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EXTERNAL PEER REVIEW OF EPA'S DRAFT AQUATIC LIFE SCREENING VALUES FOR N-(1,3-DIMETHYLBUTYL)-N′-PHENYL-P-PHENYLENEDIAMINE QUINONE (6PPD-QUINONE)

FINAL PEER REVIEW SUMMARY REPORT

November 2023

Submitted to:
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CONTENTS

1.0 INTRODUCTION

This report documents the results of an independent external peer review of the U.S. Environmental Protection Agency's (EPA) draft *Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) in Freshwater*.

ERG (a contractor to EPA) organized this review and developed this report. The report provides background on the development of the draft document (Section 1.1), describes ERG's peer reviewer selection process (Section 1.2), provides reviewers' comments organized by charge question (Section 2.0) and additional comments (Section 3.0). Appendix A provides the charge to reviewers and Appendix B presents the reviewer comments organized by reviewer.

1.1 Development of the Draft Documents

The purpose of EPA's preliminary *Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) in Freshwater* is to provide EPA's scientific rationale for the draft screening values for 6PPD-quinone focused on the protection of aquatic life. The criteria are designed to solely protect sensitive aquatic life and is based on the best available data and best professional scientific judgements on the toxicological effects of 6PPD-quinone to aquatic life.

N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine (6PPD) is an additive to vehicle tire rubber, where it functions to protect rubber from reactions with ozone and oxygen, which can lead to degradation and cracking. Recent research has determined the ozonation of (6PPD) produces the reaction product 6PPD-quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) through hydrolysis and photodegradation. 6PPD-quinone recently has been shown to present an environmental threat based on its toxicity to salmon, where it has been identified as the causal agent in urban runoff mortality syndrome (URMS) observed in the Puget Sound area of Washington state.

Relatively limited 6PPD and 6PPD-quinone toxicity studies have been conducted on various aquatic taxa, including numerous species of fish and invertebrates, indicating that exposure to 6PPD and 6PPD-quinone through the aquatic environment causes population level effects, particularly mortality to acute exposures across aquatic life in the case for 6PPD and to fish such as salmonids, which appear to be sensitive to 6PPD-quinone exposures. In these drafts, EPA provides support for and outlines the derivation of screening values for 6PPD and 6PPD-quinone that would be protective of sensitive aquatic life.

This report is a compilation of external peer review comments received for 6PPD-quinone. A separate external peer review of the *Aquatic Life Screening Values for N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in Freshwater* was conducted and the peer review comments received for 6PPD are compiled in a separate report.

1.2 Peer Reviewers

For this review, ERG identified, screened, and selected reviewers who had no conflict of interest in performing the review and who collectively met the following technical selection criteria provided by EPA:

ERG initiated a search process, asking interested candidates to describe their qualifications and respond to a series of "Conflict of Interest" (COI) analysis questions. ERG carefully screened submissions to identify a pool of qualified, COI-free candidates. From the set of candidates who met the criteria, ERG proposed a pool of five candidates to EPA on October 17, 2023. From this pool, ERG selected three experts who collectively best met the selection criteria. ERG contracted with and committed the following three experts to perform the review:

- **Jerry Diamond, Ph.D.**; Director of Ecotoxicology, TetraTech
- **David M. Janz, Ph.D.**; Professor, University of Saskatchewan
- **Richard Grippo, Ph.D.**; Emeritus Professor of Environmental Biology, Arkansas State University

ERG provided reviewers with instructions, the draft *Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) in Freshwater*, and the charge to reviewers (Appendix A of this report) prepared by EPA. Reviewers worked individually to develop written comments in response to the charge questions. After receiving reviewer comments, ERG compiled responses by charge question (see Section 2.0) and included the responses organized by reviewer (see Appendix B).

2.0 REVIEWER COMMENTS ORGANIZED BY CHARGE QUESTION

This section organizes reviewer comments by charge question (see Appendix B for reviewer comments organized by reviewer).

2.1 Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD-quinone to aquatic life.

2.1. Clarity of Document as it Relates to Assessing the Risk of 6PPD to Aquatic-Life	
Reviewer	**Comments**
Reviewer 1	Like the 6PPD document, the 6PPD-quinone document is well written and flows logically. The background summary of environmental fate and distribution of 6PPD-quinone was good. I appreciated the study summaries being described in order from most sensitive to least sensitive species, and separating quantitative and qualitative studies into separate sections. Like the 6PPD document, I have provided a marked-up version with minor editorial suggestions and comments. There are some inconsistencies in the descriptions of experimental procedures when summarizing the various studies. The document must be consistent and specific when describing the control treatments among studies. In all instances, specify "solvent control" or "solvent vehicle control" when such a control was employed, and when known state the solvent concentration occurring in the actual exposure vessel as a percentage volume/volume. In addition, please specify what is meant by a negative control. Is this water-only without solvent added? In the study descriptions (initial sentence of each) be consistent and always state whether 6PPD-quinone was measured or unmeasured.
Reviewer 2	The document for 6PPD-q follows EPA's current aquatic life criteria framework, which is organized in a similar manner to EPA 's ecological risk assessment guidance. The organization and construction of this document is easy to follow, and the writing is clearly presented for the most part. However, the document has several grammatical and typographical errors which should be corrected using a thorough editorial review before finalizing. This document summarizes what is known about the chemistry, fate, and transport properties of 6PPD-q in the environment. The document limits this discussion to freshwater as opposed to salt water as well, which is due to the paucity of data regarding fate and transport of 6PPD-q in saltwater. While the document later explains the lack of marine toxicity data, perhaps EPA

2.1. Clarity of Document as it Relates to Assessing the Risk of 6PPD to Aquatic-Life	
Reviewer	**Comments**
	should also acknowledge the current lack of fate and transport information in marine environments as well. This document mentions tire wear and wet weather runoff from roads as a major source of 6PPD-q in surface waters. There has been a fair amount of research in the past three years regarding fate and transport of 6PPD-q in surface waters, including its persistence in ice and snow moved to roadsides (and subsequent input to streams from melting snow) and from runoff of roadside dust, which may be important sources of 6PPD-q to streams. Some recent field data are coincident with Coho salmon pre-spawn mortality in the Pacific Northwest as well. EPA should consider incorporating a brief summary of relevant publications in this regard as some of those data help inform the challenges in controlling sources of 6PPD-q and the potential persistence of 6PPD-q under certain environmental conditions. This information may help support EPA's SV for 6PPD-q. I included some relevant citations of which I am aware below.
Reviewer 3	Overall this is a well-written report but it seems a bit more "hurried" than the report on the toxicity of 6PPD. Although not numerous, there are more typo's and grammatical errors than in the 6PPD document. There are also a few places where the document is unclear. For example, I am not sure what the statement from Section 2.2.1 "Given that this preliminary SSSV is intended as information to protect sensitive aquatic life species, such as salmonids, this screening value was derived without the use of aquatic plant data." is describing. The authors seem to be stating that plant data were not used because they were only interested in salmonids, yet in actuality no plant data exists. This report provides a sufficient understanding of the risk assessment of 6PPPD-quinone in the early stages of evaluation on a single taxon of organisms.

