WATER QUALITY GUIDELINE SERIES

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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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 inform decision-making in the natural resource sector. WQGs are derived 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 derived, provided sufficient toxicological data are available. Short-term acute WQGs aim 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 (ENV 2019). Long-term chronic WQGs are intended to be protective of all forms of aquatic life (all species, all life stages including multi-generational) from lethal and negative sub-lethal effects over indefinite exposures However, an exceedance of a WQG does not necessarily mean 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 (6PPD) with ozone. 6PPD is an antioxidant added to vehicle tire rubber to prevent its degradation from exposure to 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 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 (ENV 2019). 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) resulting 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 of 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

CONTENTS

LIST OF TABLES

LIST OF FIGURES

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.). WQGs provide a basis for water quality assessments and inform decision-making in the natural resource sector.

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 are derived for the protection of designated values 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). For substances with sufficient toxicological data, both short-term acute and long-term chronic guidelines are developed. Short-term acute WQGs aim 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 (ENV 2019). Long-term chronic WQGs are intended to be protective of all forms of aquatic life (all species, all life stages including multi-generational) from lethal and negative sub-lethal effects over indefinite exposures However, an exceedance of a WQG does not necessarily mean unacceptable risks are present, but that the potential for adverse effects may be increased, and additional investigation and monitoring may be warranted. To meet a WQG, both of its components (i.e., chronic long-term and acute short-term) must be met. However, an exceedance of the WQGs 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.

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

- Differences in exposure conditions between laboratory to field;
- Single contaminant tests in laboratories vs exposure to multiple contaminants in the field that may demonstrate additive, synergistic, or antagonistic effects;
- Intra- and inter-specific differences between test species used to derive the WQG and those found in the field;
- Delayed effects which may not occur within the life stage tested, or may occur across generations;
- 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; 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 (6PPD) with ozone. 6PPD is an antioxidant added to vehicle tire rubber to prevent its degradation from exposure to 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.

2. SUBSTANCE IDENTITY

N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD; 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 exposure to ozone and other oxidative species.

Its transformation product, N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone, CAS Number: 2754428-18-5; $C_{18}H_{22}N_2O_2$) (Figure 2.1), has a molecular weight of 298.39 g/mol. The reported solubility of 6PPD-quinone varies significantly across studies, with values of 38.4 µg/L (Hu *et al.* 2023), 67 µg/L (Hiki *et al.* 2021), and 51,340 µg/L (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 (logK_{ow}) of 6PPD-quinone has been estimated to be between 5 and 5.5 (Tian *et al.* 2021), 3.98 (DTSC 2022), or 4.12 (Rodgers *et al.* 2023) indicating a higher affinity for organic matter and lipids than water. The log 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. Additionally, a log K_{oc} of 3.14 has been reported by Rodgers *et al*. (2024), further indication of the compound's tendency to sorb to organic matter. 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. However, a later study reported the half-life to be in the range of 12.8 to 16.3 days, depending on the water conditions (Rodgers *et al.* 2024). Additionally, Hu *et al.* (2023) reported a 26 ± 10% decrease in 6PPD-quinone at 20–22°C and a pH of 7 after 47 days.

