United States
Environmental Protection
Agency

Office of Water
4304T

EPA-822-R-22-002
March 2022

Response to
External Peer Review Comments on

EPA’s Metals CRADA Phase I Report:
Development of an Overarching Bioavailability Modeling Approach
to Support US EPA’s
Aquatic Life Water Quality Criteria for Metals

March 2022

1.0 Introduction

In December 2017, the U.S. Environmental Protection Agency (EPA) signed a Cooperative Research and Development Agreement (CRADA) with eight metals associations (Aluminum Association, Aluminum REACH Consortium, Cobalt Institute, International Copper Association, Copper Development Association, International Lead Association, International Zinc Association, NiPERA Inc.) in order to leverage the knowledge and resources of scientists inside and outside of the agency to better protect aquatic life. EPA's Office of Science and Technology within the Office of Water (OW) is the Agency's technical lead on this CRADA which supports *EPA's FY 2018-2022 Strategic Plan* Goal: Provide for Clean and Safe Water: Protect and Restore Water Quality. EPA is using a two-phased approach to address the CRADA.

In the first phase, EPA has worked with external technical experts from the metals associations to develop a proposed modeling approach to predict the bioavailability and toxicity of metals under the range of water chemistry conditions found in aquatic environments common in freshwaters of the United States. The results of work conducted in this phase are captures in the CRADA Phase I Report: *Development of an Overarching Bioavailability Modeling Approach to Support US EPA's Aquatic Life Water Quality Criteria for Metals*. Subsequently, in the second phase, EPA will work with the metals associations to develop bioavailability models for individual metals using the overarching modeling approach. Using the resulting peer-reviewed models, EPA plans to develop updated, externally-peer reviewed Aquatic Life Ambient Water Quality Criteria for metals to better support states, territories and tribes with criteria that reflect the latest science and are easier to implement than more complex, previous approaches using metals bioavailability modeling for criteria development.

This document provides EPA responses to the results of an independent, external peer review of the U.S. Environmental Protection Agency's (EPA's) Metals CRADA Phase I Report: *Development of an Overarching Bioavailability Modeling Approach to Support US EPA's Aquatic Life Water Quality Criteria for Metals* (hereafter, CRADA Phase I Report). The peer reviewers were external expert scientists with expertise in 1) Modeling as applied to the characterization of metals bioavailability in aquatic systems, 2) Aquatic inorganic chemistry, hydrogeology, and biogeochemistry of metals in aquatic systems, 3) Aquatic toxicology of metals, aquatic ecology, and physiology of aquatic organisms, 4) Statistical analyses and data interpretation for the determination of data acceptability and/or 5) Knowledge of the Clean Water Act, especially water quality standards (WQS). Eastern Research Group, Inc. (ERG), a contractor to EPA, organized this external peer review for EPA's Office of Water (OW) and developed the external peer review report.

Section 2.0 of this document provides individual reviewer comments on the CRADA Phase I Report and EPA's responses to the peer reviewer comments. Section 3.0 provides additional reviewer comments and EPA responses.

EPA's contractor identified, screened, and selected the following five experts who met technical selection criteria provided by EPA and had no conflict of interest in performing this review:

- **David Buchwalter, Ph.D.**: Professor, Department of Biological Sciences, North Carolina State University
- **Claude Fortin, Ph.D.**: Professor, Institut National de la Recherche Scientifique (INRS), Canada
- **Erin M. Leonard, Ph.D.**: NSERC Post-Doctoral Fellow, Integrative Biology, University of Guelph
- **Christopher A. Mebane**: Water Quality Specialist, U.S. Geological Survey
- **Wilhelmus Peijnenburg, Ph.D.**: National Institute of Public Health and the Environment (RIVM), Centre for Safety of Substances and Products, The Netherlands

2.1 Please provide your scientific feedback of the strengths and weaknesses of the MLR (Multiple Linear Regression) and BLM (Biotic Ligand Model) approaches for estimating the effects of water chemistry/toxicity modifying factors on the bioavailability and toxicity of metals as discussed in the Phase I document and appendices.

2.1 General comments on the strengths and weaknesses of the MLR and BLM approaches.		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	In general terms, the MLR and BLM approaches that are presented in the documents are clearly the state of the art. It is to be noted that a major part of the models have developed in close cooperation between scientists and industry, as assisted by regulatory institutions. This cooperation has been successful and resulted in a number of sophisticated models that are suited for the derivation of water quality criteria. A pragmatic question that arises is associated to the fact that the development of the key models has been performed by a relatively small cross section of the researchers active in the field of metal bioavailability. It is therefore essential to warrant sufficient academic support regarding the scientific foundations of the models and the justification for use in regulation. **Strengths:** The approaches represent the state-of-the-art with regard to the scientific aspects of metal bioavailability quantification. A proper combination of mechanism-based knowledge (as exemplified for instance by model development based on first principles) and pragmatic approaches (as exemplified by MLR approaches) is used and integrated in the broad spectrum of models available. The basic approaches supplement each other, and the BLM approach can for instance be used to inform the correctness of the MLR approach. The overall concept is applicable to a multitude of metals and to an array of biological species of different trophic level: it is clear that the same basic principles apply across the universe of water chemistries and across the	Thank you for your comment. EPA agrees that the approaches are currently the state-of-the-science. EPA acknowledges your concern regarding the expert researchers involved in developing the models which is why we have conducted this external expert peer review of the models and associated information. EPA will also have the individual selected models for each metal externally peer reviewed before any use in criteria derivation. The criteria documents will also undergo external peer review. EPA agrees that these two approaches, MLR and BLM, complement each other, and our objective is to find a scientifically-defensible approach applicable to all metals that will be easy for states and stakeholders to implement. EPA agrees that metal bioavailability is complex and would consider non-linear relationships and interactions as well as additional toxicity modifying factors if enough data are available for their inclusion. EPA also agrees that this effort should result in the development of user-friendly tools for regulators

2.1 General comments on the strengths and weaknesses of the MLR and BLM approaches.		
Reviewer	**Comments**	**EPA Response**
	universe of biological species. This increases the credibility of the basic hypotheses related to variations in water chemistry modifying metal toxicity. The validation efforts undertaken to show that the models are capable of properly predicting toxicity across different water chemistries. **Weaknesses:** A general weakness which is inherent to metal toxicity, is that the general concept of metal bioavailability is complex. It is complex in the sense that numerous processes are non-linear and as a consequence the overall impact of water chemistry on metal toxicity is non-linear. It is therefore important to make sure that the resulting non-linear relationships as well as the interactions between the factors modifying toxicity, are properly understood and properly incorporated in the models. Although a lot of research has been performed and although various key factors have been identified, it cannot be ruled out that for specific waters, factors come into play that have not yet been identified. It is important to keep an open eye for the possible need of accounting for additional factors in toxicity assessment. The impact of carbonate that is observed for a limited number of species is an example of such an additional factor. The mere fact that numerous models have been developed for various metals and various biological species make it difficult for non-experts to have an overview of the models available, their individual strengths and weakness, as well as their domain of applicability. In practical terms the key weakness is that overall, the models might be considered as a black box by for regulators with limited background knowledge on metal bioavailability. This implies that efforts with regard to communication and development of user-friendly software tools, need to be optimized.	to be able to predict metal toxicity to aquatic organisms.
	No other reviewers provided general comments on the strength and weaknesses of MLR and BLM approaches.	

2.1.a Do you see technical/scientific advantages of using one model over the other for deriving water quality criteria?

2.1.a Technical/scientific advantages of using one model over the other for deriving water quality criteria.		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	No. The key issue in this respect is my observation that each model has its own merits and on forehand no model should be ruled out, or be classified as being better than another model. It is to be realized that each model also has its own amount of information embedded and this information is used best when using more than one model in deriving water quality criteria. Actually, a recommendation with regard to the overall set of models available and with regard to the overall knowledge available in this overall set of models, is to investigate whether transfer learning approaches can be applied to improve model performance.	Thank you for your comment.
Reviewer 2	There is validity of the chemical speciation modeling and modeling of competition between dissolved ions and complexes for binding to predict the relationship between water chemistry and metal accumulation and incipient toxicity. However, there are shortcomings in terms of neglecting that the kinetics of exposure change over time. With the BLM, the performance of the model is dependent on the parameters that are available to predict speciation reactions as well as on those that define the critical concentration of metal–biotic ligand complex at which toxicity occurs. In many cases, some of these parameters are not determined or inaccurate which leads to either inputting estimates or leaving values at the default settings. Additionally, more input parameters increase the potential and impact of human error therefore affecting the accuracy of the models. In addition, in many cases, LA50 values across all species have not been measured directly, specifically with invertebrates which are the most sensitive taxa. This should be addressed. Additionally, within the documents, biotic ligands have been defined as either the gills of fish or the respiratory surface of invertebrates, however, whole body measurements are used for determination of LA50 values for these species. For fish, although the gills are most likely the primary biotic ligand and the one driving toxicity, it should be	Thank you for your comment. EPA also agrees that it is helpful for bioavailability models to be informed by a mechanistic understanding of metal toxicity and of metal speciation but asserts that the transparency and ease of use of the MLR outweighs the mechanistic complexity of the BLM. Thank you for your suggestion to investigate the gut acting as a biotic ligand in fish as well and the gills/respiratory surface. As the reviewer mentioned, the gut can be an important uptake pathway for marine fish, however, this effort is currently focused on freshwater models. Generally, in freshwater fish, gill uptake has been shown to be more important that the gut (e.g., Niyogi et al., 2007) which is one reason the gill has been the focus of BLM development in these cases. EPA agrees that temperature could be an important toxicity modifying factor for some

2.1.a Technical/scientific advantages of using one model over the other for deriving water quality criteria.		
Reviewer	**Comments**	**EPA Response**
	included that the gut, especially in seawater, may add to the complexity by also acting as a biotic ligand (Alsop et al., 2016 Aquatic Toxicology). There is strength in an approach that simplifies the BLM model and relies on extensive toxicity data sets covering wide ranges of water chemistry parameters and ecotoxicity endpoints. I see the benefits of a MLR over a BLM approach because of its simplicity, the three input parameters (pH, DOC, and hardness), and therefore less need to collect data (or estimate parameters) on multiple water chemistry parameters to successfully run the model. However, I do see the need to include temperature as a fourth parameter. Metal accumulation in fish, pond or river water is enhanced by upsurges in temperature; therefore, it is imperative to study the detrimental effects of metals in combination with temperature to formulate accurate predictive models (Kumar et al., 2018 Int. J. Environ. Sci. Technol.). Overall, although bioavailability models should be informed by mechanistic understanding of metal toxicity and of metal speciation, I think that the transparency and ease of use of the MLR outweighs the mechanistic complexity of the BLM.	metals, but there is typically insufficient data on temperature to incorporate into the models at this time. Furthermore, while temperature is considered within the BLM, it is generally not identified as a major factor. The agreement between the predictions made by BLM and MLR models in this report can be used as a verification that even though the MLR models do not incorporate temperature, they are still able to empirically predict toxicity to aquatic organisms.
Reviewer 3	As a scientist I philosophically favor the BLM approach to the MLR approach because it has the most mechanistic validity with reference to acutely sensitive taxa. At least for the earliest derivations of the BLM, the use of real experimental data was used to parameterize the model rather than the latter approaches where they were fitted (fudged) to fit the toxicity outcome data. However, I don't think either approach is particularly defensible for the derivation of chronic criteria because it neglects the possibility the dietary metal exposures are toxic.	Thank you for your comment. EPA also agrees that it is helpful for bioavailability models to be informed by a mechanistic understanding of metal toxicity and of metal speciation. Diet is an additional route of metal exposure that is generally not considered within bioavailability models because of a lack of available data and a mechanistic complexity. Currently, data for many metals indicate that exposure of the respiratory organs via water are more sensitive to cationic metals than exposure through the gut. Furthermore, these models have been validated with long-term mesocosm studies in which the

2.1.a Technical/scientific advantages of using one model over the other for deriving water quality criteria.		
Reviewer	**Comments**	**EPA Response**
		dietary route of exposure is an operational pathway (Roussel et al. 2007; Schlekat et al. 2010; Versteeg et al. 1999). Additionally, in a dietary zinc toxicity, De Schamphelaere et al. (2004) concluded that: "the zinc BLM predicts chronic reproductive zinc bioavailability and toxicity in synthetic and field surface waters with reasonable accuracy even without explicitly directly considering the dietary toxicity pathway". For many metals, toxicity stemming from waterborne pathway has shown to occur at similar or lower concentrations than the dietary route (e.g., Evens et al. 2009 for nickel, De Schamphelaere et al. 2007 for copper, Nys et al. 2013 and Alsop et al. 2016 for lead) and indicate that ALC that are protective of aqueous metal exposure are also expected to be protective of dietary exposures.
Reviewer 4	I see several (dis)advantages to the use of either approach. Among the arguments presented, the decreased number of input parameters is cited as an advantage in favour of MLRs. I see a hidden disadvantage to that as this may introduce a bias (see response to Question 3a below). Another nuance I would like to bring forward about the "improved transparency" of the MLRs is that it may be easy to spot the driving parameters by simply looking at the equation, but it does not allow the user to understand why these parameters are important. BLM-based models are more complicated to use and require training but that results in having more informed users. MLRs do not incite users to understand the science behind the equation and in the long run this may represent a loss. It may be a question of perspective but, from my point of view, MLRs are less transparent than BLMs because I know what the speciation of a metal should be by looking at water chemistry parameters and can thus expect an output. If this output is far from	Thank you for your comment. EPA understands your argument that, from a scientific standpoint, an expert in this field can predict the output of the BLM from the presented water chemistry parameters more easily than the MLR equation. However, both the BLM program and MLR models can be used without the user understanding the science behind the equations. Furthermore, since the 2007 Copper BLM was published, only 6 states have adopted the Cu BLM statewide and 9 have adopted it as a means to develop site-specific criteria. EPA is considering the needs of end-users of these models, such as regulators and stakeholders, in our decision and as noted in the comment, both models provide good results in

2.1.a Technical/scientific advantages of using one model over the other for deriving water quality criteria.		
Reviewer	**Comments**	**EPA Response**
	my expectation, I would make additional simulations to figure out why and possibly spot a mistake in data entry for example. On the other hand, using a long equation does not trigger any expectations in terms of output. It's not clear to me how easy/hard it is to recompile a new MLR upon the addition of new data but, intuitively, it seems to me that this requires starting from scratch. On the other hand, the addition of a binding constant into the BLM should not require redefining all other constants. Also, the derivation of an MLR may be different from one user to another and may depend on the software used. This thus requires a very thorough guideline document to ensure homogeneity in data treatment and statistical approaches. On this front, the complexity seems similar. To circle back to the question, I think there is a technical advantage to using MLRs (ease of use) but a scientific advantage to using BLMs (promotes knowledge of underlying cause-effects relationships). As a scientist, I see the use of MLRs as a step back, but I can understand the motivation of using MLRs over BLMs. To be fair, they seem to provide just as good results so in terms of quality of output, they are on the same level. For regulators and stakeholders, simplicity makes sense.	most instances, the ease of use of the MLR outweighs the mechanistic complexity of the BLM. The reviewer is correct that the addition of new data to the MLR would require performing new regression analyses. However, models selected for the derivation of the criteria will be fixed in time and software to run them will be provided by EPA to address user differences.
Reviewer 5	Both the BLM and MLR approach are appropriate tools for capturing important toxicity modifying factors for the metals commonly of concern in manufacturing, mining, effluents, and runoff. The BLM excels as a research tool in that it is flexible, not as constrained to the training data as are MLRs, can be modified to address mixtures, and has good application in ecological risk assessment and other applied issues. This review provided me the first view of some of the updates to the Windward BLM software in support of single metal EU REACH or this CRADA project, and they are impressive. However, in my view, for regulatory water quality criteria, the BLM approach has fundamental key disadvantages in terms of transparency and resiliency over time. The present BLM software implementations and in some cases, the speciation models (direct implementation of the WHAM submodel from its	Thank you for your comment. EPA is also concerned about the transparency and general useability (see EPA's response to Reviewer 4's comment on the adoption of the 2007 Cu BLM above) of the BLM for criteria derivation and agrees that the MLR equations avoid these issues without sacrificing performance. The BLM does generate a detailed output file showing the concentration of chemical species in the simulation that can be compared with the BLM equations that define the model published by US EPA (2003). The CRADA partners indicate that the

2.1.a Technical/scientific advantages of using one model over the other for deriving water quality criteria.		
Reviewer	**Comments**	**EPA Response**
	developers, for example) are the intellectual property of their developers. I am not aware of any open source or public domain version of contemporary BLMs. The code cannot be directly inspected, and the specific details of calculations can only be inferred from the narrative descriptions and the outputs. For EPA to rely on software based BLMs that would require a sustained commitment to maintaining and updating the software, with updates to make the software interoperable on different and evolving computer operating systems, with a software testing and help desk to ensure it is reliable on different configurations. The push in the corporate IT culture towards enterprise software, centralized corporate control of whether individuals can load or modify software, software white lists, and off-site support can make the use of specialty software such as the BLM a hassle for many. For instance, I had to complete this review at home on personal computers because of such constraints. While there may be single-shingle consultants free of such “support” most BLM users are probably in organizations with IT controls. Does EPA really want to be in the software business or have to support software as opposed to putting their finite resources into new criteria or criteria updates? Or is it fair and reliable to rely on the free services of the model developers and their employer (or indirectly, their employer’s clients)? The MLRs sidestep all of these issues and perform fine for a wide range of water chemistries.	BLM software can be modified to include the details of the equations of each simulation with the output file to increase transparency. However, EPA recognizes the reviewer’s comment regarding the proprietary nature of most BLMs and the issues of maintaining complex model software.

2.1.b Are the models robust in their ability to accurately predict toxicity as a function of water chemistry? If not, why?

2.1.b. Are the models robust in their ability to accurately predict toxicity as a function of water chemistry?		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	In general, most models are indeed robust. This can amongst others be deduced from the statistical parameters provided with each of the models, and the validation efforts done for each of the models. These validation efforts	Thank you for your comment.

2.1.b. Are the models robust in their ability to accurately predict toxicity as a function of water chemistry?		
Reviewer	**Comments**	**EPA Response**
	include internal validation as well external validation, whereas in some cases additional field samples have been sampled and tested as part of the validation. It is also to be noted that in most cases the statistical performance of the models is well above the so-called Setubal-criteria for the acceptance of predictive models for regulatory application as derived within the OECD.	
Reviewer 2	**BLM** One of the main concepts of the BLM is that there is a strong overall correlation between log K values for gill binding and acute toxicity to the extent that measurement of binding affinity based on gill metal binding is an acceptable alternative to measurement of toxicity and vice versa. I think more information needs to be obtained to determine whether this concept can be extended to Ni bioaccumulation in the whole body of invertebrates rather than bioaccumulation on a theoretical 'biotic ligand' (target site for toxicity) such as the gills in fish. Although some studies demonstrate relatively good agreement between the log KNiBL values derived from the ionic component of the LC50 value (toxicity) with those derived from the ionic component of the Kd (ionic Ni concentration causing half saturation of Ni bioaccumulation in the whole organism – invertebrates) suggesting that whole body bioaccumulation can serve as a surrogate for Ni binding to the theoretical 'biotic ligand' which causes toxicity, further validation of the modeling approach of the BLM because estimating the concentration of Ni theoretically bound to the biotic ligand using the ionic component of the LC50 value (the BLM approach) does not in all cases correlate with the observed Ni bound to the biotic ligand (Leonard and Wood, 2013 Comparative Biochemistry and Physiology, Part C). **MLR** We know that invertebrates have greater diversity in ion transport physiology and differential responses to the TMFs laid out in the documents. Therefore, gaining more information for multiple invertebrate taxa (e.g., crustaceans,	Thank you for your comment. For the BLM, whole organism bioaccumulation typically has not been found to be a good predictor of toxicity (Amiard et al. 2006)) because aquatic organisms can sequester metals into biologically inactive fractions. When calibrating BLM binding constants, the accumulation is typically measured during short term exposures on membranes associated with the "site of action" for metal toxicity (e.g., the gills of freshwater fish) rather than whole body accumulation. The BLM was first developed for fish then applied to invertebrates and found to be empirically accurate. For the MLR, EPA agrees the available data on invertebrates and algae/aquatic plant taxa within the scientific literature is limited and, given their abundance and ecological relevance, additional toxicity studies on these taxa would benefit the MLR models. Regarding DOC, the reviewer is correct that there are different forms of DOC which may have different protective capabilities and affect the bioavailabiliity of metals in an exposure scenario (Wood et al. 2011). However, validation exercises

2.1.b. Are the models robust in their ability to accurately predict toxicity as a function of water chemistry?		
Reviewer	**Comments**	**EPA Response**
	insects, mollusks) is critical. Additionally, much less data is available for algae and aquatic plants to TMFs and the data is currently limited to a few species and much like the invertebrates their responses to TMFs is quite variable and therefore substantial the importance of gaining more insight into these taxa. **General comments:** **DOC** Although Brix et al. (2020) briefly alludes to the chemical composition of DOC affecting the metal binding capabilities and thus its effect on toxicity, there is no discussion of these difference (e.g., humic acid (HA) vs. fulvic acid (FA)). Additionally, in the modelling, HA is set to a default of 10%. I think this needs further attention and should be included in the modelling platforms or at minimum there should be reference to what is currently known regarding the various forms of DOC and how they differentially affect toxicity. For example, dark, aromatic-rich compounds of allochthonous origin, with greater humic acid content, are more effective at protecting organisms against Cu, Ag, and Pb toxicity (Wood et al., 2011 Aquatic Toxicology). In addition, the specific absorption coefficient of the DOC in the 300–350 nm range (SAC300–350) is an effective index of its protective ability. PARAFAC, a multivariate statistical technique for analysis of excitation-emission fluorescence spectroscopy data, quantifies humic-like and fulvic-like fluorophores, which tend to be positively and negatively correlated with protective ability, respectively (Wood et al., 2011 Aquatic Toxicology). **Temperature** Field temperatures are much more variable than laboratory settings which may lead to significant under-or overestimation of toxicity. This is an important component which has been drastically overlooked in the history of metal toxicity (Kumar et al., 2018 Int. J. Environ. Sci. Technol.).	have been performed using a wide range of natural waters and do not indicate that there is a need for bioavailability models to quantify the forms of DOC in order to accurately (within a factor of two(Meyer et al. 2018)) predict the toxicity within an exposure scenario (Besser et al. 2021; Deleebeeck et al. 2008). Regarding temperature, please see response to Reviewer 2's comment in section 2.1a.

2.1.b. Are the models robust in their ability to accurately predict toxicity as a function of water chemistry?		
Reviewer	**Comments**	**EPA Response**
Reviewer 3	The models are good for predicting the acute toxicity of metals in the context of acutely sensitive laboratory models. However, these lab models do not adequately represent the taxa that typically dominate stream ecosystems – aquatic insects. If the goal is to predict toxicity in simple lab tests to a limited set of laboratory models, then models are fine for acute predictions. If the goal is to protect aquatic life in nature, the models have limited value. **Copper:** What is interesting is that there can be substantial differences in HC05 estimates depending on which type of model is employed. I looked at ratios of HC05 estimates generated by the BLM relative to MLR models. Globally (combining results from synthetic and natural waters, BLMs were more protective (mean BLM:MLR HC05 = 0.916). These differences were driven by the results of synthetic water tests (mean BLM:MLR HC05 = 0.569), whereas in natural waters, the MLR approach appeared more protective (BLM:MLR HC05 = 1.292). Since most data used in the generation of WQC will likely be from tests in synthetic waters, we can conclude that for Copper, MLRs will be substantially less protective than BLMs. BLMs were at most 3.04X less protective (site 51), whereas MLR's were 2 orders of magnitude less protective at several sites relative to BLMs. **Lead:** There appears to be reasonable agreement between BLM and MLR approaches for HC05 estimates for lead. Globally the mean BLM:MLR HC05 = 1.198, with less protection afforded by the BLM in natural waters (BLM:MLR HC05 =1.42). In synthetic waters, there is general agreement with the mean BLM:MLR HC05 = 0.99. **Aluminum:** It is interesting that MLR results are slightly more protective than current EPA guidelines – and that it is shown in this table but not for the other metals. I think this comparison should be made for all of the metals so that it is transparent how adopting these models would change existing levels of protection. **Nickel:** For Nickel, BLM models were generally less protective than MLR models. Globally, the mean BLM:MLR HC05 = 1.391, with smaller differences in	Thank you for your review and comment. EPA agrees the available data on aquatic insects within the scientific literature is limited and additional toxicity studies would benefit the models given aquatic insect abundance and ecological relevance, however, the available quality data has been applied to the calibration and validation datasets of the bioavailability models. EPA and the CRADA partners are working towards a comparison of HC_{05} values using the BLM and MLRs to investigate any discrepancies and major differences between the model predictions.