2.2 Please comment on the technical approach used to derive the draft screening values presented in EPA's Preliminary Draft *Sensitive Salmonid Screening Value for Acute Exposures to 6PPD-Quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) in Freshwater*.

2.2.a. Is the technical approach used to derive the screening values logical?

2.2.b. Does the science support the conclusions?

2.2.c. Is it consistent with the protection of aquatic life?

2.2. The Technical Approach Used to Derive the Draft Screening Values	
Reviewer	**Comments**
Reviewer 1	2.2.a. The technical approach generally follows EPA's established procedures for developing a species sensitivity distribution to derive a water quality criterion. However, due to the paucity of acute and especially chronic freshwater toxicity data available at this time, some modifications of these procedures were employed to derive a draft screening value.

2.2. The Technical Approach Used to Derive the Draft Screening Values	
Reviewer	**Comments**
	I agree with using studies that determined acute toxicity (LC50) over a time period of greater than or equal to 24 hours, because this enhances the limited dataset and particularly because this represents an environmentally realistic exposure duration that would occur in freshwaters after precipitation events. I agree with the rationale for separating out the *Oncorhynchus* data and not using an overall GMAV for this Genus. Coho salmon appear to be exquisitely sensitive to 6PPD-quinone, and this is strengthened by the three independent peer-reviewed studies that reported very similar LC50 values (Tian et al. 2022; Greer et al. 2023; Lo et al. 2023). These values, generating a SMAV of 68 ng/L for coho, indicate that 6PPD-quinone may be the most acutely toxic xenobiotic known to fish, and exemplify the importance of establishing water quality guidelines for this chemical to protect aquatic biota. I also appreciated the additional analysis in section 4.1 that calculated a GMAV to show that this value would underestimate ecological risk and not be protective of coho salmon. Based on this analysis, I agree with not using an *Oncorhynchus* GMAV in deriving this SSSV. I am not familiar with the approach used to derive the FAV, but suggest that this calculation be double-checked to ensure that the 5th percentile of the SSD is truly an order of magnitude lesser than the SMAV for coho salmon. In my opinion the FAV appears to be overly conservative. 2.2.b. Yes, as mentioned above the strongest science in deriving this SSSV are the three independent peer-reviewed studies that reported very similar LC50 values for coho salmon. 2.2.c. Yes, in my opinion the screening level of 3 ng/L would be protective of aquatic life. I am not sure if this concentration can even be quantitated in water samples using modern analytical chemistry techniques.
Reviewer 2	2.2.a. The technical approach used to derive the screening value (SV) for 6PPD-q follows EPA's guidelines for deriving aquatic life criteria in general. The document discusses modifications to the guidelines in terms of minimum taxa data requirements, test duration, and some other departures from standard test protocols in terms of test data that are considered acceptable or unacceptable to use quantitatively to derive an SV. Given the fairly low persistence of 6PPD-q in water exposed to oxygen, and the few toxicity test data currently available that enable a statistically derived point estimate endpoint (for example LC or EC50), the modifications employed are scientifically justified. Specifically, an LC or EC50 based on a test duration greater than or equal to 24 hours rather than 48 or 96 hours depending on the species, should be acceptable given the chemical properties of 6PPD-q and observed toxicity responses in laboratory exposures. It may be useful to incorporate what is known regarding time to death or immobility based on test results to further support the use of 24-hour test endpoints to derive an acute SV.

2.2. The Technical Approach Used to Derive the Draft Screening Values	
Reviewer	**Comments**
	2.2.b. The study by Tian et al (2021) discussed in this document noted the rapidity with which swimming or locomotory effects on Coho salmon were observed in exposures to 6PPD-q (< 6 hours), which inevitably leads to death. This appears to be supported by field data on the urban runoff mortality syndrome affecting especially Coho salmon in the Pacific Northwest as well. While EPA's aquatic life criteria guidelines rely on apical population level effects, for example mortality or immobility, for 6PPD-q an argument could be made that a 24-hour mortality-based endpoint may not be protective enough due to the fast-acting effects on locomotion and loss of equilibrium in Coho salmon juveniles and adults. It is recommended that EPA examine whether an effect endpoint based on abnormal swimming behavior in < 12-hour exposures is protective of an endpoint based on 24 hours using mortality as the measure of effect. 2.2.c. The technical approach used to derive the SV for 6PPD-q appears to be consistent with the protection of aquatic life based on the test data currently available; that is, Coho salmon appear to be the most sensitive species of those tested thus far. Therefore, protecting Coho salmon from acute effects, with a safety margin provided by EPA's acute criterion derivation procedure used in this document, will hopefully protect aquatic life overall. However, relatively few species have been tested thus far although that is likely to change over the next few years given worldwide attention now on 6PPD-q. The document should make it even clearer that the SV for 6PPD-q is based on data obtained thus far and may have high uncertainty.
Reviewer 3	2.2.a. The technical approach in this report generally followed the US EPA guide developed for determining screening value that is outlined in the EPA's "*Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses"* (U.S.EPA 1985). Although these guidelines are becoming aged, they provide a logical, stepwise methodology to determine the screening values sought in this report. There were some technical challenges. Several toxicity tests reported only nominal 6PPD-quinone concentrations. Due to the limited availability of 6PPD-quinone toxicity data for aquatic life, these nominal concentrations were used for several studies without measuring 6PPD-quinone concentrations and were combined with studies that measured 6PPD-quinone concentrations. This approach, although not ideal, is consistent with the 1985 Guidelines. Acute toxicity tests on fish, specified by the EPA, should have at least 72 hours of exposure and ideally 96 hour exposures). However, several studies for 6PPD-quinone conducted tests with 24 hours of exposure, with the idea that reduced exposure duration modeled real-world scenarios. Also, several studies exceeded EPA's Test Quality Guidelines for biomass loading in fish toxicity studies (0.8 g/L in static tests for most fish species). However, if other study variables met test quality guidelines, and the test organisms did not appear to be stressed with acceptable levels of dissolved oxygen and ammonia, then the test was included for quantitative derivation of the acute screening value.

