6PPD Alternatives Assessment Hazard Criteria

DEPARTMENT OF ECOLOGY
State of Washington

Abstract

6PPD is an antioxidant and antiozonant used in motor vehicle tires to prevent tire cracking and promote tire longevity. Researchers have determined that 6PPD has aquatic toxicity, reproductive toxicity, environmental persistence, and bioaccumulation potential, making it a chemical of concern. 6PPD ozonation also leads to harmful breakdown products such as 6PPD-quinone (6PPD-q). This chemical has a higher toxicity than 6PPD to aquatic organisms, including species of cultural and environmental significance like the coho salmon (juveniles LC_{50} 0.095 µg/L).[1]

The Washington State Legislature has tasked the Department of Ecology with performing an Alternatives Assessment on 6PPD in motor vehicle tires to identify compounds with the potential to replace 6PPD in these products. To set a transparent standard for identifying safer alternatives, we opted to use criteria for safer alternatives similar to those created for the Safer Products for Washington program. These criteria include human health, environmental health, and fate and transport considerations. We also made three additions to better protect sensitive species.

1. Alternatives must have data on acute aquatic toxicity to coho salmon and rainbow trout, as well as data on two other trophic levels.
2. Alternatives must have data on the toxicity of transformation products after exposure to ozone.
3. We will place a limit on the acute toxicity lethal concentration 50 (LC_{50}) values allowed in the minimum criteria (>0.1 mg/L).

We originally published the 6PPD Hazard Criteria in June 2023. This version of the 6PPD Hazard Criteria is revised in response to public input received from June 14, 2023, to July 14, 2023.

[1] Lethal concentration 50 (LC_{50}) measures the amount of a substance that kills 50% of a sample population after exposure to a toxin.

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Hazard Criteria

Background and justification

As part of the 2022 state budget,[4] the Washington State Legislature directed the Department of Ecology to conduct a "full safer alternatives assessment (AA) of 6PPD compounds used in tires. The assessment shall incorporate and evaluate toxicity data of alternatives on Coho and other species." In preparation for this AA, we developed the hazard criteria described in this document. These criteria enable us to set standards for collecting data and analyzing the safety of possible replacement chemicals for 6PPD.

6PPD (*N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine) is currently used in rubber products such as vehicle tires as an antioxidant and antiozonant. Data summarized in a chemical hazard assessment of 6PPD[5] have identified 6PPD as:

- A reproductive toxicant.
- An environmental toxicant of high concern.
- Persistent in the environment.
- A skin sensitizing compound.

Use of 6PPD as an antioxidant and antiozonant within tires leads to 6PPD ozonation and the development of several breakdown products, including 6PPD-quinone (6PPD-q) (Zhao et al. 2023).

Pacific Northwest coho salmon (*Oncorhynchus kisutch)*, a species of cultural and ecological significance, are particularly sensitive to 6PPD-q (juvenile 24hr LC_{50}; 0.000095 mg/L)[6] and susceptible to urban runoff mortality syndrome (i.e., when coho salmon die prior to spawning due to chemical exposure from urban runoff) (Tian et al. 2021; Tian et al. 2022). 6PPD-q has also shown some intestinal toxicity in low concentrations to the common study species *Caenorhabditis elegans,* a type of nematode, along with a potential for prolonged intestinal effects (Hua et al. 2023). Further information on acute toxicity of 6PPD-q and 6PPD to various fish is available in Table 1.

6PPD-q is more toxic than 6PPD to many aquatic species, but there are several species where 6PPD-q has not shown acute toxic effects at the limit of solubility while 6PPD has shown effects. Some salmonids, such as rainbow trout, brook trout, and white spotted char, are susceptible to 6PPD-q toxicity at concentrations less than 1 µg/L. Other salmon species, such as chinook and sockeye salmon, have much less sensitivity. A recent study examining the sublethal effects of

[4] See substitute senate bill 5693 (38): https://lawfilesext.leg.wa.gov/biennium/2021-22/Pdf/Bills/Senate%20Bills/5693-S.pdf?q=20230427092322

[5] https://www.ezview.wa.gov/Portals/_1962/Documents/6ppd/GreenScreenExecutiveSummaryFor6PPD.pdf

[6] 24hr LC_{50} is the lethal concentration for 50% of tested organisms after 24 hours.

both 6PPD and 6PPD-q on zebrafish embryos has also shown that both compounds can have developmental toxicity, but symptoms may differ (Zhang et al. 2023).

