The Presence and Potential Impacts of the Tire-Wear-Derived Compound (6PPD-q) on NC Aquatic Ecosystems

NCDOT Technical Assistance 2024-13
FHWA/NC/TA2024-13
September 2024

Tarek N. Aziz, Ph.D.
Aaron Mann
Department of Civil, Construction and Environmental Engineering
North Carolina State University

RESEARCH &
DEVELOPMENT

Technical Report Documentation Page

1. Report No.
FHWA/NC/TA2024-13

2. Government Accession No.

3. Recipient's Catalog No.

4. Title and Subtitle
The Presence and Potential Impacts of the Tire-Wear-Derived Compound (6PPD-q) on NC Aquatic Ecosystems

5. Report Date
September 2024

6. Performing Organization Code

7. Author(s)
Tarek N. Aziz and Aaron Mann

8. Performing Organization Report No.

9. Performing Organization Name and Address
Tarek Aziz and Aaron Mann
Department of Civil, Construction and Environmental Engineering
915 Partners Way
Raleigh, NC 27695

10. Work Unit No. (TRAIS)

11. Contract or Grant No.

12. Sponsoring Agency Name and Address
North Carolina Department of Transportation
Research and Development Unit
1549 Mail Service Center
Raleigh, North Carolina 276699-1549

13. Type of Report and Period Covered
Final Report

May 1, 2024 – August 1, 2024

14. Sponsoring Agency Code
TA-2024-13

Supplementary Notes:

16. Abstract

N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is a compound widely used in the manufacturing of tires for both passenger and commercial vehicles. 6PPD is an antioxidant agent that enhances tire durability and prevents the cracking and degradation of tire rubber due to exposure to ozone and UV light. It is estimated that 6PPD comprises between 0.4 - 2% of tire rubber by mass. 6PPD works by reacting with oxidants and then transforming into transformation products (like 6PPD-quinone). Over time, 6PPD in tires migrates to the surface of the tires where it is accessible to atmospheric ozone and becomes more readily worn away. As the tires are used, the surface is abraded against the road surface and turned into tire wear particles (TWPs) (1–3). It has been estimated that the US produces 1,120,000 tons/year of tire wear particles (4). When the TWPs are released into the environment, they can leach 6PPD-q and other transformation products into the water. Researchers studying 6PPD-q release from TWPs have estimated that the minimum mass of 6PPD-q generated from tire wear particles is between 26 – 1900 tons/yr (5).

Our goal in writing this report is to help aid the NCDOT in analyzing the body of scientific literature pertaining to 6PPD-q to equip them with the necessary knowledge and tools to address the challenges posed by 6PPD-q, ensuring the protection of North Carolina's water resources and ecosystems.

We have organized this report into seven chapters. In the remainder of this chapter, we will describe the chemical properties of 6PPD and 6PPD-q. In Chapter 2 we present a brief background of methods used to measure 6PPD and 6PPD-q in the environment and to determine toxicity. In Chapter 3 we discuss literature pertaining to the potential loading of 6PPD-q into NC waters. Chapter 4 discusses the potential toxicity of 6PPD-q to NC aquatic species. In Chapter 5 we summarize our findings and make recommendations for potential next steps. The list of cited references can be found in Chapter 6 and lastly our appendix of supplemental information is contained in Chapter 7.

17. Key Words
Driving, Aquatic, Environmental

18. Distribution Statement

19. Security Classif. (of this report)
Unclassified

20. Security Classif. (of this page)
Unclassified

21. No. of Pages
37

22. Price

Form DOT F 1700.7 (8-72) **Reproduction of completed page authorized**

DISCLAIMER

The Presence and Potential Impacts of the Tire-Wear-Derived Compound (6PPD-q) on NC Aquatic Ecosystems

Prepared for
The North Carolina Department of Transportation
Hydraulics Unit

By
Tarek N. Aziz
and
Aaron Mann
Department of Civil, Construction, and Environmental Engineering
North Carolina State University

Contents

1. Introduction

1.1 Background Report Objectives

N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is a compound widely used in the manufacturing of tires for both passenger and commercial vehicles. 6PPD is an antioxidant agent that enhances tire durability and prevents the cracking and degradation of tire rubber due to exposure to ozone and UV light. It is estimated that 6PPD comprises between 0.4 - 2% of tire rubber by mass. 6PPD works by reacting with oxidants and then transforming into transformation products (like 6PPD-quinone). Over time, 6PPD in tires migrates to the surface of the tires where it is accessible to atmospheric ozone and becomes more readily worn away. As the tires are used, the surface is abraded against the road surface and turned into tire wear particles (TWPs) (1–3). It has been estimated that the US produces 1,120,000 tons/year of tire wear particles (4). When the TWPs are released into the environment, they can leach 6PPD-q and other transformation products into the water. Researchers studying 6PPD-q release from TWPs have estimated that the minimum mass of 6PPD-q generated from tire wear particles is between 26 – 1900 tons/yr (5).

Concern over 6PPD-q began around 2022 when researchers identified 6PPD-q as a primary culprit of the urban runoff mortality syndrome (URMS) plaguing Pacific Northwest coho salmon for decades (1). It was in this first study that researchers ultimately identified the median lethal concentration (LC_{50}) of 6PPD-q for coho salmon at 95 ng/L. Due to the relatively low toxicity concentration and the ubiquity of TWPs, there are now a large and growing group of scientists concerned about the aquatic toxicity of 6PPD-q (1–3,6,7).

To learn more about this potential ongoing threat to the aquatic ecosystem, the Hydraulics Unit of the North Carolina Department of Transportation (NCDOT) requested we develop a report summarizing literature about the loading and potential threat 6PPD-q may pose to North Carolina. Therefore, the objectives of this report are to:

Objective 1 – Review scientific literature to provide insights into estimates of 6PPD and 6PPD-q loading to North Carolina Surface Waters

Objective 2 - Review scientific literature to identify aquatic species that may be sensitive to 6PPD-q pollution in North Carolina

Our goal in writing this report is to help aid the NCDOT in analyzing the body of scientific literature pertaining to 6PPD-q to equip them with the necessary knowledge and tools to address the challenges posed by 6PPD-q, ensuring the protection of North Carolina's water resources and ecosystems.

We have organized this report into seven chapters. In the remainder of this chapter, we will describe the chemical properties of 6PPD and 6PPD-q. In Chapter 2 we present a brief background of methods used to measure 6PPD and 6PPD-q in the environment and to determine toxicity. In Chapter 3 we discuss literature pertaining to the potential loading of 6PPD-q into NC waters. Chapter 4 discusses the potential toxicity of 6PPD-q to NC aquatic species. In Chapter 5 we summarize our findings and make recommendations for potential next steps. The list of cited references can be found in Chapter 6 and lastly our appendix of supplemental information is contained in Chapter 7.

1.2 Properties of 6PPD and 6PPD-quinone

The chemical properties of 6PPD and 6PPD-q can help tell an important story about the fate and transport of these chemicals in the environment. Figure 1 depicts the chemical structure of 6PPD and 6PPD-q. While Table 1 lists some key details about the compounds.

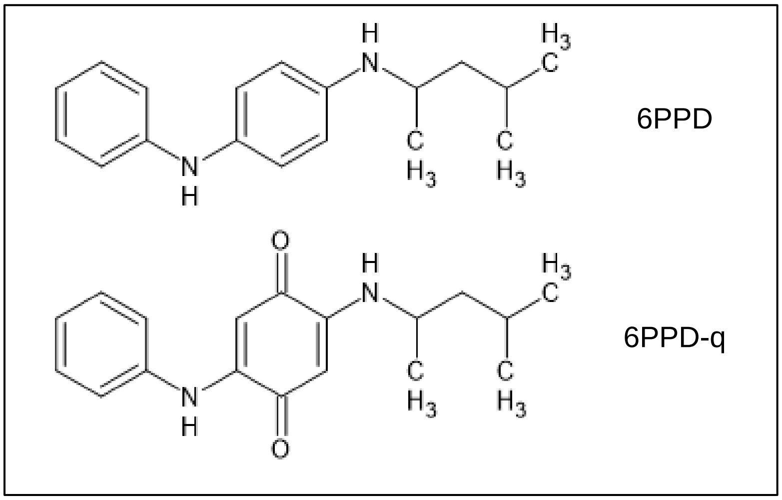

Figure 1 – Chemical structure for 6PPD and 6PPD-q

Table 1 - Summary of some chemical properties for 6PPD and 6PPD-q (3)

Name	**Abbreviation**	**CAS**	**Molecular Formula**	**MW**	**Log K_{ow}**	**Saturated Concentration in water (µg/L)**
N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine	6PPD	793-24-8	$C_{18}H_{24}N_2$	268.4	4.47	1000
N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone	6PPD-quinone (6PPD-q)	2754428-18-5	$C_{18}H_{22}N_2O_2$	298.39	3.98	85

6PPD has a saturated concentration in water of 1000 µg/L, indicating relatively higher solubility compared to 6PPD-q. However, its log K_{ow} value of 4.47 reveals its hydrophobic nature, indicating a preference for partitioning into organic phases over aqueous ones. While this lipophilicity suggests that 6PPD could potentially bioaccumulate in the fatty tissues of aquatic organisms and persist in sediments, other researchers have noticed that 6PPD degrades readily in water, undergoing various transformations influenced by factors such as pH, light, temperature, and the presence of oxidants (2). This is not surprising considering 6PPD's role as an antioxidant additive. Therefore, it seems unlikely that 6PPD would exist for very long in its original form.

In contrast, 6PPD-q has a saturated water concentration of 85 µg/L, reflecting its lower solubility in water. The log K_{ow} of 6PPD-q is 3.98, indicating it is slightly less hydrophobic than 6PPD but still shows a considerable affinity for nonpolar environments. Despite its reduced solubility, research has shown that 6PPD-q is more stable in aqueous environments than 6PPD, with a half-life of 33 hours at 23°C compared to 6PPD's half-life of just 5 hours (2). This stability allows 6PPD-q to persist longer in the environment, posing a prolonged risk to aquatic organisms. Its lower solubility and stability may indicate it is more likely to adsorb onto particulates and sediments.

For comparison's sake, it may be useful to compare 6PPD-q to another common stormwater pollutant, polycyclic aromatic hydrocarbons (PAHs). PAHs, such as phenanthrene and benzo[a]pyrene, are also known for their hydrophobic characteristics and environmental persistence. For example, phenanthrene has a log K_{ow} of approximately 4.5, and benzo[a]pyrene has a log K_{ow} of about 6.1. These values indicate strong lipophilicity and a high potential for bioaccumulation. PAHs tend to adsorb to sediments and organic matter, which can lead to prolonged environmental presence and bioaccumulation in aquatic organisms. However, PAHs are typically less soluble in water than 6PPD, leading to different dispersion and exposure patterns.

