2003 study referenced above provides direct evidence that diet derived Cd can be problematic in this aquatic insect example.	
Reviewer 4	I found the overall clarity of the document to be quite good. I especially appreciated the document being generally organized in a risk assessment format. I think this is very useful, particularly the Problem Formulation section that outlines various sources, potential exposure pathways and receptors. I hope EPA will use this overall structure for future criteria documents as well. I also like all of the comparisons to previous Cd criteria documents. This makes key changes to the criteria very transparent. My only significant criticism of the overall format is that there are a number of redundancies where information is presented multiple times, often the exact same wording (for example, Section 5.4.1 is redundant of earlier text in the document). I encourage EPA to consider consolidating and reducing these redundancies. An additional minor point is that it is unclear how the data tables in the appendices are organized. They don't seem to be listed alphabetically by either common or scientific name. It would be useful if they were.	
Reviewer 5	Generally sufficient. Problem formulation section seemed a bit of a forced fit, as if added to satisfy a new stylist protocol.	

2.2 Please comment on the technical approach used to derive the draft cadmium criteria; is it logical, does the science support the conclusion, and is it consistent with the protection of

freshwater and estuarine/marine aquatic life from acute, chronic, and bioaccumulative effects? Are the methods described in the document scientifically sound?

Reviewers 2 and 4 noted that the technical approach appeared valid, with a few exceptions. Reviewer 2 also commented that the increased number of species extending the species sensitivity distributions (SSDs) is a positive step in confirming the proposed criteria, but had concerns regarding the approach for the chronic estuarine/marine values. This reviewer noted that the use of acute-chronic ratios (ACRs) with freshwater fish or other organisms to derive estuarine/marine values is not appropriate. Reviewer 3 also questioned the "dubious" use of two ACRs from freshwater species to develop a marine chronic criterion, particularly when proper justification was not provided.

Reviewers 1 and 3 both noted significant omissions in EPA's treatment of the bioaccumulative effects of cadmium in the document. Reviewer 1 commented that studies during the past 10-15 years have shown that cadmium bioaccumulated from food can be a major, and for some species predominant, source of exposure. Once bioaccumulated from dietary sources, cadmium can reach sensitive organs in animals that cadmium taken up from an aqueous phase would not reach. The reviewer noted that the reference section contains numerous papers describing the significance of dietary sources of cadmium on aquatic animals, but EPA has chosen not to use many of these papers for reasons Reviewer 1 considered spurious or due to misinterpretations of the studies. Reviewer 3 commented that the document largely ignores the bioaccumulative effects of cadmium. He pointed out that, despite substantial evidence demonstrating the adverse effects of cadmium, the document suggests that bioaccumulated cadmium is not toxic to aquatic organisms. "What evidence can we point to suggest that bioaccumulated cadmium is not toxic to aquatic organisms?" he wrote. "This is a fundamental flaw in this document." The reviewer noted that lab tests, which use only direct aqueous cadmium exposures, generally suggest that aquatic insects are insensitive to cadmium, but field ecologists find that aquatic insects are sensitive to metal effects in natural settings. He emphasized that short-term, water-only exposures are insufficient for evaluating metal toxicity in aquatic insects.

Reviewer 3 questioned the rationale for using EC20 values for the chronic toxicity assessment, the use of only three species (all fish) to generate the hardness correction for the freshwater chronic toxicity dataset, and the removal of the most acutely sensitive marine genus from the analysis.

Reviewer 4 noted several issues. In the document, EPA concluded that most changes in cadmium toxicity can be explained by changes in hardness and that it was not necessary to incorporate the biotic ligand model (BLM) into the revision. The reviewer strongly disagreed, noting that dissolved organic carbon (DOC) and pH strongly influence cadmium toxicity, and that DOC varies widely in the natural environment. The reviewer also questioned why the document only used a multiple linear regression with alkalinity, and not with pH and/or DOC. He emphasized that DOC is a really important water quality parameter that EPA is ignoring. He also expressed concern that EPA had ignored the obvious and significant salinity effect for the *Neomysis integer* data (p. 51)—one of the four taxa used for criteria derivation—and that EPA had used the geometric mean to develop the species mean acute values.

Reviewer 5 answered "no" to both parts of the charge question. He noted that the draft document only uses data from an idealized aquaculture setting, without regard to whether the species occurrs naturally in suboptimal conditions. He emphasized that derived criteria should be suitable for diverse water bodies. Also, during criteria development, EPA excluded all long-term test endpoints for the most sensitive genus (*Hyalella*), straying from the guiding principle to protect diverse natural waters.

Reviewer 5 had significant issues with Appendix K and emphasized that EPA should state the reason why this appendix was requested. The Appendix examines the laboratory performance of *Hyalella* in great detail (establishing that *Hyalella* growth and reproductive output is greatest in waters with chloride >15 mg/L), but does not address the question of whether chloride is a factor affecting cadmium toxicity and misses the point that the comparisons of acceptable conditions should be performance in the wild. This reviewer also pointed out that EPA's quotation on page 12 of a statement from Mebane (2014) conveys an inaccurate sense that "cadmium is unlikely to accumulate in tissue to levels that would result in adverse effects to aquatic invertebrates or fish" by truncating the quote to omit important information.

Reviewer	Comments	EPA Response to Comments
Reviewer 1	This report is rather antiquated in its thinking. It basically assumes that Cd is accumulated only from the aqueous phase rather than from both the aqueous phase and ingested food. Over the past 10-15 years, it has been shown that many toxicants, including Cd and other metals, can be bioaccumulated from food as well as from the aqueous phase. Indeed, a number of laboratory, field, and modeling studies have shown that diet can be the dominant source of metals for marine invertebrates and fish. The relative importance of diet has been shown to vary with species, but it is rarely a minor source and sometimes (for some fish species, for example) the predominant source. Moreover, once accumulated from diet, Cd can reach sensitive organs within animals that are not reached by Cd taken up from the aqueous phase. Therefore, the toxic response of an animal to either ambient Cd or body burden Cd can vary considerably, depending on whether the source is ingested food or solute in ambient water. Thus, dissolved metal may be sorbed onto exoskeletons in crustacean zooplankton (often the most sensitive species, as the author points out) but this does not directly affect the animal because the metal (Cd in this case) bound to chitosan on the exoskeleton does not interact with metabolic processes, whereas metal assimilated from ingested food can enter into internal tissues where it may interfere with a variety of metabolic and reproductive processes. I saw no acknowledgement of the possible significance of dietary Cd on aquatic (freshwater or marine) animals in this report, and yet numerous papers describing such effects appeared in the reference section. In looking over appendices, many of these reports were not used, often for what appear to be spurious reasons or misinterpretations of studies. In some cases, dietary metals could be 1-2 orders of magnitude more toxic than dissolved metals to freshwater cladocerans and marine	

	copepods, for example. In the case of Cd, an EC_{50} value of 5 nM (~0.5 µg/L) was observed in copepods in a study by Hook & Fisher (cited in this report) if the animal had been fed food exposed to that Cd concentration, whereas the measured LC_{50} value based on a dissolved Cd source was 200 times greater. Also, measuring growth or mortality, as is often the case in simple toxicity tests, would have missed the effect—rather the reproductive capability of the copepods was affected by the dietary Cd, but no mortality was observed at environmentally realistic concentrations. Because dissolved Cd concentrations are typically at very low concentrations in natural waters (at least 10-fold lower in surface seawater, for example), the lower EC_{50} value derived from dietary rather than dissolved sources still indicates that Cd is unlikely to cause toxic effects in most natural waters.	
Reviewer 2	With a few notable exceptions, the technical approach for the freshwater acute and chronic derivations appear valid. Incorporation of hardness normalization is warranted given the likelihood that Cd and Ca compete for similar biological and abiotic sites. In addition, the increased number of species extending the SSDs is also an excellent step forward in confirming proposed criteria. Of concern is the approach utilized for the chronic estuarine/marine values. Utilization of ACRs with freshwater fish or other organism to derive estuarine/ marine values is not appropriate, especially when the criteria concentrations are increased. It is also unclear why freshwater salmonid values were not utilized for the ACRs, as many reside in estuarine/marine environments (see salmonid comments below).	
Reviewer 3	Bioaccumulative effects of Cd are largely ignored in this document. My comments for this section are divided into 2 parts: *1.* The technical approach according to the 1985 Guidelines, and *2.* The technical approach in light of our current understanding of cadmium bioaccumulation, effects, and deficiencies in the traditional testing approaches. 1. The technical approach according to the 1985 Guidelines A. What is the rationale for use of EC20 values for the chronic toxicity assessment? I understand	

	that a MATC approach (based on NOEC and LOECs) has its issues, and I'm generally in favor of more statistically robust approaches such as the use of an EC level based on entire datasets. But why is a 20% effect level chosen here? This value seems rather high. There should be some rationale for choosing this value, and this rationale should be clearly articulated in the text. How do we know that a 20% effect level has no impacts at the population level? B. Only 3 species (all fish) were used to generate the hardness correction for the freshwater chronic toxicity data set. *D. magna* and *P. promelas* data were not used because only MATCs were available and not EC20s. Is it not possible to estimate EC20's from these datasets? The use of only 3 species to make this very important hardness adjustment would seem to add a significant level of uncertainty to the final analysis, especially since 2 of species used have divergent slopes. ANCOVA (p=0.08) based on data from 3 species was used to say that the slopes 0.32, 1.46 and 1.08 are not different and can be pooled. Is this defensible? Shouldn't a conservative slope estimate be chosen here…. especially in light of the fact that a 20% effect level is much higher than an MATC or EC05 would be? C. The most acutely sensitive marine genus, *Tigriopus* was not used in the analysis. The rationale was that it falls below the 5th percentile of the distribution. Isn't the whole point of the SSD to determine what is protective of 95% of the species? (Not 95% of the remaining taxa after sensitive taxa are arbitrarily removed from the dataset). Shouldn't all of the data be used here? D. The use of 2 ACRs from freshwater species in the development of a marine chronic criterion is dubious on many fronts. The justification for doing this needs to be articulated. If justifiable, the authors should then justify their choices as to why these 2 species were chosen. The reason given in the text is that the freshwater species were chosen on the basis of being acutely sensitive. However the purpose of ACRs is to	

	evaluate the potential for the chemical to cause chronic toxicity. Use of an acutely sensitive species for ACR choice should theoretically result in species with low ACRs, and in this case, this is borne out. The freshwater invertebrate *L. silquoidea* has a reported ACR of 2.727, suggesting that is chronically not very toxic. However, the ACRs for most species are considerably higher: (see below) Mebane (2010) list ACRs for freshwater invertebrates: Ephemerella: 158.67 Physa: 47.6 Aplexa: 28.5 and 47.87 Ceriodapnia: 12.41 and 31.5 Dapnia: 65, 155, 112, 13 Hyalella: 17.5 This document lists the following freshwater invertebrate ACRs: Aplexa: 49.7 Lymnea: 12.81 Ceriodaphnia: 19.82 Daphnia: 57.3 With all of these values to choose from, 2.727 is clearly not a representative ACR for freshwater invertebrates. Since the use of a "mean ACR" is being applied across taxa, shouldn't the values be representative? Would it make sense to have higher ACRs apply to invertebrates and lower ACRs apply to fish since fish generally have low ACRs and inverts generally have high ACRs? 2. Technical approach based on what we understand about the world post 1985: Cadmium has been demonstrated to be toxic to practically every in vitro system it has been tested in. We strive to limit human dietary exposures in part because it is a known carcinogen and is nephrotoxic after dietary exposure. Effects of Cd on antioxidant physiology are well described in several species including aquatic insects. <u>What evidence can we point to suggest that bioaccumulated Cd is not toxic to aquatic organisms?</u> <u>This is a fundamental flaw in this document.</u> We have a major and important disconnection between what traditional laboratory tests (using	

	only direct aqueous exposures) and what field ecologists tell us about metal effects in aquatic insects. Because insects are such important players in freshwater ecosystems, and are the focus of CWA-driven biomonitoring programs, we have numerous examples of stream community structure being impaired by metal exposures. Yet lab (aqueous) tests generally suggest that insects are insensitive to Cd. Work in our laboratory has used Cd uptake and depuration kinetics to clearly demonstrate that 96 hour exposures are insufficient to elicit toxicity in aquatic insects are ecologically relevant concentrations (Buchwalter et al. 2007, Buchwalter et al. 2008, Poteat and Buchwalter 2014, Poteat and Buchwalter 2014). We have also shown that periphyton is a major sink for Cd, and is readily bioaccumulated in insects (Xie et al. 2010). We have also showed that Cd exposure does not negatively affect Ca transport in insects (Poteat and Buchwalter 2014) (as it is known to do in acutely sensitive taxa), and Ca provides little protective effects on Cd uptake(Poteat et al. 2012). Finally, we show that diet derived (but not water derived) Cd affects antioxidant physiology suggesting that dietary exposures may be more challenging to aquatic insects that aqueous exposures (Xie and Buchwalter 2011). These findings mirror those of Irving et al., 2003. All of these findings point towards short-term, water-only exposures are insufficient for evaluating metal toxicity in this important faunal group (see (Poteat and Buchwalter 2014) for discussion of these findings).	
Reviewer 4	Overall yes, I think the technical approach is scientifically sound and consistent with the protection of aquatic life. I do, however, have some specific significant comments for EPA to consider which I list below. <u>Page 15:</u> EPA concludes that most changes in Cd toxicity can be explained by changes in hardness and therefore incorporation of the BLM into this revision is not necessary. I strongly disagree with this statement. Every study I'm aware of in which a range of DOC and pH have been measured has shown that these parameters strongly influence Cd toxicity. Just because the majority of laboratory studies are conducted in laboratory waters with low DOC and do not measure dissolved organic carbon (DOC), does not provide a valid rationale for not	

	using the BLM (biotic ligand model). Obviously, in the natural environment, DOC varies widely. I would think the objective of the criteria is to ensure that they are protective/predictive of toxicity in the natural environment, not in artificial laboratory waters. Page 34: Following up on the previous comment regarding not using the BLM, why did EPA only consider a multiple linear regression with alkalinity? Why not pH and/or DOC? It is quite possible that pH autocorrelates with hardness as well given this is the case for most artificial laboratory waters (though not as consistent for natural waters), but there will not be an autocorrelation with DOC. This is a really important water quality parameter that EPA is ignoring. Page 50-51: Is the study by Voyer et al. (1974), the only study where the effects of salinity on Cd toxicity was not consistent or are there multiple studies with this problem? If it's only this one study, it's not clear why the general trend would be ignored. I don't think EPA would ignore the hardness relationship in freshwater if only a single study was inconsistent with the general trend. It is a concern that there is on obvious and significant salinity effect for the *Neomysis integer* data (p. 51), which is one of the four taxa used for the criteria derivation, and yet this obvious effect is ignored and the geometric mean is used to develop the species mean acute values (SMAV). Does EPA consider a test performed at a salinity of 1 ppt to be a marine test?	
Reviewer 5	Unfortunately some aspects of the document lead to answering both parts of the charge question 2 with answers of "no." I am only commenting on aspects which to me did not follow the available science, deviate from the principles of the 1985 "Guidelines" or otherwise have logical problems, or . While Stephan et al's (1985) Guidelines for derivation of aquatic life criteria are 30 years old and aspects of the science have progressed such that some details may not fit, they include solid principals that should continue to guide the approach. Key among Stephan et al's guiding concepts is from their p. 3: *"The guidelines were intended to provide the same level of protection as would an (infeasible) approach of conducting field tests on a wide variety of unpolluted bodies of water, adding various amounts of the material to each body of water in order to determine the highest concentration that would not cause any unacceptable*	

