MULTI-SCALE ANALYSIS OF 6PPD-QUINONE: DISTRIBUTION, ENVIRONMENTAL RISK, AND URBANIZATION INFLUENCE IN THE DELAWARE RIVER BASIN

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ABSTRACT

Tire wear particles (TWPs) represent a pervasive and chemically complex class of urban contaminants. Among these, 6PPD-quinone (6PPDQ) an oxidative transformation product of the anti-degradant i.e., N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD), have emerged as contaminant of critical concern due to their acute toxicity to aquatic species, particularly salmonids. While significant research has focused on Pacific Northwest, European, and East Asian water systems, environmental data from northeastern U.S. freshwater ecosystems remain limited. This dissertation addresses this gap through a multiscale investigation of spatiotemporal distribution, ecological risk, urbanization impact and leaching dynamics of 6PPDQ across two major urban watersheds: the Schuylkill River and the Delaware River Basin both vital for drinking water supply and ecological integrity. Field sampling was conducted across 16 sites in the Schuylkill River and 23 in the Delaware River Basin over multiple seasons (2024-25'). Using EPA Draft Method 1634 and LC-MS/MS, the analysis revealed seasonal accumulation of 6PPDQ, with elevated concentrations during summer and autumn, particularly after storm events. Multiple sites exceeded the EPA's freshwater screening value (11 ng/L), with urbanized tributaries showing the highest concentrations. Chapter 4 expands this assessment through ecological risk evaluation using both threshold-based risk quotients and species-specific toxic unit (TU) approaches, revealing significant risks to sensitive trout populations. Further analysis identified urbanization indicators like traffic volume, population density, and proximity to tire-related industries as key predictors of 6PPDQ distribution. Statistical and spatial regression modeling demonstrated strong positive correlations between these drivers and contaminant levels, especially in tributaries. Chapter 5 presents laboratory leaching

experiments with new and used tire particles, simulating environmentally relevant conditions to explore the leaching dynamics of 6PPD and 6PPDQ. Spectroscopic characterization (NMR) confirmed surface oxidation and persistence of 6PPDQ in aqueous phases. Overall, these findings provide the first regionally focused, mechanistically informed assessment of 6PPDQ in northeastern U.S. rivers. By linking occurrence, toxicity, and urban drivers, this dissertation informs regulatory science and urban watershed management. The research underscores the importance of stormwater controls, targeted monitoring during hydrologic events, and innovation in tire material design to reduce environmental and ecological risks.

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CHAPTER 1

INTRODUCTION

1.1 Overview

Tire and road wear particles (TRWPs) have emerged as a significant and ubiquitous source of environmental pollution, especially in urban ecosystems[1], [2]. Generated through friction between tires and pavement during regular vehicular activity, these particles consist of complex mixtures of rubber polymers, fillers, and chemical additives. Among the various chemicals embedded in tires, N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) is a synthetic antiozonant and antioxidant that is extensively used to prevent oxidative cracking and thermal degradation in rubber formulations[3], [4]. Typically added at concentrations of 0.4% to 2% by mass[5], 6PPD belongs to the p-phenylenediamine (PPD) class of compounds and plays a crucial role in enhancing tire longevity[6]. Beyond its primary application in tires, it is also used in various products such as hair and nail dyes, fabrics, and lubricants, thereby increasing the potential for human exposure [7]. However, 6PPD's protective function becomes a liability once released into the environment. Upon reaction with atmospheric ozone, 6PPD transforms into a potent degradation product 6PPD-Quinone (6PPDQ). Since its identification in 2020 as the toxicant responsible for acute mortality in coho salmon during stormwater runoff events in the Pacific Northwest, 6PPDQ has gained international attention as a high-risk contaminant of emerging concern.

This compound, formed primarily through ozonation, now represents a key chemical of interest in ecotoxicology and regulatory science. The history of tire derived pollution dates back to the 1970s, when early studies by Pierson, Brachaczek, and Cadle recognized the presence of particulate matter shed from vehicle tires. Initially, the focus was on physical wear and the contribution of rubber fragments to airborne or roadside dust. As tire formulations evolved and urbanization intensified, so did the complexity of TRWPs, which now carry diverse chemicals including PAHs, metals, and synthetic antioxidants like 6PPD. This compound was introduced into tire manufacturing in the 1960s and, by 1975, had become one of the most commonly used antiozonants globally. While effective for tire performance, its transformation product 6PPDQ poses significant ecological threats. The landmark study by Tian et al. found that low concentrations LC_{50} ranging as low as 41- 95 ng/L[8], [9] of 6PPDQ caused mass mortality in coho salmon (Oncorhynchus kisutch), within hours of exposure. This phenomenon, known as urban runoff mortality syndrome (URMS), underscored the risk of tire derived chemicals entering freshwater systems via rainfall and stormwater pathways.

1.1.1 Chemical properties and environmental behavior of 6PPD and 6PPDQ

Both 6PPD and 6PPDQ exhibit physicochemical properties that influence their environmental fate. 6PPD is relatively hydrophobic (log K_{OW} ~4.47-4.84), while 6PPDQ has slightly lower lipophilicity (log K_{OW} ~3.98-4.30) but increased persistence under environmental conditions. The table 1.1 summarizes key physicochemical traits. Due to their stability and transport potential, both 6PPD and 6PPDQ have been detected across various environmental media like air, soil, surface water, stormwater, and sediments [1], [2], [10] - [13]. Recent studies have also detected 6PPDQ in wastewater effluents, drinking water [14], [15], and even in human tissues, including urine, liver, and maternal blood. Atmospheric monitoring has revealed concentrations of 6PPDQ in $PM_{2.5}$ and urban dust, suggesting potential inhalation and dermal exposure risks.

Table 1: Summary of key physicochemical properties of 6PPD and 6PPDQ

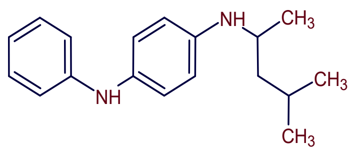
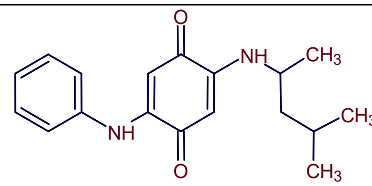
Property	6PPD	6PPDQ	Reference
Chemical Formula	$C_{18}H_{24}N_2$	$C_{18}H_{22}N_2O$	N/A
Molecular Structure			N/A
Molecular Weight	264.40 g/mol	298.38 g/mol	N/A
CAS number	793-24-8	2754428-18-5	N/A
Physical appearance	Deep brown crystalline solid	Orange crystalline solid/ oily	N/A
Water solubility	>1 mg/L (soluble)	31-67 ug/L (low solubility)	[16], [17]
Log K_{OW}, (EPI, Experimental)	4.47 - 4.84	3.98 to 4.30	[16], [17]
Log K_{OC}, (Experimental)	N/A	2.8 to 3.6	[18]
Log K_{OA} (EPI Suite)	-5.26	-7.99 to 15.319	[19]

Table 1: (continued). Summary of key physicochemical properties of 6PPD and 6PPDQ

Property	6PPD	6PPDQ	Reference
Atmospheric half-life	< day (0.64 to 2.65 hrs.)	days to weeks	[20], [21]
Photo-degradation	Moderate, forms 6PPDQ	Rapid ($t_{1/2}$ ~2.57 h)	[22]
Environmental stability	Less stable	Stable than 6PPD	[16]
Toxicity	Moderate	Highly species specific, especially to some salmonids	[23]

1.1.2 Transformation pathways and environmental mobility

6PPDQ formation is driven predominantly by the ozonation of 6PPD, especially in high-traffic urban corridor [24]. Additional transformation mechanisms include photodegradation, microbial oxidation , and hydrolysis [25]. The resulting byproducts vary in toxicity and mobility, contributing to the environmental complexity of 6PPD derived contamination. Studies have shown that temperature, pH, and dissolved organic matter significantly affect the persistence and degradation of 6PPDQ in water systems environment [26]. Once formed, 6PPDQ can be transported through stormwater runoff, adsorb to sediments, or remain in dissolved form in surface water. Seasonal dynamics such as rainfall events, snowmelt, or temperature shifts affect mobilization, and concentration could peak of 6PPDQ, with notable increase observed during summer and fall storms.

These hydrological drivers, along with urban factor patterns (e.g., traffic density, industrial activity), dictate spatial and temporal variability in exposure.

1.1.3 Toxicity and species-specific sensitivities

6PPDQ's acute toxicity to few aquatic species is well documented. Coho salmon are the most sensitive, with LC_{50} values ranging from 41-95 ng/L. Other salmonids such as brook trout, white-spotted char, and lake trout exhibit LC_{50} values ranging from 165 to 590 ng/L. Even among species, different life stages show varying sensitivity for instance, swim-up fry and juvenile stages are especially vulnerable. By contrast, species like rainbow trout and Atlantic salmon show higher tolerance, and non-salmonid species (e.g., zebrafish, medaka, Daphnia magna) often display LC_{50} values in the hundreds of μg/L range. These disparities in sensitivity necessitate site-specific ecological risk assessments, especially for rivers supporting salmonid habitats. Toxicological mechanisms include oxidative stress, endothelial dysfunction, neurotoxicity, endocrine disruption, and reproductive toxicity. In mammalian models, 6PPDQ has been associated with liver damage, metabolic disruption, and potential placental transfer. Biomonitoring efforts have detected 6PPDQ in human urine, cerebrospinal fluid, and tissues, raising broader public health concerns.

1.1.4 Regulatory attention and analytical advancements

Given the ecological risks posed by 6PPD and 6PPDQ, various mitigation strategies have been proposed. These include the development of bioretention systems designed to reduce the mass loadings of these compounds in receiving waters [27]. Additionally, research into alternative materials and additives that do not produce harmful transformation products is ongoing, aiming to minimize the environmental footprint of tire related companies [28]. Efforts to reduce the release of tire-derived contaminants into the environment have also been emphasized. The exploration of green alternatives to 6PPD in rubber production is gaining traction, with studies investigating the efficacy of natural antioxidants as substitutes [28]. The United States Environmental Protection Agency (EPA) has been actively researching 6PPD and 6PPDQ, focusing on their effects on aquatic life. In 2024, the EPA proposed a rule under the Toxic Substances Control Act (TSCA) requiring manufacturers to report health and safety studies on these chemicals. The EPA has also developed screening values for 6PPD and 6PPDQ to protect freshwater aquatic life (EPA, 2024a, 2024b) are 8900 ng/L (8.9 µg/L) and 11 ng/L, respectively with a duration of one hour average. In 2023, EPA developed a laboratory draft method (Draft EPA Method 1634) to quantify 6PPDQ using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS), [31]. Washington State is the first state to set regulatory aquatic life criteria values as 12 ng/L.

1.1.5 Relevance to the Delaware Water Basin and Schuylkill River

The Delaware Water Basin and Schuylkill River are vital freshwater resources that provide drinking water, recreation, and habitat for diverse aquatic life in the northeastern U.S. These systems are vulnerable to pollution due to urbanization, industrial activities, and transportation networks, making the study of 6PPD and 6PPDQ critical. These rivers play a vital role in supporting biodiversity and serve as critical indicators of water quality. The toxicity of 6PPDQ threatens aquatic ecosystems, particularly fish. As key drinking water sources for millions, contamination by 6PPDQ, which conventional treatment may not address, poses significant public health risks. Urban runoff, particularly in high-traffic areas and near tire related facilities, can serve as a major source of pollution through the transport of TWP. Limited data on 6PPDQ in these rivers underscores the research, we will identify contamination trends, sources, and risks while supporting mitigation strategies.

1.1.6 Dissertation motivation and scope

With growing evidence of 6PPDQ's toxicity and presence across environmental systems, significant data gaps persist. Particularly lacking are:

- Seasonal and spatial occurrence data from the northeastern United States.
- Understanding of the links between urbanization drivers and 6PPDQ loading.
- Mechanistic insights into transformation behavior under environmental versus laboratory conditions.

This dissertation addresses these gaps through a multi-pronged approach. It begins by quantifying 6PPDQ concentrations across two urban water systems like the Schuylkill River and the Delaware River Basin using field-based sampling and EPA-approved analytical methods. It then evaluates risk using species-specific toxicity thresholds and examines statistical relationships between 6PPDQ concentrations and urban infrastructure metrics such as Average Annual Daily Traffic (AADT), land use, and proximity to tire-related industries. Subsequent chapters explore the leaching and transformation behavior of 6PPD and 6PPDQ under controlled laboratory conditions using Fourier-transform infrared spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). These experiments compare new and aged tire particles to assess long-term risks and help bridge environmental exposure and mechanistic toxicity. Together, these efforts aim to enhance understanding of the environmental fate, transport, and risks associated with 6PPDQ and its parent compound, while supporting regulatory decision making and aqueous system protection strategies.

1.2 Chapter Summaries

1.2.1 Chapter 2

This chapter provides a comprehensive literature review and conceptual framework for understanding the environmental behavior, detection, toxicity, and regulatory attention surrounding 6PPD and its transformation product, 6PPDQ. It begins with the origin and use of 6PPD in tire manufacturing and its transformation into 6PPDQ via ozonation and other environmental pathways. The chapter then reviews recent studies on 6PPDQ's occurrence across environmental media including stormwater, surface water, sediment, and biota and its known toxicological effects on aquatic organisms and humans. Special attention is given to the compound's link with urban runoff mortality syndrome (URMS) in Coho salmon, its physicochemical behavior, and regulatory thresholds set by the U.S. EPA. The chapter also reviews the analytical advancements, particularly the development of EPA Draft Method 1634 for 6PPDQ detection and concludes by identifying knowledge gaps related to regional distribution, transformation mechanisms, and source-specific contributions, which are explored in the chapters that follow.

1.2.2 Chapter 3

This chapter presents a field-based quantitative assessment of 6PPDQ concentrations in two northeastern U.S. watersheds: the Schuylkill River and the Delaware River. Sampling was conducted across four seasons in 2024 using EPA Draft Method 1634, and concentrations were analyzed in the context of spatiotemporal trends. The chapter integrates data from sixteen locations per watershed and reports seasonal variations in 6PPDQ concentrations, with downstream urban areas exhibiting higher levels than upstream sites. Differences in hydrologic flow, precipitation events, and land use are considered in interpreting spatial distribution patterns. The data presented in this chapter establishes the foundation for understanding the presence and behavior of 6PPDQ in urban freshwater systems and sets the stage for risk evaluation in subsequent chapters.

1.2.3 Chapter 4

This chapter focuses on environmental risk characterization and statistical modeling of 6PPDQ contamination. It begins with an assessment of urban drivers such as average annual daily traffic (AADT), population density, and proximity to tire-related industries, and evaluates their correlation with 6PPDQ concentrations observed in both rivers. Spatial and temporal variations are examined to identify high-risk zones. The chapter then presents ecological risk assessments based on EPA's freshwater screening value for 6PPDQ (11 ng/L), and a toxic unit (TU) based approach tailored to sensitive trout species such as rainbow trout, brook trout, and white-spotted char. Risk maps and seasonal risk category heatmaps are generated to visualize vulnerability across sites. These analyses provide a deeper understanding of how urban infrastructure and hydrology influence the environmental toxicity of 6PPDQ.

1.2.4 Chapter 5

This chapter explores the leaching behavior and chemical transformation of 6PPD and 6PPDQ from tire particles under controlled laboratory conditions. Tire particles from both new and used tires were subjected to aqueous leaching tests, and the resulting leachates were analyzed using LC-MS/MS to quantify 6PPDQ release. NMR spectroscopy was used to confirm structural features and transformation pathways of 6PPD and 6PPDQ. The results reveal differences in leaching behavior between new and aged particles and suggest environmental mechanisms that could explain the persistence or transformation of

6PPDQ in natural systems. This chapter bridges field observations with laboratory evidence and informs the potential for mitigation at the source level.

1.2.5 Chapter 6

The final chapter synthesizes the major findings of the dissertation and discusses their implications for environmental monitoring, regulatory decision-making, and watershed management. It highlights the significance of combining occurrence data, risk modeling, and mechanistic insights to address emerging contaminants like 6PPDQ. The chapter also outlines practical recommendations for future monitoring programs, stormwater control strategies, and treatment interventions. Key research gaps are identified particularly the need for real-time monitoring during rain events, deeper exploration of sediment interactions, and long-term toxicity studies. The chapter concludes with a roadmap for future interdisciplinary research to support regulatory frameworks and public health protection in urban aquatic environments.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to tire wear particles (TWPs)

Tire wear particles (TWPs) are a major non-exhaust source of particulate pollution in urban environments, formed through the mechanical abrasion of tire treads against road surfaces. Although they were introduced briefly in CHAPTER 1, a more focused understanding of their chemical relevance is warranted here, given their central role as carriers and precursors of emerging contaminants like 6PPD and 6PPD-Quinone (6PPDQ). Rather than being inert physical debris, TWPs are chemically active particles embedded with a range of tire-derived additives, including antioxidants, plasticizers, and heavy metals. Among these additives, N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) is a widely used antiozonant that reacts with atmospheric ozone to form 6PPDQ, a transformation product now recognized for its acute toxicity to aquatic life.

TWPs thus function as both a source and a vector for the release and environmental transport of 6PPD and its derivatives. These particles can settle into urban soils, become resuspended in the atmosphere, or be mobilized into surface waters through stormwater runoff. The chemical interactions between TWPs and the surrounding environment particularly those involving temperature, pH, and oxidative conditions play a critical role in determining the fate and persistence of 6PPD and 6PPDQ [32]. This section frames TWPs as a functional interface through which tire-derived chemicals enter and interact with ecosystems. It sets the stage for subsequent discussions of the environmental occurrence, transformation, and toxicological behavior of 6PPDQ whose emerging ecological significance for our understanding of tire-related pollution.

2.2 Transformation mechanisms and pathways of 6PPD and 6PPDQ

The environmental persistence and mobility of 6PPD and its 6PPDQ are intricately linked to their physicochemical properties and reactivity under ambient environmental conditions. As TRWPs enter the environment, 6PPD embedded within the rubber matrix become susceptible to oxidative and photolytic processes that lead to the formation of multiple degradation products, the most prominent being 6PPDQ.

2.2.1 Primary Transformation: Ozonation

The predominant transformation mechanism of 6PPD in the environment is ozonation. As urban atmospheres contain elevated levels of ground-level ozone (O_3), especially near heavily trafficked roads, 6PPD exposed on the surface of tires or deposited TRWPs undergoes a rapid oxidation process. This transformation results in the formation of 6PPDQ, which contains a reactive quinone moiety characterized by redox activity and strong electrophilic potential. This reaction is surface-mediated and occurs more readily in the air or at solid-liquid interfaces than in purely aqueous conditions, explaining the detection of 6PPDQ in road dust, air, and stormwater runoff. Several studies have proposed multi-step reaction pathways, where intermediate hydroxylated forms of 6PPD (e.g., 6PPD-OH, 6PPD-$(OH)_2$) first form under the influence of oxidants such as ozone and hydroxyl radicals (figure 2.1). These intermediates can subsequently oxidize further to yield quinone products, including 6PPDQ and other minor byproducts like N-formyl-6PPD and quinone diamine isomers.

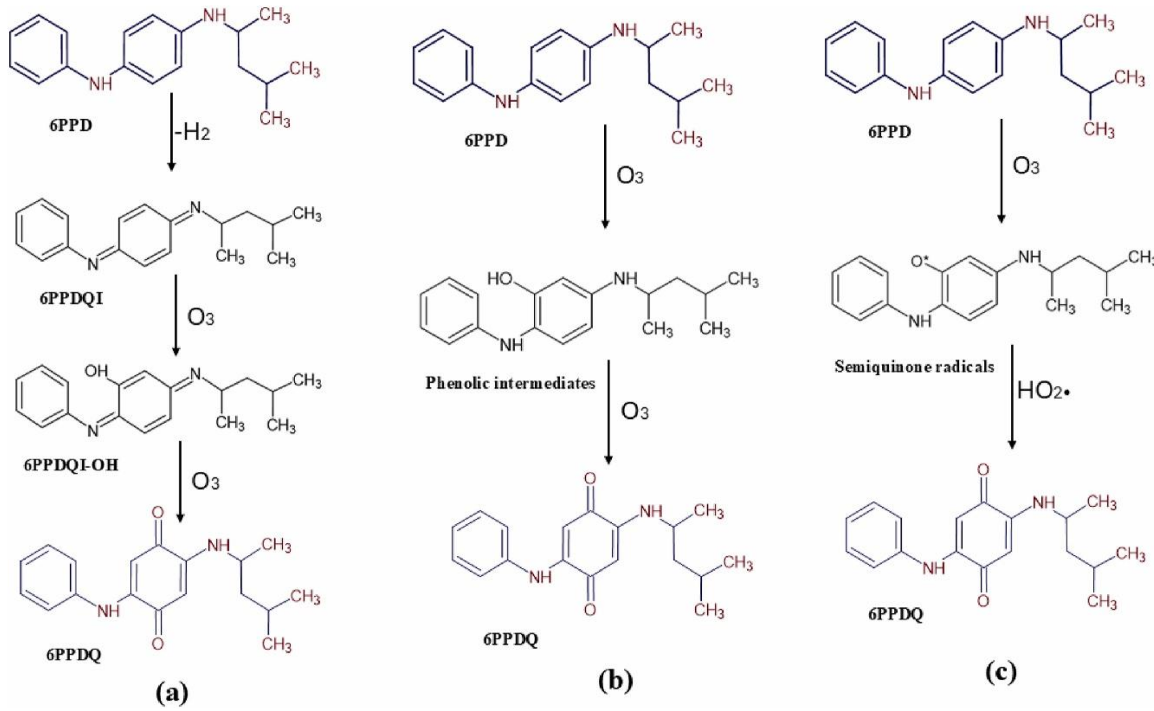


Figure 1: Oxidative transformation pathways from 6PPD to 6PPDQ: (a) QDI pathway.(b) Intermediate Phenol(IP).(c) Semiquinone radicals(SR) pathway

2.2.2 Photo transformation and indirect photolysis

Photodegradation serves as another significant transformation pathway for 6PPD, particularly in surface waters. Upon exposure to ultraviolet (UV) radiation, 6PPPD absorbs photon energy (hv) to change into an excited state (6PPD*) and subsequently reacts with dissolved oxygen (O_2), forming hydroxylated intermediates such as 6PPD-OOH ($C_{18}H_{24}N_2O_3$) and reactively hydroxyl radicals (HO•) [33]. These intermediates further react to producing stable 6PPDQ [34]. The molar yield of 6PPDQ through photodegradation has been shown to be comparable to that from ozonation, emphasizing the environmental relevance of this mechanism. Research conducted in water bodies found that hydroxylation and ring-cleavage processes in photodegradation, potentially forming byproducts including 6PPDQ, aniline (P94), 4-aminodiphenylamine (4-ADPA), 4-hydroxydiphenylamine (4-HDPA) [35]. Additionally, the generation of a fluorescent byproduct distinct from 6PPDQ has been observed, indicating the formation of novel, yet poorly characterized, transformation products [26].

2.2.3 Microbial and biotic transformation

The biotransformation of 6PPD and 6PPDQ has been studied in various organisms, including zebrafish and other aquatic species. Metabolomic analyses have shown that 6PPDQ undergoes further oxidation and conjugation, resulting in a range of metabolites that may exhibit different toxicological profiles [36], [37]. Notably, phase I and phase II metabolites have been identified, including hydroxylated derivatives and conjugates with glutathione, indicating a complex metabolic pathway that could influence the overall toxicity of these compounds [38], [39]. Various studies have shown that the metabolic profiles of 6PPD and 6PPDQ vary significantly among species, suggesting that different organisms may respond differently to exposure. This species-specific variability in metabolism is crucial for understanding the ecological risks posed by these compounds and highlights the need for comprehensive toxicological assessments across diverse species [36]. Furthermore, the identification of novel metabolites resulting from the biotransformation of 6PPD and 6PPDQ underscores the complexity of their environmental fate and potential impacts on ecosystem [38], [39].

2.2.4 Persistence and half-life

The persistence of 6PPD and 6PPDQ varies considerably with environmental compartment and condition. In water, 6PPD is generally short-lived, with half-lives ranging from 3 hours to less than a day, depending on temperature and the presence of metals or reactive oxidants [21]. In contrast, 6PPDQ demonstrates relatively great persistence, with observed half-lives of 13 to 16 days in natural river water [20], [21], and modeled values ranging from 33 to 900 hours using fugacity models [40]. In sediment or road dust, the persistence of both compounds may be significantly extended due to reduced sunlight penetration and microbial activity [26]. Importantly, the long-term behavior of 6PPDQ TPs such as hydroxylated quinones or conjugated metabolites is largely unknown, and their degradation rates, mobility, and ecotoxicological potential remain underexplored.

Overall, the transformation of 6PPD into 6PPDQ and subsequent products is governed by a combination of oxidative, photolytic, and microbial processes (figure 2.2) that are context-dependent and modulated by environmental conditions [8]. These pathways not only influence the persistence and transport of these contaminants but also shape their bioavailability and toxicological profiles in ecological systems. A nuanced understanding of these mechanisms is essential to interpreting field-based concentration data and predicting ecological risk, which will be explored in greater depth in subsequent chapters.

2.3 Occurrence and distribution of 6PPD and 6PPDQ in environmental matrices

The widespread environmental occurrence 6PPD and its oxidation product 6PPDQ reflects their pervasive use in modern tire manufacturing and their subsequent release through TRWP emissions. Since their introduction into the environment via tire abrasion, these compounds have been detected across multiple environmental compartments ranging from atmospheric particulates and road dust to stormwater runoff, surface water, sediments, and even biological tissues highlighting their multifaceted distribution and environmental persistence.

6PPD a widely used tire additive, is a significant contributor to environmental pollution in urban areas, primarily through TWPs that release microplastics, heavy metals, and polycyclic aromatic hydrocarbons (PAHs). Studies have identified 6PPD as a substantial antioxidant in urban dust and runoff, with concentrations reaching up to 468 ng/g in roadside sediments [41]. Oxidative processes, mainly interaction with atmospheric ozone, 6PPD transform into 6PPDQ, a highly toxic component known for its acute toxicity to aquatic species, especially salmonids [8], [42]. The formation of 6PPDQ, coupled with its environmental persistence and toxicity, underscores the need for effective mitigation measures and regulatory frameworks to manage its ecological and health risks.

