

Instructions for ITRC Tire Anti-degradant (6PPD) Guidance Document External Review (Comments Due on or before: Friday, March 29, 2024)



Request Snapshot & Document Goal: Our Team is excited to release this guidance document for External Review! The review/comment period for this document starts Tuesday, February 13th and ends Friday, March 29th, 2024.

The work products of the ITRC Tire Anti-degradant (6PPD) team have been prepared to provide baseline knowledge on the tire anti-degradant 6PPD and its degradation product of concern (6PPD-quinone or 6PPD-q) in terms of the following:

- background, history, and basis of concern
- physical properties, fate and transport, and sampling and analysis methods
- solutions to mitigate and manage the impacts on human health and the environment, and
- an overview of information gaps and data needs for continued research.

This information has been tailored to the needs of state program personnel tasked with making informed decisions regarding 6PPD-q as an emerging contaminant. These documents are also intended to be useful to consultants interfacing with regulatory agencies regarding 6PPD and 6PPD-q. Additionally, these documents should be helpful to public stakeholders and tribes with needs to manage the impacts of these chemicals.

Review/Comment Request: Please focus your comments on the content, thoroughness, and usefulness of the documents. While it is helpful to identify and comment on sections of text that are redundant, confusing, unclear, or unnecessary, it is not necessary to identify and provide comments on typographical errors and general grammar unless those errors have an impact on content understanding; ITRC will use a professional technical editor to review and revise this document for spelling, grammar, and consistency in format.

Notes on Highlighting Colors:

- Terms highlighted in **purple** have been identified by the subgroup for inclusion in the glossary for these documents. Please use the same convention for any additional terms you believe are warranted for inclusion in the glossary. We can also use help with review and entry of definitions in the draft glossary.
- Text highlighted in **yellow** indicates a cross-reference (existing or plan) to another section or table in the documents. These links are not active in the PDF document for external review, but will be active on the final website.
- Text highlighted in **green** or **blue** indicates a reference will be updated or may be needed to support the preceding text. These references will be added after completion of the external review.

Logistics:

- Download the Comment Spreadsheet from ITRC Connect:
<https://connect.itrcweb.org/viewdocument/itrc-tire-anti-degradant-6ppd-doc>
- Contact the Program Advisor (Steve Brauner; sbrauner@environmentalworks.com) with questions, comments, or concerns on accessing or commenting on this document.
- Submit comments using the following link (or the similar link embedded in the Comment Spreadsheet):
[Distribution List for Comments on the ITRC Tire Anti-degradant \(6PPD\) Work Products](#)

Thank you again for your time and consideration!

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In 2020, 6PPD-quinone was identified as the lethal pollutant that kills coho salmon in urbanized areas of the Puget Sound in Washington State (Tian et al. 2021). In terms of its ecological effects, it has been compared to the devastating impacts of DDT, the pesticide that caused egg-shell thinning and led to the decimation of songbirds and raptors (D. Troutt as quoted by Walgamott 2021). Since 6PPD-quinone's discovery, it is now recognized as a global contaminant and it exceeds by about 10,000 times the threshold to be categorized as having very high aquatic toxicity (United Nations 2015). 6PPD-q is now known to be acutely toxic to brook trout and rainbow trout, important ecological and recreation species throughout the U.S. (Nair et al. 2023; Brinkmann et al. 2022; Di et al. 2022). Data on human toxicity is lacking for 6PPD-q. Data on human exposure to 6PPD-q demonstrates that it has been measured in human urine (Du et al. 2022), serum (Zhang et al. 2024), cerebrospinal fluid (Fang et al. 2024), and can pass through the placenta to a fetus in mice (Zhao et al. 2023).

6PPD-quinone pollution comes from tires; however, 6PPD-quinone is a transformation product of 6PPD, the primary anti-degradant added to tires, used to prevent premature degradation of the rubber from oxygen and ozone damage, as well as other chemicals. The primary source of 6PPD-quinone is thought to be tire particles, that may contain both 6PPD and 6PPD-q. These tire wear particles are carried into waterways and picked up by the wind. Because these two chemicals are so tightly linked by fate and transport – and possibly hazard, this document discusses both chemicals. We will explain the linkage between the chemicals in each chapter and why we discussed them. To be clear, only 6PPD-q has been linked to the death of the coho salmon; 6PPD has not been.

This guidance document is intended for state and tribal agencies that may need to learn more about 6PPD and 6PPD-q to pursue their own policies and regulations regarding these chemicals. These agencies include:

- Departments of transportation
- Water quality agencies
- Fish and wildlife departments
- Solid waste agencies that manage tire reuse and recycling
- Department of health because little is known about 6PPD-quinone's potential hazard traits

- 1 • Drinking water and wastewater treatment facilities (especially combined sewerage
- 2 systems) because impacts to these facilities is not yet clear
- 3 • Chemical agencies that may seek alternative anti-degradants in tires

1 INTRODUCTION

This section serves as an introduction to the concerns arising from the recent discovery of 6PPD-quinone (6PPD-q). It begins with a summary of the problem, an explanation of transport pathways, followed by who and what are affected. We continue with how 6PPD-q is detected in the environment, and solutions to the challenge it poses. Lastly, we provide an overview of 6PPD-q topic areas for which information is lacking and a summary of how decision makers might address 6PPD-q and utilize this document as a resource.

1.1 Summary: What Is the Problem?

For decades, coho salmon (*Oncorhynchus kisutch*) in the Pacific Northwest have exhibited acute mortality in adults that seemed linked to unknown contaminants in stormwater runoff from roads, termed “urban runoff mortality syndrome” (URMS) (Tian et al. 2021). According to the WA Department of Ecology, “...for over 20 years, scientists faced a toxic mystery: coho salmon returning to urban streams and rivers in the Puget Sound region were dying before they could lay their eggs. The culprit was unknown, but it seemed linked to toxic chemicals running off our roads and highways.” (Flores 2023). Up to 100% mortality of returning coho salmon was observed (Scholz et al. 2011).

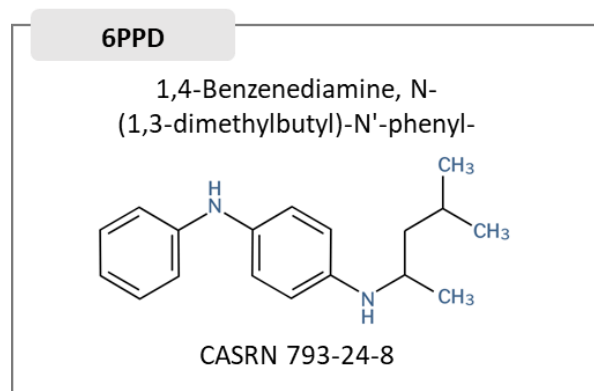


Figure 2-1 Chemical structure of 6PPD

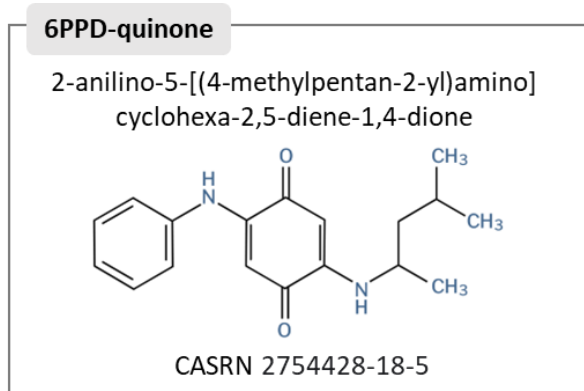


Figure 1-1 Chemical structure of 6PPD-quinone

In 2020, researchers (Tian et al. 2021) identified a transformation product of 6PPD (Figure 1-1), namely 6PPD-quinone (Figure 1-2) as the causative toxicant of premature coho salmon mass mortality events. 6PPD is within the chemical class para-phenylenediamines (PPDs). 6PPD supports the durability and safety of tires as the primary tire anti-degradant used to prevent the breakdown of rubber that results from reactions with ozone (Rossomme et al. 2023; Hu et al. 2022), oxygen (Santoso, Giese, and Schuster 2007), and free radicals (Scott 1985). The focus on 6PPD-q has also brought to the fore concerns about the toxicity of the parent 6PPD compound itself. Throughout this document, we will highlight information about each chemical as appropriate. Evaluating both these chemicals is important for estimating toxicity, for understanding the fate and transport, and for thinking about solutions to mitigate the harm caused by 6PPD-q to salmonids.

In addition to coho, also known as silver salmon, 6PPD-q has been found to be lethally toxic to *Salvelinus fontinalis* [brook trout] (Brinkmann et al. 2022); *Oncorhynchus mykiss* [rainbow trout] (Nair et al. 2023; Brinkmann et al. 2022; Di et al. 2022) which likely extends to ocean-going steelhead (French et al. 2022); and *Salvelinus leucomaenis* [white spotted char] (Hiki and Yamamoto 2022). Some acute mortality from 6PPD-q exposure is seen in juvenile *Oncorhynchus tshawytscha* [Chinook salmon or king salmon] (Lo et al. 2023), however results are mixed (Montgomery et al. 2023; Greer et al. 2023) and effects are not observed at environmentally relevant concentrations. More publications on acute toxicity among the salmonids are anticipated in 2024 (J. Greer and M. Brinkmann, pers comm). More information is available in **Section 2 (Effects Characterization and Toxicity)** of this document.

Habitat for known sensitive fish species (e.g., *O. kisutch*, *O. mykiss*, and *S. fontinalis*) are geographically dispersed across the United States, as shown in the composite map below (Figure 1-3). Native habitats for these three fish species are shown in orange on Figure 1-3 and for non-native (i.e., introduced) habitats, is shaded green. It is important to note that while this map represents the nationwide distribution of sensitive fish species, actual potential exposure in each of these habitats must be considered.

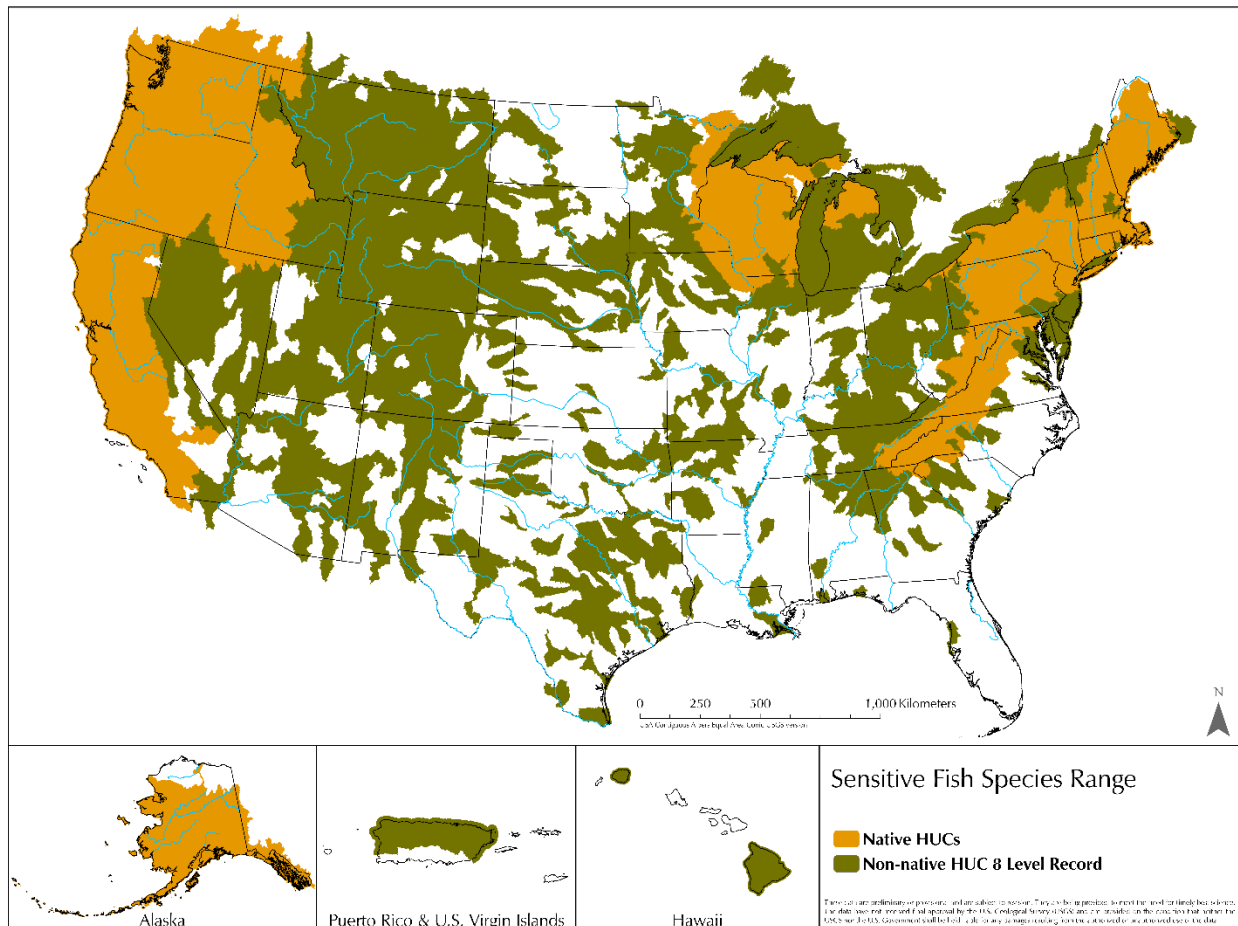


Figure 1-3. Composite map of the habitat for *Oncorhynchus kisutch* (coho), *Oncorhynchus mykiss* (rainbow trout/steelhead), and *Salvelinus fontinalis* (brook trout).
Source: USGS [Nonindigenous Aquatic Species \(NAS\)](#) database, last accessed December 2023.

1.2 Transport Pathways: How Do 6PPD and 6PPD-q get into the environment?

1.2.1 Overview

6PPD is the primary anti-degradant in tires and has been in use since the 1960s. In contrast, 6PPD-q is not intentionally added to tires during manufacturing. Rather, 6PPD-q is one of the intermediate degradation products formed by the reaction of 6PPD and ozone. 6PPD-q may be present in many places impacted by tire use. More information is available in [Section 4 \(Occurrence, Fate and Transport of 6PPD and 6PPD-q\)](#) of this document. Table 1-1 summarizes some key definitions.

Table 1-1. Key definitions for tire and road wear.

Terminology	Acronym	Meaning
Tire Wear Particles / Particulate	TWP	Friction between the road and a tire during driving, braking, and turning leads to the creation and emission of tire wear particles (TWP).
Tire and Road Wear Particles (also referred to as Tire Pavement Wear Waste)	TRWP (also TPWW)	Aggregated tire and road wear particles (TRWP) form from friction with the road surface during driving; the presence of the road wear component has an impact of the overall particle characteristics (e.g., mass, surface area, density) and therefore will impact the transport of particles in the environment (Baensch-Baltrusch et al. 2020; Kreider et al. 2010); can also include brake wear (Ha et al. 2023).
Tire Wear Contaminants	TWC	Represents a combination of 6PPD, 6PPD-q, TWP and TRWP.

As shown in Figure 1-4, TWC migrate to waterways through various transport pathways including deposition from air, direct runoff from roads, and conveyance from stormwater drains. Besides detection in stormwater and surface waters on many continents (Tian et al. 2021; Challis et al. 2021; Rauert et al. 2022; Johannessen and Metcalfe 2022; Cao et al. 2022; Maurer et al. 2023) 6PPD and 6PPD-q have been found in:

- Airborne particulates (Cao et al. 2022; Johannessen et al. 2022; Y. Zhang et al. 2022; Huang et al. 2021)
- Sediment (Zhu, Guo, Ren, et al. 2024; Zeng et al. 2023)
- Soil (Cao et al. 2022)
- Biosolids (Cao et al. 2023)
- Rubber products other than tires (Zhao et al. 2023; Sherman et al. 2024)
- Indoor dust (Huang et al. 2021; Z. Zhang et al. 2024; Zhu, Guo, Jiang, et al. 2024)
- Road dust (Huang et al. 2021; Jin et al. 2023).

In humans 6PPD-q been measured in urine (Du et al. 2022), serum (J. Zhang et al. 2024) and, cerebrospinal fluid (Fang et al. 2024).

Conceptual Transport and Exposure Model

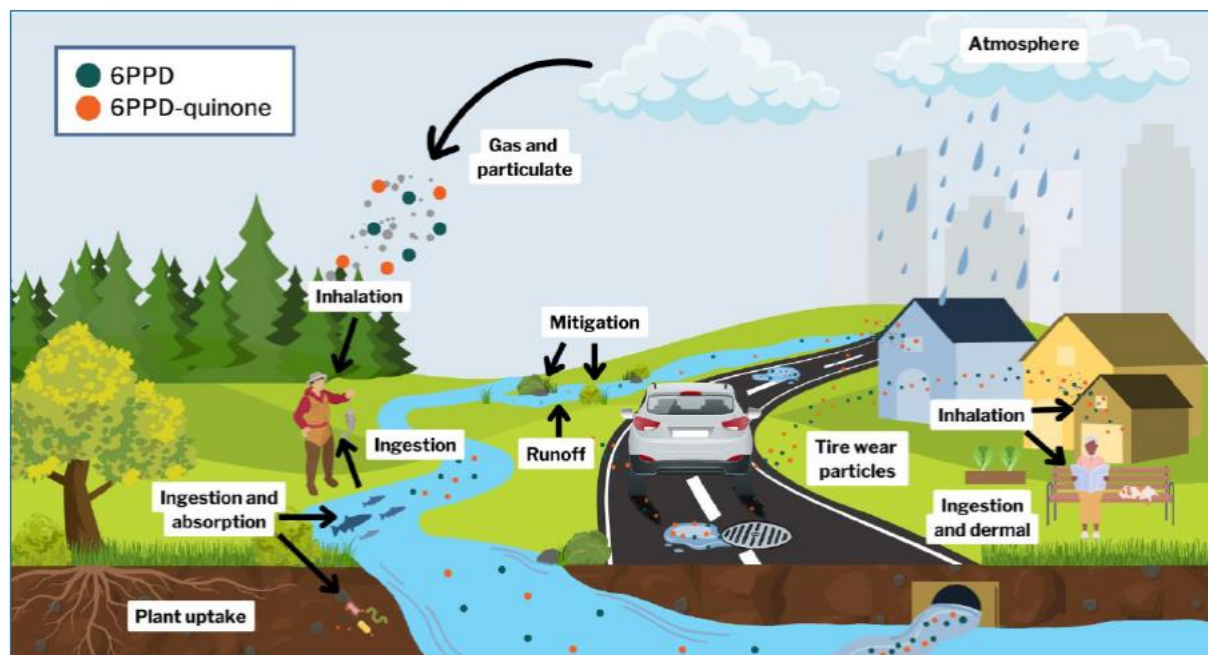


Figure 2. 6PPD in tires is converted to 6PPD-quinone (6PPD-q) when exposed to ozone. 6PPD-q is contained in tire wear particles that can be transported in the air and potentially inhaled by people. The particles can also be deposited on surfaces, soils, and plants, including foods, leading to potential plant uptake and human dermal exposure and ingestion. Tire wear particles can also stay near the roadway and be transported to surface waters through stormwater drains and runoff. 6PPD-q in surface waters can be ingested and absorbed by fishes. Exposed organisms can be ingested by humans and other species. 6PPD-q can potentially be mitigated by green stormwater infrastructure. Research is ongoing to further define 6PPD-q's environmental behaviors, exposures, and the potential development of adverse health outcomes. Figure credit: Hannah Vinyard, Washington State Department of Ecology.

1.2.2 Short History of 6PPD in Tires

PPD derivatives are among the most widely used classes of antioxidants/antiozonants in rubber products, with 6PPD being the most widely used in tires. 6PPD is an efficient anti-degradant, reacting with oxygen and ozone faster than these oxidizing compounds can react with rubber, which protects the rubber from degradation (Rossomme et al. 2023; Hu et al. 2022; Santoso, Giese, and Schuster 2007). In addition to providing direct protection to the exterior of the tire against oxygen and ozone, 6PPD also protects internal components of the tire from degradation due to the actions of heat and oxygen throughout the life of the tire (Chasar and Layer 2010; Huntink, NM and Datta, RN 2003; Kuczkowski 1990). As tires age, oxygen and ozone will attack the tire and cause cracking, hardening, and resulting loss of strength of the rubber compounds. 6PPD is consumed as it reacts with the degradants, leaving less 6PPD in the tire as it transforms into intermediate degradation products including 6PPD-q (Figure 1-5). Due to this protective effect against rubber degradation, 6PPD is currently critical to tire endurance and thus ultimately to motor vehicle safety. TWP's are generated by the friction of a tire rolling across the

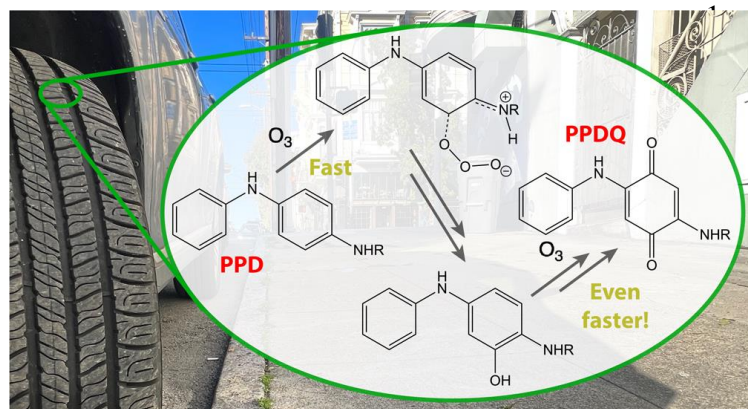


Figure 1-5: This diagram shows direct interaction between ozone and the PPD aromatic ring to yield 6PPD-d.

Source: Rossomme et al. 2023, Used with permission.

pavement surface (Ha et al. 2023). The addition of 6PPD prevents excessive abrasion of the rubber and improves tire wear life and contributes to consistent traction over the lifetime of the tire by preventing hardening of the tread compound (Pulford 1983).

Prior to the development of the paraphenylene diamine (PPD) family of antiozonants to prevent cracking and degrading of rubber compounds, there was often “rapid deterioration and loss of physical properties which caused failures in rubber goods” (Kuczkowski 1989). The effect of ozone on the degradation of tire rubber compounds was not fully understood

until the 1930s. At that time a typical tire lasted only 10,000 miles or roughly two years. In the 1930’s, waxes were identified as a static ozone protector for rubber compounds, but wax did not work for products that required dynamic applications, where the rubber stretches and flexes when in use, like a tire. Failure of tires and other rubber parts on military vehicles, which were stored from World War II and placed into service for the Korean War, led the U.S. government to sponsor research to study ways to prevent cracking and degrading of rubber compounds. This became known as the Rock Island Arsenal Technical Report (Ofner 1967) which identified a broad class of chemically modified PPDs as the most effective antiozonants for rubber compounds (Gilbert, Beck, and Calabrese 1990).

Following the Rock Island Arsenal Technical Report, the first PPDs developed were active antiozonants but they were not as effective as 6PPD as they only offered protection of rubber compounds for approximately 1.5 years. Among the first developed PPDs, IPPD (phenyl-p-phenylenediamine) and DAPD (diaryl-p-phenylene diamine) were the first to be used in rubber compounds in the mid-1960s. DAPD reacts minimally with ozone, and IPPD reacts too fast with ozone, leading to premature depletion. Thus, the speed of the reaction with ozone is a critical consideration. The final PPDs to become commercialized were 6PPD, 7PPD, and 8PPD (Kuczkowski 1989).

Some tire manufacturers began using 6PPD in tire manufacturing in the mid 1960’s and early 1970’s. In 1964, a British patent (Monsanto Company 1965) was published regarding the manufacturing of the 6PPD molecule and in 1968 a new factory was built supplying an increased volume of 6PPD to the U.S. tire industry. By 1975, 6PPD comprised 60% of the antiozonant used in tires (U.S. EPA 1975).

1.2.3 How Are Species Exposed?

A well-established route of exposure to 6PPD-q for coho salmon is via surface water from roadway runoff transported by stormwater (Tian et al. 2021; 2022). TWP containing 6PPD, that can transform to 6PPD-q in the environment (USEPA 2023), are nearly ubiquitous in the urban

environment (Wagner et al. 2018; Kole et al. 2017) (see Figure 1-4). The particles are shed along roadways and parking areas. TWC are transported via air and surface water. Stormwater is a major route of transport from the road to waterbodies where, if present, coho salmon are exposed to 6PPD-q. Coarse TWP are likely to deposit onto surfaces near roadways, while finer TWP (a smaller mass fraction of total TWP generated) can transport and disperse in air (Baensch-Baltruschat et al. 2020). Since a portion of TWC are also airborne, transport of 6PPD-q can occur over large distances, leading to atmospheric deposition of TWP containing 6PPD and 6PPD-q far from the road (Chen et al. 2023). TWP transport may result in direct 6PPD-q deposition into surface waters or onto streambanks and impervious surfaces where it can be mobilized by stormwater runoff (Johannessen et al. 2022b).

Many urban stormwater systems are designed to control flooding, not capture and treat contaminants that settle via air deposition and found in water. In separate storm sewer systems, rainwater is transported to natural receiving waters through a network of ditches and pipes without natural or engineered green spaces to remove deposited airborne particulates and water pollutants prior to entering surface waters. Additionally, some areas with installed stormwater best management practices are failing to contain stormwater discharge due to increased urbanization and storm events that are larger than the infrastructure was designed for (Levin, Howe, and Robertson 2020), leading to direct conveyance of 6PPD-q to vulnerable aquatic ecosystems.

Additional investigation is needed to determine the environmental concentrations of 6PPD-q in urban streams across the country, to understand potential impacts on sensitive species in regions beyond the Pacific Northwest (see Figures 1-3). The fate of 6PPD-q; including for example, factors that influence its formation in tires and TWPs. Additional uncertainties include: the transport and deposition of 6PPD-q from TWPs, leaching rates from TWPs, as well as 6PPD-q's persistence and bioaccumulation potential. Other potential exposure routes are being investigated, including programs that divert scrap tires from landfills recycle the tires into crumb rubber materials used on sports fields, rubber-modified asphalt, tire-derived aggregate used in civil engineering projects. The levels of 6PPD-q released from these and other recycled tire products, is also actively being researched.

Section 2 (Effects Characterization and Toxicity) of this document discusses other considerations for potential ecological and human health impacts.

1.3 What/Who Are Affected?

1.3.1 Impacts on fishes

Coho are the most sensitive species to 6PPD-q identified to date. In some observations of URMS, 100% mortality occurred in the pre-spawn coho; more typically, mortality ranged from 20%-90% (Scholz et al. 2011; Spromberg and Scholz 2011). Modelling indicates that this level of mortality could result in localized extinction of coho within 8 to 115 years, depending on whether the URMS occurs at the low end (20%) or the high end (90%) of the observed range (Spromberg and Scholz 2011). Further south, some populations of coho are already threatened and endangered. The role URMS play in their decline is unknown.

URMS has not been directly observed in brook trout or rainbow trout/steelhead. Brook trout populations are in decline in many locations within their range (Smith and Sklarew 2013; Eastern Brook Trout Venture 2024). Smith and Sklarew (2013) found a correlation with distance between a creek's distance roads and brook trout populations. This is just a correlation with roads and may not be related to 6PPD-q but instead other ways that roads can adversely affect water quality. Rainbow trout have been broadly introduced throughout the U.S. and their populations have few concerns. However, the steelhead populations that are native to the west coast are in decline and include threatened and endangered populations (NOAA 2023); there is no evidence to support or refute 6PPD-q involvement in their decline. These species of trout are popular with anglers (USFWS 2000; USGS 2023) and may substantially contribute to the recreation economy in a region (Chesapeake Bay Program 2018).

1.3.2 Ecological System Impacts

6PPD-q can increase the mortality rates of keystone species, potentially resulting in impacts on ecological systems. For example, as a keystone species, Pacific coho salmon support ecosystem health (including riverine and marine) as food for other species and bring nutrients to habitats (Field and Reynolds 2012; Holtgrieve and Schindler 2011). Their migration back from the ocean to their natal streams to spawn and ultimately die, provides marine nutrients to the terrestrial environment (Naiman et al. 2002). Salmon and ocean-going (known as anadromous) steelhead support the functioning of whole ecosystems (Holtgrieve and Schindler 2011). In Washington State's Puget Sound Southern Resident orca whales – which are listed as an endangered species – primarily rely on salmon for their diet (NOAA Fisheries 2022a). Fish can also serve as indicator species, pointing to greater problems in the ecosystem's health. With acute mortality identified in other fish species and research just getting started on sublethal or chronic impacts to fish and fisheries, ecosystem health issues in other areas may arise.

1.3.3 Tribal Nations

Indigenous communities have serious concerns over the loss of coho salmon and potentially other aquatic species due to exposure to 6PPD-q from stormwater runoff into fish-bearing watersheds. Testimony from Natural Resources Director of the Nisqually Tribe David Troutt to the U.S. House of Representatives Committee on Natural Resources on 6PPD-quinone introduces some of the deep concerns raised by some Tribal nations:

“Thank you for the opportunity to testify at today’s hearing on the impacts of 6PPD Quinone. My testimony will focus on how this toxic chemical is negatively effecting salmon populations, treaty rights, as well as solutions to address this issue . . . It is important to understand the value and role of salmon in the culture and traditions of the Nisqually Indian Tribe and other tribes in the Northwest. The tribes of the Salish Sea consider the salmon to be their brothers – family members to be honored, protected, treasured, and a gift from the creator. Salmon have been the primary source of protein for the tribes for 10,000 years. The location and movement of their villages were directly connected to the returns of salmon and steelhead. Their mythology and traditions are inextricably linked to salmon. Salmon is the central figure in Nisqually culture and traditions.

1 *Salmon and fishing for salmon on the Nisqually River is the life blood of the*
2 *Nisqually people.* ” (“Testimony of David Troutt” 2021)

3 In the Pacific Northwest in particular, salmon are significant to Tribal Nations for cultural
4 practices, food sovereignty, community health, traditional knowledge, their way of life, and their
5 identity as Salmon People ([Columbia River Inter-Tribal Fish Commission, 2021](#)). These Tribal
6 Nations’ right to fish is an inherent right, and for Tribal Nations along the West Coast (NOAA
7 Fisheries 2023b) has been affirmed through Treaty Rights and reaffirmed through court case
8 decisions. For example, the Boldt Decision (also known as United States v. Washington, 1974)
9 established that the Tribes are entitled to 50% of the fishing catch in their usual and accustomed
10 fishing grounds (U&As) within WA State; additionally, the decision requires that the Tribes and
11 WA State co-manage fisheries together ([UW Gallagher Law Library, 2023](#)). Because of this co-
12 management status, the Tribes are important decision-makers in WA State. Over 50 additional
13 court filings between 1974 – 2021 have expanded upon the Boldt Decision and have continued to
14 reaffirm Tribal Treaty Rights.

15 Although many Tribes share the common interest of protecting salmon populations, each Tribal
16 Nation has its own interests. The following information captures some, but not all, of these
17 interests and concerns as identified by Tribal Nations. There are several compounding factors
18 that already negatively impact the resilience of salmon populations, including human-made
19 blockage of waterways (e.g., dams and culverts), habitat degradation and climate change.
20 Climate change increases water temperatures, reduces the availability of cold-water refuges and
21 estuary habitats, and lowers the water level of many streams from reduced precipitation and
22 snow melt (NOAA Fisheries 2023a) . Some Tribes are federally recognized while others are non-
23 federally recognized, which can create challenges for salmon recovery efforts in the face of these
24 difficulties. 6PPD-q is an additional, compounding factor that threatens Tribally-led salmon
25 recovery efforts like reintroduction, hatchery management, and habitat restoration.

26 While the immediate focus of most research has been on acute mortality, there are many
27 unanswered questions and concerns over possible sublethal and chronic effects that may reduce
28 the resilience of the fish over time. This has important implications not just for population health,
29 but for ecosystem resilience. Another concern expressed by some Tribal members (DTSC 2021)
30 is the concern for passing 6PPD-q through the food web (DTSC 2021, 2022). Many Tribal
31 Nations harvest salmon as the salmon come back to freshwater to spawn. That migration can be
32 triggered by storms; the flow of water helps the salmon surmount stream barriers, like sand bars,
33 but that water may carry 6PPD and 6PPD-q (Challis et al. 2021; Johannessen, Helm, and
34 Metcalfe 2021; Tian et al. 2022; Tian et al. 2021). This creates the possibility that the people
35 who harvest and eat the salmon may be exposed to these chemicals. The human health impacts
36 of 6PPD-quinone are still unstudied. Another concern is that the animals that hunt or scavenge
37 on salmon or carcasses may also be exposed to 6PPD-q. Similarly, the toxicological impacts on
38 these species is poorly characterized, as is the toxicokinetics of 6PPD-q in exposed fish and their
39 carcasses. Given these many concerns, in 2023, Earthjustice submitted a petition on behalf of the
40 Yurok Tribe, the Port Gamble S’Klallam Tribe, and the Puyallup Tribe of Indians to the US EPA
41 to establish regulations prohibiting the manufacturing, processing, use, and distribution of 6PPD
42 under Section 21 of the Toxic Substances Control Act (TSCA) (Earthjustice 2023). This petition
43 was supported by the [Affiliated Tribes of Northwest Indians \(ATNI\)](#) and the states Washington,
44 Connecticut, Vermont, Oregon and Rhode Island ([EPA-HQ-OPPT 2023](#)).

While salmon are a very important part of this problem, many Tribal Nations have called for state governments to look at this problem through a holistic lens. This includes assessing the structures and institutions that caused the problem in the first place, like settler-colonist systems, transportation infrastructure and pursuing solutions that address these underlying problems.

1.3.4 Potential Economic and Community Health Concerns

The sensitive species, coho salmon, brook and rainbow trout, provide significant economic value because of their importance for commercial fishers and recreational anglers. Some communities' economic well-being relies on commercial, recreational, and tourism-based fish-related activities. The commercial salmon fishery, including coho, is economically important to coastal communities (NOAA Fisheries 2022b). Salmon caught by recreational anglers in streams contributed \$1,236 per fish to the California economy (FishBio 2017). Throughout the country, recreational fishing can be an important economic driver. For example, in the Chesapeake Bay Program (2018) states that, "brook trout are highly prized by recreational anglers who spend millions of dollars annually on related goods and services, including travel, that directly benefit local and state economies." Steelhead (*O. mykiss*) introduced to the Great Lakes, generate economic value to the region through purchasing gear, licenses, and guide services (Miller 2017). Impacts to these species may raise concerns for state fish and wildlife agencies throughout the country.

Additional communities, including **vulnerable populations**¹ and **overburdened communities**² may experience direct and indirect harm stemming from 6PPD-quinone. Many concerns are related to fish mortality (reduced fish abundance) and consumption by humans. These concerns extend from the Pacific Northwest to other parts of the country where potentially impacted fish species are important, e.g., brook trout in the mid-Atlantic and northeast. Some non-Indigenous ethnic groups are also subsistence fishers, meaning that a significant portion of their diet consists of locally harvested fish and other aquatic species (Savadatti et al. 2019; Schantz et al. 2010). Salmon and other impacted aquatic species may be of cultural importance to different communities. Additionally, the knowledge and observation of thriving salmon and other fish species in the environment can be an important part of psychological and human well-being.

6PPD-q has been found in roadway dust and airborne particulate (Huang et al. 2021; Johannessen et al. 2022). If adverse impacts of human exposure to 6PPD-q are found, there may be environmental justice concerns for near roadway communities (Rowangould 2013). The near

¹ Vulnerable populations are "...population groups that are more likely to be at higher risk for poor health outcomes in response to environmental harms..." and include, but are not limited to: racial or ethnic minorities, low-income populations, populations disproportionately impacted by environmental harms, and populations of workers experiencing environmental harms (Washington State [Provisional Community Engagement Plan for HEAL Act Implementation, 2023](#)).

² Overburdened communities "...means a geographic area where vulnerable populations face combined, multiple environmental harms and health impacts..." ([Provisional Community Engagement Plan for HEAL Act Implementation, 2023](#)).

ubiquity of tire usage and the fact that 6PPD-q exposures arise from TRWP generation from roadways highlights the close linkages between the economy, human health and safety, transportation infrastructure, ecosystem health, and protection of important aquatic species. For more information see [Section 2 \(Effects Characterization and Toxicity\)](#), [Section 4 \(Occurrence, Fate and Transport of 6PPD and 6PPD-q\)](#), and [Section 7 \(Information Gaps and Research Needs\)](#) of this document.

1.4 How Do We Find 6PPD-q?

EPA recently released a draft analytical method for detection of 6PPD-q in aqueous matrices, predominantly stormwater and surface water (U.S. EPA 2023a). The Washington Department of Ecology (2023) also has a procedure for measuring of 6PPD-q “Extraction and Analysis of 6PPD-quinone”. For more information, see the “Analytical Methods and Monitoring” section.

1.5 What Now? Once We Find It, How Do We Mitigate It? What Else Can We Do?

Implementation and expansion of stormwater management practices may reduce exposure to 6PPD-q and other pollutants in aquatic habitats in other areas of the county. State, Federal, and Tribal resources are supporting implementation of stormwater best management practices (BMPs) which are immediately available. Research efforts are looking to bolster these BMPs, such as methods for removing 6PPD-quinone and other contaminants from stormwater runoff before it enters receiving water bodies. Mitigation and stormwater management will be key to reducing the potential impacts in the short term. Bioretention filtration has been shown to be effective at preventing coho mortality (McIntyre et al. 2023; Spromberg et al. 2016). Reductions in other stormwater pollutants, such as metals, are also seen when this BMP is applied (McIntyre et al. 2023). See [Section 6 \(Mitigation Measures and Solutions\)](#) for more information. However, BMPs can be expensive to implement and maintain, thus, there is a need to ensure that implementation is focused on areas of highest risk, which includes both high concentrations of 6PPD-q in the aquatic environment in conjunction with the presence of sensitive species. Likely, solutions to 6PPD-q will include a mixture of source control, alternatives to 6PPD, stormwater BMPs, education, and more. Mitigation strategies should also consider the potential for airborne transport and exposures when designing best management practices for 6PPD-q. See [Section 6 \(Mitigation Measures and Solutions\)](#) for more information.

1.6 Knowledge and Research Gaps

As a contaminant of emerging concern (CEC), many data gaps and questions about 6PPD-q remain. These questions, while important, do not obviate the need for action based on available information to date regarding the concern and impact of 6PPD-q. For example, the US EPA granted the aforementioned citizens petition and “plans to take action to address the risk to the environment presented by 6PPD, and the degradant 6PPD-q through an advance notice of proposed rulemaking under TSCA section 6,” (U.S. EPA 2023b). In addition, [California](#) and Washington State have already initiated some regulatory actions on alternatives and more. For a detailed list with explanation see the “[Information Gaps and Research Needs](#)” section.

1.7 Governance, Litigation, and the Law

Even with research ongoing as to the full scope of rubber products containing 6PPD, the ubiquitousness of tires, TWP, and 6PPD opens the door to many potential legal, governance and litigation issues.

Stormwater laws and permits (largely stemming from Clean Water Act authority) apply to a subset of stormwater discharges, whether through construction site permits, municipal stormwater permits, combined sewer system permits, or industrial and multi-sector general permits. These permits, however, do not fully control stormwater from roads, lots, bridges, or other potential TWC sources. In New England, EPA's Region 1 office granted a petition to use the CWA's residual designation authority (40 CFR 122.26(a)(9)(i)(C) and (D)) and plans to develop permits to control pollutants in stormwater from privately-owned and unregulated sources throughout three watersheds in Eastern Massachusetts (U.S. EPA 2018). How stormwater permit are changed, used, or developed to address 6PPD-q is generally yet to be determined; two draft permits have been developed within the Puget Sound (but not yet finalized) that at least discuss 6PPD-quinone – the Washington State MS4 general permits (WA Dept of Ecology 2023) and the EPA's stormwater individual permit for Joint Base Lewis-McChord (U.S. EPA 2017). Beyond stormwater management, several other statutes may play a role in controlling, mitigating, or eliminating 6PPD-quinone depending on the considered fate and transport and/or exposure pathway. Relevant statutes may be the Clean Air Act (CAA), the Resource Conservation and Recovery Act (RCRA), and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as Superfund. From a resource management standpoint, state and federal managers of fish stocks may be required – depending on the action in front of them – to include emerging data on acute and chronic exposure risks and impacts to affected species in catch quota establishment, biological opinions, ESA reviews, or NEPA (and state equivalent) reviews. While environmental regulations may often regulate a specific media (stormwater, groundwater, solid waste, air, etc) they are not mutually exclusive - hence the challenge in regulating something like TWC. For multiple routes of exposure, including for example via aerial deposition, there may need to be a multi-regulatory approach taken to significantly impact the issue. For more details, see the “Policy” subsection of the “Solutions” Section.

In terms of recent actions, in addition to EPA granting the aforementioned citizens petition, in November 2023, two fishing groups, the Institute for Fisheries Resources and the Pacific Coast Federation of Fishermen's Associations, filed a lawsuit against 13 U.S.-based tire manufacturers under Section 9 of the Endangered Species Act (Earthjustice 2023). In June 2022, notice letters of intent to sue under the Clean Water Act were also sent to five municipalities in the Puget Sound (Seattle, Mukilteo, Normandy Park, Burien, and Seatac) by the organization Puget Soundkeeper, though no complaints have been filed in court at this time (Puget Soundkeeper 2022).

EPA's [6PPD-q website](#) includes information on available resources and Agency actions to address 6PPD-q. For more information see the “Policy” subsection of [Section 6 \(Mitigation Measures and Solutions\)](#).

1.8 Steps for Addressing 6PPD-Quinone?

- To conclude, we present here basic steps for a decision maker and environmental manager when confronting 6PPD-q and how this document can be best utilized as a resource.

Checklist for decision makers and environmental managers – do you have this problem and what are first steps?

Do I have sources of 6PPD?	See <i>Introduction</i>
Do I have potential ecological or human receptors?	See <i>Hazard/Toxicity section</i>
Do I have concentrations of 6PPD-q in my aquatic environment that can impact the sensitive species?	
Do I have ways (exposure pathways) for 6PPD/6PPD-q to reach potentially sensitive species at concentration that may cause adverse health effects?	See <i>Environmental Assessment Strategies</i>
Examples: stormwater inventory, discharges, confluence of sources (e.g., roadways) to aquatic habitats, tire-derived assets (e.g., turf fields)	
Do I have tools to interrupt exposure pathways and manage sources?	See <i>Solutions</i>

- Addressing 6PPD and 6PPD-quinone poses many challenges. These are often associated with CEC where a critical effect is observed in the environment, but full data are lacking. For more information on a framework for addressing CEC in general, see the [ITRC document on CEC](#).

2 EFFECTS CHARACTERIZATION AND TOXICITY

ITRC has developed guidance on the overview of the current understanding of 6PPD-q sources, exposure, fate, transport, toxicity, mitigation strategies, on-going research, and data needs. This section of the ITRC guidance describes:

- the toxicity of 6PPD-q and its parent compound 6PPD
- the ecological and human health effects associated with exposure to these contaminants in the environment
- how people may come into contact with 6PPD-q and 6PPD
- current knowledge gaps regarding potential the ecological and human health hazards

Section 1 (Introduction) and Section 4 (Occurrence, Fate and Transport of 6PPD and 6PPD-q) provide detailed discussions of sources, fate and transport mechanisms and levels of 6PPD-q and 6PPD in environmental compartments that are relevant to the effects and exposures discussed within this section.

2.1 Introduction

There are several different exposure pathways by which human or ecological receptors may be exposed to 6PPD-q and its parent compound 6PPD in the environment (see Figure 1-4 of the Problem Characterization section). As detailed in the Figure 1-4 and outlined in the Introduction (Section 1) and chapter on Occurrence, Fate and Transport of 6PPD and 6PPD-q (Section 4), sources of 6PPD-q and 6PPD include tire and road wear particles (TRWP), recycled tire products such as crumb rubber, and consumer products. For TRWP, stormwater runoff is believed to be the primary transport mechanism of 6PPD/6PPD-q to the aquatic environment (surface water and sediment), although there is also potential for airborne transport.

Potential aquatic ecological receptors, which include freshwater and marine organisms (vertebrates, invertebrates, and plants), may be exposed to 6PPD-q and 6PPD through direct uptake of water through respiratory surfaces, ingestion, and absorption. The route of exposure with greatest significance may vary between species or life stage. Exposure in terrestrial organisms (vertebrates, invertebrates, and plants) is poorly characterized, but it is possible that terrestrial receptors may be exposed via ingestion, inhalation, or absorption. There is evidence that 6PPD-q and 6PPD may bioaccumulate in food sources where humans may then also be exposed (Table 4-10).

Potential routes of exposures for humans include ingestion of soil, sediment, dust and water, dust inhalation, and dermal contact. At present, however, documented exposure through quantitative measure of 6PPD-q, 6PPD, or their metabolic products in humans is very limited. Biomonitoring studies of 6PPD-q or 6PPD exposure in the US are not currently available.

The effects of 6PPD-q and 6PPD on ecological and human health, as with most other chemicals, depends on the magnitude, frequency, and duration of exposure. Research on 6PPD-q toxicity is rapidly growing and, while some data is presented herein for 6PPD-q, data gaps have been

identified in our understanding of health effects. For both human and ecological receptors, the effects of 6PPD are better characterized than the effects of 6PPD-q. As a result, this section presents the currently available toxicity information for both 6PPD-q and 6PPD as a read-across substance to infer information for toxicologic endpoints where data is currently lacking for 6PPD-q. Additionally, as 6PPD is the only known source of 6PPD-q in the environment, regulatory authorities may need to mitigate the impacts of 6PPD-q through addressing the addition of 6PPD to consumer products. Therefore, understanding the toxicological effects of both 6PPD-q and 6PPD may be of benefit to interested parties.

Since the publication of Tian et al. (2021), interest in 6PPD-q has grown substantially. A search of the PubMed database¹ shows that the number of articles has risen exponentially year-over-year (Figure 2-1). It is important to note that this section is not intended to represent a comprehensive review of 6PPD-q and 6PPD toxicity studies. Additionally, a formal study reliability evaluation was not performed for the studies discussed in this section. Given the active research in this topic, it is highly likely that additional studies have been published since the release of this document. While the intent of this section is to present the most salient and recently available information on the toxicological effects of 6PPD-q and 6PPD, interested readers are encouraged to search the scientific literature for newly available information.

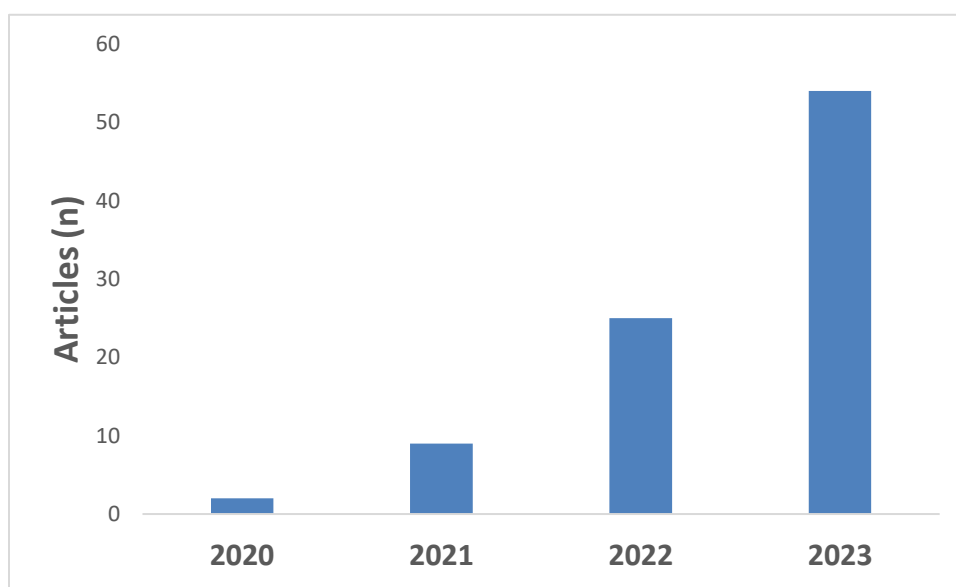


Figure 2-1. Number of articles mentioning 6PPD or 6PPD-q from 2020 – 2023.

2.2 Environmental Toxicology

This section reports the currently available toxicity information and knowledge gaps concerning the effects of 6PPD-q and 6PPD on freshwater, marine, and terrestrial organisms (vertebrates, invertebrates, and plants). Although 6PPD-q and 6PPD are similar in chemical structure, the toxicological relationship between the two chemicals is nuanced and depends highly on the

¹ Accessed December 9, 2023; search terms: (((("6ppd"[Text Word]) OR ("6ppd-q"[Text Word])) OR ("6ppd-quinone"[Text Word])) OR ("6ppdq"[Text Word]))

species and endpoints under consideration as described in the sub-sections below. Notably, when exposure occurs, both chemicals have the potential for highly potent aquatic toxicity. Given these considerations, each chemical will be discussed in its own sub-section and toxicity will be described on the basis of ecological taxa. The absence of a taxon in the following sub-sections represents a data gap at the time this document was published. Additional information is available from other previously published reports (DTSC 2022; ToxServices, LLC 2021; ECHA 2021; OSPAR Commission 2006; OECD 2004).

2.2.1 Environmental Toxicity of 6PPD-q

As noted in the Introduction (Section 1), 6PPD-q, a transformation product of the antiozonant 6PPD, was identified in stormwater samples in late 2020 as a causal toxicant in urban runoff mortality syndrome (URMS) among adult and juvenile coho salmon (*Oncorhynchus kisutch*) (Tian et al. 2021). While a considerable amount of work has focused on characterizing effects in various fish species, there are still relatively few studies on invertebrates and algae, and no data on aquatic amphibians. Research to date regarding the aquatic toxicity of 6PPD-q is discussed in the sub-sections below, and Table 2-1 and Table 2-2 summarize the range of known effect levels observed in various species for acute and chronic toxicity, respectively. Compared to those of aquatic studies, data for terrestrial wildlife is generally limited. There are currently no available data on toxicity toward birds, reptiles, and amphibians, while information for other terrestrial receptors is scarce. The following sub-sections summarize key information on the toxicological effects of 6PPD-q for taxa with available data.

2.2.1.1 Toxicity of 6PPD-q to Fish

In coho salmon, URMS begins with a common progression of behavioral symptoms which include increased surface swimming, loss of equilibrium and buoyancy, gasping at the surface, and ultimately mortality (Scholz et al. 2011; Chow et al. 2019; Tian et al. 2022). After identification of this chemical in stormwater and surface water, follow-up laboratory toxicity tests using 6PPD-q confirmed that 6PPD-q is highly acutely toxic to coho salmon, with 24-hour median lethal concentration (LC₅₀, the concentration at which 50 percent mortality of test subjects was observed) ranging from 0.040 µg/L in coho hatchlings (3 weeks post-swim up) to 0.095 µg 6PPD-q/L in juveniles (1+ year old) (Greer et al. 2023a; Lo et al. 2023; Tian et al. 2022).

Since the publication of Tian et al. (2021), acute mortality has been evaluated in over a dozen species of fish. Of the fish species studied thus far, coho salmon are the most sensitive. Lethal concentrations vary widely among fish (24-hour LC₅₀ of 0.041 µg/L in coho salmon to a 96-hour LC₅₀ greater than 500 µg/L in the Chinese rare minnow (*Gobiocypris rarus*), highlighting the likelihood of a unique species-specific physiological mechanism for URMS (Di et al. 2022; Lo et al. 2023). To date, no known adverse outcome pathway can explain the difference in sensitivities between species; however, hypothesized mechanisms of action are discussed in the next sub-section.

Variability in toxicological response to 6PPD-q exists between species, but also within species. 6PPD-q appears to exhibit age-dependent toxicity in coho salmon, with Lo et al. (2023) reporting a 24-hour LC₅₀ of 0.041 µg/L in newly feeding (approximately 3 weeks post-swim up) fish, Greer et al. (2023a) reporting a 12-hour LC₅₀ of 0.080 µg/L at a mean age of 189 days, and Tian

et al. (2022) reporting a 24-hour LC₅₀ of 0.095 µg/L in fish greater than 1 year old. Greer et al. (2023b) exposed eyed eggs (unhatched embryos) to a 24-hour pulse of 6PPD-q twice weekly (simulating intermittent rain events under laboratory conditions) until hatch at concentrations of 0.10, 0.90, and 7.22 µg/L. No significant mortality occurred in the 0.10 µg/L treatment, which was equivalent to the estimated 24-hour LC₅₀ of juveniles. Significant mortality occurred in the 7.22 µg/L treatment by the second exposure pulse with only 21% of embryos surviving to hatching and egg yolk absorption. These data suggest that the chorion, a protective membrane enveloping embryos, potentially mitigates uptake of waterborne toxicants to the embryo, such as 6PPD-q.

Sensitivity to 6PPD-q does not seem to be related to phylogenetic proximity (Figure 2-2). Effect concentrations of 6PPD-q have been reported for several salmon species, however, none are as sensitive as the coho. In fact, studies have shown 6PPD-q was not lethal to Atlantic (*Salmo salar*) and sockeye (*Oncorhynchus nerka*) salmon even at the highest test concentrations (12.16 µg/L for 48 hours and 50 µg/L for 24 hours, respectively) (Foldvik et al. 2022; Greer et al. 2023a). Lo et al. (2023) reported that the 24-hour LC₅₀ for Chinook salmon (*Oncorhynchus tshawytscha*) was greater than 67.31 µg/L, while Greer et al. (2023a) predicted a 24-hour LC₅₀ of 82.1 µg/L. It should be noted that risk to Chinook salmon is unlikely as the effect values exceed both the limit of solubility (38 µg/L) and reported environmental concentrations of 6PPD-q (less than 2.85 µg/L) (Hu et al. 2023; Johannessen et al. 2022). The white-spotted char (*Salvelinus leucomaenis pluvius*) and the brook trout (*Salvelinus fontinalis*), however, are relatively sensitive, with 24-hour LC₅₀ values of 0.51 µg/L and 0.59 µg/L, respectively (Brinkmann et al. 2022; Hiki and Yamamoto 2022). Char species are not universally sensitive to 6PPD-q, though, as no significant mortality was observed in tests with arctic char (*Salvelinus alpinus*), bull trout (*Salvelinus confluentus*) or Southern Asian dolly varden (*Salvelinus curilus*) (Brinkmann et al. 2022; Brinkmann 2023; Hiki and Yamamoto 2022). Rainbow trout (*Oncorhynchus mykiss*) are a sensitive species, with a reported 24-hour LC₅₀ of 1.96 µg/L and 96-hour LC₅₀s ranging between 0.64 and 2.26 µg/L (Brinkmann et al. 2022; Di et al. 2022; Nair et al. 2023). Given that the rainbow trout is both an established laboratory model and sensitive to 6PPD-q, it may serve as an effective sentinel for future assessment of effects on what may be a novel toxicity pathway.

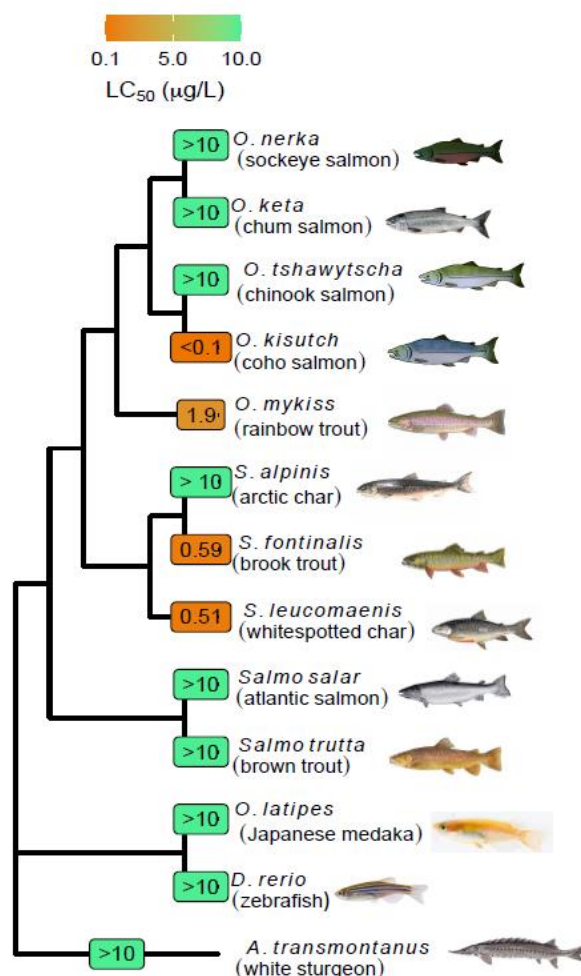


Figure 2-2. Toxicity to 6PPD-q does not follow a genetic relationship. Phylogeny of fish that have been tested for acute toxicity to 6PPD-q. For each fish, the associated LC₅₀ (based on an individual study) is represented by the color-gradient scale and numeric value. This graphic was created by Justin Greer and reproduced with permission of USGS.

Studies have reported other common model species as being poor indicators of 6PPD-q toxicity, including zebrafish (*Danio rerio*) with a 96-hour LC₅₀ greater than 132.92 μg/L for embryos, Japanese medaka (*Oryzias latipes*) with a 96-hour LC₅₀ greater than 34 μg/L for juveniles, and fathead minnows (*Pimephales promelas*) with a 168-hour LC₅₀ greater than 39.97 μg/L for embryos and 96-hour LC₅₀ greater than 9.4 μg/L for adults (Anderson-Bain et al. 2023; Hiki et al. 2021; Varshney et al. 2022). The complete list of currently known acute lethal concentrations across fish species can be found in [Table 2-1](#).

While initial research on 6PPD-q focused on acute mortality, recent work has begun investigating sublethal effects, which may be a concern for species that are not acutely sensitive to the chemical.

2.2.1.2 6PPD-q Mechanism of Action in Fish

The specific mechanism, or mechanisms, that underly the acute mortality associated with 6PPD-q are not fully understood. While apical effects are consistent with cardiorespiratory and/or neurological toxicity (changes in ventilation, surface swimming, erratic behavior, etc.), tracing them back to a discrete molecular initiating event has proven difficult. Current evidence suggests that biotransformation may play a role in driving the species- and tissue-specific toxicity observed in fish.