	long-term or short-term effects on the aquatic organisms or their uses." Further (p. 10), *These National Guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations. All North American bodies of water and resident aquatic species and their uses are meant to be taken into account."* Not bodies of water for which conditions are optimal – all bodies of water. Thus, a key concept behind the logic of criteria derivation is that criteria be suitable for diverse, natural water bodies, and laboratory data should attempt to encompass comparable field situations. The draft document instead moves towards a very different concept of only using data from an idealized aquaculture setting, without regard to whether the species occurs in the wild in waters with "suboptimal" conditions. **Drilling down on Hyalella** Most fundamentally, by throwing out all long-term test endpoints for the most sensitive genus (Hyalella) this document strays from a guiding principle of the Guidelines that criteria are to protect diverse natural waters. Criteria are indeed developed using laboratory data, but they are not intended to apply to laboratory waters; they are intended to apply to natural waters. This disconnect between laboratory-based derivation of numeric water quality criteria and application to natural waters has repeatedly debated in the literature, with me chiming in specifically with cadmium (Mebane 2010). In essence, optimal aquaculture conditions are defined for culturing *Hyalella azteca*, and chronic tests in which less than 15 mg/L chloride was present in dilution waters, or control growth, survival, and reproduction did not meet expectations. These were control growth (≥0.35 mg at 28 days and ≥0.5 mg at 42 days), survival (80% at 42d) and reproduction (≥6 per young). No explanation was found in the document why researchers were tasked to drill down on Hyalella, as any commonly used test organism could have been similarly scrutinized. Absent explanation, the inference is that Hyalella must have been chosen because it was the most sensitive organism, and there was a desire to exclude data if this heightened sensitivity could be shown to be an artifact of stressful laboratory culture conditions. In essence this logic requires the following implicit assumptions. Since	

	only Hyalella data obtained from laboratory test waters >15 mg/L are to be used for criteria development, it follows that: 1. In ambient waters, Hyalella (and presumably other freshwater amphipods) are only expected to occur in waters with >15 mg/L chloride; Alternatively if Hyalella do in fact occur in waters with lower chloride concentrations, the criteria are only intended to apply to waters with >15 mg/L. 2. Chloride is an important factor affecting the toxicity of cadmium to Hyalella (and presumably other related but less well studied amphipods or freshwater crustaceans). If so, then it follows that: a. Chloride should be included in the criteria derivation and factored into the criteria. Per the Guidelines (p32), "when enough data are available to show that the chronic toxicity is similarly related to a water quality characteristic, the relationship should be taken into account …. If two or more factors affect toxicity, multiple regression analysis should be used." b. Alternatively, while not specifically mentioned in the guidance, if data were insufficient for the covariance or multiple regression analyses endorsed, it would seem reasonable to establish different criteria in brackets, such as waters ≤15 mg/L chloride or >15 mg/L chloride. 3. Alternatively, if chloride is not an important factor affecting, then there is no reason to factor it into the criteria development. However, Appendix K does not address the question of whether chloride is a factor affecting cadmium toxicity, all that has been established is that Hyalella growth and reproductive output is greatest in waters with chloride >15 mg/L. This is not unexpected. Freshwater environments usually have an osmolarity far less than blood plasma, and energy requirements to maintain hydromineral balance increase in more dilute waters (e.g., Wendelaar Bonga and Lock 2008). Fish in dilute waters don't grow well either. For instance, about 80% of	

	the restaurant/retail rainbow trout sold in the United States come from a 30 mile stretch known as the Thousand Springs area of southern Idaho. There the constant chloride of about 20 mg/L, hardness of about 180 mg/L and temperature of 15°C provide optimal energy conversions and growth per unit feed. It would follow just as logically that only rainbow trout data that were generated from waters with chloride >15 mg/L or so should be used, because that optimizes growth? Why would it not follow that only acute data in which organisms were fed should be used, because starvation stresses organisms? This seems to be internally inconsistent logic. The reason why Appendix K was requested was never stated. It should be. I assume the reason must be a presumption that if organisms do not grow and reproduce at high rates, then they will "too sensitive" or not represent responses expected in natural conditions. It is not obvious that this is the case. McNulty et al. (1999) showed that starved amphipods exposed to low levels of cadmium survived better than controls. However, even if optimal diets do produce higher (less sensitive) growth and reproduction effects with Cd and Hyalella, the universal use of optimal diets could lead to underestimation of the toxicity risks experienced by wild populations, which may experience limited food availability. In the wild, organisms don't live in optimal conditions. Even in the center of their ranges, conditions are seldom optimal all of the time. Organisms also live in marginal conditions, for they tend to expand their ranges to the limits of their physiological tolerances. See for example France's (1996) description of Hyalella living on the margins of lakes with tolerable mineral content (France 1996). Similarly, Gibbons and Mackie (1991) showed that increasing reproductive output of H. azteca was associated with increasing sulfate, calcium hardness, sediment particle size, conductivity, alkalinity, seston, and the organic matter of the fine sediment. This consistent with Appendix K, but begs the question, what are effects of Cd in these suboptimal waters? Why assume that if Cd criteria are needed, they should only be developed from exposures in high hardness, but then blindly extrapolate results to low chloride, low hardness conditions using tests with other organisms? This is further logical problem with Appendix K's rationale – as noted in appendix K, waters with hardness less than 80 mg/L tend to have chloride less than 10 mg/L. Does the	

	hardness-toxicity relation predict safe conditions for Hyalella at low hardness? No way to know. I've poked around a bit the literature on Hyalella life histories under different environmental stresses in an effort to include extrapolate organism-level effects of Cd to potential population-level effects (Mebane 2010). While by no means exhaustive, and by now a bit dated, this leads to some other thoughts on the expected control survival, growth, and reproduction in long term tests in Appendix K. With control survival, in at least some wild populations, I estimated half-month survival rates for juveniles of about 0.9, or close to a 5% decline per week (Mebane 2010, Table II). This is higher than the 2-3% noted in Appendix K, and suggests that in the wild, survival to 42-days would likely be less than 80%. With regards to growth, while some wild populations grew as much as those in the laboratory settings discussed in Appendix (>0.5 mg at sexual maturity), this cannot be assumed in all natural waters. Cooper (1965) reported average dry weights of adults Hyalella were 0.2 mg in a population in a warm, shallow lake in Michigan. Gibbons and Mackie (1991) reported mean weights of Hyalella at maturity were only 0.1 mg, and weights of all Hyalella were only 0.3 mg. Thus the 0.35 at day 28 and 0.5 mg at day 42 may be higher than that expected in some natural settings. Gibbons and Mackie (1991) reported ranges of brood per female ranged from 6 – 15, which is consistent with appendix K. However, Strong (1972), his fig 4, showed sometimes natural brook sizes may be as low as 3 per female. In sum, the logical problems of how Appendix K's analyses are used in the document are analogous to the metaphor of not seeing the forest because of all the trees. Some trees were examined in great detail (lab performance of Hyalella) but it misses the point that the comparisons of acceptable conditions should be again performance in the wild. **Other items:** Problem formulation: It is germane to note that in natural waters, Cd is always in association with Zn, usually at about mass ratios of 1:200 (Wanty et al. 2009). p. 12, I was not quoted quite accurately. "Mebane (~~2014~~ 2006) concluded that, although there were not adequate data to establish acceptable tissue effect concentrations for aquatic life, cadmium is unlikely to accumulate in	

	tissue to levels that would result in adverse effects to aquatic invertebrates or fish, at calculated chronic criterion concentrations, which were lower than that chronic criterion concentration derived here. " This report is variously cited as Mebane (2006), Mebane (2010), or Mebane (2014). The suggested citation is, "*Mebane, C.A. 2006. Cadmium risks to freshwater life: derivation and validation of low-effect criteria values using laboratory and field studies. U.S. Geological Survey Scientific Investigation Report 2006-5245 (2010 rev.). http://pubs.usgs.gov/sir/2006/5245/*." The 2010 revision only corrected minor mistakes, and did not include any updated literature reviews. p. 28, the approach of requiring data used in the hardness-toxicity regressions to have a 3X spread and 100 mg/L absolute difference between the highest and lowest value was indeed used in the 2001 version, but was not really presented as policy. In contrast, my colleagues and I found that hardness-toxicity relations were more reliable from test series that concurrently tested the same cohort of organisms in waters with different hardness, than were ad hoc collections of found data tested under different conditions at different hardness levels (Mebane et al. 2012). Where available, giving concurrent test series data obtained at different hardnesses precedence over general hardness-toxicity compilations would be warranted.	

2.3 Please comment on the data used to derive the revised criteria, including data adequacy/comprehensiveness, and the appropriateness of the data selected and/or excluded from the derivation of the draft criteria. Is the data used correctly for the intended purpose? Are there other relevant data that you are aware of that should be included? If so, please provide the data along with supporting information.

Reviewer 4 found the data used by EPA to derive the criteria to be comprehensive and generally sound, but had a few specific concerns. He was very concerned that EPA was still using studies in which test concentrations were unmeasured (in his opinion, these studies should not be included), and he questioned the use of an ACR for *Lampsilis siliquoidea*. With respect to the suitability of chronic *Hyalella azteca* data (Section 5.2.1), the reviewer had concerns about the validity of the study EPA retained for purposes of criteria derivation. Reviewer 2 noted that the data used for derivation of the criteria for acute effects were valid and that the additional species in the SSDs reduced uncertainty and greatly improved the criteria assessments for freshwater.

Reviewers 1, 3, and 5 were concerned that the document ignored relevant information. Reviewer 1 referred to his response to charge question 2, noting that the document ignored many relevant studies that did not conform to standard EPA toxicity protocols. These protocols generally expose organisms to dissolved

cadmium in the absence of food, thus are not representative of conditions in natural waters. Reviewer 3 commented that the document ignored practically all relevant work related to bioaccumulated cadmium, and also did not consider the importance of dietary exposures. Reviewer 5 referred to his response to charge question 2, reiterating that the exclusion of most *Hyalella* data was likely not justifiable. Also, even with the Appendix K criteria as they were, he thought that the Ingersoll and Kemble reproductive data should not have been excluded.

Reviewer 2 suggested that EPA should better describe chronic effects by incorporating other targets, such as the kidney, brain, and gonad. This reviewer also suggested including more detailed discussion of the uncertainty regarding accumulation, particularly in light of limited data for chronic effects in estuarine/marine organisms. He felt that the statement that "Aquatic organisms are considered to be more susceptible to cadmium from direct aqueous exposure than through bioaccumulation and the development of criteria protective of direct exposure effects are considered more applicable to the development of criteria for aquatic life" was clearly biased toward acute toxicity, and he encouraged EPA to revisit this statement after considering reproductive effects of cadmium, which likely result from accumulation and not direct exposure.

Reviewer 2 also noted that it was unclear what endpoint data (e.g., growth, survival, reproduction) were being used to determine the effect values in the appendices. Given the potential for reproductive effects upon chronic exposure, reproduction would be expected to be the most sensitive endpoint; therefore, if other endpoints were used, then the uncertainties inherent to these endpoints should be discussed. He also found it disappointing that data from the same two species in the 1980s were the only species used to derive the 2015 values, and he found it puzzling how criteria values can be raised for estuarine/marine organisms when the same degree of uncertainty exists (only two species) in each year criteria were assessed. Adding data from freshwater organisms for ACR estimates increases uncertainty, so the 2001 value should stay as is or be reduced because of the uncertainty involved in its derivation.

Reviewer	Comments	EPA Response to Comments
Reviewer 1	As noted above, the author chose to ignore many relevant studies that did not conform with standard EPA toxicity protocols. But the problem is that these protocols basically ignore the fact that animals eat, hardly a realistic scenario and are too simplistic in looking only at growth and mortality. Typically, the test organisms are exposed to dissolved Cd at varying concentrations, but in the absence of food. Occasionally, some artificial food (fish flakes or the like) is presented once every several days (sometimes never!) to keep the animals alive. But these studies are hardly representative of what happens in natural waters.	
Reviewer 2	The use of additional species for SSD reduced uncertainty and greatly improved criteria assessments for freshwater. The QA evaluations of data usefulness was adequate and the data selected for the acute responses was correctly used for the intended purpose. The mechanistic	

	assumption that adverse effects are primarily related to calcium uptake at the gill, is accurate for acute effects. Consequently, the data used for derivation of the criteria for acute effects is valid. However, with regard to chronic effects, there are other targets once absorption of cadmium occurs, particularly the kidney, brain and gonad. In addition to specific interactions with signaling proteins, Cd clearly binds sulfhydral groups of proteins within targets disrupting cellular maintenance. The latter two tissue targets above are likely involved in the reproductive effects observed with chronic exposures. Cd clearly disrupts the Hypothalmic Pituitary Gonadal axis and gonadal function in fish (Vetillard, and Bailhache 2005). It reduces vitellogenin in females and accumulates in kidney upon chronic exposures either via diet or water (Szczerbik et al. 2006; Thomann et al. 1997). It is understood that tissue data from these organs are limited, but studies that have these data, or the fact that these data are limited should be discussion points of the uncertainty analysis. Clearly, discussions of uncertainty regarding accumulation are needed, particularly in light of limited data for chronic effects in estuarine/marine organism. The statement "**Aquatic organisms are considered to be more susceptible to cadmium from direct aqueous exposure than through bioaccumulation and the development of criteria protective of direct exposure effects are considered more applicable to the development of criteria for aquatic life**" is clearly biased toward acute toxicity and should be re-visited with particular emphasis on reproductive effects of cadmium which likely result from accumulation and not direct exposure. With regard to reproduction, it is unclear what endpoint data is being used to determine the effect values in the Appendices. Tests are provided in terms of exposure duration, but it is unclear whether growth, survival or reproduction is being utilized as the endpoint. Again, given the potential for reproductive effects upon chronic exposure, reproduction would be expected to be the most sensitive endpoint. If other endpoints were used then the uncertainties inherent to these endpoints should be discussed. Clearly growth and survival effects have likely difference mechanisms and targets than that of reproduction.	

