# Protecting Salmon from Tire Wear Toxicants:

# 6PPDQ Workshop 2025

# Post-Workshop Summary

# Protecting Salmonids from Tire Wear Toxicants: 6PPDQ Workshop 2025

## Post-Workshop Summary

Nanaimo, British Columbia

**Prepared by:**

British Columbia Conservation Foundation's Aquatic Research & Restoration Centre

&

Vancouver Island University's Centre for Health & Environmental Mass Spectrometry

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# ACKNOWLEDGEMENTS

We are grateful that the *Protecting Salmonids from Tire Wear Toxicants: 6PPDQ Workshop 2025* was able to be hosted on the traditional and unceded territory of the Snuneymuxw Frist Nation at Vancouver Island University (VIU) in Nanaimo, British Columbia (BC). We would like to extend a special thank you to Joan Brown, Snuneymuxw First Nation Elder and Chief Administrative Officer, who provided a wonderful welcome prayer and opening remarks to start the workshop off in a good way. A further thank you to VIU Interim Vice President of Research and Graduate Studies at VIU, Dr. Eve Stringham, for welcoming all presenters and attendees to the university at the start of day one.

A special thank you to Dr. Tim Rodgers (University of British Columbia), Jane Pendray (Pacific Salmon Foundation; PSF), Marissa Waddell (PSF), Jim Shinkewski (PSF), and Sylvie Spraakman (City of Vancouver) for contributing your time and efforts to assist with the planning and implementation of this workshop. We'd also like to thank the Vancouver Island University's Centre for Health & Environmental Mass Spectrometry and BC Conservation Foundation's Aquatic Research & Restoration Centre's staff for assisting with the set up and take down of the event. In particular we would like to thank Angelina Jaeger, Lily Eggert, Niki Gholamiaval, Owen Dyck, Tess Weaver, Ally Badger, Liam Whetter, and Jamieson Atkinson.

Additionally, thank you to the funders of the workshop, including the BC Salmon Restoration and Innovation Fund (BCSRIF) and the Pacific Salmon Foundation – the gathering would not have been possible without this support.

Further, thank you to all of the presenters of the event for preparing for and attending the even to share the work they are doing. Additionally, for contributing to reviewing this summery report, ensuring that all information is accurate prior to its release.

Finally, a huge thank you to all of the presenters and attendees that were able to make their way to Nanaimo for this event – it would not have been the same without such an incredibly engaged group of people!

# ACRONYM LIST

| Acronym | Definition |
| --- | --- |
| 6PPD | N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine |
| 6PPDQ | N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone |
| AA | Alternatives analysis |
| BBB | Blood-brain barrier |
| BC | British Columbia |
| BCCF ARRC | British Columbia Conservation Foundation's Aquatic Research & Restoration Centre |
| BCSRIF | British Columbia Salmon Restoration & Innovation Fund |
| BMP | Best management practices |
| CHEMS | Centre for Health & Environmental Mass Spectrometry |
| CP-MIMS | Condensed Phase Membrane Introduction Mass Spectrometry |
| DPA | Diphenylamine |
| DTSC | Department of Toxic Substances Control |
| ECCC | Environment & Climate Change Canada |
| ELS | Early life stage |
| eNGO | Environmental non-government organizations |
| EU | European Union |
| GSI | Green stormwater infrastructure |
| MF-TAP | Management framework for tire additive pollution |
| MOTT | Ministry of Transportation & Transit |
| NGO | Non-government organization |
| PSF | Pacific Salmon Foundation |
| SCPP | Safer Consumer Products Program |
| SFU | Simon Fraser University |
| TAAP | Tire additives alternatives panel |
| TAHEEP | Tire additives health and environmental effects panel |
| TWP | Tire wear particles |
| UBC | University of British Columbia |
| URMS | Urban runoff mortality syndrome |
| USA | United States of America |
| USEPA | United States Environmental Protections Agency |
| USGS | United States Geological Survey |
| VIU | Vancouver Island University |
| WQG | Water quality guidelines |

# EXECUTIVE SUMMARY

The *Protecting Salmon from Tire Wear Toxicants: 6PPDQ Workshop 2025* was hosted on May 8 and 9, 2025 at Vancouver Island University by the British Columbia Conservation Foundation's Aquatic Research & Restoration Centre (BCCF ARRC) and Vancouver Island University's Centre for Health & Environmental Mass Spectrometry (VIU CHEMS).

This workshop intended to bring together those working on understanding the fate and distribution of 6-PPDQ, its impacts on ecosystem health, and mitigation strategies. The workshop hosts see this workshop as an important knowledge-sharing gathering to ensure that science is robust, relevant, and accessible.

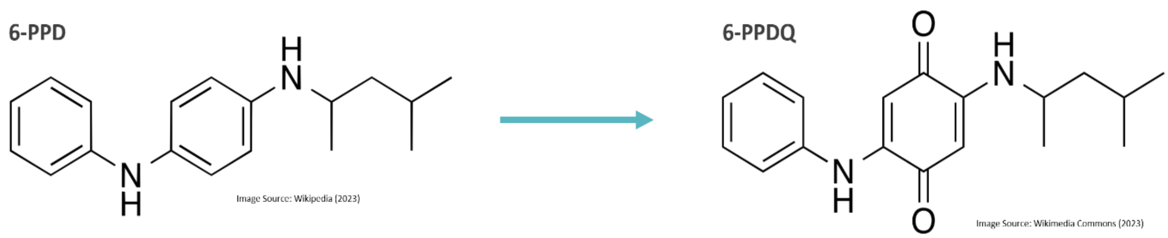
Overall, approximately 150 people, representing 75 different organizations, attended the workshop. The first day focused on policy and solutions, while the second day had presentations regarding monitoring efforts and toxicology studies. Each day began with a keynote presentation, followed by one of the day's sessions.

The first day was opened by Shane Petersen, who provided an overview of the BCSRIF. Following that, Dr. Jenifer McIntyre provided an introduction to the discovery of 6PPDQ, including current and ongoing research. Presentations by J. Richard W. Hall, Ali Azizishirazi, Kelly Grant, and Tim Rodgers provided insights into existing and proposed policies, regulations, and management strategies for 6PPDQ in Canada, USA, and internationally. The afternoon session was divided into two parts – the first half had Sylvie Spraakman and Amanda Rust reviewing current green stormwater infrastructure (GSI) and stormwater management strategies, while the second half highlighted GSI initiatives that have been initiated and completed by eNGOs and community led stewardship organizations (presentations by Jim Shinkewski, Kyle Armstrong, Carolynne Robertson, and Caitlin Pierzchalski). All three sessions from the day had separate panel discussions that took questions from workshop attendees.

The second day's first session highlighted two of the large BCSRIF funded projects (presented by Erik Krogh, Angelina Jaeger, Tanya Brown, and Mason King), as well as two other researcher's monitoring efforts (Rhea Smith and Cindy Yang). Presentations included preliminary results and lessons learned throughout their research programs to date. The afternoon session had presentations on current and recently completed toxicology-focused studies (presentations by Katie Roberts, Hui Peng, Kyle Duncan, and Nathan Ivy). Both the morning and the afternoon sessions each had separate panel discussions that took questions from the workshop attendees.

# 6PPDQ OVERVIEW

Recognized in the 1990s as urban runoff mortality syndrome (URMS), it wasn't until 2020 that the cause of the mass mortalities to returning coho salmon in Puget Sound urban creeks was identified (Scholz et al. 2011; Tian et al. 2021). A compound known as N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is added to rubber products, including tires, as an antiozonant – utilized to react with ozone and prolong the tire's life span. When 6PPD reacts with ozone, it produces N-(1,3-dimethylbutyl)-N-phenyl-p-phenylenediamine quinone (6PPDQ; Figure 1), which is the compound identified to cause URMS in coho and other salmonid species. As tires wear, particles are left on roadways until they are washed into waterways through stormwater runoff or snowmelt, where the contaminants are able to interact with and impact vulnerable species.




***N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine***

***N-(1,3-dimethylbutyl)-N-phenyl-p-phenylenediamine-quinone***

**Figure 1.** Compound added to tires as an antiozonant, known as 6PPD (left), and its transformative compound, 6PPDQ (right) that is toxic to salmonids.

Stormwater runoff appears to be the largest contributor of 6PPDQ to waterways, directed by sloped impervious surfaces and stormwater infrastructure. URMS can be induced in a matter of hours when toxic concentrations (parts-per-trillion or ng/L) enter waterways. Coho salmon were the first to be identified as vulnerable to 6PPDQ (Tian et al. 2021). After further study, similar fates have been found for rainbow trout/steelhead, brook trout (Brinkmann et al. 2022), Chinook salmon (French et al. 2022), coastal cutthroat trout (Shankar et al. 2025), and lake trout (Roberts et al. 2025).

# WORKSHOP OVERVIEW

The *Protecting Salmon from Tire Wear Toxicants: 6PPDQ Workshop 2025* was hosted at Vancouver Island University (VIU) on May 8 and 9, 2025 by the British Columbia Conservation Foundation's Aquatic Research & Restoration Centre (BCCF ARRC) and VIU's Centre for Health &

Environmental Mass Spectrometry (CHEMS). Planning for the event was supported by the University of British Columbia (UBC), Pacific Salmon Foundation (PSF), and the City of Vancouver. Funding for the event was provided by the British Columbia Salmon Restoration & Innovation Fund (BCSRIF) and PSF.

This workshop is intended to bring together those working on understanding the fate and distribution of 6PPDQ, its impacts on ecosystem health, and mitigation strategies. The workshop hosts see this workshop as an important knowledge-sharing gathering to ensure that science is robust, relevant, and accessible to all. It is important that many dimensions of this issue are thoroughly assessed, which will require a collective approach.

# ATTENDEE SUMMARY

In total, approximately 150 individuals representing 75 different organizations attended the two-day workshop. Most participants attended both days of the event. Organizations represented included environmental non-government organizations (eNGOs; 11), local First Nations and First Nations-led organizations (12), academic institutions (9), community stewardship groups (14), environmental consultants (13), provincial government (BC government – 4 ministries were represented), federal governments (2; 4 Canadian departments, 1 United States of America (USA) department), regional governments (3), municipal governments (8), and industry organizations (2).

**Figure 2.** Dr. Tim Rodgers presenting on day 1 of the workshop. Photo: VIU Communications

# WORKSHOP LAYOUT

The workshop occurred over two days, each being divided into two sections. The first day (May 8, 2025) focused on policy and solutions, while the second day (May 9, 2025) had presentations regarding monitoring efforts and toxicology studies. Each day began with a keynote presentation, followed by one of the day's sessions.

The first day had provided insights into existing and proposed policies, regulations, and management strategies for 6PPDQ in Canada, USA, and internationally. Additionally, the afternoon had speakers presenting on engineering and solutions. The afternoon session was divided into two parts – the first half reviewed current green stormwater infrastructure (GSI) and stormwater management strategies, while the second half highlighted GSI initiatives that have been initiated and completed by eNGOs and community led stewardship organizations. All three sessions from the day had separate panel discussions that took questions from workshop attendees.

The second day's first session highlighted two of the large BCSRIF funded projects, as well as two other researcher's monitoring efforts, including preliminary results and lessons learned throughout their research programs to date. The afternoon session had presentations on current and recently completed toxicology-focused studies. Both the morning and the afternoon sessions each had separate panel discussions that took questions from the workshop attendees.

Throughout the workshop, information booths and research posters were displayed in some of the surrounding break rooms. In total, there were nine information booths displayed by eNGOs, community led stewardship groups, federal government initiatives (BCSRIF), and environmental consultants. Additionally, around 15 posters were displayed and presented by current students and community group representatives (posters that were shared by presenters are included in Appendix B – ). Further, large maps of Vancouver Island and the Lower Mainland were secured to the wall; stickers were provided for participants to add their sample locations and locations where GSI currently exists.


**Figure 3.** BCSRIF information booth. Photo: VIU Communications

Prior to the initial presentations given on the first day of the workshop, welcoming remarks and prayers were provided by Joan Brown, Snuneymuxw First Nation Elder and Chief Administrative Officer, to start the workshop off in a good way. Additionally, the Interim Associate Vice President of Research and Graduate Studies at VIU, Dr. Eve Stringham, provided some words of welcome to those attending the workshop.

# SUMMARY OF OPENING REMARKS & DAY 1 KEYNOTE SPEAKER

## Shane Petersen – BCSRIF Pacific Salmon Strategy Initiative

The Manager of Partnerships & Outreach for the BC Salmon Restoration and Innovation Fund (BCSRIF), Shane Petersen, provided some opening remarks highlighting the BCSRIF program and the important work that it has been able to support over the past six years. In total, 170 projects focused on salmon habitat restoration, stock monitoring, sustainable selective fishing, catch monitoring, hatchery operations and more have been supported with $257.1 million.

This funding has allowed for diverse partnerships and salmon-focused projects to occur across much of BC. The BCSRIF funding has been applied to all stages of the salmon life cycle from the marine environment to their natal stream's headwaters. Although making up a modest portion of the portfolio, there have been two 6PPDQ-focused programs funded through BCSRIF, one on

Vancouver Island (*Mitigating Inputs of Tire Wear Toxins to Protect Salmonid Habitat on Vancouver Island*) and one in the Lower Mainland (*Identifying and Mitigating Hot Spots of Salmon Exposure to Toxic Road Runoff*). These programs have had extensive geographic coverage in their sampling and exemplify the best results of BCSRIF to date, most notably regarding collaboration. 6PPDQ research is especially important to salmon systems near urban centres and along roadways throughout BC, Canada, and the United States.

Some of BCSRIF's highlights to date include:

- Employment: Supporting an average of over 750 jobs per year, including opportunities for all employment equity groups.
- Indigenous Participation: Indigenous organizations lead 40% of BCSRIF-funded projects, making them the most represented organization type.
- Collaboration: Over seven years and three application intakes, more than 230 organizations have participated in BCSRIF funded programs.
- Salmon: In total, 45 stock management units have benefited from BCSRIF funding.

The second (and current) phase of BCSRIF is scheduled to end on March 31, 2026.

## Jenifer McIntyre – 6PPD-Quinone Toxicity Research: Retrospective to Prospective from Washington State

Dr. Jen McIntyre, from Washington State University and Puyallup Research & Extension Centre, provided the keynote presentation on the first day of the workshop, providing the background of 6PPDQ and some of the current research initiatives her team has underway.

It is well known that every fall Pacific salmon return to spawn in their natal streams – at least that's what is supposed to happen. However, in the early 2000s, they began documenting coho salmon dying in creeks before they matured or spawned, appearing to have been in good health. Although they only began discovering these mortality events in the 2000s, it had been an issue for decades. It wasn't until large-scale physical restoration efforts in urban creeks began in the late 1990s that people were paying close attention to the creeks. With a steady source (net pens operating since 1993) of coho returning to urban creeks and volunteers/researchers conducting post-construction monitoring of restored habitat in the fall, observations of sick fish were finally recorded.

In 2004, Jen was the first coordinator of pre-spawn mortality field studies to document the extent of this issue. Unfortunately, the phenomena was widespread and recurrent, with pre-spawn mortality rates as high as 60% to 100% in urban creeks. These results were first summarized in a paper in 2011. A comparison of mortality rates across 21 basins in Puget Sound showed that areas with the busiest roadways had the highest mortality rates. Exposures to stormwater runoff did result in acute behaviour changes and pre-spawn mortality. Further studies demonstrated that all life stages of free-swimming (i.e., alevin and juveniles) coho are susceptible to mortality when exposed to stormwater at low concentrations and over short durations.

Jen and her colleagues tracked the progress of the changed behaviour and ultimately the mortality in juvenile coho. When exposed, they began showing increased surfacing behaviour within the first hour, which progressed to continuous swimming at the surface before losing equilibrium and ultimately becoming incapacitated at approximately five hours of exposure. Mortality occurred around hour six in this study. Fish displaying the surface swimming were removed and placed in clean water where they dyed at approximately the same rate as those that remained in the stormwater.

By testing the blood of impacted coho, the research team found evidence of osmoregulatory imbalance (low sodium and chloride), which is a stress response, and that it is hemoconcentrated, meaning that the blood is twice the thickness of a typical coho. To further investigate this, they injected a fluorescent dye into the heart of impacted fish at different time points in their exposure. They measured the fluorescence that remained in the body, which only remains in locations where it had leaked from the vascular system into the surrounding tissues. Results indicated that the dye was able to leak into various organs, including the brain, which is normally protected by the blood-brain barrier (BBB). Leaking was present in all individuals that showed symptoms, as well as in some that were not yet symptomatic. Imaging of the brain confirmed that the leaking through the BBB is extensive and severe. Further review indicated that the cells that maintain the BBB were still intact, suggesting that it was signaled to open, rather than being damaged and leaking through.

Jen and her colleagues began investigating other species. Chum salmon were assessed, as their migration period overlaps with coho and they had been observed in the field successfully spawning next to dying coho. When exposed, no mortality or sickness was found in any tested chum; additionally, blood parameters indicated no changes after exposure to stormwater.

Further toxicology studies were conducted on juvenile steelhead, Chinook salmon, and sockeye salmon. During a 24-hour exposure steelhead and Chinook were identified as vulnerable and sockeye appeared unaffected. Other studies have now found other salmonid species to be acutely sensitive, including rainbow trout, brook trout, coastal cutthroat trout, and lake trout.

Stormwater runoff is a complex chemical soup with many of the chemicals found within having never been previously identified. To begin identifying the causative agent, chemicals were first assessed by their potential source and those associated with tires were linked to toxicity to coho. A tire leachate solution was fractioned to reduce its complexity. Each fraction was exposed to coho and the fraction identified to cause the mortality was then again fractioned further, ultimately getting to a solution of only four chemicals. The final fraction was dominated by an unknown compound, which didn't appear to be a known ingredient in tires. Zhenyu Tian ultimately associated the compound with 6PPD. The research team obtained industrial 6PPD, ozonated it, and produced 6PPDQ. Validated through a toxicity test, 6PPDQ was identified as the cause of acute mortality (6PPD produced little to no acute mortality). When compared to other toxic chemicals to aquatic life, 6PPDQ is among the most toxic chemicals we are aware of.

