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Sent via e-mail

The Honourable Steven Guilbeault, P.C., M.P.
House of Commons
Ottawa, Ontario K1A 0A6
steven.guilbeault@parl.gc.ca

Dear Minister Guilbeault,

Re: Request to assess 6PPD under s 76 of the *Canadian Environmental Protection Act, 1999*

On behalf of Raincoast Conservation Foundation, Watershed Watch Salmon Society and Pacific Salmon Foundation, we are writing with respect to the chemical N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine, commonly known as 6PPD. Used to prevent tire degradation, 6PPD breaks down with tire wear and enters the environment as 6PPD-quinone (6PPD-q). 6PPD-q is highly toxic to coho salmon, responsible for mass deaths of the fish when they spawn in urban streams. Combined with emerging research about the effect of 6PPD-q on other aquatic species and on ecosystems, it is clear that 6PPD presents a serious risk to the environment.

In light of this, we are writing pursuant to section 76 of the *Canadian Environmental Protection Act, 1999* (CEPA) to request that Environment and Climate Change Canada assess 6PPD to determine whether it is toxic or capable of becoming toxic.

The Requesters

Raincoast Conservation Foundation is comprised of scientists and conservationists whose work focuses on the land, waters and wildlife of coastal British Columbia. Their area of research includes a program addressing threats facing the survival of wild salmon, a group of species that play a foundational role in marine ecosystems. Raincoast's work also includes a Healthy Waters program, carried out through partnerships with Indigenous communities and other partners to build pollution monitoring capacity in salmon watersheds.

Watershed Watch Salmon Society works to defend and rebuild wild salmon populations and their habitats in British Columbia. Among its program areas, Watershed Watch advocates for cleaner water for fish, and works with policy experts and scientists to reform water management, healthy function and intact riparian ecosystems across BC, particularly in salmon-bearing waters.

Watershed Watch recognizes and actively supports the vital importance of salmon to the culture and food sovereignty of First Nations, and as a highly valued fish by people across our province for food, culture, commerce, and recreation.

Pacific Salmon Foundation (PSF) is a non-profit with a vision of healthy, sustainable, and naturally diverse populations of Pacific salmon for the benefit of ecosystems and Canadians for generations to come. PSF's work, carried out with its partners across BC, is focused on salmon recovery, resilience, and systems transformation. PSF supports hundreds of community-led salmon conservation projects each year, conducts research in marine and freshwater areas, supports Indigenous knowledge and works to provide open public access to the best data and tools in support of sustainable management of Pacific salmon and their habitats.

These organizations have been following the emerging research about 6PPD and its alarming impacts on coho salmon in particular, as well as on ecosystems more broadly.

Statutory Framework for Section 76 Requests

CEPA's primary purpose is pollution prevention and the protection of human health and the environment. Those goals are the lens that guides decision-making under the Act, as reflected in its preamble, which recognizes the importance of an ecosystem approach, and commits to the implementation of the precautionary principle: that a lack of full scientific certainty shall not be used as a reason to postpone cost-effective measures to prevent environmental degradation if there are threats of serious or irreversible damage.

Section 64 of CEPA defines a toxic substance as one that is entering or may enter the environment in a quantity or concentration or under conditions that:

a. have or may have an immediate or long-term harmful effect on the environment or its biological diversity;

b. constitute or may constitute a danger to the environment on which life depends; or

c. constitute or may constitute a danger in Canada to human life or health.[1]

Pursuant to section 76 of CEPA, any person may file in writing with the Minister a request to assess a substance to determine whether it is toxic or capable of becoming toxic.[2] Within 90 days after the day on which it is filed, the Minister shall inform the person who filed the request of the decision, how the Minister intends to deal with it, and the reasons for the decision.

If the request is granted, the Minister must add the substance to the plan developed under section 73 of CEPA. Pursuant to section 73, that plan "specifies the substances to which the Ministers are satisfied priority should be given in assessing whether they are toxic or capable of becoming toxic" and "specifies the activities or initiatives in relation to assessing, controlling, or otherwise

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[1] *Canadian Environmental Protection Act, 1999,* SC 1999, c 33 (**CEPA**), s 64.

[2] CEPA, s 76. Section 76(3) of CEPA states that the request "shall be filed in the form and manner and shall contain the information specified by the Minister." Ecojustice requested details on this form, manner, and information and was informed these details would not be made public until Spring 2024.

managing the risks to the environment or human health posed by substances that are or will be undertaken under an Act of Parliament for whose administration either Minister is responsible and which the Ministers are of the opinion should be prioritized."[3]

In any decision-making under CEPA, the Ministers must exercise their powers in a manner that,

- Protects the environment and human health, including the health of vulnerable populations (subsection 2(1)(a)(i));
- Applies the precautionary principle (subsection 2(1)(a)(ii)); and
- Promotes and reinforces enforceable pollution prevention approaches (subsection 2(1)(a)(iii)).

In addition, the Ministers must, among other things,

- Protect the environment, including its biological diversity, and human health, from the risk of any adverse effects from the use and release of toxic substances, pollutants, and wastes (s. 2(1)(j)); and
- Consider available information on the cumulative effects on human health and the environment that may result from exposure to the substance in combination with exposure to other substances (subsection 76.1(2)).

Beyond CEPA, Canada has a responsibility to protect and preserve fish and fish habitat as areas of the environment within federal jurisdiction. The federal *Fisheries Act* prohibits activities leading to harmful alteration, disruption or destruction of fish and fish habitat.[4] The *Fisheries Act* also prohibits the deposit of all deleterious substances into water frequented by fish.[5] A "deleterious" substance is defined as one that would degrade or alter the water quality in a manner that could directly or indirectly harm fish, fish habitat, or the use of fish by humans.[6]

Given the emerging evidence detailed below of the toxicity and exposure of salmonid species to 6PPD and 6PPD-q, this substance is deleterious and likely to cause serious harm to certain fish populations, in particular coho salmon (*Oncorhynchus kisutch*).

6PPD Merits Priority Assessment

Environment and Climate Change Canada and Health Canada evaluated 6PPD during a CEPA screening assessment in November 2018.[7] During the screening, an Ecological Risk

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[3] CEPA, *supra* note 1, s 73.

[4] *Fisheries Act*, RSC 1985, c F-14 (***Fisheries Act***), s 35.

[5] *Fisheries Act, supra* note 4, s 36(3).

[6] *Fisheries Act*, *supra* note 4, s 34(1).

[7] Environment and Climate Change Canada, Health Canada, "Screening assessment: Substances identified as being of low concern using the ecological risk classification of organic substances and the threshold of toxicological concern (TTC)-based approach for certain substances" (November 2018), online:

Classification (ERC) approach found that 6PPD posed only a moderate hazard with high exposure, resulting in an ERC classification of "moderate". 6PPD was not prioritized for further assessment.[8]

The 2018 screening assessment did not account for the transformation product 6PPD-q, which was only identified in 2020. Significant information regarding the ecological hazards posed by 6PPD, including the effects of 6PPD-q on salmonid species, has since come to light that merits a full assessment of whether 6PPD is toxic under CEPA.

Urban Runoff Mortality Syndrome

Since the late 1980s, observers have described mass deaths of coho salmon in urban streams and waterways adjacent to roads, a phenomenon that became known as urban runoff mortality syndrome.[9] It was estimated that 40-90% of adult coho salmon returning to spawn in urbanized watersheds could die from this phenomenon.[10] Coho were dying in the waterways prior to spawning, particularly following storms, when road runoff was high. Road runoff, however, contains a mixture of chemicals, and the precise cause of the mass deaths remained unknown.

That changed in December 2020, when a seminal study identified the chemical 6PPD-q in Seattle runoff waters as the cause of the repeated mass mortality events in urban coho salmon populations.[11]

As noted, 6PPD-q is a degradation product of 6PPD. 6PPD makes up .04%-2% of all passenger and commercial tires by mass and is an antioxidant and antiozonant used to extend tire life.[12]

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https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/screening-assessment-substances-ercttc.html (**2018 Screening Assessment**).

[8] 2018 Screening Assessment, *supra* note 7.

[9] Alan Ohnsman, "Car Tire Dust Is Killing Salmon Every Time It Rains" (24 January 2023), *Forbes*, online: https://www.forbes.com/sites/alanohnsman/2023/01/24/car-tire-dust-is-killing-salmon-every-time-it-rains/.

[10] Mahoney et. al. (2022), Exposure to the Tire Rubber-Derived Contaminant 6PPD-Quinone Causes Mitochondrial Dysfunction In Vitro. *Environmental Science & Technology Letters* 2022 9(9), 765-771 (**Mahoney et. al., 2022**).

[11] Tian et. al. (2021), A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon, *Science* 371(6525), 185–189 (**Tian et. al., 2021**). An erratum to Tian et. al. 2021 was published: (Tian et. al. (2022), 6PPD-quinone: Revised toxicity assessment and quantification with a commercial standard. *Environmental Science & Technology Letters*, 9(2), 140–146. https://pubs.acs.org/doi/10.1021/acs.estlett.1c00910 (**Tian et. al., 2022**)). However, the erratum was in response to a new commercial standard for 6PPD and does not affect the study's conclusions on the relationship between 6PPD and coho salmon mortality.

[12] Varshney et. al., (2022), Toxicological effects of 6PPD and 6PPD quinone in zebrafish larvae. *Journal of Hazardous Materials*, 424(Pt C), 127623 (**Varshney et. al., 2022**); Di et. al. (2022), Chiral perspective evaluations: Enantioselective hydrolysis of 6PPD and 6PPD-quinone in water and enantioselective toxicity to Gobiocypris rarus and Oncorhynchus mykiss. *Environment International*, 166, 107374 (**Di et. al., 2022**); Hu et. al. (2022), Transformation Product Formation upon Heterogeneous Ozonation of the Tire Rubber Antioxidant 6PPD (N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine). *Environmental Science & Technology Letters*, 9(5), 413–419 (**Hu et. al., 2022**).

6PPD-q is intrinsically tied to 6PPD. Where 6PPD is used, 6PPD-q is almost certainly present.[13]

Sampling of 6PPD-q in Canada

6PPD-q is being detected in aquatic environments around the world.[14] Studies of 6PPD-q in Canada are in their infancy. Nonetheless, in Canada 6PPD-q has been documented in road runoff and wastewater at levels of concern in Toronto and Saskatoon.[15] Emerging studies are also documenting its presence in BC waterways.[16]

Toronto stormwater runoff contains levels of 6PPD-q that would be toxic not only to coho salmon, but to other fish species including rainbow trout (*Oncorhynchus mykiss*), and brook trout

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[13] Varshney et. al., 2022, *supra* note 12; Johannessen, C., & Metcalfe, C. D. (2022), The occurrence of tire wear compounds and their transformation products in municipal wastewater and drinking water treatment plants. *Environmental Monitoring and Assessment*, 194(10), 731 (**Johannessen & Metcalfe, 2022**); Zhao et. al. (2023a), Screening p-Phenylenediamine Antioxidants, Their Transformation Products, and Industrial Chemical Additives in Crumb Rubber and Elastomeric Consumer Products. *Environ. Sci. Technol*, 57, 7, 2779–2791 (**Zhao et. al., 2023a**); Liang et. al. (2022), E-Waste Recycling Emits Large Quantities of Emerging Aromatic Amines and Organophosphites: A Poorly Recognized Source for Another Two Classes of Synthetic Antioxidants. *Environmental Science & Technology Letters*, 9(7), 625–631 (**Liang et. al. 2022**); Armada et. al. (2023), Assessment of the bioaccessibility of PAHs and other hazardous compounds present in recycled tire rubber employed in synthetic football fields. *The Science of the Total Environment*, 857, 159485 (**Armada et. al., 2023**).

[14] Tian et. al., 2021, *supra* note 11; Hiki & Yamamoto (2022), Concentration and leachability of N- in road dust collected in Tokyo, Japan. *Environmental Pollution* (2022), 302 (**Hiki & Yamamoto, 2022a**); Rauert et. al. (2022). Concentrations of Tire Additive Chemicals and Tire Road Wear Particles in an Australian Urban Tributary. *Environmental Science & Technology*, 56(4), 2421–2431 (**Rauert et. al., 2022a**); Rauert et. al., (2022b), Tyre additive chemicals, tyre road wear particles and high production polymers in surface water at 5 urban centres in Queensland, Australia. *The Science of the Total Environment*, 852, 158468–158468 (**Rauert et. al., 2022b**); Zhang et. al., (2023a), Occurrence and risks of 23 tire additives and their transformation products in an urban water system. *Environment International*, 171, 107715 (**Zhang H-Y. et. al., 2023a**); Zhang et. al. (2023b), Aquatic environmental fates and risks of benzotriazoles, benzothiazoles, and p-phenylenediamines in a catchment providing water to a megacity of China. *Environmental Research*, 216(Pt 4), 114721 (**Zhang R. et. al. 2023b**); Cao et al. (2022a), New Evidence of Rubber-Derived Quinones in Water, Air, and Soil. *Environmental Science & Technology*, 56(7), 4142–4150 (**Cao et. al., 2022a**); Zhao et. al. (2023), Transformation Products of Tire Rubber Antioxidant 6PPD in Heterogeneous Gas-Phase Ozonation: Identification and Environmental Occurrence. *Environmental Science & Technology*, 57(14), 5621–5632 (**Zhao et. al., 2023b**).

[15] Cao et. al. (2022b), Mass spectrometry analysis of a ubiquitous tire rubber-derived quinone in the environment. *Trends in Analytical Chemistry (Regular Ed.)*, 157, 116756 (**Cao et. al., 2022b**); Challis et. al. (2021), Occurrences of Tire Rubber-Derived Contaminants in Cold-Climate Urban Runoff. *Environmental Science & Technology Letters*, 8(11), 961–967 (**Challis et. al., 2021**).

[16] Presentation by Fisheries and Oceans Canada, “Pacific Science Enterprise Centre (PSEC) Speaker Series: the ‘tire chemical’ (6PPD-quinone) and associated risks to salmon in coastal BC streams” (14 December 2023), archived online: https://drive.google.com/file/d/1McVan_27XCU5pj6rURcNMWwri7t98h6Z/view?usp=drive_link (**DFO 6PPD Presentation**).

(*Salvelinus fontinalis*).[17] 6PPD-q has also been detected in Toronto air samples and at the discharge point of an Ontario municipal wastewater treatment plant.[18]

In Saskatchewan, 6PPD-q has been detected in 57% of Saskatoon stormwater and 80% of Saskatoon snowmelt. More than 20% of Saskatoon snowmelt reached levels considered to be lethal to coho salmon (though coho salmon are not present in this area).[19]

In Seattle, Washington, where repeated mass mortality events in coho salmon have been observed, road runoff has been found with up to 3.2 µg/L of 6PPD-q, a level comparable with Toronto stormwater.[20]

Urban runoff mortality syndrome has been anecdotally reported among coho salmon in streams in the Greater Vancouver area by Fisheries and Oceans Canada,[21] although a causal link to 6PPD-q has yet to be established. Research is underway at UBC, Vancouver Island University and at Fisheries and Oceans Canada that is likely to significantly advance our understanding of the presence, extent and effects of this contaminant in fish habitat in the near future.

BC Conservation Foundation biologists (in collaboration with Vancouver Island University chemists) have launched a citizen science campaign to collect and rapidly screen water samples from Vancouver Island streams for the presence of 6PPD-q. Sampling from fall 2022 found that smaller and more urbanized creeks were prone to higher concentrations of 6PPD-q, and four of the 14 streams studied exhibited concentrations toxic to coho salmon during at least one rain event. Stream samples collected during rain events showed a 40% detection frequency (20 of 50 samples collected).[22] Preliminary sampling conducted by Fisheries and Oceans Canada in the Lower Mainland and on Vancouver Island indicates results consistent with those found in Washington and elsewhere in Canada – detecting 6PPD-q above toxic levels.[23]

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[17] Cao et. al., 2022b, *supra* note 15.

[18] Johannessen et. al. (2022), Air monitoring of tire-derived chemicals in global megacities using passive samplers. *Environmental Pollution*, 314, 120206 (**Johannessen et. al., 2022b**); Johannessen & Metcalfe, 2022, *supra* note 13.

[19] Challis et. al., 2021, *supra* note 15.

[20] Tian et. al., 2021, *supra* note 11; Cao et. al., 2022b, *supra* note 15.

[21] Brent Richter, "Chemicals kill dozens of salmon in West Vancouver creek" (7 Nov 2023), *North Shore News*, online: https://www.nsnews.com/local-news/chemicals-kill-dozens-of-salmon-in-west-vancouver-creek-7799623; Nono Shen, "'Devastating:' B.C. stream watchers link 'unprecedented' coho salmon kill to tire toxin" (19 Nov 2023), *Vancouver Sun*, online:https://vancouversun.com/news/local-news/bc-coho-salmon-deaths-tire-toxin-drought.

[22] Monaghan et al. (2023), "Automated, High-Throughput Analysis of Tire-Derived p-Phenlyenediamine Quinones (PPDQs) in Water by Online Membrane Sampling Coupled to Ms/MS". *ACS ES&T Water* 3(10), 3293–3304 (**Monaghan et. al., 2023**).

[23] DFO 6PPD Presentation, *supra* note 16.

Globally, 6PPD-q has been found in snow,[24] soil,[25] dust,[26] drinking water,[27] and air.[28] 6PPD-q is also likely widespread in humans; it has been frequently detected in human urine samples[29] and has been found to be ubiquitous in human digestive fluids.[30] 6PPD-q has been detected in air, water, and road runoff samples around the world, including in China, Japan, France, Germany, Sri Lanka and Malaysia.[31] In the United States alone, it has been estimated between 26 and 1,900 tonnes of 6PPD-q is generated from tire tread wear.[32]

Toxicity of 6PPD-q

Research is still emerging on the implications of 6PPD-q contamination in Canada. Early findings are clear: 6PPD-q is acutely toxic to coho salmon at environmentally relevant concentrations; 6PPD-q is toxic to other salmonids and aquatic organisms at varying degrees of sensitivity; and coho salmon are dying in areas impacted by road runoff in mainland BC and on Vancouver Island before they have a chance to spawn.

For Indigenous communities in BC, salmon hold immense cultural and spiritual significance. Salmon are often revered as a symbol of sustenance, life, and interconnectedness with nature.[33] Traditional practices involve ceremonies to honour the salmon and express gratitude for their

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[24] Seiwert et. al. (2022), Abiotic oxidative transformation of 6-PPD and 6-PPD quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. Water Research, 212, 118122 (**Seiwert et. al., 2022**); Cao et. al., 2022a, *supra* note 14.

[25] Cao et. al., 2022a, *supra* note 14.

[26] Hiki & Yamamoto, 2022a, *supra* note 10; Deng et. al. (2022), Distribution patterns of rubber tire-related chemicals with particle size in road and indoor parking lot dust. *The Science of the total environment*, 844, 157144 (**Deng et. al., 2022**); Klockner et. al. (2021), Comprehensive characterization of tire and road wear particles in highway tunnel road dust by use of size and density fractionation. *Chemosphere* 279, 130530 (**Klockner et. al., 2021**); Zhang Y. et. al. (2022b), Widespread N-(1,3- dimethylbutyl)-N′ -phenyl-p-phenylenediamine quinone in size-fractioned atmospheric particles and dust of different indoor environments. *Environmental Science & Technology Letters* 9(5), 420–425 (**Zhang Y. et. al., 2022b**).

[27] Zhang H-Y. et. al., 2023a, *supra* note 14.

[28] Zhang Y. et. al. (2022a), p-Phenylenediamine Antioxidants in PM2.5: The Underestimated Urban Air Pollutants. *Environmental Science & Technology*, 56(11), 6914–6921 (**Zhang Y. et. al., 2022a**); Johannessen et. al., 2022b, *supra* note 18; Wang et. al. (2022), Beyond Substituted p-Phenylenediamine Antioxidants: Prevalence of Their Quinone Derivatives in PM2.5. *Environmental Science & Technology*, 56(15), 10629–10637 (**Wang et. al., 2022**).

[29] Du et. al. (2022), First Report on the Occurrence of N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) and 6PPD-Quinone as Pervasive Pollutants in Human Urine from South China. *Environmental Science & Technology Letters*, 9(12), 1056–1062 (**Du et. al., 2022**).

[30] Armada et. al., 2023, *supra* note 13.

[31] Benis et. al. (2023), Environmental Occurrence and Toxicity of 6PPD Quinone, an Emerging Tire Rubber-Derived Chemical: A Review. *Environmental Science & Technology Letters*, 10(10), 815−823 (**Benis et al. 2023**).

[32] Benis et. al., 2023, *supra* note 31.

[33] Syilx Okanagan Nation Alliance, "ntytyix Chief Salmon" (accessed 31 January 2024), online: https://www.syilx.org/fisheries/okanagan-sockeye/

abundance, such as through the first fish ceremony.[34] The cycle of salmon abundance and return is intricately woven into the cultural fabric of many First Nations, reflecting a deep respect for the natural world and a sustainable way of life. Coho salmon are also an important food source to a number of First Nations in the Fraser watershed.[35] Threats to the species threaten these cultural traditions.

Coho Salmon

Coho salmon are an ecologically, economically, and culturally significant species native to BC and found across its rivers, streams, and coastal areas. The seasonal migration of salmon across a variety of ecosystems, from freshwater streams, to estuaries and oceans, makes them a "keystone species", maintaining the balance of the entire ecosystem, and without which that ecosystem disappears or is dramatically altered.[36]

Coho face numerous challenges in their freshwater ecosystems. Habitat degradation due to urbanization, logging, and agriculture poses a threat to the environments in which they spawn.[37] Pollution, sedimentation, and changes in water temperature further impact survival.[38] As detailed further below, climate change is affecting river flows and increasing the frequency of extreme weather events,[39] exacerbating the impact of pollutants from road run-off.

Studies show coho salmon to be the most sensitive of fish species to 6PPD-q tested thus far. When exposed to 6PPD-q levels above 41 ng/L, 50% of three week post-swim up coho salmon experience rapid mortality, dying within hours of their initial exposure.[40] Further, when exposed to concentrations above 95 ng/L, 50% of smolts (1+ year) coho salmon experience rapid mortality.[41] Runoff waters in Seattle and Toronto both regularly exceed these lethal levels of

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[34] Andrea Reid, "Learning from Indigenous knowledge-holders on the state and future of wild Pacific salmon. *The Conversation Canada*, May 16, 2023. Online at: https://theconversation.com/learning-from-indigenous-knowledge-holders-on-the-state-and-future-of-wild-pacific-salmon-182411.

[35] COSEWIC (2016). COSEWIC assessment and status report on the Coho Salmon *Oncorhynchus kisutch*, Interior Fraser population, in Canada. Committee on the Status of Endangered Wildlife in Canada. Online: https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry/cosewic-assessments-status-reports/coho-salmon-interior-fraser-2016.html (**COSEWIC Fraser Coho Report**).

[36] Cederholm et. al. (2000), Pacific Salmon and Wildlife - Ecological Contexts, Relationships, and Implications for Management. Special Edition, Technical Report, Prepared for D.H. Johnson and T.A. O'Neill (Managing directors), Wildlife-Habitat Relationships in Oregon and Washington. *Washington Department of Fish and Wildlife*, Olympia, Washington, p. 58 (**Cederholm et. al., 2000**).

[37] Bilby & Mollot (2008), Effect of changing land use patterns on the distribution of coho salmon (*Oncorhynchus kisutch*) in the Puget Sound region. *Can. J. Fish. Aquat. Sci*. 65: 2138-2148 (**Bilby & Mollot, 2008**).

[38] Grant et. al. (2019), State of Canadian Pacific Salmon: Responses to Changing Climate and Habitats. *Can. Tech. Rep. Fish. Aquat. Sci*. 3332 (**Grant et. al., 2019**).

[39] Grant et. al., 2019, *supra* note 38.

[40] Tian et. al., 2021, *supra* note 11; DFO 6PPD Presentation, *supra* note 16.

[41] Tian et al., 2022, *supra* note 11.

6PPD-q.[42] Although coho are not found in Saskatchewan or Ontario, such findings raise important questions about the potential for harm to other valued fish species.[43]

Declines of coho and other salmon species can have ecosystem-wide impacts. They transport energy and nutrients between the ocean, estuaries, and freshwater environments. They provide a source of carbon, nitrogen and phosphorous necessary to maintain the nutritional value of the streams in which they spawn.[44] Wildlife congregate along shorelines to feed on the salmon, making these riparian habitats a bridge between upland and aquatic environments. Much of BC's coastal forests are fertilized by marine-derived nutrients.[45]

Coho declines in BC have already prompted a regulatory response. In 2016, COSEWIC identified the Interior Fraser River coho population, which represents 25% of Canadian coho salmon, as Threatened.[46] The population is currently under consideration for addition to Schedule 1 of the *Species at Risk Act*.[47] Pursuant to protections under the *Fisheries Act*, recreational fishing of wild coho is closed.[48]

Coho fishing is restricted throughout BC, and recreational coho fishing is often limited to hatchery-raised salmon.[49] Wild coho in the Interior Fraser River have not been commercially harvested since 1998 and recreational harvesting is rarely permitted.[50] Even with protections, however, coho populations are a fraction of what they once were.[51] Interior Fraser River coho populations remain low (with a marine survival rate of under 3%) and coho populations throughout southern BC are in decline.[52] 6PPD-q is almost certainly a contributing factor; the more 6PPD-q in the water, the less likely coho salmon populations will recover.

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[42] Tian et. al., 2021, *supra* note 11; Cao et. al., 2022b, *supra* note 15.

[43] Challis et. al., 2021, *supra* note 15.

[44] Cederholm et. al. 2000, *supra* note 36, p. 64.

[45] Cederholm et. al. 2000, *supra* note 36, p. 65.

[46] COSEWIC Fraser Coho Report, supra note 35.

[47] Species at risk public registry, Coho salmon (*Oncorhynchus kisutch*), Interior Fraser population, online: https://species-registry.canada.ca/index-en.html#/species/716-98#legal_list (accessed January 12, 2024).

[48] COSEWIC Fraser Coho Report, *supra* note 35; Fisheries and Oceans Canada. (2023). BC tidal area 29 – Lower mainland, Sunshine Coast, Fraser River: Recreational fishing limits, openings and closures, online: https://www.pac.dfo-mpo.gc.ca/fm-gp/rec/tidal-maree/a-s29-eng.html (accessed February 2, 2024).

[49] Fisheries and Oceans Canada, "Recreational fishing limits, openings and closures in British Columbia by fishery management area" (2023) https://www.pac.dfo-mpo.gc.ca/fm-gp/rec/bc-zones-cb-eng.html (accessed August 22, 2023).

[50] COSEWIC Fraser Coho Report, *supra* note 35.

[51] Bendriem et. al. (2019). A review of the fate of southern British Columbia coho salmon over time. *Fisheries Research, 218, 10–21* (**Bendriem et. al. 2019**).

[52] Fisheries and Oceans Canada, "Coho Update" (12 April 2022), online: https://frasersalmon.ca/wp-content/uploads/2022/05/D01-Coho-Salmon-in-2022-Fraser-River.pdf; Grant et. al., 2019, *supra* note 38.

Coho populations are also in decline on Vancouver Island, where the species can be found in the small streams along the coast. Despite the absence of large urban areas, tire dust accumulates along the coastal highways of the island. Following increasingly dry summer and fall months (exacerbated by the effects of climate change), winter flood events carry this dust and other pollutants into streams. Ongoing research by BCCF's Aquatic Research and Restoration Centre and Vancouver Island University has indicated toxic levels of 6PPD-q in a large number of these streams frequented by coho.[53]

While the coho salmon's native range is restricted to British Columbia, they are also present in Lake Ontario, along with other salmonid species. Considering the many cities that line the rivers surrounding Lake Ontario and the lethal levels of 6PPD-q in Toronto runoff, the chemical poses a threat to this coho population, as well.

Brook Trout

6PPD-q has proven toxic to brook trout,[54] the last remaining native salmonid in Toronto and the surrounding region. Brook trout populations have steadily declined since 2001. They currently occupy only 21% of their historic range in the Lake Ontario basin.[55]

Recorded levels of 6PPD-q in Toronto runoff waters are high enough to be of concern to brook trout (≥ 590 ng/L).[56] Brook trout populations decline or disappear entirely in areas of high road density, indicating a serious possibility that 6PPD-q is contributing to their overall decline, particularly in the Toronto area.[57]

Rainbow Trout

6PPD-q is also toxic to rainbow trout, a salmonid introduced to the Toronto area a century ago and now found widely through the Great Lakes and their watersheds. While the concentrations that prove lethal are higher than those for coho salmon, levels of 6PPD-q in Toronto have been recorded that meet and exceed toxic levels to this species.[58]

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[53] Monaghan et. al. 2023, *supra* note 22.

[54] Brinkmann et. al., 2022, "Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance". *Environmental Science & Technology Letters*, 9(4), 333–338 (**Brinkmann et. al., 2022**).

[55] Wood, J. (2017), "The Conservation and Management of Brook Trout in Ontario: Past, Present, and Future" (PowerPoint slides). *Latornell Conservation Symposium*, Alliston ON. http://www.latornell.ca/wp-content/uploads/files/presentations/2017/Latornell_2017_W3A_Jacquelyn_Wood.pdf.

[56] Cao et. al., 2022b, *supra* note 15.

[57] Toronto and Region Conservation Authority, "Brook Trout on the decline: What can we do?" (1 December 2017), online: https://trca.ca/news/brook-trout-decline/.

[58] Brinkmann et. al., 2022, *supra* note 54; Di et. al, 2022, *supra* note 12.

Rainbow trout support recreational, subsistence, and ceremonial fisheries, which contribute $957 million to local and national economies and employ 5,000 people.[59] Rainbow trout are also present in both the Toronto and Vancouver areas, locations where 6PPD-q levels are either proven high enough to pose a threat, or where they may reasonably be high enough to threaten the species.[60]

The Athabasca rainbow trout population is listed as endangered under the *Species at Risk Act* (SARA).[61] While its distance from urban areas mean that the Athabasca River is less likely to contain levels of 6PPD-q high enough to be lethal to rainbow trout, Canada has recognized that habitat loss and road development pose significant threats to the population,[62] along with threats from habitat loss, contaminants from mine sites, and climate change.[63] If road development in the Athabasca region continues, 6PPD-q may add to threats already facing this population.

Chinook Salmon

Chinook salmon abundance trends have been declining across BC, reaching critically low levels in the southern parts of the province.[64] Now evidence is emerging about the potential toxicity of 6PPD-q to juvenile Chinook salmon.[65] One study has found evidence of levels toxic to Chinook following storm events.[66]

While further research is needed to establish whether 6PPD-q poses a threat to Chinook at levels typically found in its habitats, precaution is urged with respect to this species, given that it is the primary prey of the highly endangered Southern Resident killer whale (SRKW) population. The presence of Chinook form part of the SRKW's critical habitat, and are therefore legally protected and required to be preserved under SARA.

Other Harms

While population-level threats associated with 6PPD-q to fish are clear, scientific understanding of potential impacts on other species, including humans, is an area of ongoing study. The mode of action for 6PPD-q toxicity remains unclear. As yet, it is difficult to predict the extent to which

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[59] Kadykalo et. al. (2022), Uncertainty, anxiety, and optimism: Diverse perspectives of Rainbow and Steelhead Trout Fisheries Governance in British Columbia. *Environmental Challenges, 9, 100610* (**Kadykalo et. al., 2022**).

[60] Cao et. al., 2022b, *supra* note 15.

[61] Fisheries and Oceans Canada, "Recovery Strategy for the Rainbow Trout (*Oncorhynchus mykiss*) in Canada (Athabasca River populations)" (2020), online: https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry/recovery-strategies/rainbow-trout.html (**Rainbow Trout Recovery Strategy**).

[62] Rainbow Trout Recovery Strategy, *supra* note 61.

[63] Rainbow Trout Recovery Strategy, *supra* note 61.

[64] Grant et. al., 2019, *supra* note 38.

[65] Lo et. al., (2023), "Acute Toxicity of 6PPD-Quinone to Early Life Stage Juvenile Chinook (*Oncorhynchus tshawytscha*) and Coho (*Oncorhynchus kisutch*) Salmon". *Environmental Toxicology and Chemistry* 42:4, 815-822 (**Lo et. al., 2023**).

[66] Lo et. al. 2023, *supra* note 65.

a given species may be harmed by 6PPD-q.[67] Consequently, findings on 6PPD-q toxicity are by necessity experimental. If exposure of an aquatic species to 6PPD-q has not been individually studied, the possibility remains that it is sensitive to 6PPD-q.[68]

6PPD-q's harmful effects were first published as recently as 2021, with salmonids appearing to be most sensitive among a number of species studied to date.[69] 6PPD-q may well pose a risk to other salmonids including coastal cutthroat trout (*Oncorhynchus clarkii*).

The mechanism of toxicity of 6PPD-q remains unknown. It has, however, been detected in the tissue of snakehead, weever and Spanish mackerel, suggesting evidence of bioaccumulation.[70] In another study, 6PPD and 6PPD-q were found to bioaccumulate in the livers of mice in a dose-dependent manner.[71] Research from 2023 indicates that 6PPD and other contaminants may be taken up and metabolized by plants consumed by humans, including lettuce.[72]

Cumulative Impacts

6PPD-q is released into the environment alongside a plethora of other contaminants, many of which are tied to road runoff, vehicle traffic and tire wear. These include tire particles, microplastics, metals, polycyclic aromatic hydrocarbons, perfluoroalkyl substances (PFS) and additional quinones.[73] It is currently unknown whether 6PPD-q interacts with these other chemicals, but these co-occurring contaminants may exacerbate the toxicity of 6PPD-q to fish.[74]

Tire wear is likely to increase as electric vehicles, whose batteries result in heavier cars, become more prevalent on the roads, exacerbating the impact of 6PPD-q. Analysts have found that adding 1,000 pounds to a midsize vehicle increases tire wear by approximately 20 percent.[75] The

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[67] Foldvik et. al. (2022), Acute Toxicity Testing of the Tire Rubber–Derived Chemical 6PPD-quinone on Atlantic Salmon (*Salmo salar*) and Brown Trout (*Salmo trutta*). *Environmental Toxicology and Chemistry*, 41(12), 3041–3045 (**Foldvik et. al., 2022**).

[68] Foldvik et. al., 2022, *supra* note 67.

[69] Brinkmann et. al., 2022, *supra* note 54.

[70] Lo, et. al., 2023, *supra* note 65.

[71] Fang, et. al. (2023), "Oral exposure to tire rubber-derived 6PPD and 6PPD-quinone induced hepatoxicity in mice". *Sci. Total Environ*, 869:161836 (**Fang, et. al. 2023**).

[72] Castan et. al. (2023), "Uptake, Metabolism, and Accumulation of Tire Wear Particle-Derived Compounds in Lettuce". *Environmental Science & Technology* 57, pp. 168-178 (**Castan et. al. 2023**).

[73] Department of Toxic Substances Control (2022), "Product - Chemical Profile for Motor Vehicle Tires Containing N-(1,3-Dimethylbutyl)-N′-phenyl-pphenylenediamine (6PPD)", (2022), online: https://dtsc.ca.gov/wp-content/uploads/sites/31/2022/05/6PPD-in-Tires-Priority-Product-Profile_FINAL-VERSION_accessible.pdf (**Department of Toxic Substances Control 2022**).

[74] Department of Toxic Substances Control 2022, *supra* note 73.

[75] Paul Krantz, " EVs are a climate solution with a pollution problem: Tire particles" (25 September 2023), *Grist*, online: https://grist.org/transportation/evs-are-a-climate-solution-with-a-pollution-problem-tire-particles/.

finding lends urgency to the need to regulate 6PPD, particularly given Canada's plan to phase out the sale of new gasoline cars by 2035.[76]

Climate change also plays a role in shaping the toxic risks associated with 6PPD-q. Rising water temperatures, for example, can prevent optimal growth in fish and increase thermal stress, which may make fish more susceptible to 6PPD-q toxicity.[77] Brook trout in Ontario and certain populations of coho salmon, including those in the interior Fraser River, are particularly vulnerable to rising temperatures, creating a potential for additional harm from 6PPD-q in the future.[78]

Droughts, most notably those affecting BC in 2022 and 2023, have made riverways in which salmon spawn more susceptible to 6PPD. When water levels are lowered by drought, and then flooded by large storm events, more roadway contaminants wash into rivers and streams, where there is less water volume to dilute them. Altered precipitation patterns will impact road runoff and consequent exposure to 6PPD-q.[79]

These effects are cumulative and compounding, degrading urban streams and rivers that are critical habitat for coho salmon and other species. In assessing this request, the Ministers must therefore consider the vulnerability of these waterways and the potential that they will no longer be able to support the species reliant on them absent efforts to regulate and contain the toxins flowing into them.[80]

Regulation in Other Jurisdictions

6PPD has, in the past year, become the subject of regulatory action in several US jurisdictions. This is a relevant consideration under CEPA, which provides that "[t]he Minister shall, to the extent possible, cooperate and develop procedures with jurisdictions, other than the Government of Canada, to exchange information respecting substances that are specifically prohibited or substantially restricted by or under the legislation of those jurisdictions for environmental or health reasons".[81]

Pursuant to subsection 75(3), where the Minister is notified of a decision made by another jurisdiction to prohibit or restrict any substance for environmental or health reasons, the Minister shall review the decision in order to determine whether the substance is toxic or capable of

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[76] Transport Canada, "Canada's Action Plan for Clean On-Road Transportation" (December 2022), online: https://tc.canada.ca/en/road-transportation/publications/canada-s-action-plan-clean-road-transportation.

[77] Department of Toxic Substances Control 2022, *supra* note 73.

[78] Irvine, J. (2004). "Climate Change, Adaptation, and 'Endangered' Salmon in Canada." Proceedings of the Species at Risk 2004 Pathways to Recovery Conference, Victoria, B.C., online: https://www.arlis.org/docs/vol1/69415913/irvine_edited_final_jan_31.pdf; Dove-Thompson et. al., (2011), "A Summary of the Effects of Climate Change on Ontario's Aquatic Ecosystems" . *Ontario Ministry of Natural Resources*, online: https://files.ontario.ca/environment-and-energy/aquatics-climate/stdprod_088243.pdf.