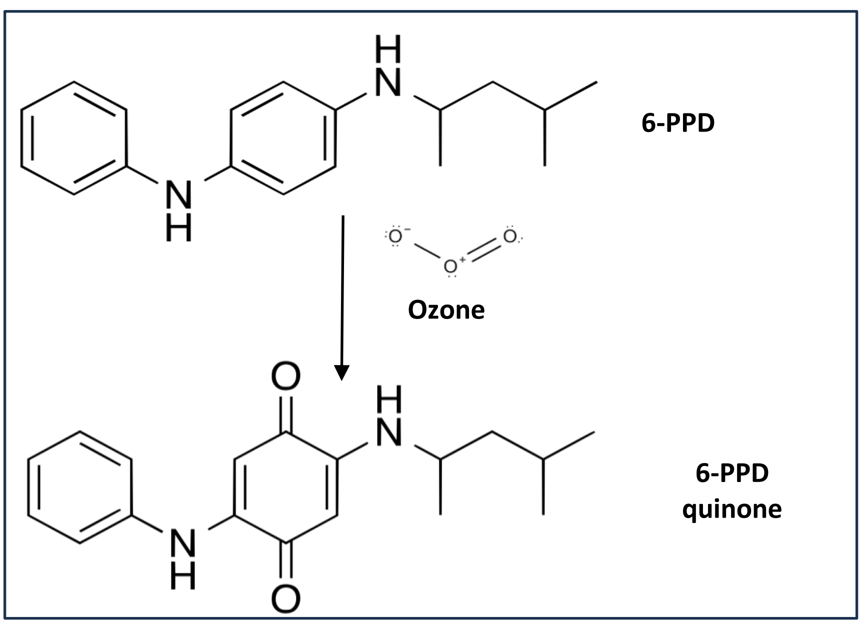

Figure 2.1. Molecular structure of 6PPD and its transformation to 6PPD-quinone.

3. SOURCES AND USES

6PPD has been used as a rubber antioxidant in vehicle tires and other rubber products around the world since the 1960s. 6PPD makes up 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, 6PPD can react with numerous reactive species such as peroxyl radicals, alkyl radicals, and ozone to form transformation products. The transformation of 6PPD to 6PPD-quinone is an ozonation reaction that takes place at the tire's surface, where 6PPD continuously migrates, and 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). With a population of 5.6 million, this would result in 14,000 to 26,320 tonnes of TWPs generated annually in B.C, assuming vehicle use is similar to that in the U.S.

4. FATE, BEHAVIOUR, AND PARTITIONING IN THE ENVIRONMENT

6PPD 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). The main transport route 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 (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 (>65% of atmospheric particles samples [Hua and Wang 2023]). Soil exposed to 6PPD-quinone through atmospheric deposition and sediments through runoff entering watercourses are sinks for 6PPD-quinone given its affinity to particulate matter.

The bioaccumulation of 6PPD-quinone has been observed in aquatic organisms, including fish and mammals, through laboratory exposure studies (Hua and Wang 2023). The Toxic Substances Management Plan (TSMP) considers substances with a log KOW greater than five to have a high potential for bioaccumulation (EC 1995), which is relevant given the estimated log KOW values for 6PPD-quinone (see Section 2).

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. <u>ENVIRONMENTAL CONCENTRATIONS OF 6PPD-QUNONE</u>

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

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 stormsewer 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 6PPD-quinone concentrations 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).

Table 6.1 Summary statistics for 6PPD-quinone concentrations (µg/L) in Canada and the U.S., including data from streams, snow, runoff, and wastewater.

Province/State	# Stations	# Samples	Date Range	Concentration Range Across All Samples	MDL Range Across All Samples *	% Samples <MDL	Distribution of Station Means		
							Median	10th Percentile	90th Percentile
Canada									
British Columbia	25	180	2021 - 2023	<0.002 - 3.75	0.002 - 0.006	58.1	0.006	0.006	0.0782
South Coast	9	39	2021 - 2023	<0.002 - 0.18	0.002 - 0.006	7.7	0.0342	0.00176	0.0981
West Coast	16	141	2021 - 2022	0.0039 - 3.75	0.006	75	0.006	0.006	0.0329
Ontario	18	96	2019 - 2023	<0.0002 - 2.85	0.0002 - 0.0065	5.2	0.0456	0.002	1.31
Saskatchewan	11	52	2019 - 2020	<0.0012 - 1.4	0.0012	23.1	0.104	0.0012	0.741
United States									
California	14	22	2018 - 2019	0.0667 - 0.407	--	0	0.132	0.036	0.263
Michigan	18	27	2021 - 2021	<0.003 - 0.66	0.003	72	0.003	0.003	0.164
Washington	7	37	2017 - 2021	0.0187 - 1.33	--	0	0.227	0.0025	0.581
All	93	414	2017 - 2023	<0.0002 - 3.75	0.0002 - 0.0065	33.7	0.017	0.0027	0.54

*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.