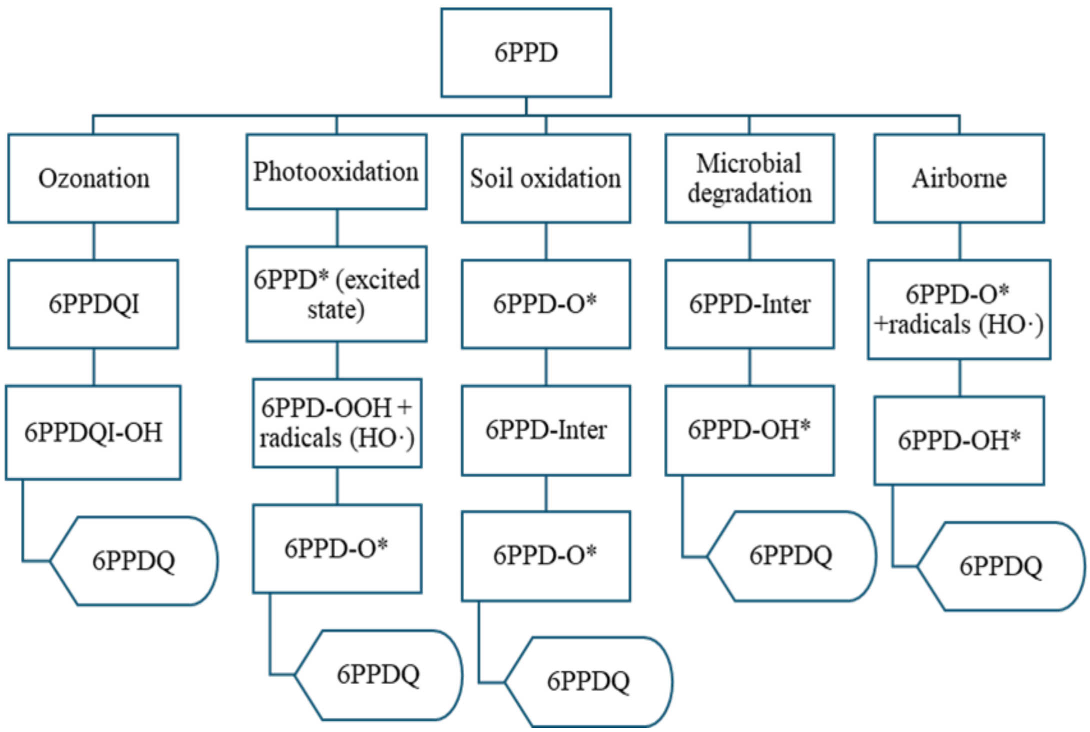


Figure 2:Transformation pathways of 6PPD to 6PPDQ

The presence of 6PPD and 6PPDQ in the air has been documented, particularly in urban environments where tire wear is prevalent. Studies have detected 6PPDQ in atmospheric particulate matter, with concentrations reported in the range of 0.1 to 1.0 ng/m³ in urban air samples [17], [23]. The occurrence of these compounds in air particles indicates that they can be transported over long distances, potentially impacting remote ecosystems.

Furthermore, the detection of 6PPD in fine particulate matter (PM2.5) has raised concerns about inhalation exposure to humans and wildlife [13], [43]. The concentrations of 6PPD in PM2.5 have been reported to reach levels of approximately 0.5 to 2.0 ng/m^3 in urban areas, highlighting the need for monitoring air quality in relation to tire-derived pollutants [44]. Indoor and outdoor dust samples have also been found to contain significant concentrations of 6PPD and 6PPDQ.

For instance, a study conducted in urban settings reported concentrations of 6PPD in house dust ranging from 10 to 100 ng/g, while 6PPDQ was detected at levels between 5 and 50 ng/g [15], [45]. The presence of these compounds in dust raises concerns about potential human exposure through inhalation and dermal contact, particularly in households with children or pets. Additionally, roadside dust samples have shown even higher concentrations, with 6PPD levels reaching up to 500 ng/g, showing that proximity to roadways significantly influences dust contamination [46], [47].

In aquatic environments, 6PPD and 6PPDQ have been detected in surface waters, stormwater runoff, and wastewater treatment plant effluents shown in Table 2.1. Previous studies have reported that 6PPDQ concentrations in urban streams ranged from 1.0 to 10.0 µg/L during storm events [27], [48]. For instance, a study in the Pacific Northwest found that 6PPDQ concentrations in urban runoff exceeded 5.0 µg/L, leading to acute mortality in coho salmon [27], [44]. Many studies showed the widespread presence of 6PPDQ in urban watersheds, with higher concentrations detected in stormwater runoff, posing risks to aquatic organisms [49], [50], [51]. The persistence of 6PPDQ in water is influenced by numerous factors, including hydrolysis, which has been reported to have a half-life of approximately 13 to 16 days [52]. Furthermore, concentrations of 6PPDQ were detected in wastewater effluents at levels ranging from 0.5 to 2.0 μg/L, contributing to the overall load of these contaminants in receiving waters [53].

Table 2: Occurrence of 6PPDQ in aquatic environment

Environmental matrix	Analytical method & Detection limit	Concentration of 6PPDQ	Location	Reference
Surface runoff and stormwater	UHPLC-MS (IQL: 0.023 ng/mL	100% detection; 0.21-2.43 μg/L	Hong Kong, China	[54]
Surface runoff and stormwater	UHPLC-MS (ng/L); (LOD: 0.05, LOQ: 0.17)	6.03-1562 ng/L(roadways), 0.53-5.58 ng/L (farmland)	Dongguan& Huizhou, China	[55]
Surface runoff and stormwater	UHPLC-MS/MS (MDL: 0.029 ng/L, MQL:0.098 ng/L	Mean: 140 ± 60 ng/L	Guangzhou, China	[55]
Surface runoff and stormwater	LC-MS/MS (LOD: 2.1 ng/L, LOQ: 5.7 ng/L)	90 ± 20 ng/L; 0.8-19 μg/L	Seattle, U.S.	[56]
Surface runoff and stormwater	Not specified	4.1-6.1 μg/L	Los Angeles, U.S.	[57]
Surface runoff	Tandem MS (LOD: 8 ng/L)	96-112 ng/L	Nanaimo, Canada	[18]
Surface runoff and stormwater	UHPLC-MS (LOD: 1.2 ng/mL, LOQ: 3.3 ng/mL	Stormwater: 86-1400 ng/L Surface water: not detected	Saskatoon, Canada	[58]

Table 2: (continued). Occurrence of 6PPDQ in aquatic environment

Environmental matrix	Analytical method & Detection limit	Concentration of 6PPDQ	Location	Reference
Receiving surface waters	UHPLC-HRMS (LOQ: 0.0065 μg/L)	0.11-2.3 μg/L	Toronto, Canada	[2]
Receiving surface waters	LC-MS/MS (MDL: 0.1 ng/L)	0.38-88 ng/L	Brisbane, Australia	[59]
Receiving surface waters	LC-MS/MS (LOD: 1.2 ng/L, LOQ: 3.1 ng/L for creek)	0.28-3.2 μg/L	Seattle, U.S.	[56]
Receiving surface waters	LOQ- 5.1 ng/L	0.02 to 0.3 μg/L	LA, U.S.	[53]
surface waters	UHPLC-MS/MS (MDL: 0.029 ng/L; MQL: 0.098 ng/L)	ND-0.75 ng/L	Liuxi River, China	[55]
Wastewater Treatment Plant (WWTP)	Not specified	Influent: 14-830 ng/L; Effluent: 2.8-140 ng/L	Hong Kong	[60]
Wastewater Treatment Plant (WWTP)	LOQ: 0.0098 μg/L	Significant increase in effluent in some WWTPs	Ontario, Canada	[61]
Snowmelt	UHPLC-MS	81–367 ng/L	Saskatoon, Canada	[62]

Soil contamination with 6PPD and 6PPDQ has been documented, particularly in areas adjacent to roadways where tire wear particles accumulate. The concentrations of 6PPD in roadside soils have been reported to range from 50 to 300 ng/g, while 6PPDQ concentrations can reach up to 100 ng/g in similar environments [11], [63]. The fate of these compounds in soil is influenced by environmental conditions such as moisture content and microbial activity, which can help their degradation or transformation [10], [23]. Research has shown that 6PPDQ can persist in anaerobic conditions, leading to its accumulation in flooded soils, thereby posing risks to terrestrial and aquatic ecosystems [13]. In urban rivers and coastal regions sediments have been found to harbor significant concentrations of 6PPD and 6PPDQ. A large-scale survey revealed that these compounds are ubiquitous in sediment samples, with concentrations of 6PPD reaching up to 200 ng/g and 6PPDQ concentrations exceeding 50 ng/g in urban sediment samples [5], [64], [65]. The accumulation of 6PPD and its transformation products in sediments raises concerns about their bioavailability and the risks they pose to benthic organisms and higher trophic levels in aquatic food webs [32], [66], [67]. The detection of these compounds in sediments highlights the long-term environmental persistence of tire-derived pollutants and their potential for ecological harm.

The potential for human exposure to 6PPD and 6PPDQ is a growing concern, particularly in areas impacted by e-waste and urban runoff. Recent studies have also reported traces of 6PPDQin the human liver [68], [69], human urine [70], human diet [71]. The reproductive toxicity of 6PPDQ has been linked to endocrine disruption, impaired sperm quality, and altered metabolism in male mice [72], [73]. In the below sections, our focus is to understand the behavior of 6PPDQ including fate and transport, sources, distribution, transformation mechanism, and toxicologic effect on aquatic species and on humans suggesting that exposure may occur through environmental pathways. The health implications of such exposure, particularly on developmental and reproductive toxicity, call for further investigation to assess the risks associated with these emerging contaminants [66]. Recent research has indicated that 6PPDQ has adverse effects on human liver cells, with studies demonstrating decreased cell viability and altered metabolic profiles in response to exposure [21], [68], [69]. Additionally, the potential for placental transfer of these compounds raises concerns about developmental risks for fetuses and young children [74]. Understanding the pathways of human exposure and the associated health risks is critical for public health policies and regulatory frameworks.

Spatially, the occurrence of 6PPDQ tends to mirror patterns of urbanization, traffic density, and proximity to road infrastructure. Higher concentrations are consistently observed in dense metropolitan areas, road tunnels, and highways, where both the rate of TWP generation and exposure to atmospheric oxidants are elevated [63], [65], [75]. Temporally, rainfall events play a critical role in mobilizing deposited TWPs and accumulated 6PPDQ into surface waters [76]. First-flush effects, coupled with seasonal variations in temperature and ozone levels, drive significant temporal variability in environmental concentrations [77]. For example, monitoring studies have documented peak 6PPDQ levels during early fall, likely due to prolonged dry periods followed by intense rain events [76]. These conditions favor accumulation on road surfaces and subsequent pulse release. In contrast, winter and early spring samples often show lower concentrations, likely due to reduced traffic activity, snow cover, and dilution [62], [78].

2.4 Removal methods of 6PPD and its transformed products

The environmental persistence and acute toxicity of 6PPD and 6PPDQ have prompted the development of removal strategies. These include chemical oxidation, biological degradation, adsorption, and membrane-based separation methods, different levels of effectiveness depending on matrix type and environmental conditions (table 2.2).

2.4.1 Advanced Oxidation Processes (AOPs)

Advanced oxidation processes (AOPs) utilize highly reactive radicals such as hydroxyl (•OH) and sulfate ($SO_4^{\bullet -}$) to break down 6PPDQ and its derivatives. Ozonation, UV-activated peroxymonosulfate (PMS) [79], and UV/H_2O_2 [80] systems have been well studied. These processes promote the formation of multiple intermediates, including hydroxylated, ring-opened, and cleaved quinone compounds. 6PPDQ degrades rapidly under UV/PMS and generates 21 TPs (DP1–DP21) through hydroxylation, quinone decomposition, ring cleavage, and deamination reactions [79], [81].

2.4.2 UV irradiation/ photolysis in surface water

While the UV/PMS method is highly effective, yet sole UV irradiation can also contribute to 6PPDQ degradation [80]. In one study only 13.3% degradation was reported in 120 minutes [80], indicating its insufficiency as a standalone method for removal process. However, one study reported that UV radiation can achieve up to 94.2% removal efficiency of 6PPDQ under 40 minutes of treatment [82]. In surface waters, photolysis leads to 6PPDQ degradation, resulting products like TP300 and TP314 through hydroxylation, and TP184 and TP201 via bond cleavage [83].

2.4.3 Disinfection

Hypochlorite and Chlorine Dioxide are the most commonly used disinfection agents in wastewater treatment which can also react with 6PPDQ. Jiao et al. [68] found that in synthetic waters both 6PPD and 6PPDQ reacted with these agents and disappeared in 5 minutes. However, in road runoff removal efficiency of 6PPDQ shown lower (30%

and 60% by chlorine and chlorine dioxide respectively), may be due to natural organic matter. In contrast, 6PPD removal efficiency was 95% [84]. A concern with this method would be Cl substitution of 6PPDQ can enhance its toxicity to aquatic species [84].

2.4.4 Microbial degradation

Biodegradation of 6PPD and its derivatives have been demonstrated under both aerobic and anaerobic conditions, with microbes utilizing oxidative enzymes to convert these compounds into less toxic forms [36], [62], [85]. In wastewater systems and constructed wetlands, microbial consortia have been shown to metabolize 6PPDQ into hydroxylated and amino phenolic derivatives. Some bacterial strains exhibit transformation capabilities through hydroxylation, deamination, and ring cleavage [61], [86].

Table 3: Removal methods of 6PPD and 6PPDQ and their efficiency

Removal Method	Mechanism	Efficiency (%)	Limitations	References
Inactivated Peroxymonosulfate (PMS system)	$^1O_2$ generation and direct PMS oxidation	39.1-92.6 (pH temperature and PMS dependent)	Efficiency influenced by pH and other factors	[79]
UV/PMS treatment	Sulfate radical oxidation via UV-activated PMS	53.4-100 (dose dependent)	Excess PMS may reduce efficiency	[80]
UV Irradiation (Photolysis)	•OH-induced photolysis (radical driven oxidation)	13.3-94.2 (UV intensity dependent)	Limited efficiency under low UV	[80]
Ozonation	Oxidation by ozone (O_3)	Around 81 (for 6PPD)	Forms toxic byproducts	[40], [90]

Table 3: (continued). Removal methods of 6PPD and 6PPDQ and their efficiency

Removal Method	Mechanism	Efficiency (%)	Limitations	References
Disinfection (Chlorine/ClO_2)	Chlorination and substitution reactions	30-60 (varies with agent used)	Chlorinated TPs can be more toxic	[84], [91]
Biological Treatment (WWTPs)	Microbial enzymatic breakdown	33.3-97.5	May form 6PPDQ during treatment	[40], [80]
Adsorption (Activated Carbon)	Surface sorption	133.8 -719.2 $\mu g.g^{-1}$ (adsorption capacity)	Non-destructive, risk of desorption	[40], [87]
Reverse Osmosis (RO)	Membrane filtration	>90	High energy demand, membrane fouling	[89]

2.4.5 Adsorption and membrane filtration technologies

Activated carbon, biochar, and engineered porous media have demonstrated high sorption capacity for 6PPDQ in aqueous solutions, effectively removing more than 85% of the compound under optimized conditions [25], [87]. Adsorption is advantageous in low-resource or decentralized treatment systems, although it did not destroy the compound and may require post-treatment handling [88]. Removal efficiency often exceeds 90%, although these systems may face operational challenges, including membrane fouling, energy requirements, and maintenance costs [89]. Integration with pre-treatment oxidation steps can enhance performance and reduce membrane burden.

2.5 Toxicological effects and ecological risk

The toxicological profile of 6PPDQ, along with its parent compound 6PPD, has rapidly emerged as a focal point in ecotoxicological and environmental health research. Since the landmark discovery of its acute lethality to Coho salmon in 2020, a growing body of literature has expanded our understanding of the effects of these compounds across a variety of aquatic and terrestrial organisms, as well as potential risks to human health. This section synthesizes key findings on both acute and sublethal toxicity, bioaccumulation, and risk classification across species and ecosystems.

2.5.1 Acute and chronic toxicity in aquatic organisms

Among all documented toxicological responses, the acute lethality of 6PPDQ to salmonids remains the most alarming. Median lethal concentration (LC_{50}) values for Coho salmon have been reported as low as 0.095 μg/L, with complete mortality observed within hours of exposure during stormwater events. Rainbow trout, brook trout, and white-spotted char also display heightened sensitivity, although LC_{50} thresholds vary by species. This specificity suggests differences in gill structure, metabolic pathways, or membrane permeability that influence susceptibility. has been implicated in significant ecological impacts, particularly concerning aquatic life. Studies have reported that exposure to 6PPDQ could lead to high mortality rates in coho salmon (Oncorhynchus kisutch) at 41-95 ng/L of LC50 [8], [17]. The toxicity of 6PPDQ is imputed to its ability to interfere with vascular permeability pathways and increase oxidative stress, leading to developmental abnormalities in fish [92].

Ecotoxicological research has expanded on understanding 6PPDQ's impact across other salmonids and trout from the year 2021. Brook trout, White-spotted char and Lake trout showed high sensitivity, with LC_{50} (24-h) values ranging from 165 to 590 ng/L [93], 510 ng/L and 510 ng/L [42], [47], respectively. Rainbow trout exhibited moderate sensitivity with LC_{50} ranging 900-2260 ng/L [94], while species like brown trout and Atlantic salmon reported much lower sensitivity, with no acute mortality observed at >12 µg/L [95]. Pink salmon has shown LC_{50} as greater than 10 µg/L [46]. Notably, life stages of salmonids/trout's exhibit varying levels of vulnerability [93]. For instance, Coastal cutthroat trout swim-up fry and parr are highly susceptible, with LC_{50} as low as 39.6 ng/L and 103.3 ng/L, respectively [96]. Beyond salmonids, several non-salmonid species including zebrafish, Japanese medaka, and fathead minnows are considerably less sensitive to 6PPDQ, with LC_{50} values often exceeding hundreds of µg/L.

In contrast, other aquatic organisms such as zebrafish (Danio rerio), Japanese medaka (Oryzias latipes), water flea (Daphnia magna), and amphipods (Hyalella azteca) exhibit higher tolerance to 6PPDQ, with LC_{50} values exceeding 10 µg/L in many cases [3], [97]. However, these species are not exempt from risk. Sublethal effects including reduced hatchability, developmental deformities, neurodevelopmental impairment, oxidative stress, and intestinal dysregulation have been observed at environmentally relevant concentrations (ng/L to low µg/L), pointing to significant chronic toxicity potential [36], [98], [99]. Phytotoxicity has also been documented. In hydroponic lettuce, exposure to 6PPDQ has resulted in reduced root growth and chlorophyll degradation [66]. These plant-level effects suggest possible risks to primary producers and food web stability in aquatic environments, particularly where stormwater or tunnel wash water discharges are frequent.

2.5.2 Mechanisms of toxic action

The mechanistic basis for 6PPDQ toxicity is still under investigation, but several plausible pathways have been identified. In fish, 6PPDQ is believed to impair mitochondrial function and energy metabolism, leading to systemic oxidative stress. Gill cell membranes appear to be particularly vulnerable, with histological studies showing edema, necrosis, and sloughing. Transcriptomic analyses in zebrafish and trout have revealed upregulation of stress-response genes, particularly those associated with inflammation, hypoxia, and apoptosis. In Daphnia, chronic exposure has led to decreased reproduction and shortened lifespan, suggesting potential interference with endocrine signaling. Neurodevelopmental studies in zebrafish embryos show reduced eye size and altered locomotor activity, indicative of neural toxicity. These findings suggest that, even at sublethal concentrations, 6PPDQ may disrupt fundamental physiological processes during early development stages.

2.5.3 Bioaccumulation and biomagnification potential

While 6PPD and 6PPDQ are not highly considered bio-accumulative compared to legacy persistent organic pollutants(POPs), evidence of tissue accumulation has been documented. 6PPDQ has been detected in fish livers and bile, with concentrations increasing in tandem with environmental exposure levels [67]. Lipophilicity, indicated by log Kow values (4.47 for 6PPD; 3.98 for 6PPDQ) [100], suggests moderate potential for bioaccumulation, particularly in lipid-rich tissues. Unlike POPs, however, 6PPDQ appears to be more hydrophilic, enhancing its potential for renal excretion. This property may limit long-term accumulation but simultaneously increases its mobility within organisms and ecosystems. Additionally, recent findings suggest trophic transfer potential. For instance, in benthic macroinvertebrates collected from contaminated urban streams, 6PPDQ has been detected in gut contents and tissue extracts, raising concerns about food web exposure pathways.

2.5.4 Human toxicity and exposure concerns

Human health implications of 6PPDQ are increasingly recognized due to its detection in urine, serum, and cerebrospinal fluid (CSF). In murine models, both 6PPD and 6PPDQ have been shown to induce liver hypertrophy, elevated triglyceride levels, and altered hepatic gene expression, particularly affecting glycolipid metabolism and immune modulation. Long-term exposure has been associated with inflammatory responses and disruptions in glutathione metabolism. Neurotoxicity is of special concern. 6PPDQ can cross the blood-brain barrier and accumulate in the central nervous system. In Parkinson's disease patients, elevated concentrations of 6PPDQ have been detected in CSF compared to healthy controls.

Experimental studies suggest that the compound may potentiate the formation of Lewy bodies by interfering with α-synuclein aggregation pathways, mitochondrial respiration, and reactive oxygen species (ROS) generation. Although these findings remain correlative, they warrant further investigation, particularly in urban populations with high vehicular exposure. Other human exposure pathways include ingestion of road dust (particularly in children), inhalation of airborne TRWPs, and dermal absorption from playgrounds and artificial turf surfaces. Pregnant women have been found to exhibit higher median urinary concentrations, raising concerns about fetal exposure. No regulatory occupational limits exist for either compound as of now, although research groups and public health agencies are increasingly calling for risk-based screening levels.

2.5.5 Ecological risk assessment approaches

The ecological risk posed by 6PPDQ is typically assessed through the Risk Quotient (RQ) method, where RQ = MEC / PNEC (measured environmental concentration / predicted no-effect concentration). Based on the U.S. EPA's freshwater screening value of 11 ng/L, many urban surface waters fall into medium or high-risk categories. For instance, road runoff concentrations during rain events frequently exceed this threshold by orders of magnitude. In the Schuylkill River, multiple sampling locations recorded 6PPDQ levels between 2-18 ng/L during peak seasons, placing them into moderate or high ecological risk zones. In addition to the RQ approach, species-specific toxic unit (TU) models have been proposed, particularly for sensitive taxa like salmonids. TU-based risk assessments incorporate species-specific LC_{50} values and provide a finer scale for risk stratification. This is especially useful in multispecies ecosystems, where community-level effects can cascade through trophic networks.

2.5.6 Knowledge gaps in toxicity assessment

Despite recent advancements, several critical knowledge gaps remain. First, long-term chronic exposure studies across multiple species are sparse. Most available data focus on acute toxicity or early developmental endpoints. Second, the interactive effects of 6PPDQ with other urban pollutants such as metals, polycyclic aromatic hydrocarbons (PAHs), or road salts remain largely unstudied. These mixtures may have additive or synergistic effects on organismal health. Third, there is limited understanding of the metabolomics and epigenetic changes induced by 6PPDQ in both aquatic and terrestrial organisms. Understanding these molecular-level effects is essential for predicting delayed or multigenerational toxicity. Lastly, dose-response relationships in humans are underdeveloped. While biomonitoring studies provide presence data, the clinical or health outcome implications of these internal concentrations are not yet established.

2.6 Detection methods and analytical advances

Accurate detection and quantification of 6PPD and its TPs in environmental matrices require the use of sensitive, selective, and robust analytical techniques, given the complexity of transformation pathways and the typically low concentrations of target analytes in the environment. Analytical methods such as liquid chromatography-mass spectrometry (LC-MS/MS), gas chromatography-mass spectrometry (GC-MS/MS), and high-resolution mass spectrometry (HRMS) have been widely adopted to support environmental monitoring and research efforts. LC-MS/MS is the most commonly used technique for detecting 6PPD, 6PPDQ, and various hydroxylated derivatives. Its high sensitivity and capacity to analyze non-volatile and thermally unstable compounds make it especially suitable for water samples . The U.S. EPA Draft Method 1634 [31], LC-MS/MS for the standardized detection of 6PPD and 6PPDQ in aqueous solutions, representing a significant step toward regulatory implementation.

GC-MS/MS is typically used to analyze volatile and semi-volatile TPs, particularly in matrices such as airborne particulates (PM2.5& PM10) and TWP's [56], [101]. Due to the low volatility and high polarity of many 6PPD-derived compounds, derivatization is often required prior to GC analysis. Despite these challenges, GC×GC-TOFMS (comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry) have been successfully used to identify complex TP mixtures, including compounds like TP207 and TP184 in atmospheric and particulate samples [102]. HRMS, often coupled with non-targeted screening (NTS) workflows, has widened the scope of environmental detection [103]. HRMS exhibited accurate mass measurements and isotopic pattern recognition, enabling the identification of known and unknown TPs. In combination with data processing software and structural elucidation tools, HRMS is particularly useful for profiling 6PPDQ degradation in stormwater runoff, urban waters, and sediments, where complex mixtures of known and unknown compounds may coexist [103], [104].

Sample preparation protocols vary by matrix. In water samples, solid-phase extraction (SPE) [105] is commonly used to isolate and concentrate analytes, reducing matrix interferences such as NOM and dissolved salts. In atmospheric or particulate samples, challenges include sorption losses and desorption variability, requiring optimized extraction conditions [101], [106]. For soil and sediment matrices, extraction efficiency is influenced by organic content and moisture levels, often necessitating techniques such as pressurized liquid extraction (PLE) or ultrasonic-assisted extraction (UAE) [107], [108]. In biological samples (e.g., fish tissue or in vitro cultures), isotope-labeled internal standards and metabolomics-based HRMS workflows are essential for assessing bioaccumulation and biotransformation [36], [109], [110]. Each method exhibits its own advantage depending on the matrix, target analyte, and required sensitivity. While LC-MS/MS excels in aqueous monitoring and is suitable for regulatory applications, GC-MS/MS provides valuable insights into airborne and volatile compounds, and HRMS-NTS is indispensable for discovery focused research and unknown TP identification.

2.6.1 Detection in human biomonitoring studies

Recent advances in bioanalytical chemistry have enabled the detection of 6PPD and 6PPDQ in human urine, blood, and cerebrospinal fluid (CSF) [70], [111]. Enzyme-assisted hydrolysis combined with LC-MS/MS has allowed researchers to quantify conjugated metabolites such as glucuronides and N-acetylcysteine derivatives [112]. These studies report key insights into human exposure, metabolism, and internal dose metrics. However, a lack of standard reference materials and limited information on pharmacokinetics restricts the broader utility of these data. Furthermore, inter-laboratory variability in detection protocols and lack of universal standards can affect comparability across studies [113], [114]. Developing reference ranges, establishing sample storage protocols, and validating methods for longitudinal studies are ongoing research needs.