2.1.b. Are the models robust in their ability to accurately predict toxicity as a function of water chemistry?		
Reviewer	**Comments**	**EPA Response**
	synthetic waters (mean BLM:MLR HC05 = 1.27), than in natural waters (mean BLM:MLR HC05 = 1.51). There were instances where HC05 estimates varied by 3-5 fold between BML and MLR approaches (e.g., sites 25, 26, 27, 29 and 36)	
Reviewer 4	As far as I can tell from the document summarising the results (Table 3) as well as from the papers provided in the Appendices, they provide results that are similar in terms of both precision and accuracy for acute values while there seems to be an advantage for the MLR for chronic values except for Ni for which both models gave good results. I would expect an MLR to do better than a BLM since there are much less constraints for the former than the latter. Based on the documents of Appendix D, the MLR provides better estimates of Aluminium toxicity than the BLM. Figure 1 of Brix et al. 2020 shows much less scatter of the data for MLR compared to BLM. In the case of copper, overall, the BLM seems to be performing slightly better than MLR for acute tests. However, for chronic data, MLR is best. It seems that the quantity of data is important. When large data sets are available, both models perform well, while for smaller data sets, MLR provide much tighter relationships than BLM (see figures 7 and 8 of Brix et al., 2020; Appendix D). However, uncertainty increases with less populated data sets. As for Lead, figures 6 and 16 of DeForest et al., 2020 (Appendix E) indicate that both models, MLR and BLM, provide similar results and scatter. Similarly for Nickel, both models seem to perform equally well. Note that in Table 3 of Croteau et al., 2021 (Appendix F), the reactions are written as dissociation (ML=M+L) reactions, but the log K value suggest a complexation (M+L=ML) reaction. Note also that the log K values in the same Table 3 suggest that the BLM is more empirical than mechanistic. Indeed, it is counter intuitive that a hydroxo-complex (log K = 4.357) would bind more strongly than the free metal (log K = 4.00). The same applies to the binding of $NiHCO_3^+$ complexes.	Thank you for your comment. Nickel CRADA partners have indicated that in Croteau et al. (2021) the reactions are indeed complexation reactions (i.e. "ML=M+L" is read as "ML is formed by M plus L"). Additionally, the reviewer calls out the log K value for the NiHCO3, but in the supporting documentation for the nickel BLM, this complex was described as an empirical adjustment to represent the added toxicity *Ceriodaphnia dubia* sees from a combination of bicarbonate and nickel at pHs above 8. It was not intended to be mechanistic. However, other parts of the model are indeed mechanistic in the sense that they are calibrated to either chemical speciation data, or metal accumulation data when those data are available. Furthermore, the argument that NiOH should bind less strongly than free Ni because it has a lower charge is not entirely accurate – the reactions for Ni and NiOH binding organic matter are given the same constants in WHAM, thus there is precedence that the binding constant for NiOH need not necessarily be lower than that of free Ni.

2.1.b. Are the models robust in their ability to accurately predict toxicity as a function of water chemistry?		
Reviewer	**Comments**	**EPA Response**
	The decrease in net charge after complexation (+2 → +1) should highly decrease affinity of the complex for the biotic ligand. The formation of these complexes depend on pH and Ni^{2+} which are also variables within the BLM. Adding the binding of these complexes to the biotic ligand seems redundant (or circular); it's a way to add weight to pH in a manner that pulls away from a purely mechanistic approach. This being said, the final goal is to have a model that predicts adequately the effects of metals on aquatic organisms and the BLM does a great job. Although less empirical than MLRs, the BLM should also be considered an empirical model.	
Reviewer 5	Yes. The performance of all of these model variations has been well described in the supporting documents, and all function well. I have had some minor quibbles with Cu BLM versions over the years, such as the handling of dissolved organic matter (DOM) has never been explained. The BLM describes implementing WHAM V within the model, which calculates organic complexation of Cu and other metals with DOM. But the BLM inputs ask for dissolved organic carbon (DOC), which is not the same as DOM. Since no adjustment is described, this implies that DOC is treated equal to DOM, which seems to make the model a little too sensitive to DOC changes (illustrated in Welsh et al (2008)). The Cu BLM also seems a little too twitchy with pH changes. By its empirical nature, the Cu MLR does not have these issues. But these are quibbles. On the whole, all of these models perform well across diverse taxa and diverse water types.	Thank you for your review and comment. Copper CRADA partners have indicated Reviewer 5 is correct that DOC concentration does not equal DOM concentration. Traditionally, DOC concentration (mg/L) is assumed to equal 0.5*DOM concentration (as mg/L; i.e., mg DOM/L = 2*mg DOC/L]), and that is how the USEPA BLM converts from measured DOC concentration to the DOM input into WHAM. This 2x conversion factor was explained in USEPA (2003: p. A1), USEPA (2007: p. C-14), and Farley et al. (2015: p. 744, in reference to the HDR model, which has the same BLM framework as the USEPA BLM). However, the reviewer's point is well taken, and such a statement could easily be added to the BLM user guide for more-prominent visibility. Because mg DOM/L = 2*mg DOC/L in the BLM (and thus mg DOM/L ≠ mg DOC/L), the model will not, in the reviewer's words, be "a little too sensitive to DOC changes".

2.1.b. Are the models robust in their ability to accurately predict toxicity as a function of water chemistry?		
Reviewer	**Comments**	**EPA Response**
		If by "twitchy" the reviewer is referring to the sensitivity of predicted toxicity concentrations as pH is changed in the Cu BLM, that twitchiness is a consequence of Cu speciation calculated in the combination of the CHESS and WHAM speciation modules in the BLM. Those speciation calculations are based on fundamental concepts of metal-ligand interactions and empirical Cu-speciation results. The predicted toxicity is then directly related to the speciation-predicted concentrations of the Cu2+ and CuOH+ bound to the biotic ligand. In contrast, the acute and chronic Cu MLR equations contain less "twitchiness" for pH; but as a consequence, they predict higher toxicity concentrations and Cu criteria at low pH (Brix et al. 2017).

2.1.c Using the information provided in Appendix G (i.e., models and example water chemistries), please provide feedback on applying the models for the specific calculations of water quality criteria presented in terms of:

i. **Complexity and transparency: are the technical details pertaining to model development and functionality clear to the user?**
ii. **Representativeness: do the models apply to a sufficient variety of taxa and range of water chemistry conditions?**
iii. **Rigor: do the modeling approaches reflect the current state-of-the-science regarding robust and unbiased data selection and analysis?**
iv. **Usability: are the models sufficiently easy to use?**

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of: i. **Complexity and transparency** ii. **Representativeness** iii. **Rigor** iv. **Usability**		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	In my opinion, a lot of effort has been put in making the models as transparent as possible, including their application to specific sets of water chemistry. Any user with a feeling for the kind of models as developed for the specific application for setting water quality criteria is likely to be able to work with the models in a technical sense as the model application in itself is fairly user-friendly. Hence, the models are sufficiently easy to use. The example water chemistries span a broad cross section of realistic water chemistries, but it is to be made sure that in all cases there is a warning when the applicability domain of the models is exceeded when a specific set of water chemistry is defined (like: extreme pH-values beyond which the bioavailability models are operational). [1] The models are in general indeed applicable to a sufficient variety of taxa although the number of taxa for which models are available, is metal-dependent. Nevertheless, the models cover a broad array of species representative for most of the aquatic ecosystem. Thereupon, the most sensitive species are commonly considered. With regard to the state of the art of the modelling approaches it is to be noted that the methods chosen (MLR), the models indeed reflect the current state of the art. Also, essential aspects of model development like model validation have been properly dealt with. On the other hand, it is to be noted that the developments within the field of informatics are progressing extremely fast nowadays and it is recommended to explore whether applications like Artificial	Thank you for your comment. EPA agrees that the domain of applicability is a key issue for all bioavailability models and the range should be published within the model user materials and the criteria. Whenever possible EPA strives to obtain data from a wide range of conditions. In previous models used in criteria, EPA has provided a warning to alert the user when the predictions fall outside of the underlying model's water chemistry data range.

---

[1] In response to a request for clarification from ERG, this reviewer clarified that, by "…it is to be made sure that in all cases there is a warning when the applicability domain of the models is exceeded when a specific set of water chemistry is defined (like: extreme pH-values beyond which the bioavailability models are operational)," he meant "…it is my suggestion that the models be equipped with such a warning in order to make sure that the user is aware of the issue of predictions outside of the strict applicability domain of the model."

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of: i. **Complexity and transparency** ii. **Representativeness** iii. **Rigor** iv. **Usability**		
Reviewer	**Comments**	**EPA Response**
	Intelligence/Machine Learning or related techniques like Transfer Learning can be exploited to improve model accuracy and to warrant that the information present in the impressive datasets, it optimally exploited.	
Reviewer 2	i. **Complexity and transparency** The information is clear and transparent. Inclusion of the R script significantly adds to the transparency and functionality of the models. Increasing the potential for these models to be used for jurisdictions other than the United States, it may be of interest to include what other endpoints (other than the FAVs for the U.S.) can be derived from these two models. ii. **Representativeness** The number of taxa included in most of the models (copper and nickel) is extensive and there is strength with the aluminum model including a wide range of invertebrates, specifically some of the more sensitive and threatened species such as *Lampsilis*. However, it is essential that the life stage assessed is disclosed because, for example, glochidia (larval stage) are much more sensitive to metals than juvenile or adult freshwater mussels (Gillis et al., 2010 Environmental Toxicology and Chemistry; Salerno et al., 2020 Environmental Pollution; Gillis et al., 2008 Aquatic Toxicology; Markich et al., 2017 Science of the Total Environment). The range of water chemistries nicely brackets environmentally relevant concentrations and combinations of TMFs. Although the models estimate the 5th percentile of the SSD (HC5) using a range of distribution models, one key issue which has not been addressed is Species at Risk (SARs) or Endangered Species. Have any of these species been included in the models? Where will they fit on the SSD? The documents should address limitations/lack of information regarding Endangered Species and their	Thank you for your review and comment. EPA agrees life stage is an important factor to consider in bioavailabiliity models and will identify lifestages for taxa used in model development in future reports on individual metals models. Regarding Endangered Species, if quality data are available for listed species they are included in EPA's water quality criteria. Frequently no, or only limited, data are available for listed species. Water quality criteria for aquatic life that are developed under the Clean Water Act Section 304(a) are intended to protect aquatic organisms broadly on a national scale. Regarding the Endangered Species Act (ESA), EPA conducts subsequent evaluations when water quality standards are submitted by individual states to EPA for approval, as these approvals are the relevant federal actions that are taken, and under which consultations related to ESA requirements are addressed. See above for EPA's responses to the inclusion of life stage of taxa in future reports and at section

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of:

- **i. Complexity and transparency**
- **ii. Representativeness**
- **iii. Rigor**
- **iv. Usability**

Reviewer	Comments	EPA Response
	sensitivities towards metals. This issue needs to be addressed in the Phase I document and appendices. **iii. Rigor** Although, the modeling approaches do reflect most of the current state of science, there are two key areas that need to be addressed: life stage/age of the species included in the modeling and DOC characteristics which impact absorption and incipient toxicity. Both issues have been outlined above. Much of the data implemented into the two frameworks are conducted by a handful of scientists who also developed the programs. This leads to potential issues with biased data. Additionally, although this may be the "state-of-the-science", in terms of an Equity, Diversity, and Inclusion (EDI) standpoint, the first authors are not representative of the states, territories, and tribes which these models will be serving. **iv. Usability** There are significant issues downloading the programs and running them on my computer. Working out the issues took a few hours to manage/mitigate. The antivirus software (AVG) was triggered with every stage of the download as well as when the program was running. The program itself once opened and working is easy to use and well organized. The user guides for all four metals were well written and helpful, especially with the screen shots. I suggest that unzipping the files before use should be included in every user guide. If this is a common issue where installing software is onerous, I see this as a major hinderance of using these models to support states, territories, and tribes.	2.1b for EPA's response to DOC characteristics which impact absorption and incipient toxicity. EPA acknowledges your concern regarding the expert researchers involved in developing the models which is why we have conducted this external expert peer review of the models and associated information to assist in identifying any issues with biased data. EPA plans to also have the individual selected models for each metal peer reviewed before use in criteria derivation The criteria documents will also undergo external expert peer review. Thank you for your feedback on the issues you encountered using the models. EPA will provide a functional and user-friendly model for criteria derivation. EPA notes that for the 2018 Aluminum criteria built upon an MLR approach, Aluminum criteria calculators (one in Excel, one in R, with identical underlying code and resultant outputs) are housed on the EPA website, eliminating the need for users to download software.

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of:

i. Complexity and transparency
ii. Representativeness
iii. Rigor
iv. Usability

Reviewer	Comments	EPA Response
Reviewer 3	**i. Complexity and transparency** There is a lack of transparency in these models overall. **ii. Representativeness** This is a significant problem. If one samples a typical flowing water freshwater ecosystem, one can expect that >90% of the sampled animal life will be insects. There is a reason that other arms of the Clean Water Act that focus on ecological integrity rely extensively on aquatic insect communities to make inferences about ecological conditions. In metals contaminated streams, alterations of aquatic insect communities are the most common and reliable source of evidence for metals associated ecological damage. Since these models likely are not applicable to insects (for reasons that science understands, but are willfully ignored by both EPA and the industry groups that generated this approach), the entire exercise is fatally flawed. Work from the Wood lab[10] demonstrated that aqueous Cd exposure resulted in the uptake of Cd but not at the expense of Ca uptake. Therefore, osmoregulatory disturbance was not associated with aqueous Cd exposure in this tolerant chironomid species. Work in my lab showed this to be generally true in other aquatic insect species[11]. Exposure to metals known to be antagonistic to Ca transport in acutely sensitive aquatic models (Cd, and Zn) did not affect Ca transport in aquatic insects described as being highly sensitive to metals exposures in nature (ephemerellids)[12]. Similar results were shown for metals associated with Na transport disturbance (Ag, and Cu)[13]. Moreover, we showed a limited protective effect of hardness on metal uptake in aquatic insects[14]. Science knows that aquatic insects are generally tolerant to acute aqueous exposures and the	Thank you for your review and comment. ii. As mentioned previously, EPA agrees the available data on aquatic insects within the scientific literature is limited and additional toxicity studies would benefit the models given their abundance and ecological relevance. However, the available data has verified that current bioavailability models are able to accurately (within a factor-of-2) predict toxicity to insects. A recent example is Besser et al. (2021) where the authors performed toxicity tests exposing nickel and zinc to the mayfly *Neocloeon triangulifera*. In addition, Mebane et al (2020) illustrated that toxicity tests of Cu, Pb, Ni and Zn with various insect communities spanned the entire SSD distribution for all of the metals tested and were not all found at the sensitive end of the SSD for any metal tested (Mebane et al. 2020b). Lastly, mesocosm data using field collected insect communities (including early life stages) are available and show HC_{05}s generated using bioavailability models are protective of insect communities (Roussel et al. 2007). iii. The CRADA partners have indicated that there is no consideration of mode of action in the selection

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of: i. **Complexity and transparency** ii. **Representativeness** iii. **Rigor** iv. **Usability**		
Reviewer	**Comments**	**EPA Response**
	reasons why[6]. This entire approach is only suitable for animals sensitive to acute aqueous exposures. **iii. Rigor** The modelling approach focuses on a very narrow set of possibilities: Taxa that are acutely sensitive to the surface binding of metals to respiratory surfaces. It does not consider bioaccumulated metals from ingestion or toxic modes of action that are not based on ionoregulatory disturbance. There are thousands of journal articles about the toxicity of metals to animal life. Relatively few of them focus on osmoregulatory disturbance as a mode of action. Metals are toxic for a host of reasons – and the biology of cells does not differ enough between different faunal groups to discount other modes of action and exposure routes as important. **iv. Usability** This question should be answered by potential end users in state agencies.	of ecotoxicity data used to develop or to validate the models. The datasets include chronic, full life cycle, and mesocosm test results. The studies in these datasets reflect observed acute and chronic toxicity, regardless of mode of action.
Reviewer 4	**i. Complexity and transparency** **Aluminium** – There were instructions for the use of the BLM but didn't find any for the MLR. It was not mentioned how hardness was calculated for the MLR from the raw data set which provided Ca and Mg. The actual equation for the MLR are not apparent and one has to refer to the Appendices to actually see it. Transparency could be improved. I was able to reproduce the results of the "Answer Key" document. I then plotted the HC5 from both models against one another and it showed a slope of	i. Thank you for the feedback on the individual models. Aluminum – Reviewers were initially provided a file that did not include the MLR equations, but this oversight was corrected after one reviewer inquired about the file, and the complete file with the MLR equations was sent to the peer reviewers Aluminum CRADA partners indicate that the reviewer is correct that, in the case of aluminum,

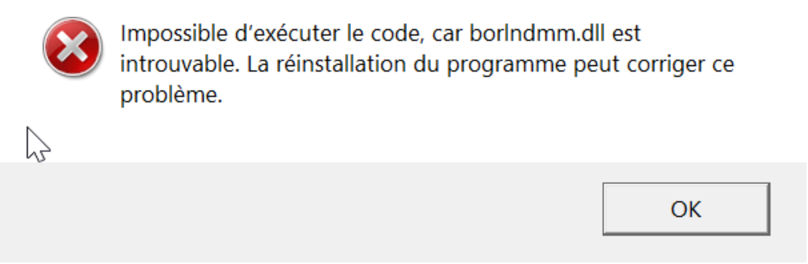
2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of:

i. **Complexity and transparency**
ii. **Representativeness**
iii. **Rigor**
iv. **Usability**

Reviewer	Comments	EPA Response
	1.33 which means that BLM HC5 values were 33% higher than MLR values. This suggests that models provide different results. **Copper** – Could not find the executable file at first but was able to recover it from FTP after sending out a request to ERG. I was able to reproduce the results from the answer key without difficulties. A few other observations: The name of the model suggests that it's chronic only, but the output file contains headers referring to "acute values". This can be a source of confusion for users. Being able to switch from one language to another is a nice option. Thanks! Program executes smoothly and quickly compared to Al or Ni. MLR equation easy to spot compared to other metals. MLR provides higher values, especially in the lower range. Models seem to agree in the higher range. **Lead** – Program (BLM_UI.exe) won't load. I tried two different computers and using different folder locations. Error message:	BLM and MLR predictions show less agreement than they do with other metals. This has also been discussed with within Brix et al. (2021) and DeForest et al. (2020). Copper – Thank you for the feedback on the confusion between the naming of the model and the output files. EPA will clarify these points in future iterations of the models so users should not encounter any potential confusion in the output from either the acute toxicity option or the chronic toxicity option. Lead -Thank you for the feedback so that EPA can make improvements in future iterations of the models. Nickel – Thank you for the feedback so that EPA can make improvements in future iterations of the models. The nickel chronic software should say "FCV" not "FAV" and will be changed in future model updates. ii. EPA acknowledges your concern about the range in diversity of taxa and agrees additional toxicity studies would benefit the models. iii. EPA acknowledges concerns about model applicability to effluent water chemistry conditions and is evaluating options to expand the range of

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of:

1. **Complexity and transparency**
2. **Representativeness**
3. **Rigor**
4. **Usability**

Reviewer	Comments	EPA Response
	 Impossible d'exécuter le code, car borlndmm.dll est introuvable. La réinstallation du programme peut corriger ce problème. Apparently, I am missing a DLL file. **Nickel** – the BLM model took about a minute to load, I was getting the impression the computer had crashed or that the program was not responding. I didn't have this problem with the Al model. I used default settings which specifies "BLM" and "Chronic". The output file was entitled "Ni test BLM_Chronic.output.xls". The headers of the last two columns were: HC5 (Lognormal Dist.) US EPA FAV There were two confusing elements here. First, this was a simulation for a chronic exposure so I assume that the last header should read "US EPA FCV". Second, when comparing with the "key" data file, the HC5 columns did not match those of the output file. But the values given in the output file under the header "US EPA FAV" had the exact same values as those of the "key" file under the header "BLM HC5". Either the header of the "key" file is wrong or the one	water chemistries to which the models can be applied. iv. Thank you for explaining the issues encountered when using software in a different language.