While chemical parameters like these can help build important insights into chemical behavior, they must also be tempered by the fact that these compounds will exist in the real world in water matrices comprised of complex organic matter, photooxidative processes, and microbial activity. As more experiments and field observations are made in relation to 6PPD and 6PPD-q we will develop greater insights into the fate and transport of these chemicals in the environment.

1.2.1 Other Transformation Products of 6PPD

While attention has been focused on the transformation product 6PPD-q, it should be noted that many other transformation products have been observed (8). Additional studies have also indicated that 6PPD-q is not the only transformation product formed from 6PPD (5,8). Hu et al. (5) noted 19 transformation products in studies that ozonated pure 6PPD and ozonated tire wear particles. Seiwert et al. (8) noted 38 transformation products of 6PPD and 6PPD-q. While detailed knowledge about these transformation products and their effects is not yet known, it will be important to understand future additional research developments in this space.

2. Methodology

2.1 Measurement of 6PPD and 6PPD-q

Sample analysis for 6PPD and 6PPD-q is relatively new and still under development. For example, researchers have noted that part of the discrepancy in sampling from early experiments was, in part, due to the absence of a commercial standard for 6PPD-q (1–3). That being said, broad sample handling workflow is similar at the high level and can provide insights into the process by which 6PPD and 6PPD-q are measured. Chen et al. (3) broke the process generally into three broad categories: sample collection, sample extraction, and instrument analysis. Depending on the medium being sampled, sample collection and extraction processes are different, however instrument analysis following successful extraction can be more process agnostic. Below is a high-level description of each of the processes that may be used:

Sample Collection

Dust - Dust samples are typically collected using vacuum cleaners or brushes and shovels, followed by sieving to remove debris. Fine particles can be collected using a wet vacuum cleaner or pressure washer, with subsequent water removal via centrifugation and freeze-drying.

Soil - Soil samples are collected using a stainless-steel shovel, then transported to the lab for weighing, freeze-drying, homogenization, and sieving.

Air - samples are collected on quartz microfiber filters using air samplers or on glass substrate membranes using cascade impactors.

Water - samples are collected using sampling poles and pre-cleaned containers, while snow samples are collected and stored in glass bottles.

Sample Extraction

Before extraction, surrogate standards are often added to estimate the recovery of 6PPD and 6PPD-quinone during the process. Common extraction techniques include:

Ultrasonic Extraction: Widely used for soil, dust, and air samples. The samples are sonicated with solvents like acetonitrile, methanol, n-hexane, or acetone. The choice of solvent can influence extraction efficiency.

Accelerated Solvent Extraction (ASE): An alternative extraction method, although sources indicate that ultrasonic extraction generally yields higher recoveries for 6PPD and 6PPD-quinone from dust.

Solid Phase Extraction (SPE): Typically used for water samples. After filtration, water samples are passed through hydrophilic-lipophilic balanced (HLB) cartridges for purification and concentration.

Instrumental Analysis

High-Performance Liquid Chromatography (HPLC) or Ultra-HPLC (UHPLC) coupled with Mass Spectrometry (MS): This is the primary analytical technique for 6PPD and 6PPD-quinone determination. Separation is usually performed on C18, C8, or HSS-T3 columns, using water and methanol or acetonitrile as mobile phases.

Mass Spectrometry Detection: Various MS detectors, including Orbitrap, triple-quadrupole (TQ), and time-of-flight (TOF), are used. The electrospray ionization (ESI) source in positive ionization mode is commonly employed. Target compounds are analyzed in selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) modes.

Quality Assurance and Quality Control

Contamination Prevention: Stringent measures are taken to minimize contamination, including using rubber-free materials, cleaning all wares, and pre-baking filters.

Recovery Assessment: Duplicate samples, spiked samples, and internal standards are used to evaluate extraction efficiency.

Blank Analysis: Laboratory blanks (Milli-Q water, filters) and field blanks are processed alongside samples to account for background contamination.

Limit of Detection (LOD) and Limit of Quantification (LOQ): These parameters are calculated to determine the sensitivity of the method.

These steps are crucial for ensuring accurate and reliable measurements of 6PPD and 6PPD-quinone in environmental samples.

Considerations

The following additional considerations have been noted by researchers.

- 6PPD appears to be somewhat difficult to measure due to its ephemeral relatively short half-life. Researchers have noted that sample storage may have relatively significant effect on the measured 6PPD in the environment (3).

- There are a significant number of transformation products formed from 6PPD. This complicates the assessment of environmental risk and chemical characterization. (8)

- The characterization of the size and type of TWPs is itself a complex process (6,9). As a result of part of this complexity, there is a wide variety of methods and results. Often TWP size classes emphasized are based on the medium being studied (e.g. air, water, soil). This is particularly important because particle size can be an important factor if, for example, 6PPD-q leaching is to be studied (more information on this in Chapter 3).

2.2 Bioassay Methods

Bioassays can be a lower effort method for assessing whole water toxicity or approximating concentrations of toxicants with known effects. Greer et al. demonstrated that colorimetric assays could be used to measure cytotoxicity and cell viability of coho salmon cell lines; however, whole water samples may include many toxicants that would cause similar effects (10). Other *In vitro* studies of salmonid and rat cell lines have observed molecular signals specific to 6PPD-quinone (11–13). Further research is required to develop a specific bioassay for 6PPD-quinone, and using molecular methods may not be lower effort than other means of quantitation.

3. Estimates of Potential 6PPD-q Loads into NC Waters

6-PPD and its transformation products are leached into the environment through tire wear particles (TWPs). Globally, around 3.1 billion tires are produced annually for over 1.4 billion vehicles, leading to an average emission of 0.81 kg of tire rubber particles per capita each year (1). These particles are a substantial source of microplastics in freshwater, with 2 to 45% of total tire particle loads entering receiving waters and freshwater sediments containing up to 5800 mg/kg of TWPs (1). TWPs, therefore, are crucial contributors to microplastic pollution and associated chemical toxicity risks (1). Researchers have estimated the quantity of 6PPD-q released from tire wear in the US to range from 26 to 1,900 tons per year (2). Thus, to understand the release of 6PPD and its transformation products, we must discuss TWPs production. In this section of the report, we will first discuss the generation of TWPs. Then we will discuss the measurements of 6PPD and 6PPD-q in the environment in various forms. Next, we will discuss the work of the few researchers that have investigated event-driven 6PPD-q concentrations in the environment. We will conclude with a section describing factors that could influence potential 6PPD-q hot spots in North Carolina based on our review of the literature.

3.1 Tire Wear Particles (TWP) – Generation, Characteristics, and Chemicals

While the investigation of 6PPD-q in the environment is a more recent research effort, the primary source of 6PPD, tire-wear particles (TWPs) or tire road wear particles (TRWPs), have been more thoroughly investigated. TWPs are the catch-all phrase used to describe the production of 'microplastics' from vehicle tires into the environment. The production and transport of TWPs in the environment is an important and active area of research. Mayer et al. has authored an extensive review article discussing several pertinent findings related to the role of tires in the environment (6). The path of TWPs in the environment is complex. After release from vehicles, TWPs can be transported in the environment via the atmosphere and stormwater runoff. Depending on particle size, atmospheric TWPs can deposit on land and/or water, sometimes great distances from their source. TWPs may also enter the environment through recycled or disposed of tires (7). There remains a number of questions related to the production of TWPs and their impacts on the environment and public health. For this report, we will operationally focus on the generation, characteristics, and properties of TWPs as the primary source of the tire-derived contaminants 6PPD-q.

TWP generation has been shown to depend on a number of transportation related factors including (6):

- **Vehicle weight and tire size:** Larger, heavier vehicles like trucks emit more TWPs than smaller, lighter vehicles.
- **Traffic speed and driving style:** Higher speeds lead to increased generation of tire particles, as do driving styles that involve a lot of braking and accelerating, such as urban stop-and-go driving versus highway driving.
- **Road surface condition:** Rougher road surfaces cause more tire wear, leading to higher TWP emissions.

As may be expected, TWPs are produced in a range of sizes. Mayer et al. (2024) categorize these size classes as: ultrafine (< 0.1 µm diameter), fine (0.1 – 2.5 µm diameter), and coarse (> 2.5 µm). Of the coarse particles, those greater than 50 µm compromise the largest volume and mass of TWPs (6).. Ultrafine and fine TWPs are subject to long-distance aerial transport and are mobilized by the turbulence generated with high-speed traffic. Coarser TWP particles deposit more quickly near the source. Particle size, like the overall production of TWPs, is influenced by vehicle traffic. As vehicles drive over roads with TWPs the abrasion changes the size and shape of those particles, ultimately making them smaller. While a greater number of small TWP particles are produced, the greater mass of TWPs are generally believed to come from the coarser size range. Though there is an overall need for more research into the particle size distribution from TWPs, the size distribution of TWPs is important for two specific reasons:

(1) **Transport in the Environment** – As noted, fine and ultrafine particles can be suspended in the atmosphere. Depending on weather conditions, these particles can travel for substantial distances. This potential long-distance transport can lead to down-wind impacts from TWPs and make management of transportation-based air and water quality impacts more complicated. Larger particles are more likely experienced in closer proximity to road surfaces and are thus the TWP size-class most likely to be influenced by stormwater mitigation strategies.

(2) **Surface Area Impacts** – Small particles have a greater surface area-to-volume ratio and more environmental mobility than larger particles. This makes them potentially more impactful on a per-mass basis.

The significance of TWP size is already demonstrated in recent studies of 6PPD and 6PPD-q from road dust. Multiple researchers have shown that finer TWPs result in a higher leachable concentration of 6PPD and 6PPD-q (14,15).

While emphasis of this report is on the tire-derived additive transformation product, 6PPD-q, it should be noted that tire wear particles produce a range of compounds of concern (discussed further in 3.6 Additional Considerations).

3.2 Quantifications of 6PPD and 6PPD-q in the Environment

Recently, there have been multiple studies investigating the measurement of 6PPD and 6PPD-q in the environment (2,3). Research has been split based on the media being studied. Samples have been collected and analyzed in water, road-side dust, atmospheric particles, and road-side soils. In the case of 'dry' samples (e.g. road dust samples and air samples), researchers had to conduct leach tests to dissolve 6PPD and 6PPD-q into the aqueous phase. These leaching experiments themselves offer important information about the potential fate of tire-derived contaminants in the environment but also influence the result obtained. For example, the size of dust particles, temperature, pH of the water, and the light conditions could all have an influence on the leach test outcome (2). It is important to take these factors into consideration when comparing results from various studies.

