WATER QUALITY GUIDELINE SERIES

DRAFT 6PPD-quinone Acute Water Quality Guidelines – Freshwater Aquatic Life

Ministry of Water, Land, and Resources Stewardship
Water Stewardship and Security Branch

BRITISH COLUMBIA

No. WQG-24

The Water Quality Guideline Series is a collection of British Columbia (B.C.) Ministry of Water, Land, and Resource Stewardship water quality guidelines. Water quality guidelines are developed to protect a variety of water values and uses: aquatic life, drinking water sources, recreation, livestock watering, irrigation, and wildlife. The Water Quality Guideline Series focuses on publishing water quality guideline technical reports and guideline summaries using the best available science to aid in the management of B.C.'s water resources. For additional information on B.C.'s approved water quality parameter specific guidelines, visit:

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ISBN: 978-1-0399-0034-9

Document citation:
B.C. Ministry of Water, Land, and Resource Stewardship. 2024. 6PPD-quinone Water Quality Guidelines – Freshwater Aquatic Life. Water Quality Guideline Series, WQG-24. Prov. B.C., Victoria B.C.

Cover Photograph:
Location: Victoria, B.C.

Acknowledgements

The Ministry of Water, Land, and Resource Stewardship (WLRS) would like to thank Annie Chalifour, Allison Schein Jessica Johnson-MacKinnon, and Jesse Sinclair from LGL Limited for their technical expertise and contributions to this WQG. WLRS would also like to thank those who provided comments during the internal and external review.

EXECUTIVE SUMMARY

The British Columbia Ministry of Water, Land and Resource Stewardship (WLRS) develops province-wide ambient Water Quality Guidelines (WQGs) for substances or physical attributes that are important for managing both the fresh and marine surface waters of British Columbia (B.C.). WQGs provide a basis for water quality assessments and to inform decision-making in the natural resource sector. WQGs may be created for the protection of designated values, including aquatic life, wildlife, agriculture, drinking water sources, and recreation. For some substances, both long-term chronic and short-term acute guidelines are recommended as provincial WQGs, provided sufficient toxicological data are available. Short-term acute WQGs aim at protecting all individuals of all aquatic species from acute, severe effects (such as lethality) over short-term exposure, for instance after a spill event or an infrequent release of a chemical (ENV 2019). However, an exceedance of a WQG does not imply that unacceptable risks are present, but that the potential for adverse effects may be increased and additional investigation and monitoring may be warranted.

N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product from the reaction of N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6-PPD) with ozone. 6-PPD is an antioxidant added to vehicle tire rubber to prevent its degradation from the effect of ozone and other oxidative species. 6PPD-quinone was identified as a highly toxic substance to Coho Salmon (Oncorhynchus kisutch) in 2020. This discovery followed decades of observations of unexplained acute mortality among Coho Salmon populations in urbanized watersheds of the Pacific Northwest, a phenomenon known as urban runoff mortality syndrome (URMS) (Tian et al. 2021). 6PPD-quinone was found to be moderately toxic to other salmonid fish.

The data compiled from recent toxicological studies met the requirements to develop a Type B short-term acute guideline. No chronic guideline could be developed due to the limited availability of long-term toxicity data. A deterministic approach was used to derive the WQG. Following the B.C. protocol for WQG derivation (ENV 2019), an assessment factor of 4 was applied to the critical effect concentration (LC_{50} value for Coho Salmon) which results in a short-term acute WQG of 0.01 µg/L. This is 2.5 times lower than the LC_{10} of Coho Salmon and is predicted to be protective for all species against lethality. This report only provides a freshwater short-term acute WQG. More research is required to derive a long-term chronic WQG as well as WQGs for the protection of marine life. It is, however, recommended to use the short-term acute WQG as an indicator of potential sub-lethal effects to non-salmonids, especially if concentrations are at or above the short-term acute WQG.

Table ES.1. Proposed water quality guideline

Designated use	WQG for 6PPD-quinone (µg/L)	
	Long-term chronic WQG	**Short-term acute WQG**
Freshwater aquatic life	--	0.01

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1. INTRODUCTION

The British Columbia Ministry of Water, Land, and Resource Stewardship (WLRS) develops province-wide ambient Water Quality Guidelines (WQGs) for substances or physical attributes that are important for managing both the fresh and marine surface waters of British Columbia (B.C.). WLRS defines a WQG as a scientifically derived numerical concentration or narrative statement considered to be protective of designated values in ambient conditions. WQGs provide a basis for water quality assessments and inform decision-making in the natural resource sector and may be derived for the protection of designated uses including aquatic life, wildlife, agriculture (livestock watering and irrigation), drinking water sources, and recreation.

In B.C., WQGs are developed to protect the most sensitive endpoint associated with a given value (e.g., aquatic life, wildlife, livestock). For substances with sufficient toxicological data, both short-term acute and long-term chronic guidelines are developed. Interim WQGs are developed when the available toxicological data are insufficient (ENV 2019).

WQGs are typically based on toxicological studies conducted under laboratory conditions. There are several uncertainties associated with applying WQGs to field conditions, including:

- Laboratory to field differences in exposure conditions;
- Single contaminant tests in laboratories vs exposure to multiple contaminants in the field that may demonstrate additive, synergistic, or antagonistic effects;
- Toxicity of metabolites;
- Intra- and inter-specific differences between test species used to derive the WQG and those found in the field;
- Indirect effects (e.g., behavioral responses, food web dynamics);
- Laboratory studies conducted on partial life cycle studies which may not include the most sensitive life stage;
- Delayed effects which may not occur within the life stage tested, or may occur across generations; and,
- Cumulative effects of the various stressors, such as habitat loss and climate change, that organisms in the field are exposed to.

Given these uncertainties, WQGs are an estimate of a no-effect concentration (i.e., no effects are expected if exposure concentrations are below the WQG). An exceedance of the WQGs presented in this document, however, does not imply that unacceptable risks are present, but that the potential for adverse effects is increased and additional investigation and monitoring may be warranted. To that end, ongoing ecological monitoring is encouraged to ensure the WQG is indeed protective under field conditions.

N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product from the reaction of N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6-PPD) with ozone. 6-PPD is an antioxidant added to vehicle tire rubber to prevent its degradation from the effect of ozone and other oxidative species. 6PPD-quinone was identified as a highly toxic substance to Coho Salmon (Oncorhynchus kisutch) in 2020. This discovery followed decades of observations of unexplained acute mortality among Coho Salmon populations in urbanized watersheds of the Pacific Northwest, a phenomenon known as urban runoff mortality syndrome (URMS) (Tian et al. 2021). 6PPD-quinone was found to be moderately toxic to other salmonid fish.

The purpose of this report is to present an acute water quality guideline for the protection of aquatic life in British Columbia.

