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Subject: Technical memorandum on aquatic toxicity of stormwater and role of 6PPD-quinone

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History of WSU studies on stormwater toxicity, impacts, treatment

In 2011, researchers from several institutions published a 7-year forensics study documenting acute mortality of coho salmon spawners (*Oncorhynchus kisutch*) in Seattle-area streams and exploring the cause (Scholz *et al.* 2011). Based on weight-of-evidence, the authors concluded that stormwater runoff was responsible for the acute mortality phenomenon. That same year, research into the toxicology of stormwater runoff began at Washington State University Puyallup Research & Extension Center (WSU). Most work was in collaboration with researchers at the NOAA Northwest Fisheries Science Center (NOAA) and the U.S. Fish & Wildlife Service. Test organisms were aquatic invertebrates (daphniids from the WSU colony and wild-caught *Baetis* spp. mayfly larvae), zebrafish embryos (*Danio rerio*) from the colony at NOAA (and later WSU), Pacific herring embryos (*Clupea pallasii*) fertilized from wild-caught spawners in Puget Sound, adult coho and chum from the Suquamish Tribe Grovers Creek hatchery, and juvenile salmonids reared at WSU including coho (*O. kisutch*), chum (*O. keta*), steelhead (*O. mykiss*), Chinook (*O. tshawytscha*) and sockeye (*O. nerka*).

Our initial attempts to study the toxicity of stormwater runoff to aquatic organisms used street dirt or mixtures of chemicals known to contaminate stormwater runoff. Pilot work with street dirt from refuse piles of street sweeping by the City of Seattle did not produce acute toxic responses in the daphniid *Ceriodaphia dubia* nor in zebrafish embryos (McIntyre *unpublished data*). Mixtures of metals and hydrocarbons did not elicit acute toxic responses in adult coho salmon (Spromberg *et al.* 2016). Clearly, these simple attempts at a synthetic stormwater were missing contaminant(s) necessary to the toxicity observed when aquatic organisms are exposed to stormwater runoff (Skinner *et al.* 1999, Kayhanian *et al.* 2008, Scholz *et al.* 2011, and others).

In contrast, collected roadway runoff re-created the acute mortality syndrome of coho spawners observed in runoff-impacted streams (Spromberg *et al.* 2016). Behaviors and blood changes associated with the mortality syndrome were observed in coho, but not in co-exposed chum (*O. keta*) salmon (McIntyre *et al.* 2018). Juvenile coho were determined to be a

suitable model for studying impacts on adults, based on acute sensitivity as well as changes in behavior and blood prior to mortality (Chow *et al.* 2019). Using juvenile coho, we elucidated the behavioral progression of the mortality syndrome: lethargy -> discrete surfacing -> continuous surface swimming -> loss of equilibrium -> immobility -> mortality (Chow *et al.* 2019). The point-of-no-return occurs before surface swimming, as transfer to clean water at this stage did not prevent or delay mortality (Chow *et al.* 2019). High dilutions and brief exposures of roadway runoff are required to prevent mortality (≥95%, ≤4h) (Prat 2019). A spectrum of sensitivity to runoff exists among tested salmonids; runoff collected from three storm events produced differential lethality in the order of *O. kisutch* > *O. mykiss* > *O. tshawytscha* >> *O. nerka* = *O. keta* (French *et al.* In review). Coho embryos exposed episodically to roadway runoff during development died upon hatching, but prior to hatching showed a variety of sub-lethal effects, including impaired growth (McIntyre *et al.* In Prep) and defects in the mechanosensory system (Young *et al.* 2018). For mode of action, we have ruled out hemoglobin as a target; oxidation does not appear to underlie the various symptoms of hypoxia in coho exposed to runoff (Blair *et al.* 2020). Elevated hematocrit associated with advanced symptoms appears to result from loss of vascular integrity, causing plasma to leak from the blood into cerebral and other tissues (Blair *et al.* 2021).