2.2. The Technical Approach Used to Derive the Draft Screening Values	
Reviewer	**Comments**
	The Lo et al. (2023) and Greer et. (2023) studies did not report how they determined the dilution series for the 24-h static renewal test, suggesting no range-finding test was employed. However, the concentration ranges used in the two studies were similar. The Tian et al. (2022) study did employ range-finding tests prior to setting up a definitive static 24-h toxicity test but the actual 6PPD-quinone concentrations used were only reported as "narrowly focused within the concentration range where partial mortality was expected. In summary, for the purposes of developing an early acute toxicity screening value the methodology appears reasonable. 2.2.b. The science does appear to support the goal of this project, which is development of an acute toxicity screening value. The value developed fills a need to quickly establish protective values for 6PPD-quinone, especially for sensitive salmonids. This Final Acute Value (FAV) was calculated using the methods described in the U.S. EPA 1985 and divided by two to develop a Sensitive Salmonid Screening Value (SSSV) for acute exposures of 6PPD-quinone in freshwater was determined to be **0.0031 µg /L** (or 3.1 ng/L). This value is expected to be protective of freshwater genera potentially exposed to 6PPD-quinone. 2.2.c. All available studies relating to the acute toxicological effects of 6PPD-quinone on aquatic life were considered. Data for possible inclusion were obtained from published literature reporting acute and chronic exposures of 6PPD-quinone that were associated with mortality, survival, growth, and reproduction. Acute data meeting quality objectives were utilized quantitatively in deriving the draft SSSV for acute exposures to 6PPD-quinone in freshwater. Thus, for the taxa already tested, the results of the identified studies allow some level of protection of aquatic life. However, not all important taxa were considered. Because of a limited time interval between when the 6PPD-quinone metabolite was identified and a literature search was conducted for freshwater alga or vascular plants, no toxicity data for these taxa were identified. Thus, EPA was unable to determine if aquatic plants may be more sensitive to 6PPD-quinone than aquatic animals. Given that this preliminary SSSV is intended as information to protect sensitive aquatic life species, such as salmonids, this screening value was derived without the use of aquatic plant data and thus photosynthesizing taxa may not be protected. It is unfortunate that no toxicity data on phytoplankton or vascular plants are available, due to their important role as the trophic base of the aquatic ecosystem.

2.3 Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD-quinone document.

2.3.a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?

2.3.b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?

2.3.c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

2.3. The Toxicity Data used to Derive the Screening Values	
Reviewer	**Comments**
Reviewer 1	2.3.a. EPA made the most out of the limited data available at this time. I agree that data were insufficient to currently derive an acute freshwater criterion, and agree with first developing a preliminary draft screening value (in this case a SSSV) for 6PPD-quinone. I am not sure why the SSSV term is being introduced in this case, because certainly the proposed screening level of 3 ng/L would be protective of all freshwater biota? I also agree that data are not sufficient to derive a chronic screening value for freshwater. Given the lack of saltwater/estuarine species data, I also concur with the inability to derive a screening level for these systems. However, a quick literature search of recently published journal articles indicates that there are some limited data available for marine invertebrates (albeit not enough to meet the MDR). 2.3.b. Yes, I agree with the EPA decisions on which data to include or exclude in the derivation of the screening value. As mentioned below in 3(c), a recent study in four additional invertebrate species strengthens the conclusion that freshwater invertebrates are relatively tolerant of acute 6PPD-quinone exposure compared to certain salmonids. 2.3.c. A recently published study determined acute toxicity data for four additional freshwater invertebrate species that could be included in derivation of the screening level (Prosser et al. 2023; cited below). This was a well conducted study published in a top tier journal within this field. Prosser RS, Salole J, Hang S. 2023. Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environmental Pollution 337: 122512. doi: 10.1016/j.envpol.2023.122512
Reviewer 2	2.3.a. The toxicity data obtained and discussed in this document appears as comprehensive as EPA can be at this time. The document is clear regarding the limitations of the data and it may be worth adding that data are currently limited because it is only very recently (the past 3 years or so) that the significance of 6PPD-q has been identified in terms of aquatic life risk. 2.3.b. The data selected or not selected to derive the SV for 6PPD-q appears scientifically justified and consistent with the goal of protecting aquatic life. As noted in my response to charge question 1, given the low persistence of 6PPD-q and the few test data

2.3. The Toxicity Data used to Derive the Screening Values	
Reviewer	**Comments**
	available, endpoints based on > 24 hour exposures, and/or higher fish loading per test chamber are acceptable to use in this case given other test acceptance rationale applied in the document (e.g., dissolved oxygen and ammonia concentration were acceptably low and control survival met EPA's test acceptability criterion for acute tests despite higher fish loading). One question I had was whether the study by Hiki and Yamamoto (2022) cited in this document, should be included as quantitatively acceptable test data. This study used only one replicate per concentration, and so It would be important in this case to confirm that the concentration-response relationship observed in this test supports the LC50 reported. 2.3.c. This reviewer is not aware of other toxicity test data that have been published and would satisfy basic requirements of acceptability according to EPA's aquatic life criteria guidelines- for example, test data based on organism exposure to the chemical of interest only and not a field study for example, or studies testing tire leachates without a toxicity identification evaluation similar to what Tian et al (2021) published.
Reviewer 3	2.3.a. To develop a comprehensive data set sufficient for representing the risks of 6PPD-quinone to sensitive aquatic life, the following taxonomic classes need to be evaluated: a. A Salmonid b. A second bony fish, preferably a commercially or recreationally important warmwater species c. A representative Chordate (may be bony fish or amphibian) d. A planktonic crustacean e. A benthic crustacean f. An insect g. A non-arthropod or chordate (e.g., Rotifera, Annelida, Mollusca) h. An insect or any phylum not already represented For the present report, only data from testing salmonids and a zebra fish were used. The salmonids used were: chinook, coho, and sockeye salmon; brook and rainbow trout; and white spotted char. All the tests were acute. The authors state in a "yes we have no bananas" format that chronic toxicity data for 6PPD-quinone were limited. In actuality, there were *no* chronic studies available. Therefore, no chronic screening value could be derived. Thus, as in the 6PPD study, a valuable data set is not available. Given the lack of sufficient testing among the required animal and plant groups, the risks to sensitive aquatic life are not adequately characterized.

