United States Environmental Protection Agency
Office of Water
EPA-822-R-24007
May 2024

# EPA Response to the

# External Peer Review of EPA's

# "*Draft Aquatic Life Screening Value for 6PPD in Freshwater*"

# May 2024

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, D.C.

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## 1.0 INTRODUCTION

The U.S. Environmental Protection Agency (EPA) Office of Water (OW) has developed draft screening values to protect aquatic life from the short-term (acute) exposures caused by the presence of 6PPD and 6PPD-quinone in freshwater. Because there are only limited data for 6PPD and 6PPD-quinone in the ecotoxicity literature, the EPA developed aquatic life screening values for acute exposures in freshwater, rather than national recommended Ambient Water Quality Criteria (AWQC). The derivation of these screening values is described in two documents: *Acute Aquatic Life Screening Value for 6PPD in Freshwater* and *Aquatic Life Screening Value for 6PPD-quinone or 6PPD-q) in Freshwater.*

An independent letter peer review of the EPA's draft *Acute Aquatic Life Screening Value for 6PPD in Freshwater* was conducted in the fall (October through November 2023) by Eastern Research Group, Inc (ERG), a contractor for the EPA OW. The external peer review report can be found at the 6PPD Aquatic Life Screening Value website (https://www.epa.gov/wqc/acute-6ppd-aquatic-life-screening-value-freshwater). Independent peer review of the draft *Acute Aquatic Life Screening Value for 6PPD-quinone in Freshwater* document is covered in a separate set of external peer review and the EPA response documents.

This document provides the EPA's responses to external peer review comments on the draft 6PPD screening value document. Section 2.0 of this report presents the individual reviewer comments and the EPA's responses organized by charge question.

### 1.1 Development of the Draft Documents

The EPA obtained toxicity studies primarily via the EPA's ECOTOXicology Knowledgebase through September 2022 (and subsequently updated following the external peer review with newer studies through December 2023). Toxicity studies were carefully evaluated and thoroughly reviewed to ensure studies were of sufficient data quality to use in the derivation of screening values. For a number of published studies, the EPA consulted primary authors for clarifications on study methods and author-reported raw toxicity data during the data quality review phase to ensure that the studies used to derive the screening values were of high quality. The screening value document identifies those instances where the EPA obtained additional information from study authors.

The purpose of the Acute Aquatic Life Screening Value for 6PPD is to provide information under Section 304(a)(2) of the Clean Water Act (CWA) that states and authorized Tribes may consider for use in their water quality protection programs.

There were insufficient data to develop CWA Section 304(a)(1) recommended Aquatic Life Ambient Water Quality Criteria according to the EPA's method for developing criteria (*Guidelines for Developing Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*, U.S. EPA 1985). Rather, acute aquatic life screening values were derived for acute exposures in freshwaters due to the paucity of 6PPD data and the fact that many published studies on 6PPD were not conducted according to standard toxicity test guidance (e.g., EPA 850 Ecological Effects Test Guidelines; https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines). Study limitations included insufficient testing duration (24 hour-tests instead of the standard 96 hour acute fish test duration), overcrowding of tanks, and lack on analytical measurements throughout the tests for this unstable compound. There were insufficient data

to develop screening value or criteria for estuarine/marine waters or long (chronic) exposures. These issues are documented in the EPA's 6PPD screening value document. Addressing data limitations and extensively reviewing toxicity studies, the EPA derived an 6PPD Acute Aquatic Life Screening Value via a comprehensive, rigorous process that included collaborations across EPA scientists in OW, Office of Research and Development (ORD), and Region 10.

Subsequently, the EPA contracted with ERG to organize an independent, external peer review of both draft documents. External peer reviewer comments on the 6PPD screening value document and the EPA's responses to those comments are described in this report. Results of the 6PPD-quinone external peer review are documented in a separate report (https://www.epa.gov/wqc/acute-6ppd-q-aquatic-life-screening-value-freshwater).

### 1.2 Peer Reviewers

The EPA contractor identified, screened, and selected three experts who met technical selection criteria provided by the EPA and were determined by the contractor to have no conflict of interest in performing this review. The External Peer Review Report for 6PPD-q, including details on the external peer reviewer selection process, can be found at (https://www.epa.gov/wqc/acute-6ppd-aquatic-life-screening-value-freshwater).