	It is also significantly disappointing that data from the same 2 species in 1980s are still the only two species being used to derive the 2015 values. In addition, it is puzzling how criteria values can be raised for estuarine/marine organisms when the same degree of uncertainty exists (only 2 species) in each year criteria were assessed. To add in data from freshwater organisms for ACR estimates ***increases*** uncertainty and does not reduce it. Therefore, the 2001 value should stay as is, or be reduced because of the uncertainty associated with its derivation.	
Reviewer 3	Practically all relevant work related to bioaccumulated Cd and the importance of dietary exposures is ignored. (see (Barata et al. 2002, Barata et al. 2002, Buchwalter et al. 2008, Cain et al. 2004, Croteau et al. 2003, Hare et al. 2001, Hare et al. 2003, Irving et al. 2003, Klaassen et al. 1999, Luoma and Rainbow 2005, Luoma et al. 2009, Luoma and Carter 1991, Martin et al. 2007, Timmermans et al. 1992, Wallace et al. 2003, Xie et al. 2010, Xie and Buchwalter 2011, Xie et al. 2008) for some examples) I suspect that there are other reviewers who can comment more directly on the issues with *Hyalella* data, so I will refrain from doing so here.	
Reviewer 4	Overall, I found the data used by EPA to derive the criteria to be comprehensive and generally sound. There are a few specific data where I have concerns that EPA should consider as described below. <u>Page 51:</u> I'm very concerned that EPA is still allowing studies in which test concentrations were unmeasured as being acceptable for WQC derivation. This is particularly concerning when they are for one of the four taxa used to calculate the criteria. In my opinion, these studies should not be included. <u>Page 68:</u> I agree with EPA's use of freshwater ACRs to supplement the limited marine ACRs for the purpose of deriving a final marine ACR. However, I question whether use of the ACR for *Lampsilis siliquoidea* is appropriate. There are obviously a number of factors that influence the ACR, but a major factor is the life history of the organism and the life stage selected for the acute toxicity test used to derive the ACR. It seems to me that freshwater mussels have a unique life history with no real analog in marine systems (marine bivalves have a	

	different life history). Consequently, use of this of the ACR for this species to derive a marine ACR seems inappropriate. I think use of an ACR for daphnids would be more appropriate and representative of the life history of the most acutely sensitive taxa in marine systems, the copepod *Tirgriopus*. Table 17: Why is the pH 6.0 test for *H. azteca* excluded? This is within the range of test pH values (6.0-9.0) normally consider by EPA. Additionally, earlier in the document it was stated that hardness was the only water quality parameter that mattered for normalizing Cd toxicity data. I disagree with that statement, but if EPA is going to argue other water quality parameters are not important, then I don't see how it can then exclude data for this reason. Table 18: I agree with EPA's re-evaluation of the *Hyalella* data and their application of water quality and performance criteria for test acceptability. However, I'm concerned about the study EPA retained for purposes of criteria derivation for several reasons. First, I do not believe use of a 10-d survival endpoint constitutes a chronic study as defined in Stephan et al. (1985). EPA has excluded a number of other studies from use in criteria derivation for this reason (e.g., the 21-d survival study on the sea starlet anemone, p. 81) in this document that creates a major internal inconsistency. Having said that, it could be argued that inclusion of this sub-chronic data is warranted given that it is the lowest toxicity value in the data set and exclusion of the data would be non-conservative in terms of environmental protection (as opposed to including sub-chronic data for insensitive species). However, using this logic why would the 7-d survival/growth data with the fountain darter then be excluded? My second concern is whether the sensitivity of *H. azteca* is real? Given that these 10-d data come from a 42-d study that fails to meet control performance criteria, how does EPA know that these animals weren't already stressed at 10 d and inappropriately sensitive? Given both the duration and performance issues associated with these data, in my opinion they should not be used for WQC derivation. However, I strongly encourage EPA to conduct a 28- or 42-d *Hyalella* study that meets the necessary performance criteria. Finally, after Table 18, EPA has descriptions of each of the chronic *H. azteca* studies and rationale for their rejection but did not	

	include a description of the Ingersoll and Kemble study that was accepted and the rationale for use of the 10-d survival endpoint. This should be added to the document.	
Reviewer 5	As noted in the response above, the exclusion of most Hyalella data is doubtfully justifiable, because the criteria for doing so are questionable. However, even with these Appendix K criteria as they are, the Ingersoll and Kemble data reproductive data should not have been excluded. The 42d reproductive endpoint from that test met the Appendix K criteria for control survival and brood size (6.35 per female). The 28 day endpoint was presumably excluded because of low growth as weight. However, the organisms were not weighed, but rather lengths were measured and weights were inferred from lengths. Regardless, by the stated logic, it would follow to exclude the 28-day endpoint with low (estimated) weight. But to then pick an acute survival endpoint (10-day) instead of the 42-day reproductive endpoint is inexplicable. The entry for this test in Table 2 is misleading. Saying the test was a life cycle test, but then using an acute endpoint, is misleading. I estimated the EC20 for reduced reproduction to be about 1.2 µg/L using logistic regression, or the MATC (geomean of LOEC and NOEC) would be 0.98 µg/L. **Other specific points on data used or not used.** **<u>Durations of tests</u>** If 30-day tests with salmonids that started with fry consistently yield more sensitive results than 60-day tests that started with eggs or embryos, why ignore all the shorter, more sensitive tests. The Guidance counsels to beware of tests in which acclimation probably occurred during resistant states. Chapman (1985) recently described this problem. It would make more sense to exclude the less sensitive data, rather exclude the more sensitive data. Likewise with Mottled Sculpin, there's doubtfully anything special about 28-day exposures over 21-day exposures. Besser et al. (2007) ran two tests, one 28-day and one 21-day test. The 28-day was less sensitive, and it was used with the other ignored. There is no established ASTM protocol for Mottled Sculpin, and the ASTM (1998) mention of "28 to 120-day (depending on species)	

	continuous exposure" tests for early-life stage tests refers back to their species-specific appendices. **Other data** (Calfee et al. 2014) and (Wang et al. 2014) report acute and chronic data with White Sturgeon and Rainbow Trout. The same data are reported in Environmental Toxicology and Chemistry, but Wang is paywalled, so I would use the open access USGS report version. An acute test with Mottled Sculpin, (Cottus bairdi) and Cd was attributed to Mebane et al. (2012). We tested Shorthead Sculpin, Cottus confusus.	

2.4 Are the derived criteria appropriately protective of listed species and commercially and recreationally important species, particularly as the criteria relates to salmonids?

Four reviewers (Reviewers 1-4) responded that the derived criteria were appropriate under certain conditions. Reviewer 5 responded that the derived criteria were not necessarily protective, noting that NMFS (2012) in Oregon concluded that the 2001 CMC of 2.0 µg/L could jeopardize some salmonids.[1]

Reviewer 1 agreed that marine animals are less at risk for cadmium toxicity than freshwater animals, primarily because of strong chloro-complexation of cadmium in seawater, and that plants are less sensitive to cadmium than animals. He noted that, while the criteria for dissolved cadmium were developed based on consideration of many key issues and are probably acceptable, they do not account for the effects of dietary cadmium, which is a large part of the picture. Consequently, the derived criteria probably overestimate the safe levels of cadmium. Reviewer 1 agreed that expressing cadmium toxicity as a function of body burden was appropriate and commented that EPA had adequately discussed the caveats associated with this approach.

Reviewer 2 commented that overall, the proposed freshwater criteria are likely safe for salmonids, but the values for estuarine/marine are highly uncertain and deserve further evaluation. This reviewer noted that salmonids are clearly one of the more sensitive species to cadmium. The proposed criteria are appropriate for freshwater conditions, but only one study evaluated cadmium toxicity in coho salmon smolts in saltwater conditions, and this was at nearly full seawater strength, which is a concern because many salmonids are anadromous and often come in contact with cadmium at lower salinities. While the Agency should be applauded for normalizing toxicity to hardness to improve freshwater criteria, there is a critical need to understand the impacts of salinity on cadmium toxicity, particularly in anadromous salmonid species.

Reviewer 2 was also concerned about the lack of discussion of the sublethal impacts of cadmium, particularly to olfaction, which significantly alters return rates of salmon. He recommended that return metrics be included in the uncertainty discussions. He also recommended that EPA consider and incorporate

[1] NMFS. 2012. Final Biological Opinion for the Environmental Protection Agency's Proposed Approval of Certain Oregon Administrative Rules Related to Revised Water Quality Criteria for Toxic Pollutants (August 14,2012). National Marine Fisheries Service, Portland, OR.

how relevant climate change effects, such as acidification, sea level rise, and temperature increases, would likely affect sensitive species.

Reviewer 3 agreed that the derived criteria are appropriately protective, but only if the assumption is made that only aqueous exposures matter. Evidence for dietary toxicity is less compelling for these fish species than for invertebrates, therefore the criteria are likely to be more protective for the species than for invertebrates. Reviewer 4 agreed that the criteria will be protective of salmonids, but was concerned that the fountain darter data were excluded from the derivation. This species is very sensitive despite test conditions that would tend to reduce their sensitivity, and the genus *Etheostoma* is widespread throughout central and eastern United States, with a number of listed species at state and federal levels. Therefore, Reviewer 4 recommends that EPA assess how inclusion of these data would impact the derivation of the freshwater criteria for cadmium.

Reviewer 5 noted that data from the long-term exposures to salmonids that began with the sensitive fry stage were excluded in favor of data from tests that began with eggs or alevins. He pointed out that the draft document evaluates protection of listed species by rolling up species data to a hardness-normalized species mean acute value (SMAV) and comparing that with the criteria, an approach that may lose sensitive life stages or strains. A more informative way to evaluate the data with listed species, he suggested, would be to compare the criteria values for the conditions of each test of interest with listed species to the magnitude of effects to listed species at a given criteria. The reviewer also noted some instances of inappropriate averaging using resistant life stages.

Reviewer	Comments	EPA Response to Comments
Reviewer 1	I agree with the author that marine animals are less at risk than freshwater animals, and this is primarily due to the strong chloro-complexation of Cd in seawater, thereby reducing the bioavailability of Cd. Consequently, marine bioconcentration factors are often 1-2 orders of magnitude higher in freshwater. I also agree that plants (e.g., phytoplankton) are less sensitive to Cd than animals, and thus it is appropriate to focus on the animals. I think that the criteria that the author generated for dissolved Cd have taken into consideration many of the key issues influencing this (e.g., water hardness) are probably ok, but by missing the effects of dietary Cd, the report is missing a large part of the overall story. This is not to suggest that ambient Cd concentrations are unsafe for animals, but the derived criteria are probably over-estimates of the safe levels of Cd. Another complicating issue is the influence of dissolved organic carbon and its effect on Cd bioavailability. Thus, expressing Cd toxicity as a function of body burden is appropriate; the caveats associated with this approach have been appropriately discussed in the report.	

Reviewer 2	Salmonids are clearly one of the more sensitive species with regard to Cd toxicity. Not only are they very sensitive, they are commercially important, and possess several species that are listed as endangered and threatened in the US. The proposed criteria are appropriate for freshwater conditions since many of the studies used to derive the criteria focused on freshwater treatments to rainbow trout. However, only one study evaluated Cd toxicity in coho salmon smolts in saltwater conditions, and this was at nearly full seawater strength (28 ppth). Of concern is the fact that many salmonids including strains of *O. mykiss* (steelhead) are anadromous and often come in contact with Cd at lower salinities (5-15 ppth). While the agency should be applauded for normalizing toxicity to hardness to improve freshwater criteria, there is a critical need to understand the impacts of salinity on Cd toxicity particularly in anadromous salmonid species. Of additional concern is the lack of discussion of sublethal impacts of Cd particularly to olfaction (Williams and Gallagher 2013) which significantly alters return rates of salmon (Baldwin et al. 2009). Return metrics are population level endpoints that should supersede standard repro/survival/growth. These should also be topics of discussion with regard to uncertainty. Lastly, the issue of climate change is largely missing from the document. Acidification (particularly with metal availability) and temperature issues are also likely to impact sensitive species (eg. salmonids). Sea level rise will also cause saltwater intrusion into salmonid spawning habitats and affect "estuarine/marine" criteria. Evaluation of these stressors should be focal points for future criteria assessment particularly for salmonids. Overall, while the values for freshwater are likely safe for salmonids, the values for estuarine/marine are highly uncertain and deserve further evaluation.	
Reviewer 3	This seems to be the case if we assume that only aqueous exposures matter. Evidence for dietary toxicity is less compelling than for invertebrates, so for these fish species, the criteria are likely more protective for these species than they are for invertebrates.	
Reviewer 4	Yes, I think the criteria as derived will be protective of salmonids. However, I'm concerned about the exclusion of the fountain darter data from the derivation. EPA	

	argues that the acute data should be excluded because the test was fed and that the chronic data should be excluded because the study was only 7 d in duration (i.e., not true chronic). Generally, I agree with both of these decisions, but from my perspective, these rules are in place to prevent the inclusion of data indicating organisms are insensitive due to inappropriate test conditions (i.e., food reducing metal bioavailability, short test durations missing sensitive endpoints). However, this is not the case with the darter data, which indicate this species is very sensitive despite test conditions that would tend to reduce their sensitivity. EPA also seems to infer (p. 86) that the fountain darter data has limited applicability because this species has a limited distribution However, the genus *Etheostoma* is widespread throughout central and eastern U.S. with a number of listed species at both the state and federal level. Hence these data a representative for a genus that is under considerable threat. Given this, I think it would be important to assess how inclusion of these data would impact derivation of the freshwater Cd WQC. <u>Page 87:</u> I don't think the statement that dividing the LC50 by two is expected to result in a concentration with effects no different than the control is correct. Dividing the LC50 by two will result in an "LC-low". I agree that across a range of species and toxicants, dividing by two equates to a values that approximates the NOEC. However, it does not equate to an LC0, which is inferred by this statement. Please clarify.	
Reviewer 5	Not necessarily, although to definitively answer this would take a considerably more thorough review to determine than was presented in the document, or could be done independently in the time available. I note that NMFS (2012) in Oregon concluded the 2001 CMC of 2.0 µg/L could jeopardize some salmonids and that the CCC of 0.25 µg/L would not jeopardize listed salmonids under their prevue. Thus the draft 2015 criterion of 2.2 µg/L would presumably be a concern. Conversely, NMFS (2011) concurred with EPA that Idaho acute and chronic criteria of 1.34 and 0.55 µg/L respectively would not jeopardize listed anadromous salmonids. I did not attempt to reconcile the three documents. However, I think part of the discrepancies may be in the manner of analyses. In the draft document, data from long-term exposures to salmonids that began with sensitive fry life stage are excluded in favor of data from tests that began	

	with eggs or alevins. While all fish have some life stage-sensitivity interaction, with at least salmonids sensitivity increases with size up to at least 0.4g ww, and maybe up to 1g or more (Hansen et al. 2002; Mebane et al. 2012). With other fish, the newly hatched stage may be more sensitive, or life events such as the onset of exogenous feeding may be related to a stressful and sensitive stage (Wang et al. 2014). There are some instances of inappropriate averaging using resistant life stages. Bull trout at the most sensitive (~1g) were averaged with results of test with yearling brook trout to produce a nonsense genus mean acute value of 126 µg/L. Stephan et al. advise against pooling species mean values when they differ by more than a factor of 10; these differed by a factor of 1000X. The draft document evaluates protection of listed species by rolling up species data to a hardness-normalized species mean acute value (SMAV) and comparing that with the criteria. Because the accuracy of hardness-normalization is uncertain, but the criteria values can be calculated with certainty for any hardness, a more informative way to evaluate the data with listed species is to compare the criteria values for the conditions of each test of interest with listed species to the effects magnitude of effects to listed species at a given criteria. If the test concentrations causing an adverse effect are close to criteria concentrations, such as if the EC50s were within a factor of 2 (or maybe 2.5 to 3 to be on the safe side), then evaluate the actual adverse effects observed at the criteria concentrations. The SMAV approach involves a lot of data manipulation and may lose sensitive life stages or strains.	