2. SUBSTANCE IDENTITY

N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6-PPD; CAS Number: 793-24-8; $C_{18}H_{24}N_2$) (Figure 2.1) is an antioxidant added to rubber tires to prevent degradation from the effect of ozone and other oxidative species. Its transformation product N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone, CAS Number: 2754428-18-4; $C_{18}H_{22}N_2O_2$) has a molecular weight of 298.39 g/mol (Figure 2.1). Its measured water solubility was only 0.067 ± 0.05 mg/L (in the study of Hiki *et al.* [2021]), despite being predicted to be 51.34 mg/L (by the United States [U.S.] Environmental Protection Agency [EPA]'s Estimation Programs Interface Suite [DTSC 2022]); therefore, more research is currently needed to explain this large difference and determine an accurate solubility value. The predicted log octanol-water partition coefficient (K_{ow}) of 6PPD-quinone has been estimated to be between 5 and 5.5 (Tian *et al.* 2021) or 3.98 (DTSC 2022), indicating a higher affinity for organic matter and lipids than water. The organic carbon-water partition coefficient, or log K_{oc}, was determined to be 2.8 ± 0.8 and 3.6 ± 0.8 in low and high organic carbon sediment, respectively (Monaghan *et al.* 2023), suggesting that 6PPD-quinone will preferentially bind to soil particles. A half-life of 33 hours for 6PPD-quinone was derived by Hiki *et al.* (2021) in experiments conducted in dechlorinated tap water at 23°C.

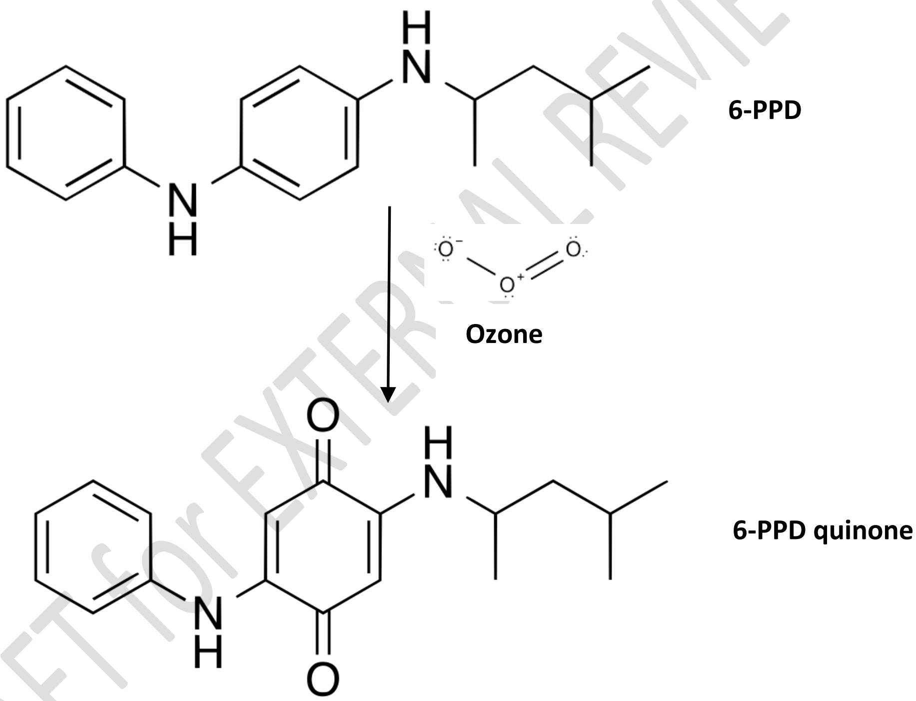

Figure 2.1. Molecular structure of 6-PPD and its transformation to 6PPD-quinone.

3. SOURCES AND USES

N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6-PPD) has been used as a rubber antioxidant in vehicle tires and other rubber products around the world since the 1960s. 6-PPD can make up from 0.5% to 2% of the content of rubber material used in the tire industry (Huntink 2003). Used to prevent cracks and increase the durability of the tires, 6-PPD can react with numerous reactive species such as peroxyl radicals, alkyl radicals, and ozone to form transformation products. 6PPD-quinone is a transformation product of the ozonation reaction with 6-PPD. This ozonation reaction can take place at the surface of the tire, where 6-PPD continuously migrates, but also in tire wear particles (TWPs). These particles are generated as tires roll on the pavement, especially during acceleration, turning, and braking (DTSC 2022). The per capita generation of TWPs in the U.S. is estimated to be between 2.5 kilograms (kg) and 4.7 kilograms kg per year (DTSC 2022). This would result in 12,700 to 23,800 metric tonnes of TWPs generated in British Columbia (B.C.) per year, assuming that the vehicle use is similar in B.C and U.S.

4. FATE, BEHAVIOUR, AND PARTITIONING IN THE ENVIRONMENT

The substance 6-PPD, can diffuse to the tire surface and be transformed to 6PPD-quinone when in contact with ozone (Hua and Wang 2023; Chen *et al*. 2023; Zoroufchi Benis *et al*. 2023), though the specific transformation pathways are still under study (Hua and Wang 2023). Tire usage on roads and other surfaces releases 6PPD-quinone and generates TWPs which are important carriers of 6PPD-quinone given their rough surfaces which allows them to carry more of the substance (Hua and Wang 2023). The main transport pathway of 6PPD-quinone to the environment is from runoff following rainfall (Hua and Wang 2023). Over time, TWPs accumulate in road dust and are washed off during rain events or snowmelt, where they reach watercourses via stormwater systems or direct runoff pathways (Johannessen *et al*. 2022). Subsequently, 6PPD-quinone can move to other water environments such as wastewater effluent and potentially drinking water (Hua and Wang 2023). The concentrations of 6PPD-quinone reported in several effluent samples from wastewater treatment plants were variable (Zoroufchi Benis *et al*. 2023).

TWPs are the main source of 6PPD-quinone in dust which is frequently detected in atmospheric particles (detection of 6PPD-quinone in > 65% of atmospheric particles samples; Hua and Wang 2023). Soil exposed to 6PPD-quinone through atmospheric deposition, and sediments through runoff entering water systems, are sinks for 6PPD-quinone given its affinity to particulate matter. The bioaccumulation of 6PPD-quinone has been documented in aquatic organisms such as fish, and in mammals through exposure in lab studies (Hua and Wang 2023).

5. ANALYSIS OF 6PPD-QUINONE IN ENVIRONMENTAL SAMPLES

Water samples are typically collected in amber glass bottles with polytetrafluoroethylene-lined caps and stored on ice at a temperature of 4°C during transport. One study showed that 6PPD-quinone is rapidly lost to the atmosphere when sampling bottles are left uncapped at room temperature, with a 50% decrease in the concentration of 6PPD-quinone after 51 hours (Monaghan *et al.* 2023). In capped vials stored at 4°C, a 55-65% reduction in concentration was observed over 3.5 months (Monaghan *et al.* 2023). Hence, analysis of samples should proceed quickly after collection.

The U.S. EPA has developed a draft standard method to measure 6PPD-quinone in aqueous environmental samples (U.S. EPA 2023). This method first concentrates 6PPD-quinone from 250 mL of water via solid phase extraction, which is then analysed by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS). A calibration curve is produced by injecting different dilutions of an analytical standard (i.e., purified 6PPD-quinone) into the LC/MS/MS. Quantification is facilitated by the addition of an isotope-labelled internal standard, such as $^{13}C_6$-6PPD-quinone or D_5-6PPD-quinone, to all samples and calibration standards (U.S. EPA 2023). 6PPD-quinone is identified by comparing the mass transitions and retention times in the samples and the calibration curve under identical conditions. Recent publications described MDLs of 0.0002 µg/L to 0.0012 µg/L, and commercial laboratories currently offer a reporting limit of 0.001 µg/L to 0.003 µg/L (e.g., Nedrich 2022; ALS Environmental Ltd personal communication 2024).