2.6.2 Limitations and challenges

Despite these advancements, several limitations remain. First, the short environmental half-life of 6PPD in aqueous environments complicates its detection, especially in field samples collected after storm events. Second, matrix effects such as ion suppression or enhancement can reduce sensitivity and precision, particularly in complex matrices like sediments and tissues. While isotope-labeled standards improve accuracy, they are not available for all transformation products. Third, current detection techniques often focus on parent compounds and well-characterized TPs, leaving many possible derivatives undetected. This is especially important given the wide range of oxidative and biotic degradation pathways reported in recent literature. Non-targeted screening methods using HRMS hold promise but require significant expertise in data processing and interpretation.

2.7 Research gaps, objectives, and hypotheses

The environmental behavior and toxicity of tire-derived compounds particularly 6PPD and its oxidative product 6PPDQ demand an integrated conceptual framework that links chemical occurrence, transformation, fate, exposure pathways, and risk to ecological and human health. The growing recognition of 6PPDQ as a critical toxicant in aquatic environments necessitates not only a multidimensional understanding of its life cycle in the environment but also the identification of scientific and regulatory blind spots that hinder mitigation. This section synthesizes the major concepts emerging from literature reviewed in prior sections and outlines a conceptual model for investigating 6PPDQ contamination in urban watersheds. It also highlights the unresolved research gaps that motivate the current dissertation.

2.7.1 Key research gaps

Despite increasing recognition of 6PPDQ's toxicity, significant gaps remain in our understanding of its environmental behavior and impact:

1. Limited quantitative data: While 6PPDQ has been detected in aquatic systems, its temporal and spatial distribution across seasons and varying watershed conditions is not well-documented.
2. Unexplored relationship with urbanization factors: The potential influence of traffic volume, population density, and industrial activities (e.g., tire related industries) on 6PPDQ concentrations remains poorly understood.
3. Inadequate risk assessment: Current risk assessments lack sufficient data to quantify the ecotoxicological risks posed by 6PPDQ to freshwater resources. Toxic unit (TU) site-specific ecological risk classification of 6PPDQ and potential for sublethal effects such as endocrine disruption or neurotoxicity, which are poorly understood.
4. Leaching potential of 6PPD& 6PPDQ from tire particles: Although waste tire rubber is increasingly being recycled in various applications, comprehensive

leachate analysis for specific 6PPD and 6PPD-Q release is lacking. Most studies focus on heavy metals and other organic contaminants from TWP's. The extent to which tire particles leach 6PPD and 6PPDQ into freshwater environments under natural sunlight conditions remain insufficiently studied.

2.7.2 Research objectives

The primary objective of this research is to investigate the environmental behavior, transformation, and risks associated with 6PPD and its toxic derivative, 6PPDQ, in freshwater systems. Specifically, the study focuses on quantitative analysis, statistical correlations, and transformation pathways to fill critical gaps in understanding their environmental fate and toxicity. The key objectives are:

1. Quantitative Analysis: Measure the concentrations of 6PPDQ in the Delaware Water Basin and the Schuylkill River using the EPA Draft Method 1634. Analyze seasonal and spatial trends in 6PPDQ concentrations.
2. Statistical Analysis and Correlation: Examine the relationship between traffic volume (Average Annual Daily Traffic data, 2024) and 6PPDQ concentrations. Investigate the correlation of 6PPDQ concentrations with population density and proximity to tire related facilities.
3. Risk Assessment: Evaluate the environmental risk posed by 6PPDQ using the Risk Quotient (RQ), calculated as the ratio of measured 6PPDQ concentrations to the EPA screening value for freshwater. Identify high-risk areas within the studied watersheds.

4. Leaching Studies: Assess the leaching potential of 6PPD and 6PPDQ from new and used tire particles in river water under environmental conditions. Perform chemical and structural analysis of these compounds using FTIR and NMR spectroscopy.

2.7.3 Research hypotheses

This research is guided by the following hypotheses:

1. The concentrations of 6PPDQ vary spatially and temporally, with higher concentrations observed in areas of greater traffic density and during seasons with higher runoff (like in spring).
2. There will be significant positive correlation between 6PPDQ concentrations and traffic volume, as well as population density and proximity to tire facilities.
3. Used tire particles might leachate higher amounts of 6PPDQ than new tire particles due to wear and degradation.

2.7.4 Guiding research questions and dissertation scope

Building upon the conceptual framework and identified knowledge gaps, this dissertation is guided by the following key research questions:

1. What are the spatiotemporal trends of 6PPDQ concentrations in urban freshwater systems across different seasons and flow regimes?
2. How do urbanization indicators such as traffic volume, population density, and industrial proximity influence 6PPDQ distribution?
3. What are the ecological risks posed by 6PPDQ, particularly to sensitive fish species like rainbow trout and brook trout, based on TU thresholds and EPA screening values?

4. How do tire particle properties (new vs. used) affect the leaching potential and transformation behavior of 6PPD and 6PPDQ?
5. What insights can surface, and molecular characterization techniques (FTIR, NMR) provide regarding the transformation pathways and chemical aging of tire particles?

By integrating environmental monitoring, statistical modeling, leaching experiments, and analytical chemistry, this research aims to provide a comprehensive and mechanistically grounded understanding of 6PPDQ behavior and its implications for ecosystem and public health management.

CHAPTER 3

QUANTITATIVE ANLAYISIS AND SPATIOTEMPORAL DISTRIBUTION OF 6PPDQ IN TWO URBAN WATERSHEDS: SCHUYLKILL RIVER AND DELWARE RIVER BASIN

3.1 Introduction

6PPD-Quinone (6PPDQ) a prominent transformation product (TPs) of the antioxidant N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) [8] has gained global attention for its acute toxicity to aquatic species, particularly salmonids [17], [42]. 6PPD has been widely used in rubber tire formulation for over 50 years to inhibit oxidative degradation and extend tire lifetime [4], [115]. However, upon reaction with atmospheric ozone 6PPD (via tire wear particles (TWPs)) converts into 6PPDQ. The compound was first identified as a cause of urban runoff mortality syndrome (URMS) in coho salmon (Oncorhynchus kisutch) in late 2020 [8] where lethal concentration (LC_{50}) as 41-95 ng/L caused acute mortality within 24 h of exposure [9].

Since this discovery, 6PPDQ has been detected across various environmental compartments like stormwater, road runoff, surface water, sediments, soils, and even air particulates [6], [44], [116]. It was also detected in drinking water and municipal water [14], [48]. Alarmingly, its presence has also been found in human tissues, including urine, liver, and maternal samples [68], [117], [118], raising concerns about potential public health risks. Toxicity studies showed significant interspecies variation: brook trout, white-spotted char, and lake trout exhibiting LC_{50} values between 165-590 ng/L, whereas rainbow trout and Atlantic salmon exhibited higher tolerance. However, early life stages (e.g., swim up, fry, parr) of salmonid and trout showed more vulnerability, highlighting the importance of monitoring in trout-bearing water systems.

The environmental occurrence of 6PPDQ is strongly associated with urban runoff/ storm mobilization. TWPs accumulate on roads and other impervious surfaces and are transported into surface waters during rainfall or snowmelt. Urban stormwater runoff, a primary pathway for TWPs into water bodies, the contamination, particularly in areas with heavy traffic volume and industrial activity [58], [75], [119]. In aquatic systems, the presence of 6PPDQ correlates strongly with precipitation events [52]. Studies in urban areas like Seattle, Tokyo, and Toronto have shown 6PPDQ concentrations ranging 1-3 µg/L, during storm events with peak levels exceeding coho salmon LC_{50} threshold. These findings underscore the compound's persistence in urban environments and the importance of rainfall-driven mobilization mechanisms.

Seasonal dynamics also play a critical role in summer, and autumn typically exhibited increased concentrations due to elevated ozone levels (enhancing 6PPD transformation), and frequent precipitation. In contrast, winter promotes accumulation of TWPs, which may later be mobilized by spring runoff. Studies also document complex flow dynamics, such as "first flush" or "middle flush" patterns, where peak 6PPDQ concentrations appear either at the onset or during the middle of rainfall events, depending on waterbody characteristics.

In 2024, the U.S. Environmental Protection Agency (EPA) has significantly advanced the monitoring of 6PPDQ contamination by establishing freshwater screening values of 8.9 ng/L for 6PPD and 11 ng/L for 6PPDQ [29], [30]. These values serve as critical thresholds for assessing the potential harm to aquatic life and identifying high-risk areas. The EPA developed Draft Method 1634 in 2023 [31], which enables precise detection and quantification of 6PPDQ, facilitating the identification of contamination hotspots.

Additionally, the EPA is funding and supporting further research to understand the fate and transport of 6PPDQ, particularly in aquatic systems through stormwater runoff. These findings can help us evaluate and mitigate the risks posed by 6PPDQ contamination. Despite these advancements, there is limited information about 6PPDQ occurrence and behavior in freshwater systems of the northeastern U.S., including two major freshwater rivers such as the Schuylkill River and the Delaware River Basin.

The Schuylkill River, a major tributary of the Delaware River, supplies drinking water to millions, supports ecological habitat, and recreational activities. Its proximity to densely urbanized and industrial areas, including Philadelphia, places it at high risk and susceptible to tire-derived pollution. Previous studies in Schuylkill have focused on pollutants like polycyclic aromatic hydrocarbons (PAHs), metals, and microplastics [2], [120], [121], but 6PPDQ has not been assessed. To address this knowledge gap, this study measured 6PPDQ concentrations sixteen locations along the Schuylkill River across four seasonal events in 2024 (February, May, August, and October).

The Delaware River Basin, spanning over 282 miles through New York, New Jersey, Pennsylvania, and Delaware, is another ecologically and hydrologically important system that supplies drinking water to nearly 15 million people, including major cities such as New York, Trenton, and Philadelphia. Its biodiversity, including sensitive salmonid and trout populations, makes it a critical watershed for toxicological assessments. Tributaries like Valley Creek and Bushkill Creek, designated as Class A trout streams, are particularly vulnerable making it a high-priority for monitoring emerging toxicants. However, till date no studies have evaluated its presence, and distribution within this basin, despite increasing concern about 6PPDQ. To fill this gap, the current study conducted six sampling periods between 2024 and 2025 including storm and spring events across 13 fixed locations and 9 boat-accessed sites, capturing both mainstem and tributary conditions.

This chapter presents the first coordinated dataset on 6PPDQ occurrence and variability in both the Schuylkill and Delaware Rivers. The data will help assess seasonal and flow-related patterns and guide future risk evaluation and pollution mitigation strategies. The primary objectives of this chapter are to quantify the spatial and temporal distribution of 6PPDQ in surface waters of the Schuylkill River and Delaware River Basin, and to evaluate seasonal patterns of its occurrence in relation to flow dynamics, pH, temperature, and precipitation events. By analyzing multi-season datasets from both watersheds, this chapter aims to identify spatiotemporal trends and contamination hotspots while highlighting how environmental variables influence the mobilization and persistence of 6PPDQ in these urban water systems.

3.2 Study area overview

3.2.1 Schuylkill River

The Schuylkill River extends approximately 135 miles from its headwaters in Pottsville to its confluence with the Delaware River in Philadelphia. It traverses a range of land-use types from forested and agricultural headwaters to heavily urbanized downstream sections passing through municipalities such as Pottstown, Norristown, and Philadelphia. The river supplies drinking water to approximately 1.5 million residents and supports both recreational use and aquatic biodiversity.

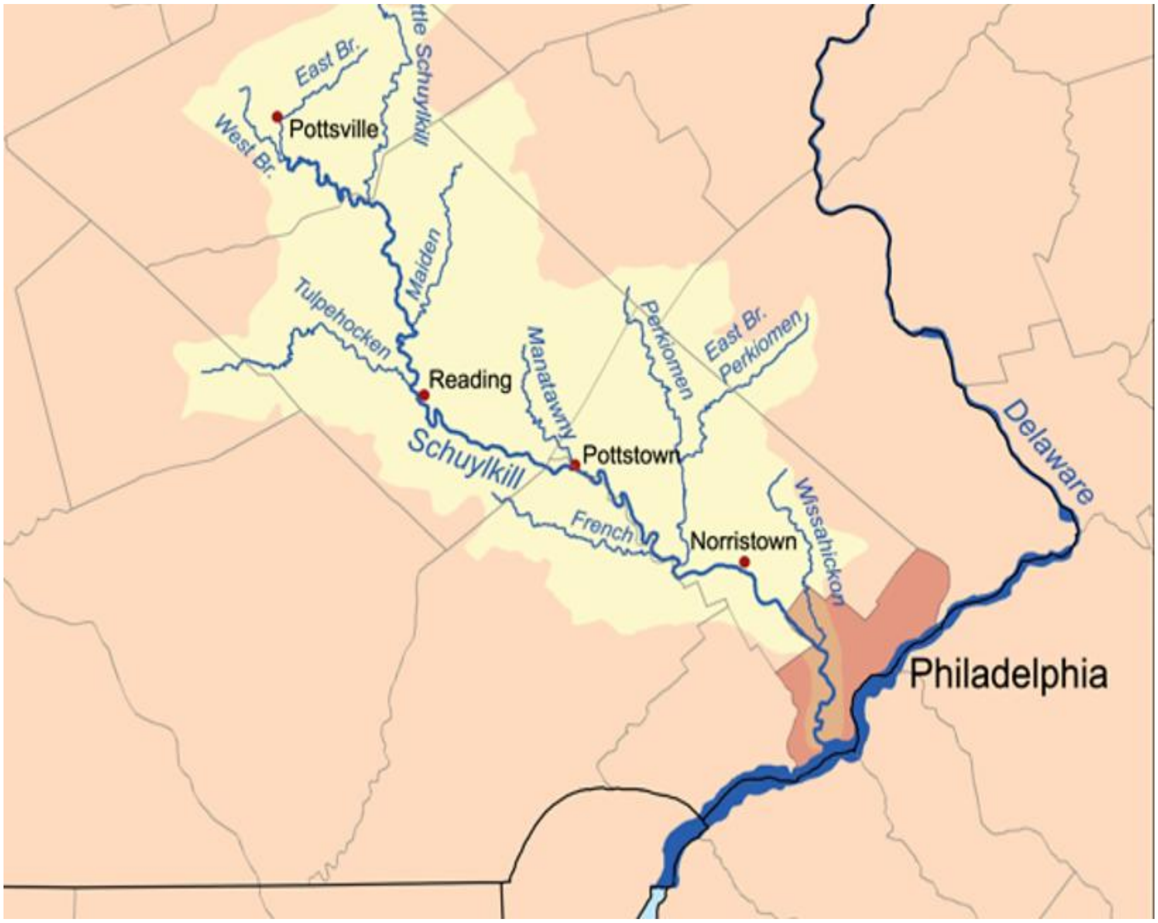


Figure 3: Schuylkill River from Pottsville to Philadelphia

Hydrologically, the Schuylkill is highly responsive to storm events, with impervious surface cover in downstream areas facilitating rapid runoff. This dynamic makes it particularly susceptible to non-point source pollution, including emerging contaminants like 6PPDQ. Urban runoff, especially following precipitation, is a known vector for TWPs and associated chemical pollutants. While prior studies have documented legacy contaminants such as heavy metals and PAHs, there remains a significant data gap regarding tire-derived TPs. The current study's spatially distributed sampling design targets this knowledge gap by capturing variation across urban pressure gradients and seasonal hydrological conditions.

3.2.2 Delaware river basin

The Delaware River spans approximately 282 miles from the Catskill Mountains in New York to Delaware Bay, forming state boundaries between New York, Pennsylvania, New Jersey, and Delaware. As one of the longest undammed rivers in the continental United States, it supports a wide array of freshwater and estuarine ecosystems along its gradient. Key tributaries include the Lehigh and Schuylkill Rivers, both of which drain heavily urbanized regions contributing to the river's pollutant burden. Provides drinking water for over 15 million people, including those in major metropolitan areas such as Philadelphia, Trenton, and NYC, the river is of exceptional hydrological and ecological significance. It also sustains extensive commercial and recreational fishing, with sensitive trout populations both native (brook trout) and non-native (like rainbow and brown trout).

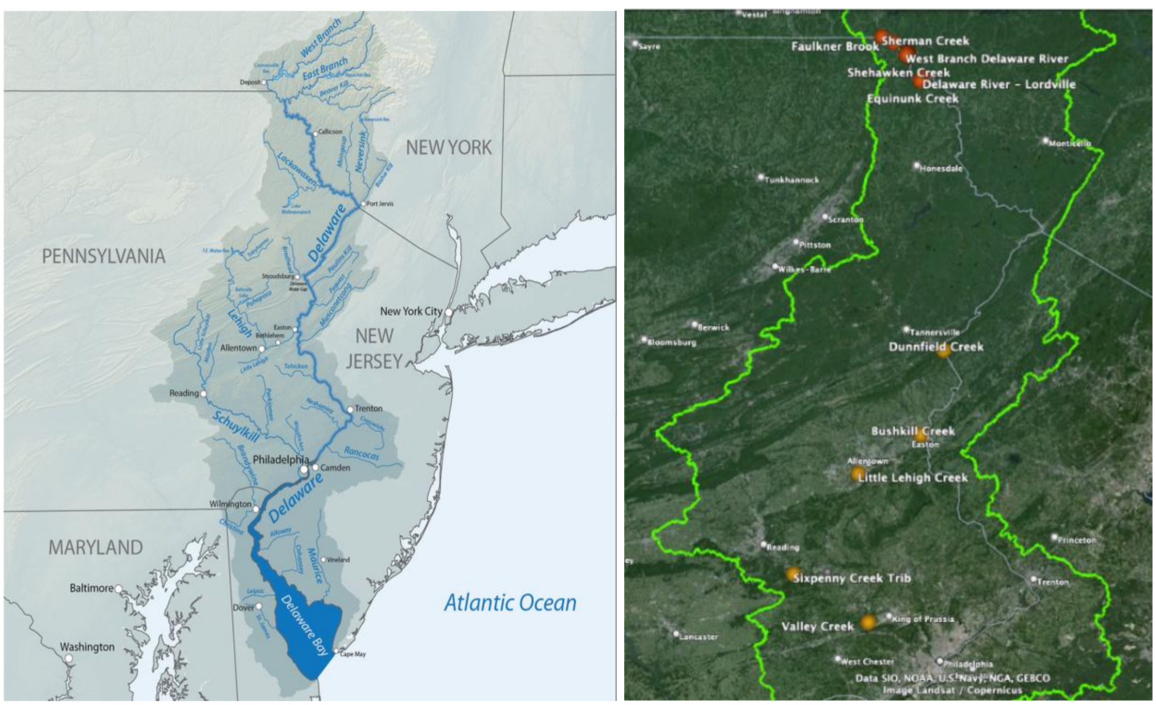


Figure 4: Fig. (a) Delaware River from New York to Delaware , Fig. (b) Delaware River Basin from Sherman Creek to Valley Creek sampling locations (courtesy of DRBC)

Despite its protected status in some stretches, the Delaware River remains vulnerable to urban stormwater runoff and atmospheric deposition of contaminants. Previous monitoring has highlighted the presence of emerging pollutants, including PFAS and heavy metals. However, the distribution and fate of tire-derived compounds such as 6PPDQ are not studied. Given its diverse land-use profile, variable hydrology, and ecological sensitivity, the Delaware River Basin presents a critical landscape for understanding the spatial and seasonal dynamics of 6PPDQ across contrasting water system conditions.

3.3 Materials and methods

3.3.1 Chemicals and reagents

HPLC grade acetonitrile (≥99.9%), methanol (≥ 99.9%), and analytical grade formic acid (ACS reagent grade) were purchased from Fisher Scientific. Reagent water (Type I) was purchased from Fisher Scientific. Standard solutions of 6PPDQ (>95%) and mass-labeled non-extracted and extracted internal standards (>98%) were obtained from Cambridge Isotope Laboratories. A complete list of chemicals and their abbreviations is provided in Table S4. We purchased Strata-XL SPE cartridges (200 mg/6 cc, 100 µm) and Kinetex XB C18 (3.6 µm, 4.6 mm x 50 mm) columns from Phenomenex.

3.3.2 Schuylkill river water sampling

Samples were collected from sixteen locations along the Schuylkill River, ranging from upstream sites near Pottstown to downstream areas near Grays Ferry Bridge (Figure 3.3). Sampling was conducted in February (n=13), May, August, and October 2024 (n=16). In February, samples were obtained from Pottstown, Linfield Bridge, Black Rock Bridge, Mont Clare Bridge, Pawling Rd Bridge, Perkiomen Bridge, Norristown Dam Bridge, Conshohocken, Manayunk Canal (Green Lane), Falls Bridge, Strawberry Mansion, Chestnut Bridge, and Grays Ferry Bridge. Additional locations like Spring City (Main St), Perkiomen Creek, and Sullivan's Bridge were added in later months to enhance spatial coverage. For a complete list of sampling locations and coordinates, refer to Table S1.

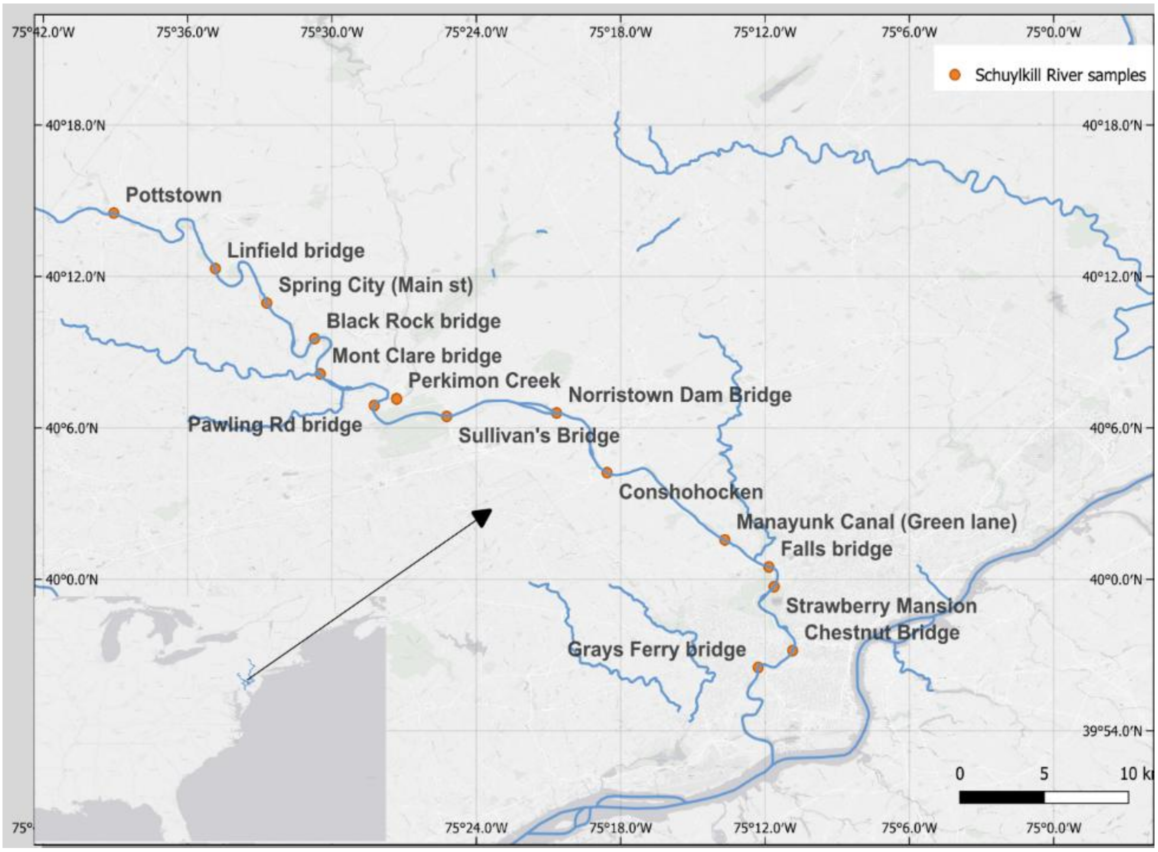


Figure 5: Schuylkill River sampling locations

Comprehensive grab sampling was conducted at each location, with samples collected at multiple points along the riverbed to ensure representative and homogeneous samples of the overall water flow. This approach ensures the capture of any spatial variability in the riverbed's flow profile, thereby enhancing the reliability and consistency of the collected data. Additionally, we collected field blanks at all the sampling locations by exposing each site to reagent water for the duration of the sample collection process. Pre-cleaned glass bottles were used to prevent contamination. Samples were immediately stored in coolers maintained at 4±2°C to preserve sample stability during transport to Temple University for analysis. No preservatives were added to the samples, as the analysis was performed promptly.

3.3.3 Delaware River basin sampling

A total of 23 distinct locations were monitored to represent a wide range of hydrological conditions, land-use gradients, and urbanization levels, including rural tributaries, mainstem Delaware River stretches, and urban-industrial zones in the lower basin. Seasonal/quarterly monitoring sampling was conducted during five periods (April, July, and November 2024, and February, April 2025). During each sampling event, triplicate grab samples were collected at 13 fixed sites across the upper and middle Delaware River basin, extending from Sherman Creek through Valley Creek. One additional site, Valley Creek Tributary, was included in July 2024 period, resulting in 14 samples. In June 2024, following a significant rainfall event (>0.75 inches/day), four tributaries (Bushkill Creek, Little Lehigh Creek, Sixpenny Creek, Valley Creek) were sampled specifically to evaluate the influence of stormwater driven 6PPDQ runoff.

These targeted rain event samples were also collected in triplicates. In addition to the land-based monitoring and expanding coverage into more urbanized areas of the Delaware River, boat sampling was conducted between May and August 2024 at nine locations. These sites expand from Paulsboro, Navy Yard, and multiple bridge crossings such as the Benjamin Franklin Bridge and Burlington Bristol Bridge to Biles channel (fig 3.4)), capturing zones of elevated environmental influence. For a complete list of sampling locations and coordinates, refer to Table S2 and S3.