Additional aquatic species are also sensitive to stormwater runoff. Studies by WSU showed that roadway runoff could be acutely toxic to daphniids, mayfly nymphs, and zebrafish embryos (McIntyre *et al.* 2014, McIntyre *et al.* 2015). Although runoff was rarely lethal to developing fish, cardiac defects are common in developing zebrafish (Wu *et al.* 2014, McIntyre *et al.* 2016) and also in developing herring (Harding *et al.* 2020) exposed to runoff. As with coho embryos, zebrafish embryos showed neurotoxicity to the mechanosensory system when developing in dilutions of runoff (Young *et al.* 2018).

Research into solutions for preventing stormwater toxicity began at WSU in 2014. We showed that lethality to coho juveniles (McIntyre *et al.* 2015), adults (Spromberg *et al.* 2016), and alevin (McIntyre 2016, McIntyre *et al.* In Prep) could be prevented by bioretention filtration, as could sublethal impairments to aquatic invertebrates (McIntyre *et al.* 2015), zebrafish (McIntyre *et al.* 2014, Young *et al.* 2018), and Pacific herring (Harding *et al.* 2020). Compost-amended bioswales (CABS) for treating runoff along roadways were moderately effective at preventing sub-lethal impacts in zebrafish embryos (Tian *et al.* 2019), as were various alternative bioretention blends designed to leach less copper and phosphorus tested at bench-scale (Herrera 2019). Similarly, field-scale bioretention installations receiving real-time inputs of runoff for two years did not always prevent sublethal toxicity (McIntyre *et al.* 2020). An innovation to use rolls of coarse sand and compost to filter incoming water as a retrofit to existing stormwater detention ponds appeared to provide toxicity reduction benefits greater than from CABS and similar or better than from bioretention (McIntyre 2021). As bioretention media ages, it may provide less protection against some types of toxicity. For example, bioretention media used to treat collected roadway runoff protected fish from sublethal impairments during a first year of use, but not during a second (Young *et al.* 2018, McIntyre *et al.* In Prep). Anecdotally, age-related loss of ability to prevent sub-lethal impairment to developing fish was also observed at WSU for experimental bioretention media containing

60% sand, 15% compost, 15% shredded bark and 10% water treatment residuals (McIntyre *unpublished data*).

Identification of 6PPD-quinone as primary causal toxicant of coho mortalities

In 2016, WSU began collaborating with analytical chemists at the University of Washington Tacoma (UWT) to learn more about the chemicals in roadway runoff causing aquatic toxicity. Using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), thousands of unique chemicals were observed in roadway runoff, many of which were also detected in the tissue of exposed coho spawners (Du *et al.* 2017). At this time, we began exploring sources of toxic chemicals to roadway runoff, including tire wear particles, windshield wiper fluid, antifreeze, used motor oil, gear oil, and transmission fluid. Sub-lethal impacts to zebrafish embryos were best explained by the presence of tire-related chemicals (McIntyre *unpublished data*), and tires were the vehicle source most similar chemically to waters that killed coho (Peter *et al.* 2018).

To explore whether tire-derived chemicals were the source of acute toxicity to coho salmon, we made a tire particle leachate by recirculating water through particles abraded from tire tread; realistic of how rainwater could interact with tires or particles worn from the tread of tires. Chemicals leached from tire tread particles were sufficient to re-create the mortality syndrome in coho salmon, including changes in blood physiology, behavior, and rapid onset of mortality, while not causing any apparent impacts in chum salmon (McIntyre *et al.* 2021). In 2018, the team began fractionating tire particle leachate to learn about the chemical properties of the toxicant(s) killing coho and support an effects direct analysis approach to toxicant identification. This involved treating the leachate to create fractions containing chemicals with different properties. These fractions were tested for toxicity to coho and analyzed by LC-HRMS. In early testing, leachate was treated by silica sand filtration, cation exchange, and chelation with ethylenediaminetetraacetic acid (EDTA). Toxicity persisted in each case, confirming that the toxicant(s) were not associated with particles, cations, or metals, respectively.