6. ENVIRONMENTAL CONCENTRATIONS OF 6PPD-QUINONE

6PPD-quinone has been measured in roadway runoff, snowmelt, surface water, municipal wastewater effluent, sediments, and dust in North America, Europe, and Asia (Hua and Wang 2023). The highest concentrations are usually found alongside roads after heavy rain events (ITRC 2023).

6.1 Methods for Estimating Environmental Concentrations

Data to characterize environmental concentrations were retrieved from published peer-reviewed literature and grey literature. All concentrations of 6PPD-quinone measured in road runoff, storm-sewer waters, snow (from municipal snow dump sites), and watercourses of Canada and the U.S. were compiled into a database; one wastewater effluent sample reported in the literature was also included. Summary statistics (Table 6.1) were calculated by Canadian province or U.S. state using the software R 4.3.1 (R Core Team 2023) and the "tidyverse" package (Wickham 2023). Non-detect data were replaced with the method detection limit (MDL). This approach was taken because of inconsistencies in the methods used to determine MDLs in different studies. Therefore, as a more conservative measure, the full MDL was used rather than 1/2 MDL to represent the potential environmental concentrations. The underlying 6PPD-quinone data used to generate summary statistics are presented in Appendix 1.

6.2 Environmental Concentrations Results

In accordance with the source of 6PPD-quinone (i.e., mostly from TWPs), the largest concentrations of 6PPD-quinone in water samples were measured in road runoff, storm sewers, and urban streams (Figure 6.1), confirming that the source of 6PPD-quinone is mostly from TWPs. Rural or suburban streams, even when located near a road, typically have lower or undetected concentrations of 6PPD-quinone (Nedrich 2022; Monaghan *et al.* 2023). The highest concentrations reported in the literature were measured in storm-sewer water collected after a rain event around Nanaimo, B.C., with concentrations ranging between 2.9 µg/L and 3.7 µg/L (Monaghan *et al.* 2021). Studies conducted around rain events showed that concentrations of 6PPD-quinone in watercourses increased during the rain event and returned to low levels after the rain event (Johannessen *et al.* 2022; Monaghan *et al.* 2023). For instance, samples collected in the Don River in the Greater Toronto area during a 7-hour rain event showed an increase from 0.93 µg/L at the beginning of the rain event to a maximum concentration of 2.85 µg/L 17 to 20 hours later, and a slow return to 1.13 µg/L 41 to 44 hours after the start of the rain event (Johannessen *et al.* 2022). Similarly, concentrations of 6PPD-quinone measured in Northfield Creek, an urban creek in Nanaimo, rose from 0.037 µg/L before a November rainfall to 0.159 µg/L during the peak streamflow and decreased to 0.023 µg/L following a dry period of > 48 hours after the rain event (Monaghan *et al.* 2023). One sample measured in Greater Toronto municipal wastewater effluent had a concentration of 0.05 µg/L 6PPD-quinone (Johannessen *et al.* 2021).

Table 6.1 Summary statistics for 6PPD-quinone concentrations in Canada and the U.S.

Province/State	# Stations	# Samples	Date Range	Concentration Range Across All Samples (µg/L)	MDL Range Across All Samples (µg/L)*	% Samples <MDL	Distribution of Station Means (µg/L)		
							Median	10th Percentile	90th Percentile
Canada									
British Columbia	24	176	2021 - 2022	<0.002 - 3.75	0.002 - 0.006	59.6	0.006	0.006	0.0816
South Coast	8	35	2021 - 2022	<0.002 - 0.18	0.002 - 0.006	8.6	0.036	0.00648	0.1
West Coast	16	141	2021 - 2022	0.0039 - 3.75	0.006	75	0.006	0.006	0.0329
Ontario	14	36	2019 - 2023	<0.0002 - 2.85	0.0002 - 0.0065	13.9	0.66	0.0065	2.76
Saskatchewan	11	52	2019 - 2020	<0.0012 - 1.4	0.0012	23.1	0.104	0.0012	0.741
United States									
California	6	6	2018 - 2019	0.0667 - 0.407	--	0	0.203	0.0733	0.34
Michigan	18	27	2021	<0.003 - 0.66	0.003	72	0.003	0.003	0.164
Washington	5	22	2017 - 2019	0.0187 - 1.33	--	0	0.337	0.031	0.586
All	78	340	2017 - 2023	<0.0002 - 3.75	0.0002 - 0.0065	42.8	0.017	0.003	0.859

*MDL: Method Detection Limit. This might be called Limit of Detection or Limit of Quantification by some authors. MDLs were determined differently by the authors. Some used the concentration of the lowest calibration standard (e.g., Johannessen *et al.* 2022), others used the concentration at signal:noise ratio of 3:1 (e.g., Monaghan *et al.* 2023; Tian *et al.* 2022), while some reported the commercial laboratory Reporting Limit, which may not be equivalent to the laboratory's MDL (e.g., Nedrich 2022; Metro Vancouver 2022). South Coast = Metro Vancouver; West Coast = Vancouver Island.