The contractor provided reviewers with instructions, the draft Acute *Aquatic Life Screening Value for 6PPD in Freshwater*, and the charge to reviewers prepared by the EPA. Reviewers worked individually to develop written comments in response to the charge questions. After receiving reviewer comments, the contractor compiled responses by charge question (see Section 2.0) and included the responses organized by reviewer.

### 1.3 Charge Questions to Peer Reviewers

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

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

## 2.0 PEER REVIEWER COMMENTS AND EPA RESPONSES ORGANIZED BY CHARGE QUESTION

This section organizes reviewer comments by charge question.

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

| 2.1. Clarity of Document as it Relates to Assessing the Risk of 6PPD to Aquatic-Life | | |
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| **Reviewer** | **Reviewer Comments** | **EPA Response** |
| **1** | The document is generally well written and flows logically. The background summary of environmental fate and distribution of 6PPD was good. The explanation of how EPA derives water quality criteria was clear. I have provided a marked-up version with minor editorial suggestions and comments.<br><br>There are several 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 and control vessels as a percentage volume/volume. For example, I was confused on page 44 lines 5-7 ("No mortality was observed in the control or treatment concentration. Mortality in the control was 5%, and mortality in the test concentration was 100%"). I am assuming that "treatment concentration" refers to the solvent control?<br><br>In addition, please specify what is meant by a negative control. Is this water-only, without solvent added? | Thank you for your comments. The EPA has made your edits as suggested.<br><br>The EPA also made edits to ensure that the inconsistencies in the descriptions of the experimental procedures were addressed so that all of the study summaries are clear and consistent. For instance, the EPA made the following edit to the study summary for the test used qualitatively and described by Reviewer 1 formally on page 44 of the 6PPD Screening Value Document now in Section 4.2.1.2 and Appendix Section E.1.1.2 of the updated document:<br><br>*"Mortality in the control was 5%, and mortality in the single test concentration was 100%."* |

| 2.1. Clarity of Document as it Relates to Assessing the Risk of 6PPD to Aquatic-Life | | |
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| **Reviewer** | **Reviewer Comments** | **EPA Response** |
| | In the study descriptions (initial sentence of each) be consistent and always state whether 6PPD was measured or unmeasured. | |
| **2** | The document for 6PPD 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.<br><br>This document summarizes what is known about the chemistry, fate, and transport properties of 6PPD 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 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.<br><br>This document mentions tire wear and wet weather runoff from roads as a major source of 6PPD in surface waters. There has been a fair amount of research in the past seven or eight years regarding fate and transport of 6PPD in surface waters and some of those data are coincident with coho salmon pre-spawn mortality in the Pacific Northwest. EPA should consider incorporating a brief summary of relevant publications in this regard as some of those data | The EPA has corrected grammatical and typographical errors throughout.<br><br>EPA thanks Reviewer 2 for the notes on the chemistry, fate, and transport information presented in the draft document. The EPA has made edits to acknowledge the lack of current fate and transport data in marine environments to better round out the information presented.<br><br>Lastly, the EPA made edits to the draft 6PPD Screening Value Document to incorporate the fate publications recommended in Reviewer 2's comments. These edits can be found in Section 2.1 of the updated 6PPD Screening Value Document. |

| 2.1. Clarity of Document as it Relates to Assessing the Risk of 6PPD to Aquatic-Life | | |
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| **Reviewer** | **Reviewer Comments** | **EPA Response** |
| | may help to support EPA's SV for 6PPD. I included some relevant citations of which I am aware below. | |
| **3** | This report has high overall clarity and good construction for reporting the results of previous studies regarding the toxic risk of 6PPD to multiple diverse taxonomic groups. Some of the reported data appear redundant. For example, Table 3-3. Ranked Freshwater Genus Mean Acute Values and Figure 0-1. Ranked Freshwater Acute 6PPD GMAVs Fulfilling the Acute Family MDR seem to be reporting the same results. This also appears to be true for Table 3-5 and Figure 3-2. Overall, I have no problem with the presentation of this report with regard to assessing the risk of 6PPD to aquatic life. | Thank you for your comments. The EPA retained the presentation of the data in Tables 3-3 and 3-5 and Figures 3-1 and 3-2 as presented in the draft 6PPD Screening Value Document that underwent external peer review since the tables and figures provide various ways to look at the data. |