2.5 Other Comments Provided

Reviewer	Comments	EPA Response to Comments
Reviewer 4	**Additional Minor Comments** **Page 11:** Note that Cd does not form complexes with Ca as stated, but rather competes with Ca for uptake and Ca channels. Please correct. **Page 11:** While Atli and Canli did observe a reduction in NKA activity in their study, it's a significant overstatement to say disruption of Na homeostasis is a mechanism of	

	action for Cd. To the best of my knowledge, it hasn't been observed in any other study that has investigated this potential mechanism. **Page 11:** If Cd inhibits catalase, glutathione reductase, SOD, etc., it seems to me this is direct inhibition of anti-oxidant processes, not indirect as stated. **Page 12:** Regarding the relationship between Cd tissue burdens and toxicity, see also the analysis by Adams et al. (2011). **Page 50:** *Tigriopus* is a copepod, not a mysid, as indicated in the second paragraph. **Page 58:** Please specific at the top of p. 58 which two freshwater ACRs were used in the calculation of the marine ACR. **Table 18:** Change the test duration for the Borgmann studies to 42 d rather than 6 w to make the units consistent with the rest of the table. **Page 83:** It should be mentioned that both BCFs and BAFs are inversely related to exposure concentration which explains much of the variation in BCFs/BAFs (McGeer et al. 2003, DeForest et al. 2007). **Table 21:** Taking a final look through Table 21 I note that EPA has included several species that are not resident to N. America (*Oreochromis spp., Danio rerio, Xenopus laevis*). Unless this requirement has changed, they should be removed from the data set.	

3.0 NEW INFORMATION PROVIDED BY REVIEWERS

This section presents all new information that reviewers provided in addition to their specific responses (presented in Section 2, above) to the charge questions.

Reviewer	Comments	EPA Response to Comments
Reviewer 2	Baldwin, DH , Spromberg, JA, Collier, TK and NL Scholz (2009) A fish of many scales: extrapolating sublethal pesticide exposures to the productivity of wild salmon populations. Ecological Applications 19:2004-2015. Szczerbik, P, Mikolajczyk, T, Sokolowska-Mikolajczyk, M, Socha, M, Chyb, J, Epler, P. (2006) Influence of long-term exposure to dietary cadmium on growth,	

	maturation and reproduction of goldfish (subspecies: Prussian carp Carassius auratus gibelio B.) Aquatic Toxicology 77:126-135. Thomann, RV, Shkreli, F., Harrison, S. (1997) A pharmacokinetic model of cadmium in rainbow trout. Environmental Toxicology and Chemistry 16:2268-2274. Vetillard, and Bailhache (2005) Cadmium: An endocrine disrupter that affects gene expression in the liver and brain of juvenile rainbow trout. Biology of Reproduction 72:119-126. Williams CR and EP Gallagher (2013) Effects of cadmium on olfactory mediated behaviors and molecular biomarkers in coho salmon (Oncorhynchus kisutch) Aquatic Toxicology 140-141:295-302.	
Reviewer 3	Barata, C., S. J. Markich, D. J. Baird, and A. M. V. M. Soares. 2002. The relative importance of water and food as cadmium sources to *Daphnia magna* Straus. Aquat. Toxicol. 61:143-154. Barata, C., D. J. Baird, S. E. Mitchell, and A. M. V. M. Soares. 2002. Among- and within-population variability in tolerance to cadmium stress in natural populations of *Daphnia magna*: implications for ecological risk assessment. Environ. Toxicol. Chem. 21:1058-1064. Buchwalter, D. B., D. J. Cain, W. H. Clements, and S. N. Luoma. 2007. Using biodynamic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. Environ. Sci. Technol. 41:4821-4828. Buchwalter, D. B., D. J. Cain, C. A. Martin, L. Xie, S. N. Luoma, and T. Garland, Jr. 2008. Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. Proc. Nat. Acad. Sci. 105:8321-8326. Cain, D. J., S. N. Luoma, and W. G. Wallace. 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. Environ. Toxicol. Chem. 23:1463-1473. Croteau, M.-N., L. Hare, and A. Tessier. 2003. Difficulties in relating Cd concentrations in the predatory insect	

Chaoborus to those of its prey in nature. Can. J. Fish. Aquat. Sci. 60:800-808.

Hare, L., A. Terrier, and L. Warren. 2001. Cadmium accumulation by invertebrates living at the sediment-water interface. Environ. Toxicol. Chem. 20:880-889.

Hare, L., A. Tessier, and U. Borgmann. 2003. Metal sources for freshwater invertebrates: pertinence for risk assessment. Human Ecol. Risk Assessment 9:779-793.

Irving, E. C., D. J. Baird, and J. M. Culp. 2003. Ecotoxicological responses of the mayfly *Baetis tricaudatus* to dietary and waterborne cadmium: implications for toxicity testing. Environ. Toxicol. Chem. 22:1058-1064.

Klaassen, C. D., J. Liu, and S. Choudhuri. 1999. Metallothionein: An intracellular protein to protect against cadmium toxicity. Annu. Rev. Pharmacol. Toxicol. 39:267-294.

Luoma, S. N., D. J. Cain, and P. S. Rainbow. 2009. Calibrating Biomonitors to Ecological Disturbance: a New Technique for Explaining Metal Effects in Natural Waters. Integrated Environmental Assessment and Management 6:199-209.

Luoma, S. N. and P. S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol. 39:1921-1931.

Luoma, S. N. and J. L. Carter. 1991. Effects of trace metals on aquatic benthos, pp. 261-300 In M. C. Newman and A. W. McIntosh (eds.), Metal Ecotoxicology Concepts & Applications. Lewis Publishers, Inc., Chelsea, Michigan.

Martin, C. A., D. J. Cain, S. N. Luoma, and D. B. Buchwalter. 2007. Cadmium ecophysiology in seven stonefly (Plecoptera) species: Delineating sources and susceptibility. Environ. Sci. Technol. 41:7171-7177.

Poteat, M. D. and D. B. Buchwalter. 2014. Calcium uptake in aquatic insects: influences of phylogeny and metals (Cd and Zn). J. Exp. Biol. 217:1180-1186.

Poteat, M. D. and D. B. Buchwalter. 2014. Four reasons why traditional metal toxicity testing with aquatic insects is irrelevant. Environ. Sci. Technol. 48:887-888.

	Poteat, M. D. and D. B. Buchwalter. 2014. Phylogeny and Size Differentially Influence Dissolved Cd and Zn Bioaccumulation Parameters among Closely Related Aquatic Insects. Environ. Sci. Technol. 48:5274-5281. Poteat, M. D., M. Diaz-Jaramillo, and D. B. Buchwalter. 2012. Divalent metal (Ca, Cd, Mn, Zn) uptake and interactions in the aquatic insect Hydropsyche sparna. J. Exp. Biol. 215:1575-1583. Timmermans, K. R., E. Spijkerman, and M. Tonkes. 1992. Cadmium and zinc uptake by two species of aquatic invertebrate predator from dietary and aqueous sources. Can. J. Fish. Aquat. Sci. 49:655-662. Wallace, W. G., B.-G. Lee, and S. N. Luoma. 2003. The subcellular compartmentalization of Cd and Zn in two bivalves. I. The significance of metal-sensitive fractions (MSF) and biologically-detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249:183-197. Xie, L. and D. B. Buchwalter. 2011. Cadmium exposure route affects antioxidant responses in the mayfly Centroptilum triangulifer. Aquat. Toxicol. 105:199-205. Xie, L., D. H. Funk, and D. B. Buchwalter. 2010. Trophic transfer of Cd from natural periphyton biofilms to the grazing mayfly *Centroptilum triangulifer* in a life cycle test. Environmental Pollution. 158:272-277. Xie, L. T., D. Lambert, C. Martin, D. J. Cain, S. N. Luoma, and D. Buchwalter. 2008. Cadmium biodynamics in the oligochaete Lumbriculus variegatus and its implications for trophic transfer. Aquat. Toxicol. 86:265-271.	
Reviewer 4	Adams, W. J., R. Blust, U. Borgmann, K. V. Brix, D. K. DeForest, A. S. Green, J. S. Meyer, J. C. McGeer, P. R. Paquin, P. S. Rainbow and C. M. Wood (2011). "Utility of tissue residues for predicting effects of metals on aquatic organisms." Integr. Environ. Assess. Manag. **7**(1): 75-98. DeForest, D. K., K. V. Brix and W. J. Adams (2007). "Assessing metal bioaccumulation in aquatic environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration." Aquat. Toxicol. **84**: 236-246.	

	McGeer, J. C., K. V. Brix, J. M. Skeaff, D. K. DeForest, S. I. Brigham, W. J. Adams and A. S. Green (2003). "The inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment." Environ. Toxicol. Chem. **22**(5): 1017-1037. Stephan, C. E., D. I. Mount, D. J. Hansen, J. H. Gentile, G. A. Chapman and W. A. Brungs (1985). Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. Duluth, U.S. Environmental Protection Agency, Environmental Research Laboratory: 98 pp.	
Reviewer 5	ASTM. 1998. Standard guide for conducting early life-stage toxicity tests with fishes. Method E1241-98. Pages 29 *in* Annual Book of ASTM Standards, volume 11.04. American Society for Testing and Materials, West Conshohocken, PA. Besser, JM, Mebane, CA, Mount, DR, Ivey, CD, Kunz, JL, Greer, EI, May, TW, and Ingersoll, CG. 2007. Relative sensitivity of mottled sculpins (*Cottus bairdi*) and rainbow trout (*Oncorhynchus mykiss*) to toxicity of metals associated with mining activities. Environ Toxicol Chem 26:1657–1665. Calfee, RD, Little, EE, Puglis, HJ, Beahan, E, Brumbaugh, WG, and Mebane, CA. 2014. Acute Sensitivity of White Sturgeon (*Acipenser transmontanus*) and Rainbow Trout (*Oncorhynchus mykiss*) to Cadmium, Copper, or Zinc in Laboratory Water-Only Exposure. Pages 5-34 *in* Ingersoll, CG, and Mebane, CA, editors. Acute and chronic sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to cadmium, copper, lead, or zinc in laboratory water-only exposures. U.S. Geological Survey Scientific Investigations Report 2013–5204. Chapman, GA. 1985. Acclimation as a factor influencing metal criteria. Pages 119–136 *in* Bahner, RC, and Hansen, DJ, editors. Aquatic Toxicology and Hazard Assessment: Eighth Symposium (STP 891-EB), volume STP 891. American Society for Testing and Materials (ASTM), Philadelphia.	

Cooper, WE. 1965. Dynamics and production of a natural population of a fresh-water amphipod, *Hyalella azteca*. Ecol Monogr 35:377-394.

France, RL. 1996. Biomass and production of amphipods in low alkalinity lakes affected by acid precipitation. Environ Pollut 94:189-193.

Gibbons, WN, and Mackie, GL. 1991. The relationship between environmental variables and demographic patterns of *Hyalella azteca* (Crustacea: Amphipoda). J N Am Benthol Soc 10:444-454.

Hansen, JA, Welsh, PG, Lipton, J, Cacela, D, and Dailey, AD. 2002. Relative sensitivity of bull trout *(Salvelinus confluentus)* and rainbow trout *(Oncorhynchus mykiss)* to acute exposures of cadmium and zinc. Environ Toxicol Chem 21:67–75.

McNulty, EW, Dwyer, FJ, Ellersieck, MR, Greer, EI, Ingersoll, CG, and Rabeni, CF. 1999. Evaluation of ability of reference toxicity tests to identify stress in laboratory populations of the amphipod *Hyalella azteca*. Environ Toxicol Chem 18:544-548.

Mebane, CA. 2010. Relevance of risk predictions derived from a chronic species-sensitivity distribution with cadmium to aquatic populations and ecosystems. Risk Anal 30:203-223.

Mebane, CA, Dillon, FS, and Hennessy, DP. 2012. Acute toxicity of cadmium, lead, zinc, and their mixtures to stream-resident fish and invertebrates. Environ Toxicol Chem 31:1334–1348.

Stephan, CE, Mount, DI, Hansen, DJ, Gentile, JH, Chapman, GA, and Brungs, WA. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency, EPA 822-R-85-100, NTIS PB85 227049, Duluth, Narragansett, and Corvallis.

Strong, DR, Jr. 1972. Life history variation among populations of an amphipod (*Hyalella azteca*). Ecology 53:1103-1111.

Wang, N, Ingersoll, CG, Dorman, RA, Kunz, JL, Hardesty, DK, Brumbaugh, WG, and Mebane, CA. 2014. Chronic Sensitivity of White Sturgeon (*Acipenser transmontanus*) and Rainbow Trout (*Oncorhynchus*

	mykiss) to Cadmium, Copper, or Zinc in Laboratory Water-Only Exposure. Pages 35-76 *in* Ingersoll, CG, and Mebane, CA, editors. Acute and chronic sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to cadmium, copper, lead, or zinc in laboratory water-only exposures. U.S. Geological Survey Scientific Investigations Report 2013–5204. Wanty, RB, Verplanck, PL, San Juan, CA, Church, SE, Schmidt, TS, Fey, DL, DeWitt, EH, and Klein, TL. 2009. Geochemistry of surface water in alpine catchments in central Colorado, USA: Resolving host-rock effects at different spatial scales. Appl Geochem 24: 600-610. Wendelaar Bonga, SE, and Lock, RAC. 2008. The osmoregulatory system. Pages 401-416 *in* Di Giulio, RT, and Hinton, DE, editors. Toxicology of Fishes. CRC Press, Boca Raton, Florida.	