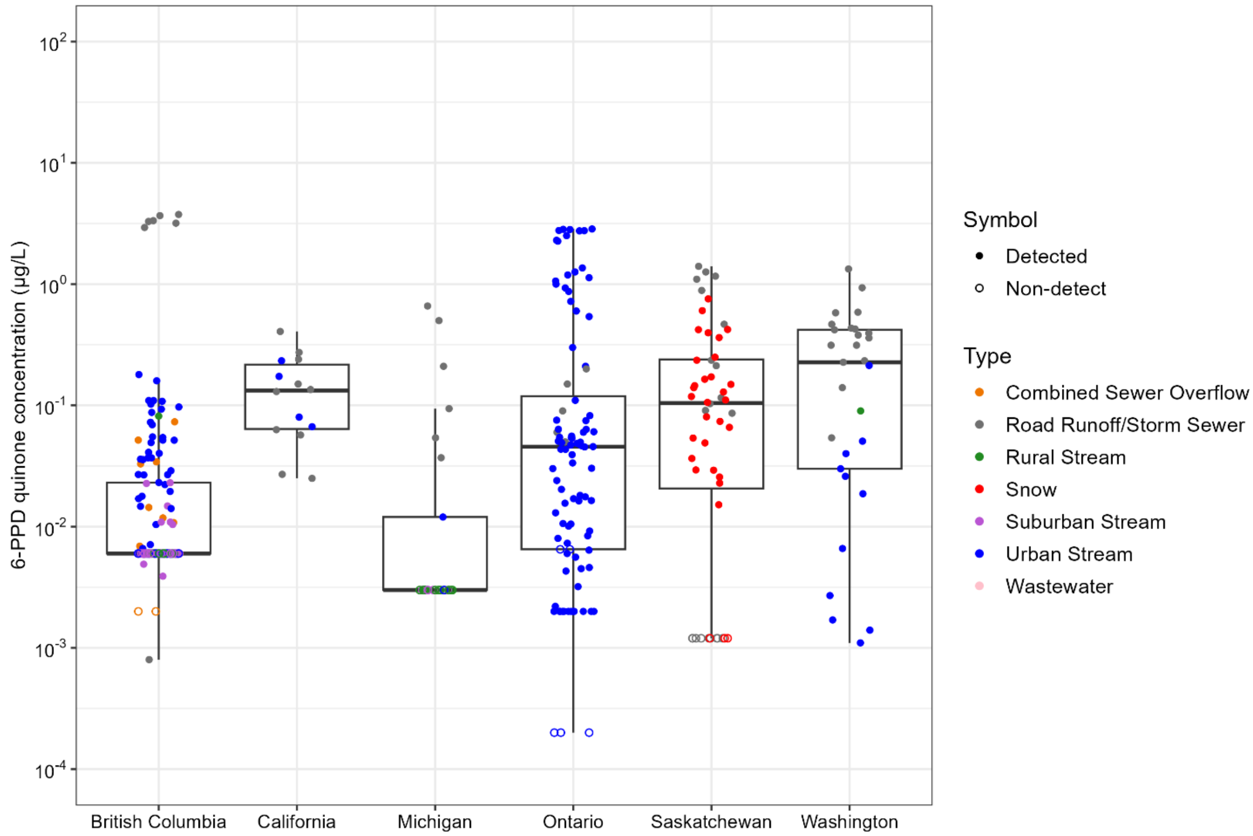

Figure 6.1 Distribution of environmental concentrations for 6PPD-quinone.

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. The data from South Coast, West Coast are pooled together.