**2.2 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 (N-(1,3-dimethylbutyl)-N′ -phenyl-p-phenylenediamine) 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** | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| **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 or "relaxing" of these procedures were employed to derive a draft screening value. Thus, I agree with the decision to use selected qualitative data from studies that did not meet all requirements. Given the current major research focus worldwide on investigating 6PPD and related TWP toxicants, no doubt there will soon be more data available to further develop water quality criteria for 6PPD (and 6PPD-quinone).<br><br>2.2.b. I have a major comment related to this question. The decision to use the Japan Ministry of the Environment (2019) acute toxicity test in medaka is questionable in my opinion. Few details are available on experimental design, including 6PPD purity, use of solvent control, nominal exposure concentrations, routine water quality parameters, or source/age of animals. Was this a static test over 96 hours? What was the loading density of animals? What were the ammonia levels? I do not think that it can be "assumed", as stated in the EPA document, that since Japan is an OECD member that this study was conducted | Thank you for your comment. The EPA's responses are arranged in a manner consistent with the Reviewer's comments by question number throughout the rest of this document.<br><br>2.2.a. While there is great interest in 6PPD toxicity, data on apical endpoints (e.g., growth, reproduction, and survival) remain relatively limited and inhibits the development of ambient water quality criteria for aquatic life. Therefore, the EPA continued to use the available data as presented in the draft acute 6PPD Screening Value that underwent external peer review.<br><br>2.2.b. The EPA acknowledges the limited details that are presented in the Japan Ministry of the Environment (2019) acute toxicity test on medaka. However, given the limited toxicity data available at this time and that the EPA is deriving a screening value as opposed to ambient water quality criteria, the EPA retained the use of the medaka data discussed in Reviewer 1's comments. The inclusion of the medaka data ensures the protectiveness of the 6PPD screening value to all aquatic life.<br><br>2.2.c. The EPA retained the use of the medaka study raised in Reviewer 1's comments since this study stated that the methods followed OECD test guidelines, was used in |

| 2.2. The Technical Approach Used to Derive the Draft Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | with sufficient rigor. Perhaps additional information can be obtained from this lab to clarify the lack of experimental details? This is important because the draft 6PPD screening value is based largely on the apparent sensitivity of medaka, which is not consistent with all other freshwater species shown in Figure 3-1 (note log scale on x-axis). Note: in the description of this medaka study (page 20, section 3.1.1.1) it states "source of daphnids", is this a typo or were daphnids used as food? Also in this section (last sentence), it states that this value was acceptable for qualitative use, but in Table 3-2 it indicates quantitative use?<br><br>In summary, given the known labile nature of 6PPD in solution, especially with respect to its transformation to 6PPD-quinone, I think there are too many unknowns in this medaka study to allow it to be used as the driver of the acute 6PPD screening level. However, despite my cautionary opinion above, the study by Hiko et al. (2021) observed 80% mortality in medaka exposed for 96 hours to 107 ug 6PPD/L (measured; only single exposure concentration used), which does provide support for the notion that medaka are very sensitive to 6PPD.<br><br>Although there were inadequate data available to derive a freshwater 6PPD screening value for chronic exposures, the EPA's decision to consider the Japan Ministry of the Environment (2019) chronic toxicity test using medaka is also fraught with uncertainty in my opinion, for the same reasons listed above. | OECD's 2004 assessment of 6PPD (OECD 2004), and was conducted by a member of OECD, Japan's Ministry of the Environment, and updated the draft 6PPD Screening Value to include recently published data that were unavailable prior to the external peer review. OECD (2004) stated that this medaka study was conducted following OECD test guidelines which require standard conditions to be maintained, similar to the EPA 850 Test Guidelines (OECD 2004) The OECD (and ECHA in 2021) both have used this medaka test in their analyses of 6PPD toxicity, and the US is a member of the OECD. Thus, it is reasonable for the EPA to include this value in the 6PPD screening value in order to ensure the agency does not disregard data that indicate medaka sensitivity to 6PPD. As such, the updated screening value for 6PPD is now 8.9. µg/L. This updated value is slightly higher than the value of 8.3 µg/L that was presented in the draft 6PPD Screening Value document that underwent external peer review. |