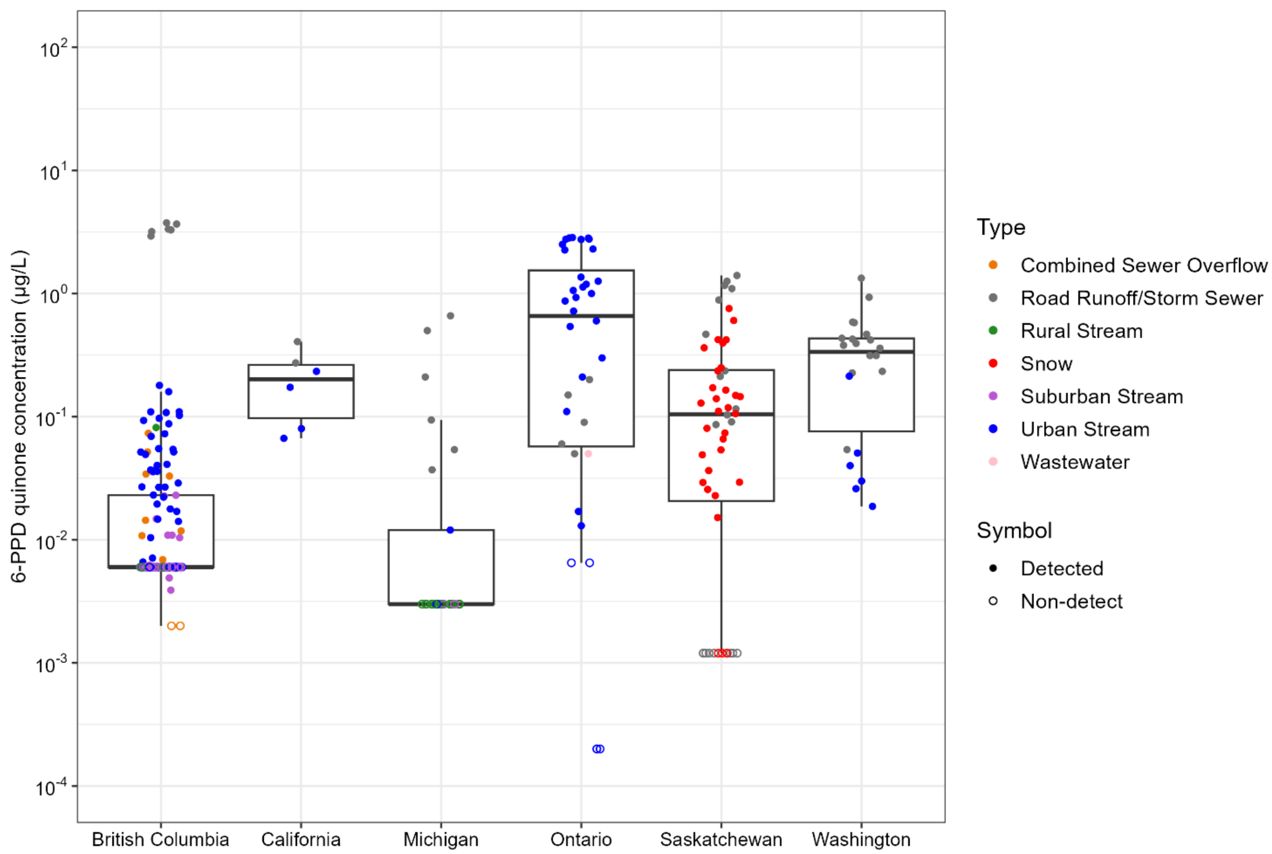

Figure 6.1 Distribution of environmental concentrations for 6PPD-quinone.

Notes: The y-axis is on a log scale. Solid horizontal bar within each box represents the median in each province or state. Points are jittered (randomly spread horizontally) to show points that otherwise would have overlapped.

7. <u>MODE OF ACTION</u>

Research into the specific mode of action of 6PPD-quinone is still ongoing as 6PPD-quinone was identified as a highly toxic substance to Coho Salmon (Oncorhynchus kisutch) in 2020. This discovery followed decades of observations of unexplained acute mortality among Coho Salmon populations in urbanized watersheds of the Pacific Northwest, a phenomenon known as urban runoff mortality syndrome (URMS), (Tian *et al.* 2021). Researchers suspected TWPs to be responsible for the URMS, and after two years and numerous chemical fractionations and toxicity tests, 6PPD-quinone was identified as the responsible molecule behind URMS (Tian *et al*. 2021). Once isolated, subsequent studies found that the toxicity of 6PPD-quinone to Coho Salmon was as high as the most toxic substances to aquatic life evaluated (e.g., neurotoxic organophosphates such as parathion and chlorpyrifos, organochlorines such as dichlorodiphenyltrichloroethane (DDT) and toxaphene, and metals such as cadmium) (Tian *et al*. 2022). However, other fish species, especially non-salmonids, did not demonstrate the same sensitivity to either 6PPD-quinone or TWPs (Brinkmann *et al*. 2022; Greer *et al*. 2023a; McIntyre *et al*. 2021). This discrepancy between species could either be due to the mode of action of 6PPD-quinone or the ability of the fish to metabolise this molecule (Mahoney *et al*. 2022).

Although the mode of action is still to be determined, effects on Coho Salmon include loss of equilibrium and buoyancy, gasping, increased hematocrit, and cerebrovascular plasm leakage, all indicators of a potential disruption of the blood-brain barrier (Blair *et al*. 2021). Based on the levels of 6PPD-quinone metabolites present in tissue, sensitivity may be linked to enzymatic expression and detoxification potential (Montgomery *et al*. 2023). A lower heart rate and increased oxygen consumption rate were also reported in Zebrafish (*Danio rerio*), albeit at much higher concentrations of 6PPD-quinone (i.e., 1 µg/L and greater) (Varshney *et al*. 2022). In contrast, 24 hours of exposure to 0.02 µg/L 6PPD-quinone significantly increased the heart rate and altered the exploratory behaviour of Zebrafish larvae while other neurobehavioral effects were found at higher 6PPD-quinone concentrations (Ricarte *et al*. 2023).

In *in-vitro* studies using Rainbow Trout (*Oncorhynchus mykiss*), 6PPD-quinone toxicity was linked to gill and liver mitochondrial dysfunction (Mahoney *et al*. 2022). However, no evidence of mitochondrial dysfunction was found in Zebrafish larvae with neurobehavioral effects from 6PPD-quinone (Ricarte *et al*. 2023). Within non-salmonid species, isomers of 6PPD-quinone have been detected in genomic DNA from the liver, gills, and roe of the marine capelin (*Mallotus villosus*) (Wu *et al*. 2023). In embryotic Zebrafish, 6PPD-quinone exposure resulted in effects to the gut including an enlarged intestine, blood coagulation, neutrophil activation, and over-expressed enteric neurons (Zhang *et al*. 2023). Evidence that 6PPD-quinone may disrupt one-carbon metabolism and cause oxidative stress in livers and gills of adult fathead minnows (*Pimephales promelas*), with no mortality, was noted in a 96-hour exposure test (Anderson-Bain *et al*. 2023).

Aquatic invertebrates were found to be tolerant to 6PPD-quinone in studies reported to date (NOEC of 232 µg/L for *Hexagenia* spp.) (Prosser *et al*. 2023).

8. <u>CRITERIA FROM OTHER JURISDICTIONS</u>

The US EPA recently released an acute aquatic life screening level for 6PPD-quinone of 11 ng/L (US EPA 2024).

9. <u>RECOMMENDED GUIDELINE</u>

9.1 Toxicity Data

9.1.1 *Effects on algae*

Currently, there is one published study on the effects of 6PPD-quinone on freshwater microalgae, the green microalgae *Chlamydomonas reinhardtii* (Wu *et al*. 2023). The lowest observed effect concentration (LOEC) for growth inhibition obtained was 0.25 mg/L (based on absorbance at 550 nm). Genomic DNA damages, measured as the number of lesions/109 nucleotides, was observed for 6PPD-quinone concentrations between 0.25 mg/L and 1 mg/L (Wu *et al*. 2023).

Different plastic leachates, including vehicle tire rubber leachate, have been tested on two phytoplankton species (Capolupo *et al*. 2020). The leachate was prepared by shaking a mass of plastic in algae growth media in a ratio of 80 g plastic/L media, in an incubator at 125 revolutions per minute, and at room temperature for 14 days in the dark. The leachate was then filtered to remove plastic particles and tested at different dilutions on the freshwater microalgae *Raphidocelis subcapitata* and the marine microalgae *Skeletonema costatum* over 72 hours. The vehicle tire rubber leachate growth inhibition EC_{50} (effect concentration affecting 50% of a test population) was obtained at 0.5% and 19% dilution of leachate for the freshwater and marine algae, respectively (Capolupo *et al*. 2020); however, the concentration of 6PPD-quinone in the vehicle tire leachate was not measured by the authors. They concluded that the toxicity observed was most likely the result of several organic and inorganic contaminants that leached out of the vehicle tire rubber material (Capolupo *et al*. 2020).