Table 1. LC_{50} values of various aquatic species exposed to 6PPD and 6PPD-quinone. References listed in table.

Species	6PPD LC_{50}	6PPD-q LC_{50}
Coho salmon (*O. kisutch*)	251 µg/L (24h-nominal) (Tian et al. 2021), calculated at 105 µg/L in (Hiki et al. 2021)	0.095 µg/L (24h) (Tian et al. 2021), 0.0804 µg/L (24h) (Greer et al. 2023), 0.041 µg/L (Lo et al. 2023), 2.26 µg/L (Di et al. 2022)
Rainbow trout (*O. mykiss*)	140 µg/L (96h) (EPA 2023)	1.0 µg/L (24h) (Brinkmann et al. 2022), 0.64 µg/L (Nair et al. 2023)
Japanese rice fish (*O. latipes*)	28 µg/L (96h) (EPA 2023); 80% mortality at 107 µg/L (96h) (Hiki et al. 2021)	No mortality <34 µg/L (96h) (Hiki et al. 2021)
Water flea (*D. magnia*)	230 µg/L (48h) (EPA 2023); 100% mortality at 138 µg/L (48h) (Hiki et al. 2021)	No mortality <46 µg/L (48h) (Hiki et al. 2021)
Fathead minnow (*P. promelas*)	450 µg/L (96hr) (EPA 2023)	No mortality <39.97 µg/L (Anderson-Bain et al. 2023)
Zebrafish (*D. rerio*)	740 µg/L (Fang et al. 2023); No 96h mortality at 137 µg/L (Hiki et al. 2021)	No mortality <54 µg/L (96h) (Hiki et al. 2021)
White spotted char (*S. leucomaenis*)	No data	0.51 µg/L (96h) (Hiki et al. 2022)
Brook trout (S. *fontinalis*)	No data	0.59 µg/L (24h) (Brinkmann et al. 2022)
Chinook salmon (*O. tshawytscha)*	No data	24h: approx 80 µg/L (Greer et al. 2023), 67 µg/L (Lo et al. 2023)
Sockeye salmon (*O. nerka)*	No data	No mortality <50 µg/L (24h) (Greer et al. 2023)

With the known toxicity of 6PPD, and our increasing understanding of 6PPD-quinone's toxicity, it is critical to identify an alternative for use in motor vehicle tires. The Department of Ecology is also separately evaluating stormwater management approaches to prevent toxicity in receiving waters. An Alternatives Assessment (AA) on 6PPD will help us identify alternatives that will protect aquatic species and prevent further environmental contamination. For this assessment,

we plan on using the hazard criteria as described in the Safer Products for Washington Regulatory Determinations Report to the Legislature[7] (June 2022), with three additions.

Due to the known effects of 6PPD and 6PPD-q on aquatic species, especially coho salmon, we want to ensure any alternative identified as safer will have less adverse effects on aquatic species. We know that any chemical used as an anti-degradant in tires will find its way into salmonid spawning streams. Researchers have detected 6PPD-q in the environment at levels such as 0.21-2.43 µg/L in Hong Kong urban runoff affected creeks (Cao et al. 2022) and 4.1-6.1 µg/L in Los Angeles runoff (Tian et al. 2021). These values are extremely toxic to the most sensitive species. In addition, 6PPD-q has been detected in sediment, which may serve as a long-term source for 6PPD-q, especially for sediment-dwelling organisms (Zeng et al. 2023).