| 2.2. The Technical Approach Used to Derive the Draft Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | 2.2.c. Given my opinion above on the suitability of the medaka studies, if the Japan Ministry of the Environment (2019) acute toxicity study in medaka was excluded then it appears the most sensitive species would be rare minnow, and the FAV/2 used to derive the screening level would be somewhat greater that the proposed value of 8.3 ug/L. Thus, I do believe that the proposed screening value of 8.3 ug/L would be protective of aquatic life. Although this value may be overly conservative, given the lack of 6PPD freshwater toxicity data available at this time it would be prudent to be precautionary. | |
| **2** | 2.2.a. The technical approach used to derive the screening value (SV) for 6PPD 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 what data are considered acceptable or unacceptable to use quantitatively to derive an SV. Given the fairly low persistence of 6PPD 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 and observed toxicity responses in laboratory exposures. It may be useful to incorporate what is known regarding time to death or | 2.2.a. Thank you for your comments supporting use of the toxicity data to derive the draft screening value for 6PPD. The EPA considered Reviewer 2's comments seeking added text to incorporate known time of death or immobility based on the results provided by individual study authors. Very few studies had this information, and the analysis is thus based on the published $LC_{50}$ results, similar to the use of 96-hour toxicity tests to yield 96-hour $LC_{50}$'s, which also often do not report time to affect. Thus, the EPA did not add text reporting specific time of death.<br><br>2.2.b. The EPA agrees with Reviewer 2 that the text of the draft 6PPD Screening Value document should more strongly state the science and understanding of 6PPD is evolving with a number of toxicity studies currently underway. The EPA thus made edits to the draft 6PPD Screening Value document to provide this context. These |

| 2.2. The Technical Approach Used to Derive the Draft Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | immobility based on test results to further support the use of 24-hour test endpoints to derive an acute SV.<br><br>2.2.b. The science presented supports the SV for 6PPD given that it is not a national criterion for reasons discussed in the document and it is clearly based on a limited data set (also discussed in the document). A caution that should be even further highlighted in this document is that the significance of 6PPD in terms of aquatic life risk has only recently come to light and there are many ongoing studies that are likely to provide more data with which to refine the SV or an eventual aquatic life criterion.<br><br>2.2.c. The technical approach used to derive the SV for 6PPD 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 and 6PPD-q. The document should make it even clearer that the SV is based on data obtained thus far and may have high uncertainty. | edits can be found in Section 3.3 of the revised 6PPD Screening Value document and include:<br><br>*"The science and understanding of the aquatic toxicity of 6PPD is relatively recent (with 6PPD transformation product 6PPD-q being attributed as the causative pollutant behind urban runoff mortality syndrome (URMS) in the past decade), and a number of toxicity studies are currently underway. As such, the EPA will continue to monitor the 6PPD literature and toxicity data to evaluate the protectiveness of this screening value."*<br><br>2.2.c. Please see the EPA's response to 2.2.b above and the corresponding edits to the draft 6PPD Screening Value document. One clarification that the EPA would like to note is that Coho salmon appear to be particularly sensitive to 6PPD-quinone, but not 6PPD itself. Two separate screening values were derived to 6PPD and 6PPD-quinone in order to provide protection to aquatic life from both compounds. |
| **3** | The U.S. Environmental Protection Agency (EPA) developed the preliminary draft screening value for 6PPD in accordance with Section 304(a) of the Clean Water Act (CWA). EPA has developed the 6PPD screening value for sensitive aquatic life in a manner that is generally | The EPA acknowledges the shortcomings presented in the toxicity data and literature for 6PPD. These inconsistencies in the individual tests are discussed in the study summaries of the draft 6PPD Screening Value document that underwent external peer review. The EPA thanks Reviewer |

| 2.2. The Technical Approach Used to Derive the Draft Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | consistent with methods described in EPA's "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" U.S.EPA (1985) and EPA's OSCPP's Ecological Effects Test Guidelines (U.S.EPA 2016) given the methodological limitations of some of the older studies. Thus, some of the methodology may not have been ideal for determining toxicity. For example, in the Monsanto Co. 1979 study, the concentrations used for a 28-d flow thru proportional diluter test were prepared as a simple arithmetic progression doubling the five concentrations from 0.066 to 1 mg/L of PPD. No mention was made of a using a range-finding test to come up with these concentrations. If this was indeed the range-finding test then the concentrations should have been prepared as a logarithmic progression. This is also true of the Monsanto Co. (1984) study. I believe for the more recent study they should have employed the correct array if concentrations. It should be noted that other studies (e.g. Peng 2022), correctly employed range-finding tests and eventually settled on a similar concentration array as Monsanto 1979 and 1984. However, they then calculated the LC50 using nominal concentration instead of the actual measured concentrations which were available. This unfortunately increases the uncertainty in predicting toxicity in natural systems. However, even given the stated reservations, the technical approach was appropriate and useful and yielded important information.<br><br>2.2.a. In arriving at preliminary draft screening value for acute exposures of 6PPD in freshwater, EPA attempted to | 3 for their comments regarding the technical approach and usefulness of the information provided for 6PPD toxicity to aquatic organisms.<br><br>2.2.a. The EPA agrees and acknowledges that the draft 6PPD Screening Value document has greater inherent uncertainty regarding risk to aquatic organisms in comparison to national ambient water quality criteria derived following The EPA's Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (hereafter referred to as the *Guidelines*; (U.S.EPA 1985)). This screening value for 6PPD is intended to provide information to states and/or Tribes to use in their water quality protection programs, while additional testing of 6PPD is conducted to better support the understanding of 6PPD toxicity.<br><br>2.2.b. The available toxicity data for 6PPD results in unevenness across the test methodologies used, interpretation of results, and analytical methods. However, this issue is not unique to 6PPD and was considered by the EPA in the derivation of the draft 6PPD Screening Value to ensure the protection of aquatic organisms. The EPA thanks Reviewer 2 for noting that the EPA produced a credible product based on the data available.<br><br>2.2.c. Thank you for your comments regarding the protectiveness of the draft 6PPD Screening Value based on the data that are currently available. |