9.1.2 *Effects on macrophytes*

There are currently no studies conducted on the effect of 6PPD-quinone on aquatic macrophytes.

9.1.3 *Effects on invertebrates*

Aquatic invertebrates were thus far found to be highly tolerant to 6PPD-quinone. Studies conducted on cladocerans, gastropods, mollusks, and mayflies did not measure toxicity impacts at environmentally relevant concentrations or concentrations below the estimated 6PPD-quinone aqueous solubility (Hiki *et al*. 2021; Klauschies and Isanta-Navarro 2022; Prosser *et al*. 2023).

In acute bioassays using *Hyalella azteca* and *Daphnia magna*, the no observed effect concentration (NOEC) was 43 µg/L and 46 µg/L, respectively (Hiki *et al*. 2021). No effects on survival of *D. magna* (48-hour), *Ceriodaphnia dubia* (48-hour), and the gastropod *Physella gyrina* (96-hour) were reported at concentrations up to 100 µg/L following the standard Environment Canada protocols (Kennedy 2023). Furthermore, tests conducted on mayfly larvae (*Hexagenia* spp.) over four days did not measure any effects on survival at up to 232 µg/L (Prosser *et al*. 2023). A 24-hour exposure to 6PPD-quinone did not affect hatching of ramshorn snail (*Planorbella pilsbryi*) embryos at concentrations up to 11.7 µg/L (Prosser *et al*. 2023).

Kennedy (2023) also conducted chronic toxicity tests on *D. magna* (21-day), *C. dubia* (8-day), and *P. gyrina* (21-day) and found no effect on survival or reproduction up to concentrations of 100 µg/L. Similarly, growth and survival of *D. magna* in a 21-day test was not affected at 6PPD-quinone concentrations up 42 µg/L and survival of the washboard mussel (*Megalonaias nervosa*) was not reduced at test concentrations up to 17.9 µg/L (Prosser *et al*. 2023). Exposure of 6PPD-quinone concentrations of up to 1,000 µg/L over eight days did not affect the growth of the rotifer *Brachionus calyciflorus* (Klauschies and Isanta-Navarro 2022).

Chronic toxicity studies performed on *Caenorhabditis elegans*, a soil nematode, demonstrated increased intestinal permeability at concentrations between 1 and 10 µg/L (Hua *et al*. 2023a). Intestinal oxidative stress, neurotoxicity, and locomotive dysfunction were observed at 0.1 µg/L of 6PPD-quinone (Hua *et al*. 2023a; Hua *et al*. 2023b). However, further studies are required to understand if the sub-lethal effects observed on *C. elegans* also apply to aquatic invertebrates.

9.1.4 *Effects on fish*

The substance 6PPD-quinone has been shown to be highly toxic to fish species, especially some salmonids. It is estimated that mass die-off of salmon due to URMS can result in over 50% mortality in returning Coho Salmon in some catchments (Chow *et al*. 2019). Fish under URMS exhibit symptoms such as increased surface swimming and loss of equilibrium and buoyancy, and eventually die only a few hours after exposure (Chow *et al*. 2019). The specific mode of action of 6PPD-quinone is not yet understood (see Section 7).

Overall, salmonid species tend to show higher levels of sensitivity to 6PPD-quinone than non-salmonid fish species. However, sensitivity to 6PPD-quinone varies among salmonids, with Coho Salmon being the most sensitive, with 24-hour LC_{50} (lethal concentration affecting 50% of a test population) values of 0.041 µg/L, 0.0804 µg/L, and 0.095 µg/L obtained in three different studies by Lo *et al*. (2023), Greer *et al*. (2023a), and Tian *et al*. (2022), respectively. Coho Salmon is followed in sensitivity by White-spotted Char (*Salvelinus leucomaenis pluvius*; 24-h LC_{50} = 0.51 µg/L; Hiki and Yamamoto 2022), Brook Trout *(S. fontinalis*; 24-hour LC_{50} = 0.59 µg/L; Brinkmann *et al*. 2022), and Rainbow Trout (24-h LC_{50} = 1.96 µg/L; Brinkmann *et al*. 2022). There is, however, no apparent phylogenetic sensitivity as species within the same genus show varying levels of sensitivity (Hiki and Yamamoto 2022; ITRC 2023). Within the *Oncorhynchus* genus, toxicity ranges from a 24-hour LC_{50} of 0.041 µg/L in juvenile Coho Salmon (Lo *et al*. 2023) to no mortality after 24-hours at 50 µg/L for Sockeye Salmon (*O. nerka*) and a projected 24-hour LC_{50} of 82.1 µg/L in Chinook Salmon (*O. tshawytscha*; Greer *et al*. 2023a). In addition, McIntyre *et al*. (2021) co-exposed pre-spawn adult Coho and Chum (*O. keta*) salmon to 320 mg/L TWP leachate for 24 hours and found complete mortality of Coho Salmon yet no mortality of Chum Salmon. Similarly, the Coho Salmon had a significant increase in hematocrit and decreases in plasma sodium and pH compared to controls after 3 hours of exposure to 320 mg/L TWP leachate, while the Chum Salmon had no blood parameters significantly altered by the TWP leachate exposure (McIntyre *et al*. 2021). This TWP leachate contained an estimated 6PPD-quinone concentration ranging from 1.3 µg/L to 2.4 µg/L, depending on the exposure replicate, along with other chemicals from the tires, such as hexa(methoxymethyl)melamine, 1,3-dicyclohexylurea, and 1,3-diphenylguanidine (McIntyre *et al*. 2021).

Although the differences in age and size of the species tested were not described in Greer *et al*. (2023a), their methods show the Coho Salmon tested were smaller and younger (mean age 189 days, mass of 1.95 ± 0.56 g [mean ± SD]) than the Chinook (mean age 582 days, mass of 12.1 ± 3.7 g) and Sockeye (mean age 625 days, mass of 6.46 ± 3.4 g) Salmon tested. Lo *et al*. (2023) found that 3 weeks post swim-up juvenile Coho Salmon were more sensitive to 6PPD-quinone than the age 1+ Coho Salmon tested in Tian *et al*. (2022). Nonetheless, adult Coho Salmon were killed by <24 hours exposure to TWP leachate containing 1.3 µg/L to 2.4 µg/L 6PPD-quinone (McIntyre *et al*. 2021). It is possible that the precise age of the fish during exposure to 6PPD-quinone affects the toxicity of this substance, though more research on this and the mechanism of 6PPD-quinone toxicity is needed.