While there may be a little more homogeneity in process handling for grab samples collected from water, there still may be methodological differences. For example, commercial standards were initially unavailable for 6PPD-q. Tien et al., the researchers that initially discovered the acute toxicity of coho salmon to 6PPD-q, had to revise their results once standards were available. This revision led to a 8.3 x decrease in the estimated LC_{50} (1).

Table 2 provides an abbreviated summary of stormwater-related concentration measurements of 6PPD-q and 6PPD. This table is an adaptation of a table developed in a review paper written by Zoroufchi et al. (2). While road dust and soil samples were not included in Table 2, they were included in the Appendix in Table 5 and Table 6. The original table also included measurements related to drinking and wastewater treatment. Those were removed as they were not seen as relevant for this report.

Table 2 is difficult to interpret when considering the massive range of values, variable locations, and previously mentioned methodological differences. However, a few important observations can be made:

- **Stormwater drives 6PPD-q loading-** Samples collected from stormwater runoff consistently have the highest concentrations of 6PPD-q. While not surprising, this points directly to runoff-driven events as a transport mechanism of 6PPD-q to receiving waters.

- **The concentration of 6PPD-q in samples is often environmentally relevant -** While concentrations vary substantially, many of the concentrations were significantly above the 95 ng/L LC_{50} specified for coho salmon.

- **6PPD-q will likely be found in runoff and receiving waters -** 6PPD-q was detected in most samples measured. This includes both stormwater runoff and in receiving waters.

- **6PPD was measured at lower concentrations -** Where measured, 6PPD appeared at a much lower concentration (despite its higher solubility). This is likely due to the reactivity of 6PPD.

There are a significant number of factors that we can reasonably assume influence the presence of 6PPD-q in the environment. A major factor is, of course, the presence of TWPs. Tire wear particle production, as noted previously, is a function of several factors (traffic density, speed, etc.). While some researchers took consideration of traffic density in their studies (15), most did not. The researchers that did include traffic noticed a higher concentration of 6PPD-q with higher traffic density too (15). It is also regionally specific. For example, it is known that US drivers travel more miles and thus likely produce more TWP per capita (4). We also know, as will be discussed in the next section, that 6PPD-q transport is driven by precipitation events (16). This may be part of the reason why a more arid environment would show lower concentrations of 6PPD-q in receiving waters (e.g. the Australian data from Table 2). In combination, these observations are intended to dissuade a reader from, for example, taking an average of the reported concentrations as an indication of what one may expect in North Carolina.

Table 2 – Abbreviated reproduction of f 6PPD and 6PPD-q concentrations in varying environments from Zoroufchi et al. (2023) (2). Emphasis here has been placed on studies that measured collected samples from runoff and receiving waters. Additional tables for road dust and other categories can be found in the Appendix (Figures 5 and 6).

		Concentration (ng/L)			% Detection Frequency		
Category	**Sampling Location**	**6PPD-q**	**6PPD**	**Sample #**	**6PPD-q**	**6PPD**	**Ref**
Runoff	Roadway Runoff, Seattle-region, USA	800 - 19,000	-	16	100	-	(1)
Runoff	Receiving waters during storm, Seattle-region, USA	< 300 - 3200	-	21	29	-	
Runoff	Roadway runoff, Los Angeles region, USA	4,100 - 6,100	-	2	100	-	
Receiving waters	Creeks affected by urban runoff, San Francisco region, USA	1,000 - 3,500	-	4	40	-	
Runoff	Road surface runoff (Hong Kong, China)	210 - 2,430	0.21 - 2.71	9	100	100	(17)
Other Runoff	Courtyard surface runoff (Dongguan and Huizhou cities, China)	6.03 - 875	0.19 - 1.10	-	100	41.7	(18)
Runoff	Road surface runoff (Gongguan and Huizhou cities, China)	38.5 - 1,562	0.41 - 7.52	-	100	37.5	
Other Runoff	Farmland surface runoff (Huizhous and Gongguan cities, China)	0.53 - 5.58	ND	-	100	0	
Receiving Waters	Zhujiang River (China)	0.26 - 11.3	0.31 - 1.07	-	100	30.8	
Receiving Waters	Dongjiang River (China)	0.29 - 8.12	0.27 - 1.29	-	100	48	
Receiving Waters	Surface water (Guangzhou, China)	ND - 0.75	ND	19	89.5	ND	(19)
Groundwater	Groundwater (Guangzhou, China)	ND - 0.75	ND	43	60.5	ND	
Runoff	Stormwater (Guangzhou, China)	0.18 - 1.42	ND	10	100	ND	
Other	Suspended Particles (Guangzhou, China)	ND - 0.07	ND - 0.74	10	90	70	
Receiving waters	Don River (Ontario, Canada)	290 - 2,300	-	14	100	-	(16)
Other	Snowmelt (Saskatoon, Canada)	15 - 756	-	32	90	-	(20)
Runoff	Stormwater (Saskatoon, Canada)	86 - 1,400	-	19	57	-	
Receiving waters	Creek (Queensland, Australia)	<0.05 - 24	-	6	83	-	(21)
Receiving waters	River (Queensland, Australia)	2.1 - 15	-	4	100	-	
Runoff	Retention Pond (Queensland, Australia)	2.9	-	1		-	
Receiving waters	Tributary (Queensland, Australia)	0.62	-	1		-	
Receiving waters	Lower Estuary (Queensland, Australia)	< 0.05 - 0.1	-	2	50	-	
Receiving waters	Middle Estuary (Queensland, Australia)	0.28	-	1		-	
Receiving waters	Lake/Reservoir (Queensland, Australia)	< 0.05 - 3.1	-	3	67	-	
Receiving waters	Creek (Brisbane, Australia)	2 - 8.4	-	3	100	-	
Receiving waters	Creek During Storm Event (Brisbane, Australia)	0.4 - 88	-	32	100	-	(20)

3.3 Event-Driven 6PPD-q Measurements

3.3.1 Precipitation

Of the research on 6PPD-q to date, few studies have specifically correlated 6PPD-q production with rainfall events to better understand the transport of 6PPD-q during precipitation events. Johannessen et al. (16) performed a retroactive investigation of archived samples collected from the greater Toronto area (GTA) of Canada. Specifically, the researchers measured both 6PPD-q and DPG, another tire-derived contaminant. The authors noted that, in their study, 6PPD-q plateaued at around 2.8 µg/L (~ 3x the LC50 for coho salmon) and sustained that plateau for around 12-18 hours. 6PPD-q did not display a first-flush behavior but rather showed a "middle flush dynamic" where the contaminant loading was maintained despite increasing cumulative runoff volumes. In other words, the researchers noted a larger mass-transfer of 6PPD-q with an increase in precipitation. The authors cited the work of other researchers (22) who attributed this type of behavior to a potential reservoir of tire-wear materials in urban watersheds. Termed the "semi-infinite reservoir" hypothesis, this idea suggests that due to the transport-limited nature of the 6PPD-q, additional runoff volumes will produce higher mass loadings of the pollutant into the environment.

3.3.2 Atmospheric Ozone

Hiki and Yamamoto (15) noted a correlation of 6PPD-q to atmospheric ozone concentration in their study of road-dust in Tokyo Japan. Specifically, they noted elevated atmospheric ozone concentrations between May-June in Tokyo, Japan. These elevated ozone levels correlated in a statistically significant way to enhance normalized 6PPD-q concentrations in road dust collected from both arterial and residential roads. It should be noted that a similar trend may be expected in North Carolina. Figure 2 shows the atmospheric ozone concentration in Raleigh, NC from January 2020-June 2024. It can be noted that, as with Tokyo, ozone concentrations in NC appear to peak in the May-June timeframe. This occurs due to a combination and interaction of seasonal and atmospheric conditions potentially including stronger sunlight, warmer temperatures, stagnant weather conditions, and an increase of emissions of ozone precursors.

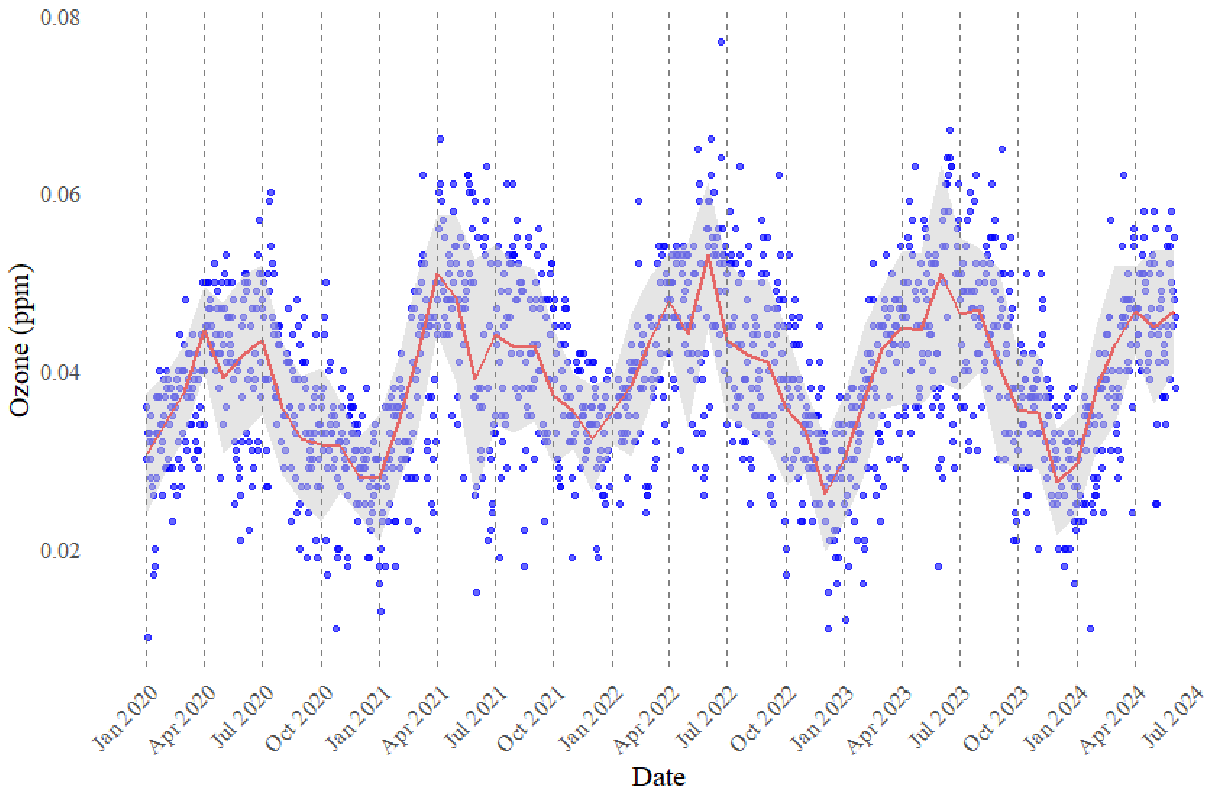

Figure 2 – Daily maximum 8-hour ozone concentrations for Raleigh, NC, from January 1st, 2020 – June 5th, 2024. The solid line represents the monthly average. The grey band represents the average ± monthly standard deviation. The vertical lines are displayed at three-month intervals. Data obtained from US EPA AirData (https://www.epa.gov/outdoor-air-quality-data/air-data-concentration-plot)

3.4 Mitigation of 6PPD-q

While there is still research to be done in understanding the transport and mitigation of 6PPD-q (and other stormwater contaminants) in the environment, there is some research that suggests that 6PPD-q can be removed via bioretention cells (23,24). The only specific mechanism discussed explicitly is soil sorption.