Following the research team's findings, legal action at both state and federal levels in the US was triggered because salmon have federal protections. The federal government has obligations to uphold tribal treaty rights and the US Clean Water Act. Failure to protect salmon from water quality threats would violate these legally binding treaties and legislation. The Clean Water Act guides water quality standards like the Aquatic Life Criteria at the State level. In 2024, Washington State adopted an Aquatic Life Criteria for 6PPDQ that states concentrations in waterways should not exceed 12 ng/L for more than one hour.

Current ongoing solutions research has included green stormwater infrastructure (GSI), which encourage runoff to spread out and infiltrate into soils instead of running directly into surface waters. Jen's team has done previous studies to assess if running stormwater through a filtration system would prevent acute mortality in coho; results indicated that there were no acute effects, which was the case for all free-swimming life stages.

A recent study assessed bioretention media of three depths (6", 12", and 18") – each was dosed with roadway runoff. The runoff was applied at an accelerated rate to simulate 13 water years of treating runoff. Toxicity tests indicated that mortality was prevented and more than 90% of 6PPDQ that was introduced was removed. The only limitations experienced throughout the simulated timeframe included some physical clogging, which was remediated by scrapping the top 1” of media off.

Although GSI is proving to be beneficial, there is still a high percentage of impervious surfaces across Puget Sound and urban centres. Some of the best chances for implementation of GSI is in new developments where construction does not need to work around pre-existing infrastructure. Due to cost and space limitations with GSI, a final solution to prevent acute mortalities is source control of 6PPDQ. The tire industry through the US Tire Manufacturers Association has been responsive and are taking the next steps to find a replacement for 6PPD. As part of this, students in Jen’s lab have begun assessing other anti-ozonants that could be considered as substitutes for 6PPD.

# SUMMARY OF 6-PPDQ POLICY OVERVIEW PRESENTATIONS & PANEL DISCUSSION

The policy panel and associated discussion was chaired by Jane Pendray, PSF’s Salmon and Climate Adaptation Program Manager.

## J. Richard W. Hall – 6PPDQ: First Nations & Environmental Toxicants: Legislative & Policy Issues

Richard Hall is a Barrister and Solicitor with over 40 years of experience practicing law in BC. Richard has worked with numerous First Nations across BC regarding environmental, legislative and policy issues.

Richard began with recognizing the importance of salmon to coastal First Nations and how integrated salmon are within First Nations culture. Impacts to salmon directly impact the people that depend on them.

Some of the present challenges with regards to policy and regulation involve the approach that is taken. Often when developing policy and regulation, they are developed in silos rather than taking a holistic approach. This involves collaboration with all different types of organizations including all levels of government, academic/research organizations, and the general public.

Existing legislative framework in Canada that could help direct 6PPDQ legislation include:

- Section 35 of the *Constitution Act* (1982): recognizing the existing Aboriginal and treaty rights of Aboriginal Peoples of Canada;

- *United Nations Declaration on the Rights of Indigenous Peoples Act*: a Federal Act that indicates 'Indigenous Peoples and individuals have the right not to be subjected to forced assimilation or destruction of culture' (with salmon being a major component of First Nation's culture on the west coast); and
- *Declaration on the Rights of Indigenous Peoples Act*: a BC Provincial Act for which the Province of BC must consider Indigenous People's languages, culture, practices, rights, legal traditions, institutions, governance structures, relationships to territories and knowledge systems.

To ensure the survival of coho salmon, regulation and policy development require more First Nations engagement, public education, funding support for research, research coordination and collaboration, an advocacy campaign, and political action. A few good examples of co-governance models that have been proven to be successful include the Mackenzie Valley Land and Water Board in the Northwest Territories, the Cowichan Watershed Board on Vancouver Island, and the Nicola Valley Watershed Governance Partnership in the BC Interior. Richard rounded out the presentation reminding everyone that "we are of one mind and heart".

## Ali Azizishirazi – 6PPDQ Water Quality Guidelines for the Protection of Aquatic Life

Ali Azizishirazi is a Water Quality Guidelines Specialist with the BC Ministry of Water, Land, and Resource Stewardship. Ali led the team that developed the recently released *6PPD-quinone Acute Water Quality Guidelines – Freshwater Aquatic Life* and this is what he presented on.

Water quality guidelines (WQG) are where science and policies meet both provincially and federally. WQG are used as benchmarks of comparison for physical, chemical, and biological parameters; they exist for water, biota, and sediment. WQG are not legally binding unless used in an authorization, such as a permit; they are most typically used for monitoring purposes. Existing policy is what defines the level of protection that occurs and guides the development of the guidelines. In BC, we need to protect 100% of species at all life stages. Long-term chronic WQGs are created to protect all forms of aquatic life from lethal and sub-lethal effects throughout indefinite exposures. Short-term acute WQGs are created to protect against severe effects like lethality over short-term intermittent and/or transient exposures to contaminants (i.e., spills, infrequent releases). There is very little chronic data that exists for 6PPDQ and more would be required for a long-term chronic WQG to be developed.

As 6PPDQ was only discovered in 2020, there had not been enough data to confidently develop a guideline until recently. In 2021, the Province of BC began supporting further toxicology studies to assess different species vulnerability to 6PPDQ to build up further data to inform WQGs. Toxicology data indicates that vulnerability varied amongst species, including at which concentrations they were impacted. All existing data that had been generated was utilized to develop the WQGs. Despite there being a growing set of data, there is still a lot of sources of uncertainty; as a result, an assessment factor of four was used to direct the guidelines. Using the most sensitive species (coho) at its most vulnerable life stage (juvenile), the lethal concentration (41 ng/L) was divided by four, resulting in a guideline of 10 ng/L, which is similar to the US Environmental Protection Act guideline.

## Kelly Grant – Driving Change: California's Approach to Safer Alternatives to 6PPD in Tires

Kelly Grant has been a Scientist for the California Department of Toxic Substances Control (DTSC) in the Safer Consumer Products Program (SCP) since 2018. She has been part of finding solutions to problematic chemicals in products, including 6PPD in tires.

The goal of SCP is to ensure the design, development, and use of products are safe for people and the environment. If DTSC lists a 'Priority Product', manufacturers are required to systematically evaluate alternatives and try to identify those that are safer for humans and/or the environment. Product manufacturers bear this responsibility because they have the knowledge of the chemistry that will work in their products. DTSC recognized the importance of Tian et al. (2021) and listed 6PPD as a Priority Product just eight months after the publication.

The primary purpose of an Alternatives Analysis (AA) is to prevent adverse impacts associated with a potential alternative. Preliminary stages of an AA require manufacturers to screen a wide range of alternatives, while the Final AA involves a more in-depth assessment of the top alternative candidates. The investigations need to consider product performance and adverse impacts across the life cycle of the product. Regrettable substitutes would be ones that reduce durability, increase rolling resistance, and similar or worse hazard traits.

6PPD is integral to the safety of tires and has been used since the 1970s. Alternatives for 6PPD had not been an active area of research and development; therefore, alternatives were not readily available. Tire-derived chemicals are broadly detected throughout the environment and have widespread exposure concerns. It is reasonably assumed that any alternative will be similarly pervasive in the environment. As a result, the AA must assess the impacts beyond fish toxicity to ensure that the new alternative won't have other adverse impacts in the environment or for human health. The Preliminary AA was reviewed in August 2024. Collectively, the global tire manufacturers selected 20 alternatives that will be assessed in the Final AA, which is due in August 2026. Part of the Final AA will involve a public comment period; all are encouraged to submit comments.

Once the Final AA is complete, regulatory responses could ensue to reduce adverse impacts, including but not limited to generation and submission of critical data, providing information to consumers, implemented restrictions and/or sales prohibition, engineered or administrative controls, and/or requirements for end-of-life management. In the event a safer alternative is not identified, there may be requirements for manufacturers to invest in or advance their green chemistry and engineering.

## Tim Rodgers – Turning the Corner on Hazardous Tire Additives: A Management Framework for Tire Additive Pollution

Tim Rodgers is a postdoctoral fellow in the Department of Civil Engineering at the University of British Columbia (UBC). Aside from his ongoing research that looks at the fate and behaviour of toxic chemicals in the environment, he has been developing a management framework for tire additive pollution, which is what he presented on at the workshop and was recently published and is freely available here.

The motivation to develop a management framework is that tires are a global pollution challenge. It is estimated that total tire particle emissions are over one million tons across the USA alone, which is twice the total emissions of pesticides. Tires work in challenging environments and are therefore made of complex mixtures. With so many different types of chemicals involved, tire particle emissions are an important source of chemical emission in the environment. The discovery of 6PPDQ's acutely toxic impacts to coho salmon is just one example of the potential for severe effects from tire additives and their transformation products through unforeseen chemical fate pathways.

Tim showed a map indicating the 'maximum tire wear particle emissions intensity' across the Greater Vancouver region. Areas with the highest emission potential are urban areas where road networks are most dense. The widespread road networks also mean that there are pervasive aquatic exposures, with the highest emissions along highway corridors and in urban areas. Expanding out, research has indicated that 6PPD and 6PPDQ are being detected in humans, as well as deep sea sediments; therefore, tire emissions are demonstrating a global emission challenge (Figure 4).

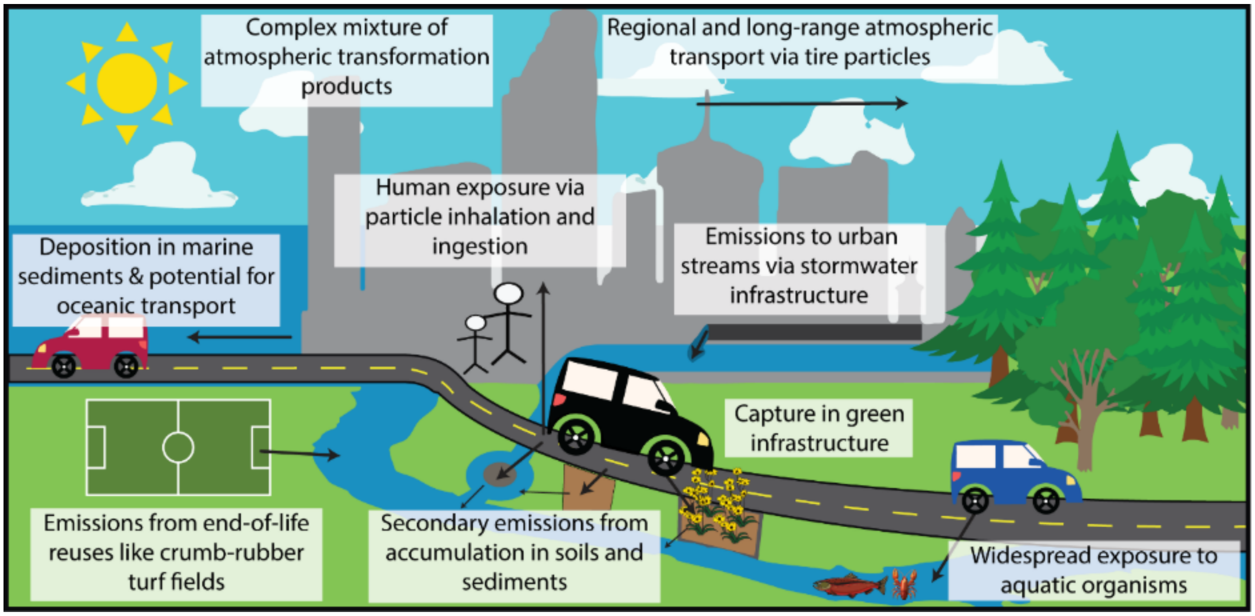



**Figure 4.** Schematic diagram of tire additive releases, exposures, and transport and fate pathways. Image: Tim Rodgers

Tim's team believes that a strong response to the global pollution challenge posed by tire additives should follow five principles:

1. Non-hazardous tire additives: Additives should be non-toxic to humans or ecological receptors and should not be persistent, bioaccumulative, or mobile through soil;
2. Life-cycle application: Management practices should apply across all phases of a tire's life cycle;
3. Transparent tire composition: A complete list of chemical ingredients (including tire additive formulations) should be made publicly available;
4. Characterizing hazards: Research and monitoring of human health and environmental effects of existing and proposed tire additives should be coordinated and expanded; and

5. International harmonization: Management of tire additives should proceed with an international agreement and approach.

At a national level, tire additives are covered under existing chemical management legislation. 6PPD in on the permitted substances list in the European Union (EU), Canada, USA, China, and Japan – it had been 'grandfathered in' and has received less scrutiny than new substance have. Canada, USA, and EU have begun processes to assess 6PPD. The national chemical management programs are moderately successful at the non-hazardous tire additives, life cycle application, and characterizing hazards principles, as some jurisdictions look at human and environmental health hazards and have led to additional resources to assess alternatives. None of the regulations meet the transparent tire composition or international harmonization principles.

International chemical management is largely driven by three main treaties. *Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal* applies at the end of the product's life – waste tires are listed as hazardous waste. *Stockholm Convention on Persistent Organic Pollutants* applies to chemicals that are persistent, bioaccumulate, and toxic. The *Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade* applies to trade in chemicals – countries have the right to know about the hazards associated with a chemical and that other countries must help to enforce national bans. All three treaties meet the 'international harmonization' principle. Further, for the chemicals listed as persistent organic pollutants, the Stockholm Convention is fairly protective. Otherwise, these three treaties don't really comply with the five principles.

Overall, current practices are insufficient at managing tire additives. To address this, Tim's team developed a 'Management Framework for Tire Additive Pollution' (MF-TAP) to match their principles. Modeled in part on the *Montreal Protocol for Ozone Depleting Substances*, there would be four operational mechanisms: [1] phased transition from hazardous tire additives; [2] robust transparency and disclosure requirements; [3] scientific oversight through independent expert panels; and [4] implementation through internationally coordinated governance.

The US Tire Manufacturers' Association has stated that it would take 8 to 13 years to phase out 6PPD after the alternatives analysis. Rather, it is suggested that a thorough review of all tire additives be completed simultaneously, prioritizing them by their hazard level. Once the review is complete, they could begin phasing out the highest priority compounds, while continuing to invest in developing safer alternatives. However, to conduct that review, more data on the chemicals used in tires is required.

Robust transparency and disclosure requirements should be required. It is proposed that an anonymized database be developed where tire manufacturers or independent analysts can submit data on tire compositions, robust human and environmental toxicological information, fate parameters, and transformation product profiles.

Scientific oversight is proposed to occur through two independent expert panels: Tire Additives Alternatives Panel (TAAP) and Tire Additives Health and Environmental Effects Panel (TAHEEP). TAAP would provide a forum to direct and encourage innovation of nonhazardous tire additives and provide technical assessments on the performance, economics, and predicted environmental and human health impacts of alternative tire additives. TAHEEP will characterize the environmental fate and exposure hazards associated with existing, legacy, and novel tire additives, and define their environmental mass-balances and exposures, so that we know where they are going and who and what are being exposed.

It is proposed that MF-TAP be implemented into the *Global Treaty to End Plastic Pollution* to be implemented through internationally coordinated governance. To incorporate it into the Plastics Treaty, it should include elastomers like tire rubber into its scope, outline strict phase-out timelines, and be directed by expert panels (TAAP, TAHEEP). Although this is likely the best option to be part of, it does seem like there has been great difficulty in getting all countries to agree to the language used and strength of provisions around chemicals of concern. Alternative to the Plastics Treaty, MF-TAP could be a standalone treaty, potentially as a bi-lateral agreement between Canada and USA.

In general, efforts to reduce tire particle emissions would complement efforts to reduce hazards associated with tire additives. By reducing total vehicles travelling through investing in public transport and making active transport more feasible, it could significantly reduce tire additive emissions, as well.

## Panel Discussion (Including Keynote)

Panelist’s responses were written based off notes taken at the workshop, not a transcript. All responses were approved by panelists prior to publishing.

***Question: When looking at coho and chum salmon – are there any biophysical processes that are similar or different? Do you know why there is a difference in vulnerability?***

[Jen] We don't know why there is a difference. We've been looking at if the uptake/processing rates are different and at this point we don't know. We're also looking at the genetics of sensitive and non-sensitive coho to try to begin understanding variation.

***Question: Do the tires that are imbedded or remain in creeks leach?***

[Jen] The chemicals that are on the surface of the tire are going to leach out pretty quickly. The parent chemical stays in the tire for a longer period of time. We haven't seen tires that remain underwater continuing to leach, but we're still learning. Tires on the road appear to be a higher source of the concentration that is flushing into creeks.

***Question: Is recycled tire used in asphalt?***

[Tim] It would be really challenging to disentangle quantities of 6PPD/6PPDQ from in or on asphalt.

[Jen] A study suggested that tires get encrusted in the asphalt, which could limit the release of 6PPD.

***Question: Do tire manufacturers share how much 6PPD is in tires?***

[Kelly] The tire manufacturers are careful about their proprietary information. In order to obtain that information, it would likely require an anonymized database.

[Tim] It took a lot of effort to ultimately isolate 6PPDQ and determine the cause of coho mortality, so it is challenging to get this information made publicly available.

[Kelly] Sometimes the product manufacturer isn't sure what is in the chemical they get from their supplier either, as the supply chain is also very complicated.

***Question: Do you think that it is realistic for the WQGs to be a requirement for permitting?***

[Ali] For a case like 6PPDQ, because it is a non-source pollutant, the WQGs will mostly be used for monitoring. The WQGs will direct you on where you may be seeing exceedances and be used more as an information tool to identify problem areas. There are known mitigation measures that are proven to be effective at reducing 6PPDQ discharges in streams that could also be applied to sites of high concentrations of the parameter. However, in very specific cases such as a development site where you could clearly identify a specific source, it may be possible to regulate with permits. This latter point would need to be confirmed with regulatory agencies.

***Question: Tim, the map of tire emission in Greater Vancouver that you showed, have you taken the next step for predictive modelling for mortality?***

[Tim] The emissions map was developed base don the number of kilometres a vehicle has travelled, it doesn't tell you what's directly connected to those streams. We're working on another project that is looking at specific point sources to begin assessing potential mortality hotspots. Mapping urban watershed is tricky, as it is a lot of stormwater infrastructure chasing and following the elevations to see where water is deposited.