| 2.2. The Technical Approach Used to Derive the Draft Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | review and consider all relevant acute toxicity test data. The goal here was to identify all data from acceptable tests that met data quality standards and thus determine acceptable data meeting the minimum data requirements (MDRs) as outlined in EPA's 1985 Guidelines (U.S.EPA 1985). The MDRs described in Section 2.1.1 were not met for acute freshwater criteria derivation. Acceptable studies of aquatic algae and vascular plants were also not available. Consequently, national 304(a) ambient water quality criteria for the protection of aquatic life could not be derived for 6PPD.<br><br>The authors were able to derive a protective acute screening value for 6PPD in freshwater by dividing the Final Acute Value (FAV) by two to obtain a concentration yielding a minimal effects acute screening value. Based on the above, the FAV/2, which is the freshwater acute water column screening value, is 8.3 µg/L 6PPD. This assessment quantifies the acute toxicity of 6PPD to aquatic organisms to protect aquatic life in freshwater. The 6PPD screening value is expected to be protective of most sensitive aquatic organisms in the community and is derived to be protective of aquatic life designated uses. It must be noted that this preliminary draft screening value for 6PPD is based on fewer empirical data than an aquatic life criterion would use and therefore has greater inherent uncertainty regarding environmental risk assessment.<br><br>2.2.b. Overall, the science does support the conclusions (derivation of a useful freshwater screening value). Because the studies evaluated are by multiple laboratories over a wide time frame (as early as 1977 and as recent as | |

| 2.2. The Technical Approach Used to Derive the Draft Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | 2022) there is an unavoidable unevenness in toxicity testing methodology, presentation of results, and interpretation. There were also some issues with chemical analyses, specifically the level of purity of N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylenediamine on Medaka, N-(1,3-dimethylbutyl)-N'-phenyl-1,4-benzenediamine and N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylenediamine on the cladoceran Daphnia magna, and N-(1,3-Dimethylbutyl)-N'-Phenyl-P-Phenylenediamine on the Fathead Minnow Pimephales promelas were all unknown. Nevertheless, the authors appeared to produce a credible product based on the data available to them.<br><br>2.2.c. All of the studies included in this report (after excluding numerous studies with insufficient useful data) were generally following multiple EPA guidelines published between 1975 and 2022. Thus, I am reasonably confident that (given the well-documented limitations of these guidelines) all except the most sensitive aquatic life taxa will be afforded a reasonable protection associated with exposure to 6PPD. | |

**2.3 Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD 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** | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| **1** | 2.3.a. Despite my concerns provided above in detail, I do think 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 for 6PPD. 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 ecosystems. However, it may be worth noting here that in the wild, the Japanese medaka is a euryhaline teleost that inhabits tidal/estuarine ecosystems in Asia so may be somewhat relevant to saltwater/brackish environments. Despite this point, I do realize that there are no studies that used saline exposure water.<br><br>2.3.b. As stated above, I think EPA made the most of out of the limited aquatic toxicity dataset and appropriately included/excluded data to derive the screening value, with the exception of my concerns on utilizing the medaka acute toxicity study (Japan Ministry of the Environment 2019). I agree with (i) using nominal exposure concentrations in studies that did not quantitate 6PPD, (ii) using studies with greater than or equal to 24-hour exposure durations, particularly due to the environmental realism of such short- | 2.3.a. Thank you for your comment regarding the adequate use of the available toxicity data. Thank you for your comment supporting the EPA's conclusion that he data for 6PPD are currently insufficient for development of ambient water quality criteria for aquatic life. and for your support of the EPA's development of a preliminary screening value for 6PPD using available data.<br><br>2.3.b. and 2.3.c. The EPA thanks Reviewer 1 for clearly stating the considerations of the toxicity data that were of concern and those that were appropriate. Please see the EPA's responses to Reviewer 1's comments above regarding the medaka study from Japan Ministry of the Environment. This study was retained in the derivation of the 6PPD screening value despite the limited exposure details provided since this test was conducted by Japan Ministry of the Environment and was used by in OECD's 6PPD assessment. Further, EPA double checked the ammonia concentrations reported in the Monsanto (1979) fathead minnow test and made needed changes to the text to correct the ammonia concentrations. This test was retained as acceptable for quantitative use. |