Rodgers et al. (23) performed experimentation and modeling to explore the feasibility of 6PPD-q removal via a bioretention cell in Vancouver, Canada. Their experimental results indicated a mass reduction of 95% of 6PPD-q to the underdrain. Using the bioretention cell model Bioretention Blues, the researchers also simulated a longer duration of storm events into their bioretention cell. The results were similarly positive with the simulated bioretention cell showing good performance under most typical storm conditions (< 2 yr. return period for the region). While the authors noted that there is still work to be done to understand the transformation of 6PPD-q in the soil, these results suggest that LID stormwater control measures that involve stormwater filtration through soil may provide a barrier of protection to surface waters. We would like to note that, while this research is promising, gaps still remain in our understanding of 6PPD-q transport in stormwater control measures. For example, the experiment conducted by Rodgers et al. (23) specifically measured a spike of 6PPD-q into the bioretention cell and not the process by which 6PPD-q is produced through TWP accumulation and transport. Future work should explore the question of 6PPD-q fate in stormwater control measures in a more mechanistic way to understand how protective this infrastructure may be.

McIntyre et al. (24), though not specifically measuring 6PPD-q, investigated the changes to the toxicity of stormwater to coho salmon before and after it was treated through a bioretention cell. In their study, while the bioretention cell did not prevent all adverse effects for the coho embryos, it greatly reduced the impacts of untreated stormwater.

Overall, these experiments show the great promise of filtration-based natural stormwater infrastructure to potentially mitigate 6PPD-q release into the environment.

3.5 Considerations for 6PPD-q in North Carolina

Considering data pertaining to tire-wear production and the measurement of 6PPD-q during rainfall events, the following factors should be considered when thinking about potential 6PPD-q hotspots in North Carolina:

- **High Volume and Speed** – Regions of high traffic volume and high vehicular speed are likely to produce increased quantities of TWPs. Though our information regarding TWP transport in watersheds is imperfect, it seems plausible that since coarser TWPs appear to deposit closer to roadways, they will be more likely to be transported into nearby waters and release 6PPD-q during precipitation events.
- **Precipitation Events** – In stream 6PPD-q concentration appears to increase during storm events in regions impacted. Therefore, it is anticipated that during a storm event, concentration will be measurable.
- **No Green Infrastructure** – Research suggests that 6PPD-q can be mitigated through use of green stormwater infrastructure like bioretention cells. Researchers have also noted that natural infiltration may also help remove and trap 6PPD-q. Therefore, it is likely that sites that do not employ such filtration-based infrastructure will be more likely to show heightened concentrations of 6PPD-q.
- **Seasonal Impacts** – Researchers have noted that there seemed to be a higher proportion of 6PPD-q in tire wear particles during seasons that had higher atmospheric ozone. This is likely to occur in late spring/early summer months (April – July).

3.6 Additional Considerations

3.6.1 Other Tire-Derived Compounds of Concern

It should be noted that 6PPD and 6PPD-q are not the only compounds of concern related to tire wear products. Mayer et al. (2024) identify a wide array of chemicals of concern that can leach from tire particles, posing potential risks to ecosystems and human health. These chemicals can be categorized into several groups (6):

- **Metals:** Tire manufacturing utilizes various metals, particularly zinc (Zn) in the vulcanization process. Consequently, Zn is a major toxicant found in tire particles and leachates. Other metals, including aluminum (Al), copper (Cu), nickel (Ni), cobalt (Co), manganese (Mn), iron (Fe), chromium (Cr), lead (Pb), titanium (Ti), strontium (Sr), barium (Ba), selenium (Se), and cadmium (Cd), are also present in tires, brakes, catalytic converters, and road asphalt, frequently detected in tire and road dust.

- **Polycyclic Aromatic Hydrocarbons (PAHs):** Generated during the incomplete combustion of organic matter, PAHs are present in tires, crude oil, and gasoline. The types and amounts of PAHs vary among tire brands. PAHs can leach from tire particles into water, soil, and even biological fluids, posing carcinogenic and non-carcinogenic risks to organisms.

- **Organic Compounds:** Besides PAHs, tires contain a multitude of other organic compounds. Notably, alkylphenols, such as bisphenol A (BPA), have been detected in tire particles and their leachates. BPA is recognized as an endocrine disruptor in various vertebrate species, including fish.
- **Tire Additives and Transformation Products:** The manufacturing process of tires involves a complex cocktail of additives, many of which are proprietary. These additives, along with their industrial and environmental transformation products, can leach into the environment.

The complexity of tire leachate composition, highlighting that the leaching magnitude is influenced by chemical properties such as diffusivity, hydrophobicity, polarity, and the structure of the chemical itself. Additionally, the tire material properties can play a significant role in leaching. As is the case with the relationship with 6PPD and 6PPD-q, the chemical composition, age, weathering, surface alterations, particle size, and surface area of the tire material can all impact leaching. Lastly, environmental factors like the liquid-to-solid ratio, time, mixing regime, temperature, pH, salinity, and the presence of co-solvents or organic matter in the surrounding environment can significantly influence the leaching process.

Due to this complexity, observed leachate concentrations vary substantially across studies. Environmentally relevant concentrations of tire particles are significantly lower than those typically used in laboratory leaching studies. This disparity, combined with the influence of weathering on leachable chemical concentrations, makes it challenging to extrapolate laboratory findings to real-world scenarios. Therefore, accurately assessing the environmental risks of tire leachate necessitates considering these various factors and their complex interactions.

3.6.2 Other Potential TWP Exposure Pathways

Concerns surrounding tire wear particles (TWPs) and their chemical additives extend beyond vehicular use, highlighting the need for a comprehensive approach to managing these risks. One major concern is the use of crumb rubber, derived from grinding used tires, as infill in artificial turf. With over 11,000 synthetic turf fields in the US alone, crumb rubber poses potential health risks by releasing hazardous substances like heavy metals, PAHs, and PFAS chemicals into the environment (6). Exposure pathways include inhalation, ingestion, and direct contact, with potential health risks such as lymphoma and leukemia among athletes prompting the EPA to develop a research action plan. Additionally, other rubber products like belts, hoses, cables, and sporting equipment can also release 6PPD and 6PPD-quinone, complicating efforts to track and mitigate their environmental impacts. The management of waste tires is another significant issue, with an annual production of 3 billion new tires and approximately 800 million becoming waste. Improper storage and disposal of these tires can create mosquito habitats, cause chemical leaching into soil and groundwater, and result in air pollution from tire burning. While recycling tires into products like crumb rubber can aid waste management, concerns remain about the potential release of harmful chemicals, especially in applications close to humans or sensitive ecosystems. These issues underscore the need for a holistic strategy to address the environmental and health risks of TWPs and their chemical additives throughout their lifecycle.

4. Potential Toxicity of 6PPD-q in NC Waters

The toxic potential of tire wear particles (TWPs) to North American fish was established in 2018 when an investigation linked TWPs as the cause of urban runoff mortality syndrome (URMS) in Coho salmon (25). 6PPD-q was later identified as the most harmful TWP toxicant for Coho salmon in the seminal 2021 study. The breadth of 6PPD-q’s toxic effects to aquatic life has been discovered through toxicological studies of closely related salmonid species and ecotoxicity model organisms (e.g. zebrafish). The earlier studies were echoed by studying URMS of the zooplankton *Daphnia pulex* (a water flea), which found 6PPD-q was also the primary causal agent (26). Other toxicants derived from TWPs may also have important ecological impacts due to higher rates of bioaccumulation, compared to 6PPD-q, which may result in chronic effects (27). The current body of research suggests that 6PPD-q is the critical TWP toxicant in road runoff inducing URMS in freshwaters and estuaries. One endemic North Carolina fish species, *Salvelinus fontinalis* (Brook trout), has been evaluated for 6PPD-q toxicity while studies performed on model organisms are used in this chapter to predict which groups of endemic aquatic life may be sensitive.

Literature Review of 6PPD-quinone Ecotoxicity in Water

This section summarizes the findings from a systematized search and review of literature published by May 2024 on the topic of 6PPD-quinone toxicity to aquatic organisms. Methods for selecting literature and collecting relevant data are included in Appendix 0. This review evaluated chemical ecotoxicity using the framework described in Bioanalytical Tools in Water Quality Assessment (28) and illustrated in Figure 3.

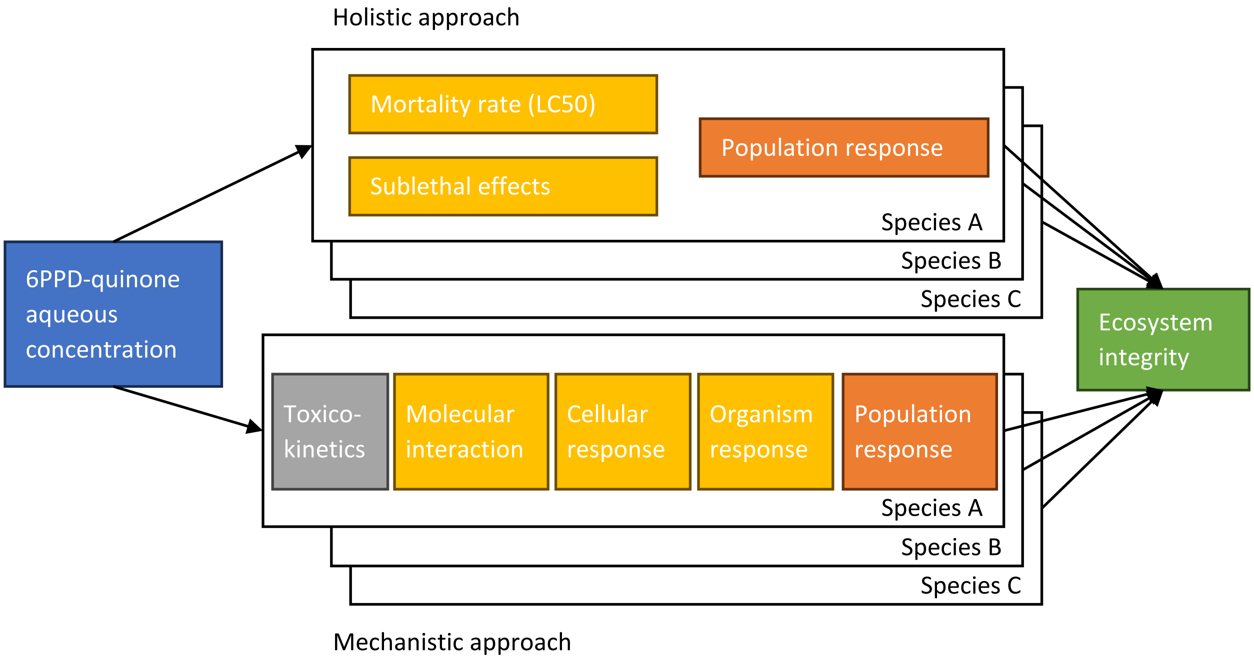

Figure 3: Comparison of the holistic approach used in this study for assessing risks to NC aquatic species from 6PPD-quinone pollution and the novel mechanistic approach. Each white box is a single adverse outcome pathway. This figure was adapted from Bioanalytical Tools in Water Quality Assessment by Escher et al. (28).