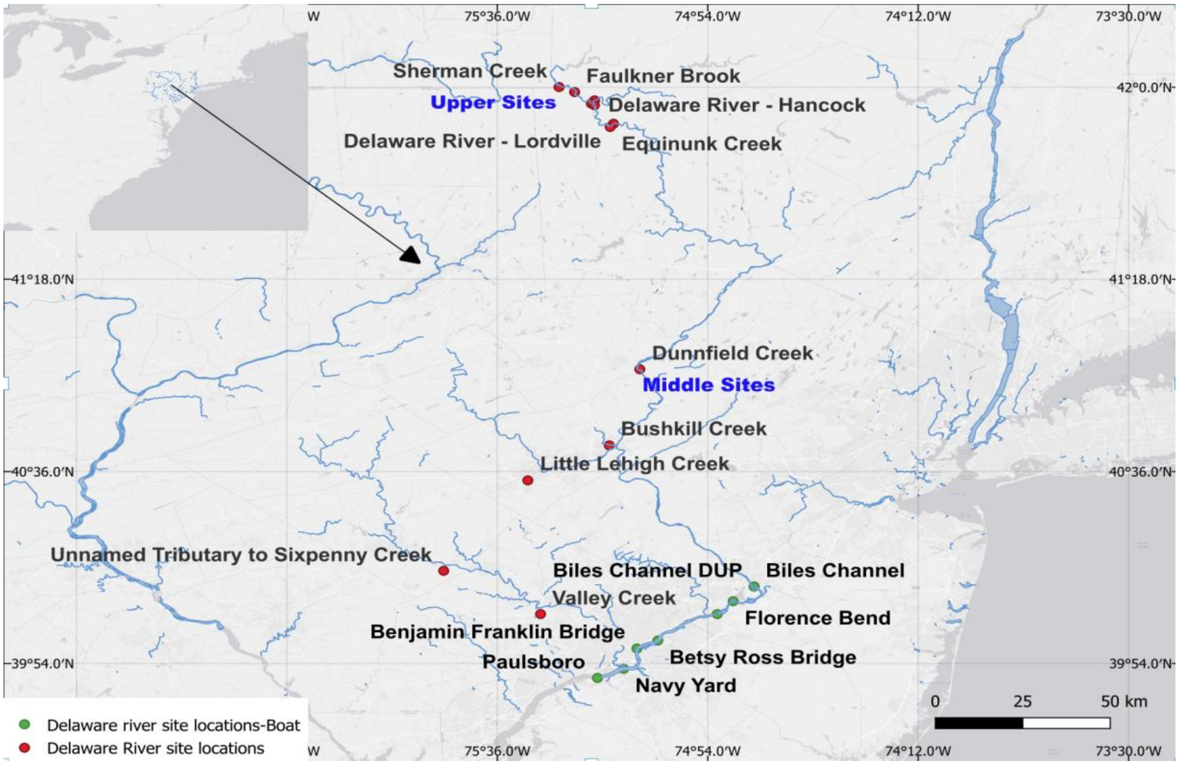


Figure 6: Delaware River Basin and boat sample locations

All samples were collected using standard grab sampling in triplicate, using pre-cleaned amber glass bottles. To ensure sample representativeness, we targeted well mixed zones near the main flow path, with lateral subsampling conducted where feasible. Field blanks were prepared on site by exposing reagent water to environmental conditions for sampling duration to check for potential field contamination. Immediately after collection, all samples were stored in ice-filled coolers (4 ± 2 °C) and transported to Temple University for extraction and quantification. No preservatives were added to avoid matrix interference, as analytical processing occurred promptly.

3.3.4 Analytical method

3.3.4.1.Extraction procedure. The sample extraction for both the river water samples was performed using the guidelines provided in EPA Draft Method 1634 [31]. We spiked aqueous samples with extracted internal standards (EIS) and homogenized them. Solid-phase extraction (SPE) cartridges were conditioned with 5 mL of acetonitrile followed by 5 mL of reagent water to maintain hydration. Samples were passed through the cartridges at a controlled flow rate (10-15 mL/min). Sample bottles were rinsed with 5 mL of 50:50 methanol and reagent water mixture, which was also passed through the cartridges. We dried the cartridges under a vacuum for 5 minutes before eluting them with 5 mL of acetonitrile. We spiked the eluate with non-extracted internal standards (NIS) and then transferred it to polypropylene vials for LC/MS/MS analysis.

3.3.4.2 Instrumental analysis. Concentrations of 6PPDQ and internal standards were analyzed using the Acquity Xevo TQ-S UHPLC system (Waters, USA) combined with the Kinetex XB C18 column (3.6 µm, 4.6 mm x 50 mm) with a column temperature of 40°C. We determined the compounds using the positive ion electrospray ionization (ESI) mode and identified them by multiple reaction monitoring (MRM). The mobile phase components are 0.2% formic acid in DI water (A) and 100% acetonitrile (B), with a flow rate of 0.3 mL/min. Detailed instrumental analysis parameters are included in Table S5. MRM settings: cone energy(V) at 20, 0.025-0.163 sec dwell times, curtain and collision gas flows set to 305 psi and 58 psi, respectively. Elaborated MS scan settings with ion transitions, collision energy (V), and retention time (RT) (min) are in Table S6.

3.3.4.3 Quality assurance and quality control (QA/QC). Method validation included calibration, sensitivity, and precision assessments. Positive identification of 6PPDQ was based on consistent retention times and MRM transition ion ratios within the average ion ratio (for standard calibrations) ± 3*Std. dev. (in calibration standards). Calibration standards ranged from the limit of quantification (LOQ) to the upper quantifiable range, with six calibration points for linear models and seven for non-linear models, following

EPA Draft Method 1634[31]. We conducted calibration verification (VER) at the start of each sequence, and after every 10 samples, we conducted instrument sensitivity checks (ISC) to ensure stable performance.

To monitor contamination, instrument blanks, method blanks, and field blanks were analyzed. Instrument blanks confirmed the absence of carryover contamination, while method blanks were processed alongside field and QC samples to detect contamination introduced during sample preparation. Field blanks were prepared by exposing reagent water to field conditions at each location from the start to the end of sampling, ensuring no contamination occurred during sample handling. Accuracy and precision were evaluated using procedural blanks, matrix spikes, and duplicates, with acceptable recoveries between 70–130% and RSD ≤ 20%. Method detection limits (MDL) were estimated using EPA standard protocol:

$$MDL = s * \ t_{(n-1,1-\propto=0.99)}$$

Where, $t_{(n-1,1-\propto=0.99)}$ is the t-values at 99% confidence level and n-1 degrees of freedom and s is the standard deviation of replicate measurements at low concentration levels. LOQ was determined at 3.3*MDL for each bath.

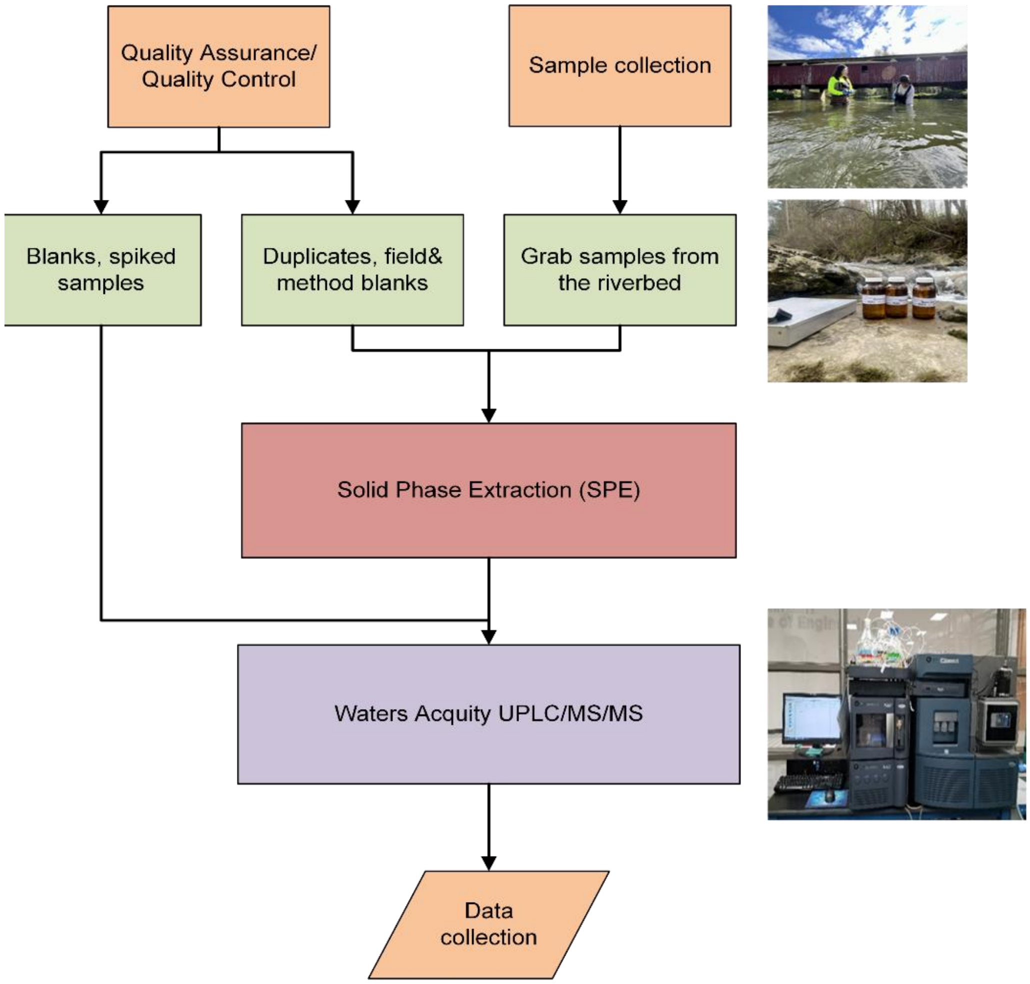


Figure 7: Workflow of sampling and analytical methods for 6PPDQ quantification

Validation involved spiking reagent water with EIS (20 ng/L), followed by extraction and analysis. Isotope dilution ensured accurate quantification. Quality control measures included retention time (RT) verification before each analytical sequence, internal standard addition to correct for matrix effects, and ongoing precision recovery (OPR) checks per batch. These QA/QC measures ensured data reliability, contamination control, and reproducibility in assessing 6PPDQ contamination. The schematic Figure 3.5 illustrates the sequential workflow applied in this study from sample collection and quality assurance/quality control (QA/QC) through SPE and instrumental analysis via Waters

Acquity UPLC-MS/MS, culminating in data collection. Figure visually complements the detailed descriptions in Sections 3.3.2 and 3.3.3 by providing a step-by-step representation of how 6PPDQ samples were managed, extracted, and analyzed.

3.4 Results and discussions

3.4.1 Distribution of 6PPDQ in the Schuylkill River

The presence of 6PPDQ was consistent throughout all sampling months, with concentrations increasing over time (Figure 3.6). The average concentrations across all sites were 0.52 ng/L in February, 1.41 ng/L in May, 1.14 ng/L in August, and 6.12 ng/L in October, indicating seasonal variation in contaminant accumulation. The highest concentrations were observed in October 2024, after a rain event, suggesting that runoff, precipitation, and other environmental factors played a role in mobilizing 6PPDQ from pavement or road surfaces into the Schuylkill River.

In February 2024, the concentrations of 6PPDQ ranged from non-detectable (ND) to 1.59 ng/L, with Strawberry Mansion (1.59 ng/L), Chestnut Bridge (1.23 ng/L), and Falls Bridge (0.87 ng/L) exhibiting the highest values. Upstream locations, such as Pottstown (0.13 ng/L) and Perkiomen Bridge (0.21 ng/L), had the lowest concentrations. Minimal contamination in winter is likely due to reduced runoff from snow and ice accumulation [78], as well as lower vehicular emissions [119] and particle resuspension. Additionally, low microbial activity at 40ºF could have slowed the biodegradation and transformation of 6PPDQ, leading to its persistence in the environment [79].

By May 2024, concentrations increased across most sampling locations, with a notable rise in downstream sites. The highest concentrations were recorded at Grays Ferry Bridge (4.83 ng/L), Chestnut Bridge (4.48 ng/L), and Strawberry Mansion (4.08 ng/L).

Locations previously that were not detected in February, such as Norristown Dam Bridge (0.84 ng/L), showed measurable concentrations, indicating a seasonal increase. However, certain sites, including Black Rock Bridge (0.45 ng/L) and Mont Clare Bridge (0.27 ng/L), exhibited slightly lower values compared to February. The rise in temperature to 65ºF likely enhanced microbial degradation and photo-transformation [79], though increased traffic-related runoff and stormwater discharge may have counteracted this effect [119]. Previous research reported that microbial activities play a significant role in the transformation of 6PPDQ, with seasonal variations in bacterial populations potentially affecting its breakdown [122].

In August 2024, a slight decline in 6PPDQ levels was observed at some locations, while others remained elevated. The Manayunk Canal, which recorded 3.79 ng/L in May, decreased to 0.55 ng/L in August, potentially due to variations in water flow and elevated photodegradation at 80ºF, which can increase the breakdown of 6PPDQ in surface waters [81]. However, locations such as Grays Ferry Bridge (4.05 ng/L), Chestnut Bridge (2.44 ng/L), and Norristown Dam Bridge (1.20 ng/L) exhibited higher concentrations compared to earlier months. The persistence of 6PPDQ may be attributed to resuspension from sediments, as previous studies indicate that tire-related products can accumulate in sediments and can be reintroduced into water bodies [5], [65]. The most substantial rise in 6PPDQ concentrations occurred in October 2024, with all sample sites reporting elevated levels compared to previous periods. The highest concentration was detected at Perkiomen Bridge (17.95 ng/L), followed by Manayunk Canal (14.1 ng/L), Falls Bridge (10.33 ng/L), and Grays Ferry Bridge (5.94 ng/L).

The widespread increase suggests a possibility of cumulative accumulation over time, possibly due to sustained contaminant input and reduced dilution capacity. Variations in river flow may partially influence the observed temporal trends in 6PPDQ concentrations. Streamflow data from United States Geological Survey (USGS) gaging stations near Norristown, Conshohocken, and Chestnut Bridge indicate a substantial decline in Schuylkill River discharge from approximately 2400 ft^3/s in February to 510 ft^3/s in October 2024 [123]. This decline in flow may reduce the river's dilution capacity, contributing to an increase in 6PPDQ concentrations. The concurrent decline in discharge and rise in contaminant levels suggest potential accumulation effects, particularly in downstream locations. A recent study [124] on 6PPDQ also reported higher 6PPDQ concentrations in downstream locations and elevated contamination levels after rain events, consistent with the findings in this study.

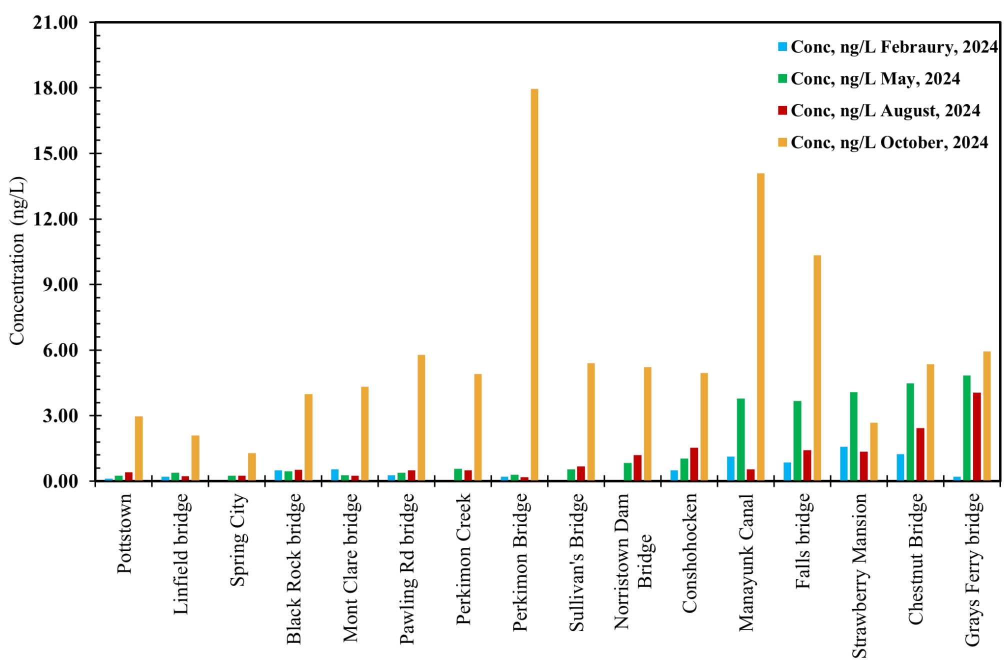


Figure 8: Concentration of 6PPDQ, ng/L vs. sampling sites in Schuylkill River

Other factors may have contributed to the resulting peak in October, like the drop in temperature (60ºF), which could have slowed microbial degradation, prolonging 6PPDQ persistence in water [11]. Additionally, pH values in May (7.5) and October (8.1), were slightly lower than in February (8.3) and August (8.0) which may have influenced 6PPDQ stability. Previous studies indicated that pH impacts the transformation of 6PPDQ, with alkaline conditions promoting degradation [79], [81]. Furthermore, dissolved organic matter (DOM) and metal ions, which likely fluctuate seasonally, influence the oxidation and transformation of 6PPDQ [34], [125]. The combined effects of stormwater retention, microbial shifts, pH values, temperature fluctuations, and seasonal hydrological changes provide a more comprehensive understanding of the October peak in 6PPDQ concentrations. These findings highlight the complex interplay of environmental factors, such as precipitation, temperature, microbial activity, and river flow, in understanding the seasonal dynamics of 6PPDQ contamination in the Schuylkill River.

3.4.2 Distribution of 6PPDQ in the Delaware River Basin

6PPDQ was detected across all sampling events at varying concentrations and spatial scales, demonstrating clear spatiotemporal heterogeneity across the Delaware River Basin. The study covered 23 monitoring locations including upper and middle basin tributaries, mainstem river points figure 3.7, and boat-accessed downstream estuarine segments figure 3.8. Concentrations ranged from below detection limits to 150 ng/L exceeding known toxicity thresholds, particularly during the summer period. In April 2024, concentrations were low overall, with 6PPDQ detected at a few headwater and midstream sites such as East Branch Delaware River (1.84 ng/L), Sixpenny Creek (1.05 ng/L), and Dunnfield Creek (1.51 ng/L). Several sites including Sherman Creek, Faulkner Brook, and

West Branch Delaware River exhibited non-detectable levels. The April pattern reflects the early spring hydrology with relatively lower runoff and colder temperatures, limiting mobilization and transformation of TWPs into 6PPDQ.

In July 2024, the sampling period was the most spike in 6PPDQ concentrations across the watershed. The Valley Creek tributary reached a peak of 150.99 ng/L, nearly 14 times the EPA screening value and well above the LC_{50} for sensitive species such as Coho salmon and Brook trout fry. Similarly, Valley Creek showed elevated values (44.97 ng/L), followed by Sixpenny Creek (1.58 ng/L) and Delaware River-Hancock (0.94 ng/L). This surge was likely influenced by summer heat, increased vehicular activity, and prior dry spells followed by rainfall, enhancing the wash-off of TWPs accumulated on road surfaces. Boat-collected samples during this period also showed elevated concentrations, with Navy Yard (2.82 ng/L), Benjamin Franklin Bridge (2.45 ng/L), and Florence Bend (1.82 ng/L) among the highest. The strong downstream signal suggests that 6PPDQ is transported into tidal and estuarine zones during high-activity months.

During the June 2024 rain event, four tributaries were sampled, confirming the role of storm-driven mobilization. Little Lehigh Creek reached 8.40 ng/L, followed by Bushkill Creek (3.71 ng/L) and Valley Creek (1.09 ng/L). These values further support the premise that rainfall following dry accumulation periods can cause episodic pollution pulses, disproportionately impacting sensitive aquatic ecosystems. November 2024 showed consistently high concentrations in many tributaries and mainstem locations, particularly at Delaware River–Lordville (6.91 ng/L), Bushkill Creek (6.25 ng/L), and Little Lehigh Creek (4.83 ng/L). This seasonal rise could be attributed to continued contaminant input during autumn storms combined with reduced dilution capacity due to lower streamflow.

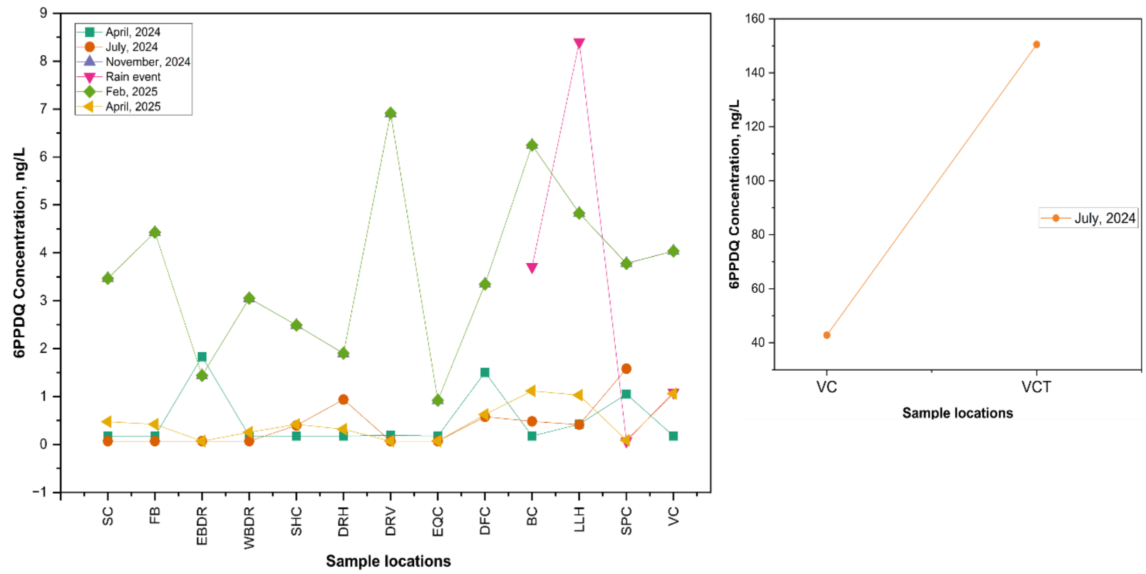


Figure 9: Concentration of 6PPDQ, ng/L vs. sampling locations in Delaware River Basin

Meanwhile, Paulsboro (1.77 ng/L) and Betsy Ross Bridge (1.98 ng/L) from the boat samples confirmed 6PPDQ presence even in more industrialized, downstream reaches. February 2025 recorded the lowest overall concentrations, with many locations showing non-detectable levels, consistent with lower runoff volumes, and reduced precipitation. However, low but measurable levels were still found in some midstream tributaries, including Hancock (0.47 ng/L), Bushkill Creek (0.50 ng/L), and Equinunk Creek (0.52 ng/L). In April 2025, concentrations rebounded moderately as spring runoff began. Elevated levels were observed at Bushkill Creek (1.12 ng/L), Little Lehigh Creek (1.03 ng/L), and Valley Creek (1.06 ng/L), suggesting resumed transport of tire-related contaminants into aquatic systems during seasonal transition.

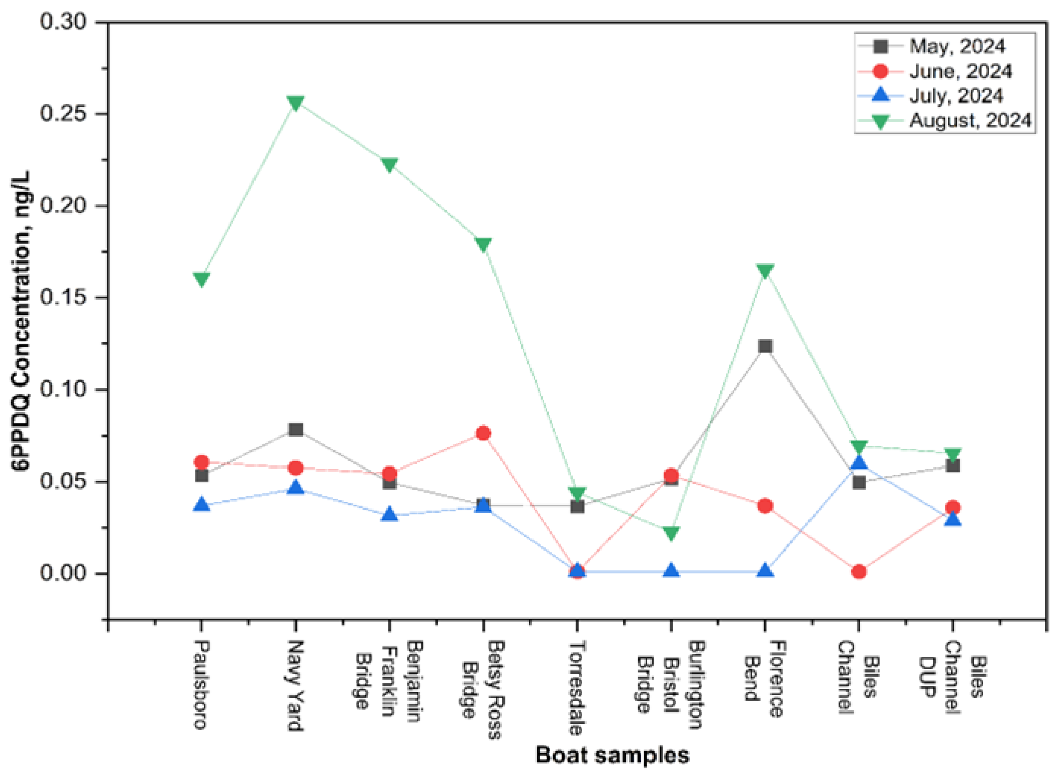


Figure 10: 6PPDQ Concentration, ng/L vs. Boat sampling locations in Delaware River Basin

Headwater locations like Sherman Creek (0.48 ng/L) and Faulkner Brook (0.42 ng/L) also showed detectable levels for the first time since prior spring. These increases may reflect a combination of thaw-induced runoff, road salt interactions, and increased vehicle abrasion in early spring. Concurrently, boat sample sites maintained measurable contamination: Florence Bend (1.20 ng/L) and Navy Yard (1.21 ng/L) continued to rank among the highest, with most estuarine locations falling between 0.4-1.2 ng/L.

Collectively, the results reveal a clear pattern of seasonal variation, with the highest concentrations in summer and post-storm periods, and lower levels during winter. Spatially, urbanized tributaries and mid-basin regions like Valley Creek, Bushkill Creek, and Little Lehigh Creek demonstrated consistent contamination, while lower estuarine sites showed persistent background levels, possibly due to cumulative inflow and delayed dispersion.

The exceptional values at Valley Creek tributary and Valley Creek in July 2024 suggest extreme episodic risk events that demand closer investigation and potentially regulatory attention. These findings emphasize the need for continuous monitoring across seasons and spatial zones.

3.4.3 Comparative analysis of 6PPDQ distribution across both rivers

The comparative analysis of 6PPDQ concentrations across the Schuylkill River and Delaware River Basin reveals both common patterns and distinct behaviors shaped by their unique hydrological, geographical, and urbanization characteristics. Although both river systems are impacted by urban runoff and host significant anthropogenic activity, their spatiotemporal profiles of 6PPDQ contamination differ in magnitude, variability, and environmental response to seasonal and meteorological events.