7. <u>TOXICITY OF 6PPD-QUINONE TO AQUATIC LIFE</u>

This section provides an analysis of the sensitivity of various taxa to 6PPD-quinone, examining differences between species and life stages within salmonids and comparing these findings to the limited data available for non-salmonid species. Most of the available toxicity data on 6PPD-quinone focuses on salmonids, as they have been consistently identified as one of the most sensitive taxa. Among salmonids, coho salmon have emerged as particularly vulnerable, with significant variation in sensitivity observed across different life stages. This focus on salmonids is not only due to their ecological and cultural importance but also because their heightened sensitivity serves as an indicator for potential risks to other aquatic species.

7.1 Effects on Algae

To date, two studies have examined the effects of 6PPD-quinone on freshwater microalgae. Wu *et al*. (2023) used the green microalga *Chlamydomonas reinhardtii* as the test species and reported a lowest observed effect concentration (LOEC) for growth inhibition of 0.25 mg/L. Genomic DNA damage, measured as the number of lesions per 10^9 nucleotides, was observed at concentrations between 0.25 mg/L and 1 mg/L.

Liu *et al*. (2024) studied the effects of 6PPD and 6PPD-quinone on *Chlorella vulgaris*, finding that 6PPD-quinone enhanced photosynthetic efficiency and promoted growth at lower concentrations but inhibited it at higher levels. The study also reported that 6PPD-quinone induced oxidative stress, disrupted cell stability, and significantly altered fatty acid metabolites.

7.2 Effects on Macrophytes

Currently, there is one published study on the effects of 6PPD-quinone on freshwater macrophytes. Ge *et al*. (2024) examined concentrations up to 10 µg/L on *Eichhornia crassipes*, reporting minimal effects on growth and root length, but noting concentration-dependent reductions in photosynthetic pigments and increased oxidative damage.

7.3 Effects on Invertebrates

Aquatic invertebrates appear 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 cause 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 to 6PPD-quinone concentrations 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.

7.4 Effects on Fish

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 affected by URMS exhibit symptoms such as increased surface swimming and loss of equilibrium and buoyancy and die only a few hours after exposure (Chow *et al*. 2019). The specific mode of action of 6PPD-quinone is not yet understood.

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 are followed in sensitivity by lake trout (*Salvelinus namaycush*) (24-h LC_{50} = 0.50 µg/L [Roberts et al. 2024]), 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 (*O. mykiss*) (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).

Only three studies 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 (*Danio rerio*) 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.

A recent study of zebrafish larvae found a 24-hour exposure to 6PPD-quinone affected the central nervous system causing behavioural shifts, along with alterations 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, larvae 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 suggest there may be sub-lethal effects on fish that are more tolerant of 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 individuals in their natural environment; therefore, fish species that appear tolerant to 6PPD-quinone may, in fact, be at risk.

Investigations on the long-term chronic effects of 6PPD-quinone to date are limited. 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 (*Pimephales promelas*) embryos exposed to concentrations of 6PPD-quinone up to 39.97 µg/L for 168 hours (i.e., seven days).

7.4.1 *Mode of Action*

Research into the specific mode of action of 6PPD-quinone in fish is still ongoing as it was only identified as a highly toxic substance to coho salmon in 2020. 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 6PPD-quinone (Brinkmann *et al*. 2022; Greer *et al*. 2023a). The discrepancy between species could be attributed to differences in the mode of action of 6PPD-quinone or the fish's ability to metabolize this compound, as indicated by Mahoney *et al*. (2022). Other factors, such as age, may also play a role and require further investigation for a complete understanding.

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 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). Considering the quick action of 6PPD-quinone, it is likely that the primary source of uptake by salmon (and fish in general) is from the water directly rather than trophic transfer from sediment. While sediments can act as a sink, the rapid movement of 6PPD-quinone in aquatic environments suggests that direct absorption from water is the most probable pathway. However, the specific uptake mechanisms in coho salmon are not fully understood and further research is needed to confirm these pathways.

In *in-vitro* studies using rainbow trout, 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, with no mortality, was noted in a 96-hour exposure test (Anderson-Bain *et al*. 2023).