| 2.3. The Toxicity Data used to Derive the Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | term exposure scenarios in the wild, and (iii) considering non-native aquatic species such as medaka and zebrafish in derivation of this screening level.<br><br>I think the study by Prosser et al. (2017c) in early life stage fathead minnow should be reconsidered. This well-conducted, peer-reviewed study was excluded due to use of 6PPD-spiked sediment in exposure vessels, thus not following EPA's data inclusion rules. However, fathead minnow embryo-larval stages were exposed aqueously in isolated "egg cups" suspended in the water column (i.e., not directly in contact with sediment), and aqueous 6PPD was measured in this 21-day study. Thus, I think the study warrants further consideration in the derivation of screening values for 6PPD.<br><br>I am concerned about the water quality reported in the Monsanto (1979) study in fathead minnow (section 3.1.1.1.7). Specifically, the reported ammonia concentration of 0.9 mg/L is near lethal levels for fish at pH7.8. I suggest double-checking this value, and if correct then consider not employing this study for quantitative use.<br><br>2.3.c. I am not aware of any further data on 6PPD aquatic toxicity. | |
| **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 that the significance of 6PPD has been identified in terms of aquatic life risk. | 2.3.a. Thank you for your comment. The EPA made an edit to the draft 6PPD Screening Value document to note that the limitations to the toxicity data can be attributed to the recent understanding of 6PPD toxicity and risk to aquatic life.<br><br>2.3.b. and 2.3.c. The EPA thanks Reviewer 2 for their comments. The EPA retained the screening criteria of the |

| 2.3. The Toxicity Data used to Derive the Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | 2.3.b. The data selected or not selected to derive the SV for 6PPD appears scientifically justified and consistent with the goal of protecting aquatic life. As noted in response to charge question 1, given the low persistence of 6PPD 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).<br><br>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, where other stressors, chemical and otherwise, may be present. | toxicity data for 6PPD and have updated the dataset to include recently published data. |
| **3** | As mentioned above, unevenness in testing protocols, toxicity data collection and interpretation of results over a 45-year span makes it challenging to coalesce the data into screening values. I believe this attempt has been a reasonable effort to address this challenge with the available data and think that the risk associated with this type of meta-analysis is acceptable for an initial pass at producing guidelines for controlling 6PPD planned and unplanned releases such as used automobile tire reefs and | 2.3.a. Thank you for your comments. The EPA developed this preliminary screening value for 6PPD to address information needs of states and Tribes. The EPA intends to continue monitoring the 6PPD toxicity data to determine if enough data are available to derive ambient water quality criteria for aquatic life following the *Guidelines*. The EPA is also aware of ongoing toxicity testing to fill data gaps for 6PPD by various researchers and will review and incorporate those data as appropriate in the future. |

| 2.3. The Toxicity Data used to Derive the Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | storm sewer associated settling ponds and wetland mitigation.<br><br>2.3.a. The authors attempted to utilize all available historical studies in this meta-analysis. However, overall aquatic life toxicity data for 6PPD were limited, especially with regard to freshwater chronic testing for which the data sets were extremely limited. This resulted in an inability to develop a freshwater chronic screening value. Additionally acute and chronic estuarine/marine data were completely unavailable. While certainly not a complete development of risks of 6PPD to sensitive aquatic life, it is a reasonable early attempt to do so.<br><br>I believe, using currently available EPA Testing facilities in Region 6 (Houston) or Region 5 (Cincinnati), or independent EPA-Certified laboratories such as the Ecotoxicology Research Facility in Jonesboro, AR, the necessary data for a well-supported environmental risk analysis of 6PPD could be completed in 6 – 9 months, perhaps in a round-robin format. This would render the current meta-analysis mostly obsolete. This approach requires slicing thru a significant amount of red tape but it could be done if a suitable project coordinator is identified.<br><br>2.3.b. The authors correctly concluded that the chronic data set for 6PPD was much more limited in scope and content in comparison to acute data. Because of the limited chronic data available for 6PPD, a chronic screening value for aquatic life in freshwater was not derivable.<br><br>While the current acute 6PPD data does not support the complete derivation of an acute water column criterion in | 2.3.b. and 2.3.c. The EPA thanks Reviewer 3 for their comments on the adequate use of the 6PPD toxicity data to develop a screening value for 6PPD. |