***Question: With regards to the Watershed Sustainability Act in BC and the notification process, new stormwater outfalls require a Section 11. Is there a way to make a connection with the WQG or put a condition in the permit? In a way, if possible, this could force municipality/developers to implement mitigation strategies.***

[Ali] In the WQGs there is a statement that says the guidelines need to be considered whenever making any water management decisions. This situation, where you have a universal substance that is everywhere and seeing how it can be worked into permitting, is all new so there is not a set protocol at the moment.

[Jane] Do things look different in other jurisdictions?

[Jen] From Washington State, we have now mandated GSI for any new development unless the ground under the property can't be infiltrated.

[Kelly] California's Protection Council had a conference on TWP generation and discussed the price and difficulty of retrofitting GSI versus source control. It may be more cost effective to try to limit the ways TWPs can get into the environment, rather than trying to deal with chemicals when they're already in the environment. However, if you identify a hotspot, finding a bioremediation solution is probably the quickest solution.

***Question: We're paying $5 to $100s of dollars per tire for recycling. What's the solution to repurposing them?***

[Jen] If we could make biochar out of tires, if that was a safe thing to do, that would be beneficial and could be repurposed into mitigation efforts.

***Question: There were no human health issues in the BC WQGs – why were they not included? I had raised concerns about this when it went out for comments, but I don't see it referenced in the document at all.***

[Ali] We received multiple comments and we still have that full list that we intend to address, when possible. At this point, as this is a relatively new discovery, there are still a lot of data gaps, one of which is evidence that clarifies how exposure to 6PPDQ may be impacting human health. The WQGs that were released were the best we could do with the data that we had. With more input and data, we can work to generate a more complete set of guidelines.

# SUMMARY OF ENGINEERING & SOLUTIONS PRESENTATIONS & PANEL DISCUSSIONS

The afternoon of day 1 was divided into two panels that focused on engineering and solutions. The first panel was comprised of representatives from municipal and provincial governments, as well as academics and consultants, while the second panel had representatives from non-government organizations (NGO), including environmental NGOs and stewardship groups.

## Panel A: Government & Academic Representatives

Panel A and associated discussion was chaired by Rachel Scholes, Assistant Professor in the Department of Civil Engineering and leads the Scholes Lab at UBC.

### Sylvie Spraakman – City of Vancouver's Green Rainwater Infrastructure

Sylvie Spraakman is a Senior Water Resources Engineer at the City of Vancouver in the Green Infrastructure Implementation Branch. She discussed the types and purpose of GSI that the City of Vancouver has, and some of the 6PPDQ focused studies they have done.

Urban environments interrupt the natural water cycle due to the extensive impervious surfaces. Rainfall in urban areas is directed quickly to local waterways, collecting a mixture of chemicals and pollutants along the way. In most cases, this runoff sees little to no filtration time before it reaches the creek. Over time, urban areas have seen stormwater management change – adding different strategies (i.e., floodplain management, water quality and erosion/sediment control, environmental concerns, etc.) as we learn more about its impacts.

There are many benefits to GSI, including but not limited to mitigation of air pollution, supporting equitable water future, increasing biodiversity and habitat connectivity, and reviving natural systems. All of these are important, but the main financial drivers for GSI is its ability to decrease water pollution, reduce urban flooding, and reduce urban heat.

In 2019, the City of Vancouver adopted the Rain City Strategy – a high level, 30-year implementation plan that aims to manage rainwater sustainably through green infrastructure that protects, restores, and mimics the natural water cycle. The two primary objectives that guide this initiative are: [1] keep rainwater out of the pipe system and let it soak into the ground, evaporate, or reuse it and [2] reduce pollutants in urban rainwater runoff by capturing and cleaning it. Currently, the City of Vancouver has over 400 assets across the city in 6 types:

1. Bioretention (bioswales/rain gardens): a shallow depression with layers of rock, engineered soils, and resilient vegetation that has rainfall flow into and percolate down through the soil, removing pollutants;
2. Subsurface infiltration: uses grey infrastructure to collect and convey rainwater to areas where it can be stored and infiltrate into soils;
3. Permeable pavement: pavement or roadways (laneway subsurface infiltration) that allows rainfall and runoff to soak into the underlying base;

4. Rainwater tree trenches: appearing like a typical tree trench, but water is collected in a nearby catch basin and is distributed through the tree trench (i.e., water storage for trees);
5. Wetlands: rainwater is collected from impermeable surfaces and discharged into wetlands where it can be held and filtered; and
6. Manufactured treatment devices: typically located at outfalls, they are designed to treat turbidity.

Ultimately, choosing the style of GSI to install depends on the area your treating, including the space you have and any other limiting factors. The first four solutions work as distributed GSI and should be distributed throughout an urban area. Wetlands and manufactured treatment devices are for end-of-pipe treatments and should at located at outfalls. The primary challenge in urban areas and with roadways is finding space because these spaces are used for so many things (i.e., water, sewer, gas, electricity, etc.).

The City of Vancouver is aiming to manage 90% of their rainfall runoff from 40% of its land area, with a design standard to capture and clean 48mm of rainfall per day. This goal was ambitious, so after more thorough development with the Healthy Waters Plan, they now have a target of all private spaces being managed to 24 mm per day and 503 hectares of public roads to be treated, which appears to be a more realistic target.

In order to assess how effectively the City's GSI is working, staff have been collecting water samples at the inlets and outlets of multiple systems. Additionally, synthetic runoff tests have been conducted, which involves adding a known volume of water into the inlets of systems and monitoring filtration/flow rates. To add to this, the team is able to monitor their GSI's ability to remove added pollutants during these tests. One example of this type of study occurred in collaboration with UBC. The team added a known mass of 6PPDQ into an existing, established rain garden. Results indicated that the effluent concentration still exceeded the lethal concentration for coho for a short period, meaning there could be some risk to fish health during larger rain events next to major sources, such as highways.

There are very few salmon-bearing streams that remain in Vancouver, therefore there are only a few places where implemented solutions could have direct benefits. One of the primary watersheds of interest to implement solutions is Still Creek as it still has salmon returning each year. The Still Creek watershed and sites along West King Edward Avenue will be the next to have GSI updates and installations.

## Amanda Rust – Transportation & Drainage Design Around Watercourses

Amanda Rust is a Registered Professional Environmental Engineer with more 18 years of experience in environmental and water resources engineering and consulting. Recently, she has worked extensively with the Ministry of Transportation and Transit (MOTT) on major transportation infrastructure designs.

Water resource and environmental services (i.e., stormwater management, water quality, erosion protection, flood management and protection, fish passage, etc.) are of the utmost importance when it comes to planning, designing, and permitting of infrastructure projects. The synergy of environmental and design professionals can help create efficiencies and possibly provide innovative ideas into the design, because environmental constraints need to be identified as early as possible. In order to identify constraints, environmental overview assessments, review of concepts throughout the design period, identification of regulatory issues, and suggestions of alternatives to ensure compliance are imperative.

When developing drainage for highway corridors, the team must consider:

- Pavement drainage: Runoff needs to drain efficiently into catch basins, spillways, curbs/gutters, and/or concrete barriers to reduce risk of hydroplaning.
- Bridge deck drainage: Runoff water from the surface of the bridges and/or approaching roads shall be conveyed to discharge at locations that are acceptable to environmental agencies and the Ministry. Discharge into rivers and creeks requires approval by an appropriate environmental regulatory agency.
- Open channels: Typically located along rural highways, where there is space, drainage ditches are located within the highway right-of-way, beyond the required clear zone, and are a maximum depth of 0.6 m. Other considerations that could be built into open channels include: low vegetation for biofiltration, riprap to reduce flow, rock check dams to slow flow, and/or infiltration trenches (i.e., installation of granular material below ditch surface) for increased infiltration.
- Drainage ponds: These can include detention, retention, and infiltration ponds, water quality (sedimentation) ponds, constructed wetlands, and/or rain gardens. These are typically harder to fit into urban spaces, but are often incorporated into interchanges.
- Storm sewers (oil/grit separators): This is an end-of-pipe treatment that is placed in tight spaces to avoid direct discharge of runoff into watercourses. They are designed to

capture and treat 90% of annual rainfall; however, removal efficiencies decrease with increased rainfall intensity.

- Highway maintenance: The Province of BC has a Best Practices for Highway Maintenance Activities document that are effective when being done consistently.
- Water quality: MOTT's best practices guide includes water quality criteria because runoff quality treatment for highway or land development drainage is good practice and often mandated by Federal, Provincial, and regional guidelines or permits.

## Panel A Discussion

In addition to Sylvie and Amanda, other government and academic representatives joined the panel, including Rachel Scholes (University of British Columbia), Lori Beaulieu (BC Transit), and Samantha Ward (City of Surrey) – each panelist that did not previously speak introduced themselves and their interest in this engineering & solutions pertaining to 6PPDQ:

Dr. Rachel Scholes is an Assistant Professor of Environmental Engineering at the University of British Columbia. Her research focuses on emerging contaminants in wastewater and stormwater, with an emphasis on nature-based treatment systems. She has conducted research on emerging contaminants for the National Oceanic and Atmospheric Administration, and at the University of Otago, New Zealand. Prior to joining UBC, she conducted postdoctoral research at the USA Department of Agriculture, where she focused on green chemistry approaches to mitigate emerging environmental contaminants.

Lori Beaulieu is a Senior Project Manager with BC Transit. Their team recently completed a new handyDART bus facility, designed to support the first fully battery electric bus facility in Victoria. They constructed a new tributary to Craigflower Creek, which runs adjacent to the property, which now has coho salmon and coastal cutthroat trout inhabiting it. The team took on the reasonability of ensuring an enhanced and protected riparian area throughout the multi-year construction project. Additionally, the site was designed with nine rain gardens to capture and treat water runoff from paved surfaces. When designing and constructing the rain gardens 6PPDQ was an emerging issue and they were able to pivot and install sampling ports throughout the rain gardens for future efficiency testing opportunities.

**Figure 5.** BC Transit handyDART facility in Victoria, designed to support a fully electric bus fleet. Photo: Lori Beaulieu

Samantha Ward is the Drainage Manager for the City of Surrey. Surrey is one of the fastest growing cities in Canada and with that comes a lot of growth pressure, but also opportunities to incorporate stormwater best management practices (BMP) into road expansion/widening projects. The City is working to meet the Provincial housing legislation that stresses land use densification, so her team is looking at how to incorporate more BMPs into these really dense areas.

Following introductions, a questions period occurred. Panelist's responses were written based off notes taken at the workshop, not a transcript. All responses were approved by panelists prior to publishing.

***Question: Lack of space was mentioned a lot – are there incentive programs for private landowners to put GSI on their own properties?***

[Sylvie] There are requirements for private property owners to have their own rainwater management. Rainwater and stormwater aren't supposed to cross boundaries (i.e., roads).

[Samantha] When developments come into a community, they must prepare on-lot designs that are based on the requirements from that specific municipality. To date, 6PPDQ is new and there haven't been any municipal regulations developed specific to it. That said, Surrey has completed several Integrated Stormwater Management Plans for watersheds in the community that prioritize the implementation of biofiltration-based BMPs such as rain gardens and bioswales on both private and public property.

***Question: All pipes belong to different jurisdictions. Is the water quality standard the same for all? Who is responsible for the pipe – the municipality at the entrance or exit of the pipe?***

[Samantha] Surrey is in the middle of a couple municipalities, with Langley upstream of us and we flow into other locations. We speak regularly with our adjacent communities about stormwater management and strive to work collaboratively to address issues as they arise.

[Sylvie] There are guidelines that the water flowing into the pipe needs to meet. Each municipality has their own Sewer Use By-Laws, so anyone discharging to a sewer must meet these water quality guidelines. This helps with ensuring there is no illegal dumping.

[Amanda] The best location to put any treatment is at the source, before it reaches a pipe.

***Question: With regards to BMP for maintenance of GSIs – when removing sediment from a catch basin or pond, where does that material go?***

[Amanda] There is a BMP for maintaining infrastructure that exists and there are criteria within that. Typically, the removal of sediment is contracted out to private companies and they are required to follow the BMP for disposal, which typically involved decanting the material and sending it to the landfill.

***Question: Is there a carrying capacity for bioretention ponds?***

[Sylvie] Most bioretention cells have a catchment at the inlet that needs to be cleaned out regularly. Studies have shown that the media in bioretention cells can last 20 to 50 years.

***Question: When large rain event occurs, some of the materials are washed out of catch basins. Are there thoughts of cleaning catch basins before large rain events?***

[Amanda] Maintenance is typically done before the winter.

[Sylvie] It could be good if it could also be scheduled more frequently throughout the winter.

[Samantha] It would be good to incorporate strategic cleaning times to ensure it's completed before anticipated September rains. Surrey has a catch basin cleaning program in place to complete regular cleaning of the City's catch basin infrastructure.

## Panel B: NGOs & Streamkeepers

Panel B and associated discussion was chaired by Jim Shinkewski, Director of Grants and Community Programs at PSF.

### Jim Shinkewski – 6PPDQ Community Funding Investments

Jim Shinkewski works as the Director of Grants and Community Programs, specifically overseeing all aspects of PSF's grants program, including the Community Salmon Program.

The Community Salmon Program provides funding support for community/volunteer stewardship, Indigenous groups, and NGOs. To date, the program has granted approximately $32 million to 3,553 projects, creating a total value of $222 million (including in-kind funds to the projects). This funding has engaged over 30,000 volunteers and salmon advocates.

PSF has provided $166,000 to 26 projects directly related to 6PPDQ, including 23 community rain gardens (~$6,300/rain garden), tire wear toxin monitoring, construction of bioswales, and a watershed overview assessment. With the community support and input, these projects are valued at approximately $1.1 million.

This type of funding supports the volunteers, professionals, and supporters of front-line salmon restoration efforts.

### Kyle Armstrong – Community Capacity for Green Stormwater Infrastructure

Kyle Armstrong is the Executive Director of Peninsula Streams & Shorelines (PSS). He is a restoration ecologist with over 10 years of experience working in coastal and aquatic ecosystems.

PSS has been working around the Saanich Peninsula for approximately 20 years. Throughout that time, their work to restore healthy streams and shorelines has been conducted in partnership with local First Nations, governments, and their extensive number of volunteers.

One of their many initiatives over the past few years has been looking to install rain gardens to benefit the many urbanized creeks in their region. Some of the barriers that they have found along the way include: design standards/permitting, costs, maintenance, retroactive planning, and over engineering. To counteract many costs, PSS enlists the help of their volunteers to help construct, plant, and maintain the rain gardens. Each year, PSS volunteers log ~7,000 hours across all their projects.

Over time, PSS's goal is to scale up green infrastructure by putting it in the hands of communities. By improving community literacy of stormwater policy and BMP, involving them in all project stages, and installing demonstration sites in strategic locations, its possible to have more people installing GSI in the future. Incorporating the community is a huge tool and resource that is often overlooked.

Kyle provided a couple of examples of rain gardens they've constructed. The first at Campus View Elementary, near Bowker Creek, was constructed as the school had significant flooding in a portion of their parking lot. Working with the local high school and elementary school, the planting was completed, and the maintenance of the garden was taken on by the Friends of Bowker Creek stewardship group. This site, funded by PSF, was very successful and has been a great demonstration site to show how well rain gardens can work.

The second example site shown was the Monterey Middle School rain garden. The parking lot drained into a large, underused piece of the property. PSS dug it out, added more porous materials, better soils, and planted it with native plants. The final rain garden example Kyle provided was the one installed at Parkdale Church and Childhood Centre in Victoria. They converted a portion of their parking lot and outdoor play area into an interactive garden. The design workshop had more than 70 community members attend to learn about the project, GSI, and help design the garden.

In addition to the continued construction of GSI, PSS has been and will continue to work with local groups like RUSH and Friends of Bowker Creek to map existing GSI locations.

## Carolynne Robertson – Wagg Creek Water Quality Monitoring Initiative

Carolynne Robertson is the Vice President of the North Shore Streamkeepers and has been involved in the monitoring of Wagg Creek.

Carolynne got involved with the North Shore Streamkeepers water monitoring initiatives because she had taken on the maintenance of a rain garden in her strata that she learned had been installed to protect Wagg Creek. In 2023, 6PPDQ was beginning to get more attention and pre-spawn coho mortalities had been observed in Wagg Creek and other North Shore creeks in previous years. Prior to 2023 had focused on habitat restoration, but there hadn't been a full assessment of water quality and stormwater management in the watershed. To advocate for change, data would be needed.

To better understand the most impacted areas of the Wagg Creek watershed, the group developed a sampling plan: they would sample at all of the outfall locations (data obtained from the City of North Vancouver's GIS department). However, lab samples were very expensive and they were looking at 17 different locations. Instead of sampling all, they first collected basic water quality parameters from each site to narrow down which sites should be tested.