Temporal Patterns and Seasonal Peaks: Both systems exhibited seasonal variation, with higher 6PPDQ concentrations generally occurring during warmer months (May-October) and following precipitation events. In the Schuylkill River, the highest concentrations were recorded in October 2024, with peak values exceeding 17 ng/L at downstream locations such as Perkiomen Bridge. This seasonal increase is consistent with prior studies suggesting enhanced mobilization of TWPs during autumn due to leaf litter interaction, declining flow rates, and reduced dilution capacity. The Delaware River Basin followed a similar trend, but its July 2024 concentrations were significantly higher in some sites particularly the Valley Creek tributary, where concentrations peaked at 150.99 ng/L, over an order of magnitude greater than values observed in the Schuylkill. This disparity may be attributed to localized land use and hydrological differences. Valley Creek, for instance, is situated in a highly urbanized area with dense impervious surfaces and receives direct roadway runoff with limited buffering capacity, making it more susceptible to acute contaminant. The presence of Class A trout habitats in this region amplifies ecological concerns, as observed concentrations far exceed known LC_{50} values for salmonid species.

Spatial Hotspots and Urban Influence: Spatially, both systems demonstrated consistent patterns of elevated 6PPDQ near urban centers and high-traffic areas. In the Schuylkill River, sites such as Grays Ferry Bridge, Chestnut Bridge, and Strawberry Mansion located in heavily developed areas of Philadelphia consistently recorded higher concentrations than upstream, less urbanized locations like Pottstown and Spring City. Similarly, in the Delaware Basin, urban tributaries such as Bushkill Creek and Little Lehigh Creek exhibited persistent contamination across seasons, while more rural or upstream sites like Sherman Creek and Faulkner Brook often showed non-detectable levels. Interestingly, while estuarine zones in the lower Delaware (e.g., Navy Yard, Florence Bend) recorded moderate 6PPDQ concentrations (1.2-2.8 ng/L), they did not experience the sharp peaks seen in mid-basin tributaries. This suggests dilution, dispersion, and possibly sedimentation processes play a more dominant role in downstream attenuation. In contrast, the Schuylkill River being a smaller system exhibited more cohesive downstream loading, particularly in late fall when discharge was lowest.

Response to Rain Events and Flow Dynamics: Both river systems responded acutely to rain-driven events, reinforcing the role of stormwater as a primary vector for 6PPDQ transport. The June 2024 targeted rain sampling in the Delaware Basin captured significant runoff-driven spikes, particularly in Little Lehigh Creek and Bushkill Creek. Similarly, the highest observed concentration in the Schuylkill occurred during October 2024 after a significant rain event, emphasizing the importance of antecedent dry conditions and impervious surface wash-off. However, the Delaware system's response appeared more spatially heterogeneous, with localized flashpoints such as Valley Creek contributing disproportionately to the overall contaminant burden. This may reflect the presence of distinct contaminant "reservoirs" in urbanized sub-catchments and the influence of hydrological complexity in a larger watershed. By contrast, the Schuylkill River's response to rainfall was more spatially uniform, consistent with its more linear watershed morphology and relatively cohesive land-use gradients.

Hydrological Modulation and Mass Flux Considerations: Flow regimes played a differential role in the two systems. The Schuylkill River, with observed discharge declining from 2400 ft^3/s in February to 510 ft^3/s in October 2024, exhibited increasing concentrations that paralleled decreasing dilution capacity. In the Delaware Basin, lower winter/spring flows were associated with reduced concentrations, but mass flux during peak rainfall events likely remained high. The extreme spike at Valley Creek in July well above EPA's 11 ng/L screening value suggests the presence of a substantial TWP reservoir mobilized by storm conditions, a pattern consistent with "middle flush" dynamics.

Implications for Monitoring and Management: These findings reinforce that 6PPDQ behavior is highly responsive to both land-use patterns and hydrological conditions. While both rivers are vulnerable to stormwater driven contamination, the Delaware River Basin particularly in smaller tributaries like Valley Creek demonstrates a higher likelihood of episodic acute toxicity events. This suggests mitigation strategies, such as targeted green infrastructure placement in high-risk sub catchments and increased sampling during dry and then wet transitions. Moreover, the contrasting responses suggest that blanket monitoring approaches may be insufficient; real-time or event triggered sampling may be necessary in urbanized tributaries, while seasonal monitoring may be less in variable systems like the upper Schuylkill. Cross system comparisons also validate the importance of integrating flow data, land-use mapping, and ecological sensitivity in future risk assessments and regulatory frameworks for tire-derived contaminants like 6PPDQ.

3.5 Chapter summary

In this chapter we conducted comprehensive assessment of the spatiotemporal distribution of 6PPDQ in two urban water systems: the Schuylkill River and the Delaware River Basin. Using EPA Draft Method 1634, multi-seasonal monitoring events were conducted across 16 sites in the Schuylkill River and 23 sites in the Delaware River Basin, including fixed tributaries and boat-accessed locations. The results showed insights into the occurrence, behavior, and influencing factors of 6PPDQ in surface waters. Seasonal variation was evident in both rivers, with higher 6PPDQ concentrations observed in warmer months and following storm events, emphasizing the critical role of urban runoff in mobilizing tire-derived contaminants. In the Schuylkill River, concentrations increased steadily from winter through fall, with the highest values recorded after a rainfall event in October 2024. Similarly, in the Delaware Basin, peak concentrations were observed in July 2024, particularly in the Valley Creek tributary, which exceeded acute toxicity thresholds for sensitive aquatic species. Spatial patterns in both systems indicated elevated 6PPDQ levels in downstream and urbanized segments. Urban tributaries in the Delaware River Basin such as Bushkill, Little Lehigh, and Valley Creek showed persistent contamination, while estuarine zones demonstrated moderate but widespread presence. The Schuylkill River exhibited consistent downstream accumulation, especially near high-traffic zones in Philadelphia. These patterns highlight the influence of land use, impervious surfaces, and hydrological connectivity in determining contamination hotspots. The cross-system comparison underscored 6PPDQ levels depends on precipitation, and seasonal temperature shifts but also revealed unique dynamics. The Delaware Basin showed greater episodic spikes and heterogeneity in response to storm events, while the Schuylkill reflected a more uniform temporal trend driven by sustained contaminants and declining river discharge. Overall, this chapter illustrates the environmental persistence and mobility of 6PPDQ in highly urbanized watersheds, reinforcing the need for stormwater-targeted mitigation strategies, sensitive site-specific monitoring, and improved modeling of seasonal transport mechanisms. These findings lay the groundwork for the risk characterization and statistical analysis to follow in Chapter 4.

CHAPTER 4

STATISTICAL ANALYSIS OF URBAN FACTORS INFLUENCING 6PPDQ CONCENTRATIONS AND ECOLOGICAL RISK ASSESSMENT

4.1 Introduction

While chapter 3 established the widespread presence and spatiotemporal variability of 6PPDQ in the Schuylkill River and Delaware River Basin, the detection of this toxicant alone does not fully capture its ecological significance. The question remains: Are these concentrations biologically meaningful, and what urban factors are driving them? To answer this, Chapter 4 shifts the focus from occurrence to consequence assessing environmental risk and exploring anthropogenic factors that shape the spatial and seasonal patterns of 6PPDQ in two urban rivers. 6PPDQ, formed primarily through the environmental transformation of the tire antioxidant 6PPD, has been associated with acute toxicity in sensitive aquatic species such as coho salmon, brook trout, and cutthroat trout. As discussed in chapter 1, LC_{50} values for these species can be as low as ng/L, while the U.S. EPA has recommended a freshwater screening benchmark of 11 ng/L for 6PPDQ.

Chapter 3 showed that multiple sites in both river systems, particularly during post-storm periods or summer months, exceeded these ecological thresholds raising concerns about the vulnerability of trout-bearing streams and downstream aquatic communities. Moreover, the data revealed strong spatial heterogeneity in 6PPDQ concentrations. Certain tributaries within the Delaware River Basin (e.g., Valley Creek, Bushkill Creek) and downstream reaches of the Schuylkill River displayed recurrent contamination, especially after rainfall events. These patterns suggest that urban infrastructure and river characteristics such as vehicle activity, population density, and proximity to tire related industries play a critical role in determining 6PPDQ loading. Contaminants from TWPs like PAHs[120], heavy metals[65], and microplastics [75] were found in aqueous systems in higher concentrations near urban areas[119], [121] and higher traffic locations. Factors, including vehicular traffic, urbanization, and industrial operations, contribute to the release and environmental distribution of 6PPD and its transformation product 6PPDQ[2], [13], [126]. Quantifying the relationship between these factors and 6PPDQ concentrations is critical for identifying contamination hotspots and understanding the environmental implications.

This chapter aims to address these gaps by conducting statistical relationships between 6PPDQ concentration and factors such as traffic volume, population density, and proximity to tire related facilities and comprehensive risk assessment. Specifically, ecological risk is evaluated using two complementary approaches: (1) a threshold-based method, which compares observed concentrations to the EPA's freshwater screening level, and (2) a toxic unit (TU) based method, which normalizes concentrations against species-specific LC_{50} values to assess relative risk across sensitive trout species. This dual framework allows for both environmental relevance and aquatic species risk interpretation.

In parallel, urbanization factors analysis is conducted to identify statistical correlations between 6PPDQ concentrations and anthropogenic variables, including average annual daily traffic (AADT), population density, and proximity to tire-related facilities. By applying regression and correlation analyses, this chapter seeks to understand patterns that explain why certain locations consistently exhibit elevated 6PPDQ levels, while others do not with similar hydrological conditions. In sum, Chapter 4 is guided by two interconnected aims: 1) To evaluate where and when 6PPDQ concentrations exceed ecological thresholds, and how these exceedances relate to known species sensitivities and seasonal flow patterns. 2) To identify which urbanization indicators most strongly correlate with 6PPDQ contamination, and how these relationships differ between the Schuylkill and Delaware River systems. Together, these efforts provide a mechanistic understanding of contamination dynamics, inform spatial risk prioritization, and build a foundation for Chapter 5, where laboratory experiments further explore the leaching behavior of 6PPDQ from tire particles under controlled environmental conditions.

4.2 Methodology overview

This section outlines the analytical approaches used to assess ecological risks associated with 6PPDQ contamination and to investigate the statistical relationships between urban infrastructure and 6PPDQ concentrations in both the Schuylkill River and Delaware River Basin. The methodology comprises two major components: (1) a two-tiered environmental risk assessment framework integrating regulatory benchmarks and species-specific toxicity, and (2) statistical analyses evaluating the influence of traffic volume, population density, and industrial proximity on 6PPDQ distribution.

4.2.1 Statistical analysis evaluating the influence of traffic volume, population density, and tire industrial proximity on 6PPDQ distribution

4.2.1.1 Schuylkill River. Through this study we evaluated the relationship between Annual Average Daily Traffic (AADT) 2023 and 6PPDQ concentrations in the Schuylkill River across four sampling periods: February, May, August, and October 2024. We collected the AADT, 2023 data from Penn DOT[127]. A combination of regression model comparisons, multiple regression analysis, interaction modeling, and correlation analysis was conducted to determine the extent to which traffic volume influences 6PPDQ concentrations and seasonal variations can contribute significantly to contaminant levels.

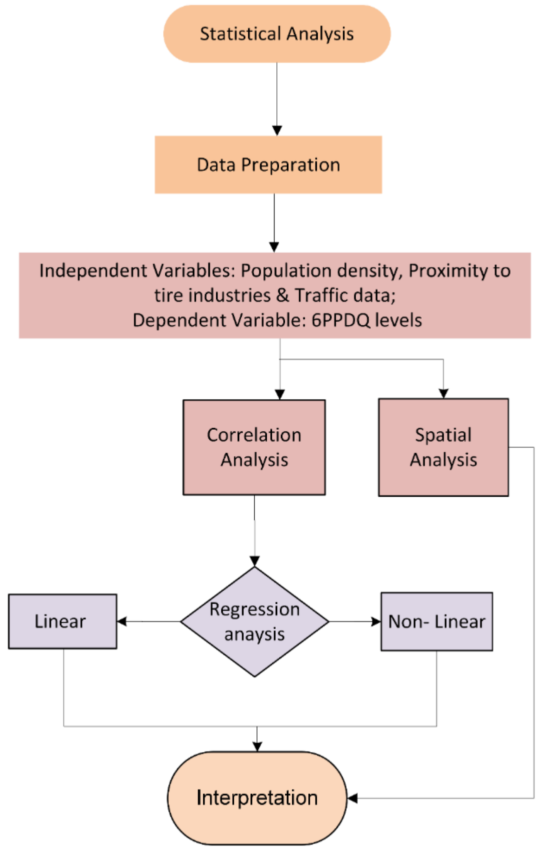


Figure 11: Methodology for statistical analysis

4.2.1.2 Delaware River Basin Assessment. To evaluate how traffic volume influenced 6PPDQ concentrations in the Delaware River, we conducted correlation and regression analyses across six sampling events from April 2024 to April 2025. The total Average Annual Daily Traffic (AADT) was derived from PennDOT (2024), NYSDOT, and NJDOT datasets. All computations were conducted using Python (v3.12) with relevant statistical libraries. Non-detect (ND) values below the method detection limit were substituted using LOD/ √2. Sampling events with missing data were excluded from correlation and regression analyses.

4.2.2 Ecological risk assessment framework and approach

To evaluate the ecological significance of 6PPDQ concentrations observed across both watersheds, we employed a dual risk assessment approach. This included both a screening values comparison and a species-specific toxic unit (TU) analysis. Together, these tools offer comprehensive understanding of high-risk zones and biological vulnerability to interpret 6PPDQ risk profiles over time.

4.2.2.1 Tier I: Threshold-based risk assessment. We applied the Risk Quotient (RQ) approach [128] to evaluate the potential environmental risk associated with 6PPDQ concentrations in freshwater systems. This approach compares the measured environmental concentration (MEC) of a contaminant to a regulatory benchmark, the predicted no effect concentration (PNEC). For 6PPDQ, the U.S. Environmental Protection Agency (EPA) recently established a freshwater screening threshold of 11 ng/L. The RQ was calculated as:

$$Risk\ Quotient\ (RQ) = \frac{MEC}{PNEC} = \frac{MEC}{11\ ng/L} \tag{4.1}$$

Based on the RQ values, risk levels were categorized as follows: very low risk (RQ < 0.01), low risk (0.01 < RQ < 0.1), medium risk (0.1 < RQ < 1), and high risk (RQ > 1). This regulatory aligned framework provides a conservative screening level assessment that accounts for a range of aquatic species and ecological scenarios. While RQ does not reflect species-specific toxicity or site-specific sensitivity, it is valuable for identifying potential contamination hotspots and prioritizing monitoring efforts.

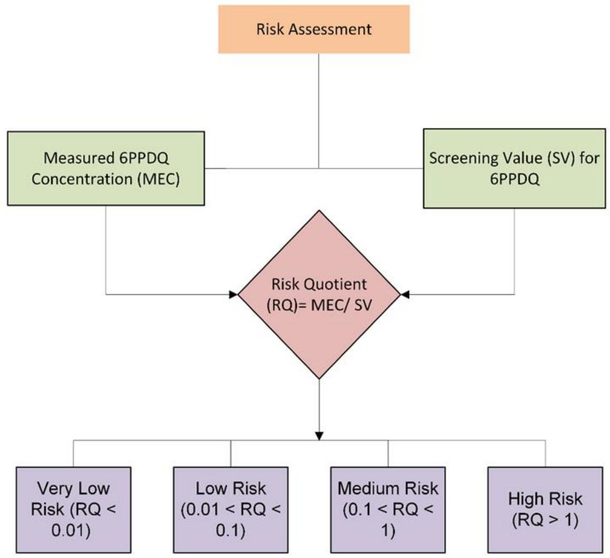


Figure 12: Methodology for environmental risk assessment

4.2.2.2 Tier II: Species-specific toxic unit (TU) analysis. To complement the RQ based environmental assessment, we conducted a Toxic Unit (TU) analysis focused on the acute toxicity of 6PPDQ to salmonid species that inhabit or migrate through the Delaware River. This includes coho salmon (Oncorhynchus kisutch), brook trout (Salvelinus fontinalis), rainbow trout (Oncorhynchus mykiss), brown trout (Salmo trutta), and white-spotted char (Salvelinus leucomaenis).

$$Toxic\ Unit\ (TU) = \frac{MEC}{\mathrm{LC50}} \tag{4.2}$$

Where, MEC = measured 6PPDQ concentration at a given site (ng/L), LC_{50}= the species-specific 50% lethal concentration (ng/L) derived from literature (see Table S5). TU thresholds were interpreted as: High Risk: TU ≥ 1 (acute lethal concentrations), Moderate Risk: 0.1 ≤ TU < 1, Low Risk: TU < 0.1. This method provides high-resolution insight into the likelihood of acute toxicity to individual species. Unlike the RQ approach, the TU method does not apply uncertainty factors, making it more precise but also more sensitive to interspecies differences and life stage variability.

4.3 Results and Discussion

4.3.1 Statistical analysis: Traffic volume to 6PPDQ concentrations

4.3.1.1 Schuylkill River Assessment. Regression model comparisons: We evaluated three regression models, including linear, quadratic, and logarithmic, to determine the most appropriate model for describing the relationship between AADT and 6PPDQ concentrations. The models were compared based on R^2 values and Akaike Information Criterion (AIC) scores, which assess the best fit while accounting for model complexity. Table summarizes the results. The quadratic model exhibited the highest R^2 value (0.502) and the lowest AIC score (76.32), indicating a nonlinear relationship between AADT and 6PPDQ concentrations. However, further examination revealed that the quadratic term introduced multicollinearity between AADT and its squared term variance inflation factor (VIF > 10), which resulted in non-significant coefficients for the quadratic term ($p = 0.764$). This indicated that higher order terms did not provide additional predictive value beyond a linear relationship. Given these findings, the linear model was ultimately selected as the most interpretable and statistically reliable model, yielding an R^2 value of 0.469 ($p = 0.001$), confirming a positive association between AADT and 6PPDQ concentrations [119].

Table 4: Regression model comparison

Model	R^2 value	AIC score
Linear	0.469	78.61
Quadratic	0.502	76.32
Logarithmic	0.489	77.45

Multiple regression and interaction model analysis: To assess the combined effect of AADT and seasonal variations on 6PPDQ concentrations, multiple regression analysis was performed using both linear and quadratic approaches. We expressed the models, Equations 4.3 and 4.4 , as follows:

Linear model:

$$Concentration, \frac{ng}{L} = \beta 0 + \beta 1\ (AADT) + \beta 2\ (Month) \tag{4.3}$$

Quadratic model:

$$Concentration, \frac{ng}{L} = \beta 0 + \beta 1\ (AADT) + \beta 2\ (AADT^2) + \beta 3\ (Month) \tag{4.4}$$

The quadratic model failed to improve predictive power, as the $AADT^2$ term was non-significant ($p = 0.492$), and the R^2 value remained nearly identical at 0.420, reinforcing the decision to use the linear model. The multiple regression model, incorporating AADT and seasonal variation as independent variables, supported that traffic volume can contribute to 6PPDQ contamination, but seasonal factors also influence contaminant distribution ($p < 0.001$).

Correlation analysis: Pearson correlation analysis revealed seasonal variations in the relationship between AADT and 6PPDQ concentrations. In February, the correlation was weak ($r = 0.3288$, $p < 0.05$), likely due to minimal runoff, less vehicular activity, and dilution from a higher discharge rate. By May, the correlation increased ($r = 0.5229$, $p < 0.05$) as precipitation enhanced pollutant mobilization into the river 85. The strongest correlation was observed in August ($r = 0.5872$, $p < 0.05$), reflecting the combined impact of peak vehicular activity and frequent rainfall. In October, despite the highest 6PPDQ concentrations, the correlation weakened ($r = 0.3375$, $p = 0.259$), suggesting dilution capacity or other factors may have had a greater impact than direct traffic volume.

The relationship between AADT and 6PPDQ concentrations across all sampling months is shown in figure 4.3. The trend lines show a consistent positive association across all months, with October (represented by red triangles) showing the highest contamination levels. The seasonal variations in correlation highlight complex pollutant transport dynamics beyond direct runoff effects. In dry months, TWPs accumulate on road surfaces, with precipitation in October mobilizing these particles into the river [58], [78]. These findings align with previous research demonstrating that stormwater retention structures delay pollutant transport, affecting seasonal variability in contaminant distribution 86. Additional hydrological & urban influences and sediment resuspension during storm events may reintroduce previously deposited 6PPDQ, further decoupling its concentrations from real time AADT values [5], [108].

While statistical analysis supports AADT as a significant contributor to 6PPDQ pollution, several limitations must be considered. AADT data reflects annual averages and does not capture short-term traffic fluctuations, seasonal congestion, or specific road maintenance events that could influence emissions. Unmeasured confounding factors, including industrial emissions, stormwater discharge variability, and construction activity, may also contribute to observed trends but were not directly accounted for in the statistical models 88,89. Moreover, the transformation and degradation of 6PPDQ in aquatic systems remain understudied. Overall, the findings demonstrate that AADT significantly impacts 6PPDQ contamination, but seasonal variations strongly modulate its impact. While higher traffic volumes correspond to increased contamination, stormwater retention, sediment resuspension, and dilution effects may also contribute to variations across seasons. Future research integrating real-time traffic data, stormwater hydrology, and transformation product analysis would provide a more comprehensive understanding of 6PPDQ transport mechanisms in urban waterways.

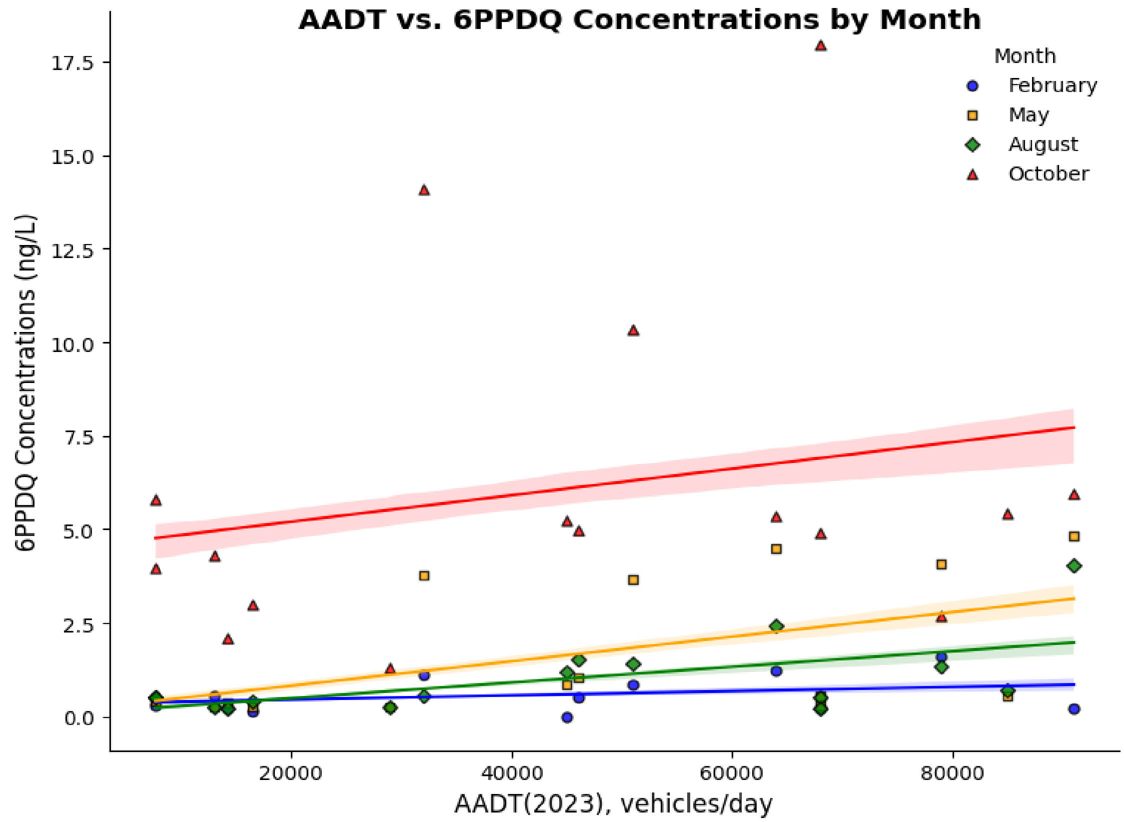


Figure 13: AADT (vehicle/day) vs. 6PPDQ concentrations (ng/L) per month in the Schuylkill River

4.3.1.2 Delaware River Basin Assessment. Correlation Analysis: Both Spearman and Pearson correlations were used to explore the relationship between AADT and 6PPDQ concentrations. Spearman's rank correlation captured monotonic associations without assuming linearity and proved more informative than Pearson in most cases. Spearman correlation coefficients were notably higher in July 2024 (rho (ρ) = 0.67, p = 0.0089) and April 2025 (ρ = 0.567, p = 0.0433), indicating a strong and statistically significant monotonic relationship during these periods. In contrast, Pearson correlation for July 2024 was weaker (r = 0.343, p = 0.2294), suggesting that while 6PPDQ concentrations increased with traffic, the trend was non-linear. Only in April 2025 did both Spearman and Pearson show strong, significant associations ($\rho = 0.567$; $r = 0.827$; $p < 0.001$), pointing to a period of robust linear and monotonic alignment. These results are presented in Figure 5, which compares Spearman and Pearson coefficients across all sampling events. This finding contrasts with the Schuylkill River study, where Pearson correlation alone was used and showed the strongest correlation during August 2024 ($r = 0.5872$, $p < 0.05$), corresponding to increased traffic and stormwater activity. However, the Schuylkill analysis may have underrepresented non-linear trends by not incorporating Spearman's method. The Delaware River study demonstrates that non-linear monotonic relationships are common, particularly under varying hydrologic conditions.

Regression Analysis: To further assess the predictive influence of AADT, we applied simple linear regression across all sampling periods. Consistent with the correlation results, linear models performed best during April 2025 (R^2=0.684, $p < 0.001$) and the June 2024 rain event ($R^{2=}$0.822, p= 094). In other periods, linear relationships were weak or non-significant, with R^2 values ranging from 0.007 (April 2024) to 0.128 (November 2024). However, when quadratic and logarithmic regression models were assessed, quadratic models consistently outperformed linear and logarithmic fits (Figure 4. 6). For example, in July 2024, the quadratic model explained 29.1% of the variance (R^2=0.291) compared to just 11.8% by the linear model. In the storm-driven June 2024 event, the quadratic fit increased to R^2=0.889 one of the highest across all models and periods.