Mahoney et al. (2022) reported that rainbow trout liver cells were not sensitive to 6PPD-q, while cell viability was decreased, and oxygen consumption rate was increased by a proposed uncoupling of the mitochondrial electron transport chain in gill cells. Monohydroxy metabolites of 6PPD-q were detected in the liver, but not gill tissues, suggesting that the chemical was more effectively metabolized in liver (Mahoney et al. 2022). In another recent study, biliary O-glucuronide metabolites of 6PPD-q were found to be present at higher levels in tolerant species than in sensitive species, such as coho salmon. It was therefore suggested that species-specific differences in expression of biotransformation enzymes may be a key factor in the variable toxicity of 6PPD-q (Montgomery et al. 2023). In a separate study, juvenile rainbow trout were exposed to seven different PPD-quinones (DTPPD-quinone, HPPD-quinone, OPPD-quinone, IPPD-quinone, DPPD-quinone, CPPD-quinone, and 6PPD-q) as well as 6PPD for 96 hours and, subsequently, whole-body homogenates were analyzed for nontargeted identification of metabolites. The LC₅₀ for 6PPD-q was 0.64 µg/L, however no toxicity occurred for any of the other PPD-quinones up to 50 µg/L. Tissue concentrations of 6PPD-q was an order of magnitude higher than the six other PPD-quinones. Fish that survived 6PPD-q exposure also exhibited lower concentrations of 6PPD-q, suggesting that survival may be dependent on an organism's ability to biotransform PPD-quinones to less toxic hydroxy-metabolites. Notably, the same study reported that two OH-6PPD-q isomers were detected in fish exposed to 6PPD-q. One isomer exhibited hydroxylation on the alkyl side chain and one on the aromatic ring. In contrast, the other six, less toxic PPD-quinones only appeared to undergo aromatic ring hydroxylation. It is unknown if this alkyl side chain isomer may actually elicit toxicity through interaction with an unknown protein target (Nair et al. 2023).

There are also some data to indicate potential enantioselective toxicity of 6PPD-q. In a study by Di et al. (2022), rainbow trout and the rare minnow (*G. rarus*) were exposed to R-enantiomer, S-enantiomer, or a racemate of both 6PPD and 6PPD-q (Figure 2-3) for 96 hours. In *G. rarus*, LC₅₀ values were identical for both R-6PPD and S-6PPD (201 µg/L), while the 6PPD racemate was slightly lower (162 µg/L), and no mortality occurred up to 500 µg/L R-6PPD-q, S-6PPD-q, or 6PPD-q racemate. In contrast, rainbow trout were not sensitive to 6PPD, with no mortality occurring at concentrations up to 400 µg/L. However, rainbow trout responded differently to R-6PPD-q, S-6PPD-q, or 6PPD-q racemate, with LC₅₀ values of 4.31, 1.66, and 2.26 µg/L, respectively. As rainbow trout are known to be sensitive to 6PPD-q, it is feasible, as the authors propose, that 6PPD-q enantiomers may bind a presently unknown molecular target with varying affinity.

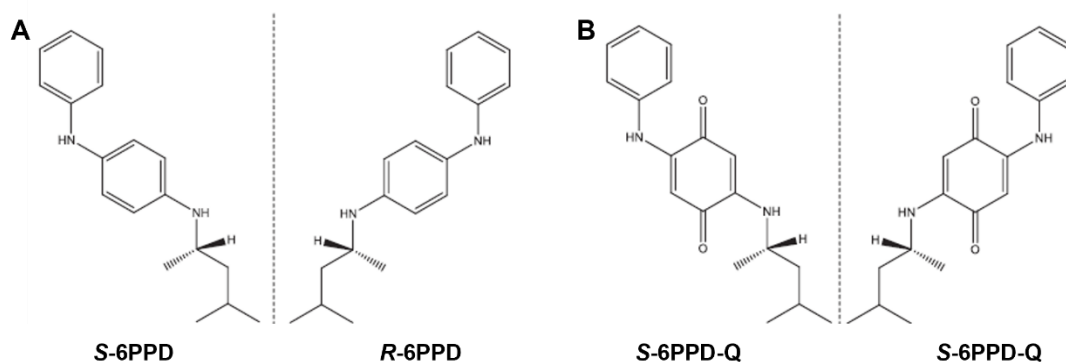


Figure 2-3. Molecular structures of 6PPD and 6PPD-q enantiomers.

2.2.1.3 Toxicity of 6PPD-q to Aquatic and Benthic Invertebrates

Acute toxicity tests using invertebrate species thus far have shown that, among the species tested, aquatic and benthic invertebrates are not acutely sensitive to 6PPD-q. No significant mortality was observed in the following freshwater species: amphipods (*Hyaella azteca*), water flea (*Daphnia magna*), rotifer (*Brachionus calyciflorus*), file ramshorn snail (*Planorbella pilsbryi*), washboard mussel (*Megaloniais nervosa*), or mayfly (*Hexagenia spp.*) (Table 2-1) (Hiki et al. 2021; Klauschies and Isanta-Navarro 2022, Prosser et al. 2023). Klauschies and Isanta-Navarro (2022) also reported no mortality in *B. calyciflorus* after 12 days of exposure to 6PPD-q, indicating no chronic toxicity to this species. Sublethal endpoints such as fecundity and oxidative stress were also not affected in *B. calyciflorus* at concentrations up to 1000 µg/L 6PPD-q. Additionally, the only two studies currently available that assessed acute toxicity of 6PPD-q to marine invertebrates also found no significant mortality in the amphipod *Parhyale hawaiiensis* and the rotifer *Brachionus koreanus* (Botelho et al. 2023; Maji et al. 2023). Therefore, it appears that aquatic and benthic invertebrates are not acutely sensitive to 6PPD-q, while sublethal effects represent a data gap.

2.2.1.4 Toxicity of 6PPD-q to Algae and Aquatic Plants

To date, one study has specifically evaluated the toxicity to 6PPD-q in algae (J. Wu et al. 2023). Cultures of the single-celled green alga *Chlamydomonas reinhardtii* were exposed to 6PPD-q for 72 hours at concentrations of 250 and 1000 µg/L, and cell viability was evaluated by calculating absorbance ratios between exposed and unexposed groups. The study observed a significant treatment effect with an increase in algal cell death between the exposed and unexposed groups, but there was no major difference in viability between the 250 and 1000 µg/L treatments (J. Wu et al. 2023). Although the exposure concentrations exceeded environmental relevance, the study provided evidence of a possible genotoxicity mechanism (J. Wu et al. 2023).

2.2.1.5 Toxicity of 6PPD-q to Mammals

Some data are available that may be used to evaluate the sublethal effects of 6PPD-q on mammalian wildlife. One study found that oral exposure at 10 and 100 mg/kg 6PPD-q to mice for six weeks resulted in fatty liver syndrome (L. Fang et al. 2023), as did the parent compound 6PPD. The authors determined that 6PPD tended to affect gene expression related to fatty acid

metabolism; in comparison, 6PPD-q affected inflammatory pathways. Mice were not monitored for apical outcomes. Although steatosis is an adverse outcome and indicator of liver damage in human health evaluations, no studies in wild mammals are available that would define steatosis as an adverse apical outcome in non-human organisms. Another study (He, Gu, and Wang 2023) evaluated toxicity to multiple organs in rodents and found that 28 days of 6PPD-q injection (intraperitoneal) exposure damaged liver and other organs. Concentrations in the livers in this study were approximately 20 ng/g, which was similar to what found in their oral exposure study. Additionally, Zhao et al. (2023a) found that oral gavage exposure to 4 mg/kg 6PPD-q in pregnant mice resulted in chemical transfer to dams, resulting in concentrations in livers (mean 200 ng/g) that were greater than studies cited above. These results may be of use for ecological risk assessment based on tissue residue values if an apical outcome was associated with organ damage as shown in the study.

2.2.1.6 Toxicity of 6PPD-q to Soil Invertebrates

There are a limited number of studies that examine the effects of 6PPD-q on terrestrial invertebrates that can be directly used for ecological risk assessments. The most relevant study was conducted in springtails (*Folsomia candida*), a commonly tested soil-dwelling organism with a global distribution (Xu et al. 2023). The authors estimated a nominal 28-day LC₅₀ of 16.31 µg/kg soil for adult springtails. Reproduction was not significantly affected by 6PPD-q exposure at nominal soil concentrations up to 5000 µg/kg. Notably, genomic analysis showed a difference in effect on microbes from soil compared to those from the guts of springtails. Exposure to 5000 µg/kg was associated with an increase in nitrogen cycling genes in soil microbes and a decrease in springtail gut microbes. Changes to microbial community structure and function in response to 6PPD-q exposure may be an important endpoint to examine with respect to the provisioning of terrestrial ecosystem services.

Further work with invertebrate taxa has been conducted in *C. elegans* that may be informative for effects in soil invertebrates (Hua et al. 2023a; 2023b; Y. Wang, Hua, and Wang 2023). Because this work involved aqueous exposures (0.1, 1, and 10 µg/L 6PPD-q), extrapolation to exposure concentrations in the terrestrial environment is uncertain. However, it is worth summarizing effects because *C. elegans* is a soil-dwelling organism. Hua et al. (2023b) found that reproductive capacity declined with increasing aqueous 6PPD-q concentration, potentially as related to gonadal development. 6PPD-q exposure at concentrations as low as 1 µg/L reduced locomotion in *C. elegans*. It also led to the development of neurodegeneration, with the former occurring at lower concentrations than the latter (Hua et al. 2023a), indicating a potential for neurobehavioral and thus apical effects in this species. The same exposure conditions also led to an accumulation of lipids *in vivo*, suggesting that 6PPD-q has the potential to alter lipid metabolism in invertebrates such as *C. elegans* (Wang et al. 2023).

2.2.1.7 Toxicity of 6PPD-q to Terrestrial Plants

Although it has been shown that 6PPD-q can be taken up in plant tissues (Castan et al. 2023) and therefore potentially expose herbivores, no published plant toxicity tests could be found as of the writing of this summary.

2.2.1.8 Toxicity of 6PPD-q to Terrestrial Microorganisms

There are limited ecologically relevant studies examining the effect of 6PPD-q on terrestrial microorganisms. One study, as described above, assessed the impact of 6PPD-q in soil exposure on microbial diversity and taxonomic abundance, with potential ramifications for nitrogen cycling (Xu et al. 2023). Given these findings, potential implications to ecosystem services, and the detected or expected presence of 6PPD-q in soils, this area requires more research.

2.2.2 Environmental Toxicity of 6PPD

2.2.2.1 Toxicity of 6PPD to Fish

Coho salmon (*O. kisutch*) are less sensitive to 6PPD compared to 6PPD-q, with a reported nominal 24-hour LC₅₀ of 251 µg 6PPD/L (Tian et al. 2021). However, this study notes a lack of confidence in the measured concentrations of 6PPD in water due to its poor solubility, high instability, and formation of transformation products during exposure. As such, only nominal values were used to estimate the LC₅₀ value. In another study using the common laboratory test species, zebrafish (*Danio rerio*), the 96-hour LC₅₀ was equal to 442.62 µg 6PPD/L (Varshney et al. 2022). Similarly, this study noted that the LC₅₀ values for 6PPD should be used with caution due to the chemical's low water solubility and stability, high K_{ow}, and formation of transformation products. Another study, conducted under good laboratory practice (GLP), reported a 96-hour LC₅₀ equal to 28 µg/L (measured) for Japanese medaka (*O. latipes*) exposed to 6PPD (ECHA 2021). A similar study using Japanese medaka corroborated these results and reported 80% mortality in medaka after a 96-hour exposure to a time weighted average (TWA) concentration of 107 µg/L 6PPD (Hiki et al. 2021), indicating that the LC₅₀ was lower than 107 µg/L. There are two long term studies that tested toxicity of 6PPD in fathead minnow (*P. promelas*), and the LC₅₀ values ranged from 35 µg/L (21-day exposure) to 150 µg/L (28-day exposure) (Ryan S. Prosser et al. 2017; Monsanto Company 1979). 6PPD was not acutely toxic to rainbow trout (*O. mykiss*) exposed to a concentration of 50 µg/L 6PPD for 96 hours (Nair et al. 2023). Overall, the reported LC₅₀ values for 6PPD acute toxicity in fish species are much higher than the maximum measured concentrations of 6PPD in river water (0.00129 µg/L) and road runoff (0.00752 µg/L) (H.-Y. Zhang et al. 2023).

Several studies using zebrafish (*D. rerio*) reported sublethal effects after exposure to 6PPD. For example, endpoints such as development, locomotor behavior, respiration and heart rate, or oxidative damage were affected after exposure to 6PPD at various concentrations tested in the literature (Ji, Huang, et al. 2022; Varshney et al. 2022; Hiki et al. 2021; S.-Y. Zhang et al. 2023; C. Fang et al. 2023).

2.2.2.2 Toxicity of 6PPD to Aquatic and Benthic Invertebrates

There are limited data on acute toxicity of 6PPD to aquatic and benthic invertebrates. In water flea (*D. magna*), a 48-hour LC₅₀ value equal to 230 µg/L was reported from a GLP study based on measured concentrations (ECHA 2021). A more recent study observed 100% mortality of *D. magna* after 48-hour exposure to 6PPD at a time weighted average concentration of 138 µg/L, indicating that the 48-hour LC₅₀ is less than 138 µg/L (Hiki et al. 2021). It is unclear why there are inconsistencies between the studies that tested *D. magna*. Physicochemical properties of

6PPD such as low water solubility and stability, high K_{OW} , and formation of transformation products may have resulted in uncertainties regarding the derived LC_{50} values.

In the epibenthic amphipod *H. azteca*, the 96-hour LC_{50} based on measured concentrations was 250 $\mu\text{g/L}$ (R. S. Prosser et al. 2017). Chronic toxicity (28-day exposure) tests were also performed as part of this study in both water and sediment and the 28-day LC_{50} was equal to 13 $\mu\text{g/L}$ for water exposures and 135 $\mu\text{g/g}$ sediment dry weight for sediment exposures. The results from the chronic water and sediment toxicity tests indicated that 6PPD is more bioavailable in water versus sediment, and the authors suggested this may be due to sorption of 6PPD to organic matter in sediment. Additionally, the concentration of 6PPD in sediment and water declined over the study duration, and the authors noted that bioavailability of 6PPD to aquatic and sediment dwelling organisms in freshwater ecosystems should be specifically investigated due to the chemical's apparent degradation. Prosser, Bartlett, et al. (2017) also exposed aquatic worms (*Tubifex* sp.) in a chronic (28-day exposure) sediment toxicity test. Their results indicated that mortality was the least sensitive endpoint (28-day LC_{50} of 67 $\mu\text{g/g}$ dry weight), while reproduction was a more sensitive endpoint with a 28-day 10 percent effect concentration (EC_{10} , the concentration at which an effect was observed in 10 percent of test subjects) for production of juvenile worms greater than 500 μm , equal to 3 $\mu\text{g/g}$ dry weight). Compared to *H. azteca* in sediment, their results suggested that *Tubifex* may be more exposed to 6PPD due to species-specific differences in behavior (e.g., *Tubifex* remains buried in sediment to feed on organic matter and is likely exposed to sediment bound 6PPD and 6PPD in pore water consumed by the worms).

In a study that investigated the effects of 6PPD on different life stages of freshwater mussels, the viability of glochidia (larvae) of two mussel species (the fatmucket mussel *Lampsilis siliquoidea* and the wavy-rayed lampmussel *Lampsilis fasciola*) was assessed after a 48-hour exposure to 6PPD in water (R.S. Prosser et al. 2017). The 48-hour median effect concentration (EC_{50} , the concentration at which an effect was observed in 50 percent of test subjects) values for larval viability were 439 $\mu\text{g/L}$ 6PPD for *L. siliquoidea* and 137 $\mu\text{g/L}$ for *L. fasciola*. For the juvenile stage of fatmucket mussels (*L. siliquoidea*), the same study exposed test organisms to 6PPD for 28 days in sediment and water. The reported 28-day LC_{50} s were 62 $\mu\text{g/g}$ dry weight sediment and 17 $\mu\text{g/L}$ for sediment and water, respectively. Adult mussels exposed for 28 days did not exhibit significant sublethal impacts, including a lack of oxidative stress in any tissue examined (gill, gonad, and digestive), no adverse effect on viability of hemocytes, and no DNA damage in hemocytes. The authors concluded that genotoxicity was not observed in mussel hemocytes at concentrations up to 115.9 $\mu\text{g/g}$ dry weight sediment (R.S. Prosser et al. 2017).

2.2.2.3 Toxicity of 6PPD to Algae and Aquatic Plants

There are very few studies on the toxicity of 6PPD to algae and aquatic plants. An algal study presented in the REACH dossier for 6PPD reported a 96-hour EC_{50} equal to 600 $\mu\text{g/L}$ and a 96-hour no-observed-effect concentration ($NOEC$) for growth rate equal to 200 $\mu\text{g/L}$ in the green algae species, *Pseudokirchneriella subcapitata* (previously *Selenastrum capricornutum*) (ECHA 2021). However, this study was categorized as not reliable (Klimisch score of 3) in the REACH dossier due to "significant methodological deficiencies" including the lack of exponential growth of the test organism during the incubation period, which is required in most standardized algal

toxicity tests. Therefore, the reported effect levels cannot reliably be interpreted as a result of exposure to 6PPD.

2.2.2.4 Toxicity of 6PPD to Mammals

Some studies on the toxicity of 6PPD to mammals were conducted in parallel with 6PPD-q; therefore, the available information is similar to that provided in Section 2.2.1.5 above.

One study found that oral exposure at 10 and 100 mg/kg 6PPD to mice for six weeks resulted in fatty liver syndrome (L. Fang et al. 2023), similar to 6PPD-q. Unlike 6PPD-q, which affected inflammatory pathways, the authors determined that 6PPD tended to affect gene expression related to fatty acid metabolism. However, mice were not monitored for apical outcomes. Although steatosis is an adverse outcome and indicator of liver damage in human health evaluations, no studies in wild mammals are available that would define steatosis as an adverse apical outcome in non-human organisms. Zhao et al. (2023a) found that oral gavage exposure to 4 mg/kg 6PPD in pregnant mice resulted in chemical transfer to dams, resulting in concentrations in livers (mean 200 ng/g) that were greater than studies cited above. These results may be of use for ecological risk assessment based on tissue residue values if an apical outcome was associated with organ damage as shown in the study.

2.3 Potential for Bioaccumulation, Biomagnification, and Adduct Formation

Additional data is required to thoroughly evaluate the bioaccumulation potential of 6PPD-q and 6PPD in aquatic organisms, as well as potential toxic effects as a result of this uptake. See the **Occurrence, Fate and Transport of 6PPD and 6PPD-q (Section 4)** for a discussion and table of concentrations of 6PPD-q measured in aquatic organisms (**Table 4-10**).

2.4 Human Health Toxicology

The most critical studies pertinent to the toxicity of 6PPD-q relevant to the evaluation of health effects in humans are presented in this Section. While some data presented herein is for 6PPD-q, additional research is needed to extrapolate these studies to potential effects on human health. Therefore, the parent compound 6PPD was used as a **read-across substance** to infer information for toxicologic endpoints where data is currently lacking for 6PPD-q.

Detailed toxicology data relevant to the assessment of human health for 6PPD-q and read-across substance 6PPD is provided in **Table 2-3**.

2.4.1 6PPD-q Toxicity

2.4.1.1 Toxicokinetics

Repeat dose intraperitoneal (IP) administration, which allows for rapid and efficient absorption and increased bioavailability (Al Shoyaib et al, 2019) of 6PPD-q, indicated accumulation in the liver and lung of mice (He et al, 2023). Following oral gavage, 6PPD-q was distributed to the brain and liver of dams and fetal body and fetal brain (Zhao et al, 2023). Urine concentrations measured in male and female mice indicated lower excretion of 6PPD-q than 6PPD (Zhao et al, 2023).

In mice fed a single dose of deuterated 6PPD-q₅, 6PPD-q was primarily distributed in the adipose tissue followed by the kidney, lung, testis, liver, spleen, heart and muscle (Zhang, J et al, 2024). Similar to the Zhao et al (2023) study, 6PPD-q also penetrated the blood-brain barrier of mice. However, fecal excretion rather than urine excretion was identified as the main excretory pathway for 6PPD-q and two newly identified novel hydroxylated metabolites of 6PPD-q (Zhang, J et al, 2023).

Human biomonitoring conducted in China (see Section 2.7.7) has measured 6PPD-q and 6PPD in urine with 6PPD-q concentrations significantly higher than 6PPD and higher concentrations of both 6PPD-q and 6PPD observed in pregnant woman than in adults or children. Daily excretion rates of 6PPD-q in urine of adults, children and pregnant women were 11.3 (ng/kg bw)/day, 2.18 (ng/kg bw)/day and 90.9 (ng/kg bw)/day, respectively (Du et al, 2022).

6PPD-q was detected in cerebrospinal fluid from a small sample of Parkinson's Disease patients and a control group (J. Fang et al, 2023). The study suggests that 6PPD-q can penetrate the central nervous system in humans.

2.4.1.2 Acute Toxicity

No acute toxicity data is available for 6PPD-q. As described by DTSC (2022), "...the expected redox potential of the quinone (Bolton et al. 2000) in 6PPD-q's chemical structure, along with toxic effects of other quinones on human lung epithelial cells (Shang et al. 2014), offers suggestive evidence that the structure of 6PPD-q imparts the respiratory toxicity hazard. Confirmation that 6PPD-q imparts toxicity at least partly through oxidative stress will require additional research."

2.4.1.3 Irritation/Sensitization

No irritation or sensitization data is available for 6PPD-q.

2.4.1.4 Chronic Toxicity and Systemic Effects

In a sub-chronic toxicity study by L. Fang et al. (2023), 6PPD-q increased lipid accumulation in the livers of mice that were given oral doses of 10 mg/kg bw/day for 6 weeks. In addition, 6PPD-q increased liver triglycerides at all doses tested (10, 30, and 100 mg/kg body weight per day) and an altered expression of liver enzymes and inflammatory markers in the liver were also reported (L. Fang et al. 2023).

In a sub-chronic toxicity study by He et al. (2023), "...significant pathological changes were formed in liver, kidney, lung, spleen, testis, and brain..." in mice repeatedly administered 0.4 and 4 mg/kg 6PPD-q via IP administration. According to He et al. (2023), "...[b]iochemical parameters of liver [metabolism] (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)) and kidney [function] (urea and creatinine) were all significantly upregulated by repeated injection with 0.4 and 4 mg/kg 6PPD-q...", indicating potential injury to the liver and kidney.

2.4.1.5 Chronic Toxicity and Tumorigenicity

In vitro 6PPD-q was mutagenic to *Parhyale hawaiiensis* and weakly mutagenic to bacteria (Botelho et al. 2023). *Caenorhabditis elegans* exposed to 1 µg/L and 10 µg/L 6PPD-q experienced increased DNA damage and impacted expression of DNA damage-related genes (Hua et al. 2023). DNA adducts were detected in 6PPD-q-treated mammalian cells (Wu et al. 2023). Removal of 6PPD-q led to decreased levels of the adduct, which suggested potential repair pathways (Wu et al. 2023).

2.4.1.6 Reproductive/Developmental Toxicity

In a reproductive toxicity study by Hua et al. (2023), exposure to 1 µg/L and 10 µg/L 6PPD-q reduced reproductive capacity and negatively affected gonad development in *Caenorhabditis elegans*. The relevance to human health of an effect in *C. elegans* worms is not clear.

In a prenatal developmental toxicity study, pregnant female mice were treated with 4 mg/kg 6PPD-q from embryonic day 11.5 to 15.5. 6PPD-q was found to cross the placenta and was detected in fetal whole-body tissue and fetal brain samples at higher concentrations than 6PPD (Zhao et al. 2023). This study did not measure fetotoxicity but distribution to the fetus and human retinoic acid receptor α (RAR α) and RXR α agonist activity in HEK cells as found in Zhao et al. (2023) indicates the potential for developmental effects.

2.4.1.7 Neurotoxicity

J. Fang et al (2024) investigated 6PPD-q's effects on mitochondrial processes in primary murine dopaminergic neurons. The study authors hypothesized that Parkinson's Disease (PD) may result in the degradation of the blood brain barrier, thus creating the possibility for toxins, such as 6PPD-q, to enter the brain and cerebral spinal fluid (CSF). In the study, CSF samples from PD patients and non-PD subjects (controls) were analyzed where they found that 6PPD-q levels were twice as high in PD patients (11.18 ng/ml) compared to controls (5.07 ng/ml). Using the levels found in the CSF, the authors went further to study if 6PPD-q exacerbates the formation of Lewy neurites induced by α -synuclein preformed fibrils (α -syn PFF), a feature in PD patients. To determine if there is an association between 6PPD-q and Lewy neurite development, primary dopaminergic neurons were used, which were exposed to 10 nM (2.98 ng/ml) or 100 nM (29.8 ng/ml) of 6PPD-q in the presence or absence of α -syn PFF. Treatment resulted in a dose-dependent decrease in overall ATP production – although that is not necessarily linked to oxidative phosphorylation. The primary neurons demonstrated a decrease in the basal respiratory capacity after treatment with 100 nM 6PPDq. In addition, 6PPDq [presumably at 100 nM] caused changes in the concentrations of some components of the citric acid cycle. There was no comparison with other molecules which may cause oxidative stress. Fang et al (2023) found some synergistic effects on mitochondrial membrane potential and mitochondrial processes when pre-seeding the cells with misfolded α -synuclein protein.

There are a few points to note with this study. The author's attempt to determine 6PPD-q levels in human CSF samples is a novel approach and the observed levels in CSF were used to set the exposure parameters *in vitro*. The CSF sample size for both the controls (n = 11) and PD patients (n = 13) was very small with significant deviations noted in the results (i.e., 2-3 data points that were much higher than the other samples) which may have a large impact on the statistical

analysis presented. Further, details on the disease progression of the PD patients, and the exposure environments of all subjects was not provided. Finally, discrepancies were noted in the exposure parameters established for the endpoints analyzed. More research is needed to understand the role of 6PPD-q on neuronal mitochondrial dysfunction.

2.4.2 6PPD Toxicity

2.4.2.1 Toxicokinetics

There are no *in vivo* absorption studies of 6PPD available. Nonetheless, toxicity studies at acute and repeated doses allow for the derivation of certain conclusions. The primary bioavailability of 6PPD through oral and dermal exposure is demonstrated by the emergence of systemic toxicity following exposure (OECD 2004; ECHA 2022). Following oral gavage in mice, 6PPD has been shown to distribute to the brain and liver of dams and body and brain of fetuses (Zhao, Thomas, et al. 2023a).

In an *in vitro* metabolism study, in the presence of simulated gastric juice, 6PPD had half-life of 36.9 hours. The major observed hydrolysis product was aniline with trace amounts of benzoquinoneimine-N-phenyl, N-1,3 dimethyl-butylamine p-phenol, quinone and 2-amino-4-methylpentane (ToxServices, LLC 2021).

In another study (Du et al, 2022), 6PPD was minorly metabolized via human liver microsomes in humans. 65% of 6PPD was depleted after 3 hours of incubation with liver microsomes under *in vitro* conditions. Interestingly, only 2% of the 6PPD was metabolized into 6PPD-q, indicating 6PPD may metabolize into other metabolites.

In mice, 6PPD has been shown to rapidly excrete in urine (Zhao, Thomas, et al. 2023a)). Human biomonitoring studies have detected 6PPD in urine (Du et al. 2022; Mao et al. 2024; ECHA 2022). Du et al. (2022) reported that pregnant women had higher daily excretion rates of 6PPD (median value of 2.13 (ng/kg bw)/day) compared with adults (0.51 (ng/kg bw)/day) and children (0.43 (ng/kg bw)/day). Daily excretion rates of 6PPD measured by Mao et al (2024) in adults were 34 (ng/kg bw)/day (mean) and 30 (ng/kg bw)/day (median). According to Rimatori and Castellino 1989, as cited in OECD 2004, biomonitoring "...workers in the rubber industry detected 6PPD in urine thereby demonstrating that the substance can be resorbed from the respiratory tract and possibly after dermal contact".

2.4.2.2 Acute Toxicity

Acute toxicity of 6PPD is moderate via the oral route of exposure and low via the dermal route of exposure. Oral LD50 values measured in rats range between 893 mg/kg and 5,000 mg/kg (ECHA 2022). Substance preparation (dosing vehicle) influences the bioavailability after oral application, which explains the broad range of oral LD50 observed (OECD 2004).

Currently, dermal exposure studies of acute toxicity following OECD guideline studies have not been completed. However, two older studies (ECHA 2022) are available to evaluate dermal acute toxicity. In one study, a dermal LD50 value of > 7,940 mg/kg, the highest dose tested, was measured in rabbits. In a second study, a lethal dose in the range between 3,160 and 5,010 mg/kg bw was established. According to OECD (2004), "...[c]linical signs of toxicity included reduced

food consumption, hypoactivity, and lethargy [...but] reliable data were [not] available to evaluate acute toxicity of 6PPD through an inhalation route of exposure”.

2.4.2.3 Irritation/Sensitization

6PPD is considered to be a skin sensitizer based on multiple animal studies (ECHA 2022). Patch tests in human populations resulted in similar observations. Those who had previously been sensitized to rubber samples showed a higher rate of sensitization to 6PPD, whereas healthy volunteers who had not previously been exposed to test rubber formulations showed no or very little sensitization (OECD 2004; ECHA 2022). Based on this animal and human data (see Table 2-3), ECHA (2022) classifies 6PPD as a Category 1 skin sensitizer without subcategorization.

Studies of rabbits determined that 6PPD was not irritating to skin, but slightly irritated eyes (ECHA 2022).

2.4.2.4 Chronic Toxicity and Systemic Effects

In a sub-chronic toxicity study by Fang et al. (2023), 6PPD increased lipid accumulation in the livers of mice that were given oral doses of 10 milligrams per kilogram body weight per day (mg/kg bw/day) for 6 weeks. Similarly, ECHA (2022) identified the liver and blood cells as targets of toxicity in a 28-day oral exposure rat study. In the rat study, effects on the liver were reversible at 20 mg/kg bw/day (the established no adverse effect level or NOAEL), and both sexes showed fat deposition in the liver and anemia at 100 mg/kg bw/day (ECHA 2022). A sex-specific sensitivity was also observed in the study data where a no observed effect level of 4 mg/kg bw/day was established for female rats only (OECD 2004).

2.4.2.5 Chronic Toxicity and Tumorigenicity

6PPD is not likely to be mutagenic or genotoxic based on negative mutagenicity data *in vitro* in bacterial or mammalian cells and negative clastogenicity data *in vivo* (OECD 2004; ECHA 2022). 6PPD has low potential for carcinogenicity as indicated in data collected from two two-year chronic feeding studies in rats and negative results in an *in vitro* cell transformation assay with BALB/3T3 cells (ECHA 2022).

2.4.2.6 Reproductive/Developmental Toxicity

6PPD is listed as a category 1B reproductive toxicant by the European Chemicals Agency (ECHA 2022). Dose-dependent dystocia (difficult birth) was found in multiple treatment groups in rats. A NOAEL of 7 mg/kg bw/day was established for female reproductive toxicity (ECHA 2022). Other studies did not identify reproductive effects in either rats or rabbits (OECD 2004; OSPAR Commission 2006).

Developmental toxicity tests on 6PPD have not been conclusive. No indications for teratogenic or developmental effects were observed in rats up to oral doses of 250 mg/kg bw/day (highest dose tested) (OECD 2004; ECHA 2022). In a prenatal developmental toxicity study, no conclusion could be drawn regarding whether the developmental effects (lower mean fetal body weights) observed in rabbits were due to decreased maternal feeding or specific effects of 6PPD on the fetus. A dosage level of 25 mg/kg/day was established as the NOAEL for embryo/fetal

development (ECHA 2022). However, this NOAEL is supported by another study where “...exposure during the gestation period demonstrated the absence of a developmental or teratogenic potential and of maternal toxicity in rabbits for doses up to 30 mg/kg bw/day (highest dose tested)” (OSPAR Commission 2006; ECHA 2022).

In a prenatal developmental toxicity study, pregnant female mice were treated with 4 mg/kg 6PPD from embryonic day 11.5 to 15.5. 6PPD was found to cross the placenta and was detected in fetal whole body tissue and fetal brain samples (Zhao et al. 2023). Retinoid X receptor α (RXR α) agonist activity in Human Embryonic Kidney (HEK) cells was also observed, which along with distribution to the fetus, indicates the potential for developmental effects.

2.5 Potential Human Exposures

As introduced in Section 1.2.3 (Problem Characterization Section), people may come into contact with 6PPD-q through many exposure pathways. This section provides a discussion of human exposure pathways for the environmental sources of 6PPD-q that are described elsewhere in this guidance (Section 4, Environmental Fate, Transport, and Occurrence). Populations of concern who could have elevated exposures are addressed briefly.

Because 6PPD-q and its parent compound 6PPD co-occur in tires and in the environment this section summarizes occurrence of both compounds, with emphasis on data for the quinone when available. In studies when only 6PPD was measured, the presence of the parent compound has the potential to serve as a proxy for the presence of 6PPD-q as well. See Occurrence, Fate and Transport of 6PPD and 6PPD-q (Section 4) for a detailed summary of the concentrations of 6PPD-q and 6PPD in environmental compartments.

2.5.1 Air and Dust

Much of the existing data on 6PPD-q in air and dust that has been collected to date comes from China. While 6PPD is the predominant anti-ozonant used in tires in China, as in the US there may be differences in 6PPD sources, concentrations in sources, and environmental fate factors that limit applicability of the findings from China to US environments.

6PPD and 6PPD-q have been detected in settled indoor dusts (see Table 4-2). The term “dust” can comprise different particle types and size fractions. We use the term here in the context of human health to indicate particles that are greater than 10 μ m in diameter, and therefore not respirable into the deep lung. Exposure to chemicals in dust can occur when wind or turbulence re-suspends dust particles into air. Dust particles >10 μ m deposit in the upper airways and can cause effects at the site of deposition, or can be transported by mucociliary action and swallowed, resulting in ingestion exposure. Dusts can also be ingested when they get onto hands or food, or in the case of young children, objects that are mouthed. People can also have dermal contact with chemicals in dust (see Section 2.1).

2.5.1.1 Road Dust

A potential route of exposure is the inhalation of resuspended tire and road wear particles that contain 6PPD and 6PPD-q. Road dust can act as a reservoir for chemical compounds in tire wear particles.

A pilot study was conducted to test whether funnels placed inside near roadway passive samplers (Sigma-6) can successfully capture tire wear particles (Olubusoye et al. 2023). The authors reported the presence of 6PPD-q in their samples collected at 5, 10, and 15 meters from a Mississippi highway, suggesting the possibility of inhalation exposure at near road locations. The findings were qualitative and must be confirmed in further US near road settings.

6PPD and 6PPD-q were detected in dust samples from a parking lot in Guangzhou, China (Huang et al. 2021). 6PPD and 6PPD-q were both detected in 100% of 83 roadside dust samples collected from a variety of settings in Hangzhou, China (Jin et al. 2023). Both compounds were present in dusts at parts per billion levels; the median 6PPD-q concentration was lower than the median for 6PPD.

6PPD-q has also been identified in roadside dust sampled from street sweepers and 6PPD in road tunnels in Germany (Klößner, Seiwert, Wagner, et al. 2021; Klößner, Seiwert, Weyrauch, et al. 2021). For the concentrations of 6PPD and 6PPD-Q in the cited studies, please see [Table 4-2](#).

Jin et al. (2023) applied their measured concentrations of 6PPD and 6PPD-Q in dust samples from urban/suburban, agricultural and forest areas to derive estimates of daily intake from ingestion and dermal contact for adults and children for each of these area types. Using standard exposure factors, Jin et al. estimated that ingestion may be the primary exposure route for roadside dust for all scenarios and age groups. The highest estimated daily intake by both adults and children was predicted to occur in urban/suburban regions (Jin et al. 2023).

2.5.1.2 Indoor Dust

6PPD and 6PPD-q are also reported in several studies of indoor dust samples. Over time, as tires wear down, particles containing 6PPD and 6PPD-q can be released into the environment, and these particles can find their way indoors, settling as dust on various surfaces. Indoor dust particles can also become airborne through activities like cleaning, vacuuming, or walking on carpeted surfaces. Some indoor environments, particularly occupational settings, may also have indoor sources that can combine with tire dust that penetrates from outdoors to cause increased indoor concentrations. While the indoor dust studies summarized here used varied sample collection and processing methods and so are not directly comparable to each other, the results taken together serve to illustrate the potential for human exposure via indoor dusts. Study findings are described qualitatively below with a focus on detection frequency in various exposure settings. For quantitative concentration data from the studies described below, please refer to [Table 4-2](#).

6PPD was detected in settled dust collected from the floor of US and Canadian residences in Indiana and Ontario, respectively. Median 6PPD levels in dust were slightly higher in residences located in Indiana compared to Ontario (Y. Wu, Venier, and Hites 2020). 6PPD-q was not analyzed in this study, but because other reports on indoor dust, described below, have generally detected the quinone where 6PPD was also present.

Composite dust samples collected from 97 residences in a large city in eastern China contained both 6PPD and 6PPD-quinone, with a 100% detection frequency (Zhu, Guo, Jiang, et al. 2024). Average levels in the dust were 17 ng 6PPD and 14 ng 6PPD-quinone per gram of dust.

6PPD and 6PPD-q were detected in settled dust samples (25um-250um particle size fraction) taken from the interior of 11 vehicles and 18 residences in Guangzhou, China (Huang et al. 2021). Median levels of 6PPD in dust samples taken from vehicle interiors were two orders of magnitude higher than median levels of 6PPD in dust samples taken from residences. For 6PPD-q, concentrations were higher in interior vehicle dusts than in dusts sampled outdoors in parking lots and roadways. 6PPD-q was not detected in residences above the limit of quantitation in this study.

In another study relevant to vehicle interiors, 6PPD-q concentrations were higher in dust sampled from surfaces in buses compared to indoor dust sampled from shopping malls, residential bedrooms, and air conditioner filters inside college dormitories and houses (Y.-J. Zhang et al. 2022).

Consumer products made of rubber or recycled crumb rubber can potentially contribute to indoor dust concentrations when they break down or release 6PPD and 6PPD-q, which then adsorb onto dust particles (Y.-J. Zhang et al. 2022; Zhao, Hu, et al. 2023). See Section 2.5.5 for further discussion of 6PPD- and 6PPD-q-containing consumer.

2.5.1.3 Air

People are potentially exposed to 6PPD and 6PPD-q by inhalation of ambient air. Several studies have published reports of 6PPD in respirable particulate matter (PM). Some studies also analyzed air samples for 6PPD-q. Findings for both chemicals from studies of PM are described qualitatively to demonstrate that inhalation of ambient air is a plausible exposure pathway for 6PPD-q. For concentration levels reported in outdoor air, please refer to Section 4: Occurrence, Fate and Transport of 6PPD and 6PPD-q and Table 4-1. Measurements of 6PPD and 6PPD-q in respirable particulate matter samples of indoor air were not identified during the preparation of this guidance. The relevance of exposure through indoor air remains a data gap.

In general, the ambient air studies below report high detection frequency of 6PPD and, when measured, 6PPD-q. The levels at which different sectors of the U.S. population are exposed to 6PPD and 6PPD-Q from outdoor air, and the relevance of exposure from air to potential health impacts are areas for further research.

Wu et al. reported 6PPD in 70% of ambient air samples from Chicago, 6PPD-q was not measured in this study (Y. Wu, Venier, and Hites 2020).

Three recent papers reported detection of both 6PPD and 6PPD-q at high frequency in PM_{2.5} collected in ambient air samples in China and Hong Kong. Zhang et al. analyzed multiple PM_{2.5} samples from each of six large cities in China (Y. Zhang et al. 2022). Detection rates of 6PPD and 6PPD-q averaged 86% and 81% respectively across samples from all six cities, with some variation across locations. The measured concentrations were in the low picogram per cubic meter range. The authors used standard exposure assumptions to compute resulting average daily intakes for residents of each city from the measured concentrations in PM_{2.5}. Estimated daily intakes ranged from 0.3 pg-7.2 pg/day for 6PPD and 2.2-18 pg/day for 6PPD-q.

The same research group reported higher levels of 6PPD and 6PPD-q in PM_{2.5} samples from two of the six cities in a different publication (W. Wang et al. 2022). Samples taken at a roadside location were higher than either city sampling site for both 6PPD and 6PPD-q.

Similarly, sixteen PM_{2.5} samples from Hong Kong all contained 6PPD and 6PPD-q (Cao et al. 2022).

The studies summarized above from China and Hong Kong reported on PM_{2.5}. However, there is some indication that compounds associated with tire particles are more highly associated with coarse PM (2.5µM-10µM), though this association was not measured specifically for 6PPD or 6PPD-q (X. Wang et al. 2023; Y.-J. Zhang et al. 2022). More investigation is needed to determine potential human exposure to 6PPD and 6PPD-q due to coarse PM.

2.5.2 Soil and Sediment

The widespread use of 6PPD in tires has led to the presence of 6PPD and 6PPD-q in soil and sediment that may be sources of human exposure through contact with these media. The primary pathways through which humans may be exposed to 6PPD and 6PPD-q in soil and sediment are ingestion and dermal contact. Sediment disturbance due to human activities such as wading and swimming can resuspend sediment particles into the water column (Section 2.5.4.2), making them available for dermal contact or accidental ingestion. Incidental ingestion can also occur when people come into direct contact with contaminated soil or sediment and engage in hand to mouth behaviors. See Table 4-4 and Table 4-5 in Occurrence, Fate and Transport of 6PPD and 6PPD-q (Section 4) for concentrations and further discussion of the presence of 6PPD and 6PPD-q in roadside soil and sediment, respectively.

6PPD and 6PPD-q were detected in roadside soil samples taken on non-rainy days from greenbelt areas in New Territories and Kowloon in Hong Kong. The median concentrations of 6PPD and 6PPD-q were similar (Cao et al. 2022).

6PPD has been detected in surficial sediment sampled from a ship canal in Chicago, Illinois (Y. Wu, Venier, and Hites 2020). 6PPD-q was not analyzed in these sediment samples.

6PPD and 6PPD-q were both detected in sediment sampled from the Jiaojiang River and the Pearl River Delta and Estuary in China, with higher levels of 6PPD detected compared to 6PPD-q (Zhu, Guo, Ren, et al. 2024; Zeng et al. 2023).

An exposure assessment conducted by Cao et al. (2022), using concentrations of total PPD compounds and their quinone transformation products in roadside soil, runoff water, and air particles in Hong Kong, estimated that ingestion of roadside soil could be the primary contributor of human exposure to PPDs and PPD-Qs, followed by dermal contact, and then inhalation. The relative importance of the exposure pathway reflected the lower concentrations of PPD chemicals and their transformation products in ambient air particulate as compared to concentrations in roadside soil and roadway runoff samples (Cao et al. 2022). It is unknown whether these exposure pathway trends are applicable to people in the US. Additional research is needed to understand the potential human health impacts from different exposure pathways of 6PPD and 6PPD-q.

2.5.3 Food

The following sections summarize what is known about the potential for exposure to 6PPD and 6PPD-q from consumption of fish and other potentially contaminated food sources. See [Table 4-10](#) for concentrations of 6PPD and 6PPD-q in aquatic and other food sources.

2.5.3.1 Aquatic Food Sources

6PPD and 6PPD-q have been detected in the organs of several different species of fish. However, a substantial knowledge gap exists regarding the presence of 6PPD and 6PPD-q within edible fish tissues and other aquatic biota consumed by humans, as well as if these levels are sufficiently elevated to pose a risk to human health. Additionally, more research is needed to understand human exposure potential to 6PPD and 6PPD-q from consuming the whole body of the fish. Results described below indicate measurements done on uncooked fish tissue. It is unknown how heat applied during cooking processes may impact 6PPD and 6PPD-q concentrations in fish tissue and other aquatic food sources.

Ji et al. (2022) analyzed for 6PPD and 6PPD-q in a small sample of fish from a market in China. Freshwater species tested included snakehead fish, mandarin fish, tilapia, crucian, yellow-head catfish and blunt-snout bream, and marine species were Spanish mackerel, weever, silver pomfret, large yellow croaker. 6PPD was detected in snakehead and weever. 6PPD-q was detected in the Spanish mackerel, but at a level below the limit of quantitation. Ji et al. (2022) did not indicate which tissue types of the fish were analyzed, nor the source waters they were fished from.

Frozen capelin caught in Canada and purchased at a supermarket contained 6PPD-q adducts in liver, gill, and roe (J. Wu et al. 2023). The authors did not state where in Canada the capelin were caught.

6PPD-q was detected in the brain and gill of white spotted char (*Salvelinus leucomaenis pluvius*), southern Asian dolly varden (*Salvelinus curilus*), and masu (*Oncorhynchus masou masou*) exposed under laboratory conditions (Hiki and Yamamoto 2022) but the relevance of this finding to human exposure through consumption of these organs is unclear. The monohydroxylated metabolite of 6PPD-q was also detected in the same tissues (Hiki and Yamamoto 2022). People who consume the whole body of the fish, such as in soup stock, may be exposed to higher concentrations of 6PPD-q due to its distribution to fish brain. It is unknown whether 6PPD and/or 6PPD-q are present in the tissues of fish that are more likely to be consumed, such as the skin and fillet.

In addition to the lack of data concerning 6PPD and 6PPD-q levels in edible fish tissue, a notable data gap concerns other aquatic biota consumed by people, including shellfish and aquatic plants, including wild rice and related foods. In addition, limited attention has been directed towards understanding how cooking processes might alter levels in the edible portions of these aquatic organisms. Determining concentrations of 6PPD and 6PPD-q in aquatic food sources and assessing the impact of various cooking techniques is important for evaluating any potential human exposure risks and establishing informed consumption guidelines.

2.5.3.2 Other Potential Food Sources

As discussed above 6PPD and 6PPD-q are present in roadside dusts. This raises potential concern for contamination of foods that are grown in near-roadway locations through uptake from soil, or deposition of particles from the air onto edible plant surfaces. To date, we are not aware of any data to show that 6PPD or 6PPD-q are present in agricultural soils in near-roadway settings or more distant environments that could be affected by atmospheric deposition of particles. Similarly, we did not identify sampling studies of food crops grown in near-road environments for 6PPD or 6PPD-q.

The uptake of 6PPD into hydroponically grown lettuce has been examined by (Castan et al. 2023). The results provide some general context for whether food crops are able to uptake 6PPD and translocate it through the plant. Interpretation is limited to the laboratory conditions, however, and not necessarily relevant for field grown crops. *Valerianella locusta* roots were exposed to 6PPD and 6PPD-q via tire wear particles in hydroponic nutrient solution. Small amounts of 6PPD and 6PPD-q were detected in the leaves. The experiments show that uptake and translocation of 6PPD-q into consumable plants is possible under some conditions. The exposure relevance of this study is limited because hydroponics is not a predominant cultivation method employed in agriculture, and so it is uncertain whether the uptake behavior of 6PPD shown in this study would occur similarly in crops grown in agricultural soil. Additionally, a conjugated 6PPD-glucoside form and three stable biotransformation products of 6PPD-q were detected, but the human health relevance of these products is unknown (Castan et al. 2023).

Only one other study examining the presence of 6PPD and 6PPD-q in food other than fish was located during preparation of this guidance document (Ji, Li, et al. 2022). Ten samples of honey purchased at a supermarket in Beijing, China were analyzed for 6PPD and 6PPD-q; neither 6PPD nor 6PPD-q were detected in any of the samples (Ji, Li, et al. 2022).

It is unknown whether the ingestion of non-aquatic food sources will expose the consumer to 6PPD or 6PPD-q. More studies need to be conducted to fill this knowledge gap.

2.5.4 Water

The following sections summarize what is known about the potential for human exposure to 6PPD and 6PPD-q from drinking water and recreational contact with surface water.

2.5.4.1 Drinking water

Water bodies used as sources for drinking water located near roadways may be vulnerable to environmental contamination with 6PPD and 6PPD-q. Surface water in streams and lakes that are drinking water sources may ~~can~~ be affected by stormwater runoff or, depending on the setting, effluent from wastewater treatment plants. Regarding groundwater sources, some analyses consider groundwater contamination unlikely because 6PPD and 6PPDq have low solubility and tend to adhere to soil particles, limiting movement through soils (OSPAR Commission 2006). However, recently, groundwater contamination by 6PPD and 6PPD-q, along with other PPD chemicals, was been reported in a shallow aquifer in China (R. Zhang et al. 2023). The authors describe the hydrogeology of the aquifer as unconfined and highly permeable to the nearby river water, potentially to septic tanks and WWTP effluent. Whether groundwaters

that serve as drinking water sources in the US could be vulnerable to a similar contamination pathway is an important data gap.

At the time this guidance was prepared, we did not identify any reports of either chemical detected in finished drinking water in the US, nor studies underway. There is a need for data on 6PPD and 6PPD-q occurrence in drinking water sources, influent and effluent from drinking water treatment plants and tap waters.

Two published studies report analysis of drinking water source samples, one in China and the other in the greater Toronto area in Canada. The Chinese study (H.-Y. Zhang et al. 2023) detected 6PPD in 30-48% of filtered river source water samples and 6PPD-q in 100%. Concentrations were in the low ng/L range. 6PPD-q concentrations were higher than the parent chemical 6PPD. Within the drinking water treatment plant (DWTP), neither chemical was detected in samples drawn at each of six treatment stages. The Canadian study sampled for 6PPD-q in four wastewater treatment plants and two drinking water treatment plants in the same region of Lake Ontario (Johannessen and Metcalfe 2022). 6PPD-q was detected in wastewater treatment plant (WWTP) influent and effluent but not in pre- or post-treatment water from either drinking water treatment plant.

One study that analyzed tap water samples was identified during preparation of this guidance, 6PPD was detected in 25% of drinking water samples collected from 20 buildings in Singapore (Marques dos Santos and Snyder 2023). 6PPD-q was not detected in any of the drinking water samples.

2.5.4.2 Surface Water

Human exposure to 6PPD and 6PPD-q during water-based recreational activities is an emerging area of concern. People who come into direct contact with water contaminated by runoff during recreational activities such as swimming, fishing, or boating may be exposed to 6PPD and 6PPD-q through skin contact and unintentional ingestion of water. Individuals may also be exposed to 6PPD and 6PPD-q if they are present in the sediments (Section 2.5.2) and particles suspended in the water. It is unknown if skin absorption of the chemicals from these particles is possible or if levels of exposure are of concern.

6PPD and 6PPD-q are ubiquitous in urban runoff which can contaminate waters that are used for recreation (Cao et al. 2022; H.-Y. Zhang et al. 2023; Tian et al. 2021). 6PPD and 6PPD-q have been detected in surface water in the US, as well as Canada, China, and Australia to date (Occurrence, Fate and Transport of 6PPD and 6PPD-q (Section 4); Table 4-7). Recreational water activities are therefore a plausible human exposure pathway that requires further investigation.

2.5.5 Consumer Products

In addition to exposure to tire-derived 6PPD and 6PPD-q via environmental media pathways (air, soil/dust, food, and water), there is also potential for exposure from manufactured consumer products other than tires that can also contain 6PPD and 6PPD-q. Consumer products are manufactured from new materials that contain 6PPD as an additive as well as from recycled rubber that derives from scrap tires. Potential routes of human exposure from contact with

consumer products may include dermal contact, inhalation of particles, and incidental ingestion of particles, depending upon the product and usage patterns. Exposure to 6PPD and 6PPD-quinone from products has not been characterized. The extent of human exposure and importance of the sources and products discussed below is not known.

2.5.5.1 Recycled Tire Rubber Products

An estimated 5 million tons of scrap tires were generated in the U.S. in 2021 (USTMA 2022). Approximately 32% or 1.4 million tons per year of scrap tires are made into ground rubber in the US. Ground rubber is then repurposed for a range of applications and manufactured products, including asphalt rubber paving, sports surfaces, loose mulch, automotive and consumer products (USTMA 2022).

USTMA estimates that 34% of the ground rubber from scrap tires is used to manufacture molded and extruded consumer products. Examples of molded products made from recycled tire rubber that can be present in indoor environments, and thus increase human exposure potential, include flooring materials such as rubber mats and tiles and accessibility ramps for indoor use (CalRecycle 2023). A small sample (n=2) of doormats made from recycled rubber were reported to contain 6PPD at an average of 630 ug/g and 6PPD-q at 18 ug/g (Zhao et al. 2023).

Some products can also be stamped or cut directly from scrap tire tread. No analytic data on samples of stamped products was located.

As of 2021, 22% of recycled tire rubber is used for sports surfaces, typically as crumb rubber infill material for artificial turf fields. Another 28% is used as playground mulch, typically as larger sized ground rubber (USTMA 2022). Recreational applications of recycled rubber from scrap tires raise the possibility of exposure concerns for people who work or play at these sports facilities and playgrounds, including children.

The US National Toxicology Program (NTP) detected 6PPD in recycled tire crumb rubber samples from manufacturing facilities (National Toxicology Program, Public Health Service U.S. Department of Health and Human Services 2019). NTP did not test for 6PPD-q. In a series of publications from a European study of crumb rubber, 86 samples from playing fields across Europe contained 6PPD at an average concentration of 571 mg/kg for all samples. Levels were higher, averaging 1000 mg/kg (0.1% by weight), for granules that had not been coated with polyurethane (Schneider, De Hoogd, Madsen, et al. 2020). Again, concentrations of the quinone were not measured. More recently both 6PPD and 6PPD-q were found in samples of crumb rubber from recreational facilities in Europe (Armada et al. 2023). Further, both compounds could be extracted from crumb rubber samples with synthetic digestive fluids.

Both 6PPD and 6PPD-q were detected in crumb rubber from a small sample of synthetic turf athletic fields in Washington state (Zhao, Hu, et al. 2023). Concentrations were highly variable. Median concentrations for 6PPD and 6PPD-q were 1.2 mg/kg and 9.8 mg/kg respectively. The difference in concentration levels noted in Washington compared to the European samples is not yet understood.

The potential for 6PPD and 6PPD-q to volatilize from crumb rubber is currently unknown. The study of recreational facilities in Europe did not detect 6PPD above the limit of quantitation in

air monitoring conducted at field locations with crumb rubber playing surface (Schneider, De Hoogd, Haxaire, et al. 2020).

Crumb rubber ingestion intake by children was estimated at roughly 100 mg/day for children who play on turf fields with crumb rubber infill (Peterson et al. 2018). Using video-taped observations of children playing on turf and in playground settings resulted in comparable estimates that children ingest 70-80 mg/day (Lopez-Galvez et al. 2022).

2.5.5.2 *Manufactured Products from New Materials Containing 6PPD*

The vast majority of 6PPD in the US is used in the manufacturing of tires, however other consumer products made from elastomeric materials may also include 6PPD as an anti-degradant additive. Manufactured products that contain 6PPD and are used indoors could contribute to human exposure to 6PPD-q. People spend the majority of their time indoors and young children are particularly vulnerable to exposure from household dusts due to behavioral factors such as crawling and playing on the floor (U.S. EPA 2011). We were unable to evaluate complete data on the extent of 6PPD additives in products. The lack of that data, and of studies that determine the rate of production of 6PPD-q from 6PPD in indoor environments, represent information needs for adequate characterization of human exposure levels.

A small sample of rubber consumer products including laboratory stoppers, sneaker soles, and rubber garden hoses were reported to contain 6PPD (Zhao, Hu, et al. 2023). The authors also tested for 6PPD-q. The laboratory stoppers, and sneaker soles contained detectable levels of 6PPD-q (Zhao, Hu, et al. 2023).

(Marques dos Santos and Snyder 2023) analyzed samples of plumbing fittings to determine if these products can be a source of 6PPD in drinking water. Seven different plumbing devices comprised of o-rings and polymer seals were tested. An oscillating kit for a faucet filter made of acrylonitrile butadiene rubber leached the highest amount of 6PPD (1 ng/mg of material). Silicone-based seals did not leach 6PPD. 6PPD-q was not detected in these samples.

6PPD and 6PPD-q were found in the dust of e-waste recycling facilities in China (Liang et al. 2022) and Ontario (Wu, Venier 2020). Liang et al suggest that the sources may be materials in consumer electronics. Whether consumer electronics expose people in the US to 6PPD or 6PPD-q is unknown at this time.

2.5.6 *Occupational Exposure Potential*

Many workplaces in the US contain tires and tire debris. As described by DTSC (2022), "...[s]ome of these occupations include tire manufacturers, mechanics, highway workers, street sweepers, car washers, and parking attendants". Workers involved in handling, shredding or otherwise processing waste tires and other products may have dermal exposure to the rubber as well as dust and particulate matter exposures.

In four solid waste recycling facilities in China, 6PPD-q was found in airborne particulate matter and settled dusts (Y. Zhang et al. 2022). 6PPD was reported in dust samples from electronic waste recycling facilities, one in China and one in Canada (Liang et al. 2022; Wu, Venier, and

Hites 2020). Neither of the electronic waste studies analyzed for 6PPD-q. Overall, there is a lack of data that can determine occupational exposure levels for US workers with potential exposure to 6PPD-q. Biomonitoring and evaluation of health impacts to worker population groups is a data gap (DTSC 2022).

2.5.7 Biomonitoring

At the time this report was prepared, there was limited information about biological measures of exposure to 6PPD and 6PPD-q in people. The kinds of samples used to characterize human exposure may vary with the chemical contaminant of concern but can include blood, hair, and urine, among other biological materials. Detection of 6PPD and 6PPD-q in urine, serum, and cerebrospinal fluid is summarized below.

6PPD and 6PPD-q were detected in the urine of adults, children, and pregnant individuals living in South China (Du et al. 2022). Pregnant women's urine had the highest levels of 6PPD and 6PPD-q out of all the demographic groups in the study, with 6PPD-q concentrations greater than 6PPD concentrations by two orders of magnitude. Du et al. (2022) only analyzed for 6PPD and 6PPD-q, but there may be other metabolic transformation products of 6PPD and 6PPD-q in urine that are not yet accounted for. Metabolic transformation products have not yet been identified in mammalian studies. Higher levels of 6PPD and 6PPD-q were detected in the urine of adult women than adult men, but this difference was not present in children. Additionally, 6PPD-q urine concentrations increased with age in children, with levels higher in children aged 4 – 6 years and 7 – 13 years compared to 1 – 3 years. Du et al. (2022) hypothesized this age difference is due to a difference in toxicokinetics between young and older children, as well as the change in diet from breast milk/formula to solid foods.

Mao et al. (2024) analyzed urine sampled from 151 adults for 6PPD, but not 6PPD-q in China. Higher levels of 6PPD were detected in the urine from adult women than adult men, which supports the findings of Du et al. (2022). A general trend of decreasing 6PPD concentration with increasing age was also noted, with significance achieved for the <20 years versus 50+ years comparison.

J. Zhang et al. (2024) collected serum from 30 volunteers and analyzed for 6PPD-q to identify environmentally relevant exposure concentrations for a laboratory study in mice. The detection frequency of 6PPD-q in these serum samples was 100%, the median concentration was 0.24 ng/mL. The quality of the data is not confirmed because the paper provides limited methodological information for the serum analysis. Further studies designed to characterize exposure and toxicokinetic properties of 6PPD and 6PPD-q in people are needed.

6PPD-q was detected in cerebrospinal fluid from Parkinson's Disease patients and a control group (J. Fang et al. 2024). While differences were noted between the populations, these could be caused by different exposure or different distribution to cerebrospinal fluid in the disease state.