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of: i. **Complexity and transparency** ii. **Representativeness** iii. **Rigor** iv. **Usability**		
Reviewer	**Comments**	**EPA Response**
	from output file. Or perhaps I did something wrong. Same story for the MLR results. **ii. Representativeness** **Taxa**: Some models are based on the results of one alga, one invertebrate and one fish. There is thus lots of room for improvement of diversity. **Chemistry:** I saw a reasonable range of pH, DOC and Ca values that would encompass a large range of natural systems. Industrial effluents could be outside of validation range. **iii. Rigor** Regarding data analysis, the approaches are rigorous, and the authors of the papers have an outstanding reputation. As for the data selection, I can't answer that. Review of data selection would require weeks (more likely months) of analysis and backtracking values and literature review. This being said, the papers were published in reputable journals and there is no reason to think that there could be a bias in data selection. **iv. Usability** I had no experience with the end-user BLMs, and I found them somewhat easy to use with the instruction manuals. I did run into some problems. When copying and pasting data from Excel to the Al-BLM software, all values after the decimal disappeared. I only realised after running the program and comparing results to the Answer Key document. The problem came from the fact that my Excel program is in French and in French, the decimal mark is a comma instead of a period for the English format. I thus had to modify the default decimal marker in order to be able to paste values correctly. An error message would	

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of: i. Complexity and transparency ii. Representativeness iii. Rigor iv. Usability		
Reviewer	**Comments**	**EPA Response**
	have been useful here. I had to investigate to find the source of the discrepancy. When using the Ni-BLM, this problem got worse. The comma/period confusion was not limited to the format in Excel. The data I copied from Excel was in "period" format but once pasted into the BLM model, it was changed to a "comma" format. To fix this, this time I had to change Windows settings to English and restart the computer. After that I could get the model to run. Not a huge problem but being forced to switch language of my operating system was irritating.	
Reviewer 5	**i. Complexity and transparency** With Al, Cu, and Pb, the MLR models are transparent and reasonably simple to use. Not so for nickel. I could not find a spreadsheet or even the text description in the articles or SI files describing the complete equation. The pooled MLR calculates the FCV as a function of hardness and DOC plus an intercept, but nowhere in the documentation or in the numerous output files could I find a value that the intercept for the HC5 or FCV. For example, the output file "Ni-inputs.ssdnormalized.xls" in column AC has "MLR intercept" values but these vary by each test and the intercept for the FCV should not vary. Obviously the intercept is in the model files somewhere since it works. This is a minor matter that likely would have quickly been cleared up in an email with the developers had the review not been explicitly sequestered by the peer review manager. The explanations of BLM development in the respective articles is reasonably detailed. **ii. Representativeness** They seem to. The draft report and most of these models may be a bit overstating the case in that they address "invertebrates" or for the MLRs, that	Thank you for your comments. EPA will display the Ni MLR in a similar, spreadsheet format as the other metals for future iterations of the models. Nickel CRADA partners responded that there was originally a question of whether the Ni MLR SSD would be normalized by one pooled model or multiple models, thus the format (since the whole SSD would need normalization to each set of chemistry and then the HC5/FAV/FCV calculated, if there were multiple models). Because of this, we did not have a single intercept for the HC5/FAV/FCV calculated. These are quite easy to calculate, however, and the final equations using the "Pooled All" slopes for each SSD should be: Acute lognormal HC5 (ug/L)=exp(0.475*ln(Hardness,mg/L)+0.148*ln(DOC,mgC/L)+2.8220)

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of:

i. **Complexity and transparency**
ii. **Representativeness**
iii. **Rigor**
iv. **Usability**

Reviewer	Comments	EPA Response
	they include “invertebrate models” when in fact, the invertebrates tested were mostly daphnids. The very different phylogeny of crustaceans from aquatic insects has led to strong criticisms of using crustaceans to represent freshwater “invertebrates” (Poteat and Buchwalter 2014). All the models are relatively rich in fish and daphnid data. To test if the models and associated EPA-style final chronic values (FCV) or 5th percentile hazardous values (HC5) values calculated from the species sensitivity distributions (SSDs) compiled as part of the model development were protective of insects, I calculated the FCV/HC5 values from the models and compared them to Cu and Ni FCV/HC5 values that my colleagues and I had recently updated by added aquatic insect chronic values from community testing (Mebane et al. 2020b). With Ni, the model FCV/HC5s appeared to scale appropriately to the test conditions and appeared to be fully protective of the aquatic insects tested. For the conditions tested (hardness 17.5 mg/L, pH 7.67, DOC 3 mg/L), the Ni MLR produced a HC5 of 3.3 µg/L Ni and the EPA FCV equation 1.3 µg/L. The Ni BLM produced similar values (4.7 and 1.4 µg/L) for the community test water conditions. The lowest NOEC (no observed effect concentration) with any insect species or insect community metric was 9.5 µg/L. Algae was affected by nickel at the lowest concentration tested, but the practice in USA criteria, hazards to algae have not been given the same level of concern as have effects to aquatic animals With Cu, the model FCV/HC5s also appeared to scale appropriately, but the SSDs updated with insect values were lower than the model FCV/HC5s. This potential underprotectiveness is a function of the different SSDs, not the models. For the same conditions tested (hardness 17.5 mg/L, pH 7.67, DOC 3 mg/L), the Cu MLR produced a HC5 of 6.2 µg/L Cu and the BLM produced a lower value (4.7 µg/L). EC10s for reductions in overall taxa richness in the Cu	US EPA FAV(ug/L)=exp(0.475*ln(Hardness,mg/L)+0.148*ln(DOC,mgC/L)+3.3418) Chronic lognormal HC5 (ug/L)=exp(0.475*ln(Hardness,mg/L)+0.148*ln(DOC,mgC/L)-0.3232) US EPA FCV(ug/L)=exp(0.475*ln(Hardness,mg/L)+0.148*ln(DOC,mgC/L)-1.2937) EPA acknowledges your concern about the range in diversity of invertebrate taxa and agrees additional toxicity studies would benefit the models given their abundance and ecological relevance. Copper CRADA partners have indicated that they agree with the reviewer that some mesocosm experiments have demonstrated that some aquatic insects are highly sensitive to Cu, and agreed that SSDs (but not the BLM binding constants) would need to be adjusted if mesocosm data were to be incorporated into criteria derivation (as demonstrated in Mebane et al. 2020). However, USEPA has not yet adopted mesocosm toxicity data as acceptable inputs for construction of SSDs. Consequently, no guidance currently exists for incorporation of the Mebane et

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of:

- **i. Complexity and transparency**
- **ii. Representativeness**
- **iii. Rigor**
- **iv. Usability**

Reviewer	Comments	EPA Response
	tests were 2.6 to 3.4 µg/L (the Cu test was repeated), with some mayfly taxa EC20 values below the BLM and MLR calculated HC5 values of 4.7 and 6.2 µg/L (Baetis, Diphetor, Ephemerella). This suggests that the model criteria adjustments are appropriate but that the Cu criteria SSD should be updated to account for sensitive insect taxa. Other non-fish, non-daphnid datasets I was familiar with and compared with include acute mayfly (Baetis) tested in natural waters with a range of hardness and pH values (Mebane et al (2012), included in the DeForest Appendix E comparisons) and acute and chronic freshwater mussels with varying hardness, pH, and DOC (Wang et al. 2009; Wang et al. 2011). The models performed well with these "nonstandard" taxa. Note also that the Pb and Ni models included Lymnaea snails in their development. I just don't see any major animal taxa for which the model performance gives great pause, and the BLMs and MLRs have been tested with pretty diverse artificial and natural waters. While MLRs have been shown to work well with a wide variety of waters, the power of the BLM approach is that due to its mechanistic underpinnings, BLMs can often function well beyond their calibration datasets. This is one more reason BLMs should be kept in the quiver of potential tools that can be employed in risk assessment or site-specific criteria development. For instance, BLMs can handle strange Ca:Mg ratios and other uncommon chemistry reasonably well (Van Genderen et al. 2007). MLRs fall apart under such scenarios. **iii. Rigor** Yes. I think the CRADA crowd should be commended for their work with primary datasets from the literature and for generating necessary data. In particular, they avoided the trap that some prominent related efforts have	al. (2020) results and other mesocosm results into the Cu SSD. A consequence of updating the toxicity database for Cu or any other metal, with or without incorporating mesocosm data, is that the MLR equations would have to be revised to fit the updated database. That would be more complex to address than for the BLM, for which only the critical accumulation value (CAV) might have to be recalculated to be consistent with the HC5 of the new SSD. EPA agrees that the development of empirical models like MLR can be informed by mechanistic models like the BLM by helping to identify the key TMFs and expected mechanistic patterns and by evaluating MLR models against existing BLMs. EPA acknowledges the comment that the MLR should optimally not be used beyond its calibration dataset and is evaluating options to expand the range of water chemistries to which the models can be applied. EPA has in the past, in limited ranges, applied the MLR model beyond the calibration dataset when the values yield lower, more protective criteria than if one used the

2.1.c Appendix G - applying the models for the specific calculations of water quality criteria presented in terms of: **i. Complexity and transparency** **ii. Representativeness** **iii. Rigor** **iv. Usability**		
Reviewer	**Comments**	**EPA Response**
	fallen into – the incautious reliance on the EPA EcoTox database. Despite the EcoTox statement that it is "*a comprehensive, publicly available Knowledgebase providing single chemical environmental toxicity data on aquatic life,..*" updates have been ad hoc on a chemical-by-chemical basis and the database does not appear to have been updated for metals in more than 10 years. **iv. Usability** Yes, mostly. The (not yet public) Windward BLM updates included in this review were clearly explained and ran without hiccups. The Al, Cu, and Pb MLR models were straightforward. Rolling the Ni MLR into the BLM software is a nice comparative touch, but the Ni MLR obviously also needs to be available as a standalone spreadsheet.	criteria at the limit, in order to address stakeholder needs (e.g., Al MLR). EPA notes that the ECOTOX Knowledgebase is updated periodically, including special targeted updates that are conducted during criteria development for specific chemicals, including metals.

2.2 Please provide your overall review of the approaches used to compare and evaluate the BLM and MLR models for the metals addressed in the Phase I document and appendices.

2.2 General comments for the approaches used to compare and evaluate the BLM and MLR models.		
Reviewer	**Comments**	**EPA Response**
Reviewer 4	This is difficult for me to say as I am not a specialist in model performance assessment but as far as I know, the approaches used were convincing and credible. I have no alternative approach to recommend.	Thank you for your comment.

2.2.a Are the approaches presented consistent with the state-of-the-science?

2.2.a Consistency with the state-of-the-science.		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	As far as I can judge, the approaches are indeed consistent with the state-of-the-science with regard to the type of modeling applied. As already indicated above, nowadays more advances informatics and bioinformatics tools are becoming increasingly available and most likely, these tools might be considered more advanced than for instance MLR models. Nevertheless, in my opinion the models developed are well suited for the purpose of quantifying metal bioavailability.	Thank you for your comment.
Reviewer 2	Yes, generally the approaches presented are consistent with the state-of-the-science, however, I feel as though certain aspects were not addressed adequately. These have been previously addressed in sections 1 b. DOC and 1. C. ii. and include the various forms of DOC and how they differentially affect toxicity and disclosing the life stage/age of the species implemented into the modeling.	Thank you for your comment. Please see EPA's responses to the reviewer's concerns above in section 2.1b and 2.1c.
Reviewer 3	The approaches are consistent with the state of the science for organisms acutely sensitive to aqueous metal exposures only. The models ignore a large body of science relating to dietary exposures because this science does suit the goal of relaxing environmental protection. It is remarkable that the possibility of dietary exposures is ignored in the main document when these industry groups have compiled a robust bibliography of references on the topic (see Appendix 1). Willfully ignoring science that does not meet set intentions will not make that science go away. It is incumbent on EPA scientists to appreciate that these models represent science with a set goal in mind, and that goal is not purely about protecting aquatic life. The fundamental underlying premise here is that if a water body can	Thank you for your comment and providing the references for EPA's review in Appendix 1. Please see responses to Reviewer 3 in sections 2.1a and 2.1c regarding dietary exposures. More information on the dietary exposure route has been added to Section III of the report. In addition, Mebane et al. (2020) suggested there is currently "insufficient evidence to conclude that bioavailability models would be under-protective if based on waterborne-only exposures" and recommended that researchers conduct concurrent exposures to strengthen the literature surrounding dietary exposure and support the development of a biodynamic modeling framework that is able to incorporate the dietary exposure route (Mebane et al. 2020a). Lastly, where it is well-established that the diet is an important exposure route,

2.2.a Consistency with the state-of-the-science.		
Reviewer	**Comments**	**EPA Response**
	absorb more pollution, then more pollution should be permissible. This is dangerous from the perspective of persistent contaminants that are very expensive to clean up after the fact.	EPA has considered this information in their criteria development. For example, the selenium water quality criteria (US EPA 2016) use fish tissue concentrations as diet is the primary route of exposure.
Reviewer 5	Yes, the comparisons are consistent with those suggested in the 2017 SETAC experts meeting, and appear to be evenhanded, and statistically robust.	Thank you for your comment.

2.2.b. Can you identify other approaches that could be used to compare the models?

2.2.b Other approaches that could be used to compare the models.		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	No doubt, other advanced tools are available from within the field of (bio)informatics. I am, however, not aware of the details of such alternative tools and approaches. For now, the comparison made with regard to the performance of the BLM and MLR models, is sufficient to warrant confidence in the models and in the selection of the best model.	Thank you for your comment.
Reviewer 2	It would be helpful to provide multiple data sets; some with common water chemistries and then highlight some more complex water chemistries for example wastewater effluent where different combinations of the TMFs are observed.	Thank you for your comment. EPA will consider adding more datasets (beyond the natural and artificial datasets provided with this review) to future peer reviews of the individual metals models.

2.2.b Other approaches that could be used to compare the models.		
Reviewer	**Comments**	**EPA Response**
Reviewer 3	I don’t have any recommendations here but I think there could be more serious treatment about model differences in synthetic vs natural waters.	Thank you for your comment. As mentioned previously in response to Reviewer 3’s comment in section 2.1b, EPA and the CRADA partners are working towards a comparison of HC_{05} values using the BLM and MLRs to investigate discrepancies and major differences between the model predictions. Furthermore, toxicity tests for metals in both synthetic and natural waters can be important sources of information for model development and testing and model application in estimating protective values under real world conditions. Most of the toxicity data used in guideline development was developed in synthetic waters, partially because EPA prefers data generated in standard toxicity testing regimes (including using waters with low DOC) to provide consistency across species and chemicals regarding relative toxicity and to reduce confounding factor interference,. The consistent exposure conditions that can be obtained with synthetic waters are also useful for chemical adjustments of modifying factors to determine how water chemistry affects metal toxicity (for example, testing over ranges of hardness, pH, or DOC) and these types of experiments are useful for model development. Natural waters are useful for validating the model in real world conditions. The CRADA partners indicate that the application of the model to synthetic and natural waters was clearly specified in the papers that were submitted in support of the nickel BLM and MLR (Appendix F). Similar breakdowns of synthetic and natural waters have not been compiled for other metals, but this information is available in the sources cited for toxicity data.

2.2.b Other approaches that could be used to compare the models.		
Reviewer	**Comments**	**EPA Response**
Reviewer 5	Well yes, there is no end to ways the models *could* be compared, but I don't know of other approaches that *should* be used. The models essentially produce paired groups and there are all sorts of statistical methods for group comparisons. Likewise, there is no end of different species and waters and speciated vs. dissolved metals models, of combined food and water pathways. I think the present set of comparisons is at the point of diminishing returns. Time to move on to other metals.	Thank you for your comment.

2.3 Please comment on the use of a limited set of toxicity modifying factors to estimate toxicity using both the MLR and BLM approaches (i.e., compared to the full parameter set used to derive ambient water quality criteria for copper in EPA 2007).

a. Please provide feedback on limiting toxicity modifying factors to a set of *a priori* determined parameters (e.g., pH, hardness, dissolved organic carbon (DOC), and potentially temperature, as appropriate).

2.3 Use of a limited set of toxicity modifying factors to estimate toxicity using both the MLR and BLM approaches.		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	There is a wealth of data showing that a limited set of toxicity modifying factors is capable of capturing most of the impacts of water chemistry on metal bioavailability. In general terms my estimate would be that over 90 % of the possible impacts of water chemistry on metal bioavailability, is properly considered. This implies that it can never be ruled out for 100 % that in specific cases not considered so far, additional toxicity modifying factors might be of importance – even apart from the full parameter set use in EPA 2007. This is inevitable, and there is no solution but to accept that models cannot be for 100 % accurate.	Thank you for your comment.

2.3 Use of a limited set of toxicity modifying factors to estimate toxicity using both the MLR and BLM approaches.		
Reviewer	**Comments**	**EPA Response**
Reviewer 2	There is strength in an approach that simplifies the BLM model from ~10 parameters to 3-4 parameters. In many cases, these additional parameters are not determined or inaccurate which leads to either inputting estimates or leaving values at the default settings. Requiring more variables also increases the potential and impact of human error for derivation of accurate water quality criteria for the protection of aquatic life. However, as mentioned above, there is a need to include temperature as a fourth parameter. Metal accumulation in fish, pond or river water is enhanced by upsurges in temperature; therefore, it is imperative to study the detrimental effects of metals in combination with temperature to formulate accurate predictive models (Kumar et al., 2018 Int. J. Environ. Sci. Technol.). This is an area which has been grossly overlooked in metal toxicology.	Thank you for your comment. EPA agrees there is strength in a simplified parameter set for end-users. Regarding temperature, please see the response to Reviewer 2's comment in section 2.1a.
Reviewer 3	There is no doubt that each of these TMFs are important. There should be balance between TMFs that relax protection with TMFs that potentially would require additional protections. It would be great if the influence of temperature was well understood in metal toxicity, but unfortunately it is not. At this time of writing the Pacific Northwest is experiencing an unprecedented heat wave. Does anyone think the effects of pollutants are not exacerbated under these extreme conditions? It is progress that temperature is recognized is a potentially important TMF, but we are nowhere close to being able to address it at the level of criteria development. When science emerges that highlight the potential risks of metals from dietary exposures for example, it is largely ignored by the metals industry groups that are promoting this modeling	Thank you for your comment. Regarding temperature, please see the response to Reviewer 2's comment in section 2.1a. Regarding dietary exposure, please see the responses to Reviewer 3's comments in sections 2.1a, 2.1c and 2.2a. Furthermore, the 1985 Guidelines do not explicitly exclude the consideration of dietary exposure. EPA believes it is important to focus on the primary drivers of toxicity, which for the metals evaluated in this effort, appear to be aquatic exposure (as noted above).

2.3 Use of a limited set of toxicity modifying factors to estimate toxicity using both the MLR and BLM approaches.		
Reviewer	**Comments**	**EPA Response**
	approach. It is remarkable that this work is being sold as state-of-the-science when there is no recognition of the contributions of Luoma, Cain, Hare, Fisher, Rainbow and others that do not fit this aqueous exposure paradigm. This is partially the fault of the antiquated 1985 guidelines for excluding dietary exposures and partially a function that considering things that could argue for strengthening environmental protection is not in the interest of these metals groups. This effort is all about reducing overprotection – not protection.	
Reviewer 4	When building an empirical model, one must be cautious about the domain of validity of the model and no extrapolation can be made. It follows that extensive documentation must be provided to guide the users for the applicability of the MLR within the conditions that were used to build the model, even for parameters that were not considered significant. If a parameter is not measured and is well outside of the range of values used for model calibration, the model may be off without the user being aware of it. For example, if the MLR for Ni does not require pH as input, it is still an important parameter as some organisms may not tolerate this pH. The same applies for any parameter that would be outside of the range of values present in the calibration data set. In other words, less input data may be convenient, but it increases the probability of a wrong conclusion. Range of applicability of water chemistries should not be limited to the parameters used in the MLR but perhaps this is already specified, and I missed it in my review of the numerous documents provided. **Temperature** – I think temperature is only pertinent for Al which may often exceed solubility. Adequate prediction of the	Thank you for your comment. EPA acknowledges the need for understanding the range of conditions that correspond to model development and testing and that caution and scientific judgement must be applied if application of the MLR beyond its calibration dataset is considered. EPA is evaluating options to expand the range of water chemistries to which the models can be applied. Furthermore, tables of water chemistry boundaries are included in the BLM and noted in the user guide, and this information will be provided with any future models used for criteria derivation. Lead CRADA partners have indicated that the reviewer is correct that Pb solubility is affected by the presence of phosphate and phosphate depletion can cause growth inhibition in plants and algae. To address this issue, algae and plant tests conducted for lead toxicity were optimized for phosphate content in the test media and lead speciation was calculated where needed. The Pb MLR model for algae was based on 15 *Pseudokirchneriella subcapitata* tests reported in De Schamphelaere et al. (2014) and 2 *P. subcapitata* tests reported in Nys and De Schamphelaere (2017). In De Schamphelaere et al. (2014), tests were conducted with organic phosphorous (i.e,. glycerol-2-phosphate) to prevent

2.3 Use of a limited set of toxicity modifying factors to estimate toxicity using both the MLR and BLM approaches.

Reviewer	Comments	EPA Response
	dissolved concentration is key. I don't see any other elements in the given list of metals for which temperature would be critical. I would point out, as an example, that Pb is poorly soluble in the presence of phosphate. Phosphate has never been mentioned in the documents (my apologies if I missed it) but it is a required nutrient for plants and usually present at high concentrations in standard tests for plants and algae. Growth inhibition can be wrongly interpreted as an effect of Pb while in reality it could be the lack of available phosphorus that would decrease growth. Speciation calculations would flag this while an MLR wouldn't.	lead-phosphate mineral precipitation. Those authors conducted Pb speciation calculations that "strongly indicated" addition of glycerol-2-phosphate to the alga test waters had no effect on free Pb^{2+} activities. In the Nys and De Schamphelaere (2017) study, Pb toxicity tests were conducted under both low and high phosphorus conditions (10 and 100 µg P/L, respectively). Phosphorus was added in these tests as $\mathrm{NaH2PO4 \cdot 2H2O}$. The 2 tests with low phosphorus concentrations did not meet test validity criteria and were excluded from the MLR model evaluation. The 2 tests with high phosphorus concentration did meet test criteria and were included in the MLR evaluation. The MLR model was "driven" by the 15 tests from De Schamphelaere et al. (2014), and the predicted Pb EC20s for the 2 tests from Nys and De Schamphelaere (2017) were a factor of 1.0 and 1.8 different from the observed EC20s. Based on the Pb speciation calculations previously described in De Schamphelaere et al. (2014), it is not believed that the growth effects in the Pb toxicity tests with *P. subcapitata* were potentially caused by an absence of bioavailable phosphorus due to formation of insoluble lead-phosphate. Further, the Pb MLR model that was largely based on tests from De Schamphelaere et al. (2014) accurately predicted Pb toxicity in 2 tests in which phosphorus was added as phosphate. This provides an example where speciation calculations can be used to inform selection of data sets used for MLR model development.

2.3 Use of a limited set of toxicity modifying factors to estimate toxicity using both the MLR and BLM approaches.		
Reviewer	**Comments**	**EPA Response**
Reviewer 5	Hardness, pH, and DOC have been shown able to capture the majority of metals toxicity variability in laboratory settings. I have never seen a quantitative analysis of why hardness is better than Ca. No BLM uses hardness. Yes, there is some evidence that Mg offers some protection to daphnids, but there is lots of evidence of Ca giving greater protection (Welsh et al. 2000; Naddy et al. 2002). I suspect that the real reason for relying on hardness rather than Ca is the policy desire to keep a lineage to the old hardness-based criteria. I also suspect that the empirical performance of MLRs with Ca or hardness would be similar for most waters. If this is the case, some quantitative comparison and a statement of policy heredity might be appropriate. In regard to temperature, there is evidence that animals may be more sensitive to metals when tested either well below or well above their temperature optimums (I can dig out references upon request). However, I question whether this is a metals toxicity modifying factor or a multiple stressor, or if this fine distinction even matters. Adding more factors really complicates implementation, for temperatures can swing >10°C over the course of the day, and we already have an underappreciated problem with daily pH cycles that commonly swing over 0.5 units in waters and up to at least 2 units. A 0.5 pH swing is a big deal in any of these models, and diurnal variability in pH has not been considered in any of these approaches. It should be.	Thank you for your comment. As a user-friendly modeling version, MLRs use hardness because most end-users monitor hardness rather than Ca. One line of evidence that validates the use of hardness instead of Ca and/or Mg concentrations is the consistency in the result from cross-validation exercises comparing the BLM and MLR predictions. Consideration of Ca:Mg ratios could be included in future guidance for criteria development and implementation, but the practical utility of models and criteria would be reduced by introducing the need for additional data that is not typically collected in state sampling programs. EPA plans to develop criteria that can be broadly implemented. Diurnal variability in various parameters including pH (diurnal fluctuations in temperature was previously discussed by Reviewer 5 in Section 2.3) can be a consideration within the EPA's conceptual models being developed as part of the criteria derivation process. EPA agrees that temperature could be an important toxicity modifying factor for some metals, but there is not enough data on temperature to incorporate into the models at this time. Please also see response to Reviewer 2's comment in section 2.1a.

2.4 Please provide recommendations on potential software platforms/tools (e.g., Excel, R, or other freestanding programs) that could/should be used to perform MLR and BLM calculations.

a. Please discuss advantages and disadvantages of any software platforms/tools.

2.4 Recommendations on potential software platforms/tools that could/should be used to perform MLR and BLM calculations.		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	What I experience is that the number of data and the number of models for individual metals (and hence the overall set of data and models) is increasing. In my experience this means that tools like Excel cannot be used anymore given their limitations when dealing with large amounts of (complex) data. Instead, the number of R-applications as well as the number of advanced modelling platforms is quickly increasing. Also, modelling platforms are in development which allow the user to systematically store data and models, and to use this information to develop and integrate models and data according to the wish of the users. It is recommended to explore the new generation of software platforms and tools which are quickly becoming increasingly user-friendly.	Thank you for your recommendations. Both an Excel and R application were provided for use in the 2018 EPA Aluminum criteria. EPA will also consider other new software platforms.
Reviewer 2	There are many advantages of using R over Excel. R can handle very large datasets and automate and calculate much faster than Excel. The reproducibility of R source code is much more advanced and easier to use than Excel and there are community libraries of R source code which are available to all. R has more complex and advanced data visualization. Lastly, which may have the most significance with broad demographics of people who will be using these models, R is free and Excel is not. However, Excel is still a powerful tool for smaller datasets, basic data entry, simpler functions and formulas, and viewing raw data. I tend to think that more of the general population is familiar with Excel and will more readily use Excel. R is overwhelming and may cause more mental barriers in using the models. I cannot comment on programs such as Python, Matlab, SAS, and SQL which may be arguably better.	Thank you for your recommendations that EPA consider R-applications for large datasets and potentially Excel for a user-friendly option for smaller datasets.