A recent study of Zebrafish larvae found 6PPD-quinone exposure for only 24 hours affected the central nervous system, causing behavioural shifts, along with alteration in the sleep/wake cycle and electrical conduction of the heart (i.e., increased heartbeat rate; Ricarte *et al*. 2023). At concentrations of 0.02 µg/L, embryos exhibited exploratory behavior and habituation change; exposure to the highest concentration tested (2 µg/L) altered the wake/sleep cycle and the expression of circadian clock genes (Ricarte *et al*. 2023). Although this study used an acute exposure period, the results show that there may be sub-lethal effects on more tolerant fish that are not killed at the concentrations of 6PPD-quinone typically found in the environment. Ricarte *et al*. (2023) concluded that the changes to Zebrafish found in their study could be lethal to Zebrafish in their natural environment; therefore, fish species that appear tolerant to 6PPD-quinone may, in fact, be threatened by it.

Only three papers reported LC_{10} values in addition to LC_{50} values. Lo *et al*. (2023) reported a 24-hour LC_{10} for Coho Salmon of 0.021 µg/L, and 20.9 µg/L for Chinook Salmon, and 24-hour LC_{50} values of 0.041 µg/L and >67.3 µg/L for Coho Salmon and Chinook Salmon, respectively. Greer *et al*. (2023a) reported similar results, with a 24-hour LC_{10} of 0.0292 µg/L for Coho Salmon and 24.6 µg/L for Chinook salmon, and 24-hour LC_{50} values of 0.0804 µg/L for Coho Salmon and 82.1 µg/L for Chinook Salmon. Brinkmann *et al*. (2022) reported a 24-h LC_{10} of 0.477 µg/L for Brook Trout and a 96-hour LC_{10} of 0.8 µg/L for Rainbow Trout.

Non-salmonid fish species were found to be much more tolerant to 6PPD-quinone than salmonids, with 96-hour NOEC values ranging from >12.7 µg/L for juvenile White Sturgeon (*Acipenser transmontanous*) (Brinkmann *et al*. 2022) to >54 µg/L for Zebrafish embryos (Hiki *et al*. 2021). In addition, Varshney *et al*. (2022) reported 96-hour and 24-hour LC_{50} values for Zebrafish larvae of 132.92 µg/L and 308.67 µg/L, respectively.

Investigations on the long-term chronic effects of 6PPD-quinone to date have been limited. Only two studies using a long-term chronic duration test on fish could be found. Kennedy (2023) reported a NOEC of >100 µg/L for 30-day tests conducted on Sockeye Salmon fry. Anderson-Bain *et al*. (2023) found no effects on survival, hatching success, or developmental malformations of Fathead Minnow embryos exposed to concentrations of 6PPD-quinone up to 39.97 µg/L for 168 hours (i.e., seven days).

While no short-term mortality was observed in embryotic Coho Salmon exposed at environmental concentrations that are lethal to juveniles and adults (i.e., 0.1 µg/L), growth was inhibited in embryos exposed to 0.9 µg/L and 7.22 µg/L, and some of the alevins that emerged from these exposed eggs died five to eight days after hatching (Greer *et al*. 2023b).

9.1.5 *Effects on amphibians*

There are currently no studies conducted on the effect of 6PPD-quinone on amphibians.

9.2 Toxicity Modifying Factors

To date, no toxicity modifying factors have been identified in the scientific literature.

9.3 Derivation of 6PPD-quinone Water Quality Guidelines

WQGs were derived using the guidance in *Derivation of Water Quality Guidelines for the Protection of Aquatic Life in British Columbia* (ENV 2019). A search of the current scientific literature for studies on 6PPD-quinone toxicity to freshwater aquatic organisms in water-only exposures under laboratory conditions was conducted. Thirteen acceptable studies were identified, of which four had long-term toxicity endpoints and twelve had short-term toxicity endpoints (three studies included both). Most studies were conducted on Canadian species (indigenous and non-invasive exotic species); however, if available, studies on exotic species (e.g., Japanese Medaka) were also selected and evaluated to complement the dataset. The toxicity data evaluated for use in the derivation of the 6PPD-quinone WQG are presented in Appendix 2.

9.3.1 *Long-term chronic water quality guideline*

Only four studies examined the long-term effects of 6PPD-quinone on fish and invertebrate species (Klauschies and Isanta-Navarro 2022; Anderson-Bain *et al.* 2023; Kennedy 2023; Prosser *et al.* 2023). These authors were unable to measure toxicity effects on the studied organisms; hence, the maximum concentration used in each toxicity test, ranging from 11.7 µg/L to 1,000 µg/L, was reported as the NOEC. This dataset is insufficient to derive a long-term chronic WQG. Other approaches were considered to calculate a long-term chronic WQG, for instance using an acute to chronic ratio, but were disregarded due to the absence of studies demonstrating both acute and chronic effects on the same organisms.

While no long-term studies were available to develop a long-term chronic guideline, sub-lethal effects to Zebrafish have been documented (e.g., 20% increase in heartbeat rate or malformation rate) at concentrations that are near the lethal concentrations to sensitive salmonids (i.e., 0.02 µg/L - 0.025 µg/L) (Ricarte *et al.* 2023; Zhang *et al.* 2023). Therefore, the short-term acute guideline could be used as an indicator of potential for sublethal effects to non-salmonids when environmental concentrations are at or above this WQG.

9.3.2 *Short-term acute water quality guideline*

The short-term acute WQG aims to protect all individuals of all aquatic species from acute, severe effects (such as lethality) over short-term exposure, for instance after a spill event or an infrequent release of a chemical. For the derivation of the short-term WQG, concentrations of 6PPD-quinone that caused 50% mortality (i.e., median lethal concentration or LC_{50}) were used. LC_{50} values were only available for five salmonid species and one non-salmonid species (Table 9.1). The standard exposure period for acute toxicity tests on fish is 96-hours, however many studies only reported 24-hour LC_{50} values because mortality of all individuals of sensitive species occurred during the first 24 hours of a 96-hour test (Brinkmann *et al.* 2022; Hiki and Yamamoto 2022). Both 24-hour and 96-hour LC_{50} were included in the toxicity dataset.

The available toxicity data did not meet the requirements for derivation of either a short-term acute Type A1 or A2 WQG due to an insufficient number of primary studies, lack of reported LC_{50} values and absence of plant, invertebrate, and amphibian data (ENV 2019).

The toxicity data included only LC_{50} values for six fish species and therefore did not meet the minimum requirement for Type B WQG which requires four LC_{50} values: two for fish and two for invertebrates (ENV 2019). However, NOEC values were available for an additional nine fish species and six invertebrate species (Table 9.1). From the collected date in can be concluded that fish are considerably more sensitive to 6-PPD-Quinone compared to invertebrates. Therefore, it can be concluded that by following the deterministic approach and protection of fish as most sensitive taxa, more tolerant invertebrates will also be protected.

In this approach, the short-term acute guideline is extrapolated from the critical endpoint which is the severe effects datapoint for the most sensitive species. All LC_{50} and NOEC concentrations were plotted, and the critical value was for Coho Salmon (LC_{50} = 0.041 µg/L; Figure 9.1). An assessment factor (AF) of 4 was applied to this critical value to obtain short-term acute WQG of 0.01 µg/L. This WQG is two and a half times lower than the LC_{10} of Coho Salmon (0.025 µg/L) and therefore is considered protective of this species against lethality.

Table 9.1 Toxicity endpoints used to derive the short-term acute 6PPD-quinone water quality guideline.