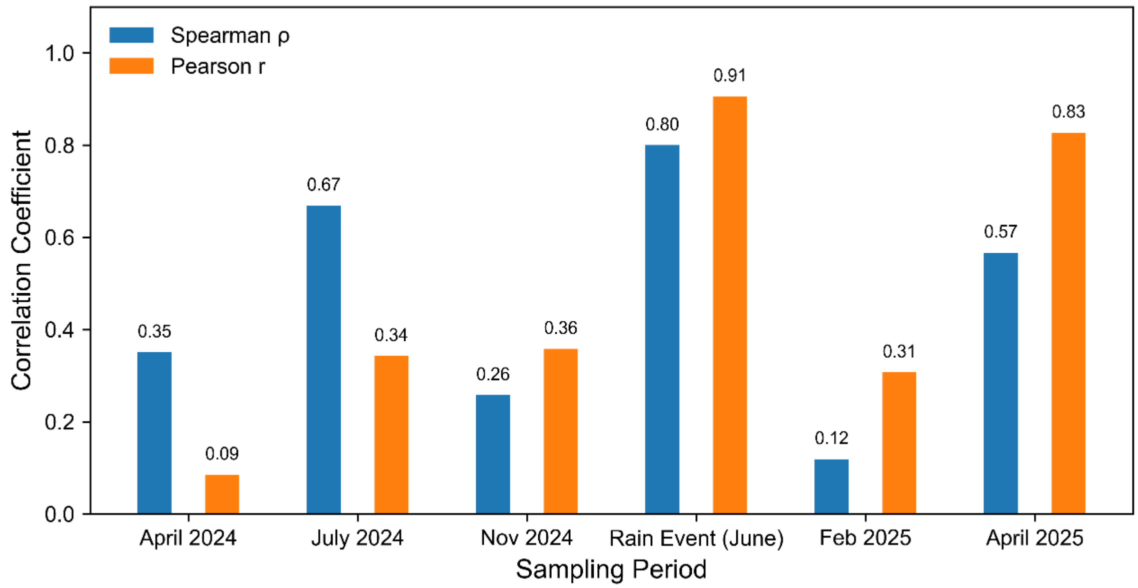


Figure 14: Comparison of correlation coefficient vs sampling period

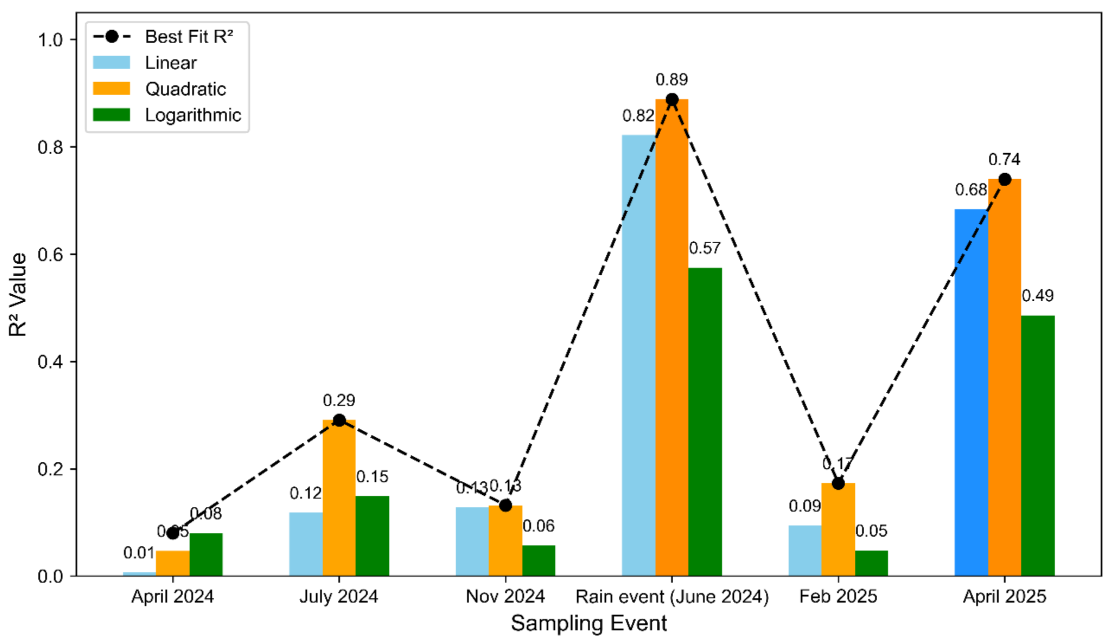


Figure 15: Regression models (linear, quadratic, & logarithmic) vs sampling event

These improvements suggest that traffic-intensity effects on 6PPDQ are not strictly linear. Instead, they likely reflect non-linear environmental responses such as contaminant accumulation. followed by threshold wash-off during runoff events. These dynamics align with observed urban runoff patterns are better captured by parabolic models that account for rate of change variability. In contrast, the Schuylkill River study found that while quadratic models yielded slightly higher R^2 values, they were ultimately rejected due to multicollinearity (VIF > 10) and non-significant coefficients [105]. The linear model was retained there for statistical parsimony. In the Delaware River study, however, quadratic models provided meaningful, interpretable fits without multicollinearity concerns, as only a single predictor (AADT) was used. This highlights a methodological evolution from the Schuylkill to Delaware study i.e., statistical reliability in Schuylkill was prioritized over model performance, whereas Delaware leverages environmental reasoning to support more flexible modeling. Furthermore, logarithmic models underperformed in nearly all cases, with the lowest R^2 values observed during most sampling events. This suggests that 6PPDQ loading does not follow a diminishing-return pattern with traffic volume, but rather one of cumulative buildup and abrupt mobilization, further justifying the use of quadratic models.

Interpretation**:** Overall, the statistical analysis reveals that traffic volume is a significant driver of 6PPDQ concentration in certain hydrological contexts, particularly during high-flow periods or storm events. However, the strength and nature of the relationship vary seasonally and are often non-linear. Quadratic regression best represents these dynamics, especially in April 2025 and June 2024, where AADT explained a substantial proportion of the variance in 6PPDQ concentrations and regression slopes were statistically significant. Incorporating both Spearman and non-linear regression models offered a more nuanced understanding of traffic contaminant relationships than traditional linear only approaches. This aligns more closely with the physical processes governing urban runoff and contaminant transport in real world systems.

4.3.2 Impact of population density on 6PPDQ concentration

The relationship between population density and 6PPDQ contamination in the Schuylkill River and Delaware River Basin was examined the role of urbanization in influencing 6PPDQ distribution. Population density data were obtained from the U.S. Census Track 2022 dataset (2019-2023), and spatial overlays with sampling locations.

4.3.2.1 Schuylkill River. The spatial distribution of population density and sampling locations (Figure 4.6) indicates that downstream locations with higher population densities reported elevated 6PPDQ concentrations across all the sampling periods. Strawberry Mansion, Chestnut Bridge, and Grays Ferry Bridge had the highest population densities (24,320-41,875 people per square mile) and exhibited elevated concentrations, ranging from 1.35 ng/L to 4.83 ng/L in May and August, increasing further to 5.35-5.94 ng/L in October. In contrast, upstream locations such as Pottstown, Linfield Bridge, and Perkiomen Bridge, with lower population density (470-3,500 people per square mile), consistently exhibited lower concentrations in all sampling periods, ranging from 0.13 ng/L to 0.42 ng/L in earlier months. However, in October, these sites showed an increase, with Pottstown reaching 2.98 ng/L and Linfield Bridge 2.09 ng/L, suggesting that seasonal changes may have influenced contaminant levels.

To quantify this relationship, Pearson correlation analysis was performed, resulting in a moderate to strong positive correlation ($r=0.645$, $p=0.007$) between population density and 6PPDQ concentrations. This statistically significant correlation suggests that stormwater runoff, vehicular emissions, and the volume of roadways & vehicle usage in densely populated areas can contribute to elevated contamination. These findings align with Hiki [91] and Seiwert [92], that 6PPDQ accumulates in urban roads & road dust. Additionally, Zeng et al [5] research identified that environmental factors, including temperature fluctuations, microbial degradation, and sediment transport, influence 6PPDQ persistence in urban rivers.

Figure 16: Spatial distribution of population density vs. sampling sites in the Schuylkill River

Moreover, population density correlates with traffic volume, which further influences 6PPDQ concentrations [75]. As discussed in Section 4.3, traffic-related emissions and road runoff may play a major role in 6PPDQ distribution. Moderate to strong correlation, indicating that stormwater retention, reduced river dilution, and contaminant resuspension from sediments play a role in contamination distribution93. Although population density does not directly generate 6PPDQ, high-density urban areas typically have more roadways, vehicular traffic, and temperature changes [92], all of which enhance

TWP generation and contaminant fate. Increased stormwater runoff in urbanized areas accelerates contaminant mobilization into nearby water bodies [48]. In contrast, lower-density upstream locations experience less road runoff but may still accumulate contaminants due to seasonal hydrological variations. The moderate to strong correlation between population density and 6PPDQ levels highlights the importance of implementing stormwater management strategies, particularly near urban areas.

4.3.2.2 Delaware River Basin. The spatial distribution highlights that downstream and urban locations consistently exhibit elevated 6PPDQ concentrations relative to rural and upstream sites (Figure 4.7). Sites located in densely populated regions such as Valley Creek, Little Lehigh Creek, Sixpenny Creek, Bushkill Creek, and Paulsboro, Florence Bend, and Navy Yard reported higher contaminant loads across multiple sampling periods. Notably, Valley Creek and its tributary, situated near heavily urbanized areas (>6,475 people per square mile), showed concentrations exceeding 44.97 ng/L and 150.99 ng/L, respectively, during summer 2024 storm events. Similarly, Little Lehigh Creek, which flows through suburban Allentown, exhibited 6PPDQ concentrations up to 8.40 ng/L during the rain event, and a consistent presence (>4 ng/L) during fall and winter. Paulsboro, Florence Bend, and Navy Yard located in the southern, urbanized stretch of the river also found concentrations ranging from 0.86 ng/L to 2.82 ng/L, with peak observed during summer.

In contrast, upper basin sites such as Sherman Creek, Faulkner Brook, and East Branch Delaware River, which are situated in low-density rural areas (<1,303 people/sq. mile), showed non-detectable or minimal 6PPDQ concentrations in most sampling periods. Although Faulkner Brook recorded a small spike (4.43 ng/L) in November 2024, overall, these headwater streams exhibited far lower 6PPDQ contamination compared to their urban counterparts. A preliminary statistical analysis using Pearson correlation showed a moderate positive relationship ($r = 0.59$, $p < 0.05$) between population density and 6PPDQ concentration across all sampling periods. This correlation supports the hypothesis that population density through its association with land use, impervious surfaces, and traffic volume plays a significant role in the spatial variability of 6PPDQ contamination. Urban areas with dense road networks and high impervious cover tend to generate greater volumes of stormwater runoff, which mobilize tire-derived pollutants into receiving water.

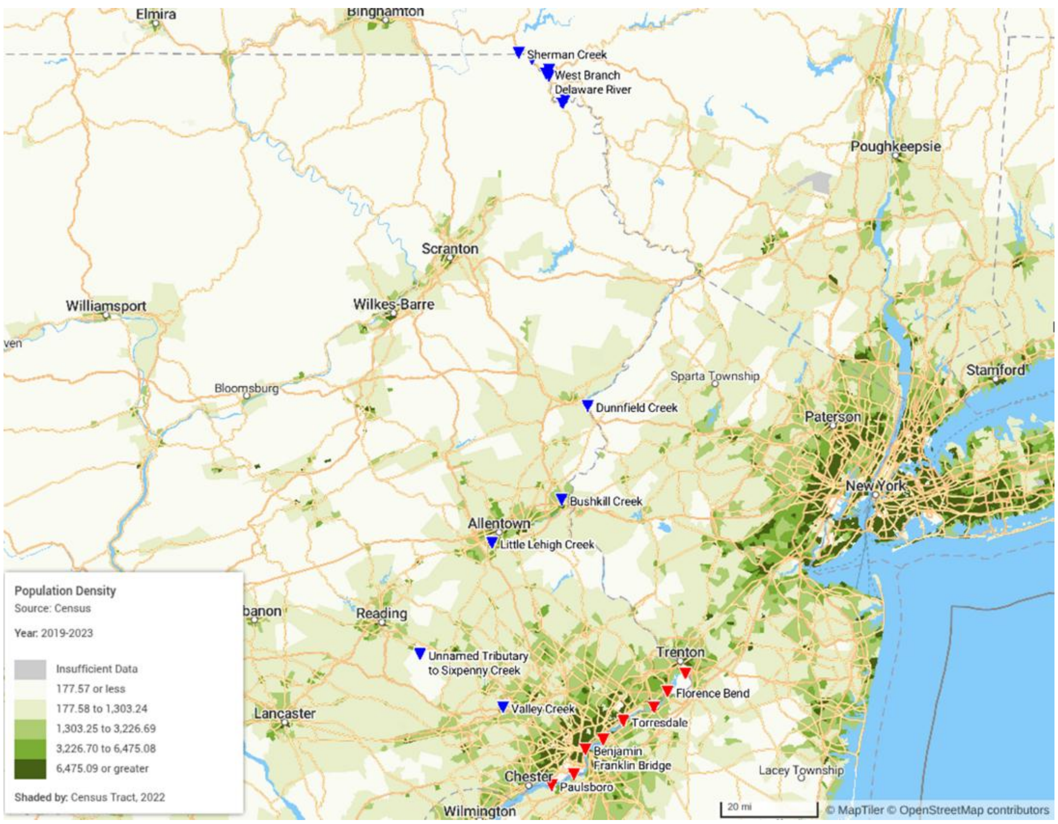


Figure 17: Spatial distribution of population density vs. sampling sites in the Delaware River Basin (Quarterly samples-Blue, Boat samples- Red)

These findings align with recent studies that observed enhanced accumulation of 6PPDQ in road dust and runoff from urbanized zones. Additionally, population density serves as a surrogate for other urbanization metrics such as traffic emissions, industrial activity, and thermal shifts all of which may influence the fate and transport of tire-related contaminants. Seasonal rainfall further amplifies the runoff potential in dense areas, explaining the heightened concentrations during summer rain events observed in sites like Valley Creek and Sixpenny Creek. The observed gradient from low 6PPDQ levels in forested upper headwaters to high concentrations in downstream urban areas illustrates the compounded impact of anthropogenic activity. These insights emphasize the importance of integrating population-based spatial analysis with contaminant monitoring to identify at-risk zones. Future watershed management strategies must prioritize stormwater control in densely populated areas to mitigate tire-derived chemical inputs into freshwater systems.

4.3.3 Influence of tire-related industry proximity to sampling sites on 6PPDQ

Tire-related industries, including tire manufacturing plants, tire shops, recycling, and distribution facilities, could be potential sources of 6PPDQ contamination in freshwater systems. These industries manage large volumes of tire-derived materials, potentially releasing contaminants via direct industrial runoff, airborne particulate deposition, and leachate from improperly stored material [16]. Previous research consistently reported the presence of 6PPDQ in road dust, atmospheric particulate matter (PM2.5), and stormwater runoff, suggesting that tire-related pollutants can be transported via both dry and wet deposition mechanisms [50,94,95].

To comparatively assess the influence of tire-related industries on 6PPDQ concentrations, spatial proximity analysis was conducted in both the Schuylkill and Delaware Rivers, measuring Euclidean distance between each sampling site and nearby tire-related facility using Google Maps business listings. The analyses revealed strong negative correlations for both rivers, though the Schuylkill River exhibited a slightly stronger correlation (Pearson's r = -0.703, p = 0.003) compared to the Delaware River (Pearson's r = -0.649, p = 0.007). These results consistently indicate that sampling sites closer to tire-related industries experienced higher 6PPDQ concentrations.

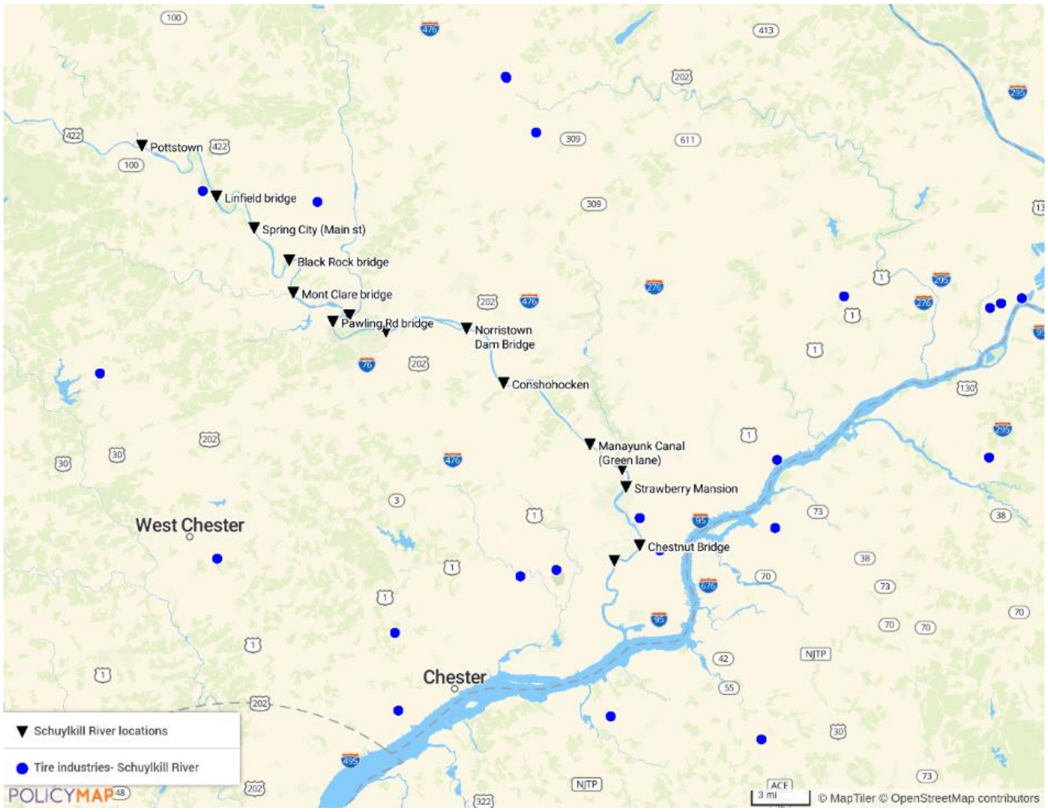


Figure 18: Spatial distribution of tire related companies vs. sampling sites in the Schuylkill River

In the Schuylkill River, sampling sites adjacent to prominent industrial zones, such as Grays Ferry Bridge, Chestnut Bridge, and Falls Bridge, showed elevated 6PPDQ concentrations (3.76-4.08 ng/L), whereas distant upstream locations, such as Pottstown (0.94 ng/L) and Linfield Bridge (0.73 ng/L), presented significantly lower levels. In the Delaware River, locations near tire-related industrial areas, notably Little Lehigh Creek (0.42-8.40 ng/L), Valley Creek (0.18-44.97 ng/L), and Bushkill Creek (0.18-6.25 ng/L), exhibited considerably higher concentrations across different sampling events. Conversely, more remote sites such as Sherman Creek (0.07-3.47 ng/L), Delaware River at Lordville (0.07-6.91 ng/L), and Equinunk Creek (0.07-0.92 ng/L) generally lower concentrations, although seasonal variability was noted.

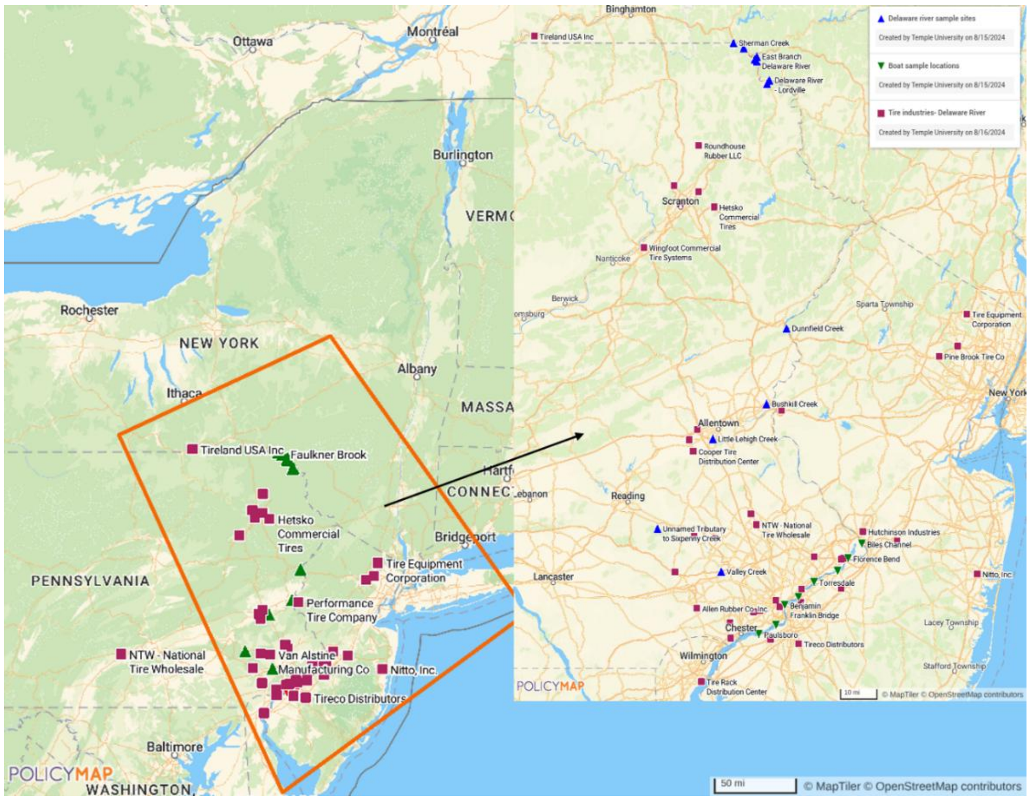


Figure 19: Spatial distribution of tire related companies vs. sampling sites in the Delaware River Basin

The similarities in spatial trends between the two river systems strongly suggest that proximity effects are driven primarily by localized stormwater runoff from impervious industrial surfaces, enhancing contaminant transport during precipitation events 96. Airborne particulate emissions from tire-related facilities, deposited onto adjacent land surfaces, further contribute to contaminant influx following rainfall.[13]. Despite similar mechanisms of contamination, subtle differences observed between the Schuylkill and

Delaware Rivers may be attributed to variations in industrial density, hydrological conditions, and urbanization patterns within their respective watersheds. Areas with lower hydrological flow or enhanced stormwater retention capacity, particularly near urbanized industrial clusters, potentially facilitate prolonged persistence of 6PPDQ in sediments. Additionally, seasonal fluctuations in precipitation may differentially modulate contaminant transport across the two river basins, affecting temporal concentration variability [124]. Overall, the consistent correlations observed in both rivers reinforce the critical localized influence of tire-related industries on freshwater 6PPDQ contamination. While urbanization, vehicular traffic, and population density undoubtedly contribute additional pollutant sources, the pronounced relationship between industry proximity and elevated 6PPDQ levels highlights industrial activities as significant and distinct contributors to water quality impairment in urban freshwater environments.

4.3.4 Ecological risk assessment

4.3.4.1 Tier I: Threshold-based risk assessment. Schuylkill River. The Risk Quotient (RQ) analysis revealed spatial and temporal variations in 6PPDQ contamination risk, with shifting risk classifications across months. As shown in Figure 6, in February, May, and August, low-risk locations ($0.01 < RQ < 0.1$) dominated, with 9 to 10 locations falling within this category. During these months, 5 to 9 locations fell into the medium risk range ($0.1 < RQ < 1$). A significant shift occurred in October, where no locations remained in the low-risk category. However, contrary to initial observations, two sites (Perkiomen Bridge and Manayunk Canal) (12%) were classified as high risk ($RQ > 1$), while the remaining locations (88%) were in the medium risk category ($0.1 < RQ < 1$). This pattern suggests that while contamination levels increased across all locations in October, the risk was not uniformly high across the study area. Figure 3.7 further supports this finding, as it highlights the concentration of elevated RQ values in Perkiomen Bridge and Manayunk Canal, while other downstream locations, including Falls Bridge, Chestnut Bridge, and Grays Ferry Bridge exhibited moderate RQ levels, indicating medium risk. The spatial trends observed suggest that downstream sites, particularly those near urban and industrial zones, consistently exhibited elevated risk levels, while upstream locations such as Pottstown and Linfield Bridge, which were predominantly low risk in earlier months, transitioned to medium risk in October.