2.4 Recommendations on potential software platforms/tools that could/should be used to perform MLR and BLM calculations.		
Reviewer	**Comments**	**EPA Response**
Reviewer 3	I have no comments or recommendations about which platforms should be used to make these calculations.	Thank you for your comment.
Reviewer 4	Ideally, online tools should be provided to prevent misuse of user-owned platforms. This could also prevent issues related to regional settings (see answer to Question 1c above).	Thank you for your recommendation. EPA aims to prevent misuse and general user issues of the tools.. EPA notes that for the 2018 Aluminum criteria (MLR approach), criteria calculators (one in Excel, one in R) are housed on the EPA website, eliminating the need for users to download software.
Reviewer 5	A major feature of MLRs is that they don't need a specific software platform. An equation yields the same answer for given inputs no matter whether it is calculated in an xlsx spreadsheet, Google Sheets, Open Office, R script, Python, C code, hand calculator or longhand. It doesn't matter. Imagine if EPA had provided software to calculate the 1984 Pb criterion. I think the Mac debuted that year, some precursor to MS-DOS was going, ... Certainly, when it comes time to publish MLR based criteria, certainly providing some calculation tools such as in xlsx spreadsheet format and R would be helpful. At present, I think spreadsheet formats have the advantage since they can readily hold data in most a human-readable format as long as some care to structure tables in lightly formatted forms that are easily exported to csv and R. Note that "Excel" and "xlsx" are not the same thing. "Excel" is a proprietary Microsoft application; "xlsx" is a non-proprietary spreadsheet open standad, part of the Open Office XML standard. At the present, I would say that the "xlsx" Open XML spreadsheet format would be most widely accessible and transparent to most users, but that R users are closing the	Thank you for your recommendations and clarification on Excel vs xlsx format. EPA will consider continuing to provide R-applications and Excel applications for various users.

2.4 Recommendations on potential software platforms/tools that could/should be used to perform MLR and BLM calculations.		
Reviewer	**Comments**	**EPA Response**
	gap. It would not be a big lift for R aficionados to pull information in from spreadsheets to work with.	

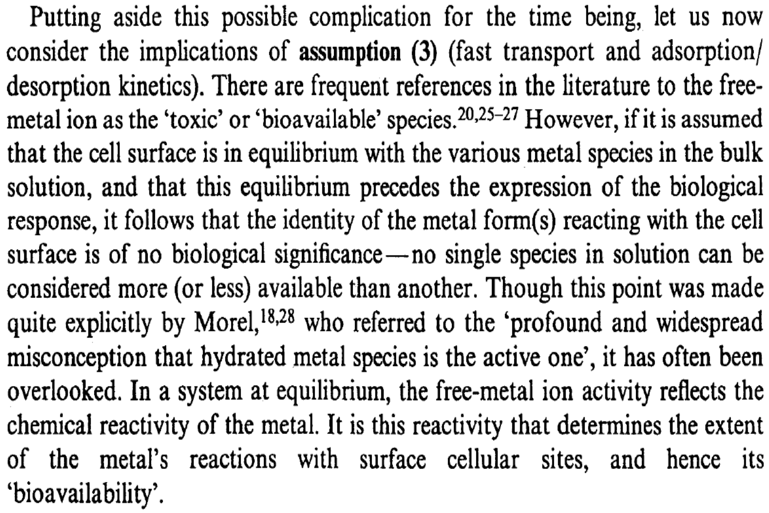
2.5 Please provide any additional suggestions that you feel would improve the report.

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	My key suggestion is that one overarching approach is chosen for deriving water quality criteria for metals that take account of the most important toxicity modifying factors. What is important, if only to gain sufficient confidence of non-experts, is to not only indicate the merits of the overarching approach, but to also mention the limitations and the 'domain of applicability' of the models underlying the overarching approach. These domains may be metal-dependent, and do not include extreme water chemistries (the more as physiological limitations of most biota limit the applicability of the models in extreme environments). A final suggestion is to take count of interactions between toxicity modifying factors as such interactions are likely to affect toxicity.	Thank you for your comment. EPA will discuss both the advantages and limitations of the chosen overarching approach in both this report and when developing the individual metals models/criteria. As exemplified in the case studies presented with the report (Appendices D, E, and F), EPA will consider interactions between toxicity modifying factors (as was also demonstrated in the 2018 Freshwater Aluminum Criteria) in the development of all metals models.
Reviewer 2	p.3 section a. pH – bioavailability should be changed to bioavailability (remove extra "i"). In Canada, the government has a duty to consult (https://www.rcaanc-cirnac.gc.ca/eng/1331832510888/1609421255810), and where appropriate, accommodate Indigenous groups when it considers conduct that might adversely impact potential or established Aboriginal or treaty rights. The goal is to listen to the views and concerns of affected Indigenous groups and, where necessary and possible, modify	This edit has been made to the text. Thank you for your comments.

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	the action or decision to avoid unlawful infringement of those rights. This may be an important consideration when using these models to support states, territories, and tribes.	
Reviewer 3	The report should provide a table showing what the WQC would be under different water chemistry conditions for the different metals with columns for the current criteria, what a BLM based criteria would be, and that the MLR based criteria would be. There should be transparency about how WQC would be altered from the current values under a wide range of water chemistry conditions. I have never seen any proof or analysis that demonstrate that current criteria are egregiously over protective. I think this is important to show. This exercise is using taxpayers' dollars to revisit metals criteria yet again, when the agency is woefully behind in establishing criteria for thousands of relevant pesticides, industrial pollutants and personal care products. On p. 3, section II, there is a statement that toxicity is dependent on route of exposure, however the entire modeling approach is only based on direct aqueous exposures. This is a regrettable byproduct of the 1985 Guidelines document's focus on aqueous exposures only. This issue should be fixed immediately. In Mebane et al, 2020[8], there is the recommendation that "for best practice in the future, that during chronic tests combined waterborne and dietary matched exposures should be performed. These should be based on natural live diets that have undergone full biological equilibration with the waterborne metal through pre-exposure." These authors comment that very few data of this type exist. The reason more of these data don't exist is because there is no market for this information. EPA should require these data rather than excluding them in the criteria process. My laboratory has shown a path forward for these type of experiments with a relevant aquatic insect model[4,15–20] as both an end receptor and as a food source,	EPA is considering development of a table like the one described by the reviewer. Regarding dietary exposure, please see EPA's responses to Reviewer 3's comments in sections 2.1a, 2.1c and 2.2a. Regarding synthetic vs natural waters, please see response to Reviewer 3's comment in sections 2.1b and 2.2b. EPA agrees that invertebrates and fish are the most common species within toxicity databases, however, the impact of TMFs has been studied in numerous species including invertebrates, fish, amphibians, insects, plants, and algae.

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	but WQC constructed with the antiquated 1985 guidelines would exclude these data from consideration for having dietary exposures associated with them. It is remarkable that a scientific flaw as egregious as this is allowed to persist in criteria derivation. There is little attention given to the differences between BLM and MLR approaches in natural waters vs synthetic waters (e.g., see copper results above). It is not clear to me what the relative proportions of toxicity data exist for synthetic vs natural waters, but this should probably be addressed quantitatively in more detail in a final report. Finally, there needs to be more attention given to the extrapolation of TMFs based on 2 taxa to represent thousands of other species. The distinction between fish and invertebrates is a nice start, but I don't know how people could be comfortable with these extrapolations. I have similar discomfort with the application of Acute to Chronic Ratios (ACRs) in situations where chronic data are limited. Some of Chris Mebane's work on this area[21] needs to be studied by EPA scientists.	
Reviewer 4	The document refers to "binding sites on the gill surface or respiratory surface" on two occasions. This is a too narrow description of the biotic ligands that only applies to animals. A more generic description would be "surface binding sites leading to internalization and effect". On page 2, "…simple linear regression models…", I think several of these are not linear. On page 3: "The effect of a number of metals on aquatic organisms is not well predicted by the total metal concentration (or total dissolved concentration), but rather the bioavailable forms (e.g., the free metal ion) which is a function of many modifying factors that affect the speciation, bioavailability, and toxicity of metals." This is an incorrect wording. Although widely used in the literature, I would like to (at least try to) convince the authors to refrain from using these terms.	Thank you for your comments and suggestions. Added suggested text on page 1 of the report regarding the description of biotic ligands. Text regarding the discussion of bioavailability was refined on page 3 of the report.

2.5 Additional suggestions that would improve the report.

Reviewer	Comments	EPA Response
	Bioavailability is a relative concept, not an absolute one. A metal can be more or less bioavailable depending on ambient conditions, but one cannot identify a “bioavailable form” or “fraction”. In fact, I would argue that <u>all forms</u> are bioavailable because all forms can dissociate. Overall, there is a mathematical relationship between the free metal ion concentration and uptake / toxicity, but this does not mean that only the free species is bioavailable. A metal complex can also react with a binding site and, by a ligand-exchange reaction, release the original ligand prior to internalisation. In such a case, the mathematical relationship between the binding surface and the free ion remains the same even though the complex was the reacting species. I refer the authors to page 55 of Campbell (1995) for a development of this point:  Putting aside this possible complication for the time being, let us now consider the implications of **assumption (3)** (fast transport and adsorption/desorption kinetics). There are frequent references in the literature to the free-metal ion as the ‘toxic’ or ‘bioavailable’ species.[20,25–27] However, if it is assumed that the cell surface is in equilibrium with the various metal species in the bulk solution, and that this equilibrium precedes the expression of the biological response, it follows that the identity of the metal form(s) reacting with the cell surface is of no biological significance—no single species in solution can be considered more (or less) available than another. Though this point was made quite explicitly by Morel,[18,28] who referred to the ‘profound and widespread misconception that hydrated metal species is the active one’, it has often been overlooked. In a system at equilibrium, the free-metal ion activity reflects the chemical reactivity of the metal. It is this reactivity that determines the extent of the metal’s reactions with surface cellular sites, and hence its ‘bioavailability’. Another good paper on this topic is that of Meyer (2002). An easy fix to this would be to replace “bioavailable form / fraction” by “metal bioavailability”. In other words, one can say that the bioavailability is	

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	greater / lower in experiment A vs B, but one cannot say that there are more or less bioavailable forms in A vs B.	
	Suggested rewording: "The effect of a number of metals on aquatic organisms is not well predicted by the total metal concentration (or total dissolved concentration). Metal bioavailability is a function of many modifying factors that affect the speciation and toxicity of metals."	
	Page 3: "In addition, the BLM also accommodates temperature as a modifying factor for some metals, such as for aluminum (Santore et al. 2018)". It's not clear how temperature influences bioavailability of Aluminium without reading Santore. This is related to Al solubility which is sensitive to T in a range pertinent to a natural exposure scenario. Role of T should be clarified as the reader may think this is a physiological parameter.	The discussion of temperature as a TMF was moved to page 4 of the report.
	Page 3: "The second way is by competing with metal ions for binding sites on organisms (e.g., competition from H^+, Ca^{2+}, and Mg^{2+}) ~~which interferes with essential ions (Na^+, K^+, and Cl^-) needed by organisms for osmoregulation~~". Somewhat confusing here. Interference with an essential ion can be a toxicity mechanism but the beginning of the sentence is about competition between two cations for a binding site; the sentence is thus deviating from its original purpose. Also, why focus on H, Ca and Mg if Na and K are the essential ions that are affected? Deleting this part of the sentence would make the sentence much clearer.	Suggested deletion was made in text on page 3 of the report.
	Page 4: ". In addition, higher Ca:Mg ratios have a greater protective effect by modifying toxicity than waters with similar hardness that had lower Ca:Mg ratios (Welsh et al. 2000)". I would delete this sentence. This repeats the observation about fish being sensitive to Ca and is in contradiction with the observation about invertebrates.	Sentence was deleted from text on page 4 of the report. "Usually was added" in the text to provide clarification on page 4 of the report.

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	Page 4: “An increase in sodium (Na$^+$) cations generally decreases toxicity by competition at metal binding sites, however Na$^+$ provides less protection than Ca^{2+} and Mg^{2+}”. For fish and silver, sodium is a better protecting parameter than calcium. Add “usually” before “provides”.	Per the comment of Reviewer 5 below regarding humic acid, this portion of the Table 1 footnote was removed.
	Table 1: First mention of humic acid. This may need an explanatory sentence perhaps in the DOC section. I understand what is meant by the 10% default, but the average reader won’t.	
	Page 5: “The approaches used by these models fall within a continuum between empirical (e.g., Water Effects Ratio [WER] and hardness equations) and mainly mechanistic (e.g., biokinetic BLM) (see Textbox 3 in Adams et al. 2020 and Figure 1 in Brix et al. 2020). In the middle of the continuum are the empirically-based MLR and mechanistically-based BLM”. I would argue that MLR are very close to entirely empirical models and not in the middle of the continuum. It’s however reasonable for the BLM. Although the BLM was initially a purely mechanistic conceptual model based on the Free-Ion Activity Model, it has evolved into a more empirical model over time (see also response to Question 1b above).	The continuum presented in Brix et al. (2020) represents the relative empiricism/mechanistic basis of various bioavailability models. There may be some variation in the specific placement of the models along this continuum, the positions of each model relative to one another is accurate. In addition, since this diagram has been published in the peer-reviewed literature, it is reasonable to keep the text regarding the Figure "as is" for illustrative purposes.
	Table 2: 4 in SO$_4$, should be in subscript (also in the main text) Alkalinity and hardness sometimes have a capital letter, sometimes not	The suggested changes were applied to Table 2.
	Page 15: “It is important to note that, the Cu BLM is not optimized for toxicity observations (neither chronic nor acute)”. What is it optimised for? Accumulation?	The text has been clarified to say, “It is important to note that, the Cu BLM is optimized for measured Cu accumulations and not for toxicity observations (neither chronic nor acute).”

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	Page 15: What does "without interactions" mean? I found out by reading the paper in the Appendices, but this should be understandable for people who read the report only. Page 16: About bicarbonate toxicity, from reading Santore 2021, this conclusion lacks nuance. Bicarbonate toxicity is one possible explanation for the poor reproduction of C. dubia at high pH. It would be preferable to say that C. dubia does not tolerate pH > 8 and that other factors are at play and that Santore speculated that this could be due to bicarbonate toxicity. The reader needs to be guided here.	Clarification was made in text regarding TMF interactions in both the section referring to MLR models and in the discussion of the metal case studies. The text was modified to provide clarification regarding bicarbonate toxicity in the discussion of the Ni case study.
Reviewer 5	**Specific comments on the draft CRADA report** These comments refer to the draft report entitled "*Development of an Overarching Bioavailability Modeling Approach to Support US EPA's Aquatic Life Water Quality Criteria for Metals*" (21 pp) hereafter "bioavailability report." Appendixes B and C are integral to the report, and I also have some comments on those. Overall, I thought the "bioavailability report" and Appendix B were very good. They will doubtlessly be influential for years, and so should get more vetting with attention to referencing and supporting all statements before final publication. There are some unreferenced statements that seem like overstatements in Section II. p. 2, paragraph b, under "Overview of EPA's metals criteria," consider adding a sentence or so on why some metals have criteria but most do not. Cobalt is prominent by its absence. Maybe something along these lines? '*Of the 56 elements commonly classified as metals on the periodic table, currently EPA has developed recommended AWQC for 9 metals (aluminum, cadmium, chromium (III and IV), copper, iron, lead, nickel, silver, and zinc). This list of metals requiring criteria dates to a 1976*	Thank you for your comments, your detailed analyses, and citations on the effect of TMFs across several metals.

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	negotiation among parties to a settlement agreement (NRDC et al. vs Train, 6 ELR 20588, D.D.C. June 9, 1976). In setting priorities for establishing new or revised criteria EPA may consider the changing societal uses of metals that could affect potential prevalence in aquatic environments. For example, cobalt has come into wide use in rechargeable lithium-ion batteries which are ubiquitous in consumer electronics, electric vehicles, and in other uses that did not exist in 1976. These demands might increase the prevalence of cobalt mining and processing, and potential exposure to aquatic life. Likewise, silver uses have changed. In the 1970s silver was widely used in the photographic film industry, which has been supplanted by digital imagery. Another current use of silver, manufactured nanoparticles, did not exist in the 1970s.'	
	Btw, arsenic (and selenium) are not metals in any periodic table I've consulted.	Arsenic has been removed from the text on page 2 of the report.
	Section II. "Metal Toxicity Modifying Factors (TMFs) and their relative importance", starting on p. 3	
	p. 3 *"These factors include pH, hardness ions (primarily Ca and Mg), alkalinity, temperature, sodium, chloride, fluoride,..."* This statement is attributed to Adams et al 2020. I don't believe that is entirely accurate. I did not see the term "hardness ions" in Adams. As noted in my response to questions, I recommend adding some explanation how hardness got into recent MLRs instead of Ca. I have never seen a quantitative analysis of why hardness is better than Ca. No BLM uses "hardness ions." I suspect that the real reason for relying on hardness rather than Ca is the policy desire to keep a lineage to the old hardness-based criteria. Brix et al (2017) started this and subsequent MLRs have followed suit. I don't question the approach, but if this is the case, I would mention this policy heredity.	The text has been adjusted to "water hardness (primarily Ca and Mg ions)" rather than hardness ions on page 3 of the report. The suggestion that hardness was used in recent criteria (cadmium 2016, aluminum 2018) was a "policy desire to keep a lineage to the old hardness-based criteria" is incorrect. These recent criteria use hardness because most end-users monitor hardness rather than Ca. Regarding the use of hardness in MLR models, please see response to Reviewer 5's comment in section 2.3.

2.5 Additional suggestions that would improve the report.

Reviewer	Comments	EPA Response
	p.3 “*Meyer et al. (2007) described two ways in which these factors can affect the bioavailability and toxicity of metals*” I don’t follow attributing this to Meyer et al, as they discuss more than two ways. In particular, the factors themselves, particularly pH and major ions, affect the vigor of aquatic organisms. See Meyer et al, (2007), their chapter 6. My impression of this body of work is that the energy requirements of osmoregulation is the biggest factor. Fish become leaky in low ionic strength water requiring much energy to counteract this and maintain internal mineral balance and metals seem to compound this problem. The much greater resistance of fish to metals in marine waters vs. freshwaters cannot solely be attributed to competition and complexation, but that the increased Na marine environment adds physiological protections. As a practical matter, it matters not to the organism whether they get killed or not by metals toxicity or whether they get killed by increased susceptibility to ionic disruption secondary to metals. People like Chris Wood, Mike Wilkie, Martin Grosell, and Kevin Brix have published much on this. Most research on this has been with fish. Meyer et al. (2007) have a good discussion of these issues in their ch. 6. Wood (2012) gives a more recent overview with fish and we briefly touched on it in our introduction to BLM mechanisms (Mebane et al. 2020a). Buchwalter touches on this with aquatic insects (Buchwalter et al. 2008).	Clarification was made to the text to include “Meyer et al. (2007) described two ways in which these modifying factors can affect whether metals result in bioavailable concentrations that can cause toxicity by affecting the physiological responses of aquatic organisms” on page 3 of the report.
	p. 3 *“Specifically, the effects of the most commonly studied TMFs are described below (see Meyer et al. 2007 for more information)”* If this entire section is attributed to Meyer et al (Meyer et al. 2007), then the end of each paragraph should include “(Meyer et al. 2007).” There are some sweeping statements that presently are either unattributed or ambiguously attributed to Meyer et al. While the authors may have considered this an “overview” of metal toxicity modifying factors, uncluttered by references, rather than a “review” I think more precision on the basis of some of these statements would be helpful	A more citations were added to the text of Section II of the report as well as a reference to more detailed information in Appendix B.

2.5	**Additional suggestions that would improve the report.**	
Reviewer	**Comments**	**EPA Response**
	p. 3 "*a. pH*" The discussion only addressed speciation changes and not the role of proton competition. It makes a difference. Al and Cu toxicity often increase (lower ECx values) at lower pH (but see Cusimano et al (1986) for an opposite result with Cu) but almost all studies I've seen show Cd and Zn toxicity increasing at increasing pH, at least within the range commonly encountered in natural waters, 5.5 to 9 or so (Bradley and Sprague 1985; Cusimano et al. 1986; Schubauer-Berigan et al. 1993; Bervoets and Blust 2000; Hansen et al. 2002; Heijerick et al. 2003; De Schamphelaere and Janssen 2004a; Tan and Wang 2011). Some studies showed no consistent effect at all of pH on toxicity, which might be the two factors (speciation and competition) cancelling each other out (Niyogi et al. 2008; Clifford and McGeer 2009, 2010). These sorts of details might better go into Appendix B, but if so the paragraph attribution should be to Appendix B, and not solely to Meyer et al. 2007.	A general reference to find more metal-specific details in Appendix B was added to the text of Section II of the report.
	p. 4 Hardness: "*…however Mg^{2+} is generally as or more protective than Ca^{2+} in invertebrates."* Generally? That's generally too sweeping. I do not believe there are enough data on this point to say "generally." I would remove this statement, or explicitly support it. From my readings, I do not believe it is supportable. If this refers to Naddy et al. (2002) it overstates their results. Yes, they found hardness with a 1:1 Ca:Mg ratio was more protective to Ceriodaphnia and Daphnia compared to the same hardness with a 4:1 Ca:Mg ratio, but they also tested Gammarus and found it was *better protected* at the higher Ca:Mg ratios same as fish. Gammarus are just as much invertebrates as daphnids. (Heijerick et al. 2002; Heijerick et al. 2005) found Ca and Mg were approximately equal in protectiveness to Daphnia magna from acute Zn toxicity, and De Schamphelaere and Janssen (2002) found the same for protection from acute Cu toxicity.	This statement was removed from the text in the hardness section of the report.
	p. 4, Dissolved Organic Carbon – Paragraph is good, but citation needed. Suggest Wood et al. (2011).	

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	p. 4. d. Other – "... *however* Na^+ *provides less protection than* Ca^{2+} *and* Mg^{2+}." Citation needed. I doubt anyone would challenge that for Ca, but it's not obvious to me that Na provides less protection than Mg. Certainly some Na log(K) values in BLMs are lower than Mg, and that arguments could be invoked if direct evidence is less obvious. I looked through Meyer et al, as that was the implied source. It might be in there, but I did not quickly find it. **Table 1,** p4-5. *"Table 1 illustrates the relative importance of the most studied TMFs for several metals."* Table 1 doesn't really do that - capture the relative importance of TMFs. Most are the same, and since nothing's cited it's hard to evaluate the evidence behind this interpretation. I would change the table as follows: put it on a three part qualitative scale, instead of the present two parts (that is, change to +, ++, +++ scale). Shading indicates where I removed a mark that I didn't think had strong support in the literature, red marks are my additions. To show more relative importances, I suggest change the scoring as follows:	The citation Wood et al. 2011 was added to the DOC section of the report. Made reviewer's suggested changes in Table 1 to the qualitative scale and clarified that the scoring illustrates the relative importance of the TMFs within metals but not across metals.