Older studies of occupational exposure to 6PPD detected levels in the urine of rubber industry workers. 6PPD-q was not recognized at the time and was not measured. 15% of urine samples of 21 Italian rubber industry workers who were exposed by both inhalation and dermal contact contained 6PPD (maximum was 1.3 µg/L 6PPD in urine). 6PPD was detected in air samples

taken in the work area at a concentration range of 0.01 – 1 µg/m³ (Carlucci et al. 1984). A peak value of 580 µg/g 6PPD was detected in another biomonitoring study of 341 Italian rubber industry workers (1982 – 1987). The urine concentration of 6PPD was found to correlate with the level of 6PPD detected in the air, which reached a maximum concentration of 6.6 mg/m³ (Rimatori and Castellino 1989, as cited in OECD 2004).

2.5.8 Populations of Concern

People may be more likely to experience health effects from a chemical if they are biologically susceptible, more highly exposed due to either behavioral exposure factors or increased contact with contaminated environmental media, or because they are more vulnerable to the effects of exposure due to other health stressors. At present, there is limited data to assess biological susceptibility factors that may be relevant for 6PPD and 6PPD-Q. Until human health hazards are better characterized, and we have a clear understanding of who is exposed, biological susceptibility factors can't be addressed. This section identifies some possible populations of concern based on potential exposure patterns and possible vulnerable populations who may be more affected by declining health of salmon and other fish.

Occupationally exposed people: People who work in rubber manufacturing, processing, handling, and recycling industries, occupations that routinely handle tires, and manufacturing or use of recycled rubber products may be at higher risk of exposure due to their direct contact with rubber materials containing 6PPD.

People with prior skin sensitization to 6PPD or other phenylenediamine compounds may be more highly susceptible to allergic skin responses upon exposure to 6PPD.

Children: Children who live, play, attend school and sports near roadways or in environments that use crumb rubber could be at a heightened risk of exposure to 6PPD and 6PPD-q in TRWPs and road dusts. Children who play on fields amended with crumb rubber or with playground structures made from recycled tires may also be at elevated risk for exposure to 6PPD and 6PPD-q released from these materials, although exposure levels that result from these sources is not yet documented. Children's lower body weight, greater dust ingestion rate, and dust to skin adherence factor result in a higher dose from exposure compared to adults, which might make them more vulnerable to any potential adverse effects (Jin et al. 2023). Additionally, older children may be at higher risk of exposure compared to younger children, as indicated by higher urine concentrations of 6PPD-q in 4 – 13 year olds compared to 1 – 3 year olds (Du et al. 2022).

Residents living near busy roads and highways might experience increased exposure to 6PPD and 6PPD-q. Resuspension and dispersion of tire and road particles containing 6PPD and 6PPD-q could potentially contaminate air and dust in near-road residences, schools, and workplaces, impacting the health of individuals in these communities.

Pregnant people may have higher exposure levels as indicated by the biomonitoring results of (Du et al. 2022) that reported the highest levels of 6PPD and 6PPD-q in the urine of pregnant women.

1 Women may also be at risk for increased exposure to 6PPD and 6PPD-q than men. (Du et al.
2 2022) showed there is a gender difference in adults in China wherein higher levels of 6PPD and
3 6PPD-q were detected in the urine of adult women than adult men.

4 Environmental justice concerns arise when vulnerable communities, often including low-income
5 and minority populations, are disproportionately exposed to environmental hazards. This can
6 result in unequal health impacts and socioeconomic disparities. Populations living near roadways
7 are disproportionately non-white and low-income and may be at higher risk for exposure and
8 health effects of 6PPD and 6PPD-q.

9 Mortality to coho salmon and declining fish populations directly and indirectly impact
10 populations who rely on these fish. Tribal members, recreational fishers, and communities that
11 harvest salmon for subsistence in urbanized areas may experience higher exposure if it is found
12 that these chemicals accumulate in edible fish tissue. See **Section 1.3.3 (Tribal Nations)** and
13 **Section 1.3.4 (Potential Economic and Community Health Concerns)** for further discussion of
14 environmental justice.

Table 2-1. Summary of acute aquatic toxicity data for 6PPD and 6PPD-q

	Receptor (specific)	Receptor (general)	Chemical	Benchmark Value (L(E)C50)	Units (ug/l)	Duration	Endpoint	Reference
1	Algae, Selenastrum capricornutum	Plant/algae	6PPD	600	ug/l	96h	Cell number	Monsanto 1978 as cited in UNEP 2004
2	Amphipod, Hyalella azteca	Invertebrate	6PPD	250	ug/l	96h	Mortality	Prosser, R. S., Bartlett, A. J., Milani, D., Holman, E. A. M., Ikert, H., Schissler, D., Toito, J., Parrott, J. L., Gillis, P. L., & Balakrishnan, V. K. (2017). Variation in the toxicity of sediment-associated substituted phenylamine antioxidants to an epibenthic (Hyalella azteca) and endobenthic (Tubifex tubifex) invertebrate. Chemosphere, 181, 250-258. https://doi.org/10.1016/j.chemosphere.2017.04.066
3	Coho salmon, Oncorhynchus kisutch	Fish	6PPD	251	ug/l	24h	Mortality	Tian, Z., Zhao, H., Peter, K. T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A. E., Biswas, R. G., Kock, F. V. C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., . . . Kolodziej, E. P. (2021). A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. Science, 371(6525), 185-189. https://doi.org/doi:10.1126/science.abd6951
4	Fatmucket mussel, Lampsilis siliquoidea	Invertebrate	6PPD	439	ug/l	48h	Viability	Prosser, R. S., Gillis, P. L., Holman, E. A. M., Schissler, D., Ikert, H., Toito, J., Gilroy, E., Campbell, S., Bartlett, A. J., Milani, D., Parrott, J. L., & Balakrishnan, V. K. (2017). Effect of substituted phenylamine antioxidants on three life stages of the freshwater mussel Lampsilis siliquoidea. Environ Pollut, 229, 281-289. https://doi.org/10.1016/j.envpol.2017.05.086
5	Japanese medaka, Oryzias latipes	Fish	6PPD	28	ug/l	96h	Mortality	Japanese Ministry of Environment, 2001; as cited in ECHA 2023 and UNEP 2004
6	Japanese medaka, Oryzias latipes	Fish	6PPD	< 107	ug/l	96h	Mortality	Hiki, Kyoshiro,Asahina, Kenta,Kato, Kota,Yamagishi, Takahiro,Omagari, Ryo,Iwasaki, Yuichi,Watanabe, Haruna,Yamamoto, Hiroshi. "Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species". Environmental Science & Technology Letters 8 (2021): 779-784
7	Rainbow trout, Oncorhynchus mykiss	Fish	6PPD	> 50	ug/l	96h	Mortality	Nair, P.,Sun, J. ,Xie, L.,Kennedy, L.,Kozakiewicz, D.,Kleywegt, S,Kleywegt, S.,Hao, C.,Byun, H.,Barrett, H.,Baker, J.,Monaghan, J.,Krogh, E.,Song, D.,peng, h. (2023). Synthesis and Toxicity Evaluation of Tire Rubber-Derived Quinones ChemRxiv. Open Engage. Cambridge: Cambridge. This content is a preprint and has not been peer-reviewed
8	Water flea, Daphnia magna	Invertebrate	6PPD	230	ug/l	48h	Mortality	Japanese Ministry of Environment, 2019; as cited in ECOTOX database
9	Water flea, Daphnia magna	Invertebrate	6PPD	< 138	ug/l	48h	Mortality	Hiki, Kyoshiro,Asahina, Kenta,Kato, Kota,Yamagishi, Takahiro,Omagari, Ryo,Iwasaki, Yuichi,Watanabe, Haruna,Yamamoto, Hiroshi. "Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species". Environmental Science & Technology Letters 8 (2021): 779-784
10	Wavy-rayed lampmussel, Lampsilis fasciola	Invertebrate	6PPD	137	ug/l	48h	Viability	Prosser, R. S., Gillis, P. L., Holman, E. A. M., Schissler, D., Ikert, H., Toito, J., Gilroy, E., Campbell, S., Bartlett, A. J., Milani, D., Parrott, J. L., & Balakrishnan, V. K. (2017). Effect of substituted phenylamine antioxidants on three life stages of the freshwater mussel Lampsilis siliquoidea. Environ Pollut, 229, 281-289. https://doi.org/10.1016/j.envpol.2017.05.086
11	Zebrafish, Danio rerio	Fish	6PPD	442.62	ug/l	96h	Mortality	Varshney, S.,Gora, A. H.,Siriyappagouder, P.,Kiron, V.,Olsvik, P. A.. "Toxicological effects of 6PPD and 6PPD quinone in zebrafish larvae". J Hazard Mater 424 (2022): 127623
12	Zebrafish, Danio rerio	Fish	6PPD	2200	ug/l	96h	Mortality	Peng, W., Liu, C., Chen, D., Duan, X., & Zhong, L. (2022). Exposure to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) affects the growth and development of zebrafish embryos/larvae. Ecotoxicol Environ Saf, 232, 113221. https://doi.org/10.1016/j.ecoenv.2022.113221
13	Zebrafish, Danio rerio	Fish	6PPD	737	ug/l	96h	Mortality	Fang, C., Fang, L., Di, S., Yu, Y., Wang, X., Wang, C., & Jin, Y. (2023). Characterization of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD)-induced cardiotoxicity in larval zebrafish (Danio rerio). Sci Total Environ, 882, 163595. https://doi.org/10.1016/j.scitotenv.2023.163595
14	Zebrafish, Danio rerio	Fish	6PPD	>137	ug/l	96h	Mortality	Hiki, Kyoshiro,Asahina, Kenta,Kato, Kota,Yamagishi, Takahiro,Omagari, Ryo,Iwasaki, Yuichi,Watanabe, Haruna,Yamamoto, Hiroshi. "Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species". Environmental Science & Technology Letters 8 (2021): 779-784
15	Algae, Chlamydomonas reinhardtii	Plant/algae	6PPD-Q	0.84*	uM	72h	Relative growth rate	Wu, J.,Cao, G.,Zhang, F.,Cai, Z.. "A new toxicity mechanism of N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone: Formation of DNA adducts in mammalian cells and aqueous organisms". Sci Total Environ #volume# (2023): 161373
16	Amphipod, Hyalella azteca	Invertebrate	6PPD-Q	> 43	ug/l	96h	Mortality	Hiki, Kyoshiro,Asahina, Kenta,Kato, Kota,Yamagishi, Takahiro,Omagari, Ryo,Iwasaki, Yuichi,Watanabe, Haruna,Yamamoto, Hiroshi. "Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species". Environmental Science & Technology Letters 8 (2021): 779-784

Table 2-1. Summary of acute aquatic toxicity data for 6PPD and 6PPD-q

	Receptor (specific)	Receptor (general)	Chemical	Benchmark Value (L(E)C50)	Units (ug/l)	Duration	Endpoint	Reference
17	Arctic char, Salvelinus alpinus	Fish	6PPD-Q	> 14.2	ug/l	96h	Mortality	Brinkmann, Markus,Montgomery, David,Selinger, Summer,Miller, Justin G. P.,Stock, Eric,Alcaraz, Alper James,Challis, Jonathan K.,Weber, Lynn,Janz, David,Hecker, Markus,Wiseman, Steve. "Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance". Environmental Science & Technology Letters 9 (2022): 333-338
18	Atlantic salmon, Salmo salar	Fish	6PPD-Q	> 12.16	ug/l	48h	Mortality	Foldvik, A.,Kryuchkov, F.,Sandodden, R.,Uhlig, S.. "Acute toxicity testing of the tire rubber-derived chemical 6PPD-quinone on Atlantic salmon (Salmo salar) and brown trout (Salmo trutta)". Environ Toxicol Chem #volume# (2022): #pages#
19	Brook trout, Salvelinus fontinalis	Fish	6PPD-Q	0.59	ug/l	24h	Mortality	Brinkmann, Markus,Montgomery, David,Selinger, Summer,Miller, Justin G. P.,Stock, Eric,Alcaraz, Alper James,Challis, Jonathan K.,Weber, Lynn,Janz, David,Hecker, Markus,Wiseman, Steve. "Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance". Environmental Science & Technology Letters 9 (2022): 333-338
20	Brown trout, Salmo trutta	Fish	6PPD-Q	> 12.16	ug/l	48h	Mortality	Foldvik, A.,Kryuchkov, F.,Sandodden, R.,Uhlig, S.. "Acute toxicity testing of the tire rubber-derived chemical 6PPD-quinone on Atlantic salmon (Salmo salar) and brown trout (Salmo trutta)". Environ Toxicol Chem #volume# (2022): #pages#
21	Bull trout, Salvelinus confluentus	Fish	6PPD-Q	> 13	ug/l	24h	Mortality	Brinkmann et al in prep
22	Chinese rare minnow, Gobiocypris rarus	Fish	6PPD-Q	> 500	ug/l	96h	Mortality	Di, S.,Liu, Z.,Zhao, H.,Li, Y.,Qi, P.,Wang, Z.,Xu, H.,Jin, Y.,Wang, X.. "Chiral perspective evaluations: Enantioselective hydrolysis of 6PPD and 6PPD-quinone in water and enantioselective toxicity to Gobiocypris rarus and Oncorhynchus mykiss". Environ Int 166 (2022): 107374
23	Chinook salmon, Oncorhynchus tshawytscha	Fish	6PPD-Q	> 2.5	ug/l	24h	Mortality	Montgomery, D., Ji, X., Cantin, J., Philibert, D., Foster, G., Selinger, S., Jain, N., Miller, J., McIntyre, J., de Jourdan, B., Wiseman, S., Hecker, M., & Brinkmann, M. (2023). Toxicokinetic Characterization of the Inter-Species Differences in 6PPD-Quinone Toxicity Across Seven Fish Species: Metabolite Identification and Semi-Quantification. bioRxiv, 2023.2008.2018.553920. https://doi.org/10.1101/2023.08.18.553920
24	Chinook salmon, Oncorhynchus tshawytscha	Fish	6PPD-Q	> 67.307	ug/l	24h	Mortality	Lo, Bonnie,Marlatt, Vicki,Liao, Xiangjun,Reger, Sofya,Gallilee, Carys,Brown, Tanya. "Acute toxicity of 6PPD-quinone to early life stage juvenile Chinook (Oncorhynchus tshawytscha) and coho (Oncorhynchus kisutch) salmon". Environmental toxicology and chemistry #volume# (2023): #pages#
25	Chinook salmon, Oncorhynchus tshawytscha	Fish	6PPD-Q	82.1	ug/l	24h	Mortality	Greer, Justin B.,Dalsky, Ellie M.,Lane, Rachael F.,Hansen, John D.. "Establishing an In Vitro Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products". Environmental Science & Technology Letters #volume# (2023): #pages#
26	Coho salmon, Oncorhynchus kisutch	Fish	6PPD-Q	0.041	ug/l	24h	Mortality	Lo, Bonnie,Marlatt, Vicki,Liao, Xiangjun,Reger, Sofya,Gallilee, Carys,Brown, Tanya. "Acute toxicity of 6PPD-quinone to early life stage juvenile Chinook (Oncorhynchus tshawytscha) and coho (Oncorhynchus kisutch) salmon". Environmental toxicology and chemistry #volume# (2023): #pages#
27	Coho salmon, Oncorhynchus kisutch	Fish	6PPD-Q	0.0804	ug/l	24h	Mortality	Greer, Justin B.,Dalsky, Ellie M.,Lane, Rachael F.,Hansen, John D.. "Establishing an In Vitro Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products". Environmental Science & Technology Letters #volume# (2023): #pages#
28	Coho salmon, Oncorhynchus kisutch	Fish	6PPD-Q	0.095	ug/l	24h	Mortality	Tian, Zhenyu,Gonzalez, Melissa,Rideout, Craig A.,Zhao, Haoqi Nina,Hu, Ximin,Wetzel, Jill,Mudrock, Emma,James, C. Andrew,McIntyre, Jenifer K.,Kolodziej, Edward P.. "6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard". Environmental Science & Technology Letters 9 (2022): 140-146
29	Fathead minnow, Pimephales promelas	Fish	6PPD-Q	>9.65	ug/l	96h	Mortality	Anderson-Bain, Katherine,Roberts, Catherine,Kohlman, Evan, Ji, Xiaowen,Alcaraz, Alper J.,Miller, Justin,Gangur-Powell, Tabitha,Weber, Lynn,Janz, David,Hecker, Markus,Montina, Tony,Brinkmann, Markus,Wiseman, Steve (2023). Apical and mechanistic effects of 6PPD-quinone on different life-stages of the fathead minnow (Pimephales promelas) Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 271(#issue#), 109697
30	Freshwater rotifer, Brachionus calyciflorus	Invertebrate	6PPD-Q	> 10000	ug/l	NR	Mortality	Klauschies, T.,Isanta-Navarro, J.. "The joint effects of salt and 6PPD contamination on a freshwater herbivore". Sci Total Environ 829 (2022): 154675
31	Japanese medaka, Oryzias latipes	Fish	6PPD-Q	> 34	ug/l	96h	Mortality	Hiki, Kyoshiro,Asahina, Kenta,Kato, Kota,Yamagishi, Takahiro,Omagari, Ryo,Iwasaki, Yuichi,Watanabe, Haruna,Yamamoto, Hiroshi. "Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species". Environmental Science & Technology Letters 8 (2021): 779-784
32	Lake trout, Salvelinus namaycush	Fish	6PPD-Q	0.51	ug/l	24h	Mortality	Brinkmann et al in prep

Table 2-1. Summary of acute aquatic toxicity data for 6PPD and 6PPD-q

	Receptor (specific)	Receptor (general)	Chemical	Benchmark Value (L(E)C50)	Units (ug/l)	Duration	Endpoint	Reference
33	Lake trout, Salvelinus namaycush	Fish	6PPD-Q	0.5	ug/l	96h	Mortality	Brinkmann et al in prep
34	Marine amphipod, Parhyale hawaiiensis	Invertebrate	6PPD-Q	> 500	ug/l	96h	Mortality	Botelho, M. T., Militao, G. G., Brinkmann, M., & Umbuzeiro, G. A. (2023). Toxicity and mutagenicity studies of 6PPD-quinone in a marine invertebrate species and bacteria. Environ Mol Mutagen, 64(6), 335-341. https://doi.org/10.1002/em.22560
35	Marine rotifer, Brachionus koreanus	Invertebrate	6PPD-Q	> 1000	ug/l	24h	Mortality	Maji, Usha Jyoti,Kim, Kyuhyeong,Yeo, In-Cheol,Shim, Kyu-Young,Jeong, Chang-Bum. "Toxicological effects of tire rubber-derived 6PPD-quinone, a species-specific toxicant, and dithiobisbenzanilide (DTBBA) in the marine rotifer Brachionus koreanus". Marine Pollution Bulletin 192 (2023): 115002
36	Masu salmon, Oncorhynchus masou masou	Fish	6PPD-Q	> 3.5	ug/l	96h	Mortality	Hiki, Kyoshiro,Yamamoto, Hiroshi. "The Tire-Derived Chemical 6PPD-quinone Is Lethally Toxic to the White-Spotted Char Salvelinus leucomaenis pluvius but Not to Two Other Salmonid Species". Environmental Science & Technology Letters #volume# (2022): #pages#
37	Mayfly, Hexagenia spp	Invertebrate	6PPD-Q	>53.4	ug/l	4d	Mortality	Prosser, R. S., Salole, J., & Hang, S. (2023). Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environmental Pollution, 337, 122512. https://doi.org/https://doi.org/10.1016/j.envpol.2023.122512
38	Mayfly, Hexagenia spp	Invertebrate	6PPD-Q	>232	ug/l	4d	Mortality	Prosser, R. S., Salole, J., & Hang, S. (2023). Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environmental Pollution, 337, 122512. https://doi.org/https://doi.org/10.1016/j.envpol.2023.122512
39	Rainbow trout, Oncorhynchus mykiss	Fish	6PPD-Q	0.64	ug/l	96h	Mortality	Nair, P.,Sun, J. ,Xie, L.,Kennedy, L.,Kozakiewicz, D.,Kleywegt, S,Kleywegt, S.,Hao, C.,Byun, H.,Barrett, H.,Baker, J.,Monaghan, J.,Krogh, E.,Song, D.,peng, h. (2023). Synthesis and Toxicity Evaluation of Tire Rubber-Derived Quinones ChemRxiv. Open Engage. Cambridge: Cambridge. This content is a preprint and has not been peer-reviewed
40	Rainbow trout, Oncorhynchus mykiss	Fish	6PPD-Q	0.47	ug/l	96h	Mortality	Brinkmann et al in prep
41	Rainbow trout, Oncorhynchus mykiss	Fish	6PPD-Q	1	ug/l	96h	Mortality	Brinkmann, Markus,Montgomery, David,Selinger, Summer,Miller, Justin G. P.,Stock, Eric,Alcaraz, Alper James,Challis, Jonathan K.,Weber, Lynn,Janz, David,Hecker, Markus,Wiseman, Steve. "Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance". Environmental Science & Technology Letters 9 (2022): 333-338
42	Rainbow trout, Oncorhynchus mykiss	Fish	6PPD-Q	2.26	ug/l	96h	Mortality	Di, S.,Liu, Z.,Zhao, H.,Li, Y.,Qi, P.,Wang, Z.,Xu, H.,Jin, Y.,Wang, X.. "Chiral perspective evaluations: Enantioselective hydrolysis of 6PPD and 6PPD-quinone in water and enantioselective toxicity to Gobiocypris rarus and Oncorhynchus mykiss". Environ Int 166 (2022): 107374
43	Sockeye salmon, Oncorhynchus nerka	Fish	6PPD-Q	> 50	ug/l	24h	Mortality	Greer, Justin B.,Dalsky, Ellie M.,Lane, Rachael F.,Hansen, John D.. "Establishing an In Vitro Model to Assess the Toxicity of 6PPD-Quinone and Other Tire Wear Transformation Products". Environmental Science & Technology Letters #volume# (2023): #pages#
44	Southern Asian dolly varden, Salvelinus curilus	Fish	6PPD-Q	> 3.8	ug/l	96h	Mortality	Hiki, Kyoshiro,Yamamoto, Hiroshi. "The Tire-Derived Chemical 6PPD-quinone Is Lethally Toxic to the White-Spotted Char Salvelinus leucomaenis pluvius but Not to Two Other Salmonid Species". Environmental Science & Technology Letters #volume# (2022): #pages#
45	Washboard mussel, Megaloniaias nervosa	Invertebrate	6PPD-Q	>11.4	ug/l	8d	Mortality	Prosser, R. S., Salole, J., & Hang, S. (2023). Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environmental Pollution, 337, 122512. https://doi.org/https://doi.org/10.1016/j.envpol.2023.122512
46	Washboard mussel, Megaloniaias nervosa	Invertebrate	6PPD-Q	>17.9	ug/l	8d	Mortality	Prosser, R. S., Salole, J., & Hang, S. (2023). Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environmental Pollution, 337, 122512. https://doi.org/https://doi.org/10.1016/j.envpol.2023.122512
47	Water flea, Daphnia magna	Invertebrate	6PPD-Q	> 46	ug/l	48h	Mortality	Hiki, Kyoshiro,Asahina, Kenta,Kato, Kota,Yamagishi, Takahiro,Omagari, Ryo,Iwasaki, Yuichi,Watanabe, Haruna,Yamamoto, Hiroshi. "Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species". Environmental Science & Technology Letters 8 (2021): 779-784
48	Westslope cutthroat trout, Oncorhynchus clarkii lewisi	Fish	6PPD-Q	>10	ug/l	24h	Mortality	Montgomery, D., Ji, X., Cantin, J., Philibert, D., Foster, G., Selinger, S., Jain, N., Miller, J., McIntyre, J., de Jourdan, B., Wiseman, S., Hecker, M., & Brinkmann, M. (2023). Toxicokinetic Characterization of the Inter-Species Differences in 6PPD-Quinone Toxicity Across Seven Fish Species: Metabolite Identification and Semi-Quantification. bioRxiv, 2023.2008.2018.553920. https://doi.org/10.1101/2023.08.18.553920
49	White spotted char, Salvelinus leucomaenis pluvius	Fish	6PPD-Q	0.51	ug/l	96h	Mortality	Hiki, Kyoshiro,Yamamoto, Hiroshi. "The Tire-Derived Chemical 6PPD-quinone Is Lethally Toxic to the White-Spotted Char Salvelinus leucomaenis pluvius but Not to Two Other Salmonid Species". Environmental Science & Technology Letters #volume# (2022): #pages#

Table 2-1. Summary of acute aquatic toxicity data for 6PPD and 6PPD-q

	Receptor (specific)	Receptor (general)	Chemical	Benchmark Value (L(E)C50)	Units (ug/l)	Duration	Endpoint	Reference
50	White sturgeon, Acipenser transmontanus	Fish	6PPD-Q	> 12.7	ug/l	96h	Mortality	Brinkmann, Markus,Montgomery, David,Selinger, Summer,Miller, Justin G. P.,Stock, Eric,Alcaraz, Alper James,Challis, Jonathan K.,Weber, Lynn,Janz, David,Hecker, Markus,Wiseman, Steve. "Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone to Four Fishes of Commercial, Cultural, and Ecological Importance". Environmental Science & Technology Letters 9 (2022): 333-338
51	Zebrafish, Danio rerio	Fish	6PPD-Q	> 54	ug/l	96h	Mortality	Hiki, Kyoshiro,Asahina, Kenta,Kato, Kota,Yamagishi, Takahiro,Omagari, Ryo,Iwasaki, Yuichi,Watanabe, Haruna,Yamamoto, Hiroshi. "Acute Toxicity of a Tire Rubber-Derived Chemical, 6PPD Quinone, to Freshwater Fish and Crustacean Species". Environmental Science & Technology Letters 8 (2021): 779-784
52	Zebrafish, Danio rerio	Fish	6PPD-Q	132.92	ug/l	96h	Mortality	Varshney, S.,Gora, A. H.,Siriappagouder, P.,Kiron, V.,Olsvik, P. A.. "Toxicological effects of 6PPD and 6PPD quinone in zebrafish larvae". J Hazard Mater 424 (2022): 127623
53	Zebrafish, Danio rerio	Fish	6PPD-Q	> 1000	ug/l	12h	Mortality	Ji, J.,Huang, J.,Cao, N.,Hao, X.,Wu, Y.,Ma, Y.,An, D.,Pang, S.,Li, X.. "Multiview behavior and neurotransmitter analysis of zebrafish dyskinesia induced by 6PPD and its metabolites". Sci Total Environ 838 (2022): 156013

* LOEC

Table 2-2. Summary of chronic aquatic toxicity data for 6PPD and 6PPD-q

	Receptor (specific)	Receptor (general)	Chemical	Benchmark Value (NOEC, LC10)	Units	Duration	Endpoint	Reference
1	Algae, Selenastrum capricornutum	Plant/algae	6PPD	200	ug/l	96h	Cell number	Monsanto 1978 as cited in UNEP 2004
2	Japanese medaka, Oryzias latipes	Fish	6PPD	3.7	ug/l	30d		National Institute of Technology and Evaluation, Japan, 2003 as cited in ECHA 2023
3	Aquatic worm, Tubifex tubifex	Invertebrate	6PPD	63	ug/g dw	28d	Mortality	Prosser, R. S., Bartlett, A. J., Milani, D., Holman, E. A. M., Ikert, H., Schissler, D., Toito, J., Parrott, J. L., Gillis, P. L., & Balakrishnan, V. K. (2017). Variation in the toxicity of sediment-associated substituted phenylamine antioxidants to an epibenthic (Hyalella azteca) and endobenthic (Tubifex tubifex) invertebrate. Chemosphere, 181, 250-258. https://doi.org/10.1016/j.chemosphere.2017.04.066
4	Fatmucket mussel, Lampsilis siliquoidea	Invertebrate	6PPD	47	ug/l	14d	Mortality	Prosser, R. S., Gillis, P. L., Holman, E. A. M., Schissler, D., Ikert, H., Toito, J., Gilroy, E., Campbell, S., Bartlett, A. J., Milani, D., Parrott, J. L., & Balakrishnan, V. K. (2017). Effect of substituted phenylamine antioxidants on three life stages of the freshwater mussel Lampsilis siliquoidea. Environ Pollut, 229, 281-289. https://doi.org/10.1016/j.envpol.2017.05.086
5	Fatmucket mussel, Lampsilis siliquoidea	Invertebrate	6PPD	17	ug/l	28d	Mortality	Prosser, R. S., Gillis, P. L., Holman, E. A. M., Schissler, D., Ikert, H., Toito, J., Gilroy, E., Campbell, S., Bartlett, A. J., Milani, D., Parrott, J. L., & Balakrishnan, V. K. (2017). Effect of substituted phenylamine antioxidants on three life stages of the freshwater mussel Lampsilis siliquoidea. Environ Pollut, 229, 281-289. https://doi.org/10.1016/j.envpol.2017.05.086
6	Fatmucket mussel, Lampsilis siliquoidea	Invertebrate	6PPD	188	ug/g dw	14d	Mortality	Prosser, R. S., Gillis, P. L., Holman, E. A. M., Schissler, D., Ikert, H., Toito, J., Gilroy, E., Campbell, S., Bartlett, A. J., Milani, D., Parrott, J. L., & Balakrishnan, V. K. (2017). Effect of substituted phenylamine antioxidants on three life stages of the freshwater mussel Lampsilis siliquoidea. Environ Pollut, 229, 281-289. https://doi.org/10.1016/j.envpol.2017.05.086
7	Fatmucket mussel, Lampsilis siliquoidea	Invertebrate	6PPD	62	ug/g dw	28d	Mortality	Prosser, R. S., Gillis, P. L., Holman, E. A. M., Schissler, D., Ikert, H., Toito, J., Gilroy, E., Campbell, S., Bartlett, A. J., Milani, D., Parrott, J. L., & Balakrishnan, V. K. (2017). Effect of substituted phenylamine antioxidants on three life stages of the freshwater mussel Lampsilis siliquoidea. Environ Pollut, 229, 281-289. https://doi.org/10.1016/j.envpol.2017.05.086
8	Amphipod, Hyalella azteca	Invertebrate	6PPD	68	ug/g dw	28d	Mortality	Prosser, R. S., Bartlett, A. J., Milani, D., Holman, E. A. M., Ikert, H., Schissler, D., Toito, J., Parrott, J. L., Gillis, P. L., & Balakrishnan, V. K. (2017). Variation in the toxicity of sediment-associated substituted phenylamine antioxidants to an epibenthic (Hyalella azteca) and endobenthic (Tubifex tubifex) invertebrate. Chemosphere, 181, 250-258. https://doi.org/10.1016/j.chemosphere.2017.04.066
9	Amphipod, Hyalella azteca	Invertebrate	6PPD	6	ug/l	28d	Mortality	Prosser, R. S., Bartlett, A. J., Milani, D., Holman, E. A. M., Ikert, H., Schissler, D., Toito, J., Parrott, J. L., Gillis, P. L., & Balakrishnan, V. K. (2017). Variation in the toxicity of sediment-associated substituted phenylamine antioxidants to an epibenthic (Hyalella azteca) and endobenthic (Tubifex tubifex) invertebrate. Chemosphere, 181, 250-258. https://doi.org/10.1016/j.chemosphere.2017.04.066
10	Fathead minnow, Pimephales promelas	Fish	6PPD	86	ug/g dw	21d	Mortality	Prosser, R. S., Parrott, J. L., Galicia, M., Shires, K., Sullivan, C., Toito, J., Bartlett, A. J., Milani, D., Gillis, P. L., & Balakrishnan, V. K. (2017). Toxicity of sediment-associated substituted phenylamine antioxidants on the early life stages of Pimephales promelas and a characterization of effects on freshwater organisms. Environ Toxicol Chem, 36(10), 2730-2738. https://doi.org/10.1002/etc.3828
11	Fathead minnow, Pimephales promelas	Fish	6PPD	20	ug/l	21d	Mortality	Prosser, R. S., Parrott, J. L., Galicia, M., Shires, K., Sullivan, C., Toito, J., Bartlett, A. J., Milani, D., Gillis, P. L., & Balakrishnan, V. K. (2017). Toxicity of sediment-associated substituted phenylamine antioxidants on the early life stages of Pimephales promelas and a characterization of effects on freshwater organisms. Environ Toxicol Chem, 36(10), 2730-2738. https://doi.org/10.1002/etc.3828
12	Nematode, Caenorhabditis elegans	Invertebrate	6PPD-Q	10	ug/l	4.5 d	Mortality	Hua, X.,Feng, X.,Liang, G.,Chao, J.,Wang, D.. "Long-term exposure to tire-derived 6-PPD quinone causes intestinal toxicity by affecting functional state of intestinal barrier in Caenorhabditis elegans". Sci Total Environ 861 (2023): 160591
13	Nematode, Caenorhabditis elegans	Invertebrate	6PPD-Q	1	ug/l	4.5 d	Paralysis	Hua, Xin,Feng, Xiao,Liang, Geyu,Chao, Jie,Wang, Dayong. "Exposure to 6-PPD Quinone at Environmentally Relevant Concentrations Causes Abnormal Locomotion Behaviors and Neurodegeneration in Caenorhabditis elegans". Environmental Science & Technology #volume# (2023): #pages#
14	Nematode, Caenorhabditis elegans	Invertebrate	6PPD-Q	0.1	ug/l	4.5 d	Brood size	Hua, Xin,Feng, Xiao,Liang, Geyu,Chao, Jie,Wang, Dayong. "Long-term exposure to 6-PPD quinone reduces reproductive capacity by enhancing germline apoptosis associated with activation of both DNA damage and cell corpse engulfment in Caenorhabditis elegans". Journal of Hazardous Materials 454 (2023): 131495
15	Freshwater rotifer, Brachionus calyciflorus	Invertebrate	6PPD-Q	> 1000	ug/l	12d	Mortality	Klauschies, T.,Isanta-Navarro, J.. "The joint effects of salt and 6PPD contamination on a freshwater herbivore". Sci Total Environ 829 (2022): 154675

Table 2-2. Summary of chronic aquatic toxicity data for 6PPD and 6PPD-q

	Receptor (specific)	Receptor (general)	Chemical	Benchmark Value (NOEC, LC10)	Units	Duration	Endpoint	Reference
16	Rainbow trout, Oncorhynchus mykiss	Fish	6PPD-Q	0.56	ug/l	28d	Mortality	Brinkmann et al in prep
17	Lake trout, Salvelinus namaycush	Fish	6PPD-Q	0.33	ug/l	45d	Mortality	Brinkmann et al in prep
18	Water flea, Daphnia magna	Invertebrate	6PPD-Q	> 42	ug/l	21d	Mortality and growth	Prosser, R. S., Salole, J., & Hang, S. (2023). Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environmental Pollution, 337, 122512. https://doi.org/https://doi.org/10.1016/j.envpol.2023.122512
19	File ramshorn snail, Planorbella pilsbryi	Invertebrate	6PPD-Q	> 11.7	ug/l	10d	Mortality and hatching	Prosser, R. S., Salole, J., & Hang, S. (2023). Toxicity of 6PPD-quinone to four freshwater invertebrate species. Environmental Pollution, 337, 122512. https://doi.org/https://doi.org/10.1016/j.envpol.2023.122512
20	Coho salmon, Oncorhynchus kisutch	Fish	6PPD-Q	0.1	ug/l	16d, pulsed exposure for 24h	Mortality	Greer, Justin B.,Dalsky, Ellie M.,Lane, Rachael F.,Hansen, John D. (2023). Tire-Derived Transformation Product 6PPD-Quinone Induces Mortality and Transcriptionally Disrupts Vascular Permeability Pathways in Developing Coho Salmon Environmental Science & Technology, #volume#(#issue#), #Pages#
21	Fathead minnow, Pimephales promelas	Fish	6PPD-Q	> 39.27	ug/l	7d	Mortality	Anderson-Bain, Katherine,Roberts, Catherine,Kohlman, Evan, Ji, Xiaowen,Alcaraz, Alper J.,Miller, Justin,Gangur-Powell, Tabitha,Weber, Lynn,Janz, David,Hecker, Markus,Montina, Tony,Brinkmann, Markus,Wiseman, Steve (2023). Apical and mechanistic effects of 6PPD-quinone on different life-stages of the fathead minnow (Pimephales promelas) Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 271(#issue#), 109697
22	Marine rotifer, Brachionus koreanus	Invertebrate	6PPD-Q	> 1000	ug/l	24h	Fecundity	Maji, Usha Jyoti,Kim, Kyuhyeong,Yeo, In-Cheol,Shim, Kyu-Young,Jeong, Chang-Bum. "Toxicological effects of tire rubber-derived 6PPD-quinone, a species-specific toxicant, and dithiobisbenzanilide (DTBBA) in the marine rotifer Brachionus koreanus". Marine Pollution Bulletin 192 (2023): 115002
23	Springtail, Folsomia candida	Invertebrate	6PPD-Q	16.31	mg/kg	28d	Mortality	Xu, Q., Wu, W., Xiao, Z., Sun, X., Ma, J., Ding, J., Zhu, Z., & Li, G. (2023). Responses of soil and collembolan (Folsomia candida) gut microbiomes to 6PPD-Q pollution. Science of The Total Environment, 900, 165810. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.165810

Table 2-3. Summary of Human Health Toxicity Studies

	Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Measured Effects	Author
1	6PPD-q	ADME, Repeat Dose (Subchronic)	Two exposure modes were performed, single and repeated intraperitoneal injections. 0.4 mg/kg bw, 4 mg/kg bw. Repeated dose was every 4 days for 28 days	Male BALB/c mice (n=36)	Serum, liver, kidney, lung, spleen, testis, brain, and heart were collected for injury evaluation by organ index, histopathology analysis and biochemical parameters. In 0.4 and 4 mg/kg 6PPD-q single injected mice, no significant changes in organ indexes and biochemical parameters were detected, and only moderate pathological changes were observed in organs of liver, kidney, lung, and brain. In 0.4 and 4 mg/kg 6PPD-q repeated injected mice, observed an increase in organ indexes of liver, kidney, lung, testis, and brain, and the decrease in spleen index. Significant pathological changes were formed in liver, kidney, lung, spleen, testis, and brain in 0.4 and 4 mg/kg 6PPD-q repeated injected mice. Biochemical parameters of liver (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)) and kidney (urea and creatinine) were all significantly upregulated by repeated injection with 0.4 and 4 mg/kg 6PPD-q. After repeated exposure, most of 6PPD-q was accumulated in liver and lung of mice.	He et al 2023
2	6PPD-q	ADME, Neurotoxicity	<p>Testing concentrations of 10 nM (2.98 ng/ml) or 100 nM (29.8 ng/ml) 6PPD-q were used to evaluate the potential to exacerbate Lewy neurite formation. α-syn PFF and 6PPD-q were added to neurons at 10 days in vitro (DIV) for 7 days.</p> <p>To determine if 6PPD-Q increased α-syn PFF-induced p-α-synSer129 levels, the authors exposed primary dopaminergic cells to 10 nM or 100 nM 6PPD-Q and / or 1 μg/ml of α-syn PFF. However, when measuring effects on mitochondrial respiration, the cells were exposed to 10 nM or 100 nM 6PPD-Q and/or 2 μg/ml of α-syn PFF.</p>	Human (n=24 from Shenzhen, South China) E13.5 pregnant C57BL/6 mice	<p>Cerebrospinal fluid (CSF) samples from Parkinson’s disease (PD) patients and non-PD subjects (controls) were analyzed. The average age of all participants was 57.71 years old, and the age of PD patients and the control population did not differ significantly. The PD patients had an average of 7 years of disease. Average concentrations of 6PPD-q were twice as high in PD patients (11.18 ng/ml) compared to controls (5.07 ng/ml).</p> <p>Treatment with 10 nM (2.98 ng/mL) and 100 nM (29 ng/mL) 6PPD-q resulted in a dose-dependent decrease in overall ATP production – although that is not necessarily linked to oxidative phosphorylation. The primary neurons demonstrated a decrease in the basal respiratory capacity after treatment with 100 nM 6PPD-q. In addition, 6PPD-q [presumably at 100 nM] caused changes in the concentrations of some components of the citric acid cycle. There was no comparison with other molecules which may cause oxidative stress. The study authors found some synergistic effects on mitochondrial membrane potential and mitochondrial processes when pre-seeding the cells with misfolded α-synuclein protein.</p>	Fang, J et al 2023
3	6PPD-q	ADME	<p>Mice were fed with a single human-relevant dose (400 μg/kg) of deuterated 6PPD-q-d5 dissolved in corn oil by gavage.</p>	<p>To determine a human-relevant dose, human serum samples were collected from 30 healthy volunteers. Participants were selected randomly from the general population without any occupational exposure to 6PPD-Q or other rubber chemicals. In total, 30 volunteers, of which 12 were males and 18 females (mean age 40.5 years, age range 23-66 years), were sampled.</p> <p>Adult male and female ICR mice (7–8 weeks old) (n=4 for each sex)</p>	<p>In human serum, 6PPD-q was found with a detection frequency (DF) of 100 % and concentrations ranging from 0.11 to 0.43 ng/mL (mean: 0.21 ng/mL, median: 0.24 ng/mL). Animals were fed at the oral dose that achieved an average 24 h serum concentration of contaminants within this range of human bio-monitoring concentrations.</p> <p>At specified intervals of 0.5 h, 1 h, 3 h, 6 h, 12 h, 24 h, 72 h, and 1 week after dosing, mice were humanely euthanized, and the whole-blood was collected to prepare serum samples. Selected tissues of male mice including the heart, liver, spleen, lung, kidney, brain, testis, adipose, and muscle were collected. The urine and feces of each post- gavage male mouse were collected in glass metabolic cages after a period (ranging from 6 h to 1 week).</p> <p>At a dose of 400 μg/kg, the 24 h serum concentrations of 6PPD-q (0.34 ± 0.06 ng/mL) in exposed mice were within the range of human serum levels (0.11–0.43 ng/mL). Results indicated that 6PPD-q was quickly assimilated and distributed into bloodstream and main organs of mice, with the concentrations reaching peaks under 1 h following administration. 6PPD-q was primarily distributed in the adipose tissue followed by the kidney, lung, testis, liver, spleen, heart, and muscle. In addition, 6PPD-q was demonstrated to penetrate the blood-brain barrier of mice within 0.5 h after exposure. The half-lives ($t_{1/2}$) of 6PPD-q in serum, lung, kidney, and spleen of mice were measured at 12.7 ± 0.3 h, 20.7 ± 1.4 h, 21.6 ± 5.3 h, and 20.6 ± 2.8 h, respectively. Two novel hydroxylated metabolites of 6PPD-q in the mice liver were also identified (6PPD-q-d5-OH and 6PPD-q-d5-2OH) for the first time using high resolution mass spectrometry. Fecal excretion was identified as the main excretory pathway for 6PPD-q and its hydroxylated metabolites (35.1 ± 1.9 % of the initial dose at one week after administration).</p>	Zhang et al 2024

Table 2-3. Summary of Human Health Toxicity Studies

Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Measured Effects	Author
6PPD-q	Reproductive Toxicity	L1-larval nematodes were exposed to environmentally relevant concentrations of 0.1, 1 and 10 µg/L 6PPD-q for 4.5 days (till development of adult day-1) to assess long-term exposure.	C. Elegans: Wild-Type N2 and transgenic strain WS1433 (30 nematodes per treatment)	<p>Reproductive capacity: Exposure to 1 and 10 µg/L 6PPD-q significantly decreased both the number of fertilized eggs in utero and the number of hatched eggs.</p> <p>Gonad development: At concentrations of 1 µg/L 6PPD-q the number of mitotic cells per gonad and area of gonad arm were significantly decreased. At concentrations of 10 µg/L 6PPD-q the number of mitotic cells per gonad, length of gonad arm and area of gonad arm were significantly reduced. The study authors concluded that these results indicated that 6PPD-q exposure caused impairments on gonad development in <i>C. elegans</i> .</p> <p>Induction of germline apoptosis was enhanced and expressions of ced-3, ced-4 and egl-1 were also significantly increased. While ced-9 expression was significantly decreased.</p> <p>Inducation of germline DNA damage was also enhanced. Exposure to 1 and 10 µg/L 6PPD-q caused significant increase in the expression of DNA damage checkpoints CLK-2, HUS-1 and MRT-2 and an increase in expression of ced-1 and ced-6, which govern cell corpse engulfment process.</p>	Hua et al 2023
6PPD or 6PPD-q	ADME, Developmental	<p>Urinary Excretion: 4 mg/kg (dissolved in corn oil) via oral gavage in mice age 10-15 weeks; Urine samples collected prior to treatment and 1, 5 and 24 hours post-treatment</p> <p>Maternal Transfer: 4 mg/kg (dissolved in corn oil); Pregnant dams treated from E11.5 to E15.5 once per day via oral gavage.</p>	Male/Female C57Bl/6 mice (n=12/group)	Female and male mice exhibited sex difference in excretion profiles of 6PPD and 6PPD-q. Urine concentrations of 6PPD-q were one order of magnitude lower than those of 6PPD, suggesting lower excretion and higher bioaccumulation of 6PPD-q. In pregnant mice treated with 6PPD or 6PPD-q from embryonic day 11.5 to 15.5, 6PPD-q showed ~1.5–8 times higher concentrations than 6PPD in placenta, embryo body, and embryo brain, suggesting higher placental transfer of 6PPD-q. Using in vitro dual-luciferase reporter assays, we revealed that 6PPD-q activated the human retinoic acid receptor α (RAR α) and retinoid X receptor α (RXR α) at concentrations as low as 0.3 µM, which was ~10-fold higher than the concentrations detected in human urines. 6PPD activated the RXR α at concentrations as low as 1.2 µM.	Zhao et al 2023
6PPD or 6PPD-q	Repeat Dose (Subchronic)	6PPD and 6PPD-q were dissolved in acetone (five parts per thousand by volume) and mixed with corn oil. Mice in the six treatment groups were orally administered 10 mg/kg, 30 mg/kg, or 100 mg/kg 6PPD or 6PPD-q for six weeks	Male C57BL/6 mice (n = 56)	6PPD and 6PPD-q were found to bioaccumulate in the liver in a dose-dependent manner. A high dose of 6PPD and 6PPD-q exposure increased not only the liver weights but also liver triglyceride levels, indicating that 6PPD and 6PPD-q exposure induced hepatotoxicity in mice. Transcriptomic analysis revealed that 6PPD and 6PPD-q induced differential expression of genes mainly enriched in glycolipid metabolism, immune-related, and glutathione metabolism pathways. Therefore, 6PPD and 6PPD-q altered hepatic metabolism in mice. Furthermore, 6PPD-q could induce an immune response by upregulating the transcription of immune-related genes and promoting macrophage infiltration in the liver.	Fang, L et al 2023
6PPD or 6PPD-q	ADME	N/A	Human (n=150 from Guangzhou, South China; adult (50), children (50), pregnant women (50))	Both 6PPD and 6PPD-Q were detected in the urine samples, with detection frequencies between 60% and 100%. Urinary 6PPD-Q concentrations were significantly higher than those of 6PPD and correlated well with those of 6PPD (p < 0.01), indicating coexposure to 6PPD and 6PPD-Q in humans. In vitro metabolic experiments demonstrated rapid depletion of 6PPD by human liver microsomes, which should be responsible for the lower concentrations of 6PPD in human urine. Additionally, pregnant women exhibited apparently higher concentrations of 6PPD and 6PPD-Q (median 0.068 and 2.91 ng/mL, respectively) than did adults (0.018 and 0.40 ng/mL) and children (0.015 and 0.076 ng/mL). The high daily urinary excretion of 6PPD-Q in pregnant women was estimated to be 273 (ng/kg bw)/day.	Du et al 2022
6PPD	Basic Toxicokinetics	N/A	Not indicated	An <i>in vitro</i> hydrolysis study was conducted to determine the hydrolysis rate and hydrolysis products of 6PPD in simulated gastric juice. At the 48-hour observation period, 60% of 6PPD was hydrolyzed. Based on this data the hydrolysis rate was reported as -0.0188 with a calculated half-life of 36.9 hours. The approximate solubility of 6PPD in gastric juice was reported as 173 mg/l. The major observed hydrolysis product was aniline. A trace of two intermediate hydrolysis products, benzoquinoneimine-N-phenyl, and N-1,3 dimethyl-butylamine p-phenol were detected.	Unnamed study reported in ECHA
6PPD	Basic Toxicokinetics	Urine biomonitoring was conducted between 1982 and 1987.	Human	6PPD was measured in the urine of workers from the rubber industry which demonstrates that 6PPD can be resorbed from the respiratory tract and possibly after dermal contact In environmental air levels were in the range of < 0.01 - 260 µg/mc (peak 6600) and in urine levels were in the range of < 1 - 300 µg/g creatinine (peak 580).	Carlucci 1984, Rimatori and Castellino, 1989

Table 2-3. Summary of Human Health Toxicity Studies

	Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Measured Effects	Author
10	6PPD	Basic Toxicokinetics	Urine biomonitoring was conducted in April 2019 (one spot morning fasting sample per person).	Human (n=151 adults in Quzhou, China; 58% were female; mean age 50 years (male) and 46 years(female))	9 PPDs, including 6PPD, were measured in human urine samples. Total concentrations of PPDs ranged from 0.41 to 38 ng/mL. 6PPD was detected at the highest concentration (mean 1.2 ng/mL, range < LOD – 3.8 ng/mL) and exhibited the highest detection frequency (82 %). Female adults exhibited significantly higher mean urinary concentrations than their male counterparts (1.4 ± 0.29 ng/mL compared to 1.0 ± 0.18 ng/mL). In addition, urinary concentrations showed a general decreasing trend with the age of participants. Daily excretion rates for 6PPD were estimated as 34 ng/kg bw/day (mean value).	Mao et al 2024
11	6PPD	Acute toxicity	In an OECD guideline 401 (acute toxicity study), male and female Sprague-Dawley rats (5 per sex per dose) were exposed to 6PPD in corn oil via oral gavage at the following doses: 0, 250, 500, 1000, and 2000 mg/kg bw.	Sprague-Dawley rat	The acute oral LD ₅₀ was reported as 893 mg/kg bw/day for females and 1005 mg/kg bw/day for males based on clinical signs and pathological lesions in digestive organs and respiratory system. Clinical signs included: reduced volume of feces (500 mg/kg dose for male and females); hypoactivity, diarrhea, bradypnea, hypothermia, prone position (1000 mg/kg dose); abnormal gait, lacrimation, and weakness of limbs (2000 mg/kg dose). In addition to this, male rats were less sensitive than female rats.	Hatano Research Institute 1999
12	6PPD	Acute toxicity	An acute oral toxicity study was conducted under GLP according to EPA OTS 798.1175 (Acute Oral Toxicity). Sprague-Dawley rats were dosed with warmed 6PPD. The solid test substance was warmed to 55°C to produce a liquid and then allowed to cool to 50°C for dosing. The density of the liquid test article was determined to be 1.00 g/ml. A dose-rage finding study was conducted up to 5000 mg/kg bw with one animal per dose and sex. One male died at 5000 mg/kg bw; whereas no death occurred in treated females up to 5000 mg/kg bw. Following the range-finding test, a limit test was performed in which one group of five male and five female rats received a single oral dose of the test substance at a dose of 5000 mg/kg bw. Following dosing, the rats were observed daily and weighed weekly.	Sprague-Dawley rat	A gross necropsy examination was performed on all animals at the scheduled necropsyon day 15. Death occurred in 2/5 males and in 1/5 females. The most notable clinical signs included decreased fecal output, fecal/urine stains, rough coat, piloerection, diarrhea/soft stools and dark material around the facial area. Body weight loss was noted in one surviving male and three surviving females during the test period. Body weight gain was exhibited by all other surviving animals during the study. The most notable internal necropsy finding consisted of black, hard (rock-like) material contents in the stomach. This finding occurred in the surviving animals as well as in those that died. Additional findings which occurred in the animals that died included black/yellow-green mucoid contents throughout the digestive tract eroded areas with perforations of the stomach and reddened mucosa/dark red foci of the stomach. Based on findings from this study an acute oral LD ₅₀ greater than 5000 mg/kg bw was estimated.	Monsanto Co., 1991
13	6PPD	Acute toxicity	In an acute dermal toxicity study, male and female New Zealand White rabbits were exposed to 3160, 5010, or 7940 mg/kg bw of 6PPD undiluted via semiocclusive dermal exposure for 24 hours.	New Zealand White rabbits	The dermal LD ₅₀ was reported as > 7940 mg/kg bw/day based on reduced appetite and reduced activity for three to seven days. At necropsy viscera appeared normal.	Monsanto Co. 1973, Randall and Bannister, 1990
14	6PPD	Skin irritation	In a dermal irritation study, male and female albino New Zealand White rabbits were exposed to undiluted 6PPD (500 ml warmed to 46oC to liquefy) via semi-occlusive dressings to clipped intact and abraded skin at a concentration of 0.5 ml for 24 hours. The rabbits were observed for 7 days.	New Zealand White rabbits (n=6)	The mean overall irritation score was reported as 0/8 based on no effects being observed.	Monsanto Co. 1973, Randall and Bannister 1990
15	6PPD	Skin irritation	A Draize test was conducted using rabbits (strain not specified) exposed to undiluted 6PPD via occlusive dermal exposure for 24 hours. The rabbits were observed for 120 hours.	Unspecified strain of rabbits (n=3)	At the 4-hour observation timepoint, very slight erythema was noted in 2/3 rabbits. The mean overall irritation score was reported as 0.6/8 for the 4-hour observation timepoint. At the 24-hour observation timepoint, very slight to well defined erythema was observed with a mean overall irritation score of 1.6. At the 48-hour observation timepoint, erythema was observed with a mean irritation score of 1.3. At the 72-hour observation timepoint, very slight erythema was observed with a mean irritation score of 1. All of the observed effects at each time point were fully reversible within 120-hours.	Monsanto Co. 1962
16	6PPD	Eye irritation	An undiluted 0.1ml dose of 6PPD (warmed to 46°C to liquefy) was applied into the conjunctiva sac of the rabbit eye for 24 hours. A 7-day observation period followed the application of the 6PPD.	New Zealand White rabbits	The eye irritating potential of 6PPD was evaluated in an eye irritation study using New Zealand albino rabbits. 6PPD was slightly irritating to the rabbit eye (irritation mean score at 24, 48, and 72 hours: 1.2/110.0). Effects on the conjunctivae were noted in all animals at 24 hours (mean score 24 h: 5.6/110) but were reversible within 72 hours.	Monsanto Co. 1973

Table 2-3. Summary of Human Health Toxicity Studies

	Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Measured Effects	Author
17	6PPD	Eye irritation	An undiluted 6PPD (0.1 ml) applied to the eyes of rabbits (strain not specified) for 24 hours followed by a 5 day post exposure period.	Unspecified strain of rabbits (n=3)	After 1 hour slight edema and erythema, copious discharge and slight dullness of the corneal area were observed with an average score of 20.6/110 according to the method of Draize. Iris and cornea cleared somewhat in 24 hours and within 72 hours iris clarity was normal. Very slight redness and edema disappeared by the 5th day	Monsanto Co.1962
18	6PPD	Skin sensitisation	A guinea pig maximization test was conducted using doses of 50 ppm and 5000 ppm. Acetone was used as the vehicle for dosing.	Hartley guinea pig, female (n=4)	6PPD was reported as sensitizing to the skin of four female guinea pigs in a guinea pig maximization test. All four guinea pigs showed positive skin reactions for both doses of 6PPD (50 ppm (0.005%) and 5000 ppm (0.5%)).	Yamano, T. et al. 2009
19	6PPD	Skin sensitisation	Female Balb/c mice were exposed to the following doses of 6PPD: 0, 0.1, 0.3, 1, and 3%.	Balb/c mice (n=4)	6PPD was reported as a positive skin sensitizer in a mouse local lymph node assay (LLNA). The stimulation index (SI) score was reported as 2.34 for the 1% dose and 5.06 for the 3% dose.	Yamano, T. et al. 2009
20	6PPD	Skin sensitisation	Over a period of 32 years (1980-2012) data from a manufacturing site in Brunsbüttel (Germany) were collected and analyzed by the company medical department. Workers were examined at regular intervals to determine the skin sensitization potential to the airborne dust of Vulkanox 4020 LG (6PPD).	Human (n=70)	Airborne dust of Vulkanox 4020 LG (6PPD) may arise from the manufacturing process. During processing the workers may come into direct contact with the airborne dust, which requires wearing personnel protective equipment to avoid any possible skin sensitization. Since 1993 close meshed indoor measurements have been conducted to investigate the room air on the airborne dust of Vulkanox 4020 LG (6 PPD). In regular intervals concentrations of 0.4 mg/m3 room air were measured. The results showed no evidence of a sensation effects related to the skin exposure to 6PPD suggesting that the personal protective equipment (PPE) worn during the process of manufacturing was adequate to prevent exposure.	Unnamed study reported in ECHA
21	6PPD	Skin sensitisation	Not provided	Human	Repeated insult patch tests (method of Shelanski or a modified Schwartz patch test) in humans indicate that healthy volunteers not previously exposed to test rubber formulations experienced no sensitization or only a low sensitization rate to 6PPD was while the sensitization rate was much higher in persons who had been previously sensitized to rubber samples.	OECD, 2004.
22	6PPD	Repeat Dose (Subchronic)	A 28-day repeat dose toxicity study was conducted using male and female Sprague-Dawley rats. Test subjects were exposed to 0, 4, 20, 100 mg/kg bw/day of 6PPD followed by a 14 day recovery period.	Sprague-Dawley rat (5 animals per sex per dose group)	No effects on survival or body weight gain were reported. The NOAEL was reported as 20 mg/kg bw/day based on the following effects on females: reversible periportal fatty changes of the liver without an increase of liver weight and increased total serum protein. A LOAEL of 100 mg/kg bw/day was reported based on the following effects on both sexes: an increase in relative liver weights accompanied by periportal fatty change, clinical chemistry, haematological parameters changed indicating an existing anemia.	OECD, 2004.
23	6PPD	Repeat Dose (Chronic)	An OECD guideline 452 (Chronic Toxicity) study was conducted using male and female Sprague-Dawley rats exposed to 0 ppm, 50 ppm, 250 ppm, or 1500 ppm 6PPD in their feed daily. At the beginning of the experiment all of the rats in the group were dosed with 6PPD. At 12 months of treatment approximately 20 rats/sex/group were sacrificed. After 24 months of treatment all survivors were sacrificed.	Sprague-Dawley rat (n=70)	The NOEL of this study is 50 ppm in males and females (2.6 mg/kg/day in males, 3.2 mg/kg/day in females), based on reduced body weight in females, increased food consumption in females and increased liver weight at 250 ppm in both sexes (13.5-16.5 mg/kg/day). At the high dose (1500 ppm; 84.8-109.5 mg/kg/day) liver histopathology in females and slight effects on hematology are observed in both sexes.	Monsanto Co. 1993
24	6PPD	Genotoxicity	µg/plate: TA1535, TA1537/-S9mix: up to 19.5; TA98, 100/-S9mix: up to 78.1; TA100, TA1535, TA1537/+S9mix: up to 156; TA98/+S9mix: up to 625 E. coli: 313, 625, 1250, 2500, 5000 µg/plate	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 E. coli WP2 uvr A	The data from the bacterial mutation assays indicated no genotoxic potential of 6PPD. 6PPD did not induce gene mutation in S. typhimurium tester strains or E. coli WP2 uvr A with and without metabolic activation	Shibuya et al, 1999
25	6PPD	Genotoxicity	pre-test: 0.33 to 333 µg/ml, pre-test II -S9: 0.5 to 5 µg/ml, +S9: 1 to20 µg/ml, main experiment I: -S9 1 to 5 µg/ml, +S9: 1 to24 µg/ml, main experiment II and III: -S9 1 to 5 µg/ml, +S9: 3 to 15 µg/ml	Chinese hamster Ovary (CHO)	The data from the <i>in vitro</i> mutation study in cultured Chinese Hamster Ovary (CHO) cells indicated that 6PPD did not induce mutagenicity in the presence of metabolic activation or in the absence of metabolic activation. An ambiguous result for clastogenicity was noted in CHO cells treated with 6PPD.	Monsanto, 1987
26	6PPD	Genotoxicity	continuous treatment/-S9mix: 0, 0.0025, 0.0050, 0.010 mg/ml; short-term treatment: -S9-mix: 0, 0.00063, 0.0013, 0.0025 mg/ml; +S9mix: 0, 0.0038, 0.0075, 0.015 mg/ml	Chinese hamster lung cells (CHL/IU)	The data from the <i>in vitro</i> chromosome aberration assay in Chinese hamster lung (CHL/IU) cells, without metabolic activation, revealed that 6PPD exposure led to a dose-dependent increase in chromosomal aberrations after 24 hours (0.005 mg/ml) and 48 hours (0.01 mg/ml). No chromosomal aberrations were observed ,with metabolic activation, after 6 hours of exposure to 6PPD. This study indicates that 6PPD exposure has clastogenic effects on Chinese hamster lung cells.	Tanaka et al., 1999