Holistic Aquatic Toxicity Assessment

Toxins have unique modes of action in the cells of each species that lead to population and ecosystem effects. The traditional holistic assessments characterize the ultimate population-level effects by exposing organisms to the toxin at varying aqueous concentrations to estimate concentrations of no effect for sublethal impacts and concentrations for inducing 50% mortality (LC50). The data from these toxicity studies can be used to fit holistic concentration-response models. The range of LC_{50} values and minimum concentration to elicit sublethal effects reported in literature are summarized in Table 3. Organisms with a common evolutionary history are expected to have similar concentration-response curves when the mode of action targets a conserved cellular mechanism. As other studies have discussed, the modes of action inducing acute mortality in 4 salmonids (genus *Oncorhynchus*, *Salmo*, and *Salvelinus*) may not be highly conserved since 9 other salmonids were found to be not sensitive (29–32). Many fish species summarized in Table 3 were studied at various life-stages. Developing concentration-response assessments for larvae and adults would be used for quantifying seasonal risk based on spawning patterns. The screening procedure in this report does not distinguish toxicity data by life-stage. This analysis does not consider the different toxicities of 6PPD-quinone enantiomers because only Di et al. identified which enantiomers were tested and investigated multiple chiral forms (33).

Table 3: List of species with toxicological data for 6PPD-quinone

TAXONOMIC GROUP	SCIENTIFIC NAME	COMMON NAME	NC PRESENCE	LC$_{50}$ (ng/L)	SUBLETHAL CONC. (ng/L)	SUBLETHAL EFFECTS	SOURCES
CRUSTACEAN	*Daphnia magna*	Water flea	Endemic				(34)
	Hyalella azteca		Endemic				(34)
	Parhyale hawaiensis		Not present				(35)
FRESHWATER FISH	*Acipenser transmontanus*	White sturgeon	Not present				(36)
	Danio rerio	Zebrafish	Not present		1,000	impaired locomotion, malformation, intestinal inflammation, oxidative stress, dysregulated metabolism	(13,34,37–40)
	Gobiocypris rarus		Not present				(33)
	Oncorhynchus gorbuscha	Pink salmon	Not present				(32)
	Oncorhynchus kisutch	Coho salmon	Not present	33.6 - 960			(10,41–46)
	Oncorhynchus masou masou	Masu salmon	Not present				(29)
	Oncorhynchus mykiss	Rainbow trout	Introduced	1860 – 2,060	2,780	oxidative stress	(33,36)
	Oncorhynchus nerka	Sockeye salmon	Introduced				(10,44)
	Oncorhynchus tshawytscha	Chinook salmon	Not present	80,000			(10,42,44)
	Oncorhynchus keta	Chum salmon	Not present				(43)
	Oryzias latipes	Japanese medaka	Not present				(34)
	Pimephales promelas	Fathead minnow	Introduced		200	oxidative stress, dysregulated metabolism	(47)
	Salmo salar	Atlantic salmon	Not present				(30)
	Salmo trutta	Brown trout	Introduced				(30)
	Salvelinus curilus	Southern Dolly Varden trout	Not present				(29)
	Salvelinus fontinalis	Brook trout	Endemic	480 - 630	720	oxidative stress	(36)
	Salvelinus leucomaenis pluvius	Whitespotted char	Not present	410 – 2,100	1,000	oxidative stress	(29)
	Salvelinus alpinus	Arctic char	Not present				(36)
MARINE FISH	*Sciaenops octellatus*	Red drum	Endemic				(48)
GASTROPOD	*Planorbella pilsbryi*	File ramshorn snail	Endemic				(49)
BIVALVE	*Megalonaias nervosa*	Washboard mussel	Not present				(49)
MAYFLY	*Hexagenia bilineata*	Burrowing mayfly	Not present				(49)
NEMATODE	*Caenorhabditis elegans*		Endemic		100	paralysis, neurodegeneration, DNA damage, reduced fecundity, oxidative stress	(50–55)
ROTIFER	*Brachionus koreanus*		Not present		250,000	reduced fecundity	(31)
	Brachionus calyciflorus		Endemic				(56)
GREEN ALGAE	*Chlamdomonas reinhardtii*		Endemic		250,000	DNA damage, reduced growth	(57)

Mechanistic Aquatic Toxicity Assessment

Mechanistic toxicity models may result in more accurate risk assessments of untested species. The mechanistic approach has been standardized by environmental agencies in the United States (USEPA Tox21) and Europe (EU's ITS) (28). A mechanistic approach would begin with modeling the concentration of a toxicant within an organism (i.e. dose) based on aqueous concentrations and known toxicokinetics. The predicted dose would be related to concentration-response assessments for a known adverse outcome pathway (AOP). A complete model would ultimately predict the population response to surface water pollution. This report does not use mechanistic models for the risk assessment of 6PPD-quinone. The Tox21 framework for computing the chemical hazard for individual species may be more time and resource efficient than conducting *in vivo* holistic toxicity studies for all species of concern.

Toxicokinetics considers the internal mass balance of 6PPD-quinone in an organism. In fish, toxicant enters through the gills, exits through excretion, and is transformed in the liver (11). 6PPD-quinone has been found to accumulate in some invertebrates because the hydrophobic compound avoids excretion by remaining in fatty tissue (27). In contrast, the principal factor for 6PPD-quinone dose in fish for the same study was the instantaneous aqueous concentration due to transformation in the liver.

An adverse outcome pathway includes the molecular interactions of 6PPD-quinone, the resulting cellular and organ level responses, and the ultimate organism and population level responses. Adverse outcome pathways often affect well-conserved cellular systems, so they can be extrapolated to untested species. Table 4 lists adverse outcome pathways proposed by other authors or inferred from related modes of action for organisms exposed to dissolved 6PPD-quinone. The adverse outcome pathway ending in acute mortality for fish includes the generation of reactive oxygen species (ROS) in gill cells which causes oxidative stress and cell death. Other pollutants that produce ROS can exacerbate oxidative stress as observed by *in vivo* toxicity tests (38,58). Except for acute mortality in fish, the sublethal organism responses require further investigation to extrapolate population-level responses.

Table 4: Proposed adverse outcome pathways (AOPs) for organisms exposed to dissolved 6PPD-quinone. Blank cells indicate unknown responses.

MOLECULAR INTERACTION	**CELLULAR/ORGAN RESPONSE**	**ORGANISM RESPONSE**
→ Blocking of quinone sites (59) → Dissociation of mitochondrial membrane potential (11,60) → Activation of AhR signaling pathway (61)	→ Dysregulated mitochondrial respiration in gills (11,30,47) → Generation of ROS (59,60) → DNA damage (61) → Enterotoxicity (52)	→ Acute mortality → Oxidative stress (62) → Swimming to surface and gasping
→ Bulky DNA adducts (57) → Mutagenicity (35,56)	→ Loss of genetic stability	→ Genotoxicity
→ Dysregulation of membrane permeability genes (13,37,46) → Alterations of neurotransmitter profiles (40)	→ Malformation of eyes, intestines, and swim bladders (13,37,46)	→ Larval mortality (46) → Impaired locomotion (37,38)
→ Calcium channel inhibition	→ Cardiotoxicity (60)	

→ Myocardial contraction suppression (60)		
→ Dysregulation of dopaminergic genes → Promotion of D-motor proteins (55)	→ Reduced dopamine content → Degeneration of motor neurons (55)	→ Impaired locomotion (nematode) (50,55)
→ Dysregulation of insulin signaling pathway (53)		→ Lifespan reduction (53)
→ Activation of apoptosis and phagocytosis signaling pathways	→ Reduced egg fertility (51)	→ Reduced hatch rate (51)

Interspecies and ecosystem effects

The concentration-response models could be used to predict ecosystem responses to 6PPD-quinone aqueous concentrations. Ecosystem models can be complex by including interspecies interactions. A model for evaluating 6PPD-quinone risks at the scale of a watershed or stream ecosystem may contribute important insights because of potential impacts to lower trophic level organisms (e.g. nematodes, zooplankton, minnows). However, such a model would require greater knowledge of the final population-level stages of sublethal AOPs. For example, the sublethal impacts of 6PPD-quinone on Fathead Minnow populations have not been studied *in situ*. The existing library of data that could be used for an ecosystem model include acute mortality results by species (Table 3, LC_{50}) and *in situ* studies of Coho salmon and zooplankton URMS with 6PPD-quinone measurements (25,26). One study of bioaccumulation of TWP pollutants through and estuarine food chain begins to elucidate interspecies mass transfer of 6PPD-quinone, but does not provide sufficient data to model how species populations are impacted by pollution (27).

Assessment of Potentially Sensitive NC Species

To evaluate the risk of 6PPD-quinone to NC aquatic life, endemic species were assessed for potential sensitivity based on the holistic concentration-response data (Table 3). A dataset of species in taxonomic groups with toxicity testing (crustaceans, freshwater bivalves, freshwater fish, freshwater/terrestrial gastropods, and mayflies) was acquired from the North Carolina Natural Heritage Program (NHP) open-source record. Each species was cross-referenced with Table 3 using taxonomic classifications to identify the closest taxonomic relative with a toxicity study. While this is similar to phylogenetic approaches used to assess origins of toxicity in the Salmonidae family (29–32), taxonomic classifications only indirectly imply genetic and physiological similarities.