<table>
<tr><th colspan="3">2.5 Additional suggestions that would improve the report.</th></tr>
<tr><th>Reviewer</th><th>Comments</th><th>EPA Response</th></tr>
<tr><td></td><td>
<table>
<tr><th rowspan="2">Metal</th><th rowspan="2">Type</th><th colspan="4">Most Important Parameters[1]</th></tr>
<tr><th>Hardness</th><th>pH</th><th>DOC</th><th>Other</th></tr>
<tr><td>Aluminum</td><td>Freshwater</td><td>+</td><td>++</td><td>++</td><td>temperature</td></tr>
<tr><td>Cadmium</td><td>Freshwater</td><td>+++</td><td>+</td><td>+</td><td></td></tr>
<tr><td>Cobalt</td><td>Freshwater</td><td>++</td><td>+</td><td>+</td><td></td></tr>
<tr><td>Copper</td><td>Freshwater</td><td>+</td><td>++</td><td>+++</td><td>sodium</td></tr>
<tr><td>Copper</td><td>Marine</td><td></td><td>+</td><td>+</td><td>salinity</td></tr>
<tr><td>Lead</td><td>Freshwater</td><td>+</td><td>+</td><td>+++</td><td></td></tr>
<tr><td>Nickel</td><td>Freshwater</td><td>+</td><td></td><td>+</td><td></td></tr>
<tr><td>Silver</td><td>Freshwater</td><td></td><td></td><td>+</td><td>chromium reducible sulfur, sodium, chloride</td></tr>
<tr><td>Zinc</td><td>Freshwater</td><td>+++</td><td>++</td><td>+</td><td></td></tr>
</table>
</td><td>Thank you for the suggestion. This summary information has been added as Appendix H.</td></tr>
</table>

2.5 Additional suggestions that would improve the report.

Reviewer	Comments	EPA Response
	I suggest adding a short rationale for the different qualitative rankings below the table, since many readers won't delve into Appendix B **Aluminum:** Hardness has a moderate role in modifying Al toxicity; pH has a strong role but the direction of effect can change with different organisms, and DOC consistently reduced Al toxicity (DeForest et al. 2018). **Cadmium:** Hardness regressions predict acute and chronic toxicity well in natural waters (Mebane 2006; USEPA 2016a). pH effect appears weak and ambiguous (Niyogi et al. 2008; Clifford and McGeer 2010). The threshold for a DOC effect appears to be >5 mg/L (Niyogi et al. 2008). **Cobalt:** Hardness is clearly important (Diamond et al. 1992; Borgmann et al. 2005). pH at least affected gill uptake, with uptake increasing with increasing pH up to 8.7. DOM reduced Co gill binding, but Co-DOM affinity was much lower than that of Cd, Cu, or Ag (Richards and Playle 1998). **Copper, freshwater:** DOC has a strong binding affinity to Cu and predictably reduces Cu toxicity, even at low concentrations (Erickson et al. 1996; Welsh et al. 2008). pH has a strong effect on Cu toxicity, with toxicity tending to decrease with increasing pH in alkaline conditions, but toxicity decreasing with decreasing pH in acidic conditions (Cusimano et al. 1986; Erickson et al. 1996). Hardness is a comparatively minor factor in natural waters (Markich et al. 2005). **Copper, marine:** DOC and salinity tend to reduce Cu toxicity in marine and estuarine waters (Grosell et al. 2007; Hall et al. 2008). **Pb:** Similar to Cu, DOC and pH have strong effects on the bioavailability and toxicity of Pb (DeForest et al. 2017) Hardness may be an important factor in natural waters, especially when DOC is low (Mebane et al. 2012).	

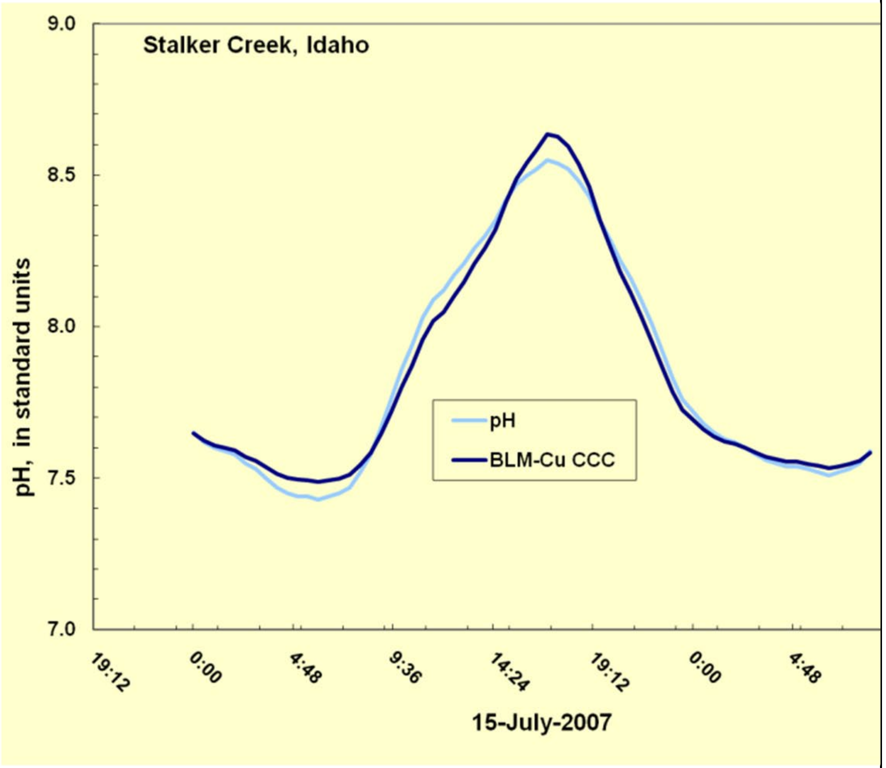
2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	Ni: Ni toxicity tends to decrease as hardness increased and decrease with increasing DOC. pH has inconsistent influence on toxicity (Croteau et al. 2021; Santore et al. 2021). **Silver**: DOC reduces toxicity but pH and hardness influences may be inconsistent (Naddy et al. 2018). **Zinc:** Similar to Cd, hardness has a strong influence on Zn toxicity, with decreasing toxicity with increasing hardness (Clifford and McGeer 2009; Mebane et al. 2012; CCME 2018); with fish, toxicity generally increases with increasing pH but relations may be inconsistent in other taxa (De Schamphelaere and Janssen 2004a). DOC reduces Zn toxicity but influence may be nonlinear, with a threshold of >≈10 mg/L DOC required to reduce toxicity (Bringolf et al. 2006; Ivey et al. 2019). Footnote to Table 1. *"Additionally, the bioavailability of metals such as cadmium, copper, nickel, and silver has been shown to be sensitive to humic acid and scientific advances are beginning to shed light on options that may be more representative than using the default of 10% generally recommended for BLM applications (Glover et al. 2005; Nadella et al. 2009; Al-Reasi et al. 2012; Blewett et al. 2016)."* I recommend deleting this part of the footnote. First, I would argue that if a footnote caution/caveat is warranted, it should first be about pH which can change by more than a unit depending on the time of day sampled. A 1-unit change in any of these BLMs or MLR based criteria is huge – I appended an example showing that the BLM Cu chronic criteria would swing from about 8 to 26 µg/L, just from the time of day that pH was measured. Regarding DOC, there are lots of practical issues with DOC in BLMs that might be at least as important as the humic/fulvic – the DOM/DOC conversion & active fraction, contamination from capsule filters or tubing. I appended an example of likely filter artifacts in USGS data toward the end of these comments. Further, I don't think the footnote is fully accurate. Three of the 4 references cited studied DOM	This portion of the footnote has been deleted from the footnote to Table 1.

2.5 Additional suggestions that would improve the report.

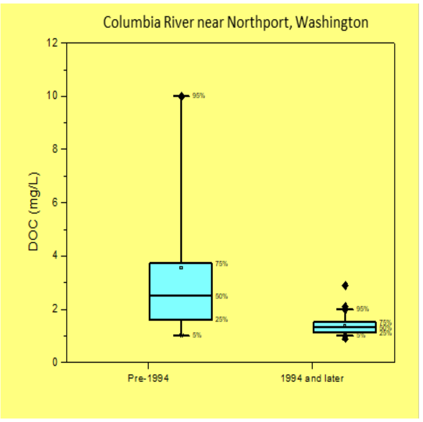
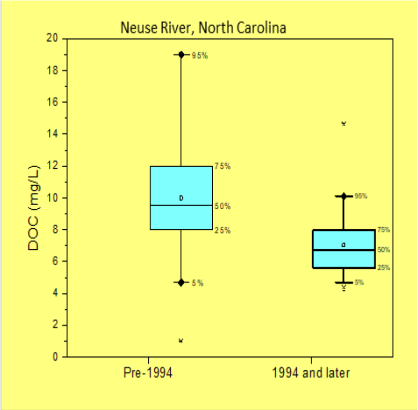
Reviewer	Comments	EPA Response
	with Cu and 1 studied DOM with Ni, so Cd and Ag? True, Nadella found that NOMs with high humic acid offered less protection to Cu toxicity than those dominated by fulvic acid, but that is the *opposite* of the effect of the humic acid selection in the Windward BLMs. In the Windward BLMs, higher humic acid fraction adds a slightly greater protective effect. Plus, it's hard to generalize Nadella's results - testing a marine species in saltwater with NOMs from different freshwaters.	
	Table 2: Very nice compilation.	Thank you for your comment.
	p. 13 *"Multiple Linear Regression Models"* Somewhere in this first paragraph I would mention that EPA put out its first MLR-type criteria in 1984 with ammonia, in which the criteria varied with a relatively complicated nonlinear equation as a function of temperature and pH. At least some states (Idaho and Colorado come to mind) dealt with the calculation complexity by publishing table values of criteria values for every tenth of a pH unit or degree that could be used in permitting in lieu of calculating the values directly. While it's a lot easier now than it was in the 1980s when PCs and spreadsheets were scarcer, this MLR level of complexity did not seem a big deal with ammonia.	Thank you for your comment.
	p. 17 "*... as EPA moves forward with updating the metals AWQC, it is desirable to have a single software platform.*" Some people prefer R scripts, some prefer spreadsheets, over time something else might become widely used. At the present, I'd say the "xlsx" format would be most accessible and it isn't that hard for R users to export carefully assembled xlsx to a R friendly format. A core, common syntax would be helpful, but it's easy enough to put out criteria datasets and equations in say both xlsx and R	

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	Appendix B comments Appendix B reflects a big effort and is a very helpful, concise guide to much relevant information for the subset of metals supported through the CRADA efforts. While hardness is hard to screw up, I do suggest adding a bit on the importance of data quality in pH and DOC data. pH probes are notoriously finicky. More importantly, in some waters the daily cycles of production and respiration can cause pH swings high enough to skew criteria a lot. Even ~0.2 units can make noticeable differences in criteria calculations and natural swings of >1 unit aren't unheard of. Figure 1 gives an example calculation where the criteria would swing 3-fold from 8 to 25 µg/L over the course of a day. So when should waters be sampled? Depending on the desired answer? Most likely, whenever it's most convenient for the person doing the sampling which might not give the most representative results. In a stream contaminated with Zn (primarily) and subject to daily Zn and pH swings, the observed toxicity to trout corresponded best to the daily average conditions, not the daily maximum (worst case) concentrations (Balistrieri et al. 2012). I recommend saying something about the uncertainty of daily pH cycles and the need to resolve the most representative time of day (or daily average) for sampling. With DOC, there has been lots of research and debate on different characteristics that affect metal binding and bioavailability, such as that terrestrial sources with high fulvic/humic acid content reduce Cu bioavailability more than autochthonous sources such as algae senescence. However, I have seen much less in the BLM and metals bioavailability literature about the importance of basic QC in collecting and analyzing DOC. In particular, filtration and tubing can be a real bugaboo that introduces DOC at biologically and BLM-relevant concentrations. I show a few examples of the issue in figure 2 and figure 3. In my group, while we think we are reasonably careful and attuned to the issue, we still sometimes see DOC in filter blanks at 0.2 to 0.3 mg/L,	A brief paragraph about the importance of collecting high quality data was added to page 5 of the report.

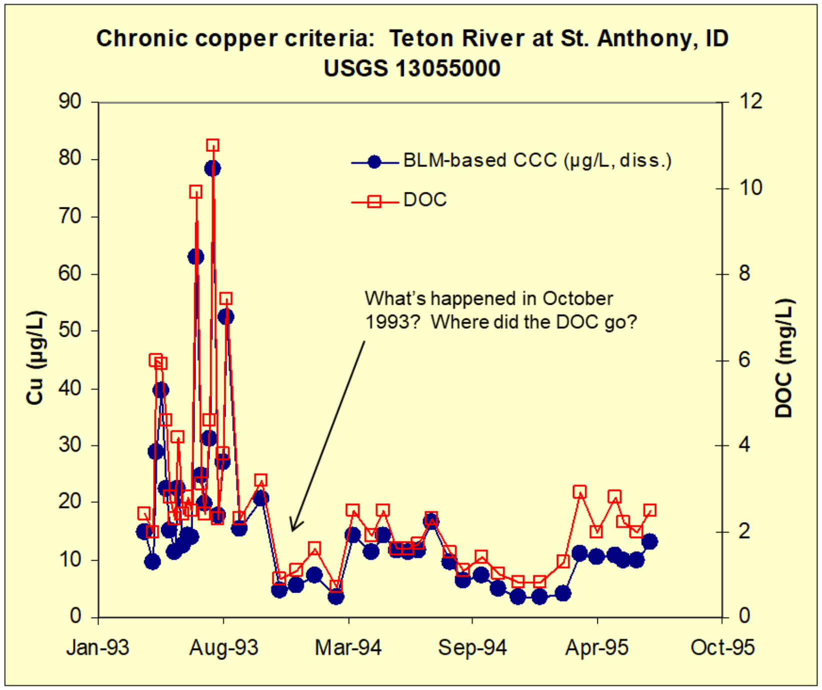
2.5 Additional suggestions that would improve the report.

Reviewer	Comments	EPA Response
	even though the manufacturer of the organic blank water that we purchase certifies that the water contains <0.05 mg/L TOC. We're probably picking up some DOC through the filters and tubing during filtering. Yoro et al. (1999) is a good citable citation on this point.  An example of how natural, daily swings in pH can cause wild swings in criteria that rely on pH as a modifying factor. If the discharger wants a high criteria value that's easy to comply with, they should sample in late afternoon (pH 8.7, Cu CCC 26 µg/L). If zealous regulators want a low criterion value, they should sample late at night or early in the morning	

2.5 Additional suggestions that would improve the report.

Reviewer	Comments	EPA Response
	when pH is low (pH 7.5, BLM based CCC 8 µg/L). So what to do? Take the average?   A couple of examples of differences in DOC concentrations likely influenced by sampling contamination through filters and bottles, one from a low DOC river (Columbia River by the US/Canada border) and one from a high DOC stream (the piedmont Neuse River). In 1993, the USGS began pushing so-called “clean sampling” methods for trace metals and this hygiene emphasis seemed to carry over to DOC. We still see occasional DOC filter blank contamination from modern capsule filters a biologically and BLM-relevant concentrations.	

2.5 Additional suggestions that would improve the report.

Reviewer	Comments	EPA Response
	 What's happened in October 1993? Where did the DOC go? Another example of how filtration and cleaning practices can create bad DOC data, which can be hard to catch on a sample by sample basis. In this case, DOC contamination was suspected to have been caused surfactants residual to the capsule filter manufacturing process and inadequate flushing before the sample was taken. My point in all this is that either in the main document or in appendix B it would be prudent to say something about the importance of good sampling and measurement practices with the inputs to these models, and in particular pH and DOC. I suggest it could be a lot shorter than my examples and cite on the pH issue studies like Balistrieri et al (2012) and maybe Nimick et al (2011), and Yoro et al. (1999) on the DOC issue. As these models move towards criteria, it would be good to include some	

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	recommended practices on these mundane but important issues of data representativeness and quality. **Copper** **Cu and Hardness.** *"There is a consistent protective effect of water hardness on Cu toxicity in acute and chronic exposures to fish and invertebrates ... with equivocal results or no protection in only a few studies."* That seems a little overstated and I would reword it to be more even handed. Something like 'Many studies reviewed have shown some protective effect of water hardness on Cu toxicity in acute and chronic exposures to fish and invertebrates (for example, cite; cite; cite;...). However, inconsistent results or no protection were reported in some studies, for example (Chapman et al. 1980; Richards and Playle 1999; De Schamphelaere and Janssen 2004b; Hyne et al. 2005; Markich et al. 2005; Wang et al. 2009)	Appendix B text was modified based on Copper CRADA partner's suggestion.
	Zinc **Zn and Hardness** – I can't help but chime in with a "us too." In Mebane et al. (2012), we reported 4 tests with rainbow trout, each with fish from the same cohort in different natural waters. Hardness explained between 90% to 99% of the variability in EC50s in these natural waters where pH was allowed to covary. **Zn and DOC**. I think the story with DOC protecting against Zn toxicity is more nuanced and equivocal than this paragraph would lead readers to believe. In particular the sentence "*In freshwaters, dissolved organic matter (DOM) – quantified as dissolved organic carbon (DOC) – generally decreases Zn bioavailability (e.g., Hyne et al. 2005; Clifford and McGeer 2009; Heijerick et al. 2003).*" First, that is not what Hyne et al (2005) reported. Rather, they reported that the addition of 10 mg/L DOC only resulted in a very small (1.3-fold) reduction in the toxicity of zinc to Ceriodaphnia, whereas the same DOC addition resulted in a 45-	Zinc CRADA partners indicated that they appreciate the input provided by the reviewer. Indeed, Mebane et al. (2012) did demonstrate the ameliorative effect of hardness in natural waters, but as the reviewer indicated: "pH was allowed to covary". The reviewer is also correct that the DOC effects on Zn bioavailability are nuanced, which is why we indicated that DOC "generally decreases Zn bioavailability". The general trend in tests specifically investigating the effect of DOC on Zn toxicity is that increasing DOC concentrations increase Zn effect concentrations. Although for MLR model development purposes, it is recognized that the slope of the relationship between Zn effect concentration and DOC concentration is shallow (i.e., low slope) for some datasets (i.e., individual studies). All data suitable for MLR model development will be used to evaluate the effect of DOC on Zn bioavailability. The reviewer makes an

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	fold reduction in Cu toxicity. I have seen no reports of DOC having an important role in reducing Zn toxicity until DOC concentrations are fairly high (greater than at least 5 mg/L DOC and probably greater than 10 mg/L DOC). The minimum DOC tested by Heijerick et al was 9 mg/L. Clifford and McGeer (2009) tested a base condition with 0.6 mg/L DOC, 6-7 mg/L DOC additions, and 10-11 mg/L DOC added. Only the pair of high DOC additions (10-11 mg/L) reduced toxicity beyond the range of the base conditions with 0.6 mg/L DOC. In tests of the acute toxicity of Zn to sturgeon, DOC in the range of 1 to 5 mg/L had no effect (Ivey et al. 2019). In tests with fathead minnow and Zn under different organic carbon conditions, a threshold concentration of 11 mg/L DOC was required to reduce acute toxicity to (Bringolf et al. 2006). The take home on Zn-DOC toxicity relations from published research is that DOC concentrations <10 mg/L are sparse, and from what I can find indicates little protective effect for Zn toxicity. The significance of this to the MLR approach is that if there is a threshold effect for DOC reductions at around 10 mg/L, a linear regression that predicts a linear response may be misleading and underprotective in the low range between say 0.5 and 10 mg/L. A regression that fits a straight line from controls with say 0.5 mg/L to 40 mg/L, will show a strong response, and give the same slope in the 0.5 to 10 mg/L DOC range of the regression as in the higher DOC range, even though no data were in the low range. It's just fitting a straight line. For instance, in the Heijerick et al. (2003) study mentioned above, they have a very clean plot predicting a linear response between DOC and Daphnia toxicity (their figure 3). However, the underlying data included test pairs with huge ranges. One test pair had pH 7.25, hardness 240 and DOC of 2 vs DOC 40 mg/L; one test pair had pH 6, hardness 110 and DOC of 9.7 vs 32 mg/L; and the third test pair was with pH 8, hardness 370 and DOC 9.7 vs 32 mg/L. None of those tests tell us anything about what is going	interesting suggestion regarding a nonlinear or piecewise nonlinear function to represent the effect of DOC. We agree that this is something that should be explored during MLR model development. Ultimately, regardless of model formulation, if DOC is shown to be an important TMF, it will be retained in MLR models. The converse will also be true.

2.5 Additional suggestions that would improve the report.

Reviewer	Comments	EPA Response
	on at the low 1-5 mg/L DOC values, although one wouldn't immediately realize that from the pretty model plot in their Figure 5. The reason for this concern with the potential overextrapolation of DOC-Zn toxicity relations to the range of ≈ 0 to 10 mg/L, is that that is the range where the vast majority of flowing waters in the US fall. USEPA (2016b) included a summary of DOC values collected from 1,392 sites sampled across the 84 ecoregions of the United States using a probability-based sample design from the EMAP Wadable Stream Assessment (WSA). The median values for each of the 84 ecoregions were reported. The 90th percentile of the 84 ecoregions was 8.4 mg/L, the 75th percentile was 5.2 mg/L, and the national ecoregional median was 2.7 mg/L DOC (calculated from table 17 of USEPA (2016a)). Thus >90% of the streams in the United States would be expected to have DOC values in the range of questionable Zn-DOC relations. Thus, the usual MLR straight line approach may not be the most appropriate for Zn and DOC and a nonlinear function or a piecewise 'nonlinear' function may need to be explored.	
	Appendix C comments I just glanced through "*Appendix C, Table 2: Supporting Information for Bioavailability Model Comaprison Table*" First, I think "comaprison" is a fine new word that should be added to the spell checker and kept in the report, applicable to the state of mind in many an office cube. Well, maybe it should be hyphenated, coma-prison. A couple other items that caught my eye... First row, Aluminum BLM: No reference is given, but the version "3.18.2.42" looks like a Windward numbering version. Santore et al (2018) describe using CHESS and WHAM V, not WHAM 7. To my knowledge, no Windward BLM version has incorporated WHAM 7.	This change has been made to Appendix C. The Aluminum BLM, similar to other BLMs developed uses WHAM Model V for speciation calculations. This change has been applied to Appendix C. The Cobalt BLM, similar to other BLMs developed uses WHAM Model V for speciation calculations. This change has been applied to Appendix C.

2.5 Additional suggestions that would improve the report.		
Reviewer	**Comments**	**EPA Response**
	Cobalt BLM says it is “complete” but to my knowledge no Co BLM has been formally published or publicly released online. The version “3.15.2.41” also looks like Windward numbering, which makes me wonder whether it actually used “WHAM 6” for speciation, since as with WHAM 7, that would have been a big coding project. I would check this.	

3.0 ADDITIONAL COMMENTS PROVIDED

3.0 Additional comments.		
Reviewer	**Comments**	**EPA Response**
Reviewer 1	**General considerations** With much interest I have read the documentation that was send as part of the assignment on the evaluation of EPA’s draft report on the development of an overarching bioavailability modeling approach to support US EPA’s aquatic life water quality criteria for metals. This brief draft report properly describes the information available as the basis for the overarching bioavailability modeling approach. It is to be noted that the report and the underlying documentation are a reflections of decades of work by scientists across the globe on bioavailability modeling. Nevertheless it is clear from the draft report that proper care needs to be taken with regard to actual implementation of the various complex models (independent of them being BLM- or MRL-based) in derivation of water quality criteria and it is especially clear that it is essential to make sure that the complexities and the interactions of the various toxicity modifying factors are properly incorporated in the software platform that is likely to be the	Thank you for your review. EPA strives to properly incorporate all the complexities and the interactions of the various toxicity modifying factors in models and accompanying software platform.