[79] Monaghan et. al., 2023, *supra* note 22.

[80] CEPA, *supra* note 1, s 76.1(2).

[81] CEPA, *supra* note 1, s 75(2).

becoming toxic.[82] "Jurisdiction" used in section 75 includes the government of a foreign state or of a subdivision of a foreign state that is a member of the Organization for Economic Co-operation and Development (OECD).

The United States, an OECD country, has taken steps toward regulating 6PPD in response to the dangers posed by 6PPD-q contamination.

On November 2, 2023, the US Environmental Protection Agency (EPA) granted a petition from the Yurok Tribe, the Port Gamble S'Klallam Tribe, and the Puyallup Tribe of Indians under the federal *Toxic Substances Control Act* (TSCA). The petitioners submitted a petition under section 21 of the TSCA, asking the EPA to consider establishing regulations prohibiting the manufacturing, processing, use and distribution of 6PPD in tires. The EPA now plans to publish an advanced notice of proposed rulemaking under section 6 of the TSCA by fall 2024, in order to gather more information that could be used to inform a subsequent regulatory action.[83] This process is comparable to what the Requesters are seeking under CEPA.

Two individual states have also taken action.

The California Department of Toxic Substances Control (DTSC) promulgated a regulation to add motor vehicle tires containing 6PPD to its Priority Product List, effective October 1, 2023.[84] This requires domestic and foreign manufacturers of tires that contain 6PPD and are placed into the stream of commerce in California to notify the DTSC of their 6PPD use by November 30, 2023.[85] Manufacturers must take steps to reduce 6PPD use and/or evaluate alternatives by March 29, 2024.[86]

Following publication of a technical memo outlining the hazards posed by 6PPD-q,[87] the Washington State legislature tasked its Department of Ecology with assessing potential alternatives to 6PPD in motor vehicle tires. It published hazard criteria for assessing alternatives in June of 2023.[88]

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[82] CEPA, *supra* note 1, s 75(3).

[83] EPA Press Office, "EPA Grants Tribal Petition to Protect Salmon from Lethal Chemical" (2 November 2023), online: https://www.epa.gov/newsreleases/epa-grants-tribal-petition-protect-salmon-lethal-chemical.

[84] Department of Toxic Substances Control, "Listing Motor Vehicle Tires Containing N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) as a Priority Product" (2023), online: https://dtsc.ca.gov/listing-motor-vehicle-tires-containing-n-13-dimethylbutyl-n%E2%80%B2-phenyl-p-phenylenediamine-6ppd-as-a-priority-product/ (**Department of Toxic Substances Control 2023**).

[85] Department of Toxic Substances Control 2023, *supra* note 84.

[86] Department of Toxic Substances Control 2023, *supra* note 84.

[87] Washington State Department of Ecology, "6PPD Alternatives Assessment Hazard Criteria" (June 2023), online: https://apps.ecology.wa.gov/publications/SummaryPages/2304036.html (**Washington Alternatives Criteria 2023**).

[88] Washington Alternatives Criteria 2023, *supra* note 87.

The research into alternatives is part of a suite of actions being undertaken by the state of Washington, which include evaluating stormwater management approaches to treat tire debris before it reaches streams, and developing methods to measure 6PPD-q in the environment.[89]

The European Union has proposed the Euro 7 regulations to reduce vehicle emissions. In a first, the emissions targeted will go beyond tailpipe exhaust and include standards for tires, setting specific limits on particulates they release.[90] EU lawmakers have said the regulations could be agreed upon by 2024.[91]

6PPD Must be Assessed

The requesters urge the Ministers to add 6PPD to the plan developed under section 73 of CEPA, giving priority to the assessment of its toxicity.

Although the research with respect to 6PPD-q and its effect on aquatic and other species is still emerging, pursuant to the precautionary principle, a lack of full scientific certainty should not be an impediment to regulatory action where there are threats of serious harm.

That principle is enshrined not only in the preamble to CEPA and in section 2 with respect to the administration of the Act, but in the provision under which this petition is being submitted. Subsection 76.1(1) of CEPA requires the Ministers to apply a weight of evidence approach and the precautionary principle when conducting an assessment in order to determine whether a substance is toxic or capable of becoming toxic.[92]

With respect to 6PPD-q, scientific studies have clearly linked its presence to mass deaths of coho salmon. That alone is enough to merit prioritizing it for assessment. And while the full scope of threats to other species and to aquatic environments is still emerging, the threat of serious harm is clear.

6PPD-q may pose a significant risk to Canadian ecosystems by threatening the health of fish and other organisms. Because 6PPD-q is an inevitable product of 6PPD's use in rubber tires, 6PPD-q cannot be regulated or addressed in isolation. The newly established evidence of 6PPD-q's toxicity constitutes substantial new evidence that 6PPD is toxic.

None of the information about 6PPD-q's harmful effects on the environment and its biological diversity has been considered in a CEPA assessment of 6PPD. The Requesters therefore ask that you urgently initiate an assessment to determine whether 6PPD is toxic or has the potential to

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[89] Washington State Department of Ecology, "Tire anti-degradant (6PPD) and 6PPD-quinone", online: https://ecology.wa.gov/waste-toxics/reducing-toxic-chemicals/addressing-priority-toxic-chemicals/6ppd.

[90] European Commission, "Commission proposes new Euro 7 standards to reduce pollutant emissions from vehicles and improve air quality" (10 November 2022), online: https://ec.europa.eu/commission/presscorner/detail/en/ip_22_6495. The proposed regulation will also include performance requirements for battery durability in electric vehicles.

[91] Nick Carey and Barbara Lewis, "Insight: Tyre-makers under pressure as too much rubber hits the road" (17 May 2023), *Reuters*, online: https://www.reuters.com/business/autos-transportation/tyre-makers-under-pressure-too-much-rubber-hits-road-2023-05-17/.

[92] CEPA, *supra* note 1 at s 76.1(1).

become toxic under CEPA so that appropriate regulatory action may be taken. As other jurisdictions are showing, this may take the form of requiring tire-makers to report on alternatives within a specified timeframe, stormwater management approaches, or vehicle regulations that include tire standards.

We look forward to hearing from you and would be pleased to engage in further dialogue on this matter.

Respectfully,

LBeck

Lindsay Beck
Barrister & Solicitor

Daniel Cheater
Barrister & Solicitor

Encl. Appendix "A": Tian, Z., Zhao, H., Peter, K. T., Gonzalez, M., Wetzel, J., Wu, C., et al. (2021). A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon, *Science* 371(6525), 185–189. https://www.science.org/doi/10.1126/science.abd6951.

Appendix "B": Grant et. al. (2019), State of Canadian Pacific Salmon: Responses to Changing Climate and Habitats. *Canadian Journal of Fisheries and Aquatic Sciences*, 3332. https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/40807071.pdf.

Appendix "C": Brinkmann, M., Montgomery, D., Selinger, S., Miller, J. G. P., Stock, E., Alcaraz, A. J., Challis, J. K., Weber, L., Janz, D., Hecker, M., & Wiseman, S. (2022). Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance. *Environmental Science & Technology Letters*, 9(4), 333–338. https://doi.org/10.1021/acs.estlett.2c00050.

Appendix "D": Benis, K., Behnami, A., Minaei, S., Brinkmann, M., McPhedran, K., & Soltan, J. (2023), Environmental Occurrence and Toxicity of 6PPD Quinone, an Emerging Tire Rubber-Derived Chemical: A Review. *Environmental Science & Technology Letters*, 10(10), 815−823. https://doi.org/10.1021/acs.estlett.3c00521.

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Appendix "F": U.S. Environmental Protection Agency, "Petition ID No. 001845: Toxic Substances Control Act Section 21 Petition Regarding N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (CASRN 793-24-8, aka 6PPD) in Tires – Final EPA Response to Petition" (2 November 2023), online: https://www.epa.gov/system/files/documents/2023-11/pet-001845_tsca-21_petition_6ppd_decision_letter_esigned2023.11.2.pdf

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ECOTOXICOLOGY

A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon

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In U.S. Pacific Northwest coho salmon (*Oncorhynchus kisutch*), stormwater exposure annually causes unexplained acute mortality when adult salmon migrate to urban creeks to reproduce. By investigating this phenomenon, we identified a highly toxic quinone transformation product of *N*-(1,3-dimethylbutyl)-*N*′-phenyl-p-phenylenediamine (6PPD), a globally ubiquitous tire rubber antioxidant. Retrospective analysis of representative roadway runoff and stormwater-affected creeks of the U.S. West Coast indicated widespread occurrence of 6PPD-quinone (<0.3 to 19 micrograms per liter) at toxic concentrations (median lethal concentration of 0.8 ± 0.16 micrograms per liter). These results reveal unanticipated risks of 6PPD antioxidants to an aquatic species and imply toxicological relevance for dissipated tire rubber residues.

Humans discharge tens of thousands of chemicals and related transformation products to water (*1*), most of which remain unidentified and lack rigorous toxicity information (*2*). Efforts to identify and mitigate high-risk chemical toxicants are typically reactionary, occur long after their use becomes habitual (*3*), and are frequently stymied by mixture complexity. Societal management of inadvertent, yet widespread, chemical pollution is therefore costly, challenging, and often ineffective.

The pervasive biological degradation of contaminated waters near urban areas (“urban stream syndrome”) (*4*) is exemplified by an acute mortality phenomenon that has affected Pacific Northwest coho salmon (*Oncorhynchus kisutch*) for decades (*5*–*9*). “Urban runoff mortality syndrome” (URMS) occurs annually among adult coho salmon returning to spawn in freshwaters where concurrent stormwater exposure causes rapid mortality. In the most urbanized watersheds with extensive impervious surfaces, 40 to 90% of returning salmon may die before spawning (*9*). This mortality threatens salmonid species conservation across ~40% of the Puget Sound land area despite costly societal investments in physical habitat restoration that may have inadvertently created ecological traps through episodic toxic water pollution (*9*). Although URMS has been linked to degraded water quality, urbanization, and high traffic intensity (*9*), one or more causal toxicants have remained unidentified. Spurred by these compelling observations and mindful of the many other insidious sublethal stormwater impacts, we have worked to characterize URMS water quality (*10*, *11*).

Previously, we reported that URMS-associated waters had similar chemical compositions relative to roadway runoff and tire tread wear particle (TWP) leachates, providing an opening clue in our toxicant search (*10*). In this work, we applied hybrid toxicity identification evaluation and effect-directed analysis to screen TWP leachate for its potential to induce mortality (a phenotypic anchor) in juvenile coho salmon as an experimental proxy for adult coho (*6*). Using structural identification by means of ultrahigh-performance liquid chromatography–high-resolution tandem mass spectrometry (UPLC-HRMS/MS) and nuclear magnetic resonance (NMR), we discovered that an antioxidant-derived chemical was the primary causal toxicant. Retrospective analysis of runoff and receiving waters indicated that detected environmental concentrations of this toxicant often exceeded acute mortality thresholds for coho during URMS events in the field and across the U.S. West Coast.

Aqueous TWP leachate stock (1000 mg/liter) was generated from an equal-weight mix of tread particles (0.2 ± 0.3 mm^2 average surface area) (fig. S1) from nine used and new tires (table S1). TWP leachate (250 mg/liter positive controls) was acutely and rapidly (~2 to 6 hours) lethal to juvenile coho (24 hours exposures, 98.5% mortality, $n = 135$ fish from 27 exposures) (data file S1), even after heating (80°C, 72 hours; 100% mortality, $n = 10$ fish from two exposures), indicating stability during handling. Behavioral symptomology (circling, surface gaping, and equilibrium loss) (fig. S2 and movie S1) of TWP leachate exposures mirrored laboratory and field observations of symptomatic coho (*5*, *6*). No mortality occurred in negative controls, including solvent- and process-matched method blanks subjected to identical separations (0 of 80 fish, 16 exposures) or exposure water blanks (0 of 45 fish, nine exposures).

Mixture complexity [measured here as number of UPLC-HRMS electrospray ionization (ESI+) chemical features] was a substantial barrier to causal toxicant identification because 250 mg/liter TWP leachate typically contained more than 2000 ESI+ detections. Our fractionation studies, optimized over 2-plus years through iterative exploration of toxicant chemical properties, focused on reducing these detection numbers to attain a simple, yet toxic, fraction amenable to individual compound identifications. Throughout this fractionation procedure, observed toxicity remained confined to one narrow fraction, which is consistent with a single compound or a small, structurally related family of causal toxicants. In initial studies, TWP leachate toxicity was unaffected by silica sand filtration, cation and anion exchange, and ethylenediaminetetraacetic acid (EDTA) (114 μM) addition (*12*), indicating that toxicant(s) were not particle-associated, strongly ionic, or metals, respectively, and validating prior studies that eliminated candidate pollutants (*13*, *14*) as primary causal toxicants.

Mixture complexity was reduced by using cation exchange, two polarity-based separations (XAD-2 resin and silica gel), and reverse-phase high-performance liquid chromatography (HPLC) on a semipreparative C18 column (250 by 4.2 mm ID, 5 μm particle size). After C18-HPLC generated 10 fractions, only C18-F6 (10 to 11 min) was toxic; it contained ~225 ESI+ and ~70 ESI– features (Fig. 1). Having removed ~90% of features, we began to prioritize and identify candidate toxicants by abundance (peak area), followed by fish exposures with commercial standards at fivefold higher concentrations (mixtures at 1 to 25 μg/liter) than those estimated in C18-F6. We identified 11 plasticizers, antioxidants, emulsifiers, and various transformation products, including some well-known environmental contaminants [such as tris(2-butoxyethyl) phosphate] and some that are rarely reported [such as di(propylene glycol) dibenzoate and 2-(1-phenylethyl)phenol] (table S2). We also detected several bioactive, structurally related phenolic antioxidants and their transformation products (2,6-di-*t*-

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butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone, 3,5-di-*t*-butyl-4-hydroxybenzaldehyde, and 7,9-di-*tert*-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione) (*15*). However, over many rounds of identification and subsequent exposure to juvenile coho, none of these identified chemical exposures reproduced URMS symptoms or induced mortality. Because these identifications used exhaustive environmental scientific literature searches (*10*, *16*, *17*), we suspected a previously unreported toxicant.

To sharpen our search, we used multidimensional semipreparative HPLC using two additional structurally distinct column phases [pentafluorophenyl (PFP) and phenyl]. Parallel fractionations (same column dimensions, mobile phase, and gradient as for C18-HPLC) (*18*) of the toxic silica gel fraction generated toxic fractions of PFP-F6 (10 to 11 min; ~204 ESI+, 60 ESI– features) and phenyl-F4 (8 to 9 min; ~237 ESI+, 75 ESI– features); all other fractions were nontoxic. Across these separations (C18, PFP, phenyl), only four ESI+ and three ESI– HRMS features co-occurred in all three toxic fractions (fig. S3). Of these, one unknown compound [mass/charge ratio (*m*/*z*) 299.1752, $C_{18}H_{22}N_2O_2$, RT 11.0 min on analytical UPLC-HRMS] dominated the detected peak area (10-fold higher intensity in both ESI+ and ESI–). To further resolve candidate toxicants for synthetic efforts, we converted the three-dimensional chromatography workflow from parallel to serial through sequential C18, PFP, and phenyl columns (C18-F6 to PFP-F6 to phenyl-F4; with solvent removal by means of centrifugal evaporation and toxicity confirmation between separations). The purified final fraction was chemically simple (four ESI+, three ESI– detections), highly lethal (100% mortality in 4 hours; *n* = 15 coho, three exposures), and was again dominated by $C_{18}H_{22}N_2O_2$. Drying this fraction yielded a pink-magenta precipitate (Fig. 1).

Published characterizations of crumb rubber (*16*) and receiving waters (*10*, *17*) did not mention $C_{18}H_{22}N_2O_2$. UPLC-HRMS/MS spectra indicated C_4H_{10} and C_6H_{12} alkyl losses (M-58 and M-84 fragments) (Fig. 2B), but MS^3 and MS^4 fragmentation yielded no additional structural insights (fig. S4). Additionally, in silico fragmentation (MetFrag, CSI:FingerID) of $C_{18}H_{22}N_2O_2$ compounds in PubChem and ChemSpider (15,624 and 17,105 structures, respectively) failed to match observed fragments. Thus, to the best of our knowledge, $C_{18}H_{22}N_2O_2$ was not described in environmental literature or databases and posed a "true unknown" identification problem (*19*). We then assumed a transformation product; industrial manufacturing (such as high heat or pressure, or catalysis) and diverse reactions in environmental systems generate many undocumented transformation products, most of which lack commercial standards.

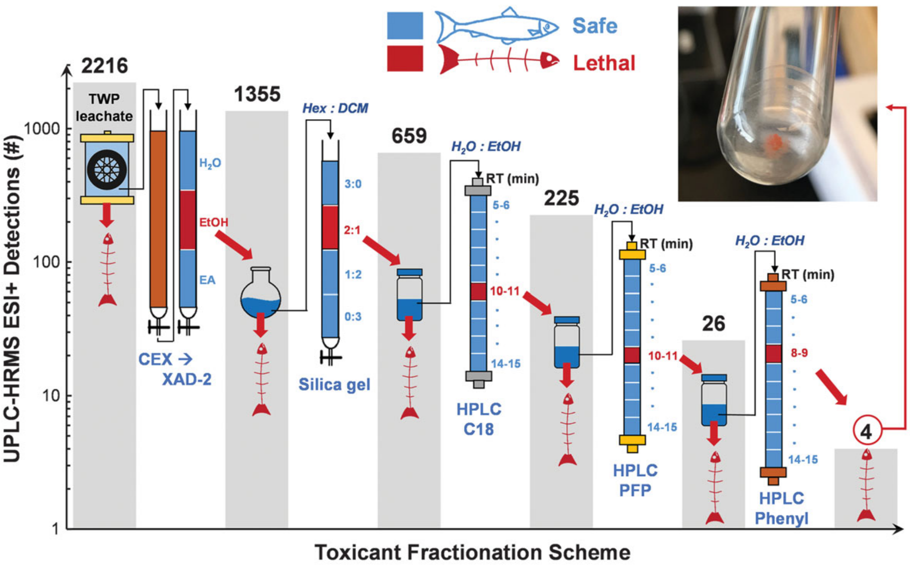

Fig. 1. Tire rubber leachate fractionation scheme. As a metric of mixture complexity and separation efficiency, the numbers above gray bars represent distinct chemical features detected in solid-phase extracted fish exposure water (1 liter) and subsequent fractions by means of UPLC-HRMS. Blue indicates nonlethal fractions; red indicates lethal fractions. All fractionation steps and exposures were replicated at least twice; positive and negative controls were included throughout fractionations. (**Inset**) Purified product (~700 μg from 30 liter of TWP leachate) in the final lethal fraction. TWP, tire tread wear particles; CEX, cation exchange; EA, ethyl acetate; EtOH, ethanol; H_2O, water; Hex, hexane; DCM, dichloromethane; RT, retention time.

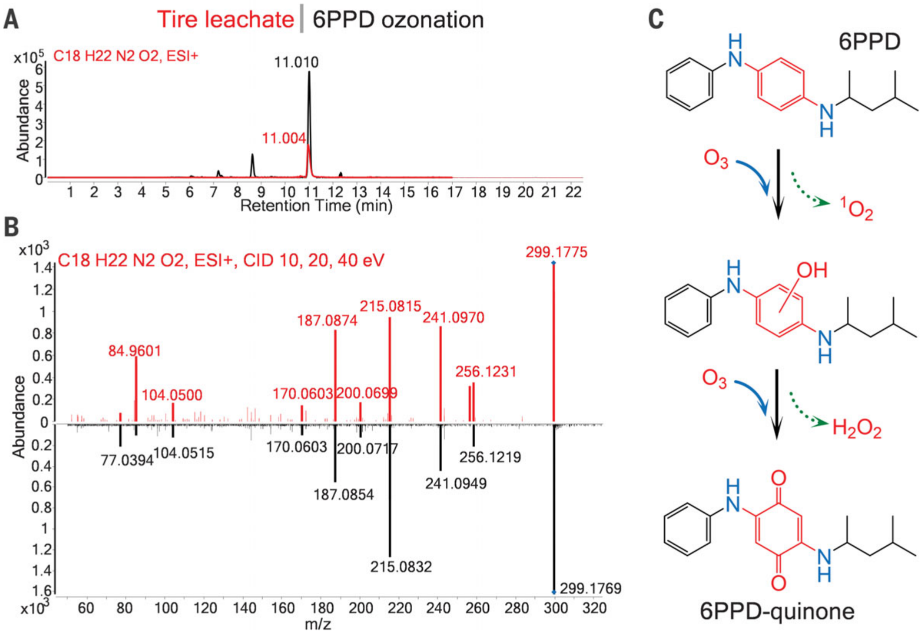

Fig. 2. 6PPD-quinone identification and a proposed formation pathway. (**A**) Extracted ion chromatograms of 6PPD-quinone from UPLC-HRMS (ESI+); red data indicate the final fraction from TWP leachate, and black data indicate the purified 6PPD ozonation mixture. (**B**) Observed MS/MS fragmentation (integrated from 10, 20, and 40 eV) of 6PPD-quinone in the final toxic fraction from TWP leachate (red spectra) and 6PPD ozonation (black spectra). (**C**) One proposed reaction pathway from 6PPD to 6PPD-quinone (alternate proposed formation pathways are provided in fig. S13). Red highlights indicate key changes in the diphenylamine structure during ozonation.

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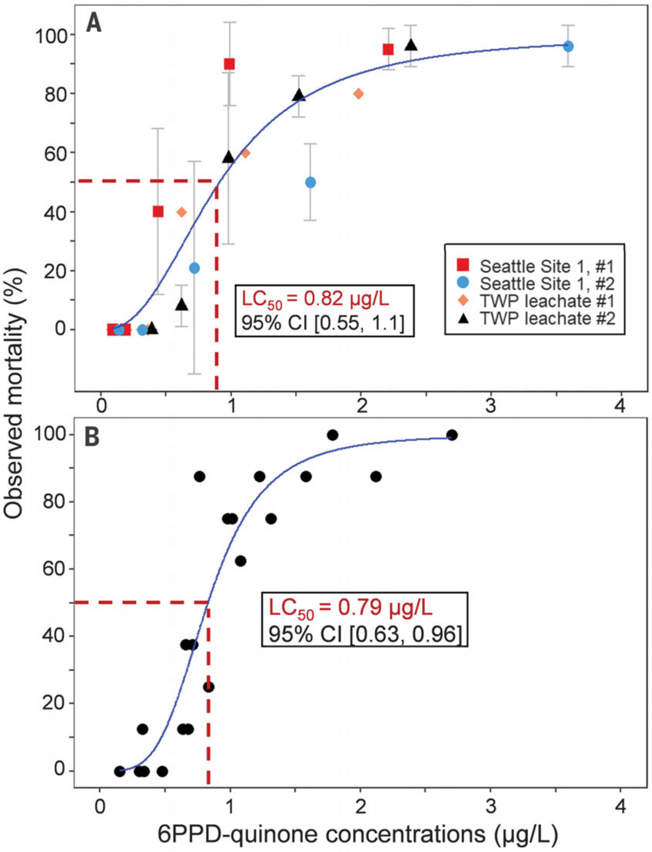

Fig. 3. Dose-response curves. (**A**) Dose-response curve for 24-hour juvenile coho exposures to roadway runoff and TWP leachate (*n* = 365 fish). Error bars represent three replicates of eight fish (except TWP leachate 2, *n* = 5 fish; Seattle site 1, duplicate of *n* = 10 fish). 6PPD-quinone concentrations were from retrospective quantification. (**B**) Dose-response curves for 24-hour juvenile coho exposures to ozone-synthesized 6PPD-quinone (10 concentrations, two replicates, *n* = 160 fish). Curves were fitted to a four-parameter logistic model. CI, confidence interval.

Our breakthrough came by assuming that abiotic environmental transformations commonly modify active functional groups by preferentially altering the numbers of hydrogen and oxygen atoms relative to carbon and nitrogen. By searching a recent U.S. Environmental Protection Agency (EPA) crumb rubber report (*16*) for related formulas ($C_{18}H_{0\text{-}x}N_{2\text{-}4}O_{0\text{-}y}$), several characteristics of the $C_{18}H_{24}N_2$ anti-ozonant "6PPD" [*N*-(1,3-dimethylbutyl)-*N*′-phenyl-p-phenylenediamine] matched necessary attributes. First, 6PPD is globally ubiquitous (0.4 to 2% by mass) in passenger and commercial vehicle tire formulations (*20*), indicating sufficient production to explain mortality observations within large and geographically distinct receiving water volumes. 6PPD was present in TWP leachate but was completely removed during fractionation through cation exchange. 6PPD crystals are purple, similar to the pink-magenta precipitate obtained after fractionation. Most compellingly, neutral losses in 6PPD gas chromatography (GC)–MS spectra matched the $C_{18}H_{22}N_2O_2$ GC-HRMS spectra (fig. S5), and the predicted logK_{ow} of 6PPD (5.6) (K_{ow}, *n*-octanol-water partition coefficient) was close to that for $C_{18}H_{22}N_2O_2$ (5 to 5.5) (*11*). Last, literature detailing the industrial chemistry of 6PPD reactions with ozone [7 days, 500 parts per billion volume (ppbv)] described a $C_{18}H_{22}N_2O_2$ product (*21*), leading us to hypothesize that 6PPD was the likely protoxicant (Fig. 2C).

We tested this hypothesis with gas-phase ozonation (500 ppbv O_3) of industrial grade 6PPD (96% purity) (*21*). A $C_{18}H_{22}N_2O_2$ product formed; UPLC-HRMS analysis demonstrated exact matches of retention time (11.0 min) and MS/MS spectra between this synthetic $C_{18}H_{22}N_2O_2$ and the TWP leachate fractionation-derived $C_{18}H_{22}N_2O_2$ (Fig. 2, A and B). When purified, the ozone-synthesized $C_{18}H_{22}N_2O_2$ formed a reddish-purple precipitate. One-dimensional ^{1}H NMR structural analysis confirmed identical TWP leachate–derived and ozone-synthesized $C_{18}H_{22}N_2O_2$ structures (figs. S6 to S7). Two-dimensional NMR spectra and related simulations revealed isolated tertiary carbons and carbonyl groups (figs. S8 to S12), clearly indicating a quinone structure for $C_{18}H_{22}N_2O_2$ rather than the dinitrone structure reported in the past 40 years of literature describing 6PPD ozonation products (*21*). Therefore, the $C_{18}H_{22}N_2O_2$ candidate toxicant was unequivocally "6PPD-quinone" {2-anilino-5-[(4-methylpentan-2-yl)amino]cyclohexa-2,5-diene-1,4-dione}. Consistent with environmental 6PPD ozonation, reported 6PPD ozonation products $C_{18}H_{22}N_2O$ (formula-matched) and 4-nitrosodiphenylamine ($C_{12}H_{10}N_2O$, standard-confirmed) (*21*) also were detected in ozonation mixtures and nontoxic TWP leachate fractions.

Exposures to ozone-synthesized and tire leachate–derived 6PPD-quinone (~20 μg/liter nominal concentrations) both induced rapid (<5 hours, with initial symptoms evident within 90 min) mortality (*n* = 15 fish, three exposures) (fig. S2 and movie S2), which matched the 2 to 6 hours mortality observed for positive controls. Behavioral symptomology in response to synthetic 6PPD-quinone exposures matched that from field observations, roadway runoff, bulk TWP leachate, and final toxic TWP fraction exposures, confirming the phenotypic anchor (*5*–*9*). Using synthetic 6PPD-quinone (purity ~98%), we performed controlled dosing experiments (10 concentrations, *n* = 160 fish in two independent exposures). 6PPD-quinone was highly toxic [median lethal concentration (LC_{50}) 0.79 ± 0.16 μg/liter] to juvenile coho salmon (Fig. 3B). Estimates of LC_{50} through controlled exposures closely matched estimates derived from bulk roadway runoff and TWP leachate exposures (LC_{50} 0.82 ± 0.27 μg/liter), indicating the primary contribution of 6PPD-quinone to observed mixture toxicity (Fig. 3A). Direct comparisons with 6PPD were performed (LC_{50} 250 ± 60 μg/liter through nominal concentrations) (fig. S14), but confident assessment of 6PPD toxicity was precluded by its poor solubility, high instability, and formation of products during exposure.

To assess environmental relevance, we used UPLC-HRMS to retrospectively quantify 6PPD-quinone in archived extracts from roadway runoff and receiving water sampling (fig. S15 and table S4) (*10*). In Seattle-region roadway runoff (*n* = 16 of 16 samples), 0.8 to 19 μg/liter 6PPD-quinone was detected (Fig. 4A). During seven storm events in three Seattle-region watersheds highly affected by URMS, 6PPD-quinone occurred at <0.3 to 3.2 μg/liter (*n* = 6 of 7 discrete storm events; *n* = 6 of 21 samples when including samples collected across the full hydrograph). These samples included three storms with documented URMS mortality in adult coho salmon; 6PPD-quinone was not detected in pre- and poststorm samples, but concentrations were near or above LC_{50} values during storms. We also detected 6PPD-quinone in Los Angeles region roadway runoff (*n* = 2 of 2 samples, 4.1 to 6.1 μg/liter) and San Francisco region creeks affected by urban runoff (*n* = 4 of 10 samples, 1.0 to 3.5 μg/liter).

These data implicate 6PPD-quinone as the primary causal toxicant for decades of stormwater-linked coho salmon acute mortality observations. Although minor contributions from other constituents in these complex mixtures are possible, 6PPD-quinone was both necessary (consistently present in and absent from toxic and nontoxic fractions, respectively) and, when purified or synthesized as a pure chemical exposure, sufficient to produce URMS at environmental concentrations. Over the product

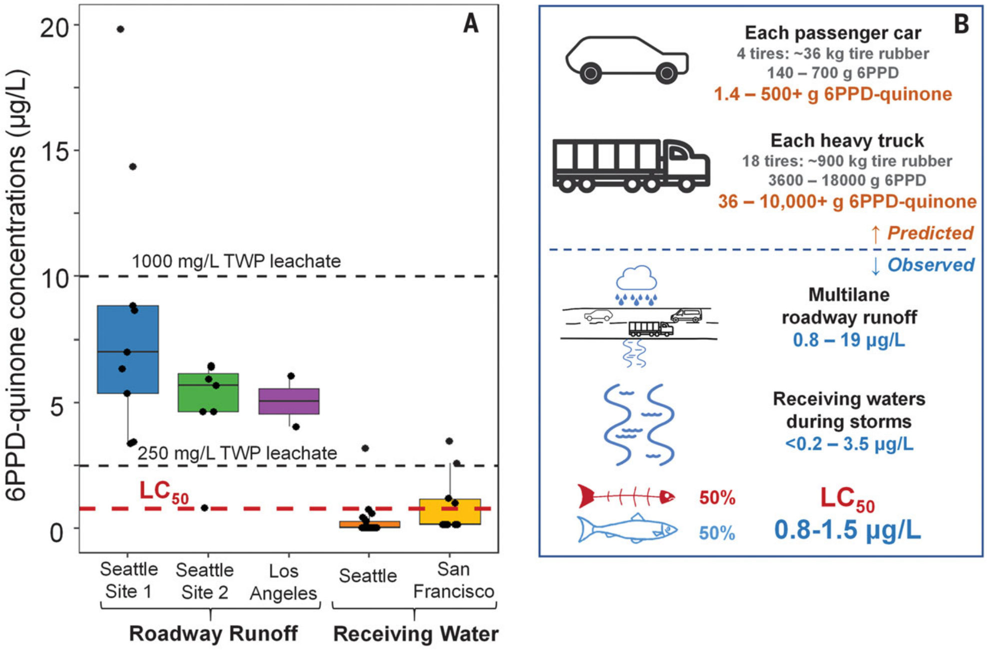

Fig. 4. Environmental relevance of 6PPD-quinone. (**A**) Using retrospective UPLC-HRMS analysis of archived sample extracts, 6PPD-quinone was quantified in roadway runoff and runoff-affected receiving waters. Each symbol corresponds to duplicate or triplicate samples, and boxes indicate first and third quartiles. For comparison, the 0.8 µg/liter LC$_{50}$ value for juvenile coho salmon and detected 6PPD-quinone levels in 250 and 1000 mg/liter TWP leachate are included. (**B**) Predicted ranges of potential 6PPD-quinone mass formation in passenger cars (for example, four tires, ~36 kg tire rubber mass) and heavy trucks (for example, 18 tires, ~900 kg of tire rubber) (represented in orange) and measured 6PPD-quinone concentrations in affected environmental compartments (represented in blue, with experimental data italicized). Predicted ranges reflect calculations applying 0.4 to 2% 6PPD per total vehicle tire rubber mass followed by various yield scenarios (1 to 75% ultimate yields) for 6PPD reaction with ground-level ozone to form 6PPD-quinone.

life cycle, antioxidants [such as PPDs, TMQs (2,2,4-trimethyl-1,2-dihydroquinoline), and phenolics] are designed to diffuse to tire rubber surfaces, rapidly scavenge ground-level atmospheric ozone and other reactive oxidant species, and form protective films to prevent ozone-mediated oxidation of structurally important rubber elastomers (*21*, *22*). Accordingly, all 6PPD added to tire rubbers is designed to react, intentionally forming 6PPD-quinone and related transformation products that are subsequently transported through the environment. This anti-ozonant application of 6PPD inadvertently, yet drastically, increases roadway runoff toxicity and environmental risk by forming the more toxic and mobile 6PPD-quinone transformation product. On the basis of the ubiquitous use and substantial mass fraction (0.4 to 2%) of 6PPD in tire rubbers and the representative detections across the U.S. West Coast (table S4), which include many detections near or above LC$_{50}$ values, we believe that 6PPD-quinone may be present broadly in peri-urban stormwater and roadway run-off at toxicologically relevant concentrations for sensitive species, such as coho salmon.

Globally, ~3.1 billion tires are produced annually for our more than 1.4 billion vehicles, resulting in an average 0.81 kg per capita annual emission of tire rubber particles (*23*). TWPs are one of the most substantial microplastics sources to freshwaters (*24*); 2 to 45% of total tire particle loads enter receiving waters (*25*, *26*), and freshwater sediment contains up to 5800 mg/kg TWP (*23*, *24*, *27*). Supporting recent concerns about microplastics (*24*, *28*), 6PPD-quinone provides a compelling mechanistic link between environmental microplastic pollution and associated chemical toxicity risk. Although numerous uncertainties exist regarding the occurrence, fate, and transport of 6PPD-quinone, these data indicate that aqueous and sediment environmental TWP residues can be toxicologically relevant and that existing TWP loading, leaching, and toxicity assessments in environmental systems are clearly incomplete (*25*). Tire rubber disposal also represents a major global materials problem and potential potent source of 6PPD-quinone and other tire-derived transformation products. In particular, scrap tires repurposed as crumb rubber in artificial turf fields (17) suggest both human and ecological exposures to these chemicals. Accordingly, the human health effects of such exposures merit evaluation.

Environmental discharge of 6PPD-quinone is particularly relevant for the many receiving waters proximate to busy roadways (Fig. 4B). It is unlikely that coho salmon are uniquely sensitive, and the toxicology of 6PPD transformation products in other aquatic species should be assessed. For example, used tires were more toxic to rainbow trout (75% lower 96 hours LC$_{50}$) relative to new tires (*29*), an observation that is consistent with adverse outcomes mediated by transformation products. If management of 6PPD-quinone discharges is needed to protect coho salmon or other aquatic organisms, adaptive regulatory and treatment strategies (*17*, *30*, *31*) along with source control and "green chemistry" substitutions [identifying demonstrably nontoxic and environmentally benign replacement antioxidants (*22*, *32*)] can be considered. More broadly, we recommend more careful toxicological assessment for transformation products of all high-production-volume commercial chemicals subject to pervasive environmental discharge.

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/371/6525/185/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S15
Tables S1 to S5
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Movies S1 and S2
Data File S1

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Post date 18 February 2022

Erratum

Erratum for the Report "A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon," by Z. Tian, H. Zhao, K T. Peter, M. Gonzalez, J. Wetzel, C. Wu, X. Hu, J. Prat, E. Mudrock, R. Hettinger, A. E. Cortina, R. G. Biswas, F. V. C. Kock, R. Soong, A. Jenne, B. Du, F. Hou, H. He, R. Lundeen, A. Gilbreath, R. Sutton, N. L. Scholz, J. W. Davis, M. C. Dodd, A. Simpson, J. K. Mcintyre, E. P. Kolodziej

After publication of the Report "A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon," which revealed 6PPD-quinone to be the primary causal toxicant toward coho salmon, a commercial standard of this molecule became available, and the authors developed and published an isotopic analytical method for more accurate quantification of extracts, environmental samples, and fish exposures (*1*). The authors found a ~15-fold increase in peak areas using the commercial standard, indicating that the previous standards overestimated both the reported median lethal concentration (LC_{50}) and the environmental concentrations of 6PPD-quinone in the study by a factor of 8.3. Using new exposures with the commercial standard and the isotopic method for quantification, LC_{50} values to juvenile coho salmon were subsequently revised to a lower value of 95 ng/L. Although the absolute concentrations in Figs. 3 and 4 of the Report shift lower when using the updated calibration, the relative relationship between environmental concentrations and LC_{50} presented in Fig. 4A are not changed, and the conclusions and implications of the paper are otherwise not affected.