Table 2-3. Summary of Human Health Toxicity Studies

	Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Measured Effects	Author
27	6PPD	Genotoxicity	Male and female Sprague-Dawley rats were exposed to one dose of 900, 1300, 1790 mg/kg bw/day (preliminary study) or 1000 mg/kg bw/day (main study) of 6PPD in an in vivo bone marrow chromosome aberration assay.	Sprague-Dawley rat (5 rats per sex per dose)	<p>Clinical signs were observed for all 1000 mg/kg treated animals at 6 and 18 and 30 hours after application. The clinical signs observed were abnormal gait and decreased body tone with most of the rats also exhibiting decreased activity and abnormal stance. Several animals also exhibited piloerection, diarrhea, lacrimation, tremor, body drop, staining of the anal-genital region and poor grooming around the oral, nasal region. No pharmacotoxic signs were observed in rats administered the vehicle or positive controls with the exception of one male in the 6 hour corn oil group which exhibited diarrhea prior to colchicine administration. Results from the 6, 18 and 30 hours sacrifice data show that no statistically significant increase in the frequency of chromosome aberrations compared to control values was seen in the groups treated with 6PPD.</p> <p>6PPD (1000 mg/kg bw/day) did not induce structural chromosomal aberrations to hemopoietic cells of the rat bone marrow in this assay.</p>	Monsanto Co. 1988
28	6PPD	Carcinogenicty	A two year OECD guideline 451 (carcinogenicity) study was conducted using male and female Sprague-Dawley rats were exposed to 0, 50 (2.6 mg/kg bw/day for males and 3.2 mg/kg bw/day for females), 250 (13.5 mg/kg bw/day for males and 16.5 mg/kg bw/day for females) or 1500 (84.8 mg/kg bw/day for males and 109.5 mg/kg bw/day females) ppm of 6PPD via their feed. At the beginning of the experiment all rats in the group were dosed with 6PPD. At 12 months of treatment approximately 20 rats/sex/group were sacrificed.	Sprague-Dawley rat (n=70)	<p>The NOEL for this oral chronic toxicity study (for non-neoplastic toxicity) is 50 ppm in males and females (2.6 mg/kg/day in males, 3.2 mg/kg/day in females), based on reduced body weight in females, increased food consumption in females and increased liver weight at 250 ppm in both sexes (13.5-16.5 mg/kg/day). At the high dose (1500 ppm; 84.8-109.5 mg/kg/day) liver histopathology in females and slight effects on hematology are observed in both sexes.Follicular cell hyperplasia was slightly but not statistically significant increased among treated males. Slightly increased follicular carcinoma was observed in males of the mid and high dose group.</p> <p>The follicular cell carcinoma of the thyroid observed in this study are considered to be equivocal, based on 1) low, statistically not significant incidence in males, 2) no increase in females, 3) no effect on thyroid in an earlier chronic study (1978) in another rat strain (Charles River CD). Data on animals for which follicular cell carcinoma and liver weight data are available indicate that both observations are correlated and the effects on the thyroid gland are most likely to be high dose, secondary effects caused by increased liver activity. Together with the evidence that humans may not be as sensitive quantitatively to thyroid cancer development from thyroid-pituitary disruption as rodents (EPA/630/R-97/002, March 1998), the high-dose observations are considered to be of limited relevance for human risk assessment.</p> <p>Overall It can be concluded that it is unlikely that 6PPD possess a carcinogenic risk for humans.</p>	Monsanto Chemical Co. 1993
29	6PPD	Carinogenicity	A 2 year chronic feeding study was conducted using male and female CD Outbred Charles River rats exposed to 0,100, 300, or 1000 ppm (approx. 0, 8,23, or 75 mg/kg bw/day) of 6PPD in their diet.	Charles River (CD Outbred) rat (n=50)	A NOAEL (for systemic toxicity) of 300 ppm (23 mg/kg bw/day) was reported based on no adverse effects in male and female rats. A LOAEL of 1000 ppm (75 mg/kg bw/day) was reported for male and female rats based on reduced body weight, body weight gain, reduced erythrocyte counts, hemoglobin concentrations and hematocrit values. A NOAEL (for carcinogenicity) of 1000 ppm (75 mg/kg bw/day) was reported for male and females based on neoplasms found in treated mice compared to control and or within the historical control range.	Stevens, M.W., et al. 1981
30	6PPD	Carcinogenicty	An in vitro cell transformation assay was conducted using Balb/3T3 cells exposed to 0.165, 0.33, 0.495, 0.66, or 0.99 ug/ml of 6PPD.	Balb/3T3 cells	There was no significant increase in the number of transformed foci, therefore 6PPD was considered negative for carcinogenicity in this assay.	Unnamed study reported in ECHA

Table 2-3. Summary of Human Health Toxicity Studies

	Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Measured Effects	Author
31	6PPD	Toxicity to reproduction	An OECD Guideline 443 (extended one-generation reproductive toxicity) study was conducted using male and female Sprague-Dawley rats exposed to 7, 20, 60 mg/kg bw/day of 6PPD via oral gavage (corn oil as vehicle). F0 males were dosed orally by gavage for 70 consecutive days prior to mating and continuing through the day prior to euthanasia (for a minimum of 10 weeks). F0 females were dosed orally by gavage for 70 consecutive days prior to mating and continuing throughout mating, gestation, and lactation, through the day prior to euthanasia (following completion of weaning for all F1 litters). The offspring selected for the F1 generation began dosing on the day of weaning until the day prior to euthanasia (PND 21 [Cohort 2B], PND 91 [Cohort 1A], PND 78 [Cohort 2A], and PND 98 [Cohort 1B]). All animals were dosed at approximately the same time each day.	Sprague-Dawley rat	<p>F0 male and female mating and fertility, male copulation, and female conception indices, mean estrous cycle lengths, mean precoital intervals, and sperm parameters in the 6PPD treated groups were comparable to the control group. 6PPD-related dystocia and/or prolonged labor was noted for 2 and 5 females in the 20 and 60 mg/kg/day groups, respectively, although mean gestation lengths for the surviving females in these 2 groups and the 7 mg/kg/day group were between 22.0 to 22.2 days and were comparable to the control group mean (22 days).</p> <p>The key effect is dystocia, which was found in multiple treatment groups in this study.</p> <p>Based on the lack of adverse systemic effects in the F0 males and females at dosages up to 60 mg/kg/day, a dosage level of 60 mg/kg/day (the highest dosage level tested) was considered to be the NOAEL for F0 systemic toxicity of 6PPD when administered orally to CrI:CD(SD) rats. Based on the lack of F0 male reproductive effects at any dosage level, 60 mg/kg/day was considered to be the NOAEL for F0 male reproductive toxicity. Based on dystocia in the F0 20 and 60 mg/kg/day groups, 7 mg/kg/day was considered to be the NOAEL for F0 female reproductive toxicity. Based on effects of F1 pup survival and body weight gains at 60 mg/kg/day, a dosage level of 20 mg/kg/day was considered be the NOAEL for F1 neonatal toxicity. Based on the lack of adverse effects on the F1 adult males and females or on neurobehavioral parameters at any dosage level, 60 mg/kg/day was considered to be the NOAEL for F1 adult systemic and neurotoxicity.</p>	Unnamed study reported in ECHA
32	6PPD	Developmental toxicity	The potential maternal, embryotoxic and teratogenic effects of 6PPD were evaluated in a teratology study with Sprague-Dawley rats. 6PPD was administered orally by gavage to three groups of bred Sprague-Dawley female rats. The rats were treated once daily from gestation day 6 through 15. Dose levels of 50, 100 and 250 mg/kg bw/day were selected. For comparative purpose, a concurrent control group composed of 25 bred females was dosed with corn oil, the vehicle control material.	Sprague-Dawley rat (n=25)	<p>On day 20 of gestation, all females were sacrificed for Cesarean section. Fetuses were weighed, sexed and examined for external, skeletal and soft tissue anomalies and developmental variations. Survival was 100 % in all study groups. The pregnancy rate in this study was 80% in the control group and 92 % in each of the treated groups. Clinical signs which could be attributed to the administration of 6PPD occurred in a dose-related trend in the mid and high dose groups. Salivation prior to dosing, soft stool, diarrhea, decreased defecation and green fecal discoloration were the most remarkable clinical signs. Single observations of soft stool, salivation prior to dosing and clear wet material around the mouth in the 50 mg/kg/day group cannot be directly attributed to test material administration. Maternal body weights and body weight gain were not adversely affected by the administration of 6PPD. Food consumption was slightly reduced in the 250 mg/kg/day group during the first three days of treatment. Food intake among maternal animals in this group was slightly increased for the remainder of gestation with statistical significance occurring from days 16-20 of gestation. No pathological changes occurred in the dams which could be considered treatment-induced in this study. Intrauterine survival as well as the fetal weights was comparable between the control and all 6PPD treated groups. The type and frequency of fetal malformations and developmental variations observed in this study were not indicative of a teratogenic response. One malformation occurred in the control group and two fetuses with malformations were found in the mid dose group. More importantly, no malformations were observed in the 250 mg/kg/day group. Developmental variations occurred with similar frequency between the control and the treated groups.</p> <p>In conclusion, 6PPD administered to pregnant rats during the period of major organogenesis, was neither teratogenic nor embryo/fetotoxic at dose levels of 250 mg/kg/day and less. An increase in clinical signs occurred at dose levels of 100 and 250 mg/kg/day in a dose-dependent manner. The 50 mg/kg/day dosage was considered a NOAEL for any maternal toxicity. The 100 mg/kg bw/day dosage was considered the LOAEL for maternal toxicity. The 250 mg/kg bw/day dosage was considered the NOAEL for teratogenicity.</p>	Monsanto 1987

Table 2-3. Summary of Human Health Toxicity Studies

	Chemical	Endpoint	Dose/Duration	Species (number of test subjects)	Measured Effects	Author
33	6PPD	Developmental toxicity	An OECD Guideline 414 (Prenatal Developmental Toxicity) study was conducted using New Zealand White rabbits exposed to 0, 25,50, or 100 mg/kg bw/day of 6PPD (vehicle 1% Methylcellulose in deionized water) via oral gavage for 28 days (gestation day 7-28 inclusively).	New Zealand White rabbit	<p>Abortions at 100 mg/kg/day were noted in the presence of mean body weight losses, lower mean body weight gains, and corresponding reduced food consumption for females at this dosage level. Mean body weight losses, lower mean body weight gains, and lower food consumption were also noted at 50 mg/kg/day. In addition, higher maternal liver weights were noted at 50 and 100 mg/kg/day. Based on these results, a dosage level of 25 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity. A higher mean litter proportion of postimplantation loss and corresponding lower mean litter proportion of viable fetuses were noted at 100 mg/kg/day and lower mean fetal weights were noted at 50 and 100 mg/kg/day. Given the relationship between reduced maternal food consumption and increase in post-implantation losses and decrease in fetal weights, and given the severity and time period of the reduced food consumption in individual animals in this study, as well as the postimplantation loss during the implantation period (Gestation Days 7–20), it is not possible to assign the increase in mean litter proportion of postimplantation loss (and lower mean litter proportion of viable fetuses) in the 100 mg/kg/day group to either the 6PPD or the treatment-related decreases in food consumption.</p> <p>Based on the aforementioned developmental effects at 50 and 100 mg/kg bw/day dosages, the 25 mg/kg bw/day dose was considered to be the NOAEL for embryo/fetal development when 6PPD was administered orally by gavage to time-mated New Zealand White rabbits.</p>	Unnamed study reported in ECHA
34	6PPD	Developmental toxicity	In an early teratogenicity study Albino rabbits were exposed to the 0, 10 or 30 mg/kg bw/day of 6PPD from gestation day 6 to 18 (organogenesis) and sacrificed on day 29 of gestation. Based on the results of a pilot study showing high maternal toxicity at 100 and 300 mg/kg bw/day, the animals were dosed with 0, 10 or 30 mg/kg bw/day 6PPD in gelatine capsulaes.	New Zealand Albino rabbit	<p>Mortality occurred throughout all groups (5/17, 3/17, and 6/23 in control, 10, and 30 mg/kg bw groups) mainly caused by respiratory insufficiency. The number of pregnant animals that survived until gestation day 29 was 10 in the control group, 13 in the low-dose group (10 mg/kg bw/day) and 11 in the high dose (30 mg/kg bw/day) group. Reduced body weights were observed in dosed animals and also in controls. In the high-dose group (30 mg/kg bw/day) the relative resorption rate was 38.6% compared with 31.4% in controls. The value for the low dose (10 mg/kg bw/day) animals was in the upper range of historical control data. The relative number of live offspring (based on 100 implantation sites) was decreased in both treatment groups compared with controls (68.8 %, 48.3% or 38%, respectively). The application of 6PPD did not cause increased incidences of external, visceral or skeletal abnormalities.</p> <p>Based on the findings obtained, a NOAEL for maternal toxicity and developmental toxicity of 30 mg/kg bw/day is suggested for 6PPD related findings.</p>	Unnamed study reported in ECHA

3 PROPERTIES OF 6PPD AND 6PPD-Q

This chapter reviews the modeled and measured physicochemical properties of 6PPD and 6PPD-quinone. This information is needed to inform analytical field and lab methods, best management practices and remediation actions. In general, more research is needed to verify the characteristics of tire contaminants.

This section reports on the physicochemical properties, sources, and transport for 6PPD and 6PPD-q in the environment. This is an emerging area of research, and widespread sampling for 6PPD and 6PPD-q has yet to begin, so many assumptions must be made. In addition, the presence of 6PPD in many products, transformation to 6PPD-q, and the leaching characteristics are not yet clear (See Section 7.3). This section will attempt to summarize the current state of knowledge but is not intended to represent a comprehensive review of detections. Additionally, reliability evaluation and comparison of analytical methods have not been performed for studies discussed in this guidance (Table 5-2 and 5-3). A range of PPDs and transformation products originating from tires are known to exist and occur, but we are not addressing them in this guidance document. More research is needed to understand PPDs and quinones.

A summary of physicochemical characteristics of 6PPD and 6PPD-q are listed in Table 3-1. Studies highlighted in the table were performed with an isolated 6PPD-q standard in a lab setting and not with field collected samples (for example, stormwater or tire leachate) unless otherwise specified. Therefore, the physicochemical properties of 6PPD-q may be different in the environment.

3.1 Solubility

One notable characteristic is the solubility of 6PPD and 6PPD-q. 6PPD needs to be unbound and mobile in the rubber matrix of tires as part of the manufacturing process, and the migration rate from inside the tire to the outside layer where it will react with ozone is vital to its performance (Razumovskii & Batashova, 1970; Huntink and Datta, 2003). The solubility of 6PPD in water is between 0.5 and 2 milligrams per liter (mg/L) (Department of Toxic Substances Control, 2022; ECHA, 2021; Hiki et al., 2021; Klöckner et al., 2020).

Although isolated 6PPD-q is modeled to be more soluble, testing a 6PPD-q commercial standard has shown that it is much less soluble than 6PPD, with reported solubilities ranging from 0.04-0.07 mg/L (DTSC, 2022; Hu et al., 2023). This has consequences on the toxicity and transport of these compounds in aquatic environments, as well as analytical methods, where they are often dissolved in other solvents such as methanol

Solubility

- *6PPD is more soluble than 6PPD-q in water (mg/L vs ug/L)*
- *6PPD-q preferentially binds to organic matter and can sorb to containers, so it has some hydrophobic properties.*
- *Conversely, 6PPD-q is sufficiently soluble to be transported by water until it is captured by an organic media.*

or acetonitrile before use in the lab. 6PPD-q preferentially binds to organic matter and can sorb to containers, so it has some hydrophobic properties. Yet it dissolves enough to be transported by water until it is captured by an organic media.

3.2 Half-Life

6PPD and 6PPD-q also have different stabilities. In water, 6PPD has a half-life of less than a day under aerobic conditions (OECD SIDS, n.d.), whereas 6PPD-q has been shown to be relatively stable in water, degrading ~25% in 47 days (DTSC, 2022; ECHA, 2021; Hu et al., 2023; OSPAR Commission, 2006). The half-lives of 6PPD and 6PPD-q have been shown to change with temperature and pH, and also be affected by sunlight and biological processes (Hiki et al., 2021; Redman et al., 2023; Qian et al., 2023). More research is needed to predict stability under variable environmental conditions (for example, anaerobic, temperature, pH) and matrices (for example, water, soil, sediment).

Half-life

- 6PPD-q is more stable than 6PPD in water (1/2 life of weeks vs. <1 day)
- More research is needed to understand the half-life under variable conditions and formations

3.3 Transformation Products and Processes

The fate and transport of 6PPD and several transformation products from TRWP (fresh and weathered/aged) has been investigated under various benchtop leaching and sediment incubator conditions (Unice et al., 2015). 6PPD-q is estimated to be 10% of the transformation products when 6PPD is exposed to ozone (Lattimer et al., 1983; Zhao, Hu, Tian, et al., 2023). Other transformation products (TPs) have been identified in roadway-impacted environmental samples (Seiwert et al., 2022). Unice et al. (2015) investigated 6PPD in all ages of TRWP (up to 3.3 years of simulated aging), but there was a decrease in mass detected as the age increased, with the largest difference occurring between fresh (0 years) and 0.1 years of aging. As expected, there was limited leaching of 6PPD to water (i.e., low solubility) and a higher fraction that was released to sediment. This study was conducted prior to the discovery of 6PPD-quinone.

Transformation Products

- Tires contain approximately 1% 6PPD.
- 10% of the 6PPD in a tire transforms to 6PPD-q when exposed to ozone
- 6PPD in solution transforms to 6PPD-q when exposed to ultraviolet radiation
- The amount of 6PPD in tires decreases with age of the tire
- Initial studies have found 2-3 times more 6PPD in the tires' side walls than the tread
- More research is needed to characterize additional PPDs and their transformation products.

Rapid photodegradation has been observed when 6PPD (in solution) was exposed to illumination in lab water experiments, especially UV region irradiation (Li et al., 2023). The photodegradation of 6PPD was observed to be accelerated under acidic conditions, due to the

increased absorption of long wavelength irradiation by ionized 6PPD. Li et al (2023) identified nine photodegradation products, as identified by ultra-performance liquid chromatography quadrupole Time-of-Flight (QTOF) mass spectrometry. Reported mechanisms involved in photodegradation include photoexcitation, direct photolysis, self-sensitized photodegradation, and O₂ oxidation (Li et al., 2023). Sunlight has been shown to transform 6PPD in water to 6PPD-q with a molar yield of ~1.01% within 90 min at pH 7.0 under simulated sunlight irradiation (Zhou et al., 2023). As discussed in section 3.1.2, research suggests that the degradation of 6PPD-q by sunlight is temperature-dependent. This is an area of active research, and the **formation pathways** and mechanisms of these transformation products in the environment are still considered largely unknown. Evaluating the photodegradation pathway will help us understand the fate of 6PPD and 6PPD-q in the environment.

3.4 Biological Uptake

The **bioconcentration factor** for 6PPD is predicted to range from 617 to 801 (CompTox, U.S EPA, 2023; OSPAR Commission, 2006), suggesting a low to moderate potential for bioaccumulation. For 6PPD-q, the bioconcentration factor has been calculated as 20.9 (CompTox, U.S EPA, 2023) yet, more research is needed to confirm the **bioavailability** and **bioaccumulation**.

Bioconcentration factors (BCFs) of 6PPD-q in rainbow trout were calculated at 2.9, 19, 25, and 17.2 liters per kilogram (L/kg) at the water concentrations of 0.8, 3, 12, and 25 µg/L, respectively, and concentrations of 6PPD in tissue were similar at the same water concentrations (Nair et al., 2023). These BCF factors are 1-2 orders of magnitude lower than other contaminants with similar **K_{ow}** values, suggesting that 6PPD-q may be rapidly metabolized in rainbow trout (and other susceptible salmonids). Several studies have demonstrated moderate uptake of 6PPD and 6PPD-q in zebrafish from laboratory water, with uptake levels generally higher with 6PPD compared to 6PPD-q (Fang et al., 2023; Grasse et al., 2023; S.-Y. Zhang et al., 2023). Fang et al. (2023) estimated accumulation factors for 6PPD in zebrafish larvae of 265 and 103 based on concentrations in water of 1.35 and 28.2 µg/L, respectively. Grasse et al. (2023) estimated accumulation concentration factors of 6PPD and 6PPD-q in zebrafish embryo over 96 hours of exposure. Exposure concentrations were 1.28 and 6.3 µg/L for 6PPD and 4.8, 11.3, and 20.9 µg/L for 6PPD-q. At 72 hours (when 6PPD reached steady state in all experiments), the concentrations factors ranged from 142 to 2,447 for 6PPD. 6PPD-q never reached steady state; however, the concentration factors associated with the

Biological Uptake

- *6PPD is thought to have a low to moderate potential for bioaccumulation*
- *6PPD-q is modeled to have a lower bioaccumulation than 6PPD*
- *Research supports the biological uptake of both 6PPD and 6PPD-q.*
- *See the toxicity chapter for more information on the mode of action*
- *More research is needed to confirm relevant bioaccumulation and toxicity.*
- *Studies thus far have been conducted in the lab, more in situ studies are needed to capture environmental and stormwater variability.*

highest internal concentrations (at 48 hours) ranged from 75 to 216. Overall, the decrease in internal concentration of 6PPD-q suggested that there were biotransformation reactions in the zebrafish embryo.

S.Y. Zhang et al. (2023) observed statistically significant accumulation of 6PPD and 6PPD-q in zebrafish embryo following exposures of 0.2 and 0.8 mg/L of each compound from 8 to 120 hours post fertilization (hpf), with 6PPD having a greater magnitude of accumulation. There was no statistically significant accumulation of either compound at an exposure concentration of 0.025 mg/L. With the exception to the minimum exposure concentrations in Fang et al. (2023) and Grasse et al. (2023), these experimental exposure levels were higher than those detected in surface water. Uptake has also been detected in lettuce in hydroponic solution in a laboratory (Castan et al., 2023), and in fish purchased at a local market in China (Ji et al., 2022).

Another study assessed the toxicity and accumulation of 6PPD-q at environmentally relevant concentrations in three different fish species (Hiki & Yamamoto, 2022b). The concentration of 6PPD-q in the target tissues (brain and gills) increased with exposure concentration in the most sensitive species tested (*S. leucomaenis pluvius*), yielding internal median lethal concentration (ILC₅₀) estimates of "...4.0 µg/kg wet weight in brain and 6.2 µg/kg wet weight in gill for *S. leucomaenis pluvius*, while the tissue concentration of 6PPD-q in the two other non-surviving species (*S. curilus* and *O. masou masou*) exceeded the ILC₅₀ value for *S. leucomaenis Pluvius*" (Hiki & Yamamoto, 2022b).

The toxicological significance of any accumulation is currently unknown (For more information, see Section 7.3.1). See Section 2.3 in the toxicity section for more information about the mode of action.

3.5 Biodegradation

More research is needed to understand the biodegradation processes of tires, tire wear particles and related contaminants. Whole tires, tire debris and tire wear particles are all potential sources of 6PPD and 6PPD-q in the environment. Biodegradation of tire-related compounds, including 6PPD and 6PPD-q, may occur within the TWP itself or in the environment after leached (Calarnou et al., 2023). There are some studies on the biodegradation of tire wear particles or tire-

related compounds, however, these did not assess the leaching or biodegradation of 6PPD or 6PPD-q (Klun et al., 2023; Saifur & Gardner, 2023). 6PPD-q has been shown to be formed in wetted soil from 6PPD, presumably by bacteria (Qian et al., 2023). Calarnou et al. (2023) investigated the biodegradation of roadway particles (of which TWP is a component) and several tire-related compounds, including 6PPD-q. They found a significant decrease in 6PPD-q in the presence of *S. phaeofaciens* (Calarnou et al., 2023). Similarly, Xu et al. (2023) suggested that biodegradation was attributable to the decrease of 6PPD-q in soil under wet conditions compared to anaerobic flooded conditions (Xu et al., 2023). Better understanding of the biodegradation and the fate of 6PPD and 6PPD-q is a major data gap.

Biodegradation

- Studies suggest that 6PPD can transform to 6PPD-q in wetted soils (sediments) in anaerobic conditions presumable by bacteria
- More research is needed to understand the biodegradation and the fate and transport of 6PPD and 6PPD-q.

Table 3-1. Properties of 6PPD and 6PPD-q.

	Property	6PPD	6PPD-q	Reference
1	Molecular Formula	C ₁₈ H ₂₄ N ₂	C ₁₈ H ₂₂ N ₂ O ₂	ChemIDplus 2021; Tian et al. 2021, 2022
2	CAS Number[1]	793-24-8	2754428-18-5	OPSAR 2006
3	SMILES	CC(C)CC(C)NC1=CC=C(C=C1)NC2=CC=CC=C2	CC(C)CC(C)NC1=CC(=O)C(=CC1=O)NC2=CC=CC=C2	
4	Molecular Name	N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine	2-((4-Methylpentan-2-yl)amino)-5-(phenylamino)cyclohexa-2,5-diene-1,4-dione	ChemIDplus 2021; Hu et al. 2023
5	Molecular Weight	268.402 g/mol	298.39 g/mol	
6	Solubility	1 mg/L at 50 C	38 +/- 10 ug/L	Hu et al. 2023
7	Physical State	Solid (brown or violet)	Solid (from dark red/deep yellow red/dark orange to black)	
8	Melting Point	49.2 C	66.0 C	EPA Comptox
9	Boiling Point	163-165 C	341 C	EPA Comptox
10	Vapor Pressure	0.004 Pa at 25 C (Estimated)	0.0000421 Pa (Estimated)	EPA Comptox
11	Dissociation constant	pKa=6.7 at 20 C	pKA=9.14 (Estimated)	EPA Comptox
12	Octanol Air Partition coefficient, logKow[2]	4.68	4.3 ± 0.02	PubChem 2021;Hu et al. 2023
13	Soil Absorbion Coefficient	Koc: 11000 L/kg	Koc: 2400 L/kg	EPA Comptox
14	logK _{oc}	4,84	3.928	OSPAR 2006; EPI Suite
15	Sw[3]	1000 µg/L	38±10 µg/L	OSPAR 2006; Hu et al. 2023
16	Bioaccumulation[4]	Given the short half life of 6PPD in the environment, minimal bioaccumulation is assumed	6PPD-q is suspected to moderately bioaccumulate	OSPAR 2006; Hu et al. 2023
17	Bioaccumulation	avg zebrafish BCF 103-265 in 10-100 ng/g water; ~3-4x concentrations in zebrafish larva compared to 6PPD-q	Rainbow Trout BCF 2.9-17.2 in 0.8-25 ug/L; s. leucomaenis 8.6-24 in 0.1-3.5 ug/L	Fang et al., 2023; Hiki and Yamamoto 2022; Zhang et al., 2023
18	Sorption	Readily sorbs to organics and soils.	Readily sorbs to organics and soils. High sorption losses are observed during sampling and lab analysis.	OSPAR 2006; Hu et al. 2023
19	Half life	1 day (aerobic conditions and dissolved)	days (aerobic conditions and dissolved), characteristics suggest more persistent if bound to soils/organics	OSPAR 2006; Johannessen et al. 2022, Hu et al. 2023
20	Stability in Water	Abiotic Half life less than a day under most conditions. Faster degradation in biologically active water.	1/2 Life= 33 hrs in 23 C tap water; 26+/-10% loss over 47 days at ph 5, 7, and 9	Hiki et al. 2021; Hu et al. 2023; OSPAR 2006; Johannessen et al. 2022,
21	Stability in Air	Half life under two hours		
22	Stability in Sediment			
23	Stability in Soil	Half life modeled at 1800 hours		

4 OCCURRENCE, FATE AND TRANSPORT OF 6PPD AND 6PPD-Q

This chapter reviews the fate, transport and occurrence of tire particles containing 6PPD and 6PPD-q in the environment. More research is needed on the fate, transport, occurrence, and persistence of 6PPD-quinone once released from tires and other rubber products to inform toxic reduction actions.

Recent studies have investigated the occurrence of 6PPD and 6PPD-q in various environmental matrices across the globe. Lists of peer-reviewed studies are provided by medium in the following tables (included at the end of this section):

- Table 4-1: Outdoor air
- Table 4-2: Indoor air and (non-road) settled dust
- Table 4-3: Road dust, roadside snow, and road puddles
- Table 4-4: Roadside soil
- Table 4-5: Sediment
- Table 4-6: Stormwater
- Table 4-7: Surface water
- Table 4-8: Groundwater
- Table 4-9: Wastewater and drinking water treatment plants
- Table 4-10: Aquatic organisms and food

The list of studies in these tables are from peer-reviewed literature and do not capture the preliminary monitoring efforts by State and Local agencies. Some of these studies also measured a range of other PPDs and transformation products, which are not addressed in this initial guidance document. Recent efforts have been made to summarize occurrence data in peer-reviewed literature (Benis et al., 2023; Chen et al., 2023; Hua & Wang, 2023). Given the regional-specific conditions that impact the fate and transport of 6PPD-q (and TRWP and 6PPD), considerations should be made when interpreting these data. Further, the sampling and analytical methods used should be reviewed (for example, collection and storing material, use of commercially available standard) given the continued advancements made in quantifying this compound in environmental matrices. The transport pathways and exposure risk to aquatic, terrestrial and human systems is poorly understood (See Section 7.3). The

Occurrence, Fate and Transport

- *The occurrence and persistence of 6PPD, 6PPD-q, TWP, TRWP in the environment is poorly understood.*
- *The fate and transport mechanisms that control occurrence vary among landscapes, regions and environmental conditions.*
- *Dissolved 6PPD-q and TRWP are released from tire surfaces and transported by stormwater.*
- *TWP are emitted and become TRWP or carried beyond pavement and deposited directly to aquatic or terrestrial systems.*
- *More research is needed to standardize methods and fill in data gaps, initial studies have only scratched the surface.*

mechanisms of toxicity exposure, including the duration and mode of action, is needed to understand environmental exposure risk (See also [Section 2: Effects Characterization and Toxicity](#)).

The transformation of 6PPD-q from tires depends on the amount of 6PPD in tires and the release of tire wear particles (TWP) into the environment during the primary or secondary product life cycle. After TWP are released from the tire product and dispersed to the roadway, they become mixed with road debris and referred to as tire road wear particles (TRWP). While the road component of TRWP is not expected to be a source of 6PPD or 6PPD-q and does not change the overall mass of tire wear emitted into the environment, this road component has an impact on the characteristic and transport of the whole TRWP in the environment (for example, particle size, particle mass, surface area of the tire fraction, overall TRWP morphology, and mineralogical content of the TRWP particle (Kreider et al., 2010; Lokesh 2023). Elsewhere in this document TWP will be referred to as the tire fraction of the overall TRWP or the TWP released to non-road surfaces.

An estimated 4.7 kg/year of tire wear particles is released to the environment in the US (Kole et al., 2017). The mass loading of 6PPD and 6PPD-q in the environment is expected to vary spatially given the differences in 6PPD-q and TWP release rates, tire manufacturing, tire age, and vehicle attributes and operation (for example, weight, speed, and braking) (Baensch-Baltruschat et al., 2020; Kreider et al., 2010; Wagner et al., 2018). Further, once released to roads and parking lots, the fate and transport of 6PPD-q and TWP depends on many factors including tire particle characteristics (for example, size, shape, and density), road characteristics as well as regional weather and environmental characteristics (Unice et al., 2019; Wagner et al., 2018). Environmental and transportation characteristics include [traffic amount and type, road surface, size and type \(local roads versus highways\) and gradient](#). Watershed characteristics and stormwater conveyance are expected to influence the transport of TRWP and 6PPD-q including [roadside slope and conveyance \(curb and gutter, grass ditch, paved ditch, presence/absence of stormwater drain and pipe, presence/absence of stormwater filtration or catchment\)](#), land use, seasonal traffic trends and weather patterns, regional stormwater and wastewater management practices, soil types, [watershed size and flood risk](#). Research is underway to understand the extent and significance of environmental variability on the mass loading and fate of 6PPD-q, yet we know enough to support [stormwater, wastewater, and drinking water treatment and mitigation](#) management efforts (Johannessen, Helm, et al., 2022; Mayer et al., 2024; Rodgers et al., 2023; Seiwert et al., 2022).

In addition to surface and stormwater water transport, TRWP, containing 6PPD are released and transported by atmospheric processes. In a road dust and sediment study (Klöckner et al., 2020), more coarse particles were found closer to the roadway, while smaller particles more readily transported away from the road. As mentioned previously, TRWP are generally found more frequently and in higher quantities near roadways and urban areas, particularly those with high-volume traffic patterns (Unice et al., 2013). The majority of TRWP by volume are less than 100 µm (Kreider et al., 2010). Smaller particles represent a greater surface area relative to size compared to larger particles and therefore a greater chemical release rate per particle. Larger TRWP are regularly found in [PM2.5](#) or [PM10](#) air quality monitoring stations (Baensch-Baltruschat et al., 2020; Panko et al., 2013, 2019; Unice et al., 2019). Sources of TWP are also discussed in Section 2.2.2.1 of the [ITRC Microplastics Guidance Document](#) (ITRC, 2023).

Consideration of exposure pathways and their intersection with tire-derived products could help reduce the prevalence of 6PPD-q in the environment. In addition to tires, 6PPD can be found in many different primary and secondary consumer products, where it can then react to form 6PPD-q (Zhao, Hu, Gonzalez, et al., 2023). For example, initial studies have found 6PPD-q in windshield wipers and that the mass of 6PPD decreases with age (per communications Patterson 2024). While tires have been identified as a significant contributor of 6PPD-q in the environment, less is understood about the risk posed by reuse of tires or end of life tire management. Tires are commonly used in a wide array of industrial and consumer applications, which may contribute 6PPD-q to the environment. These tire products currently represent another data gap in 6PPD-q research. Table 4.11 provides a summary of tire contaminants over the life cycle of a tire. Secondary consumer products, such as crumb rubber used in playgrounds and sports fields, represents an additional source of tire contaminants (U.S. EPA & ATSDR, 2019; Zhao, Hu, Gonzalez, et al., 2023). Direct measurements of primary and secondary consumer product chemical compositions are needed to identify and verify additional sources of rubber-derived contaminants.

Table 4-11. List of used tire products containing 6PPD.

Rubber product	Description
Building Construction	Accessibility ramps, flooring, sealant, roofing, waterproof membranes, and other construction materials can be produced from used tires.
Synthetic Fields, Tracks, and Playgrounds	Track and field facilities may utilize crumb rubber recycled from old tires in new synthetic surfaces.
Road and Traffic Maintenance	Erosion control, weed abatement, seismic transition coverings, traffic control cones, wheel stops, and curb ramps are all practical applications for used tires.
Rubberized Asphalt Concrete	Rubberized asphalt concrete (RAC) produces a durable surface by blending ground up recycled tires with asphalt prior to mixing in conventional materials.
Tire-Derived Aggregate	Chipped tires are often repurposed in civil engineering for fill, drainage, and vibration mitigation in construction projects.
Retread	Worn tires receive new tread.
Material Feedstock	Ground tires are used to feed industrial processes as rubber or fuel.
Beneficial Reuse	Aquatic bumpers, art projects, Earthship homes, misc. repurpose
Disposal	Tires may be disposed of whole, burned, exported, or dumped.

4.1 Water

Surface water is presumed to be a major transport pathway of TWC to streams and where it causes coho mortality (Chow et al., 2019; Du et al., 2017; French et al., 2022; Hiki et al., 2021; Johannessen et al., 2021; McIntyre et al., 2018, 2021, 2023; Peter et al., 2020; Scholz et al., 2011; Seiwert et al., 2020; Spromberg et al., 2016; Tian et al., 2021). Rain and snow melt pick up and transport dissolved and particulate contaminants from impervious surfaces and deliver them to natural waterbodies or to stormwater treatment structures or wastewater treatment plants (Challis et al., 2021; Seiwert et al., 2022). The development of methods to accurately measure 6PPD and 6PPD-q will help investigate the fate, transport, and occurrence data gaps to inform management actions. 6PPD and 6PPD-q can be released directly from tires and readily bind to

particulates (Hu et al., 2023); these particulates are transported by stormwater (Seiwert et al., 2022). 6PPD and 6PPD-q can also be released from TRWP shed from tires, deposited along roadways, and transported by stormwater to waterbodies or treatment facilities. Detected environmental concentrations of 6PPD and 6PPD-q have been highest in urban runoff (Table 4-6). Studies that measure and compare dissolved and suspended fractions of 6PPD and 6PPD-q in water are needed to understand the fate and transport of these contaminants' pathways from impervious surfaces to natural waterbodies. Finally, groundwater measurements are needed to confirm assumptions based on physicochemical characteristics that suggest minimal movement through soils.

4.2 Soil

Soil is the loose surface material that covers dry land and often supports plant growth. Modeled and measured physicochemical characteristics of 6PPD and 6PPD-q suggest that these compounds readily bind to soils and organics (Cao, Wang, et al., 2022; OSPAR Commission, 2006) along the side of the road rather than staying in the dissolved phase, but occurrence data are limited and more studies are needed. Sampling protocols for measuring and understanding the occurrence and persistence of tire contaminants in soils along roadways is needed to address data gaps. 6PPD-q in soils is eventually biodegraded, unless transported to sediments by water or air (Xu et al., 2023).

Occurrence in Water

- Surface and stormwater are the major transport mechanism for TRWP, 6PPD and 6PPD-q (TWC).
- More studies are needed to understand how environmental, landscape, and stormwater characteristics effect the fate and transport of TWCs.
- More studies are needed to understand what stormwater, wastewater and drinking water treatment technologies are most effective at preventing the transport of 6PPD, 6PPD-q and TRWC.
- More studies are needed to understand if 6PPD or 6PPD-q are transported by subsurface water and groundwater.

4.3 Sediment

In hydrology practices, sediment often refers to the benthic media at the bottoms of streams, rivers, estuaries, oceans, and lakes. In stormwater practices, the dirt and debris transported from impervious surfaces to stormwater catchments is referred to as sediments as well. In the San Francisco Bay Area alone, an estimated 0.3 to 2.4 million kilograms of TRWP wash off roads and parking lots into stormwater systems and surface waters per year (Moran et al., 2023). Other urban areas likely have similar wash off rates. Similarly with soil, 6PPD and 6PPD-q will readily bind to sediment instead of the water-phase; however, occurrence data are limited, and more studies are needed. A study conducted in China found both 6PPD and 6PPD-q as the most dominant PPD and PPDq chemicals of concern in a large-scale survey of urban rivers, estuaries, coasts, and deep-sea sediments (Zeng et al., 2023). The

concentration of 6PPD and 6PPD-q decreased with distance from the urban areas. The detection of 6PPD may suggest that TRWP are a continual source and/or that the half-life of 6PPD varies between air and water, environmental conditions, and chemical phases (dissolved and suspended fractionation). More studies are needed to continue understanding the variability in partitioning and other physicochemical characteristics. Klockner et al. (2021) suggested using organic markers, including 6PPD and 6PPD-q, to measure TRWPs in sediments and soils, because TRWP are challenging to measure directly (Klößner, Seiwert, Wagner, et al., 2021). TRWP in sediments may also represent a major secondary source of 6PPD and 6PPD-q to the environment following aging and biodegradation processes more studies are needed to verify hydrophobicity and solubility to estimate leaching rates (Unice et al. 2015). Anaerobic sediment conditions have been shown to produce more 6PPD-q over time (Xu et al., 2023). More research is needed to understand the ecological risks associated with TWC in sediments.

Occurrence in Sediment

- *Tire, road, and soil are transported by storm and surface waters. The allocation between what stays suspended in water and what is deposited to the sediments is unknown.*
- *Standardized methods for measuring small TRWP in water and sediments is challenging. 6PPD-q may provide a proxy for tire-derived microplastics that could represent a continued source of 6PPD and 6PPD-q.*
- *More studies are needed to understand the fate and transport of TRWP in sediments including deposition, composition, biodegradation, and transformation processes.*

4.4 Air

4.4.1 Outdoor air

In outdoor air, 6PPD has been observed in fine particulate matter (PM_{2.5}), while 6PPD-q has been found in both outdoor ambient air and in the PM_{2.5} fraction (Table 4-1). In PM_{2.5}, 6PPD has been observed at concentrations ranging from about 0.02 - 9340 pg/m³ (picograms/cubic meter) (Cao, Wang, et al., 2022; Wang et al., 2022; Y. Zhang et al., 2022). 6PPD was not detected in ambient air during a three-month study where passive air samplers were deployed across 18 major cities comprising the Global Atmospheric Passive Sampling (GAPS) Network (Johannessen, Saini, et al., 2022). 6PPD-q has been observed at concentrations ranging from about 0.1 - 7250 pg/m³ in PM_{2.5} (Cao, Wang, et al., 2022; Wang et al., 2022; Y. Zhang et al., 2022) and 0.17 - 1.75 pg/m³ in ambient air (Johannessen, Saini, et al., 2022). Detection frequencies of 6PPD and 6PPD-q were generally 100%, indicating widespread prevalence in urban PM_{2.5} with trends suggesting that seasonal, geographic, and economic conditions may impact 6PPD and 6PPD-q occurrence in urban air (Cao, Wang, et al., 2022; Wang et al., 2022; Y. Zhang et al., 2022).

Occurrence in Air

- *6PPD and 6PPD-q have been observed in outdoor ambient air and fine particulate matter.*
- *6PPD and 6PPD-q have been observed in dust along roads and highways, parking lots and garages, rubber playgrounds, recycling facilities, 6PPD and 6PPD-q have been observed in dust along roads and highways, parking lots and garages, rubber playgrounds, recycling facilities, and homes.*
- *Tire dust has been observed in snow along roadways.*
- *More research is needed to understand the toxicity and exposure to humans.*

More research is needed to understand the toxicity and exposure of tire dust to terrestrial and aquatic ecosystems.

4.4.2 Indoor air

6PPD and 6PPD-q have also been observed in dust in indoor environments (Table 4.2). 6PPD and 6PPD-q in indoor dust, have been detected within the range of 13.8 – 1020 ng/g and 0.97 - 2850 ng/g, respectively (Liang et al., 2022; Liu et al., 2019; Y.-J. Zhang et al., 2022).

4.4.3 Road dust

6PPD and 6PPD-q has been observed along roads (Table 4-4), including urban roads, a parking garage, a highway tunnel, electronic-waste recycling facilities, rubber playgrounds, and homes (Deng et al., 2022; Hiki & Yamamoto, 2022a; Klöckner, Seiwert, Weyrauch, et al., 2021; Liang et al., 2022; Liu et al., 2019; Y.-J. Zhang et al., 2022). In road dust, 6PPD concentrations have been found ranging from 11.4 - 5359 ng/g (nanograms/gram) and 6PPD-q concentrations ranging from 4.02 - 2369 ng/g (Deng et al., 2022; Hiki & Yamamoto, 2022a). Higher concentrations of

6PPD and 6PPD-q were found on finer particles than coarser particles in road dust collected within a highway tunnel (Klöckner, Seiwert, Weyrauch, et al., 2021).

6PPD and 6PPD-q have also been found in roadside snow (Table 4-3). 6PPD concentrations have been detected up to 783.79 ng/L (nanograms/liter) and 6PPD-q from about 110 – 428 ng/L (Maurer et al., 2023). Studies to date have not evaluated the relative contribution in snow between potential atmospheric sources and road dust sources. Snow melt has been shown to transport the road dust deposition into surface waters.

TRWP are regularly found in PM_{2.5} air monitoring stations along roadways (U.S. EPA, 2023). Studies have demonstrated the transport of TRWP by resuspension and deposition along roadways. Air monitoring is needed along transportation corridors and in near-road environments to estimate the mass loading, chemical composition, and transport of TRWP non-exhaust emissions and volatile forms of tire contaminants.

4.5 Biota

The ability to develop consistent and reliable standardized methods for measuring 6PPD and 6PPD-q in biological matrices will allow us to investigate biouptake and potentially, exposure history.

Table 4-1. Studies of 6PPD and 6PPD-Q concentrations in outdoor air

	Location	Information	Concentration (pg/m³)	Method	Detection Limit
1	Hong Kong Baptist University, Hong Kong (Cao et al 2022)	Between September 2020 and August 2021, researchers collected 16 samples and analyzed them for a range of antioxidants and transformation products, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q in air were found to be [median (range)]: 6PPD: 1.78 (0.82-6.30) 6PPD-Q: 1.18 (0.54-13.8)	UPLC Orbitrap Mass Spectrometry & UHPLC Triple Quadrupole Mass Spectrometry	IQL (ng/mL): 6PPD: 0.035 6PPD-Q: 0.023
2	Global megacities (Johannessen et al 2022)	Researchers measured a range of antioxidants and transformation products, including 6PPD and 6PPD-Q in archived extracts collected at representative sites (away from major roadways) on polyurethane foam (PUF) disks from passive air samplers deployed across 18 major cities that comprise the Global Atmospheric Passive Sampling (GAPS) Network (GAPS-Megacities) during a three-month period in 2018.	The concentrations of 6PPD and 6PPD-Q in ambient air were found to be [mean (range)]: 6PPD: (ND-<LOQ) 6PPD-Q: 0.85 (ND-1.75)	UPLC Q-Exactive Orbitrap HRMS	IQL (ng/mL): 6PPD: 1.95 6PPD-Q: 0.122 MQL (pg/m³): 6PPD: 2.71 6PPD-Q: 0.169
3	Guangzhou and Taiyuan, China (Wang et al 2022)	Between May 2017 and April 2018, researchers collected 72 samples of PM _{2.5} from air at three sites. Each site was sampled 24 times and samples were analyzed for a range of antioxidants and transformation products, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q in air (PM _{2.5} fraction) were found to be [median (range)]: 6PPD: Guangzhou: 1820 (22.2-6050) Roadside in Guangzhou: 4040 (2.23-9340) Taiyuan: 81 (1.02-3190) 6PPD-Q: Guangzhou: 1100 (3.04-2350) Roadside in Guangzhou: 2810 (2.96-7250) Taiyuan: 744 (2.44-1780)	ESI/ Ultrahigh-resolution Orbitrap MS/triple quadrupole MS	MQL (pg/m³): 6PPD: 0.25 6PPD-Q: 0.08 MDL (pg/m³): 6PPD: 0.07 6PPD-Q: 0.02
4	Taiyuan, Zhengzhou, Shanghai, Nanjing, Hangzhou, and Guangzhou, China (Zhang et al 2022a)	Researchers collected 81 samples of PM _{2.5} from ambient air in six megacities in China between 2018 and 2019 and analyzed them for a range of antioxidants and transformation products, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q in air (PM _{2.5} fraction) were found to be [median (range)]: 6PPD: Guangzhou: 0.9 (0.3-10) Hangzhou: 4.6 (0.1-6.0) Nanjing: 2.1 (0.4-75) Shanghai: 4.4 (0.5-135) Taiyuan: 6.9 (0.02-487) Zhengzhou: 8.4 (1.2-109) 6PPD-Q: Guangzhou: 1.7 (0.1-15) Hangzhou: 6.7 (0.8-26) Nanjing: 2.3 (1.1-68) Shanghai: 5.9 (0.3-39) Taiyuan: 3.3 (1.1-84) Zhengzhou: 2.9 (0.3-32)	Liquid chromatograph with UHPLC-MS/MS	LOD (pg/mL): 6PPD: 1 6PPD-Q: 5

Table 4-1. Studies of 6PPD and 6PPD-Q concentrations in outdoor air

	Location	Information	Concentration (pg/m³)	Method	Detection Limit
5	Guangzhou, China (Zhang et al 2022b)	Researchers measured airborne 6PPD-Q concentrations in eight size-segregated particles (0.43–10 µm) at four waste recycling plants (n = 160) in September and December 2020. At the same time settled dust samples (n = 24) were collected from the plants and analyzed for 6PPD-Q. As part of this study, researchers also collected dust samples from different indoor environments from April – August 2021 and analyzed them for 6PPD-Q, including air conditioner filters in college male (n = 16) and female (n = 16) dormitories and residential houses (n = 18), as well as the settled dust in residential bedrooms (n = 16), buses (n = 17), and shopping malls (n = 20).	The reported concentrations of 6PPD-Q in atmospheric particles were [median (range)]: 0.43 – 0.65 µm size: 2.75 (ND – 9.52) 0.65 – 1.1 µm size: 2.07 (ND – 12.2) 1.1 – 2.1 µm size: 1.17 (ND – 6.81) 2.1 – 3.3 µm size: 2.22 (0.17 – 13.1) 3.3 – 4.7 µm size: 4.1 (0.89 – 18.9) 4.7 – 5.8 µm size: 4.66 (1.46 – 17.0) 5.8 – 9.0 µm size: 9.43 (2.37 – 39.2) 9.0 – 10.0 µm size: 16.65 (3.12 – 36.0) Overall: 3.73 (ND – 39.2)	LC-MS/MS	LOD (pg/m³): 0.03
6	United States and Canada (Wu et al. 2020)	In 2016, researchers collected 21 e-waste dust samples in an e-waste dismantling facility located in Ontario, Canada. Residential samples were collected in Ontario, Canada in 2015 (n = 20) and in Indiana, United States in 2013 (n = 12). In 2013, 10 sediment samples were collected in the Chicago Sanitary and Ship Canal. From 2018 to 2019, 20 air samples were collected in Chicago. Samples were tested for various antioxidants and UV filters, including 6PPD. 6PPD was detected in 100% of the e-waste dust samples, and in 70-75% of all other sampled media.	The concentrations of 6PPD were found to be [median (range)]: Air particles (particle size not specified): 0.06 (<MDL-0.41)	LC-MS/MS	MDL: Air: 0.02 pg/m³ Dust and sediment: 0.06 ng/g
7	Oxford Mississipi	Researchers used passive samplers for 10 days along US Highway 278.			
8	Olubusoye et al. 2023	The abundance of airborne TWPs increased with proximity to the road with deposition rates (TWPs cm–2 day–1) of 23, 47, and 63 at 30 m, 15 m, and 5 m from the highway, respectively. Two common TWP compounds (6PPD-Q and 4-ADPA) were detected in all samples, except the field blank, at levels above their limits of detection, estimated at 2.90 and 1.14 ng L–1, respectively.			

HRMS = high-resolution mass spectrometry

Table 4-2. Studies of 6PPD and 6PPD-Q concentrations in indoor settled dust

	Location	Information	Concentration (ng/g)	Method	Detection Limit
1	Guangzhou, China (Liang et al. 2022)	In August 2020, researchers collected one sample each from 45 different e-waste recycling workshops within a larger e-waste recycling complex. The samples were analyzed for a range of antioxidants and transformation products, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q in e-waste dust were found to be [median (range)]: 6PPD: 113 (13.8 – 1,020) 6PPD-Q: 375 (87.1 – 2,850)	LC-MS/MS	MDL (ng/g): 6PPD: 0.96 6PPD-Q: 1.8
2	Beijing, China (Liu et al. 2019)	In March 2016, researchers collected dust samples from 30 different rubber playgrounds and from living rooms in 30 different homes in Beijing and analyzed them for three PPDs, including 6PPD.	The concentrations of 6PPD in playground dust and indoor dust were found to be [geometric mean (range)]: Playground dust: 6PPD: 30.4 (<MQL – 685) Indoor dust: 6PPD: 16.4 (<MQL – 180)	ASE & UHPLC	Not available*
3	Guangzhou, China (Zhang et al. 2022b)	From April – August 2021, researchers collected dust samples from different indoor environments and analyzed them for 6PPD-Q, including air conditioner filters in college male (n = 16) and female (n = 16) dormitories and residential houses (n = 18), as well as settled dust in residential bedrooms (n = 16), buses (n = 17), and shopping malls (n = 20). As part of this study, researchers also measured airborne 6PPD-Q concentrations in eight size-segregated particles (0.43–10 µm) at four waste recycling plants (n = 160) in September and December 2020. At the same time settled dust samples (n = 24) were collected from the plants and analyzed for 6PPD-Q.	The concentrations of 6PPD-Q in indoor dust were found to be [mean ± SD (range)]: Air conditioner filters: Male dormitories: 4.76 ± 2.81 (1.95-13.4) Female dormitories: 6.78 ± 2.98 (2.85-12.6) Residential houses: 11.4 ± 8.11 (0.62-31.7) Settled dust: Residential bedrooms: 10.7 ± 7.58 (0.97-26.1) Buses: 43.0 ± 12.9 (19.7-71.4) Shopping malls: 23.5 ± 23.4 (3.92-106)	LC-MS/MS	LOD (ng/g): 0.03
4	Guangzhou, China (Huang et al. 2021)	In 2020, researchers collected 20 road dust samples, 10 parking lot samples, 11 vehicle dust samples, and 18 house dust samples in homes located in an e-waste dismantling area in South China. These samples were analyzed for a range of p-phenylenediamines, including 6PPD, and 6PPD-Q. Both compounds were detected in 100% of the road dust, parking lot dust, and vehicle dust samples. For house dust, 6PPD and 6PPD-Q were detected in 56% 33% of the samples, respectively.	The concentrations of 6PPD and 6PPD-Q in vehicle and house dust were found to be [median (range)]: 6PPD: Vehicle dust: 19.3 (5.0-41.9) House dust: 0.3 (<LOQ-6.1) 6PPD-Q: Vehicle dust: 80.9 (17.9-146) House dust: <LOQ (<LOQ-0.4)	HPLC-MS/MS	LOD (ng/g): 6PPD: 0.11 6PPD-Q: Value not reported, but estimated on the basis of the calibration curve of 6PPD
5	United States and Canada (Wu et al. 2020)	In 2016, researchers collected 21 e-waste dust samples in an e-waste dismantling facility located in Ontario, Canada. Residential samples were collected in Ontario, Canada in 2015 (n = 20) and in Indiana, United States in 2013 (n = 12). In 2013, 10 sediment samples were collected in the Chicago Sanitary and Ship Canal. From 2018 to 2019, 20 air samples were collected in Chicago. Samples were tested for various antioxidants and UV filters, including 6PPD. 6PPD was detected in 100% of the e-waste dust samples, and in 70-75% of all other sampled media.	The concentrations of 6PPD were found to be [median (range)]: E-waste dust: 15.4 (7.31-37.7) House dust (Canada): 0.083 (<MDL-6.65) House dust (United States): 1.84 (<MDL-23.7)	LC-MS/MS	MDL: Air: 0.02 pg/m ³ Dust and sediment: 0.06 ng/g
6	Hangzhou, China (Zhu et al. 2024)	During July–August 2022, indoor dust samples were collected from residential apartments (n = 97) in Hangzhou, China, and analyzed for various PPDs and PPDQs. 6PPD and 6PPDQ were detected in 100% of the indoor dust samples collected.	The concentrations of 6PPD and 6PPD-Q in indoor dust were found to be [median (range)]: 6PPD: 10 (0.48-135) 6PPD-Q: 9.5 (0.33-82)	HPLC-MS/MS	LOD: PPDs 0.051–0.39 ng/g PPDQs 0.081–0.27 ng/g
ASE = Accelerated solvent extraction HPLC = high performance liquid chromatography LC-MS/MS = liquid chromatography mass spectrometry / mass spectrometry LOQ = limit of quantification MDL = method detection limit MQL = method quantification limit ND = nondetect SD = standard deviation UHPLC = ultra performance liquid chromatography					
*This information may be available in the supplemental information section, but we were unable to obtain access.					