APPENDIX A

CHARGE TO REVIEWERS

EPA-HQ-2016-005391_00000156

Technical Charge to External Peer Reviewers

Contract No. EP-C-13-009
Task Order 2015-26
August 2015

External Letter Peer Review of EPA's Draft Aquatic Life Ambient Water Quality Criteria (AWQC) for Cadmium – 2015

BACKGROUND

The U.S. Environmental Protection Agency (EPA) Office of Water is charged with protecting ecological integrity and human health from adverse anthropogenic, water-mediated effects, under the purview of the Clean Water Act (CWA). In support of this mission, EPA is working to update water quality criteria to protect aquatic life and aquatic-dependent wildlife from the presence of cadmium in freshwater and estuarine/marine environments.

EPA's document presents draft acute and chronic criteria expressed as concentrations of cadmium in fresh and estuarine/marine waters (dissolved). The document represents an update to the draft cadmium water quality criteria that was last published in 2001, and incorporates additional toxicological data, while using the same criteria derivation process that was used in 2001. The purpose of this independent external peer review is to provide EPA a focused, objective evaluation of the draft criteria document and supporting rationale.

REVIEW MATERIALS PROVIDED

- Internal Draft Cadmium AWQC_ 042115 (081315).pdf
- Internal Draft Cadmium AWQC_Appendicies_7 1 15 (081315).pdf
- Appendix K Issue Summary Regarding Test Conditions and Methods...H. Azteca.pdf
- Internal Draft Cadmium AWQC_References_11 4 14 (081315).pdf

Background/Supplemental Material (not for review, reference only)

- Cadmium Risks to Freshwater (Mebane 2010).pdf

Additional background reference material may be provided upon request to ERG.

CHARGE QUESTIONS

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

2. Please comment on the technical approach used to derive the draft cadmium criteria; is it logical, does the science support the conclusion, and is it consistent with the protection of freshwater and

estuarine/marine aquatic life from acute, chronic, and bioaccumulative effects? Are the methods described in the document scientifically sound?

3. Please comment on the data used to derive the revised criteria, including data adequacy/comprehensiveness, and the appropriateness of the data selected and/or excluded from the derivation of the draft criteria. Is the data used correctly for the intended purpose? Are there other relevant data that you are aware of that should be included? If so, please provide the data along with supporting information.

4. Are the derived criteria appropriately protective of listed species and commercially and recreationally important species, particularly as the criteria relates to salmonids?

APPENDIX B

INDIVIDUAL REVIEWER COMMENTS

EPA-HQ-2016-005391_00000159

COMMENTS SUBMITTED BY

Reviewer 1

External Letter Peer Review of EPA's Draft Aquatic Life Ambient Water Quality Criteria (AWQC) for Cadmium – 2015

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

This report makes for very dull reading, but it is well-written and it is usually clear what the author is trying to say. There are no insightful comments or new ideas presented in this report, but the report is laid out in a clear, logical fashion.

2. Please comment on the technical approach used to derive the draft cadmium criteria; is it logical, does the science support the conclusion, and is it consistent with the protection of freshwater and estuarine/marine aquatic life from acute, chronic, and bioaccumulative effects? Are the methods described in the document scientifically sound?

This report is rather antiquated in its thinking. It basically assumes that Cd is accumulated only from the aqueous phase rather than from both the aqueous phase and ingested food. Over the past 10-15 years, it has been shown that many toxicants, including Cd and other metals, can be bioaccumulated from food as well as from the aqueous phase. Indeed, a number of laboratory, field, and modeling studies have shown that diet can be the dominant source of metals for marine invertebrates and fish. The relative importance of diet has been shown to vary with species, but it is rarely a minor source and sometimes (for some fish species, for example) the predominant source. Moreover, once accumulated from diet, Cd can reach sensitive organs within animals that are not reached by Cd taken up from the aqueous phase. Therefore, the toxic response of an animal to either ambient Cd or body burden Cd can vary considerably, depending on whether the source is ingested food or solute in ambient water. Thus, dissolved metal may be sorbed onto exoskeletons in crustacean zooplankton (often the most sensitive species, as the author points out) but this does not directly affect the animal because the metal (Cd in this case) bound to chitosan on the exoskeleton does not interact with metabolic processes, whereas metal assimilated from ingested food can enter into internal tissues where it may interfere with a variety of metabolic and reproductive processes. I saw no acknowledgement of the possible significance of dietary Cd on aquatic (freshwater or marine) animals in this report, and yet numerous papers describing such effects appeared in the reference section. In looking over appendices, many of these reports were not used, often for what appear to be spurious reasons or misinterpretations of studies. In some cases, dietary metals could be 1-2 orders of magnitude more toxic than dissolved metals to freshwater cladocerans and marine copepods, for example. In the case of Cd, an EC_{50} value of 5 nM (~0.5 µg/L) was observed in copepods in a study by Hook & Fisher (cited in this report) if the animal had been fed food exposed to that Cd concentration, whereas the measured LC_{50} value based on a dissolved Cd source was 200 times greater. Also, measuring growth or mortality, as is often the case in simple toxicity tests, would have missed the effect—rather the reproductive capability of the copepods was affected by the dietary Cd, but no mortality was observed at environmentally realistic concentrations. Because dissolved Cd concentrations are typically at very low concentrations in natural waters (at least 10-fold lower in surface seawater, for example), the lower EC_{50} value derived from dietary rather than dissolved sources still indicates that Cd is unlikely to cause toxic effects in most natural waters.

3. Please comment on the data used to derive the revised criteria, including data adequacy/ comprehensiveness, and the appropriateness of the data selected and/or excluded from the derivation of the draft criteria. Is the data used correctly for the intended purpose? Are there other relevant data that you are aware of that should be included? If so, please provide the data along with supporting information.

As noted above, the author chose to ignore many relevant studies that did not conform with standard EPA toxicity protocols. But the problem is that these protocols basically ignore the fact that animals eat, hardly a realistic scenario and are too simplistic in looking only at growth and mortality. Typically, the test organisms are exposed to dissolved Cd at varying concentrations, but in the absence of food. Occasionally, some artificial food (fish flakes or the like) is presented once every several days (sometimes never!) to keep the animals alive. But these studies are hardly representative of what happens in natural waters.

4. Are the derived criteria appropriately protective of listed species and commercially and recreationally important species, particularly as the criteria relates to salmonids?

I agree with the author that marine animals are less at risk than freshwater animals, and this is primarily due to the strong chloro-complexation of Cd in seawater, thereby reducing the bioavailability of Cd. Consequently, marine bioconcentration factors are often 1-2 orders of magnitude higher in freshwater. I also agree that plants (e.g., phytoplankton) are less sensitive to Cd than animals, and thus it is appropriate to focus on the animals. I think that the criteria that the author generated for dissolved Cd have taken into consideration many of the key issues influencing this (e.g., water hardness) are probably ok, but by missing the effects of dietary Cd, the report is missing a large part of the overall story. This is not to suggest that ambient Cd concentrations are unsafe for animals, but the derived criteria are probably over-estimates of the safe levels of Cd. Another complicating issue is the influence of dissolved organic carbon and its effect on Cd bioavailability. Thus, expressing Cd toxicity as a function of body burden is appropriate; the caveats associated with this approach have been appropriately discussed in the report.

COMMENTS SUBMITTED BY

Reviewer 2

External Letter Peer Review of EPA's Draft Aquatic Life Ambient Water Quality Criteria (AWQC) for Cadmium – 2015

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

Overall the document is relatively clear with formatting in a risk assessment format which allows the reader to evaluate each criteria. Of minor concern was the lack of inclusion of emerging materials as sources of cadmium such as quantum dots which do make up photovoltaic substances (mentioned). However, the increased use of these materials as "inorganic" Cd sources and the uncertainties surrounding the potential absorption and effects of these materials to aquatic organisms needs some discussion.

In addition, some inconsistencies were noted with regard to sub-lethal effects mentioned in the Estuarine/Marine Acute section. While present in this section, discussions of sublethal effects were largely omitted in the Freshwater sections and chronic sections of both water types. There was also inconsistencies with regard to the use of flow-through vs. static exposures and whether more or less uncertainty is involved in utilization of the values. For example, flow-through methods were stated for *Salmo trutta*, but methods for *Morone* were static or static-renewal. One would clearly suggest the flow through values should be given greater weight with regard to uncertainty assessments. As it reads right now, it appears there are no differences between using static or flow-through exposures.

The inability to determine salinity relationships to toxicity is also a concern but it is likely due to varied salinity regimes confounded with temperature and solute constituents in experimental designs (see comments below). It is noteworthy that a 1ppt value is considered "estuarine" for the *Morone* value, when there are "freshwater" systems that likely have higher conductance than this value. There should also be some statement or better clarity documenting the lack of a standard salinity value being utilized to compare toxicity values. It appears that the most sensitive toxicity value is being used regardless of the salinity.

Overall, the uncertainty analysis section should be extended to include aspects of uncertainty with the data used for the derivation of the criteria. As it stands presently, the emphasis seems to be more on justification of data not utilized for the derivations.

2. Please comment on the technical approach used to derive the draft cadmium criteria; is it logical, does the science support the conclusion, and is it consistent with the protection of freshwater and estuarine/marine aquatic life from acute, chronic, and bioaccumulative effects? Are the methods described in the document scientifically sound?

With a few notable exceptions, the technical approach for the freshwater acute and chronic derivations appear valid. Incorporation of hardness normalization is warranted given the likelihood that Cd and Ca compete for similar biological and abiotic sites. In addition, the increased number of species extending the SSDs is also an excellent step forward in confirming proposed criteria.

Of concern is the approach utilized for the chronic estuarine/marine values. Utilization of ACRs with freshwater fish or other organism to derive estuarine/marine values is not appropriate, especially when the

criteria concentrations are increased. It is also unclear why freshwater salmonid values were not utilized for the ACRs, as many reside in estuarine/marine environments (see salmonid comments below).

3. **Please comment on the data used to derive the revised criteria, including data adequacy/ comprehensiveness, and the appropriateness of the data selected and/or excluded from the derivation of the draft criteria. Is the data used correctly for the intended purpose? Are there other relevant data that you are aware of that should be included? If so, please provide the data along with supporting information.**

The use of additional species for SSD reduced uncertainty and greatly improved criteria assessments for freshwater. The QA evaluations of data usefulness was adequate and the data selected for the acute responses was correctly used for the intended purpose. The mechanistic assumption that adverse effects are primarily related to calcium uptake at the gill, is accurate for acute effects. Consequently, the data used for derivation of the criteria for acute effects is valid.

However, with regard to chronic effects, there are other targets once absorption of cadmium occurs, particularly the kidney, brain and gonad. In addition to specific interactions with signaling proteins, Cd clearly binds sulfhydral groups of proteins within targets disrupting cellular maintenance. The latter two tissue targets above are likely involved in the reproductive effects observed with chronic exposures. Cd clearly disrupts the Hypothalmic Pituitary Gonadal axis and gonadal function in fish (Vetillard, and Bailhache 2005). It reduces vitellogenin in females and accumulates in kidney upon chronic exposures either via diet or water (Szczerbik et al. 2006; Thomann et al. 1997).

It is understood that tissue data from these organs are limited, but studies that have these data, or the fact that these data are limited should be discussion points of the uncertainty analysis. Clearly, discussions of uncertainty regarding accumulation are needed, particularly in light of limited data for chronic effects in estuarine/marine organism. The statement "**Aquatic organisms are considered to be more susceptible to cadmium from direct aqueous exposure than through bioaccumulation and the development of criteria protective of direct exposure effects are considered more applicable to the development of criteria for aquatic life**" is clearly biased toward acute toxicity and should be re-visited with particular emphasis on reproductive effects of cadmium which likely result from accumulation and not direct exposure.

With regard to reproduction, it is unclear what endpoint data is being used to determine the effect values in the Appendices. Tests are provided in terms of exposure duration, but it is unclear whether growth, survival or reproduction is being utilized as the endpoint. Again, given the potential for reproductive effects upon chronic exposure, reproduction would be expected to be the most sensitive endpoint. If other endpoints were used then the uncertainties inherent to these endpoints should be discussed. Clearly growth and survival effects have likely difference mechanisms and targets than that of reproduction.

It is also significantly disappointing that data from the same 2 species in 1980s are still the only two species being used to derive the 2015 values. In addition, it is puzzling how criteria values can be raised for estuarine/marine organisms when the same degree of uncertainty exists (only 2 species) in each year criteria were assessed. To add in data from freshwater organisms for ACR estimates ***increases*** uncertainty and does not reduce it. Therefore, the 2001 value should stay as is, or be reduced because of the uncertainty associated with its derivation.

4. Are the derived criteria appropriately protective of listed species and commercially and recreationally important species, particularly as the criteria relates to salmonids?

Salmonids are clearly one of the more sensitive species with regard to Cd toxicity. Not only are they very sensitive, they are commercially important, and possess several species that are listed as endangered and threatened in the US. The proposed criteria are appropriate for freshwater conditions since many of the studies used to derive the criteria focused on freshwater treatments to rainbow trout. However, only one study evaluated Cd toxicity in coho salmon smolts in saltwater conditions, and this was at nearly full seawater strength (28 ppth). Of concern is the fact that many salmonids including strains of *O. mykiss* (steelhead) are anadromous and often come in contact with Cd at lower salinities (5-15 ppth). While the agency should be applauded for normalizing toxicity to hardness to improve freshwater criteria, there is a critical need to understand the impacts of salinity on Cd toxicity particularly in anadromous salmonid species. Of additional concern is the lack of discussion of sublethal impacts of Cd particularly to olfaction (Williams and Gallagher 2013) which significantly alters return rates of salmon (Baldwin et al. 2009). Return metrics are population level endpoints that should supersede standard repro/survival/growth. These should also be topics of discussion with regard to uncertainty.

Lastly, the issue of climate change is largely missing from the document. Acidification (particularly with metal availability) and temperature issues are also likely to impact sensitive species (eg. salmonids). Sea level rise will also cause saltwater intrusion into salmonid spawning habitats and affect "estuarine/marine" criteria. Evaluation of these stressors should be focal points for future criteria assessment particularly for salmonids. Overall, while the values for freshwater are likely safe for salmonids, the values for estuarine/marine are highly uncertain and deserve further evaluation.

References

Baldwin, DH , Spromberg, JA, Collier, TK and NL Scholz (2009) A fish of many scales: extrapolating sublethal pesticide exposures to the productivity of wild salmon populations. Ecological Applications 19:2004-2015.

Szczerbik, P, Mikolajczyk, T, Sokolowska-Mikolajczyk, M, Socha, M, Chyb, J, Epler, P. (2006) Influence of long-term exposure to dietary cadmium on growth, maturation and reproduction of goldfish (subspecies: Prussian carp Carassius auratus gibelio B.) Aquatic Toxicology 77:126-135.