The influence of age on sensitivity to 6PPD-quinone has been minimally studied; however, existing data indicates that sensitivity varies across life stages, with younger fish being the most sensitive, followed by adults, and embryos showing the least sensitivity. Lo et al. (2023) reported that 3-week-old juvenile coho salmon were more sensitive to 6PPD-quinone (24-hour LC_{50} of 0.041 µg/L) compared to older coho salmon tested by Greer et al. (2023a), which had a mean age of 189 days (24-hour LC_{50} of 0.08 µg/L). Similarly, Tian et al. (2022) found that even older coho salmon (1+ year) were less sensitive, with a 24-hour LC_{50} of 0.095 µg/L.
In contrast, embryonic coho salmon showed no short-term mortality when exposed to environmental concentrations lethal to juveniles and adults (e.g., 0.1 µg/L). However, growth inhibition occurred at higher concentrations of 0.9 µg/L and 7.22 µg/L, and some surviving alevins died five to eight days after hatching under these conditions (Greer et al. 2023b). These findings suggest a general trend of decreasing sensitivity from juveniles to adults to embryos, though further research is needed to fully understand the underlying mechanisms and age-related differences in toxicity.

7.5 Effects on Amphibians

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

7.6 Toxicity Modifying Factors

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

8. CRITERIA FROM OTHER JURISDICTIONS

The US EPA recently released an acute aquatic life screening level for 6PPD-quinone of 0.011 µg/L (US EPA 2024). Washington State adopted an acute freshwater quality criterion for 6PPD-quinone, establishing a maximum concentration of 0.012 µg/L to protect aquatic ecosystems (Washington State Department of Ecology 2023).

9. DERIVATION OF 6PPD-QUINONE WATER QUALITY GUIDELINES

9.1 Toxicity Data

WQGs were derived following 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 on 6PPD-quinone toxicity to freshwater aquatic organisms, including fish and invertebrates, in water-only exposures under laboratory conditions was conducted using the search terms "6PPD-quinone," "toxicity," "aquatic life," "fish," and "invertebrates." 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 introduced species); however, studies on exotic species (e.g., Japanese medaka) were also selected and evaluated to complement the dataset. The toxicity data evaluated are presented in Appendix 2.

9.1.1 *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 (e.g., using an acute to chronic ratio) but were disregarded because no studies demonstrated both acute and chronic effects on the same organisms.

While no data 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 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 presented below may provide an indicator of potential risk for sublethal effects to non-salmonid fish when environmental concentrations are at or above this level.

9.1.2 *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 exposures such as spill events or an infrequent release of a chemical. The derivation of the short-term WQG is based on LC_{50} values from the acceptable studies. LC_{50} values were only available for six 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). Therefore both 24-hour and 96-hour LC_{50} were included in the toxicity dataset. The B.C. guideline derivation protocol (ENV 2019) specifies using the geometric mean if multiple comparable data records are available for the same species (i.e., same species, life stage, endpoint, and exposure duration). However, this condition did not apply to the 6PPD-quinone data; therefore, no geometric mean was used.

The available toxicity data did not meet the requirements for derivation of either a short-term acute Type A1 or A2 WQG due to the 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 seven 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) which were included to satisfy the Type B data requirement. The available data suggest that fish are considerably more sensitive to 6-PPD-quinone than invertebrates. Following the deterministic Type B approach with fish as the most sensitive taxa, the more tolerant invertebrates will also be protected.

In this approach, the short-term acute guideline is based on the lowest scientifically defensible acceptable effects concentration from a short-term exposure study (i.e., the critical data point). All LC_{50} and NOEC concentrations were plotted, and the critical data point was for coho salmon (LC_{50} = 0.041 µg/L). An assessment factor (AF) of 4 was applied to obtain a short-term acute WQG of 0.01 µg/L (Figure 9.1).