Table 4-3. Studies of 6PPD and 6PPD-Q concentrations in road dust, roadside snow, and road puddles

	Location	Information	Concentration (varies by study)	Method	Detection Limit
1	Guangzhou, China (Deng et al 2022)	In January 2021, researchers collected one sample each of dust from 10 roads and 10 indoor parking lots using a handheld vacuum and analyzed them samples for a range of antioxidants and transformation products, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q in dust were found to be [median (range), ng/g]: Road dust: 6PPD: 356 (15.1-1508) 6PPD-Q: 122 (10.5-509) Indoor parking lot dust: 6PPD: 323 (11.4-5359) 6PPD-Q: 154 (4.02-2369)	HPLC with triple-quadrupole mass spectrometry	Not available*
2	Tokyo, Japan (Hiki and Yamamoto 2022)	From May to October 2021, researchers collected a total of 22 samples of road dust from 2 arterial and 8 residential roads and analyzed them for 6PPD and 6PPD-Q.	The total concentrations of 6PPD and 6PPD-Q collected in road dust were found to be [median (range), ng/g]: 6PPD: 329 (45-1175) 6PPD-Q: 809 (116-1238) The concentrations of 6PPD and 6PPD-Q were generally higher in road dust collected from the arterial roads compared to the residential roads.	LC-MS/MS	LOQ (µg/L): 6PPD: 0.63 6PPD-Q: 0.18
3	Germany (Klößner et al 2021)	Two road dust samples were obtained from a tunnel in 2020 using a pressure washer and wet vacuum (three samples of each location). Particles were analyzed for various tire-related chemicals, including 6PPD and 6PPD-Q. Concentrations were also reviewed by particle diameter. 6PPD and 6PPD-Q increased from coarser (500-100 µm) to finer particles (<50 µm).	The concentrations of 6PPD and 6PPD-Q in road dust were found to be [mean ± SD]: 6PPD: 1.5 ± 0.044 and 1.9 ± 0.14 ng/mg dw 6PPD-Q: 220 ± 9.5 and 270 ± 27 peak area/mg dw	UPLC-ToF-MS	Not reported
4	Leipzig, Germany (Maurer et al 2023)	In February 2021, researchers collected 20 snow samples along roadsides and 3 background snow samples in urban areas away from roads and analyzed them for 489 chemicals of concern, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q in roadside snow were found to be [mean (range), ng/L]: 6PPD: 329 (ND-784) 6PPD-Q: 259 (110-428)	LC-HRMS	MDL (ng/L): 6PPD: 60 6PPD-Q: 4
5	Leipzig, Germany (Seiwert et al 2022)	As part of a study that included a series of laboratory ozonation experiments to evaluate the abiotic transformation of 6PPD, and sampling of the influent and effluent of a wastewater treatment plant (during snow melt, rainfall event, and dry conditions), three samples of snow were collected from urban streets after a week of cold weather following a snow event in February 2021. The snow samples were separated into water and particulates and analyzed for the presence of a range of tire and road wear particles and transformation products, including 6PPD and 6PPD-Q.	38 transformation products of 6PPD were detected in the laboratory study, of which 26 were reported for the first time. Snow samples contained 32 transformation products of 6PPD, including 9 transformation products of 6PPD-Q. 90% - 99% of the load of 6PPD and most of its transformation products were present in the particulate phase. 6PPD-Q and some of its transformation products had higher proportions in the water phase than 6PPD and its transformation products. Although snow samples were collected on different roads, their compositions were comparatively similar.	UHPLC-HRMS/UHPLC-ToF-MS	LOQ (ng/L): 6PPD-Q: 25

Table 4-3. Studies of 6PPD and 6PPD-Q concentrations in road dust, roadside snow, and road puddles

	Location	Information	Concentration (varies by study)	Method	Detection Limit
6	Guangzhou, China (Huang et al. 2021)	In 2020, researchers collected 20 road dust samples, 10 parking lot samples, 11 vehicle dust samples, and 18 house dust samples in homes located in an e-waste dismantling area in South China. These samples were analyzed for a range of p-phenylenediamines, including 6PPD, and 6PPD-Q. Both compounds were detected in 100% of the road dust, parking lot dust, and vehicle dust samples. For house dust, 6PPD and 6PPD-Q were detected in 56% 33% of the samples, respectively.	The concentrations of 6PPD and 6PPD-Q in road and parking lot dust were found to be [median (range)]: 6PPD: Road dust: 52.5 (4.1-238) ng/g Parking lot dust: 241 (13.5-429) ng/g 6PPD-Q: Road dust: 32.2 (3.0-88.1) ng/g Parking lot dust: 41.8 (5.7-277) ng/g	HPLC-MS/MS	LOD (ng/g): 6PPD: 0.11 6PPD-Q: Value not reported, but estimated on the basis of the calibration curve of 6PPD
7	Hangzhou, China (Jin et al. 2023)	In 2018, researchers collected 16 urban/suburban road dust samples, 32 agricultural road dust samples, and 35 road dust samples in Hangzhou in East China. These samples were analyzed for a range of aminoaccelerators and antioxidants and their transformation products, including 6PPD and 6PPD-Q. Both compounds were detected in 100% of the dust samples. 6PPD-Q was one of the most abundant analytes detected, comprising 41.4% of the total concentration of transformation product analytes.	The concentrations of 6PPD and 6PPD-Q in road dust were found to be [median (range)]: 6PPD: 18.8 (0.46-245) ng/g 6PPD-Q: 9.75 (0.46-143) ng/g	UHPLC-HRMS	MDL (ng/g): 6PPD: 0.019 6PPD-Q: 0.019
8	Norway (Kryuchkov et al., 2023)	Samples were collected from tunnel wash runoff (n=4) and tunnel runoff treatment plant (n=3). One artificial turf runoff sample (from soccer field) and one puddle sample were also collected. Samples were analyzed for 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q in various media were found to be [range, ng/L]: Tunnel wash runoff: 49.5-143 (n=4) Wastewater treatment facility near tunnel: 7.00-23.0 (n=2) Culvert at treatment facility: <LOQ (n=1) Road puddle: 258 (n=1)	LC-MS/MS	LOQ: 5 ng/L
9	Canada (Challis et al. 2021)	Sampling was conducted in the City of Saskatoon in Canada. Snowmelt samples were collected in 2019 and 2020 from the City's snow dumps. Stormwater samples were collected at seven outfalls representing residential, industrial, and retail developments. Nine surface water samples were collected from the South Saskatchewan River. Samples were analyzed for 6PPD-Q.	The concentrations of 6PPD-Q were found to be [mean (range), ng/L]: Snowmelt - 2019: 367 (74-756) Snowmelt - 2020: 81 (15-172)	UHPLC-MS	LOD: 1.2 ng/mL LOQ: 3.3 ng/mL
10	Michigan (Nedrich, 2022)	The Michigan Department of Environment performed a preliminary investigation of 6PPD-Q occurrence. Seventeen surface water samples were collected in rivers or creeks adjacent to roadways and five samples were collected from roadway puddles. Surface water samples included two from known salmon spawning rivers and two from locations downstream of industrial crumb rubber storage facilities. Samples were collected within 35 hours of a significant rain event. Two of the 17 surface water samples had detections of 6PPD-Q, whereas 100% of puddle samples had detectable levels of 6PPD-Q.	The concentrations of 6PPD-Q were found to be [range, ng/L]: Puddles: 54-660	LC-MS	MDL: 3 ng/L

Table 4-3. Studies of 6PPD and 6PPD-Q concentrations in road dust, roadside snow, and road puddles

Location	Information	Concentration (varies by study)	Method	Detection Limit
HRMS = high resolution mass spectrometry IDL = instrument detection limit LC-MS/MS = liquid chromatography mass spectrometry / mass spectrometry LOQ = limit of quantification MDL = method detection limit ND = nondetect SD = standard deviation UHPLC = ultra performance liquid chromatography UHPLC-ToF-MS = ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry				
*This information may be available in the supplemental information section, but we were unable to obtain access.				

Table 4-4. Studies of 6PPD and 6PPD-Q concentrations in roadside soil

Location	Information	Concentration (ng/g)	Method	Detection Limit
New Territories and Kowloon, Hong Kong (Cao et al. 2022)	In August and September 2021, researchers collected 12 samples of roadside soil on non-rainy days and analyzed them for a range of antioxidants and transformation products, including 6PPD and 6PPD-Q. 6PPD-quinone is the primary quinone present in soil, comprising 75.7% of the total PPD-Qs detected in roadside soil samples.	The concentrations of 6PPD and 6PPD-Q in roadside soil were found to be [median (range)]: 6PPD: 309 (31.4-831) 6PPDQ: 234 (9.50-936)	Analytical: UHPLC Triple Quadrupole Mass Spectrometry	IQL: 6PPD: 0.035 ng/mL 6PPDQ: 0.023 ng/mL
IQL = Instrument quantification limit				

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Table 4-5. Studies of 6PPD and 6PPD-Q concentrations in sediment

Location	Information	Concentration (ng/g)	Method	Detection Limit
1 China [Pearl River Delta, Pearl River Estuary, South China Sea] (Zeng et al. 2023)	In 2021, researchers collected 32 samples along the Pearl River Delta, 21 samples in the Pearl River Estuary, 20 samples on the coast of the South China Sea, and 12 samples in the deep-sea regions of the South China Sea. These samples were analyzed for a range of p-phenylenediamines and quinone transformation products, including 6PPD and 6PPD-Q. Both compounds were detected in at least 75% or more of the samples collected, with the highest concentrations found in the Pearl River Delta.	The concentrations of 6PPD and 6PPD-Q in sediment were found to be [median (range)]: 6PPD: River sediment: 14.4 (0.585-468) Estuary sediment: 3.92 (1.49-5.71) Coastal sediment: 1.82 (1.07-11.1) Deep sea sediment: 2.66 (<MDL-2.69) 6PPD-Q: River sediment: 9.03 (1.87-18.2) Estuary sediment: 2.00 (<MDL-4.88) Coastal sediment: 1.27 (0.431-2.98) Deep sea sediment: 2.71 (<MDL-3.02)	LC-MS/MS	MDL (ng/g): 6PPD: 0.015 6PPDQ: 0.043
2 United States and Canada (Wu et al. 2020)	In 2016, researchers collected 21 e-waste dust samples in an e-waste dismantling facility located in Ontario, Canada. Residential samples were collected in Ontario, Canada in 2015 (n = 20) and in Indiana, United States in 2013 (n = 12). In 2013, 10 sediment samples were collected in the Chicago Sanitary and Ship Canal. From 2018 to 2019, 20 air samples were collected in Chicago. Samples were tested for various antioxidants and UV filters, including 6PPD. 6PPD was detected in 100% of the e-waste dust samples, and in 70-75% of all other sampled media.	The concentrations of 6PPD were found to be [median (range)]: Sediment: 0.14 (<MDL-1.93)	LC-MS/MS	MDL: Air: 0.02 pg/m ³ Dust and sediment: 0.06 ng/g
MDL = method detection limit				

Table 4-6. Studies of 6PPD and 6PPD-Q concentrations in stormwater

	<u>Location</u>	<u>Information</u>	<u>Concentration (varies by study)</u>	<u>Method</u>	<u>Detection Limit</u>
1	New Territories and Kowloon, Hong Kong (Cao et al., 2022)	Nine urban runoff water samples were collected in a dense traffic urban area in Kowloon, Hong Kong in August 2021. Samples were analyzed for PPDs and PPD-quinones.	The concentrations of 6PPD and 6PPD-Q in stormwater were found to be [median (range), µg/L]: 6PPD: 0.32 (0.21-2.71) 6PPD-Q: 1.12 (0.21-2.43)	UHPLC-MS	IQL (ng/mL): 6PPD: 0.035 6PPD-Q: 0.023
2	Norway (Kryuchkov et al., 2023)	Samples were collected from tunnel wash runoff (n=4) and tunnel runoff treatment plant (n=3). One artificial turf runoff sample (from soccer field) and one puddle sample were also collected. Samples were analyzed for 6PPD-Q.	The concentrations of 6PPD-Q in runoff were found to be [range, ng/L]: Artificial turf runoff: 159 (n=1)	LC-MS/MS	LOQ: 5 ng/L
3	Canada (Challis et al., 2021)	Sampling was conducted in the City of Saskatoon in Canada. Snowmelt samples were collected in 2019 and 2020 from the City's snow dumps. Stormwater samples were collected at seven outfalls representing residential, industrial, and retail developments. Nine surface water samples were collected from the South Saskatchewan River. Samples were analyzed for 6PPD-Q.	The concentrations of 6PPD-Q were found to be [mean (range), ng/L]: Stormwater - 2019: 593 (86-1,400)	UHPLC-MS	LOD: 1.2 ng/mL LOQ: 3.3 ng/mL
4	Canada (Monaghan et al., 2021)	Surface water (n=2) and stormwater (n=4) samples were collected in May and June of 2021 in Nanaimo, BC, and analyzed for 6PPD-q in Nanaimo, BC, Canada. The analytical method provides semiquantitative results of 6PPD-Q.	The concentrations of 6PPD-Q were found to be [range, ng/L]: Stormwater: 48-5,580	direct sampling tandem mass spectrometry method for <i>semiquantitative</i> 6-PPDQ determinations using a thin polydimethylsiloxane membrane immersion probe (CP-MIMS)	LOD: 8 ng/mL
5	Seattle, LA, San Francisco (Tian et al., 2022)	Roadway runoff and roadway runoff-impacted receiving water samples were re-analyzed for 6PPD-Q using a commercial standard. Concentrations were visually approximated from the boxplots provided in the publication.	The concentrations of 6PPD-Q in stormwater were found to be [µg/L]: Stormwater: approximately 1.3 (maximum)	UHPLC-MS with SPE	LOQ: 5.1 ng/L

Table 4-6. Studies of 6PPD and 6PPD-Q concentrations in stormwater

<u>Location</u>	<u>Information</u>	<u>Concentration (varies by study)</u>	<u>Method</u>	<u>Detection Limit</u>
6 Pearl River Delta, China (Zhang et al., 2023)	Surface runoff samples (courtyard [n=2], road [n=3], and farmland [n=2]) were collected in Dongguan and Huizhou across multiple events in 2015. Three Guangzhou WWTP influents and effluents were sampled in 2016. Surface water samples from the Zhujiang (n=13) and Dongjiang (n=13) Rivers were collected in 2015. Six samples were collected from a DWTP in Guangzhou in 2020. All samples were analyzed for 23 tire additives and their transformation products, including 6PPD-q and 6PPD.	The concentrations of 6PPD and 6PPD-Q were found to be [median (range), ng/L]: 6PPD: Stormwater: 3.05 (0.41–7.52) Courtyard runoff: 0.89 (0.19–1.10) Farmland runoff: ND 6PPD-Q: Stormwater: 576 (38.5-1,562) Courtyard runoff: 51.6 (6.03-875) Farmland runoff: 0.73 (0.53-5.58)	UHPLC-MS	6PPD: LOD: 0.04 ng/L LOQ: 0.12 ng/L 6PPD-Q: LOD: 0.05 ng/L LOQ: 0.17 ng/L
7 Guangzhou, China (Zhang et al., 2023)	Surface water (n=19), groundwater (n=43), and stormwater (n=10) samples were collected along the Liuxi River and analyzed for p-phenylenediamines, including 6PPD and 6PPD-Q. Suspended particles from stormwater samples were also analyzed. As expected, 6PPD was only detected in the particle phase.	The concentrations of 6PPD and 6PPD-Q in were found to be [median (range), ng/L]: 6PPD: Stormwater: ND Suspended particles: 0.20 (ND-0.74) 6PPD-Q: Stormwater: 0.34 (0.18-1.42) Suspended particles: 0.01 (ND-0.07)	UHPLC-MS/MS	6PPD: MDL: 0.048 ng/L MQL: 0.160 ng/L 6PPD-Q: MDL: 0.029 ng/L MQL: 0.098 ng/L
8 Seattle Area (Zhao et al., 2023)	Roadway runoff (n=4; grab samples) and roadway-impacted creek water (n=5; composite samples) samples were collected and analyzed for 6PPD and 6PPD-Q. Both compounds were detected in 100% of the samples. 6PPD measurements were considered semiquantitative given the compounds instability in water.	The concentrations of 6PPD and 6PPD-Q in stormwater were found to be [mean ± SD, ng/L]: 6PPD: Stormwater: 75 ± 40 6PPD-Q: Stormwater: 140 ± 60	LC-MS/MS	6PPD: LOD - Creek: 3.3 ng/L LOQ - Creek: 4.9 ng/L LOD - Runoff: 2.4 ng/L LOQ - Runoff: 3.9 ng/L 6PPD-Q: LOD - Creek: 1.2 ng/L LOQ - Creek: 3.1 ng/L LOD - Runoff: 2.1 ng/L LOQ - Runoff: 5.7 ng/L

Table 4-7. Studies of 6PPD and 6PPD-Q concentrations in surface water

Location	Information	Concentration (varies by study)	Method	Detection Limit
1 Canada (Challis et al., 2021)	Sampling was conducted in the City of Saskatoon in Canada. Snowmelt samples were collected in 2019 and 2020 from the City's snow dumps. Stormwater samples were collected at seven outfalls representing residential, industrial, and retail developments. Nine surface water samples were collected from the South Saskatchewan River. Samples were analyzed for 6PPD-Q.	The concentrations of 6PPD-Q were found to be [mean (range), ng/L]: Surface water - 2020: not detected	UHPLC-MS	LOD: 1.2 ng/mL LOQ: 3.3 ng/mL
2 Canada (Monaghan et al., 2021)	Surface water (n=2) and stormwater (n=4) samples were collected in May and June of 2021 in Nanaimo, BC, and analyzed for 6PPD-q in Nanaimo, BC, Canada. The analytical method provides semiquantitative results of 6PPD-Q.	The concentrations of 6PPD-Q were found to be [range, ng/L]: Surface water: 96-112	direct sampling tandem mass spectrometry method for <i>semiquantitative</i> 6-PPDQ determinations using a thin polydimethylsiloxane membrane immersion probe (CP-MIMS)	LOD: 8 ng/mL
3 Seattle, L.A. San Francisco (Tian et al., 2022)	Roadway runoff and roadway runoff-impacted receiving water samples were re-analyzed for 6PPD-Q using a commercial standard. Concentrations were visually approximated from the boxplots provided in the publication.	The concentrations of 6PPD-Q in stormwater were found to be [µg/L]: Surface water (receiving water): approximately 0.3 (maximum)	UHPLC-MS with SPE	LOQ: 5.1 ng/L
4 Pearl River Delta, China (Zhang et al., 2023)	Surface runoff samples (courtyard [n=2], road [n=3], and farmland [n=2]) were collected in Dongguan and Huizhou across multiple events in 2015. Three Guangzhou WWTP influents and effluents were sampled in 2016. Surface water samples from the Zhujiang (n =13) and Dongjiang (n=13) Rivers were collected in 2015. Six samples were collected from a DWTP in Guangzhou in 2020. All samples were analyzed for 23 tire additives and their transformation products, including 6PPD-q and 6PPD.	The concentrations of 6PPD and 6PPD-Q were found to be [median (range), ng/L]: 6PPD: Zhujiang River: 0.48 (0.31-1.07) Dongjiang River: 0.36 (0.27-1.29) 6PPD-Q: Zhujiang River: 1.51 (0.26-11.3) Dongjiang River: 0.91 (0.29-8.12)	UHPLC-MS	6PPD: LOD: 0.04 ng/L LOQ: 0.12 ng/L 6PPD-Q: LOD: 0.05 ng/L LOQ: 0.17 ng/L
5 Guangzhou, China (Zhang et al., 2023)	Surface water (n=19), groundwater (n=43), and stormwater (n=10) samples were collected along the Liuxi River and analyzed for p-phenylenediamines, including 6PPD and 6PPD-Q. Suspended particles from stormwater samples were also analyzed. As expected, 6PPD was only detected in the particle phase.	The concentrations of 6PPD and 6PPD-Q in were found to be [median (range), ng/L]: 6PPD: Surface water: ND 6PPD-Q: Surface water: 0.18 (ND-0.75)	UHPLC-MS/MS	6PPD: MDL: 0.048 ng/L MQL: 0.160 ng/L 6PPD-Q: MDL: 0.029 ng/L MQL: 0.098 ng/L

Table 4-7. Studies of 6PPD and 6PPD-Q concentrations in surface water

Location	Information	Concentration (varies by study)	Method	Detection Limit
6 Seattle Area (Zhao et al., 2023)	Roadway runoff (n=4; grab samples) and roadway-impacted creek water (n=5; composite samples) samples were collected and analyzed for 6PPD and 6PPD-Q. Both compounds were detected in 100% of the samples. 6PPD measurements were considered semiquantitative given the compounds instability in water.	The concentrations of 6PPD and 6PPD-Q in stormwater were found to be [mean ± SD, ng/L]: 6PPD: Surface water: 99 ± 64 6PPD-Q: Surface water: 90 ± 20	LC-MS/MS	6PPD: LOD - Creek: 3.3 ng/L LOQ - Creek: 4.9 ng/L LOD - Runoff: 2.4 ng/L LOQ - Runoff: 3.9 ng/L 6PPD-Q: LOD - Creek: 1.2 ng/L LOQ - Creek: 3.1 ng/L LOD - Runoff: 2.1 ng/L LOQ - Runoff: 5.7 ng/L
7 Canada (Johannessen et al., 2022)	Archived extracts of surface water composite samples originally collected in 2019 and 2020 from the Don River were analyzed for 6PPD-Q. The composite samples comprised of 3-hour flow-weighted composites collected over 42 hours.	The concentrations of 6PPD-Q were found to be [μg/L]: 42-hr composite sample: 2.30 ± 0.05 (maximum) 3-hr composites of one rain event: 0.93 to 2.85	HRMS-UPLC	LOQ: 0.0098 μg/L
8 Canada (Johannessen et al., 2021)	Surface water samples were collected from the Don River and Highland Creek during 2020. These samples included grab and composite samples. Samples were also collected upstream, downstream, and immediately below the effluent outfall of a WWTP. Samples were analyzed in triplicate for tire-related compounds, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q were found to be [mean ± SD, μg/L]: 6PPD: Surface water (grab samples): ND Surface water (composite samples): NA 6PPD-Q: Surface water (grab samples): 0.72 ± 0.26 (Highland Creek; maximum triplicate 0.91) and 0.54 ± 0.04 (Don River) Surface water (composite samples): 0.21 ± 0.02 (Highland Creek) and 0.11 ± 0.02 (Don River)	UHPLC-HRMS	6PPD: LOD: NR LOQ: NR 6PPD-Q: LOD: NR LOQ: 0.0065 μg/L
9 Nedrich, 2022	The Michigan Department of Environment performed a preliminary investigation of 6PPD-Q occurrence. Seventeen surface water samples were collected in rivers or creeks adjacent to roadways and five samples were collected from roadway puddles. Surface water samples included two from known salmon spawning rivers and two from locations downstream of industrial crumb rubber storage facilities. Samples were collected within 35 hours of a significant rain event. Two of the 17 surface water samples had detections of 6PPD-Q, whereas 100% of puddle samples had detectable levels of 6PPD-Q.	The concentrations of 6PPD-Q were found to be [range, ng/L]: Surface water: ND-37	LC-MS	MDL: 3 ng/L

Table 4-7. Studies of 6PPD and 6PPD-Q concentrations in surface water

Location	Information	Concentration (varies by study)	Method	Detection Limit
10 Rauert et al., 2022a	Thirty-two surface water grab samples were collected in a tributary to the Brisbane River during storm events in 2020 and analyzed for tire-related compounds, including 6PPD-Q. 6PPD was not included in this study because of its instability. 6PPD-Q was detected in 100% of the grab samples.	The concentrations of 6PPD-Q were found to be [median (range), ng/L]: Surface water: 17.5 (0.38-88)	LC-MS/MS	MDL: 0.1 ng/L
11 Rauert et al., 2022b	Surface water grab samples from 21 sites, including two estuary and one marine sites, were collected in 2021 in Queensland and Brisbane. Samples were analyzed for tire-related chemicals, including 6PPD-Q. 6PPD was not included in this study. 6PPD-Q was detected in 18 of the 21 sites.	The concentrations of 6PPD-Q were found to be [median (range), ng/L]: Surface water: <0.05-24 Estuary: <0.05-0.28 (two sites) Marine water: 0.10 (one site)	LC-MS/MS	MDL: 0.05 ng/L
MDL = method detection limit				

Table 4-8. Studies of 6PPD and 6PPD-Q concentrations in groundwater

Location	Information	Concentration (ng/L)	Method	Detection Limit
Guangzhou, China (Zhang et al., 2023)	Surface water (n=19), groundwater (n=43), and stormwater (n=10) samples were collected along the Liuxi River and analyzed for p-phenylenediamines, including 6PPD and 6PPD-Q. Suspended particles from stormwater samples were also analyzed. As expected, 6PPD was only detected in the particle phase.	The concentrations of 6PPD and 6PPD-Q in were found to be [median (range)]: 6PPD: Groundwater: ND 6PPD-Q: Groundwater: 0.11 (ND-0.70)	UHPLC-MS/MS	6PPD: MDL: 0.048 ng/L MQL: 0.160 ng/L 6PPD-Q: MDL: 0.029 ng/L MQL: 0.098 ng/L

Table 4-9. Studies of 6PPD and 6PPD-Q concentrations in wastewater and water treatment plants

Location	Information	Concentration (varies by study)	Method	Detection Limit
1 WWTPs in Hong Kong (Cao et al., 2023)	Tested influent, effluent, and biosolids at four WWTPs in Hong Kong for PPDs and PPD-Qs, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q were found to be [median (range)]: 6PPD: Influent: 12 (1.1-59) ng/L Effluent: 0.30 (<LOQ-15) ng/L Biosolids: 5.5 (2.1-71) ng/g 6PPD-Q: Influent: 53 (1.9-470) ng/L Effluent: 3.4 (1.1-37) ng/L Biosolids: 6.4 (2.6-7.3) ng/g	Extraction with DCM, Envi-carb SPE, HPLC-MS	6PPD: LOQ - Influent: 0.12 ng/L LOD - Influent: 0.037 ng/L LOQ - Other: 0.06 ng/L LOD - Other: 0.018 ng/L LOQ - Biosolids: 0.31 ng/g LOD - Biosolids: 0.092 ng/g 6PPD-Q: LOQ - Influent: 0.02 ng/L LOD - Influent: 0.005 ng/L LOQ - Other: 0.01 ng/L LOD - Other: 0.002 ng/L LOQ - Biosolids: 0.04 ng/g LOD - Biosolids: 0.012 ng/g
2 WWTPs and Drinking Water Treatment Plants in Ontario (Johannessen and Metcalfe, 2022)	Samples were collected at the influent and effluent of four WWTPs and two DWTPs in Ontario and analyzed for 6PPD-Q. Removal efficiencies of 6PPD-Q varied by WWTP, and in twp WWTPs, the effluent mass exceeded that in the influent.	The concentrations of 6PPD-Q were found to be [range, ng]: WWTP influent: 64.8 ± 5.3-145.7 ± 46.7 WWTP effluent: <LOD-446.5 ± 37.7 DWTP untreated and treated: ND	UHPLC-HRMS	LOQ: 0.5 ng/mL
3 Influent and Effluent from WWTP in Leipzig Germany (Maurer et al., 2023)	Influent and effluent of a WWTP in Germany were collected for 24-hour intervals during snow melting. Samples were analyzed for various chemicals, including 6PPD-Q; 6PPD was not included.	The concentrations of 6PPD-Q in WWTP were found to be [mean (range), ng/L]: Influent: 777 (592-1,100) Effluent: 50 (41-66)	LC-HRMS	MDL (ng/L): 6PPD: 60 6PPD-Q: 4
4 Influent and Effluent from five WWTPs in Malasia and Sri Lanka (Zhang et al., 2023)	Tested influent and effluent at three WWTPs in Malaysia (n=93) and two WWTPs in Sri Lanka (n=28). Samples were analyzed for 6PPD, 6PPD-q and other PPDs.	The concentrations of 6PPD and 6PPD-Q in WWTP were found to be [median (range), ng/L]: 6PPD: Malaysia WWTP influent: ND Malaysia WWTP effluent: ND Sri Lanka WWTP influent: ND Sri Lanka WWTP effluent: ND 6PPD-Q: Malaysia WWTP influent: ND Malaysia WWTP effluent: ND (ND-0.11) Sri Lanka WWTP influent: ND (ND-0.37) Sri Lanka WWTP effluent: ND (ND-0.37)	LC-MS/MS	LOQ (ng/L): 6PPD: 0.120 6PPD-Q: 0.098

Table 4-9. Studies of 6PPD and 6PPD-Q concentrations in wastewater and water treatment plants

Location	Information	Concentration (varies by study)	Method	Detection Limit
5 Pearl River Delta, China (Zhang et al., 2023)	Surface runoff samples (courtyard [n=2], road [n=3], and farmland [n=2]) were collected in Dongguan and Huizhou across multiple events in 2015. Three Guangzhou WWTP influents and effluents were sampled in 2016. Surface water samples from the Zhujiang (n =13) and Dongjiang (n=13) Rivers were collected in 2015. Six samples were collected from a DWTP in Guangzhou in 2020. All samples were analyzed for 23 tire additives and their transformation products, including 6PPD-q and 6PPD.	The concentrations of 6PPD and 6PPD-Q were found to be [median (range), ng/L]: 6PPD: WWTP Influent: ND WWTP Effluent: ND DWTP (all samples): ND 6PPD-Q: WWTP Influent: 14.2 ± 0.80 to 69.8 ± 2.40 WWTP Effluent: ND to 2.09 ± 0.16 DWTP source water: 0.25 DWTP (all other samples): ND	UHPLC-MS	6PPD: LOD: 0.04 ng/L LOQ: 0.12 ng/L 6PPD-Q: LOD: 0.05 ng/L LOQ: 0.17 ng/L
6 Drinking water in Singapore (Marques dos Santos and Snyder 2023)	Drinking water samples were collected from 20 buildings in Singapore ranging in age from seven to 44 years. Samples were analyzed for various tire-related compounds including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q were found to be [median (range), ng/L]: 6PPD: <10 (approximated based on boxplot; 25% detection frequency) 6PPD-Q: ND	LC-MS/MS	6PPD: LOD: 0.075 ng/mL MRL: 0.94 ng/L 6PPD-Q: LOD: 0.050 ng/mL MRL: 0.61 ng/L
7 Canada (Johannessen et al., 2021)	Surface water samples were collected from the Don River and Highland Creek during 2020. These samples included grab and composite samples. Samples were also collected upstream, downstream, and immediately below the effluent outfall of a WWTP. Samples were analyzed in triplicate for tire-related compounds, including 6PPD and 6PPD-Q.	The concentrations of 6PPD and 6PPD-Q were found to be [mean \pm SD, μ g/L]: 6PPD: WWTP discharge: ND Upstream and downstream of WWTP: ND 6PPD-Q: WWTP discharge: 0.05 ± 0.02 Upstream and downstream of WWTP: ND	UHPLC-HRMS	6PPD: LOD: NR LOQ: NR 6PPD-Q: LOD: NR LOQ: 0.0065 μ g/L
8 Leipzig, Germany (Seiwert et al 2022)	As part of a study that included a series of laboratory ozonation experiments to evaluate the abiotic transformation of 6PPD, sampling of the influent and effluent of a wastewater treatment plant (during snow melt, rainfall event, and dry conditions) was conducted. Samples were analyzed for the presence of a range of tire and road wear particles and transformation products (TPs), including 6PPD and 6PPD-Q. Concentrations of 6PPD were reported as 6PPD and its TPs.	The concentrations of 6PPD + TPs and 6PPD-Q were found to be [mean \pm SD, μ g/L]: 6PPD + TPs: Snowmelt - influent: 4.4 Snowmelt - effluent: 2.4 Rainfall - influent: 14.3 Rainfall - effluent: 11.2 Dry weather - influent: 0.9 Dry weather - effluent: 0.3 6PPD-Q: Snowmelt - influent: 0.105 ± 0.037 Snowmelt - effluent: ND Rainfall - influent: 0.052 ± 0.022 Rainfall - effluent: ND Dry weather - influent: ND Dry weather - effluent: ND	UHPLC-HRMS/UHPLC-ToF MS	LOQ (ng/L): 6PPD: 100 6PPD-Q: 25

Table 4-10. Studies of 6PPD and 6PPD-Q concentrations in aquatic organisms and food

Location	Information	Concentration	Method	Detection Limit
1 Beijing, China, Ji et al., 2022	Fish and honey were purchased from a local supermarket and fish market. Samples were analyzed for 6PPD and 6PPDq	Fish: 6PPD in snakehead (0.669 µg/Kg) weever (0.481 µg/Kg), 6PPDQ in the Spanish mackerel (< LOQ) Honey: ND	modified QuEChERS methods with (HPLC/MS-MS)	LOD: 6PPDQ: 0.0003 mg/Kg 6PPD: 0.00025 mg/Kg in fish, 0.0003 mg/Kg in honey LOQ: 6PPDQ: 0.001 mg/Kg, 6PPD: 0.00043 mg/Kg in fish, 0.0001 mg/Kg in honey
2 Laboratory in Hangzhou, China Fang et al, 2023	Zebrafish were analyzed after laboratory exposure to 6PPD	After 7 Days 6PPD in Zebrafish Larvae: TWA in Water 351ng/g; 1.35ng/g BAF 265 2685ng/g; 28.2ng/g BAF 103	QuEChERS with (HPLC/MS-MS)	LOQ: 0.1 ng/mL
3 Laboratory in Wenzhou, China Zhang et al, 2023	Zebrafish were analyzed after laboratory exposure to 6PPD or 6PPDq	After 10 Days 6PPD and 6PPDq in larvae was significantly higher at 0.2 and 0.8 mg/L exposure vs. control, but not at 0.025 mg/L exposure. Levels of 6PPD were higher than 6PPDq. BAFs were not calculated.	Homogenization, poly filtration, and UPLC/MS-MS	Not Specified
4 Laboratory in Norway Hagg et al., 2023	Lumpfish were exposed to seawater with fish feed mixed with crub rubber in the lab for 7 days then fed uncontaminated feed for 14 days. Blood was analyzed for 6PPD and 6PPD-q (among other chemicals) at various timepoints throughout.	6PPD Max on Day 9 of 1206 pg/g 6PPD-q not detected in blood DTPD and TPPD also detected in blood	Blood was spiked with 6PPD-q-D5, centrifuged, and ran by GC-HRMS	Instrumental LOD 6PPD LOD: 0.1 pg 6PPD-q LOD: 0.5 pg
5 Laboratory in Germany Grasse et al., 2023	Zebrafish were analyzed after 24, 48, 72, and 96 hpf of exposure in the lab	Ratio of internal:external concentration 6PPD (exposure of 6.3 and 1.28 ug/L) Max of ~3000 at 48h 6PPD-q (exposure of 20.0, 11.3., and 4.8 ug/L) Max of ~225 at 48h	HPLC/MS-MS. Fish: FastPrep homogenizer, sonication, and centrifuging	LOD (ng/mL) 6PPD-q: 0.089 6PPD: 0.130 LOQ (ng/mL) (6PPD-q: 6PPD) 0.439: 0.638
6 Laboratory in Austria Castan et al., 2023	Hydroponic solutions of lettuce were spiked with 1 mg/L of 6PPD-q or constantly leaching TWP over 14 days to analyze uptake and metabolism.	Spiked compounds max: 6PPD 0.78 ug/g, 6PPD-q 2.19 ug/g. Tire leachate max:: 6PPD 0.4ug/g, 6PPD-q 0.02 ug/g	Tissues extracted using acetonitrile, then ran on LC-MS	Not Specified
7 Laboratory in Toronto Nair et al., 2023	Rainbow trout were exposed to 6PPD-q (and other PPD-quinones) for 96 h at 0.2, 0.8, 3, 12, and 50 µg/L, then the whole fish body was analyzed for 6PPD-q	Dose dependent increase of 6PPD-q concentration (n.34-432 ng/g). Whole-body BCFs of 6PPD-Q were calculated as 2.9, 19, 25, and 17.2 293 L/kg at the water concentrations of 0.8, 3, 12, and 25 µg/L, respectively	Tissues extracted using acetonitrile, centrifuged, then ran on LC-MS	Not Specified
8 Laboratory in Japan Hiki and Yamamoto 2022	S.l.pluvius, S.curilus, and O.m.masou were exposed up to 3.5-3.8ug/L 6PPD-q for 24 hours, then brain and gill were analyzed for 6PPD-q	6PPD-q max (brain/gill) (ug/kg-wet) S.l.pluvius ~50, S.curilus 25/70, O.m.masou 4.7/38 6PPD-q-OH (more in brain vs gill) (ug/kg-wet) S.l.pluvius ~50, S.curilus ~50, O.m.masou ~25 ILC50 in S.l.pluvius of 4.0ug/kg (brain) and 6.2 ug/kg (gill)	LC/MS/MS in the exposure solution. Tissue extracted using acetonitrile	Not Specified
9 Canada Wu et al., 2023	Researchers measured levels of 6PPDQ-dG in from tissue of frozen capelin from a Canadian supermarket. 6PPDQ-dG is isomer of 3-hydroxy-1, N2-6PPD-etheno-2'-deoxyguanosine	Liver: median = 6.69 (4.24 - 8.03) lesions/10 ⁸ nucleosides Roe: median = 10.9 (4.45 - 16.8) lesions/10 ⁸ nucleosides Gill: median = 11.2 (8.47 - 15.5) lesions/10 ⁸ nucleosides	UPLC-ESI-MS/MS	LOD: 0.017 ng/ml LOQ: 0.056 ng/ml

5 MEASURING 6PPD AND 6PPD-QUINONE

This chapter reviews the variety of methods for measuring 6PPD and 6PPD-quinone among variable environmental matrices and landscapes. The most appropriate sampling method will depend on study objectives and medias. The matrix and available laboratories will often determine the analytical method. The discovery of a new chemical has spurred collaborations and sharing of scientific innovations to develop standard operating procedures in record time. Continued innovations and coordination between technical staff are needed to further develop analytical methods to understand the scope and scale of the tire contaminant problem. This chapter includes a section on using desktop mapping (GIS) tools to help focus reconnaissance efforts when dealing with such a wide spread contaminant. In addition, this chapter provides an overview of mapping tools to help identify potential 6PPD-quinone hot spots near vulnerable ecosystems.

5.1 Sampling Methods

Monitoring efforts by local, state, and federal agencies and universities are in the method development stage of sampling and measuring 6PPD-quinone. Therefore, the following sampling methods are based on initial efforts by toxicology and chemistry professionals, many of which have not been published and are still under development. In this section we focus on measuring 6PPD-q because it is more stable and longer lasting than the short lived 6PPD once released from tires. The ability to measure more PPDs and PPD-quinones will likely be needed as more information becomes available on tire contaminants and transformation products. A summary of the pros and cons of commonly used sampling methods is available ([Table 5-1](#)).

To assess the exposure risk of TWP to humans and terrestrial and aquatic life, more research is needed to understand the many [data gaps](#) including tire contaminant leaching dynamics, transport mechanisms and allocations between air and water, half-lives, and toxicity impacts. Bulk-based and single particle-based methods are used to attempt to measure TWP and TRWP; however, the complex and variable characteristics of TWP and TRWP present analytical challenges and many data gaps persist (Rødland et al., 2023).

5.1.1 Desktop Tools

[Desktop GIS exercises](#) are useful for visualizing potential exposure hot spots before heading out to the field to sample. In the case of 6PPD-q, mapping roads, traffic counts, sensitive species habitat, impervious surfaces, and precipitation will help guide where to focus reconnaissance efforts. The need to focus on suspected hot spots is particularly important when addressing ubiquitous contaminants like tires and 6PPD. [See section 5.3 for more details on mapping and modeling methods.](#)

Understanding areas of concern to local communities, for instance traditional fishing areas or already threatened populations to which the contaminant 6PPD-Q is acutely toxic, is another important consideration and method for focusing sampling efforts. The more sampling conducted, the more data will be available to help focus management actions.

Sampling across urban and traffic gradients and variable conveyance from road to natural waterbodies is an example of a monitoring strategy to understand the mass loading and transport of road runoff contaminants to vulnerable aquatic ecosystems (Ettinger et al., 2021; Feist et al., 2017; Geiger, 2021).

5.1.2 Grab Sampling

Grab samples can be an effective sampling method for screening for 6PPD-q given its flexibility and low cost. Yet, 6PPD-q grab samples often require logistically challenging storm chasing at all hours and days of the week. Glass bottles are recommended to avoid 6PPD-q loss from binding, polyurethane bottles can be used for short hold times but samples should be transferred to glass for longer hold times (Hu et al., 2023).

Preferred Container for 6PPD-q Samples

- *Amber glass bottles avoid photodegradation and affinity to bind to some plastics, minimize head space.*
- *250 mL bottles work well for whole sample extractions.*

5.1.3 Storm Event Sampling

Tire derived contaminants are washed into waterbodies during storm events which leads to temporary peaks in 6PPD-q concentrations (Johannessen, Helm, et al., 2022). Single time-point grab samples may not represent an ecologically relevant concentration due to 6PPD-q mobility through the aquatic environment. In larger rivers and watersheds, peak concentration of 6PPD-q may not be observed for many hours after peak discharge (Johannessen, Helm, et al., 2022). In smaller tributaries with high amounts of impervious surfaces, referred to the **urban stream syndrome** (Walsh 2005), peak concentrations may occur in the first hour. The greater the percent impervious surface within the stream catchment, the flashier the stream and more difficult to capture the pollutant peak (Gulliver et al., 2010).

5.1.4 Autosamplers

Autosamplers are an effective **active sampling** method for measuring organic contaminants in stormwater. However, they require an equipment investment and technical staff. Most autosamplers can be programmed to composite the samples into one jar or collect discrete sequential samples in separate containers.

Special considerations for 6PPD-q autosampling include tubing and bottle type choices. The preferred bottle type is glass, however, polyurethane bottles are acceptable for short periods of time based on the measured stickiness of lab and field

Autosampler considerations

- *PTFE tubing or glass bottles are preferred to minimize 6PPD-q loss due to affinity to some plastics.*
- *If polyethylene (PE) containers are used, the sample should be transferred to amber glass bottles as soon as possible.*
- *Store samples on ice until transferred to the lab.*
- *Remote samplers are an effective method that reduce logistically challenging storm chasing.*

equipment (Hu et al., 2023). If polyurethane bottles are used, samples transfer to amber glass bottles is recommended as soon as possible. The preferred tubing is polytetrafluoroethylene (PTFE) lined tubing, as 6PPD-q has been shown to readily adhere to other types of tubing (Hu et al., 2023). Samples should be kept on ice until transported from the field to the lab for extractions.

According to Gulliver et al. (2010), "...[r]emote sampler capabilities reduce collection costs and make it possible to obtain many more samples per storm event than is possible with current manual sampling techniques". For example, USEPA has created an in-stream remote sampling tool that uses a micropump to collect water samples (Kahl et al., 2014). Other groups have developed similar remote samplers (for example, Schönenberger et al., 2020). These types of samplers may prove more practical for stormwater monitoring needs, as they cost less while also offering greater flexibility on sampling location relative to traditional approaches using manual sampling.

5.1.5 Passive Samplers

Passive samplers have gained popularity over the last decade as an effective method for assessing the spatial and temporal occurrence of organic contaminants. The passive samplers are deployed in the field and left for days to weeks and are relatively affordable and often mimic the bioavailable form of contaminants. This is particularly useful for 6PPD-q that is transported during storm events and therefore difficult to measure. Preliminary investigations are underway to evaluate the efficacy of several passive samplers for quantifying 6PPD-q.

Passive Samplers

Passive samplers provide a cost-effective method for assessing the most bioavailable dissolved form of a contaminant. Passive samplers for 6PPD-q are being evaluated.

5.1.6 Biota

The measurement of 6PPD and 6PPD-q in biotic materials may provide an effective measure of exposure to aquatic organisms. Coho salmon mortality events can be difficult to catch given the short exposure duration period and low concentrations required to cause mortality. The ability to measure 6PPD and 6PPD-q in biota may help us understand the occurrence, exposure, and sublethal impacts to more tolerant species.

5.1.6.1 Bioassessments

Status and trends monitoring is an important type of assessment that tracks whether overall water quality and habitat conditions are improving or degrading and is most appropriate when done on a regional scale over a long period of time (decades). Biological assessments have become a widely accepted metric of overall stream health; however, it can be a challenging metric to connect to a single contaminant over a short period of time (Larson et al., 2019). For agencies or programs that rely on bioassessments, it might be advantageous to revisit past studies that used biological assessments that were unable to determine a cause of poor stream health by correlating with 6PPD-q detections.

5.1.6.2 Bioassays/Biomarkers

Risk from 6PPD-q exposure to organisms can be assessed in two primary ways. The analysis of 6PPD-q in water provides a surrogate measurement of exposure and with toxicity screening values or literature that provides LC50 or other toxicity values for comparison, this method is helpful for assessments. Yet, there is some uncertainty with respect to how the dynamic nature of 6PPD-q concentrations in-stream effects fish. For example, when is a good time to sample streams in conjunction with stormwater runoff, rainfall, and other factors? Additionally, other environmental factors may affect toxicity in yet unknown ways. Furthermore, most ambient waters contain several to thousands of chemicals forming complex and dynamic mixtures in many rivers and streams; this situation is challenging because many of the co-occurring chemicals have not been toxicologically tested and the way that chemicals in mixtures interact is highly challenging to predict. Therefore, bioassays can be used alone or in conjunction with 6PPD-q analysis to provide an integrated means of assessing mixtures and their dynamic behavior. Several bioassay types exist that vary in complexity and logistical demands. Depending on the goals of a monitoring event or study, careful consideration of their pros and cons is essential in order to produce data responsive to the question being addressed. Table 5-1 provides a set of common approaches that may be considered for research or regulatory purposes.

5.1.7 Sampling in Natural Environments

5.1.7.1 Wetlands

The impact and vulnerability of wetlands to tire contaminant exposure is currently unknown. The fish species that are thought to be most sensitive to 6PPD-q do not typically reside in wetlands. Yet, more research is needed to understand the transport of tire contaminants to wetlands and the toxicity to its inhabitants. The effluent of a wetland to adjacent streams and estuaries is needed as well.

Environmental Data Gaps

- *Few published studies have conducted 6PPD or 6PPD-q sampling.*
- *The majority of sampling has been along roadways and in streams or rivers.*
- *More sampling is needed to understand the occurrence, fate and transport of TWC in the terrestrial and aquatic environments.*

5.1.7.2 Lakes/Ponds

The land use and discharge types to a lake or pond will determine the exposure risk to resident fish species that are acutely impacted by tire contaminants. GIS desktop assessments and communication with the local managing jurisdiction will help identify areas of highest concern when determining where to start sampling. Lakes can provide an effective record of contamination over time by collecting and processing sediment cores. Sampling biota, such as fish tissues is another useful contaminant monitoring tool. Methods are being developed for measuring 6PPD-q in fish tissues and plasmas to support this type of work. Research is needed to evaluate the presence of tire wear particles and if they continue leaching over time.

5.1.7.3 *Streams/Rivers*

Coho salmon are the most acutely impacted by 6PPD-q and reside in small streams throughout the lowlands. Coho salmon bearing streams and rivers at lower elevations are often subjected to urban runoff mortality syndrome (URMS). The best sampling method depends on the size of the stream, accessibility, flow, mixing rates and sampling duration. For small, well mixed, shallow streams a well-timed grab sample from the stream bank or a well-placed autosampler is effective. For larger rivers with higher banks, deep, fast-moving waters and difficult terrain, a cable, bridge, or boat are more appropriate for sampling. For longer-term monitoring an autonomous stream sampling station with an autosampler triggered by rain and flow metrics. Autonomous moorings are another great option for larger rivers. The sampler should be aware of upstream and downstream point and non-point sources such as highway bridges, stormwater or wastewater treatment discharges when choosing the best site to sample or deploy sampling equipment. The goal is to collect a sample that is representative of the whole stream, however, streams and rivers with stormwater and wastewater discharges are not always homogenous (Shelton, 1994). The timing and location of coho exposure to TWC is poorly understood, more research is needed.

5.1.7.4 *Estuaries*

Salmon and trout species most sensitive to 6PPD-q spend a considerable time in estuaries. The juveniles spend several months to years getting ready to leave their natal watershed before they venture out to sea. Toxics tend to accumulate in estuaries, exposing juvenile fish (Carey et al., 2023; Gipe et al., n.d.). Sampling of water, sediments and biota in estuaries is needed to evaluate the fate and transport of tire contaminants in estuaries. Like streams and rivers, the characteristics of the estuary will determine the best sampling method.

5.1.7.5 *Marine*

Studies are available that verify the presence of tire wear particles as a major component of microplastics (Boucher & Friot, 2017; Kole et al., 2017; Sieber et al., 2020). The fate and transport of these particles and the ability to continue leaching tire wear particles is unknown, more research is needed to evaluate the presence of 6PPD and 6PPD-q in the marine environment. Coastal marine environments are logistically more complex to sample and often require boats to support collections. Sediments and biota are effective methods for measuring contaminant exposure. Methods for measuring 6PPD-q in marine matrices are being evaluated.

5.1.8 Sampling in Built Environments

5.1.8.1 Drinking Water

Special considerations for collecting 6PPD-q drinking water samples include using an amber glass bottle with a PE or PTFE lid and minimize head space, place sample on ice during transport to lab. Otherwise, methods are similar to standard procedures (for example, U.S. EPA, 2017). The impact of 6PPD-q to human health is unknown, the presence of 6PPD-q in drinking water is unknown (see also Section 2.5.4.1).

Contamination and Management Data Gaps

- *Few studies have been published regarding the efficacy of current drinking water, wastewater and stormwater filtration technologies.*
- *More sampling is needed to determine if, how often and when 6PPD and 6PPD-q are removed with current technology.*
- *And what new technologies can be added to existing infrastructure if and when needed.*

5.1.8.2 Wastewater

Wastewater is a combination of freshwater, raw water, drinking water or saline water post processing. Some municipal and industrial stormwater systems discharge to wastewater treatment facilities. A study conducted in Toronto, Canada detected 6PPD-q in the influent and effluent of a wastewater treatment plant (Johannessen & Metcalfe, 2022). Many transportation operation centers and car washing sites collect and dispose of their grey water to wastewater treatment facilities. More research is needed to measure the efficacy of wastewater treatment facilities ability to remove contaminants of emerging concern, including newly discovered organic contaminants like 6PPD-q. Sampling methods will depend on the study goal and available resources. Passive samplers deployed downstream from the effluent is an effective method for comparing between sites. Choosing between autosamplers and grab samples will partially depend on the residence time of the influent prior to discharge (McHugh, 2023).

5.1.8.3 Groundwater

There is very little information regarding the transport of 6PPD and 6PPD-q from surface water to groundwater. The transport potential will ultimately depend on the organic content and soil type present. More research is needed to evaluate the assumptions that 6PPD and 6PPD-q stay bound to particulates rather than be unbound and move through soils and sediments (Cunningham & Schalk, 2011).

5.1.8.4 *Hatcheries*

The first prespawn coho salmon mass mortality events were observed in a fish hatchery in Washington State. The hatchery diverted stream water that ran through a moderately urbanized area. Unexplained mass die offs would occur among coho salmon, but not other salmonids. WA Department of Ecology conducted a three year ecoforensic investigation and determined that the timing of the mortality events occurred during or after storm events (Kendra & Willms, 1990). The analytical capabilities hadn't been established to pinpoint the contaminant causing the mortality. The hatchery switched from using stream water to well water and the coho mortality events stopped. Any hatcheries that use creek or river water that are impacted by urbanization or transportation runoff are at risk of tire contaminant exposure to sensitive species. There may be a need to sample the influent of hatcheries that observe mass coho salmon mortality events or learn from the effectiveness of their filtration systems.

Hatcheries

- *Larger hatcheries have developed filtration systems to protect rearing salmon and trout populations from contaminants.*
- *Do these filtration methods remove 6PPD and 6PPD-q?*
- *More studies are needed to support larger hatcheries as refugia from urban pollution.*
- *Smaller tribal hatcheries do not have the same institutional support as state and federal hatcheries.*
- *Hatcheries near urban areas are most likely more vulnerable to 6PPD-q exposure, sampling is needed to detect 6PPD-q toxicity.*

Hatcheries have been proposed as a refuge for salmon and trout populations that are impacted by urbanization. For example, Elwha hatcheries on the Olympic Peninsula in Washington provided refuge after the Elwha river dam was removed and built up sediment smothered spawning grounds. Eventually the sediment was transported out of the river, but the hatcheries helped sustain the population.

In the case of 6PPD and 6PPD-q the hatcheries often have outdated infrastructure that may need to be retrofitted to control toxic exposures. Studies that coordinate fish population trends and 6PPD-q are needed to understand the impact on returning adults that provide future broodstocks.

5.1.8.5 Stormwater (Built Surface Water)

Stormwater management filtration technologies, mimics natural filtration processes capture tire wear particles and bind contaminants from roadways (McIntyre et al., 2014, 2023; Rodgers et al., 2023; Spromberg et al., 2016). Customized treatment trains that employ multiple methods to reduce and control tire wear and other contaminants is another potential effective strategy. Further research is needed to study the effectiveness of new and existing stormwater control devices and features. The conveyance and presence or absence of stormwater control devices (SCDs) will determine the discharge of 6PPD-q to vulnerable aquatic habitats. Inventories of these discharge outfalls and the type of conveyance and stormwater control devices will help identify areas to sample.

Stormwater Management Efficacy

- *Stormwater management is customized to land uses.*
- *More studies are needed to identify effective stormwater management practices to protect aquatic life.*

The following is for paired influent and effluent sampling to evaluate SCD effectiveness. Paired sampling is most appropriate for SCD with a relatively short (in the order of minutes) detention time. For longer detention time SCDs (ponds, etc.) TAPE, the Washington State Department of Ecology's process for evaluating and approving emerging stormwater treatment BMPs (<https://www.wastormwatercenter.org/stormwater-technologies/tape/>), suggests an alternative sampling method.

5.1.8.5.1 Selecting a Stormwater Control Measure

This section provides a typical stormwater effectiveness sampling protocol. When choosing a SCD to monitor be sure to get permission from the property owner and/or jurisdiction. It is also best to: find a SCD that is in an area that is not a safety hazard (steep slopes, in a roadway, etc.); avoid sites affected by backwater, tidal influence, or high groundwater; ensure land use/drainage basin will produce enough pollutants of interest to evaluate the effectiveness. Sampling equipment will require either A/C or battery power. When selecting a SCD to monitor consideration should be made to whether staff will need to carry batteries and sample bottles a far distance. It is also best to avoid sites that require confined space entry for sample collection or other routine activities.

Stormwater Control Device Efficacy

- *There is a very prescribed method for testing and approving new technologies to avoid unintended consequences and maximize the treatment co-benefits for other pollutants of concern.*
- *In addition, exploratory sampling is needed for existing infrastructure and will require less stringent efficacy methods as well. Understanding the inventory of existing conveyance and treatment are good first steps.*

SCD Information

The following information about the SCD should be recorded/documented, where applicable.

- If this is an existing SCD, how long has the system been in operation?
- If this is an existing SCD, how frequently has it needed maintenance or been maintained?
- Was the SCD sized following the regional stormwater management guidance?
- What is the design flow rate?
- What are the treatment mechanisms (HDS, sand filter, membrane filter, media filter, etc.).
- What is the size of the system (area of filter bed, number of cartridges, etc.)? For media filters, what type of media is it?
- What is the primary land use in the drainage basin?
- How big is the drainage basin?

5.1.8.5.2 Precipitation Monitoring

Rainfall should be measured within the drainage basin or adjacent to the monitoring equipment. Rainfall monitoring should be performed to measure and record rainfall continuously throughout the study duration (Washington State Department of Ecology, 2018).

5.1.8.5.3 Flow Monitoring

The following recommendations on current best practices for treatment system flow monitoring are summarized from (Washington State Department of Ecology, 2018). As stated in that technical guidance document, it is important to continuously monitor influent, effluent, and bypass flow rates throughout the duration of each project or study .

Influent and effluent flows should be measured as practicable to the inlet and outlet, respectively, for a treatment system inlet to ensure that the recorded depth and flow rate represent actual conditions experienced by the system. It is important to select an effluent monitoring location that avoids measuring effluent flow rates in portions of the conveyance system where this flow mixes with bypass flow.

Bypass flows should be measured in areas that represent only the water bypassing the treatment system. It is best practice to avoid bypass flow measurement in the portions of the conveyance system where bypass flow mixes with effluent (treated) flow.

Each flow measurement device should be installed in a place that is both secure and convenient to access. This equipment needs to be properly calibrated and maintained on a regular basis, and should be easily accessible for the entire duration of monitoring activities.

5.1.8.5.4 Water Quality Sampling

Technical guidance from WA Ecology recommends collecting a minimum of 15 samples from both the influent and effluent of the treatment system during separate storm events (Washington State Department of Ecology, 2018). That guidance also recommends use of automated samplers for sample collection for target analytes unless that analyte requires manual grab samples (for example, NWTPH-Dx, fecal coliform, E. coli).

When choosing monitoring locations, keep in mind that in insufficient mixing may cause stratification across the flow column for settleable or floating solids and the bound pollutants associated with these solids. Best practice is to gather influent and effluent samples where stormwater flow is well-mixed and unaffected by pollutants that have accumulated or been stored in stagnant areas. When an automated sampler is used, the sampling locations should be located sufficiently far from the flow monitoring apparatus to prevent skewed depth and flow measurements (Washington State Department of Ecology, 2018).

Influent samples should be collected as close as possible to the treatment system inlet to ensure that the samples are representative of actual conditions. To collect samples that are representative of treated water, it is recommended that effluent samples be collected as close as practicable to the outlet for the treatment system. Sample collection should not occur from locations where the conveyance system and bypass flow mixes (Washington State Department of Ecology, 2018).

5.1.8.5.5 Valid Sample/Storm Event

Current best practice is for samples and storms to meet the following criteria to be considered an Event Mean Concentration and a valid sample:

- Storm Size: $\geq 0.15''$
- Antecedent dry period: 6 hours with less than $0.04''$
- Storm duration: ≥ 1 hour
- Range of rainfall intensities
- Number of aliquots per sample: ≥ 10
- Storm coverage: aliquots should be collected over $\geq 75\%$ of the first 24 hours of the hydrograph
- Sample duration: ≤ 36 hours
- Number of samples: 15

5.1.8.5.6 Study Duration

The study should continue until at least 15 samples have been collected. The duration of sample should span at least one and a half maintenance cycles or, for systems with more than a 2-year maintenance cycle, over two wet seasons (Washington State Department of Ecology, 2018).

5.1.8.5.7 Maintenance inspections and activities

The study should include regular maintenance inspections to track and record the maintenance needs of the test system. Maintenance indicators are generally specific to the BMP, but typical indicators include an accumulation of sediment and a reduction in effluent (treated) flow rate through the system. All maintenance inspections and activities should be documented.

5.2 Laboratory Methods

This section summarizes published analytical methods and laboratory approaches for the analysis of 6PPD and 6PPD-q. This review does not address methods or techniques for TWP or TRWP in the environment as using 6PPD or 6PPD-q as markers for is imperfect given the analytical challenges with quantifying TRWP in various matrices and the uncertain and variable transformation rate of 6PPD to 6PPD-q. For example, because TRWP are heterogenous and dark in color, there are uncertainties associated with quantifying the particles using methods such as Fourier-transform infrared spectroscopy (FTIR) and Raman (Werbowski et al., 2021). The density of TRWP is a sampling challenge when collecting water samples for 6PPD and 6PPD-q because TRWP can sink to sediments and the density of particles is variable, particularly throughout the age of the TRWP (Klöckner et al., 2020). Further, some chemical markers used in GC-MS analyses (for example, zinc, benzothiazoles) have sources to the environment in addition to TRWP, which could overpredict the concentrations of TRWP (Unice et al., 2012; Wagner et al., 2018). An investigation with TRWP from a range of environmental samples found that N-formyl-6-PPD, hydroxylated N-1,3- dimethylbutyl-N-phenyl quinone diimine and 6PPD-q are potential organic markers for TRWPs in the environment, but further research is needed to confirm the relationship (Klöckner, Seiwert, Wagner, et al., 2021).