2.3. The Toxicity Data used to Derive the Screening Values	
Reviewer	**Comments**
	The available acute data for salmonids and the zebra fish were, however, available for developing a SSSV value as a "first-pass" evaluation for the toxicity of 6PPD-quinone and thus are useful for protecting what may turn out to be the most sensitive aquatic taxa, salmonid fishes, especially coho salmon. 2.3.b. Acute freshwater toxicity tests with 6PPD-quinone exposures were considered for calculating the 6PPD-quinone screening value. Qualitative studies not included in the numeric screening value derivation were either rejected outright or used as supporting information. Those data deemed acceptable were evaluated for meeting the threshold of the EPA guidelines (1985) for minimum data requirements (MDRs). The MDRs were not met for acute freshwater criteria derivation for either animals or plants. Thus, it is appropriate that the national 304(a) ambient water quality criteria for the protection of aquatic life was not derived for 6PPD-quinone. However, EPA was able derive a protective acute SSSV for 6PPD-quinone in freshwater. Although this report states that this assessment was sufficient to protect aquatic life, at this time it can only protect sensitive salmonids from acute toxic effects of 6PPD-quinone. The only unused data reported was from the "Mahoney, H et al. (2022) study on mitochondrial dysfunction. The reason offered for this exclusion was that it was an invitro exposure on excised cell, rather than a whole-body exposure like the other toxicity tests. 2.3.c. I am not aware of additional relevant data that should be considered for this report.

2.4 Are the derived screening values appropriately protective of sensitive aquatic life?

2.4. The Derived Screening Values Protectiveness to Sensitive Aquatic Life	
Reviewer	**Comments**
Reviewer 1	Yes, in my opinion the screening level of 3 ng/L would be protective of aquatic life.
Reviewer 2	The EPA chose to separate the different species acute values for the genus *Oncorhynchus* in deriving the final acute value (FAV) rather than base the FAV on genus mean acute values (GMAVs) as typically calculated using EPA's aquatic life criteria guidelines. While the document provides a reasonable rationale for calculating the FAV using the separate species mean acute values (SMAVs) for *Oncorhynchus* I am concerned that the SV may be too conservative given even the lowest species value available for Coho salmon. Note that the proposed SV is more than 10 times lower than the lowest LC50 reported thus far and may be biased low because SMAVs were used. An alternative approach that EPA considered in Section 4.1 of this document is calculating the FAV based on genus mean acute values (GMAV's) as is typically done by EPA in developing aquatic life criteria; but in this case, the FAV should be lowered to protect the commercially important and most sensitive species, Coho salmon. The resulting FAV would then be 67 ng/L based on the Coho salmon SMAV and the SV would be one half the

2.4. The Derived Screening Values Protectiveness to Sensitive Aquatic Life	
Reviewer	**Comments**
	FAV or 34 ng/L, which is still lower than the most sensitive Coho salmon acute value. Lowering the FAV to protect the most sensitive species is in keeping with EPA's aquatic life criteria guidelines. Furthermore, there is fairly high confidence in the SMAV for Coho salmon because all three published studies obtained very similar LC50s. Field studies that have examined 6PPD-q concentration and pre-spawn mortality of Coho salmon in streams may shed light on an appropriate SV that is protective of aquatic life and yet not overly conservative.
Reviewer 3	There are insufficient data to derive a national 304(a) freshwater criteria for 6PPD-quinone. There are currently three quantitatively acceptable Genus Mean Acute Values (GMAVs), and the FAV calculation requires at least four (GMAVs) to calculate a criterion value. Thus, sensitive aquatic life *in toto* are not protected by the derived screening values. However, to establish protective values for 6PPD-quinone for sensitive salmonids alone, a preliminary draft SSSV for acute exposures to 6PPD-quinone was derived and the stated procedure for determining this value were appropriate. It is a bit disappointing that the available data for assessing 6PPD-quinone are so limited for the toxic agent causing Urban Runoff Mortality Syndrome, considering the problem was noted as early as 2011 (Spromberg, & Scholz) and the causal agent identified in 2018 (Peter et al.) and clearly linked in 2021 (Tian et al.). It would seem that a toxicant with significant effects on local aquatic ecosystem ecology, the commercial fishing industry, recreational angling and tourism, and native American subsistence groups would have received a faster track to comprehensively determining the environmental risks. Nevertheless, the present report remains a valuable tool for early evaluation of such risks.

3.0 ADDITIONAL REVIEWER COMMENTS

Reviewer	**Comments**
Reviewer 1	See minor edits provided in the draft document.
Reviewer 2	Additional literature regarding fate and transport for EPA's consideration • Baensch-Baltruschat, B., Kocher, B., Stock, F., & Reifferscheid, G. 2020. Tire and road wear particles (TRWP) - A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. *Science of The Total Environment*, 137823. • Challis, J. K., H. Popick, S. Prajapati, P. Harder, J. P. Giesy, K. McPhedran, and M. Brinkmann. 2021. Occurrences of Tire Rubber-Derived Contaminants in Cold-Climate Urban Runoff *Environmental Science & Technology Letters,* 8: 961-967 • French, B.F., D. H. Baldwin, J. Cameron, J. Prat, K. King, J. W. Davis, J. K. McIntyre, and N. L. Scholz. 2022. *Environmental Science & Technology Letters 9* (9), 733-738

Reviewer	Comments
	• Johannessen, C. P., Helm, P., Lashuk, B., Yargeau, V., & Metcalfe, C. D. 2021. The Tire Wear Compounds 6PPD-Quinone and 1,3-Diphenylguanidine in an Urban Watershed. Archives of Environmental Contamination and Toxicology. doi:https://doi.org/10.1016/j.envpol.2021.117659 • Johannessen, C., & Parnis, J. 2021. Environmental modelling of hexamethoxymethylmelamine, its transformation products, and precursor compounds: an emerging family of contaminants from tire wear. Chemosphere. doi:https://doi. org/10. 1016/j.chemo sphere.130914 • Johannessen, C., Helm, P., & Metcalfe, C. D. 2021. Detection of selected tire wear compounds in urban receiving waters. *Environ. Pollut.* • McIntyre, J.F., J. Prat, J. Cameron, J. Wetzel, E. Murdock, K.T. Peter, Z. Tian, C. Mackenzie, J. Lundin, J. D. Stark, K. King, J.W. Davis, E.P. Kolodziej, and N. L. Scholz. 2021. Treading Water: Tire Wear Particle Leachate Recreates an Urban Runoff Mortality Syndrome in Coho but Not Chum Salmon. Environ. Sci. Technol. 2021, 55, 11767−11774 • Peter, K.T., F. Hou, Z. Tian, C. Wu, M. Goehring, F. Liu, and E. P. Kolodziej. 2020. More Than a First Flush: Urban Creek Storm Hydrographs Demonstrate Broad Contaminant Pollutographs. Environ. Sci. Technol. 2020, 54, 10, 6152–6165. • Seiwert, B., Nihemaiti, M., Troussier, M., Weyrauch, S., & Thorsten, R. 2022. Abiotic oxidative transformation of 6-PPD and 6-PPD quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. *Water Research, 212*. • Unice, K. M., Bare, J. L., Kreider, M. L., & Panko, J. M. 2015. Experimental methodology for assessing the environmental fate of organic chemicals in polymer matrices using column leaching studies and OECD 308 water/sediment systems: Application to tire and road wear particles. *The Science of the Total Environment, 533*, 476-487.