Subsequent fractionation focused on organic contaminants in tire particle leachate. Utilizing techniques including cation exchange, polarity-based separation, reverse phase high performance liquid chromatography (HPLC), and multi-dimensional HPLC in series, the chemical complexity of the toxic fraction was reduced from >2000 chemicals to just four (Tian *et al.* 2021). The most abundant chemical in the final fraction was a previously unknown chemical with molecular formula $C_{18}H_{22}N_2O_2$, which was determined to be a transformation product of 6PPD (N-(1,3- dimethylbutyl)-N'-phenyl-p- phenylenediamine) – the most widely used anti-ozonant in tire rubber. By exposing commercial 6PPD ($C_{18}H_{24}N_2$) to ozone, the team produced $C_{18}H_{22}N_2O_2$ with mass spectral properties identical to those of the $C_{18}H_{22}N_2O_2$ purified from tire wear particle leachate, confirming the origin of this previously unknown transformation product. The structure of the unknown chemical was identified by nuclear magnetic resonance (NMR) to be 6PPD-quinone (Tian *et al.* 2021).

Purified 6PPD-quinone, isolated from 6PPD subjected to ozone, was highly acutely lethal to coho salmon. The median lethal concentration (LC_{50}) was originally estimated as 0.79 µg/L (95% C.I. = 0.63-0.96 µg/L). The LC_{50} of 6PPD-quinone in unfractionated/unpurified tire tread particle leachate and roadway runoff was 0.82 µg/L (95% C.I.: 0.56-1.10 µg/L), supporting that 6PPD-quinone was the primary causal toxicant for coho salmon exposed to roadway runoff (Tian *et al.* 2021). These concentrations were determined using a 6PPD-quinone standard isolated and purified from tire wear particle leachate. Recent availability of a commercial standard and an isotope-labeled standard (D5-6PPD-Q; HPC Standards Inc.) revealed a substantially higher (15-fold) peak area response for the commercial standard relative to the in-house standard. This difference was attributed to less 6PPD-quinone mass in the in-house stocks than previously thought. On-going studies are examining possible causes of the loss of mass (including 6PPD-quinone solubility, sorption to laboratory materials, or possible oxidative polymerization) (Tian *et al.* 2022). Additionally, use of an internal standard revealed that recovery of 6PPD-quinone during sample processing was 60-70%. The net result of the refined methodology in Tian *et al*. 2022 was an 8-fold reduction of reported environmental and effect concentrations.

6PPD-quinone sources

Sources of 6PPD-quinone to the environment are expected to be primarily from tires. The parent compound, 6PPD, from which 6PPD-quinone is derived by reaction with ozone, is recommended for use as an anti-ozonant in the tread, sidewall, and rim strip of tires, as well as the rubber cover of conveyor belts (Sheridan 2010). All tires produced by the 12 member companies of the U.S. Tire Manufacturers Association use 6PPD as the primary antiozonant (https://www.ustires.org/6ppd-and-tire-manufacturing). Tire stored uncovered outdoors and re-uses of tires such as crumb rubber in synthetic turf, playgrounds, and incorporation into materials such as rubberized asphalt may continue to contribute 6PPD-quinone to the environment, but direct investigation is required for confirmation.

Rubbers used as seals may also contain 6PPD, depending on the ozone resistance of the elastomer (Sheridan 2010). For example, whereas ethylene-propylene rubbers (EPM or EPDM) are resistant to ozone, and isobutylene-based elastomers and neoprenes are moderately resistant, natural rubber, styrene-butadiene, polybutadiene, and nitrile elastomers readily degrade in the presence of ozone and require an anti-ozonant such as 6PPD to protect the rubber from cracking as it ages (Sheridan 2010). A recent report has identified dust generated from e-waste recycling (<100 µm) as a potentially important source of 6PPD-quinone, containing concentrations of 87-2,850 ng/g (Liang *et al.* 2022). For comparison, 6PPD-quinone in various other dusts (25-250 µm) was reported at up to 0.4 ng/g for households, 88 ng/g for roads, 146 ng/g for vehicles, 277 ng/g for parking lots (Huang *et al.* 2021), and for roadside soils (<250 µm) was 9.5-936 ng/g (Cao *et al.* 2022).