| 2.3. The Toxicity Data used to Derive the Screening Values | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | freshwater, the dataset does enable the calculation of an acute screening value for freshwaters. Thus, I believe the available data were appropriately utilized.<br><br>2.3.c. I do not believe other relative data exists that should have been included in the evaluation of 6PPD toxicity. The authors appear to have completed an exhaustive literature review which identified most/all of the previous studies on this compound. These were all carefully evaluated using a specific set of acceptability criteria to determine if the results should be included in the derivation of the final screening value. | |

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

| 2.4. The Derived Screening Values' Protectiveness to Sensitive Aquatic Life | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| **1** | Yes, despite the relative lack of 6PPD toxicity data in freshwater, I do believe the proposed screening values would be protective of aquatic life.<br><br>Some final thoughts for consideration: Given the labile nature of 6PPD (half-life in the environment of less than one day), its known susceptibility to hydrolysis and photolysis, and particularly a study that reported detection of 6PPD-quinone 4 hours after spiking water with 6PPD (Hiki et al. 2021; cited in the EPA document), it seems to me that this creates a large uncertainty in evaluating aquatic toxicity testing of this specific chemical for the following reasons: (i) if purity is reported as 95-98%, what is the remaining 2-5%? Could some of this be the more toxic 6PPD-quinone? (ii) if purity is not reported, how can it ever be determined whether the results truly represent actual 6PPD toxicity? (iii) in 96-hour static-renewal experiments with replacement every 24 or 48 hours (or not at all) how can it ever be determined whether the results truly represent actual 6PPD toxicity? My point here is related to implementation of the 6PPD screening level. Is a 6PPD screening level needed? Would valuable resources be wasted measuring 6PPD in aquatic systems when the real culprit is 6PPD-quinone (or potentially other degradation products of 6PPD)? | Thank you for your comment on the protectiveness of the draft 6PPD Screening Value to aquatic life.<br><br>Following external peer review, the EPA ensured that the screening values were derived based on averaged concentrations over the exposure duration to account for observed loss of both 6PPD and 6PPD-q. In cases where study authors reported averaged concentrations or they could be calculated from initial and final concentrations, the EPA calculated $LC_{50}$ values based on average concentrations. In cases where average concentrations were not available or if a test was unmeasured, the EPA adjusted $LC_{50}$ values to account for loss by reducing the concentrations by 40% for 6PPD and 20% for 6PPD-quinone. These adjustments were based on reported losses of 6PPD and 6PPD-quinone in the toxicity literature and are fully detailed in Sections 2.2.2.3 of the 6PPD and 6PPD-quinone screening value documents.<br><br>The EPA considered the questions raised by Review 1 regarding the potential toxicity of 6PPD-quinone (6PPD-q) in the 6PPD toxicity studies and the overall usefulness of a screening value for the parent compound 6PPD. While some species of aquatic life, particularly salmonids, are more sensitive to 6PPD-q compared to 6PPD, some species appear to be more sensitive to 6PPD than to 6PPD-q. It is for these reasons that the EPA has developed a separate screening value for 6PPD-q itself. However, the screening value for the parent compound 6PPD was still determined to be useful information to share with state, Tribes, |