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A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon

Zhenyu Tian, Haoqi Zhao, Katherine T. Peter, Melissa Gonzalez, Jill Wetzel, Christopher Wu, Ximin Hu, Jasmine Prat, Emma Mudrock, Rachel Hettinger, Allan E. Cortina, Rajshree Ghosh Biswas, Flvio Vinicius Crizstomo Kock, Ronald Soong, Amy Jenne, Bowen Du, Fan Hou, Huan He, Rachel Lundeen, Alicia Gilbreath, Rebecca Sutton, Nathaniel L. Scholz, Jay W. Davis, Michael C. Dodd, Andre Simpson, Jenifer K. McIntyre, and Edward P. Kolodziej

Science, **371** (6525), .
DOI: 10.1126/science.abd6951

Tire tread particles turn streams toxic

For coho salmon in the U.S. Pacific Northwest, returning to spawn in urban and suburban streams can be deadly. Regular acute mortality events are tied, in particular, to stormwater runoff, but the identity of the causative toxicant(s) has not been known. Starting from leachate from new and aged tire tread wear particles, Tian *et al.* followed toxic fractions through chromatography steps, eventually isolating a single molecule that could induce acute toxicity at threshold concentrations of #1 microgram per liter. The compound, called 6PPD-quinone, is an oxidation product of an additive intended to prevent damage to tire rubber from ozone. Measurements from road runoff and immediate receiving waters show concentrations of 6PPD-quinone high enough to account for the acute toxicity events.

Science, this issue p. 185

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State of Canadian Pacific Salmon: Responses to Changing Climate and Habitats

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2019

Canadian Technical Report of Fisheries and Aquatic Sciences 3332

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Canadian Technical Report of Fisheries and Aquatic Sciences

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Rapport technique canadien des sciences halieutiques et aquatiques

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Canadian Technical Report of Fisheries and Aquatic Sciences 3332

2019

STATE OF CANADIAN PACIFIC SALMON: RESPONSES TO CHANGING CLIMATE AND HABITATS

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ABSTRACT

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At DFO’s first State of the Salmon meeting in 2018, scientists concluded that Canadian Pacific salmon and their ecosystems are already responding to climate change. Northeast Pacific Ocean warming trends and marine heatwaves like “The Blob” are affecting ocean food webs. British Columbia and Yukon air and water temperatures are increasing and precipitation patterns are changing, altering freshwater habitats. The effects of climate change in freshwater are compounded by natural and human-caused landscape change, which can lead to differences in hydrology, and increases in sediment loads and frequencies of landslides. These marine and freshwater ecosystem changes are impacting Pacific salmon at every stage of their life-cycle.

Some general patterns in Canadian Pacific salmon abundances are emerging, concurrent with climate and habitat changes. Chinook numbers are declining throughout their B.C. and Yukon range, and Sockeye and Coho numbers are declining, most notably at southern latitudes. Salmon that spend less time in freshwater, like Pink, Chum, river-type Sockeye, and ocean-type Chinook, are generally not exhibiting declines. These recent observations suggest that not all salmon are equally vulnerable to climate and habitat change.

Improving information on salmon vulnerability to changing climate and habitats will help ensure our fisheries management, salmon recovery, and habitat restoration actions are aligned to future salmon production and biodiversity. To accomplish this, we must integrate and develop new research across disciplines and organizations. One mechanism to improve integration of salmon-ecosystem science across organizations is the formation of a Pacific Salmon-Ecosystem Climate Consortium, which has been recently initiated by DFO’s State of the Salmon Program.

RÉSUMÉ

Grant, S.C.H., MacDonald, B.L., and Winston, M.L. 2019. State of Canadian Pacific Salmon: Responses to Changing Climate and Habitats. Can. Tech. Rep. Fish. Aquat. Sci. 3332. ix + 50 p.

Lors de la première réunion du MPO en 2018 sur la situation du saumon, des scientifiques ont conclu que le saumon du Pacifique canadien et ses écosystèmes réagissaient déjà au changement climatique. Les tendances au réchauffement du nord-est de l'océan Pacifique et les vagues de chaleur marines telles que « The Blob » affectent les réseaux alimentaires des océans. La température de l'air et de l'eau augmente en Colombie-Britannique et au Yukon, et les régimes de précipitations changent, modifiant les habitats d'eau douce. Les effets du changement climatique sur les eaux douces sont aggravés par les modifications du paysage d'origine naturelle et humaine. Ils peuvent entraîner des différences hydrologiques, une augmentation de la charge en sédiments et des glissements de terrain bien plus fréquents. Ces changements dans les écosystèmes marins et d'eau douce ont des répercussions sur le saumon du Pacifique à tous les stades de son cycle de vie.

Certaines tendances générales de l'abondance du saumon du Pacifique canadien apparaissent parallèlement aux changements climatiques et de l'habitat. Les saumons quinnats sont en déclin dans l'ensemble de leur aire de répartition en Colombie-Britannique et au Yukon, tandis que les saumons rouges et cohos le sont plus particulièrement dans les régions du sud. Les populations de saumons qui passent moins de temps en eau douce, comme le saumon rose, le kéta, le saumon rouge de rivière et le saumon quinnat de l’océan, n’ont généralement pas une tendance à décliner. Ces observations récentes suggèrent que tous les saumons ne sont pas vulnérables au changement du climat et de l’habitat de la même façon.

L’amélioration de la collecte d’informations sur la vulnérabilité du saumon et de ses habitats aux changements climatiques permettra de garantir que les mesures de gestion de la pêche, le rétablissement du saumon et la restauration de l’habitat correspondent à la production et à la biodiversité futures du saumon. Pour ce faire, nous devons développer de nouvelles recherches dans plusieurs disciplines et les intégrer aux organisations. La création d’un consortium sur le climat et les écosystèmes du saumon du Pacifique, récemment mis en place par le programme sur la situation du saumon du MPO, est un des mécanismes permettant d’améliorer l’intégration de la science des écosystèmes du saumon à toutes les organisations.

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We gratefully acknowledge the following for participating in Fisheries and Oceans Canada's first State of the Salmon meeting May 15 & 16, 2018. This report was constructed from input provided by these individuals during this meeting (Agenda provided in Appendix 1). These participants represented expertise on B.C. and Yukon Pacific salmon populations, B.C. and Yukon freshwater and the Northeast Pacific Ocean ecosystems, and salmon population modeling.

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HEADLINES

These headlines summarize observed changes in Canada's Pacific salmon populations and their ecosystems. They reflect the integration of expert judgement and results provided by Fisheries and Oceans Canada (DFO) Science participants at DFO's first State of the Salmon meeting, held in May 18-19, 2019. Background and relevant references supporting these headline statements are provided in the main body of this report. This report represents a starting point for more detailed work, broader participation, and peer-review processes.

The planet is warming, the last five years have been the warmest on record. The increase in global temperatures above pre-industrial levels is irreversible over the coming centuries. The extent that we are able to curb our CO_2 and other greenhouse gas emissions will determine the magnitude of future warming. There is still time to moderate climate change impacts on salmon and people.

Canada's climate warming has been double the global average, and warming at northern latitudes has been even greater.

Canadian Pacific salmon and their ecosystems are responding to global climate change. Marine heatwaves, warmer rivers and lakes, food web changes, increased floods and droughts, and other freshwater habitat changes are all affecting salmon.

An unprecedented marine heatwave nicknamed 'The Blob' persisted from 2013 to 2017 in the Northeast Pacific Ocean, where most Canadian Pacific salmon growth occurs. Ocean temperatures were 3-5°C above seasonal averages, extending down to depths of 100 m. After a one year hiatus, a marine heatwave re-developed in the Northeast Pacific in 2018. A strong El Niño event further increased temperatures in late 2015 to early 2016, to the hottest observed throughout the 137 years of ocean monitoring.

Less nutritious zooplankton species, typically found in latitudes south of B.C., dominated the Northeast Pacific Ocean during these warming periods. The heatwaves altered physical and biological ocean processes, with considerable effects on the marine food webs on which salmon rely.

Unusual fish species from Mexico and other southern latitudes appeared in local marine waters, along with the proliferation of gelatinous plankton, known as pyrosomes.

Above average temperatures have been observed in B.C. and Yukon rivers and lakes that salmon use for migration, spawning and early development. In some months and southern locations these temperatures exceed upper thermal limits of migrating salmon, having lethal and sub-lethal consequences.

Climate change and habitat alteration are destabilizing salmon freshwater habitats. Many freshwater habitats are becoming less productive for early salmon life stages due to increased sedimentation and landslides, and alteration of river hydrology. These changes are caused by an increased frequency of extreme rain events and droughts, coupled with deforestation and other human activities.

No single factor can explain all of the recent observed patterns in salmon abundances. Along with ecosystem changes, fisheries, hatcheries, disease, and contaminants can also affect salmon. There are many gaps in our understanding regarding how all factors act alone or cumulatively to affect salmon population trends, and how these factors will interact with climate change.

Chinook salmon abundances are declining throughout their B.C. and Yukon range. Chinook are also returning to spawn at younger ages, their sizes are decreasing for a given age, and egg numbers and egg sizes are decreasing. Chinook might be particularly sensitive to changes in freshwater, given their site-specific adaptations to spawning and rearing habitats. There are exceptions to these declines, such as populations that spawn on the East Coast of Vancouver Island.

Many Sockeye and Coho population abundances are declining in southern latitudes. A number of these populations are considered Endangered or Threatened by COSEWIC. Some Sockeye in southern B.C. are faring better than others, including Sockeye that occupy more remote, high altitude lake habitats for juvenile rearing. More recently, some northern Sockeye populations have also declined.

Pink and Chum are generally doing better than other salmon species throughout their ranges, with exceptions. Most of these populations have not declined in numbers, although there are some exceptions of populations that are doing poorly, such as Skeena and Nass Chum.

Some salmon populations and species have been more resilient to recent warm conditions, which may provide insights into salmon responses to future climate change. Traits of salmon generally not exhibiting long-term abundance declines include populations that: spawn in northern Canadian latitudes; have limited to no freshwater rearing stages; use largely undisturbed freshwater habitats; and are not specifically adapted to a particular spawning site. Other factors, such as adult upstream migration distances, migration timing, ocean distributions, and unique physiologies, also contribute to salmon responses to climate change.

Continued, consistent, and expanded monitoring and research on Pacific salmon, their ecosystems, and climate science is required. This is critical at this time of rapid environmental change.

Sustaining future salmon populations in the face of climate change requires collaboration. A Pacific Salmon-Ecosystem Climate Consortium has been initiated by DFO to foster integration of our collective science on salmon, their ecosystems, and climate. Integrating information across experts through collaborative, structured processes will rely on existing and evolving methods. This work will build on efforts in countries like the U.S., as well as recent DFO activities conducted to improve our understanding of Pacific salmon vulnerability to climate change. This work can help ensure that fisheries management, habitat restoration, and salmon recovery efforts are aligned to future salmon production and diversity under climate change.

1 INTRODUCTION

The planet is warming. Earth's average land-ocean temperature has risen by 1˚C over the last century, and the last five years were the warmest on record (Morice et al. 2012, Hartmann et al. 2013). Global temperatures are projected to rise 1.5° to 3.7°C above the 1850-1900 average by the end of this century. The extent that human society curbs our CO_2 and other greenhouse gas emissions will determine where in this range future temperatures fall (IPCC 2013).

The Intergovernmental Panel on Climate Change (IPCC) recommends we do not exceed temperature increases of 1.5°C above pre-industrial levels, since the predicted planet's responses above this level are significant (IPCC 2018). As human activities are already estimated to have caused 1.0°C warming to date, ranging from 0.8°C to 1.2°C, we will reach the IPCC limit between 2030 and 2052 unless emissions are significantly curbed (IPCC 2018).

Temperature increases in Canada have been double the global average, with even higher rates of warming in the north (Bush and Lemmen 2019). Over half of this warming is due to human-caused CO_2 emissions (Bush and Lemmon 2019).

Observed and projected climate change impacts include increased temperatures and more severe weather events, such as heavy precipitation and droughts (IPCC 2018, Bush and Lemmen 2019). Since human caused warming overlays natural climate variability, environmental changes will not be constant or homogenous across time and space. However, the net global temperature trend is upward.

These climate changes are already altering the ecosystems that Canadian Pacific salmon rely on throughout their life-cycle. Broadly, the Northeast Pacific Ocean is warming, affecting salmon and their food webs. British Columbia and Yukon freshwater temperatures are also increasing, and the associated habitat changes contribute to observed salmon trends.

Pacific salmon populations are uniquely adapted to the conditions they have experienced historically in the diverse river, lake, and ocean habitats they rely on throughout their lives. The current rate of ecosystem change is likely exceeding the adaptation potential of many salmon populations. For this reason, it is essential to understand which populations are more or less resilient to the effects of climate and habitat change, in order to optimize habitat restoration, and fisheries management actions.

Currently, salmon recovery, habitat restoration and fisheries management actions operate under the assumption that future salmon production will function similarly to how it has in the past. However, under rapidly changing climate conditions this assumption is no longer valid. Changing climate means that conditions are increasingly falling outside the bounds of historical observations. This can alter the effectiveness of activities currently relied upon to manage salmon and their ecosystems, and ultimately puts salmon populations at risk.

This report presents the first high-level overview of Canadian Pacific salmon responses to a rapidly changing world, and provides a foundation for further detailed work required to assess the vulnerability of salmon populations. Here we compile observations, and expert opinion, contributed by DFO Science staff working on the different salmon life-stages and ecosystems, during a meeting held in Nanaimo, B.C., May 15-16, 2018. References are also provided where readily available.

Results from this science process provide the first integrated overview of B.C./Yukon Pacific salmon and ecosystems changes observed during the recent period of notable warming and longer term habitat alteration. This report does not represent an exhaustive literature review, and the contents of this report have not gone through a formal peer review process. Instead it provides a starting point for future work that will expand on the observations presented, through data compilation, analyses, and the integration of expert judgement.

This first State of the Salmon meeting (Agenda: Appendix 1) included DFO staff only, with the intention of expanding to experts external to the organization in subsequent meetings. This paper is grouped into the following sections:

1. **Pacific Salmon Background**: This section includes background on the five Pacific salmon species managed by DFO: Sockeye, Chinook, Coho, Pink and Chum. An overview of the general biology of each species is provided.
2. **Ecosystem Trends:** Trends and observations are presented for the Northeast Pacific Ocean, and B.C./Yukon freshwater ecosystems.
3. **Canadian Pacific Salmon Trends:** Here we present trends for the five species of Pacific salmon managed by DFO: Sockeye, Chinook, Coho, Pink and Chum.
4. **Lessons learned from the recent period of warm ocean and freshwater conditions:** This section highlights salmon populations that have not exhibited declining abundances during the recent warm period, and salmon traits that might have contributed to these positive outcomes. This early exploration may provide preliminary insights into factors that may make particular salmon populations more resilient to future climate change.
5. **Conclusions and Next Steps:** This section presents conclusions of this report, and identifies the next steps required to expand our understanding of salmon vulnerability to a changing climate.
6. **References:** This section provides key literature relevant to this report, but does not represent an exhaustive literature review related to salmon and climate change.
7. **Agenda:** The 2018 State of the Salmon meeting agenda is provided here.
8. **Wild Salmon Policy and COSEWIC status assessments:** Statuses are available for Fraser River Sockeye, Southern B.C. Chinook and Interior Fraser Coho.

2 CANADIAN PACIFIC SALMON BIOLOGY

2.1 PACIFIC SALMON DIVERSITY

Five species of Pacific salmon are assessed and managed by Fisheries and Oceans Canada (DFO): Sockeye (*Oncorhynchus nerka*), Chinook (*O. tshawytscha*), Coho (*O. kisutch*), Pink (*O. gorbuscha*) and Chum (*O. keta*). These salmon are anadromous and semelparous, meaning they migrate from the ocean to freshwater to spawn, and die shortly after spawning.

Over 10,000 years ago, there were no salmon in B.C., since these areas were covered in ice from the last ice age. Ice-free areas during this period provided refuges for the salmon populations that formed the foundation for all current Pacific salmon diversity in B.C. and the Yukon (McPhail and Lindsey 1970).

Pacific salmon species and populations exhibit considerable biological variation. Many Sockeye and Chinook populations, and all Coho populations, rear in freshwater for one to two years as juveniles, before migrating to the ocean. Other Sockeye and Chinook populations, and all Chum and Pink populations, migrate to the ocean shortly after hatching and emergence, with only a limited freshwater juvenile stage. Salmon are adapted to the particular freshwater and marine conditions they experience throughout their life-cycles. Adaptive traits that are unique to each population include age of maturity, distribution in freshwater and the ocean, timing of migration and spawning, and thermal tolerance ranges, among others.

The population structure of Pacific salmon is complex and hierarchical, where each level of organization is the aggregate of its subcomponent parts. The spawning site is the smallest unit of organization, and this level forms increasingly broader groupings from the population, to the conservation unit (CU) or designatable unit (DU), to the species (DFO 2005, Holtby and Ciruna 2007). The precision of salmon fidelity to their natal spawning locations affects the extent of gene flow among populations, and determines the basic genetic organization of Pacific salmon.

The Conservation Unit (CU) has been identified as the fundamental unit of Canadian Pacific salmon biodiversity under DFO's Wild Salmon Policy (WSP) (DFO 2005). A CU is defined as 'a group of salmon living in an area sufficiently isolated from other groups that, if the salmon were to become extirpated it is unlikely that area would be recolonized naturally in a human life time.' Individual CUs are genetically and ecological distinct (Holtby and Ciruna 2007). In B.C. and the Yukon, 377 CUs currently have been identified: 184 Sockeye CUs, 76 Chinook CUs, 43 Coho CUs, 32 Pink CUs, and 42 Chum CUs (Table 1; Wade et al. 2019).

It is critical to understand which characteristics of these salmon populations make them more resilient, and might mitigate the effects of climate and habitat changes on future salmon production and biodiversity.

Table 1. Summary of Canadian Pacific Salmon Conservation Units (CUs), reprinted from Wade et al. (2019).

	Sockeye (lake-type)	Sockeye (river-type)	Chinook	Coho	Pink (odd year)	Pink (even year)	Chum	**Total**
Current CUs	165	19	76	43	19	13	42	**377**
Extirpated CUs	6							**6**

The Committee for the Endangered Wildlife in Canada (COSEWIC) identifies designatable units (DUs) as the fundamental units of biodiversity for Canadian wildlife species. A DU is defined as “discrete and evolutionarily significant units of the taxonomic species’, where ‘significant’ means that the unit is important to the evolutionary legacy of the species as a whole, and if lost would likely not be replaced through natural dispersion.”

DFO WSP CUs and COSEWIC DUs are identical for Fraser Sockeye, and there are slight variations for Southern B.C. Chinook and Interior Fraser Coho (Appendix 2; COSEWIC 2016, 2017). Very few DU’s have been currently identified, as this work only occurs preceding COSEWIC status assessments for particular groups of Pacific salmon, though it is likely they will largely align with DFO’s CUs (Table 1).

2.2 SOCKEYE

Sockeye age of maturity varies by latitude; most Sockeye in Southern B.C. mature at four years of age, while further north in B.C. and the Yukon, they mature at five years. Most Sockeye populations rear for one to two years as juveniles in lakes, after their egg stage, and are referred to as lake-type Sockeye. Since lake-type populations are reproductively isolated and adapted to their particular lake systems, they comprise the largest numbers of CUs in B.C. and the Yukon, totalling 165 (Table 1; Wade et al. 2019). River-type populations are a second ecotype of Sockeye, and these 19 CUs spend limited time rearing in freshwater after emergence (Table 1; Wade et al. 2019).

Lake-type Sockeye remain in near-shore areas upon entering the ocean in the spring. By their first winter they reach offshore rearing sites in the Gulf of Alaska, following a northwest migration (Tucker et al. 2009). River-type Sockeye are smaller than their lake-type counterparts when they first enter the ocean in late-spring to early-summer, and spend more time rearing in river estuaries before migrating offshore (Beamish et al. 2016). Upon reaching maturity, Sockeye salmon migrate back to their natal freshwater spawning habitats from spring to fall, depending on the population. This species is iconic to Canadians, due to its distinctive red body and green head, which develop when they reach spawning maturity.

2.3 CHINOOK

Chinook are the largest bodied of the salmon species, and can reach up to seven years of age before returning to spawn. Their large size, along with their high fat content and year round availability make them a preferred prey species for some resident Killer Whale (*Orcinus orca*) populations (Ford et al. 1998, Ford and Ellis 2005).

Chinook populations exhibit considerable variability in their life-histories. They are uniquely adapted to their particular spawning, freshwater, and marine rearing habitats. Ocean-type Chinook populations migrate to the ocean shortly after they emerge from the gravel as under one year old fry. River-type Chinook rear in freshwater rivers as juveniles for one to two years before they migrate to the ocean. There are 76 Chinook CUs in B.C. and the Yukon (Table 1; Wade et al. 2019), exhibiting a range of life-histories.

Most ocean-type Chinook migrate to the ocean earliest in the spring, from March to May, followed by river-type Chinook, which migrate from April to May (Healey 1991). Ocean distributions vary among Chinook populations. Chinook may remain in coastal marine areas, near their natal rivers, for one to three years after they enter the ocean (Orsi and Jaenicke 1996; Trudel et al. 2009). The length of time they spend in coastal areas depends on the Chinook population, where some populations may remain in nearshore waters for their entire marine period, or they may migrate from these areas either into deeper offshore waters or north to Alaska, to rear. Most populations that enter the marine environment in the Strait of Georgia remain there for three to five months (Beamish et al. 2011), although some leave earlier (Tucker et al. 2011, 2012). West Coast of Vancouver Island populations remain coastal for one year after ocean entry, before migrating north along the continental shelf. Meanwhile, northern populations remain in coastal Alaskan waters until their second year in the ocean (Orsi and Jaenicke 1996).

Chinook salmon complete their life-cycle by migrating back to their natal freshwater spawning habitats between spring and fall, depending on the population.

2.4 COHO

Coho age-at-maturity varies by latitude; most Coho in B.C. mature at three years old, while further north and in the Yukon, they mature at four years. This species shares a common early life-history across populations. Coho rear as fry for one year in small rivers and creeks near, or downstream from, where they were spawned. Coho fry prefer rearing in structurally complex streams, in back eddies, log jams, and undercuts. Fry are territorial, and if densities are too high in preferred habitats, new arrivals will use less optimal habitat downstream. Fry are also vulnerable to stream flows. High flows can sweep them downstream, out of suitable habitats, while low flows and droughts can reduce habitat availability, or result in stranding of fry if their habitat becomes isolated. There are 43 Coho CUs in B.C. and the Yukon (Table 1; Wade et al. 2019).

In the ocean, there are two key types of Coho salmon: those that migrate rapidly northwest to the Gulf of Alaska after ocean entry, and others that remain near their ocean entry location in coastal waters over winter (Morris et al. 2007, Beacham et al. 2016). Most populations that enter the ocean on the West Coast of Vancouver Island and in the Strait of Georgia tend to be slower migrators, while more northern populations migrate more rapidly northwest upon ocean entry (Morris et al. 2007, Beacham et al. 2016). However, there are a few exceptions to these slower migrators in the south, like Thompson River Coho in the Fraser watershed, which migrate more rapidly northward in the Strait of Georgia after ocean entry, exiting via the Johnstone Strait and moving into the Northeast Pacific Ocean (Beacham et al. 2016). Many other populations that enter the Strait of Georgia remain there from spring to fall, subsequently moving to the West Coast of Vancouver Island via the Juan de Fuca Strait (Beamish et al. 2010).

Coho salmon complete their life-cycle by migrating back to their natal freshwater habitats as adults during late-summer to fall, and spawn from October to March, depending on the population. They generally begin migrating to their spawning grounds when there is an increase in river flow, and typically have longer upstream migrations than Pink and Chum salmon, but do not migrate as far as Sockeye and Chinook (Sandercock 1991).

2.5 PINK

Pink salmon mature at two years of age, and are the smallest of the Pacific salmon species. There are two distinct and genetically isolated brood lines of Pink that return in odd versus even years (Heard 1991). The odd year brood line dominates central and Southern B.C. populations, and the even year dominates northern B.C. and Yukon populations (Irvine et al. 2014). Although there are a number of proposed causes for dominance of one brood line over the other, there is insufficient evidence to make any broad conclusions as to why this occurs (Heard 1991).

Pink salmon immediately migrate to the ocean after their egg incubation stage, similar to river-type Sockeye, ocean-type Chinook, and Chum salmon. Genetic evidence indicates that the population structure of Pink salmon is less differentiated across broader areas in freshwater (Holtby and Ciruna 2007). These salmon also have the simplest age structure, maturing consistently at two years of age. For these reasons, Pink salmon have the fewest number of CUs. There are 13 even year Pink CUs and 19 odd year Pink CUs in B.C. and the Yukon (Table 1; Wade et al. 2019).

There has been relatively little work conducted to understand the distribution of Pink salmon in the Northeast Pacific Ocean (Trudel and Hertz 2013). Generally, Pink salmon remain near shore, or in areas protected from waves and currents, for several months in their early sea life, similar to Chum (Heard 1991, Salo 1991). Pink fry are often observed schooling with Chum fry during their early ocean stages. At larger sizes, Pink salmon move from nearshore to offshore waters, although the exact size that triggers this shift varies by area. Pink salmon migrate rapidly northward following coastlines up to the Gulf of Alaska, where they rear with Sockeye and Chum, although Pinks may overwinter farther south than Sockeye (Heard 1991).

Pink salmon return to their spawning grounds in the fall. Spawning migrations are relatively short in freshwater, since fish from this species have a limited capacity to leap over obstacles or swim through heavy flows (Heard 1991). During spawning, Pink salmon are characterized by a large hump on their back, hooked jaws, and teeth on their lower and upper jaws.

2.6 CHUM

Chum salmon mature at predominantly four or five years of age, and are the second largest bodied species, following Chinook. Chum immediately migrate to the ocean after their egg incubation stage, similar to river-type Sockeye, ocean-type Chinook, and Pink salmon. There are 42 Chum CUs in B.C. and the Yukon (Table 1; Wade et al. 2019).

There has been relatively little work conducted to understand the distribution of Chum salmon in the Northeast Pacific Ocean, similar to Pink salmon (Trudel and Hertz 2013). The limited information available indicates that Chum salmon remain near-shore for several weeks in their early sea life, similar to Pink salmon (Heard 1991, Salo 1991). Chum fry are often observed schooling with Pink fry during early ocean stages (Heard 1991). Unlike Pink, however, Chum remain in near-shore waters into the summer months, before migrating offshore (Holtby and Ciruna 2007). They migrate north and rear in the Gulf of Alaska with Pink and Sockeye salmon.

Despite being strong swimmers, Chum spawning migrations are relatively short in freshwater, because they are not leapers and, therefore, generally do not move past barriers in a river (Salo 1991). There are exceptions among northern Chum populations, such as Yukon River Chum, which have spawning migration distances up to 2,500 km (Holtby and Ciruna 2007). During spawning, Chum salmon are characterized by mottled burgundy/black/green colouration, and canine teeth on their upper and lower jaws.

3 ECOSYSTEM TRENDS

3.1 CLIMATE CHANGE: GLOBAL, REGIONAL, AND LOCAL

The planet is warming. Earth's average land-ocean temperature has risen by close to 1˚C over the last century (Figure 1), with the last five years registering as the warmest on record (Morice et al. 2012, Hartmann et al. 2013). Global surface temperatures are projected to rise by 1.5° to 3.7°C on average by the end of this century compared to the 1850-1900 average, depending on the extent humans moderate our CO_2 emissions (IPCC 2013). Climate change is effectively irreversible. Even with cessation of human-caused CO_2 gas emissions, temperatures will remain at the current elevated levels over the coming centuries (Solomon et al. 2009, IPCC 2014).

Climate is responding on global, regional, and local scales, through increased temperature extremes, changes in precipitation, and more severe weather events. Since human caused warming overlays natural climate variability, temperature increases will not be constant or homogenous across time and space. However, the net global temperature trend is upward.

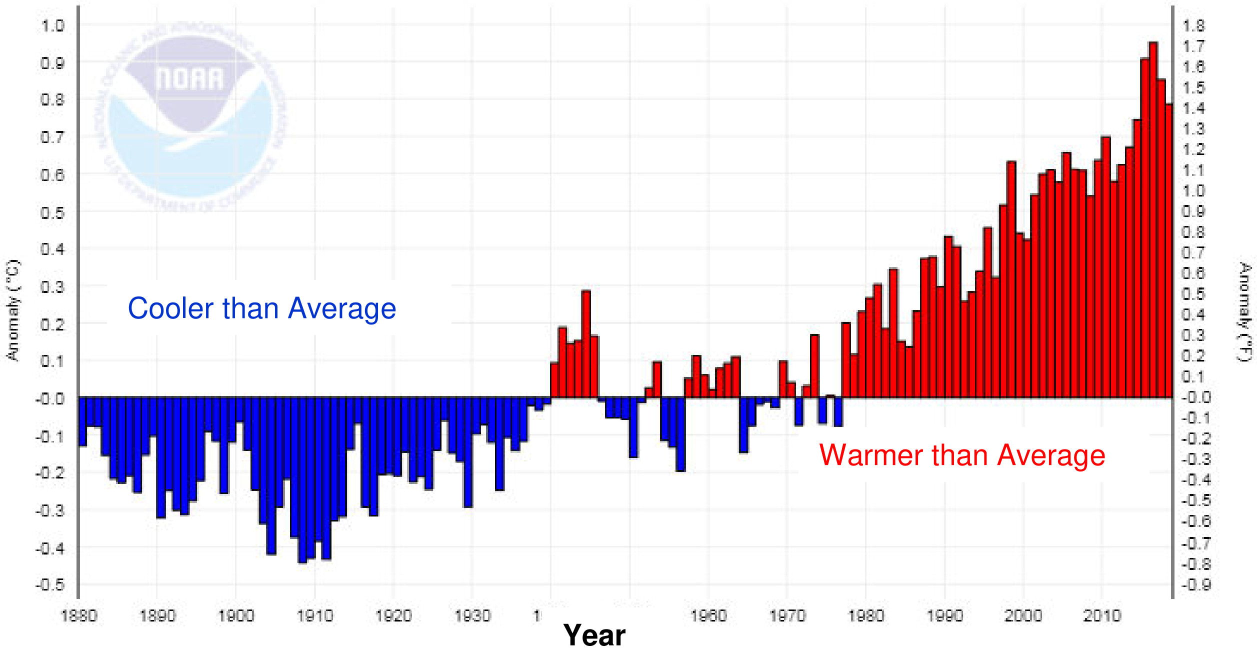


Figure 1. Global land and air temperature anomalies. Normalization period includes 1981-2010. Source: NOAA: https://www.ncdc.noaa.gov/cag/global/time-series/globe/land_ocean/ytd/12/1880-2019).

The rate of global warming is greater at northern latitudes. For this reason, Canada's current and projected warming is double the global average, and in more than double in the north (Bush and Lemmen 2019). Precipitation in Canada is also responding to global climate change. Rainfall has increased and has become more extreme, except in summer months, while snowfall has decreased in the west.

3.2 MARINE HEATWAVES IN THE NORTHEAST PACIFIC OCEAN

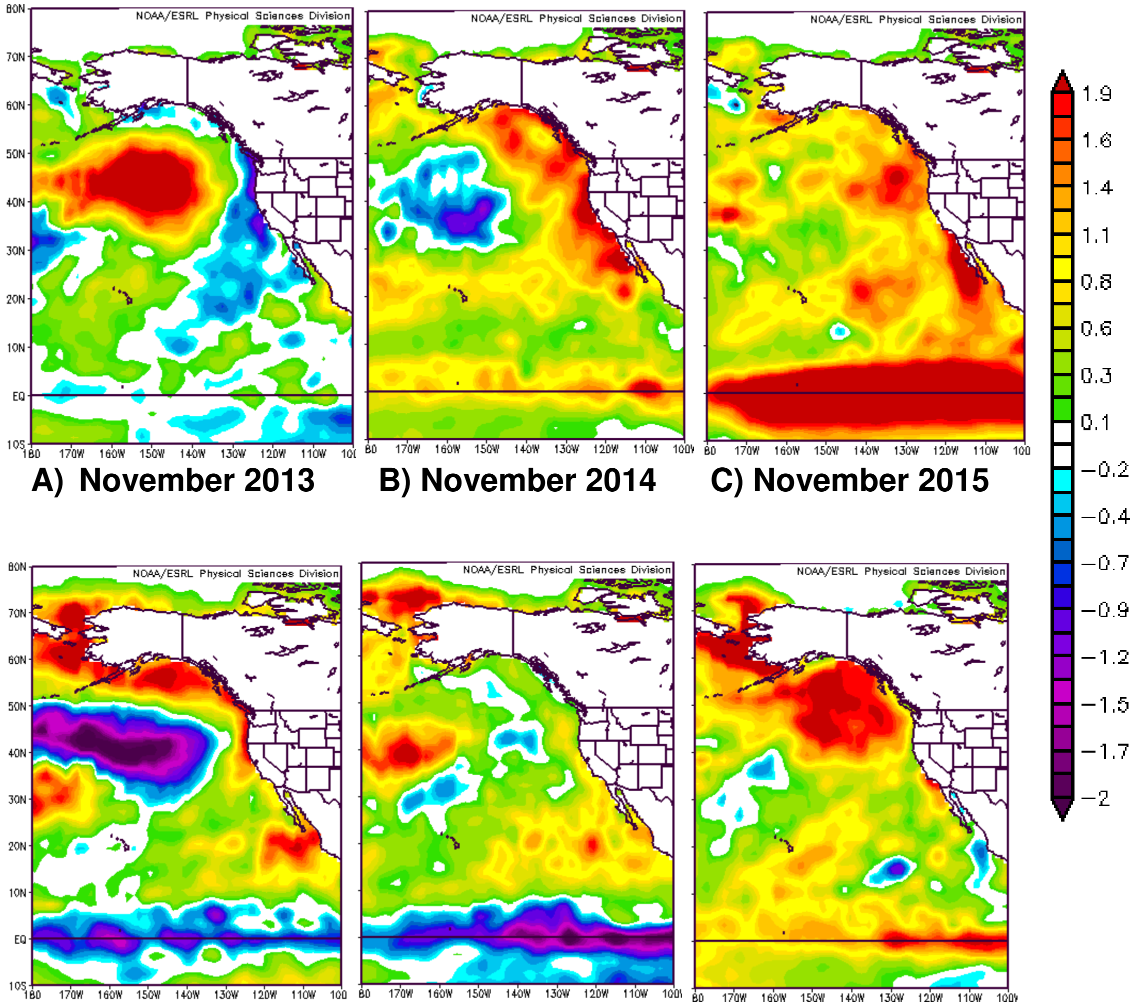
An unprecedented heatwave, nicknamed “The Blob”, dominated the Northeast Pacific Ocean from 2013-2016 (Figure 2). This ocean warming contributed to physical and biological changes, some of which continue to persist. Sea-surface-temperatures (SST) during the heatwave were 3-5°C above seasonal averages, extending down to depths of 100 m (Bond et al. 2015, Ross and Robert 2018, Smale et al. 2019). Climate modeling has shown that this heatwave can best be explained by human-caused warming (Walsh et al. 2018).

Concurrently, a strong El Niño event further increased temperatures in late 2015 to early 2016, to the hottest observed throughout the 137 years of ocean temperature monitoring (Figure 2). The frequency of extreme El Niño events is expected to increase with climate change (Cai et al. 2014, 2015, Santoso et al. 2017, Wang et al. 2017).

Although SSTs in the Northeast Pacific cooled towards the second half of 2016, warm temperatures persisted at depths of 100-200 m until early 2018 (Ross 2017, Ross and Robert 2018). Any reprieve from abnormal ocean temperatures was short-lived, as warmer than average seasonal temperatures were again observed in the Northeast Pacific and Bering Sea in the fall of 2018 (Britten 2018, Livingston 2018).

Underlying these heatwaves has been a steady increase in North Pacific Ocean temperatures of 0.1°C/year to 0.3°C/year from 1950 to 2009 (Poloczanska et al. 2013, Holsman et al. 2018).

Detailed information on the State of the Pacific Ocean is reported annually (Chandler et al. 2015, 2016, 2017, 2018).



A) November 2013 **B) November 2014** **C) November 2015**

D) November 2016 **E) November 2017** **F) November 2018**

Figure 2. Sea-surface-temperature anomalies in the Northeast Pacific Ocean are presented for the month of November from 2013 to 2018. These maps do not show absolute temperatures, but indicate how much above (red) or below (blue) average the ocean surface temperatures were, compared to a thirty year average from 1981 and 2010. The coloured bar on the right of the maps provides greater detail to interpret the monthly deviations from average.

A) 'The Blob' formed in the latter half of 2013; **B)** 'The Blob' moved into coastal waters in 2014; **C)** an El Niño formed in 2015 and remained until early 2016, adding to warming of 'The Blob'; **D)-E)** 'The Blob' was no longer a prominent feature in surface waters in 2016-2017, although it remained at depth; **F)** Warmer sea-surface-temperatures similar to 'The Blob' re-developed in 2018.

Data and map tools are from the U.S. National Centers for Environmental Prediction (NCEP) and from the National Oceanic and Atmospheric Administration (NOAA).

Warm ocean temperatures may be harmful to salmon through their effect on zooplankton composition, a key pathway potentially linking reduced salmon survival to temperatures in the Northeast Pacific Ocean (Mackas et al. 2007). Warmer temperatures cause shifts in the distribution of southern prey species northward, to occupy habitats previously too cold for them (Mackas et al. 2004). Zooplankton communities near the base of the food web in the Northeast Pacific Ocean shifted in warm “Blob” years towards a greater abundance of lipid-poor southern copepods, as these animals moved northward, and fewer lipid-rich subarctic and boreal copepods (Galbraith and Young 2018, Young et al. 2018). The warmer water species are considered to be poorer quality food for species higher up the food chain, due to their smaller size and lower fat content (Mackas et al. 2007).

Salmon metabolic demands also increase with temperature, therefore, food consumption must increase accordingly. Without a concurrent increase in prey quality or quantity, salmon growth and survival will decrease under warming conditions (Holsman et al. 2018). For example, in recent years Chinook body weight for a given length declined (Daly et al. 2017). Predation also can intensify in warmer ocean conditions, increasing mortality of salmon during these periods (Holsman et al. 2012).

Highly unusual and sporadic observations in Northeast Pacific Ocean food webs are less understood with regard to their effects on salmon survival. Southern Pacific fish species such as Louvar (*Luvaris imperialis*), Finescale Triggerfish (*Balistes polylepis*), and Pacific Pompano (*Peprilus medius*) were observed in Northeast Pacific waters during recent research cruises (King et al. 2019). These foreign species can potentially disrupt ecosystems as competitors and/or predators of local fish communities.