APPENDIX A

CHARGE TO REVIEWERS

Technical Charge to External Peer Reviewers

Contract GSA GS-00F-079CA; BPA #68HERH23A0019
Call Order 68HERH23F0365 (ERG Call Order #04)
October 2023

External Peer Review of EPA's Draft Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) in Freshwater

BACKGROUND

The purpose of EPA's Preliminary Draft Screening Value for Acute Exposures to 6PPD (N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine) in Freshwater and Preliminary Draft Sensitive Salmonid Screening Value for Acute Exposures to 6PPD-Quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) in Freshwater is to provide EPA's scientific rationale for the draft screening values for 6PPD and 6PPD-quinone focused on the protection of aquatic life. The criteria are designed to solely protect sensitive aquatic life and is based on the best available data and best professional scientific judgements on the toxicological effects of 6PPD and 6PPD-quinone to aquatic life.

N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine (6PPD) is an additive to vehicle tire rubber, where it functions to protect rubber from reactions with ozone and oxygen, which can lead to degradation and cracking. Recent research has determined the ozonation of (6PPD) produces the reaction product 6PPD-quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) through hydrolysis and photodegradation. 6PPD-quinone recently has been shown to present an environmental threat based on its toxicity to salmon, where it has been identified as the causal agent in urban runoff mortality syndrome (URMS) observed in the Puget Sound area of Washington state.

Relatively limited 6PPD and 6PPD-quinone toxicity studies have been conducted on various aquatic taxa, including numerous species of fish and invertebrates, indicating that exposure to 6PPD and 6PPD-quinone through the aquatic environment causes population level effects, particularly mortality to acute exposures across aquatic life in the case for 6PPD and to fish such as salmonids, which appear to be sensitive to 6PPD-quinone exposures in particular. In these drafts, EPA provides support for and outlines the derivation of screening values for 6PPD and 6PPD-quinone that would be protective of sensitive aquatic life.

CHARGE QUESTIONS

1) Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD-quinone to aquatic life.

2) Please comment on the technical approach used to derive the draft screening vales presented in EPA's preliminary *Draft Sensitive Salmonid Screening Value for Acute Exposures to 6PPD-Quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) in Freshwater*.

    a. Is the technical approach used to derive the screening values logical?
    b. Does the science support the conclusions?
    c. Is it consistent with the protection of aquatic life?

3) Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD-quinone document.

   a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?
   b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?
   c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

4) Are the derived screening values appropriately protective of sensitive aquatic life?

APPENDIX B

INDIVIDUAL REVIEWER COMMENTS

COMMENTS SUBMITTED BY

REVIEWER 1

External Peer Review of EPA's Draft Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) in Freshwater

CHARGE QUESTIONS

1. **Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD-quinone to aquatic life.**

Like the 6PPD document, the 6PPD-quinone document is well written and flows logically. The background summary of environmental fate and distribution of 6PPD-quinone was good. I appreciated the study summaries being described in order from most sensitive to least sensitive species, and separating quantitative and qualitative studies into separate sections. Like the 6PPD document, I have provided a marked-up version with minor editorial suggestions and comments.

There are some inconsistencies in the descriptions of experimental procedures when summarizing the various studies. The document must be consistent and specific when describing the control treatments among studies. In all instances, specify "solvent control" or "solvent vehicle control" when such a control was employed, and when known state the solvent concentration occurring in the actual exposure vessel as a percentage volume/volume. In addition, please specify what is meant by a negative control. Is this water-only without solvent added? In the study descriptions (initial sentence of each) be consistent and always state whether 6PPD-quinone was measured or unmeasured.

2. **Please comment on the technical approach used to derive the draft screening values presented in EPA's Preliminary Draft Screening Value for Acute Exposures to 6PPD-Quinone (N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine quinone) in Freshwater.**

    a. **Is the technical approach used to derive the screening values logical?**

    The technical approach generally follows EPA's established procedures for developing a species sensitivity distribution to derive a water quality criterion. However, due to the paucity of acute and especially chronic freshwater toxicity data available at this time, some modifications of these procedures were employed to derive a draft screening value.

    I agree with using studies that determined acute toxicity (LC50) over a time period of greater than or equal to 24 hours, because this enhances the limited dataset and particularly because this represents an environmentally realistic exposure duration that would occur in freshwaters after precipitation events.

    I agree with the rationale for separating out the Oncorhynchus data and not using an overall GMAV for this Genus. Coho salmon appear to be exquisitely sensitive to 6PPD-quinone, and this is strengthened by the three independent peer-reviewed studies that reported very similar LC50 values (Tian et al. 2022; Greer et al. 2023; Lo et al. 2023). These values, generating a SMAV of 68 ng/L for coho, indicate that 6PPD-quinone may be the most acutely toxic xenobiotic known to fish, and exemplify the importance of establishing water quality guidelines for this chemical to protect aquatic biota.

I also appreciated the additional analysis in section 4.1 that calculated a GMAV to show that this value would underestimate ecological risk and not be protective of coho salmon. Based on this analysis, I agree with not using an Oncorhynchus GMAV in deriving this SSSV.

I am not familiar with the approach used to derive the FAV, but suggest that this calculation be double-checked to ensure that the 5th percentile of the SSD is truly an order of magnitude lesser than the SMAV for coho salmon. In my opinion the FAV appears to be overly conservative.

b. Does the science support the conclusions?

Yes, as mentioned above the strongest science in deriving this SSSV are the three independent peer-reviewed studies that reported very similar LC50 values for coho salmon.

c. Is it consistent with the protection of aquatic life?