Lethal/sub-lethal impacts concentrations and species

In the published scientific literature, 6PPD-quinone toxicity has been directly tested on nine species of fish and two aquatic invertebrates (Table 1). As summarized above, Tian *et al.*

(2021) initially reported a 24-h LC_{50} for juvenile coho salmon of 0.8 µg/L. Tian *et al.* (2022) recently revised that estimate to be 8-fold lower (0.95 µg/L, 95% C.I. = 0.80-1.10 ng/L) based on refined analytical chemistry methods (described above). In contrast, Hiki *et al.* (2021) reported no mortality in four aquatic species tested at initial concentrations of 40-94 µg/L. Significant decreases in concentration were measured over the 48-96-hour exposures by Hiki *et al.*, resulting in time-weighted average exposure concentrations of 34-54 µg/L. Additionally, similar to Tian *et al.* (2021), Hiki *et al.* conducted their studies before a commercial standard was available for 6PPD-quinone. As such, they may have similarly over-estimated exposure concentrations. Varshney *et al.* (2021) reported a 24-h LC_{50} for zebrafish embryos of 308.7 µg/L, with sub-lethal impacts described for 96-h exposure at concentrations as low as 10 µg/L (eye size, heart rate, locomotion), based on nominal concentrations of 6PPD-quinone. Pilot studies in our lab have indicated that adsorption to polymers and presence of biota can be sources of significant loss of concentration during an exposure. More recent studies include measurement with a commercial standard, similar to Tian et al. (2022). Concentrations of 6PPD-quinone did not decrease appreciably (i.e., <20%) during an exposure by Brinkmann *et al.* (2022) likely due to high exposure volumes. The study identified two additional salmonids sensitive to 6PPD-quinone (*O. mykiss* and *Salvelinus fontinalis*), with LC50s of 0.6 µg/L and 1.0 µg/L, respectively (Table 1). The sensitivity of *O. mykiss* to 6PPD-quinone was also supported by Di et al. (2022).

6PPD-quinone concentrations in stormwater tested to date

Published studies documenting 6PPD-quinone concentrations in stormwater or surface waters are increasing in number but are still limited. Available data are summarized in Table 2. Likewise, limited evaluations of observed 6PPD-quinone concentrations with respect to land-use data have been performed to date. However, Challis *et al.* (2021) included a linear regression of 6PPD-quinone mass loads observed in Saskatoon, Canada stormwater outfalls as a function of land-use area, finding a strong positive correlation to roads and residential areas, but no correlation with industrial areas or green spaces (Challis *et al.* 2021). This result supports previous modeling efforts by NOAA and USFWS researchers linking increased risk of coho mortality with roads/traffic intensity in Seattle, WA area watersheds (Feist *et al.* 2017).

6PPD-quinone of statewide concern

Based on 6PPD-quinone concentrations observed by our research teams and those available in the literature, we anticipate statewide 6PPD-quinone concentrations in road runoff and surface waters similar to observations elsewhere (see Table 2). However, additional sampling is needed to evaluate 6PPD-quinone variability with respect to different land use and land cover characteristics, distinct watershed hydrology, and roadway types/traffic intensities.

6PPD-quinone toxicity to typical indicator species

There is very limited information on whether typical indicator species are or will be sensitive to 6PPD-quinone. Zebrafish embryos are a commonly used test species for environmental toxicants. This species does not have an acute lethal response to 6PPD-quinone (Table 1), but shows evidence of sublethal impairments. Based on the low sensitivity that we have

documented in coho and herring embryos exposed to stormwater (described above), and of zebrafish embryos exposed to 6PPD-quinone (Varshney *et al.* 2021), we suspect that the chorion reduces bioavailability of 6PPD-quinone to fish embryos. All fish species typically used in early life stage testing may therefore show similar protection compared with free swimming life stages.

The relative sensitivity of various indicator species to 6PPD-quinone may be related to their sensitivity to complex mixtures containing 6PPD-quinone, including stormwater and tire leachate, but this association needs to be tested empirically. Species and endpoints shown to be sensitive to stormwater, including reproduction in *Ceriodaphnia dubia* and survival of wild *Baetis* spp (McIntyre et al. 2015) should be explored as potential indicators for detrimental effects from 6PPD-quinone exposure. Notably, species not sensitive to 6PPD-quinone may yet be sensitive to the parent compound 6PPD (Di et al. 2022), or to other chemicals derived from tires. The toxicity of other antiozonants should also be considered, particularly as we move towards identifying a replacement for 6PPD in tires.