Other noteworthy observations in 2017 included vast numbers of pyrosomes (*Pyrosoma atlanticum*), a colonial tunicate typically found off the coast of California, which clogged fishing gear in coastal B.C. waters (Brodeur et al. 2017). Pyrosomes filter feed phytoplankton, the base of the food web, which could have a negative effect on the abundance of higher trophic level animals, including salmon.
Unusual phytoplankton blooms that can kill or harm migrating salmon (McCabe et al. 2016, Peña and Nemcek 2017) have been observed recently in coastal waters, linked to climate change (McKibben et al. 2017). It is difficult to predict salmon responses to such unusual and sporadic events. However, as the effects of climate change intensify, we can expect the frequency and magnitude of such events to increase.

Warmer regional temperatures also influence interactions between freshwater and marine ecosystems. Earlier snowmelt, increased precipitation, and melting of ice on land are some of the factors contributing to a freshening of the coastal Northeast Pacific surface waters (Bonsal et al. 2019, Greenan et al. 2019). Fresher and warmer surface waters increase ocean stratification, which limits the supply of nutrient rich deep ocean waters to the sunlit surface waters in the spring-to-fall growing season. This limits the nutrients available to support algal growth at the base of the salmon food web (Bush and Lemmen 2019).

3.3 CLIMATE RELATED CHANGES IN FRESHWATER

Air temperatures over British Columbia and the Yukon have reached record highs in recent years, with the Yukon warming twice as fast as southern Canadian latitudes (Streicker 2016, Bush and Lemmen 2019). Local air temperatures were particularly warm from 2015 to 2018 (Figure 3), coinciding with the marine heatwave in the Northeast Pacific Ocean (Figure 2).

Precipitation patterns are also more extreme in response to climate change, with greater variation between wet and dry conditions in the summer, and increased frequency and magnitude of storms and rainfall events (Pike et al. 2010a). Increased temperatures and precipitation, and a greater frequency of droughts, floods, and landslides are already being observed in B.C. (Pike et al. 2010b) and the Yukon, in response to climate change.

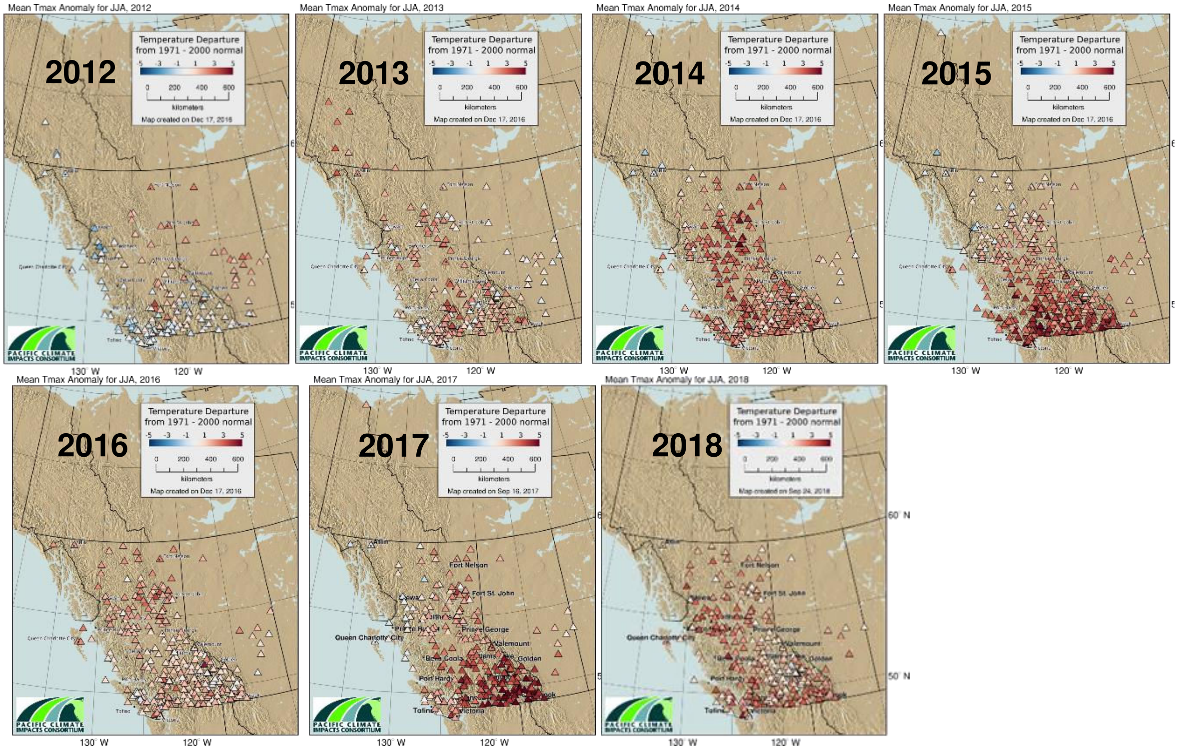


Figure 3. Air temperature anomalies in British Columbia. These are seasonal average temperatures for the summer months of June, July and August minus the total mean from 1971-2000. Data are from the University of Victoria's Pacific Climate Impacts Consortium. The colour table at the top of each map indicate the deviations from average; warmer colours are above average and cooler colours are below average temperatures. In recent decades, air temperatures have been above average for most season and years.

Changes in air temperatures and precipitation affect river temperatures and flows (Holsman et al. 2018), and also contribute to increased erosion and landslides (Pike et al. 2010a, 2010b). The impacts of these changes on salmon freshwater ecosystems are not homogenous, but vary depending on the latitude, altitude, and physical characteristics of these habitats, such as watershed vegetation, geology, groundwater flows, hydrology, size, etc.

Warmer air temperatures, lower spring snow packs, and receding glaciers are causing river temperatures to rise well above seasonal averages. Observations of river temperatures exceeding 18°C - 20°C in summer months are becoming more common in Southern B.C., including in the lower Fraser, and Somass watersheds (Eliason et al. 2011, Martins et al. 2011, Hyatt et al. 2015b, MacDonald et al. 2018).

Salmon that migrate to their spawning grounds in summer months are experiencing more stress and greater depletion of their energy reserves, negatively impacting swim performance and survival (Tierney et al. 2009, Burt et al. 2011, Eliason et al. 2011, Sopinka et al. 2016). Temperatures above 18°C can result in decreased adult swimming performance, and above 20°C can increase adult pre-spawn mortality and disease, reduce egg viability, and cause legacy effects that negatively impact juvenile condition (Tierney et al. 2009, Burt et al. 2011, Eliason et al. 2011, Sopinka et al. 2016). Salmon upstream migration is energetically demanding even in optimal conditions. These migration demands are exacerbated when temperatures fall outside the optimal range for salmon.

Climate changes are also affecting stream flows. In snow dominated hydrological systems in the B.C. Interior and/or northern latitudes, the snow-to-rain ratio is decreasing overall, glacier retreats are accelerating, and lake ice is melting earlier in the spring. Another key change in these systems is earlier than average peak river flows in the spring (Pike et al. 2008, 2010a).

Juvenile outmigration data for Sockeye indicate that in warmer spring seasons when Fraser River flows peak early, Sockeye smolts migrate downstream to the ocean several weeks earlier than normal (MacDonald et al. 2018). Shifts in salmon migration timing could lead to mismatches with the start of the plankton growing season in freshwater or marine ecosystems. If timing does not align, juvenile salmon will encounter suboptimal feeding conditions, grow more slowly, and face higher predation risk.

Rain-dominated hydrographic systems in coastal B.C. are experiencing more extreme conditions, reflecting the greater variability in climate conditions. These rivers are exhibiting more flash flooding, likely leading to increased egg losses from scouring (Holtby and Healey 1986, Lisle 1989, Lapointe et al. 2000). Droughts are also becoming more frequent, creating migration barriers to salmon and losses of incubating eggs and juveniles.

Erosion and landslides are increasing within watersheds. More prolonged periods of precipitation and warming temperatures are increasing the vulnerability of hillsides to landslides, and also increase the frequency of slide triggers from more intense rain events, changes in the freeze-thaw cycle, and severe shifts from dry to wet conditions (Pike et al. 2010a, Cloutier et al. 2016).

Increased sediment inputs into salmon-bearing watersheds reduce the quality and amount of available spawning and juvenile rearing habitat. Incubating salmon eggs can get smothered by increased sediment and debris loads, and juveniles may also have less relief from higher temperatures from the loss of deep pool refuges.

In severe cases, landslides can restrict access to suitable spawning and rearing habitats, and in some cases result in blockages of portions of river systems. There are several examples of major landslides in recent years, including the 2011 Kwinageese River rockfall in the Nass River watershed (Gaboury et al. 2015), and landslides in the Fraser River watershed including at Mount Meager in 2010 (Guthrie et al. 2012), Seton-Portage in 2015/2016 (K. Benner, Fraser Sockeye Stock Assessment, DFO, pers. comm., Dec. 11, 2018), and Big Bar on the Fraser mainstem in 2019 (DFO 2019). The latter landslide has largely blocked upstream access for critical Sockeye and Chinook populations. At the time of completing this report, options to mitigate this barrier were being considered. All of these landslides negatively affected spawning, migration or rearing habitat of a number of Pacific salmon populations.

Lake habitats are also changing. This is particularly important for the Sockeye salmon juvenile rearing stage. Thermal stratification and primary productivity in lakes consistently vary in recent assessments compared to historical data, where these data exist. These changes have had both positive (Chandler et al. 2018, MacDonald et al. 2018) and negative effects (Bradford et al. 2011, DFO 2018a) on juvenile Sockeye survival, for the two populations where these data are available, specifically Chilko and Cultus Fraser Sockeye populations.

3.4 HUMAN-CAUSED HABITAT CHANGES IN FRESHWATER

Human-caused changes to salmon-bearing watersheds can amplify the effects of climate change. The sum of such combined changes in freshwater ecosystems can affect overall salmon survival (Nelitz et al. 2007, McDaniels et al. 2010, Crozier et al. 2019).

Agriculture, mining, urbanization, forestry practices, and other land use activities have long been altering the freshwater habitats salmon rely on for part of their life cycles (Pike et al. 2010a, 2010b). These activities contribute to deforestation and water extraction, and increase inputs of nutrients into freshwater ecosystems. The combined effects of climate change and human land-use activities can result in even warmer river temperatures, greater changes in river flows, and even higher frequencies of erosion and landslides.

Deforestation is increasing, driven by human use, as well as climate change factors, such as the expansion of mountain pine beetle and the increasing magnitude of forest fires. Loss of forest canopies can reduce the capacity of rivers and lakes to buffer warmer temperatures through the cooling effects of shade.

Removal of forest canopies, combined with more extreme rain events caused by climate change, can further increase peak river flows. Loss of trees increases the amount of snow and rain reaching the ground, contributing to runoff. It also increases water volumes in the soil, since water is not removed from the soil to the atmosphere through evapotranspiration (Pike et al. 2010a).

Deforestation also amplifies slope instability caused by changing climate, since the stabilizing effect of tree root systems on soil is removed. Although climate change alone can increase the frequency of erosion and landslides, human landscape changes may be a more significant driver overall (Cloutier et al. 2016). The effects of increased erosion and landslides on salmon are described in the previous section.

Similar to forest canopy losses, water extraction magnifies climate-driven temperature increases in rivers and lakes. Increased water extraction from urbanization, agriculture, and industry, reduces stream flows and inputs of cooler groundwater into freshwater systems. This can diminish the capacity of aquatic systems to moderate higher air temperatures from climate change. It also interacts with increased prevalence of droughts, putting further strain on water availability, particularly during summer months.

Freshwater ecosystems closer to human development are especially challenged by human impacts. Increased nutrient inputs from human sources, particularly from agricultural activities, can have severe impacts on water quality for salmon. Cultus Lake provides an example of large changes in salmon productivity, linked to synergies between climate and habitat degradation (DFO 2018a, Putt et al. 2019).

Cultus Lake is located near Western Canada’s largest urban center, Vancouver, and is adjacent to areas of agricultural and residential land-use (Putt et al. 2019). This lake is experiencing severely depleted oxygen levels in deep water, due to the combined effects of rising lake temperature and increased nutrient inputs from agriculture, and other factors (COSEWIC 2003, DFO 2010, 2018a, Putt et al. 2019).

Juvenile Sockeye salmon that inhabit the deep water environment of Cultus Lake through winter are exhibiting extremely high freshwater mortality, as a result of oxygen depletion (DFO 2018a). The Cultus Lake Sockeye population is now facing an imminent risk of extirpation, with wild fish contributing negligible numbers to the small recent annual returns (COSEWIC 2017, DFO 2018b). As the climate continues to warm, responses across lakes will vary depending on local characteristics.

3.5 OTHER FACTORS THAT AFFECT SALMON

In the 2018 State of the Salmon meeting, participants identified many other factors that can affect salmon, acting alone or cumulatively. However, since the main purpose of this workshop was to identify the current state of salmon and their ecosystems during this recent warming period, the factors identified were not exhaustive. Here we present a short list of some of the factors that should be considered in subsequent iterations of this work, including how their effects on salmon will interact with climate change.

Fisheries and hatcheries directly influence salmon numbers through, respectively, removals from catch, and additions of juvenile salmon to supplement natural production. Considerable stock assessment and hatchery enhancement monitoring and research supports the management of these activities. Critical science inputs will be required to improve our understanding of the role of these two factors moving forward. This can help shape current fisheries and hatchery management practices to prepare and adapt to future salmon production and diversity.

Other factors that can affect salmon include disease, invasive species, contaminants, competition, increased predation, and ocean acidification, to name a few. The effects of these factors are less well understood, particularly in how they affect salmon population numbers, and also how they will interact with climate change. However, there is a growing body of research that can provide a greater understanding of these factors.

4 CANADIAN PACIFIC SALMON TRENDS

4.1 SUMMARY

Recent trends in Canadian Pacific salmon abundances were collated among participants attending the 2018 State of the Salmon meeting. These trends coincide with the recent warm period observed in salmon ecosystems. Many of these salmon trends vary along a north-south gradient, where northern populations of particular species are generally doing better than their southern counterparts. Northern salmon populations are defined in this report, as those that enter the ocean above the northern tip of Vancouver Island.

Chinook salmon populations are declining throughout B.C., the Yukon, and the Northern Transboundary region (B.C.-Southeast Alaska) (Table 2). Many southern Chinook CUs are doing particularly poorly (DFO 2016), while multiple Sockeye and Coho CUs in the south have also declined and are doing poorly (Table 2; Appendix 2; Grant and Pestal 2012, DFO 2015, 2018b). COSEWIC has identified many of these Sockeye, Chinook and Coho DUs as Endangered, meaning they are facing an imminent threat of extinction, or as Threatened (Appendix 2; COSEWIC 2016, 2017). Though there are exceptions to these generalizations.

This contrasts with abundance trends for northern Sockeye populations, which have shown declines only very recently. Pacific salmon populations that have generally not exhibited declines in recent years include northern Coho, apart from the Northern Transboundary populations, most Chum, and odd year Pink populations (Table 2).

Catch for all five DFO managed salmon species has declined, due to declines in target population abundances, or due to constraints placed on fisheries in order to protect co-migrating populations in poor status in mixed-stock fisheries (Figure 4).

4.2 SALMON DATA

Salmon abundance trends are presented qualitatively, based on expert input provided at the 2018 State of the Salmon meeting. Abundance information generally includes catch plus escapement. For the purpose of this report, populations in the north are those that enter the ocean above the northern tip of Vancouver Island, and southern populations enter below this boundary. Consolidation and standardization of salmon abundance data sets is on-going through both internal DFO data management initiatives, and through the Pacific Salmon Foundation-Pacific Salmon Explorer initiative.

There is higher certainty associated with Sockeye and Chinook trends, particularly at southern latitudes, and the Northern Transboundary systems, since these are more complete, higher-quality data sets. Northern Sockeye and Chinook, and most Coho, Pink and Chum data sets, in contrast, have more gaps, and/or are of lower precision, increasing uncertainty in trends reported for these populations.

Hatchery enhancement information is not presented in this report, since experts on hatchery production were not present at the 2018 State of the Salmon meeting to provide their input. This information will be included in subsequent processes.

4.3 SOCKEYE

Northern Canadian Sockeye populations in B.C., the Yukon, and Northern Transboundary systems have generally not exhibited declines in recent decades, contrasting with many Southern B.C. populations that have exhibited longer term declines. Only in very recent years have Northern B.C. and Northern Transboundary populations also started to decline (Table 2). Fecundity has also generally declined for some Sockeye populations, such as those in the Skeena and Fraser watersheds.

Longer term declines have been identified for Fraser River Sockeye populations. Almost half of Fraser Sockeye CUs have been placed in the WSP Red status zone (Grant and Pestal 2012, DFO 2018b), with most of these WSP Red CUs identified as Endangered by COSEWIC in their recent assessment (COSEWIC 2017; Appendix 2, Table A2-3). This group of salmon was the focus of the Cohen Inquiry into the declines of Sockeye salmon from the mid-1990's to 2009 (Cohen 2012a, 2012b, 2012c).

There are exceptions to the declining trend in Southern B.C. Sockeye populations. For example, Barkley Sound Sockeye on the West Coast of Vancouver Island (Hyatt et al. 2018), and Chilko and Shuswap Sockeye in the Fraser watershed (DFO 2018b, Grant and MacDonald 2018), have not declined in abundance. There are also cases, like Okanagan Sockeye, where declining population trends have been reversed when informed human interventions have coincided with favourable environmental conditions (Hyatt et al. 2015a). Another exception is the river-type Harrison Sockeye population in the Fraser watershed, which migrates to the ocean shortly after they emerge from their spawning gravel. This population has exhibited dramatic increases in abundances in recent decades, while many lake-type populations in the Fraser watershed have declined (Grant and MacDonald 2011, Chandler et al. 2018).

Fraser Sockeye are one group of salmon where freshwater and marine survival data have been tracked for two populations: Chilko and Cultus Lake Sockeye. These two populations potentially reflect bookends for Sockeye freshwater survival in Canada. Chilko Sockeye rear in a remote, high altitude, glacial lake, while Cultus Sockeye rear in a southern lake situated close to Vancouver, B.C. In contrast with Chilko Lake, Cultus Lake is subject to considerable recreational use, human development, and agricultural runoff. Marine survival has declined in recent decades for both populations, while freshwater survival has been above average for Chilko (Chandler et al. 2018, Grant and MacDonald 2018) and conversely, critically low for Cultus

(DFO 2018a). Differences in their freshwater habitats are potentially contributing to these large variations in overall abundance and survival trends between these populations.

Sockeye is a highly valued salmon species in B.C. and transboundary fisheries. Data quality and quantity are relatively high for a subset of productive Sockeye populations that are actively managed, compared to other salmon species (Table 2). However, this subset accounts for less than half of the total number of Sockeye populations within B.C. and the Yukon.

4.4 CHINOOK

Chinook salmon abundance trends are unique across Canadian Pacific salmon species, synchronously declining throughout B.C., Yukon and Northern Transboundary systems (Table 2). Synchrony in Chinook survival trends has been reported more broadly, from Oregon up to Alaska (Sharma et al. 2013, Kilduff et al. 2014, Dorner et al. 2018). Declining Chinook abundances are exacerbated by decreases in Chinook size-at-age, age-at-return, and reproductive potential, including reductions in the numbers of eggs-per-female and in egg size.

Abundances of Chinook salmon are reaching critically low levels in Southern B.C., where recent status assessments have placed over half of assessed Southern B.C. Chinook CUs in the WSP Red status zone (DFO 2016; Appendix 2, Table A2-4). COSEWIC has determined that many of the DUs in the B.C. Interior are Endangered or Threatened (Appendix 2, Table A2-4).

There are only a few exceptions to these declines in recent years, such as East Coast of Vancouver Island Chinook populations. Decreasing marine and freshwater survival are contributing to these trends, though data on freshwater survival is limited (Brown et al. 2019).

Chinook, similar to Sockeye, is a highly valued salmon species in B.C./Yukon fisheries. More data are collected on Chinook salmon than other species, apart from Sockeye, including escapement, catch, size, and age (Table 2).

4.5 COHO

Coho, like Sockeye, are currently experiencing better abundance trends in the north compared to the south (Table 2). Southern populations have had consistently low abundances for the past two decades, and Interior Fraser River populations were recently placed in the Amber WSP status zone (DFO 2015), or identified as Threatened by COSEWIC (COSEWIC 2016) (Appendix 2, Table A2-5).

There are many data gaps for Coho, particularly at northern latitudes (Table 2). As a result, there is greater uncertainty in these reported trends.

4.6 PINK

Odd year Pink salmon have not exhibited any declines in recent years, while even year brood lines have exhibited declines in some areas (Table 2; Irvine et al. 2014, Malick and Cox 2016). On a broader scale, this species dominates numbers of salmon in the Northeast Pacific Ocean (Ruggerone and Irvine 2018).

Pink can be resilient, rebounding from weak to strong run strength within regional populations in one or two generations (Heard 1991). They have been observed spawning in new locations within the Yukon, Skeena, and the Fraser watersheds, indicating a potential expansion of their range. Prevalence of Pink salmon has also increased in the Northeast Bering and Beaufort Seas in the Arctic, likely straying from more southern locations as these areas warm (K. Dunmall, DFO Arctic Region, pers. comm.).

Pink salmon populations can contribute large numbers to commercial, recreational and First Nations fisheries. However, in recent years, Pink catch has declined, due to concerns for at-risk co-migrating salmon populations (Figure 4). Declining stock assessments and the lower importance of this species to fisheries have limited the data available for Pink salmon (Table 2). Existing abundance estimates are highly aggregated and generally do not provide detail at the scale of individual populations.

4.7 CHUM

Chum populations in the Yukon, Northern Transboundary, and Northern B.C. regions have generally not exhibited declines (Table 2). Meanwhile, southern Chum populations are showing mixed abundance trends, with some very recent declines in the Fraser watershed. There are some exceptions to these general trends, such as Skeena and Nass Chum, which are located in the north and have been doing poorly. On a broader scale, Chum dominate the overall biomass of salmon in the Northeast Pacific, due to contributions from populations in other countries (Ruggerone and Irvine 2018).

Chum salmon contribute lower numbers to all fisheries in B.C. and the Yukon, although they are particularly important to First Nations fisheries. Limited data exist for Chum salmon (Table 2). As a result, there is greater uncertainty in these reported trends.

Table 2. Recent abundance trends for the five species of Pacific salmon managed by DFO: Sockeye, Chinook, Coho, Pink and Chum, based on input from DFO participants at the May 2018 State of the Salmon meeting. These generally include catch and escapement

These trends are reported for four geographic areas from north to south: Yukon includes salmon populations spawning in the Yukon River; Northern Transboundary includes salmon populations that spawn and migrate through rivers that cross SE Alaska and B.C.; Northern B.C. includes B.C. salmon populations that enter the ocean north of the northern tip of Vancouver Island; and Southern B.C. includes salmon populations that enter the ocean south of the northern tip of Vancouver Island.

There are exceptions to these general trends, where some populations are not exhibiting the same overall patterns.

Area	Sockeye	Chinook	Coho	Pink-Odd Year	Pink-Even Year	Chum
Yukon	No trend (M)	Decline (M)	No trend (L)	NA	NA	No trend (L)
Northern Transboundary-B.C./SW Alaska	Recent decline (H)	Decline (H)	Recent decline (M)	No trend (VL)	Decline (VL)	No trend (VL)
Northern B.C.	Very recent declines (M)	Decline (M)	No trend (L)	No trend (L)	No trend (L)	No trend (L)
Southern B.C.	Decline (H)	Decline (M)	Decline (M)	No trend (L)	Decline (VL)	Mixed (L)

The data quality and quantity vary across areas and species and are indicated in brackets below: H (high); M (medium); L (low); VL (very low).

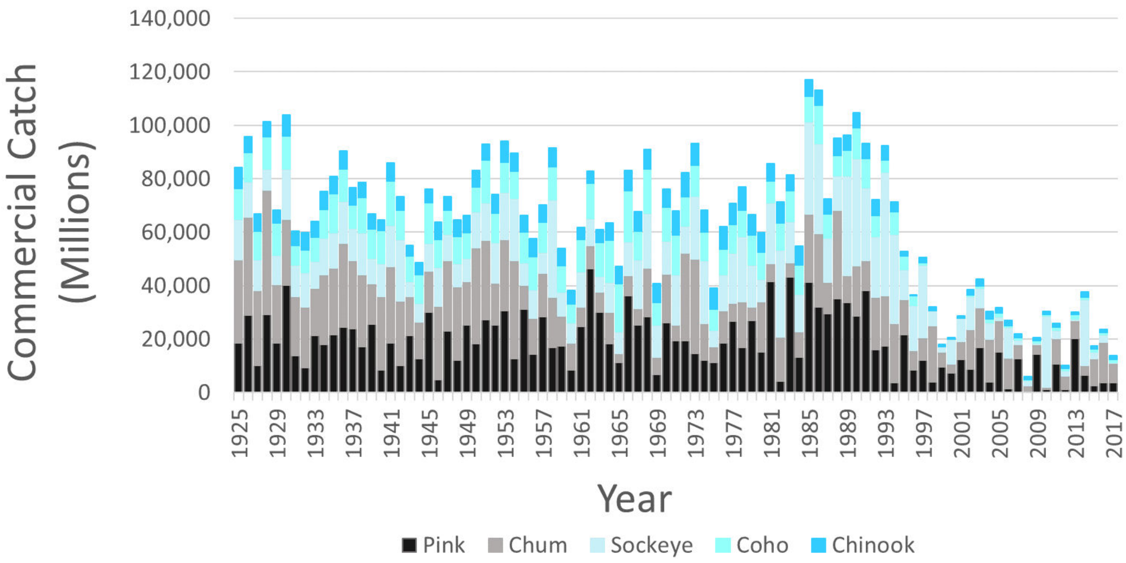


Figure 4. Canadian commercial catch numbers for Pink, Chum, Sockeye, Coho, and Chinook salmon. Data Source: North Pacific Anadromous Fish Commission (NPAFC). 2018. NPAFC Pacific salmonid catch statistics (updated 31 July 2018). North Pacific Anadromous Fish Commission, Vancouver. Available from www.npafc.org.

5 LESSONS LEARNED FROM THE RECENT PERIOD OF WARM CONDITIONS

5.1 SUMMARY

Responses of Canadian Pacific salmon to recent warm freshwater and marine conditions may provide insights into salmon resiliency to climate and habitat change. Current salmon trends indicate that northern Sockeye and Coho populations are doing better than their southern counterparts, and that salmon populations that spend little time in freshwater, including river-type Sockeye, ocean-type Chinook, and Pink and Chum populations, are not exhibiting persistent declines.

Spawning, rearing, and early ocean distributions in northern latitudes appears to be particularly advantageous to Sockeye and Coho populations. Although the rate of climate warming is greater in the north, temperatures remain cooler at northern latitudes relative to the south. Cooler temperatures in the north, relative to the south, can result in more beneficial marine food webs at these latitudes. In freshwater, these cooler northern temperatures, combined with generally better habitat quality, may also be contributing to more positive outcomes for northern salmon populations.

Another attribute of salmon populations that are not declining, is shorter periods of time spent rearing as juveniles in freshwater. River-type Sockeye, ocean-type Chinook, and Pink and Chum populations all migrate to the ocean shortly after gravel emergence. Short freshwater residence times decrease the exposure of these populations to temperature and habitat changes that are occurring in their freshwater habitats, and may be contributing to their overall improved survival.

Particular population traits such as adult upstream migration timing and distances, distribution in freshwater, physiology, fishery encounters, and/or ocean distribution can also affect a salmon population’s ability to survive climate and habitat change.

5.2 POPULATIONS IN THE NORTH

Sockeye, Chinook and Coho population trends vary by latitude. Sockeye and Coho are generally doing better in the north. Chinook are declining throughout their Canadian range, but are doing particularly poorly in southern latitudes (Table 2; DFO 2015, 2016, 2018). Two key factors that vary by latitude are temperature and the degree of human-caused habitat alteration.

Temperature is a key variable that influences Pacific salmon growth and survival. Although the rate of climate change is greater in the north, temperatures at these latitudes are still cooler compared to the south.

In the ocean, cooler temperatures in the north can lower salmon metabolism, reducing energy demands. This can contribute to better outcomes for salmon that enter the ocean at northern latitudes, particularly when combined with the presence of more energy rich zooplankton relative to the south (more details provided in Section 3.2 Marine Heatwaves in the Northeast Pacific Ocean).

Freshwater may present even greater temperature-related challenges for Pacific salmon. With a warming climate, summer river temperatures in some southern B.C. systems are now annually exceeding thermal tolerance ranges of migrating adult salmon (Eliason et al. 2011, Martins et al. 2012, MacDonald et al. 2018). This can have many effects on migrating adults, and can also affect egg viability and the fitness of their offspring (see previous section 3.3 Climate Related Changes in Freshwater for more details).

Other factors like increased deforestation, water extraction, and nutrient loading are all more concentrated at southern latitudes, where human populations are larger. Habitat alteration can amplify the impacts of climate-driven changes on salmon freshwater ecosystems (see previous section 3.4 Human-Caused Habitat Changes in Freshwater for more details). Northern freshwater ecosystems are less impacted by these changes, and therefore, may have better conditions for Pacific salmon compared to southern freshwater habitats. These factors may contribute to the north-south trends in Sockeye, Chinook and Coho salmon.

5.3 SPECIES AND POPULATIONS SPENDING LESS TIME IN FRESHWATER

Salmon populations with no prolonged juvenile rearing stage in freshwater, referred to as immediate migrants, are generally not exhibiting persistent declines. Their trends either vary around a mean, or are increasing. This includes many river-type Sockeye, ocean-type Chinook, Pink and Chum salmon populations.

Immediate migrants avoid longer exposure to the acute temperature and habitat changes that are occurring freshwater ecosystems. Since temperatures are higher, and habitat changes are greater in the south, this may be an important reason why immediate migrant Sockeye and Chinook populations at these latitudes are doing better than their freshwater rearing counterparts.

Immediate migrant salmon populations may have other unique traits that are providing advantages to their survival under changing climate and habitat conditions.

For example, the one river-type Fraser Sockeye population, Harrison Sockeye, has exhibited improving trends in survival and abundance, counter to most lake-rearing populations (Grant and Pestal 2012, Chandler et al. 2018, Grant and MacDonald 2018). This river-type Sockeye population has a limited freshwater rearing stage, migrates a relatively short distance upstream, has a later-timed downstream juvenile migration, and exhibits a different ocean distribution, compared to lake-type Fraser Sockeye (Birtwell et al. 1987, Tucker et al. 2009, Beamish et al. 2016). Any of these traits acting alone, or cumulatively, may be contributing to differences in survival between this immediate migrant population and those with longer freshwater rearing stages.

Pink and Chum salmon also have no freshwater rearing stage, migrating to the ocean after emerging from their spawning gravel. Their populations are generally doing well throughout their Canadian range, with the exception of even year Pinks that spawn at southern latitudes (Irvine et al. 2014), and some Chum populations, such as those originating in the Skeena and Nass River systems. Pink and Chum also generally have shorter migration distances to their spawning grounds. In the Fraser system, few Pink and Chum salmon spawn upstream of Hells Gate, located near Hope, B.C., whereas most of the Sockeye, Chinook and Coho production in this system occurs upstream of Hells Gate, in the B.C. Interior.

5.4 CONSERVATION UNITS WITH BROADER DISTRIBUTIONS IN FRESHWATER

Pink and Chum are generally exhibiting better recent survival than Sockeye, Chinook, and Coho salmon. Each Chum and Pink CU covers a wider spawning distribution in freshwater compared to Sockeye, Chinook, or Coho CUs, and this trait may provide them with greater adaptability to deteriorating conditions. A broader geographic distribution of populations within a CU may be able to maintain a CU, if the quality of some freshwater spawning locations declines. There are fewer Chum and Pink CUs compared to Sockeye, Chinook, and Coho. Chum and Pink CUs combined comprise only 20% of all Canadian Pacific Salmon CUs, (Table 1).

In contrast, Sockeye, Chinook, and Coho are the most highly adapted species to specific freshwater habitats, and are exhibiting declines in abundances across many populations. Together, they comprise 80% of all the Canadian Pacific Salmon CUs, which emphasizes the degree of specialization they represent (Table 1). Specific adaptations to particular habitats restrict their ability to redistribute to more optimal spawning or rearing habitats in the event of poor local conditions, and may limit the adaptability of these species to changing climate and habitats.

5.5 UPSTREAM MIGRATION TIMING AND OTHER SALMON POPULATION CHARACTERISTICS

Since salmon generally return to their natal rivers or lakes to spawn, populations are reproductively isolated from one another to varying degrees. Individual salmon share similar traits within a population, such as those related to their behaviors, body shapes, and thermal tolerances. These traits reflect genetic adaptations to the unique set of conditions these fish have encountered in their past (Hess and Narum 2011; Drinan et al. 2012; Narum et al. 2013), and have resulted in their persistence as populations to date. However, since salmon habitats are now rapidly changing, not all these traits will be suited to new conditions, and salmon populations may not have sufficient flexibility to adapt in time.

One example of a trait that may affect a salmon population's resilience to climate change is their upstream migration timing. This trait can vary from summer to winter months depending on the salmon species and population. Among Fraser Sockeye populations for example, some migrate during the summer, when river temperatures are at their hottest. Increasingly, river temperatures are exceeding the 18°C to 20°C upper thermal limits of these salmon in the summer (MacDonald et al. 2018). As a result, summer migrating populations are experiencing greater stress and greater depletion of their energy reserves, which reduces their ability to swim and survive to spawn (Tierney et al. 2009; Burt et al. 2011; Eliason et al. 2011; Sopinka et al. 2016), among other impacts (see section 3.3 Climate Related Changes in Freshwater for more details). This contrasts with later timed Fraser Sockeye populations, and other species like Chum, which migrate in the fall when cooler river temperatures provide more optimal conditions for their upstream migrations.

As river temperatures continue to warm, upstream migration distances, and population-specific physiology and body shapes, might moderate some of the impacts on summer migrating salmon. Shorter migration distances upstream in freshwater, for example, may reduce the exposure of a salmon population to high temperatures.

5.6 MORE SALMON POPULATIONS ARE EXHIBITING NEGATIVE TRENDS IN RECENT YEARS

Synchrony in salmon survival trends across populations is increasing (Peterman and Dorner 2012, Kilduff et al. 2015, Malick and Cox 2016, Dorner et al. 2018). This suggests that large-scale mechanisms are having stronger, or more consistent, effects on salmon survival (Malick and Cox 2016, Dorner et al. 2018). Large-scale, climate patterns have been identified as a potential driver of greater synchrony across populations, and have been increasing in variability and intensity in recent years

(Peterman and Dorner 2012, Kilduff et al. 2015, Dorner et al. 2018). These broad climate patterns can affect both marine and freshwater ecosystems.

The degree of synchrony varies by species. Synchronous declines in Chinook salmon survival throughout their range, from Oregon to Western Alaska, are of particular concern, and their degree of synchrony has been increasing (Dorner et al. 2018). Sockeye also are exhibiting greater synchrony, but at smaller spatial scales than Chinook. Sockeye show opposite survival patterns between Canadian and Southeast Alaskan populations, and those from central and western Alaska. Populations from Canada to Southeast Alaska are generally declining in survival, while central and western Alaska populations are increasing or showing no trend (Peterman and Dorner 2012).

Increasing synchrony in survival trends across populations puts salmon species at risk, due to the loss of portfolio effects (Schindler et al. 2010, Griffiths et al. 2014). Synchronization of salmon trends produces greater volatility in the short term. Increasing synchrony means that declines in one population will not be offset by concurrent increases in other salmon populations. The stability provided by variability across populations is critically important for maintaining ecosystem and fisheries resources (Schindler et al. 2010), and is a concern now that we are seeing more synchronization in salmon trends across areas and species.

The deterioration of diversity in survival responses reduces the overall resilience of salmon to changing conditions (Kilduff et al. 2015, Dorner et al. 2018). As climate patterns in Pacific salmon ecosystems continue to increase in variability and intensity, due to climate change, synchrony in regional salmon survival is expected to increase, as is the prevalence of more extreme highs and lows in salmon survival (Dorner et al. 2018).

6 CONCLUSIONS AND NEXT STEPS

“There are two major environmental crises facing the planet, climate change and catastrophic losses to nature” (CPAWS 2019). A number of global reports are alerting us to accelerating climate change and biodiversity losses on the planet (IPCC 2014, 2018, IPBES 2018, 2019). These warnings have been strongly echoed for Canada (WWF 2017, Bush and Lemmen 2019, CPAWS 2019). Climate change impacts may be particularly acute in Canada, since rates of warming at northern latitudes are double the global average (IPCC 2014, Bush and Lemmen 2019).

The impacts of recent, unprecedented, heatwaves in the Northeast Pacific Ocean, coupled with extremely warm freshwater temperatures, provide insight into the responses of Canadian Pacific salmon and their ecosystems to climate change. These changes are amplified by local salmon habitat changes in freshwater, such as deforestation and water extraction. As the climate continues to warm and precipitation patterns change, conditions observed during the recent period of high temperatures will likely become more common, and more extreme.

Fisheries have been identified as one of the major climate change risks to Canada, which could contribute to ‘significant losses, damages or disruptions over the next 20 years’ (Council of Canadian Academies 2019). There is still time to moderate the severity of climate change and its impacts, through mitigation and adaptation. The extent that we are able to curb our net CO_2 and other greenhouse gas emissions will determine the magnitude of future warming. We must also adapt to current and expected climate conditions and their effects, though research, planning, and actions.

Recent trends in salmon abundances yield a growing, but still incomplete, view of salmon vulnerability to climate change. This vulnerability is determined by multiple factors, including salmon spawning and rearing locations, warming water temperatures, ecosystem changes, freshwater habitat alteration, salmon traits, and more. All these factors acting alone or cumulatively increase our current uncertainty related to salmon population responses to climate change.