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
Lake trout (*Salvelinus namaycush*)	24-h LC_{50}	0.50	Roberts *et al.* 2024
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)*	24-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	>9.65	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)*	24-h LC_{50}	309	Varshney *et al.* 2022
Zebrafish *(Danio rerio)*	96-h NOEC	>54	Hiki *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

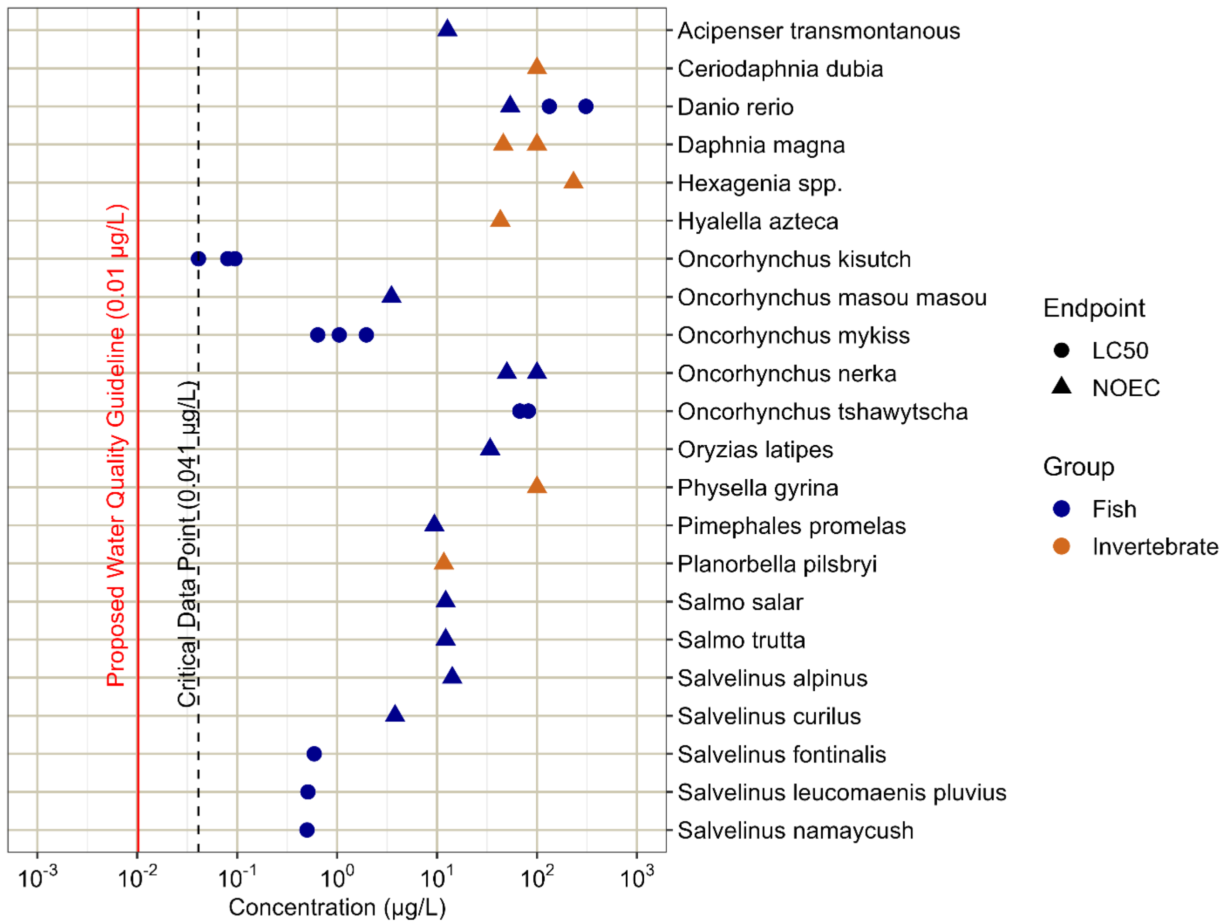

Figure 9.1 Short-term acute water quality guideline for 6PPD-quinone.