Receptor Group/Species	Exposure Duration/Endpoint	Effect Value (µg/L)	Reference
Fish - salmonid species			
Coho Salmon (*Oncorhynchus kisutch*)	24-h LC_{50}	0.095	Tian *et al.* 2022
Coho Salmon (*Oncorhynchus kisutch*)	24-h LC_{50}	0.0804	Greer *et al.* 2023a
Coho Salmon (*Oncorhynchus kisutch*)	24-h LC_{50}	0.041	Lo *et al.* 2023
Whitespotted Char (*Salvelinus leucomaenis Pluvius*)	24-h LC_{50}	0.51	Hiki and Yamamoto 2022
Brook Trout (*Salvelinus fontinalis*)	24-h LC_{50}	0.59	Brinkmann *et al.* 2022
Rainbow Trout (*Oncorhynchus mykiss)*	96-h LC_{50}	1.05	Brinkmann et al. 2022
Rainbow Trout (*Oncorhynchus mykiss)*	96-h LC_{50}	1.96	Brinkmann et al. 2022
Rainbow Trout (*Oncorhynchus mykiss)*	96-h LC_{50}	0.64	Nair *et al.* 2023
Masu Salmon (*Oncorhynchus masou masou*)	48-h NOEC	>3.5	Hiki and Yamamoto 2022
Southern Dolly Varden *(Salvelinus curilus)*	48-h NOEC	>3.8	Hiki and Yamamoto 2022
Atlantic Salmon *(Salmo salar)*	48-h NOEC	>12.2	Foldvik *et al.* 2022
Brown Trout (*Salmo trutta)*	48-h NOEC	>12.2	Foldvik *et al.* 2022
Arctic Char (*Salvelinus alpinus)*	96-h NOEC	>14.2	Brinkmann *et al.* 2022
Chinook Salmon (*Oncorhynchus tshawytscha)*	24-h LC_{50}	82.1	Greer *et al.* 2023a
Chinook Salmon (*Oncorhynchus tshawytscha)*	24-h LC_{50}	>67.3	Lo *et al.* 2023
Sockeye Salmon (*Oncorhynchus nerka)*	96-h NOEC	>50	Greer *et al.* 2023a
Sockeye Salmon (*Oncorhynchus nerka)*	96-h NOEC	>100	Kennedy 2023
Fish - non-salmonid species			
Fathead Minnow *(Pimephales promelas)*	96-h NOEC	>9.4	Anderson-Bain *et al.* 2023
White Sturgeon (*Acipenser transmontanous)*	96-h NOEC	>12.7	Brinkmann *et al.* 2022
Japanese Medaka *(Oryzias latipes)*	96-h NOEC	>34	Hiki *et al.* 2021
Zebrafish *(Danio rerio)*	96-h LC_{50}	133	Varshney *et al.* 2022
Zebrafish *(Danio rerio)*	96-h LC_{50}	309	Varshney *et al.* 2022
Zebrafish *(Danio rerio)*	96-h NOEC	>54	Varshney *et al.* 2022
Invertebrate species			
Planorbella pilsbryi	24-h NOEC	>11.7	Prosser *et al.* 2023
Hyalella Azteca	96-h NOEC	>43	Hiki *et al.* 2021
Daphnia magna	48-h NOEC	>46	Hiki *et al.* 2021
Daphnia magna	48-h NOEC	>100	Kennedy 2023
Ceriodaphnia dubia	48-h NOEC	>100	Kennedy 2023
Physella gyrina	96-h NOEC	>100	Kennedy 2023
Hexagenia spp.	96-h NOEC	>232	Prosser *et al.* 2023

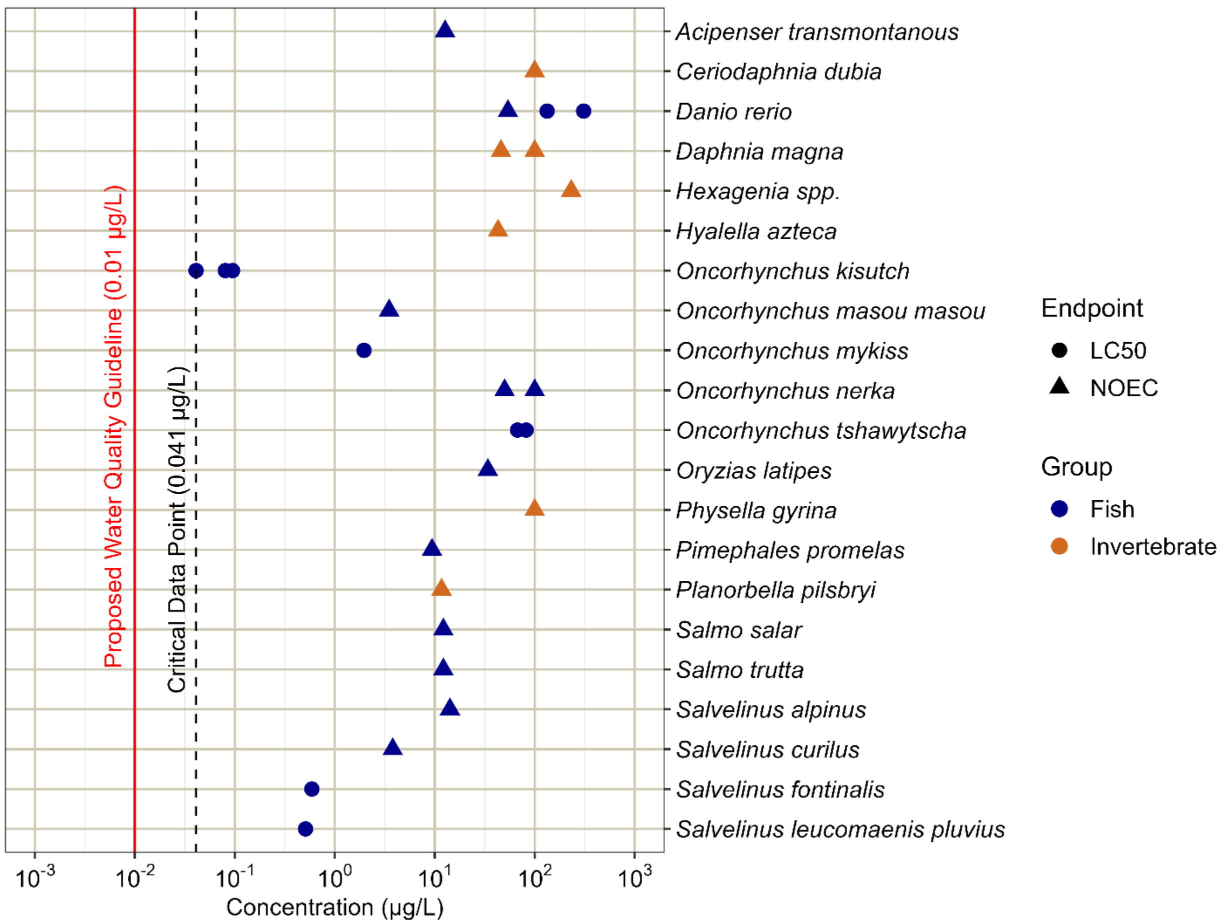

Figure 9.1 Short-term acute water quality guideline for 6PPD-Quinone.