More detailed assessments of salmon vulnerability to climate change are required to understand and predict future trends in salmon populations. Work was initiated on Canadian Pacific salmon vulnerability assessments in 2007 (PFRCC 1999, Nelitz et al. 2007), and reinvigorated in 2015 more broadly on a number of fish species (Hunter and Wade 2015, Hunter et al. 2015). U.S. scientists recently completed vulnerability assessments for their own Pacific salmon populations (Hare et al. 2016, Urban et al. 2016, Crozier 2017, Crozier and Siegel 2018, Crozier et al. 2019). Other work, through the International Year of the Salmon initiative, is also fostering global research to help improve our understanding of the status and responses of Atlantic and Pacific salmon to climate change and other factors (Irvine et al. (*eds*) 2019, Young et al. (*eds*) 2019).

Improved integration across a wide variety of organizations that study and manage Canadian Pacific salmon, their habitats, and local climate change predictions, would advance efforts to address existing knowledge gaps in these areas.

In DFO's Pacific Region, participants attending a recent second annual State of the Salmon meeting, held in March 2019, agreed that a Pacific Salmon-Ecosystem-Climate Consortium would be one mechanism to assist with integration of scientific expertise across organizations. This Consortium is currently being initiated by DFO to advance our own assessments of Pacific salmon vulnerability to climate and habitat change.

This integrative work is critical to support changes to management, habitat restoration, and salmon recovery activities required now to prepare for future salmon production and diversity.

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APPENDIX 1. MAY 15 & 16 2018 STATE OF THE SALMON MEETING AGENDA

TUESDAY MAY 15 2018

Start	End	Time	Item	Lead
8:30 AM	9:00 AM	0:30	Registration & Getting Organized	MacDonald
9:00 AM	**9:05 AM**	**0:05**	**Welcome**	**Grant/MacDonald**
9:05 AM	**9:25 AM**	**0:20**	**FIRST ICEBREAKER**	**ALL**
9:25 AM	**9:30 AM**	**0:05**	**SOS MEETING DELIVERABLES: SOS Technical Report; Other Session Deliverables Identified**	**Grant**
9:30 AM	**9:50 AM**	**0:20**	**HOW WE ARE GOING TO ENGAGE TODAY & MEETING APPROACH**	**Grant**
9:50 AM	**9:55 AM**	**0:05**	**Theme 1: RECENT SALMON TRENDS (ABUNDANCE, PRODUCTIVITY, SIZE, FECUNDITY, ETC.)**	**Grant**
9:55 AM	10:00 AM	0:05	**Theme 1:** RECENT SALMON TRENDS: in the Yukon/TB Area Highlights	Foos
10:00 AM	10:05 AM	0:05	**Theme 1:** RECENT SALMON TRENDS: in the North Coast Area Highlightss	Cox-Rogers/Winther
10:05 AM	10:10 AM	0:05	**Theme 1:** RECENT SALMON TRENDS: in the South Coast Area Highlights	Luedke
10:10 AM	10:15 AM	0:05	**Theme 1:** RECENT SALMON TRENDS: in the Fraser Area Highlights	Whitehouse & Team
10:15 AM	10:20 AM	0:05	**Theme 1:** RECENT TRENDS: Pacific Chinook-maturation, age, fecundity and productivity	Brown
10:20 AM	**10:30 AM**	**0:10**	**CLARIFICATION QUESTIONS ONLY**	**Grant**
10:30 AM	**10:50 AM**	**0:20**	**BREAK**	
10:50 AM	**11:50 AM**	**1:00**	**Theme 1 BO Groups: SALMON TRENDS**	**Each BO group**
			1. Identify recent key salmon trends abundance, productivity, size-at age…	1 facilitator
			2. Are there similarities among species, watersheds, latitidue, life-history types, etc? Exceptions?	1 note taker
			3. Identify key gaps in assessments and knowledge: time lags in data avail, spatial gaps, etc.	
11:50 AM	**12:30 PM**	**0:40**	**Theme 1 PLENARY: SALMON TRENDS**	**Grant**
12:30 PM	**1:30 PM**	**1:00**	**LUNCH**	
1:30 PM	**1:35 PM**	**0:05**	**Theme 2: ECOSYSTEM: PHYSICAL & BIOLOGICAL IN RECENT YEARS**	**Grant**
1:35 PM	1:40 PM	0:05	**Theme 2:** ECOSYSTEM: Observations from E-Watch: temps, discharge, & salmon in freshwater	Patterson*/Robinson
1:40 PM	1:45 PM	0:05	**Theme 2:** ECOSYSTEM: Freshwater Lakes & Juvenile Rearing	Selbie
1:45 PM	1:50 PM	0:05	**Theme 2:** ECOSYSTEM: Pacific Ocean Ecosystem	Boldt/King*
1:50 PM	1:55 PM	0:05	**Theme 2:** ECOSYSTEM: Pacific Fish Surveys	King
1:55 PM	2:00 PM	0:05	**Theme 2:** ECOSYSTEM: Strait of Georgia Juvenile Salmon Surveys	Neville
2:00 PM	**2:10 PM**	**0:10**	**CLARIFICATION QUESTIONS ONLY**	**Grant**
2:10 PM	**3:10 PM**	**1:00**	**Theme 2 BO Groups: ECOSYSTEM: PHYSICAL & BIOLOGICAL RECENT TRENDS**	**Each BO group**
			1. Identify recent key ecosystem observations that are tracked by salmon life-stage	1 facilitator
			2. Are there ways to group these observations: latitude, altitidue, coastal, in-land, etc.?	1 note taker
			3. What can the recent warming period in FW/Mar tell us about salmon trends in the future	
			4. Identify Gaps in ecosystem assessments	
3:10 PM	**3:30 PM**	**0:20**	**BREAK**	
3:30 PM	**4:15 PM**	**0:45**	**Theme 2: PLENARY: ECOSYSTEM**	**Grant**
4:15 PM	**4:30 PM**	**0:15**	**Wrap up day 1 Exercise**	**Grant**

WEDNESDAY MAY 16 2018

Start	End	Time	Item	Lead
8:30 AM	9:00 AM	0:30	Getting organized: new seating assignments etc.	MacDonald
9:00 AM	**9:05 AM**	**0:05**	**Review yesterday**	**Grant**
9:05 AM	**9:10 AM**	**0:05**	**Theme 3: LINKAGES: SALMON & ECOSYSTEM & OTHER FACTORS**	**Grant**
9:10 AM	9:15 AM	0:05	Theme 3: LINKAGES: NE Pacific Salmon Abundances: Role of Hatcheries	Irvine
9:15 AM	9:20 AM	0:05	Theme 3: LINKAGES: Cumulative Effects	Hyatt
9:20 AM	9:25 AM	0:05	Theme 3: LINKAGES: Overview of Modeling Approaches	Holt/Bradford
9:25 AM	9:30 AM	0:05	Theme 3: LINKAGES: Fisheries	Dobson
9:30 AM	**9:40 AM**	**0:10**	**CLARIFICATION QUESTIONS ONLY**	**Grant**
9:40 AM	**11:00 AM**	**1:20**	**Theme 3 BO Groups: how much do we know about how factors we can control are influencing the salmon trends we are observing (Facilitators schedule 20 minute break)**	**Each BO group**
			1. List factors you think are contributing to salmon trends: group into what we can control or not	1 facilitator
			2. How do these different factors we can't control interact with those we can control?	1 note taker
			3. Identify Gaps and Potential Future Strategies to Address Gaps	
11:00 AM	**11:45 AM**	**0:45**	**Theme 3: PLENARY: LINKAGES**	**Grant**
11:45 AM	**12:45 PM**	**1:00**	**LUNCH**	
12:45 PM	**12:50 PM**	**0:05**	**HOW THE SCIENCE ORGANIZATION FITS TOGETHER**	**Grant**
12:50 PM	12:55 PM	0:05	Current Science Organization	Holmes
12:55 PM	1:00 PM	0:05	Salmon Coordinator	Dobson
1:00 PM	1:05 PM	0:05	Stock Asssessment Core Program	Thiess
1:05 PM	1:10 PM	0:05	State of the Salmon Program	Grant
1:10 PM	1:15 PM	0:05	Salmon Communications	Sloan
1:15 PM	**1:30 PM**	**0:15**	**General Discussion on how everything fits together**	**Grant**
1:30 PM	**1:45 PM**	**0:15**	**Break**	
1:45 PM	**1:50 PM**	**0:05**	**Key Questions on Collaboration & Communication on SOS**	**Grant**
1:50 PM	2:20 PM	0:30	Exercise on Communication	
2:20 PM	3:05 PM	0:45	SOS meeting: who, what, when, where, why, and how???	
3:05 PM	**3:20 PM**	**0:15**	**CLOSING ACTIVITY**	**Grant**
3:20 PM			**AJOURN**	

APPENDIX 2. DFO WILD SALMON POLICY AND COSEWIC STATUS ASSESSMENTS FOR CANADIAN PACIFIC SALMON

Fisheries & Oceans Canada (DFO) and the Committee on the Endangered Wildlife in Canada (COSEWIC) have both conducted status assessments for three groups of Canadian Pacific salmon, including Fraser Sockeye, Southern B.C. Chinook, and Interior Fraser Coho.

DFO's WSP status assessments are conducted on Conservation Units (CU) (Holtby and Ciruna 2007; Grant et al. 2011; DFO 2013; Wade et al. 2019). CUs are placed into one of five WSP status zones: Red, Red/Amber, Amber, Amber/Green, and Green. Definitions of the three key status zones are provided in Table A-1, and Red/Amber and Amber/Green status zones are intermediate between these (DFO 2005; Grant & Pestal 2012). DFO WSP status can also include a data deficient category for CUs where there is insufficient data available to determine status.

COSEWIC groups salmon populations into Designatable Units (DUs), which are identical or very similar to DFO's CUs. They place DUs into five status zones: Endangered, Threatened, Special Concern, Data Deficient, and Not at Risk. Definitions are presented in Table A-2.

Table A2-1. Wild Salmon Policy biological status zones (DFO 2005; Grant and Pestal 2012)

Status	**Definition**
Red	"… established at a level of abundance high enough to ensure there is a substantial buffer between it and any level of abundance that could lead to a CU being considered at risk of extinction by COSEWIC"
Amber	"While a CU in the Amber zone should be at low risk of loss, there will be a degree of lost production. Still, this situation may result when CUs share risk factors with other, more productive units"
Green	"identif[ies] whether harvests are greater than the level expected to provide on an average annual basis, the maximum annual catch for a CU, given existing conditions…there would not be a high probability of losing the CU"

Table A2-2. The Committee on the Endangered Wildlife in Canada (COSEWIC) biological status zones and their definitions (COSEWIC 2010).

Status	**Definition**
Endangered (E)	A wildlife species facing imminent extirpation or extinction.
Threatened (T)	A wildlife species that is likely to become an endangered if nothing is done to reverse the factors leading to its extirpation or extinction.
Special Concern (SC)	A wildlife species that may become threatened or endangered because of a combination of biological characteristics and identified threats.
Data Deficient (DD)	A category that applies when the available information is insufficient (a) to resolve a wildlife species' eligibility for assessment or (b) to permit an assessment of the wildlife species' risk of extinction.
Not At Risk (NAR)	A wildlife species that has been evaluated and found to be not at risk of extinction given the current circumstances.

Fraser Sockeye WSP and COSEWIC statuses

There are 24 Fraser Sockeye CUs that were first assessed by DFO in 2012 (DFO 2012; Grant and Pestal 2012). These were re-assessed in 2017 (DFO 2018b). There are currently seven Fraser Sockeye CUs in the Red status zone, two in the Red/Amber status zone, four in the Amber status zone, six in the Amber/Green status zone, three in the Green status zone, and one data deficient CU (Table A-3, first column). COSEWIC aligned their Fraser Sockeye DUs exactly with DFO's WSP CUs. COSEWIC statuses also align with DFO's WSP statuses for Fraser Sockeye and COSEWIC identifies eight Endangered DUs, two Threatened, five Special Concern, and eight Not-at-Risk (Table A-3, last column).

*Table A2-3: The 2017 Integrated status designations for **the 24 Fraser River sockeye salmon CUs**, ranked from poor (Red zone) to healthy (Green zone) status based on the current 2017 assessment. Cyclic CU statuses are determined including abundance benchmarks estimated using the Larkin model* (DFO 2018b). *For each CU, more commonly used stock names are presented. An asterisks (*) indicates provisional status designations; R/A: Red/Amber; A/G: Amber/Green; DD: data deficient; Undet: undetermined. The previous assessment's integrated statuses are also listed in the 2012* (DFO 2012; Grant and Pestal 2012). *The COSEWIC 2017 status designations are presented in the final column (released 2018).*

2017		**2012**		**Conservation Unit**	**Stock**	**COSEWIC 2017**
R		R		Bowron-ES	Bowron	**Endangered**
R		R		Cultus-L	Cultus	**Endangered**
R		R		Takla-Trembleur-EStu	Early Stuart	**Endangered**
R		R*		Taseko-ES	Miscellaneous E. Summ	**Endangered**
R		R		Widgeon – River*	Miscellaneous Lates	**Threatened**
R		A		Harrison (U/S)-L	Weaver	**Endangered**
R		UD		Seton-L	Portage	**Endangered**
R	A	R	A	Quesnel-S	Quesnel	**Endangered**
R	A	R	A	Takla-Trembleur-Stuart-S	Late Stuart	**Endangered**
A		R		Nahatlatch-ES	Miscellaneous E. Summ	**SC**
A		A		North Barriere-ES	Fennel & Miscellaneous E. Summ	**Threatened**
A		A		Kamloops-ES	Raft & Miscellaneous E. Summ	**SC**
A		A	G	Shuswap-ES	Scotch, Seymour, Mis. E. Summ	**NAR**
A		G*		Lillooet-Harrison-L	Birkenhead	**SC**
A	G	R		Nadina-Francois-ES	Nadina	**NAR**
A	G	R	A	Chilliwack-ES	Miscellaneous E. Summ	**NAR**
A	G	R	A	Francois-Fraser-S	Stellako	**SC**
A	G	A		Anderson-Seton-ES	Gates	**NAR**
A	G	G		Harrison (D/S)-L	Miscellaneous Lates	**SC**
A	G	G		Shuswap Complex-L	Late Shuswap	**NAR**
G		A	G	Pitt-ES	Pitt	**NAR**
G		G*		Chilko-S & Chilko-ES agg.	Chilko	**NAR**
G		G		Harrison River – River Type	Harrison	**NAR**
DD		DD		Chilko-ES	Chilko	**NA**

Abbreviations: EStu: Early Stuart; ES: Early Summer; S: Summer; L: Late; Mis: miscellaneous;
**Widgeon (river-type) CU has a small distribution, therefore, this CU will be consistently in the Red status zone;*

Southern B.C. Chinook WSP and COSEWIC statuses

There are 34 Southern B.C. Chinook CUs that were assessed by DFO in 2016 (DFO 2016). There are currently 11 Red, one Red/Amber, one Amber, two Green, 10 to-be-determined, and 9 data deficient CUs. COSEWIC has identified 28 DUs that are slightly different from DFO's CUs (Table A-4), although most DUs align with DFO's WSP CUs. COSEWIC identifies 11 Endangered, four Threatened, one Special Concern, one Not-at-Risk DU, and two data deficient DUs. A number of status assessments for both DFO and COSEWIC are pending further work. Nuances with the data and hatchery contributions are currently being resolved in data sets to support status assessments.

*Table A2-4: The 2016 Integrated status designations for **the 34 Southern B.C. Chinook CUs**, ranked from poor (Red zone) to healthy (Green zone) status based on the current 2016 assessment (DFO 2016). For each CU, their name and CU ID is provided. The COSEWIC 2017 status designations for 28 DUs are presented in the final column (released Dec 4 2017).*

CU Name	CU	WSP 2016	DU	COSEWIC 2018	COSEWIC 2019
Okanagan_1.x	CK-01	Red	--	Endangered*	--
Middle Fraser River-Portage_FA_1.3	CK-09	Red	DU08	Endangered	--
Middle Fraser River_SP_1.3	CK-10	Red	DU09	Threatened	--
Upper Fraser River_SP_1.3	CK-12	Red	DU11	Endangered	--
South Thompson-Bessette Creek_SU_1.2	CK-16	Red	DU14	Endangered	--
Lower Thompson_SP_1.2	CK-17	Red	DU15	--	TBD
North Thompson_SP_1.3	CK-18	Red	DU16	Endangered	--
North Thompson_SU_1.3	CK-19	Red	DU17	Endangered	--
East Vancouver Island-North_FA_0.x	CK-29	Red	DU23	--	TBD
West Vancouver Island-South_FA_0.x	CK-31	Red	DU24	--	TBD
West Vancouver Island-Nootka & Kyuquot_FA_0.x	CK-32	Red	DU25	--	TBD
South Thompson_SU_1.3	CK-14	Red Amb	DU13	--	TBD
Middle Fraser River_SU_1.3	CK-11	Amber	DU10	Threatened	--
Lower Fraser River_FA_0.3	CK-03	Green(p)	DU02	Threatened	--
South Thompson_SU_0.3	CK-13	Green	DU12	Not At Risk	--
Shuswap River_SU_0.3	CK-15	TBD			
Lower Fraser River_SP_1.3	CK-04	TBD	DU03	Sp. Concern	--
Lower Fraser River-Upper Pitt_SU_1.3	CK-05	DD	DU04	Endangered	--
Lower Fraser River_SU_1.3	CK-06	DD	DU05	Threatened	--
Middle Fraser-Fraser Canyon_SP_1.3	CK-08	DD	DU07	Endangered	--
Southern Mainland-Georgia Strait_FA_0.x	CK-20	DD	DU18	--	TBD
East Vancouver Island-Nanaimo_SP_1.x	CK-23	DD	DU19	Endangered	--
Southern Mainland-Southern Fjords_FA_0.x	CK-28	DD	DU22	--	TBD
Homathko_SU_x.x	CK-34	DD	DU27	DD	--
Klinkaklini_SU_1.3	CK-35	DD	DU28	DD	--
Upper Adams River_SU_x.x	CK-82	DD	--	--	--
Boundary Bay_FA_0.3	CK-02	TBD	DU01	--	TBD
Maria Slough_SU_0.3	CK-07	TBD	DU06	--	TBD
Vancouver Island-Georgia Strait_SU_0.3	CK-83	TBD	DU20	--	TBD
East Vancouver Island-Goldstream_FA_0.x	CK-21	TBD	DU21	--	TBD
East Vancouver Island-Cowichan & Koksilah_FA_0.x	CK-22	TBD			
East Vancouver Island-Nanaimo & Chemainus_FA_0.x	CK-25	TBD			
East Vancouver Island-Qualicum & Puntledge_FA_0.x	CK-27	TBD			
West Vancouver Island-North_FA_0.x	CK-33	TBD	DU26	--	TBD
Fraser-Harrison fall transplant_FA_0.3	CK-9008	TBD	--	--	--

**CK-01 has been assessed by COSEWIC as a single DU under a separate process. The last assessment date for this DU was April 2017.*

Interior Fraser Coho WSP and COSEWIC statuses

There are five B.C. Interior Fraser Coho CUs that were assessed by DFO in 2015 (DFO 2015). There are currently three Amber and two Amber/Green CUs. COSEWIC has grouped these five CUs into one DU and assessed it's status as Threatened.

Table A2-5: The 2015 Integrated status designations for ***the five Interior Fraser Coho CUs****. The COSEWIC 2017 status designation groups these five CUs into a single DU and has assessed this DU as Threatened (released 2016).*

CU Name	***WSP 2016***	**DU**	***COSEWIC 2019***
Middle Fraser	***Amber***	**Interior Fraser Coho**	***Threatened***
Fraser Canyon	***Amber***		
Lower Thompson	***Amber***		
North Thompson	***Amber***		
South Thompson	***Amber***		

Environmental Science & Technology LETTERS

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Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance

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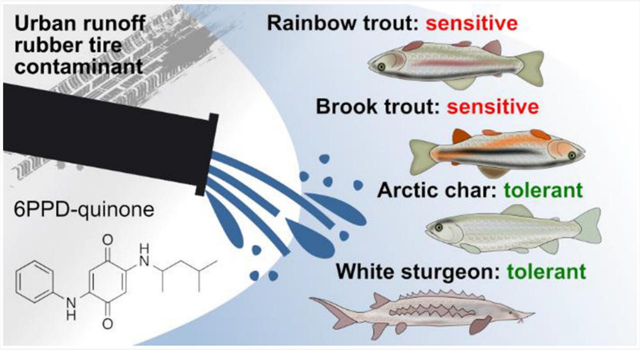
ABSTRACT: *N*-(1,3-Dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone), a transformation product of the rubber tire antioxidant 6PPD, has recently been identified as the chemical responsible for urban runoff mortality syndrome in coho salmon, with a median lethal concentration (LC_{50}) of <0.1 μg/L. Subsequent studies have failed to confirm comparable sensitivity in other fish species. Here, we investigated the acute toxicity of 6PPD-quinone to rainbow trout, brook trout, Arctic char, and white sturgeon. Fish were exposed under static renewal conditions, and exposure concentrations were verified analytically. Mortalities in brook trout occurred between 1.2 and 20 h, while mortalities began after 7 h and spanned 60 h in rainbow trout. The LC_{50}s in brook trout (24 h) and rainbow trout (72 h) were 0.59 and 1.00 μg/L, respectively. Both species showed characteristic symptoms (increased ventilation, gasping, spiraling, and loss of equilibrium) shortly before death. No mortalities were observed after exposure of either char or sturgeon for 96 h at measured concentrations as high as 14.2 μg/L. This is the first study to demonstrate the acute toxicity of 6PPD-quinone to other fishes of commercial, cultural, and ecological importance at environmentally relevant concentrations and provides urgently needed information for environmental risk assessments of this contaminant of emerging concern.



■ INTRODUCTION

Stormwater runoff from urban landscapes has long been a cause for environmental concern due to its chemical complexity, toxicity to aquatic organisms, and temporal and spatial dynamics.[1] In addition to road salt, organic contaminants from vehicle emissions and leakage, and toxic metals from brake pad abrasion,[2] tire wear particles (TWPs) have recently become the focus of scientific and public interest.[3] Earlier research into the causes of fish kills following rainfall events along the west coast of the United States, termed coho salmon (*Oncorhynchus kisutch*) urban runoff mortality syndrome (URMS), suggested that rubber tire-derived chemicals might be responsible for this effect because they co-occurred with these mortality events.[4] In a landmark study, Tian et al.[5] applied a combination of fractionation, chemical analysis, and biological testing to pinpoint the causative chemical. The authors found that *N*-(1,3-dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone) was generated through the environmental oxidation of the common rubber tire antidegradant 6PPD and can cause lethality in coho salmon at a median lethal concentration (LC_{50}) of <0.8 μg/L. Using a commercial standard, a revised LC_{50} in coho salmon of <0.10 μg/L was reported in a follow-up study.[6] Tian et al.[5,6] and subsequent studies have demonstrated the widespread occurrence of 6PPD-quinone in stormwater runoff and surface waters at concentrations of ≤19 μg/L,[7,8] indicating that 6PPD-quinone exposure poses an immediate risk to coho salmon populations. However, it was unknown whether exposure to this pollutant would also affect other aquatic species.

Two follow-up studies have determined the acute toxicity of 6PPD-quinone to a variety of species, including fish (zebrafish, *Danio rerio*; Japanese medaka, *Oryzias latipes*) and invertebrates (*Daphnia magna* and *Hyalella azteca*).[9,10] All tested species were significantly less sensitive than coho salmon: exposure to 6PPD-quinone did not cause lethality in any of the four species studied by Hiki et al.[10] up to concentrations as high as the maximum water solubility, which the authors estimated to range between 34 and 54 μg/L. Varshney et al.[9] observed an LC_{50} of 309 μg/L for zebrafish larvae when ethanol was used as the solvent vehicle. Because of the

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alarmingly high sensitivity of coho salmon to 6PPD-quinone, environmental risk assessors urgently require data on the acute toxicity of 6PPD-quinone across a greater diversity of fish species, with an emphasis on additional salmonid species.

This study investigated the acute toxicity of 6PPD-quinone across four species of commercial, cultural, and ecological importance to North America: rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), Arctic char (*Salvelinus alpinus*), and white sturgeon (*Acipenser transmontanus*). Additionally, rainbow trout are an important model fish species used in chemical risk assessment across many jurisdictions.[11,12] This research provides important information for the environmental risk assessment of urban runoff and has the potential to inform regulatory controls of the use of 6PPD in rubber tires.

■ MATERIALS AND METHODS

Chemicals and Reagents. Native and mass-labeled (d_5) 6PPD-quinone were purchased from Toronto Research Chemicals (Toronto, ON). Stock solutions for exposure of fish to 6PPD-quinone were prepared using dimethyl sulfoxide (DMSO) to achieve a final solvent concentration of 0.01% (v/v) during exposures. Analytical standard solutions of native and mass-labeled 6PPD-quinone were prepared in HPLC-grade methanol.

Fish Source and Culture. Brook trout were from Allison Creek Trout Hatchery (Coleman, AB), were ~1 year old, were 17.1 ± 1.1 cm in length, and weighed 52.8 ± 7.6 g. Fish were housed in the Aquatic Research Facility (ARF) at the University of Lethbridge and acclimated in 150 L inert glass-fiber Krescel tanks (four fish per tank, 30% daily water renewal) for 2 weeks prior to exposures. Fish were fed a commercial salmonid feed at a daily rate of 1% of body weight during acclimation. Studies were approved by the University of Lethbridge Animal Welfare Committee (Protocol 2111).

Rainbow trout (from Lyndon Hatcheries, New Dundee, ON), Arctic char (from Miracle Springs Inc., North Vancouver, BC), and white sturgeon (wild fish spawned at the Nechako White Sturgeon Conservation Centre, Vanderhoof, BC) were from in-house cultures raised from embryos in the Aquatic Toxicology Research Facility (ATRF) at the University of Saskatchewan. Fish were cultured under flow-through conditions in facility water until they reached the juvenile stage (rainbow trout, ~2 years, 19.6 ± 1.9 cm, 97.5 ± 28.9 g; Arctic char, ~3 years, 13.8 ± 1.7 cm, 28.3 ± 9.8 g; white sturgeon, ~4.5 years, 42.4 ± 4.5 cm, 462.3 ± 159.3 g) and fed with a commercial fish feed at a daily rate of 1% of body weight during acclimation. Even though fish were somewhat larger than recommended according to various guidelines for acute toxicity tests, all fish were sub-adult and the larger size was selected due to availability considerations and to provide sufficient tissues for downstream analyses. Experiments were approved by the University of Saskatchewan Animal Care Committee (Protocol 20070049). A Species at Risk Act (SARA) permit for culture of and experimentation with white sturgeon was obtained from the Department of Fisheries and Oceans Canada (Permit 20-PPAC-00026).

Exposure Experiments. Pilot studies were conducted for each species to establish upper concentration bounds for acute lethality studies. For brook trout, fish were fasted for 24 h, moved to aerated 45 L rectangular glass tanks (two fish per tank) at 10 °C, and exposed for 24 h to nominal concentrations of 0, 0.02, 0.2, 2, or 20 μg/L 6PPD-quinone under static conditions (10 fish total). For the other species, two fish per species were each exposed under static conditions in individual tanks at either 6 or 20 μg/L (two fish total per species). Brook trout and rainbow trout became moribund at 2 and 6 μg/L, respectively, within 4 h of the onset of exposure. Arctic char and white sturgeon did not show any response to concentrations as high as 20 μg/L within 96 h.

Accordingly, in the main experiment, brook trout and rainbow trout were exposed to nominal concentrations of 6PPD-quinone ranging from 0.1 to 6 μg/L (see Table S1 for details). Tanks were cleaned with a series of detergents, disinfectants, and/or ethanol, carefully rinsed, and left to dry before experiments. Due to their lower sensitivity, Arctic char and white sturgeon were exposed to only one nominal concentration (20 μg/L) that could be achieved using the limited amount of chemical available and that was nearing water solubility, while still being environmentally relevant.[5,7,8] Exposures of brook trout were performed in 150 L inert glass-fiber Krescel tanks at 10 ± 1 °C for 24 h (two replicate tanks with four fish each; two controls at five concentrations, 56 fish total). A shorter exposure period was chosen for brook trout due to a much faster onset of symptoms compared to rainbow trout. Test solutions were continuously aerated, recirculated, and temperature controlled. Rainbow trout, white sturgeon, and Arctic char were exposed in 700 L glass-fiber Min-o-Cool tanks containing 500, 500, and 300 L of test solution, respectively, at 12 ± 1 °C for 96 h under static renewal conditions. Water was exchanged at 40−60% (white sturgeon) or 75% (rainbow trout and Arctic char) daily (two replicate tanks and one extra control replicate with five fish each for rainbow trout, 65 fish total; two replicate tanks with five fish each for Arctic char, 20 fish total; three replicate tanks with two fish each for white sturgeon, 12 fish total). Control tanks were dosed with the DMSO solvent vehicle at the same level as all other tanks [0.01% (v/v)]. Average (±SD) water quality parameters were as follows for brook trout: temperature, 10.3 ± 0.7 °C; pH, 6.74 ± 0.13; DO, 99.8 ± 11.5%; ammonia, 0.13 ± 0.11 mg/L; hardness, 131 ± 2.33 mg/L. Average (±SD) water quality parameters were as follows for other species: temperature, 12.8 ± 0.8 °C; pH, 8.35 ± 0.45; DO, 92.8 ± 13.2%; ammonia, 0.14 ± 0.15 mg/L; hardness, 132 ± 6.80 mg/L. Water samples were collected for analytical confirmation of concentrations of 6PPD-quinone ~1 h after the initial dosing of tanks, which occurred after acclimation of fish for 48−96 h. For rainbow trout, Arctic char, and white sturgeon, a water sample was also taken every 24 h prior to water changes or after most fish in a tank became moribund. Samples were immediately spiked at 50 μg/L with 6PPD-quinone-d_5 and stored at −20 °C until they were analyzed. Fish were observed during most of the exposure duration, immediately removed once they became moribund, and humanely euthanized using >250 mg/L buffered MS-222. Characteristic signs of 6PPD-quinone exposure leading to brook trout and rainbow trout becoming moribund (increased ventilation rate, gasping on the water surface, permanent loss of equilibrium, and spiraling motion) were observed during regular tank inspections and noted and would have resulted in death within 0.5 h if fish were not euthanized.

Biological Sampling. The fork length (millimeters) and weight (grams) of each fish were determined after euthanasia. Blood samples were obtained from the caudal vein using heparinized syringes, and blood glucose concentrations determined using hand-held meters (brook trout, OneTouch

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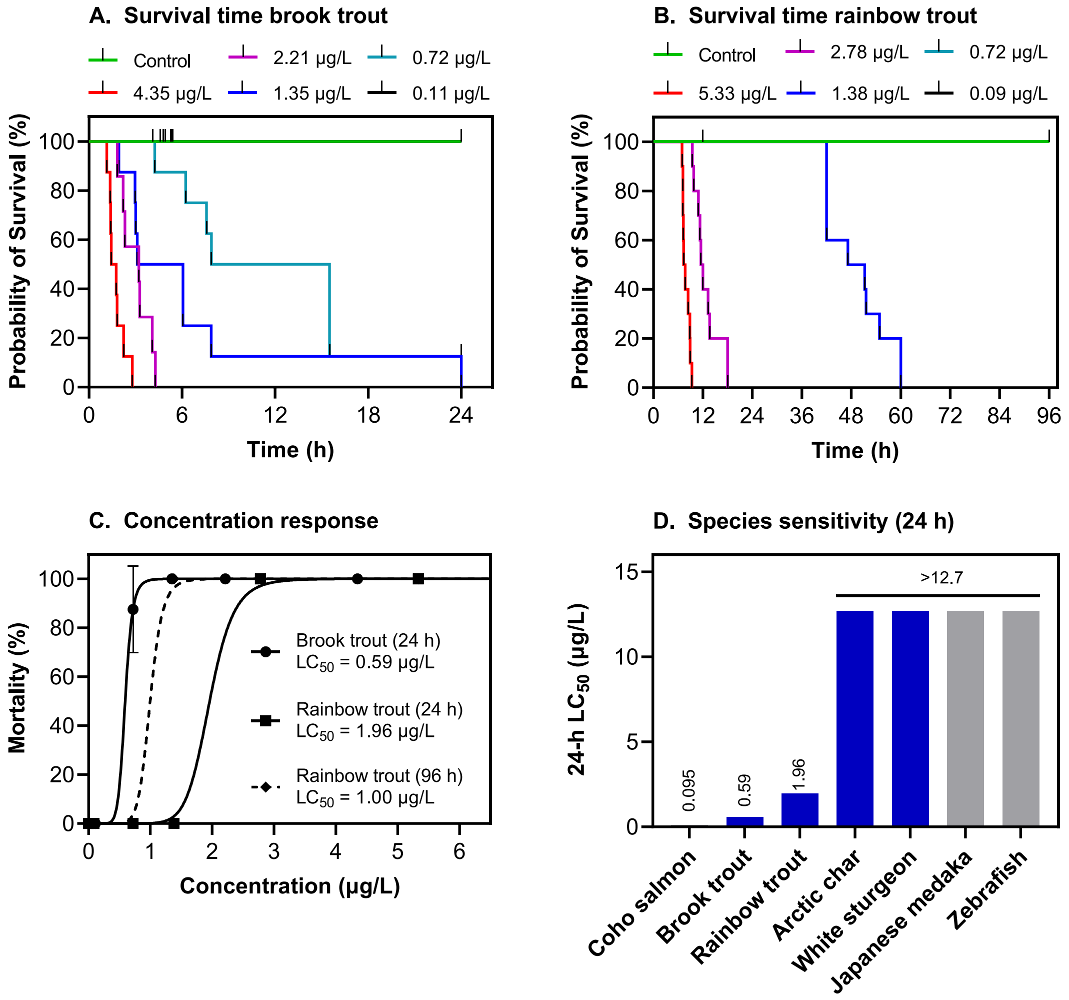

Figure 1. Relationships among exposure time, exposure concentration, and survival in (A) brook trout and (B) rainbow trout over exposure durations of 24 and 96 h, respectively. Median lethal concentrations at 24 and 96 h of exposure were interpolated for both species using (C) two-parameter logistic regression and (D) compared with those of other previously studied species. All concentrations are based on measured concentrations. Blue bars in panel D are from this study, while values for coho salmon, Japanese medaka, and zebrafish have been previously published.[6,9,10]

Ultra 2 m, LifeScan, Malvern, PA; all other species, Contour Next meter, Ascensia, Basel, Switzerland). The percent hematocrit was determined in brook trout using a StatSpin CritSpin microhematocrit centrifuge (StatSpin, Norwood, MA).

Analytical Chemistry. Instrumental verification of exposure concentrations of 6PPD-quinone followed the method outlined by Challis et al.[7] with modifications. Briefly, samples were analyzed on a Vanquish UHPLC instrument coupled with a Q-Exactive HF Quadrupole-Orbitrap hybrid mass spectrometer (Thermo-Fisher). An isotope dilution strategy using 6PPD-quinone-d_5 was applied for quantification. Average measured exposure concentrations were calculated and used for subsequent data analysis instead of nominal exposure concentrations. A detailed description of the analytical methods is provided in the Supporting Information.

Data Analysis and Statistics. The percent mortality for each concentration and replicate was calculated at 24 h for brook trout and at 24 and 96 h for rainbow trout to account for differences in time to death between both species. LC_{50}s were interpolated for each time point using logistic regression of the percent mortality versus average measured exposure concentrations. Blood glucose measurements were analyzed for normality and heteroscedasticity using Kolmogorov−Smirnov's test and Levene's test, respectively. Because the data sets violated the assumptions for one-way analysis of variance (ANOVA), a nonparametric Kruskal−Wallis's test with Dunn's post hoc test was performed. A p value of ≤ 0.05 was considered indicative of statistically significant differences. All plots were created and statistically analyzed using Prism 9 (GraphPad, La Jolla, CA).

RESULTS AND DISCUSSION

Analytical Verification of Exposure Concentrations. Average concentrations of 6PPD-quinone measured over the exposure periods deviated <16% from nominal values across all species with the exception of the low-treatment groups for brook trout and rainbow trout (Table S1). There was an average loss of 14% (1.7% and 32% in the high- and low-treatment groups, respectively) of the test chemical over the 24 h window between water changes, suggesting exposure levels were stable throughout the experiments. Losses were slightly greater at the higher exposure concentrations used for Arctic char and white sturgeon. Hiki et al.[10] reported a 17−73% decrease in 6PPD-quinone concentrations over 48 h between water changes for zebrafish and medaka, confirming the

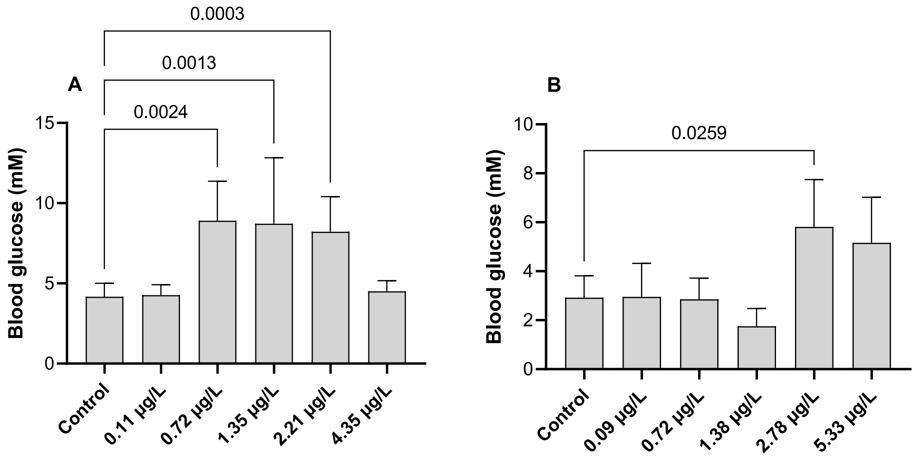

Figure 2. Blood glucose concentrations in (A) brook trout and (B) rainbow trout in moribund animals and those that survived until termination of the experiment following exposure to graded nominal concentrations of 6PPD-quinone. Bars depict the mean, and error bars the standard deviation of 4−15 fish per treatment and species, rather than tank replicates. Because control fish taken down at different sampling times did not differ significantly, individuals were pooled in a single control group for this analysis. Concentrations are based on measured exposure concentrations. Numbers above the brackets indicate the *p* value of statistical comparisons of blood glucose concentrations in 6PPD-quinone-exposed fish with those of the control group (Kruskal−Wallis ANOVA on ranks with Dunn's post hoc test).

relative stability of this chemical under static renewal conditions.