The potential sensitivity of each endemic species is summarized by the minimum and maximum LC_{50} calculated (if acute mortality was observed in relatives), the minimum concentration observed for sublethal effects, and descriptors of the sublethal adverse outcomes. Only one endemic species, *Salvelinus fontinalis* (Brook Trout), has published toxicity studies. Four species have a close relative in their same genus with a toxicity study and 75 matched with toxicity data at the family level. The remaining 218 species matched at the level of order, class, or phylum and are discussed as data gaps in the next section. The complete table of results and the R scripts used for analysis are listed in Appendix 0.

Brook Trout have been identified as one of the four salmonid species sensitive to 6PPD-quinone. Although this is the only endemic freshwater salmonid species, the introduced sport fish *Oncorhynchus tshawytscha* (Chinook Salmon), *Oncorhynchus mykiss* (Rainbow Trout), and *Salmo Trutta* (Brown Trout) have published toxicity data. Rainbow Trout (LC_{50} 1860-2060 ng/L) (33,36) has been found to be less sensitive than Brook Trout (LC_{50} 480-630 ng/L), while Chinook Salmon and Brown Trout have been classified as not sensitive (36). Other endemic species that have been studied but were not included in the NHP list include many widespread species of invertebrates and the marine fish *Sciaenops octellatus* (Red Drum). As discussed in the previous section, sublethal effects have been observed in some model invertebrates, while Red Drum is not sensitive.

The four species that matched at the genus level included three species of sturgeon and one species of snail. None of the related species were found to be sensitive to 6PPD-quinone.

Four taxonomic families had matching results. Most species matching at the family level belong to Unionidae which includes 45 of all 46 freshwater bivalves. The studied relative, *Megalonaias nervosa* (Washboard mussel), was not found to be sensitive to 6PPD-quinone. The next largest family is Leuciscidae (27 true minnows) which includes minnows, chubs, and shiners. The closest studied minnow relative is *Pimephales promelas* (Fathead minnow) which experienced oxidative stress and disruption to critical metabolic cycles in the liver and gills at water concentrations as low as 200 ng/L (47). The remaining three species with a family level match were two snails and the Freshwater Drum, none of which related to a sensitive species.

These findings raise concerns for Brook Trout, non-endemic Rainbow Trout, and all true minnows as potentially sensitive species. Brook trout are a “significantly rare”, but “apparently secure” species distributed throughout the mountain region in headwater streams according to the NHP ranking system. All 27 Leuciscidae family members in the species list are more threatened with 6 at the highest rank of “critically imperiled” and the remainder “imperiled” or “vulnerable”. State legally protected statuses apply to 16 Leuciscidae including 2 endangered, 7 threatened, and 7 carrying special concern. Two Leuciscidae species are federally protected. All counties and basins have endemic members of the Leuciscidae family. This analysis also provides evidence that sturgeons and freshwater bivalves are less likely to be sensitive; however, both groups only have toxicity data for one representative species.

Toxicology Data Gaps for NC Species

This exercise revealed the dearth of relevant toxicity data for many endemic species. Amphibians, reptiles, semi-aquatic mammals (discussed in section 4.4), dragon/damselflies, caddisflies, and stoneflies were excluded from the initial dataset because there are no representative concentration-response assessments.

Within the included species that did not have a family match or lower, many (72) matched by order including 48 fish, 21 mayflies, and 3 small crustaceans. The larger fish families in this selection include Percidae (25 darters, ruffes, and perches) and Catostomidae (14 suckers). Sensitivity may be more likely for suckers since they are related by order Cypriniformes to Fathead minnow.

The majority (132) of the less-studied species matched by class including 88 gastropods, 29 crustaceans, 14 fish, and 1 bivalve. A vast majority of these gastropods are air-breathing land snails, but 9 of the understudied gastropods are aquatic. The largest family of crustaceans in this selection is Camaridae (27 freshwater crayfish). The most notable groups of fish are toothcarps and catfish.

The remaining 14 species that only matched by phylum included 11 small arthropods and 3 fish (all lampreys).

5. Conclusions and Recommendations

5.1 Summary of Findings

In this report we summarized literature pertaining to the properties, measurement, production, transport, mitigation, and toxicity of 6PPD-q. In the development of this report, we learned that the threats our aquatic ecosystems face from tire wear particles are substantial. While attention has rightly been given to 6PPD-q based on recent toxicity findings in the Pacific Northwest, the cocktail of contaminants derived from tire wear particles is undoubtedly adversely impacting our ecosystems.

Through our review of literature, we have identified several relevant conclusions and suggestions about the loading of 6PPD-q and its potential toxicity to important NC organisms. While the research we reviewed was performed under conditions non-specific to NC, we believe our review has provided us useful insights to make recommendations about future research needs to better protect our waters.

Loading Findings

- 6PPD-q is very likely present at measurable levels in transportation stormwater influencing NC waters
- 6PPD-q load in road dust has been correlated to to precipitation event intensity, traffic density, and higher atmospheric ozone concentrations. In the case of each factor, researchers have proposed physiochemical mechanisms for increased 6PPD-q levels. However, more work is needed to definitively link these drivers to 6PPD-q levels.
- During a storm event, 6PPD-q follows a middle flush dynamic where the pollutant load increased with increasing runoff volumes.
- TWP production and properties are a function of several interconnected factors (traffic density, weight, driving style, road conditions, etc.) many of those factors could be used to identify potential 6PPD-q hotspots in North Carolina
- According to preliminary studies, 6PPD-q appears to be removed in bioretention cells. More holistically, bioretention cells appear to reduce the toxicity of urban stormwater.

Toxicity Findings

- 6PPD-q is the primary pollutant causing urban runoff mortality syndrome for some fishes and zooplankton
- The modes of action of 6PPD-q can be exacerbated by other water quality conditions such as microplastics and toxicants that induce oxidative stress
- 6PPD-q has toxic effects on some fish, crustaceans, and a nematode. Effects include oxidative stress, genotoxicity, and neurotoxicity which have outcomes such as acute mortality and reduced fecundity.
- Two species of N.C. salmonids (endemic Brook trout and introduced Rainbow trout) are sensitive to 6PPD-q (sensitive means they experience acute mortality at environmentally relevant concentrations).
- Sublethal effects have been observed in a true minnow at environmentally relevant concentrations. NC true minnow populations all have concerning conservation statuses.

5.2 Future Research Needs

Here we propose a series of future research needs based on the findings of our literature review.

6PPD-q Loading Related

- Survey of NC receiving waters for 6PPD-q
- Leveraging existing geospatial data sets to identify potential 6PPD-q/TWP hotspots
- Investigate the performance of existing bioretention cells in regions of high potential 6PPD-q loadings

Toxicity/Risk Related

- Create a risk assessment model of 6PPD-q to ecosystem integrity

  - → Holistic approach
    - o Additional *in vivo* toxicity tests of unstudied genuses; with both 6PPD-q and TWP leachate
  - → Mechanistic approach (Tox21)
    - o Additional *in vitro* toxicity tests to clarify relevant modes of action
  - → Models of population responses to sublethal effects
  - → Ecological models of target surface waters
  - → Investigate whether population declines in biological assessments correlate with proximity to traffic

- Assess temporal and spatial risk to target species across NC

  - AOPs of target species at all life-stages
  - Temporal/spatial distribution of target species at all life-stages
  - Temporal/spatial model of 6PPD-q aqueous concentrations

- *In vivo* toxicity testing of NC true minnows to identify sensitive species

  - *In vitro* testing of sensitive true minnows

- Develop bioassays

  - NC specific

6. References

1. Tian Z, Zhao H, Peter KT, Gonzalez M, Wetzel J, Wu C, et al. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science. 2021 Jan 8;371(6525):185–9.

2. Zoroufchi Benis K, Behnami A, Minaei S, Brinkmann M, McPhedran KN, Soltan J. Environmental Occurrence and Toxicity of 6PPD Quinone, an Emerging Tire Rubber-Derived Chemical: A Review. Environ Sci Technol Lett. 2023 Oct;10(10):815–23.

3. Chen X, He T, Yang X, Gan Y, Qing X, Wang J, et al. Analysis, environmental occurrence, fate and potential toxicity of tire wear compounds 6PPD and 6PPD-quinone. J Hazard Mater. 2023 Jun 15;452:131245.

4. Wagner S, Hüffer T, Klöckner P, Wehrhahn M, Hofmann T, Reemtsma T. Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects. Water Res. 2018 Aug 1;139:83–100.

5. Hu X, Zhao HN, Tian Z, Peter KT, Dodd MC, Kolodziej EP. Transformation Product Formation upon Heterogeneous Ozonation of the Tire Rubber Antioxidant 6PPD (N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine). Environ Sci Technol Lett. 2022 May 10;9(5):413–9.

6. Mayer PM, Moran KD, Miller EL, Brander SM, Harper S, Garcia-Jaramillo M, et al. Where the rubber meets the road: Emerging environmental impacts of tire wear particles and their chemical cocktails. Sci Total Environ. 2024 Jun 1;927:171153.

7. Moran K, Miller E, Mendez M, Moore S, Gilbreath A, Sutton R, et al. A Synthesis of Microplastic Sources and Pathways to Urban Runoff. Richmond, CA: San Francisco Estuary Institute; 2021.

8. Seiwert B, Nihemaiti M, Troussier M, Weyrauch S, Reemtsma T. Abiotic oxidative transformation of 6-PPD and 6-PPD quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. Vol. 212, WATER RESEARCH. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND: PERGAMON-ELSEVIER SCIENCE LTD; 2022.

9. Gao Z, Cizdziel JV, Wontor K, Clisham C, Focia K, Rausch J, et al. On airborne tire wear particles along roads with different traffic characteristics using passive sampling and optical microscopy, single particle SEM/EDX, and µ-ATR-FTIR analyses. Front Environ Sci. 2022 Oct 25;10:1022697.

10. Greer JB, Dalsky EM, Lane RF, Hansen JD. Establishing an In Vitro Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products. Environ Sci Technol Lett. 2023 Jun 13;10(6):533–7.

11. Mahoney H, da Silva Junior FC, Roberts C, Schultz M, Ji X, Alcaraz AJ, et al. Exposure to the Tire Rubber-Derived Contaminant 6PPD-Quinone Causes Mitochondrial Dysfunction In Vitro. Environ Sci Technol Lett. 2022 Sep 13;9(9):765–71.

12. Dudefoi W, Ferrari BJD, Breider F, Masset T, Leger G, Vermeirssen E, et al. Evaluation of tire tread particle toxicity to fish using rainbow trout cell lines. Sci Total Environ. 2024 Feb 20;912:168933.

13. Zhang SY, Gan X, Shen B, Jiang J, Shen H, Lei Y, et al. 6PPD and its metabolite 6PPDQ induce different developmental toxicities and phenotypes in embryonic zebrafish. J Hazard Mater. 2023 Aug 5;455:131601.