Due to the high toxicity of 6PPD and its breakdown products, we must place greater emphasis on aquatic toxicity analyses and endpoints as compared to other chemicals and products considered under Safer Products for Washington (Safer Products). Additionally, in Safer Products we are protecting against the theoretical potential for adverse impacts from use of priority chemicals with other products. In this case, we *know* that the current use of 6PPD in tires leads directly to pre-spawn mortality in coho salmon. Recent detection of 6PPD within biomonitoring samples suggest it could be a hazard for human health as well, where 6PPD and 6PPD-q were identified within human urine samples (Du et al. 2022). Therefore, we aim to find alternatives that are a lower hazard than 6PPD in our endpoints of concern.

Criteria for safer alternatives to 6PPD

During the first Safer Products for Washington cycle, the Safer Products team developed criteria for identifying safer alternatives. Safer Products criteria, which serve as the foundation for our 6PPD hazard criteria, require data on both mammalian and aquatic toxicity endpoints (Table 2). Safer Products criteria include minimum and additional requirements to identify progressively safer alternatives, as described below:

1. Potential alternative chemical has data on required hazard endpoints, as outlined in Table 2.
2. Data shows the chemical aligns with the GreenScreen® Benchmark 2 category or better. Carcinogens, mutagens, reproductive/developmental (CMR) toxicants and persistent, bioaccumulative, and toxic (PBT) chemicals will not pass this criterion.
3. All known data will be used, even if it is outside of the required endpoints.

[7] https://apps.ecology.wa.gov/publications/documents/2204018.pdf

Table 2: Hazard endpoint and data requirement for alternative chemicals within Safer Products for Washington criteria for safer chemicals.

Hazard endpoint	Requirement	Human or Environmental Health
Carcinogenicity	Required	Human health
Mutagenicity/genotoxicity	Required	Human health
Reproductive or developmental toxicity	Required	Human health
Endocrine disruption	Not required	Human health
Acute toxicity	Not always required*	Human health (acute mammalian toxicity)
Single or repeat systemic toxicity	Not always required*	Human health
Single or repeat neurotoxicity	Not always required*	Human health
Skin or respiratory sensitization	Required	Human health
Skin or eye irritation	Not required	Human health
Acute or chronic aquatic toxicity	Required	Environmental health
Persistence	Required	Environmental health
Bioaccumulation	Required	Environmental health

*Two of the three required.

Safer Products criteria rely on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) scoring system for aquatic toxicity. The aquatic toxicity of 6PPD-quinone is 1000 times higher than the acute “very high” score in the GHS scoring system. Therefore, a chemical could be 1000 times better than 6PPD-q and the Safer Products (and GHS) scoring systems would not discern the difference. Further, the data requirements for Safer Products do not include coho salmon. While 6PPD is toxic to other aquatic species, the toxicity of its breakdown product 6PPD-q to coho salmon is much more severe. Therefore, due to the high toxicity of 6PPD-q to species of concern, the 6PPD AA uses Safer Products criteria with three additions that focus on aquatic toxicity. Our 6PPD AA will follow all criteria used in Safer Products, including those for mammalian based endpoints, plus these three aquatic toxicology additions.

First addition: Acute aquatic toxicity to coho salmon

To meet the minimum criteria for safer in the 6PPD AA, data is required on acute aquatic toxicity to coho salmon. This aligns with a requirement set by the Washington State Legislature

in the state budget ("The assessment shall incorporate and evaluate toxicity data of alternatives on Coho and other species").[8] All data will be assessed for best scientific practice before it is considered. Evaluating for best scientific practice includes reviewing for standardized methods, quality assurance and control (QA/QC) within the study, and overall quality (e.g., Klimisch score).

We will also require data on rainbow trout (*Oncorhynchus mykiss*) and two other trophic levels (e.g., daphnia and algae) to obtain adequate data on species sensitivity. Inclusion of rainbow trout as a required study species will allow for greater comparability, as it is a commonly used test species. Additionally, established rainbow trout cell lines allow for *in-vitro* analyses, making it relatively straightforward to conduct toxicity testing.