Acute Toxicity of 6PPD-quinone in Fish. Exposure to 6PPD-quinone resulted in significant acute effects in two of the four tested species, which varied as a function of chemical concentration, exposure time, and species (Figure 1). Brook trout were most sensitive with 100% of mortalities in the high-treatment group occurring within 3 h of exposure and a 24 h LC_{50} of 0.59 μg/L [95% confidence interval (CI) of 0.48−0.63 μg/L], which is similar to previous observations in coho salmon.[5] A slightly greater LC_{50} of 1.00 μg/L (95% CI of 0.95−1.05 μg/L) was recorded for rainbow trout after 72−96 h (1.96 μg/L after 24 h, 95% CI of 1.86−2.06 μg/L), while no mortalities were observed for either Arctic char or white sturgeon at measured concentrations as high as 14.2 and 12.7 μg/L after 96 h. Interestingly, in rainbow trout, the first signs of morbidity did not manifest until 7 h after commencing exposures and maximum mortalities occurred at 60 h, which was significantly longer than the times for brook trout and coho salmon.[5] The LC_{50} values reported here for brook trout (0.59 μg/L) and rainbow trout (1.00 μg/L) were ∼6-10-fold greater than that of coho salmon (0.10 μg/L) and are well within ranges of environmental concentrations of 6PPD-quinone previously reported in Canadian and U.S. surface waters after stormwater runoff events.[5−8] While no mortality of endangered white sturgeon or Arctic char was observed after exposure to 6PPD-quinone, potential subchronic or chronic impacts have not been fully studied and cannot be excluded at this time.

These results support earlier reports that identified marked differences in the sensitivity of fishes to exposure with 6PPD-quinone and TWP leachates.[3,5,10] Previous studies have hypothesized that sensitivity to 6PPD-quinone may be unique to salmonids.[9] These authors, who assessed the acute toxicity of this chemical to Japanese medaka and zebrafish, did not observe any significant mortalities up to the limit of the water solubility of 6PPD-quinone, which was estimated to range between 34 and 54 μg/L. While this is in accordance with the lack of effects reported in white sturgeon in this study, our results for Arctic char as well as those reported for TWP leachates by McIntyre et al.[3,5] for chum salmon (*Oncorhynchus keta*) clearly demonstrate the tolerance of these two salmonid species. Thus, we can conclude that sensitivity to acute exposure with 6PPD-quinone is highly variable among fishes in general, and salmonids specifically, even among species from the same genus such as brook trout and Arctic char representing the genus *Salvelinus*, and rainbow trout, chum salmon, and coho salmon representing the genus *Oncorhynchus*.

In cases in which mortalities occurred, both brook trout and rainbow trout exhibited behaviors consistent with those observed in coho salmon,[3,5] including hovering close to the water surface, accelerated opercular movements, gasping, and spiraling motion. This is in accordance with the hypothesis by McIntyre et al.[3] and Varshney et al.[9] that these types of behavior are suggestive of 6PPD-quinone causing cardio-respiratory distress. A significant increase in blood glucose concentrations observed at 0.72−2.21 μg/L in brook trout and 2.78 μg/L in rainbow trout (Figure 2) indicates that 6PPD-quinone impacted energy metabolism, although the underlying mechanisms for this increase are currently unclear. Additionally, hematocrit of brook trout exposed to 0.72−4.35 μg/L 6PPD-quinone significantly increased from an average of 42% in the control group to 68% at 4.35 μg/L (Figure S4). This agrees with observations by Blair et al.,[13] who found even more pronounced increases in hematocrit in coho salmon following exposure to urban runoff. The authors also provided evidence of the disruption of the blood−brain barrier in exposed fish, which might be one of the reasons for the observed increases in hematocrit. However, it is currently unclear if this is the key event ultimately responsible for causing death or if other processes are involved.

Environmental Implications and Risk Assessment. Salmonids are of significant ecological, commercial, and recreational importance in many countries around the world, and this study highlights that the acute toxicity of 6PPD-quinone previously reported for coho salmon[3,5] is also of significant concern for other key receptors, including rainbow trout and brook trout. While there have only been a limited number of studies that characterized the presence of 6PPD-quinone in surface waters and urban runoff,[7,8] available reports clearly highlight that commonly found concentrations of this emerging contaminant exceed toxicity thresholds reported here and by Tian et al.[5] Hence, 6PPD-quinone appears to pose a significant and widespread ecological risk to these species, and potentially other salmonids, especially downstream of urban areas and in smaller water bodies receiving roadway runoff.[5,7,8] However, other ecologically relevant genera and families of fishes have not been studied to date, which represents an important uncertainty at this point.

The observed differences in the temporal dynamics of time to death among the three species for which acute effects of 6PPD-quinone have been observed to date are interesting. While in coho salmon[5] and brook trout morbidities at the greatest concentrations were observed as early as 1−2 h after initiation of exposure, in rainbow trout the first mortalities did not occur until ∼7 h at comparable concentrations. As exposure conditions were comparable among experiments in terms of temperature (10−13 °C), pH (6.7−8.3), and DO (>90% saturation), it is unlikely that these parameters would have been a driving factor. Despite the similar LC_{50} values observed for all three species, these differences may have significant implications for ecological risk assessment of urban runoff events. The shorter time to death for coho salmon and brook trout may increase their risk of mortality prior to dilution of stormwater in receiving water bodies over time after rain events.

Future Research Needs. For more comprehensive future risk assessments of 6PPD-quinone in aquatic ecosystems, it is imperative to study its acute and sublethal effects in a broad range of fish species. More research into the potential respiratory or cardiovascular mechanisms of action is needed to conclusively and comprehensively elucidate the specific mechanism by which 6PPD-quinone triggers URMS in select salmonids and possibly other fishes. Most importantly, drivers of species differences in sensitivity need to be studied; i.e., why are some salmonids more sensitive than others? Several native salmonid fish species (e.g., cutthroat trout, *Oncorhynchus clarkii*; bull trout, *Salvelinus confluentus*)[14−16] are at risk of extinction in parts of their native range, and the contribution of 6PPD-quinone to their stock status needs to be urgently investigated.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.2c00050.

Additional details of the chemical analytical methods (Text S1) and results (Table S1 and Figures S1 and S2) and results of hematocrit measurements in brook trout (Figure S3) (PDF)

Video of characteristic symptoms of brook trout exposed to 6PPD-quinone, here loss of equilibrium and spiraling (MOV)

Video of characteristic symptoms of rainbow trout exposed to 6PPD-quinone, here gasping (MOV)

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Notes

The authors declare no competing financial interest.

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Review

Environmental Occurrence and Toxicity of 6PPD Quinone, an Emerging Tire Rubber-Derived Chemical: A Review

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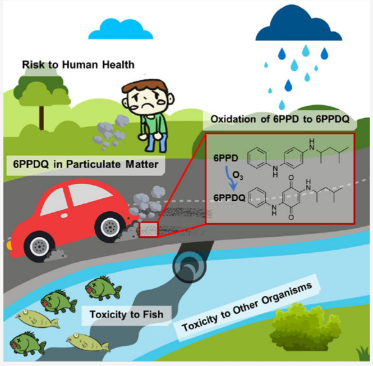
ABSTRACT: *N*-(1,3-Dimethylbutyl)-*N*′-phenyl-p-phenylenediamine (6PPD) is a chemical added to tires to prevent their oxidative degradation. 6PPD is highly reactive with ozone and oxygen, leading to the formation of transformation products such as 6PPD quinone (6PPDQ) on the tire surfaces and, subsequently, in tire and road wear particles. 6PPDQ is a toxicant that has been found in roadway runoff and receiving water systems. Its presence in municipal stormwater has led to the acute mortality of coho salmon during their migration to urban creeks to reproduce, generating global interest in studying its occurrence and toxicity in the environment. This review aims to provide a critical overview of the current state of knowledge of 6PPDQ, assisting researchers and policymakers in understanding the potential impacts of this emerging chemical on the environment and human health. As there are many unanswered questions surrounding 6PPDQ, further research is needed. This review highlights the importance of including transformation products in regulations for 6PPD, as well as all emerging synthetic chemicals of concern.



KEYWORDS: *6PPD, 6PPDQ, Transformation Product, Stormwater, Toxicity, Rubber Tires*

1. INTRODUCTION

Over the years, the production of synthetic chemicals for various applications has surged, but their environmental consequences remain a concern.[1] *N*,*N*′-Disubstituted phenylenediamines (PPDs) are one such class of compounds added to tires to prevent rubber from undergoing oxidative degradation, thereby preserving its structure and function.[2] PPDs are highly reactive with ozone and oxygen resulting in transformation products (TPs) on the tire surfaces and, subsequently, in tire and road wear particles (TRWPs).[3] One commonly used PPD is *N*-phenyl-*N*′-(1,3-dimethylbutyl)-p-phenylenediamine (6PPD).

Initial research in 1983 investigating the reaction of 6PPD with ozone identified five TPs with higher molecular weights and an additional five with lower molecular weights than 6PPD.[4] One of the TPs was previously misidentified as a nitrone,[3,4] but it has since been confirmed to be 6PPD quinone.[5] The newly discovered toxicant, known as 6PPDQ, has been identified as a widespread pollutant in roadway runoff and receiving water bodies, resulting in the acute mortality of coho salmon before spawning in freshwater streams.[5] This phenomenon, known as the "urban runoff mortality syndrome (URMS)", has sparked global interest in studying the occurrence and toxicity of 6PPDQ in the environment. It is imperative to emphasize that the implications of 6PPDQ exposure extend beyond the acute mortality observed in coho salmon. While initial studies have primarily focused on its effects on specific salmon species, recent research has shed light on the broader environmental and health concerns associated with this emerging chemical. For instance, human exposure to 6PPDQ can occur through various routes such as inhalation of fine particles,[6,7] dietary intake,[5,8] and consumption of contaminated water,[9] raising concerns about potential adverse effects on human health. Additionally, its detection in human urine samples underscores the urgent need to address the health risks associated with exposure to these substances.[10] Since the initial report on its toxicity in December 2020,[5] extensive global research has been conducted worldwide to investigate the effects of 6PPDQ on aquatic life (14 studies), human health (five studies), and plants (one study). In addition, 14 studies have reported the presence of 6PPDQ in various cities and water systems around the world, and eight studies have reported its occurrence in air.

Given the numerous unanswered questions surrounding the fate and transport of this emerging chemical, further research is expected to be conducted worldwide in the coming years. Current knowledge gaps include the need for investigation of the 6PPDQ behavior in different environments, factors impacting persistence and degradation, and transformation

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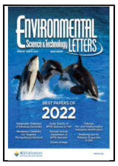

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mechanisms. Assessing ecological effects on organisms and ecosystems, potential cascading effects, health risks, toxicological effects, and vulnerable populations is also important. While a recent review[11] provided comprehensive coverage of analytical methods and environmental occurrences of 6PPD/6PPDQ, the current review brings a unique perspective by focusing on the environmental transformation of 6PPD to 6PPDQ and the potential environmental impacts of 6PPDQ. Therefore, this review aims to provide a critical overview of the current knowledge on 6PPDQ (and 6PPD), aiding researchers and policymakers in the understanding of the potential impacts of this emerging chemical on the environment and human health.

2. 6PPDQ IN THE ENVIRONMENT

Several studies have reported the presence of TPs in rubber antioxidants under environmental conditions. The widely used amine antioxidants, such as 6PPD, have been found to react with ozone and produce PPD-quinones.[12,13] 6PPD is typically used in the rubber industry at concentrations ranging from 0.4% to 2% by mass in tire manufacturing.[14] Once incorporated into rubber materials, 6PPD slowly migrates to the surfaces of the tires, where it becomes accessible for gas-phase reactions. These reactions are responsible for the antioxidant function of 6PPD which protects the rubber from oxidative damage. However, as 6PPD continues to diffuse to the tire surface it also becomes susceptible to environmental release.[15] The transportation of 6PPD can be influenced by different factors that are inherent in the rubber structure and its associated microenvironments. However, it is important to note that all 6PPD added to tires is ultimately intended to react with oxidants such as ozone leading to the formation of TPs such as 6PPDQ.[16]

Previous evaluations of the 6PPD transformation through oxidant exposure have predominantly centered on its industrial utilization in rubber formulations, neglecting its relevance in environmental systems and employing less comprehensive analytical methodologies. However, recent studies have confirmed that the reaction of tire rubber antioxidants with ozone results in the formation and release of various TPs into the environment. For example, lab-scale reactions demonstrated the formation of 38 TPs from the reaction of solid and aqueous 6PPD with ozone gas.[3] The transformation pathway involves the oxidation of 6PPD to benzoquinone imine, which subsequently forms 6PPDQ as a major product through oxygen additions. Further degradation of 6PPDQ occurred in the presence of oxidants resulting in the detection of 12 additional TPs.[3] Time−concentration profiles of 6PPD and 6PPDQ have been examined under different thermo and/or photoaging conditions.[17] Overall, the decay of 6PPD followed an exponential pattern with shorter half-lives observed during photoaging compared to thermal aging. The 6PPDQ concentration profiles within TRWPs exhibited distinct patterns under natural aging conditions. Thermal aging induced a slower decay of 6PPDQ within TRWPs, indicating greater stability and persistence.[17] Another study identified 19 probable 6PPD-derived TPs in ozonated 6PPD and TRWPs, highlighting the widespread presence of these TPs in roadway-impacted air, soil, sediment, and water.[18] Additional information regarding the TPs of 6PPD can be found in Supporting Information 2: Transformation products of 6PPD. Recent research has advanced our understanding of 6PPD transformation and its resulting TPs. However, several key research gaps need attention. Investigating rate constants and 6PPDQ yield across varying temperatures is pivotal for understanding environmental fate. Additionally, exploring the influence of different environmental conditions, such as exposure to light, and co-occurring pollutants, on 6PPD transformation pathways can provide deeper insights. Addressing these gaps will refine our assessment of the environmental impact of 6PPD and implications of its TPs.

The amount of 6PPDQ generated from tire tread wear in the USA is estimated to fall between 26 and 1,900 tons per year.[13] It is postulated that water gradually removes 6PPDQ from exposed and intact rubber surfaces, resulting in its continuous release throughout the material lifespan until 6PPD is completely exhausted.[13] Additionally, recent studies indicated the presence of 6PPDQ in ambient air $PM_{2.5}$ particles and roadside soil that was attributed to vehicle rubber tire abrasion.[1,19] According to estimated C_w^{sat} and log K_{ow} values, the neutral form of 6PPDQ is expected to be more easily leached or transported than the neutral form of 6PPD.[13] However, at neutral pH 6PPD (estimated pK_a of 6.46) is expected to exist mainly in its cationic form (more soluble), whereas 6PPDQ is expected to be predominantly in its neutral form due to its estimated pK_a value of −4.02.[13] Recent measurements indicated that the C_w^{sat} value for 6PPDQ is 38 ± 10 μg/L,[20] which is significantly lower than the value of 1 mg/L reported for 6PPD.[13] However, the C_w^{sat} value for 6PPDQ is still around 900-times higher than the LC_{50} value of 95 ng/L for coho salmon.[21,22]

The following section provides an overview of recent studies on the occurrence of 6PPDQ in environmental media, including water, air/dust, and others, with a compilation of detected concentration ranges provided in Supporting Information: Occurrence of 6PPDQ was observed in water, air/dust, and other media (Table S1).

2.1. Occurrence in Water.

6PPD and its transformation product 6PPDQ are released into urban water systems, including surface runoff, receiving surface waters, drinking water treatment plants (DWTPs), and wastewater treatment plants (WWTPs).[23] Among the different water matrices, the highest 6PPDQ concentrations were observed in road surface runoff samples (2.43 μg/L), while no concentration was detected in the DWTP effluents (Supporting Information: Occurrence of 6PPDQ in water, air/dust, and other media (Table S1)). The fate of 6PPDQ in different water systems is discussed below.

6PPDQ was detected in all surface runoff samples taken from an urban traffic area in Hong Kong, China.[1] Among the five investigated PPD compounds and their quinone derivatives, 6PPDQ was dominant (48.8%) in the samples. Similarly, 6PPDQ was observed in 100% of the surface runoff samples taken from roads, courtyards, and farmland in Dongguan and Huizhou cities, China, while the detection frequency of 6PPD was 0 to 41.7%.[23] Furthermore, the 6PPDQ concentrations were considerably higher than 6PPD. Similarly, while 6PPD was not detected in road runoff water samples taken in Paris, France, 6PPDQ was present in most samples.[24] Also, 6PPDQ was found to be more stable than 6PPD in ultrapure water containing 10% v/v methanol. Moreover, recent stability experiments indicated that 6PPDQ remains fairly stable over 14 days but experiences around 26% loss over 47 days in deionized water systems.[20] The study's sorption results suggest using glass for lab operations and storage, chemically inert plastics like PTFE and FEP for short-term contact, and being cautious with rubber and silicon materials due to strong sorption of 6PPDQ.[20] These highlight the need for careful consideration during sample pretreatment, as the measurement of 6PPD and its quinone concentrations in water samples may underestimate their actual concentrations.[9] Likewise, oxidation was observed instantly after making an aqueous 6PPD solution in contact with air.[3] These results indicate that the degradation of tire additives is a crucial pathway in determining their environmental fate, further complicating the situation with respect to tire additives and related compounds found on roads.[23,25] The ecological risk quotient (RQ) of 6PPDQ was the highest among 16 compounds investigated in the Dongjiang and Zhujiang rivers in China.[23] Similarly, the estimated RQ of 6PPDQ was the highest among eight investigated chemicals in groundwater, surface water, and stormwater in Guangzhou, China.[9]

In a temporal study during storm events, the concentration of 6PPDQ in Don River (Ontario, Canada) samples reached 2.30 μg/L after a lag time of 17−20 h from the start of the rain. Interestingly, concentrations remained constant at this peak level for 12−18 h.[26] Samples collected during a COVID-19 lockdown period in this study showed a lower 6PPDQ concentration compared to samples collected before the lockdown, likely due to reduced vehicular traffic and subsequently reduced tire wear.[26] In another temporal study conducted in the Brisbane River of Australia, complex concentration patterns were observed during the individual storms. The concentrations peaked after a storm in June, while in October, the concentrations peaked at the beginning or middle of the storm. This was attributed to several factors such as rainfall duration, the water level in the river, and soil saturation. A 6PPDQ mass loading of 3 g/storm was estimated in this study.[27]

In urban settings, it is typical for snow to accumulate particles produced on roads during the period between snowfall and snowmelt. Snow samples collected from urban roads in Leipzig, Germany, revealed the presence of 32 TPs of 6PPD, including 6PPDQ.[3] While 6PPDQ is only one of the 6PPD TPs, the detection of nine 6PPDQ TPs shows that 6PPDQ is not a dead-end product. The majority of 6PPD and its TPs were found in the particulate phase rather than in the water phase of snow samples, indicating that they are stabilized by their embedding into tire wear particles.[3] Therefore, controlling the particle emission from road runoff could effectively limit their release (e.g., 6PPDQ) into the receiving waters. Moreover, it is important to analyze both the water and particulate phases of discharges containing particles to accurately measure the quantities of 6PPD/6PPDQ transferred to surface waters. A study conducted in Saskatoon, Canada, found that concentrations of 6PPDQ were higher in stormwater samples than in snowmelt samples.[28] The estimated 6PPDQ mass loading was 1.7−384 g/storm during a heavy rainfall in Saskatoon,[28] which was comparable with 34−416 g/storm reported for Toronto, Canada.[29]

Surface runoff caused by rainfall is a significant source of pollution in receiving rivers. Additionally, in some instances or combined sewer systems, this runoff is directed to WWTPs with effluents becoming a significant point source.[3,23] Moreover, it has been suggested that TRWPs are transported via stormwater overflows into sewer systems, where WWTPs act as sinks for these pollutants and may subsequently release them into receiving waters over time.[3,30] Typically, PPDs are not efficiently removed by WWTPs, which means these chemicals end up being released into water bodies that receive effluents.[23,29,31] A study conducted in Sri Lanka and Malaysia investigated the concentrations of eight PPDs in five industrial, hospital, and municipal WWTPs. Among the investigated PPDs, only three chemicals, including 6PPDQ, were detected in the WWTPs influents and effluents.[31] Like prior research that found low or negligible concentrations of 6PPD in runoff water samples,[23,24] 6PPD was not found in either the influent or effluent of WWTPs.[31] The estimated RQ and no-effect concentration (PNEC) indicated that the 6PPDQ in the WWTP effluents presents a significant risk to aquatic ecosystems.[31] Similarly, no 6PPD was detected in influents and effluents of three WWTPs in Guangzhou, China, while the 6PPDQ removal rate of the plants was 85.3−100%.[23] The study also found that DWTP could eliminate the minute quantity (0.25 ng/L) of 6PPDQ present in the source water.[23]

High levels of 6PPDQ were detected in the influents of four WWTPs in Ontario, Canada, carrying mostly residential wastewater with no significant industrial contributions.[32] Two plants utilizing activated sludge, extended air sequencing batch reactor, tertiary sand filtration, and UV-disinfection processes were able to completely remove 6PPDQ. In contrast, two plants using activated sludge, extended air plug flow reactors, tertiary sand filtration, and UV-disinfection processes showed a significant increase in 6PPDQ concentration (up to 7-fold) in their effluents. The higher concentration of 6PPDQ in the effluent of the plants with extended air plug flow reactors was linked to the possible oxidation of 6PPD to 6PPDQ during treatment.[32] In this study, 6PPDQ was not detected in the influent and effluent of two DWTPs that receive their water supply from Lake Ontario.[32] A study conducted in Leipzig, Germany, examined the presence of 6PPD and its 32 TPs in the influent and effluent of a WWTP. The analysis was carried out over the course of a day, including periods of rainfall, snowmelt, and dry weather.[3] The results showed that 6PPD was detected only during snowmelt, while 6PPDQ was detected during both rainfall and snowmelt, with no detection during dry weather. This supports the theory that these chemicals originate from surface runoff and TRWPs rather than other wastewater sources like industry and residences.[3]

Overall, the presence of 6PPDQ in water systems highlights its widespread occurrence and the need for effective measures to limit its release. Controlling particle emissions from road runoff is crucial to preventing the introduction of 6PPDQ into receiving environments. Stormwater, which is often untreated, can contribute to the release of this chemical into waterbodies.

2.2. Occurrence in Air. Chemical accumulation in dust is a growing concern in indoor and outdoor environments as it poses a risk to human health through inhalation, ingestion, and skin contact.[33,34] Fine particulate matter can bind various organic and inorganic chemicals due to their large specific surface area.[35] The widespread presence of 6PPDQ in both indoor and outdoor atmospheres has been highlighted in recent studies.

6PPDQ was detected in atmospheric particles for 15 of 18 megacities with an average concentration of 0.847 pg/m^3.[36] Comparable concentrations of 6PPD and 6PPDQ were detected in air samples from various locations, including Taiyuan, Zhengzhou, and Guangzhou in China,[37,38] and Tokyo, Japan.[39] This finding indicates that the transformation of 6PPD to 6PPDQ is a widespread phenomenon that is influenced by common environmental factors. Samples from road dust, parking lot dust, and vehicles had a lower 6PPD/

6PPDQ ratio than house dust, suggesting a higher degree of ozonation in vehicle dust due to sunlight irradiation and increased temperatures.[6] Unexpectedly high concentrations of 6PPDQ were detected in e-waste dust, waste disposal areas, and enclosed environments such as vehicles, exceeding levels of their precursor 6PPD compounds.[19,40] Roadside dust samples were found to be an important source of exposure to 6PPDQ compared to indoor environments.[1,6] Environmental factors, such as the presence of ozone or elevated temperatures, can facilitate the conversion of 6PPD to 6PPDQ in dust samples, leading to increased environmental 6PPDQ.

The presence of 6PPDQ and 6PPD in samples collected from Hong Kong's roadsides was widespread indicating the significant impact of vehicular traffic.[1] The levels of 6PPDQ in road dust samples were found to be comparable in Tokyo, Japan,[39] and Guangzhou, China.[41] However, the distribution of PPDQs and PPDs in different cities may vary due to economic structure and topography. For instance, Guangzhou, which had higher traffic and tire wear levels, exhibited more significant concentrations of 6PPDQ than Taiyuan,[36] a valley basin city that is driven by mining and heavy industries but with lower traffic levels. Furthermore, a roadside site in Guangzhou displayed more PPDQ emissions than a campus building site suggesting that tire wear is a possible source in dust samples.[38]

It is crucial to consider seasonal fluctuations of PPDQs in air pollution across diverse environmental regions that exhibit seasonality. Observations of temporal and seasonal variations of 6PPDQ in two Chinese megacities showed differing patterns, with Guangzhou exhibiting a fluctuating trend and Taiyuan showing a clear peak in winter.[38] Positive linear relationships were found between 6PPDQ, photochemical species, and environmental oxidants in Chinese cities.[19] Elevated concentrations of normalized 6PPDQ levels were also observed in road dust in Japan during the late spring, attributed to higher atmospheric ozone concentrations. No seasonal trends were observed for 6PPD, which is likely due to its easy degradation at high temperatures or via photooxidation.[39] 6PPDQ has been widely detected in indoor dust samples including vehicles, bedrooms, and shopping malls from Guangzhou.[42] Other rubber-related products, such as clothes and furniture, could also be potential sources of 6PPDQ. Vehicle dust samples showed higher concentrations suggesting that solar radiation and high temperatures may accelerate the transformation of 6PPD.[42]

Research has shown that 6PPDQ is widely present in air in various locations, indicating the significant impact of rubber, tire wear, and vehicular traffic on its distribution. The high 6PPDQ concentrations found in e-waste dust, waste disposal areas, and enclosed environments raise concerns about the potential health risks associated with exposure to this emerging chemical. Additionally, the seasonal and temporal variations of 6PPDQ levels highlight the importance of considering the complex interactions among photochemical species, environmental oxidants, and atmospheric conditions in assessing its environmental fate.

The size distribution of dust particles is important for determining their mobility and pollutant concentration. The concentration of 6PPDQ increased with the proportion of fine particles (<75 μm) in road dust samples, consistent with prior findings demonstrating higher concentrations of 6PPD and 6PPDQ in fine particles (<100 μm) compared to coarse particles in tunnel dust.[39,43] Indoor parking lot dust had a higher proportion of fine particles (<53 μm) compared to road dust, with a higher proportion of medium-sized particles (53–250 μm).[41] Dust particle size distribution varied depending on environmental factors, and the presence of 6PPD and 6PPDQ in fine particulate matter was due to their origin in tire wear products found in the 6–120 μm size range.[41,44] The elevated presence of 6PPDQ in indoor parking can be attributed to the finer particulate matter compared to roadways.[6] The consistent composition of indoor parking dust particles mainly consisted of tire wear products and vehicle dust, while the composition of roadway dust particles was more complex. It encompassed tire wear products, naturally occurring particles originating from soil, anthropogenic particles originating from road construction materials, industrial sources, or atmospheric deposition.[1] It should be noted that the variation in the particle size distribution contributed to the potential for bias in experimental data collected in studies assessing the composition of particulate matter containing 6PPD and 6PPDQ.

Further research is needed to understand the fate and distribution of 6PPDQ under different environmental conditions such as varying temperatures and ozone levels. Investigation into the influence of other antioxidants and reactive chemical additives in rubbers on the transformation of 6PPD to 6PPDQ is also crucial. By focusing on these specific research areas, we can gain a deeper understanding of the sources, transport mechanisms, and transformation pathways of 6PPDQ can be gained. This knowledge will enable the development of targeted strategies to mitigate its environmental impact and safeguard human and environmental health.

2.3. Occurrence in Other Media. Although TRWPs, as one of the largest sources of microplastics, are the main contributors to terrestrial microplastics (45%),[45] there has been insufficient research on the presence of 6PPDQ in soil. In a study investigating the occurrence of rubber-derived quinones in air, water, and soil in Hong Kong, China, 6PPDQ was detected in roadside soil samples. The ratio of 6PPD/6PPDQ in roadside soil was similar to that in airborne particles but with considerably higher runoff waters. The study also concluded that ingesting roadside soil dust was the primary source of human exposure to PPDs and their quinones.[1]

There is a growing concern over the use of recycled crumb rubber, which has a chemical composition similar to that of road wear particles, as infill in synthetic turf football fields.[46] In the first study of its kind, 6PPD and 6PPDQ were found in all analyzed *in vitro* human gastrointestinal fluids taken following the digestion of recycled crumb rubber materials.[46] However, this study did not investigate particulate matter that could potentially be inhaled, deposited, or ingested. Rubber hoses in contact with food are commonly utilized in large-scale dairies to transport milk. 6PPD is an antiaging agent for rubber articles intended for repeated food-contact use.[47] However, a study investigating milk lines confirmed that milk safety and quality were not affected by 6PPD migration and degradation.[47]

6PPD is an additive used to prevent oxidative degradation and to extend the lifespan of plastics, rubbers, and other polymeric products used in electronics.[48] High concentrations of 6PPD and 6PPDQ were detected in the presence of amine antioxidants in dust collected from 45 e-waste recycling workshops.[40] A significant correlation was found between 6PPDQ and its parent, 6PPD, suggesting significant indoor conversion of 6PPD to 6PPDQ. The revealed presence of 6PPDQ and its precursor in different environmental media underscores the pressing need for targeted research. Future research can explore their prevalence, behavior, and potential risks in different environments such as soil, recycled materials, and indoor spaces.

Overall, 6PPD is frequently utilized as an antiaging agent in the rubber manufacturing sector to prolong the life of various rubber articles. Recent research has revealed the widespread presence of seven PPDs and four PPDQs in riverine, coastal, estuary, and deep-sea sediments. Among these, 6PPD and 6PPDQ were identified as the most prevalent and concerning chemicals of interest.[49] Nevertheless, the need persists for targeted investigations, such as comprehensive fate studies, toxicity assessments, and ecological impact evaluations, to deepen our understanding of 6PPD and its quinone.

3. POTENTIAL IMPACTS OF 6PPDQ ON THE ENVIRONMENT

The first identification of 6PPDQ in stormwater was in December 2020,[5] thus, the environmental risks of this substance are not yet well understood. Nonetheless, an increasing number of studies have highlighted the toxic effects of tire rubber antioxidants. Exposure to 6PPDQ has been correlated with acute mortality,[50] population reduction,[51] unnatural behavior,[52] and nerve system damage in aquatic organisms.[53] As indicated by the majority of the studies discussed in Section 2, human exposure to 6PPDQ is primarily through inhalation. Nevertheless, a recent study has introduced the possibility of human ingestion of 6PPD and its TPs through vegetables.[54] The presence of 6PPDQ in vegetables raises concerns about whether this chemical can enter the food chain and negatively impact human health. Additional research is needed to answer this important question. This section provides an overview of studies investigating the presence of 6PPDQ in plant and animal communities as well as its potential occurrence in human organs.

3.1. Aquatic Life. The main source of 6PPDQ is urban runoff after rainfall events.[55] Therefore, in the absence of a system for collecting and treating urban runoff, 6PPDQ enters waterbodies and can endanger aquatic life. Since the identification of 6PPDQ in 2020, worldwide research has focused on the potential effects of 6PPDQ on aquatic life, particularly various fish species[21,56−59] (Supporting Information: Acute lethality (LC50) in fish (adult and larvae) species during exposure to 6PPDQ (Table S2)). Furthermore, limited studies have been conducted on investigating the toxicity of 6PPDQ on freshwater crustaceans (e.g., *Daphnia magna* and *Hyalella azteca*),[60] fish larvae,[53] and also *in vitro* studies.[61,62]

Of all the fish species investigated for their susceptibility to 6PPDQ, *Oncorhynchus kisutch* (Coho salmon) has been the subject of the most extensive research due to its high sensitivity. An approximate median lethal concentration (LC_{50}) of 800 ng/L was initially estimated for Coho salmon;[5] however, more recently this LC_{50} was updated to 95 ng/L.[21] Notably, the juvenile coho LC_{50} was 2.3-times lower than the previous report for 1+-year-old coho (95 ng/L), underscoring the significance of considering age-related variations in sensitivity to this hazardous tire-related compound.[63] Further, 6PPDQ was also found to be toxic to other salmonid species including *Oncorhynchus mykiss* and *Salvelinus fontinalis*.[56,64] In contrast to its toxicity toward specific salmonids, 6PPDQ was not toxic toward other species such as *Salvelinus curilus* and *Oncorhynchus masou masou*,[59] and *Oryzias latipes*,[60] at environmental concentrations. It is not yet fully understood how 6PPDQ causes acute mortality in *Salmonidae*. However, research suggests that the main factors behind its toxicity may include blood-brain barrier disruption, mitochondrial dysfunction, and oxidation.[61,65] When fish are exposed to 6PPDQ their responses are species dependent. For instance, when subjected to 6PPDQ concentrations that induced lethality, *Salvelinus leucomaenis pluvius* exhibited atypical swimming behaviors such as hovering close to the water surface, erratic movements, and tumbling. These behaviors are consistent with responses observed in sensitive salmonids like *Oncorhynchus kisutch*, *S. fontinalis*, and *O. mykiss*.[59]

In addition, studies show that different fish exhibit different sensitivities to 6PPDQ even when they are closely related. For instance, among *O. kisutch*, *O. mykiss*, *Oncorhynchus nerka*, and *O. tshawytscha* salmonids exposed to the same 6PPDQ concentration, *O. kisutch* showed 100% mortality in 24 h compared to *O. nerka* having 100% survival.[58] Similarly, a study in Japan showed that salmonids including *S. leucomaenis*, *S. curious*, and *O. masou masou* had distinct sensitivity toward 6PPDQ. For instance, the estimated internal LC_{50} (ILC_{50}) of 6PPDQ in the brain and gill tissues of *O. masou masou* and *S. curilus* species was considerably higher than ILC_{50} values for *S. leucomaenis*.[59] The ability of the brain and gill to metabolize 6PPDQ can be attributed to the low ILC_{50}, which was confirmed by the detection of monohydroxylated metabolites in the brain and gill. These results are consistent with other *in vitro* studies that measured the liver's ability to metabolize 6PPDQ.[61] The findings imply that the dissimilarities in how different species respond to toxins may be influenced by both toxicokinetic and toxicodynamic factors.[59]

6PPDQ not only can harm adult fish but also has adverse impacts on fish larvae, leading to negative effects and mortality. It was found that normal zebrafish larvae development was significantly disrupted when they were exposed to 6PPD and 6PPDQ.[53] Particularly, larvae exposed to 10 and 25 μg/L 6PPDQ displayed symptoms of intestinal reddening. Additionally, when the larvae were exposed to 25 μg/L 6PPDQ, physical abnormalities and a notable decrease in eye size were observed.[53]

In addition to fish, 6PPDQ can have detrimental effects on other aquatic organisms. Sodium chloride from deicing roads, along with 6PPD and 6PPDQ released from urban runoff, showed a negative synergistic effect on a freshwater herbivore rotifer *Brachionus calyciflorus*.[51] It was reported that, when 6PPD and 6PPDQ concentrations exceed the levels typically found in the environment, the presence of NaCl increases the vulnerability of freshwater herbivores to 6PPD exposure. This heightened susceptibility results in more pronounced detrimental effects, even at lower concentrations.[51] In contrast, even at its highest water solubility ($\lesssim$100 μg/L), 6PPDQ did not cause significant acute mortality in *Daphnia magna* and *Hyalella azteca*.[60]

Although there have been studies on the negative impacts of 6PPDQ on different fish species, the exact mechanisms leading to high mortality rates are still unknown. Therefore, further research is necessary to validate previous findings and gain a deeper understanding of this issue. Moreover, the 6PPDQ story highlights the need to investigate the transformation mechanisms and potential impacts of other PPDs and their TPs.

3.2. Humans and Other Mammalians. There are many ways that humans come into contact with 6PPDQ including diet,[5,8] inhalation of fine particles,[6,7] and consumption of contaminated water.[9] Human exposure to 6PPDQ primarily occurs through the inhalation of $PM_{2.5}$-bound quinones. The daily intake of PPDQs among different subgroups and exposures was estimated to be within 0.16−1.25 ng/(kg_{bw} day), similar to the parent PPDs' daily intake of 0.19−1.41 ng/(kg_{bw} day). This suggests that exposure to PPDQs might be important but has been overlooked.[38] Similar to other $PM_{2.5}$-bound quinones that have been demonstrated to induce oxidative stress and DNA damage, 6PPDQ may also show comparable effects.[66] The daily intake of PPDQs through oral ingestion for adults and children in Hong Kong, China, was estimated to be 1.08 and 7.3 ng/(kg_{bw} day), respectively, which was higher than their parent compounds.[1] Implementing an acellular dithiothreitol (DTT) assay revealed that PPDQs (particularly 6PPDQ) enhance the oxidative potential of $PM_{2.5}$ by 15.6−42.2% prompting DNA damage via oxidative stress.[7] The reaction between 6PPDQ and deoxyguanosine creates a specific isomer called 3-hydroxy-1,N2-6PPD-etheno-2′-deoxyguanosine (6PPDQ-dG). Genomic DNA obtained from mammalian cells exposed to 6PPDQ exhibited detectable levels of 6PPDQ-dG, and a direct relationship between the levels of externally introduced 6PPDQ and the quantities of 6PPDQ-dG present was observed.[67] A recent study also revealed that 6PPD and 6PPDQ can bioaccumulate in liver, trigger disturbances in lipid metabolism, and cause inflammatory reaction in mice.[68]

Recently, 6PPD and 6PPDQ were monitored for the first time in 150 urine samples of children, pregnant women, and adults. Both 6PPD and 6PPDQ were detected in 60−100% urine samples, indicating high exposure of humans to these compounds. Surprisingly, pregnant women showed the highest concentration of 6PPDQ (2.91 ng/mL) compared to that of adults (0.40 ng/mL) and children (0.076 ng/mL). 6PPD was detected in lower levels in human urine due to its depletion in the liver, as demonstrated by *in vitro* metabolic experiments.[10] Currently, research on the biological effects caused by exposure to 6PPDQ is limited, with further research necessary to thoroughly understand its actual toxicities and associated risks to human health.