Laboratory Methods

Commercial and research laboratories are developing methods for the analysis of 6PPD and 6PPD-q in a range of environmental matrices and consumer products. When selecting a laboratory aspects to consider for data quality and reproducibility includes method accreditation, detection limits, sample holding times, and quality control parameters

5.2.1 Physicochemical Property Considerations in the Laboratory

Physicochemical chemical properties are discussed in section 3.1.1 and summarized in **Table 3-1**.

The main considerations for 6PPD analysis is the a relatively short half-life of several hours (OSPAR Commission, 2006), solubility of 0.5 to 2 mg/L, and relatively high log Kow and log Koc (Department of Toxic Substances Control, 2022; ECHA, 2021; Hiki et al., 2021; Klöckner et al., 2020). If 6PPD is present in environmental water samples there is a short window of analysis time before the compound degrades. In waterways, with mg/L solubility and the short half live, it is anticipated that 6PPD well be less frequently found in the dissolved phase than 6PPD-Q. When investigating the leaching of 6PPD directly from rubber or tire wear particles, the solvent for leaching studies and approach used can influence the rate and number of tire related compounds leached (Zhao, Hu, Tian, et al., 2023).

A challenging aspect for 6PPD-q analysis is the relatively high log K_{ow} and log K_{oc} causing it to partition to organic or solid materials. Materials used by the laboratories should be investigated to be sure sorption is minimized when filtering or processing samples and that laboratory carryover is minimized on equipment. With observations of sorption to organic material (Hu et al., 2023), methods that provide a whole-water analysis (R. Zhang et al., 2023) are important as a filtered direct-inject water method could miss concentrations in the sorbed fraction. With observed prolonged water stability in tap water, laboratory stock solutions, and stormwater (Hiki et al 2021; Hu et al, 2023; USGS-placeholder) and the ug/L solubility, 6PPD-q is observed in the aqueous phase before binding to organic or solid materials. Additional research is needed to understand rates of adsorption and the fate and transport of the dissolved versus suspended particle fractions of 6PPD-q and 6PPD.

5.2.2 Bottle Types and Laboratory Holding Times

6PPD: A thorough bottle comparison study has not been performed for 6PPD as this is challenging to investigate due to the short half-life. Approaches that have been investigated to prolong the 6PPD half-life includes the use of free ozone scavengers, such as ascorbic acid, but the preservatives can cause stability issues during the analysis of 6PPD-q (Woudneh, 2023).

6PPD-q: For longer-term storage, glass is recommended, and plastics should be avoided; stainless steel and polytetrafluoroethylene (PTFE) can be used for shorter holding times (Hu et al., 2023; USGS-placeholder). If plastic containers are used, the bottles can be rinsed with organic solvents to recover some sorbed 6PPD-q but glass is recommended to avoid the losses and work of trying to recover the sorbed fraction. In 5-month holding time studies with laboratory generated 6PPD-q solutions, significant sorption to HDPE, polypropylene, and polystyrene were observed, no significant sorption to glass or differences observed from light, headspace, or storage temperature. Holding times in laboratory solutions can be significantly different than environmental water samples and in 75-day holding times in stormwater variability in the stability was observed with differences noted for headspace and storage temperature; based on that data the conditions recommended are glass bottles with minimal headspace and refrigeration (5C) or freezing, [USGS-placeholder].

5.2.3 Analytical Method Approaches for 6PPD and 6PPD-q

The most frequently developed analytical technique for 6PPD and 6PPD-q is liquid chromatography mass spectrometry (LC/MS) (Table 5-2 and Table 5-3) gas chromatography (Tian et al 2021, Science; Rauert et al 2002) can also be used but has not been as widely applied. LC/MS has been frequently used because of low nanogram per liter (ng/L) method detection limits, the ability to analyze a variety of biological and environmental samples and flexibility in quantitation and providing structural elucidation for unknowns (Cao, Zhang, et al., 2022). LC/MS has been used to analyze 6PPD and 6PPD-q from abiotic and biotic matrices. 6PPD-q has been analyzed in surface waters, stormwater, groundwater, road runoff, snow, suspended particles, dust, fine particulate matter (PM_{2.5}), influent and effluent from wastewater treatment plants (WWTPs), drinking water treatment plant (DWTP), soil, sediment, biosolids, human urine, mammalian cells, fish tissues, fish bile, fish plasma, lettuce, and honey (Tables 4-1 through 4-10) with corresponding reporting limits listed in the (Table 5-2 and Table 5-3). 6PPD has been analyzed in surface waters, stormwater, road runoff, snow, influent and effluent from wastewater treatment plants (WWTPs), dust, fine particulate matter (PM_{2.5}), soil, sediment, fish

tissues, human urine, mammalian cells, lettuce, and honey (Tables 4-1 through 4-10) with corresponding reporting limits listed in the (Table 5-2 and Table 5-3).

5.2.3.1 MSMS Targeted and Non-targeted (HRMS, Qtof, etc.)

Liquid chromatography or gas chromatography with targeted, semi-targeted, suspect screening, and non-targeted analysis have been used in the analysis of 6PPD-q and 6PPD; commonly monitored ions for 6PPD and 6PPD-q during multiple reaction monitoring and targeted screenings are listed in the Table 5-2 and Table 5-3, respectively. High resolution mass spectrometry can be used for targeted analysis and is a mass spectrometry technique that is gaining popularity because a full scan is collected (even during a targeted analysis), which results in capturing additional information so that transformation products of unknown compounds related to 6PPD or 6PPD-q or tire chemicals can be elucidated (Cao, Zhang, et al., 2022). As was demonstrated by Müller et al. (2022), this can be an advantageous in screening a large number of unknowns related to tire chemicals while also identifying 6PPD and 6PPD-q.

5.2.3.2 Other Techniques

Other techniques that have been investigated for the sampling or detection of 6PPD-q and 6PPD but not widely applied. For the sampling of 6PPD-q a CP-MIMS with a semipermeable polydimethylsiloxane capillary hollow fiber membrane has been used (Monaghan et al., 2021). ASTM-D5666 standard method is available for the purity of PPDs by HPLC UV detection (ASTM International, 2023). Researchers at the University of Toronto have developed a colorimetric method for the instrument free, naked-eye, rapid detection of 6PPD in rubber products wastes which results from the phenyl-p-phenylenediamine core structure forming a red colored product during oxidation (Hui Peng, Victor Li, Linna Xie, manuscript to be submitted). UV/VIS spectrometry analysis has been used for some 6PPD research and in the identification of transformation products (Cataldo et al., 2015; Li et al., 2023; Zhao, Hu, Tian, et al., 2023) and in measurement of 6PPD-q standards (Tian et al., 2021).

5.2.3.3 Rubber Products

Analytical techniques have been applied to understand the leaching of particles from rubber products. When preparing rubber products for analysis consideration should be given to the preparation technique; techniques such as sample grinding can increase surface area and the formation of 6PPD-Q. Recycled tire rubber used in synthetic football fields has been ultrasound-assisted extracted and with an *in-vitro* simulation of digestion extraction to obtain the bioaccessible fraction and analyzed with GC/MS suspect screening (Armada et al., 2023). Tire particles have also been investigated with the fish *in vitro* digestive model and co-ingestion experiments to create digestate solutions that were analyzed by UHPLC-HRMS (Masset et al., 2022). Tire wear particles leaching has also been explored with solvent and water extractions and analyzed by LC-MS/MS (Zhao, Hu, Tian, et al., 2023).

5.2.4 Considerations for Measuring 6PPD

Methods for 6PPD have not been as widely reported due to the relatively short half-life of 6PPD in both environmental and laboratory conditions. Transformation products (including hydrolysis products) are quickly formed once 6PPD is in water or to a lesser degree in organic solvents (for

example, methanol, acetonitrile, toluene). While there is limited information on identifying all transformation products of 6PPD, it is known that 6PPD-q may occur as a transformation product in the environment (Cao, Wang, et al., 2022; Seiwert et al., 2022; Zeng et al., 2023). Therefore, due to the relative reactivity of 6PPD in the environment and laboratory, the methods can only generate an estimated level of 6PPD in environmental samples. Preservatives and pH modifiers have been investigated as ways to stabilize 6PPD, but these additives negatively impact the analysis of 6PPD-q, compromising the sample; the current recommendation for 6PPD analysis by LC/MS is to minimize holding times and direct-injection to minimize both processing and losses (Woudneh, 2023). Monitoring the ions for 6PPD in mass spectrometry methods can provide presence/absence information. In consumer goods and TRWP laden samples that show positive detections for 6PPD that can act as a reservoir for increased 6PPD-Q formation.

5.2.4.1 Standard or Approved Methods for 6PPD

Currently there are no standardized or approved methods for 6PPD. NOAA and the U.S. Geological Survey have included 6PPD in their 6PPD-q methods and provide an estimate of 6PPD concentrations. (NOAA and USGS placeholder).

5.2.4.2 Detecting 6PPD in Consumer Products

Techniques that can be applied to define and quantify 6PPD in consumer products includes, high performance liquid chromatography (HPLC) (Ikarashi & Kaniwa, 2000; Lokesh et al., 2023; Poldushova et al., 2016), liquid chromatography/mass spectrometry (LC/MS) (Cao, Wang, et al., 2022; Ikarashi & Kaniwa, 2000; Johannessen, Helm, et al., 2022; Kryuchkov et al., 2023), gas chromatography mass spectrometry (GC/MS) (Armada et al., 2022; Llompert et al., 2013; Skoczyńska et al., 2021), quadrupole time-of-flight/mass spectrometry (QTOF/MS) (Cao, Wang, et al., 2022), raman spectroscopy (Santini et al., 2022), fourier transform infrared (FTIR) (Olubusoye et al., 2023; Santini et al., 2022), thermal desorption (TD), and pyrolysis-gas chromatography/mass spectrometry (pyro-GC/MS) (Rauert et al., 2022).

Consumer products are cut down to micro sized particles via cryogenic grinding (or cryomilling) to a length less than 5 mm. The apparatus is dependent upon the specific instrumentation used; for example, ICP/MS would use glass vials and then transferred into polyethylene sample holders for analysis where TD would use quartz sampling tubes. Density separation is not necessary when working with a known consumer product. Procedural steps are based on which specific instrumentation is used to analyze the sample. One of the least complicated methods is GC/MS. For example, with HPLC, LC/MS and ICP/MS, acid digestion may be a necessary first step depending upon the type of polymer rubber of the consumer product, adding time to the analysis process and less green.

For LC/MS, the consumer sample is prepared with solvent extraction via centrifugation, shaking or sonication and concentrated with via blowdown, solvent accelerator extraction, or plastic-free stream with nitrogen, and cleanup steps can include syringe filter or SPE. With TD and pyro-GC/MS, there are three types of quartz sample tubes to choose from depending on the volatility of the consumer sample: the double open-ended, single open-ended, and slit. The tube is conditioned by passing through a hot flame, such as a Bunsen burner or butane flame, and small amount of consumer sample added to the quartz tube and pyrolyzed. A well characterized NIST

rubber sample is used as a reference and total ion count (TIC) chromatogram to identify patterns of ions and major chemical markers of tire rubber.

5.2.4.3 6PPD Commercial Standards and Isotopically Labeled Surrogate or Internal Standards

6PPD is available from a range of commercial vendors including HPC Standards, Cambridge Isotopes Laboratories, Sigma-Aldric, J&K Scientific Ltd, AccuStandard, Tokyo Chemical Industry (TCI), Toronto Research Chemical (TRC) , Dr. Ehrenstorfer, Aladdin Biochemical Technology Co., Fluorochem, Novachem, Toyko Kasei, Jiaxuan Biotechnology, and Usof Chemicals [Table 5-2] . An isotopically labeled 6PPD readily available and the following have been used as surrogate and internal standards 6PPD-q-D5, 6PPDQ-13C6, aniline-d5, atrazine-d5, benzophenone-d10, benzothiazole-d4, caffeine-13C3, coumaphos-d10, 16diphenylamine-d10, diphenylurea-d10, 5-methylbenzotriazole-d6, melamine-13C3, progesterone-d9, and pyrene-d10 (Table 5-3).

5.2.5 Considerations for Measuring 6PPD-q

The discovery of 6PPD-q (Tian et al., 2021) used a variety of analytical techniques to isolate and identify 6PPD-q. To simplify the complexity of the roadway runoff sand filtration, ion exchange, xad-2 fractionation, silica gel fractionation, parallel HPLC fractionation, and sequential HPLC fractionation were used to isolate fractions that were toxic to coho salmon. Analysis that was used to elucidate the compounds in the toxic fraction included liquid chromatography and gas chromatography for separation with quadruple time-of-flight (qTOF) and orbitrap high resolutions mass spectrometry (UPLC-QTOF-MS/MS, UPLC-Orbitrap-MSN, GC-QTOF-HRMS). These techniques for investigating unknown compounds in complex mixtures along with NMR data for confirmation identified 6PPD-q.

With observations of sorption to organic material (Hu et al., 2023), methods that provide a whole-water analysis, including suspended materials, is important to understanding exposures as stormwaters and surface waters can have high turbidities. Laboratory rubber materials, silicone, and parafilm also have a higher capacity for sorption and should be avoided (Hu et al., 2023; Woudneh, 2023).

5.2.5.1 Standard or Approved Methods for 6PPD-q

Options for the analysis of 6PPD-q include commercial and research laboratories. Methods of analysis in water have been published by several organizations and laboratory accreditation in the state of Washington is offered by the Washington State Department of Ecology (WSDOE). WSDOE has published a standard operating procedure for the detection of 6PPD-q in water, the U.S. EPA's Office of Water (OW) has published Draft Method 1634 for the analysis of 6PPD-q in storm and surface waters, and the U.S. Geological Survey a direct-inject method in water. The National Oceanic and Atmospheric Administration (NOAA) has pending method publications for 6PPD-q analysis in biotic and abiotic media.

5.2.5.1.1 EPA Draft Method 1634

EPA's Office of Water (OW) Draft Method 1634 for the analysis of 6PPD-q in storm and surface waters, 250-mL samples are collected headspace-free in amber glass bottles with 26eflon

lined caps and stored at 0 – 6 °C, but not frozen. Samples, which may be held for up to 14 days, are then extracted using a solid-phase extraction (SPE) column after adding an aliquot of the extracted internal standard (EIS), 13C6-6PPD-q. The SPE column is rinsed with a 5-mL aliquot of 50:50 methanol:water and then, extracted with two 5-mL aliquots of acetonitrile. The non-extracted internal standard, D5-6PPD-q, is added to the extract, and a 50-uL aliquot injected on to the LC/MS/MS system. Analyte separation is accomplished on a C18 column using a 0.2% formic acid in water and acetonitrile mobile phase that has a 10-minute run time. The isotope dilution method is used to quantitate 6PPD-q. The EIS recoveries are also determined using the NIS and are used as an indicator of analytical data quality. The minimum level of quantification (ML) for the method is 2 ng/L.

5.2.5.1.2 Washington State Department of Ecology

Standard Operating Procedure (SOP): Extraction and Analysis of 6PPD-q (Mel730136, Version 1.2) contains procedures for the extraction and analysis of water for 6PPD-q by triple quadrupole mass spectrometry. The standard operating procedure recommends the sample collection, preservation, storage, and holding times. 250-mL samples are collected headspace-free in amber glass bottles with 27eflon lined caps and stored above freezing to 6 °C. Samples, which may be held for up to 28 days, are then extracted using a solid-phase extraction (SPE) column. Compounds that can be used as the extracted internal standard (EIS) or injected internal standard (IIS) are D5-6PPD-q and 13C6-6PPDQ. The SPE column is conditioned with a 5-mL aliquot of acetonitrile and 10-mL of Milli-Q water, rinsed with 5-mL of 1:1 methanol:water and 5-mL of hexane, then extracted with two 5-mL aliquots of acetonitrile. Analyte separation is accomplished on a biphenyl column using a 0.1% Formic acid/water and 0.1% Formic acid/Acetonitrile mobile phase that has a 10.5-minute run time. The suggested reporting limit is 1 ng/L and Lower Limit of Quantitation for the instrument is suggested to be 0.025 ng/mL.

5.2.5.1.3 National Oceanic and Atmospheric Administration, NOAA

The NOAA method is a LC-MS/MS method for the detection of 6PPD-q in water, fish bile, fish plasma, blubber, fish tissue, and shellfish. The procedure recommends the sample collection in glass vials or bottles. Water samples are prepared with liquid/liquid extraction, concentrated, and the solvent exchange. The surrogate standard is D5-6PPD-q and the internal standard is progesterone-D9 Bile and plasma are diluted and then extracted with SPE. Blubber, fish, and shellfish are ASE extracted with gravity-flow column cleanup & fractionation, then GPC cleanup. Analyte separation is accomplished on a C18 column. The detection limit for plasma is 0.0075-0.025 ng/mL, bile: 0.015-0.05 ng/mL, and fish tissue is 0.07 ng/g.

5.2.5.1.4 U.S. Geological Survey

The U.S. Geological Survey direct-inject method is a UPLC-MS/MS method for the analysis of 6PPD-q in surface waters. Samples are collected in headspace-free in amber glass bottles with 27eflon lined caps and stored at 0 – 6 °C for less than 14 days or frozen until analysis. Samples are 0.7-micron glass-fiber filtered and the surrogate internal standard D5-6PPD-q added. Analyte separation is accomplished on a C18 column using a 0.1% formic acid in water and acetonitrile mobile phase that has a 5-minute run time. The isotope dilution method is used to quantitate 6PPD-q and the method reporting limit is 2 ng/L.

5.2.5.2 Finding a Laboratory

Commercial and research laboratories are available for the analysis of 6PPD-q. When selecting a laboratory, be sure it provides the analytical rigor required for project or program goals. Laboratory method accreditation, method detection limits, sample holding times, and quality control parameters (laboratory blanks, laboratory duplicate samples, spike recoveries, method verification standards) should be reviewed to ensure data will meet the desired quality and reproducibility. A list of Washington State Department of Ecology accredited labs is available at [Home - Lab Search \(wa.gov\)](#)

5.2.5.3 6PPD-q Commercial Standards and Isotopically Labeled Surrogate or Internal Standards

One of the initial limitations of 6PPD-q research was the lack of a reference standard for consistent accurate concentrations, which are important for correlating exposure concentrations to environmental detections. 6PPD-q is available from a range of commercial vendors HPC Standards, Cambridge Isotopes Laboratories, Sigma-Aldrich, J&K Scientific Ltd., AccuStandard, Tokyo Chemical Industry (TCI), Toronto Research Chemical (TRC), Dr. Ehrenstorfer, Jiaxuan Biotechnology Co., Ltd, Novachem, and Aladdin Biochemical Technology [Table 5-2] Isotopically labeled standards are commercially available for 6PPD-q and include D5-6PPD-q, $^{13}\text{C}_{12}$ -6PPD-q, and $^{13}\text{C}_6$ -6PPD-q. An interferent with the $^{13}\text{C}_{12}$ -6PPD-q analogue has been observed with some stormwaters (Eurofins 2023). These labeled standards along with aniline-d5, atrazine-d5, benzophenone-d10, benzothiazole-d4, coumaphos-d10, caffeine-13C3, diphenylamine-d10, diphenylurea-d10, 5-methylbenzotriazole-d6, progesterone-d9, pyrene-d10 have been used as surrogate or internal standard [Table 5-2]

5.2.6 Laboratory Extraction Techniques for 6PPD and 6PPD-q

For water samples, filtration, liquid/liquid extraction or solid phase extraction can be used for sample clean-up and concentration (Table 5-2 and Table 5-3). For biological fluids such as bile, urine or plasma, solid-phase extraction is commonly used as an extraction/cleanup technique. (place holder for da Silva's manuscript – update in April 2024) For biological or other solid samples other techniques have been used include salting out, serial sonication, accelerated solvent extractions, and QuEChERS extractions (Table 5-2 and Table 5-3).

5.3 Mapping Vulnerable Ecosystems

This chapter provides guidance on tools that can help focus sampling and mitigation efforts on 6PPD and 6PPD-q hot spots and vulnerable ecological populations.

Although the causal link between 6PPD-q and pre-spawn coho salmon mortality was first identified in the Pacific Northwest (Kendra & Willms, 1990; McIntyre et al., 2021; Scholz et al., 2011; Spromberg & Scholz, 2011), the potential global extent of effects of 6PPD-quinone on aquatic life is unknown. 6PPD-quinone has been measured in human urine (Du et al., 2022), but toxicity in mammals have been measured in only a few studies (Chen et al., 2023). Although the concentrations and distribution of 6PPD and 6PPD-q also has not been well-characterized, 6PPD and 6PPD-q have been found in various environmental media (Link to Occurrence Section and Tables) (Cao, Wang, et al., 2022; Challis et al., 2021; Johannessen, Saini, et al., 2022; Monaghan

et al., 2021; Nedrich, 2022; Rauert et al., 2022; Seiwert et al., 2022; Tian et al., 2022). The spatial distribution of 6PPD-q is not uniform across landscapes and depends on multiple factors (Link to Occurrence Section that discusses TWP emissions and F/T). Areas where prespawn coho salmon mortality has been observed in streams near high traffic areas in the Pacific Northwest (Ettinger et al., 2021; Feist et al., 2017; Spromberg & Scholz, 2011) are assumed to correspond with high 6PPD-q concentrations. To better characterize potential risks and to strategically monitor pre-spawn coho salmon mortality, scientists are developing spatially explicit sampling and assessment approaches (Chen et al., 2023).

5.3.1 Data Layers and Tools

To date, there are no national databases nor mapping tools dedicated to assessing 6PPD-q concentrations, effects to sensitive fish species, or associations between them. However, there are national data sets and mapping tools for landscape metrics that may help visualize stream crossing, traffic intensity, impervious surfaces, and other parameters that may be associated with 6PPD-q levels and delivery to streams (Table 3-14). The occurrences of sensitive fish are available from national data sets via the Water Quality Portal and a lower density coverage via the EPA National Aquatic Resource Surveys for lakes and rivers (NLA, NRSA). However, additional data processing is needed before mapping, and available data will not provide a census of every stream or waterbody. Furthermore, extirpation of sensitive fish species may have already occurred due to other causes (Smith & Sklarew, 2013). Researchers have also developed coverages for projects not originally designed to address questions regarding 6PPD-q (for example, storm drain locations): but nevertheless, may be useful for local and regional assessments.

Some local and regional data sets, mapping tools, and spatially explicit assessments are available especially in the Pacific Northwest (Washington State Department of Ecology, 2022) and efforts have increased to better assess the scope of the problem both locally and nationally. Table 5-4 lists some of the resources that are currently available.

Table 5-4. Selected mapping tools and other resources that may be useful for visualizing water quality conditions in support of resource and stormwater management

Spatial Resource	Developer	Region
Puget Sound Stormwater Heatmap	TNC Geosyntecs	Puget Sound
Puget Sound Watershed Characterization Project	WA Ecology	Puget Sound
EnviroAtlas	EPA	US
Freshwater Explorer	EPA	US
StreamCAT	EPA	US
Water Quality Portal	EPA USGS	US
National Aquatic Resource Surveys	EPA	US
Puget Sound Stream Benthos	King County	Puget Sound
SWIFD Salmonscape	NWIFC WDFW	Statewide
Washington State Fish Passage	WDFW	Statewide
Tire Contaminant in Coho Watersheds Story Map	WA Ecology	Statewide
High Resolution Change Detection	WDFW	Statewide
Water Quality Atlas	WA Ecology	Statewide
Washington Geospatial Open Data Portal	WA State	Statewide
Urban Canopy	City of Seattle	Puget Sound
NorWeST project	USDA	Statewide
Stream flow	USGS	Statewide
WSDOT Online Map Center	WSDOT	Statewide
Washington Geospatial Open Data	WOCIO	Statewide
Puget Sound Mapping Project	WDOC	Puget Sound
WA's National Hydrography Dataset Program	Ecology USGS	Statewide
Visualizing Ecosystem Land Management Assessments	EPA	Flexible
GridMET	Climatology Lab	CONUS
Physical Solar Model V3	NREL	CONUS
Coho Urban Runoff Mortality in Puget Sound	USFWS, NOAA, WSU	Puget Sound

5.3.2 Spatial Data for Relevant Land Surface Features

The nearness to impervious surfaces, roads, and their proximity to streams are factors that have been linked to pre-spawn mortality and higher levels of 6PPD-quinone (Feist et al. 2017, Tian et al 2022). Because 6PPD-quinone is released from tires, traffic density, impervious surfaces, roads, and their proximity to streams have been used as indicators of relative potential concentrations of 6PPD-quinone in water. However, quantitative models for predicting 6PPD-quinone concentrations have not been developed or validated nationally (Tian et. al 2022).

Good national coverages of impervious surfaces, roads, and their proximity to streams are readily available; however, traffic density data is not available for all roads or may not be available from open access datasets. Rainfall and other geographical factors that affect surface run-off and dilution from USGS and has been conveniently curated by USEPA (StreamCat) and can be modeled (for example, Soil and Water Assessment Tool (SWAT), (Arnold et al., 1998). These data can be visualized with commercial mapping software or free open-source languages such as R and Python. These GIS software programs require some experience with GIS computing. No GIS experience is needed to visualize impervious surfaces, traffic, and road proximity to streams with the USEPA Freshwater Explorer mapping tool which can be accessed from the USEPA Freshwater Explorer Landing page: <https://www.epa.gov/water-research/freshwater-explorer> from the add data widget.

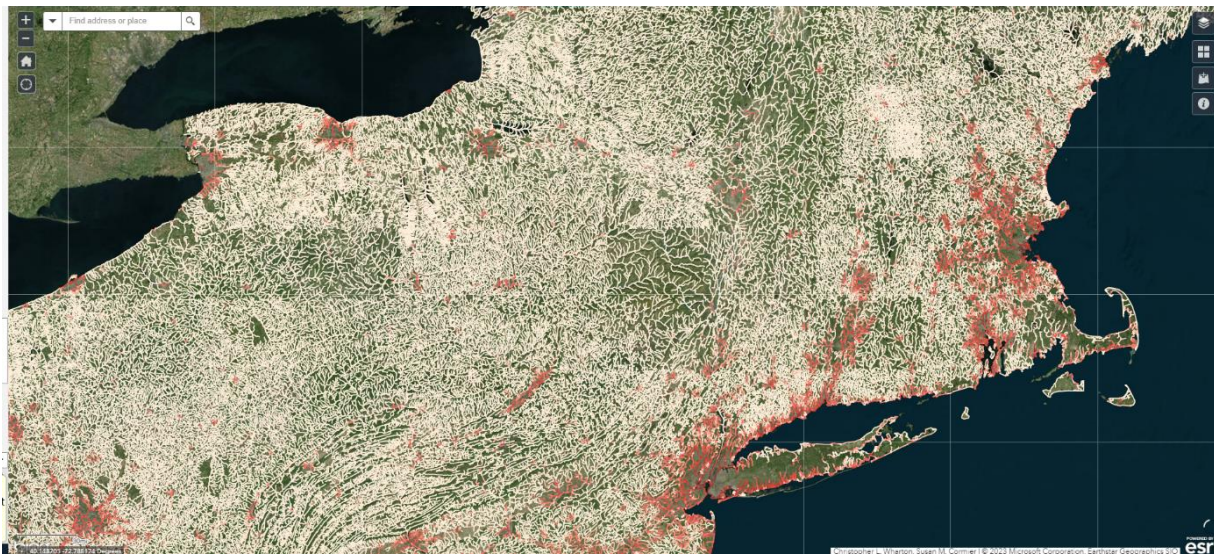


Figure 5-1. Depiction of streams in the Northeastern United States from the National Hydrography Dataset (NHD).

For example, in Figure 5-1 the yellow lines are streams with less impervious surfaces in their catchments and the red areas have more impervious surfaces. Impervious surface has been linked with coho vulnerability (Feist et al., 2017). As can be seen in this image, the resolution of small streams artificially varies where rectangular areas are depicted with fewer streams, i.e., fewer yellow lines. No data set perfectly depicts all the small streams that often serve as spawning habitat. In general, streams in urban areas near Boston, MA, New York City, NY, and Pittsburgh, PA have greater amounts of impervious in their watersheds than other areas.

At a more local scale, more detail can be seen. The screenshot from the USEPA Freshwater Explorer (Dec. 2023) illustrates how spatial data can be used to depict information that may help to inform monitoring designs. The graphic shows streams color-coded for proximity to roads, warmer colors being closer or crossed by a road (Figure 5-2). The inset box provides the data from a selected stream. In this example, the associated data box shows information for a segment of Beaverton Creek, Portland, Oregon (highlighted in turquoise) which has minor roads that cross it and is 147 m (about 482.28 ft) from a major road with 27,775 cars reported as local traffic. Impervious cover is 52% and 63% for the watershed and the smaller catchment, respectively. Green areas in this satellite image are forests and tan and gray areas are agricultural or urban areas.

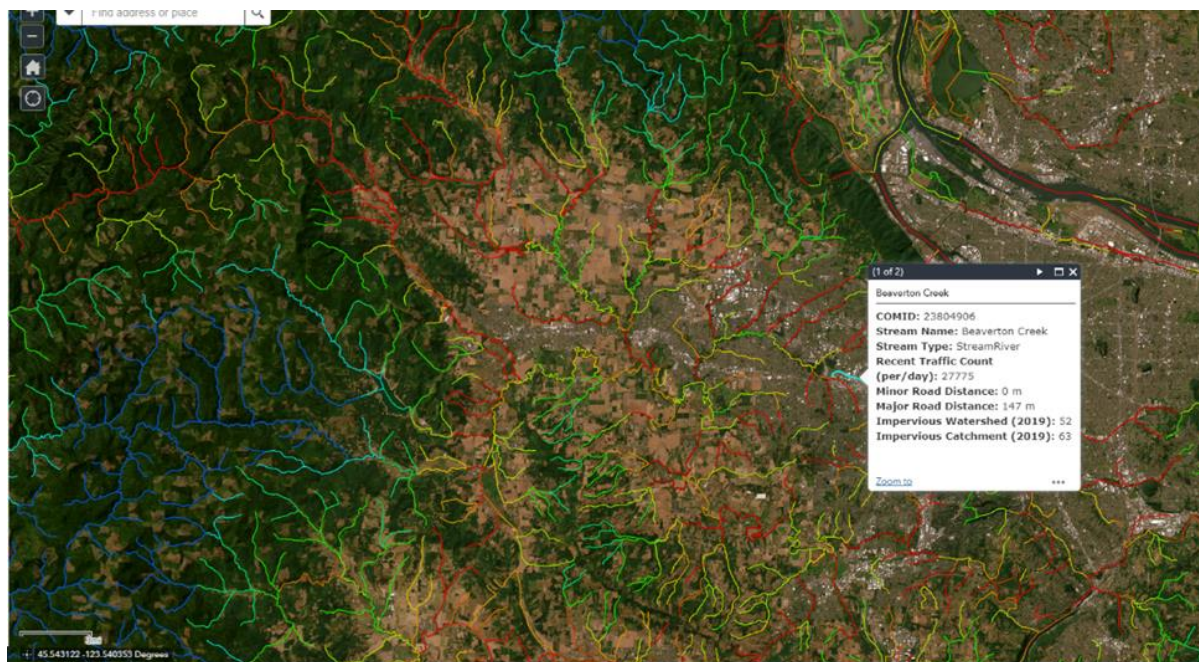


Figure 5-1. Streams color-coded indicating proximity of stream to roads, a spatial data analysis that highlights the sources from which 6PPD-q enters streams.

Source: Screenshot from USEPA Freshwater Explorer, 20231005, Portland, OR.

5.3.3 Fish Distribution and Ecological Risk Mapping

National data sets of the native range of fish species are available as well as occurrence data of fish species within and outside their native ranges. Distribution maps are shown for coho salmon, rainbow trout, and brook trout (Figures 5-4 and 5-5). The native ranges are shown in yellow. Shown in green are areas where one or more individuals were observed outside their native range. The boundaries of the geographic area drained by a river system or hydrologic unit (HU) used to make the maps are at the USGS 8-digit HU level. For example, Coho are native to the Pacific Northwest, but have been introduced to the Great Lakes basin and the Northeast (Figure 5-3) (USGS 2023). Although an order of magnitude less sensitive to coho salmon, rainbow trout and brook trout have more extensive distributions. A composite of native (blue) and introduced (tan) ranges of Coho salmon, rainbow trout, and brook trout shows that sensitive fish are not limited to the Pacific Coast (Figure 5-6). Data was provided by USGS from their [Nonindigenous Aquatic Species](#) (NAS) database December 2023.



Figure 5-4. Native and non-native geographical distribution of Rainbow Trout.
Source: USGS Nonindigenous Aquatic Species (NAS) database.



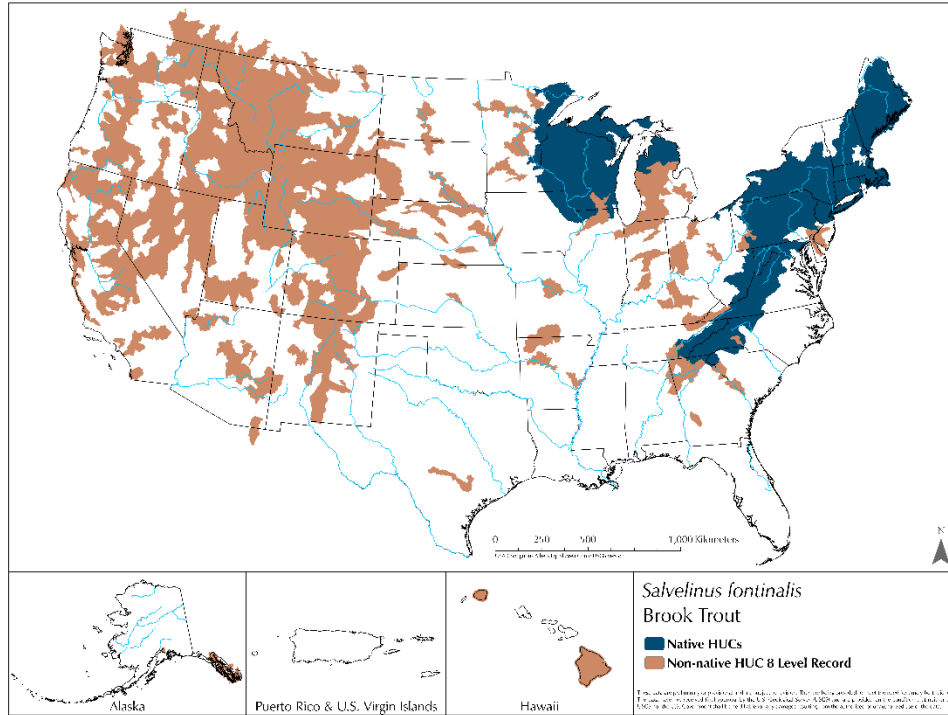


Figure 5-5. Native and non-native geographical distribution of Brook Trout.
Source: USGS Nonindigenous Aquatic Species (NAS) database.

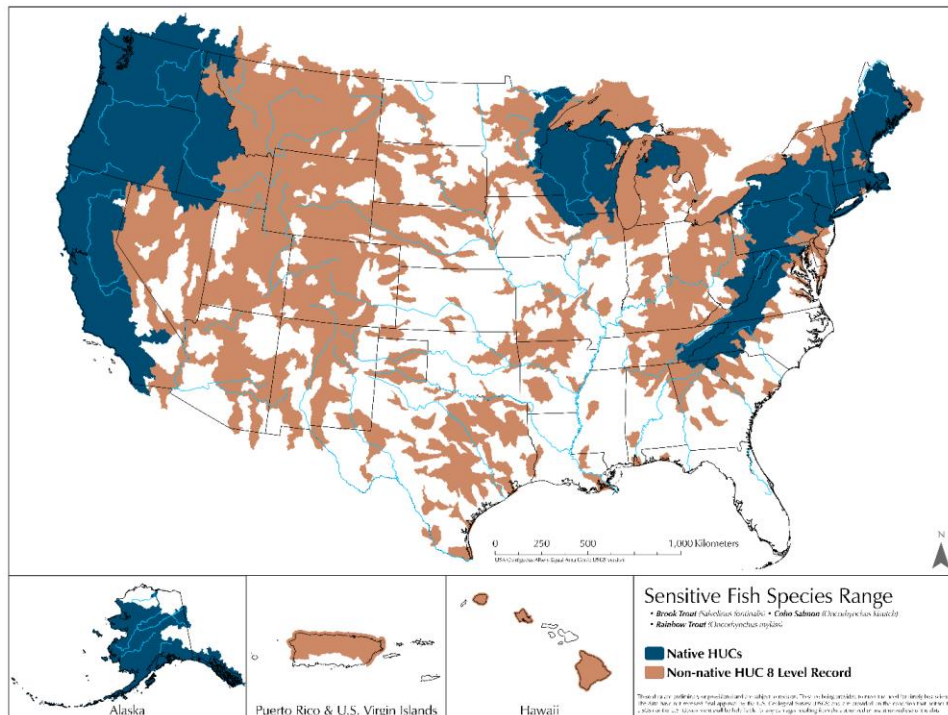


Figure 5-6. Native and non-native geographical distribution of known sensitive fish species.
Source: USGS Nonindigenous Aquatic Species (NAS) database.

5.3.4 Scales of Occurrence, Exposure, and Risk

Desktop geographic information systems (GIS) can be applied at different scales to help identify toxic hotspots. These efforts are most effective when there are data infrastructure and resources to maintain the tools as new data layers are developed and new information becomes available.

As indicated in [Section 5.3.1](#), there are currently no national databases or mapping tool dedicated for assessing the impact or prevalence of 6PPD or 6PPD-q in our nation's waterways. While local data are available for select areas, the issue of data scarcity is further exacerbated by the variability of coverages among datasets with relevant environmental inputs. The lack of pertinent and analytically compatible information may limit the areas in which traditional modeling techniques can be applied. Alternatively, adjusting modeling approaches to available data may be more pragmatic and more broadly applicable. This is of particular interest because environmental proxies such as: nationwide datasets for streamflow, traffic, and fish populations are more readily available than measured 6PPD or 6PPD-q. These parameters to estimate 6PPD loadings may be used in data driven modeling techniques to estimate the potential exposure and effects of 6PPD in areas with little monitoring and 6PPD-q data.

For example, the model used to generate maps in Feist et al. (2017) paper "Roads to ruin: conservation threats to a sentinel species across an urban gradient" were used to develop an interactive visualization of the Predicted Mean Annual Coho Urban Runoff Mortality Syndrome Rates Across the Puget Sound Predicted (USFW, NOAA, and Washington State University 2019) (Figure 5-7). Users can visualize differences across the area and select drainage basins to see information such as the predicted mean percentage annual average coho spawner mortality and a brief characterization of the area. In both the published map and the interactive map (Figure 5-7), red areas are predicted to have high mortality and yellow areas less mortality. The manuscript details the science whereas information on the interactive map is more granular and adaptable to different assessment needs.

A variety of geographic data tools may be needed to address specific goals and scales of assessment. For example, a federal agency may be interested in a country scale map and a state agency in a state scale map. A tribe may want watershed-scale maps to design salmon recovery programs. Initial goals may be related to mapping biological or environmental parameters or reconnaissance efforts. As data layers become more complete, spatially explicit data may become sufficient to build models to predict contaminant concentrations and estimate coho mortality in areas where sampling has not been done.

An additional example for a statewide scale tool is the tire contaminant in coho salmon watershed story map that hosts an interactive web map to help guide sampling efforts across urban and traffic gradients and considering salmon and trout distributions (Figure 5-8).

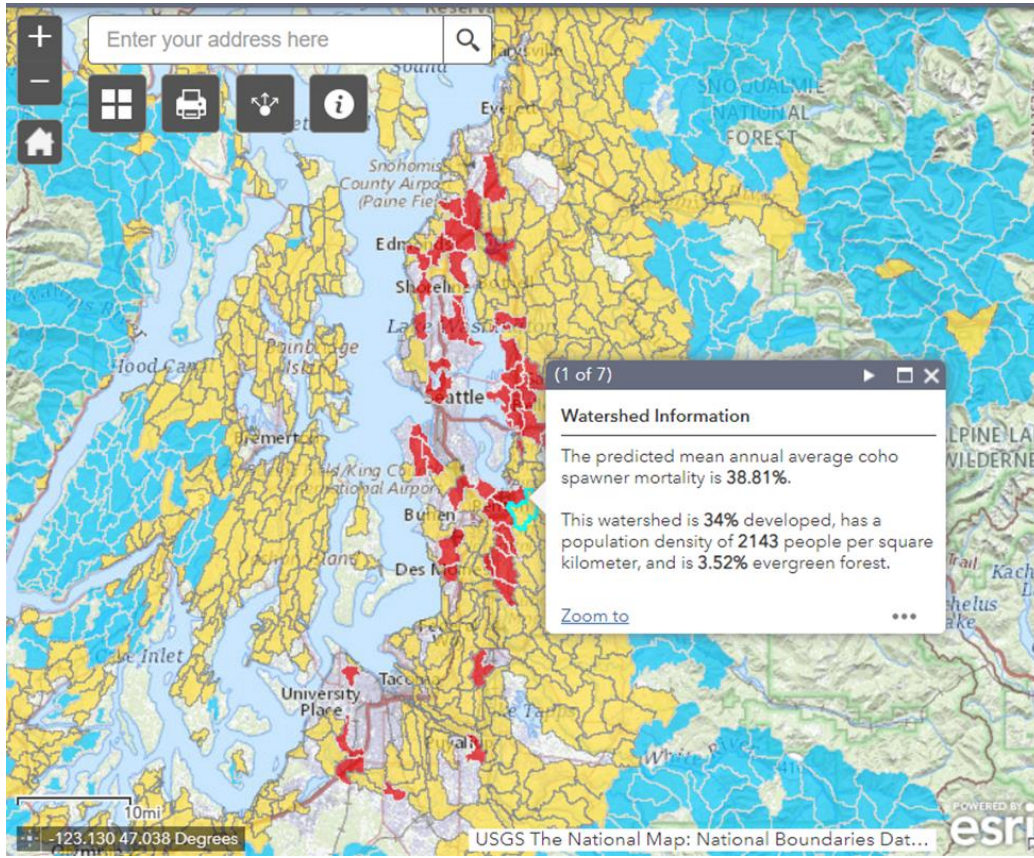


Figure 5-7. Mean Annual Spawner Mortality Urban runoff mortality syndrome
Source: <https://fws.maps.arcgis.com/apps/MapSeries/index.html?appid=5dd4a36a2a5148a28376a0b81726a9a4>

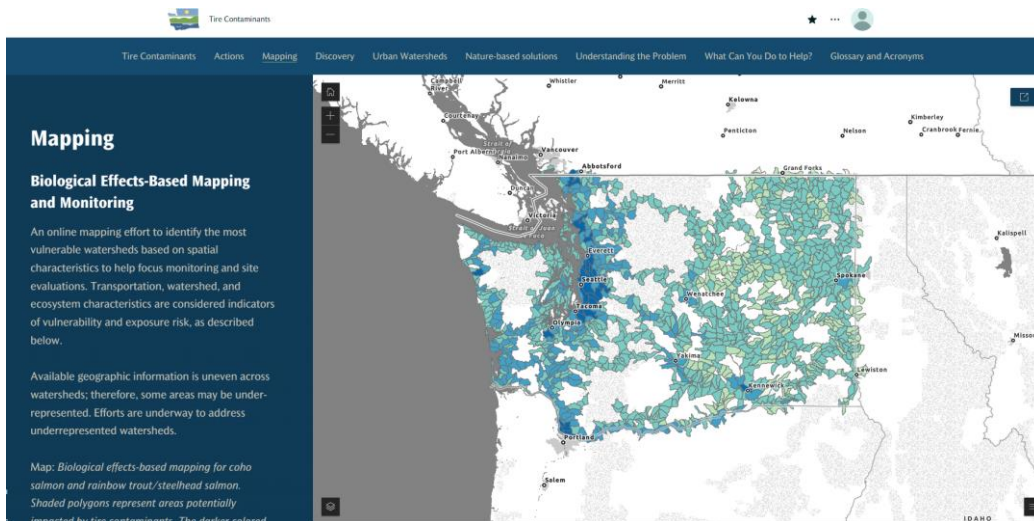


Figure 5-8. Washington Department of Ecology Storymap to help visualize the potential occurrence of 6PPD-q along roadways near salmon bearing waterbodies and focus sampling efforts.
Source: Washington Department of Ecology website

5.3.5 Modeling to Support Regulation and Planning

5.3.5.1 6PPD/Q Fate and Transport modeling

5.3.5.1.1 TMDL Modeling for Sinks, Sources, Occurrence, and Exposure

When a potentially harmful exposure or an adverse biological effect is observed, the Clean Water Act requires steps to reduce exposures and restore the aquatic life use (Clean Water Act 303d). Under Section 303(d) of the CWA and in accordance with supporting regulations in 40 CFR Part 130.7, states are required to develop biennial lists of waters impaired or threatened by a pollutant. Under this regulation, “impaired” has been defined as failing to meet one or more applicable water quality standards, and states are further required to prepare a list of impaired waters that need a total maximum daily load (TMDL). TMDLs are calculated as the maximum pollutant quantity that a waterbody can receive and still meet water quality standards (also referred to as a water quality target). As part of developing TMDLs, allocation loads among the various point and/or nonpoint sources of the pollutant are also calculated. (For more information on CWA Section 303(d) Impaired Waters and TMDLs, visit: <https://www.epa.gov/tmdl>). The pollutants in this example are tire anti-degradants and the adverse effect that requires remediation is fish survival and reproduction.

There are many strategies for identifying a water quality target for a TMDL, but 6PPD-q is particularly challenging because of its acute toxicity and ubiquitous occurrence on and along transportation infrastructure (Washington State Department of Ecology, 2023). Furthermore, EPA has not yet recommended aquatic life criteria for 6PPD-q. As a result, where 6PPD-q is the identified cause of a biological impairment, States and authorized Tribes can develop and then obtain EPA approval for mitigation targets by translating their narrative aquatic life criteria. If EPA develops aquatic life criteria for 6PPD-quinone at some future date, then states and tribes may adopt the recommended protective levels as their regulatory standards, or they may propose alternative values based on their own scientifically defensible methods.

Typically, a TMDL follows a sequence of assessments, as shown in the Figure 5-9 below. (U.S. EPA, 2002). As described in Cormier and Suter (2008) and adapted from USEPA (2002), the “...corresponding components of the assessment and management framework are indicated in grey oblongs: condition assessment (listing process), causal assessment (problem/pollutant identification), risk assessment of effects from exposure (target analysis), source assessment, risk assessment of sources (linkage of sources and target), management assessment (allocation to sources), and outcome assessment (update next listing cycle)”. Identification of adverse effects to Coho could be the driver that initiates a TMDL. However, 6PPD-quinone may not be the cause of the adverse effects based on a causal assessment. However, when a causal assessment identifies 6PPD-quinone as a probable contributing cause, then allowable concentrations that protect the resource, for example, Coho populations, are estimated in a risk assessment. After mitigation, an outcome assessment evaluates the performance of the intervention and effectiveness in the ecological goal of protecting Coho populations. Maps are useful for each type of assessment to scope the extent and patterns of adverse effects, to develop evidence for and against 6PPD-q as a cause, to model and allocate loadings, to design mitigation strategies and placement, and to map progress in achieving environmental goals.

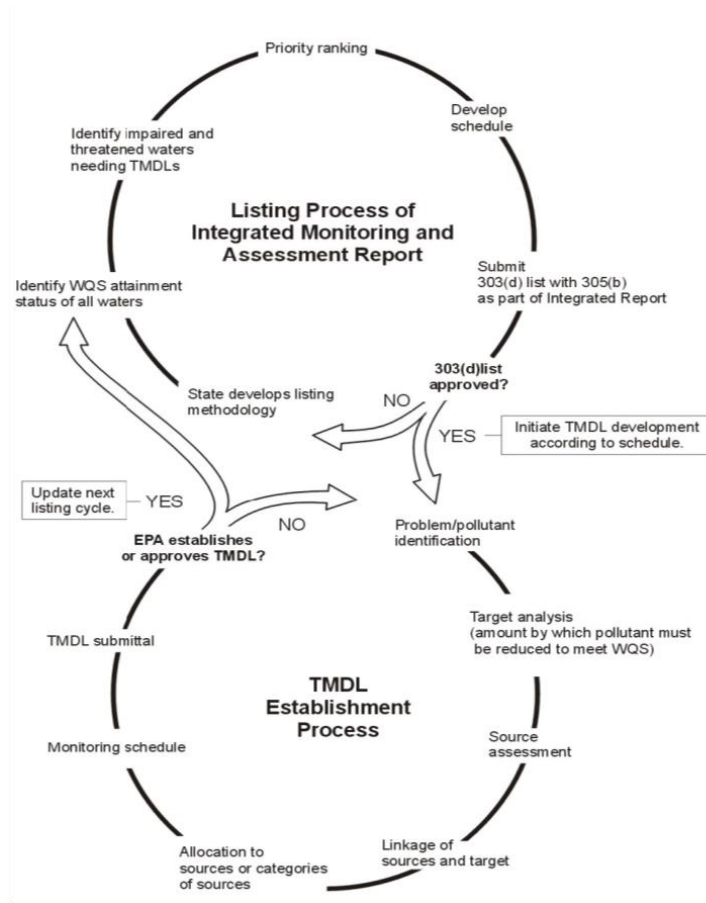


Figure 5-9. A diagram of the assessments and decisions involved in the listing of waters as impaired and the determination of total maximum daily loads (USEPA 2002).

Source: USEPA, 2002.

TMDLs can also be associated with stormwater permits administered under the National Pollutant Discharge Elimination System (NPDES). According to USEPA (2015), “[s]imilar to traditional stormwater management authorities (cities and counties) and transportation authorities are also responsible for managing the stormwater runoff that discharges to our nation’s waters via regulated municipal separate storm sewer systems (MS4s) along streets, roads, and highways...”

For example, if the adverse effect requiring remedy might impair Coho survival and reproduction, the probable cause might be 6PPD-q from a combination of non-point source road run-off and from several NPDES storm water point sources. The TMDL would describe the total amount of 6PPD-q allowed in any part of the stream where Coho could be exposed and management options for mitigating point and non-point sources of road run-off.

Maps of stormwater distribution systems and outfalls can play an important role in TMDL management. For example, VELMA was demonstrated as capable of identifying 6PPD-q hotspots at fine scales (5m), with results potentially useful when prioritizing the green infrastructure treatment placement, type, and amounts (at the watershed-scale) that could be used to reduce coho mortality (Halama et al., 2022). (For additional information on VELMA and its

application to decision-making for environmental issues, visit: <https://www.epa.gov/water-research/visualizing-ecosystem-land-management-assessments-velma-model>).

There are many strategies for establishing TMDL thresholds, but 6PPD-q is particularly challenging because of its acute toxicity and ubiquitous occurrence on and along transportation infrastructure (Washington State Department of Ecology, 2023).

The major transport and exposure of 6PPD-q to aquatic life is thought to be stormwater runoff. There are many other contaminants of concern associated with transportation infrastructure and motor vehicles beyond 6PPD-q. Thus, the efforts to model stormwater and 6PPD-q have become entwined. Natural resource scientists and modelers recognized that existing runoff models developed for forestry practices and reducing sedimentation in rivers could be re-purposed for stormwater modeling.

5.3.5.1.2 Groundwater Plume Modeling

Due to the limited amount of groundwater studies involving 6PPD and 6PPD-q, groundwater plume modeling has not been extensively examined.

5.3.5.2 Air transport modeling

5.3.5.2.1 Vapor

Significant knowledge gaps exist concerning contaminants associated with TRWP that may be present in the gas phase (Johannessen, Saini, et al., 2022). However, 6PPD and 6PPD-q have low vapor pressures (reported as 6.85×10^{-3} Pa and 6.57×10^{-6} Pa, respectively, at 25°C), which means these chemicals are unlikely to volatilize at typical conditions in the natural environment (Washington State Department of Ecology, 2022). Due to the tendency for 6PPD to sorb to soil, sediments, and suspended particulates, 6PPD can be present on suspended particles in the air (OSPAR Commission, 2006). Based on an estimated Henry's Law constant of 7.43×10^{-4} at 25 °C, 6PPD has moderate potential to volatilize from surface waters (OSPAR Commission, 2006). Research summarized by OSPAR (2006) did not indicate gaseous emissions of 6PPD from tires, but it is unclear if the absence of these emissions is due to lack of volatility from tires or rapid degradation of 6PPD after release.

5.3.5.2.2 Deposition (PM_{2.5})

Fine TWP suspended into the air can travel varying distances before depositing onto other surfaces. The fate of airborne TWP strongly depends on particle size. Only a small fraction of TWP generated become airborne. Larger particles, greater than 10 micrometers, constitute a major part of mass being released to the environment and are likely to settle on or near the roadway and constitute only a small fraction of airborne particles (Kole et al., 2017). As cited in Kole et al. (2017), Grigoratos and Marini (2015) concluded that the airborne fraction of TWP typically varies between 0.1% and 10% by mass while acknowledging that some studies reported fractions as high as 30%. TWP ranging in size from 1 to 10 µm are likely to remain airborne for minutes to hours and can be transported from hundreds of meters to 50 km depending on particle characteristics and local conditions (Kole et al., 2017). These researchers further state that "...[s]pecific transport data on the 0.1–1 µm fraction are lacking, but it is well known that PM_{2.5}

particles (i.e., particles <2.5 µm) can stay in the air for days or weeks and travel more than a thousand kilometers.”

5.3.6 Modeling Tools and Strategies to Support Planning – VELMA

Contaminant hotspot maps help identify locations for source control and prioritize GI treatments (i.e., rain gardens, bioswales, pervious pavements) that can most effectively reduce contaminant loadings to threatened aquatic habitats. Models that help identify such hotspots can be a useful tool in planning to help this load reduction.

The U.S. EPA’s Visualizing Ecosystem Land Management Assessments ([VELMA](#)) high-resolution spatial and temporal analysis of 6PPD-q hotspots provides a tool for prioritizing the locations, amounts, and types of green infrastructure that can most effectively reduce 6PPD-q stream concentrations to levels protective of coho salmon and other aquatic species (Hallama et al.; In review).

Table 5-1. 6PPD-q Sampling Methods Pros and Cons Assessment

Method name	Collection type	Sample type	Media	Description	Pro	Con	Example Reference	Comments
Grab sample	Manual	Discreet	Surface water	Collecting samples at a discreet time point from shore, boat or bridge. Certified amber glass bottles are recommended. PTFE tubing and containers can be used for short term storage (Hu et al. 2023). Some studies pre-rinse uncertified, non-glass bottles with methanol prior to collection (Rauert et al. 2022).	Accessible and low technology and cost option that offers a lot of flexibility. An amber glass bottle can be used to minimize loss of 6PPD-q.	Transport of 6PPD-q from surfaces occurs during storm events, some waterbodies are flashier than others making it easy to miss the 6PPD-q peak concentration. Stormwater sampling is logistically challenging and takes a lot of boots in the field chasing storms at all hours and days of the week. The mass loading of 6PPD and 6PPD-q is on and along roadways and stormwater infrastructure that are inherently dangerous areas to sample without proper safety precautions. Sampling near and within transportation and stormwater infrastructure often requires permission and coordination. Sampling in and around streams can also present dangerous conditions and ecological impacts (the spread of invasive species).	Tian et al. 2021/23; Rauert et al. 2022; Challis et al. 2021; Unpublished data (check with PI's); Lane 2023 (Rachael's presentation); Nedrich (Sara's presentation and white paper report); Holzer 2023. (Katie's presentation); Smith 2023	Please add your name or a reference if you agree with each assessment. Samples can be collected with a variety of water sampling devices depending on target environment including a sampling pole or bottle harness. Deeper water can be sampled using self-flushing sampling bottles on a cable triggered by a messenger.
Portable sampler	Active	Sequential	Water	Collecting samples using an autosampler from shore, many autosamplers can be programmed to take multiple samples at set intervals and duration.	The sampler can be programmed to sample throughout a storm event to capture the pollutagraph when coupled with flow measurements. Sequential sampling provides information on when 6PPD-q is transported to waterbodies and how long it persists (resident time) by collecting samplers, for instance, every hour into separate sample containers. Portable and programmable samplers are a good tool for measuring contaminants associated with storm events for identifying hotspots and determining the effectiveness of stormwater management and other toxic reduction actions.	Procurement, operation and maintenance of portable samplers can be time costly. Theft and vandalism of deployed equipment in urban areas is of concern. The equipment necessary to set an automated sampling criteria adds another layer of complexity to the deployments. There is a chance that the portable sampler will malfunction and the samples won't be collected. Sequential, discreet samples over each storm can add up to 24 samples per site, increasing the projects analytical costs.	Johannessen et al. 2021; Cao et al. 2023; Tian et al. 2022 (SFEI used Iscos, double check with Ezra)	
Portable sampler	Active	Composite	Water	Collecting samples using an autosampler that combines interval samples into one large container or jar.	The sampler can be programmed to sample throughout a storm event and combine the samples taken, for instance, every hour into one composited container providing an average concentration over time. Sample composites helps keep analytical costs down, especially during the screening stage of a new contaminant.	The temporal resolution of 6PPD-q concentration trends throughout a storm event is lost. The portable samplers need to be procured, operated and maintained by trained technical staff. The equipment needs to be secured in the field to minimize theft and vandalism.		
Bailer	Manual	Discreet	Groundwater	A portable grab sampler for measuring groundwater	Relatively simple and inexpensive method for measuring groundwater once a sampling well is established.	Need to establish a sampling well, groundwater sampling often requires technical staff and equipment.	Zhang et al. 2023	The modeled physicochemical properties suggest minimal movement through soils because of 6PPD and 6PPD-q affinity for sorbing to soils, but research is needed to verify the modeled information.
Grab sample	Manual	Discreet	Sediment and Soils	Collecting samples at a discreet time point from shore, boat or bridge using a scoop	Accessible and low technology and cost option that offers a lot of flexibility. Less opportunities to loose 6PPD-q during the sampling process.	Considerable transport of 6PPD-q from surfaces occurs during storm events, during storms the surface sediments are dynamic and may or may not represent the most recently deposited particles.	Cao et al. 2022 (Soils)	
Grab sample	Grab device	Discreet	Sediment	Collection of sediments using specialized devices including box corers, grabs, etc.	Allows the collection of sediments from deeper, more stable benthic environments. Grab devices or box corers come in a variety of sizes and can help standardize the sediment collection process. The sampler is able to homogenize large grabs and take replicate samples. 6PPD and 6PPD-q has been shown to readily sorb onto particles, collecting and measuring 6PPD-q will help us further understand the contaminants fate and transport.	Sediment grab devices work best off boats and larger devices require specialized winches or davets, and trained technical staff.		