Next steps for toxicology research on 6PPD-quinone to limit impacts from stormwater

This section will focus on the toxicology of 6PPD-quinone. The relevant chemistry affecting toxicology of 6PPD-quinone will be described in a separate memo by researchers at UWT. Briefly however, to understand toxicity of 6PPD and 6PPD-quinone, an accurate and reliable method is needed for measurements in water and tissues. Methods have been developed for accurately and reliably measuring 6PPD-quinone in water, but we will need to be able to accurately measure 6PPD-quinone in fish tissues – a more complex matrix than water.

Defining toxicity of 6PPD and 6PPD-quinone to coho salmon and other sensitive species relevant for Washington State requires understanding the toxicokinetics and toxicodynamics of these contaminants. Toxicokinetics are the fate and transport of a chemical within the tissues of an organism, including the pathways of uptake (e.g., gills, skin, gastrointestinal tract), biotransformation (i.e., metabolites), distribution (e.g., concentrations in various tissue compartments), and elimination (e.g. *via* gills, kidney, feces). Toxicodynamics describe the way(s) in which toxicity is manifest in the organism, including target tissues and modes of action (e.g., liver via necrosis). A first step towards understanding toxicokinetics of 6PPD-quinone is underway at WSU by testing toxicity to coho under varying water quality conditions (temperature, pH, ionic strength). We will additionally test toxicity for different life stages (embryo, alevin, juvenile, adult) and under different water flow conditions.

For toxicodynamics, ongoing work at WSU is exploring impacts to the blood brain barrier (BBB) of coho exposed to roadway runoff. We have shown that BBB disruption is concurrent with advanced symptoms of the mortality syndrome (Blair *et al.* 2021). Pilot studies confirm that 6PPD-quinone alone is sufficient to cause the observed impacts. Continued work is needed to confirm that BBB disruption is the proximal cause of death, rather than a co-occurring condition, and much more work is needed to determine the molecular initiating

event leading to BBB disruption. Determining safe levels of 6PPD and 6PPD-quinone will also require evaluating sublethal impairments that are likely to impact fitness, including growth, behavior, and reproduction for coho and other species. A potential role for 6PPD-quinone in the toxic response of other aquatic species sensitive to roadway runoff remains to be determined.

Although the discovery of 6PPD-quinone allows us to begin ascribing some toxic impacts from stormwater to 6PPD-quinone specifically, it does not change our need to further understand stormwater toxicity. We must continue to elucidate the ways in which the complex chemical mixture of stormwater affects aquatic communities and develop solutions to reduce those impacts. As we learn more about organisms affected by stormwater contaminants, and the specific contaminants driving those impacts, we can work towards optimizing treatment systems and reducing inputs.

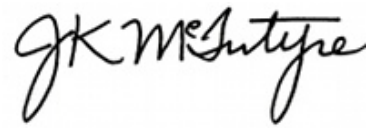
To optimize treatment systems, we first need to understand how they work. For example, bioretention is arguably the best understood of the green stormwater infrastructure (GSI) technologies for reducing stormwater contaminants and preventing toxicity. We know that contaminants are treated in bioretention by a variety of pathways including physical filtration, chemical adsorption, and microbial degradation. However, our lack of knowledge about the relative importance of these pathways, the factors affecting their efficacy, and their long-term effectiveness demonstrate that this research is still in its infancy.

At WSU we are quantifying 6PPD-quinone treatment for three ongoing research studies and one that will begin this fall: 1) a study of bioretention funded by Stormwater Action Monitoring (SAM), 2) a permeable pavement study in collaboration with IDEA School (Tacoma, WA) funded by The Boeing Foundation, 3) VIS system field test in collaboration with Long Live the Kings (LLTK), the Nisqually Tribe, and Cedar Grove Composting, and 4) a study of the role of compost in bioretention. In the SAM study, we are researching the effects on chemistry and toxicology of treatment by bioretention for various media depths aged to 10 years under accelerated conditions with collected stormwater. We preserved influent and effluent water samples for analysis of 6PPD-quinone from the final 3 events. Water samples from an additional 5 events are planned for an assessment of performance at water years 6 to 13. At IDEA School, effluents from permeable pavements to which tire particles have been applied have been analyzed for 6PPD-quinone and several other tire-derived compounds. The transport of applied tire wear particles through porous asphalt and pervious concrete have also been quantified. Two additional synthetic storms spiked with a 6PPD-quinone standard are also planned. In total, three storms will be completed to quantify removal efficiencies of 6PPD-quinone by permeable pavement. Results are expected to be published in early 2023. With LLTK, we are testing toxicity of influent and effluent waters from a VIS system treating roadway runoff over Ohop Creek, a tributary to the Nisqually River. Tire-derived chemicals, including 6PPD-quinone, are being measured by UWT as part of evaluating chemical performance. Finally, a 2022 WA State legislative proviso is funding a 3-year study of the role of compost in treating tire wear particles and tire-derived chemicals in bioretention systems.