14. Klöckner P, Seiwert B, Weyrauch S, Escher BI, Reemtsma T, Wagner S. Comprehensive characterization of tire and road wear particles in highway tunnel road dust by use of size and density fractionation. Chemosphere. 2021 Sep 1;279:130530.

15. Hiki K, Yamamoto H. Concentration and leachability of *N*-(1,3-dimethylbutyl)-*N′*-phenyl-*p*-phenylenediamine (6PPD) and its quinone transformation product (6PPD-Q) in road dust collected in Tokyo, Japan. Environ Pollut. 2022 Jun 1;302:119082.

16. Johannessen C, Helm P, Lashuk B, Yargeau V, Metcalfe CD. The Tire Wear Compounds 6PPD-Quinone and 1,3-Diphenylguanidine in an Urban Watershed. Arch Environ Contam Toxicol. 2022 Feb 1;82(2):171–9.

17. Cao G, Wang W, Zhang J, Wu P, Zhao X, Yang Z, et al. New evidence of rubber-derived quinones in water, air, and soil. Vol. 56, ENVIRONMENTAL SCIENCE & TECHNOLOGY. 1155 16TH ST, NW, WASHINGTON, DC 20036 USA: AMER CHEMICAL SOC; 2022. p. 4142–50.

18. Zhang HY, Huang Z, Liu YH, Hu LX, He LY, Liu YS, et al. Occurrence and risks of 23 tire additives and their transformation products in an urban water system. Environ Int. 2023 Jan 1;171:107715.

19. Zhang R, Zhao S, Liu X, Tian L, Mo Y, Yi X, et al. Aquatic environmental fates and risks of benzotriazoles, benzothiazoles, and *p*-phenylenediamines in a catchment providing water to a megacity of China. Environ Res. 2023 Jan 1;216:114721.

20. Challis JK, Popick H, Prajapati S, Harder P, Giesy JP, McPhedran K, et al. Occurrences of Tire Rubber-Derived Contaminants in Cold-Climate Urban Runoff. Environ Sci Technol Lett. 2021 Nov 9;8(11):961–7.

21. Rauert C, Vardy S, Daniell B, Charlton N, Thomas KV. Tyre additive chemicals, tyre road wear particles and high production polymers in surface water at 5 urban centres in Queensland, Australia. Sci Total Environ. 2022 Dec 15;852:158468.

22. Peter KT, Hou F, Tian Z, Wu C, Goehring M, Liu F, et al. More Than a First Flush: Urban Creek Storm Hydrographs Demonstrate Broad Contaminant Pollutographs. Environ Sci Technol. 2020 May 19;54(10):6152–65.

23. Rodgers TFM, Wang Y, Humes C, Jeronimo M, Johannessen C, Spraakman S, et al. Bioretention Cells Provide a 10-Fold Reduction in 6PPD-Quinone Mass Loadings to Receiving Waters: Evidence from a Field Experiment and Modeling. Environ Sci Technol Lett. 2023 Jul 11;10(7):582–8.

24. McIntyre JK, Spromberg J, Cameron J, Incardona JP, Davis JW, Scholz NL. Bioretention filtration prevents acute mortality and reduces chronic toxicity for early life stage coho salmon (*Oncorhynchus kisutch*) episodically exposed to urban stormwater runoff. Sci Total Environ. 2023 Dec 1;902:165759.

25. Peter KT, Tian Z, Wu C, Lin P, White S, Du B, et al. Using High-Resolution Mass Spectrometry to Identify Organic Contaminants Linked to Urban Stormwater Mortality Syndrome in Coho Salmon. Environ Sci Technol. 2018 Sep 18;52(18):10317–27.

26. Li J, Xu J, Jiang X. Urban runoff mortality syndrome in zooplankton caused by tire wear particles. Environ Pollut. 2023 Jul 15;329:121721.

27. Wei LN, Wu NN, Xu R, Liu S, Li HX, Lin L, et al. First Evidence of the Bioaccumulation and Trophic Transfer of Tire Additives and Their Transformation Products in an Estuarine Food Web. Environ Sci Technol. 2024 Apr 9;58(14):6370–80.

28. Escher B, Neale P, Leusch F. Bioanalytical Tools in Water Quality Assessment: Second Edition.

29. Hiki K, Yamamoto H. The Tire-Derived Chemical 6PPD-quinone Is Lethally Toxic to the White-Spotted Char Salvelinus leucomaenis pluvius but Not to Two Other Salmonid Species. Environ Sci Technol Lett. 2022 Dec 13;9(12):1050–5.

30. Foldvik A, Kryuchkov F, Sandodden R, Uhlig S. Acute Toxicity Testing of the Tire Rubber–Derived Chemical 6PPD-quinone on Atlantic Salmon (Salmo salar) and Brown Trout (Salmo trutta). Environ Toxicol Chem. 2022;41(12):3041–5.

31. Maji UJ, Kim K, Yeo IC, Shim KY, Jeong CB. Toxicological effects of tire rubber-derived 6PPD-quinone, a species-specific toxicant, and dithiobisbenzanilide (DTBBA) in the marine rotifer *Brachionus koreanus*. Mar Pollut Bull. 2023 Jul 1;192:115002.

32. Foldvik A, Kryuchkov F, Ulvan EM, Sandodden R, Kvingedal E. Acute Toxicity Testing of Pink Salmon (Oncorhynchus gorbuscha) with the Tire Rubber–Derived Chemical 6PPD-Quinone. Environ Toxicol Chem. 2024;43(6):1332–8.

33. Di S, Liu Z, Zhao H, Li Y, Qi P, Wang Z, et al. Chiral perspective evaluations: Enantioselective hydrolysis of 6PPD and 6PPD-quinone in water and enantioselective toxicity to *Gobiocypris rarus* and *Oncorhynchus mykiss*. Environ Int. 2022 Aug 1;166:107374.

34. Hiki K, Asahina K, Kato K, Yamagishi T, Omagari R, Iwasaki Y, et al. Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species. Environ Sci Technol Lett. 2021 Sep 14;8(9):779–84.

35. Botelho MT, Militão GG, Brinkmann M, Umbuzeiro G de A. Toxicity and mutagenicity studies of 6PPD-quinone in a marine invertebrate species and bacteria. Environ Mol Mutagen. 2023;64(6):335–41.

36. Brinkmann M, Montgomery D, Selinger S, Miller JGP, Stock E, Alcaraz AJ, et al. Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance. Environ Sci Technol Lett. 2022 Apr 12;9(4):333–8.

37. Varshney S, Gora AH, Siriyappagouder P, Kiron V, Olsvik PA. Toxicological effects of 6PPD and 6PPD quinone in zebrafish larvae. J Hazard Mater. 2022 Feb 15;424:127623.

38. Varshney S, O'Connor OL, Gora AH, Rehman S, Kiron V, Siriyappagouder P, et al. Mixture toxicity of 6PPD-quinone and polystyrene nanoplastics in zebrafish*. Vol. 348, ENVIRONMENTAL POLLUTION. 125 London Wall, London, ENGLAND: ELSEVIER SCI LTD; 2024.

39. Jiao M, Luo Y, Zhang F, Wang L, Chang J, Croué JP, et al. Transformation of 6PPDQ during disinfection: Kinetics, products, and eco-toxicity assessment. Water Res. 2024 Feb 15;250:121070.

40. Ricarte M, Prats E, Montemurro N, Bedrossiantz J, Bellot M, Gómez-Canela C, et al. Environmental concentrations of tire rubber-derived 6PPD-quinone alter CNS function in zebrafish larvae. Sci Total Environ. 2023 Oct 20;896:165240.

41. Tian Z, Zhao H, Peter KT, Gonzalez M, Wetzel J, Wu C, et al. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science. 2021 Jan 8;371(6525):185–9.

42. Lo BP, Marlatt VL, Liao X, Reger S, Gallilee C, Ross ARS, et al. Acute Toxicity of 6PPD-Quinone to Early Life Stage Juvenile Chinook (Oncorhynchus tshawytscha) and Coho (Oncorhynchus kisutch) Salmon. Environ Toxicol Chem. 2023;42(4):815–22.

43. McIntyre JK, Prat J, Cameron J, Wetzel J, Mudrock E, Peter KT, et al. Treading Water: Tire Wear Particle Leachate Recreates an Urban Runoff Mortality Syndrome in Coho but Not Chum Salmon. Environ Sci Technol. 2021 Sep 7;55(17):11767–74.

44. French BF, Baldwin DH, Cameron J, Prat J, King K, Davis JW, et al. Urban Roadway Runoff Is Lethal to Juvenile Coho, Steelhead, and Chinook Salmonids, But Not Congeneric Sockeye. Environ Sci Technol Lett. 2022 Sep 13;9(9):733–8.

45. Tian Z, Gonzalez M, Rideout CA, Zhao HN, Hu X, Wetzel J, et al. 6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard. Environ Sci Technol Lett. 2022 Feb 8;9(2):140–6.

46. Greer JB, Dalsky EM, Lane RF, Hansen JD. Tire-Derived Transformation Product 6PPD-Quinone Induces Mortality and Transcriptionally Disrupts Vascular Permeability Pathways in Developing Coho Salmon. Environ Sci Technol. 2023 Aug 1;57(30):10940–50.

47. Anderson-Bain K, Roberts C, Kohlman E, Ji X, Alcaraz AJ, Miller J, et al. Apical and mechanistic effects of 6PPD-quinone on different life-stages of the fathead minnow (*Pimephales promelas*). Comp Biochem Physiol Part C Toxicol Pharmacol. 2023 Sep 1;271:109697.

48. Ackerly KL, Roark KJ, Lu K, Esbaugh AJ, Liu Z, Nielsen KM. Acute toxicity testing of 6PPD-quinone on the estuarine-dependent sport fish, *Sciaenops ocellatus*. Ecotoxicology (London, England). VAN GODEWIJCKSTRAAT 30, 3311 GZ DORDRECHT, NETHERLANDS: SPRINGER; 2024.

49. Prosser RS, Salole J, Hang S. Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environ Pollut. 2023 Nov 15;337:122512.

50. Hua X, Feng X, Liang G, Chao J, Wang D. Exposure to 6-PPD Quinone at Environmentally Relevant Concentrations Causes Abnormal Locomotion Behaviors and Neurodegeneration in Caenorhabditis elegans. Environ Sci Technol. 2023 Mar 28;57(12):4940–50.

51. Hua X, Feng X, Liang G, Chao J, Wang D. Long-term exposure to 6-PPD quinone reduces reproductive capacity by enhancing germline apoptosis associated with activation of both DNA damage and cell corpse engulfment in *Caenorhabditis elegans*. J Hazard Mater. 2023 Jul 15;454:131495.