Table 5-1. 6PPD-q Sampling Methods Pros and Cons Assessment

	Method name	Collection type	Sample type	Media	Description	Pro	Con	Example Reference	Comments
7	Core sample	Coring device	Composite	Sediment	Collection of a sediment core that can be sectioned and possibly dated using geochronology	Stable benthic environments can provide a deposition history from pre-industry to post-industry conditions. Sediment cores might be a good tool. Most suitable in lakes or backwater areas.	Coring devices often require technical water crafts and personnel. The collection and processing of cores is time intensive. Coring works best in stable depositional environments such as lakes and not in as well in hydrologically dynamic environments such as rivers and streams or tidally active environments.	Nipen et al. 2022 (not specific for 6PPD-q, general reference for sediment coring and emerging organic contaminants)	
8	Mobile Centrifuge	Active	Composite	Sediment	Collection and consolidation of particulates from water	6PPD-q has been shown to readily bind to soils and sediments, therefore high volume collections of ambient water and consolidation could be an effective method for measuring 6PPD-q where it exists in trace amounts.	Mobile centrifuging requires specialized equipment and trained technical field and lab staff. You can only measure one site at a time per mobile centrifuge making site comparisons under similar conditions challenging and time consuming.	No reference available	High volume centrifuging may be a useful tool for screening sites for 6PPD-q between and during storms. More research is needed.
9	Sediment trap	Sediment sampler	Composite	Sediment	A sediment trap collects modern sediments that are suspended or resuspended in the water column and deposited to the benthic environment	Sediment traps can be deployed for days to weeks to capture the particulate sedimentation that is washed into waterbodies during storms and eventually settles. 6PPD-q has been shown to readily sorb to particulates, deploying, capturing and measuring these settled particles may provide a tool for understanding the mass loading.	High carbon environments make sediment traps challenging to deploy for longer durations because of bacterial activity and the break down of carbon that may release the sorbed 6PPD-q.	No reference available	Sediment traps show promise for being an useful tool for estimating partial bound 6PPD-q. More research is needed to verify this method.
10	Solid Phase Micro-Extraction (SPME), Polyethylene (PE) and Polyoxymethylene (POM)	Passive sampler	Composite	Water or sediments	SPME samplers are made of fiber-optic cable with a glass core surrounding by a polydimethylsiloxane (PDMS) that is absorptive to many hydrophobic chemicals. PE and POM samplers are made of organic polymer, or more simply a piece of plastic.	The analytical costs of passive samplers are often lower than active and discreet sample analysis. The samplers can be deployed for days to weeks and provides a greater chance of measuring contaminants over several storm events. The sampler targets the dissolved form of a contaminant that is the most bioavailable. 6PPD-q is hyrophobic and may adhere to this media. If a model was developed, this method can be used to compare field concentrations with risk-based values or criteria. Passive samplers can be deployed in freshwater or saltwater environments, however, 6PPD-q in marine matrices is still under development.	Passive samplers provide an average concentration over time. They are most helpful when the rates of absorption have been estimated and a model verified to provide an estimate of concentration. A model for 6PPD-q has not been established to date. These types of passive samplers uptake hydrophobic contaminants until an equilibrium is reached. The small amount of data available for 6PPD-q through a storm event supports spikes in concentrations correlated with storm events, so these types of passive samplers may not represent the maximum concentration that coho salmon and other sensitive species are exposed to in the environment. Preliminary research suggests that if coho are exposed to high levels of 6PPD-q for hours, they do not recover. Understanding how long the resident fish are exposed to the highest concentrations will help prioritize areas of concern. Lastly, these materials can be difficult to work with in the field and lab.	McIntyre et al. (toxicity paper that describes the exposed salmon being placed back in clean water to test recovery). For equilibrium process see Schwarzenbach et al. 2003. For passive sampler methods Hawthorne et al. 2005, 2009; Burgess et al. 2001; Mayer et al. 2000; Vinturella et al. 2004.	
11	Semi-permeable membrane device (SPMD)	Passive sampler	Composite	Water	A polyethylene passive sampler that contains a lipid, used to measure trace levels of organic compounds.	Provides a time weighted average for nonpolar or hydrophobic organic contaminants with Kow > 3. Avoids logistically challenging storm chasing, works best in high flow environments.	The time weighted average may miss the 6PPD-q concentration peaks that are helpful to understand the exposure risk and impact to sensitive species such as coho salmon. There is no absorption model for 6PPD or 6PPD-quinone. Passive samplers deployments often require boats and technical field staff.	General method: Huckins and others 1993, 2006; Schubauer-Berigan et al. 2012. No references for 6PPD-q specific studies to date, research is needed to compare passive sampler effectiveness.	SPMDs to measure and compare 6PPD-q before and after toxic reduction actions or to compare sites within the same region (with similar environmental conditions) are worth investigating.
12	Polar Organic Chemical Integrative Sampler (POCIS)	Passive sampler	Composite	Water	microporous (0.1µm pore size) polyethersulfone membrane encasing a solid phase sorbent (Oasis HLB) which retains sampled chemicals. The Oasis HLB is a universal solid-phase extraction sorbent widely used for sampling a large range of hydrophilic to lipophilic organic chemicals from water.	Passive samplers helps avoid storm event sampling that can be logistically challenging. The analytical costs are often lower than more traditional discreet sampling. It can provide a time integrated comparison between sites and treatment types.	Sampling rates have not been determined for 6PPD-q. The deployment, retrieval and data interpretation require technical field and lab staff.	Johannessen & Metcalfe 2022	WA department of ecology is currently evaluating the effectiveness of POCIS to detect 6PPD-q across sites and toxic reduction actions.

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	Method name	Collection type	Sample type	Media	Description	Pro	Con	Example Reference	Comments
13	DGT Device	Passive sampler	Composite	Water	A passive sampler that is widely used to measure contaminants in freshwater, marinewater and assessing soil and sediment. It is a low cost plastic device that mimics biological uptake.	These samplers can be deployed for days to weeks at a time avoiding storm event sampling. They provide a time-weighted average concentration for select contaminants. DGT is considered a dynamic rather than an equilibrium method because it continously removes analyte from the sampling media.	This device has not been tested for measuring 6PPD-q to date. A sampling rate for 6PPD-q has not been estimated.	No reference available	
14	Remote Samplers	In situ active sampler	Continuous	Water	These devices actively pump water through an SPE media, in a stainless or polyethylene casing, in situ for several hours per filter, another type fills a carboy container.	These samplers can be deployed remotely and left for several hours. The filter version has no water sample to deal with and the filters can be frozen until ready for extraction and analysis. These devices can be secured on a remote mooring.	These samples tend to get clogged after several hours and may not last for an entire storm event. The device could malfunction and miss the sampling window.	No reference available	WA department of ecology is currently evaluating the effectiveness of remote in situ active samplers to detect 6PPD-q across sites and toxic reduction actions.
15	Benthic Index of Biotic Integrity (B-IBI)	Macroinvertebrate	Bioassessment	Biota	Benthic invertebrates are collected and identified. If the taxonomy reveals only tolerant species composition and the absence of sensitive species then there is some sort of water quality impact.	B-IBI is the standard for measuring stream health and might be useful to correlate with 6PPD-q as an indicator of stormwater impacts.	B-IBI is difficult to pin point one disturbance among many.	Larson et al. 2019	WA Department of Ecology and a network of partners regularly use B-IBI to estimate the overall health of a water body.
16	Biofilms	Periphyton and microbial communities	Bioassessment	Biota					
17	<i>In vitro</i> using primary cells		Bioassay	Biota	Cells isolated from an organism that are subsequently cultured and aliquots frozen (-80C or liquid nitrogen vapor phase) for longer term sample testing.	•Each cell line representative of one individual •Cell lines contain normal genetic structure •Genetic diversity and biological replication can be increased by obtaining cells from more than one individual •Contains multiple cells types more representative of in vivo compared to immortalized cells	•Requires sacrificing or invasively sampling more than one animal to obtain cells over time •Requires access to living animals •Ambient samples can harm cell viability and much be treated prior to use in an assay (ISO protocol) •More time intensive, less consistent than immortalized cells	Fibroblasts from killer whale (Yajing et al. 2018), and liver cells from mice (Arora et al. 2009).	https://www.iso.org/obp/ui/#iso:std:iso:5667:-16:ed-2:v1:en
18	<i>In vitro</i> using an immortalized cell line		Bioassay	Biota	Primary cells that do not undergo senescence and can be propagated indefinitely . Immortalized cell lines are available for many species and tissue types.	•Simplified model compared to in vivo •Greatly reduces use of animals in testing •Simple for many biological laboratories to integrate •Easy access through repositories such as ATCC •Well-established assays available to assess cell health	•Aberrant genetic structure •No biological replication •Ambient samples can harm cell viability and much be treated prior to use in an assay (ISO protocol) •May not retain all physiological functions of the original cell •Not available for all species/tissues	RTgill-W1 (OECD 2021), Embryonic cells isolated from coho salmon (Greer et al. 2023)	
19	<i>In vivo</i> , In situ		Bioassay	Biota	Direct testing of live animals in the area under investigation	•Direct field assessment of the environmental conditions experienced by organism	•Many uncontrolled variables (pH, temperature, etc) compared to laboratory studies •Logistically challenging	Caged fathead minnow (Ankley et al. 2021)	
20	<i>In vivo</i> , laboratory		Bioassay	Biota	Testing of animals in the laboratory using field collected water samples	•Represents the whole conduit of chemicals present in stormwater •Interactions between organ systems are maintained	•Requires access to living animal	Methods like whole effluent toxicity (WET) testing in rainbow trout and other species (40 CFR 136.3)	https://www.epa.gov/cwa-methods/whole-effluent-toxicity-methods
21	<i>Ex vivo</i> , laboratory		Bioassay	Biota	Extraction and isolation of tissues and cells from field exposed animals for subsequent laboratory exposures	Allows for the evaluation of effects in wild species exposed to a stimulant introduced in a controlled manner	Intricate procedures required highly trained personnel	Short-term culture and stimulation of immune cells (Rehberger et al. 2021)	
22	Fish samples	Collection and processing of fish tissues and plasmas	Composite	Biota		Fish can act as natural passive samplers that represent 6PPD and 6PPD-q concentrations. 6PPD and 6PPD-q have been found in both published and unpublished studies. 6PPD and 6PPD-q in snakehead, weever, and Spanish mackerel fish. 6PPD and 6PPD-q has been found in English sole and mussels (unpublished, check with Denis if OK to share).	Fishing often requires specialized electro fishing equipment and technical field and lab staff to collect and process samples.	Ji et al. 2023	Fish tissues is a promising method for detecting 6PPD and 6PPD-q in aquatic environments, more research is needed.

Table 5-1. 6PPD-q Sampling Methods Pros and Cons Assessment									
	Method name	Collection type	Sample type	Media	Description	Pro	Con	Example Reference	Comments
23	Quartz fiber filters using a moderate-volume air sampler	Active	Composite	Air and particulates					
24	Polyurethane Foam sampler (PUF)	Active	Composite	Air and particulates	A high volume air sampler that measures airborne contaminants			Gaga 2019 (General PUF reference).	
25				Dust				Huang et al. 2021	
26	Ambient air sample	Active	Composite	Air	Large volume air sample collected on a quartz glass fiber filter (GFF) over a relatively long duration (~24-hour). Cascade impactor can be used in sample collection, which would allow for determining concentrations in differet size fractions (e.g., PM2.5).	Captures particle-bound fraction. If a cascade impactor is used, the concentration is different size fractions can be determined.	Due to relatively low concentrations in air, large sample volumes are required. Monitoring necessary to ensure sample integrity and security. Sampling equipment can be large and noisy because of the need for a high flowrate fan. Continuous power supply needed. Would not capture all of the fraction that may be present in the vapor phase.	Cao et al. 2022, Zhang et al. 2022a, Zhang et al. 2022b	Researchers have reported 6PPD and 6PPD-q have low vapor pressures and are not likely to volatilize at 25C, but signififant data gaps exist concerning their presence in the vapor phase.
27	Ambient air sample	Passive	Composite	Air	Passive polyurethane foam (PUF) disk samplers deployed over an extended period (e.g., months).	Samples represent the whole air mixture (i.e., particle-bound and vapor fractions). No fan required. Lower cost and complexity compared to active air sampling.	Samplers must be deployed for extended periods. Monitoring necessary to ensure sample integrity and security.	Gaga 2019 (General PUF reference), Johannessen et al. 2022	
28	Dust sample	Manual	Grab	Dust	Collection of dust from roadways, parking garages, tunnels, waste management facilities, and indoor environemnts using a variety of collection methods, such as vacuum cleaner, brush and shovel, pressure washing and wet vaccum.	Sample collection is relatively easy. Pressure washing and wet vacuum repoted to be more efficient in collecting smaller particles.	Dry vacuum sampling may be less efficient in collecting smaller particles.	Liang et al. 2022, Liu et al. 2019, Deng et al. 2022, Hiki and Yamamoto 2022, Klöckner et al. 2021, Zhang et al. 2022b	There are gaps in our understanding how the use of dry vs. wet sample collection methods could impact the transformation of 6PPD or 6PPD-q.
29	Grab sample	Manual	Discrete	Snow	Collection of snow along roadways and associated particulates.	Snow captures and consolidates the tire wear particles and 6PPD-q overtime. Snow is relatively straight forward to collect.	Snow samples can only be collected opportunistically, and works better in areas with regular snow events. Method would not distinguish between contribution from road dust vs. atmospheric deposition. See comments.	Challis et al. 2021, Maurer et al. 2023, Seiwert et al 2022	Studies to date have not evaluated the relative contribution in snow between potential atmospheric sources and road dust sources.

Table 5-2. 6PPD Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
1	Water, Fish bile & plasma, Blubber, Fish, Shellfish	Plasma: 0.0075-0.025 ng/mL Bile: 0.015-0.05 ng/mL Fish: 0.07 ng/g	Glass vials and bottles	HPC Standards	Surrogate: DPPD-d14 Internal: Progesterone-d9	Water: SPE or dilution in MeOH, analysis Bile & Plasma: Water dilution, SPE, analysis	Water, Bile, Plasma: LC-MS/MS	Phenomenex Kinetex C18 EVO (100 x 2.1mm, 1.7 µm particle size)	ESI+ (MRM)	268>211	268 > 167	NOAA, placeholder for Denis (for bile/plasma) and Li-Jung Kuo (fish tissues)
2	Storm and Surface Waters	2 ng/L	Amber glass bottles, no headspace. Samples held up to 14-days in the fridge or frozen if hold times would exceed 14 days	HPC Standards and Cambridge Isotopes Lab	Surrogate internal standard: D5-6PPD-Q	0.7-micron glass-fiber filter and then direct-injection	UPLC-MS/MS	Waters Acquity UPLC BEH C18 (1.7 µm, 2.1 × 50 mm) plus Vanguard Pre-column (1.7 µm, 2.1- × 5-mm) column with 0.1 % formic acid in water and ACN mobile phase	ESI+	269.1/184.1	269.1/93.0	USGS, placeholder for Rachael publication
3	influent and effluent of WWTP	MDL: 60 ng/L	large volume solid phase extraction (LVSPE)	Not available as of the publication date	Not available as of the publication date	filtered using a Sartopure GF + MidiCap, 0.65 µm deep filter and extracted using cartridges filled with 10 g of Chromabond HR-X, eluted with 5 mL of ethyl acetate, 5 mL of methanol, 5 mL of methanol containing 1% of formic acid, 5 mL of methanol containing 2% of 7 N ammonia in methanol, evaporated under a nitrogen to near dryness, brought to 1 mL with methanol	LC-HRMS	Kinetex C18 EVO column (50 × 2.1 mm, 2.6 µm particle size, 83 Phenomenex, pre-column 4 x 2.1 mm and in-line filter 0.2 µm) and a gradient elution with 0.1% 84 of formic acid and methanol containing 0.1% of formic acid at a flow rate	Ion Mode ESI+	M+H = 269.2012		Maurer, 2023. https://doi.org/10.1021/acs.est.2c05784
4	Snow from urban street	LOQ: 100 ng/L	250 mL glass bottles	Sigma-Aldrich	Not available as of the publication date		UPLC-TOF-MS	HSS T3 column; 100 × 2.1 mm, 1.7 µm and Atlantis T3 (3µm, 2,1x 100mm); (A) water with 0.1% formic acid (v/v) and (B) methanol with 0.1% formic acid (v/v)	ESI(+) mode MRM	269.1/184.0	269.1/106.9	Seiwart_2022 https://doi.org/10.1016/j.watres.2022.118122
5	Influent and effluent WWTP during snow melt, rain, and dry condtions	LOQ: 100 ng/L	250 mL glass bottles	Sigma-Aldrich	Not available as of the publication date		UPLC-TOF-MS	HSS T3 column; 100 × 2.1 mm, 1.7 µm and Atlantis T3 (3µm, 2,1x 100mm); (A) water with 0.1% formic acid (v/v) and (B) methanol with 0.1% formic acid (v/v)	ESI(+) mode MRM	269.1/184.0	269.1/106.9	Seiwart_2022 https://doi.org/10.1016/j.watres.2022.118122
6	Air from 18 major cities that comprise the Global Atmospheric Passive Sampling (GAPS) Network	Instrument LOQ: 1.95 ng/mL Method LOQ: 2.71 pg/m3	PUF disk samplers collecting both collect both gas- and particle-phase chemicals	Sigma-Aldrich	Not available as of the publication date	Accelerated solvent extractor (ASE) extraction with petroleum ether and acetone (83/17, v/v), rotary evaporation, reconstituted with iso-octane, silica column cleanup	UPLC-HRMS	Phenomenex (Torrance, CA, USA) Kinetex C18 column (2.6 µm in particle size, 50 × 4.6 mm in length and inner diameter HPLC grade methanol, with 0.1% of formic acid in both, were used as the mobile phase	Positive ionization mode with a heated electrospray ionization source (HESI-II) Parallel Reaction Monitoring (PRM)	269.2012/185.1068		Johannessen 2022 MegaCity https://doi.org/10.1016/j.envpol.2022.120206
7	fine particulate matter PM _{2.5}	MQL: 0.25 pg/m ³ MDL: 0.07 pg/m3	quartz fiber filter	J&K, AccuStandard, TCI, TRC (PPD-Q standards were lab synthesized)	Surrogate standard diphenylamine-d10 and internal standard 6PPD-Q-d5	Serial ultrasonication with dichloromethane and acetonitrile, concentrated with nitrogen in acetonitrile and PTFE filtered	HPLC-HRMS	Waters Acquity HSS T3 column (1.8 µm, 2.1×100 mm) with 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile mobile phase	data-dependent MS2 mode MRM	269.2/93.1	269.2/184.1 269.2/212.1	Wang 2022 https://doi.org/10.1021/acs.est.2c02463

Table 5-2. 6PPD Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
8	fine particulate matter PM _{2.5} from megacity	LOD: 1 pg/mL	Whatman medium-volume quartz fiber filters	J&K Scientific Ltd. and Sigma-Aldrich	Pyrene-d10 and benzophenone-d10	ultrasonication acetonitrile and dichloromethane/hexane, taken to near dryness with nitrogen, redissolved in methanol and filtered with PTFE membrane	UHPLC-MS/MS	A Waters ACQUITY UPLC C18 column (1.7µm, 2.1 mm×100 mm) with 0.4 mM CH3COONH4 (A)/ MeOH (B)	ESI(+) mode MRM	<u>269/184</u>	<u>269/107</u>	Zhang 2021 https://doi.org/10.1021/acs.est.1c04500
9	Airborne Particulate Matter Along a Highway in Mississippi, USA	LOD: 0.58 ng/L	Airborne PM was collected using Sigma-2 passive samplers	Not available as of the publication date	Not available as of the publication date	methanol and hexane extraction shaker table, filtered with polycarbonate gold-coated filters, rotary evaporation to near dryness and redissolved in 66% methanol	UHPLC-HRMS	online filter cartridge with a 2.1 mm ID × 0.2 µm porosity stainless steel filter, an Eclipse Plus C18 RRHD (5 mm × 2.1 mm ID; 1.8 µm) guard column followed by the analytical column with the same stationary; 1mM ammonium formate and 0.1% formic acid (A) and methanol 0.1% formic acid (B) phase (100 mm × 2.1 mm, 1.8 µm)	Heated electrospray ionization mode (HESI) Data dependent product scan	Not available as of the publication date	Not available as of the publication date	Olubusoye 2023 https://doi.org/10.1007/s00128-023-03820-7
10	Dust: Road dust, Interior car dust , Parking lot dust , Indoor dust from homes near E-waste dismantling area	LOQ: 0.11 ng/g	precleaned nylon bag (pore size of 25 µm)	TCI, Accustandard, Sigma-Aldrich, Toronto Research Chemical	ISTD: Coumaphos-d ₁₀ SSTD: Benzophenone-d10	Serial sonication with acetonitrile and 1:1 dichloromethane: hexane. Concentrated by nitrogen into methanol and filtered	HPLC-MS/MS	<u>HPLC</u> : C18 column (100mm × 2 mm, Luna 3 µm, Phenomenex) with 0.3 g/L ammonium acetate (A) and methanol (B) mobile phase	ESI(+) mode MRM	<u>269.0 / 184.1</u>	<u>269.0 / 212.4</u> <u>269.0 / 92.8</u>	Huang et al. 2021 https://doi.org/10.1021/acs.est.1c00148
11	Sediments across UrbanRivers, Estuaries, Coasts, and Deep-Sea Regions	Sediments across UrbanRivers, Estuaries, Coasts, and Deep-Sea Regions	sediment packed in aluminum foil and stored in polypropylene tubes; freeze dried and 1.0 mm mesh screened	Tokyo Chemical Industry, AccuStandard, J&K Scientific, Dr. Ehrenstorfer	6PPD-d5	Transferred to glass tube, ultrasonicated with acetonitrile, concentrated and filtered with PTFE membrane.	LC–MS/MS	C8 column (Waters Xbridge BEH, 2.5µm,2.1 mm×100mm) 0.1%formic acid in water and (B) methanol at a flowrate of 0.3 mL/min	ESI(+) mode MRM	269.2/184.1	269.2/212.1	Zeng 2023 https://doi.org/10.1021/acs.est.2c07652
12	Fish	LOD: 0.00025 mg/Kg LOQ: 0.00043 mg/Kg	homogenized by the electric blender, frozen until extraction in centrifuge tube	Aladdin Biochemical Technology Co., Ltd	Not available as of the publication date	Modified QuEChERS	HPLC-MS/MS	Athena C18-WP chromatographic column (2.1 mm x 50 mm, 3.0 µm) Mobile Phase: MeOH: Deionized water/90:10	ESI(+) mode MRM	269.3/184.1	269.3/211.0	Ji 2022. https://doi.org/10.1016/j.foodchem.2022.133640
13	larval zebrafish & Water	LOQ: 0.1 ng/mL	Glass beakers	Aladdin	Not available as of the publication date	QuEChERS	HPLC/MS-MS	Luna Omega C18, 100 mm × 2.1 mm, 1.7 µm; b) mobile phase: 5 mmol/L ammonium formate solution (A), methanol (B);	ESI(+) mode MRM	269.40 > 184.25	269.40 > 107.15	Fang 2023 https://doi.org/10.1016/j.scitotenv.2023.163595
14	zebrafish embryo	LOD: 0.130 ng/mL LOQ: 0.638 ng/mL	Glass trays	abcr GmbH	Not available as of the publication date	FastPrep homogenizer, sonication, and centrifuging	HPLC/MS-MS	Atlantis T3 C18-phase column(2.1 mm×50 mm, 3µm; Waters) with an Atlantis T3 Security Guard column (2.1×10 mm, Waters)	ESI(+) mode MRM	269.079/106.90	269.079/184.0	Grasse 2023 https://doi.org/10.1021/acs.est.3c02819
15	Embryonic zebrafish	See Zhang https://doi.org/10.1021/acs.estlett.2c00193 .	well plate exposures	Toronto Research Chemicals	13C6-6PPDQ	Homogonization, polyfiltration, sonication, concentrated to near dryness, and redissolved in methanol and filtrated through a 0.22 µm poly (ether sulfone) membrane	UPLC/MS-MS	See Zhang https://doi.org/10.1021/acs.estlett.2c00193 .	ESI(+) mode MRM	See Zhang https://doi.org/10.1021/acs.estlett.2c00193 .		Zhang 2023 https://doi.org/10.1016/j.jhazmat.2023.131601

Table 5-2. 6PPD Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
16	Rainbow trout tissue & exposure water samples	MDLs: 0.1 – 0.6 ng/g in tissue	Plastic and glass	Toronto Research Chemicals	d5-6PPD-Q	whole fish body was homogenized into Eppendorf tube, serial sonication with acetonitrile and centrifuged	UHPLC-HRMS	Hypersil GOLD C18 column (50×2.1 mm, 1.5 µm). 0.1% formic acid in ultrapure water (A) and 0.1% formic acid in methanol	ESI(+/-) full scan mode	Not available as of the publication date	Not available as of the publication date	Nair 2023 https://doi.org/10.26434/chemrxiv-2023-pmxvc
17	Lumpfish blood	LOD: 0.5 pg	Not available as of the publication date	Fluorochem	Internal: 6PPD-Quinone-D5 Recovery: 6PPD-Quinone-13C6	vortex-sonication and centrifuged	HRGC/HRMS	TG-5SILMS column (30m, 0.25 mm i.d., film thickness—0.25 µm)	Non-targeted screening Full-scan mode	Not available as of the publication date	Not available as of the publication date	Hagg_ 2023 DOI 10.3389/fenvs.2023.1219248
18	Human Urine from general adults, children, and pregnant women	MDL: 0.012 ng/mL	After urine samples collected, immediately transferred to the laboratory, stored at –40 °C until analysis. Glass used during laboratory extractions	Cambridge Isotope Laboratories, Toronto Research Chemical	¹³ C ₆ -6PPD-Q	salting-out assisted liquid–liquid extraction, concentrated with nitrogen and 0.22 µm filtered	LC-MS/MS	Ultra-Fast LC: Waters XBridge C8 column (2.1 mm×100 mm, 2.5µm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	ESI(+) mode MRM	269.2/184.1	268.2/107.1	Du 2022 https://doi.org/10.1021/acs.estlett.2c00821
19	Honey	LOD: 0.0003 mg/Kg LOQ: 0.0001 mg/Kg	stored at room temp until extraction in centrifuge tube	Aladdin Biochemical Technology Co., Ltd	Not available as of the publication date	Modified QuEChERS	HPLC-MS/MS	Athena C18-WP chromatographic column (2.1 mm x 50 mm, 3.0 µm) Mobile Phase: MeOH: Deionized water/90:10	ESI(+) mode MRM	269.3/184.1	269.3/211.0	Ji 2022. https://doi.org/10.1016/j.foodchem.2022.133640
20	Lettuce (Valerianella locustaL) plant and roots & TWPs in nutrient solution	Not available as of the publication date	Glass vials	Sigma-Aldrich	Not available as of the publication date	Plant:serial bead beater with stainless steel beads and acetonitrile extraction, centrifuged, filtered with nylon filter Roots: Freeze dried roots, suspended in nutrient solution, reciprocal shaker, centrifuge, nylon syringe filter Nutrient Solution: serial liquid–liquid extraction, nylon syringe filter	UPLC-MS/MS	C18 column (Acquity HSS T3, 1.8 µm, Waters), ultrapure water (Phase A) and acetonitrile (Phase B), both containing 0.1% formic acid	ESI(+) mode MRM	269/184	269/107 269/93	Castan_2022 https://doi.org/10.1021/acs.est.2c05660
21	soil, water, atmospheric particles; urban runoff water samples were collected in a dense traffic urban area	IQL: 0.035 ng/mL	Soil: stainless steel shovel, transported to lab within 2 hours, freeze dried, homogenized, sieved through a 60 mesh Atmospheric particle collect on quartz fiber filters and stored at -20C Water: 200mL collected in Teflon tubes, glass microfiber filter, acidified with 2% formic acid	J&K, TCI Chemicals, Sigma-Aldrich, and Toronto Research Chemicals	Internal: diphenylamine-d ₁₀ Surrogate: 6PPD-quinone-d5	Soil: serial ultrasonication with acetonitrile, concentrated to dryness with nitrogen, redissolved in methanol and 0.45 µm nylon filtered Atmospheric particles: serial ultrasonication with dichloromethane and acetonitrile, concentrated to near dryness with nitrogen, redissolved in acetonitrile and filtered Water: HLB SPE Cartridge (60 mg, 3 mL), eluted with methanol–dichloromethane (1:9, v/v), concentrated to dryness with nitrogen, redissolved in acetonitrile and 0.45 µm nylon filtered	UPLC-HRMS	Waters Acquity HSS T3 (1.8 µm, 2.1 × 100 mm) column with 0.1 % formic acid in water and 0.1 % formic acid in ACN mobile phase	ESI(+) mode full scan and data-dependent acquisition mode	269.2/212.1	269.2/93.1	Cao 2022 DOI: 10.1021/acs.est.1c07376

Table 5-2. 6PPD Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
22	recycled tire rubber employed in synthetic football fields	Suspect screening	Glass vial aluminum cap, stored in the dark at room temperature	TCI Europe	Not available as of the publication date	<i>In-vitro</i> simulation of digestion extraction, then solid phase extraction or the bioaccessible fraction: 50 mg of Oasis HLB eluted with ethyl acetate. Ultrasound-assisted extraction for PAHs: crumb rubber in ethyl acetate, ultrasonic bath at 50 kHz for 20 min, PTFE filtered	GC/MS	Phenomenex Zebron ZB-Semivolatiles capillary column (30 m × 0.25 mm × 0.25 µm film)	Selected Reaction Monitoring (SRM)	<u>Suspect screening</u>	Not available as of the publication date	Armada_2023 http://dx.doi.org/10.1016/j.scitotenv.2022.159485
23	Solubilization of organic compounds from tire particles using Fish <i>In Vitro</i> Digestive Model	<u>Digestate</u> LOD: 0.2 ug/L LOQ: 0.5 ug/L <u>Cryogenically milled tire tread</u> LOD: 0.3 ug/L LOQ: 0.9 ug/L	Chemie Brunschwig AG	Not available as of the publication date	6PPD-Q-d ₅ , benzothiazole-d ₄ , aniline-d ₅ , and diphenylurea-d ₁₀ ,	Fish <i>In Vitro</i> Digestive Model, then liquid/liquid extracted twice DCM, then concentrated for analysis	UHPLC-HRMS	ACQUITY UPLC HSS T3 (100 × 2.1 mm, 1.8 µm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	ESI(+) mode MRM	269.05/106.99	269.05/184.09	Masset 2022 https://doi.org/10.1021/acs.est.2c04291
24	Road runoff	Not available as of the publication date	Water extracted within 48 hours of collection; glass bottles used for spikes	Toronto Research Chemicals	6PPD-quinone-d ₅	filtered with 0.7-mm glass microfiber filters (GF/F, cytiva), then SPE with Oasis 6 cc, 500 mg HLB cartridges, eluted with methanol	HPLC-MS/MS	Agilent InfinityLab Poroshell 120 EC-C8 LC (30 mm, 2.1 mm, 2.7 µm) column with 1mM ammonium formate in water and methanol mobile phase	ESI(+) mode MRM	<u>269.3/184.2</u>	Not available as of the publication date	Rodgers 2023 https://doi.org/10.1021/acs.estlett.3c00203
25	Urban river with stormwater-influenced flows; upstream, downstream, and near WWTP discharge	0.0098 ug/L	PET bottle, stored frozen (−18 °C) <i>protocol established prior to discover of 6PPD-Q</i>	Sigma-Aldrich	Not available as of the publication date	SPE with Waters HLB cartridges, 6 cc, 500 mg, eluted with methanol and concentrated	UPLC-HRMS	Kinetex 2.6 µm C18 column (50×4.6 mm). Solvent A, MilliQ water (pH =7) with 0.1% of formic acid, and Solvent B, methanol with 0.1% of formic acid	Orbitrap HRMS, positive ionization mode with a heated electrospray ionization source (HESI/II). Parallel reaction monitoring (PRM) for data acquisition	targeted select ion monitoring: 269.20123	Not available as of the publication date	Johannessen 2022 https://doi.org/10.1007/s00244-021-00878-4
26	surface water at 5 urban centres in Queensland, Australia; Surface waters and stormwater Australian Urban Tributary	Not available as of the publication date	600 mL polypropylene jars, frozen (−20 °C) until analysis	UCA, Tokyo Chemical Industry, Novachem, Cambridge Isotope Laboratories	Internals: d ₆ -5-methylbenzotriazole and d ₅ -atrazine Inject Internal: 13C3-Caffeine	Water: filtered through Whatman 47 mm, 1 µm, GFF/B glass fibre filter, SPE with Waters Oasis 6 cm3 HLB cartridges, eluted with methanol concentrated with nitrogen Particles: Filter papers with particles dried in an incubator at 60 °C for 3 h and stored at 4 °C for analysis; filters were cut into 1/8th segments, and one segment loaded into an 80 µL pyrolysis cup	Water: LC-MS/MS Particles: PYR-GC/MS (not analyzed for 6PPD-Q)	LC: Phenomenex Kinetex biphenyl 100 Å analytical column (2.6 µm, 50 mm × 2.1 mm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase Pyr-GCMS: Particulates captured on the 1 µm filter analyzed for TRWPs and polymers with pyrolysis gas chromatography mass spectrometry	ESI(+) mode MRM	LC-MS/MS: 269/184 PYR-GC/MS: Full scan mode over a mass range of 40 to 600 m/z	269/107 269/93	Rauert_2022 https://doi.org/10.1016/j.scitotenv.2022.158468 AND https://doi.org/10.1021/acs.est.1c07451
27	Exposure concentrations during acute toxicity studies	LOD: 0.5 ug/L LOQ: 1.8 ug/L	Glass tanks, beakers, and bottles	Cambridge Isotope Laboratories and Toyko Kasei	Not available as of the publication date	Direct-inject and direct-inject with dilutions	LC-MS/MS	Shim-pack VP ODS C18 (150 mm × 2.0 mm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	ESI(+) mode MRM	269/185		Hiki 2021 https://doi.org/10.1021/acs.estlett.1c00453

Table 5-2. 6PPD Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
28	Exposure concentrations during zebrafish behavior and neurotransmitter studies	Not available as of the publication date	Not available as of the publication date	Aladdin Biochemical Technology and Jiaxuan Biotechnolog	Not available as of the publication date	Extracted with ACN	HPLC-MS/MS	Athena C18-WP (2.1 mm × 50 mm, 3.0 μm) column with water and methanol mobile phase	ESI(+) mode MRM	269.3/184.1	269.3/211.0	Ji 2022 http://dx.doi.org/10.1016/j.scitotenv.2022.156013
29	surface water from two urbanized watersheds	LOQ: 0.0065 μg/L	polyethylene (PE) bottles, held for 72 hours refrigerated, and then frozen	Sigma-Aldrich	atrazine-d5 and melamine-13C3	To ensure efficient extraction of TPs with unknown chemical structures, three different solid phase extraction (SPE) methods were employed.	UPLC-MS/MS	Kinetex 2.6 μm C18 column (50 × 4.6 mm), mobile phase A consisting of MilliQ water (pH = 7) and mobile phase B consisted of methanol.	heated electrospray ionization source (HESI-II) operated in positive ionization mode. Data acquisition was achieved using parallel reaction monitoring (PRM)	269.20123		Johannessen 2021 https://doi.org/10.1016/j.envpol.2021.117659
30	Surface water, groundwater, stormwater & suspended particles from stormwater	MDL: 0.048 ng/L MQL: 0.160 ng/L	stainless-steel bucket, 0.7 μm glass-fiber filters to collect suspended particles, water sampels in HDPE bottles NaN3 (0.05%) to inhibit microbial activity, stored at 4°C	J&K & HPC	6PPD-Q-D ₅	<p>Filtered through 0.7 μm glass-fiber filters (GF/F; Whatman, UK), NaN3 (0.05%) was added into water samples to inhibit microbial activity. The water samples and suspended particles were stored at 4 °C until they were extracted.</p> <p>SPE Water samples: adjusted to pH 2 and EDTA added, then Waters Oasis HLB cartridges (6 mL, 200 mg), eluted with methanol, taken to dryness with nitrogen and redissolved in 10% methanol and filtered with 0.22 μm nylon microfiltration membrane</p> <p>Suspended Particles: Freeze dried and sequential ultrasonicated with methanol, dryness with nitrogen and redissolved in 10% methanol and filtered with 0.22 μm nylon microfiltration membrane</p>	UPLC-MS/MS	Waters XBridge BEH C18 column (2.1 mm × 100 mm, 2.5 μm) column with 0.05 % formic acid in water and 0.05 % formic acid in ACN mobile phase	ESI(+) mode MRM	269.20/184.10	269.20/185.15	Zhang 2023 https://doi.org/10.1016/j.envres.2022.114721
31	Surface water, groundwater, and stormwater, and suspended material	MDL: 0.048 ng/L MQL: 0.160 ng/L	stainless-steel bucket, 0.7 μm glass-fiber filters to collect suspended particles, water sampels in HDPE bottles NaN3 (0.05%) to inhibit microbial activity, stored at 4°C	Tokyo Chemical Industry	D5-6PPD-Q	Water samples were adjusted to pH = 2 using 3 mol/L HCl, SPE Oasis HLB cartridges, eluted with methanol, evaporated to almost dryness, redissolved in 10% methanol and nylon filtered.	LC-MS/MS	Column: Poroshell HPH-C18 column (2.1×100 mm, 2.7 μm) with Mobile Phase: water (0.1%formic acid) and methanol (0.1% formic acid)	ESI(+) mode MRM	269.20/184.10	269.20/185.15	Zhang_2023 Megacity China https://doi.org/10.1016/j.envres.2022.114721
32	Mammalian Cells	LOQ: 0.11 ng/g	Not available as of the publication date	Not available as of the publication date	Internal: Coumaphos-d10 (ISTD) Surrogate: Benzophenone-d10	Digestion mixtures were extracted by SPE with Waters Oasis HLB 1 cc 30 mg cartridges, eluted with 8:2 methanol: acetonitrile, and concentrated by vacuum concentrator	UPLC-HRMS	Waters Acquity BEH C18 UPLC column (2.1 × 100 mm, 1.7 μm in particle size and 130 Å in pore size) with 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile mobile phase	positive-ion mode parallel reaction monitoring (PRM) mode	269.0/184.1	269.0/212.4 269.0/92.8	Wu 2023 http://dx.doi.org/10.1016/j.scitotenv.2022.161373

Table 5-2. 6PPD Analytical Methods

Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
33 influent,effluent,and biosolids in four WWTPs in Hong Kong	<u>Influent</u> LOQ: 0.12 ng/L LOD: 0.037 ng/L <u>Other</u> LOQ: 0.06 ng/L LOD: 0.018 ng/L <u>Biosolids</u> LOQ: 0.31 ng/g LOD: 0.092 ng/g	Glass bottles, held on ice and transferred to lab within 2 hours Wastewater: glass microfiber filtered (1.2µm, Whatman,Hillsboro,USA) to remove suspended particulate matter, added 5% (v/v) methanol to inhibiting microbial growth, stored in the dark at 4°C until extraction. Biosolids & filtered suspended particulate matter: freeze-dried, homogenized, 60-mesh sieve, stored at-20°C until extraction	TCI Chemicals and TRC	Surrogate: diphenylamine-d10 Internal: 6PPD-Q-d5	Glass bottles, held on ice and transferred to lab within 2 hours Wastewater: serial liquid/liquid dichloromethane extraction, purification with Envi-carbSPE cartridge and eluted with ethanol/dichloromethane(2:8, v/v), taken to near dryness with nitrogen, redissolved with acetonitrile and nylon filtered <u>Biosolids & filtered suspended particulate matter</u> : serial ultrasonication with dichloromethane and acetonitrile, purification with Envi-carbSPE cartridge and eluted with ethanol/dichloromethane(2:8, v/v), taken to near dryness with nitrogen, redissolved with acetonitrile and nylon filtered	LC-MS/MS	Waters Acquity HSS T3 column(1.8µm, 2.1×100 mm), where the mobilephase consistedof 0.1%formic acid in deionizedwater (A) and 0.1% formic acid inacetonitrile(B)	ESI(+) mode MRM	269.2/93.1	269.2/184.1 269.2/212.1	Cao 2023 https://doi.org/10.1021/acs.est.3c03758
34 Influent and effluent from municipal, hospital, and industrial WWTPs	LOD: 0.120 ng/L	Upon arrival at lab hydrochloric acid added to a pH 2, stored at -20°C	J&K & HPC	6PPD-Q-D5	0.7µm glass fiber filter then SPE Oasis HLB (6mL, 200mg) eluted with methanol, evaporated to almost dryness with nitrogen, redissolved with 10% methanol	LC-MS/MS	Waters Xbridge BEH C18 column (2.1mm i.d., 100 mm, 2.5 µm), 0.05% formic acid in Milli-Q water (mobile phase A) and acetonitrile(mobile phase B)	MRM	269.2/184.10	269.2/185.15	Zhang 2023_Malaysia and Sri Lanka https://doi.org/10.1021/acsestwater.2c00410
35 urban water system: surface water, surface rainfall runoff (hardened pavement, road, farmland), influents and effluents WWTP, and six points along drinking water treatment sections	LOD: 0.04 ng/L LOQ: 0.12 ng/L	Glass amber bottles, immediately adjusted to pH 3.0 with 4 M H2SO4, added 5% methanol (v/v) to inhibit microbial growth, transported in cold ice boxes, stored at (4 °C) before processing and extracted within 48 hours	Tokyo Chemical Industry	Not available as of the publication date	filtered through 0.7 µm glass-fiber filter membranes, filter membrane serial sonication extraction with methanol and 0.1% formic acid, added to filtered water. SPE Oasis HLB cartridges (500 mg, 6 mL), eluted methanol ethyl acetate, and dichloromethane. Taken to dryness with nitrogen and redissolved with methanol and PTFE filtered	UPLC-MS/MS	Column not listed; 0.1 % formic acid; A) and methanol (B)	ESI(+) mode MRM	269.05/92.7	269.05/185 269.05/183.8	Zhang 2023_Urban Water System Pearl River Delta region, South China https://doi.org/10.1016/j.envint.2022.107715
36 TWP solvent extracts, TWP aqueous leachate, roadway runoff, roadway-impacted creek samples	<u>Creek</u> LOD: 3.3 ng/L LOQ: 4.9 ng/L <u>Roadway Runoff</u> LOD: 2.4 ng/L LOQ: 3.9 ng/L <u>TWP Leachate</u> LOD: 2.8 ng/L LOQ: 4 ng/L <u>TWP Methanolic Extracts</u> LOD: 3.4 ug/g LOQ: 11 ug/g	Roadway Runoff: grab and ISCO sampler, stored at 4 °C and extracted within 24 hours of sample collection	Usolf Chemicals	6PPDQ-d5	TWP samples methanol-extracted; TWP leachate, roadway runoff, and creek water samples were SPE extracted (Oasis HLB cartridges, eluted with methanol and concentrated to 1 mL)	LC-MS/MS	Agilent Poroshell HPH-C18 column (2.1×100 mm, 2.7 µm) preceded with a C18 guard column (2.0 × 4 mm). LC-MS grade water (A) and methanol (B), both with 0.1% formic acid, were used as mobile phases	ESI+ mode Dynamic multiple reaction monitoring mode (dMRM)	269.2/184.0	269.2/107.1	Zhao 2023 https://doi.org/10.1021/acs.est.2c08690

Table 5-3. 6PPD-Q Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
1	Storm and Surface Waters	2 ng/L	Aqueous: full 250 mL amber glass bottle, samples held up to 14-days in the fridge		extracted internal standard (EIS): 13C6-6PPD-q non-extracted internal standard: D5-6PPD-q	SPE with acetonitrile elution	LC-MS/MS	C18 column using a 0.2% formic acid in water and acetonitrile mobile phase				Placeholder for EPA citation
2	Storm, Surface Water, and Solids	Aqueous: 2 ng/L Solids: 0.25 ng/g	<u>Aqueous</u> : full 250 mL amber glass bottle, samples held up to 14-days in the fridge <u>Solids</u> : Full 120 mL amber glass jar		extracted internal standard (EIS): 13C6-6PPD-q non-extracted internal standard: D5-6PPD-q	Serial sonication with cetonitrile and SPE with acetonitrile elution	LC-MS/MS	C18 column using a 0.2% formic acid in water and acetonitrile mobile phase				Eurofins
3	Water, Fish bile & plasma, Blubber, Fish, Shellfish	Plasma: 0.0075-0.025 ng/mL Bile: 0.015-0.05 ng/mL Fish: 0.07 ng/g	glass vials or bottles	HPC Standards	Surrogate: 6PPD-Q-D ₅ Internal: Progesterone-d9	<u>Water</u> : Liquid/liquid extraction: concentration/solvent exchange <u>Bile & Plasma</u> : Water dilution, SPE, analysis <u>Blubber, Fish, Shellfish</u> : ASE, gravity-flow column cleanup & fractionation, then GPC cleanup for 6PPD-Q analysis	LC-MS/MS	Phenomenex Kinetex C18 EVO (100 x 2.1mm, 1.7 µm particle size)	ESI+ (MRM)	299 > 241	299 > 215	NOAA, placeholder for Denis (for bile/plasma) and Li-Jung Kuo (fish tissues)
4	Storm, Surface Waters, Effluent, Soil and Biosolid	0.1 ng/L and 0.05 ng/g	Amber glass bottles (HDPE not recommended), store aqueous sample at 4°C and solids at -20°C. Aqueous sample stable for atleast 35 days. Solids and biosolids are stable for at least 40 days. No significant recovery difference in river water store at -20°C and 4°C for 35 days	HPC Standards	D ₅ - 6PPD-Q	Water Extraction : Liquid-liquid extraction. with DCM. Soil and biosolid: Ultrasonic extraction. with MeCN Cleanup: 2 g, 6 mL Silica SPE	UPLC-MS/MS	Waters Acquity UPLC BEH C18 (1.7 µm, 2.1 × 50 mm) plus Vanguard Pre-column (1.7 µm, 2.1- × 5-mm) column with 0.1 % formic acid in water and 0.1 % formic acid in ACN mobile phase	Positive mode ionization	299.4/241.0	299.4 > 215.0	SGS AXYS Analytical Ltd
5	Water	1 ng/L (0.025 ng/mL on instrument)	250 mL bottles, no preservative, stored above freezing to 6°C for 28 days	HPC Standards, Cambridge Isotopes	D ₅ -6PPD-Q 13C6-6PPD-Q	SPE: Waters Oasis HLB 6cc (200mg) SPE cartridge (WAT 106202) or Bakerbond Speedisk H2O-Philic DVB (8072-07) cartridges, eluted with ACN	LC-MS/MS	Phenomenex 00D-4622-AN (100 mm x 2.1 mm, 2.6 um Biphenyl 100 Å) with 0.1% Formic acid/water and 0.1% Formic acid/Acetonitrile	ESI+ Dynamic MRM	299.1/215.1	299.1/215.1	Standard Operating Procedure MEL730136, Version 1.2
6	Storm and Surface Waters	2 ng/L	Amber glass bottles, no headspace. Samples held up to 14-days in the fridge or frozen if hold times would exceed 14 days	HPC Standards and Cambridge Isotopes Labratories	Surrogate internal standard: D5-6PPD-Q	0.7-micron glass-fiber filter and then direct-injection	UPLC-MS/MS	Waters Acquity UPLC BEH C18 (1.7 µm, 2.1 × 50 mm) plus Vanguard Pre-column (1.7 µm, 2.1- × 5-mm) column with 0.1 % formic acid in water and ACN mobile phase	ESI+	299.1/241.2	299.1/215.1	USGS, placeholder for Rachael publication
7	influent and efflent of WWTP	MDL: 4 ng/L	large volume solid phase extraction (LVSPE)	Not available as of the publication date	Not available as of the publication date	filtered using a Sartopure GF + MidiCap, 0.65 µm deep filter and extracted using cartridges filled with 10 g of Chromabond HR-X, eluted with 5 mL of ethyl acetate, 5 mL of methanol, 5 mL of methanol containing 1% of formic acid, 5 mL of methanol containing 2% of 7 N ammonia in methanol, evaporated under a nitrogen to near dryness, brought to 1 mL with methanol	LC-HRMS	Kinetex C18 EVO column (50 × 2.1 mm, 2.6 µm particle size, 83 Phenomenex, pre-column 4 x 2.1 mm and in-line filter 0.2 µm) and a gradient elution with 0.1% 84 of formic acid and methanol containing 0.1% of formic acid at a flow rate	Ion Mode ESI+	M+H = 299.1754		Maurer, 2023. https://doi.org/10.1021/acs.est.2c05784
8	Snow from urban street	LOQ: 25 ng/L	250 mL glass bottles; kept frozen until further treatment and analysed on the same day	HPC Standards	Not available as of the publication date	Snow: Thawed, centrifuge, supernatant direct injection, remaining snow particles ultrasonic bath with methanol, diluted 1:1 with water	UPLC-TOF-MS	HSS T3 column; 100 × 2.1 mm, 1.7 µm and Atlantis T3 (3µm, 2,1x 100mm); (A) water with 0.1% formic acid (v/v) and (B) methanol with 0.1% formic acid (v/v)	ESI(+) mode MRM	299.0/215.1	299.0/241.1	Seiwart_2022 https://doi.org/10.1016/j.watres.2022.118122

Table 5-3. 6PPD-Q Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
9	Influent and effluent WWTP during snow melt, rain, and dry conditions	LOQ: 25 ng/L	250 mL glass bottles	HPC Standards	Not available as of the publication date	Two mL of wastewater filtered using syringe filters (0.45 µm, RC membrane, Minisart RC4, Sartorius)	UPLC-TOF-MS	HSS T3 column; 100 × 2.1 mm, 1.7 µm and Atlantis T3 (3µm, 2,1x 100mm); (A) water with 0.1% formic acid (v/v) and (B) methanol with 0.1% formic acid (v/v)	ESI(+) mode MRM	299.0/215.1	299.0/241.1	Seiwart_2022 https://doi.org/10.1016/j.watres.2022.118122
10	Air from 18 major cities that comprise the Global Atmospheric Passive Sampling (GAPS) Network	Instrument LOQ: 0.122 ng/mL Method LOQ: 0.169 pg/m3	PUF disk samplers collecting both collect both gas- and particle-phase chemicals	Sigma-Aldrich	Not available as of the publication date	Accelerated solvent extractor (ASE) extraction with petroleum ether and acetone (83/17, v/v), rotary evaporation, reconstituted with iso-octane, silica column cleanup	UPLC-HRMS	Phenomenex (Torrance, CA, USA) Kinetex C18 column (2.6 µm in particle size, 50 × 4.6 mm in length and inner diameter HPLC grade methanol, with 0.1% of formic acid in both, were used as the mobile phase	Positive ionization mode with a heated electrospray ionization source (HESI-II) Parallel Reaction Monitoring (PRM)	299.1754/187.0866		Johannessen 2022 MegaCity https://doi.org/10.1016/j.envpol.2022.120206
11	fine particulate matter PM _{2.5}	MQL: 0.08 pg/m ³ MDL: 0.02 pg/m3	quartz fiber filter	J&K, AccuStandard, TCI, TRC (PPD-Q standards were lab synthesized)	Surrogate standard diphenylamine-d10 and internal standard 6PPD-Q-d5	Serial ultrasonication with dichloromethane and acetonitrile, concentrated with nitrogen in acetonitrile and PTFE filtered	<u>UHPLC-HRMS</u>	Waters Acquity HSS T3 column (1.8 µm, 2.1×100 mm) with 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile mobile phase	data-dependent MS2 mode MRM	299.2/241.1	299.2/215.1 299.2/187.1	Wang 2022 https://doi.org/10.1021/acs.est.2c02463
12	fine particulate matter PM _{2.5} from megacity	LOD: 5 pg/mL	Whatman medium-volume quartz fiber filters	J&K Scientific Ltd. and Sigma-Aldrich	Pyrene-d10 and benzophenone-d10	ultrasonication acetonitrile and dichloromethane/hexane, taken to near dryness with nitrogen, redissolved in methanol and filtered with PTFE membrane	UHPLC-MS/MS	A Waters ACQUITY UPLC C18 column (1.7µm, 2.1 mm×100 mm) with 0.4 mM CH3COONH4 (A)/ MeOH (B)	ESI(+) mode MRM	299/187	299/ 215	Zhang 2021 https://doi.org/10.1021/acs.est.1c04500
13	Size-Fractioned Atmospheric Particles and Dust of Different Indoor Environments	Ambient Particles LOD: 0.03 pg/m3 Dust LOD: 0.03 ng/g	eight-stage nonviable Anderson cascade impactor (TISCH-Model TE-20-800, USA) with a glass substrate membrane for ambient particle collection	Cambridge Isotope Laboratories	Internal standard: 13C6-6-PPD quinone	ultrasonic extraction with n-hexane and acetone, centrifuged, and concentrated to near dryness, redissolved in methanol, and filtered (0.22 µm poly(ether sulfone) membrane	UPLC-MS/MS	Betasil C18 column (100 × 2.1 mm, particle size 3 µm, Thermo Scientific) with water (A) and acetonitrile (B) w	ESI(+) mode MRM	299.2/241.1	299.2/187.1	Zhang, 2022. https://doi.org/10.1021/acs.estlett.2c00193
14	Airborne Particulate Matter Along a Highway in Mississippi, USA	LOD: 0.58 ng/L	Airborne PM was collected using Sigma-2 passive samplers	Not available as of the publication date	Not available as of the publication date	methanol and hexane extraction shaker table, filtered with polycarbonate gold-coated filters, rotary evaporation to 3 mL, nitrogen evaporation to near dryness and redissolved in 66% methanol	UHPLC-HRMS	online filter cartridge with a 2.1 mm ID × 0.2 µm porosity stainless steel filter, an Eclipse Plus C18 RRHD (5 mm × 2.1 mm ID; 1.8 µm) guard column followed by the analytical column with the same stationary; 1mM ammonium formate and 0.1% formic acid (A) and methanol 0.1% formic acid (B) phase (100 mm × 2.1 mm, 1.8 µm)	Heated electrospray ionization mode (HESI) Data dependent product scan	Not available as of the publication date	Not available as of the publication date	Olubusoye 2023 https://doi.org/10.1007/s00128-023-03820-7
15	Dust: Road dust, Interior car dust , Parking lot dust , Indoor dust from homes near E-waste dismantling area	Not available as of the publication date	precleaned nylon bag (pore size of 25 µm)	TCI, Accustandard, Sigma-Aldrich, Toronto Research Chemical	ISTD: Coumaphos-d ₁₀ SSTD: Benzophenone-d10	Serial sonication with acetonitrile and 1:1 dichloromethane: hexane. Concentrated by nitrogen into methanol and filtered	<u>HPLC-MS/MS</u>	<u>HPLC</u> : C18 column (100mm × 2 mm, Luna 3 µm, Phenomenex) with 0.3 g/L ammonium acetate (A) and methanol (B) mobile phase	ESI(+) mode MRM	299.18 / 215.08	299.18/241.09 299.18/256.12 299.18/187.09	Huang 2023 https://doi.org/10.1021/acs.estlett.1c00148
16	Sediments across UrbanRivers, Estuaries, Coasts, and Deep-Sea Regions	MDL: 0.043 ng/g	sediment packed in aluminum foil and stored in polypropylene tubes; freeze dried and 1.0 mm mesh screened	Tokyo Chemical Industry, AccuStandard, J&K Scientific, Dr. Ehrenstorfer	13C6-6PPD-Q	Transferred to glass tube, ultrasonicated with acetonitrile, concentrated and filtered with PTFE membrane.	LC-MS/MS	C8 column (Waters Xbridge BEH, 2.5µm,2.1 mm×100mm) 0.1%formic acid in water and (B) methanol at a flowrate of 0.3 mL/min	ESI(+) mode MRM	299.2/241.1	299.2/215.1	Zeng 2023 https://doi.org/10.1021/acs.est.2c07652

Table 5-3. 6PPD-Q Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
17	Fish	LOD: 0.0003 mg/Kg LOQ: 0.001 mg/Kg	homogenized by the electric blender, frozen until extraction in centrifuge tube	Jiaxuan Biotechnology Co., Ltd	Not available as of the publication date	Modified QuEChERS	HPLC-MS/MS	Athena C18-WP chromatographic column (2.1 mm x 50 mm, 3.0 µm) Mobile Phase: MeOH: Deionized water/80:10	ESI(+) mode MRM	299.2/215.2	299.2/187.2 299.2/241.2	Ji 2022. https://doi.org/10.1016/j.foodchem.2022.133640
18	larval zebrafish & Water	LOQ: 0.1 ng/mL	Glass beakers	Not available as of the publication date	Not available as of the publication date	QuEChERS	HPLC/MS-MS	Luna Omega C18, 100 mm × 2.1 mm, 1.7 µm; b) mobile phase: 5 mmol/L ammonium formate solution (A), methanol (B);	ESI(+) mode MRM	299.25/215.25	299.25/187.15	Fang 2023 https://doi.org/10.1016/j.scitotenv.2023.163595
19	Embryonic zebrafish	LOD: 0.089 ng/mL LOQ: 0.439 ng/mL	Glass trays	HPC Standards GmbH	Not available as of the publication date	FastPrep homogenizer, sonication, and centrifuging	HPLC/MS-MS	Atlantis T3 C18-phase column(2.1 mm×50 mm, 3µm; Waters) with an Atlantis T3 Security Guard column (2.1×10 mm, Waters)	ESI(+) mode MRM	298.979/241.10	298.979/215.10	Grasse 2023 https://doi.org/10.1021/acs.est.3c02819
20	Embryonic zebrafish	See Zhang https://doi.org/10.1021/acs.estlett.2c00193 .	well plate exposures	Toronto Research Chemicals	13C6-6PPDQ	Homogonization, polyfiltration, sonication, concentrated to near dryness, and redissolved in methanol and filtrated through a 0.22 µm poly (ether sulfone) membrane	UPLC/MS-MS	See Zhang https://doi.org/10.1021/acs.estlett.2c00193 .	ESI(+) mode MRM			Zhang 2023 https://doi.org/10.1016/j.jhazmat.2023.131601
21	Rainbow trout tissue & exposure water samples	MDLs: 0.1 – 0.6 ng/g in tissue	Plastic and glass	Toronto Research Chemicals	d5-6PPD-Q	whole fish body was homogenized into Eppendorf tube, serial sonication with acetonitrile and centrifuged	UHPLC-HRMS	Hypersil GOLD C18 column (50×2.1 mm, 1.5 µm). 0.1% formic acid in ultrapure water (A) and 0.1% formic acid in methanol	ESI(+/-) full scan mode	Not available as of the publication date	Not available as of the publication date	Nair 2023 https://doi.org/10.26434/chemrxiv-2023-pmxvc
22	Fish Tissue (S.l.pluvius, S.curilus, and O.m.masou)	Not available as of the publication date	Not available as of the publication date	Cambridge Isotope Laboratories	6PPD-quinone-13C6	wet tissue into polypropylene tube, homogenized with glass beads, acetonitrile centrifugation	LC-MS/MS	Shim-pack VP ODS column (150 mm×2.0 mm, silica-based C18stationary phase). The mobile phase was 0.1% ammonium acetate (pH: about 5) and methanol (1:8 v/v)	full-scan mode	transitions from m/z 299 to 241, m/z 305 to 247, and m/z 315 to 231 were used for the quantification of 6PPD-Q	Not available as of the publication date	Hiki 2022 https://doi.org/10.1021/acs.estlett.2c00683
23	Lumpfish blood	LOD: 0.1 pg	Not available as of the publication date	ASCA GmbH	Internal: 6PPD-Quinone-D5 Recovery: 6PPD-Quinone-13C6	vortex-sonication and centrifuged	HRGC/HRMS	TG-5SILMS column (30m, 0.25 mm i.d., film thickness—0.25 µm)	Non-targeted screening Full-scan mode	Not available as of the publication date	Not available as of the publication date	Hagg_ 2023 DOI: 10.3389/fenvs.2023.1219248
24	Exposure concentrations in toxicity studies	0.05 ug/L	aerated 45 L rectangular glass tanks, 150 L inert glass-fiber Krescel tanks, 700 L glass-fiber Min-o-Cool tanks	Toronto Research Chemicals	6PPD-quinone-d ₅	Direct injection of exposure concentrations	UPLC-HRMS	Phenomenex Kinetex 1.7 µm XB-C18 column and SecurityGuard C18 guard column) with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	positive heated electrospray ionization (HESI) mode	299.1754/215.0819	299.1754/187.0869 299.1754/243.1132	Brinkmann_2022 DOI: 10.1021/acs.estlett.2c00050
25	Human Urine from general adults, children, and pregnant women	MDL: 0.021 ng/mL	After urine samples collected, immediately transferred to the laboratory, stored at –40 °C until analysis. Glass used during laboratory extractions	Cambridge Isotope Laboratories, Toronto Research Chemical	¹³ C ₆ -6PPD-Q	salting-out assisted liquid–liquid extraction, concentrated with nitrogen and 0.22 µm filtered	<u>LC-MS/MS</u>	<u>Ultra-Fast LC:</u> Waters XBridge C8 column (2.1 mm×100 mm, 2.5µm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	ESI(+) mode MRM	299.2/241.1	299.2/215.1	Du 2022 https://doi.org/10.1021/acs.estlett.2c00821
26	Honey	LOD: 0.0003 mg/Kg LOQ: 0.001 mg/Kg	stored at room temp until extraction in centrifuge tube	Jiaxuan Biotechnology Co., Ltd	Not available as of the publication date	Modified QuEChERS	HPLC-MS/MS	Athena C18-WP chromatographic column (2.1 mm x 50 mm, 3.0 µm) Mobile Phase: MeOH: Deionized water/80:10	ESI(+) mode MRM	299.2/215.2	299.2/187.2 299.2/241.2	Ji 2022. https://doi.org/10.1016/j.foodchem.2022.133640