Yes, in my opinion the screening level of 3 ng/L would be protective of aquatic life. I am not sure if this concentration can even be quantitated in water samples using modern analytical chemistry techniques.

3. Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD-Quinone document.

a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?

EPA made the most out of the limited data available at this time. I agree that data were insufficient to currently derive an acute freshwater criterion, and agree with first developing a preliminary draft screening value (in this case a SSSV) for 6PPD-quinone. I am not sure why the SSSV term is being introduced in this case, because certainly the proposed screening level of 3 ng/L would be protective of all freshwater biota?

I also agree that data are not sufficient to derive a chronic screening value for freshwater. Given the lack of saltwater/estuarine species data, I also concur with the inability to derive a screening level for these systems. However, a quick literature search of recently published journal articles indicates that there are some limited data available for marine invertebrates (albeit not enough to meet the MDR).

b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?

Yes, I agree with the EPA decisions on which data to include or exclude in the derivation of the screening value. As mentioned below in 3(c), a recent study in four additional invertebrate species strengthens the conclusion that freshwater invertebrates are relatively tolerant of acute 6PPD-quinone exposure compared to certain salmonids.

c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

A recently published study determined acute toxicity data for four additional freshwater invertebrate species that could be included in derivation of the screening level (Prosser et al. 2023; cited below). This was a well conducted study published in a top tier journal within this field.

Prosser RS, Salole J, Hang S. 2023. Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environmental Pollution 337: 122512. doi: 10.1016/j.envpol.2023.122512

4. Are the derived screening values appropriately protective of sensitive aquatic life?

Yes, in my opinion the screening level of 3 ng/L would be protective of aquatic life.

COMMENTS SUBMITTED BY

REVIEWER 2

External Peer Review of EPA's Draft Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) in Freshwater

CHARGE QUESTIONS

1. Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD-q-quinone to aquatic life.

The document for 6PPD-q follows EPA's current aquatic life criteria framework, which is organized in a similar manner to EPA 's ecological risk assessment guidance. The organization and construction of this document is easy to follow, and the writing is clearly presented for the most part. However, the document has several grammatical and typographical errors which should be corrected using a thorough editorial review before finalizing.

This document summarizes what is known about the chemistry, fate, and transport properties of 6PPD-q in the environment. The document limits this discussion to freshwater as opposed to salt water as well, which is due to the paucity of data regarding fate and transport of 6PPD-q in saltwater. While the document later explains the lack of marine toxicity data, perhaps EPA should also acknowledge the current lack of fate and transport information in marine environments as well.

This document mentions tire wear and wet weather runoff from roads as a major source of 6PPD-q in surface waters. There has been a fair amount of research in the past three years regarding fate and transport of 6PPD-q in surface waters, including its persistence in ice and snow moved to roadsides (and subsequent input to streams from melting snow) and from runoff of roadside dust, which may be important sources of 6PPD-q to streams. Some recent field data are coincident with Coho salmon pre-spawn mortality in the Pacific Northwest as well. EPA should consider incorporating a brief summary of relevant publications in this regard as some of those data help inform the challenges in controlling sources of 6PPD-q and the potential persistence of 6PPD-q under certain environmental conditions. This information may help support EPA's SV for 6PPD-q. I included some relevant citations of which I am aware below.

2. Please comment on the technical approach used to derive the draft screening vales presented in EPA's Preliminary Draft Sensitive Salmonid Screening Value for Acute Exposures to 6PPD-q-Quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) in Freshwater.

a. Is the technical approach used to derive the screening values logical?

The technical approach used to derive the screening value (SV) for 6PPD-q follows EPA's guidelines for deriving aquatic life criteria in general. The document discusses modifications to the guidelines in terms of minimum taxa data requirements, test duration, and some other departures from standard test protocols in terms of test data that are considered acceptable or unacceptable to use quantitatively to derive an SV. Given the fairly low persistence of 6PPD-q in water exposed to oxygen, and the few toxicity test data currently available that enable a statistically derived point estimate endpoint (for example LC or EC50), the modifications employed are scientifically justified. Specifically, an LC or EC50 based on a test duration greater than or equal to 24 hours rather than 48 or 96 hours depending on the species, should be acceptable given the chemical properties of 6PPD-q and observed toxicity responses in laboratory exposures. It may be useful to incorporate what is

known regarding time to death or immobility based on test results to further support the use of 24-hour test endpoints to derive an acute SV.

b. Does the science support the conclusions?

The study by Tian et al (2021) discussed in this document noted the rapidity with which swimming or locomotory effects on Coho salmon were observed in exposures to 6PPD-q (< 6 hours), which inevitably leads to death. This appears to be supported by field data on the urban runoff mortality syndrome affecting especially Coho salmon in the Pacific Northwest as well. While EPA's aquatic life criteria guidelines rely on apical population level effects, for example mortality or immobility, for 6PPD-q an argument could be made that a 24-hour mortality-based endpoint may not be protective enough due to the fast-acting effects on locomotion and loss of equilibrium in Coho salmon juveniles and adults. It is recommended that EPA examine whether an effect endpoint based on abnormal swimming behavior in < 12-hour exposures is protective of an endpoint based on 24 hours using mortality as the measure of effect.

c. Is it consistent with the protection of aquatic life?

The technical approach used to derive the SV for 6PPD-q appears to be consistent with the protection of aquatic life based on the test data currently available; that is, Coho salmon appear to be the most sensitive species of those tested thus far. Therefore, protecting Coho salmon from acute effects, with a safety margin provided by EPA's acute criterion derivation procedure used in this document, will hopefully protect aquatic life overall. However, relatively few species have been tested thus far although that is likely to change over the next few years given worldwide attention now on 6PPD-q. The document should make it even clearer that the SV for 6PPD-q is based on data obtained thus far and may have high uncertainty.

3. Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD-q-Quinone document.

a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?

The toxicity data obtained and discussed in this document appears as comprehensive as EPA can be at this time. The document is clear regarding the limitations of the data and it may be worth adding that data are currently limited because it is only very recently (the past 3 years or so) that the significance of 6PPD-q has been identified in terms of aquatic life risk.

b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?

The data selected or not selected to derive the SV for 6PPD-q appears scientifically justified and consistent with the goal of protecting aquatic life. As noted in my response to charge question 1, given the low persistence of 6PPD-q and the few test data available, endpoints based on > 24 hour exposures, and/or higher fish loading per test chamber are acceptable to use in this case given other test acceptance rationale applied in the document (e.g., dissolved oxygen and ammonia concentration were acceptably low and control survival met EPA's test acceptability criterion for acute tests despite higher fish loading).