3.0 Additional comments.		
Reviewer	**Comments**	**EPA Response**
	future means of user-friendly implementation of the decades of metal bioavailability research.	
Reviewer 3	**Preface:** There is scientific consensus that water chemistry profoundly affects the bioavailability and toxicity of trace metals in freshwaters. My own research career started with studies of the effects of dissolved organic carbon and pH on the speciation and acute toxicity of Cu to developing amphibian eggs and larvae[1]. I am predisposed to appreciate the development of mechanistic understanding of how trace metal toxicity occurs from a purely scientific perspective, and I also feel strongly that regulatory approaches to protecting aquatic life should be based on defensible science. I recognize the scientific achievements and conceptual advancements embodied by Biotic Ligand Models, and understand how their complexity contributed to their limited adoption by regulatory end users. I can appreciate the frustration of the metal industry groups who put substantial efforts into these scientific developments and not have them widely adopted. Indeed, the science has progressed considerably and regulatory approaches for protecting the environment need to be modernized (see [2]). That said, I think it is important to articulate that BLM and MLR models primarily have the shared goal of accounting for Toxicity Modifying Factors (TMFs) such that "overprotection" is avoided. As more TMFs are considered, protection levels will generally be more relaxed. The goal of these approaches is not protection – it is the avoidance of overprotection.	Thank you for your review. In regard to accounting for TMFs to avoid overprotection, the updated Cu and Al criteria both have values that are lower than previous criteria for high metal bioavailability conditions and accounting for TMFs can result in criteria that can more accurately achieve EPA's targeted level of protection over a wide range of water chemistry conditions. Regarding dietary exposure, please see the responses to Reviewer 3's comments in sections 2.1a, 2.1c and 2.2a. Furthermore, the 1985 Guidelines do not explicitly exclude the consideration of dietary exposure. EPA believes it is important to focus on the primary drivers of toxicity, which for the metals evaluated in this effort, appear to be aquatic exposure (as noted above).

3.0 Additional comments.		
Reviewer	**Comments**	**EPA Response**
	The models at the heart of this review are driven by the perspective that metals are problematic or toxic in freshwater environments as surface-active, aqueous toxicants. While this perspective is largely accurate and scientifically supported for acute exposures to many aquatic animals, it is unfortunately not complete. Dietary exposures are extremely important to aquatic insects[2–7] – the faunal groups that largely drives the ecology of the ecosystems that EPA is charged with protecting. Aquatic insects were recognized by Workgroup 2 of the 2017 SETAC Metal Bioavailability Workshop as a faunal groups that might not be adequately covered by the models under consideration[8] – likely because dietary exposure pathways predominate from a toxicity perspective. Thus, the models which are the focus of this review are likely not applicable to the most ecologically important faunal group in freshwater ecosystems. A complete exposure perspective that includes aqueous and dietary exposure pathways is required for scientifically defensible Water Quality Criteria. This fact is extremely problematic in the context of Water Quality Criteria development because the 1985 Guidance document[9] requires the exclusion of data that deviate from strict aqueous exposures. Until this changes, even the best aqueous based models will represent an incomplete understanding of metal toxicity in aquatic ecosystems.	
Reviewer 5	**The End** I realize these comments are longer than I intended. I hope they are useful and that they did not come across as giving a negative perspective on the project. Quite the opposite was intended. These models in appendices D-F are remarkable and this project has taken a huge step towards the goal of updating and	Thank you for your review.

3.0 Additional comments.		
Reviewer	**Comments**	**EPA Response**
	expanding metals criteria in the US. I look forward to seeing good progress with Co and Zn as well. The summary report and appendices B and C will be influential and valuable. Well done to all.	

4.0 NEW INFORMATION PROVIDED BY REVIEWERS

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

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
Reviewer 3	As noted in comments above: (1) Buchwalter, D. B.; Linder, G.; Curtis, L. R. Modulation of Cupric Ion Activity by PH and Fulvic Acid as Determinants of Toxicity in Xenopus Laevis Embryos and Larvae. Environ. Toxicol. Chem. 1996, 15 (4), 568–573. https://doi.org/10.1002/etc.5620150423. (2) Buchwalter, D. B.; Clements, W. H.; Luoma, S. N. Modernizing Water Quality Criteria in the United States: A Need to Expand the Definition of Acceptable Data. Env. Toxicol Chem 2017, 36 (1552-8618 (Electronic)), 285–291. https://doi.org/10.1002/etc.3654. (3) Cain, D. J.; Luoma, S. N.; Wallace, W. G. Linking Metal Bioaccumulation of Aquatic Insects to Their Distribution Patterns in a Mining-Impacted River. Environ. Toxicol. Chem. 2004, 23 (0730-7268 (Print)), 1463–1473. (4) Xie, L. T.; Lambert, D.; Martin, C.; Cain, D. J.; Luoma, S. N.; Buchwalter, D. Cadmium Biodynamics in the Oligochaete Lumbriculus Variegatus and Its Implications for Trophic Transfer. Aquat. Toxicol. 2008, 86 (2), 265–271.	Thank you for providing the following references for EPA's review.

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	(5) Xie, L.; Buchwalter, D. B. Cadmium Exposure Route Affects Antioxidant Responses in the Mayfly Centroptilum Triangulifer. Aquat. Toxicol. 2011, 105 (1879-1514 (Electronic)), 199–205. (6) Poteat, M. D.; Buchwalter, D. B. Four Reasons Why Traditional Metal Toxicity Testing with Aquatic Insects Is Irrelevant. Environ. Sci. Technol. 2014, 48 (1520-5851 (Electronic)), 887–888. https://doi.org/10.1021/es405529n. (7) Soucek, D. J.; Dickinson, A.; Schlekat, C.; Van Genderen, E.; Hammer, E. J. Acute and Chronic Toxicity of Nickel and Zinc to a Laboratory Cultured Mayfly (Neocloeon Triangulifer) in Aqueous but Fed Exposures. Environ. Toxicol. Chem. 2020, 39 (6), 1196–1206. https://doi.org/10.1002/etc.4683. (8) Mebane, C. A.; Chowdhury, M. J.; De Schamphelaere, K. A. C.; Lofts, S.; Paquin, P. R.; Santore, R. C.; Wood, C. M. Metal Bioavailability Models: Current Status, Lessons Learned, Considerations for Regulatory Use, and the Path Forward. Environ. Toxicol. Chem. 2020, 39 (1), 60–84. https://doi.org/10.1002/etc.4560. (9) Stephan, C. E.; Mount, D. I.; Hansen, D. J. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses.; U.S. Environmental Protection Agency: Washington D.C. USA, 1985. (10) Gillis, P. L.; Wood, C. M. Investigating a Potential Mechanism of Cd Resistance in Chironomus Riparius Larvae Using Kinetic Analysis of Calcium and Cadmium Uptake. Aquat. Toxicol. 2008, 8. (11) Poteat, M. D.; Buchwalter, D. B. Calcium Uptake in Aquatic Insects: Influences of Phylogeny and Metals (Cd and Zn). J.Exp.Biol. 2014, 217 (1477-9145 (Electronic)), 1180–1186. https://doi.org/10.1242/jeb.097261. (12) Clements, W. H.; Carlisle, D. M.; Lazorchak, J. M.; Johnson, P. C. Heavy Metals Structure Benthic Communities in Colorado Mountain Streams. Ecol. Appl. 2000, 10 (2), 626–638. (13) Scheibener, S. A.; Richardi, V. S.; Buchwalter, D. B. Comparative Sodium Transport Patterns Provide Clues for Understanding Salinity and Metal Responses in Aquatic Insects. Aquat. Toxicol. 2016, 171, 20–29. https://doi.org/10.1016/j.aquatox.2015.12.006.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	(14) Poteat, M. D.; Diaz-Jaramillo, M.; Buchwalter, D. B. Divalent Metal (Ca, Cd, Mn, Zn) Uptake and Interactions in the Aquatic Insect Hydropsyche Sparna. J.Exp.Biol. 2012, 215 (1477-9145 (Electronic)), 1575–1583. https://doi.org/10.1242/jeb.063412. (15) Kim, K. S.; Funk, D. H.; Buchwalter, D. B. Dietary (Periphyton) and Aqueous Zn Bioaccumulation Dynamics in the Mayfly Centroptilum Triangulifer. Ecotoxicology. 2012, 21 (1573-3017 (Electronic)), 2288–2296. https://doi.org/10.1007/s10646-012-0985-1. (16) Xie, L.; Funk, D. H.; Buchwalter, D. B. Trophic Transfer of Cd from Natural Periphyton Biofilms to the Grazing Mayfly Centroptilum Triangulifer in a Life Cycle Test. Environ. Pollut. 2010, 158, 272–277. (17) Conley, J. M.; Funk, D. H.; Buchwalter, D. B. Selenium Bioaccumulation and Maternal Transfer in the Mayfly Centroptilum Triangulifer in a Life-Cycle, Periphyton-Biofilm Trophic Assay. Environ.Sci.Tech. 2009, 43, 7952–7957. (18) Conley, J. M.; Funk, D. H.; Cariello, N. J.; Buchwalter, D. B. Food Rationing Affects Dietary Selenium Bioaccumulation and Life Cycle Performance in the Mayfly Centroptilum Triangulifer. Ecotoxicology 2011, 20 (1573-3017 (Electronic)), 1840–1851. (19) Conley, J. M.; Funk, D. H.; Hesterberg, D. H.; Hsu, L. C.; Kan, J.; Liu, Y. T.; Buchwalter, D. B. Bioconcentration and Biotransformation of Selenite versus Selenate Exposed Periphyton and Subsequent Toxicity to the Mayfly Centroptilum Triangulifer. Environ. Sci. Technol. 2013, 47 (1520-5851 (Electronic)), 7965–7973. https://doi.org/10.1021/es400643x. (20) Conley, J. M.; Watson, A. T.; Xie, L.; Buchwalter, D. B. Dynamic Selenium Assimilation, Distribution, Efflux, and Maternal Transfer in Japanese Medaka Fed a Diet of Se-Enriched Mayflies. Environ. Sci. Technol. 2014, 48 (1520-5851 (Electronic)), 2971–2978. https://doi.org/10.1021/es404933t. (21) Mebane, C. A.; Hennessy, D. P.; Dillon, F. S. Developing Acute-to-Chronic Toxicity Ratios for Lead, Cadmium, and Zinc Using Rainbow Trout, a Mayfly, and a Midge. Water. Air. Soil Pollut. 2008, 188 (1–4), 41–66. **Appendix 1. Diet relevant metals citations compiled by ETAP**	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	References – Survival, Growth, Reproduction, and Feeding Behavior (note: additional silver references may be added, if confirmed to be of interest to ETAP) Abdel-Tawwab M, Mousa MAA, Abbass FE. 2007. Growth performance and physiological response of African catfish, Clarias gariepinus (B.) fed organic selenium prior to the exposure to environmental copper toxicity. Aquacult 272:335-345. Ashanullah M, Williams AR. 1991. Sublethal effects and bioaccumulation of cadmium, chromium, copper and zinc in the marine amphipod Allorchestes compressa. Mar Biol 108:59-65. Alsop D, Brown S, Van Der Kraak G. 2007. The effects of copper and benzo(a)pyrene on retinoids and reproduction in zebrafish. Aquat Toxicol 82:281-295. Ashley LM. 1972. Nutritional pathology. Pages 439-535 in Halver JE, ed. Fish nutrition. Chapter 10. Academic Press, New York. Baker RTM, Handy RD, Davies SJ, Snook JC. 1998. Chronic dietary exposure to copper affects growth, tissue lipid peroxidation, and metal composition of the grey mullet, Chelon labrosus. Mar Environ Res 45:357-365. Ball AL, Borgmann U, Dixon DG. 2006. Toxicity of a cadmium-contaminated diet to Hyalella azteca. Environ Toxicol Chem 25:2526-2532. Bennett WN, Brooks AS, Boraas ME. 1986. Selenium uptake and transfer in an aquatic food chain and its effects on fathead minnow larvae. Arch Environ Contam Toxicol 15:513-517. Berntssen MHG, Hylland K, Bonga SEW, Maage A. 1999a. Toxic levels of dietary copper in Atlantic salmon (Salmo salar L.) parr. Aquat Toxicol 46:87-99. Berntssen MHG, Lundebye AK, Maage A. 1999b. Effects of elevated dietary copper concentrations on growth, feed utilization and nutritional status of Atlantic salmon (Salmo salar L.) fry. Aquacult 174:167-181. Besser JM, Canfield TJ, La Point TW. 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. Environ Toxicol Chem 12:57-72.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Besser JM, Brumbaugh WG, Brunson EL, Ingersoll CG. 2005. Acute and chronic toxicity of lead in water and diet to the amphipod Hyalella azteca. Environ Toxicol Chem 24:1807-1815. Beyers DW, Sodergren C. 2002. Assessment of exposure of larval razorback to selenium in natural waters. Arch Environ Contam Toxicol 42:53-59. Bielmyer GK. 1999. Toxicity of ligand-bound silver to Ceriodaphnia dubia [M.S. Thesis]. Pendleton, SC, USA: Clemson University. 71 p. Bielmyer GK, Gatlin D, Isely JJ, Tomasso J, Klaine SJ. 2005. Responses of hybrid striped bass to waterborne and dietary copper in freshwater and saltwater. Comp Biochem Physiol Part C 140:131-137. Bielmyer GK, Grosell M, Brix KV. 2006. Toxicity of silver, zinc, copper, and nickel to the copepod Acartia tonsa exposed via a phytoplankton diet. Environ Sci Technol 40:2063-2068. Bowen L, Werner I, Johnson ML. 2006. Physiological and behavioral effects of zinc and temperature on coho salmon (Oncorhynchus kisutch). Hydrobiologia 559:161-168. Brix KV, Gillette P, Pourmand A, Capo TR, Grosell M. 2012. The effects of dietary silver on larval growth in the echinoderm Lytechinus variegatus. Arch Environ Contam Toxicol DOI 10.1007/s00244-012-9757-4. Bryan GW, Gibbs PE. 1983. Heavy metals in the Fal estuary, Cornwall: a study of long-term contamination by mining waste and its effects on estuarine organisms. Mar Biol Assoc UK Occasional Publication 2:1-112. Bryson WT, Garrett WR, Mallin MA, MacPherson KA, Partin WE, Woock SE. 1984. 1982 Environmental monitoring studies, Volume II: Hyco Reservoir bioassay studies. New Hill, NC, USA Carolina Power and Light Company. 65 pp + appendices. Bryson WT, Garrett WR, MacPherson KA, Mallin MA, Partin WE, Woock SE. 1985a. Hyco Reservoir 1983 bioassay report. New Hill, NC, USA Carolina Power and Light Company. 55 pp.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Bryson WT, MacPherson KA, Mallin MA, Partin WE, Woock SE. 1985b. Hyco Reservoir 1984 bioassay report. New Hill, NC, USA Carolina Power and Light Company. 51 pp. Brown BE. 1977. Uptake of copper and lead by a metal tolerant isopod Asellus meridianus Rac. Freshw Biol 7:235-244. Canli M. 2005. Dietary and water-borne Zn exposures affect energy reserves and subsequent Zn tolerance of Daphnia magna. Comp Biochem Physiol Part C 141:110-116. Chou CL, Uthe JF, Castell JD, Kean JC. 1987. Effect of dietary cadmium on growth, survival, and tissue concentrations of cadmium, zinc, copper and silver in juvenile American lobster (Homarus americanus). Can J Fish Aquat Sci 44:1443-1450. Cleveland L, Little EE, Buckler DR, Wiedmeyer RH. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (Lepomis macrochirus). Aquat Toxicol 27:265-280. Cockell KA, Bettger WJ. 1993. Investigations of the gallbladder pathology associated with dietary exposure to disodium arsenate heptahydrate in juvenile rainbow trout. Toxicol 77:233-248. Cockell KA, Hilton JW. 1988. Preliminary investigation on the comparative chronic toxicity of four dietary arsenicals to juvenile rainbow trout (Salmo gairdneri R.). Aquat Toxicol 12:73-82. Cockell KA, Hilton JW, Bettger WJ. 1991. Chronic toxicity of dietary disodium arsenate heptahydrate to juvenile rainbow trout (Oncorhynchus mykiss). Arch Environ Contam Toxicol 21:518-527. Conley JM, Funk DH, Buchwalter DB. 2009. Selenium bioaccumulation and maternal transfer in the mayfly Centroptilum triangulifer in a life-cycle, periphyton-biofilm trophic assay. Environ Sci Technol 43:7952-7957. Conley JM, Funk DH, Cariello NJ, Buchwalter DB. 2011. Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly Centroptilum triangulifer. Ecotoxicol 20:1840-1851. Coughlan DJ, Velte JS. 1989. Dietary toxicity of selenium-contaminated red shiners to striped bass. Trans Am Fish Soc 118:400-408.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Coyle JJ, Buckler DR, Ingersoll CG, Fairchild JF, May TW. 1993. Effect of dietary selenium on the reproductive success of bluegills (Lepomis macrochirus). Environ Toxicol Chem 12:551-565. CP&L. 1997. Largemouth bass selenium bioassay. Roxboro, NC, USA. Carolina Power & Light Company. 