Thomann, RV, Shkreli, F., Harrison, S. (1997) A pharmacokinetic model of cadmium in rainbow trout. Environmental Toxicology and Chemistry 16:2268-2274.

Vetillard, and Bailhache (2005) Cadmium: An endocrine disrupter that affects gene expression in the liver and brain of juvenile rainbow trout. Biology of Reproduction 72:119-126.

Williams CR and EP Gallagher (2013) Effects of cadmium on olfactory mediated behaviors and molecular biomarkers in coho salmon (Oncorhynchus kisutch) Aquatic Toxicology 140-141:295-302.

EPA-HQ-2016-005391_00000169

COMMENTS SUBMITTED BY

Reviewer 3

External Letter Peer Review of EPA's Draft Aquatic Life Ambient Water Quality Criteria (AWQC) for Cadmium – 2015

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

In general, the document language is reasonably clear. However, throughout the document, there are several instances where certain decisions are made that appear to be rather arbitrary without sufficient justification as to how or why these decisions were made (see details below).

Minor comments:

p. 8 and elsewhere: use mass units rather than ppm, ppb etc.

p. 9: quantify concentrations found in impaired water ("several micrograms per liter" is vague)

p. 10: is the suggestion that precipitated/particulate forms of Cd that ultimately end up in sediments are not bioavailable?

p. 19: do data exist for any other salts of Cd that has been excluded?

P.63: Please be explicit about how the constants in the equations are derived for both the CMC and CCC.

P. 67: Define the values listed under the two tables: (S^2, L, A)

Major comments:

p. 12: "Mebane (2014) conclude that, although there were not adequate data to establish acceptable tissue effects concentrations for aquatic life, <u>cadmium is unlikely accumulate in tissue to levels that would result in adverse effects to aquatic invertebrates or fish</u>. The evaluation of direct exposure effects is therefore considered to be more applicable to the development of criteria for aquatic life."

This line of reasoning is questionable on many levels. Establishing critical tissue effects thresholds that work across species is problematic, especially in invertebrates, because species vary in their abilities to store/sequester Cd in physiologically inert forms. However, this does not mean that bioaccumulated metals are non-toxic as is implied by the language in this document. I think Mebane is being grossly misquoted here (aside from the fact that there is no 2014 reference). Here are some quotes from his 2010 document that directly refute the underlined text above:

- "Thus the consequences of elevated tissue residues or effects of dietary exposures may be important when estimating protective thresholds for cadmium and other pollutants (McCarty and Mackay, 1993; Meyer and others, 2005)." P. 32
- "A diet of cadmium-contaminated green algae *Chlorella* sp caused reduced growth in the amphipod *Hyalella azteca* in a recent study (Ball and others, 2006)." P. 38

- "Dietary cadmium exposures appear to be an important risk for at least some invertebrates. The data reviewed on dietary effects of cadmium to invertebrates indicated that adverse effects could occur at concentrations realistic in cadmium-polluted waters". P. 38
- "Toxicity to mayflies from feeding on cadmium-contaminated algal mats at environmentally realistic concentrations was observed (Irving and others, 2003). P. 38

I understand that dealing with dietary exposures is incredibly inconvenient in the context of the 1985 Guidelines, but pretending that they are not important in 2015 is irresponsible because we know better. The Irving et al., 2003 study referenced above provides direct evidence that diet derived Cd can be problematic in this aquatic insect example.

2. Please comment on the technical approach used to derive the draft cadmium criteria; is it logical, does the science support the conclusion, and is it consistent with the protection of freshwater and estuarine/marine aquatic life from acute, chronic, and bioaccumulative effects? Are the methods described in the document scientifically sound?

Bioaccumulative effects of Cd are largely ignored in this document.

My comments for this section are divided into 2 parts: *1.* The technical approach according to the 1985 Guidelines, and *2.* The technical approach in light of our current understanding of cadmium bioaccumulation, effects, and deficiencies in the traditional testing approaches.

1. The technical approach according to the 1985 Guidelines

   A. What is the rationale for use of EC20 values for the chronic toxicity assessment? I understand that a MATC approach (based on NOEC and LOECs) has its issues, and I'm generally in favor of more statistically robust approaches such as the use of an EC level based on entire datasets. But why is a 20% effect level chosen here? This value seems rather high. There should be some rationale for choosing this value, and this rationale should be clearly articulated in the text. How do we know that a 20% effect level has no impacts at the population level?

   B. Only 3 species (all fish) were used to generate the hardness correction for the freshwater chronic toxicity data set. *D. magna* and *P. promelas* data were not used because only MATCs were available and not EC20s. Is it not possible to estimate EC20's from these datasets? The use of only 3 species to make this very important hardness adjustment would seem to add a significant level of uncertainty to the final analysis, especially since 2 of species used have divergent slopes. ANCOVA (p=0.08) based on data from 3 species was used to say that the slopes 0.32, 1.46 and 1.08 are not different and can be pooled. Is this defensible? Shouldn't a conservative slope estimate be chosen here....especially in light of the fact that a 20% effect level is much higher than an MATC or EC05 would be?

   C. The most acutely sensitive marine genus, *Tigriopus* was not used in the analysis. The rationale was that it falls below the 5th percentile of the distribution. Isn't the whole point of the SSD to determine what is protective of 95% of the species? (Not 95% of the remaining taxa after sensitive taxa are arbitrarily removed from the dataset). Shouldn't all of the data be used here?

D. The use of 2 ACRs from freshwater species in the development of a marine chronic criterion is dubious on many fronts. The justification for doing this needs to be articulated. If justifiable, the authors should then justify their choices as to why these 2 species were chosen. The reason given in the text is that the freshwater species were chosen on the basis of being acutely sensitive. However the purpose of ACRs is to evaluate the potential for the chemical to cause chronic toxicity. Use of an acutely sensitive species for ACR choice should theoretically result in species with low ACRs, and in this case, this is borne out. The freshwater invertebrate *L. silquoidea* has a reported ACR of 2.727, suggesting that is chronically not very toxic. However, the ACRs for most species are considerably higher: (see below)

Mebane (2010) list ACRs for freshwater invertebrates:

Ephemerella: 158.67
Physa: 47.6
Aplexa: 28.5 and 47.87
Ceriodapnia: 12.41 and 31.5
Dapnia: 65, 155, 112, 13
Hyalella: 17.5

This document lists the following freshwater invertebrate ACRs:

Aplexa: 49.7
Lymnea: 12.81
Ceriodaphnia: 19.82
Daphnia: 57.3

With all of these values to choose from, 2.727 is clearly not a representative ACR for freshwater invertebrates. Since the use of a "mean ACR" is being applied across taxa, shouldn't the values be representative? Would it make sense to have higher ACRs apply to invertebrates and lower ACRs apply to fish since fish generally have low ACRs and inverts generally have high ACRs?

2. Technical approach based on what we understand about the world post 1985:

Cadmium has been demonstrated to be toxic to practically every in vitro system it has been tested in. We strive to limit human dietary exposures in part because it is a known carcinogen and is nephrotoxic after dietary exposure. Effects of Cd on antioxidant physiology are well described in several species including aquatic insects. <u>What evidence can we point to suggest that bioaccumulated Cd is not toxic to aquatic organisms? This is a fundamental flaw in this document.</u>

We have a major and important disconnection between what traditional laboratory tests (using only direct aqueous exposures) and what field ecologists tell us about metal effects in aquatic insects. Because insects are such important players in freshwater ecosystems, and are the focus of CWA-driven biomonitoring programs, we have numerous examples of stream community structure being impaired by metal exposures. Yet lab (aqueous) tests generally suggest that insects are insensitive to Cd. Work in our laboratory has used Cd uptake and depuration kinetics to clearly demonstrate that 96 hour exposures are insufficient to elicit toxicity in aquatic insects are ecologically relevant concentrations (Buchwalter et al. 2007, Buchwalter et al. 2008, Poteat and Buchwalter 2014, Poteat

and Buchwalter 2014). We have also shown that periphyton is a major sink for Cd, and is readily bioaccumulated in insects (Xie et al. 2010). We have also showed that Cd exposure does not negatively affect Ca transport in insects (Poteat and Buchwalter 2014) (as it is known to do in acutely sensitive taxa), and Ca provides little protective effects on Cd uptake (Poteat et al. 2012). Finally, we show that diet derived (but not water derived) Cd affects antioxidant physiology suggesting that dietary exposures may be more challenging to aquatic insects that aqueous exposures (Xie and Buchwalter 2011). These findings mirror those of Irving et al., 2003. All of these findings point towards short-term, water-only exposures are insufficient for evaluating metal toxicity in this important faunal group (see (Poteat and Buchwalter 2014) for discussion of these findings).

3. **Please comment on the data used to derive the revised criteria, including data adequacy/ comprehensiveness, and the appropriateness of the data selected and/or excluded from the derivation of the draft criteria. Is the data used correctly for the intended purpose? Are there other relevant data that you are aware of that should be included? If so, please provide the data along with supporting information.**

Practically all relevant work related to bioaccumulated Cd and the importance of dietary exposures is ignored. (see (Barata et al. 2002, Barata et al. 2002, Buchwalter et al. 2008, Cain et al. 2004, Croteau et al. 2003, Hare et al. 2001, Hare et al. 2003, Irving et al. 2003, Klaassen et al. 1999, Luoma and Rainbow 2005, Luoma et al. 2009, Luoma and Carter 1991, Martin et al. 2007, Timmermans et al. 1992, Wallace et al. 2003, Xie et al. 2010, Xie and Buchwalter 2011, Xie et al. 2008) for some examples)

I suspect that there are other reviewers who can comment more directly on the issues with *Hyalella* data, so I will refrain from doing so here.

4. **Are the derived criteria appropriately protective of listed species and commercially and recreationally important species, particularly as the criteria relates to salmonids?**

This seems to be the case if we assume that only aqueous exposures matter. Evidence for dietary toxicity is less compelling than for invertebrates, so for these fish species, the criteria are likely more protective for these species than they are for invertebrates.

Reference List

Barata, C., S. J. Markich, D. J. Baird, and A. M. V. M. Soares. 2002. The relative importance of water and food as cadmium sources to *Daphnia magna* Straus. Aquat. Toxicol. 61:143-154.

Barata, C., D. J. Baird, S. E. Mitchell, and A. M. V. M. Soares. 2002. Among- and within-population variability in tolerance to cadmium stress in natural populations of *Daphnia magna*: implications for ecological risk assessment. Environ. Toxicol. Chem. 21:1058-1064.

Buchwalter, D. B., D. J. Cain, W. H. Clements, and S. N. Luoma. 2007. Using biodynamic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. Environ. Sci. Technol. 41:4821-4828.

Buchwalter, D. B., D. J. Cain, C. A. Martin, L. Xie, S. N. Luoma, and T. Garland, Jr. 2008. Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. Proc. Nat. Acad. Sci. 105:8321-8326.

Cain, D. J., S. N. Luoma, and W. G. Wallace. 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. Environ. Toxicol. Chem. 23:1463-1473.

Croteau, M.-N., L. Hare, and A. Tessier. 2003. Difficulties in relating Cd concentrations in the predatory insect *Chaoborus* to those of its prey in nature. Can. J. Fish. Aquat. Sci. 60:800-808.

Hare, L., A. Terrier, and L. Warren. 2001. Cadmium accumulation by invertebrates living at the sediment-water interface. Environ. Toxicol. Chem. 20:880-889.

Hare, L., A. Tessier, and U. Borgmann. 2003. Metal sources for freshwater invertebrates: pertinence for risk assessment. Human Ecol. Risk Assessment 9:779-793.

Irving, E. C., D. J. Baird, and J. M. Culp. 2003. Ecotoxicological responses of the mayfly *Baetis tricaudatus* to dietary and waterborne cadmium: implications for toxicity testing. Environ. Toxicol. Chem. 22:1058-1064.

Klaassen, C. D., J. Liu, and S. Choudhuri. 1999. Metallothionein: An intracellular protein to protect against cadmium toxicity. Annu. Rev. Pharmacol. Toxicol. 39:267-294.

Luoma, S. N., D. J. Cain, and P. S. Rainbow. 2009. Calibrating Biomonitors to Ecological Disturbance: a New Technique for Explaining Metal Effects in Natural Waters. Integrated Environmental Assessment and Management 6:199-209.

Luoma, S. N. and P. S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol. 39:1921-1931.

Luoma, S. N. and J. L. Carter. 1991. Effects of trace metals on aquatic benthos, pp. 261-300 In M. C. Newman and A. W. McIntosh (eds.), Metal Ecotoxicology Concepts & Applications. Lewis Publishers, Inc., Chelsea, Michigan.

Martin, C. A., D. J. Cain, S. N. Luoma, and D. B. Buchwalter. 2007. Cadmium ecophysiology in seven stonefly (Plecoptera) species: Delineating sources and susceptibility. Environ. Sci. Technol. 41:7171-7177.

Poteat, M. D. and D. B. Buchwalter. 2014. Calcium uptake in aquatic insects: influences of phylogeny and metals (Cd and Zn). J. Exp. Biol. 217:1180-1186.

Poteat, M. D. and D. B. Buchwalter. 2014. Four reasons why traditional metal toxicity testing with aquatic insects is irrelevant. Environ. Sci. Technol. 48:887-888.

Poteat, M. D. and D. B. Buchwalter. 2014. Phylogeny and Size Differentially Influence Dissolved Cd and Zn Bioaccumulation Parameters among Closely Related Aquatic Insects. Environ. Sci. Technol. 48:5274-5281.

Poteat, M. D., M. Diaz-Jaramillo, and D. B. Buchwalter. 2012. Divalent metal (Ca, Cd, Mn, Zn) uptake and interactions in the aquatic insect Hydropsyche sparna. J. Exp. Biol. 215:1575-1583.

Timmermans, K. R., E. Spijkerman, and M. Tonkes. 1992. Cadmium and zinc uptake by two species of aquatic invertebrate predator from dietary and aqueous sources. Can. J. Fish. Aquat. Sci. 49:655-662.

Wallace, W. G., B.-G. Lee, and S. N. Luoma. 2003. The subcellular compartmentalization of Cd and Zn in two bivalves. I. The significance of metal-sensitive fractions (MSF) and biologically-detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249:183-197.

Xie, L. and D. B. Buchwalter. 2011. Cadmium exposure route affects antioxidant responses in the mayfly Centroptilum triangulifer. Aquat. Toxicol. 105:199-205.

Xie, L., D. H. Funk, and D. B. Buchwalter. 2010. Trophic transfer of Cd from natural periphyton biofilms to the grazing mayfly *Centroptilum triangulifer* in a life cycle test. Environmental Pollution. 158:272-277.