3.3. Plants.

Plants are one of the final destinations where 6PPDQ can bioaccumulate in the environment. It is anticipated that TRWPs, upon entering farmland soils will release their compounds on the upper soil layers rather than moving them down to deeper soil horizons.[69] In the case of TRWPs, only one study has examined 6PPD and 6PPDQ absorption in plants, specifically assessing lettuce.[54] Lettuce plants were irrigated with a hydroponic solution containing 1 mg/L TRWPs such as 6PPD and 6PPDQ for 2 weeks, finding 6PPD in lettuce leaves at 0.75 μg/g. Surprisingly, among all studied compounds only 6PPDQ continued its accumulation in lettuce leaves over 2 weeks with a concentration of up to 2.19 μg/g. The study found that edible plants could act as pathways for 6PPDQ to enter the food chain and cause harm. As stated above, only one hydroponic study has been conducted on the bioaccumulation of 6PPDQ in edible plants (lettuce), which demonstrated 6PPDQ bioaccumulation. However, given the limited scope of this study, it is crucial to conduct comprehensive research involving various plant species to establish a more robust understanding of the bioaccumulation processes. Previous studies have investigated the bioaccumulation of different pollutants in edible plants,[70,71] and similar investigations should be conducted regarding 6PPDQ to broaden our knowledge in this area.

4. FUTURE PERSPECTIVES

- Implementing effective measures to control particle emissions from road runoff is crucial to mitigate the environmental impact of 6PPDQ. Given the limitations of stormwater treatment, it is essential to specifically address the challenge of 6PPDQ contamination in runoff. Further research can explore the potential of improved stormwater management practices, such as green infrastructure solutions, filtration systems, and bioretention[72,73] in preventing the release of 6PPDQ into aquatic environments.
- Although studies have reported the 6PPDQ toxicity to specific aquatic species, such as Coho salmon, its impact on other organisms and ecosystems, such as freshwater invertebrates, amphibians, and aquatic plants, remains uncertain. The detection of 6PPDQ in urine samples emphasizes the need for further research to evaluate its potential toxicity and health risks in both human and wildlife populations.
- A comprehensive approach is essential to prevent the introduction of emerging chemicals, such as transformation products (TPs) like 6PPDQ, into the environment and protect the well-being of humans and ecosystems. Recognizing the potential toxic effects of TPs, it is crucial to include them in regulations for effective monitoring and control, mitigating environmental risks and safeguarding health. Further investigation is required to explore the pathways of TPs derived from 6PPD and 6PPDQ, as they have not been addressed adequately. The formation of these TPs involves various reactions beyond oxidation. Understanding specific pathways requires additional research to expand our knowledge in this area.
- Exposure risks and health effects of 6PPDQ are poorly understood. In complex environmental conditions, other additives in rubbers may contribute to the release of mobile 6PPDQ into air, water, soil, and sediments. Research is needed to understand the reactivity, transformation mechanisms, and behavior of 6PPD and associated 6PPDQ in tire rubber and recycled rubber products.
- While a recent study[74] has provided principles that guide the development of safe and effective antiozonants as alternatives to 6PPD in tires, further research is needed to fully explore this area.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.3c00521.

Occurrence of 6PPDQ in water, air/dust, and other media; Acute lethality (LC_{50}) in fish (adult and larvae) species during exposure to 6PPDQ; Brief background on transformation products of 6PPDQ parent compound 6PPD (PDF)

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Notes

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Automated, High-Throughput Analysis of Tire-Derived *p*-Phenylenediamine Quinones (PPDQs) in Water by Online Membrane Sampling Coupled to MS/MS

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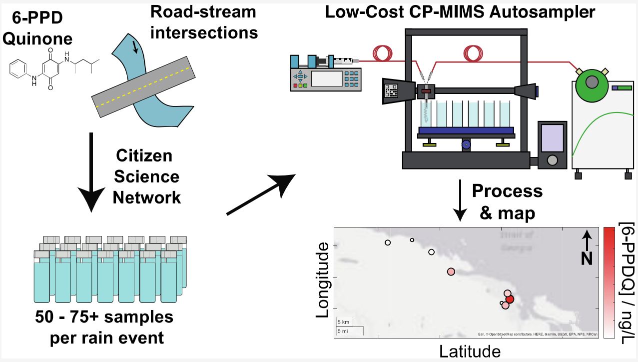

ABSTRACT: The tire-derived contaminant *N*-(1,3-dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine quinone (6-PPDQ) was recently identified as a potent toxin to coho salmon (*Oncorhynchus kisutch*). Studies investigating 6-PPDQ have employed solid-phase extraction (SPE) or liquid−liquid extraction (LLE) with liquid chromatography−mass spectrometry (LC-MS), providing excellent sensitivity and selectivity. However, cleanup and pre-enrichment steps (SPE/LLE) followed by chromatographic separation can be time- and cost-intensive, limiting sample throughput. The ubiquitous distribution of 6-PPDQ necessitates numerous measurements to identify hotspots for targeted mitigation. We recently developed condensed phase membrane introduction mass spectrometry (CP-MIMS) for rapid 6-PPDQ analysis (2.5 min/sample), with a simple workflow and low limit of detection (8 ng/L). Here, we describe improved quantitation using isotopically labeled internal standards and inclusion of a suite of PPDQ analogues. A low-cost autosampler and data processing software were developed from a three-dimensional (3D) printer and Matlab to fully realize the high-throughput capabilities of CP-MIMS. Cross-validation with a commercial LC-MS method for 10 surface waters provides excellent agreement (slope: 1.01; R^2 = 0.992). We employ this analytical approach to probe fundamental questions regarding sample stability and sorption of 6-PPDQ under lab-controlled conditions. Further, the results for 192 surface water samples provide the first spatiotemporal characterization of PPDQs on Vancouver Island and the lower mainland of British Columbia.

KEYWORDS: *6PPD-quinone, citizen science, para-phenylenediamines, direct mass spectrometry, CP-MIMS, rapid screening, tandem mass spectrometry, tire wear leachates*

■ INTRODUCTION

Urban runoff represents an ongoing threat to aquatic life. As rainwater washes over anthropogenic surfaces, both inorganic and organic contaminants are released and carried to receiving waterways. In 2020, Tian et al. published a landmark study identifying *N*-(1,3-dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine quinone (6-PPDQ) as a potent toxin to coho salmon (*Oncorhynchus kisutch*) in the ng/L range.[1,2] The parent compound, *N*-(1,3-dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine (6-PPD), is added to tire rubber at 0.4−2.0% by mass as an antiozonant. 6-PPD reacts with ground-level ozone to produce 6-PPDQ and other oxidized byproducts.[3] Since the discovery of 6-PPDQ, acute toxicity at environmentally relevant (ng to μg/L) concentrations has been established for several species including white-spotted char (*Salvelinus leucomaenis*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalus*), and early-life stage Chinook (*Oncorhynchus tshawytscha*).[4−6] Further, several groups have identified sublethal effects of 6-PPDQ (intestinal, neural)[7,8] across multiple species including *Caenorhabditis elegans*, mice,[9] and fathead minnow (*Pimephales promelas*).[10] While the mechanism of 6-PPDQ toxicity is still being uncovered, Mahoney et al. recently reported *in vitro* evidence that 6-

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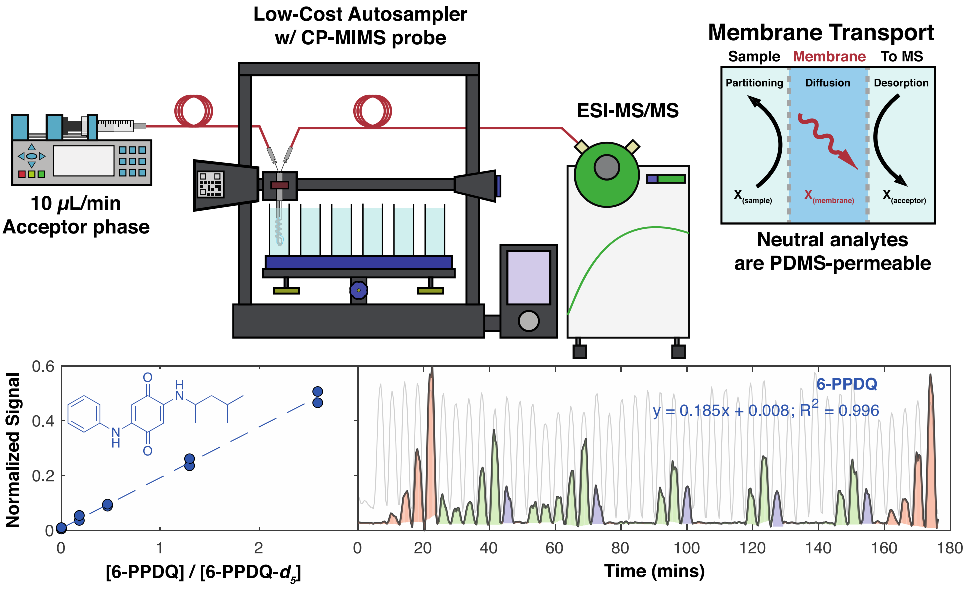

Figure 1. Instrumental schematic illustrating the CP-MIMS probe, 3D printer-based autosampler, and MS. The chronogram shows automatic peak detection for standards (red), samples (green), and calibration verification standards (blue). Normalized signal intensity for the sample-phase internal standard is shown in gray.

PPDQ toxicity may be mediated by disruption of mitochondrial respiration[11] and Blair et al. have observed disruption of the blood−brain barrier in coho salmon treated with roadway runoff.[12]

Several targeted studies have identified 6-PPDQ in various matrices beyond storm- and surface water, including fish tissue,[13] snow,[3] urine,[14] air particulates,[15,16] and sediments[15] suggesting that this contaminant is widely distributed in the environment. Additionally, 6-PPD is only one compound from a family of *para*-phenylenediamine (PPD) preservatives. Several of the corresponding quinone analogues (Figure S1) have been identified in the environment, with unknown implications for human and environmental health.[15]

Most investigations to date have employed solid-phase extraction (SPE) or liquid−liquid extraction (LLE) coupled to liquid chromatography−tandem mass spectrometry (LC-MS/MS).[2,3,6,15] This approach can provide excellent sensitivity and selectivity, and was especially valuable in the initial discovery of 6-PPDQ as the acute toxin responsible for "urban runoff mortality syndrome."[1] However, the cleanup and pre-enrichment steps (SPE/LLE) can be cost- and labor-intensive and coupled with chromatographic separation time, limit sample throughput. Given the diffuse and ubiquitous nature of 6-PPDQ sources, large sample sets need to be analyzed to characterize regional inputs and identify hotspots for targeted mitigation.[17−19] Therefore, direct mass spectrometry approaches that obviate sample cleanup and pre-concentration steps provide a faster, "*fit-for-purpose*" approach for analyzing 6-PPDQ and analogues in storm- and surface water.

CP-MIMS interfaces liquid or slurry samples directly with a mass spectrometer (MS) via a semipermeable membrane.[20,21] Analytes that permeate the membrane are carried to the MS ion source by a continuous flow of organic solvent acceptor phase through the lumen of a capillary hollow-fiber membrane immersed in the sample. Generally, a poly-dimethylsiloxane membrane is employed, which allows permeation of neutral, hydrophobic analytes (e.g., 6-PPDQ $\log K_{ow}$ ∼4)[15] while rejecting bulk matrix components and particulate matter. The acceptor phase composition, ion source, and MS parameters can be optimized for a specific analyte class. This approach has been implemented for polycyclic aromatic hydrocarbons,[22−24] phthalates,[23] UV filters,[25] and naphthenic acids.[22,26,27] CP-MIMS provides continuous, real-time data allowing dynamic processes such as organic synthesis[28] or sorption/desorption phenomena[27] to be studied directly. We have previously reported a preliminary CP-MIMS method using tandem MS for analysis of 6-PPDQ exhibiting environmentally relevant detection limits (8 ng/L) and a short analytical duty cycle (2.5 min/sample).[29]

Here, we describe significant improvements and validation for 6-PPDQ analysis with CP-MIMS. A low-cost 3D printer was repurposed and reprogrammed for use as an autosampler (Figure 1) and accompanying software for automated data processing was developed in Matlab. Isotopically labeled internal standards were used to improve quantitative performance, and the technique is expanded to include five additional PPD-quinone analogues (Figure S1). Cross-validation with a commercial LC-MS method exhibited excellent quantitative agreement in a series of real-world samples. We apply the improved CP-MIMS method to probe fundamental questions about the aqueous stability of 6-PPDQ as well as sorption behavior on natural sediments. Further, 192 water samples collected as part of a citizen science campaign were rapidly screened with the presented approach to provide the first spatiotemporal survey of 6-PPDQ and analogues on Vancouver Island and the lower mainland of British Columbia, Canada.

■ MATERIALS AND METHODS

Standards and Samples. Analytical-grade 6-PPDQ and $^{13}C_6$-6-PPDQ (both ≥95%; 100 μg/mL in acetonitrile) were purchased from ACP Chemicals (Montreal, QC). Neat standards of DTDPQ, DPPDQ, IPPDQ, CPPDQ, 77PDQ (10 mg each), and 100 μg/mL 6-PPDQ-d_5 (in acetonitrile) were purchased from HPC Chemicals (Atlanta, GA) at ≥92% purity. Full analyte names and structures are available in Figure S1. Combined substocks of the suite of analytes were prepared

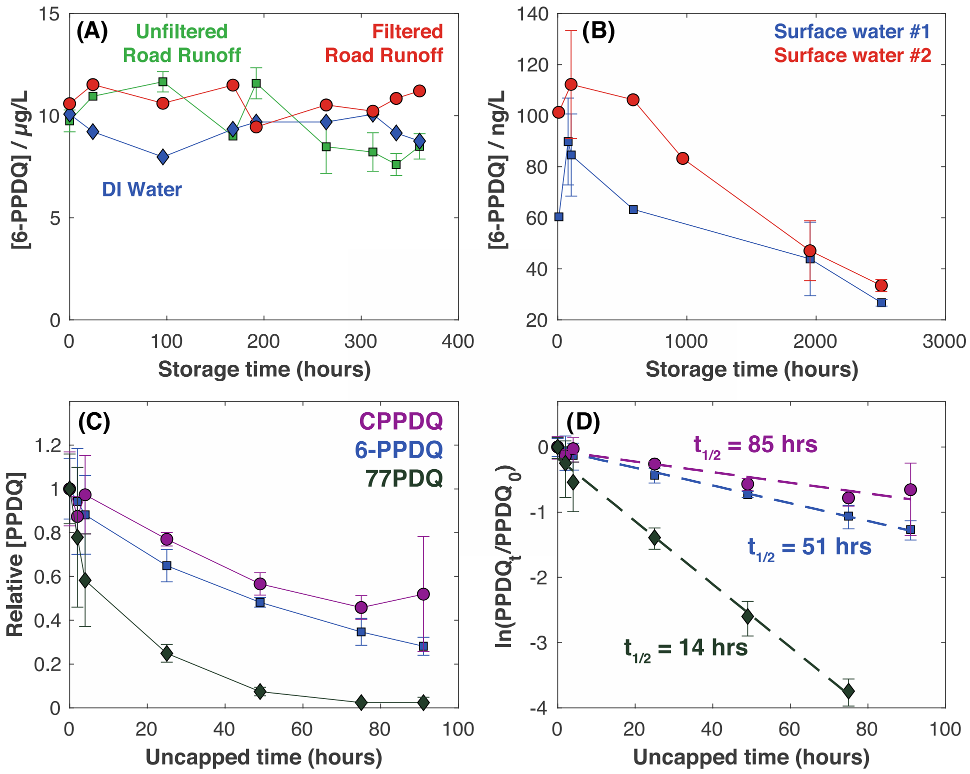

Figure 2. (A) Shelf life of 6-PPDQ in DI water (blue diamonds), unfiltered road runoff (green squares), and filtered road runoff (red circles) over a 2-week period. Minimal if any loss is observed throughout this window beyond the precision limits of the method (±15%). (B) Shelf life of 6-PPDQ in two real-world samples measured over a ca. 3.5-month period. Concentrations appear generally stable within the precision limits of the method for the first 400−500 h (ca. 3 weeks) followed by a slow decay during months-long storage. (C) Loss of 6-PPDQ (blue squares), CPPDQ (purple circles), and 77PDQ (gray diamonds) from uncapped solutions of 6-PPDQ over 120 h. Fitting the process to a first-order decay (D) yields $t_{1/2}$ ranging from 14 to 108 h for different PPDQ analogues. For analysis of samples shown in (C) and (D), the vials were kept capped until immediately before analysis to minimize atmospheric losses during the analytical run. The full dataset for (C) and (D) is available in Table S2; the $t_{1/2}$ values for the compounds not shown here were 108 h (DTPDQ), 69 h (DPPDQ), and 65 h (IPPDQ).

gravimetrically in HPLC-grade methanol (Fisher Scientific, Ottawa, ON) at ca. 15−30 μg/kg. Calibration standards were prepared between 8 and 1600 ng/L by spiking this combined standard into deionized water (Facility scale reverse Osmosis/Ion Exchange Water Purification System, Applied Membranes, Inc., Vista, CA).

Water samples (n = 192 total; 24 road runoff samples and 168 stream water samples) were collected at sites on Vancouver Island and in Vancouver, British Columbia, Canada. Samples were collected during October−November 2022 as part of a citizen science campaign led by the British Columbia Conservation Foundation and Fisheries and Oceans Canada's Whale Contaminants Program. Where possible, samples were collected before, during, and after a rain event (≥5 mm) following a dry period (≥48 h). For "during" samples, volunteers were instructed to collect during estimated peak stream flow. Prior to sampling, bottles were thoroughly rinsed with tap water, then subsequently rinsed 3× with deionized water and finally with a small volume of HPLC-grade methanol. Environmental grab samples were collected by completely filling (i.e., headspace < 2% overall volume) amber glass bottles with PTFE-backed septa (40−1000 mL; Fisher Scientific) after rinsing three times with the sample. All samples were kept at 4 °C until they were picked up for transport in a cooler with ice packs (<1.5 days transit time). Given the limited stability of 6-PPDQ in water observed by others,[5,30] surface water samples were prioritized and analyzed as soon as possible, generally within 5 days for CP-MIMS experiments unless otherwise mentioned. Road runoff samples were held for up to 100 days prior to analysis.

Samples were measured "as is," with no filtration or sample cleanup. Where high concentrations were anticipated (e.g., roadway runoff), samples were diluted 2- to 10-fold in deionized water to bring them into the working calibration range (ca. 8−250 ng/L) for general sample analysis. As needed, higher calibration standards were also included for storage time and sorption experiments. The linear dynamic range for CP-MIMS analysis of PPDQs extends to the μg/L range (up to 32 μg/L for 6-PPDQ).[27] Immediately prior to measurement, all samples and standards were spiked with ca. 100 ng/L 6-PPDQ-d_5 internal standard. Sample analysis was carried out in clean (as described above) 40 mL TOC vials, using both clear and amber glass vials as available.

Storage Time Experiments. For initial evaluation of aqueous stability (Figure 2A), 6-PPDQ was added at ca. 10 μg/L to deionized water and both a filtered (0.45 μm Nylon syringe filter, 33 mm, Fisher Scientific) and unfiltered aliquot of roadway runoff. These solutions were subsampled and diluted 40- to 100-fold in deionized water for manual CP-MIMS analysis using a 2- to 3-point standard addition methodology described previously.[29] This was repeated at several time points over the 2-week (340 h) test period. Samples were kept in clear, capped glass 40 mL vials with PTFE-backed septa at room temperature (ca. 22 °C; headspace 10−20% overall volume). Stability in real-world stream samples (Figure 2B) was evaluated opportunistically.

Where sufficient sample was available (≥500 mL) and significant 6-PPDQ was detected during initial measurement (≥50 ng/L), fresh aliquots of the sample (35−40 mL) were withdrawn and measured over time to monitor analyte stability. The bulk solution was stored at 4 °C in amber glass bottles sealed with PTFE-backed septa between analyses. These were all measured as described below for general sample analysis using the autosampler and sample-phase internal standard (Figure S2C). Headspace in these containers increased over time as the sample was withdrawn, starting at <2% overall volume and reaching up to ∼50% by the final time point. For the uncapped experiments (Figure 2C,D), a 1 L aqueous solution containing all of the PPDQ analogues was prepared at ca. 1−5 μg/L and transferred into clear, 40 mL vials. The vials were kept uncapped for the indicated time up to 120 h at ambient temperature (22 °C) in a fume hood. Relative concentrations were then measured directly (no dilution) against a capped control (<5% headspace volume; sealed with PTFE-backed rubber septa) after the addition of internal standard. To mitigate losses during sample analysis, in this experiment, vials on the autosampler were kept capped until immediately prior to analysis. This workflow is illustrated in Figure S3. Relative quantification was carried out using the same sample-phase internal standard calibration model employed for real-world samples (Figure S2C).

Sorption Experiments. A 1 L aqueous solution of 6-PPDQ at ca. 0.5−1 μg/L with 60 mM concentration phosphate buffer (pH = 7.16) was prepared. This solution was transferred into 40 mL vials, and low-organic-content sediment (clean loam soil; f_{OC} = 1.85%, Sigma-Aldrich, Oakville, ON), high-organic-content sediment (clean sandy loam; f_{OC} = 5.96%; Sigma-Aldrich), Ottawa sand (Chromatographic Specialties, Inc., Brockville, ON), and activated carbon (Euroglas, Delft, Holland) were added in the 75−1500 mg range to individual, clear 40 mL vials (equivalent to 3−60 g/L loading). These solutions were capped and allowed to equilibrate for 2 days under gentle shaking (200 rpm, multiplatform shaker table, Fisher Scientific) at room temperature. Manual CP-MIMS analysis was then performed directly on the heterogeneous sediment slurry using the acceptor phase internal standard calibration method (Figure S2B). The fraction of 6-PPDQ remaining in solution was calculated against a control vial that underwent the same equilibration time and analysis method, but with no sorbent added. To calculate the organic carbon binding constant log(K_{OC}), pairwise calculations between the control vial and each different loading of sediment (n = 5) were calculated as: K_{OC} = [6-PPDQ]$_{sediment}$/([6-PPDQ]$_{water}\cdot f_{OC}$), where K_{OC} is the binding constant (L/kg organic carbon), [6-PPDQ]$_{sediment}$ is the concentration of 6-PPDQ on the sediment in ng/kg, [6-PPDQ]$_{water}$ is the aqueous concentration (ng/L), and f_{OC} is the mass fraction of organic carbon in the sediment. The results from the five different loadings were then averaged for each sediment and transformed into logarithm space. The uncertainty associated with the resulting log(K_{OC}) values was then increased to reflect the potential for nonequilibrium conditions (only 2-day equilibration time) and relatively low levels of sorption for the low-f_{OC} sediment.

Condensed Phase Membrane Introduction Mass Spectrometry. The CP-MIMS apparatus has been described in detail previously.[29] Briefly, a 7.6 cm length of 55 μm thick PDMS hollow-fiber membrane (Permselect, MedArray, Inc., Ann Arbor, MI) was mounted onto 31-gauge stainless steel capillaries (Microgroup, Medway, MA). A stainless steel wire support was epoxy-potted into 1/4″ stainless steel tubing alongside the capillaries to improve probe ruggedness. The acceptor phase was composed of 15/85/0.03 heptane/methanol/formic acid (v/v/v; Fisher Scientific) and was flowed through the membrane lumen at 10 μL/min using a syringe pump (Chemyx Fusion 100, Stafford, TX) and 10 mL gas-tight syringe (Hamilton 1000 series, Fisher Scientific). Due to the limited stability of formic acid in methanol,[31] fresh acceptor phase was prepared for each sampling batch (2−3 days of analysis) unless otherwise mentioned. To monitor ionization suppression and instrument drift, 20 μg/kg $^{13}C_6$-6-PPDQ internal standard was also included in the acceptor phase. Membrane sampling was carried out under ambient laboratory conditions (ca. 22 °C; 1 atm). For CP-MIMS, the system should be refreshed periodically with clean solvent flushes and/or membrane replacements, particularly after complex or high-concentration samples (e.g., roadway runoff). The membranes employed here exhibited stable analytical performance for weeks to months (hundreds of samples) and were generally only replaced as part of a preemptive cleaning routine or due to operator error during setup (e.g., membrane inadvertently dislodged).

Detection was carried out with an electrospray ionization (ESI) triple quadrupole mass spectrometer (QSight 220, PerkinElmer, Waltham, MA) in positive-ion mode. Global instrument parameters include: nebulization gas = 120 psi, hot-surface induced desolvation (HSAID) source = 320 °C, and capillary voltage = +4.0 kV. For MS/MS monitoring of 6-PPDQ, atypical qualifier/quantifier MS/MS ion ratios were observed using the dominant m/z 299 → 215 transition. This may be due to co-occurring isomeric ozonation products of 6-PPD recently characterized by Zhao et al.[32] Instead, 6-PPDQ was monitored using m/z 299 → 256 as quantifier ion and both 299 → 241 and 299 → 100 as qualifier ions. MS/MS scan parameters for quantifier and qualifier ion transitions of other PPDQs are provided in Table S1. A dwell time of 1000 ms was used for each MS/MS transition. Additionally, full-scan mass spectra were collected between m/z 100 and 500 with step size 1 m/z and 3 ms per step.

Autosampler Construction and Operation. The autosampler was constructed from a base-model 3D printer (Ender 3 V2, Creality, Shenzhen, China). Conversion to a CP-MIMS autosampler was performed by removal of the print nozzle and fan. A 1/4″ hole was drilled through the aluminum cooling housing, allowing the CP-MIMS probe to slide in and be secured using the existing set screw. This places the CP-MIMS probe at approximately the same XY coordinates as the print nozzle would be, providing 220 mm × 220 mm area of programmable XY motion. The Z-stop sensor was moved up such that the bottom of the probe would not contact the print bed. Photos of the cooling housing, raised Z-stop sensor, and overall setup are shown in Figure S4. A 6 × 6 sample tray was 3D-printed with polylactic acid (PLA) filament (Creality) to cover the print bed surface and accommodate 40 mL TOC vials (1 1/8″ holes). The sample tray was secured to the print bed with tape for early experiments, and later with a 3D-printed base, which held the sample tray in place. These prints were made using a larger working area 3D printer (CR-10 V3, Creality); however, in principle, the trays could be printed on the smaller Ender 3 by printing the trays vertically rather than laid flat. The XY coordinates for the four corner sample slots were measured manually and the remaining slots were interpolated from these coordinates.

To control the autosampler, custom Matlab script (version 2021b; MathWorks, Natick, MA) was written which takes a user-defined runlist, coordinate map, sampling time, and rinse time and writes G-code for a given run. For routine sample analysis, only full trays were measured, containing one rinse solution (HPLC-grade methanol), 5 calibration standards, 25 samples, and 5 calibration verification standards. The system was programmed to give sample/standard membrane exposure for 2.5 min, stirring the solution by moving the probe in a square motion about the center of the vial. A 1 min rinse in the methanol vial with the same stirring motion was programmed to start the run and after each sample/standard analysis. Overall, this gave a total measurement time of ca. 4 min/sample. A full calibration was performed at the start and end of each run (Figure 1). Additional QA/QC samples included a calibration standard and deionized water blank interspersed after every batch of 5 samples. This process includes a total of 44 analyses and takes ca. 3 h to run, allowing 3−4 trays to be processed per day (i.e., 132−176 total analyses).

Data Processing. Matlab scripts were developed to import a raw data file and sample info file and output quantitative results. A flow diagram outlining the overall process is available in Figure S5. Briefly, a CSV file was exported from the MS vendor software (Simplicity 3Q, version 1.4.1806.29651; PerkinElmer, Waltham, MA) and imported into Matlab alongside an info file with sample list and calibrant concentrations. Peaks were picked and identified from the raw data based on the donor phase internal standard peak (6-PPDQ-d_5) and specified runlist. Calibration models were then constructed using the maximum signal intensity after smoothing in each sample/standard window normalized to that of the internal standard in the same window. The intra-run limit of detection (LoD) was calculated using the blank calibration standard (n = 6) and lowest calibration standard exhibiting a signal-to-noise ratio ≥3 (n = 2). A more robust evaluation of the LoD and limit of quantitation (LoQ) was also performed using n = 7 analyses of a blank, 25 ng/L, and 60 ng/L combined standard of the PPDQ suite. The intra-run LoD was used as reporting limit for environmental samples as it accounts for day-to-day variability in performance. Data is flagged for a possible interferent if the relative abundance of the qualifier ion transitions deviates significantly from expected values. The sample flag threshold for qualifier ion ratios was set at three standard deviations observed for calibration check solutions, applied to samples that exhibit 6-PPDQ concentrations ≥50 ng/L (i.e., above LoQ).

Commercial Analysis with Liquid Chromatography−Mass Spectrometry. Water samples (n = 12) collected from the Nanaimo area on Vancouver Island were sent to SGS AXYS Analytical Ltd. (Sydney, BC) within 48 h of sampling for commercial analysis of aqueous 6-PPDQ. Samples were preserved with dichloromethane upon arrival and held at 4 °C in the dark for 23−24 days until analysis. The LoD was 0.05 ng/L in a sample size of 1 L. Spike recovery experiments at 80 ng/L 6-PPDQ for a blank stream sample (n = 5) yielded 103 ± 1% recovery. Analysis was carried out according to the methods outlined in Lo et al.[6] with minor modifications. Briefly, 6-PPDQ-d_5 was added to aqueous samples prior to liquid−liquid extraction (LLE) with dichloromethane. The extracts were then analyzed on a Waters ACQUITY UPLC I-Class System and a Xevo TQ-S tandem mass spectrometer operated in positive-ion mode. A Waters ACQUITY UPLC BEH C18 (1.7 μm, 2.1 mm × 50 mm) column was used for analytical separation, with an ACQUITY UPLC BEH C18 Vanguard (1.7 μm, 2.1 mm × 5 mm) employed as guard column. Gradient elution was performed with UPLC-grade water containing 0.1% formic acid (solvent A) and 1:1 acetonitrile/methanol (solvent B). The gradient started at 70% A and was held for 1 min, and then increased to 100% B by 10 min. This mobile phase composition was maintained for 2 min and then returned to original conditions over another minute for a total time of 13 min. The column was then held at these conditions for another minute to equilibrate before the next injection. 6-PPDQ was monitored using two MRM transitions at m/z 299 → 241 (quantifier ion) and 299 → 215 (qualifier ion).

RESULTS AND DISCUSSION

Development of a Low-Cost Autosampler for CP-MIMS Analysis of PPDQs. Rapid analysis techniques offer significant advantages, including higher sample throughput and reduced cost. However, as the sample analysis time decreases, manual operation becomes impractical. In anticipation of large sample sets collected during multiple rain events, the need to automate CP-MIMS became apparent. Early efforts in our lab employed a rotary tray autosampler from a flow injection analyzer.[33] While reasonably effective, this approach was limited by the lack of full user control (e.g., limited sampling time), inflexible geometry (e.g., sample bottle types), and did not accommodate stirring. To leverage the high sample throughput of the technique, we have developed a custom autosampler from a repurposed 3D printer, enabling fully automated data acquisition and processing. Others have used this approach for similar challenges including spatially resolved mapping of uneven surfaces for mass spectrometry imaging[34] and nucleic acid isolation/amplification.[35] The printer employed here (Creality Ender 3) met our basic criteria of being low cost (<$500 CAD) and supporting precise motion (±0.1 mm). Further, the heated print bed lends itself to future temperature-controlled experiments to probe physicochemical properties and/or improve analytical sensitivity.[36,37] With relatively minor physical modification of the printer (replacement of print nozzle with CP-MIMS immersion probe, reposition Z "zero" position; Figure S4) and a simple Matlab program to translate user inputs into printer instructions, a functional autosampler was constructed for CP-MIMS (Figure 1).

A typical signal chronogram is shown in Figure 1. The system demonstrated stable operation for 3 h, and we have conducted up to 4 of these runs in a single day (100 samples, 176 total analyses after incorporation of calibrators, QA/QC, and blank samples). Sample analysis is bracketed by a full calibration suite at the beginning and end of the run, with a calibration check and DI blank measured after every 5 samples. Currently, the system is operated with a ca. 4 min duty cycle consisting of 2.5 min in a sample solution, 1 min in a methanol rinse, and roughly 30 s to transition between vials. This can be reduced to as little as 2.5 min/sample for steady-state membrane transport measurements or even faster using a non-steady-state approach.[38,39] Beyond the eased burden on the operator, an additional advantage of automating data acquisition is that signal peaks are more reproducibly structured (width, distance) facilitating automated data analysis. The colored peaks in Figure 1 are identified and processed automatically based on the specified acquisition order and time stamp of the internal standard peak. The combination of automatic data acquisition and processing has been transformative, with same- or next-day reporting possible for the large number of samples associated with each rain event. This is particularly important for community-based projects where engagement with citizen scientists and other project partners is crucial. This basic platform can be expanded to any membrane permeable analyte, allowing for a simple, high-throughput, and automated workflow for the analysis of a wide range of trace organic contaminants.

Adaptation to PPDQ Analogues and Laboratory Experiments. Electrospray ionization (ESI) in positive-ion mode is a particularly sensitive technique for primary and secondary amines,[40] including PPDQs. However, it can be prone to ion suppression/enhancement effects due to both the sample matrix and long-term signal drift. While membrane extraction largely excludes matrix components that can affect ionization, signal drift across long run times necessitates use of an internal standard to compensate.[39] During our previous study, isotopically labeled standards of 6-PPDQ were not commercially available, so quantitation relied on a standard addition methodology to compensate for the above effects.[29] In this work, 6-PPDQ-d_5 and $^{13}C_6$-6-PPDQ were included in the aqueous sample phase and methanolic acceptor phase, respectively. Normalizing signal response to either internal standard improves calibration stability (Figure S2); however, the sample-phase internal standard was favored where possible as it captures both sample partitioning and ionization effects.

Given the similar structural features of the PPDQ analogues (Figure S1), their physicochemical properties[15] are favorable for membrane transport through poly(dimethylsiloxane) (low pK_a of the conjugate acid, high K_{ow}). After initial MS/MS optimization (Table S1), the limit of detection (LoD) was assessed by replicate analyses ($n = 7$) of a blank, 25 ng/L, and 60 ng/L standard. LoDs and limits of quantitation were in the low ng/L range (Table S1) for 6-PPDQ (LoD: 6.0 ng/L; LoQ: 20 ng/L), IPPDQ (11; 36), CPPDQ (2.0; 6.6), DTDPQ (3.3; 11), and 77PDQ (4.8; 16). DPPDQ showed the poorest performance with an LoD of 28 ng/L (LoQ: 94). We attribute this to less efficient ionization by ESI due to the lower basicity of the amine moieties in DPPDQ, as electron density can be more delocalized into the phenyl rings. While DTDPQ may also be expected to exhibit this behavior, similar sensitivity to 6-PPDQ was observed for DTDPQ. Steric hindrance of the ortho-methyl groups may reduce the pi-orbital overlap and reduce electron delocalization into the aromatic system.[41] Due to the proximity of parent $[M + H]^+$ ions (e.g., m/z 299 and 297 for 6-PPDQ and CPPDQ, respectively) and the potential for source fragmentation of 6-PPDQ into IPPDQ, we conducted a control experiment and observed no signal intensity in the CPPDQ or IPPDQ MS/MS channels in response to several spike additions of 6-PPDQ (Figure S6).

The detection limits achieved here are within an order of magnitude of those achieved by conventional workflows[2,15] (e.g., LC-MS) and are below the acute toxicity values reported for aquatic organisms (41−95 ng/L LC_{50} for coho salmon).[2,6] We believe that given the simplicity and high-throughput capabilities of CP-MIMS, it provides a “fit-for-purpose” complement to conventional workflows. For instance, CP-MIMS provides an excellent avenue to probe the behavior of 6-PPDQ under controlled conditions as illustrated with the following two examples:

(1) The aqueous stability of 6-PPDQ remains an open question in the literature and has important implications for sample hold times, operation of exposure tank toxicological studies, and optimization of engineered mitigation strategies. An early report from Hiki et al. suggested a half-life as short as $t_{1/2}$ = 33 h,[30] which would challenge comprehensive environmental monitoring campaigns significantly. To verify this result, we monitored the 6-PPDQ concentration in several fortified samples over 2 weeks at room temperature (Figure 2). Minimal loss of 6-PPDQ was observed beyond the precision limits of the method (ca. ± 15%). Real-world samples stored at 4 °C exhibited similar stability over the short term, with significant losses only occurring after months of storage (55−65% concentration reduction over 3.5 months; 4 °C). While these results do indicate some loss of 6-PPDQ during extended storage, it is considerably slower than that observed by Hiki et al.[30] However, when we repeated the experiment with a series of standards left uncapped (open to the air), we observed loss of 6-PPDQ with $t_{1/2}$ = 51 h (Figure 2 C,D). Given that the partitioning rate between water and air will depend on a number of factors (e.g., surface area, agitation, temperature), this result is in better agreement with that reported by Hiki et al.[30] When we examined the rate of loss across the series of PPDQ analogues (Table S2) in the uncapped vials, 77PDQ exhibited the shortest half-life ($t_{1/2}$ = 14 h) and DTDPQ had the longest ($t_{1/2}$ = 108 h). It is unclear whether loss of PPDQs from the aqueous phase is due to physical and/or chemical processes. In either case, the rapid loss of PPDQ analogs from aqueous solutions open to the atmosphere has important implications for the environmental fate and distribution of these contaminants, as well as the design of experiments involving PPDQs.