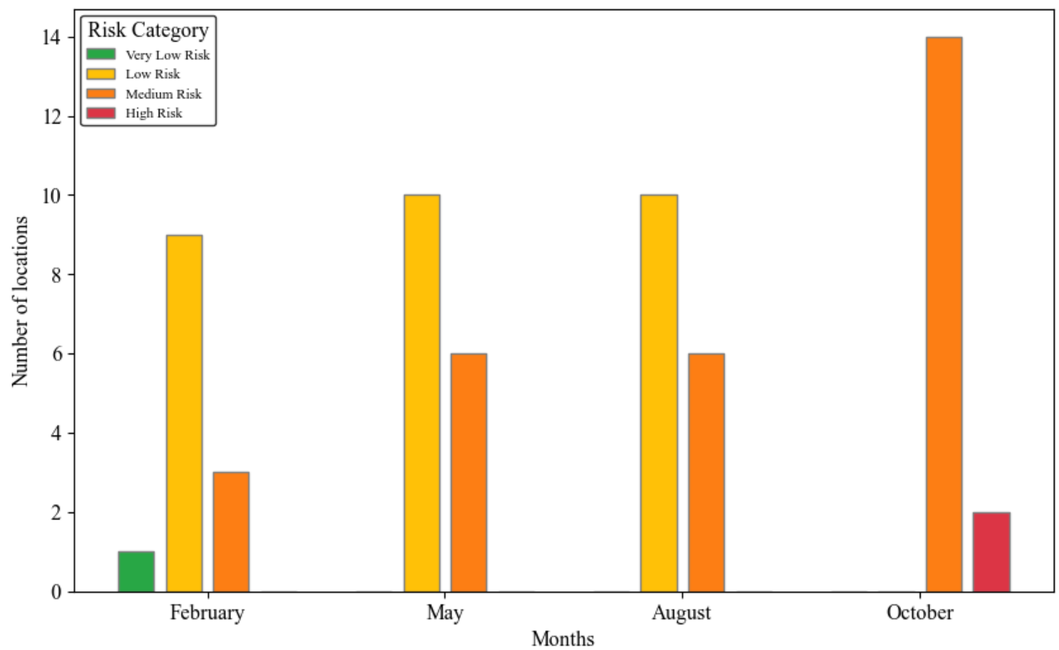


Figure 20: Risk categories across months in the Schuylkill River

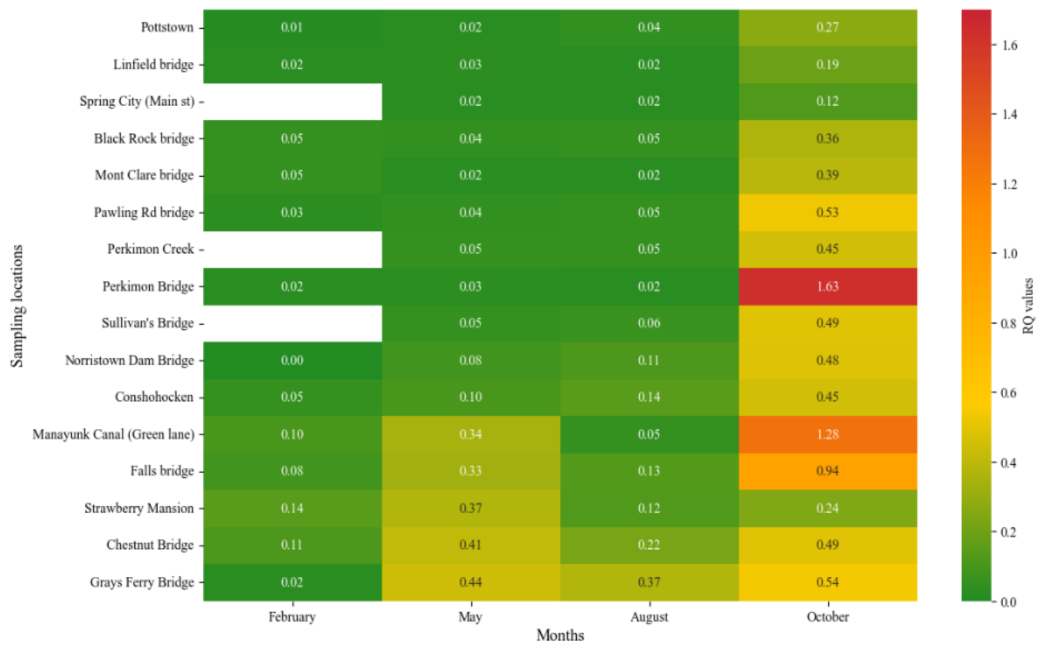


Figure 21: Risk Quotient (RQ) values by sample locations and month (heatmap)

The progression from low to medium risk across most locations suggests a seasonal accumulation effect, likely influenced by hydrological changes, reduced river flow, and prolonged contaminant retention in October. These findings emphasize the importance of seasonal hydrodynamics in influencing 6PPDQ risk trends in the Schuylkill River. While traffic volume, population density, and industrial proximity may contribute to elevated contamination levels, seasonal factors could have modulated the extent of risk, preventing all locations from reaching high-risk zones despite overall increases in 6PPDQ concentrations.

4.3.4.2 Tier I: Threshold-based risk assessment: Delaware River Basin. The Risk Quotient (RQ) analysis for the Delaware River revealed notable spatial and temporal variations in 6PPDQ contamination risk. As shown in Figures 4.10& 4.11, risk classifications shifted across sampling periods, indicating dynamic environmental conditions and pollutant loading. In April and July 2024, most sampling sites were classified as low risk (0.01 < RQ < 0.1), with only Valley Creek exhibiting notably high RQ values in July (RQ = 4.088) and its tributary (RQ = 13.726), suggesting isolated but severe contamination. The RQ values significantly increased during the November sampling, transitioning many sites from low to medium risk (0.1 < RQ < 1). Notably, Delaware River at Lordville (RQ = 0.628), Bushkill Creek (RQ = 0.568), and Faulkner Brook (RQ = 0.403) exhibited relatively high medium-risk values, emphasizing localized contamination spikes.

Boat sampling conducted in May through August further highlighted temporal variations, with most locations maintaining a low-risk status, though some increases in risk were observed in August. Specifically, Navy Yard (RQ = 0.257), Benjamin Franklin Bridge (RQ = 0.223), and Florence Bend (RQ = 0.165) demonstrated higher medium risk levels, underscoring potential urban influences and runoff impacts from adjacent areas. This dynamic pattern indicates a seasonal accumulation and hydrological influence on contaminant distribution.

Figure 22: Risk Quotient (RQ) values by Delaware river basin quarterly locations and sampling months.

Higher RQ values during certain months and specific locations likely reflect hydrological factors such as reduced river flow, increased stormwater runoff, and potential seasonal deposition of airborne particulates. The temporal and spatial variability highlights the importance of continuous monitoring and adaptive management practices, particularly in locations exhibiting periodic high-risk classifications. Overall, the environmental risk assessment underscores the influence of hydrological conditions, seasonal dynamics, and proximity to urban and industrial sources in shaping the contamination profile of the Delaware River, similar to trends observed in the Schuylkill River.

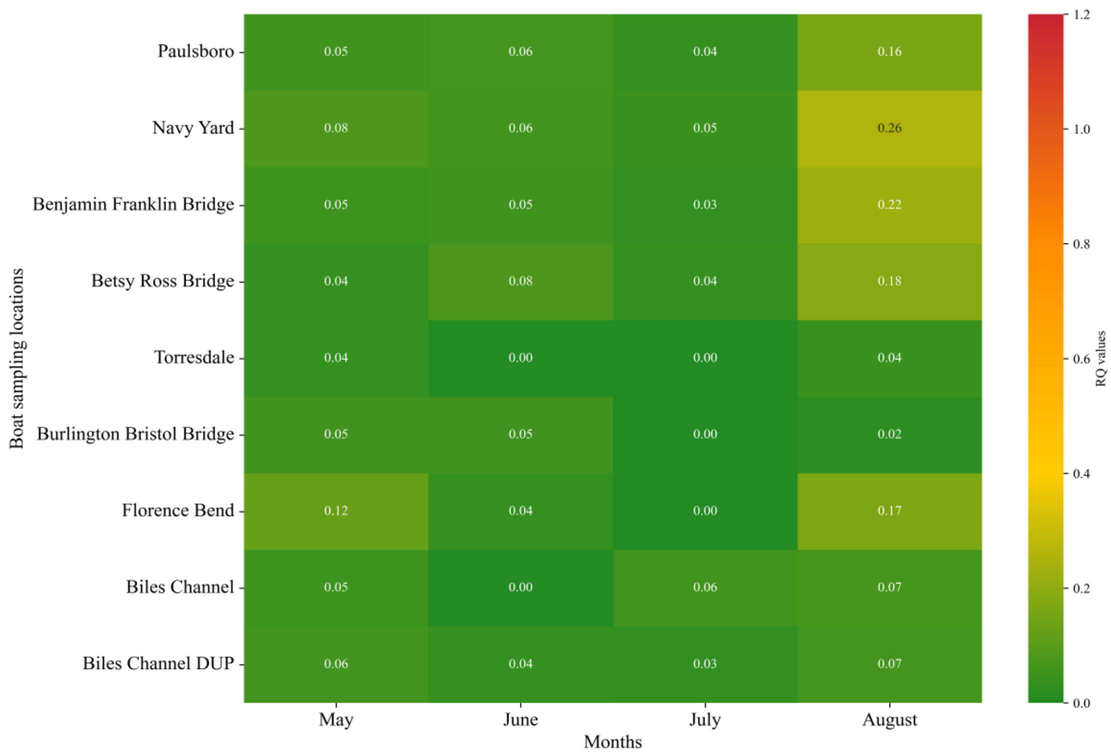


Figure 23: Risk Quotient (RQ) values by boat sample locations in Delaware river and sampling months

4.3.4.3 Tier II: Toxic Unit (TU) risk assessment: Delaware River Basin. Overall Risk Distribution (All Sites, All Species, All Seasons Combined) are the following:

- High Risk, approximately 12% of location-species combinations exhibited high risk (TU ≥ 1), primarily concentrated in Valley Creek and Valley Creek Tributary during the summer and fall (notably July and November 2024), often associated with storm-driven 6PPDQ spikes.
- Medium Risk, nearly 29% of observations fell into the medium-risk category, particularly affecting Coho salmon, Brook trout fry, and White-spotted char in creeks such as Bushkill, Little Lehigh, Dunfield, and Delaware River Lordville.
- Low Risk, around 59% of the total combinations remained below TU= 0.1, indicating minimal immediate threat. Sites like Sherman Creek, West Branch

Delaware River, and Shehawken Creek consistently showed low-risk values across all species and seasons.

These percentages reflect a composite evaluation across 14 locations, 6 species, and 6 time points, capturing both spatial and temporal variability in 6PPDQ risk dynamics. Notably, early spring and winter months (February and April 2024/25) showed predominantly low-risk profiles, while summer and post-storm periods drove elevated TU values in urban-adjacent tributaries. Full toxic unit calculations, species sensitivity thresholds, and site-by-season breakdowns are presented in Supplementary Table S8. These data provide a foundation for spatial risk mapping and temporal prioritization of monitoring and remediation in the Delaware River Basin.

4.4 Chapter conclusion

This chapter presents a comprehensive, data driven evaluation of the environmental behavior and ecological risks of 6PPDQ across two urban-impacted watersheds, the Schuylkill River and Delaware River Basin. Through integrated spatiotemporal analyses, the findings highlight how urbanization gradients, traffic intensity, population density, and industrial proximity collectively shape the environmental footprint of 6PPDQ.

Statistical analyses demonstrated a robust association between traffic volume (AADT) and 6PPDQ levels, with the Schuylkill River exhibiting a strong linear relationship ($R^2 = 0.469$, $p = 0.001$), while the Delaware River showed improved predictive strength using quadratic models, especially during storm events (R^2 up to 0.889). This indicates a shift from direct runoff-driven accumulation in the Schuylkill to more nonlinear, threshold-driven mobilization dynamics in the Delaware Basin, particularly under high-flow or precipitation-intensive periods. Population density showed a significant positive correlation with 6PPDQ concentrations in both systems (Schuylkill: $r = 0.645$, $p = 0.007$; Delaware: $r = 0.59$, $p < 0.05$), supporting the role of urban population. Sites near tire-related industries consistently exhibited elevated contaminant levels, with Pearson correlation coefficients -0.703 (Schuylkill) and -0.649 (Delaware), highlighting spatial influence of industrial hotspots.

From an ecological perspective, 12% of all species-location combinations in the Delaware River exhibited high toxic unit (TU ≥ 1) risk, primarily affecting salmonids in urban creeks during warmer, storm-prone months. An additional 29% were classified as medium risk, and 59% remained low risk, reflecting both seasonal exposure dynamics and species sensitivity. Risk Quotient (RQ) analysis echoed these findings, with increasing temporal risk from February to October, and medium to high risk increasingly concentrated at downstream and industrial-adjacent sites.

Overall, this chapter establishes that 6PPDQ contamination is a function of both chronic urban pressure and acute hydrological events. Traffic and population density act as broad predictors, while proximity to tire-related industries and storm events generate localized risk amplification. The results emphasize the need for targeted stormwater interventions and industry-specific controls, while also setting the stage for laboratory-based leaching studies to further dissect the environmental persistence and transformation of tire-derived pollutants.

CHAPTER 5

EXPERIMENTAL STUDIES ON LEACHING BEHAVIOUR, AND SURFACE CHARACTERIZATION

5.1 Introduction

While the preceding chapters established the environmental presence and ecological risks of 6PPDQ across two urban freshwaters, a critical unanswered question remains: what are the mechanisms by which this compound enter aquatic environments, and to what extent can TWPs act as ongoing sources? Chapter 5 addresses this by transitioning from field based spatiotemporal observations (Chapter 3) and risk modeling (Chapter 4) to laboratory-controlled experimentation that elucidates the leaching dynamics of 6PPD and 6PPDQ from tire derived microparticles. TWPs generated through mechanical abrasion of tire treads are increasingly recognized not only as a dominant form of microplastic contamination[19], [134] but also as other embedded chemicals such as antiozonants, vulcanization agents, and their toxic transformation products. Among these, 6PPD and its byproduct 6PPDQ are of particular concern due to their acute toxicity to aquatic ecosystems[8]. However, the extent to which these compounds leach from the TWP matrix under realistic environmental conditions remains poorly quantified.

This chapter focuses on experimentally quantifying the release of 6PPDQ from both new and aged (used) tire particles submerged in river water under controlled agitation, mimicking natural flow dynamics. By comparing leaching behavior across tire types, the study investigates the influence of pre-existing wear and environmental aging on chemical mobilization. Importantly, the findings build upon the environmental concentrations and spatial trends observed in Chapter 3 and offer mechanistic insight into persistent exceedances noted in risk assessments (Chapter 4), especially during hydrologically dynamic periods. Additionally, surface and molecular characterization techniques, including NMR spectroscopy used to validate the presence of leached compounds and probe structural transformation pathways. This integrated approach not only strengthens the link between environmental observations and particle chemistry but also informs future research directions and mitigation efforts discussed in Chapter 6.

5.2 Method and material: Leaching experiment

To understand the leaching dynamics of 6PPD and 6PPDQ from TWPs, both new and used tire samples were subjected to controlled aqueous leaching experiments designed to simulate environmental conditions relevant to urban freshwater systems.

5.2.1 Sample preparation

New tire particles were sourced from commercial suppliers to ensure consistency in chemical composition and manufacturing history. These tires were mechanically shredded to achieve uniform particle sizes in the range of 0.5-2 mm using a standardized rotary milling process. Used tire samples were collected from the local recycling center and manually scraped using a standardized abrasion protocol to maintain comparable particle sizes. The used tires were characterized via scanning electron microscopy (SEM) to evaluate surface morphology and quantify the degree of wear and environmental aging. All particles were stored in inert conditions prior to testing to minimize pre-leaching degradation. Leaching tests were conducted using river water collected from Schuylkill River to reflect environmental conditions and water chemistry relevant to the regional aquatic ecosystems studied in previous chapters. Water was collected in pre-cleaned glass containers and stored at 4 °C until use.

5.2.2 Experimental setup

Each experiment was conducted in triplicate for both new and used tire particle samples to ensure statistical robustness. In each sample, 500 mg of tire particles were placed in individual 50 mL polypropylene centrifuge tubes, each containing 50 mL of river water (liquid-to-solid ratio = 100 mL/g). Control samples containing only river water (no tire particles) were prepared in parallel to account for background contamination of 6PPDQ presence. To simulate natural hydrodynamic mixing in aquatic environments, samples were mounted on a rotary shaker set at 30 rpm and maintained room temperature (25 °C) for a continuous leaching period of 7 days. This duration was chosen to account short-term surface desorption & time dependent transformation dynamics of tire related contaminants.

5.2.3 Post-leaching processing and chemical analysis

After the leaching period, all samples were centrifuged at 3500 rpm for 10 minutes to separate suspended solids and tire particles. The supernatant was filtered using 0.45 μm PTFE filters to remove remaining particles. To stabilize analyte recovery and enhance quantification accuracy, non-interfering internal standards (NIS) were spiked into each sample. Chemical quantification of 6PPD and 6PPDQ was performed using ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The analytical method was based on EPA Draft Method 1634 (Chapter 3 method). Calibration curves were prepared using certified reference standards, and method detection limits (MDL) were determined prior to sample analysis. The quantification was performed using isotope dilution similar to chapter 3.

5.3 Results and discussion

This section presents and interprets the results of the controlled leaching experiments designed to quantify the release of 6PPDQ from new and used TWPs into river water. The data shows significant differences in leaching behavior between used and new tire particles, understanding the transformation potential of 6PPD to 6PPDQ under environmental conditions, and providing molecular level synthesis from NMR characterization. Together, these results provide critical insights into the leaching dynamics of tire-derived quinones in aquatic ecosystems.

5.3.1 Leaching behavior of 6PPDQ from new and used tire particles

The leaching test, conducted over 7 days under controlled conditions (30 rpm) at room temperature, resulted that 6PPDQ is released from both new and used tire particles into river water, though at different concentrations. As shown in Table 5.1, used tire particles released substantially higher levels of 6PPDQ, ranging from 804 to 2756 ng/L, compared to new tire particles, which ranged from 460 to 564 ng/L. The control samples, which consisted only of river water with no tire particles, showed a negligible background concentration of 0.05 ng/L, confirming minimal contamination. These results show that used tires released 4-6 times more 6PPDQ per gram of rubber than new tires.

The elevated leaching observed from aged tires can be attributed to cumulative environmental exposure, surface oxidation, and structural degradation factors known to enhance chemical release and transformation in TWP. Aging not only increases the surface roughness and porosity of the rubber matrix but also accelerates the oxidative transformation of residual 6PPD to 6PPDQ[135], [136]. These findings are consistent with prior research, including [8], [132], who demonstrated elevated 6PPDQ formation in environmentally weathered tire particles.

Table 5: Leaching concentrations of 6PPDQ from tire particles

Sample Type	6PPDQ Concentration (ng/L)	Mass of Tire (mg)	Volume of River water(L)	Mass of 6PPDQ leached (ng)	6PPDQ released per (g) tire (ng/g)
Control	0.053	500	0.05	0.003	0.005
New tire-1	460.57	500	0.05	23.03	46.06
New tire-2	516.77	500	0.05	25.84	51.68
New tire-3	564.98	500	0.05	28.25	56.50
Used tire -1	2756.61	500	0.05	137.83	275.66
Used tire -2	804.04	500	0.05	40.20	80.40
Used tire- 3	2044.82	500	0.05	102.24	204.48

5.3.2 Presence of 6PPDQ in new tire particles: evidence of inherent oxidation

While used tires understandably exhibited higher 6PPDQ release, the detection of 460-565 ng/L 6PPDQ from new tires indicates that transformation of 6PPD can begin early even before significant on-road aging occurs. This supports recent findings by Kolodziej's group and [16], [82], who observed that small amounts of 6PPDQ can form during tire storage, manufacturing, or short-term environmental exposure. Minor pre-oxidation during milling, handling, or transportation (e.g., ambient ozone exposure) may also contribute. Additionally, synthetic leaching studies [77], [137] have shown that the transformation of

6PPD to 6PPDQ can be initiated under ambient conditions in as little as 24 hours when exposed to aqueous oxidants.

5.3.3 NMR Analysis: molecular characterization of 6PPD and 6PPDQ

To better understand the structural distinction between the parent compound 6PPD and its transformation product 6PPDQ, comparative [1]H NMR spectra were examined (Figures 5.2 and 5.3). The spectrum for 6PPD (in CH_3CN) showed sharp aromatic proton signals in the region of 6.5-7.5 ppm and strong aliphatic signals near 1.0-1.5 ppm, consistent with its alkyl-phenylamine backbone. In contrast, 6PPDQ, analyzed in the same solvent, displayed a distinct set of peaks shifted downfield (5.5-7.5 ppm), corresponding to the quinonoid and oxidized aromatic environment.

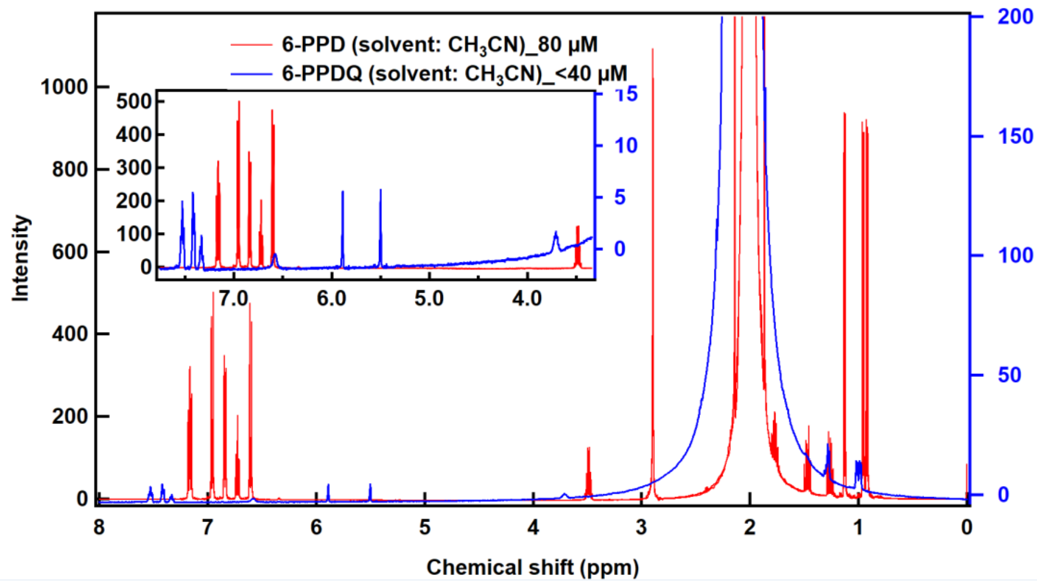


Figure 24: Overlay spectrum of 6PPD and 6PPDQ in Deuterated acetonitrile (CH_3CN)

The overlay spectrum (Figure 5.2) clearly differentiates 6PPD (red) from 6PPDQ (blue), with a notable broadening and flattening of the aromatic region in 6PPDQ due to its redox-active quinone structure. The absence of strong aliphatic multiples and emergence of shielded protons in 6PPDQ further confirms oxidation of the amino groups to quinone moieties. These spectral features corroborate field data and analytical measurements by confirming the transformation pathway of 6PPD to 6PPDQ under mild oxidative conditions and insight aligned with transformation kinetics observed by [53] and supporting the leachate composition in this study.

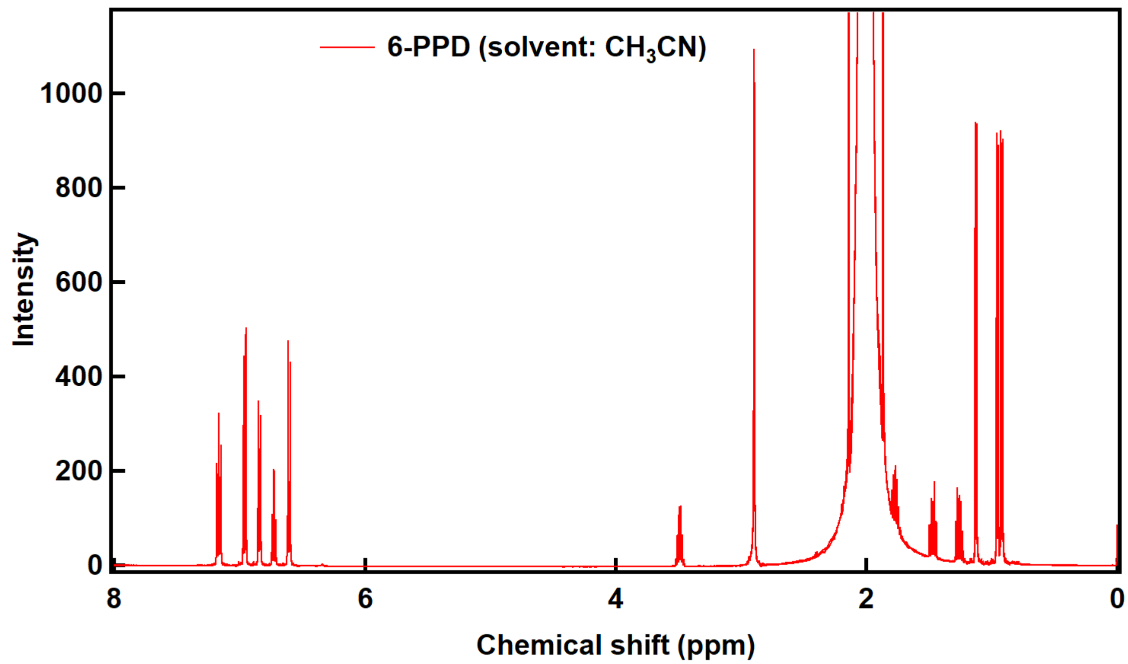


Figure 25: NMR spectrum for 6PPD in deuterated acetonitrile (CH_3CN)

5.3.4 Environmental relevance and comparison to field concentrations

The concentrations of 6PPDQ observed in leachates particularly from used tires are consistent with or even exceed levels reported in urban stormwater and runoff. For instance, urban roadway runoff in Seattle has reported 6PPDQ levels of 90-19,000 ng/L [8], and Don River and Highland Creek in Toronto showed wet-weather values of up to 2.3 µg/L [14]. The 2045-2757 ng/L values observed in used tire leachates are within this range, supporting the ecological significance of TWPs as a source of bioavailable 6PPDQ. Moreover, the observed release profiles suggest that TWPs represent a "pulse" source of

6PPDQ during storm events, especially when accumulated particles are mobilized by surface runoff. The release from new tires may contribute to chronic background levels, while used tires and aged TWPs may cause acute exposures in urban freshwater systems.

Given that the EPA freshwater screening value for 6PPDQ is 11 ng/L, the concentrations observed in both new and used tire leachates far exceed this threshold, particularly for salmonid-sensitive regions. These findings underscore the critical need for improved tire design, regulation of chemical additives, and stormwater infrastructure capable of intercepting TWPs before they reach receiving water. Further, the results suggest that current tire material formulations, even when new, present a latent risk due to the rapid formation and leaching of 6PPDQ under mild environmental conditions. Management strategies must consider both freshly generated TWPs and legacy particles that continue to leach contaminants during sediment resuspension or rainfall mobilization.

5.4 Chapter summary

This chapter examined the leaching behavior of 6PPD and its toxic transformation product, 6PPD-quinone (6PPDQ), from new and used tire wear particles (TWPs) under controlled aqueous conditions. Using a 7-day leaching experiment with river water from the Delaware or Schuylkill River basin and standardized agitation, we quantified the actual release of 6PPDQ from both particle types. Results revealed a clear distinction in leaching intensity: used tire particles released significantly higher concentrations of 6PPDQ (up to 2757 ng/L) compared to new particles (ranging 461-565 ng/L), consistent with the hypothesis that environmental aging enhances oxidative transformation and surface desorption of 6PPD derived quinones. The presence of 6PPDQ in new tire particles, although at lower levels, confirms that oxidation begins early potentially during manufacturing, handling, or initial road exposure supporting literature on pre-environmental transformation mechanisms.

These findings indicate that even unused tires serve as a source of 6PPDQ under ambient conditions, raising concern for early-stage environmental loading. Spectroscopic characterization using ^{1}H NMR further validated the molecular transition from 6PPD to 6PPDQ, with spectral shifts and broadened peaks in the aromatic region consistent with quinonoid structures. These molecular-level changes support bulk chemical evidence and provide mechanistic insight into the oxidative fate of 6PPD in tire matrices. Comparisons with field studies show that the concentrations of 6PPDQ released in this study align closely with or exceed levels observed in urban stormwater and runoff, highlighting the environmental relevance of tire-derived contamination. The leaching profiles support the conceptual framework in which TWPs serve as both chronic and acute sources of 6PPDQ, depending on particle age, accumulation, and runoff dynamics.