Several uncertainties were considered when assigning the AF for the short-term acute WQG. No toxicity data are currently available for plants or amphibians, both of which are highly desirable to include in the derivation of WQGs. Although the toxicological data suggest that invertebrates and non-salmonid species are not sensitive to 6PPD-quinone, the mode of action of 6PPD-quinone has still not been elucidated. In addition, 6PPD-quinone is ubiquitous in urban environments due to its continuous release from vehicle tires, and it is unclear whether species that have not been tested are also sensitive to this contaminant. Currently, the LC_{50}, the preferred endpoint for short-term acute WQGs, has been characterized for only six species, four of which are Canadian. Furthermore, there is some evidence that younger and smaller fish may be more sensitive to 6-PDD quinone than older fish (Greer *et al.* 2023a; Lo *et al.* 2023), but more research is needed, particularly because studies testing “juvenile” fish can include a fairly large size and age range (e.g., Greer *et al.* 2023a). While most of the available fish acute toxicity data are from tests conducted with embryos, alevins, or juveniles, the only acute toxicity data available for Fathead Minnow were for adults (Appendix 2). It is unknown whether younger Fathead Minnow would be more acutely sensitive to 6PPD-quinone, although embryonic Fathead Minnow were not sensitive in a 7-day chronic exposure (Anderson-Bain *et al.* 2023). In addition, there is no apparent phylogenetic predictability to 6PPD-quinone because species within the same genus shows varying levels of sensitivity (Hiki and Yamamoto 2022; ITRC 2023). Thus, the degree of toxicity of 6PPD-quinone to untested species is very uncertain.

9.3.3 *Protectiveness Assessment*

Following the B.C. WQG derivation protocol (ENV 2019), the WQG was compared against the LC_{10} of the five most sensitive species (based on their LC_{50}). The LC_{10} reported for salmonid species varied between 0.025 µg/L and 22.7 µg/L, with the most sensitive species being the Coho Salmon (Table 9.2). The short-term acute WQG is 2.5 times lower than the geometric mean LC_{10} of Coho Salmon (0.025 µg/L) and therefore is predicted to be protective of Coho Salmon against lethality. There is no available information regarding the effects of repeated exposures to concentrations below the acute WQG. The Coho Salmon is a species of ecological, cultural, and economic importance in B.C. waters and the Interior Fraser population of this species is designated as Threatened under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC).

Table 9.2 LC_{10} values used to meet the protection clause.

Species	**Exposure Duration/Endpoint**	**Concentration (µg/L)**	**Reference**
Oncorhynchus kisutch	24-h LC_{10}	0.025*	Greer *et al.* 2023a, Lo *et al.* 2023
Salvelinus fontinalis	24-h LC_{10}	0.48	Brinkmann *et al.* 2022
Oncorhynchus mykiss	96-h LC_{10}	0.80	Brinkmann *et al.* 2022
Oncorhynchus tshawytscha	24-h LC_{10}	22.7*	Greer *et al.* 2023a, Lo *et al.* 2023

* Geomean of 2 values

10. COMPARION OF ENVIRONMENTAL CONCENTRATIONS TO WATER QUALITY GUIDELINES

Water quality guidelines are commonly used to help determine the potential risk of toxicity to aquatic life from a given substance in environmental conditions. In general, if environmental concentrations are below the WQG, the potential for risk is assumed to be low. However, an exceedance of a WQG does not imply that unacceptable risks are present, but that the potential for adverse effects may be increased and additional investigation and monitoring may be warranted.

The environmental concentrations measured in road runoff, storm- sewer water, snow, and streams were compared to the short-term acute WQG of 0.01 µg/L (Figure 6.1). Any non-detect data above the proposed WQG of 0.01 µg/L were removed from the comparison.

Overall, 55% of samples reported in the literature exceeded the guideline. In British Columbia, 36% of the samples collected were above 0.01 µg/L. Seven out of 11 samples collected in combined sewer overflows of Metro Vancouver had concentrations above 0.01 µg/L (Metro Vancouver 2022; 2023). Samples collected in Cougar Creek, a Vancouver urban creek, had concentrations above the acute WQG in 22 of 24 samples collected between October and November. Although the highest concentrations were measured during rain events, concentrations were often above the WQG before and > 48 hours after the rain event (Monaghan *et al.* 2023). In Ontario, 86% of the samples were above the guideline. These samples were mostly collected in urban creeks passing through the Greater Toronto Area (Johannessen *et al.* 2021; Johannessen *et al.* 2022). Of 21 samples collected in storm sewers in Saskatoon, 12 samples were above the acute guideline (Challis *et al.* 2021). Snow collected in snow dumps around Saskatoon had concentrations of 6PPD-quinone above the WQG in 90% of samples (Challis *et al.* 2021). In California and Washington states, where samples were mostly collected in storm sewers, 100% of samples were above the guideline (Tian *et al.* 2021). In Michigan, seven of the 25 samples collected (28%) were above the WQG (Nedrich 2022).

11. APPLYING THE 6PPD-QUINONE WATER QUALITY GUIDELINE

The short-term acute WQG is designed to protect aquatic species against severe effects, such as lethality. To meet the short-term acute WQG, there should be no exceedances at any given time. Short-term acute WQGs are intended to assess risks associated with infrequent and transient exposure events such as spills.

Although studies have shown that 6PPD-quinone is released in higher concentrations during rain events, it is still unclear how long the concentrations may remain elevated in watercourses after rain events. Results from Monaghan *et al.* (2023) showed that concentrations of 6PPD-quinone were still elevated (e.g., up to 2.8 times above the short-term acute WQG) 48 hours after the rain event ended. Therefore, when sampling for 6PPD-quinone, repeat sampling is needed to set baseline conditions, identify peaks during rain events and to document the return period to baseline conditions.

The range of Coho Salmon covers a large portion of the province of B.C. (Figure 11.1). The short-term acute WQG applies to all watercourses, regardless of whether they are considered coho-bearing or non-coho-bearing streams because: 1) not all streams have not been studied for the presence of Coho Salmon; 2) the stream could be a tributary to a Coho Salmon-bearing streams and the duration required for 6PPD-quinone concentrations to fall below the short-term WQG, or the dilution that would be needed to achieve the short-term WQG, can only be established on a case-by-case basis; and 3) there is no chronic WQG yet but there is some evidence that 6PPD-quinone could have a chronic effect at concentrations near the acute WQG level. For example, higher basal locomotor activity and heartbeat rate, and an increase in neurotransmitters (acetylcholine, serotonin, norepinephrine, and epinephrine) was measured in Zebrafish larvae at 0.02 µg/L of 6PPD-quinone (Ricarte *et al.* 2023). In addition, in the absence of a clear mechanism of action and with very few species studied at the time of writing this report, it is appropriate to be more conservative and account for the protection of all species in all watercourses. Since a long-term chronic WQG could not be determined based on the studies available to date, it is recommended to use the short-term acute WQG as an indicator of potential sub-lethal effects to fish.

Figure 11.1. Coho Salmon range in British Columbia

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