(2) Given the relatively high octanol-water partition coefficient ($\log K_{ow}$ = 3.98) of 6-PPDQ,[15] some fraction is expected to sorb to sediments rather than remaining in the aqueous phase. Because CP-MIMS provides an online membrane cleanup, the aqueous phase concentration can be probed directly from a heterogeneous slurry containing sediment or sorbent.[29] Capitalizing on this, we set up a series of samples with the same initial concentration of 6-PPDQ and increasing loadings of a low (1.85%)- and high (5.96%)-organic-content sediment. After 2 days of equilibration under gentle shaking, the remaining fraction of 6-PPDQ in the aqueous phase was measured directly within the heterogeneous sample (Figure S7). Relative to negative control (Ottawa sand), significant sorption occurred for both sediments, with a greater extent observed for the high organic content sediment (up to 90% removal from the aqueous phase). Preliminary organic carbon−water partition coefficients $\log K_{OC}$ (L/kg) can be derived from these data, yielding values of 2.8 ± 0.8 and 3.6 ± 0.5 for the low- and high-organic-carbon sediment, respectively. These values are in good agreement with both calculated values (3.14)[42] and experimental reports (3.2−3.5);[43] however, given the relatively short equilibration time (2 days) and modest extent of sorption for the low-organic-carbon sediment, caution should be exercised when interpreting these values. We are currently conducting a more exhaustive study to probe heterogeneous partitioning

behavior using longer equilibration times and a broader array of sorbent materials.

Quantitative Performance for PPDQ Analogues. With any new analytical tool, performance should be thoroughly evaluated in order to understand the reliability of results in real-world samples. In this work, performance was assessed with calibration check solutions interspersed throughout regular samples. Additionally, spike recovery experiments were evaluated in a series of surface water samples.

Figure S8 shows the results for the calibration check solutions (n = 81; ca. 60 ng/L of each PPDQ). 6-PPDQ showed excellent performance, with a 73/81 (90%) calibration check solutions exhibiting bias <±30% and a relative standard deviation of ±21%. The most similar structural analogues IPPDQ and CPPDQ also performed well, both with 64/81 (79%) exhibiting bias < ± 30%. However, DPPDQ, DTDPQ, and 77PDQ showed poorer performance with inconsistent (DPPDQ, DTDPQ) or systematically biased recoveries (77PDQ). For 77PDQ, the observed concentration of the calibration check solution drops over the course of individual sample runs (Figure S9), with the first calibration check solution of each run exhibiting an average bias of 7 ± 28% and the final one (ca. 110 min later) exhibiting an average bias of −54 ± 44% (n = 10 runs). In contrast, for 6-PPDQ, these values were 10 ± 26 and −2.0 ± 26%, respectively (n = 15 runs). This suggests loss of 77PDQ from the uncapped vials during analysis, which is consistent with the uncapped vial stability study where 77PDQ exhibited the shortest half-life ($t_{1/2}$ = 14 h, Figure 2D). The more rapid loss observed here may be attributed to the continuous agitation on the autosampler tray. For DPPDQ and DTDPQ, some sample batches produced relatively reproducible and accurate quantitation of the calibration check solution (e.g., November 1st run with 9.5 ± 8.7% bias for DTDPQ and −7 ± 46.8% for DPPDQ, n = 5) punctuated by later batches exhibiting more inconsistent and/or inaccurate results (e.g., November 22nd run with −29.6 ± 40 and 61 ± 119% for DTDPQ and DPPDQ, respectively). We realized that the periods of good performance corresponded to when fresh acceptor phase had been prepared. We believe this is due to the sensitivity of these analogues to the formic acid concentration and the limited stability of formic acid in methanol, as it can be slowly esterified to form methyl formate.[31]

From this point onward, care was taken to ensure formic acid was added to the acceptor phase immediately before analysis. This approach improves the performance of these compounds as demonstrated by a spike recovery experiments for five real-world samples using fresh acceptor phase (Figure S10 and Table S3). Water quality parameters for these stream samples are available in Table S4. A low- (60 ng/L) and mid-concentration (125 ng/L) spike of the PPDQ suite were added to samples, and the recovery was measured against an unfortified sample. 6-PPDQ, IPPDQ, and CPPDQ again show excellent performance, exhibiting mean recoveries of 109 ± 14, 105 ± 13, and 100 ± 16%, respectively. With the fresh formic acid in the acceptor phase, DTDPQ now also shows excellent recoveries (mean of 106 ± 11%). For DPPDQ, the lower-concentration spike did not always produce a signal above the limit of detection. However, the mid-concentration spike shows good performance with recoveries between 66 and 120%. The loss of 77PDQ to the atmosphere was also apparent in this experiment, with the recovery dropping steadily as the run progressed.

Based upon the above results for the calibration check solutions (Figure S8) and spike recovery experiments (Figure S10 and Table S3), we are confident in the quantitative results for 6-PPDQ, IPPDQ, and CPPDQ in environmental samples. For DPPDQ and DTDQ, performance was significantly improved by the inclusion of fresh formic acid in the acceptor phase. However, because this was only implemented partway through the sampling campaign, our reporting for these analogues in this work should be considered semiquantitative. Similarly, experimental results suggest significant loss of 77PDQ to the atmosphere even during the analysis of a single autosampler tray (Figure S9). As such, reporting for 77PDQ using CP-MIMS should also be considered semiquantitative or even be relegated to presence/absence. While not evaluated systematically here, this effect can be mitigated by reducing the time samples are open to the atmosphere as was employed for Figure 2C. Alternatively, inclusion of an isotopically labeled standard for 77PDQ could compensate for this effect. We are working to obtain isotopically labeled standards for the full suite of PPDQ analogues to improve quantitative reporting.

Spatiotemporal Monitoring of PPDQs. Roadway runoff is an intrinsically diffuse problem, benefiting from local

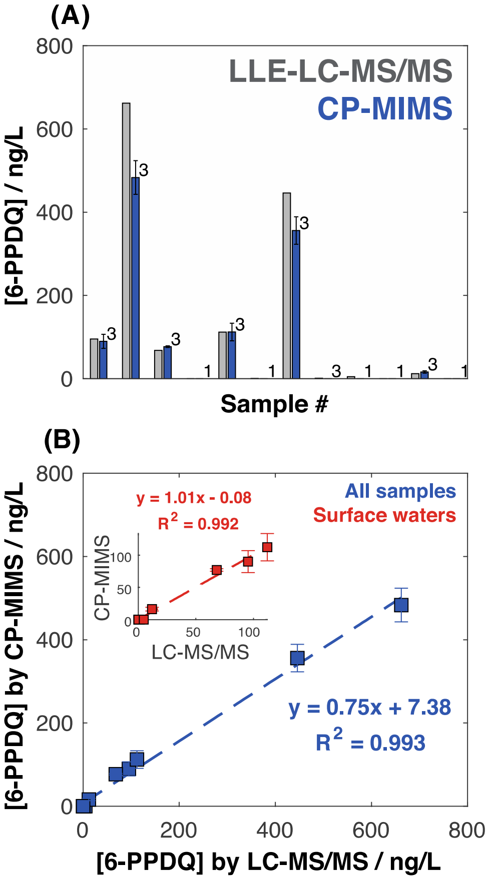

Figure 3. Quantitative agreement between CP-MIMS (blue, right bars) and a commercial LLE-LC-MS/MS method (gray, left bars) for analysis of trace 6-PPDQ. Good correlation is observed when comparing all samples (n = 12; R^2 = 0.993), and the two methods exhibit quantitative agreement for surface waters (n = 10; slope = 1.01, R^2 = 0.992). The numbers above CP-MIMS results in (A) indicate the number of replicate measurements taken for that sample.

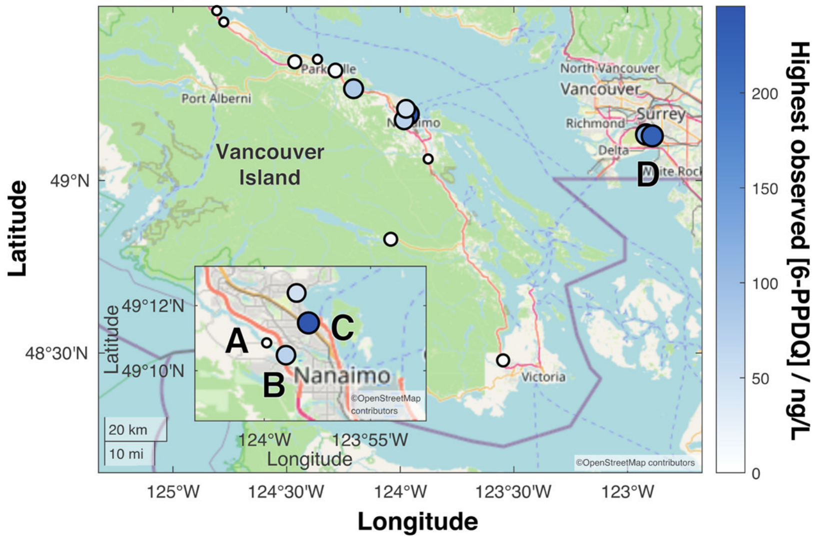

Figure 4. Map of eastern Vancouver Island, British Columbia (BC), Canada, and the lower mainland of BC summarizing observed concentration of 6-PPDQ in streams over the fall 2022 sampling campaign. Dots are sized and colored according to the maximum concentration of 6-PPDQ observed at each site across all sampling times. Indicated sites A−D are Millstone river up (A) and downstream (B) of a highway, Northfield creek (C), and Cougar creek (D).

expertise to identify threatened streams and potential inputs. This information can then be employed by regional authorities and municipalities to mitigate the risks of 6-PPDQ entering waterways and coastal environments. We partnered with 10 volunteer groups across coastal British Columbia, including local First Nations and stream keeper groups as part of a citizen science campaign to collect stream samples before, during, and after rain events. Where available, roadway runoff samples were also collected near stream sites, resulting in *ca.* 75 samples per rain event and 192 samples (168 stream samples and 24 roadway runoff samples) over the Fall 2022 season.

Concentrations of 6-PPDQ in stream water samples were relatively low (<150 ng/L; Table S5), and 6-PPDQ was detected in 20 of the 50 samples (40%) collected during rain events. Four of the 14 streams studied (28%) exhibited concentrations exceeding the LC_{50} for coho salmon (41−95 ng/L)[2,6] during at least one rain event. Substantial variability in concentration was observed both between sites (Figure 4) and for the same site over time (Figure 5). Where appreciable 6-PPDQ was observed, a distinct concentration pulse was generally observed across the before-during-after stream sample series. Smaller and/or more urbanized creeks were more prone to high concentrations, even during relatively small rain events. IPPDQ and CPPDQ were also observed, often co-occurring with 6-PPDQ at somewhat lower concentrations (20−80 ng/L; Tables S6 and S7). Higher concentrations were observed in the roadway runoff samples ($n = 24$; Figure S11), with the average 6-PPDQ concentration (360 ng/L) an order of magnitude higher than in surface waters (28 ng/L) collected during a rain event. These roadway runoff samples also exhibited concentration variability both site-by-site and across different rain events, but the average concentrations of 6-PPDQ (360 ng/L), IPPDQ (110 ng/L), and CPPDQ (200 ng/L) are generally in line with those observed by Cao et al.[15] In that work, runoff water in Hong Kong was measured, exhibiting median concentrations of 1120 ng/L (6-PPDQ), 560 ng/L (IPPDQ), and 60 ng/L (CPPDQ).

To validate the quantitative results obtained with CP-MIMS, 12 duplicate samples were collected at select sites for additional conventional analysis of 6-PPDQ using a liquid−liquid extraction−liquid chromatography−tandem mass spectrometry method (LLE-LC-MS/MS). Across these 12 samples (10 surface water samples and 2 roadway runoff samples), an excellent correlation was observed between the LLE-LC-MS/MS method and CP-MIMS (Figure 3; $R^2 = 0.993$). For the roadway runoff samples, CP-MIMS exhibited a negative bias of 22 and 30% relative to the LLE-LC-MS/MS results. This difference may be attributable to the fact that CP-MIMS probes the concentration of analytes that are free in solution. Given that these samples were unfiltered and that conventional LLE methods are more exhaustive, it is likely that they are recovering analyte bound to fine suspended solids (including tire particulates) prevalent in roadway runoff samples. It should be further noted that the stream water samples were measured within 5 days of collection (i.e., minimal if any loss expected; Figure 2B) while the roadway runoff was held for up to 100 days (some instability expected). When we compare quantitation for only the stream samples (inset in Figure 3B), excellent quantitative agreement is observed ($n = 10$; slope = 1.01, $R^2 = 0.992$). Residual plots for both the "all samples" and "surface waters" plots are available in Figure S12. We believe that this cross-validation reaffirms the value of CP-MIMS as a platform to quickly probe and/or answer sample-intensive research questions. As with any direct mass spectrometry method, we recommend prudence when interpreting results given the greater risk of interference in complex, real-world samples. In this work, multiple MS/MS transitions are monitored for each PPDQ analog. Atypical ion ratios between these transitions can indicate the presence of an interferent. This revealed that the m/z 299 → 215 MS/MS transition previously employed for quantitation[29] can lead to positive bias in the quantitation of 6-PPDQ. As such, we no longer recommend this transition for analysis of 6-PPDQ using CP-MIMS. Interference at m/z 299 → 215 may be due to the presence of isomeric ozonation products of 6-PPD. Zhao et al. recently characterized several such products, revealing three 6-PPDQ isomers.[32] Product ion scans for all three isomers exhibited m/z 215 fragment ions, but do not exhibit the

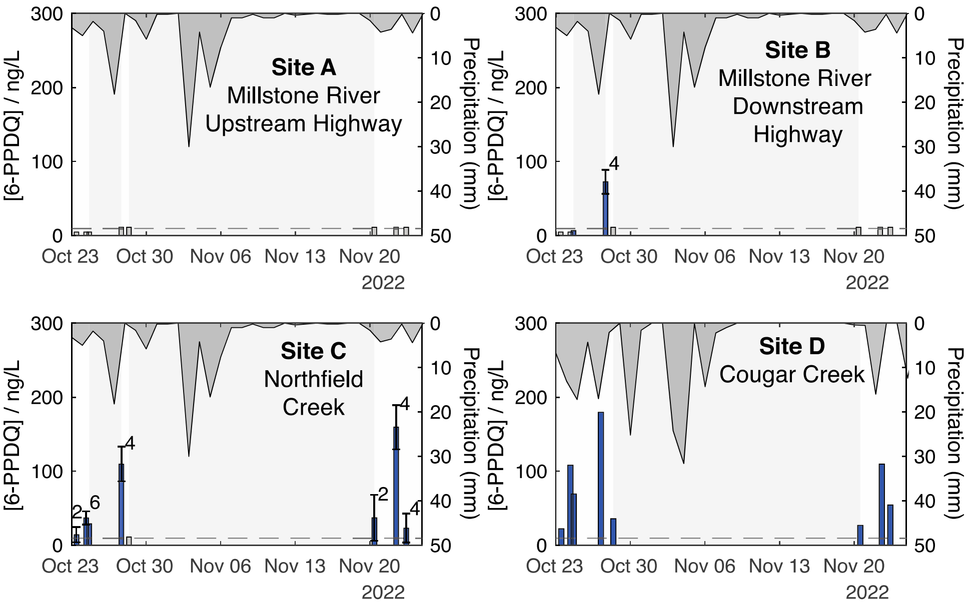

Figure 5. Concentration of 6-PPDQ over time for the 4 stream sites (A−D) indicated in Figure 4. The shaded gray area indicates the precipitation over time (reverse axis) for the nearest weather station at each site. Sites A and B are along the same river (Millstone), up- and downstream of a highway, respectively. While the upstream site (A) exhibits no 6-PPDQ above the limit of detection, the downstream site (B) reaches concentrations ≥50 ng/L during a ≥15 mm rain event. (C) and (D) are small urban creeks in a small (Nanaimo; Northfield) and large (Vancouver; Cougar Creek) city in British Columbia. In these urban creeks, even small rain events (5−10 mm) introduce significant levels of 6-PPDQ. Bars represent the average of *n* replicates (*n* indicated next to error bar), and the error bars represent the standard deviation across those replicates. Lighter gray shading in the background indicates periods where no samples were collected, most notably over the period of October 29−November 19.

fragment ions employed herein for MS/MS quantitation (*m*/*z* 256) or qualification (*m*/*z* 243 and 100). CP-MIMS can be employed as a rapid "first-tier" analysis to identify and quantify a large suite of samples, allowing for more efficient allocation of LC-MS resources to samples with highly consequential or suspect results (Figure S13). Here, we have reported all quantitative results and flag those with unusual qualifier ion ratios (blue italics in Table S5).

Figure 4 presents a geospatial map summarizing quantitative data for 6-PPDQ in streams across the sampling region. To visualize local "hotspots," points are sized and colored according to the maximum concentration observed for 6-PPDQ at each site. Similar plots for IPPDQ and CPPDQ are available in Figure S14. Numerous factors are expected to contribute to the PPDQ concentration in a given stream at a particular point in time, including the surrounding topology, overall stream flow,[44] and traffic density. Here, larger streams and those outside of city centers were generally observed to have lower 6-PPDQ concentrations (<20 ng/L); however, those within urban areas reached concentrations up to 180 ng/L. The time-course data for 4 stream sites (indicated as A−D in Figure 4) are provided in Figure 5. The shaded area indicates the precipitation from the nearest weather station for each site. A and B are sites along the same stream (Millstone), upstream (A) and downstream (B) from a highway. C and D are smaller, more urbanized streams in Nanaimo (Northfield creek) and Vancouver (Cougar Creek), BC, respectively. During the same rain events, these sites show significantly differing behavior. Throughout the first (~6 mm, October 23−24th) rain event, the larger system (B; Millstone) shows low concentrations of 6-PPDQ (<15 ng/L) while the more urbanized sites C and D rise to ca. 35 and 110 ng/L, respectively. However, during the second, larger (~20 mm, October 27−28th) rain event, the concentrations at all three sites (B−D) exceeded the coho LC_{50} by a factor of ca. 2−4.5. Through the third rain event captured (November 23−24th), only the two small, urbanized creeks (C and D) showed appreciable 6-PPDQ. The site upstream of the highway (A; Millstone) remains below the detection limit during all three rain events supporting the source as roadway runoff. At this stage, it is unclear whether the differences in concentration we observe in different streams are due to road runoff inputs (e.g., traffic density) and/or stream hydrology (e.g., flow). Further, the concentration of 6-PPDQ throughout a rain event is dynamic.[45] Given the nature of collecting samples through a citizen science network, there is some variability in precise sampling time relative to the rain event, which confounds detailed comparisons between sites. We are currently expanding sampling campaigns for wider surveillance to identify persistent hotspots and characterize site-specific flush dynamics for 6-PPDQ. Capturing this type of fine spatiotemporal scale data would be both time- and cost-prohibitive without the high-throughput measurement capacity afforded by CP-MIMS.

■ CONCLUSIONS

Direct mass spectrometry measurements employing tandem MS provide unique opportunities for high-throughput analysis, enabling greater spatial and temporal resolution in environmental assessments. Here, CP-MIMS is used to provide quantitative information on PPDQs at low ng/L levels at a throughput of up to 100 samples/day with only minor sample preparation (i.e., addition of internal standard). Quantitative comparison between a conventional method (LC-MS) and CP-MIMS for a series of surface waters shows excellent agreement (n = 10, slope: 1.01, R^2 = 0.992). The high-throughput capability of CP-MIMS is applied to answer fundamental questions about this emerging class of toxins (sample storage time, partitioning behavior), revealing significant loss to the atmosphere for PPDQs ($t_{1/2}$ = 14−108 h). These results suggest the need for careful experimental design and ongoing stability evaluation when studying 6-PPDQ and analogues to ensure reliable results. The method is employed to analyze 192 real-world stream and road runoff samples, representing the first spatiotemporal survey of PPDQs on Vancouver Island and the lower mainland of British Columbia (Canada). The resulting spatial and temporal variability of large sample sets can be followed up with conventional analysis, if and when required.

An overarching objective of this work is to provide molecular-level information when and where it is needed through the development of simple, high-throughput analytical techniques. Future work includes adapting the simple workflow enabled by CP-MIMS to fieldwork, allowing for real-time streamside measurements to characterize pulse dynamics and/or stormwater inputs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.3c00275.

> Full analyte names and structures (Figure S1), calibration data using different internal standard strategies (Figure S2), workflow for probing loss of PPDQs to the atmosphere (Figure S3), photos of autosampler construction and setup (Figure S4), overview of automated data processing (Figure S5), MS/MS cross-talk evaluation (Figure S6), preliminary sorption data (Figure S7), bias on calibration check solutions (Figure S8), bias on calibration check solutions as a function of analytical run time (Figure S9), spike recovery experiments (Figure S10), roadway runoff concentrations (Figure S11), residual plot for comparison of LC-MS and CP-MIMS (Figure S12), proposed workflow for complementary use of CP-MIMS and LC-MS (Figure S13), and "hotspot" mapping for IPPDQ and CPPDQ (Figure S14), MS/MS instrument parameters (Table S1), tabulated atmospheric loss data (Table S2), tabulated spike recovery results (Table S3), water quality data for spike recovery experiments (Table S4), quantitative results for 6-PPDQ (Table S5), IPPDQ (Table S6), and CPPDQ (Table S7) (PDF)

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Notes

The authors declare the following competing financial interest(s): Chris G. Gill and Erik T. Krogh hold patent US 9583,325 issued to none.

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ASSISTANT ADMINISTRATOR FOR CHEMICAL SAFETY AND POLLUTION PREVENTION
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Re: Petition ID No. 001845: Toxic Substances Control Act Section 21 Petition Regarding N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (CASRN 793-24-8, aka 6PPD) in Tires
- Final EPA Response to Petition

Dear Ms. Forsyth and Ms. O'Brien:

The U.S. Environmental Protection Agency received your petition dated August 1, 2023, submitted on behalf of the Yurok Tribe, the Port Gamble S'Klallam Tribe, and the Puyallup Tribe of Indians, requesting that the EPA "establish regulations prohibiting the manufacturing, processing, use, and distribution of N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine, CASRN 793-24-8, for and in tires under the EPA's TSCA Section 6(a) authority, 15 U.S.C. 2605(a), with such regulation to take effect as soon as practicable, in order to eliminate the unreasonable risk 6PPD in tires presents to the environment."

The EPA acknowledges that the Yurok, Port Gamble S'Klallam, and Puyallup Tribes are federally recognized Tribes with whom the EPA maintains a government-to-government relationship. The EPA recognizes that the Port Gamble S'Klallam Tribe is a signatory to the Treaty of Point No Point, while the Puyallup Tribe is a signatory of the Medicine Creek Treaty, and that under these treaties, the Port Gamble S'Klallam and Puyallup Tribes reserved the right to fish, hunt and gather. The EPA further acknowledges the importance of healthy and abundant salmon populations to these Tribes and to Tribal treaty rights.

This letter is to advise you that the EPA grants the petition. Specifically, the EPA plans to in the coming months: (a) commence a proceeding through issuance of an advance notice of proposed rulemaking for 6PPD under TSCA Section 6; and (b) initiate additional data gathering activities under TSCA to address data needed to understand and characterize risk associated with 6PPD-quinone and potential risks associated with 6PPD.

Statutory Requirements

TSCA Section 21(b)(1), 15 U.S.C. 2620(b)(1), requires that the petition "set forth the facts which it is claimed establish that it is necessary" to initiate the proceeding requested. 15 U.S.C. 2620(b)(1). TSCA Section 21's "necessary" language implicitly incorporates the statutory standards that apply to the requested actions. Accordingly, the EPA has reviewed this TSCA Section 21 petition by considering whether petitioners have established it is "necessary" to initiate a proceeding for a rule under TSCA Section 6. Notwithstanding that the burden is on the petitioners to present "the facts which it is claimed establish that it is necessary" for the EPA to initiate the proceeding sought, the EPA in its discretion also considered relevant information that was reasonably available to the agency during the 90-day petition review period.

TSCA prescribes the circumstances under which a TSCA Section 6(a) rulemaking may occur absent a TSCA Section 6(b)(4) risk evaluation. Thus, if a petitioner requests the initiation of a rulemaking under TSCA Section 6, the petitioner must establish that it is "necessary" for the agency to undertake a TSCA Section 6 rulemaking, and the relevant standard is found in TSCA Section 6(a), which specifies the Administrator must formally "determine" there is unreasonable risk before it may issue a rule under TSCA section 6. The purpose of the risk evaluation is to determine whether a chemical substance presents an unreasonable risk of injury to health or the environment, under the conditions of use, including an unreasonable risk to a relevant potentially exposed or susceptible subpopulation identified as relevant by the Administrator. As part of this process, the EPA must evaluate both hazard and exposure, exclude consideration of costs or other non-risk factors, use scientific information and approaches in a manner that is consistent with the requirements in TSCA Section 6 to use the best available science, and ensure decisions are based on the weight-of-scientific-evidence. As part of this process, the EPA must evaluate both hazard and exposure, exclude consideration of costs or other non-risk factors, use scientific information and approaches in a manner that is consistent with the requirements in TSCA to use the best available science, and ensure decisions are based on the weight-of-scientific-evidence. 15 U.S.C. 2605(b)(4)(F); 15 U.S.C. 2625(h) and (i). A TSCA Section 21 citizen petitioner need only present facts demonstrating that a chemical substance poses an unreasonable risk due to one or more conditions of use, not all conditions of use. See *Food & Water Watch, Inc. v. EPA*, 291 F. Supp. 3d 1033, 1052 (N.D. Cal. 2017).

Under TSCA Section 6(a), if the EPA determines that the manufacture, processing, distribution in commerce, use, or disposal of a chemical substance or mixture, or that any combination of such activities, presents an unreasonable risk of injury to health or the environment, the EPA conducts a rulemaking to apply one or more of TSCA Section 6(a) requirements to the extent necessary so that the chemical substance or mixture no longer presents such risk. In proposing and promulgating rules under TSCA Section 6(a), the EPA considers, among other things, the provisions of TSCA Sections 6(c)(2), 6(d), 6(g), and 9. In addition, to the extent that the EPA makes a decision based on science, TSCA Section

26(h) requires the EPA, in carrying out TSCA Sections 4, 5, and 6, to use "scientific information, technical procedures, measures, methods, protocols, methodologies, or models, employed in a manner consistent with the best available science," while also taking into account other considerations, including the relevance of information and any uncertainties. TSCA Section 26(i) requires that decisions under TSCA Sections 4, 5, and 6 be "based on the weight of scientific evidence." TSCA Section 26(k) requires that the EPA consider information that is reasonably available in carrying out TSCA Sections 4, 5, and 6.

Agency's Existing Commitment to Take Action on 6PPD

Notwithstanding the petition, the agency is firmly committed to fully protecting human health and the environment from adverse effects of exposure to 6PPD-quinone, a degradant of 6PPD. The EPA formed a cross-agency workgroup to facilitate inter-program office coordination for 6PPD-quinone in November 2022. This senior level workgroup is currently coordinating initiatives for addressing information gaps and commencing actions to address concerns regarding the use of 6PPD and adverse effects of the degradant 6PPD-quinone, including coordinating with external entities such as other federal agencies, Tribes, states, industry, and academia. Externally, the National Science and Technology Council's Joint Subcommittee on Environment, Innovation and Public Health offers the potential for cross-governmental coordinated research on human health effects. Further, EPA staff are actively involved with the Interstate Technology and Regulatory Council 6PPD-quinone workgroup, which has the goal of sharing information and coordinating among states and Tribal Nations. To learn more about how the EPA is addressing 6PPD and 6PPD-quinone, please see: *https://www.epa.gov/chemical-research/6ppd-quinone*.

The EPA's research activities to address these issues include planned studies in the Office of Research and Development's 2023-2026 research cycle, continued leveraging of the EPA Regional partnerships, and potential research collaborations with external entities. In the current ORD Strategic Research Action Plan (2023-26), there are multiple efforts which focus solely or in part on further investigation of 6PPD-quinone, including work on fate and transport, ecotoxicity, and green infrastructure solutions for stormwater contamination. Research activities include: Emission rates from motor vehicle brake and tire wear; ecological effects of tire wear particles and 6PPD-quinone on marine benthic communities; high-throughput hazard screening for 6PPD-quinone; development of metrics, models, and monitoring techniques to determine optimal green infrastructure placement and size for urban stormwater control; identification, assembly, and curation of toxicity data for ecologically relevant species for risk assessment (known as "ECOTOX"); and remediation of tire-related pollutants in stormwater.

ORD will continue leveraging EPA Regional partnerships to better understand the hazard of and potential exposure to 6PPD-quinone. Current ORD-Regional research collaborations include the following: Understanding airborne emissions and health impacts of 6PPD from tires conducted by EPA Region 3 and ORD; evaluating the bioactivity of the ubiquitous tire preservative 6PPD-quinone conducted by EPA Region 10 and ORD; fate, transport, and treatment of tire-derived pollutants in stormwater conducted by EPA Region 4 and ORD; and development of a rapid, low-cost bioassay to guide stormwater management and evaluate the potential toxicity of 6PPD alternatives conducted by EPA Region 10 and ORD.

In addition, EPA Region 10 has supported stormwater research via a federal interagency agreement with the U.S. Fish and Wildlife Service. EPA Region 10 also has funded state and academic research teams. Research publications resulting from EPA Region 10 funding contributions include: the seminal articles titled *A ubiquitous tire rubber-derived chemical induces acute morality in coho salmon* (Tian, et. al. 2021) that concluded mass pre-spawn mortality of coho salmon is linked to 6PPD-quinone found in stormwater runoff, and *Urban roadway runoff is lethal to juvenile coho, steelhead, and chinook salmonids, but not congeneric sockeye* (French et. al. 2022) that investigated additional salmonids. The EPA Region 10, through the Puget Sound Geographic Program, continues to fund relevant work on 6PPD and 6PPD-quinone, for example, via interagency agreements with the National Oceanic and Atmospheric Administration and the U.S. Geological Survey, and via cooperative agreement with the Washington Department of Ecology's Stormwater Strategic Initiative. Additionally, EPA Region 10 is working with the EPA's Office of Water to develop an analytical method for the detection of 6PPD-quinone as no standard method currently exists.

Summary of the Petition

The petition requests that the EPA promulgate a rule under TSCA Sections 6(a)(2)(A)(i) and 6(a)(5) to prohibit the manufacture, processing, use, and distribution of 6PPD in and for tires (Petition, pp. 1 and 16). The petition notes that 6PPD is present in "most if not all tires" and has been used in such products for more than six decades as an antioxidant and antiozonant to prevent tire degradation (Petition, pp. 1 and 6). The petition mentions that 6PPD is "highly reactive" by design, and can transform to the degradant 6PPD-quinone at the surface of a tire or when released into the environment (Petition, pp. 1 and 6). The petition describes the lethal effects for coho salmon exposed to 6PPD-quinone, as well as the presence of 6PPD-quinone in stormwater runoff and urban watersheds at levels "that can kill salmon, steelhead trout, and other aquatic organisms" (Petition, pp. 2 and 6). The petition also references the presence of 6PPD-quinone in "sediments and soils, road and household dust, and the urine of pregnant women, with emerging science pointing to toxicity in mammals and therefore potential risk to human health" (Petition, pp. 2 and 14).

EPA's Evaluation of the Petition

The petition, taken together with information reasonably available to the EPA, sets forth facts establishing that it is necessary to initiate a TSCA Section 6(a) rule to address risk to the environment from 6PPD-quinone, a degradant of 6PPD. The petitioners submitted sufficient evidence to show that 6PPD-quinone, a degradant of 6PPD, presents lethal hazards to coho salmon in the Pacific Northwest. This evidence supports a finding that 6PPD-quinone is acutely toxic to coho salmon at very low concentrations and additionally harms other fish species, with coho salmon being the most sensitive species studied to date. The petition also submitted evidence, in the form of measured exposure monitoring data, that there are exceedances of the LC_{50}, which is the concentration at which exposure results in mortality of 50 percent of animals in laboratory tests for coho salmon in the Pacific Northwest. Specifically, available information on 6PPD-quinone cited by petitioners indicates that concentrations in stormwater were found to be lethal for coho salmon following exposures that lasted only a few hours.

While the petition has shown that there is hazard from 6PPD-quinone, a degradant of 6PPD, and exposure to it, the petition alone does not demonstrate the facts which would establish that it is necessary to issue a TSCA Section 6(a) rule. TSCA Section 26(h)-(i) require the EPA to evaluate and impose requirements under section 6 using the best available science and based on the weight of the scientific evidence. Thus, a risk determination consistent with the scientific standards required by TSCA Section 26 must contain sufficient scientific evidence and analysis.

However, the EPA in its discretion may consider all reasonably available information when evaluating a petition, and has done so in this instance. The agency has and is developing (or can readily develop) additional scientific information on 6PPD and its transformation products, including 6PPD-quinone. For example, OW and ORD have worked collaboratively to conduct comprehensive literature searches and screening of ecotoxicity data for 6PPD and 6PPD-quinone. The literature searches are conducted as part of regular updates to the agency's ECOTOX Knowledgebase, a publicly available resource providing single chemical environmental toxicity data on aquatic and terrestrial species. OW is also currently developing draft screening values for 6PPD-quinone and 6PPD to protect sensitive salmon and other aquatic life, and is evaluating data quality as part of this effort.

To increase certainty associated with the exposure data, it would be beneficial to have data showing 6PPD in tires is the primary source for 6PPD-quinone in stormwater and surface water in the Pacific Northwest. To do so, the agency would need to collect and assess data on other products and processes where 6PPD is found, like footwear, synthetic turf infill, and playgrounds, including the relative volume or mass of 6PPD used in each product type, and additional research on fate and transport of tire wear particles. However, taken together, the data that are currently reasonably available to the EPA suggest a link between 6PPD use in tires and the presence of 6PPD-quinone in urban streams in the Pacific Northwest and warrants granting the petition.

Additionally, in proposing and promulgating rules under TSCA Section 6(a), the EPA considers the provisions of TSCA Sections 6(c)(2), 6(d), 6(g), and 9. When deciding whether to prohibit or ban a use, as requested by petitioners, TSCA Section 6(c)(2)(C) requires EPA to "consider, to the extent practicable, whether technically and economically feasible alternatives that benefit health or the environment, compared to the use so proposed to be prohibited or restricted, will be reasonably available as a substitute when the proposed prohibition or other restriction takes effect." The petition merely suggests that a TSCA Section 6(a) ban would "spur the technological innovation needed to develop alternatives to 6PPD." Nevertheless, the agency has met with the California Department of Toxic Substances Control to better understand the California Safer Consumer Products regulation which requires manufacturers of motor vehicle tires for sale in California to evaluate safer alternatives to 6PPD. Under this regulation, domestic and foreign manufacturers of motor vehicle tires that contain 6PPD and whose products are placed into the stream of commerce in California must submit a Priority Product Notification for those products by November 2023. Thereafter, manufacturers have the option to submit by March 2024 one of several notifications related to removal/replacement of or alternatives to 6PPD in products. The agency also met with the Washington Department of Ecology regarding similar efforts to assess 6PPD in products and the environment, including a cross-agency 6PPD Action Plan, a hazards assessment, an alternatives assessment, and the inclusion of 6PPD in the most recent five-year cycle to of the Safer Products for Washington program. EPA intends to coordinate its own efforts to develop information on alternatives to 6PPD with federal agencies, states, Tribes, industry, and academia, who are already engaged in this arena, including the aforementioned ITRC.

Expected Actions on 6PPD under TSCA
While the agency will promptly commence an appropriate proceeding under TSCA Section 6(a), the agency cannot commit to a specific rulemaking timeframe or outcome. The statute does not dictate the precise timing of any the agency actions and the EPA will decide on the details and scheduling during subsequent stages of the proceeding. The EPA also retains discretion to determine the content of any regulation that may be issued subsequent to a grant of the petition, which need not conform precisely to the petitioner's requested action(s). The agency intends to publish by Fall 2024 an ANPRM for 6PPD under TSCA Section 6(a) associated with risk management of 6PPD and 6PPD-quinone.

Currently, there are limited data to inform a human health risk assessment for 6PPD-quinone. The agency is committed to working with federal partners on coordinated research on human health effects. The EPA plans to utilize other TSCA authorities to collect data to understand and characterize risk associated with 6PPD-quinone and potential risks associated with 6PPD. Such actions will build on efforts underway among governments (e.g., federal, Tribal, and state), non-governmental organizations, academia, and industry, to ensure that any risk associated with 6PPD-quinone, and any potential risks associated with 6PPD are appropriately evaluated and managed. For example, the EPA intends to pursue a rulemaking under TSCA Section 8(d) to require persons who manufacture (including import) 6PPD to submit certain lists and copies of available unpublished health and safety studies conducted or initiated by, known to, or reasonably ascertainable by such manufacturers (including importers). The EPA aims to finalize the rule before 2025 with required reporting to occur 90 days after publication. Based on the information received through this reporting, the agency, as necessary, would consider requiring by rule(s), order(s), or consent agreement(s) the development of new information related to 6PPD pursuant to TSCA Section 4. Such information will serve to inform the EPA's subsequent decisions on how to proceed with any evaluation and any necessary mitigation of risks associated with 6PPD and its degradant 6PPD-quinone under TSCA.

The EPA appreciates Tribal leadership on the 6PPD-quinone issue, and is committed to considering the interests (including treaty reserved rights) of the Tribal governments described in the petition. The agency intends to offer consultation to federally recognized Tribal governments in accordance with the EPA Policy on Consultation and Coordination with Indian Tribes.

Thank you for you continued interest in reducing exposure to 6PPD and 6PPD-quinone. If you have any questions relating to your petition or the EPA's guidelines for TSCA section 21 petitions, feel free to contact Thomas Groeneveld of my staff at (202) 566-1188 or *groeneveld.thomas@epa.gov.*

Sincerely,

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