In summary, this chapter establishes that TWPs are significant sources of 6PPDQ in freshwater systems, even in their unused state, and that environmental aging exacerbates this threat. These results underscore the urgent need for sustainable tire formulations, environmental monitoring, and effective stormwater interventions to mitigate the release and transport of toxic tire-derived contaminants. The mechanistic insights gained here also set the stage for future studies aimed at characterizing transformation products and assessing their ecological impacts in complex environmental matrices.

CHAPTER 6

SUMMARY AND FUTURE RESEARCH

6.1 Summary

This dissertation presents a multiscale investigation into the environmental occurrence, risk, and mechanistic behavior of 6PPDQ, an oxidative byproduct of rubber tire antiozonant 6PPD, across urban freshwater systems in the United States. By bridging field level monitoring, statistical and spatial analyses, ecological risk assessment, and controlled laboratory experiments, the study advances both empirical understanding and methodological rigor in assessing tire-related contaminants in the environment.

The first half of this research focused on spatiotemporal occurrence patterns and contaminant dynamics of 6PPDQ in two major urban watersheds i.e., the Schuylkill River and the Delaware River Basin. Across 39 sampling sites and multiple seasonal timepoints, 6PPDQ was ubiquitously detected, with concentrations peaking during storm events and summer months. In the Delaware Basin, values reached over 150 ng/L, highlighting episodic but severe contamination. Chapter 3 established foundational insights into the concentration ranges and seasonal shifts, while Chapter 4 expanded the analysis to assess the influence of urbanization factors and ecological risk assessment.

The integration of urban indicators showed that traffic volume (AADT), population density, and proximity to tire-related industries were significant predictors of 6PPDQ contamination. In the Schuylkill River, traffic intensity showed a strong linear association with 6PPDQ concentrations ($R^2 = 0.469$), whereas in the Delaware River Basin, a nonlinear quadratic relationship better captured the seasonal accumulation and runoff dynamics (R^2 up to 0.889). Population density showed a statistically significant positive correlation in both basins (r > 0.59), indicating that urbanized zones are primary hotspots. Proximity to tire-related industries exhibited strong negative correlations (r = -0.703 and -0.649 for Schuylkill and Delaware, respectively), confirming that industrial runoff and airborne emissions are likely contributors. Both population density and tire-facilities proximity consistently linked to higher contaminant levels, with downstream, urban, and areas near industrial sites exhibiting disproportionately elevated levels.

A two-tiered ecological risk assessment framework, combining threshold-based Risk Quotient (RQ) screening and species-specific Toxic Unit (TU) analysis, was applied across both rivers. The RQ analysis indicated that 88% of Schuylkill River sites exhibited medium risk, while 12% posed high risk during the fall sampling. In the Delaware River Basin, TU-based assessments revealed that 12% of species-site-season combinations exceeded TU ≥ 1, highlighting acute risk to sensitive salmonids, especially coho salmon and brook trout fry. Medium TU risk accounted for 29%, reflecting widespread sublethal exposure potential, especially during storm events.

Chapter 5 transitioned from field-based analysis to controlled laboratory experiments examining the leaching potential of new and used tire wear particles (TWPs). Both particle types released measurable 6PPDQ under environmental conditions, though used tires leached up to 5 times more, reflecting the influence of surface aging and oxidative transformation. NMR spectroscopy confirmed the structural presence of 6PPD and its oxidative byproducts, reinforcing the mechanistic link between rubber chemistry and environmental toxicity. This chapter demonstrated that TWPs act not only as persistent microplastic sources but also as dynamic chemical reservoirs capable of sustained contaminant release under realistic environmental conditions.

6.2 Research contributions

This dissertation makes several novel contributions:

- Comprehensive Environmental Profiling of 6PPDQ in Urban Watersheds: This study is among the first to conduct high-resolution, multi-seasonal monitoring of 6PPD-quinone (6PPDQ) in two distinct but interconnected urban freshwaters: the Schuylkill River and the Delaware River Basin, revealing the compound's spatiotemporal dynamics, concentration peaks, and seasonal patterns across hydrological regimes.
- Integrated ecological risk assessment frameworks: The dissertation advances environmental risk science by applying a two-tiered ecological risk assessment including Risk Quotients (RQ) and Toxic Unit (TU) based species-specific thresholds for 6PPDQ. It provides quantitative insights into species-specific vulnerability, especially for sensitive salmonid species, across urban and semi-urban(tributaries) watersheds.
- Urbanization indicators affecting contamination level of 6PPDQ: Through statistical modeling and geospatial analyses, the research quantitatively links 6PPDQ concentrations to Average Annual Daily Traffic (AADT), population density, and proximity to tire-related industries, demonstrating how urbanization metrics can predict contaminant loading in freshwater systems. These insights inform the development of urban contaminant transport models.
- Laboratory based leaching characterization of TWPs: The work includes controlled leaching experiments using both new and aged tire particles in real river water

matrices, showing that used tires leach significantly more 6PPDQ, supports how aging and environmental exposure amplify chemical release from TWP.

6.3 Future directions

The findings of this dissertation establish a foundational framework for understanding the environmental occurrence, risk, and behavior of 6PPDQ in urban freshwater ecosystems. However, this work also opens several new avenues for future research both to refine scientific understanding and to support sustainable environmental management strategies. These directions include the following interconnected themes, many of which naturally extend from the methodologies and outcomes of this dissertation.

Expand longitudinal monitoring and spatiotemporal modeling: While this study captured critical seasonal and storm-driven dynamics of 6PPDQ, long-term data across multiple years will be vital to quantify interannual variability, long-term accumulation, and the potential effects of climate change (e.g., altered precipitation and hydrology). Future efforts should implement flow-normalized sampling combined with event-based monitoring to better capture peak contamination and mobilization phases. Integrating these datasets with hydrological models such as SWAT, HSPF, or SWMM will enhance predictions of contaminant loads under different land use and climatic scenarios.

Deepen source attribution and fate modeling: The dissertation identified strong associations between 6PPDQ concentrations and urbanization indicators traffic volume, population density, and proximity to tire-related industries. Future research should advance this by developing source apportionment models that differentiate between diffuse sources (e.g., general traffic wear) and point sources (e.g., industrial discharges or crumb rubber fields). Fate and transport modeling across air-soil-water interfaces, including particulatebound vs. dissolved-phase behavior, will help resolve unanswered questions about deposition, resuspension, and transformation over time.

Investigate mixture of toxicity and sublethal biological effects: This dissertation used both EPA threshold and species-specific Toxic Unit (TU) frameworks to characterize ecological risks. Building on this, future toxicological studies should examine mixture toxicity of 6PPDQ in combination with other tire-derived transformation products (e.g., 4-HDPA, 4-ADPA, benzothiazoles, DPG). Sublethal effects such as reproductive dysfunction, immune suppression, and behavioral alteration in fish and invertebrates should be prioritized. Mesocosm experiments and in vivo chronic exposure trials can help link environmental concentrations to real-world biological outcomes across trophic levels.

Characterize environmental transformation pathways in field conditions: Leaching and NMR characterization work demonstrated clear transformation of 6PPD into 6PPDQ under controlled settings. Future research should investigate in-situ transformation dynamics under variable environmental conditions, including temperature, microbial activity, photolysis, and redox gradients. This includes field tracking of transformation products over time in stormwater systems, riverbed sediments, and groundwater recharge zones. Combined chemical and microbial profiling will also illuminate biodegradation mechanisms and persistence in different ecological compartments.

Quantify bioavailability and bioaccumulation potential: The potential for bioaccumulation and trophic transfer of 6PPDQ and related compounds remains largely unknown. Future studies should use this study leaching dataset as a baseline to explore uptake into aquatic organisms, including macroinvertebrates, mussels, and fish, under realistic exposure conditions. Research should also quantify body burden levels, tissue partitioning, and potential biomagnification within food webs. Understanding how hydrophobicity, molecular structure, and transformation state affect bioavailability will be key for ecological risk modeling.

Evaluate the performance of green infrastructure for 6PPDQ retention and degradation: Given that storm-driven pulses are a primary delivery mechanism for 6PPDQ to rivers, there is a growing need to assess the ability of green stormwater infrastructure (GSI) to intercept or degrade tire-derived contaminants. Findings from this study suggest high seasonal and site-specific variability, which can inform targeted GSI placement. Future research should monitor removal efficiencies in bioswales, retention ponds, and permeable pavements, and explore media amendments that enhance sorption or catalytic degradation of 6PPDQ and its transformation products.

Develop safer antioxidant alternatives and evaluate their environmental behavior: While this dissertation focused on 6PPDQ, the environmental legacy of tire manufacturing depends on the broader class of antiozonants and rubber additives. Future research should focus on developing, screening, and validating alternative compounds with lower environmental persistence and toxicity. Natural compounds like polyphenols, synthetic polymer-bound antioxidants, and nano-encapsulated formulations merit investigation. Environmental transformation and the leaching behavior of these new compounds must be assessed using the analytical and spectroscopic protocols refined in this dissertation.

Inform Policy Through Risk-Based Thresholds and Predictive Tools: Building on the TU-based ecological risk assessment, future work should support the establishment of regulatory guidelines by combining environmental concentration data with species sensitivity distributions (SSDs), predicted no-effect concentrations (PNECs), and probabilistic risk models. Collaborations with regulatory agencies and municipalities will be essential to translate this science into practical monitoring frameworks and to establish enforceable limits for tire-related chemicals in drinking water sources, stormwater discharge permits, and air quality standards.

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APPENDIX

SUPPORTING INFORMATION FOR CHAPTERS 3& 4

Table 6: Coordinates of sample locations in the Schuylkill River

Sample location	Latitude	Longitude
Pottstown	40.2420062	-75.6511802
Linfield bridge	40.2053433	-75.5808074
Spring City (Main St)	40.1826168	-75.5451945
Black Rock bridge	40.1591915	-75.5120615
Mont Clare bridge	40.1358385	-75.5081728
Pawling Rd bridge	40.1149878	-75.4707849
Perkiomen Creek	40.11953	-75.4553615
Perkiomen Bridge	40.119247	-75.4552275
Sullivan's Bridge	40.1077845	-75.4206094
Norristown Dam Bridge	40.1100563	-75.344477
Conshohocken	40.0705623	-75.309514
Manayunk Canal (Green lane)	40.0262337	-75.2279186
Falls bridge	40.0083739	-75.1974499
Strawberry Mansion	39.9953581	-75.1938314
Chestnut Bridge	39.9531386	-75.1810111
Greys Ferry bridge	39.9420119	-75.2048251

Table 7: Coordinates of boat sample locations in the Delaware River Basin

Site ID	Boat sample location	Coordinates	
		Latitude	Longitude
15	Paulsboro	39.848061	-75.267146
16	Navy Yard	39.881679	-75.18019
17	Benjamin Franklin Bridge	39.955502	-75.135818
18	Betsy Ross Bridge	39.984701	-75.066603
19	Torresdale	40.040199	-74.988048
20	Burlington Bristol Bridge	40.081067	-74.868852
21	Florence Bend	40.128025	-74.816028
22	Biles Channel	40.181566	-74.746191
23	Biles Channel DUP	40.181566	-74.746191

Table 8: Coordinates of quarterly sample locations in the Delaware River Basin

Sites	Delaware River basin sample location	Latitude	Longitude
Upper Sites	Sherman Creek	42.001718	-75.395038
	Faulkner Brook	41.983374	-75.343208
	East Branch Delaware River	41.952832	-75.276983
	West Branch Delaware River	41.942253	-75.287896
	Shehawken Creek	41.941757	-75.287643
	Delaware River - Hancock	41.935612	-75.277422
	Delaware River - Lordville	41.867839	-75.213903
	Equinunk Creek	41.855874	-75.224954
Middle Sites	Dunnfield Creek	40.972081	-75.126457
	Bushkill Creek	40.696557	-75.228127
	Little Lehigh Creek	40.568954	-75.498921
	Unnamed Tributary to Sixpenny Creek	40.239000	-75.77878
	Valley Creek	40.081309	-75.456595
	Valley Creek tributary	40.080554	-75.456111

Table 9: Chemicals and abbreviation

Compound	Molecular Formula	Full Name	CAS Number
6PPD	$C_{18}H_{24}N_2$	N^1-(1,3-dimethylbutyl)-N^4-phenyl-1,4-benzenediamine	793-24-8
6PPDQ (6PPD-Quinone)	$C_{18}H_{22}N_2O_2$	2-[(1,3-dimethylbutyl)amino]-5-(phenylamino)-2,5-cyclohexadiene-1,4-dione	2754428-18-5
$^{13}C_6$ 6PPDQ	$C_{12}[^{13}C_6]H_{22}N_2O_2$	2-[(1,3-dimethylbutyl)amino]-5-(phenyl-$^{13}C_6$-amino)-2,5-cyclohexadiene-1,4-dione	N/A
D_5 6PPDQ	$C_{18}H_{17}D_5N_2O_2$	2-[(1,3-dimethylbutyl)amino]-5-(phenyl-2,3,4,5,6-d_5-amino)-2,5-cyclohexadiene-1,4-dione	2750119-14-1

Table 10: Acquity Xevo TQ-S UHPLC flow conditions

Time (min)	Flow rate mL/min	% A (0.2% formic acid in DI water)	% B (Acetonitrile)	Curve
Initial	0.3	90.0	10.0	6
1.00	0.3	90.0	10.0	6
3.00	0.3	45.0	55.0	6
6.00	0.3	1.0	99.0	6
8.00	0.3	1.0	99.0	6
8.50	0.3	90.0	10.0	6
10.00	0.3	90.0	10.0	6

Table 11: MRM transitions and settings for mass spectrum scans

Compound	Molecular Weight (g/mol)	MRM Reaction	Retention time (min)	Collision energy (V)
6PPD	269.30	184.3 212.3	3.9- 4.9	25 15
6PPDQ	299.20	215.2 241.1	5.5- 7.5	15 28
$^{13}C_6$ 6PPDQ	305.30	221.2	5.5- 7.5	12
D_5 6PPDQ	304.30	220.2 246.2	5.5- 7.5	20 30

Table 12: 6PPDQ concentration- sample locations in the Schuylkill River

Samples	6PPDQ Concentration, ng/L				
	February, 2024	May, 2024	August, 2024	October, 2024	Average Concentration
Pottstown	0.13	0.25	0.42	2.98	0.94
Linfield bridge	0.21	0.38	0.23	2.09	0.73
Spring City	N/A	0.26	0.26	1.3	0.61
Black Rock bridge	0.51	0.45	0.53	3.98	1.37
Mont Clare bridge	0.55	0.27	0.26	4.32	1.35
Pawling Rd bridge	0.29	0.39	0.51	5.79	1.74
Perkiomen Creek	N/A	0.56	0.51	4.9	1.99
Perkiomen Bridge	0.21	0.31	0.20	17.95	4.67
Sullivan's Bridge	N/A	0.55	0.69	5.41	2.22
Norristown Dam Bridge	ND	0.84	1.20	5.23	2.42
Conshohocken	0.51	1.05	1.54	4.96	2.02
Manayunk Canal	1.13	3.79	0.55	14.1	4.89
Falls bridge	0.87	3.68	1.43	10.33	4.08
Strawberry Mansion	1.59	4.08	1.35	2.68	2.42
Chestnut Bridge	1.23	4.48	2.44	5.35	3.38
Grays Ferry bridge	0.22	4.83	4.05	5.94	3.76

Table 13: 6PPDQ concentration- quarterly locations in the Delaware River Basin

Sample location	6PPDQ Concentration, ng/L																	
	April, 2024			July, 2024			Rain event, 2024			November, 2024			February, 2025			April, 2025		
Sherman Creek	ND	ND	N/D	ND	ND	ND	N/A	N/A	N/A	3.23	4.83	2.34	ND	ND	ND	0.48	0.45	0.50
Faulkner Brook	ND	ND	ND	ND	ND	ND	N/A	N/A	N/A	5.78	4.89	2.62	ND	ND	0.15	0.40	0.33	0.53
East Branch Delaware River	1.81	1.86	ND	ND	ND	ND	N/A	N/A	N/A	1.03	1.59	1.7	ND	ND	ND	ND	ND	ND
West Branch Delaware River	ND	ND	ND	ND	ND	ND	N/A	N/A	N/A	2.45	3.68	3.03	ND	ND	ND	0.24	0.20	0.34
Shehawken Creek	ND	ND	N/A	0.35	0.35	0.49	N/A	N/A	N/A	2.09	2.47	2.91	ND	ND	ND	0.48	0.37	0.41
Delaware River - Hancock	ND	ND	ND	0.78	0.89	1.15	N/A	N/A	N/A	2.25	1.65	1.81	0.24	0.66	0.51	0.20	0.40	0.35
Delaware River - Lordville	0.17	0.22	ND	ND	ND	ND	N/A	N/A	N/A	7.55	6.12	7.07	ND	ND	ND	ND	ND	ND
Equinunk Creek	ND	ND	ND	ND	ND	ND	N/A	N/A	N/A	0.95	0.9	0.91	0.52	ND	ND	ND	ND	ND
Dunfield Creek	1.51	ND	ND	0.51	0.63	0.59	N/A	N/A	N/A	3.51	2.48	4.05	ND	ND	ND	0.57	0.67	0.64
Bushkill Creek	ND	ND	ND	0.39	0.54	0.52	3.83	3.39	3.91	4.96	7.48	6.3	ND	ND	0.50	1.24	0.88	1.24
Little Lehigh Creek	0.83	0.19	0.24	0.57	0.34	0.34	8.53	7.17	9.50	5.65	4.51	4.32	ND	0.17	0.30	1.24	1.00	0.85
Sixpenny Creek	1.84	0.27	ND	2.63	1.34	0.77	ND	ND	ND	3.01	4.73	3.59	ND	ND	ND	0.11	0.08	0.07
Valley Creek	ND	ND	ND	42.83	47.90	44.19	1.05	1.03	1.19	5.12	3.24	3.75	0.66	0.83	1.08	1.02	1.18	1.00
Valley Creek tributary	NA	NA	NA	150.52	151.5	150.97	N/A	N/A	N/A	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 14: Average 6PPDQ concentration- quarterly locations in the Delaware River Basin

Sample locations	Average 6PPDQ Concentration, ng/L					
	April, 2024	July, 2024	Nov, 2024	Rain event (June 2024)	Feb, 2025	April, 2025
Sherman Creek	0.18	0.07	3.47	N/A	0.07	0.48
Faulkner Brook	0.18	0.07	4.43	N/A	0.15	0.42
East Branch Delaware River	1.84	0.07	1.44	N/A	0.07	0.07
West Branch Delaware River	0.18	0.07	3.05	N/A	0.07	0.26
Shehawken Creek	0.18	0.40	2.49	N/A	0.07	0.42
Delaware River - Hancock	0.18	0.94	1.90	N/A	0.47	0.32
Delaware River - Lordville	0.19	0.07	6.91	N/A	0.07	0.07
Equinunk Creek	0.18	0.07	0.92	N/A	0.52	0.07
Dunnfield Creek	1.51	0.58	3.35	N/A	0.07	0.63
Bushkill Creek	0.18	0.48	6.25	3.71	0.50	1.12
Little Lehigh Creek	0.42	0.42	4.83	8.40	0.24	1.03
Sixpenny Creek	1.05	1.58	3.78	0.07	0.07	0.09
Valley Creek	0.18	44.97	4.04	1.09	0.86	1.06
Valley Creek Tributary	N/A	150.99	N/A	N/A	N/A	N/A

Table 15: 6PPDQ concentration- Boat locations in the Delaware River Basin

Site ID	Locations	Concentration of 6PPDQ, ng/L				
		May, 2024	June 2024	July 2024	August 2024	Average
15	Paulsboro	0.59	0.666	0.41	1.77	0.86
16	Navy Yard	0.86	0.632	0.51	2.82	1.21
17	Benjamin Franklin Bridge	0.55	0.599	0.35	2.45	0.99
18	Betsy Ross Bridge	0.41	0.840	0.40	1.98	0.91
19	Torresdale	0.40	ND	ND	0.49	0.44
20	Burlington Bristol Bridge	0.57	0.589	ND	0.25	0.47
21	Florence Bend	1.36	0.406	ND	1.82	1.20
22	Biles Channel	0.55	ND	0.66	0.77	0.66
23	Biles Channel DUP	0.65	0.395	0.32	0.72	0.52

Table 16: Risk quotient (RQ)- Boat locations in the Delaware River Basin

Site ID	Boat samples	Risk Quotient (RQ)			
		May, 2024	June, 2024	July, 2024	August, 2024
15	Paulsboro	0.053	0.061	0.037	0.161
16	Navy Yard	0.078	0.057	0.046	0.257
17	Benjamin Franklin Bridge	0.050	0.054	0.032	0.223
18	Betsy Ross Bridge	0.037	0.076	0.036	0.180
19	Torresdale	0.036	ND	ND	0.044
20	Burlington Bristol Bridge	0.052	0.054	ND	0.023
21	Florence Bend	0.124	0.037	ND	0.165
22	Biles Channel	0.050	ND	0.060	0.070
23	Biles Channel DUP	0.059	0.036	0.029	0.065

Table 17: Risk quotient (RQ)- quarterly locations in the Delaware River Basin

Sample locations	Risk Quotient (RQ)					
	April, 2024	July, 2024	Rain event, 2024	Nov, 2024	Feb, 2025	April, 2025
Sherman Creek	0.016	0.006	N/A	0.315	0.006	0.043
Faulkner Brook	0.016	0.006	N/A	0.403	0.014	0.038
East Branch Delaware River	0.167	0.006	N/A	0.131	0.006	0.006
West Branch Delaware River	0.016	0.006	N/A	0.278	0.006	0.023
Shehawken Creek	0.016	0.036	N/A	0.226	0.006	0.038
Delaware River - Hancock	0.016	0.085	N/A	0.173	0.043	0.029
Delaware River - Lordville	0.018	0.006	N/A	0.628	0.006	0.006
Equinunk Creek	0.016	0.006	N/A	0.084	0.047	0.006
Dunnfield Creek	0.137	0.053	N/A	0.304	0.006	0.057
Bushkill Creek	0.016	0.044	0.337	0.568	0.045	0.102
Little Lehigh Creek	0.038	0.038	0.764	0.439	0.021	0.094
Sixpenny Creek	0.096	0.144	0.006	0.343	0.006	0.008
Valley Creek	0.016	4.088	0.099	0.367	0.078	0.096
Valley Creek Tributary	N/A	13.726	N/A	N/A	N/A	N/A

Table 18: Risk quotient (RQ)- sample locations in the Schuylkill River

Sample locations	Risk Quotient (RQ)			
	February, 2024	May, 2024	August, 2024	October, 2024
Pottstown	0.01	0.02	0.04	0.27
Linfield bridge	0.02	0.03	0.02	0.19
Spring City (Main st)	0.00	0.02	0.02	0.12
Black Rock bridge	0.05	0.04	0.05	0.36
Mont Clare bridge	0.05	0.02	0.02	0.39
Pawling Rd bridge	0.03	0.04	0.05	0.53
Perkiomen Creek	0.00	0.05	0.05	0.45
Perkiomen Bridge	0.02	0.03	0.02	1.63
Sullivan's Bridge	0.00	0.05	0.06	0.49
Norristown Dam Bridge	0.00	0.08	0.11	0.48
Conshohocken	0.05	0.10	0.14	0.45
Manayunk Canal (Green lane)	0.10	0.34	0.05	1.28
Falls bridge	0.08	0.33	0.13	0.94
Strawberry Mansion	0.14	0.37	0.12	0.24
Chestnut Bridge	0.11	0.41	0.22	0.49
Grays Ferry bridge	0.02	0.44	0.37	0.54

Table 19: Lethal Concentration (LC50) of aquatic species

Species	LC_{50} (µg/L)	LC_{50} (ng/L)	Sensitivity
Coho Salmon	0.041-0.095	41-95	Extremely sensitive
Brook trout (fry)	0.165	165	Highly sensitive
Brook trout (fingerlings)	0.59	590	Sensity
Rainbow trout	0.90-2.30	900-2300	Sensitive
White-spotted char	0.51	510	Sensitive
Brown trout	>12.0	12000	Tolerant

Table 20: Coordinates for tire related companies

Tire related companies	Latitude	Longitude
Van Alstine Manufacturing Co	40.20187	-75.4853
Allen Rubber Co. Inc.	39.944	-75.58
Yokohama Tire Manufacturing Virginia, LLC	37.27689	-80.0413
Sumitomo Rubber	42.96974	-78.9182
Performance Tire Company	40.67064	-75.152
NTW - National Tire Wholesale	40.25197	-75.2788
Goodyear Tire & Rubber Plant	35.16977	-78.8557
Tireco Distributors	39.81285	-75.066
Tire Rack Distribution Center	39.67319	-75.5574
Cooper Tire Distribution Center	40.52126	-75.5984
BF Goodrich Fort Wayne Manufacturing	41.1346	-84.8991
Wheel & Tire Distributors	39.93118	-75.2937
Goodyear	36.54818	-79.3744
McCarthy Tire Service	39.83379	-75.4089
Specialty Tires of America Inc.	40.61813	-79.1695
Mavis Discount Tire	40.07808	-75.6907
Industrial Tire Solutions	39.96612	-75.053
Wheel & Tire Distributors	39.93113	-75.2937

Table 20: (continued). Coordinates for tire related companies

Tire related companies	Latitude	Longitude
Tire Giants	39.97333	-75.1809
Pro Torque Performance Products	39.93575	-75.2597
Priority Tire	40.60149	-75.5776
Bergey’s Tire Warehouse	40.29146	-75.3069
Simple Tire	39.94996	-75.162
American Tire Distributors	40.01718	-74.8509
Bergey's Commercial Tire Centers	40.29221	-75.3076
Mitchell Industrial Tire Co	40.13233	-74.8199
Tire Hub	39.82947	-75.2083
Falken Tire Corporation	40.19206	-74.5677
Superior Tire & Rubber Corp	41.83793	-79.1741
Hutchinson Industries	40.22709	-74.7411
McCarthy Tire Service	40.12861	-74.8395
Wingfoot Commercial Tire Systems	40.12535	-74.8501
JWT Wholesale	40.1338	-74.9879
Gallagher Tire	40.20982	-75.5937
USA One Tire	40.01548	-75.0511
Goodyear Commercial Tire & Service Centers	39.89016	-75.4123