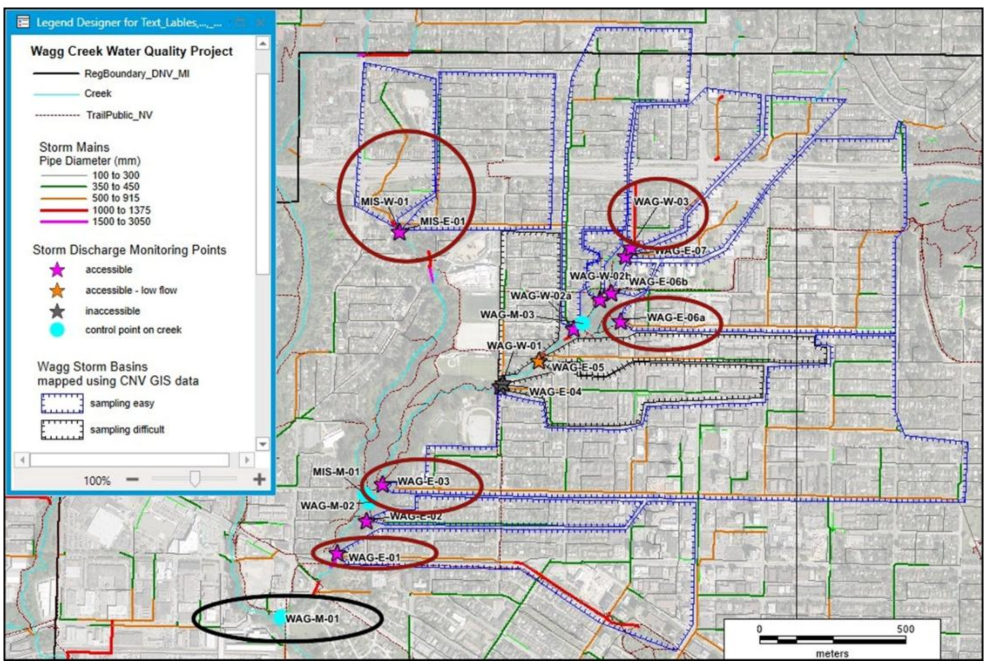



**Figure 6.** North Shore Streamkeepers sample locations along Wagg Creek, identified by purple stars. Image: Paul Lhotka

In late summer 2024, Carolynne, co-lead Paul Lhotka, and 14 other volunteers were ready and waiting to sample the first flush. One particularly memorable flush occurred on Thanksgiving

Monday. Starting in December 2024, samples were sent to both Bureau Veritas Lab to test for numerous parameters and UBC for 6PPDQ. In addition to the water samples, flow measurements were taken at 11 sample locations to assess quantity of water flowing in during low flow and high flow events.

Sampling results indicated that water quality following a flush of rainfall was very poor relative to base flow measurements. Every site sampled had multiple exceedances. Results also indicated that the timing of sampling is critical to the results as municipal sampling results had very few exceedances, whereas the streamkeepers' first flush samples had mostly exceedances. The 6PPDQ sample results also indicated that Wagg Creek regularly exceeded the lethal concentration for coho throughout the fall and winter following rain events. Near one of the stormwater point sources, a sample was nearly 700 ng/L.

Currently, the streamkeepers are at the tail end of their sampling phase. Next steps will include compiling the data to be shared with stakeholders, communication and summarizing feedback from stakeholders, and work towards developing concepts for mitigation at a catchment level, which is planned for the end of 2025.

## Caitlin Pierzchalski – Strategies to Encourage Widespread Green Infrastructure

Caitlin Pierzchalski is restoration ecologist, watershed steward, and the Executive Director of Project Watershed Society. The work that she presented at the workshop is based on a project that she did previously with Simon Fraser University's North Shore Rain Garden Project.

Through the North Shore Rain Garden Project, the six major strategies for success to constructing successful rain gardens included: identifying 'hot spots', identifying rightsholders, stakeholders, and practitioners, completing a gap analysis, closing the gaps, having a value-informed design, and celebrating/sharing stories and successes.

Different proxies, such as copper, have been used over time to identify stormwater hot spots. Acute and long-term WQGs helped indicate which areas were exceeding and having issues. Today, you could use 6PPDQ concentrations, large contaminant catchments, fish mortality events, etc. Ultimately, the site that rain gardens are constructed in should aim to infiltrate in catchments that lead to hot spots.

When an area is identified, you next need to identify the rightsholders, stakeholders, and practitioners within the problematic catchment and start engaging with them and the associated governments. By bringing everyone together to learn, they can work together to carry out the rain garden construction.

Next, mapping the gap aids in identifying where there are barriers or gaps that will change the implementation of rain gardens. Some examples of gaps could be the lack of political will, lack of planning, regulations, or bylaws, lack of trained professionals, lack of locations for installs, and/or lack of support/interest from stakeholders.

Before moving ahead, you would then need to address the app. Engage with local elected officials to obtain a champion. Work with municipal staff to inform legal documents (i.e., Official Community Plans) to close regulatory gaps and/or potentially collaborate on installs with local municipalities to share or reduce costs. Introduce yourself to local developers and property managers, as they often have area that GSI could be installed. Host workshops for practitioners for design and implementation planning and/or with community members to gain support.

Use your stakeholders to inform your design – you can do this in interactive and engaging ways. Always ensure that your design addresses the concerns, such as prioritization of native species, maintenance requirements, site lines, etc.

Finally, share your success stories through social media, websites, blogs, and data visualization. Target communication to communities or stakeholder groups that could be engaged for future installs. Celebrate the small victories and take time to reflect on the process.

## Panel B Discussion

Panelist's responses were written based off notes taken at the workshop, not a transcript. All responses were approved by panelists prior to publishing.

***Question: Is there a way to make a publicly accessible toolbox of rain garden designs to help direct people that want to build rain gardens? Who would fund that?***

[Caitlin] There are some good tools that are based out of Washington, including the 12,000 rain gardens in Puget Sound project.

[Kyle] What's the RUSH is also a good resource. Additionally, the Cougar Creek Streamkeepers have a lot of great resources on their website.

[Carolynne] Western Washington has a rain garden handbook that you can download for free. Green Communities Canada has a free online master class for rain gardens – it's designed for property owners in Ontario, but there are a lot of lessons that are applicable to here.

***Question: What conditions make a simple rain garden applicable versus a more complex design?***

[Kyle] For municipal properties, they seem to want to hold themselves to a higher standard, whereas civil properties seem to have less restrictions. Largely the biggest determining factor is the amount of water you need to filter and the slope of the surrounding area.

# SUMMARY OF DAY 2 KEYNOTE SPEAKER

## Ed Kolodziej – Occurrence & Dynamics of 6PPDQ in the Aquatic Environment: Lessons from Miller Creek

Dr. Ed Kolodziej, from the University of Washington (Tacoma/Seattle) and Centre for Urban Waters, provided a keynote presentation outlining some of his team's previous and ongoing work, focusing largely on the Miller Creek watershed (Burien/Normandy Park, WA, USA). Some of the Miller Creek results have been recently published in Zhao et al. 2025.

Approximately 90% of roadway runoff doesn't have any treatment or it is only very rudimentary. Mortality from roadway runoff does not appear to be limited to very urban watersheds, instead, small watersheds that do not have high capacity to dilute pollutants next to busy roadways appear to be the places of highest concern. Miller Creek has been a representative study site watershed for the Washington State researchers, where they have been learning how to sample and identify relationships between roadway runoff and watershed impacts. Upon first look, Miller Creek has a lot of quality salmon habitat – large woody debris, spawning gravel, pools, and the temperature remains cool. Located halfway between Seattle and Tacoma, Miller Creek is in a mixed commercial and residential area, with the Seattle-Tacoma International Airport in its headwaters.

Notably, citizen scientists have been a critical part of the fieldwork in Miller Creek. Volunteers conduct daily surveys from October to December to count spawning fish, redds, and document any observed URMS mortality. When mortalities were observed, they would assess females for eggs and document all pre-spawn mortality.

In 2018, a spatial survey was conducted to identify the major pollution sources. Sampling occurred upstream and downstream of point sources along the creek during both summer base flows and during storm events. When sampling during storm events, the team identified sites where the strongest runoff sources were entering – one site collects runoff from more than 1,000 m of busy multilane highway, and is likely responsible for most of the water quality degradation in Miller Creek.

Using autosamplers to account for dynamic changes in water quality during storms, they monitored 17 storms, collecting samples hourly for 24-36 hours. Results indicated that there are a lot of different storm dynamics, and no two storms are the same. Elevated 6PPDQ concentrations can lead the hydrograph (i.e., rise as the hydrograph rises), as well as trail it. In a few circumstances, small storms resulted in horrible water quality, so it may not always require a lot of rain to be detrimental to fish. Since 2021, sampling has occurred here in all different seasons; in that time, there was only one storm that had 6PPDQ concentrations less than 80 to 90 ng/L. At no point during storm events have 6PPDQ concentrations been below the Environmental Protection Agency's base value of 11 ng/L.

From all the data collected, Ed's team worked to identify correlations to a variety of different parameters such as peak precipitation, storm duration, antecedent dry periods, cumulative rainfall, etc. When making the comparisons, there were no situations that saw a good correlation (p-value) with any single variable, though some multivariable associations were evident (see Zhao et al. 2025 ES&T).

Unlike some of the watersheds on Vancouver Island, sampling indicated that Miller Creek has 'middle flushes', not 'first flushes' meaning that the more it rains, the more 6PPDQ that gets washed into the creek. This is the case for most contaminants that have been monitored here – it does not appear to have a clear dilution effect in small watersheds. That said, it appears that many contaminants are more transport limited than mass limited.

Notably, 6PPDQ concentrations were also high in the spring, indicating year-round risks for juvenile coho salmon, and other sensitive species like coastal cutthroat trout, in rearing and migratory habitats for young fish. In spring 2024, juvenile coho exposures during storms were conducted at the small hatchery/lab facility on Miller Creek in collaboration with Dr. Jen McIntyre, Nathan Ivy, and US Geological Survey (USGS). There were observations near the Miller Creek estuary of juvenile coho mortality following the April 2024 rain event. Results from these studies are soon to be published.

Investigations into Puget Sound tire reefs and their impacts have been underway since 2024. There are some estimates that there are more than one million tires in the Salish Sea. Ed's team worked with scientific dive teams (Annie Crawley, Ocean Annie) to pull them out of Puget Sound in four locations. They've been storing them in different ways to determine which chemicals will continue to leach from these tires. At most sites the tires are from the 1970s and onward and most remain intact. The big question that has come up is – should these be pulled out? Sampling of the tires, water, and sediment all occurred and low concentrations of 6PPD and 6PPDQ were detected. 6PPDQ has yet to be seen to form underwater; therefore, it's probably not necessary to pull them out if 6PPDQ is the only concern, although this data is still preliminary.

Another phenomena that are of concern is the partially immersed tire embankments – tire dumps, or tires that have been used to stabilize a bank and/or act as a retaining wall. As these areas are tidally influenced, they are being worn and re-exposed to ozone, likely resulting in more 6PPDQ generation. Of the two concerns (reefs or embankments), it seems that embankments should be of greater concern, although this data is not yet developed.

Since 2024, the Washington research teams have now begun taking what they've learned in Miller Creek and expanding to other watersheds. The team is now monitoring Little Skookum Creek, Skookum Creek, Percival Creek, Red Salmon Creek, and Hylebos Creek in South Puget Sound. There are further intentions to expand to the Puyallup, Nisqually, and Deschutes because of their ecological importance.

The existing treatment options that are being tested are highly variable in their performance and require further testing and optimization. Bioinfiltration systems appear to remove 90% to 95% of 6PPDQ, while strategies that were previously designed to remove metals average removals of approximately 40%. When constructing mitigation strategies, it will be important to have deposition areas that can be easily cleaned out, reducing the amount of tire wear particles that run into the system to continue to be a source of 6PPDQ.

Tires are a special product – they are constantly generated, have widespread dispersal, and make up a large mass of waste product. For this reason, we need to think about their management with especially high standards and responsibility. It's great to re-use products, but we want to ensure we're not doing it somewhere that it could be harmful or continue to release harmful chemicals into sensitive environments.

# SUMMARY OF MONITORING & LESSONS LEARNED PRESENTATIONS & PANEL DISCUSSIONS

The monitoring and lessons learned panel and associated discussion was chaired Dave Scott of the Salish Sea Indigenous Guardians Association.

## Erik Krogh & Angelina Jaeger – Characterizing Fate & Distribution of 6PPDQ, Insights from High Intensity & Real-Time Sampling

Dr. Erik Krogh and Angelina Jaeger, from the Centre for Health and Environmental Mass Spectrometry (CHEMS) in the Department of Chemistry at Vancouver Island University, presented on the research they have been conducting in partnership with BCCF's Aquatic Research & Restoration Centre.

The gold standard analysis of 6PPDQ in stream or storm waters is both selective and sensitive; however, it can be exceedingly costly in time and supplies, which can limit the number of samples measured and hence the scale and scope of projects. This work utilized a direct mass spectrometry method to expand sampling for 6PPDQ in an effort to characterize its fate and distribution on eastern Vancouver Island. Condensed phase membrane introduction mass spectrometry (CP-MIMS) involves dipping a semi-permeable hollow fibre membrane directly into stream samples to extract the chemical of interest, 6PPDQ. In-lab comparisons have shown good agreement between CP-MIMS and conventional solid/liquid sample prep connected with liquid chromatography mass spectrometry (LC-MS).

Given CP-MIMS's quick sample analysis time (2.5 min), it has been used to measure over 5,000 samples from approximately 170 locations across Vancouver Island. Sampling up and down the island has been made possible by the guidance and willingness of a large network of First Nations and volunteer stream keepers. Trends among these samples include heightened concentrations around the Island's urban centres of Victoria, Duncan, and Nanaimo. Overall, 40% of samples collected 'during' rain events have shown in-stream 6PPDQ concentrations above the BC WQG of 10 ng/L.

Given the high sample throughput, sampling sites were expanded for the 2024-2025 wet season. Additionally, unique sampling campaigns were undertaken. A simultaneous sampling event on November 22nd, 2024, at 11:00 am captured 14 samples across 2 connected waterways at the same time to observe the site-specific concentrations. Given the large dataset, comparing 6PPDQ concentrations to additional water quality parameters provides predictive models consistent with transport-limited versus mass-limited 6PPDQ sources. That is, 6PPDQ concentrations in streams can be limited by the amount of rain able to wash material into the aquatic environment, versus concentrations being dependent on the amount of tire wear material accumulating on road surfaces in the stream's catchment. Insights into 6PPDQ pulse dynamics during rain have been studied with intensive hand grab and automated samplers on hourly time scales. This has indicated for some systems that concentrations change quickly with the onset of rain, whereas other sites or systems with larger riparian zones have longer lag or delay times.

Monitoring was taken a step further recently with the first streamside real-time 6PPDQ analysis. With an outfitted mobile lab positioned next to Chase River in Nanaimo, BC, stream water was pumped up to a flow-through cell fitted with a CP-MIMS immersion probe. Measured 6PPDQ concentrations began rising quickly, shortly after the onset of precipitation with the rising edge of the stream discharge. This continuous in-stream monitoring technique provides detailed dynamics of 6PPDQ and could be used for on-site efficacy assessments of mitigation strategies. The lessons learned from this work demonstrated the wide variation of aqueous 6PPDQ in time (minutes to hours) and geography (meters to kilometers) in eastern Vancouver Island waterways. Ultimately, the information from these high-intensity samplings aims to inform mitigation strategies that remove or minimize the 6PPDQ reaching the watercourses.

## Tanya Brown & Mason King – The Timing, Magnitude, and Extent of 6PPDQ Pulses in Salmon Streams During Wet Weather

Dr. Tanya Brown and Dr. Mason King, from the Marine Mammal Ecotoxicology Lab at Simon Fraser University (SFU), presented on their work that is trying to better characterize the timing, magnitude, and extent of 6PPDQ pulses in salmon streams during wet weather in Metro Vancouver, Squamish, and Vancouver Island. This work had been carried out in collaboration with First Nations, as well as DFO, UBC, and numerous NGOs.

There are significant knowledge gaps regarding contaminant concentrations and their effects on adult and juvenile Pacific salmon; they have complex life histories and habitat needs that leave them vulnerable to contaminant exposures at multiple points through their life cycle. The contaminant of concern, 6PPDQ, has been identified as a concern to all life stages.

Toxicity studies have found that different species of salmonids appear to have different levels of sensitivity to 6PPDQ. Bonnie Lo and co-authors conducted toxicity tests on coho and Chinook and found similar results to other researchers. However, results indicated a lethal concentration for three-week post swim-up coho that was 2.3 times lower than the previously identified lethal concentration determined on coho that were one year or older.

Their team's water sampling of road runoff and salmon-bearing streams for tire-associated chemicals began in 2021 and has included 98 different waterways. Sampling occurs after at least 48 hours of dry weather, allowing for TWPs to build up on roadways, and a minimum of 5 mm of rainfall. To date, some sample locations have up to 20 rain events sampled. To obtain a more holistic look at some of the watersheds, they have been collecting other data at select sites, including flow, water quality, diphenylamine (DPA), and metals.

To date, 1,803 samples have been collected, with 1,141 (63%) of these samples analyzed. Concentrations detected range from 0.06 to 453 ng/L and 93% of samples had 6PPDQ concentrations that exceeded the detection limit (0.03 ng/L). Samples collected from the North Shore exceeded the coho salmon lethal concentration and WQGs (BC and US EPA) in most 'during' samples. Residual concentrations were observed approximately 24 hours after the rainfall had stopped.

The team has begun characterizing their first 41 sites with the data collected. Overall, 78% of the sites had concentrations that exceed the WQGs and 55% of the sites exceeded the juvenile coho lethal concentration (41 ng/L). Data has indicated positive associations between 6PPDQ concentrations in salmon bearing streams and land use characteristics for the respective watershed. There was a positive association between 6PPDQ concentrations and the amount of rainfall/length of dry period prior to rainfall (i.e., higher concentrations with larger rainfall amounts and longer dry periods prior to rainfall). Results indicated that elevated concentrations have been observed throughout the year. Further, 6PPDQ was found to have a positive association with zinc, copper, and DPA concentrations.

The next step the team is doing is adapting a Bayesian model that was used in a 2017 paper that assessed risk in the Puget Sound Basin based on observed coho mortality events and landscape attributes (i.e., land use, land cover, population densities, roadways, traffic intensity, etc.). Rather than coho mortalities, SFU will be using their 6PPDQ concentrations. To test the model, they have been using data from the first 41 sites, landscape variables, and climatic variables.

The systems that they work in are innately hydrologically dynamic, which can add challenges to data collection and interpretation. Methods used to characterize 6PPDQ as it relates to hydrologic states include: stage and discharge, rainfall, and collecting a time series of stream water samples. One of the main urban systems SFU and UBC have been monitoring is the Serpentine River at the Tynehead Hatchery. At the time of the workshop, they had monitored four rain events there, and eight elsewhere. Results indicated repeatedly elevated concentrations of 6PPDQ above the lethal concentration for coho in all but one rainy period sampled. Further, concentrations exceeded WQGs for 3.5 to 22.5 hours.

Other ongoing work in the Serpentine River includes field experiments with local hatchery-sourced coho salmon to evaluate the effects following acute exposure to road runoff contaminants.