One question I had was whether the study by Hiki and Yamamoto (2022) cited in this document, should be included as quantitatively acceptable test data. This study used only one replicate per concentration, and so It would be important in this case to confirm that the concentration-response relationship observed in this test supports the LC50 reported.

c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

This reviewer is not aware of other toxicity test data that have been published and would satisfy basic requirements of acceptability according to EPA's aquatic life criteria guidelines- for example, test data based on organism exposure to the chemical of interest only and not a field study for example, or studies testing tire leachates without a toxicity identification evaluation similar to what Tian et al (2021) published.

4. Are the derived screening values appropriately protective of sensitive aquatic life?

The EPA chose to separate the different species acute values for the genus Oncorhynchus in deriving the final acute value (FAV) rather than base the FAV on genus mean acute values (GMAVs) as typically calculated using EPA's aquatic life criteria guidelines. While the document provides a reasonable rationale for calculating the FAV using the separate species mean acute values (SMAVs) for Oncorhynchus I am concerned that the SV may be too conservative given even the lowest species value available for Coho salmon. Note that the proposed SV is more than 10 times lower than the lowest LC50 reported thus far and may be biased low because SMAVs were used. An alternative approach that EPA considered in Section 4.1 of this document is calculating the FAV based on genus mean acute values (GMAV's) as is typically done by EPA in developing aquatic life criteria; but in this case, the FAV should be lowered to protect the commercially important and most sensitive species, Coho salmon. The resulting FAV would then be 67 ng/L based on the Coho salmon SMAV and the SV would be one half the FAV or 34 ng/L, which is still lower than the most sensitive Coho salmon acute value. Lowering the FAV to protect the most sensitive species is in keeping with EPA's aquatic life criteria guidelines. Furthermore, there is fairly high confidence in the SMAV for Coho salmon because all three published studies obtained very similar LC50s. Field studies that have examined 6PPD-q concentration and pre-spawn mortality of Coho salmon in streams may shed light on an appropriate SV that is protective of aquatic life and yet not overly conservative.

Additional literature regarding fate and transport for EPA's consideration

- Baensch-Baltruschat, B., Kocher, B., Stock, F., & Reifferscheid, G. 2020. Tire and road wear particles (TRWP) - A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. Science of The Total Environment, 137823.
- Challis, J. K., H. Popick, S. Prajapati, P. Harder, J. P. Giesy, K. McPhedran, and M. Brinkmann. 2021. Occurrences of Tire Rubber-Derived Contaminants in Cold-Climate Urban Runoff Environmental Science & Technology Letters, 8: 961-967
- French, B.F., D. H. Baldwin, J. Cameron, J. Prat, K. King, J. W. Davis, J. K. McIntyre, and N. L. Scholz. 2022. Environmental Science & Technology Letters 9 (9), 733-738

- Johannessen, C. P., Helm, P., Lashuk, B., Yargeau, V., & Metcalfe, C. D. 2021. The Tire Wear Compounds 6PPD-Quinone and 1,3-Diphenylguanidine in an Urban Watershed. Archives of Environmental Contamination and Toxicology. doi:https://doi.org/10.1016/j.envpol.2021.117659
- Johannessen, C., & Parnis, J. 2021. Environmental modelling of hexamethoxymethylmelamine, its transformation products, and precursor compounds: an emerging family of contaminants from tire wear. Chemosphere. doi:https://doi. org/10. 1016/j.chemo sphere.130914
- Johannessen, C., Helm, P., & Metcalfe, C. D. 2021. Detection of selected tire wear compounds in urban receiving waters. Environ. Pollut.
- McIntyre, J.F., J. Prat, J. Cameron, J. Wetzel, E. Murdock, K.T. Peter, Z. Tian, C. Mackenzie, J. Lundin, J. D. Stark, K. King, J.W. Davis, E.P. Kolodziej, and N. L. Scholz. 2021. Treading Water: Tire Wear Particle Leachate Recreates an Urban Runoff Mortality Syndrome in Coho but Not Chum Salmon. Environ. Sci. Technol. 2021, 55, 11767−11774
- Peter, K.T., F. Hou, Z. Tian, C. Wu, M. Goehring, F. Liu, and E. P. Kolodziej. 2020. More Than a First Flush: Urban Creek Storm Hydrographs Demonstrate Broad Contaminant Pollutographs. Environ. Sci. Technol. 2020, 54, 10, 6152–6165.
- Seiwert, B., Nihemaiti, M., Troussier, M., Weyrauch, S., & Thorsten, R. 2022. Abiotic oxidative transformation of 6-PPD and 6-PPD quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. Water Research, 212.
- Unice, K. M., Bare, J. L., Kreider, M. L., & Panko, J. M. 2015. Experimental methodology for assessing the environmental fate of organic chemicals in polymer matrices using column leaching studies and OECD 308 water/sediment systems: Application to tire and road wear particles. The Science of the Total Environment, 533, 476-487.

COMMENTS SUBMITTED BY

REVIEWER 3

External Peer Review of EPA's Draft Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) in Freshwater

CHARGE QUESTIONS

1. **Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD-quinone to aquatic life.**

Overall this is a well-written report but it seems a bit more "hurried" than the report on the toxicity of 6PPD. Although not numerous, there are more typo's and grammatical errors than in the 6PPD document. There are also a few places where the document is unclear. For example, I am not sure what the statement from Section 2.2.1 "Given that this preliminary SSSV is intended as information to protect sensitive aquatic life species, such as salmonids, this screening value was derived without the use of aquatic plant data." is describing. The authors seem to be stating that plant data were not used because they were only interested in salmonids, yet in actuality no plant data exists. This report provides a sufficient understanding of the risk assessment of 6PPPD-quinone in the early stages of evaluation on a single taxon of organisms.

2. **Please comment on the technical approach used to derive the draft screening vales presented in EPA's Preliminary Draft Sensitive Salmonid Screening Value for Acute Exposures to 6PPD-Quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) in Freshwater.**

   a. **Is the technical approach used to derive the screening values logical?**

   The technical approach in this report generally followed the US EPA guide developed for determining screening value that is outlined in the EPA's "*Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses"* (U.S.EPA 1985). Although these guidelines are becoming aged, they provide a logical, stepwise methodology to determine the screening values sought in this report.

   There were some technical challenges. Several toxicity tests reported only nominal 6PPD-quinone concentrations. Due to the limited availability of 6PPD-quinone toxicity data for aquatic life, these nominal concentrations were used for several studies without measuring 6PPD-quinone concentrations and were combined with studies that measured 6PPD-quinone concentrations. This approach, although not ideal, is consistent with the 1985 Guidelines.