All applicable data will be considered and no particular methods or testing duration are required. However, all data will be evaluated regarding suitability for our assessment.

Examples of standardized methods may include:

- American Society for Testing and Materials (e.g., ASTM E729-96 (2014) for acute aquatic toxicity).
- Organization for Economic Cooperation and Development (OECD) guidelines for toxicity testing (e.g., tests included under the OECD guidelines for testing of chemicals, section 2 such as Test No. 203: Fish, Acute Toxicity Test or Test No. 230: 21 Day Fish Assay).
- Environmental Protection Agency's guidelines for acute aquatic toxicity testing (e.g., methods approved under Title 40, Chapter I, Subchapter D).

All data must be obtained by *in-vivo* testing, except for data from rainbow trout (*O. mykiss*). Researchers may obtain data for rainbow trout *in-vitro* provided that the *in-vitro* assay also shows a response to 6PPD-q. One example includes the use of RTG-2 cells, as shown in Greer et al. 2023, but we may accept other methods.

If using an *in-vitro* assay, an alternative and its ozonation products must have the same or less response as 6PPD-q at greater than 100x the concentration to meet the criteria for safer. This approach ensures the applicability of results while still allowing for use of *in-vitro* testing as a screening level assessment. Researchers may contact us at 6PPD@ecy.wa.gov with any questions concerning our criteria for safer.

Second addition: Toxicity of transformation products

Any alternative chemical that meets the minimum criteria must also have data showing acute toxicity information for transformation products when the potential alternative is exposed to ozone to be considered as an alternative to 6PPD. This is because the potential alternative still

[8] See substitute senate bill 5693 (38): https://lawfilesext.leg.wa.gov/biennium/2021-22/Pdf/Bills/Senate%20Bills/5693-S.pdf?q=20230427092322

needs to act as an antiozonant whose purpose is to transform after ozone exposure. Therefore, transformation products will, by definition, occur with use.

Transformation product mixtures will have the same testing requirements as parent compounds: four species, including coho salmon and rainbow trout, as well as two other trophic levels. *In-vivo* testing is required for all results, except for rainbow trout, where *in-vitro* data is allowed. Data produced on toxicity of transformation products will also be subject to the same rigor and evaluation as any data on the parent compounds. The method of ozonation will also be considered when evaluating toxicity of transformation products. Examples of possible ozonation methods include solid phase ozonation used by the Kolodziej lab, as shown in Zhao et al. 2023 and Hu et al. 2022, or solution phase ozonation as described in Seiwert et al. 2022.

Third addition: Requirements for hazard scores

We will place stricter requirements on acceptable hazard scores for acute aquatic toxicity than the limits in Safer Products criteria. The upper bound on the LC_{50} values allowed in the minimum criteria is >0.1 mg/L for the 6PPD AA.

6PPD-quinone has an LC_{50} value towards coho salmon of ~0.1 µg/L (Tian et al. 2022). Due to the extreme toxicity of 6PPD-q, even chemicals that are 1000 times less toxic would still score as a very high hazard for this endpoint. Therefore, during the 6PPD AA, chemicals with LC_{50} values of less than 0.1 mg/L (100µg/L) for acute aquatic toxicity will not pass the minimum criteria to be identified as a safer alternative to 6PPD. This includes both parent compounds and transformation product mixtures.

Chemicals with LC_{50} values of greater than 0.1mg/L may pass the minimum criteria defined for 6PPD alternatives, provided they meet the requirements in other endpoints, even though they would still score as very high for acute aquatic toxicity. No other changes will be placed on the limits defining categories for aquatic toxicity. As described in GHS criteria, chemicals with no acute toxicity at levels up to the water solubility limit are considered a low hazard for this endpoint.