### Rhea Smith – Road Runoff Assessment Strategies

Rhea Smith is a Natural Resource Scientist with the Environmental Assessment Program at the Washington Department of Ecology. Her current research focuses on conducting integrated, field-based studies to understand when and where aquatic species are impacted by priority contaminants.

The first mass coho mortality event was observed at a hatchery in the 1980s, approximately two decades after the use of 6PPD in tires began. By 2011, coho mortality events were regularly observed throughout Puget Sound urban areas. After multiple years of research and narrowing down the contaminant of concern, the US EPA granted a Tribal petition to have 6PPD banned in 2024. Additionally in 2024, Washington State enacted Substitute Bill 5931 to reduce sources and uses of 6PPD in Washington and the US EPA issued an Advance Notice of Proposed Rulemaking under the Toxic Substances Control Act, Section 6.

The 6PPD life cycle has complex pathways and can be transported in multiple ways. However, with more research, paired with multiple management and policy strategies implemented, solutions are possible. Identifying the areas of concern is important to inform mitigation locations in the future. Mapping sensitive species presence and their proximity to tire contaminant loading helps focus studies and site evaluations to support solution-based modeling and risk assessments.

Sampling for 6PPDQ, a toxicant associated with stormwater runoff, is challenging. To understand the impacts of 6PPDQ on aquatic populations requires integrated watershed studies that assess all aspects of urbanization, including toxics, habitat, and hydrology data. One method to do this is by setting up data collection stations, equipped with instrumentation to collect the necessary data. The Department of Ecology has installed automated systems on urban waterways that can be deployed remotely, limiting any storm chasing. All instrumentation data is then sent to an online dashboard hourly. Results have indicated that storm duration, intensity, and frequency can all drive the peak of 6PPDQ. Another strategy that is used to initially screen for 6PPDQ and to understand how it varies among watersheds is by using passive samplers that provide a time weighted average mass load at standardized, simultaneously sampled deployment times.

Primary take aways from existing data indicates that green infrastructure is effective at treating, diverting, and capturing 6PPDQ and other contaminants from stormwater, sampling helps support focused geographic strategies and avoids a patchwork of projects, and it will take continued coordination and dedication among disciplines to solve this problem.

## Cindy Yang – A New Approach to Estimate Pollutant Concentrations in Stormwater Runoff for Tire Wear Particles, 6PPD, and 6PPDQ

Cindy Yang is a Senior Engineer with Environment and Climate Change Canada (ECCC). Her responsibility is to conduct ecological exposure analysis in the risk assessment of chemical substances for the Chemicals Management Plan Program at ECCC.

Cindy and her colleagues in ECCC have been working on a new model to estimate pollutant load, specifically tire wear particles (TWPs), 6PPD, and 6PPDQ in urban watersheds. The TWP model and Monte Carlo simulation can be used to estimate event mean concentrations, the weighted average concentration (total loading divided by total runoff volume) in rain events in urban watersheds. The event mean concentrations can then be used to develop predicted environmental concentrations. The team has come up with a few equations and parameters to include in the simulation to estimate predicted environmental concentrations in the receiving waters.

These parameters include:

- *TWP emission loadings* in an urban watershed come from vehicle traffic running on all highways and roads. Mapping was used to estimate urban watershed sizes and land use types. Residual TWP loadings from previous rain events and TWP from atmospheric deposition were not included in the TWP model.
- Patterns associated with *antecedent dry days*; > 5 mm per day is considered a storm event.
- *Daily rainfall amount*.
- *Rain event runoff volumes:*
  - *Total daily rain amounts* consider minimum daily rain amount to trigger runoff (~5 to 50 mm; >50 mm assumed to cause flooding).
  - *Runoff coefficient* is the land use data used to calculate runoff coefficients for urban watersheds.
  - *Watershed size* was obtained from provincial data.
- *6PPD and 6PPDQ presence* in the environment
  - Ozone concentrations, sunlight, heat, long antecedent dry days, humidity, and other factors impact 6PPD conversion rates in the environment.
- *Converstion of 6PPD to 6PPDQ:*
  - *Leaching* – refers to 6PPD can migrate to the surface of the tire and react with ozone during its lifetime and leaches into water.
  - *6PPD conversion rate to form 6PPDQ* – a range of 10% to 20% is used in the TWP model to reflect conversion rates when favourable reaction conditions present in the environment.

The Monte Carlo simulation picks a value from the data ranges for each of the parameters and run for 1,000,000 iterations, resulting with 1,000,000 predicted environmental concentrations. The simulation results represent possible predicted environmental concentrations for rain events in urban watersheds.

At the time of presentation at this workshop, Monte Carlo simulation results for Vancouver, Greater Toronto Area, and Montreal are all pretty similar, indicating that 6PPD-quinone concentrations in a local watershed can range from 1 to 3,500 ng/L, with median values ranging from 50 to 70 ng/L and the 90th percentile values being 300 to 500 ng/L. The 50th to 90th percentile concentrations for 6PPD-quinone are 50 to 500 ng/L.

## Panel Discussion

Rachel Scholes from UBC also joined the panel for the questions and answers period.

Panelist's responses were written based off notes taken at the workshop, not a transcript. All responses were approved by panelists prior to publishing.

***Question: What do you think the biggest data gaps are?***

[Ed] I don't think we're close to understanding the mass balances for tire rubber-derived contaminants. We understand some dynamics, but still a lot of information is missing for some of these models, especially about the amounts of these chemicals that are on our roads.

[Mason] Often when referring to toxicity data, it comes from laboratory models. There are a lot of disconnects from the lab and what we see in dynamic stream environments. Trying to explore factors that may lead to or affect test results will be important.

[Tanya] We see a lot of variability between different sites on the same stream. How can we better characterize that in a more efficient manner to guide mitigation planning and reduce concentrations flowing into streams? It really requires people on the ground to help pinpoint the most impactful point sources.

[Erik] There is a big data gap in processes that take place at the particle-water interface. Where is the mass loading happening? We're measuring 6PPDQ in the aqueous phase; however, 6PPDQ can be attached to particles, which could be acting as both a source and a sink. We need to learn more about the dynamics and the impacts of those effects.

[Cindy] Efforts have been made to track down all the parameters that impact 6PPD/6PPD-quinone analysis. The conversion rate was challenging to find.

***Question: Has anyone looked at the collection basins in stormdrains?***

[Erik] Yes. We have received catchment basin samples from the CRD. We're still characterizing these samples and how quickly 6PPDQ is leaching off. Catch basins do need to be cleaned out, characterized, and properly disposed. This could be another data gap worth mentioning.

***Question: What's going to be the responsible end-of-life plan for tires?***

[Rhea] There are pre-existing progressive tire waste regulations in Washington State. A tire life cycle report was recently written and will be published soon that evaluates current practices and identifies important data gaps and recommendations for adaptive management. The most recent development for an industrial permit requires permittees to sample around their facilities starting in 2028; this includes tire recycling centres, airports, etc. that will help gauge how effective current end-of-life practices are and if where adjustments need to be applied.

***Question: Can SPATT (solid phase adsorption toxin tracking) sampling help determine where contaminants are coming into the watershed?***

[Erik] This sounds similar to a passive sampler and is a great idea to get some preliminary information and characterize the mass loads of 6PPDQ coming in to or leaving a system.

[Rhea] Passive samplers are used to screen for contaminants including 6PPDQ. They are good for collaborative spatial studies where a sampling network of collaborators deploys them at the same time for the same duration to compare loadings between watersheds with different characteristics and such as impervious surfaces. They need to be extracted within 14 days based on current standard operating procedures or frozen. We're hoping to get a standard operating procedure published to help standardize methods among counties, states and countries. We've conducted stream reconanaissance using grab and composite sampling, but its hard to verify whether you caught the 6PPDQ pulse or not, so we feel confident when we detect 6PPDQ, but less confident about the non-detects. Passive samplers helps build confidence in non-detects and helps identify false non-detects. We have a standardized GIS field form for our "integrated watershed studies".

***Question: Is there potential for 6PPDQ to be a chromophore (i.e., absorb light)? If so, could you then use chromatography to test for 6PPDQ?***

[Erik] It does have a chromophore, it's photochemically active. That said, you'd run out of sensitivity by a couple orders of magnitude and may only be able to detect really high samples.

[Rachel] There are lots of things in these samples that can absorb and emit light. We have looked into using fluorescence for detection but haven't gotten very far because of interference from other substances.

# SUMMARY OF TOXICOLOGY PRESENTATIONS & PANEL DISCUSSIONS

The toxicology panel and associated discussion was chaired by Chris Gill, a co-director of VIU's CHEMS and a professor in the Department of Chemistry.

## Katie Roberts – Toxicity of 6PPDQ to Key Salmonid Species & Putative Toxicity Pathways

Katie Roberts has been a Graduate student in the Toxicology Centre at the University of Saskatchewan since 2022. Katie's research has focused on the effects of 6PPDQ on early-life stage (ELS) salmonids, in both sub-chronic and acute exposures, as well as assessing 6PPDQ effects at the transcriptome level.

Similar to variability amongst species, there is also variability amongst life stages in vulnerability to 6PPDQ. ELS are typically ~60% more sensitive than adult life stages. When initiating the ELS work, there was very little information about any life stages outside of adults; however, this was an important area to study, as it is not just those swimming in the water column that may be impacted.

The primary objectives of Katie's work were to determine sub-chronic toxicity of 6PPDQ in ELS rainbow trout and lake trout, as well as determine sub-lethal effects of 6PPDQ exposure to rainbow trout and lake trout, assessing developmental changes and transcriptomic pathway disruption.

Rainbow trout and lake trout had the same experimental design, but each had species-specific differences. In 100 tanks, the research team began dosing alevin right at hatch; rainbow were exposed for 28 days (hatch to swim up) and lake trout were exposed for 45 days (hatch to swim up). ELS chronic exposures resulted in developmental abnormalities, including yolk sac edema (i.e., swelling and fluid accumulation around the yolk sac), spinal curvatures, and caudal fin hemorrhaging. Additionally, lake trout alevin had blood pooling in their eye. Sub-chronic mortality was monitored in the rainbow trout and lake trout. URMS symptoms were not observed within the first four days. However, around day four is when a sharp increase in mortality was observed. To compare, acute mortality tests conducted on rainbow trout and lake trout fry exposed each species to their known lethal concentration. Both species exhibited the typical URMS behaviours and mortality occurred relatively quickly (hours).

To begin investigating 6PPDQ at the transcriptome level, RNA was extracted from the rainbow trout and lake trout exposed in the ELS studies. RNA was assessed for any differential gene expression, which indicates changes at the molecular level. In total, approximately 1,200 dysregulated genes were identified, largely involved with inflammation and cell death.

Looking at identifying the lethal concentration for lake trout without having to expose the fish for 45 days, the team assessed at which concentration an increase in genes were disrupted. At ~350 ng/L is when the most genes were disrupted in a lot of the same ways that rainbow trout ELS were (i.e., inflammation, gene changes, cell death).

Other studies at the Toxicology Centre have looked at mitochondrial dysfunction. Mitochondrial uncoupling can increase reactive oxygen species. When combined with other changes imposed by 6PPDQ exposure, the resulting inflammation, membrane permeability, and oxidative damage could result in cell differential and development disruption, vascular and neurological effects, and increased apoptosis/necroptosis, ultimately leading to mortality.

In conclusion, both rainbow trout and lake trout are sensitive to 6PPDQ in the ELS, with post swim-up fry life stages exhibiting the greatest acute morality. Additionally, exposure to 6PPDQ during the developmental period results in sub-lethal effects, including significant transcriptome disruption. Further, differences in metabolism may play a role in species sensitivity.

## Hui Peng – Structure-related Toxicity of 6PPDQ & its Hydroxylated Metabolites

Dr. Hui Peng is an Associate Professor in the Department of Chemistry at the University of Toronto. His research group's interests for 6PPDQ are focused on identifying the structure-activity relationship of 6PPDQ and the molecular toxicity mechanism (i.e., proteins) of 6PPDQ.

Hui's research team synthesized seven PPDQ analogues and hypothesized that 6PPDQ is mediated by selective protein binding and the toxicity of 6PPDQ can be reduced by tuning its side chain (not the benzene ring), as protein binding is highly selective to even minor structural changes. It took the group one year to synthesize everything and toxicity was only observed for 6PPDQ in two-month-old juvenile rainbow trout. The other PPDQs, despite similar structures, did not appear to be toxic to exposed rainbow trout. The structurally selective toxicity of 6PPDQ was also observed in the cell line of coho salmon, demonstrating that the toxicity mechanism is maintained between rainbow trout and coho salmon. Therefore, the toxicity of 6PPDQ is structurally selective, strongly suggesting that its toxicity is mediated by a selective toxicity mechanism. Results demonstrate that other PPDs might be possible replacement chemicals; however, further testing would be required to rule out any other harmful impacts.

The research team also looked at toxicity of oxidized PPDs under environmentally relevant conditions. The project involved selecting PPD compounds, reacting them with ozone, extracting that compound and then exposing it to rainbow trout for 96 hours. In total 72 exposure tanks were used as there were 3 different concentrations of 4 different chemicals under 3 different reaction conditions (3 replicates of each). Water samples and dead fish were collected every day. Results indicated that at zero days, none of the PPDs were toxic, whereas by day three and seven, 6PPD was highly toxic. This was the only PPD that appeared to become toxic of those tested (IPPD, CPPD, DPPD). The alkyl hydroxylation metabolite was only observed for 6PPDQ; therefore, it may be related to 6PPDQs selective toxicity.

To assess the alkyl hydroxylation metabolite, the structure and toxicity characterization were also assessed by Hui's research group. Results indicated that the C4-alkyl-OH-6PPDQ was confirmed as a metabolite and that hydroxylation of 6PPDQ was catalyzed by CYP450. 6PPDQ has a unique tertiary carbon, a weaker carbon-hydrogen bond, which explains the selective hydroxylation of 6PPDQ. Toxicity was not observed for C4-alkyl-OH-6PPDQ, demonstrating that alkyl hydroxylation is a detoxification pathway for 6PPD-Q. The results suggest that C4 tertiary carbon is the key moiety for both toxicity and metabolism of 6PPD-Q.

In summary, 6PPDQs toxicity is very selective and uses a very selective toxic mechanism. Additionally, it may be possible (with more testing) to use other PPDs as a safe replacement, at least in the short-term.

## Kyle Duncan – Providing New Insights into Spatially Resolved 6PPDQ Toxicology with Ambient Mass Spectrometry Imaging

Dr. Kyle Duncan is an Assistant Professor in the Department of Chemistry at Vancouver Island University, where he leads the VIU Metabolomics Group that is developing, constructing, and applying new technology to map biomolecules directly in tissue.

In general, metabolomics is the study of small biomolecules that are the precursors, intermediates, and products of metabolism. This research can give clues regarding the mechanisms for toxicity, observing very fast reactions (scale of milliseconds to seconds). There are estimated to be more than 200,000 metabolites. There is a high level of isomerism, meaning they are metabolites that have the same chemical makeup, but different structures, which can be challenging to tease apart. Low concentration metabolites or toxins can have very important biological roles, but are often difficult to measure.

Typically to screen an organism's metabolome, you must extract metabolites through blood, urine, cultured cells, or tissue, separate them chromatographically, analyze with a mass spectrometer, and then process the data. In addition to this method, Kyle's lab creates thin sections of and then analyzes it to find the localization of metabolites in heterogeneous tissue.

The lab uses nano-DESI to map molecules in tissue. In brief, this method works by pushing solvent through a syringe pump onto the surface of the issue and extract present chemicals back into the mass spectrometer using another capillary. This is a custom technique that they have built and designed in-house. Ongoing improvements include 3D printed capillary mounts for easy adjustments, updated stage control software, laser sensor for height feedback, and pulled capillaries for single-cell scale imaging.

One of the ongoing questions Kyle's team is looking at is if they can visualize 6PPDQ metabolism in exposed fish. When they trialed visualizing 6PPDQ on a slide, they were able to, so they then tried with fish, obtained from the University of Saskatchewan and no 6PPDQ was detected. To investigate this further, tissue memetic molds were made by homogenizing coho liver tissue spiked it with various levels of 6PPDQ. The spiked tissue homogenates were deposited into gelatin molds, sectioned and mounted it to glass slides and imaged with nano-DESI MSI. On glass, they are able to detect below 10 picomolar of 6PPDQ. In tissue, they can only detect above one micromolar of 6PPDQ, which equates to six orders of magnitude. As a result, this has opened up a number of other questions, including is 6PPDQ particularly susceptible to matrix effects, is it binding to proteins or tissue matrix, or is it degrading?

It was observed that hydroxylated biotransformation products in exposed fish appear in the liver, meaning that the metabolite is in the liver. It's likely abundant throughout the fish, but at much lower levels than what can currently be detected with this technology.

A full scan to assess metabolite changes found that there were quite a few differences. Long chain polyunsaturated fatty acids accumulated in the central nervous system (i.e., brain and spine) of exposed fry.

In summary, they can detect that a primary biotransformation product of 6PPDQ (OH-6PPDQ) is accumulated in the liver and gut regions of exposed fry and that spatial metabolomics can add another dimension to toxicological studies.

### Nathan Ivy – Canary in the Creekbed: Quantifying Toxicity of Continuous Urban Stream Exposure on Coho Salmon Early & Juvenile Life Stages

Nathan Ivy is a graduate researcher in the Aquatic Ecotoxicology Research Lab at Washington State University, working with Dr. Jen McIntyre. His work focuses on the how chronic stormwater exposure constrains salmonid physiological development, altering life-stage transitions and migration timing.

Salmon are a keystone species – where they run, life flourishes. Therefore, declines in salmon populations can signal a broader ecological instability. In particular, coho salmon have been identified as the 'canary in the coal mine', but rather the creekbed. Coho spend over a year in freshwater; as a result, they are susceptible to stormwater contaminants for longer periods of time. Unfortunately, they have also been found to be highly vulnerable to URMS, triggered by 6PPDQ exposure, in all life stages. 6PPDQ exposure is causing 60% to 90% pre-spawn mortality in many of Puget Sound's urban creeks.