Xie, L. T., D. Lambert, C. Martin, D. J. Cain, S. N. Luoma, and D. Buchwalter. 2008. Cadmium biodynamics in the oligochaete Lumbriculus variegatus and its implications for trophic transfer. Aquat. Toxicol. 86:265-271.

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COMMENTS SUBMITTED BY

Reviewer 4

External Letter Peer Review of EPA's Draft Aquatic Life Ambient Water Quality Criteria (AWQC) for Cadmium – 2015

1. **Please comment on the overall clarity of the document and construction as it relates to the derivation of each criterion.**

Response: I found the overall clarity of the document to be quite good. I especially appreciated the document being generally organized in a risk assessment format. I think this is very useful, particularly the Problem Formulation section that outlines various sources, potential exposure pathways and receptors. I hope EPA will use this overall structure for future criteria documents as well. I also like all of the comparisons to previous Cd criteria documents. This makes key changes to the criteria very transparent.

My only significant criticism of the overall format is that there are a number of redundancies where information is presented multiple times, often the exact same wording (for example, Section 5.4.1 is redundant of earlier text in the document). I encourage EPA to consider consolidating and reducing these redundancies.

An additional minor point is that it is unclear how the data tables in the appendices are organized. They don't seem to be listed alphabetically by either common or scientific name. It would be useful if they were.

2. **Please comment on the technical approach used to derive the draft cadmium criteria; is it logical, does the science support the conclusion, and is it consistent with the protection of freshwater and estuarine/marine aquatic life from acute, chronic, and bioaccumulative effects? Are the methods described in the document scientifically sound?**

Response: Overall yes, I think the technical approach is scientifically sound and consistent with the protection of aquatic life. I do, however, have some specific significant comments for EPA to consider which I list below.

Page 15: EPA concludes that most changes in Cd toxicity can be explained by changes in hardness and therefore incorporation of the BLM into this revision is not necessary. I strongly disagree with this statement. Every study I'm aware of in which a range of DOC and pH have been measured has shown that these parameters strongly influence Cd toxicity. Just because the majority of laboratory studies are conducted in laboratory waters with low DOC and do not measure DOC, does not provide a valid rationale for not using the BLM. Obviously, in the natural environment, DOC varies widely. I would think the objective of the criteria is to ensure that they are protective/predictive of toxicity in the natural environment, not in artificial laboratory waters.

Page 34: Following up on the previous comment regarding not using the BLM, why did EPA only consider a multiple linear regression with alkalinity? Why not pH and/or DOC? It is quite possible that pH autocorrelates with hardness as well given this is the case for most artificial laboratory waters (though not as consistent for natural waters), but there will not be an autocorrelation with DOC. This is a really important water quality parameter that EPA is ignoring.

Page 50-51: Is the study by Voyer et al. (1974), the only study where the effects of salinity on Cd toxicity was not consistent or are there multiple studies with this problem? If it's only this one study, it's not clear why the general trend would be ignored. I don't think EPA would ignore the hardness relationship in freshwater if only a single study was inconsistent with the general trend. It is a concern that there is on obvious and significant salinity effect for the *Neomysis integer* data (p. 51), which is one of the four taxa used for the criteria derivation, and yet this obvious effect is ignored and the geometric mean is used to develop the SMAV. Does EPA consider a test performed at a salinity of 1 ppt to be a marine test?

3. **Please comment on the data used to derive the revised criteria, including data adequacy/comprehensiveness, and the appropriateness of the data selected and/or excluded from the derivation of the draft criteria. Is the data used correctly for the intended purpose? Are there other relevant data that you are aware of that should be included? If so, please provide the data along with supporting information.**

Response: Overall, I found the data used by EPA to derive the criteria to be comprehensive and generally sound. There are a few specific data where I have concerns that EPA should consider as described below.

Page 51: I'm very concerned that EPA is still allowing studies in which test concentrations were unmeasured as being acceptable for WQC derivation. This is particularly concerning when they are for one of the four taxa used to calculate the criteria. In my opinion, these studies should not be included.

Page 68: I agree with EPA's use of freshwater ACRs to supplement the limited marine ACRs for the purpose of deriving a final marine ACR. However, I question whether use of the ACR for *Lampsilis siliquoidea* is appropriate. There are obviously a number of factors that influence the ACR, but a major factor is the life history of the organism and the life stage selected for the acute toxicity test used to derive the ACR. It seems to me that freshwater mussels have a unique life history with no real analog in marine systems (marine bivalves have a different life history). Consequently, use of this of the ACR for this species to derive a marine ACR seems inappropriate. I think use of an ACR for daphnids would be more appropriate and representative of the life history of the most acutely sensitive taxa in marine systems, the copepod *Tirgriopus*.

Table 17: Why is the pH 6.0 test for *H. azteca* excluded? This is within the range of test pH values (6.0-9.0) normally consider by EPA. Additionally, earlier in the document it was stated that hardness was the only water quality parameter that mattered for normalizing Cd toxicity data. I disagree with that statement, but if EPA is going to argue other water quality parameters are not important, then I don't see how it can then exclude data for this reason.

Table 18: I agree with EPA's re-evaluation of the *Hyalella* data and their application of water quality and performance criteria for test acceptability. However, I'm concerned about the study EPA retained for purposes of criteria derivation for several reasons. First, I do not believe use of a 10-d survival endpoint constitutes a chronic study as defined in Stephan et al. (1985). EPA has excluded a number of other studies from use in criteria derivation for this reason (e.g., the 21-d survival study on the sea starlet anemone, p. 81) in this document that creates a major internal inconsistency. Having said that, it could be argued that inclusion of this sub-chronic data is warranted given that it is the lowest toxicity value in the data set and exclusion of the data would be non-conservative in terms of environmental protection (as opposed to

including sub-chronic data for insensitive species). However, using this logic why would the 7-d survival/growth data with the fountain darter then be excluded?

My second concern is whether the sensitivity of *H. azteca* is real? Given that these 10-d data come from a 42-d study that fails to meet control performance criteria, how does EPA know that these animals weren't already stressed at 10 d and inappropriately sensitive? Given both the duration and performance issues associated with these data, in my opinion they should not be used for WQC derivation. However, I strongly encourage EPA to conduct a 28- or 42-d *Hyalella* study that meets the necessary performance criteria. Finally, after Table 18, EPA has descriptions of each of the chronic *H. azteca* studies and rationale for their rejection but did not include a description of the Ingersoll and Kemble study that was accepted and the rationale for use of the 10-d survival endpoint. This should be added to the document.

4. Are the derived criteria appropriately protective of listed species and commercially and recreationally important species, particularly as the criteria relates to salmonids?

Response: Yes, I think the criteria as derived will be protective of salmonids. However, I'm concerned about the exclusion of the fountain darter data from the derivation. EPA argues that the acute data should be excluded because the test was fed and that the chronic data should be excluded because the study was only 7 d in duration (i.e., not true chronic). Generally, I agree with both of these decisions, but from my perspective, these rules are in place to prevent the inclusion of data indicating organisms are insensitive due to inappropriate test conditions (i.e., food reducing metal bioavailability, short test durations missing sensitive endpoints). However, this is not the case with the darter data, which indicate this species is very sensitive despite test conditions that would tend to reduce their sensitivity. EPA also seems to infer (p. 86) that the fountain darter data has limited applicability because this species has a limited distribution. However, the genus *Etheostoma* is widespread throughout central and eastern U.S. with a number of listed species at both the state and federal level. Hence these data a representative for a genus that is under considerable threat. Given this, I think it would be important to assess how inclusion of these data would impact derivation of the freshwater Cd WQC.

Page 87: I don't think the statement that dividing the LC50 by two is expected to result in a concentration with effects no different than the control is correct. Dividing the LC50 by two will result in an "LC-low". I agree that across a range of species and toxicants, dividing by two equates to a values that approximates the NOEC. However, it does not equate to an LC0, which is inferred by this statement. Please clarify.

Additional Minor Comments

Page 11: Note that Cd does not form complexes with Ca as stated, but rather competes with Ca for uptake and Ca channels. Please correct.

Page 11: While Atli and Canli did observe a reduction in NKA activity in their study, it's a significant overstatement to say disruption of Na homeostasis is a mechanism of action for Cd. To the best of my knowledge, it hasn't been observed in any other study that has investigated this potential mechanism.

Page 11: If Cd inhibits catalase, glutathione reductase, SOD, etc., it seems to me this is direct inhibition of anti-oxidant processes, not indirect as stated.

Page 12: Regarding the relationship between Cd tissue burdens and toxicity, see also the analysis by Adams et al. (2011).

Page 50: *Tigriopus* is a copepod, not a mysid, as indicated in the second paragraph.

Page 58: Please specific at the top of p. 58 which two freshwater ACRs were used in the calculation of the marine ACR.

Table 18: Change the test duration for the Borgmann studies to 42 d rather than 6 w to make the units consistent with the rest of the table.

Page 83: It should be mentioned that both BCFs and BAFs are inversely related to exposure concentration which explains much of the variation in BCFs/BAFs (McGeer et al. 2003, DeForest et al. 2007).

Table 21: Taking a final look through Table 21 I note that EPA has included several species that are not resident to N. America (*Oreochromis spp., Danio rerio, Xenopus laevis*). Unless this requirement has changed, they should be removed from the data set.

References

Adams, W. J., R. Blust, U. Borgmann, K. V. Brix, D. K. DeForest, A. S. Green, J. S. Meyer, J. C. McGeer, P. R. Paquin, P. S. Rainbow and C. M. Wood (2011). "Utility of tissue residues for predicting effects of metals on aquatic organisms." Integr. Environ. Assess. Manag. **7**(1): 75-98.

DeForest, D. K., K. V. Brix and W. J. Adams (2007). "Assessing metal bioaccumulation in aquatic environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration." Aquat. Toxicol. **84**: 236-246.

McGeer, J. C., K. V. Brix, J. M. Skeaff, D. K. DeForest, S. I. Brigham, W. J. Adams and A. S. Green (2003). "The inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment." Environ. Toxicol. Chem. **22**(5): 1017-1037.

Stephan, C. E., D. I. Mount, D. J. Hansen, J. H. Gentile, G. A. Chapman and W. A. Brungs (1985). Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. Duluth, U.S. Environmental Protection Agency, Environmental Research Laboratory: 98 pp.

COMMENTS SUBMITTED BY

Reviewer 5

External Letter Peer Review of EPA's Draft Aquatic Life Ambient Water Quality Criteria (AWQC) for Cadmium – 2015

Unfortunately, the compressed time period for this review (2 weeks, which works out to several hours on evenings and weekends for volunteer reviewers), makes a comprehensive review of a document of this length and complexity infeasible.

1. **Please comment on the overall clarity of the document and construction as it relates to the derivation of each criterion.**

Generally sufficient. Problem formulation section seemed a bit of a forced fit, as if added to satisfy a new stylist protocol.

2. **Please comment on the technical approach used to derive the draft cadmium criteria; is it logical, does the science support the conclusion, and is it consistent with the protection of freshwater and estuarine/marine aquatic life from acute, chronic, and bioaccumulative effects? Are the methods described in the document scientifically sound?**

Unfortunately some aspects of the document lead to answering both parts of the charge question 2 with answers of "no." I am only commenting on aspects which to me did not follow the available science, deviate from the principles of the 1985 "Guidelines" or otherwise have logical problems, or . While Stephan et al's (1985) Guidelines for derivation of aquatic life criteria are 30 years old and aspects of the science have progressed such that some details may not fit, they include solid principals that should continue to guide the approach. Key among Stephan et al's guiding concepts is from their p. 3: *"The guidelines were intended to provide the same level of protection as would an (infeasible) approach of conducting field tests on a wide variety of unpolluted bodies of water, adding various amounts of the material to each body of water in order to determine the highest concentration that would not cause any unacceptable long-term or short-term effects on the aquatic organisms or their uses."* Further (p. 10), *These National Guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations. All North American bodies of water and resident aquatic species and their uses are meant to be taken into account."* Not bodies of water for which conditions are optimal – all bodies of water.

Thus, a key concept behind the logic of criteria derivation is that criteria be suitable for diverse, natural water bodies, and laboratory data should attempt to encompass comparable field situations. The draft document instead moves towards a very different concept of only using data from an idealized aquaculture setting, without regard to whether the species occurs in the wild in waters with "suboptimal" conditions.

Drilling down on Hyalella

Most fundamentally, by throwing out all long-term test endpoints for the most sensitive genus (Hyalella) this document strays from a guiding principle of the Guidelines that criteria are to protect diverse natural waters. Criteria are indeed developed using laboratory data, but they are not intended to apply to

laboratory waters; they are intended to apply to natural waters. This disconnect between laboratory-based derivation of numeric water quality criteria and application to natural waters has repeatedly debated in the literature, with me chiming in specifically with cadmium (Mebane 2010).

In essence, optimal aquaculture conditions are defined for culturing *Hyalella azteca*, and chronic tests in which less than 15 mg/L chloride was present in dilution waters, or control growth, survival, and reproduction did not meet expectations. These were control growth (≥0.35 mg at 28 days and ≥0.5 mg at 42 days), survival (80% at 42d) and reproduction (≥6 per young). No explanation was found in the document why researchers were tasked to drill down on Hyalella, as any commonly used test organism could have been similarly scrutinized. Absent explanation, the inference is that Hyalella must have been chosen because it was the most sensitive organism, and there was a desire to exclude data if this heightened sensitivity could be shown to be an artifact of stressful laboratory culture conditions. In essence this logic requires the following implicit assumptions. Since only Hyalella data obtained from laboratory test waters >15 mg/L are to be used for criteria development, it follows that:

1) In ambient waters, Hyalella (and presumably other freshwater amphipods) are only expected to occur in waters with >15 mg/L chloride; Alternatively if Hyalella do in fact occur in waters with lower chloride concentrations, the criteria are only intended to apply to waters with >15 mg/L.

2) Chloride is an important factor affecting the toxicity of cadmium to Hyalella (and presumably other related but less well studied amphipods or freshwater crustaceans). If so, then it follows that:

    a. Chloride should be included in the criteria derivation and factored into the criteria. Per the Guidelines (p32), "when enough data are available to show that the chronic toxicity is similarly related to a water quality characteristic, the relationship should be taken into account …. If two or more factors affect toxicity, multiple regression analysis should be used."

    b. Alternatively, while not specifically mentioned in the guidance, if data were insufficient for the covariance or multiple regression analyses endorsed, it would seem reasonable to establish different criteria in brackets, such as waters ≤15 mg/L chloride or >15 mg/L chloride.

3) Alternatively, if chloride is not an important factor affecting, then there is no reason to factor it into the criteria development.