| 2.4. The Derived Screening Values' Protectiveness to Sensitive Aquatic Life | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | | stakeholders and the public, in addition to the screening value for the more toxic 6PPD-q. |
| **2** | The derived SV for 6PPD appears appropriate given the toxicity data available currently. However, the fact that that one of its degradates, 6PPD-q, is clearly more toxic than 6PPD, and 6PPD-q occurs under ordinary aerobic conditions in surface waters (albeit in low concentrations typically), protection of aquatic life may or may not be assured based on the proposed 6PPD SV. Other than setting the SV for 6PPD equal to the SV for 6PPD-q (or some other concentration that ensures the SV for 6PPD-q is not exceeded under typical surface water conditions), it is not certain that meeting the SV for 6PPD will in fact protect aquatic life due to toxic concentrations of its degradate 6PPD-q. | Please see the EPA's response to Review 1's comments above. In addition to the 6PPD Screening Value, the EPA has developed a separate screening value for 6PPD-q, which captures that certain species, particularly coho salmon, are more sensitive to 6PPD-q than to 6PPD. Further, the EPA determined that both of these screening values (one for 6PPD and the other for 6PPD-q) would be useful to states and Tribes in informing their water quality programs. Further, the EPA will continue to monitor the data for 6PPD and its degradates, including 6PPD-q, to determine if updates and/or additional screening values are needed to protect aquatic life from exposures to 6PPD and its related compounds. |
| **3** | The US EPA developed the final screening value following the general approach 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). Additional toxicity data (especially with taxa that are missing) and repeated toxicity studies for taxa using current methods for data generation are needed to better understand the toxicity of 6PPD and to derive national ambient water quality criteria to protect aquatic life to 6PPD. | Thank you for your comment. The EPA agrees that additional toxicity data are needed to better understand the toxicity of 6PPD to aquatic life. The EPA derived the draft 6PPD Screening Value to provide information for states and authorized tribal on the toxicity of 6PPD for potential consideration in their water quality programs. This 6PPD screening value is based on the current information available and the EPA will continue to monitor the data to determine if aquatic life AWQC can be developed at a future date. |

## 3.0 ADDITIONAL REVIEWER COMMENTS

| 3.0 Additional Reviewer Comments | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| **1** | See minor edits provided in the draft document. | The EPA addressed needed edits. |
| **2** | Additional literature regarding fate and transport for EPA's consideration:<br>• 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.<br>• 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: 733-738<br>• 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. 55: 11767−11774<br>• 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., 54: 6152–6165.<br>• Seiwert, B., Nihemaiti, M., Troussier, M., Weyrauch, S., & Thorsten, R. 2022. Abiotic oxidative transformation of 6-PPD and 6-PPD | The EPA reviewed and considered these publications in the updated 6PPD Screening Value document. Most of these papers provided information related to fate and transport. Therefore, most are cited in the Problem Formulation. |

| 3.0 Additional Reviewer Comments | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. Water Research, 212.<br>• 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. | |
| **3** | (Note: only publications not already listed in the two reports are listed here)<br>• J. A. Spromberg, N. L. Scholz, Estimating the future decline of wild coho salmon populations resulting from early spawner die-offs in urbanizing watersheds of the Pacific Northwest, USA. Integr. Environ. Assess. Manag. 7, 648–656 (2011).<br>• K. T. Peter, Z. Tian, C. Wu, P. Lin, S. White, B. Du, J. K. McIntyre, N. L. Scholz, E. P. Kolodziej, Using high-resolution mass spectrometry to identify organic contaminants linked to urban stormwater mortality syndrome in coho salmon. Environ. Sci. Technol. 52, 10317–10327 (2018).<br>• Tian, Z., H. Zhao, K. T. Peter, M. Gonzalez, J. Wetzel, C. Wu, X. Hu, J. Prat, E. Mudrock and R. Hettinger. 2021. A ubiquitous tire rubber–derived | The EPA reviewed and considered these publications in the updated 6PPD Screening Value document. Most of these papers provided information related to fate and transport. Most are cited in the Problem Formulation. |

| 3.0 Additional Reviewer Comments | | |
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| **Reviewer** | **Comments** | **EPA Response** |
| | chemical induces acute mortality in coho salmon. Science. 371(6525): 185-189. | |

## 4.0 REFERENCES CITED BY EPA IN RESPONSES

Japan Ministry of the Environment. 2019. Results of aquatic toxicity tests of chemicals conducted by Ministry of the Environment in Japan. Japan Ministry of the Environment. pp.31.

ECHA (European Chemicals Agency). 2021. 1,4-Benzenediamine, N1-(1,3-dimethylbutyl)-N4-phenyl- Registration Dossier. https://echa.europa.eu/registration-dossier/-/registered-dossier/15367/5/1

OECD (Organisation for Economic Co-operation and Development ). 2004. SIDS Initial Assessment Report for N-(1,3-Dimethylbutyl)-N´-phenyl-1,4-phenylenediamine (6PPD). https://hpvchemicals.oecd.org/UI/handler.axd?id=5e1a446c-5969-479c-9270-7ced8726952e

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