Lab studies have been showing coho salmon are highly vulnerable to 6PPDQ, succumbing to URMS within a few hours. However, no one had confirmed whether large-scale mortality was happening in the wild. To observe this, Miller Creek was selected as the location, as previous testing had shown that stream concentrations consistently exceed the lethal concentration for coho salmon during storm events throughout the year and has a documented history of adult coho dying pre-spawn.

To conduct this initial study, there were 8 tanks with 30 coho juveniles in each. Four of the tanks were exposed to filtered well water (control conditions) and the other four tanks were exposed to live, unfiltered water from Miller Creek during storm events. This set up allowed for direct comparison of survival, development, and performance outcomes between the two groups.

Storm conditions brought heavy sediment and a toxic surge, providing a real-time look at how stormwater overwhelms the juvenile coho. In each storm event, Nathan's team observed 80% mortality that can be correlated to URMS based on the symptoms expressed by the coho. Further, the University of Washington team supported this study by collecting water samples throughout the storm events. Results indicated that 6PPDQ sustained lethal concentrations for approximately 24 hours during these spring storm events.

Stormwater causes mortalities for coho salmon at both ends of their life cycle – adults die as they return to spawn and juveniles die before or during their out-migration. Each storm event pushes survival lower and over time, these aren't isolated events – they're part of a downward spiral toward localized extinction.

Other ongoing research that Nathan and his colleagues are working on include assessing different life stages exposed to 6PPDQ. Preliminary results are indicating variation in growth and development. Stormwater exposed fish show subtle changes in eye structure, including blood pooling, which may be impairing vision. Additionally, stormwater exposed fish are showing delayed yolk sac absorption, indicating slower growth, later swim-up, and less competitive advantages. Exposed fry also expressed visible differences (i.e., altered shape and surface area) in their caudal fin, which can affect their swimming, reducing their propulsion and maneuverability abilities. Chronically exposed juvenile coho were trialed in a swim tunnel, they showed impaired performance across the board with less capacity to recover with increasing flow stress.

When assessing mortality in the chronic exposure tests, the research team observed reduced mortality in each subsequent storm event, raising questions with regards to early sublethal exposure and if it can prime individuals for survival. Mortality rates for fry appear lower than in chronically exposed alevin, which may be a result of physiological changes between different life stages.

Other studies conducted assessed 6PPDQ exposures to fish that were reared in clean water compared to stream-experienced fish (i.e., reared in urban creeks). Results have indicated that the naïve fish reared in clean well water suffered significant mortality at 6PPDQ concentrations that had virtually no effect on stream-experienced fish. Again, this raises questions regarding early sublethal exposure effects.

## Panel Discussion

Panelist's responses were written based off notes taken at the workshop, not a transcript. All responses were approved by panelists prior to publishing.

***Question: When you visualize the -OH in the fish, can you know the amount of 6PPDQ it was exposed to?***

[Kyle] This is a challenging question to fully understand because there are several different isomers of OH-6PPDQ that may have different instrumental responses, and the rate of biotransformation in fish is largely unknown. We do know that the levels of OH-6PPDQ in the fish tissue in our study are higher than the exposure level of 6PPDQ, suggesting accumulation of OH-6PPDQ in the liver and surrounding organs.

***Question: Nathan, what happened to the other 20% in your study?***

[Nathan] One study involved one storm exposure. The 20% that survived were culled after exposure. In our next study with coastal cutthroat trout and coho, we'll be doing chronic exposures to see what happens to the 20% (survivors).

***Question: Have you looked at human exposure in exposure mechanisms?***

[Hui] No, not at this point – there is no solid conclusion at this point.

***Question: Are the deformations in the tail structure permanent or delayed development?***

[Nathan] It appears to be substantial delayed development, which is impactful to them as they're having to compete with hatchery coho that are twice their size. They're going to be mingling and eventually competing for food, which is why the development of the caudal fin is so important. The swim tests we did really highlighted the implications to their swimming ability and stamina.

***Question: Is anyone doing any histology regarding the caudal hemorrhaging?***

[Nathan] Some of this has been done in the lab before, but nothing from the Miller Creek project yet. We're looking to map all physiological changes at this one specific project site first.

***Question: When you were seeing the storm surge, were you also testing the water at the same time to see 6PPDQ concentrations in the water?***

[Nathan] Yes, Dr. Ed Kolodziej's group was conducting the water testing.

***Question: When you cull the fish, do you freeze them or dispose of them?***

[Nathan] We image, measure, and weigh all the fish. As this was the first study, we didn't keep them; however, we did extract the brains and livers in two-hour timeseries.

***Question: There was a lot of dysregulated genes, is that saying that it's impacting the genes permanently, or is it a delay in expression?***

[Katie] When talking about transcript abundance, genes are transcribed into proteins. Therefore, we're looking at the number of transcripts in that fish. You may see 8,000 copies in a control fish, but only 200 in an experimental fish – so that one would be downregulating.

***Question: Will dysregulated genes have generational impacts?***

[Katie] Potentially – the ones that survive exposure can go back and spawn. The change in the number of copies of genes may not affect the way the gene is expressed. Changes in the genome are likely to happen. If over a short term, they may not be able to cope; however, we may see some coho return that are more tolerant.

***Question: Utilizing previous databanks, can you do any background analysis? Could we use this to fill in some data gaps while advocating for policy/regulation?***

[Nathan] My understanding is we're experiencing jurisdictional chaos. With countless borders, there is a lot of overlap and very little collaboration. It comes down to increasing public awareness. People need to know about this – it's an important issue and has implications for our overall populations.

***Question: What's the source water for Miller Creek? Just wondering about the pre-conditioning (i.e., hatchery) and if they look different.***

[Ed] It is mostly rainfall that keeps Miller Creek flowing, but groundwater is there for the baseflow. Groundwater is recharged over the winter, but the creek can get pretty low and dry by the end of summer. Stormwater runoff can greatly increase the flows because the system is so rainfall dependent.

[Nathan] The control group is similar to hatchery fish or a rural proxy. Exposure to 6PPDQ has cascading effects on growth and development to all coho, just seems more impactful to those that are less regularly exposed.

***Question: There are a lot of people looking at alternatives. What's your prospective for screening levels for alternatives?***

[Nathan] We looked at 6PPDQ compared to IPPDQ and saw that 6PPDQ was the most sensitive – not the same for IPPDQ. The main question I think we need to ask is: what's the experience of the fish and how's that sensitivity going to change for each chemical?

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# APPENDIX A – SPEAKER BIOGRAPHIES

Speaker biographies are listed in alphabetical order.

***Ali Azizishirazi – Ministry of Water, Lands & Resource Stewardship (Province of BC)***

Ali Azizishirazi is a Water Quality Guideline Specialist with the BC Ministry of Water, Land and Resource Stewardship, where he has worked for the past nine years. In this role, he helps develop water and sediment quality guidelines for the province, ensuring they are informed by the latest scientific research. With a background in ecotoxicology, Ali also represents British Columbia on the Canadian Council of Ministers of the Environment (CCME) Water Quality Guidelines Working Group, contributing to the development of national environmental guidelines.

Contact: Ali.Azizishirazi@gov.bc.ca

***Amanda Rust - Binnie***

Amanda is a Registered Professional Environmental Engineer with more than 18 years of experience in environmental and water resources engineering and consulting. Amanda joined Binnie in early 2017 and worked her way up to Division Manager of the Water Resources Division and Climate Resiliency Service Area. She primarily leads climate change assessments, hydrologic studies, hydrotechnical assessment, and detailed drainage designs for the BC MOTT, various municipalities, and private developers across the province. Her recent experience includes working closely with highway design engineers and the MOTT in the development of major transportation infrastructure designs. Amanda specializes in leading drainage design projects, but also has extensive experience in water quality studies, environmental impact assessments, environmental permitting, and contaminated site assessment and remediation.

Contact: arust@binnie.com

***Angelina Jaeger – Vancouver Island University***

Angelina is a master's in chemistry student. Her research focuses on continued rapid-detection 6-PPDQ monitoring and method development, as well as investigating chemical characteristics and reactions of 6-PPDQ.

Contact: Angelina.jaeger@viu.ca

***Caitlin Pierzchalski – Project Watershed***

Caitlin is a restoration ecologist, watershed steward, and non-profit leader. She was born and raised on the traditional territory of the Sylix-speaking Peoples and is currently working with and learning on the traditional territory of the K'ómoks First Nation. She is inspired by her love of oceans and has been working in marine and coastal environments throughout the Salish Sea for the past 10 years. Her experience includes coastal and marine restoration (eelgrass, marshes and kelp), freshwater restoration, and implementation of green infrastructure & nature-based solutions in urban environments.

Contact: Caitlin.Pierzchalski@projectwatershed.ca

***Carolynne Robertson – North Shore Streamkeepers***

Carolynne is Vice-President of North Shore Streamkeepers. She has a B.A. in English and a Technical Communication Certificate from SFU. A City of North Van resident, Carolynne is keenly interested in improving the health of North Van watersheds through nature-based solutions and community action.

Contact: forcarolynne@shaw.ca

***Cindy Yang – Environment & Climate Change Canada***

Cindy Yang works at ECCC as a senior engineer. Her responsibility is to conduct ecological risk assessment of chemical substances for the Chemical Management Plan (CMP) program at ECCC. She holds a Master of Engineering degree from Carleton University in Canada. She has been working at ECCC for over 10 years.

Contact: cindy.yang@ec.gc.ca

***Ed Kolodziej – University of Washington***

Ed Kolodziej is a Professor at the University of Washington with joint faculty appointments at Environmental Sciences at UW-Tacoma and in Civil and Environmental Engineering at UW-Seattle. He also is a Principal Investigator at the Center for Urban Waters (Tacoma, WA) where Ed and his research group use advanced mass spectrometry and hard work to investigate contaminant fate and transport, build effective treatment systems, and ensure ecosystem health.

Contact: koloj@uw.edu

***Erik Krogh – Vancouver Island University***

Dr. Krogh completed his undergraduate studies at the University of Toronto with a specialization in Physical Organic Chemistry and Environmental Studies. He went on to obtain his Ph.D. in Chemistry at the University of Victoria, where he focused on structure-activity relationships in the photochemistry of organic molecules in aqueous media. He is currently a faculty member in the Department of Chemistry at Vancouver Island University and co-Director of the Applied Environmental Research Laboratories. He teaches environmental and analytical chemistry courses and maintains an active group of undergraduate and graduate student researchers. He has published over 60 papers in the areas of environmental analytical chemistry. His current research interests include the development and application of real-time, on-line mass spectrometry to directly investigate environmental chemical processes in complex and reactive media.

Contact: erik.krogh@viu.ca

***Hui Peng – University of Toronto***

Hui Peng is an Associate Professor at the Department of Chemistry, University of Toronto, Canada. He received his B.Sc. and Ph.D. at Peking University in 2008 and 2013, respectively. Following postdoctoral fellowships at the University of Toronto and University of Saskatchewan, Hui Peng joined the department of chemistry at the University of Toronto as a faculty member in 2017. His research focuses on establishing the environmental Chemical-Protein Interaction Network (eCPIN) by pursing two research directions: 1) Identification of exogenous and endogenous ligands using protein affinity selection coupled with untargeted mass spectrometry (AS-UMS). 2) Identification of protein targets through chemical proteomics. He has published over 100 peer-reviewed papers and has received multiple awards including the CIC EN Early Career Research Award (2024), James J. Morgan Early Career Award (2023) and Ontario Early Researcher Award (2022).

Contact: hui.peng@utoronto.ca

***Jenifer McIntyre – Washington State University***

Dr. Jenifer McIntyre is an associate professor of aquatic toxicology at the Washington State University's School of the Environment. Located at the Puyallup Research & Extension Center and collaborating with the Washington Stormwater Center, Dr. McIntyre's current research focuses on the ecotoxicology of urban stormwater runoff and the biological effectiveness of green stormwater infrastructure. In 2020, Dr. McIntyre and colleagues discovered a novel chemical leaching from vehicle tires that is one of the most acute toxicants known to science, explaining acute die-offs of coho salmon in roadway-impacted watersheds. She currently focuses on the ecotoxicology of urban stormwater runoff and the biological effectiveness of green stormwater infrastructure.

**Jim Shinkewski – Pacific Salmon Foundation**

Jim is the Director of Grants and Community Programs for the Pacific Salmon Foundation. He is responsible for overseeing all elements of PSF's grantmaking programs, including directing the Community Salmon Program, Stewardship Community Bursary, Partnered Initiatives, and other strategic functions within the Foundation. His department works with DFO's Salmonid Enhancement Program and the network of salmon stewardship volunteers throughout B.C. and the Yukon.

Contact: jshinkewski@psf.ca

***Katie Roberts – University of Saskatchewan***

Katie has been a grad student of the Toxicology Centre at the University of Saskatchewan since 2022. She recently completed her Masters and is currently a PhD student. Katie's research focuses on the effects of 6PPD-quinone on early-life stage salmonids, in both sub-chronic and acute exposures. She also looks at effects at the transcriptome level, with a goal to further elucidate both sub-lethal effects, as well as mechanism(s) of action of 6PPD-quinone.

Contact: Catherine.roberts@usask.ca

***Kelly Grant – California's Department of Toxic Substances Control***

Kelly has served as a scientist in California's Department of Toxic Substances Control in the Safer Consumer Products (SCP) program since 2018. At SCP, she helps to identify and find solutions to problematic chemicals in products, including 6PPD in tires. She served as the co-leader of the Interstate Technology Regulatory Council team producing technical guidance for government agencies on 6PPD and 6PPD-quinone. Prior to joining SCP, Kelly was a biology professor at a small university on the shores of Lake Erie, where her research used molecular biology to answer questions about toxicology, ecology, and environmental science.

Contact: Kelly.Grant@dtsc.ca.gov

***Kyle Armstrong – Peninsula Streams & Shorelines***

Kyle is the Executive Director of Peninsula Streams & Shorelines. He is a restoration ecologist with over 10 years of experience working in coastal and aquatic ecosystems. He is passionate about the restoration and enhancement of Social Ecological Systems through community-driven restoration and stewardship, research, and environmental education.

Contact: kyle.armstrong@peninsulastreams.ca

***Kyle Duncan – Vancouver Island University***

Dr. Kyle Duncan received a B.Sc. in Biology from VIU in 2010 and a Ph.D. in Chemistry from the University of Victoria. Following, he moved to Sweden for a postdoctoral fellowship at Uppsala University in the lab of Prof. Ingela Lanekoff. Currently, Dr. Duncan is an Assistant Professor in the Department of Chemistry at Vancouver Island University, where he leads a research program developing, constructing, and applying new technology to map biomolecules directly in tissue. This technology can be used to uncover the contextual distributions of biomolecules in complex tissues and help elucidate molecular mechanisms of tissue dysfunction and disease.

Contact: kyle.duncan@viu.ca

***Lori Beaulieu – BC Transit***

Lori brings expertise and a strong foundation in the construction industry to her role as a Senior Project Manager at BC Transit. Her career has evolved into a passionate journey of promoting sustainable development, fostering neighbourhood integration, and championing meaningful community and Indigenous engagement. Lori excels in navigating the complexities of infrastructure projects while staying rooted in values that prioritize environmental stewardship and being open to incorporating new learning as projects develop. Lori values the connections that make communities thrive and brings a ridiculous amount of enthusiasm to every project she leads.

Contact: LBeaulieu@BCTransit.Com

***Mason King – Simon Fraser University***

Dr. Mason King is a Post-Doctoral Fellow in the Brown Lab within Simon Fraser University's Department of Biological Sciences. His current research focuses on road runoff in salmon streams and its effects on salmon. Mason's past research examined persistent contaminants in seabirds, avian petroleum ecotoxicology, flame retardant effects in raptors, coastal watershed nutrient pollution, and ocean acidification. He is an active member of the Society of Environmental Toxicology and Chemistry and sits on the Wildlife Ecotoxicology Interest Group Steering Committee.

Contact: mason_king@sfu.ca

***Nathan Ivy – Washington State University***

Nathan is a graduate researcher in the Aquatic Ecotoxicology Research Lab at Washington State University, under the mentorship of Dr. Jen McIntyre. He studies how chronic stormwater exposure constrains salmonid physiological development, altering life-stage transitions and migration timing. His early fieldwork in steelhead habitat restoration revealed the long-term consequences of riparian degradation, prompting him to pursue a B.S. in Forest Ecology at WSU. Now, through in-situ toxicology research at Miller Creek, he investigates how repeated exposure to urban runoff disrupts salmonid development and decouples migration behavior from environmental cues, thereby reducing adaptive capacity under shifting ecological baselines.

Contact: Nathan.ivy@wsu.edu

***Rachel Scholes – University of British Columbia***

Dr. Rachel Scholes is an Assistant Professor of Environmental Engineering at the University of British Columbia. Her research focuses on emerging contaminants in wastewater and stormwater, with an emphasis on nature-based treatment systems. She has conducted research on emerging contaminants as a Hollings Scholar for the National Oceanic and Atmospheric Administration, and as a Fulbright Graduate Research Fellow at the University of Otago, New Zealand. She earned an M.S. and Ph.D. in Environmental Engineering from the University of California, Berkeley, and a B.S. in Chemical Engineering from Northwestern University. Prior to joining UBC, she conducted postdoctoral research at the United States Department of Agriculture, where she focused on green chemistry approaches to mitigate emerging environmental contaminants. As an early-career faculty member, she has been highlighted as an Emerging Investigator by the Royal Society of Chemistry, and was appointed by the Peter Wall Institute for Advanced Studies at UBC as a Catalyst Scholar addressing the Climate and Nature Emergency.

Contact: rachel.scholes@civil.ubc.ca

***Rhea Smith – Washington State Department of Ecology***

Rhea Smith is a natural resource scientist with the Environmental Assessment Program at the Washington State Department of Ecology. Her current research focuses on conducting integrated, field-based studies to understand when and where aquatic species are impacted by priority contaminants. Recently, her research has focused on understanding the scope and scale of the 6PPD-quinone impacts to aquatic life. She has a graduate degree in Chemical Oceanography and Benthic Ecology from Moss Landing Marine Laboratories, CA and over 20 years of experience in aquatic disturbance ecology.