Research needs beyond toxicology

Analyses are needed to evaluate the relative inputs of tire-derived chemicals to surface waters from various sources and the merits of various types of source controls. For example, what reductions might be achieved by increased telework to reduce daily commuting by individuals? How much reduced tire wear could be achieved by a program encouraging the purchase of lighter vehicles? What contributions do re-uses of tires make to tire chemicals in receiving waters, e.g., from playgrounds, artificial turf fields, and tire-modified asphalt?

Ultimately, tires need to be re-designed for environmental safety, while maintaining standards for road safety and tire durability. A group of experts should be conferred to define environmental safety for tires. This definition should ultimately include the health of diverse members of aquatic ecosystems, as well as humans, over acute and chronic exposures, during both intended use and end-of-life re-use applications. In the meantime, we should evaluate among currently available tires which are 'best-in-class' that could be recommended for governmental and/or commercial vehicle fleets. Finally, safe disposal options for existing tires should be identified.



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[Tables and Cited References to Follow]

Table 1. 6PPD-quinone aquatic toxicity in peer-reviewed literature. LC50 = median lethal concentration, C.L. = confidence limits

Species	pH	Conductivity (µS/cm)	Temp. (℃)	Exposure Time	Solution renewal	LC50 (µg/L; 95% C.L.)	Ref.
Oncorhynchus kisutch	7.6-7.8	1250-1300	10-12	24 h	None	0.79 (0.6-1.0)[a,*]	1
Oncorhynchus kisutch	7.6-7.8	1250-1300	10-12	24 h	None	0.10 (0.08-0.11)[a,+]	2
Danio rerio	7.7 ± 0.0	3090 ± 200	25.9 ± 0.1	96 h	48 h	>70[b,^]	3
Danio rerio	7.4 ± 0.1	ISO[c]	27 ± 1	24 h	24 h	308.7 (258.3-368.9)[d]	4
Oryzias latipes	7.9 ± 0.1	3420 ± 1100	24.4 ± 0.2	96 h	48 h	>40[b,^]	3
Daphnia magna	8.0-8.4	6380-6430	21.6-21.9	48 h	None	>60[b,^]	3
Hyallela azteca	8.0 ± 0.1	3100 ± 1100	23.5 ± 0.2	96 h	48 h	>90[b,^]	3
Salvelinus fontinalis	6.7 ± 0.1	131 ± 2.33[e]	10.3 ± 0.7	24 h	24 h	0.59 (0.48-0.63)[b,+]	5
Oncorhynchus mykiss	8.4 ± 0.5	132 ± 6.80[e]	12.8 ± 0.8	72 h	24 h	1.00 (0.95-1.05)[b,+]	5
Oncorhynchus mykiss	6.5 ± 0.0	143 ± 2	16 ± 1	96 h	48 h	2.26 (2.13-2.44)[a]	6
Salvelinus alpinus	8.4 ± 0.5	132 ± 6.80[e]	12.8 ± 0.8	96 h	24 h	>14.2[b,+]	5
Acipenser transmontanus	8.4 ± 0.5	132 ± 6.80[e]	12.8 ± 0.8	96 h	24 h	>12.7[b,+]	5
Gobiocypris rarus	8.0 ± 0.0	149 ± 2	25 ± 1	96 h	48 h	>500[a]	6