14 pp. Croteau MN, Luoma SN. 2008. A biodynamic understanding of dietborne metal uptake by a freshwater invertebrate. Environ Sci Technol 42:1801-1806. Dai W, Fu L, Du H, Jin C. 2009. Changes in growth performance, metabolic enzyme activities, and content of Fe, Cu, and Zn in liver and kidney of tilapia (Oreochromis niloticus) exposed to dietary Pb. Biol Trace Elem Res 128:176-183. Dang F, Wang W-X. 2009. Assessment of tissue-specific accumulation and effects of cadmium in a marine fish fed contaminated commercially produced diet. Aquat Toxicol 95:248-255. Dang F, Wang WX, Rainbow PS. 2012. Unifying prolonged copper exposure, accumulation, and toxicity from food and water in a marine fish. Environ Sci Technol 46:3465-3471. Davis DA, Gatlin III DM. 1996. Dietary mineral requirements of fish and marine crustaceans. Rev Fish Sci 4:75-99. De Schamphelaere KAC, Janssen CR. 2004. Effects of chronic dietary copper exposure on growth and reproduction of Daphnia magna. Environ Toxicol Chem 23:2038-2047. De Schamphelaere KAC, Canli M, Van Lierde V, Forrez I, Vanhaecke F, Janssen CR. 2004. Reproductive toxicity of dietary zinc to Daphnia magna. Aquat Toxicol 70:233-244. De Schamphelaere KAC, Forrez I, Dierckens K, Sorgeloos P, Janssen CR. 2007. Chronic toxicity of dietary copper to Daphnia magna. Aquat Toxicol 81:409-418. Dobbs MG, Cherry DS, Cairns Jr K. 1996. Toxicity and bioaccumulation of selenium to a three-trophic level food chain. Environ Toxicol Chem15:340-347. Doroshov S, Eenennaam JV, Alexander C, Hallen E, Bailey H, Kroll K, Restreppo C. 1992. Development of water quality criteria for resident aquatic species of the San Joaquin River. Report to the	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	California State Water Resources Control Board for Contract No. 7-197-250-0. Department of Animal Science, University of California, Davis, CA, USA. Ebenso IE, Ologhobo AD. 2009. Effects of lead pollution against juvenile Achatina achatina fed on contaminated artificial diet. Bull Environ Contam Toxicol 82:583-585. Eid AE, Ghonim SI. 1994. Dietary zinc requirement of fingerling Oreochromis niloticus. Aquacult 119:259-264. Erickson RJ, Mount DR, Highland TL, Hockett JR, Leonard EN, Mattson VR, Dawson TD, Lott KG. 2010. Effects of copper, cadmium, lead, and arsenic in a live diet on juvenile fish growth. Can J Fish Aquat Sci 67:1816-1826. Evens RE, De Schamphelaere KAC, Janssen CR. 2009. The effects of dietary nickel exposure on growth and reproduction of Daphnia magna. Aquat Toxicol 94:138-144. Evens R, De Schamphelaere KA, Balcaen L, Wang Y, De Roy K, Resano M, Flórez Mdel R, Van der Meeren P, Boon N, Vanhaecke F, Janssen CR. 2011. Liposomes as an alternative delivery system for investigating dietary metal toxicity to Daphnia magna. Aquat Toxicol 105:661-668. Evens R, De Schamphelaere KA, Balcaen L, Wang Y, De Roy K, Resano M, Flórez M, Boon N, Vanhaecke F, Janssen CR. 2012. The use of liposomes to differentiate between the effects of nickel accumulation and altered food quality in Daphnia magna exposed to dietary nickel. Aquat Toxicol 109:80-89. Evens RE, De Schamphelaere KAC, De Laender F, Janssen C. 2012. The effects of Zn-contaminated diets on Daphnia magna reproduction may be related to Zn-induced changes of the dietary P content rather than to the dietary Zn content itself. Aquat Toxicol 110-111:9-16. Evens R, De Schamphelaere KA, De Samber B, Silversmit G, Schoonjans T, Vekemans B, Balcaen L, Vanhaecke F, Szaloki I, Rickers K, Falkenberg G, Vincze L, Janssen CR. 2012. Waterborne versus dietary zinc accumulation and toxicity in Daphnia magna: a synchrotron radiation based X-ray fluorescence imaging approach. Environ Sci Technol 46:1178-1184.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Farag AM, Woodward DF, Brumbaugh W, Goldstein JN, MacConnell E, Hogstrand C, Barrows FT. 1999. Dietary effects of metals-contaminated invertebrates from the Coeur d'Alene River, Idaho, on cutthroat trout. Trans Am Fish Soc 128:578-592. Ferard JF, Jouany JM, Truhaut R, Vasseur P. 1983. Accumulation of cadmium in a freshwater food chain experimental model. Ecotoxicol Environ Saf 7:43-52. Finley KA. 1985. Observations of bluegills fed selenium-contaminated Hexagenia nymphs collected from Belews Lake, North Carolina. Bull Environ Contam Toxicol 35:816-825. Fort DJ, Stover EL, Rogers RL, Copley HF, Morgan LA, Foster ER. 2000. Chronic boron or copper deficiency induces limb teratogenesis in Xenopus. Biol Trace Elem Res 77:173-187. Franklin NM, Glover CN, Nicol JA, Wood CM. 2005. Calcium/cadmium interactions at uptake surfaces in rainbow trout: waterborne versus dietary routes of exposure. Environ Toxicol Chem 24:2954-2964. Galvez F, Wood CM. 1999. The physiological effects of dietary silver sulphide exposure in rainbow trout. Environ Toxicol Chem 18:84-88. Gatlin III DM, Wilson RP. 1986. Dietary copper requirement of fingerling channel catfish. Aquacult 54:277-285. Gatlin III DM, Phillips HF, Torrans EL. 1989. Effects of various levels of dietary copper and zinc on channel catfish. Aquacult 76:127-134. Geffard O, Geffard A, Chaumot A, Vollat B, Alvarez C, Tusseau-Vuillemin MH, Garric J. 2008. Effects of chronic dietary and waterborne cadmium exposures on the contamination level and reproduction of Daphnia magna. Environ Toxicol Chem 27:1128-1134. Golding LA, Borgmann U, Dixon DG. 2011. Validation of a chronic dietary cadmium bioaccumulation and toxicity model for Hyalella azteca exposed to field-contaminated periphyton and lake water. Environ Toxicol Chem 30:2628-2638.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Gusso-Choueri PK, Choueri RB, Lombardi AT, Melão MGG. 2012. Effects of dietary copper on life-history traits of a tropical freshwater cladoceran. Arch Environ Contam Toxicol 62 :589-598. Haesloop U, Schirmer M. 1985. Accumulation of orally administered cadmium by the eel (Anguilla anguilla). Chemosphere 14:1627-1634. Hamilton SJ, Holley KM, Buhl KJ, Bullard FA. 2005. Selenium impacts on razorback sucker, Colorado River, Colorado. III. Larvae. Ecotoxicol Environ Saf 61:168-189. Hamilton SJ, Buhl KJ, Faerber NL, Wiedmeyer RH, Bullard FA. 1990. Toxicity of organic selenium in the diet of chinook salmon. Environ Toxicol Chem 9:347-358. Handy RD. 1993. The effect of acute exposure to dietary Cd and Cu on organ toxicant concentrations in rainbow trout, Oncorhynchus mykiss. Aquat Toxicol 27:1-14. Handy RD, Sims DW, Giles A, Campbell HA, Musonda MM. 1999. Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (Oncorhynchus mykiss) during chronic dietary exposure to copper. Aquat Toxicol 47:23-41. Hansen JA, Lipton J, Welsh PG, Cacela D, MacConnell B. 2004. Reduced growth of rainbow trout (Oncorhynchus mykiss) fed a live invertebrate diet pre-exposed to metal-contaminated sediments. Environ Toxicol Chem 23:1902-1911. Hardy RW, Oram L, Möller G. 2010. Effects of dietary selenomethionine on cutthroat trout (Oncorhynchus clarki bouvieri) growth and reproductive performance over a life cycle. Arch Environ Contam Toxicol 58:237-245. Hatakeyama S, Yasuno M. 1981. The effects of cadmium-accumulated Chlorella on the reproduction of Moina macrocopa (Cladocera). Ecotoxicol Environ Saf 5:341-350. Hatakeyama S, Yasuno M. 1982. Accumulation and effects of cadmium on guppy (Poecilia reticulata) fed cadmium-dosed cladocera (Moina macrocopa). Bull Environ Contam Toxicol 29:159-166.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Hatakeyama S, Yasuno M. 1987. Chronic effects of Cd on the reproduction of the guppy (Poecilia reticulata) through Cd-accumulated midge larvae (Chironomus yoshimatsui). Ecotoxicol Environ Saf 14:191-207. Hermanutz RO, Allen KN, Detenbeck NE, Stephan CE. 1996. Exposure of bluegill (Lepomis macrochirus) to selenium in outdoor experimental streams. Duluth, MN, USA. USEPA Mid-Continent Ecology Division. Hilton JW, Bettger WJ. 1988. Dietary vanadium toxicity in juvenile rainbow trout: a preliminary study. Aquat Toxicol 12:63-71. Hodson PV, Blunt BR, Spry DJ. 1978. Chronic toxicity of water-borne and dietary lead to rainbow trout (Salmo gairdneri) in Lake Ontario water. Water Res 12:869-878. Hook SE, Fisher NS. 2001a. Sublethal effects of silver in zooplankton: Importance of exposure pathways and implications for toxicity testing. Environ Toxicol Chem 20:568-574. Hook SE, Fisher NS. 2001b. Reproductive toxicity of metals in calanoid copepods. Mar Biol 138:1131-1140. Hook SE, Fisher NS. 2002. Relating the reproductive toxicity of five ingested metals in calanoid copepods with sulfur affinity. Mar Environ Res 53:161-174. Hoyle I, Shaw BJ, Handy RD. 2007. Dietary copper exposure in the African walking catfish, Clarias gariepinus: Transient osmoregulatory disturbances and oxidative stress. Aquat Toxicol 83:62-72. Huang X, Guo F, Wang W-X. 2010. Responses of abalone Haliotis diversicolor to sublethal exposure of waterborne and dietary silver and cadmium. Ecotoxicol Environ Saf 73:1130-1137. Irving EC, Baird DJ, Culp JM. 2003. Ecotoxicological responses of the mayfly Baetis tricaudatus to dietary and waterborne cadmium: Implications for toxicity testing. Environ Toxicol Chem 22:1058-1064. Jeng SS, Sun LT. 1981. Effects of dietary zinc levels on zinc concentrations in tissues of common carp. J Nutr 111:134-140.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Julshamn K, Andersen K-J, Ringdal O, Brenna J. 1988. Effect of dietary copper on the hepatic concentration and subcellular distribution of copper and zinc in the rainbow trout (Salmo gairdneri). Aquacult 73:143-155. Kamunde CN, Grosell M, Lott JNA, Wood CM. 2001. Copper metabolism and gut morphology in rainbow trout (Oncorhynchus mykiss) during chronic sublethal dietary copper exposure. Can J Fish Aquat Sci 58:293-305. Kamunde C, Grosell M, Higgs D, Wood CM. 2002. Copper metabolism in actively growing rainbow trout (Oncorhynchus mykiss): interactions between dietary and waterborne copper uptake. J Exp Biol 205:279-290. Kamunde C, Wood CM. 2003. The influence of ration size on copper homeostasis during sublethal dietary copper exposure in juvenile rainbow trout, Oncorhynchus mykiss. Aquat Toxicol 62:235-254. Kang J-C, Kim S-G, Jang SW. 2005. Growth and hematological changes of rockfish, Sebastes schlegeli (Hilgendorf) exposed to dietary Cu and Cd. J World Aquacult Soc 36:188-195. Kim S-G, Kang J-C. 2004. Effect of dietary copper exposure on accumulation, growth and hematological parameters of the juvenile rockfish, Sebastes schlegeli. Mar Environ Res 58:65-82. Kim S-G, Kim J-W, Kang J-C. 2004. Effect of dietary cadmium on growth and hematological parameters of juvenile rockfish, Sebastes schlegeli (Hilgendorf). Aquacult Res 35:80-86. Kjoss VA, Grosell M, Wood CM. 2005. The influence of dietary Na on Cu accumulation in juvenile rainbow trout exposed to combined dietary and waterborne Cu in soft water. Arch Environ Contam Toxicol 49:520-527. Kjoss VA, Wood CM, McDonald DG. 2006. Effects of different ligands on the bioaccumulation and subsequent depuration of dietary Cu and Zn in juvenile rainbow trout (Oncorhynchus mykiss). Can J Aquat Sci 63:412-422. Knox D, Cowey CB, Adron JW. 1982. Effects of dietary copper and copper:zinc ratio on rainbow trout Salmo gairdneri. Aquacult 27:111-119.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Knox D, Cowey CB, Adron JW. 1984. Effects of dietary zinc intake upon copper metabolism in rainbow trout (Salmo gairdneri). Aquacult 40:199-207. Köck G, Bucher F. 1997. Accumulation of zinc in rainbow trout (Oncorhynchus mykiss) after waterborne and dietary exposure. Bull Environ Contam Toxicol 58:305-310. Kolts JM, Boese CJ, Meyer JS. 2009. Effects of dietborne copper and silver on reproduction by Ceriodaphnia dubia. Environ Toxicol Chem 28:71-85. Kumada H, Kimura S, Yokote M. 1980. Accumulation and biological effects of cadmium in rainbow trout. Bull Jap Soc Sci Fish 46:97-103. Lanno RP, Slinger SJ, Hilton JW. 1985a. Maximum tolerable and toxicity levels of dietary copper in rainbow trout (Salmo gairdneri Richardson). Aquacult 49:257-268. Lanno RP, Slinger SJ, Hilton JW. 1985b. Effect of ascorbic acid on dietary copper toxicity in rainbow trout (Salmo gairdneri Richardson). Aquacult 49:269-287. Lapointe D, Gentes S, Ponton DE, Hare L, Couture P. 2009. Influence of prey type on nickel and thallium assimilation, subcellular distribution and effects in juvenile fathead minnows (Pimephales promelas). Environ Sci Technol 43:8665-8670. Lee SC, Lee J-H, Bai SC. 2008. A preliminary study on effects of different dietary selenium (Se) levels on growth performance and toxicity in juvenile black seabrearn, Acathopagrus schlegeli (Bleeker). Asian-Aust J Anim Sci 21:1794-1799. Lee RF, Noone T. 1995. Effect of reproductive toxicants on lipovitellin in female blue crabs, Callinectes sapidus. Mar Environ Res 39:151-154. Lin YH, Shiau SY. 2007. The effects of dietary selenium on the oxidative stress of grouper, Epinephelus malabaricus, fed high copper. Aquacult 267:38-43. Lin YH, Shie YY, Shiau SY. 2008. Dietary copper requirements of juvenile grouper, Epinephelus malabaricus. Aquacult 274:161-165.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Lorentzen M, Maage A, Julshamn K. 1998. Supplementing copper to a fishmeal-based diet fed to Atlantic salmon parr affects liver copper and selenium concentrations. Aquacult Nutr 4:67-72. Lundebye A-K, Berntssen MHG, Wendelaar Bonga SE, Maage A. 1999. Biochemical and physiological responses in Atlantic salmon (Salmo salar) following dietary exposure to copper and cadmium. Mar Pollut Bull 39:137-144. Maage A. 1990. Comparison of cadmium concentrations in Atlantic salmon (Salmo salar) fry fed different commercial feeds. Bull Environ Contam Toxicol 44:770-775. Maage A, Julshamn K. 1993. Assessment of zinc status in juvenile Atlantic salmon (Salmo salar) by measurement of whole body and tissue levels of zinc. Aquacult 117:179-191. Mai K, Li H, Ai Q, Duan Q, Xu W, Zhang C, Zhang L, Tan B, Liufu Z. 2006. Effects of dietary squid viscera meal on growth and cadmium accumulation in tissues of Japanese seabass. Aquacult Res 37:1063-1069. Malchow DE, Knight AW, Maier KJ. 1995. Bioaccumulation and toxicity of selenium in Chironomus decorus larvae fed a diet of seleniferous Selenastrum capricornutum. Arch Environ Contam Toxicol 29:104-109. Metzler D. 2003. Effect of food-borne cadmium on the reproduction of Ceriodaphnia dubia [MS thesis]. Newark, DE, USA: University of Delaware. 151 p. Miller PA, Lanno RP, McMaster ME, Dixon DG. 1993. Relative contributions of dietary and waterborne copper to tissue copper burdens and waterborne-copper tolerance in rainbow trout (Oncorhynchus mykiss). Can J Fish Aquat Sci 5:1683-1689. Mount DR, Barth AK, Garrison TD, Barten KA, Hockett JR. 1994. Dietary and waterborne exposure of rainbow trout (Oncorhynchus mykiss) to copper, cadmium, lead and zinc using a live diet. Environ Toxicol Chem 13:2031-2041. Munger C, Hare L. 1997. Relative importance of water and food as cadmium sources to an aquatic insect (Chaoborus punctipennis): Implications for predicting Cd bioaccumulation in nature. Environ Sci Technol 31:891-895.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Murai T, Andrews JW, Smith RG. 1981. Effects of dietary copper on channel catfish. Aquacult 22:353-357. Ng TY-T, Wood CM. 2008. Trophic transfer and dietarytoxicity of Cd from the oligochaete to the rainbow trout. Aquat Toxicol 87:1-8. Ng TY, Klinck JS, Wood CM. 2009. Does dietary Ca protect against toxicity of a low dietborne Cd exposure to the rainbow trout? Aquat Toxicol 91:75-86. Ogino C, Yang G-Y. 1978. Requirement of rainbow trout for dietary zinc. Bull Jpn Soc Sci Fish 44:1015-1018. Ogle RS, Knight AW. 1989. Effects of elevated foodborne selenium on growth and reproduction of the fathead minnow (Pimephales promelas). Arch Environ Contam Toxicol 18:795-803. Overnell J, McIntosh R. 1988. The effect of supplementary dietary copper on copper and metallothionein levels in the fish, dab, Limanda limanda. Mar Environ Res 26:237-247. Overnell J, Fletcher TC, McIntosh R. 1988a. Factors affecting hepatic metallothionein levels in marine flatfish. Mar Environ Res 24:155-158. Overnell J, Fletcher TC, McIntosh R. 1988b. The apparent lack of effect of supplementary dietary zinc on zinc metabolism and metallothionein concentrations in the turbot, Scophthalmus maximus (Linnaeus). J Fish Biol 33:563-570. Pinho GLL, Pedroso MS, Rodrigues SC, De Souza SS, Bianchini A. 2007. Physiological effects of copper in the euryhaline copepod Acartia tonsa: Waterborne versus waterborne plus dietborne exposure. Aquat Toxicol 84:62-70. Powell ML, Jones WT, Gibbs VK, Hammer HS, Lawrence JM, Fox J, Lawrence AL, Watts SA. 2010. Dietary copper affects survival, growth, and reproduction in the sea urchin Lytechinus variegates. J Shellfish Res 29:1043-1049.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Rainbow PS, Poirier L, Smith BD, Brix KV, Luoma SN. 2006. Trophic transfer of trace metals from the polychaete worm Nereis diversicolor to the polychaete N. virens and the decapod crustacean Palaemonetes varians. Mar Ecol Prog Ser 321:167-181. Reynders H, Campenhout KV, Bervoets L, De Coen WM, Blust R. 2006. Dynamics of cadmium accumulation and effects in common carp (Cyprinus carpio) during simultaneous exposure to water and food (Tubifex tubifex). Environ Toxicol Chem 25:1558-1567. Richardson NL, Higgs DA, Beames RM, McBride JR. 1985. Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile Chinook salmon (Oncorhynchus tshawytscha). J Nutr 115:553-567. Roberts DA, Poore AGB, Johnston EL. 2006. Ecological consequences of copper contamination in macroalgae: Effects on epifauna and associated herbivores. Environ Toxicol Chem 25:2470-2479. Rodgher S, Lombardi AT, Melão MGG, Tonietto AE. 2008. Change in life cycle parameters and feeding rate of Ceriodaphnia silvestrii Daday (Crustacea, Cladocera) exposure to dietary copper. Ecotoxicol 17:826-833. Rodgher S, Lombardi AT, Melão MGG. 2009. Evaluation onto life cycle parameters of Ceriodaphnia silvestrii submitted to 36 days dietary copper exposure. Ecotoxicol Environ Saf 72:1748-1753. Rowe CL, Heyes A, Hopkins W. 2009. Effects of dietary vanadium on growth and lipid storage in a larval Anuran: Results from studies employing ad libitum and rationed feeding. Aquat Toxicol 91:179-186. Rozon-Ramilo LD, Dubé MG, Squires AJ, Niyogi S. 2011. Examining waterborne and dietborne routes of exposure and their contribution to biological response patterns in fathead minnow (Pimephales promelas). Aquat Toxicol 105:466-481. Ruangsomboon S, Wongrat L. 2006. Bioaccumulation of cadmium in an experimental aquatic food chain involving phytoplankton (Chlorella vulgaris), zooplankton (Moina macrocopa), and the predatory catfish Clarias macrocephalus X C. gariepinus. Aquat Toxicol 78:15-20.	