However, Appendix K does not address the question of whether chloride is a factor affecting cadmium toxicity, all that has been established is that Hyalella growth and reproductive output is greatest in waters with chloride >15 mg/L. This is not unexpected. Freshwater environments usually have an osmolarity far less than blood plasma, and energy requirements to maintain hydromineral balance increase in more dilute waters (e.g., Wendelaar Bonga and Lock 2008). Fish in dilute waters don't grow well either. For instance, about 80% of the restaurant/retail rainbow trout sold in the United States come from a 30 mile stretch known as the Thousand Springs area of southern Idaho. There the constant chloride of about 20 mg/L, hardness of about 180 mg/L and temperature of 15°C provide optimal energy conversions and growth per unit feed. It would follow just as logically that only rainbow trout data that were generated from waters with chloride >15 mg/L or so should be used, because that optimizes growth? Why would it not follow that

only acute data in which organisms were fed should be used, because starvation stresses organisms? This seems to be internally inconsistent logic.

The reason why Appendix K was requested was never stated. It should be. I assume the reason must be a presumption that if organisms do not grow and reproduce at high rates, then they will "too sensitive" or not represent responses expected in natural conditions. It is not obvious that this is the case. McNulty et al. (1999) showed that starved amphipods exposed to low levels of cadmium survived better than controls. However, even if optimal diets do produce higher (less sensitive) growth and reproduction effects with Cd and Hyalella, the universal use of optimal diets could lead to underestimation of the toxicity risks experienced by wild populations, which may experience limited food availability. In the wild, organisms don't live in optimal conditions. Even in the center of their ranges, conditions are seldom optimal all of the time. Organisms also live in marginal conditions, for they tend to expand their ranges to the limits of their physiological tolerances. See for example France's (1996) description of Hyalella living on the margins of lakes with tolerable mineral content (France 1996). Similarly, Gibbons and Mackie (1991) showed that increasing reproductive output of H. azteca was associated with increasing sulfate, calcium hardness, sediment particle size, conductivity, alkalinity, seston, and the organic matter of the fine sediment. This consistent with Appendix K, but begs the question, what are effects of Cd in these suboptimal waters? Why assume that if Cd criteria are needed, they should only be developed from exposures in high hardness, but then blindly extrapolate results to low chloride, low hardness conditions using tests with other organisms? This is further logical problem with Appendix K's rationale – as noted in appendix K, waters with hardness less than 80 mg/L tend to have chloride less than 10 mg/L. Does the hardness-toxicity relation predict safe conditions for Hyalella at low hardness? No way to know.

I've poked around a bit the literature on Hyalella life histories under different environmental stresses in an effort to include extrapolate organism-level effects of Cd to potential population-level effects (Mebane 2010). While by no means exhaustive, and by now a bit dated, this leads to some other thoughts on the expected control survival, growth, and reproduction in long term tests in Appendix K. With control survival, in at least some wild populations, I estimated half-month survival rates for juveniles of about 0.9, or close to a 5% decline per week (Mebane 2010, Table II). This is higher than the 2-3% noted in Appendix K, and suggests that in the wild, survival to 42-days would likely be less than 80%. With regards to growth, while some wild populations grew as much as those in the laboratory settings discussed in Appendix (>0.5 mg at sexual maturity), this cannot be assumed in all natural waters. Cooper (1965) reported average dry weights of adults Hyalella were 0.2 mg in a population in a warm, shallow lake in Michigan. Gibbons and Mackie (1991) reported mean weights of Hyalella at maturity were only 0.1 mg, and weights of all Hyalella were only 0.3 mg. Thus the 0.35 at day 28 and 0.5 mg at day 42 may be higher than that expected in some natural settings. Gibbons and Mackie (1991) reported ranges of brood per female ranged from 6 – 15, which is consistent with appendix K. However, Strong (1972), his fig 4, showed sometimes natural brook sizes may be as low as 3 per female.

In sum, the logical problems of how Appendix K's analyses are used in the document are analogous to the metaphor of not seeing the forest because of all the trees. Some trees were examined in great detail (lab performance of Hyalella) but it misses the point that the comparisons of acceptable conditions should be again performance in the wild.

Other items:

Problem formulation: It is germane to note that in natural waters, Cd is always in association with Zn, usually at about mass ratios of 1:200 (Wanty et al. 2009).

p. 12, I was not quoted quite accurately. "Mebane (~~2014~~ 2006) concluded that, although there were not adequate data to establish acceptable tissue effect concentrations for aquatic life, cadmium is unlikely to accumulate in tissue to levels that would result in adverse effects to aquatic invertebrates or fish, at calculated chronic criterion concentrations, which were lower than that chronic criterion concentration derived here. "

This report is variously cited as Mebane (2006), Mebane (2010), or Mebane (2014). The suggested citation is, "*Mebane, C.A. 2006. Cadmium risks to freshwater life: derivation and validation of low-effect criteria values using laboratory and field studies. U.S. Geological Survey Scientific Investigation Report 2006-5245 (2010 rev.). http://pubs.usgs.gov/sir/2006/5245/*."

The 2010 revision only corrected minor mistakes, and did not include any updated literature reviews.

p. 28, the approach of requiring data used in the hardness-toxicity regressions to have a 3X spread and 100 mg/L absolute difference between the highest and lowest value was indeed used in the 2001 version, but was not really presented as policy. In contrast, my colleagues and I found that hardness-toxicity relations were more reliable from test series that concurrently tested the same cohort of organisms in waters with different hardness, than were ad hoc collections of found data tested under different conditions at different hardness levels (Mebane et al. 2012). Where available, giving concurrent test series data obtained at different hardnesses precedence over general hardness-toxicity compilations would be warranted.

3. **Please comment on the data used to derive the revised criteria, including data adequacy/ comprehensiveness, and the appropriateness of the data selected and/or excluded from the derivation of the draft criteria. Is the data used correctly for the intended purpose? Are there other relevant data that you are aware of that should be included? If so, please provide the data along with supporting information.**

As noted in the response above, the exclusion of most Hyalella data is doubtfully justifiable, because the criteria for doing so are questionable. However, even with these Appendix K criteria as they are, the Ingersoll and Kemble data reproductive data should not have been excluded. The 42d reproductive endpoint from that test met the Appendix K criteria for control survival and brood size (6.35 per female). The 28 day endpoint was presumably excluded because of low growth as weight. However, the organisms were not weighed, but rather lengths were measured and weights were inferred from lengths. Regardless, by the stated logic, it would follow to exclude the 28-day endpoint with low (estimated) weight. But to then pick an acute survival endpoint (10-day) instead of the 42-day reproductive endpoint is inexplicable.

The entry for this test in Table 2 is misleading. Saying the test was a life cycle test, but then using an acute endpoint, is misleading. I estimated the EC20 for reduced reproduction to be about 1.2 µg/L using logistic regression, or the MATC (geomean of LOEC and NOEC) would be 0.98 µg/L.

Other specific points on data used or not used.

Durations of tests

If 30-day tests with salmonids that started with fry consistently yield more sensitive results than 60-day tests that started with eggs or embryos, why ignore all the shorter, more sensitive tests. The Guidance counsels to beware of tests in which acclimation probably occurred during resistant states. Chapman (1985) recently described this problem. It would make more sense to exclude the less sensitive data, rather exclude the more sensitive data.

Likewise with Mottled Sculpin, there's doubtfully anything special about 28-day exposures over 21-day exposures. Besser et al. (2007) ran two tests, one 28-day and one 21-day test. The 28-day was less sensitive, and it was used with the other ignored. There is no established ASTM protocol for Mottled Sculpin, and the ASTM (1998) mention of "28 to 120-day (depending on species) continuous exposure" tests for early-life stage tests refers back to their species-specific appendices.

Other data

(Calfee et al. 2014) and (Wang et al. 2014) report acute and chronic data with White Sturgeon and Rainbow Trout. The same data are reported in Environmental Toxicology and Chemistry, but Wang is paywalled, so I would use the open access USGS report version.

An acute test with Mottled Sculpin, (Cottus bairdi) and Cd was attributed to Mebane et al. (2012). We tested Shorthead Sculpin, Cottus confusus.

4. Are the derived criteria appropriately protective of listed species and commercially and recreationally important species, particularly as the criteria relates to salmonids?

Not necessarily, although to definitively answer this would take a considerably more thorough review to determine than was presented in the document, or could be done independently in the time available. I note that NMFS (2012) in Oregon concluded the 2001 CMC of 2.0 µg/L could jeopardize some salmonids and that the CCC of 0.25 µg/L would not jeopardize listed salmonids under their prevue. Thus the draft 2015 criterion of 2.2 µg/L would presumably be a concern. Conversely, NMFS (2011) concurred with EPA that Idaho acute and chronic criteria of 1.34 and 0.55 µg/L respectively would not jeopardize listed anadromous salmonids. I did not attempt to reconcile the three documents. However, I think part of the discrepancies may be in the manner of analyses. In the draft document, data from long-term exposures to salmonids that began with sensitive fry life stage are excluded in favor of data from tests that began with eggs or alevins. While all fish have some life stage-sensitivity interaction, with at least salmonids sensitivity increases with size up to at least 0.4g ww, and maybe up to 1g or more (Hansen et al. 2002; Mebane et al. 2012). With other fish, the newly hatched stage may be more sensitive, or life events such as the onset of exogenous feeding may be related to a stressful and sensitive stage (Wang et al. 2014).

There are some instances of inappropriate averaging using resistant life stages. Bull trout at the most sensitive (~1g) were averaged with results of test with yearling brook trout to produce a nonsense genus mean acute value of 126 µg/L. Stephan et al. advise against pooling species mean values when they differ by more than a factor of 10; these differed by a factor of 1000X.

The draft document evaluates protection of listed species by rolling up species data to a hardness-normalized species mean acute value (SMAV) and comparing that with the criteria. Because the accuracy of hardness-normalization is uncertain, but the criteria values can be calculated with certainty for any hardness, a more informative way to evaluate the data with listed species is to compare the criteria values for the conditions of each test of interest with listed species to the effects magnitude of effects to listed species at a given criteria. If the test concentrations causing an adverse effect are close to criteria concentrations, such as if the EC50s were within a factor of 2 (or maybe 2.5 to 3 to be on the safe side), then evaluate the actual adverse effects observed at the criteria concentrations. The SMAV approach involves a lot of data manipulation and may lose sensitive life stages or strains.

ASTM. 1998. Standard guide for conducting early life-stage toxicity tests with fishes. Method E1241-98. Pages 29 *in* Annual Book of ASTM Standards, volume 11.04. American Society for Testing and Materials, West Conshohocken, PA.

Besser, JM, Mebane, CA, Mount, DR, Ivey, CD, Kunz, JL, Greer, EI, May, TW, and Ingersoll, CG. 2007. Relative sensitivity of mottled sculpins (*Cottus bairdi*) and rainbow trout (*Oncorhynchus mykiss*) to toxicity of metals associated with mining activities. Environ Toxicol Chem 26:1657–1665.

Calfee, RD, Little, EE, Puglis, HJ, Beahan, E, Brumbaugh, WG, and Mebane, CA. 2014. Acute Sensitivity of White Sturgeon (*Acipenser transmontanus*) and Rainbow Trout (*Oncorhynchus mykiss*) to Cadmium, Copper, or Zinc in Laboratory Water-Only Exposure. Pages 5-34 *in* Ingersoll, CG, and Mebane, CA, editors. Acute and chronic sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to cadmium, copper, lead, or zinc in laboratory water-only exposures. U.S. Geological Survey Scientific Investigations Report 2013–5204.

Chapman, GA. 1985. Acclimation as a factor influencing metal criteria. Pages 119–136 *in* Bahner, RC, and Hansen, DJ, editors. Aquatic Toxicology and Hazard Assessment: Eighth Symposium (STP 891-EB), volume STP 891. American Society for Testing and Materials (ASTM), Philadelphia.

Cooper, WE. 1965. Dynamics and production of a natural population of a fresh-water amphipod, *Hyalella azteca*. Ecol Monogr 35:377-394.

France, RL. 1996. Biomass and production of amphipods in low alkalinity lakes affected by acid precipitation. Environ Pollut 94:189-193.

Gibbons, WN, and Mackie, GL. 1991. The relationship between environmental variables and demographic patterns of *Hyalella azteca* (Crustacea: Amphipoda). J N Am Benthol Soc 10:444-454.

Hansen, JA, Welsh, PG, Lipton, J, Cacela, D, and Dailey, AD. 2002. Relative sensitivity of bull trout *(Salvelinus confluentus)* and rainbow trout *(Oncorhynchus mykiss)* to acute exposures of cadmium and zinc. Environ Toxicol Chem 21:67–75.

McNulty, EW, Dwyer, FJ, Ellersieck, MR, Greer, EI, Ingersoll, CG, and Rabeni, CF. 1999. Evaluation of ability of reference toxicity tests to identify stress in laboratory populations of the amphipod *Hyalella azteca*. Environ Toxicol Chem 18:544-548.

Mebane, CA. 2010. Relevance of risk predictions derived from a chronic species-sensitivity distribution with cadmium to aquatic populations and ecosystems. Risk Anal 30:203-223.

Mebane, CA, Dillon, FS, and Hennessy, DP. 2012. Acute toxicity of cadmium, lead, zinc, and their mixtures to stream-resident fish and invertebrates. Environ Toxicol Chem 31:1334–1348.

Stephan, CE, Mount, DI, Hansen, DJ, Gentile, JH, Chapman, GA, and Brungs, WA. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency, EPA 822-R-85-100, NTIS PB85 227049, Duluth, Narragansett, and Corvallis.

Strong, DR, Jr. 1972. Life history variation among populations of an amphipod (*Hyalella azteca*). Ecology 53:1103-1111.

Wang, N, Ingersoll, CG, Dorman, RA, Kunz, JL, Hardesty, DK, Brumbaugh, WG, and Mebane, CA. 2014. Chronic Sensitivity of White Sturgeon (*Acipenser transmontanus*) and Rainbow Trout (*Oncorhynchus mykiss*) to Cadmium, Copper, or Zinc in Laboratory Water-Only Exposure. Pages 35-76 *in* Ingersoll, CG, and Mebane, CA, editors. Acute and chronic sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to cadmium, copper, lead, or zinc in laboratory water-only exposures. U.S. Geological Survey Scientific Investigations Report 2013–5204.

Wanty, RB, Verplanck, PL, San Juan, CA, Church, SE, Schmidt, TS, Fey, DL, DeWitt, EH, and Klein, TL. 2009. Geochemistry of surface water in alpine catchments in central Colorado, USA: Resolving host-rock effects at different spatial scales. Appl Geochem 24: 600-610.

Wendelaar Bonga, SE, and Lock, RAC. 2008. The osmoregulatory system. Pages 401-416 *in* Di Giulio, RT, and Hinton, DE, editors. Toxicology of Fishes. CRC Press, Boca Raton, Florida.

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