Contact: rhsm461@ECY.WA.GOV

***Richard Hall – Barrister & Solicitor***

Richard became a Member of the Law Society of BC in 1984, he has held senior Executive and CEO positions and In House Counsel roles in the downstream oil and gas industry, education, quasi judicial tribunals specializing in compensation, correctional services, labour relations and transportation, as well as health care, manufacturing and real estate development.

***Samantha Ward – City of Surrey***

Samantha has over 23 years of progressive engineering and project management experience in both the public and private sectors. She has led and supported clients, communities, colleagues, stakeholders and the public in solving complex engineering issues. Samantha's passion for engineering is founded in her desire to provide the best service she can to her community. I am a strategic thinker and excellent communicator who employs creativity and innovation, together with sound technical knowledge, to produce effective engineering strategies and solutions.

Contact: SWard@surrey.ca

***Shane Petersen – Department of Fisheries & Oceans***

Shane has worked for DFO for the last 24 years with positions in Science, Aquaculture, Resource Management and currently Reconciliations & Partnerships for the BC Salmon Restoration and Innovation Fund where he manages engagement and the dedicated technical staff.

Contact: shane.petersen@dfo-mpo.gc.ca

***Sylvie Spraakman – City of Vancouver***

Sylvie Spraakman is a Senior Engineer at the City of Vancouver in the Green Infrastructure Implementation Branch. She has a PhD in Civil Engineering from the University of Toronto where her research focused on the long-term performance of bioretention systems.

Contact: Sylvie.Spraakman@vancouver.ca

***Tanya Brown – Simon Fraser University***

Dr. Tanya Brown is an Assistant Professor in the Department of Biological Sciences at Simon Fraser University, British Columbia, Canada. She holds an Adjunct Professor position in the School of Environment at the University of Windsor, Ontario. Prior to her current position, she was a Research Scientist at Fisheries and Oceans Canada (DFO) with the Ocean Sciences Division from 2019-2023, where she led DFO's Whale Contaminants Program in support of the Government of Canada's Whales Initiative. She has 20 years of experience in the study of the ecology and ecotoxicology of marine mammals and fish. Her research has focused largely on understanding the influence of habitat use and feeding ecology on contaminant exposure, accumulation, and health effects in a changing marine environment. She leads a team of graduate students and researchers seeking to identify principal routes of exposure to priority contaminants and to understand how environmental changes and anthropogenic stressors are impacting valued ecosystem components, such as killer whales, beluga whales, ringed seals, Arctic char, and Pacific salmonids. She employs a combination of habitat use modelling, dietary tracers, and 'omics technologies to characterize contaminant pathways and assess the health of fish and marine mammals. Dr. Brown has applied her expertise in support of contaminant site remediation, green infrastructure, and the recovery of at-risk species.

***Tim Rodgers – University of British Columbia***

Tim is a postdoctoral fellow in the Department of Civil Engineering at UBC. His research looks at the fate and behaviour of toxic chemicals in the environment, with a focus on chemicals emissions, transport, and mitigation through green infrastructure. Tim is currently working on understanding how 6PPDQ moves through urban watersheds in the Metro Vancouver area. The goal of this project is to help design and implement engineered systems or policy interventions that can protect coho salmon and other aquatic organisms from 6PPD-quinone and other toxic road runoff compounds.

Contract: tim.rodgers@ubc.ca

# APPENDIX B – RESEARCH POSTERS

AERL
Applied Environmental Research Lab

# Intensive temporal sampling for the emerging salmon toxin 6PPD quinone by condensed phase membrane introduction mass spectrometry

**Angelina Jaeger**[1,2], Joseph Monaghan[1], Haley Tomlin[3], Jamieson Atkinson[3], Chris G Gill[1,2,4,5], Erik T Krogh[1,2]

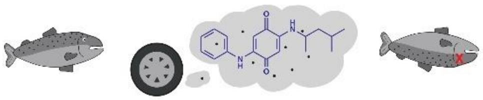
[1] Dept. of Chemistry Vancouver Island University, Nanaimo, BC; [2] Dept. of Chemistry University of Victoria, Victoria, BC; [3] British Columbia Conservation Foundation, Nanaimo, BC; [4] Simon Fraser University, Burnaby, BC; [5] University of Washington, Seattle, WA

University of Victoria

VANCOUVER ISLAND UNIVERSITY

## Background

6PPD quinone is an emerging toxin derived from tire antiozonant 6PPD. It is acutely toxic to coho salmon with **$LC_{50}$'s of 40-95 ng/L.**[1,2] Other fish species like rainbow and brook trout show sensitivity in 600 ng/L range.[3]



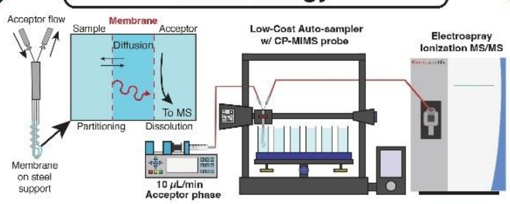
The compound has been **detected ubiquitously in the environment.**[4] However less is known about how representative current grab samples are. Finding hot spots and understanding time above toxicity can inform where and how mitigation strategies should be implemented.

[1]Tian et al. *Science*, 2021 [2]Lo et al. *Environ. Toxicol. Chem.*, 2023 Brinkmann et al. *Environ. Sci.Technol. Lett.*, 2022 [3]Cao et al. *Environ. Sci. Technol.*, 2022

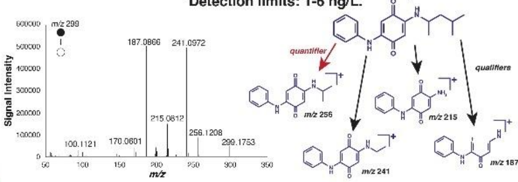
## Research Questions

1. Characterize tire wear toxins accumulation or dissipation through watercourses.
2. Trace 6PPD quinone concentrations throughout rain events for time resolved understanding of when to sample and the duration of exposure to organisms.

## Methodology

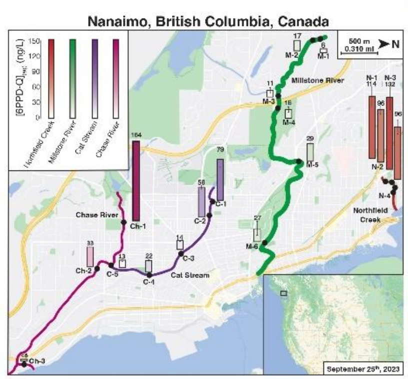


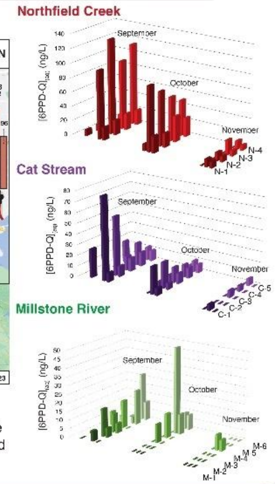

Direct from aqueous samples.
Total analytical duty cycle: 4 minutes per sample.
**Detection limits: 1-6 ng/L.**




**See Joseph at poster WP 248 for more details.**

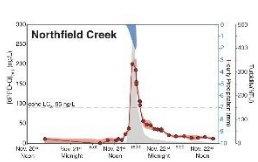
## Spatial Sampling



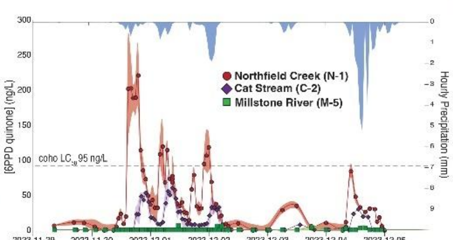




Concentrations vary with input source, distance, and landscape. September to November shows general decline in concentrations, likely related to increased water flows and the washing effect of frequent rain events.

## High Frequency Sampling








Points represent mean concentration of sample. Standard deviation of 3 ≥ sample measurements shown as shaded area.

Grab samples were taken every 1-6 hours and analyzed within 24 hours. 6PPD quinone concentrations changed rapidly depending on sample location. Concentrations were dynamic with precipitation and Northfield Creek showed several pulses above $LC_{50}$.

## Project Information

>2000 environmental samples measured for 6PPD quinone since September 2023. 30% detected above coho $LC_{50}$.

Automation & Monitoring Paper
Monaghan et al. *ACS Waters*, 2023

Project Interactive 6PPD quinone database

## Conclusions

1. We observed 6PPD quinone site **concentrations were largely influenced by surrounding inputs of road runoff and stormwater.** Potential loss through downstream may be a result of dilution, sorption or chemical transformation.
2. 6PPD quinone **concentrations are extremely dynamic with rain** in the studied watercourses. If a relationship between turbidity and 6PPD quinone concentration is characterized for a given waterway, turbidity could be used as a proxy for tire wear runoff.

High throughput analysis enables unprecedented spatiotemporal analysis used to identify significant inputs and prioritize mitigation efforts.

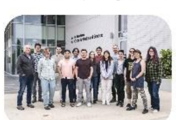
## Future Work

- Real-time monitoring during rain event with on-site mobile mass spectrometry.
- Pair CP-MIMS with field asymmetric ion mobility MS for improved selectivity in complex matrices.
- Examine particle bound fraction via direct MS.

## Acknowledgments

We would like to thank the AERL, British Columbia Conservation Foundation, and volunteer teams for around the clock sampling. We would like to thank the funding bodies for supporting this work, including the BC Salmon Restoration and Innovation Fund.
The authors declare no competing financial interest.

PACIFIC SALMON FOUNDATION · NSERC CRSNG · Fisheries and Oceans Canada · INNOVATION.CA · BRITISH COLUMBIA CONSERVATION FOUNDATION · BC Salmon Restoration and Innovation Fund · mitacs

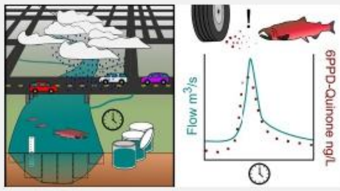


# *Portable autosamplers are suitable for monitoring 6PPD-Q in the field over several days*

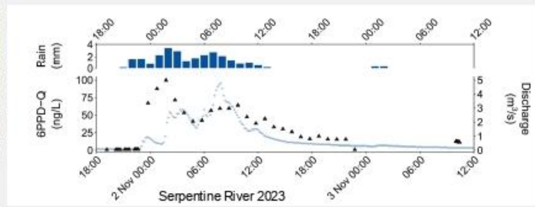
Mason D. King[1], Timothy F. M. Rodgers[2], Gopal Sharma[2], Xiangjun Liao[3], Andrew R. S. Ross[3], Mackenzie Mueller[3], Simon Drew[2], Rachel C. Scholes[2], & Tanya M. Brown[1]

1 Simon Fraser University, Department of Biological Sciences
2 University of British Columbia, Department of Civil Engineering
3 Fisheries and Oceans Canada, Institute of Ocean Sciences

## *Background - Streams are dynamic and 6PPD-quinone flushing is transient*

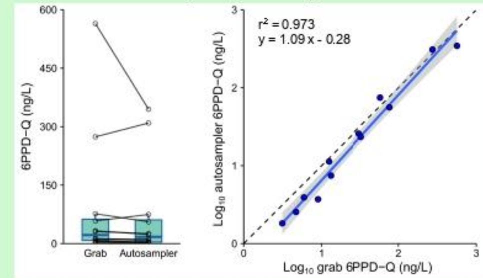



Road-source contaminants like 6PPD-quinone (Q) are washed into streams by precipitation, and in-stream concentrations are flow-dependent. Portable autosamplers are a common water quality monitoring tool to collect time-paced or flow-paced samples.




- We use portable autosamplers (Teledyne ISCO Model 6712) to study 6PPD-Q dynamics in urban salmon streams.
- A hose (5–8 m) draws water from the stream, with a rinse between samples.
- The water is stored in a carousel of glass bottles (350 mL).
- We use ice so that a meltwater bath keeps the samples (175 mL) at a stable, cool temperature until we retrieve them within 1 to 3 days to store frozen at our lab.

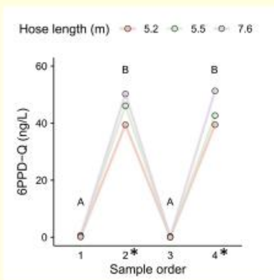
## *Procedural checks with time-matched grab samples in the field*




We took a time-matched water sample by hand (grab) for comparison with the portable autosampler.

## *Lab and field validation tests*

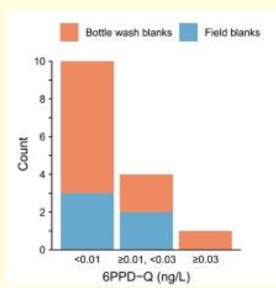
The ISCO autosampler passes water through three lengths of hose. To evaluate potential between-sample bias caused by residue from previous samples in the hoses, we conducted a lab test. An autosampler drew samples that alternated between solutions low (tap water) and high (spiked stream water) in 6PPD-Q.




Letters indicate post hoc contrasts on LMM $X^2 = 542.2$, DF = 3, $p < 0.0001$

## *Summary - Portable autosamplers are fit-for-purpose*

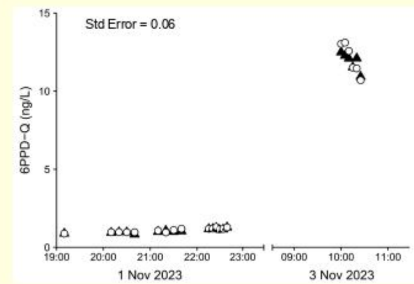
1. Lab tests indicate that
   a) Observations are unlikely to be biased by the preceding sample
   b) Glass bottles re-used after cleaning are free of contamination
   c) Concentrations are stable in the open bottles over several days
2. Field data shows that
   a) Field blanks indicate that background contamination is negligible
   b) Time-matched grab samples agree well with autosampler results




We used bottle wash blanks and field blanks (ultrapure water, 18Ω) to check whether procedures were adequate for quantifying 6PPD-Q in the field. Glassware cleaning consisted of three steps:

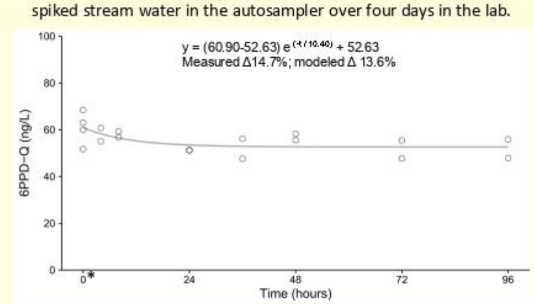
1) washing with detergent and tap water,
2) using a glassware washing machine with desalinated water, and,
3) double solvent rinsing with acetone and n-hexanes.

Bottle wash blanks contained 6PPD-Q-containing samples prior to cleaning (*) in plots at left and right). Autosampler hoses are flushed and dried thoroughly between uses.




In the field, stream water was simultaneously collected with both an autosampler (▲) and manually into a jar (○). Samples were frozen within hours.

In some instances, 6PPD-Q may not be stable in water. To test target stability over several days, we measured concentrations in spiked stream water in the autosampler over four days in the lab.




### *Acknowledgements & Funding*

We thank Mina Hassan Aghaei, Carys Gallilee, Katerina Colbourne, Steve Healy, Elliott Evans, Nikki Kroetsch, Katie Moloney, Nicole LaForge, Lisa Loseto, Neil Dangerfield, Kristy Gabelhouse, Diane Sutherland, Yvonne Lam, Rosie Barlak, Felix Ouellet, Agnes Richards, Jay Guo, Lesley England and the Tynehead Hatchery volunteers.

This work was supported by The Fisheries and Oceans Canada Pacific Salmon Strategy Initiative (2504) and the British Columbia Salmon Restoration & Innovation Fund (BCSRIF_2022_361), Mitacs Elevate (IT38465), and Raincoast Conservation Foundation.

# Uncovering spatially resolved 6PPDQ metabolism in rainbow trout fry with nano-DESI mass spectrometry imaging

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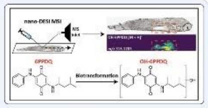
## Introduction:




**Background:** 6PPDQ (*N*-(1,3-Dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine-quinone), an oxidation product of the stabilizing and anti-cracking rubber additive 6PPD, is an emerging environmental contaminant of widespread concern. Of interest are the high species-specificity, concerningly low $LC_{50}$, and unknown mechanism for mortality that have been observed in select salmonids. To help uncover 6PPDQ bioaccumulation, metabolism, and the mechanisms of toxicity, it is of great interest to spatially resolve 6PPDQ and its biotransformation products (bioTPs) throughout whole body tissue sections of exposed fish. Herein, we use nanospray desorption electrospray ionization (nano-DESI) mass spectrometry imaging (MSI) to reveal the localization of 6PPDQ bioTPs, including monohydroxy 6PPDQ (OH-6PPDQ), in exposed Rainbow Trout (*Oncorhynchus mykiss*) fry.

**Results:** Mass spectrometry images were acquired at µm resolution for technical and biological replicates of 6-week post-hatch rainbow trout fry exposed to time-weighted average concentrations of 0.61 µg/L 6PPDQ for 96 hours, as well as control fry. Our results reveal that 6PPDQ undetectable in control and exposed tissue, potentially indicating rapid metabolism of 6PPDQ below the limit of detection (LOD) for nano-DESI MSI. However, imaging experiments reveal the accumulation of OH-6PPDQ in the liver and surrounding organs of exposed rainbow trout fry. A spiked tissue mimetic model was constructed to estimate 6PPDQ LOD by nano-DESI MSI and to explain the discrepancy between observable OH-6PPDQ and absent 6PPDQ. In addition to targeted MSI of 6PPDQ metabolites, investigation of non-targeted broadband full scans reveals disparate localization of endogenous metabolite perturbations resulting from 6PPDQ exposure.