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. In addition, 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. Furthermore, there is evidence suggesting younger and smaller fish may be more sensitive to 6PDD-quinone than older fish (Greer *et al.* 2023a; Lo *et al.* 2023); however more research is needed, recognizing studies on “juvenile” fish can include a fairly large size and age range (e.g., Greer *et al.* 2023a). Most of the available acute toxicity data for fish are from tests conducted with embryos, alevins, or juveniles, however 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 6-PPD-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 6-PPD-quinone because species within the same genus show varying levels of sensitivity (Hiki and Yamamoto 2022; ITRC 2023). Thus, the degree of toxicity of 6PPD-quinone to untested species is very uncertain.

9.1.3 *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). Lake trout was the second most sensitive species, although LC_{10} values for lake trout were not available. The short-term acute WQG (0.01 µg/L) is 2.5 times lower than the geometric mean LC_{10} of coho salmon (0.025 µg/L) and therefore is assumed 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. COMPARISON 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 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, 46% of samples reported in the literature exceeded the guideline. In British Columbia, 24% 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, 57% 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; Helm *et al.* 2024). 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 6PPD-quinone concentrations above the WQG in 90% of samples (Challis *et al.* 2021). In California and Washington states, where samples were mostly collected in storm sewers, 93% and 70% of samples, respectively, were above the guideline (Tian *et al.* 2021; Zhao *et al.* 2023; Halama *et al.* 2024; Peter *et al.* 2024). 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 afterwards. Results from Monaghan *et al.* (2023) showed that concentrations of 6PPD-quinone were still elevated up to 2.8 times above the 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 document the return period to baseline conditions.

The range of coho salmon covers a large portion of B.C. (Figure 11.1). The short-term acute WQG applies to all B.C. watercourses within and outside of the coho range, regardless of whether they are considered coho-bearing or not 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 stream; and 3) while derivation of a chronic WQG was not possible at this time, there is some evidence that 6-PPD-quinone could have a chronic effect on fish 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.

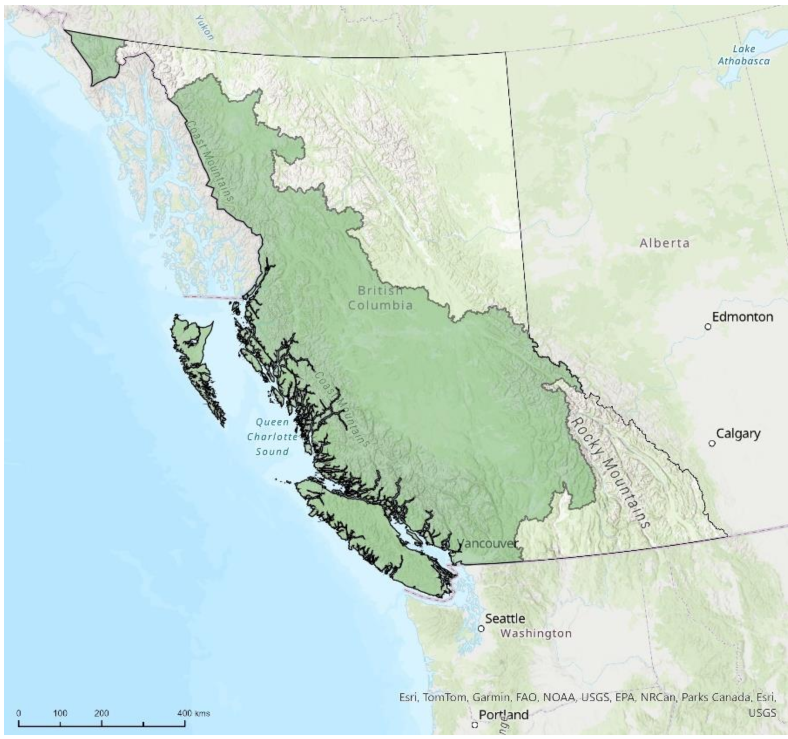

Figure 11.1. Coho salmon range in British Columbia.

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