Process for identifying a safer alternative to 6PPD

6PPD does not meet the minimum criteria outlined in Safer Products criteria (Table 2) for safer. 6PPD scored as a GreenScreen® Benchmark 1 chemical in a hazards assessment[9] and demonstrates human and environmental hazards post-exposure that are not consistent with our minimum criteria for safer. These hazards include data for 6PPD-quinone, since it is a transformation product of 6PPD. Using Safer Products criteria, alternatives to 6PPD must meet minimum criteria for safer (Figure 1).

[9] https://www.ezview.wa.gov/Portals/_1962/Documents/6ppd/6PPD Alternatives Technical Memo.pdf

However, if none of the alternative chemicals we evaluate in our 6PPD AA meet the minimum criteria, we will evaluate special considerations (Figure 1). Therefore, just because a chemical does not meet the minimum criteria for safer does not mean that we cannot find it to be a safer alternative in the 6PPD AA. For example, we may consider a chemical that does not meet our minimum criteria to be safer than 6PPD if the chance of exposure is lower than 6PPD and 6PPD-q. When assessing exposure potential, we consider the relevance of known and potential exposure routes and the magnitude of exposure. Details on special considerations and evaluations on exposure pathway, and an example of our use of special considerations, is available in the Phase 3 Regulatory Determinations Report to the Legislature[10] (fragrances section).

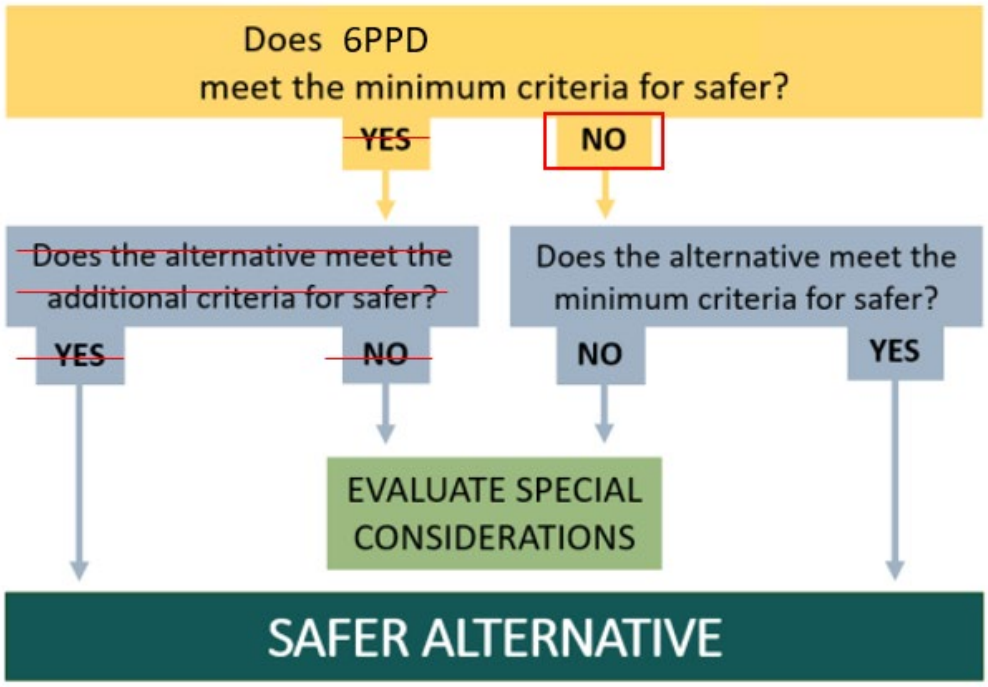

Figure 1: Flowchart demonstrating how we will identify safer alternatives to 6PPD for the 6PPD Alternatives Assessment. Because we know 6PPD does not meet the minimum criteria for safer, we are evaluating whether alternatives meet the minimum criteria. If yes, it meets the requirements as a safer alternative. If no, we will evaluate special considerations.

[10] https://apps.ecology.wa.gov/publications/summarypages/2204018.html

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