## Experimental:

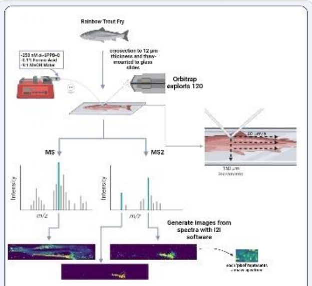



**Figure 1.** Schematic detailing nano-DESI MSI experimental workflow.

**Fish samples:** Rainbow trout were selected as a model species with known morbidity and mortality to environmentally relevant concentrations of 6PPDQ. Juvenile fish are expected to have a lower tolerance for contaminants, and their fragility and potentially prolonged exposure to 6PPDQ are important metrics to study. 6-week post-hatch fry were exposed to time-weighted average concentrations of 0.61 ppb (2.0 nM) for 96 hours. The chosen fry displayed no signs of adverse effects.

**6PPDQ and OH-6PPDQ ionization:** Standard solutions of 6PPDQ and 6PPD-1-OH were directly infused to assess the ionization efficiency of 6PPDQ and OH-6PPDQ. Comparison between the 6PPDQ and 6PPDQ-1-OH standard curves reveals similar ESI efficiency (figure 2). Given that the *in vivo* hydroxylation of 6PPDQ is known to occur at several positions, with each isomer displaying a unique ESI ionization efficiency, accurate quantification of OH-6PPDQ *in situ* remains an avenue to explore.

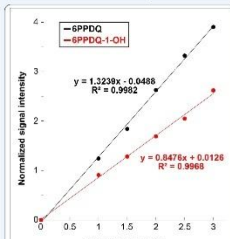



**Figure 2.** Standard solution curves for 6PPDQ (black) and 6PPDQ-1-OH (red) directly infused into the H-ESI source and normalized to 1 µM 6PPDQ-$d_5$ internal standard.

## Localization of 6PPDQ Metabolites:

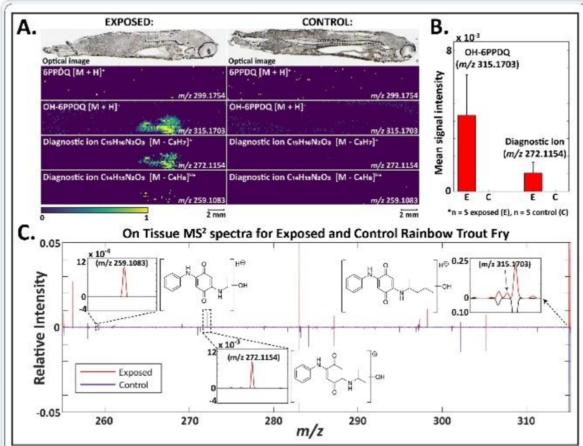



Figure 3. **A.** Optical image and corresponding ion images of 6PPDQ (SIM), OH-6PPDQ (MS²), and two diagnostic ions of OH-6PPDQ, m/z 272.1161 and 259.1083 (MS²). **B.** ROI analysis for the OH-6PPDQ bioTP and most prevalent diagnostic fragment ion (m/z 272.1154) in exposed (E) and control (C) rainbow trout fry. **C.** On tissue MS² spectra of an averaged line scan of exposed (top, red) and control (inverted bottom, blue) rainbow trout fry with highlighted precursor and diagnostic ion peaks and their associated structures. Relative intensities are displayed as a fraction of the largest intensity in the spectrum.

**Spatially resolved 6PPDQ and 6PPDQ biotransformation:** To investigate 6PPDQ metabolism *in situ*, nano-DESI MSI was performed on thin, whole-body tissue sections of 6PPDQ-exposed and control rainbow trout fry, with targeted selected ion monitoring (SIM) and $MS^2$ scans for 6PPDQ and several previously reported bioTPs. No 6PPDQ was detectable (Figure 3A); however, OH-6PPDQ was found to localize in the liver and surrounding organs of exposed fry (Figure 3A). The identity of OH-6PPDQ was verified with on-tissue $MS^2$, where observed product ions represent the partial loss of an alkyl chain and retention of the hydroxy group at *m/z* 272.1154 and 259.1083 (Figure 3C). Site-specific and regio-specific OH-6PPDQ hydroxylation have been linked to toxicity and mortality. Unfortunately, it is not currently possible to distinguish OH-6PPDQ isomers with nano-DESI MSI, and all data represent cumulative isomer classes. The localization of OH-6PPDQ to the liver, gut, and other proximal tissue (Figure 3A) is intriguing, as cytochrome P450 enzymes are known to metabolize xenobiotics in the liver. Given the extent of OH-6PPDQ accumulation in extrahepatic organs, it is unclear whether we are observing liver-derived OH-6PPDQ perfusing into nearby tissues, or 6PPDQ-metabolism is occurring simultaneously in many organs.

**6PPDQ tissue detection:** A tissue mimetic model was constructed from Chinook liver and spiked with increasing concentrations of 6PPDQ to assess the impact of matrix suppression on 6PPDQ ionization and to explain the discrepancy in 6PPDQ and OH-6PPDQ signal intensity (Figure 4A). From the calibration curve, an on-tissue LOD for 6PPDQ using the nano-DESI platform was estimated to be 1 µM, which corresponds to ~0.3 µg/g of tissue (Figure 4B). An ion image for the endogenous metabolite Arachidonic acid was included to demonstrate consistency between homogenate wells (Figure 4A).

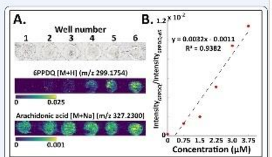



Figure 4. **A.** Optical image of Chinook salmon liver homogenate imbedded in gelatin with corresponding TIC-normalized ion images for the spiked 6PPDQ calibration curve (SIM) and Arachidonic acid (full-scan). **B.** Spike recovery calibration curve for 6PPDQ generated from ROI mean signal intensities and normalized to 6PPDQ-$d_5$ internal standard in the nano-DESI solvent.

## Non-targeted MSI:

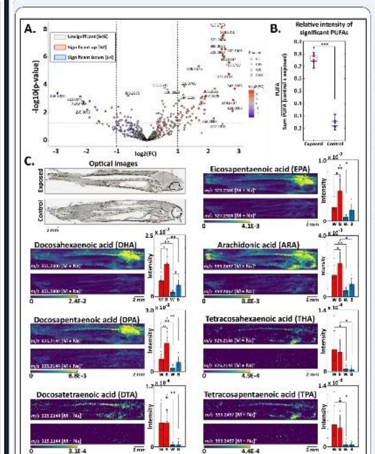



Figure 5. **A.** Volcano plot for non-targeted analysis comparing exposed and control rainbow trout fry. **B.** Box and whisker plot of seven long-chain polyunsaturated fatty acids (LC-PUFAs) upregulated in exposed rainbow trout fry. **C.** Optical images and corresponding TIC normalized ion images and intensity analysis of seven LC-PUFAs in exposed (top/red) and control (bottom/blue) rainbow trout fry. Mean intensities were pulled from regions-of-interest for the whole fish (W) and in the brain (B) and averaged across replicates.

**Non-targeted MSI:** In addition to targeted MS scans, we included a broadband full scan to evaluate spatial metabolomic profiles of exposed and control fry. Using these data, a volcano plot was generated for 711 identified molecular feature, revealing 52 upregulated and 14 downregulated metabolites (Figure 5A). Analysis of these features uncovered the accumulation of seven long-chain polyunsaturated fatty acids (LC-PUFAs) in exposed fry (Figure 5B, 5C).

**LC-PUFA localization:** All of the identified LC-PUFAs were found to localize in the central nervous system of exposed fry (Figure 5C). 6PPDQ has been implicated in the disruption of the blood-brain barrier, which has been associated with neuronal dysfunction, neuroinflammation, and death, and may help to explain this increase of free LC-PUFAs.

## Conclusions:

- 6PPDQ was not observed in 6PPDQ exposed fry tissue, suggesting that it is rapidly metabolized and/or below the limit the of detection on our nano-DESI platform.
- OH-6PPDQ was readily observed on tissue, with the highest degree of accumulation in the liver and surrounding organs. $MS^2$ fragment ion images corroborate these observations.
- A tissue mimetic model was constructed to estimate a 1 µM (to ~0.3 µg/g) LOD for 6PPDQ using nano-DESI MSI.
- Non-targeted analysis reveals the upregulation of seven LC-PUFAs in the central nervous system. This may be explained through 6PPDQ-induced disruption of the blood-brain barrier.
- These data provide the first spatial mapping of 6PPDQ metabolism in exposed fishes.

## Acknowledgements:

NSERC CRSNG · BC Salmon Restoration and Innovation Fund · Fisheries and Oceans Canada · INNOVATION.CA Canada Foundation for Innovation | Fondation canadienne pour l'innovation

**We acknowledge the Applied Environmental Research Laboratory at VIU for critical infrastructure support.**

# 6PPD-Quinone Mystery Solved. Regulatory Momentum & Analytical Advances for 6PPD-Quinone & 6PPD

Presenting Author: Louis Wagner, National Technical Specialist, ALS, Canada
Email: *louis.wagner@alsglobal.com*

## Introduction

ALS announced the first commercial testing service for 6PPDquinone (6PPD-Q) in Canada in June 2021. Since then, there has been extreme focus and activity on 6PPD-Q by the research community and by regulators in the US and Canada, due to its prevalence in the environment and its extreme toxicity to coho salmon at sub part per billion levels.

Below is a timeline showing important new regulatory updates over 13 months:

- Jan 30, 2024: US EPA releases Draft Method 1634 for 6PPD-Q (LC-MS/MS).
- June 13, 2024: US EPA publishes freshwater acute Aquatic Life (AL) screening values for 6PPD-Q (0.011 µg/L) and 6PPD (8.9 µg/L).
- Sept 14, 2024: Washington State Rule for 6PPD-Q (0.012 µg/L) becomes effective freshwater acute AL).
- Feb 11, 2025: BC Water Quality Guideline (short-term freshwater acute AL) released for 6PPD-Q (0.010 µg/L).

This pace of regulatory attention is unprecedented for an emerging contaminant of concern, and additional criteria and/or standards will almost certainly follow. In support of these developments, the ALS' R&D team in Canada has now extended our services to add a lower-level est option for 6PPD-Q and a new ISO 17025 accredited test method for 6PPD (another first in Canada).

## Mystery Salmon Toxicant Identified – 6PPD-Q

"Urban Runoff Mortality Syndrome" (URMS) causes up to 90% of coho salmon to die before spawning in some urban creeks impacted by stormwater runoff in the US Pacific Northwest, particularly in the Puget Sound region. For decades, scientists were baffled by the cause of these high mortality rates, which occur after heavy rainfall events. In December 2020, a research group led by Dr. Zhenyu Tian at the University of Washington reported some incredible scientific detective work (published in *Science*) that conclusively identified the mystery toxicant as 6PPDquinone.

6PPD-Q was shown to be acutely toxic to coho salmon at very low concentrations (below 0.1 ppb), but it is not yet known why other species of salmon, such as chinook, sockeye, Atlantic, and chum, are far less sensitive to this chemical. The UW researchers initially identified 6PPD-Q in several streams in Washington and California. Since that time, considerable research has been done to measure and characterize 6PPD-quinone in salmon-bearing streams of BC and the US Pacific Northwest. Fish-bearing streams around the world are likely to be impacted by this chemical due to its ubiquitous nature and source, and because 6PPD-Q has now been shown to affect many other fish species such as rainbow trout, steelhead, brook trout, lake trout, and coastal cutthroat trout.



## Transformation Byproduct from Tire Anti-Oxidant 6PPD

Tian's research group confirmed in 2020 that 6PPD-quinone is an ozonation byproduct of 6PPD, a widely used antioxidant which is added to car and truck tires at relatively high levels of 0.4-2%. 6PPD is a very reactive compound, and is intended to preferentially react with ozone at the road surface to prevent tire degradation, which improves tire lifespan and safety characteristics. When 6PPD reacts with ozone, it converts to 6PPD-quinone.

As tires wear, tire wear particles (TWPs – a category of microplastics) are deposited on and around road surfaces. 6PPD-Q is water soluble up to approximately 38 µg/L (20°C) and tends to dissolve into road runoff, finding its way into urban creeks, streams, and rivers during storms and high rainfall events. After a long dry summer, heavy rainfall in early fall often coincides with the migration of coho salmon into streams, when spawning grounds become accessible, which can create a deadly combination.

Measured LC-50 values for 6PPD-quinone to coho salmon are extremely low, ranging from 0.041 to 0.095 µg/L. Concentrations lethal to coho salmon have been shown to occur frequently in Washington State and British Columbia streams during high stormwater runoff events. In November 2023, an unprecedented die-off of coho salmon occurred in West Vancouver, BC at Brothers Creek, where returning coho salmon died before being able to spawn, which was linked to 6PPD-quinone.

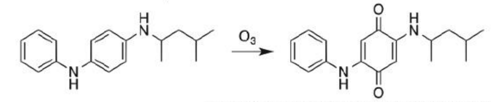



*Figure 1. Ozonation Transformation of 6PPD to 6PPD-quinone*

## Long-Term Environmental Solutions

Given the ubiquitous global usage and distribution of 6PPD today, a long term solution to this problem likely requires the re-engineering of automobile tire formulations to use antidegradants that do not generate toxic byproducts like 6PPDquinone. Alternative short-term solutions in highly impacted fish-bearing streams could involve stormwater treatment or diversion, but these strategies may be cost-prohibitive on a large scale.

## LC-MS/MS Analysis of 6PPD-Quinone

ALS recognized the urgency of this issue when the story first broke in December 2020, and immediately began work to develop a robust and sensitive test method for 6PPD-Q. Testing for 6PPD-Q in environmental waters is now offered by our Vancouver laboratory by Draft EPA Method 1634 with LC-MS/MS triple quadrupole technology, using Multiple Reaction Monitoring (MRM) of three independent mass transitions (see Figure 1). Quantitation is by isotope dilution where a deuterium-labelled 6PPD-quinone analog is added to all samples prior to extraction to correct for sample matrix effects or sample extraction losses. Method 1634 uses a selective Solid Phase Extraction and cleanup protocol to deliver definitive, confirmed identification and measurement of 6PPD-Q down to trace levels.

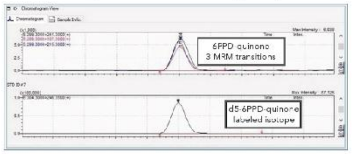



*Figure 2. LC-MS/MS MRM Chromatogram*

## Testing for the Pre-Cursor: 6PPD

Environmental testing for 6PPD, the 6PPD-Q pre-cursor and source material, is particularly challenging because 6PPD is highly reactive by design. 6PPD is added to tires specifically because it is sacrificially oxidized to prevent degradation of tire rubber. The aqueous half-life of 6PPD has been reported 6PPD-quinone 3 MRM transitions d5-6PPD-quinone labeled isotope as being only a few hours under neutral pH conditions, even in sterile water, which ALS has verified (see Figure 3). However, ALS studies identified an optimal anti-oxidant preservative material and concentration that effectively stabilizes 6PPD for at least 14 days, facilitating its analysis with the same hold time as 6PPD-Q (see Figure 4). The ALS PPD stability studies observed its degradation in water to unknown byproducts other than 6PPD-Q, likely due to oxidation.

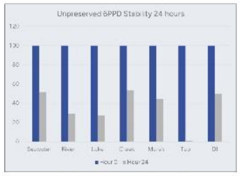



*Figure 3. ALS 6PPD Unpreserved Stability Study – 24 hours*

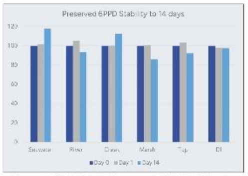



*Figure 4. ALS 6PPD Preserved Stability Study – 14 days*

## Sampling Requirements

Sampling requirements and test code details are shown in Table 1. Environmental water samples for 6PPD-Q or 6PPD may be conveniently collected in 100 mL amber glass bottles. Separate sample bottles are required for 6PPD, which are supplied pre-charged with anti-oxidant (non-hazardous). Samples should be chilled to ≤ 6°C prior to shipment to the laboratory if possible. ALS has adopted a hold time for these tests of 14 days (from sampling to extraction), based on US EPA Draft Method 1634 and on ALS stability studies for 6PPD.

Current ALS Canada test method options for 6PPD-quinone include a routine Limit of Reporting (LOR) of 0.001 µg/L, and a low-level LOR of 0.0002 µg/L (50x lower than freshwater acute aquatic life guidelines). A trace-level method is currently under development to support better characterization of chronic effects and background levels. Recent studies have shown that effective characterization of background levels in urban streams can be challenging due to temporal concentration surges which can occur within minutes or hours of significant rainfall events; the use of lowlevel or trace-level methods allows better characterization of background levels between surges.

***Table 1. 6PPD-Q & 6PPD Reporting Limits & Sampling Details***

| | 6PPD-Quinone | | | 6PPD |
|---|---|---|---|---|
| | Routine | Low-Level | Trace-Level | Routine |
| Limit of Reporting | 0.001 µg/L | 0.0002 µg/L | under development | 0.005 µg/L |
| Test Method | LC-MS/MS | | | |
| ALS Test Codes | E744B | E744B-L | pending | E744C |
| Sample Containers | 2 x 100 mL amber glass Teflon-lined (separate containers for 6PPD-Q and 6PPD) | | | |
| Preservation | none | | | anti-oxidant (pre-charged) |
| Storage Temp. | ≤ 6°C (do not freeze) | | | |
| Holding Time | 14 days (Draft EPA 1634) | | | 14 days |

Our Vancouver laboratory maintains ISO 17025 accreditation for 6PPD-quinone and 6PPD in water through CALA. Please refer to our Vancouver's *Scope of Accreditation* for current status. Please contact your ALS' Project Manager in Canada for further details about these important tests.

References

- *Z. Tian et al., 2020. A ubiquitous tire rubber-derived chemical inducesacute mortality in coho salmon, Science 10.1126/science.abd6951.*
- *US EPA Federal Register, June 13, 2024. Acute Aquatic Life ScreeningValues for 6PPD and 6PPD-Quinone in Freshwater.*
- *BC Ministry of Water, Land, and Resource Stewardship, 2025. 6PPDquinone Water Quality Guidelines – Freshwater Aquatic Life. WaterQuality Guideline Series, WQG-24. Prov. BC, Victoria BC.*

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**Funding & Workshop Support Provided By:**

Fisheries and Oceans Canada / Pêches et Océans Canada

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