4.0 New Information.

Reviewer	Comments	EPA Response
	Sabatini SE, Chaufan G, Juárez ÁB, Coalova I, Bianchi L, Eppis MR, del Carmen Ríos de Molina M. 2009. Dietary copper effects in the estuarine crab, Neohelice (Chasmagnathus) granulata, maintained at two different salinities. Comp Biochem Physiol Part C 150:521-527. Serra R, Isani G, Cattani O, Carpené E. 1996. Effects of different levels of dietary zinc on the guilthead, Sparus aurata during the growing season. Biol Trace Elem Res 51:107-116. Shaw BJ, Handy RD. 2006. Dietary copper exposure and recovery in Nile tilapia, Oreochromis niloticus. Aquat Toxicol 76:111-121. Shim KF, Lee TL. 1993. Zinc requirement of the guppy (Poecilia reticulate Peters). J Aqua Trop 8:81-90. Sofyan A, Shaw JR, Birge WJ. 2006. Metal trophic transfer from algae to cladocerans and the relative importance of dietary metal exposure. Environ Toxicol Chem 25:1034-1041. Sofyan A, Rosita G, Price DJ, Birge WJ. 2007. Cadmium uptake by Ceriodaphnia dubia from different exposures: Relevance to body burdens and toxicity. Environ Toxicol Chem 26:470-477. Spry DJ, Hodson PV, Wood CM. 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, Salmo gairdneri. Can J Fish Aquat Sci 45:32-41. 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 Carrassium auratus gibelio B.). Aquat Toxicol 77:126-135. Takeda H, Shimma Y. 1977. Effects of toxic amounts of dietary zinc on the growth and body components of rainbow trout at two levels of calcium. Bull Freshw Fish Res 27:103-109. Tashjian DH, Teh SJ, Sogomonyan A, Hung SSO. 2006. Bioaccumulation and chronic toxicity of dietary l-selenomethionine in juvenile white sturgeon (Acipenser transmontanus). Aquat Toxicol 79:401-409. Teh SJ, Deng X, Deng D-F, Teh F-C, Hung SSO, Fan TW-M, Liu J, Higashi RM. 2004. Chronic effects of dietary selenium on juvenile Sacramento splittail (Pogonichthys macrolepidotus). Environ Sci Technol 38:6085-6093.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Thomas JK, Janz DM. 2011. Dietary selenomethionine exposure in adult zebrafish alters swimming performance, energetics and the physiological stress response. Aquat Toxicol 102:79-86. Vidal D, Bay SM, Schlenk D. 2005. Effects of dietary selenomethionine on larval rainbow trout (Oncorhynchus mykiss). Arch Environ Contam Toxicol 49:71-75. Wallace WG, Brouwer TMH, Brouwer M, Lopez GR. 2000. Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ Toxicol Chem 19:962-971. Wekell JC, Shearer KD, Houle CR. 1983. High zinc supplementation of rainbow trout diets. Prog Fish-Cult 45:144-147. Wekell JC, Shearer KD, Gauglitz EJ. 1986. Zinc supplementation of trout diets: tissue indicators of body zinc status. Prog Fish-Cult 48:205-212. Woock SE, Garrett WR, Partin WE, Bryson WT. 1987. Decreased survival and teratogenesis during laboratory selenium exposures to bluegill, Lepomis macrochirus. Bull Environ Contam Toxicol 39:998-1005. Woodward DF, Brumbaugh WG, DeLonay AJ, Little EE, Smith CE. 1994. Effects on rainbow trout fry of a metals-contaminated diet of benthic invertebrates from the Clark Fork River, Montana. Trans Am Fish Soc 123:51-62. Woodward DF, Farag AM, Bergman HL, DeLonay AJ, Little EE, Smith CE, Barrows FT. 1995. Metals-contaminated benthic invertebrates in the Clark Fork River, Montana: Effects on age-0 brown trout and rainbow trout. Can J Fish Aquat Sci 52:1994-2004. Xie L, Funk DH, Buchwalter DB. 2010. Trophic transfer of Cd from natural periphyton to the grazing mayfly Centroptilum triangulifer in a life cycle test. Environ Pollut 158:272-277. Yoshimatsu T, Higuchi T, Zhang D, Fortes NR, Tanaka K, Yoshimura K. 2006. Effect of dietary cobalt supplementation on the population growth of rotifer Brachionus rotundiformis. Fish Sci 72:214-216.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Physiological and Other Endpoints Alves LC, Wood CM. 2006. The chronic effects of dietary lead in freshwater juvenile rainbow trout (Oncorhynchus mykiss) fed elevated calcium diets. Aquat Toxicol 78:217-232. Baker RTM, Handy RD, Davies SJ, Snook JC. 1998. Chronic dietary exposure to copper affects growth, tissue lipid peroxidation, and metal composition of the grey mullet, Chelon labrosus. Mar Environ Res 45:357-365. Baldisserotto B, Chowdhury MJ, Wood CM. 2005. Effects of dietary calcium and cadmium on cadmium accumulation, calcium and cadmium uptake from the water, and their interactions in juvenile rainbow trout. Aquat Toxicol 72:99-117. Barata C, Markich SJ, Baird DJ, Soares AMVM. 2002. The relative importance of water and food as cadmium sources to Daphnia magna Straus. Aquat Toxicol 61:143-154. Berntssen MHG, Aspholm OØ, Hylland K, Wendelaar Bonga SE, Lundebye A-K. 2001. Tissue metallothionein, apoptosis and cell proliferation responses in Atlantic salmon (Salmo salar L.) parr fed elevated dietary cadmium. Comp Biochem Physiol Part C 128:299-310. Berntssen MHG, Hylland K, Wendelaar Bonga SE, Maage A. 1999. Toxic levels of dietary copper in Atlantic salmon (Salmo salar L.) parr. Aquat Toxicol 46:87-99. Berntssen MHG, Lundebye A-K. 2001. Energetics in Atlantic salmon (Salmo salar L.) parr fed elevated dietary cadmium. Comp Biochem Physiol Part C 128:311-323. Brafield AE, Koodie AV. 1991. Effect of dietary zinc on the assimilation efficiency of carp (Cyprinus carpio L.). J Fish Biol 39:893-895. Campbell HA, Handy RD, Sims DW. 2002. Increased metabolic cost of swimming and consequent alterations to circadian activity in rainbow trout (Oncorhynchus mykiss) exposed to dietary copper. Can J Fish Aquat Sci 59:768-777.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Campbell HA, Handy RD, Sims DW. 2005. Shifts in a fish's resource holding power during a contact paired interaction: The influence of a copper-contaminated diet in rainbow trout. Physiol Biochem Zool 78:706-714. Cockell KA, Hilton JW, Bettger WJ. 1992. Hepatobiliary and hematological effects of dietary disodium arsenate heptahydrate in juvenile rainbow trout (Oncorhynchus mykiss). Comp Biochem Physiol Part C Toxicol Pharmacol 103:453-458. Galvez F, Hogstrand C, McGeer JC, Wood CM. 2001. The physiological effects of a biologically incorporated silver diet in rainbow trout (Oncorhynchus mykiss). Aquat Toxicol 55:95-112. Handy RD, Sims DW, Giles A, Campbell HA, Musonda MM. 1999. Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (Oncorhynchus mykiss) during chronic dietary exposure to copper. Aquat Toxicol 47:23-41. Handy RD. 1993b. The accumulation of dietary aluminium by rainbow trout, Oncorhynchus mykiss, at high exposure concentrations. J Fish Biol 42:603-606. Kamunde C, MacPhail R. 2011. Metal-metal interactions of dietary cadmium, copper and zinc in rainbow trout, Oncorhynchus mykiss. Ecotoxicol Environ Saf 74:658-667. Lanno RP, Hicks B, Hilton JW. 1987. Histological observations on intrahepatocytic copper-containing granules in rainbow trout reared on diets containing elevated levels of copper. Aquat Toxicol 10:251-263. Overnell J, Fletcher TC, McIntosh R. 1988. The apparent lack of effect of supplementary dietary zinc on zinc metabolism and metallothionein concentration in the turbot, Scophthalmus maximus (Linnaeus). J Fish Biol 33:563-570. Park CW, Shimizu C. 1989. Quantitative requirements of aluminium and iron in the formulated diets and its interrelation with other minerals in young eel. Nippon Suisan Gakkashi 55:111-116. Poston HA. 1991. Effects of dietary aluminium on growth and composition of young Atlantic salmon. Progressive Fish-Culturist 53:7-10.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Pratap HB, Fu H, Lock RAC, Wendelaar Bonga SE. 1989. Effect of waterborne and dietary cadmium on plasma ions of the teleost Oreochromis mossambicus in relation to water calcium levels. Arch Environ Contam Toxicol 18:568-575. Pratap HB, Wendelaar Bonga SE. 1993. Effect of ambient and dietary cadmium on pavement cells, chloride cells, and Na+/K+-ATPase activity in the gills of the freshwater teleost Oreochromis mossambicus at normal and high calcium levels in the ambient water. Aquat Toxicol 26:133-150. Szebedinszky C, McGeer JC, McDonald DG, Wood CM. 2001. Effects of chronic Cd exposure via the diet or water on internal organ-specific distribution and subsequent gill Cd uptake kinetics in juvenile rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem 20:597-607. Zhang L, Wang X-L. 2005. Effects of Zn pre-exposure on Cd and Zn bioaccumulation and metallothionein levels in two species of marine fish. Aquat Toxicol 73:353-369. Zhang L, Wang X-L. 2006. Alteration of dissolved cadmium and zinc uptake kinetics by metal pre-exposure in the black sea bream (Acanthopagrus schlegeli). Environ Toxicol Chem 25:1312-1321.	
Reviewer 4	**References cited:** Campbell PGC. 1995. Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. In: Metal speciation and bioavailability in aquatic systems, (Tessier A, Turner DR, eds). New York, NY, USA:John Wiley & Sons, 45-102. Meyer JS. 2002. The utility of the terms "bioavailability" and "bioavailable fraction" for metals. Marine Environmental Research 53:417-423.	
Reviewer 5	**References cited in the review** Balistrieri, L.S., D.A. Nimick, and C.A. Mebane. 2012. Assessing time-integrated dissolved concentrations and predicting toxicity of metals during diel cycling in streams. Science of the Total Environment. 425: 155–168. https://doi.org/10.1016/j.scitotenv.2012.03.008	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Bervoets, L. and R. Blust. 2000. Effects of pH on cadmium and zinc uptake by the midge larvae Chironomus riparius. Aquatic Toxicology. 49(1): 145-157. https://doi.org/10.1016/S0166-445X(99)00066-1 Borgmann, U., Y. Couillard, P. Doyle, and D.G. Dixon. 2005. Toxicity of sixty-three metals and metalloids to Hyalella azteca at two levels of water hardness. Environmental Toxicology and Chemistry. 24(3): 641–652 Bradley, R.W. and J.B. Sprague. 1985. The influence of pH, water hardness, and alkalinity on the acute lethality of zinc to rainbow trout (Salmo gairdneri). Canadian Journal of Fisheries and Aquatic Sciences. 42: 731-736. https://doi.org/10.1139/f85-094 Bringolf, R.B., B.A. Morris, C.J. Boese, R.C. Santore, H.E. Allen, and J.S. Meyer. 2006. Influence of dissolved organic matter on acute toxicity of zinc to larval fathead minnows (Pimephales promelas). Archives of Environmental Contamination and Toxicology. 51(3): 438-444. https://doi.org/10.1007/s00244-005-0088-6 Brix, K.V., D.K. DeForest, L.M. Tear, M. Grosell, and W.J. Adams. 2017. Use of multiple linear regression models for setting water quality criteria for copper: A complementary approach to the Biotic Ligand Model. Environmental Science & Technology. 51(9): 5182–5192. https://doi.org/10.1021/acs.est.6b05533 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. Proceedings of the National Academy of Sciences of the United States of America. 105(24): 8321-8326. https://doi.org/10.1073/pnas.0801686105 CCME. 2018. Canadian Water Quality Guidelines: Zinc. Scientific Criteria Document. Canadian Council of Ministers of the Environment, ISBN 978-1-77202-043-4 PDF, Winnipeg. 127 pp. Chapman, G.A., S. Ota, and F. Recht. 1980. Effects of water hardness on the toxicity of metals to Daphnia magna. U.S. EPA, Office of Research and Development, Corvallis, Oreg. 23 pp.	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Clifford, M. and J.C. McGeer. 2009. Development of a biotic ligand model for the acute toxicity of zinc to Daphnia pulex in soft waters. Aquatic Toxicology. 91(1): 26-32 https://doi.org/10.1016/j.aquatox.2008.09.016 Clifford, M. and J.C. McGeer. 2010. Development of a biotic ligand model to predict the acute toxicity of cadmium to Daphnia pulex. Aquatic Toxicology. 98(1): 1-7. https://doi.org/10.1016/j.aquatox.2010.01.001 Croteau, K., A.C. Ryan, R. Santore, D. DeForest, C. Schlekat, E. Middleton, and E. Garman. 2021. Comparison of Multiple Linear Regression and Biotic Ligand Models to Predict the Toxicity of Nickel to Aquatic Freshwater Organisms. Environmental Toxicology and Chemistry. n/a(n/a). https://doi.org/https://doi.org/10.1002/etc.5063 Cusimano, R.F., D.F. Brakke, and G.A. Chapman. 1986. Effects of pH on the toxicities of cadmium, copper, and zinc to steelhead trout (Salmo gairdneri). Canadian Journal of Fisheries and Aquatic Sciences. 43(8): 1497-1503. https://doi.org/10.1139/f86-187 De Schamphelaere, K.A.C. and C.R. Janssen. 2002. A biotic ligand model predicting acute copper toxicity for Daphnia magna: the effects of calcium, magnesium, sodium, potassium, and pH. Environmental Science and Technology. 36(1): 48 -54. https://doi.org/10.1021/es000253sS0013-936X(00)00253-4 De Schamphelaere, K.A.C. and C.R. Janssen. 2004a. Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (Oncorhynchus mykiss): comparison with other fish species and development of a Biotic Ligand Model. Environmental Science and Technology. 38(23): 6201 - 6209. https://doi.org/10.1021/es049720m De Schamphelaere, K.A.C. and C.R. Janssen. 2004b. Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to Daphnia magna. Environmental Toxicology and Chemistry. 23(5): 1115–1122. https://doi.org/10.1897/02-593 DeForest, D.K., K.V. Brix, L.M. Tear, and W.J. Adams. 2018. Multiple linear regression models for predicting chronic aluminum toxicity to freshwater aquatic organisms and developing water	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	quality guidelines. Environmental Toxicology and Chemistry. 37(1): 80-90. https://doi.org/10.1002/etc.3922 DeForest, D.K., R.C. Santore, A.C. Ryan, B.G. Church, M.J. Chowdhury, and K.V. Brix. 2017. Development of biotic ligand model–based freshwater aquatic life criteria for lead following US Environmental Protection Agency guidelines. Environmental Toxicology and Chemistry. 36(11): 2965-2973. https://doi.org/10.1002/etc.3861 Diamond, J.M., E.L. Winchester, D.G. Mackler, W.J. Rasnake, J.K. Fanelli, and D. Gruber. 1992. Toxicity of cobalt to freshwater indicator species as a function of water hardness. Aquatic Toxicology. 22: 163-180. https://doi.org/10.1016/0166-445X(92)90038-O Erickson, R.J., D.A. Benoit, V.R. Mattson, H.P. Nelson, and E.N. Leonard. 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Environmental Toxicology and Chemistry. 15(2): 181-193. https://doi.org/10.1002/etc.5620150217 Grosell, M., J. Blanchard, K.V. Brix, and R. Gerdes. 2007. Physiology is pivotal for interactions between salinity and acute copper toxicity to fish and invertebrates. Aquatic Toxicology. 84(2): 162-172. https://doi.org/https://doi.org/10.1016/j.aquatox.2007.03.026 Hall, L.W., R.D. Anderson, B.L. Lewis, and W.R. Arnold. 2008. The Influence of Salinity and Dissolved Organic Carbon on the Toxicity of Copper to the Estuarine Copepod, Eurytemora affinis. Archives of Environmental Contamination and Toxicology. 54(1): 44-56. https://doi.org/10.1007/s00244-007-9010-8 Hansen, J.A., P.G. Welsh, J. Lipton, D. Cacela, and A.D. Dailey. 2002. Relative sensitivity of bull trout (Salvelinus confluentus) and rainbow trout (Oncorhynchus mykiss) to acute exposures of cadmium and zinc. Environmental Toxicology and Chemistry. 21(1): 67–75. https://doi.org/10.1002/etc.5620210110 Heijerick, D.G., K.A.C. De Schamphelaere, and C.R. Janssen. 2002. Predicting acute zinc toxicity for Daphnia magna as a function of key water chemistry characteristics: development and validation of a biotic ligand model. Environmental Toxicology and Chemistry. 21(6): 1309–1315	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Heijerick, D.G., K.A.C. De Schamphelaere, P.A. Van Sprang, and C.R. Janssen. 2005. Development of a chronic zinc biotic ligand model for Daphnia magna. Ecotoxicology and Environmental Safety. 62(1): 1-10. https://doi.org/10.1016/j.ecoenv.2005.03.020 Heijerick, D.G., C.R. Janssen, and W.M. De Coen. 2003. The combined effects of hardness, pH, and dissolved organic carbon on the chronic toxicity of Zn to D. magna : development of a surface response model. Archives of Environmental Contamination and Toxicology. 44(2): 210-217. https://doi.org/10.1007/s00244-002-2010-9 Hyne, R.V., F. Pablo, M. Julli, and S.J. Markich. 2005. Influence of water chemistry on the acute toxicity of copper and zinc to the cladoceran Ceriodaphnia cf dubia. Environmental Toxicology and Chemistry. (7): 1667–1675. https://doi.org/10.1897/04-497R.1 Ivey, C.D., J.M. Besser, J.A. Steevens, M.J. Walther, and V.D. Melton. 2019. Influence of Dissolved Organic Carbon on the Acute Toxicity of Copper and Zinc to White Sturgeon (Acipenser transmontanus) and a Cladoceran (Ceriodaphnia dubia). Environmental Toxicology and Chemistry. 38(12): 2682-2687. https://doi.org/10.1002/etc.4592 Markich, S.J., G.E. Batley, J.L. Stauber, N.J. Rogers, S.C. Apte, R.V. Hyne, K.C. Bowles, K.L. Wilde, and N.M. Creighton. 2005. Hardness corrections for copper are inappropriate for protecting sensitive freshwater biota. Chemosphere. 60(1): 1-8. https://doi.org/ 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.). 130 pp. https://doi.org/10.3133/sir20065245. Mebane, C.A., M.J. Chowdhury, K.A.C. De Schamphelaere, S. Lofts, P.R. Paquin, R.C. Santore, and C.M. Wood. 2020a. Metal bioavailability models: current status, lessons learned, considerations for regulatory use, and the path forward. Environmental Toxicology and Chemistry. 39(1): 60-84. https://doi.org/10.1002/etc.4560 Mebane, C.A., F.S. Dillon, and D.P. Hennessy. 2012. Acute toxicity of cadmium, lead, zinc, and their mixtures to stream-resident fish and invertebrates. Environmental Toxicology and Chemistry. 31(6): 1334–1348. https://doi.org/10.1002/etc.1820	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Mebane, C.A., T.S. Schmidt, J.L. Miller, and L.S. Balistrieri. 2020b. Bioaccumulation and toxicity of cadmium, copper, nickel, and zinc to aquatic insect communities. Environmental Toxicology and Chemistry. 39(4): 812-833. https://doi.org/10.1002/etc.4663 Meyer, J.S., S.J. Clearwater, T.A. Doser, M.J. Rogaczewski, and J.A. Hansen. 2007. Effects of Water Chemistry on Bioavailability and Toxicity of Waterborne Cadmium, Copper, Nickel, Lead, and Zinc to Freshwater Organisms. SETAC, Pensacola, Fla. 352 pp Naddy, R.B., W.A. Stubblefield, R.A. Bell, K.B. Wu, R.C. Santore, and P.R. Paquin. 2018. Influence of Varying Water Quality Parameters on the Acute Toxicity of Silver to the Freshwater Cladoceran, Ceriodaphnia dubia. Bulletin of Environmental Contamination and Toxicology. 100(1): 69-75. https://doi.org/10.1007/s00128-017-2260-x Naddy, R.B., W.A. Stubblefield, J.R. May, S.A. Tucker, and J.R. Hockett. 2002. The effect of calcium and magnesium ratios on the toxicity of copper to five aquatic species in freshwater. Environmental Toxicology and Chemistry. 21(2): 347–352. https://doi.org/10.1002/etc.5620210217 Nimick, D.A., J.R. Gammon, and S.R. Parker. 2011. Diel biogeochemical processes and their effect on the aqueous chemistry of streams: A review. Chemical Geology. 283(1-2): 3-17. https://doi.org/10.1016/j.chemgeo.2010.08.017 Niyogi, S., R. Kent, and C.M. Wood. 2008. Effects of water chemistry variables on gill binding and acute toxicity of cadmium in rainbow trout (Oncorhynchus mykiss): A biotic ligand model (BLM) approach. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 148(4): 305-314. https://doi.org/10.1016/j.cbpc.2008.05.015 Poteat, M.D. and D.B. Buchwalter. 2014. Four reasons why traditional metal toxicity testing with aquatic insects is irrelevant. Environmental Science & Technology. 48(2): 887–888. https://doi.org/10.1021/es405529n Richards, J.G. and R.C. Playle. 1998. Cobalt binding to gills of rainbow trout (Oncorhynchus mykiss): an equilibrium model. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 119(2): 185-197. https://doi.org/10.1016/S0742-8413(97)00206-5	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Richards, J.G. and R.C. Playle. 1999. Protective effects of calcium against the physiological effects of exposure to a combination of cadmium and copper in rainbow trout (Oncorhynchus mykiss). Canadian Journal of Zoology. 77(7): 1035–1047. https://doi.org/doi:10.1139/cjz-77-7-1035 Santore, R.C., K. Croteau, A.C. Ryan, C. Schlekat, E. Middleton, and E. Garman. 2021. A Review of Water Quality Factors that Affect Nickel Bioavailability to Aquatic Organisms: Refinement of the Biotic Ligand Model for Nickel in Acute and Chronic Exposures. Environmental Toxicology and Chemistry. n/a(n/a). https://doi.org/https://doi.org/10.1002/etc.5109 Santore, R.C., A.C. Ryan, F. Kroglund, H.C. Teien, P.H. Rodriguez, W.A. Stubblefield, A.S. Cardwell, W.J. Adams, and E. Nordheim. 2018. Development and application of a Biotic Ligand Model for predicting the toxicity of dissolved and precipitated aluminum. Environmental Toxicology and Chemistry. 37(1): 70-79. https://doi.org/10.1002/etc.4020 Schubauer-Berigan, M.K., J.R. Dierkes, P.D. Monson, and G.T. Ankley. 1993. pH-dependent toxicity of Cd, Cu, Ni, Pb, and Zn to Ceriodaphnia dubia, Pimephales promelas, Hyalella azteca, and Lumbriculus variegatus. Environmental Toxicology and Chemistry. 12(7): 1261-1266. https://doi.org/10.1002/etc.5620120715 Tan, Q.-G. and W.-X. Wang. 2011. Acute Toxicity of Cadmium in Daphnia magna under Different Calcium and pH Conditions: Importance of Influx Rate. Environmental Science & Technology. 45(5): 1970–1976. https://doi.org/10.1021/es102453b USEPA. 2016a. Aquatic Life Ambient Water Quality Criterion for Cadmium – 2016. EPA-820-R-16-002. 721 pp. USEPA. 2016b. Draft Technical Support Document: Recommended Estimates for Missing Water Quality Parameters for Biotic Ligand Model. EPA 820-R-15-106. Van Genderen, E.J., R.W. Gensemer, C. Smith, R.C. Santore, and A.C. Ryan. 2007. Evaluation of the Biotic Ligand Model relative to other site-specific criteria derivation methods for copper in surface waters with elevated hardness Aquatic Toxicology. 84(2): 279-291. https://doi.org/10.1016/j.aquatox.2007.02.024	

4.0 New Information.		
Reviewer	**Comments**	**EPA Response**
	Wang, N., C.A. Mebane, J.L. Kunz, C.G. Ingersoll, W.G. Brumbaugh, R.C. Santore, J.W. Gorsuch, and W.R. Arnold. 2011. Influence of dissolved organic carbon on toxicity of copper to a unionid mussel (Villosa iris) and a cladoceran (Ceriodaphnia dubia) in acute and chronic water exposures. Environmental Toxicology and Chemistry. 30(9): 2115–2125. https://doi.org/10.1002/etc.596 Wang, N., C.A. Mebane, J.L. Kunz, C.G. Ingersoll, T.W. May, W.R. Arnold, R.C. Santore, T. Augspurger, F.J. Dwyer, and M.C. Barnhart. 2009. Evaluation of acute copper toxicity to juvenile freshwater mussels (fatmucket, Lampsilis siliquoidea) in natural and reconstituted waters. Environmental Toxicology and Chemistry. 28(11): 2367–2377. https://doi.org/10.1897/08-655.1 Welsh, P.G., J. Lipton, C.A. Mebane, and J.C.A. Marr. 2008. Influence of flow-through and renewal exposures on the toxicity of copper to rainbow trout. Ecotoxicology and Environmental Safety. 69(2): 199-208. https://doi.org/10.1016/j.ecoenv.2007.04.003 Welsh, P.G., J. Lipton, T.L. Podrabsky, and G.A. Chapman. 2000. Relative importance of calcium and magnesium in hardness-based modification of copper toxicity. Environmental Toxicology and Chemistry. 19(6): 1624–1631. https://doi.org/10.1002/etc.5620190620 Wood, C.M. 2012. An introduction to metals in fish physiology and toxicology: Basic principles. Fish Physiology: Homeostasis and Toxicology of Essential Metals. 31(PART A): 1-51. https://doi.org/10.1016/S1546-5098(11)31001-1 Wood, C.M., H.A. Al-Reasi, and D.S. Smith. 2011. The two faces of DOC. Aquatic Toxicology. 105S(3-4): 3-8. https://doi.org/10.1016/j.aquatox.2011.03.007 Yoro, S.C., C. Panagiotopoulos, and R. Sempéré. 1999. Dissolved organic carbon contamination induced by filters and storage bottles. Water Research. 33(8): 1956-1959. https://doi.org/10.1016/S0043-1354(98)00407-2	

References cited in EPA's Response

Alsop D, Ng TY-T, Chowdhury MJ, Wood CM. 2016. Interactions of waterborne and dietborne Pb in rainbow trout, *Oncorhynchus mykiss*: bioaccumulation, physiological responses, and chronic toxicity. Aquat Toxicol 177:343-354.

Amiard JC, Amiard-Triquet C, Barka S, Pellerin J, Rainbow PS. 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat Toxicol 76(2): 160-202.

Besser JM, Ivey CD, Steevens JA, Cleveland D, Soucek D, Dickinson A, Van Genderen EJ, Ryan AC, Schlekat CE, Garman E, Middleton E, Santore R. 2021. Modeling the Bioavailability of Nickel and Zinc to *Ceriodaphnia dubia* and *Neocloeon triangulifer* in Toxicity Tests with Natural Waters. Environ Toxicol Chem. 40(11):3049-3062. DOI: 10.1002/etc.5178.

Brix KV, DeForest DK, Tear L, Grosell M, Adams WJ. 2017. Use of multiple linear regression models for setting water quality criteria for copper: A complementary approach to the biotic ligand model. Environ Sci Technol 51:5182–5192.

Brix, K. V., Tear, L., Santore, R. C., Croteau, K., & DeForest, D. K. 2021. Comparative Performance of Multiple Linear Regression and Biotic Ligand Models for Estimating the Bioavailability of Copper in Freshwater. Environ Toxicol Chem. 40(6):1649-1661. DOI: 10.1002/etc.5012.

Croteau K, Ryan A, Santore R, DeForest D, Schlekat C, Middleton E, Garman, E. 2021. Comparison of Multiple Linear Regression and Biotic Ligand Models to Predict the Toxicity of Nickel to Aquatic Freshwater Organisms. Environ Toxicol Chem. 40(8):2189-2205. DOI: 10.1002/etc.5063

DeForest, DK, Brix KV, Tear LM, Cardwell AS, Stubblefield WA, Nordheim E, Adams WJ. 2020. Updated multiple linear regression models for predicting chronic aluminum toxicity to freshwater aquatic organisms and developing water quality guidelines. Environ Toxicol Chem. 39(9), 1724-1736.

Deleebeeck NME, De Schamphelaere KAC, Janssen CR. 2008. A novel method for predicting chronic nickel bioavailability and toxicity to Daphnia magna in artificial and natural waters. Environ Toxicol Chem. 27:2097-2107.

De Schamphelaere KA, Nys C, Janssen CR. 2014. Toxicity of lead (Pb) to freshwater green algae: development and validation of a bioavailability model and inter-species sensitivity comparison. Aquat Toxicol. 155:348-59.

De Schamphelaere K, Canli M, Van Lierde V, Forrez I, Vanhaecke F, Janssen C. 2004. Reproductive toxicity of dietary zinc to *Daphnia magna*. Aquat Toxicol. 70:233-244.

De Schamphelaere K, Forrez I, Dierckens K, Sorgeloos P, Janssen C. 2007. Chronic toxicity of dietary copper to *Daphnia magna.* Aquat Toxicol. 81:409-418.

Evens R, De Schamphelaere KA, Janssen CR. 2009. The effects of dietary nickel exposure on growth and reproduction of *Daphnia magna.* Aquat Toxicol. 94:138-144 doi:10.1016/j.aquatox.2009.06.011

Farley KJ, Meyer JS, Balistrieri LS, Iwasaki Y, Kamo M, Lofts S, Mebane CA, Naito W, Ryan AC, Santore RC, Tipping E. 2015. Metal mixtures modeling evaluation: 2. Comparison of four modeling approaches. Environ Toxicol Chem. 34:741-753.

Mebane CA, Dillon FS, Hennessy DP. 2012. Acute toxicity of cadmium, lead, zinc, and their mixtures to stream-resident fish and invertebrates. Environ Toxicol Chem. 31(6): 1334–1348. https://doi.org/10.1002/etc.1820

Mebane CA, Chowdhury MJ, De Schamphelaere KAC, Lofts S, Paquin PR, Santore RC, Wood CM. 2020. Metal bioavailability models: Current status, lessons learned, considerations for regulatory use, and the path forward. Environ Toxicol Chem. 39:60–84.

Mebane CA, Schmidt TS, Miller JL, Balistrieri LS. 2020. Bioaccumulation and toxicity of cadmium, copper, nickel, and zinc to aquatic insect communities. Environ Toxicol Chem. 39:812-833.

Meyer JS, Traudt EM, Ranville JF. 2018. Is the Factor-of-2 Rule Broadly Applicable for Evaluating the Prediction Accuracy of Metal-Toxicity Models? Bull Environ Contam Toxicol. 100:64-68.

Niyogi S,Pyle GG, Wood CM. 2007. Branchial versus intestinal zinc uptake in wild yellow perch (*Perca flavescens*) from reference and metal-contaminated aquatic ecosystems. Can. J. Fish. Aquat. Sci. 64: 1605–1613

Nys C, Janssen C, De Schamphelaere KAC. 2017. Validating the chronic Pb algae bioavailability model at high pH: Single-species evaluation. Prepared for International Lead Association, Durham, NC, U.S.A.

Nys C, Janssen C, De Schamphelaere KAC. 2013. An investigation of the potential toxicity of dietary Pb to *Ceriodaphnia dubia*. Durham, NC, U.S.A.

Roussel H, Joachim S, Lamothe S, Palluel O, Gauthier L, Bonzom J-M. 2007. A long-term copper exposure on freshwater ecosystem using lotic mesocosms: individual and population responses of three-spined sticklebacks (*Gasterosteus aculeatus*). Aquat Toxicol 82:272-280.

Schlekat CE, Van Genderen E, De Schamphelaere KA, Antunes PM, Rogevich EC, Stubblefield WA. 2010. Cross-species extrapolation of chronic nickel Biotic Ligand Models. Sci Total Environ 408:6148-6157.

U.S. Environmental Protection Agency. 2003. The Biotic Ligand Model: Technical Support Document for Its Application to the Evaluation of Water Quality Criteria for Copper. EPA 822-R-03-027. Washington, DC.

US Environmental Protection Agency. 2007. Aquatic life ambient freshwater quality criteria—Copper. EPA 822-R-07-001. Washington, DC.

US Environmental Protection Agency. 2016. Final Aquatic Life Ambient Water Quality Criterion for Selenium - Freshwater 2016. EPA 822-R-21-006. Washington, DC.

US Environmental Protection Agency. 2018. Final aquatic life ambient water quality criteria for aluminum. EPA-822-R-18-001. Washington, DC.

Versteeg DJ, Belanger SE, Carr GJ. 1999. Understanding single-species and model ecosystem sensitivity: Data-based comparison. Environ Toxicol Chem. 18:1329-1346.

Wood CM, Al-Reasi HA, Smith DS. 2011. The two faces of DOC. Aquat Toxicol. 105S(3-4): 3-8. https://doi.org/10.1016/j.aquatox.2011.03.007