   Acute toxicity tests on fish, specified by the EPA, should have at least 72 hours of exposure and ideally 96 hour exposures). However, several studies for 6PPD-quinone conducted tests with 24 hours of exposure, with the idea that reduced exposure duration modeled real-world scenarios.

   Also, several studies exceeded EPA's Test Quality Guidelines for biomass loading in fish toxicity studies (0.8 g/L in static tests for most fish species). However, if other study variables met test quality guidelines, and the test organisms did not appear to be stressed with acceptable levels of dissolved oxygen and ammonia, then the test was included for quantitative derivation of the acute screening value.

   The Lo et al. (2023) and Greer et. (2023) studies did not report how they determined the dilution series for the 24-h static renewal test, suggesting no range-finding test was employed. However, the concentration ranges used in the two studies were similar. The Tian et al. (2022) study did employ

range-finding tests prior to setting up a definitive static 24-h toxicity test but the actual 6PPD-quinone concentrations used were only reported as "narrowly focused within the concentration range where partial mortality was expected.

In summary, for the purposes of developing an early acute toxicity screening value the methodology appears reasonable.

b. Does the science support the conclusions?

The science does appear to support the goal of this project, which is development of an acute toxicity screening value. The value developed fills a need to quickly establish protective values for 6PPD-quinone, especially for sensitive salmonids. This Final Acute Value (FAV) was calculated using the methods described in the U.S. EPA 1985 and divided by two to develop a Sensitive Salmonid Screening Value (SSSV) for acute exposures of 6PPD-quinone in freshwater was determined to be **0.0031 µg /L** (or 3.1 ng/L). This value is expected to be protective of freshwater genera potentially exposed to 6PPD-quinone.

c. Is it consistent with the protection of aquatic life?

All available studies relating to the acute toxicological effects of 6PPD-quinone on aquatic life were considered. Data for possible inclusion were obtained from published literature reporting acute and chronic exposures of 6PPD-quinone that were associated with mortality, survival, growth, and reproduction. Acute data meeting quality objectives were utilized quantitatively in deriving the draft SSSV for acute exposures to 6PPD-quinone in freshwater. Thus, for the taxa already tested, the results of the identified studies allow some level of protection of aquatic life. However, not all important taxa were considered. Because of a limited time interval between when the 6PPD-quinone metabolite was identified and a literature search was conducted for freshwater alga or vascular plants, no toxicity data for these taxa were identified. Thus, EPA was unable to determine if aquatic plants may be more sensitive to 6PPD-quinone than aquatic animals. Given that this preliminary SSSV is intended as information to protect sensitive aquatic life species, such as salmonids, this screening value was derived without the use of aquatic plant data and thus photosynthesizing taxa may not be protected. It is unfortunate that no toxicity data on phytoplankton or vascular plants are available, due to their important role as the trophic base of the aquatic ecosystem.

3. Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD-Quinone document.

a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?

To develop a comprehensive data set sufficient for representing the risks of 6PPD-quinone to sensitive aquatic life, the following taxonomic classes need to be evaluated:

a. A Salmonid
b. A second bony fish, preferably a commercially or recreationally important warmwater species

c. A representative Chordate (may be bony fish or amphibian)
d. A planktonic crustacean
e. A benthic crustacean
f. An insect
g. A non-arthropod or chordate (e.g., Rotifera, Annelida, Mollusca)
h. An insect or any phylum not already represented

For the present report, only data from testing salmonids and a zebra fish were used. The salmonids used were: chinook, coho, and sockeye salmon; brook and rainbow trout; and white spotted char. All the tests were acute. The authors state in a "yes we have no bananas" format that chronic toxicity data for 6PPD-quinone were limited. In actuality, there were *no* chronic studies available. Therefore, no chronic screening value could be derived. Thus, as in the 6PPD study, a valuable data set is not available. Given the lack of sufficient testing among the required animal and plant groups, the risks to sensitive aquatic life are not adequately characterized.

The available acute data for salmonids and the zebra fish were, however, available for developing a SSSV value as a "first-pass" evaluation for the toxicity of 6PPD-quinone and thus are useful for protecting what may turn out to be the most sensitive aquatic taxa, salmonid fishes, especially coho salmon.

b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?

Acute freshwater toxicity tests with 6PPD-quinone exposures were considered for calculating the 6PPD-quinone screening value. Qualitative studies not included in the numeric screening value derivation were either rejected outright or used as supporting information. Those data deemed acceptable were evaluated for meeting the threshold of the EPA guidelines (1985) for minimum data requirements (MDRs). The MDRs were not met for acute freshwater criteria derivation for either animals or plants. Thus, it is appropriate that the national 304(a) ambient water quality criteria for the protection of aquatic life was not derived for 6PPD-quinone. However, EPA was able derive a protective acute SSSV for 6PPD-quinone in freshwater. Although this report states that this assessment was sufficient to protect aquatic life, at this time it can only protect sensitive salmonids from acute toxic effects of 6PPD-quinone.

The only unused data reported was from the "Mahoney, H et al. (2022) study on mitochondrial dysfunction. The reason offered for this exclusion was that it was an invitro exposure on excised cell, rather than a whole-body exposure like the other toxicity tests.

c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

I am not aware of additional relevant data that should be considered for this report.

4. Are the derived screening values appropriately protective of sensitive aquatic life?

There are insufficient data to derive a national 304(a) freshwater criteria for 6PPD-quinone. There are currently three quantitatively acceptable Genus Mean Acute Values (GMAVs), and the FAV calculation

requires at least four (GMAVs) to calculate a criterion value. Thus, sensitive aquatic life *in toto* are not protected by the derived screening values. However, to establish protective values for 6PPD-quinone for sensitive salmonids alone, a preliminary draft SSSV for acute exposures to 6PPD-quinone was derived and the stated procedure for determining this value were appropriate.

It is a bit disappointing that the available data for assessing 6PPD-quinone are so limited for the toxic agent causing Urban Runoff Mortality Syndrome, considering the problem was noted as early as 2011 (Spromberg, & Scholz) and the causal agent identified in 2018 (Peter et al.) and clearly linked in 2021 (Tian et al.). It would seem that a toxicant with significant effects on local aquatic ecosystem ecology, the commercial fishing industry, recreational angling and tourism, and native American subsistence groups would have received a faster track to comprehensively determining the environmental risks. Nevertheless, the present report remains a valuable tool for early evaluation of such risks.