52. Hua X, Feng X, Liang G, Chao J, Wang D. Long-term exposure to tire-derived 6-PPD quinone causes intestinal toxicity by affecting functional state of intestinal barrier in *Caenorhabditis elegans*. Sci Total Environ. 2023 Feb 25;861:160591.

53. Hua X, Wang D. Exposure to 6-PPD Quinone at Environmentally Relevant Concentrations Inhibits Both Lifespan and Healthspan in C. elegans. Environ Sci Technol. 2023 Dec 5;57(48):19295–303.

54. Hua X, Wang D. Polyethylene nanoparticles at environmentally relevant concentrations enhances neurotoxicity and accumulation of 6-PPD quinone in *Caenorhabditis elegans*. Sci Total Environ. 2024 Mar 25;918:170760.

55. Hua X, Wang D. Disruption of dopamine metabolism by exposure to 6-PPD quinone in *Caenorhabditis elegans*. Environ Pollut. 2023 Nov 15;337:122649.

56. Klauschies T, Isanta-Navarro J. The joint effects of salt and 6PPD contamination on a freshwater herbivore. Sci Total Environ. 2022 Jul 10;829:154675.

57. Wu J, Cao G, Zhang F, Cai Z. A new toxicity mechanism of *N*-(1,3-Dimethylbutyl)-*N′*-phenyl-*p*-phenylenediamine quinone: Formation of DNA adducts in mammalian cells and aqueous organisms. Sci Total Environ. 2023 Mar 25;866:161373.

58. Chibwe L, Parrott JL, Shires K, Khan H, Clarence S, Lavalle C, et al. A Deep Dive into the Complex Chemical Mixture and Toxicity of Tire Wear Particle Leachate in Fathead Minnow. Environ Toxicol Chem. 2022;41(5):1144–53.

59. Jiang Y, Wang C, Ma L, Gao T, Wāng Y. Environmental profiles, hazard identification, and toxicological hallmarks of emerging tire rubber-related contaminants 6PPD and 6PPD-quinone. Environ Int. 2024 May 1;187:108677.

60. Fang J, Wang X, Cao G, Wang F, Ru Y, Wang B, et al. 6PPD-quinone exposure induces neuronal mitochondrial dysfunction to exacerbate Lewy neurites formation induced by α-synuclein preformed fibrils seeding. J Hazard Mater. 2024 Mar 5;465:133312.

61. Fu B, Chen T, Jiang B, Feng H, Zhu Z, Li M, et al. 6PPDQ induces cardiomyocyte senescence via AhR/ROS-mediated autophagic flux blockage. Environ Pollut. 2024 May 15;349:123872.

62. Wang W, Cao G, Zhang J, Chen Z, Dong C, Chen J, et al. p-Phenylenediamine-Derived Quinones as New Contributors to the Oxidative Potential of Fine Particulate Matter. Environ Sci Technol Lett. 2022 Sep 13;9(9):712–7.

63. Johannessen C, Saini A, Zhang X, Harner T. Air monitoring of tire-derived chemicals in global megacities using passive samplers. Vol. 314, ENVIRONMENTAL POLLUTION. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND: ELSEVIER SCI LTD; 2022.

64. Zhang Y, Xu C, Zhang W, Qi Z, Song Y, Zhu L, et al. p-Phenylenediamine Antioxidants in PM2.5: The Underestimated Urban Air Pollutants. Environ Sci Technol. 2022 Jun 7;56(11):6914–21.

65. Huang W, Shi Y, Huang J, Deng C, Tang S, Liu X, et al. Occurrence of substituted ¡i¿p¡/i¿-phenylenediamine antioxidants in dusts. Vol. 8, ENVIRONMENTAL SCIENCE & TECHNOLOGY LETTERS. 1155 16TH ST, NW, WASHINGTON, DC 20036 USA: AMER CHEMICAL SOC; 2021. p. 381–5.

66. Deng C, Huang J, Qi Y, Chen D, Huang W. Distribution patterns of rubber tire-related chemicals with particle size in road and indoor parking lot dust. Sci Total Environ. 2022 Oct 20;844:157144.

67. Wang W, Cao G, Zhang J, Wu P, Chen Y, Chen Z, et al. Beyond Substituted p-Phenylenediamine Antioxidants: Prevalence of Their Quinone Derivatives in PM2.5. Environ Sci Technol. 2022 Aug 2;56(15):10629–37.

68. Liang B, Li J, Du B, Pan Z, Liu LY, Zeng L. E-waste recycling emits large quantities of emerging aromatic amines and organophosphites: a poorly recognized source for another two classes of synthetic antioxidants. Vol. 9, ENVIRONMENTAL SCIENCE & TECHNOLOGY LETTERS. 1155 16TH ST, NW, WASHINGTON, DC 20036 USA: AMER CHEMICAL SOC; 2022. p. 625–31.

7. Appendices

7.1 Literature Review Methods

The systematized search and review of 6PPD-quinone toxicity studies began with searching Web of Science with the search phrase “toxicity AND 6ppd” on June 10, 2024 which yielded 70 results. The results were screened to 54 peer-reviewed articles that included toxicity testing of organisms to aqueous 6PPD-quinone. 17 additional articles were included based on citations in screened articles.

7.2 List of Attached Files from the Holistic Toxicity Risk Assessment

- R scripts
    - nhpImport.R: transforms NC Natural Heritage Program species county data into data frames for individual species descriptions, individual species conservation statuses, and current species distribution by county.
    - writeTaxa.R: adds taxonomic data to species lists.
    - closestToxicRelative.R: finds the closest taxonomic relative with toxicity testing for each endemic NC species, summarizes the toxicity data, and creates taxonomic trees to illustrate data gaps.
    - countyConservation.R: summarizes the conservation statuses of a given taxonomic rank.
- Data
    - species_toxic.csv: table of species with holistic toxicity data.
    - species_ncToxicTable.csv: summary of toxicity data for NC species by closest taxonomic match.
    - species_leuciscidae.csv: conservation statuses of all NC endemic true minnows.
- Figures
    - Tree_gapFamily.svg: taxonomic tree with species counts of NC endemic species with a family rank match for closest relative.
    - Tree_gapClass.svg: taxonomic tree with species counts of NC endemic species with a class rank match for closest relative.
    - Tree_gapOrder.svg: taxonomic tree with species counts of NC endemic species with an order rank match for closest relative.
    - Tree_gapPhylum.svg: taxonomic tree with species counts of NC endemic species with a phylum rank match for closest relative.

7.3 Table of Air/Dust measurements for 6PPD and 6PPD-q

Table 5 – Summary of Air/Dust measurements of 6PPD and 6PPD-q. Data collected originally in Zoroufchi Benis et al. (2023) (2).

	Concentration (pg/m^3)			**Detection Frequency**		
Sampling Location	**6PPD-q**	**6PPD**	**Sample #**	**6PPD-q**	**6PPD**	**Ref**
Toronto (Canada), New York (USA), Istanbul (Turkey), Madrid (Spain), Kolkata (India), Bangkok (Thailand), Tokyo (Japan), New Delhi (India), Santiago (Chile), Lagos (Nigeria), Cairo (Egypt), Beijing (China)	< LOQ	< LOQ	-	-	-	(63)
Sydney, Australia	0.170	< LOQ	-	-	-	
London, UK	0.367	ND	-	-	-	
Sao Paulo, Brazil	1.75	-	-	-	-	
Bogota, Colombia	0.680	< LOQ	-	-	-	
Buenos Aires, Argentina	1.27	-	-	-	-	
Taiyuan, (North China Plain, China)	1.38-41.0	-	14	-	-	(62)
Zhengzhou, (North China Plain, China)	2.60 – 21.3	-	14	-	-	
Shanghai (Yangtze River Delta, China)	10.3 – 69.1	-	4	-	-	
Hangzhou (Yangtze River Delta, China)	1.38 – 110	-	7	-	-	
Guangzhou (Pearl River Delta, China)	1.50 – 64.4	-	22	-	-	
Shanxi University (Taiyuan, China)	1.1 – 84	0.02 – 487	24	79	100	(64)
Zhengzhou University (Zhengzhou, China)	0.3 – 32	1.2 – 1099	24	92	67	
Fudan University (Shanghai, China)	0.3 – 39	0.5 – 135	8	88	100	
Jiangsu Provincial Center for Disease Control and Prevention (Nanjing, China)	1.1 – 68	0.4 – 75	6	100	100	
Government of Hangzhou Binjiang District (Hangzhou, China)	0.8 – 26	0.1 – 6.0	7	100	43	
Guangdong University of Technology (Guangzhou, China)	0.1 – 15	0.3 – 10	24	67	88	
Shanxi University (Taiyuan, China)	1.1 – 84	0.02 – 487	24	79	100	
Campus of Hong Kong Baptist University (Hong Kong, China)	0.54 – 13.8	0.82 – 6.30	16	100	100	(17)
Road dust (within 20 km in the city of Gangzhou, China)	3.0 – 88.1	4.1 – 238	20	100	100	(65)
Vehicle Dust from Different Brands, Gangzhou, China	17.9 – 146	5.0 – 41.9	11	100	100	
Underground Parking dust of 10 malls, Guangzhou, China	5.7 – 277	13.5 – 429	10	100	100	
House dust, Guangzhou, China	< LOQ – 0.4	< LOQ – 6.1	18	33.3	55.6	
Road samples (Tokyo, Japan)	116 – 1238 ng/g	45 – 1,175 ng/g	33	-	-	(34)
Indoor Parking (Gungzhu, China)	4.02 – 2,369 ng/g	11.4 – 5,359 ng/g	60	100	100	(66)

Road dust (Gungzhu, China)	10.5 – 509 ng/g	15.1 – 1,508 ng/g	60	100	100	(66)
Shanxi University (Taiyuan, China)	2.44 – 1,780	1.02 – 3,190	24	100	100	(67)
Guangdong University of Technology (Guangzhou, China)	3.04 – 2,350	22.2 – 6,050	24	100	100	
South China Institute of Environmental Protection (Guangzhou, China)	2.96 – 7,250	1.39 – 9,340	24	100	100	

Table 6 – Summary of Other Media measurements of 6PPD and 6PPD-q. Data collected originally in Zoroufchi Benis et al. (2023) (2).

	Concentration (ng/g)			**Detection Frequency**		
Sampling Location	**6PPD-q**	**6PPD**	**Sample #**	**6PPD-q**	**6PPD**	**Ref**
Roadside soil, New Territories and Kowloon (Hong Kong, China)	9.50 – 936	31.4 – 831	12	100	100	(17)
E-waste dust (e-waste recycling workshops, China)	87.1 – 2,850	13.8 – 1,020	45	100	100	(68)