1 Tian et al. 2021
2 Tian et al. 2022
3 Hiki et al. 2021
4 Varshney et al. 2022
5 Brinkmann et al. 2022
6 Di et al. 2022

[a] Based on measured concentration at the start of exposure
[b] Time weighted average concentration
[c] ISO (International Standards Organization) Standard Fish Media
[d] Based on nominal concentrations
[e] Hardness as mg/L $CaCO_3$
[*]Concentrations measured using in-house standards without internal standard normalization. Tian et al. (2022) observed that use of a commercial standard yielded ~15-fold higher peak area response for the same 6PPD-quinone concentration in the UWT in-house standard, and 6PPD-Q recovery without internal standard normalization was ~60-70%.
[+]Concentrations measured using a commercial standard.
[^]Hiki et al. (2021) also created their own in-house standard and did not use an internal control when measuring exposure concentrations. Therefore, their reported concentrations may be overestimated, similar to Tian et al. 2021.

Table 2. 6PPD-Q concentrations in stormwater and surface waters

Location	Water Type	Grab/Composite (duration)	6PPD-quinone Concentrations [ng/L]	Land Use	Ref.
Seattle, WA, USA	Roadway runoff	Grab (24 h)	50-1270 ng/L*	Urban highway	1, 2
Los Angeles, CA, USA	Roadway runoff	Grab (unspecified)	270-400 ng/L *	Urban highway	1, 2
Seattle, WA, USA	Surface water (creeks during storm events)	Grab (unspecified) or composite (4 h)	<20-210 ng/L *	Highly urbanized residential watersheds (Miller Creek, Longfellow Creek, Thornton Creek)	1,2
San Francisco, CA, USA	Surface water (creeks during storm events)	Grab (unspecified)	65-230 ng/L*	Not available	1,2
Saskatoon, SK, Canada	Stormwater runoff (outfall sampling)	Grab (unique)	86-1400 ng/L+	Urban (residential / light industrial)	3
Saskatoon, SK, Canada	Snowmelt	Composite (8-12 locations in pile)	15-756 ng/L+	Urban (residential / light industrial)	3
Toronto, ON, Canada	Surface water (river during storm events)	Composite (42 h)	930-2850 ng/L+	Urban, downstream of high-traffic corridor (Don River)	4
Toronto, ON, Canada	Surface water (river during storm events)	Composite (42 h)	720 ± 260 ng/L (July 2020)+ 210 ± 20 ng/L (Aug 2020)+	Urban, downstream of high-traffic corridor/roadway input (Don River, Highland Creek)	5
Nanaimo, BC, Canada	Surface water (stream, during storm event)	Grab (unspecified)	96-112 ng/L	Not available	6
Nanaimo, BC, Canada	Stormwater	Grab (unspecified)	48-5580 ng/L	Not available	6
Australia	Urban tributary	Grab (unique)	0.44-88 ng/L	Sub-urban (low density residential, open space)	7
Michigan, USA	Road puddles	Grab (unique)	54-660 ng/L	Various	8
Michigan, USA	Surface water (during storm)	Grab (unique)	<9-37 ng/L	Various	8
Hong Kong	Urban runoff	Grab (unspecified)	21-243 ng/L	Dense traffic, urban area	9

1 (Tian *et al.* 2021)
2 (Tian *et al.* 2022)
3 (Challis *et al.* 2021)
4 (Johannessen *et al.* 2021)
5 (Johannessen *et al.* 2022)
6 (Monaghan *et al.* 2021)
7 (Rauert *et al.* 2022)
8 (Nedrich 2022)
9 (Cao *et al.* 2022)

*Concentration adjusted by 15-fold relative to originally reported concentration (Seattle roadway: 800-19000 ng/L; LA roadway: 4100-6100 ng/L; Seattle creeks: <300-3200 ng/L; San Francisco creeks: 1000-3500 ng/L) to account for response-factor difference in commercial vs. In-house analytical standard. Resulting concentrations may be underestimated by 30-40% because an internal standard was not used during sample extraction and quantification.

+Concentrations quantified using potentially impure in-house standard for 6PPD-quinone, and thus may overestimate actual concentration. However, Challis et al. 2021 noted potential underestimation of 50% due to matrix effects evaluated using an internal standard.

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