Table 5-3. 6PPD-Q Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
27	Lettuce (Valerianella locustaL) plant and roots & TWP's in nutrient solution	Not available as of the publication date	Glass vials	HPC Standards GmbH	Not available as of the publication date	Plant:serial bead beater with stainless steel beads and acetonitrile extraction, centrifuged, filtered with nylon filter Roots: Freeze dried roots, suspended in nutrient solution, reciprocal shaker, centrifuge, nylon syringe filter Nutrient Solution: serial liquid–liquid extraction, nylon syringe filter	UPLC-MS/MS UPLC-HRMS	C18 column (Acquity HSS T3, 1.8 µm, Waters), ultrapure water (Phase A) and acetonitrile (Phase B), both containing 0.1% formic acid	ESI(+) mode MRM	299/256.1	299/241 299/215 299/187	Castan_2022 https://doi.org/10.1021/acs.est.2c05660
28	Soil, water, atmospheric particles; urban runoff water samples were collected in a dense traffic urban area	IQL: 0.023 ng/mL	Soil: stainless steel shovel, transported to lab within 2 hours, freeze dried, homogenized, sieved through a 60 mesh Atmospheric particle collect on quartz fiber filters and stored at - 20C Water: 200mL collected in Teflon tubes, glass microfiber filter, acidified with 2% formic acid	J&K, TCI Chemicals, Sigma-Aldrich, and Toronto Research Chemicals	Internal: diphenylamine-d ₁₀ Surrogate: 6PPD-quinone-d5	Soil: serial ultrasonication with acetonitrile, concentrated to dryness with nitrogen, redissolved in methanol and 0.45 µm nylon filtered Atmospheric particles: serial ultrasonication with dichloromethane and acetonitrile, concentrated to near dryness with nitrogen, redissolved in acetonitrile and filtered Water: HLB SPE Cartridge (60 mg, 3 mL), eluted with methanol–dichloromethane (1:9, v/v), concentrated to dryness with nitrogen, redissolved in acetonitrile and 0.45 µm nylon filtered	UPLC-HRMS	Waters Acquity HSS T3 (1.8 µm, 2.1 × 100 mm) column with 0.1 % formic acid in water and 0.1 % formic acid in ACN mobile phase	ESI(+) mode full scan and data-dependent acquisition mode	299.2/241.1	299.2/215.1	Cao 2022 DOI: 10.1021/acs.est.1c07376
29	recycled tire rubber employed in synthetic football fields	Suspect screening	Glass vial aluminum cap, stored in the dark at room temperature	Toronto Research Chemicals (6PPD-Q)	Not available as of the publication date	<i>In-vitro</i> simulation of digestion extraction, then solid phase extraction or the bioaccessible fraction: 50 mg of Oasis HLB eluted with ethyl acetate. Ultrasound-assisted extraction for PAHs: crumb rubber in ethyl acetate, ultrasonic bath at 50 kHz for 20 min, PTFE filtered	<u>GC/MS</u>	Phenomenex Zebron ZB-Semivolatiles capillary column (30 m × 0.25 mm × 0.25 µm film)	Selected Reaction Monitoring (SRM)	Suspect screening	Not available as of the publication date	Armada_2023 http://dx.doi.org/10.1016/j.scitotenv.2022.159485
30	Solubilization of organic compounds from tire particles using Fish <i>In Vitro</i> Digestive Model	<u>Digestate</u> LOD: 0.1 ug/L LOQ: 0.3 ug/L <u>Cryogenically milled tire tread</u> LOD: 0.2 ug/L LOQ: 0.5 ug/L	amber glass vessels	Lab synthesized	6PPD-Q-d ₅ , benzothiazole-d ₄ , aniline-d ₅ , and diphenylurea-d ₁₀ ,	Fish <i>In Vitro digestive model and coingestion experiments</i> , then serial liquid/liquid extraction with dichloromethane DCM, then concentrated for analysis	<u>UHPLC-HRMS</u>	ACQUITY UPLC HSS T3 (100 × 2.1 mm, 1.8 µm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	ESI(+) mode MRM	299.00/187.00	299.00/241.00	Masset 2022 https://doi.org/10.1021/acs.est.2c04291
31	Road runoff	98% Confidence MDL: 13.98 ng/L	Water extracted within 48 hours of collection; glass bottles used for spikes	Toronto Research Chemicals	6PPD-quinone-d ₅	filtered with 0.7-mm glass microfiber filters (GF/F, cytiva), then SPE with Oasis 6 cc, 500 mg HLB cartridges, eluted with methanol	<u>HPLC-MS/MS</u>	Agilent InfinityLab Poroshell 120 EC-C8 LC (30 mm, 2.1 mm, 2.7 µm) column with 1mM ammonium formate in water and methanol mobile phase	ESI(+) mode MRM	299.0/215.1	299.0/187.0	Rodgers 2023 https://doi.org/10.1021/acs.estlett.3c00203

Table 5-3. 6PPD-Q Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
32	Urban river with stormwater-influenced flows; upstream, downstream, and near WWTP discharge	0.0098 ug/L	PET bottle, stored frozen (−18 °C) <i>protocol established prior to discover of 6PPD-Q</i>	HPC Standards	Not available as of the publication date	SPE with Waters HLB cartridges, 6 cc, 500 mg, eluted with methanol and concentrated	UPLC-HRMS	Kinetex 2.6 µm C18 column (50×4.6 mm). Solvent A, MilliQ water (pH =7) with 0.1% of formic acid, and Solvent B, methanol with 0.1% of formic acid	Orbitrap HRMS, positive ionization mode with a heated electrospray ionization source (HESI/III). Parallel reaction monitoring (PRM) for data acquisition	targeted select ion monitoring: 299.17540	Not available as of the publication date	Johannessen 2022 https://doi.org/10.1007/s00244-021-00878-4
33	Urban runoff from cold climate: stormwater, snowmelt, river water	LOD: 1.2 ng/mL LOQ: 3.3 ng/mL	4 L and 1 L nalgene bottles	Sigma-Aldrich, Toronto Research Chemical	6PPD-quinone-d5	Whatman GF/F glass microfiber filters (0.7 µm) SPE with Waters Oasis HLB (500 mg, 6cc), eluted with methanol and DCM, concentrated to dryness with nitrogen, redissolved in 1:1: methanol:water	<u>UHPLC-HRMS</u>	<u>UHPLC</u> : Phenomenex Kinetex 1.7 µm XB-C18-LC (100 × 2.1 mm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	positive mode heated electrospray ionization (HESI) parallel reaction monitoring (PRM) Suspect screening: full MS/ddMS2	299.1754/215.0819	Ions monitored during the suspect screening 299.1754/187.0869 299.1754/241.0974 299.1754/256.1210 299.1754/200.007	Challis 2021 DOI: 10.1021/acs.estlett.1c00682
34	surface water at 5 urban centres in Queensland, Australia; Surface waters and stormwater Australian Urban Tributary	MDL: 0.05 ng/L	600 mL polypropylene jars, frozen (−20 °C) until analysis	UCA, Tokyo Chemical Industry, Novachem, Cambridge Isotope Laboratories	Internals: d ₆ -5-methylbenzotriazole and d ₅ -atrazine Inject Internal: 13C3-Caffeine	Water: filtered through Whatman 47 mm, 1 µm, GFF/B glass fibre filter, SPE with Waters Oasis 6 cm3 HLB cartridges, eluted with methanol concentrated with nitrogen Particles: Filter papers with particles dried in an incubator at 60 °C for 3 h and stored at 4 °C for analysis; filters were cut into 1/8th segments, and one segment loaded into an 80 µL pyrolysis cup	Water: LC-MS/MS Particles: PYR-GC/MS (not analyzed for 6PPD-Q)	LC: Phenomenex Kinetex biphenyl 100 Å analytical column (2.6 µm, 50 mm × 2.1 mm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase Pyr-GCMS: Particulates captured on the 1 µm filter analyzed for TRWPs and polymers with pyrolysis gas chromatography mass spectrometry	ESI(+) mode MRM	LC-MS/MS: 299/241 PYR-GC/MS: Full scan mode over a mass range of 40 to 600 m/z	299/215 299/187	Rauert_2022 https://doi.org/10.1016/j.scitotenv.2022.158468 AND https://doi.org/10.1021/acs.est.1c07451
35	Fish media during acute toxicity studies with Atlantic Salmon (<i>Salmo salar</i>) and Brown Trout (<i>Salmo trutta</i>) alevins	LOD: 0.006 ug/L LOQ: 0.020 ug/L	Water samples were collected in preprepared probes 1.5-ml polypropylene tube with a 2 × 2-cm piece of dust-free paper wipe; 1-ml aliquots of fish media were collected and transferred to individual probes. Probes were kept at 5 °C, and were analyzed 5 days after the exposure trial.	Cambridge Isotope Laboratories	¹³ C ₆ -6PPD-Q	Direct-injection and analysis of probes	LC-MS/MS	Agilent Eclipse Plus C18 RRHD ultra(U)HPLC column (3 × 50-mm, 1.8-µm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	Jet Stream electrospray interface operated in positive ion mode	299.1/187.3	299.1/77.3	Foldvik 2022 DOI: 10.1002/etc.5487

Table 5-3. 6PPD-Q Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
36	Exposure concentrations during acute toxicity studies of freshwater fish and crustacean species (Danio rerio, Oryzias latipes, Daphnia magna, and Hyalella azteca)	LOD: 0.05 ug/L LOQ: 0.17 ug/L	Glass tanks, beakers, and bottles	Cambridge Isotope Laboratories and Toyko Kasei	Not available as of the publication date	Direct-inject and direct-inject with dilutions	LC-MS/MS	Shim-pack VP ODS C18 (150 mm × 2.0 mm) column with 0.1 % formic acid in water and 0.1 % formic acid in methanol mobile phase	ESI(+) mode MRM	299/241	299/187 299/215	Hiki 2021 https://doi.org/10.1021/acs.estlett.1c00453
37	Exposure concentrations during zebrafish behavior and neurotransmitter studies	Not available as of the publication date	Not available as of the publication date	Aladdin Biochemical Technology and Jiaxuan Biotechnolog	Not available as of the publication date	Extracted with ACN	HPLC-MS/MS	Athena C18-WP (2.1 mm × 50 mm, 3.0 μm) column with water and methanol mobile phase	ESI(+) mode MRM	299.2/215.2	299.2/187.2 299.2/241.2	Ji 2022 http://dx.doi.org/10.1016/j.scitotenv.2022.156013
38	surface water from two urbanized watersheds	LOQ: 0.0065 μg/L	polyethylene (PE) bottles, held for 72 hours refrigerated, and then frozen	HPC Standards, Sigma-Aldrich	atrazine-d5 and melamine-13C3	To ensure efficient extraction of TP _s with unknown chemical structures, three different solid phase extraction (SPE) methods were employed.	UPLC-MS/MS	Kinetex 2.6 μm C18 column (50 × 4.6 mm), mobile phase A consisting of MilliQ water (pH = 7) and mobile phase B consisted of methanol.	heated electrospray ionization source (HESI-II) operated in positive ionization mode. Data acquisition was achieved using parallel	299.1754		Johannessen 2021 https://doi.org/10.1016/j.envpol.2021.117659
39	Surface water, groundwater, and stormwater, and suspended material	MDL: 0.029 ng/L MQL: 0.098 ng/L	stainless-steel bucket, 0.7 μm glass-fiber filters to collect suspended particles, water sampels in HDPE bottles NaN ₃ (0.05%) to inhibit microbial activity, stored at 4°C		D5-6PPD-Q	Water samples were adjusted to pH = 2 using 3 mol/L HCl, SPE Oasis HLB cartridges, eluted with methanol, evaporated to almost dryness, redissolved in 10% methanol and nylon filtered.	LC-MS/MS	Column: Poroshell HPH-C18 column (2.1×100 mm, 2.7 μm) with Mobile Phase: water (0.1%formic acid) and methanol (0.1% formic acid) mm × 100 mm, 2.5 μm)	ESI(+) mode MRM	299.15/241.10	299.15/187.10	Zhang_2023 Megacity China https://doi.org/10.1016/j.envres.2022.114721
40	Water samples from coexposures of coho and chum with nominal 320 mg/L TWP	Not available as of the publication date	4-L precleaned amber glass bottles	Not available as of the publication date	Not available as of the publication date	SPE with s (3 mL, 100 mg Infinity Osorb), eluted with methanol, and concentrated to 1 mL with nitrogen	LC-QTOF-HRMS	Agilent ZORBAX Eclipse Plus 2.1×100 mm (analytical), 2.1×5 mm (guard), 1.8-μm particle size gradient 5 mM ammonium acetate plus 0.1% acetic acid in each of deionized water and methanol	Two methods were applied to estimate the ‘equivalent concentration’ of TWP _s in exposure water: nontarget HRMS features and measured concentrations tire-derived chemicals	Not available as of the publication date	Not available as of the publication date	McIntyre 2021 https://doi.org/10.1021/acs.est.1c03569

Table 5-3. 6PPD-Q Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
41	Mammalian Cells	Not available as of the publication date	Not available as of the publication date	Not available as of the publication date	Internal: Coumaphos-d10 (ISTD) Surrogate: Benzophenone-d10	Digestion mixtures were extracted by SPE with Waters Oasis HLB 1 cc 30 mg cartridges, eluted with 8:2 methanol: acetonitrile, and concentrated by vacuum concentrator	<u>UPLC-HRMS</u>	Waters Acquity BEH C18 UPLC column (2.1 × 100 mm, 1.7 μm in particle size and 130 Å in pore size) with 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile mobile phase	positive-ion mode parallel reaction monitoring (PRM) mode	299.18/215.08	299.18/241.09 299.18/256.12 299.18/187.09	Wu 2023 http://dx.doi.org/10.1016/j.scitotenv.2022.161373
42	influent,effluent,and biosolids in four WWTPs in Hong Kong	<u>Influent</u> LOQ: 0.02 ng/L LOD: 0.005 ng/L <u>Other</u> LOQ: 0.01 ng/L LOD: 0.002 ng/L <u>Biosolids</u> LOQ: 0.04 ng/g LOD: 0.012 ng/g	Glass bottles, held on ice and transferred to lab within 2 hours <u>Wastewater</u> : glass microfiber filtered (1.2μm, Whatman,Hillsboro,USA) to remove suspended particulate matter, added 5% (v/v) methanol to inhibiting microbial growth, stored in the dark at 4°C until extraction. <u>Biosolids & filtered suspended particulate matter</u> : freeze-dried, homogenized, 60-mesh sieve, stored at−20°C until extraction	Cambridge Isotope Laboratories and TRC	Surrogate: diphenylamine-d10 Internal: 6PPD-Q-d5	Glass bottles, held on ice and transferred to lab within 2 hours Wastewater: serial liquid/liquid dichloromethane extraction, purification with Envi-carbSPE cartridge and eluted with ethanol/dichloromethane(2:8, v/v), taken to near dryness with nitrogen, redissolved with acetonitrile and nylon filtered <u>Biosolids & filtered suspended particulate matter</u> : serial ultrasonication with dichloromethane and acetonitrile, purification with Envi-carbSPE cartridge and eluted with ethanol/dichloromethane(2:8, v/v), taken to near dryness with nitrogen, redissolved with acetonitrile and nylon filtered	LC-MS/MS	Waters Acquity HSS T3 column(1.8μm, 2.1×100 mm), where the mobilephase consistedof 0.1%formic acid in deionizedwater (A) and 0.1% formic acid inacetonitrile(B)	ESI(+) mode MRM	299.2/241.1	299.2/215.1 299.2/187.1	Cao 2023 https://doi.org/10.1021/acs.est.3c03758
43	runoff samples from tunnel washing, runoff treatment plant, and downstream of the plant drain; two water samples from puddles were included: one was run-off from an artificial soccer turf field and one from a puddle on a country road	LOQ: 5 ng/L	Water samples were kept at 5°C prior to sample preparation and were analyzed within 2 weeks after collection	Cambridge Isotope Laboratories	13C6-6PPD-quinone	Clean Samples: transfer to polypropylene tube, centrifuge, SPE with Oasis HLB 30 mg/1 mL, eluted with methanol, evaporated to dryness with nitrogen, redissolved in acetonitrile Dirty Water Samples: transfer to polypropylene tube, centrifuge, lipophilic constituents extracted with dichloromethane and orbital shaker, centrifuged. Organic phase collected and diluted with hexane and SPE Phenomenex Strata Si-1 silica (55 μm, 70 Å, 100 mg/1mL), eluted with dichloromethane, evaporated to dryness with nitrogen, redissolved in acetonitrile	LC-MS/MS	Eclipse Plus C18 RRHD LC column (1.8 μm, 3.0 × 50 mm, Agilent Technologies). Mobile phase solvent A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile.	ESI(+) mode MRM	299.1/187.3	299.1/77.3	Kryuchkov 2023 doi: 10.3389/fenvc.2023.1194664
44	Surface water and stormwater samples and tire/artificial turf particle suspensions	LOQ: 8 ng/L	amber glass bottles; raw samples stored at 4°C, analyzed within 1 week of sampling	Sigma-Aldrich, ACP Chemicals	Not available as of the publication date	A CP-MIMS immersion probe was constructed from a 7.6 cm length of dense PDMS hollow fiber membrane (inside diameter of 190μm, outside diameter of 300μm, Permelect, Medarray Inc., Ann Arbor,MI).	HRMS	CP-MIMS membrane permeate was collected offline and directly infused	ESI(+) mode HSAID source	299/215	299/243 299/256 299/100	Monaghan 2021 https://doi.org/10.1021/acs.estlett.1c00794

Table 5-3. 6PPD-Q Analytical Methods

	Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference
45	Influent and effluent from municipal, hospital, and industrial WWTPs	LOD: 0.098 ng/L	Upon arrival at lab hydrochloric acid added to a pH 2, stored at −20°C	HPC	6PPD-Q-D5	0.7µm glass fiber filter then SPE Oasis HLB (6mL, 200mg) eluted with methanol, evaporated to almost dryness with nitrogen, redissolved with 10% methanol	LC-MS/MS	Waters Xbridge BEH C18 column (2.1mm i.d., 100 mm, 2.5 µm), 0.05% formic acid in Milli-Q water (mobile phase A) and acetonitrile(mobile phase B)	MRM	299.15/241.10	299.15/187.10	Zhang 2023_Malaysia and Sri Lanka https://doi.org/10.1021/acsestwater.2c00410
46	urban water system: surface water, surface rainfall runoff (hardened pavement, road, farmland), influents and effluents WWTP, and six points along drinking water treatment sections	LOD: 0.05 ng/L LOQ: 0.17 ng/L	Glass amber bottles, immediately adjusted to pH 3.0 with 4 M H ₂ SO ₄ , added 5% methanol (v/v) to inhibit microbial growth, transported in cold ice boxes, stored at (4 °C) before processing and extracted within 48 hours	Toronto Research Chemicals	Not available as of the publication date	filtered through 0.7 µm glass-fiber filter membranes, filter membrane serial sonication extraction with methanol and 0.1% formic acid, added to filtered water. SPE Oasis HLB cartridges (500 mg, 6 mL), eluted methanol ethyl acetate, and dichloromethane. Taken to dryness with nitrogen and redissolved with methanol and PTFE filtered	UPLC-MS/MS	Column not listed; 0.1 % formic acid; A) and methanol (B)	ESI(+) mode MRM	299.28/241.10	299.28/214.98	Zhang 2023_Urban Water System Pearl River Delta region, South China https://doi.org/10.1016/j.envint.2022.107715
47	TWP solvent extracts, TWP aqueous leachate, roadway runoff, roadway-impacted creek samples	Creek LOD: 1.2 ng/L LOQ: 3.1 ng/L Roadway Runoff LOD: 2.1 ng/L LOQ: 5.7 ng/L TWP Leachate LOD: 2.4 ng/L LOQ: 6.8 ng/L TWP Methanolic Extracts LOD: 0.12 ug/g LOQ: 0.4 ug/g	Roadway Runoff: grab and ISCO sampler, stored at 4 °C and extracted within 24 hours of sample collection	HPC Standards	6PPDQ-d5	TWP samples methanol-extracted; TWP leachate, roadway runoff, and creek water samples were SPE extracted (Oasis HLB cartridges, eluted with methanol and concentrated to 1 mL)	LC-MS/MS	Agilent Poroshell HPH-C18 column (2.1×100 mm, 2.7 µm) preceded with a C18 guard column (2.0 × 4 mm). LC-MS grade water (A) and methanol (B), both with 0.1% formic acid, were used as mobile phases	ESI+ mode Dynamic multiple reaction monitoring mode (dMRM)	299.2/215.1	299.2/187.1	Zhao 2023 https://doi.org/10.1021/acs.est.2c08690
48	Stormwater runoff	LOQ Stormwater: 5.1 ng/L	For exposures, glass aquaria	HPC Standards	D ₅ -6PPD-Q	SPE: Waters Oasis HLB (200 mg, 6 mL) cartridges, with 0.5 g pre-cleaned micro glass beads (Filter Aid 400, 3M, MN) to prevent clogging, eluted with methanol, concentrated with nitrogen	UHPLC-MS/MS	Agilent Poroshell HPH-C18 (2.1 × 100 mm, 2.7 µm) with C18 guard column and gradient with 0.1 % formic acid in water and 0.1 % formic acid in methanol	ESI+ MRM	299.2/215.1	299.2/287.1	Tian 2022 Revised https://doi.org/10.1021/acs.estlett.1c00910

Table 5-3. 6PPD-Q Analytical Methods

Matrix	Detection Limit	Container & Storage	Analyte Vendor	Internal or Surrogate Standards	Sample/Pretreatment, Extraction, and Clean-Up	Instrumental Analysis	LC or GC	MS	Quantitation Ion	Confirmation Ion	Reference	
Stormwater runoff					Sand filtration, Ion exchange, XAD-2 fractionation, Silica gel fractionation, Parallel HPLC fractionation, Sequential HPLC fractionation	<p>UPLC-QTOF-MS/MS Reversed-phase C18 analytical column (Agilent ZORBAX Eclipse Plus 2.1×100 mm, 1.8 μm) with a C18 guard column (2.1×5 mm, 1.8 μm). For ESI+: 0.1% formic acid in each of water (A) and methanol (B). for ESI-: 1 mM ammonium fluoride in water (A) and methanol (B)</p> <p>UPLC-Orbitrap-MSn Agilent InfinityLab Poroshell 120 EC-C18 column (2.1 × 100 mm, 1.9 μm) with 0.1% formic acid in each of DI water (A) and MeOH (B)</p> <p>GC-QTOF-HRMS Agilent HP-5MS UI column (30m x 0.25 mm x 0.25 μm film thickness)</p>	<p>UPLC-QTOF-MS/MS ESI+/- Full scan HRMS data acquired at the range of 100-1700 m/z in 2 GHz Extended Dynamic Range mode. For structure elucidation, MS/MS data was acquired by data-dependent acquisition (m/z 50-1700, collision induced dissociation at 10, 20, and 40 eV) using lists of preferred precursors prioritized during initial MS-only screening</p> <p>UPLC-Orbitrap-MSn ESI+ with targeted MS2 and MS3 acquisition</p> <p>GC-QTOF-HRMS The acquisition rate was 200 spectra/s over the range of m/z 45–550. EPA Method 8270 internal standards were added to monitor the analytical performance</p>					Tian Discovery DOI:10.1126/science.a bd6951

6 MITIGATION MEASURES AND SOLUTIONS

ITRC has developed guidance on the overview of the current understanding of 6PPD-quinone (6PPD-q) sources, exposure, fate, transport, toxicity, mitigation strategies, on-going research, and data needs. This section of the ITRC guidance describes:

- Assessment of chemical alternatives to 6PPD in tires
- Mitigating the impacts of 6PPD-q in the environment through pollution prevention, air particulate mitigation, and stormwater source control measures
- What is known and unknown about remediating 6PPD-q if it persists in the environment
- Laws, regulations and policies that might need be considered by decision-makers when addressing 6PPD and 6PPD-q

6.1 Introduction

Near and long-term solutions are being evaluated to reduce and prevent harm to aquatic organisms and ecosystems from 6PPD and 6PPD-q. Additionally, many of the proposed solutions also could address human health impacts if 6PPD-q is found to negatively affect human populations. This chapter will describe the status of identifying potential alternatives to 6PPD in tires, evaluating and implementing mitigation strategies such as stormwater control measures (SCMs) and air particulate mitigation, and determining the need for remediation of media that have been impacted by 6PPD-q. The chapter also provides a description of policies, regulations, and laws that could be considered by decision-makers when assessing potential 6PPD planning activities. **Table 6-1** below summarizes the anticipated timescales of implementing 6PPD solutions, the duration of effects, and the scale of effort that will be required to achieve that solution at a meaningful scale.

Some of the research described throughout this section addresses tire wear particles (TWPs) instead of 6PPD-q explicitly. Due to how recent the discovery of 6PPD-q was, TWP research can be a helpful resource for understanding mitigation effectiveness. Additionally, TWP research included in this document was limited to studies where preventing or mitigating TWP pollution is also likely to address 6PPD and 6PPD-q.

Table 6-1: Predicted Solutions Timescales and Level of Effort

Solution	How soon could this solution be implemented?	How long would impacts be felt from this solution?	What is the effort of change required for this solution to be implemented?
<u>Alternative chemicals to 6PPD in tires</u>	Long-term – It could take several years to identify a safer alternative to 6PPD and even longer to phase 6PPD into new tires.	Long-term – This solution directly reduces the source of 6PPD-q.	Moderate effort to systemic change – Most of the burden of effort would be on the tire production

Solution	How soon could this solution be implemented?	How long would impacts be felt from this solution?	What is the effort of change required for this solution to be implemented?
			industry, but change would be widescale.
Driver behavior (e.g. tire care, braking and accelerating practices)	Near-term – Individual drivers could start implementing changes now.	Short to mid-term – Since this solution does not address root problems, it is only impactful if many drivers continue to practice changes.	Status quo to moderate effort – Only effective if large numbers of drivers change driving norms.
Driving alternatives to single occupancy vehicles in transportation planning	Mid to long-term – Transportation planning changes often require several years to plan and implement.	Long-term – Reducing the number of tires on the road could have long-lasting effects.	Systemic change – Would need to fundamentally change how our transportation system works, shifting the dominant mode of transportation from single occupancy vehicles to other options.
Vehicle design (weight, load distribution, location/number of drive wheels, suspension type, regenerative braking, specialized tires, etc.)	Long-term – Would take several years to innovate new vehicle designs.	Long-term – Would reduce or eliminate the amount 6PPD-q entering the environment.	Moderate change – The burden of effort would be on the vehicle manufacturing industry to test innovative designs and address potential negative impacts from electric vehicles (e.g. heavier vehicles).
Road Surfaces (e.g. road surface composition, porous pavement)	Long-term – Would take several years to repave surfaces.	Mid to long-term – Would reduce or eliminate the amount 6PPD-q entering the environment for several years.	Moderate effort – Would require a high degree of effort to implement but would not change many systems.
Traffic management (e.g. road design, roundabouts)	Near to mid-term – Depends on the traffic management change.	Long-term – Traffic management changes could reduce the generation of TWP for several years.	Status quo – Most traffic management changes could take place within current systems.
Road maintenance (e.g. street sweeping, regularly reducing road roughness)	Near to mid-term – Is location-dependent.	Short to long-term – Impacts are variable and often depend on the frequency of street sweeping and maintenance.	Status quo to moderate effort – Is location dependent. Many areas of the US have street sweeping, while others would need to implement new street sweeping practices.
Air particulate mitigation (e.g.	Near-term – Many infrastructural changes that help	Long-term – Changes could have long-lasting	Status quo to moderate effort – Many

Solution	How soon could this solution be implemented?	How long would impacts be felt from this solution?	What is the effort of change required for this solution to be implemented?
<u>roadside vegetation, solid structures)</u>	with air particulate mitigation are commonly installed.	benefits for air and water quality.	infrastructural changes are commonly installed but would take a degree of effort to install at a larger scale.
<u>Capture and retain and capture and treat stormwater control measures (SCMs)</u>	Near to mid-term – Depends on if SCMs are installed through new development, redevelopment, or retrofits and if it is required through regulations.	Mid to long-term – The duration of impacts depends on the longevity of the SCM used and maintenance practices.	Status quo or moderate effort – Depending on the location, SCMs are either commonly installed as part of new or redevelopment, or this is not a common practice. More funding and policy changes are required to implement SCMs at a meaningful scale.
<u>Remediation of 6PPD and 6PPD-q. However, if these chemicals are discovered to not persist in the environment, then remediation is not needed.</u>	Mid to long-term – Mid-term implementation could be achieved through voluntary actions. It will take several years to develop regulatory criteria.	Long-term – Remediation would permanently remove or capture 6PPD and 6PPD-q at contaminated sites.	Systemic change – There is a high degree of effort that is needed to develop appropriate cleanup levels for media, including regulatory processes.

Table 6-1 summarizes the predicted timescales of implementation and impacts and the degree of change for each proposed 6PPD solution other than laws, policies, and regulations. This information was determined based on the best available science and perspectives from subject matter experts. Due to the limited availability of information on 6PPD to-date, these timescales and impacts are subject to change as new knowledge emerges.

The factors considered in Table 6-1 were qualitatively assessed using the following criteria:

- ***How soon could this solution be implemented?***
Near-term = could be implemented in less than 5 years
Mid-term = 5-10 years
Long-term = greater than 10 years to implement.
- ***How long would impacts be felt from this solution?***
Short-term = impacts would last no longer than 5 years
Mid-term = 5-10 years
Long-term = impacts would last for longer than 10 years.
- ***What is the effort of change required for this solution to be implemented?***
Status quo = the solution could be implemented feasibility within existing policies and

societal norms
Moderate effort = the solution might require some policy or societal change, but largely still could be achieved within current systems
Systemic change = many policies and societal norms would need to change to implement this solution.

This analysis does not explicitly consider the cost of changes, which would need to be determined on a location-by-location basis.

6.2 Tire Alternatives and Innovation

6PPD is a widely used **antiozonant** and **antioxidant** in almost all automotive tires and serves a critical role in tire safety and performance by preventing cracking and the degradation of rubber compounds. For a less toxic alternative to be identified and deployed for use in tires, it would need to be tested for multiple characteristics. A chemical alternative to 6PPD would have to mix well with the **rubber matrix**, be compatible with the manufacturing and **vulcanization** process, and provide protection from ozone for the life of the tire. Potential alternatives to 6PPD in tires would need to meet relevant regulatory requirements to ultimately be brought to market. Chemicals that will be entered into commerce in the United States are regulated by EPA under the **Toxic Substances Control Act (TSCA)**. Under TSCA, USEPA “...evaluates potential risks from new and existing chemicals and acts to address any unreasonable risks chemicals may have on human health and the environment” (USEPA 2023). Tire formulations are also regulated for safety and performance by the Department of Transportation Federal Highway Administration (DOT-FHWA) under the [Federal Motor Vehicle Safety Standards](#).

6.2.1 Potential 6PPD Alternatives

Other p-Phenylenediamine chemicals (PPDs) may be the most promising replacements for 6PPD from a performance perspective due to their similar structure. They are most likely to have the proper **solubility**, **migration rate**, rate of reaction with ozone, and compatibility with **vulcanization**. However, just as with 6PPD, they are also likely to form **quinones**. This is a potential concern, but showed that several other PPD quinones do not have similar toxicity to 6PPD-q towards rainbow trout, and the other PPDs form different **metabolites** compared to 6PPD-q, suggesting that they may be applied as safer replacements to 6PPD. This was supported by research showing that 77PD-quinone, the polymer additive C₂OH₃4N₂O₂, did not cause acute toxicity in coho salmon, while 77PD did ([Chaplet et al., 2023](#)). However, more toxicity testing is needed to further explore the toxicity of transformation products to salmonids. Additionally, a suitable alternative needs to be evaluated to ensure that it meets tire performance standards.

In addition to other PPDs, a [2021 UC Berkeley study](#) investigated several other options, including modification of 6PPD to prevent quinone formation, food preservatives, **lignin** derived chemicals, and alternative rubber formulation which would require less protection against ozone. These alternatives all require significant work to develop, however, as there remain data gaps as to toxicity towards fish and viability in tires.

The California Department of Toxic Substances Control (DTSC) included a section on potential alternatives to 6PPD as part of their [Priority Product Report](#), which in addition to the options

mentioned above, also discusses reducing the formation of TWP and the release of 6PPD-q via other methods. These include a coating to prevent ozone from reaching tires, airless/non-pneumatic tires, and spring tires.

Other efforts are underway to develop novel antiozonants as well, but more time is needed before results may be realized. In April 2023, Flexsys, a major 6PPD manufacturer, and the United States Department of Agriculture (USDA) announced a [Cooperative Research and Development Agreement](#) to design and evaluate novel alternatives to 6PPD. In addition, the EPA's Small Business Innovation Research (SBIR) Program 2023 funding solicitation included a topic area on rubber anti-degradant technologies for tires and other rubber products that are lower concern for human health and the environment. Washington's Ecology sponsored the Tire Innovation Forum in December 2022, facilitated by the Sustainable Chemistry Catalysts. The Forum helped to create a road map for developing safer alternatives to 6PPD (Sustainable Chemistry Catalyst 2023).

To ensure that these alternatives do not have equivalent or worse hazard traits or other regrettable tradeoffs, Washington's Ecology will conduct and California's DTSC will require tire manufacturers to conduct an alternatives assessment (AA). The National Research Council defines AA as the "process for identifying and comparing potential chemical and non-chemical alternatives that could replace chemicals of concern on the basis of their hazards, comparative exposure, performance, and economic viability" (National Research Council 2014). The careful consideration of replacement chemicals and other types of alternatives will help to avoid regrettable substitutes. For more information see section 6.8 (State actions on 6PPD & q).

CASE STUDY: Source Control for Roadway Stormwater Pollutants

Stormwater pollutants from vehicles can be addressed through source control initiatives. Source control means reducing or removing the pollutant from its source, rather than treating or removing it once it is released. The following case study on copper brake pads demonstrates how states can work with the USEPA to reduce stormwater pollutants.

Copper at elevated concentrations in stormwater runoff is highly toxic to fish and other aquatic organisms. Copper can interfere with a fish's olfaction, thereby harming the ability of migratory species—especially salmon—to navigate to their spawning grounds and making them more vulnerable to predation. Other studies show that young salmon are especially vulnerable to the toxic effects of copper. Since the 1990s, states and municipalities have recognized that vehicle brake pads were a significant source of copper contamination in urban stormwater runoff. As they wear, brake pads release fine dust containing copper and other metals that are deposited on roadways where stormwater can suspend and transport them to streams and other waterways.

To address the copper stormwater contamination problem, the states of California and Washington enacted legislation in 2010 to phase out the use of copper in vehicle brake pads and shoes by 2025. Washington adopted the “Better Brakes Law” (Chapter 70A.340 RCW) that phased out the sale of brake material containing copper exceeding 5% (by weight) by 2021, with a further phase-down to no more than 0.5% (by weight), if low-copper alternatives are available. Similarly, in 2010 California adopted its Brake Pad Law (SB346) with the same deadline for reducing copper concentrations to no more than 5% (by weight) by 2021. Further, California set a 2025 deadline for limiting copper concentrations to no more than 0.5% (by weight).

Both statutes addressed the concern over finding suitable alternatives to copper brake pads by directing the state regulatory agencies to study alternatives and determine whether low-copper pads were available and if extensions of the deadlines were needed. The statutes also addressed the problem of stranding inventories of copper brake pads exceeding limits by allowing manufacturers to sell their existing inventories of non-compliant materials after the deadlines.

Spurred by the laws in California and Washington, in January 2015, the brake manufacturers executed a Memorandum of Agreement with the U.S. Environmental Protection Agency and the Environmental Council of the States that adopted national, but voluntary standards for brake pads based on the California and Washington standards, that is, reductions to <5% (by weight) by 2021 and to <0.5% by 2025. The agreement also called for reductions in mercury, lead, cadmium, asbestiform fibers, and chromium-six salts in brake pads. The Agreement established a LeafMark rating system whereby brake pads would be labeled according to a three-tier system based on the amount of copper in brake pads being sold.

These measures have shown significant reductions in copper concentrations in urban stormwater run-off. In a [2022 report to the legislature](#), the California regulatory agencies reported that more than 60% of brake pads on the market contained less than 0.5% copper (by weight), which resulted in an estimated 28% decrease in copper entering urban stormwater run-off.

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Both statutes addressed the concern over finding suitable alternatives to copper brake pads by directing the state regulatory agencies to study alternatives and determine whether low-copper pads were available and if extensions of the deadlines were needed. The statutes also addressed the problem of stranding inventories of copper brake pads exceeding limits by allowing manufacturers to sell their existing inventories of non-compliant materials after the deadlines.

Spurred by the laws in California and Washington, in January 2015, the brake manufacturers executed a Memorandum of Agreement with the U.S. Environmental Protection Agency and the Environmental Council of the States that adopted national, but voluntary standards for brake pads based on the California and Washington standards, that is, reductions to <5% (by weight) by 2021 and to <0.5% by 2025 (OW US EPA 2015b). The agreement also called for reductions in mercury, lead, cadmium, asbestiform fibers, and chromium-six salts in brake pads. The Agreement established a LeafMark rating system whereby brake pads would be labeled according to a three-tier system based on the amount of copper in brake pads being sold.

These measures have shown significant reductions in copper concentrations in urban stormwater run-off. In a [2022 report to the legislature](#), the California regulatory agencies reported that more than 60% of brake pads on the market contained less than 0.5% copper (by weight), which resulted in an estimated 28% decrease in copper entering urban stormwater run-off.

6.2.2 Hazard Assessment

A [technical memorandum](#) released in November 2021 from the Washington State Department of Ecology (WA Ecology) under the direction of the Washington State Legislature that evaluated the known hazards of several potential alternatives. Although some potential alternatives were identified with lower hazard than 6PPD, none of the alternatives have been tested for toxicity towards coho salmon. In addition, the suitability of these alternatives from a performance perspective compared to 6PPD was questioned in comments by industry members.

6.2.3 Hazard Criteria

No state or federal regulations currently specify toxicity criteria for potential alternatives to 6PPD. Lack of regulatory certainty may hinder the development of alternatives to 6PPD. In an effort to address this problem, WA Ecology has released the [hazard criteria](#) that they will be using for their Alternatives Assessment. This hazard criteria uses the Safer Products for Washington criteria, which includes several human health and environmental health criteria like **carcinogenicity**, reproductive or developmental toxicity, skin or respiratory **sensitization**, acute or chronic aquatic toxicity, and **bioaccumulation**. In addition to these criteria, this assessment will include additional aquatic toxicity criteria. First, to be considered a safer alternative to 6PPD, toxicity testing must be performed on coho salmon, rainbow trout, and two other **trophic levels** for both alternative chemicals and their **degradants** after exposure to ozone. Since it is known that these chemicals will find their way into salmon-bearing streams, WA Ecology has also set an upper limit on the toxicity of these potential alternatives (that is, LC50, or the concentration at which 50% of test subjects experience mortality, must be greater than 0.1mg/L) **(Citation needed?)**.

6.3 Mitigation Practices

This section describes many efforts to understand how to mitigate the effects of 6PPD-q on the environment by preventing, treating, or managing 6PPD-q before it causes negative environmental impacts. Mitigation actions include pollution prevention efforts and control measures for stormwater and air quality mitigation.

6.3.1 Pollution Prevention

Pollution prevention consists of actions taken to stop a pollutant from entering the environment or from reaching sensitive **receptors**. Implemented actions could involve separating locations physically and/or managing activities that are pollutions sources. Some entities, such as WA Ecology, refer to this type of mitigation as **source control**.

The Washington State [Stormwater Management Manuals](#) define source control measures as: “A structure or operation intended to prevent pollutants from coming into contact with stormwater through physical separation of areas or careful management of activities that are sources of pollutants.”

In WA Ecology’s [BMP effectiveness report](#), street sweeping and cleaning roadside ditches, catch basins, and storm drains were ranked as having a high potential of preventing tire wear particles from entering waterways. Moderately effective measures included education and outreach

practices. The following information describes the current state of knowledge on the effectiveness of pollution prevention measures on the mitigation of 6PPD-q in stormwater.

6.3.2 Driver Behavior

Driver behavior has a direct impact on the generation rate of **tire wear particles**. Frequent or abrupt acceleration and braking, as well as sharp high-speed turns can all increase tire wear rate ([Zhang et al. 2024](#)). Proper tire inflation also plays a role in the wear rate of both passenger vehicle and heavy truck and bus tires. For example, truck and bus tires which are underinflated by 15% may experience an 8% decrease in **tread mileage** over the lifetime of a tire. Research by Verschoor and de Valk (2018) indicate that tire wear particle emissions could potentially be reduced by 14% through the use of tire pressure monitors. Improper vehicle suspension alignment can also increase tire wear rates. Education regarding driver practices and vehicle maintenance can reduce the generation rate of tire wear particles ([ACU Tread 2017](#)), which may reduce 6PPD-q in the environment.

The [Puget Sound Starts Here campaign](#) (September – October 2023) was an effort in WA State to cultivate more awareness over how proper tire care can reduce 6PPD-q in stormwater. The campaign will use the Terry the Tire mascot as an attention grabber, message recall, videos, and social media posts to show how easy it is to check and inflate your tires. Figure 6-2 presents an example of how Terry the Tire will be used in this campaign.

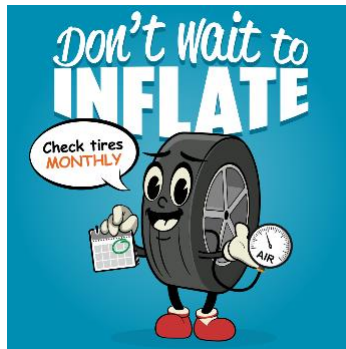


Figure 6-2. Example how Terry the Tire is used in the Puget Sound Starts Here campaign.

6.3.3 Driving Alternatives

According to the [U.S. Department of Energy](#), 75% of people in the U.S. drive a personal vehicle to work, 9% carpool in a personal vehicle, 7% work from home, 5% take public transit, 3% walk, and 1% bike to work. Increasing transportation options and reducing barriers to accessing those options reduces TWP pollution in the environment through the reduced use of personal vehicles. Additional alternatives to driving personal vehicles include increased telework options and increasing the number of walkable and accessible communities.

6.3.4 Vehicle Design

The vehicle characteristics that have an impact on tire wear include weight, load distribution, type/condition of suspension, and the drive wheel location. Several companies are developing devices to capture **tire wear particles** as they are generated. These include [The Tyre Collective](#),

[GelBko](#), and Nexen's Pureback tire. Currently, none of these broader types of collection techniques have been sufficiently developed and evaluated to be commercially viable, and the extent to which they would reduce 6PPD and 6PPD-q release to the environment remains to be seen.

Though **electric vehicles (EVs)** can be seen as a promising alternative for climate change and some air pollution mitigation, it is unclear whether electric vehicles will result in increased or decreased tire wear ([OECD 2023](#)). The average EV is heavier than its gas-powered counterpart due to the weight of the batteries. Some studies suggest that EVs will have increased tire wear; however, it is possible that **regenerative braking** and specialized tires would cancel the impacts of increased vehicle weight and torque. In addition, promoting smaller, more lightweight vehicle designs, including 2- and 3-wheel vehicles, could reduce overall vehicle weights and the amount of tire wear associated with the electrification of the motor vehicle fleet.

6.3.5 Road Surfaces

Road surface composition can affect tire wear. The roughness and frictional characteristics of a paved surface play an important role in tire wear. An improvement in roughness was found in **asphalt rubber – asphaltic concrete friction courses (AR-ACFC)** over Portland cement concrete (PCC), resulting in reduced tire wear ([Allen 2006](#)). The addition of ground tire rubber in road surfaces was found to not result in a release of 6PPD-q above the **LC50** for coho salmon (Lokesh et al. 2023; Nedrich 2022). Porous pavement has also been found to improve water quality from arterial road runoff (Holzer and Lindbo 2018, Holzer 2023)) and have been found to remove the majority of TWP mass and a significant portion of 6PPD-q from stormwater ([Mitchell and Jayakaran 2024](#)).

6.3.6 Traffic Management

The design of the road itself impacts driving characteristics and tire wear. Reducing torque and friction, which are actions that contribute to tire wear, is the goal of traffic management as a mitigation effort. Actions to achieve this might include minimizing the need for high speeds, sharp turns, and reducing the frequency of acceleration and deceleration. Roundabouts and syncing up stop lights to increase traffic flow could also reduce tire wear particles, so long as harsh braking and acceleration are avoided ([Liu et al. 2022](#)).

6.3.7 Road Maintenance

Reducing road roughness (fewer bumps, potholes, etc.) through regular maintenance can result in reduced tire wear. The effectiveness of **street sweeping** for controlling tire wear particles and potentially 6PPD-q is uncertain. However, based on a literature review, a WA Ecology report determined that street sweeping could have a high potential effectiveness ([WA Ecology 2022](#)) at capturing tire wear particles. The effectiveness of street sweeping is dependent on the efficiency in collecting various particle sizes, the frequency of street sweeping and timing relative to rain events, type of street sweeping equipment used, and other impervious surfaces downwind of the road ([Evergreen StormH2O 2023](#)). Studies to assess the effectiveness of street sweeping are underway, including a [paired watershed study](#) from the Stormwater Action Monitoring program in Washington State.

6.4 Air Particulate Mitigation

Tire wear emissions initially occur as airborne particles ([Kuraishi et al. 2018](#)). The extent of transport of these particles at or away from the road depends on many factors including the size of particles emitted, the number and type of vehicles on the road, the activity of vehicles on the road, local meteorology, and local terrain conditions. Because a portion of tire wear particles containing 6PPD-q have been identified in airborne particulate matter (PM) samples collected away from the road, a holistic 6PPD-q mitigation strategy will target both air particulates and stormwater runoff.

Roadside vegetation and solid structures (e.g. noise walls, buildings) can be used to mitigate air pollution near the road. Some of this reduction is through dilution, while some forms and characteristics of vegetation can also capture and remove airborne particles. Solid structures located adjacent to roadways, such as noise barriers or K-rail barriers, can inhibit air flow away from the roadway and increase deposition of larger tire wear particles, thus decreasing transport of tire wear particles that may contain 6PPD-q. However, studies on K-rail barriers are limited, and to have this effect they must be next to the travel lane. Particles across a range of sizes, from larger coarse PM to small nano-size particles, can also be removed by impaction and diffusion onto vegetation surfaces. These particles typically remain on the vegetation until removal by precipitation or as debris from falling leaves or needles. Certain vegetation characteristics can have positive effects, reducing the number of particles by 50% or more; however other vegetation characteristics (that is, highly porous, gaps between trees, high canopy trees) can lead to increased air pollution concentrations near the road. [An EPA report](#) titled “Recommendations for Constructing Roadside Vegetation Barriers to Improve Near-Road Air Quality” describes the characteristics of vegetation needed to achieve air pollution control and the characteristics to avoid (Baldauf 2016). Placing properly designed vegetation and solid structures along the roadside may help capture airborne 6PPD-q containing tire particles at the roadside that can be treated using the stormwater runoff mitigation strategies described in the next section. This could prevent transport of 6PPD-q over large distances that will require treatment at the watershed level rather than focused on the roadside.

Beyond mitigating for 6PPD-q, implementing air particulate mitigation strategies could lead to co-benefits, or multiple environmental and social benefits. Communities near roadways are disproportionately lower-income and non-white and often experience disproportionate environmental harms (Rowangould 2013). Air particulate mitigation could both reduce airborne 6PPD-q and address existing environmental justice concerns (Tian and Barzyk 2023). These mitigation strategies could also reduce noise pollution and additional toxics in the environment ([USEPA 2017](#)). Installing roadside vegetation could also reduce the urban heat island effect, which is a climate change mitigation and adaptation measure ([USEPA 2023](#)).

1

CASE STUDY: Roadside Green Infrastructure Characteristics that Benefit Air Quality, USEPA

Provided by the USEPA, the following three field studies demonstrate how roadside green infrastructure can increase air quality. The strategies used in these studies to mitigate particulate matter (PM) could also be beneficial for 6PPD-quinone mitigation, though this remains untested. These studies are ongoing and unpublished.

The first study was conducted at Detroit Kemeny Park in Michigan. Roadside vegetation was planted along the boundary between Kemeny Park and a major highway (I-75) in Detroit. PM and other air pollutant measurements were collected before the planting and measurements are being collected currently as the vegetation grows. Lessons learned are that planting new vegetation for air quality benefits can be difficult since mature plants have a lower survivability rate and require some spacing to allow growth. However, air quality benefits can be achieved after 3-5 years of growth. To achieve air quality benefits right away, hedges and bushes, or a solid structure like a fence, are needed to get initial air quality benefits while trees and larger vegetation grows.

The second case study took place at Brookfield Elementary School in Oakland, California. Roadside vegetation was planted along the boundary of a school playground and a major highway (I-880) in Oakland. PM and other air pollutant measurements were collected before planting and continue as the vegetation grows. Lessons learned include planting next to an existing noise barrier provides immediate air quality benefits from the solid wall, and these benefits increase as the vegetation grows. The case study also highlights the need to adequately assess soil quality and provide stormwater management infrastructure simultaneously as approximately half the vegetation originally planted did not survive a flood event that occurred during the first year of planting.

A third field study was conducted that compared air quality downwind of a large highway and how PM and other air pollution varied behind differing vegetation characteristics. Lessons learned include that vegetation must have low porosity and have coverage from the ground to the top of the canopy to achieve air quality benefits. Gaps in the vegetation, along with high porosity bushes and trees, can actually lead to increased downwind air pollution levels. ([Deshmukh et al. 2019](#))

2 6.5 Stormwater Runoff Mitigation: Stormwater Control Measures (SCMs)

3 Stormwater runoff can be defined as rain or snow melt that flows on the land surface rather than
4 infiltrates into the subsurface. When flowing over impervious surfaces like paved streets, parking
5 lots, and rooftops, runoff picks up numerous pollutants, including 6PPD-q. If left untreated,
6 runoff can transport those pollutants into streams and other waterways, degrading water quality.
7 Stormwater controls, known as **stormwater control measures (SCM)** are helpful tools to slow the
8 flow of runoff and remove pollutants. Some entities refer to SCMs as **best management practices**
9 **(BMPs)**. This document will use the term SCM for consistency, except when referring to a
10 source document that uses the term BMP.

6.5.1 Potential SCM Effectiveness

The Washington State Department of Ecology commissioned a literature review to assess different mitigation strategies and predict potential SCM effectiveness for 6PPD-q (Environmental Assessment Program and Water Quality Program 2022; Navickis-Brasch et al. 2022).

The document divided SCMs into two categories: capture and retain or capture and treat measures.

- **Capture and retain SCMs** capture runoff and only release it through infiltration into the ground, evaporation, or evapotranspiration.
- **Capture and treat SCMs** capture runoff and treat it as it flows through soils or vegetation prior to exiting the facility.

The report evaluated currently published SCMs for presumed effectiveness at capturing and treating tire wear particles, 6PPD, and 6PPD-q based on a literature review and the best judgement of subject matter experts (See also Table 6-2). Key findings from this report included:

- **Capture and Retain SCMs:** Bioretention and infiltration basins were SCMs that ranked high for the potential mitigation of 6PPD-q. Permeable pavement was presumed to be a moderately effective SCM, which is supported by recent work done at the Washington Stormwater Center (Mitchell and Jayakaran 2023) and sampling done by City of Gresham, OR (Holzer 2023) (link to road surfaces section above).
- **Capture and Treat SCMs:** Bioretention soil mixes specifically designed for water quality treatment, such as the WA Ecology 60:40 mix (60% sand, 40% stormwater compost), are reported to reduce 6PPD-q exposure and mortality in fish (McIntyre, et al, 2015). Other SCMs, like sorbent medias and media filters, are ranked as likely to have high effectiveness (WA ECY 2022). SCMs relying solely on sedimentation and filtration are only expected to remove particles larger than fine silt (25 to 62.5 µm) and thus provide only a medium level of 6PPD-q removal. Therefore, SCMs with medium potential for 6PPD-q removal include detention ponds and sand filters.

Table 6-2: Example CSMs by potential treatment effectiveness

Source: [Stormwater Treatment of Tire Contaminants / Best Management Practices Effectiveness](#)

Treatment Potential Category	Examples of SCMs
High	Bioretention, infiltration basins, media filter drain, dispersion
Medium	Sand filter, detention ponds, permeable pavements, education and outreach
Low	Perforated stub-out connection, vegetated roofs, tree retention, tree planting

6.5.2 Testing and Verifying SCM Effectiveness

There are several research efforts underway testing the effectiveness of SCMs at retaining and treating 6PPD-q. Some of these studies build off of [WA Ecology's effectiveness literature review](#) and are seeking to verify and optimize the effectiveness of SCMs noted as having moderate to

high potential effectiveness. Other studies are investigating the effectiveness of new stormwater SCMs with different specifications or configurations. The following describes several studies aimed at testing and verifying SCM effectiveness.

6PPD SCM Effectiveness Research – WA Ecology:

- The Department of Ecology’s Water Quality Program has contracted for five BMP effectiveness studies supported by WA State Legislative funds. In addition to Stormwater Work Group 6PPD Subgroup meeting support and a particle size distribution study, Osborn & Evergreen StormH2O Consulting conducted a literature review of existing SCMs and their potential effectiveness at managing 6PPD-q in stormwater.
- The University of Washington-Tacoma is actively studying different engineered medias, commercial sorbent components, and native and engineered soils to gauge their effects on 6PPD-q in stormwater samples.
- King County is performing column tests with stormwater samples to help determine which mixtures of high performance bioretention soil mixes, known as HPBSM, might be effective at reducing 6PPD-q ([King County, 2024](#)). This HPBSM and default bioretention mixes are defined by the Washington State Department of Ecology Stormwater Manuals and can be applied in sensitive watersheds.
- Herrera Environmental Consultants, in collaboration with King County Environmental Lab (KCEL), are testing TAPE, or Technology Assessment Protocol-Ecology, treatment technologies via the influent and effluent of stormwater samples. Additionally, Herrera is developing sampling protocol for 6PPD-q and comparing results across two laboratories – Manchester Environmental Laboratory and KCEL.
- KCEL is working to characterize stormwater in a mixed highway and residential area and are testing for a wide range of contaminants, including 6PPD-q.
- Lastly, Washington State University, in partnership with Evergreen StormH2O, is working to create a street sweeping manual that will help stormwater permittees across Washington state create new or improve existing street sweeping programs. This manual is slotted for release in 2025, ensuring permittees have an additional resource to aid in meeting proposed street sweeping requirements currently in the stormwater permit at the draft stage. It is anticipated that street sweeping will prove an effective source control SCM for 6PPD-q, as outlined in the [BMP Effectiveness Report](#).

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CASE STUDY: 6PPD-q in Highway Runoff, BMP Effectiveness, and Field Protocol Development

Supported by the Washington State Department of Ecology, Herrera Environmental Consultants (Herrera) is conducting 6PPD-q sampling at both the Interstate-5 Ship Canal Testing Facility and the ODOT Stormwater Technology Testing Center. Herrera is partnering with King County Environmental Lab, who is conducting the laboratory analyses of over 400 6PPD-q samples. This sampling occurs throughout “storm events,” or when it rains for at least one hour following a somewhat dry period. Contaminants like 6PPD-q can accumulate on roads when there is little rain, and then can be flushed into stormwater during storm events and discharged into salmon bearing streams.

Herrera’s sampling will:

- 1. Help inform if existing field sampling protocols for collecting stormwater treatment samples, like the use of carboys, type of bottle and pump tubing material, for 6PPD-quinone need to be refined. To ensure that protocols are reliable in different conditions, Herrera will test these field protocols over three storm events.*
- 2. Characterize 6PPD-q in untreated highway runoff in Seattle and Portland. Stormwater characterization helps stormwater managers understand the full chemical profile of contaminants in runoff throughout a storm event, including if there is variability in when 6PPD-q concentrations are highest during the storm. This information is essential for managing and regulating 6PPD-q.*
- 3. Evaluate the ability of emerging stormwater treatment technologies (TAP-E) to reduce 6PPD-q concentrations. Depending on the device, TAP-E devices have been proven to reduce the concentrations of phosphorus, oil, suspended solids, heavy metals, and other contaminants in stormwater. This study will test some of these implemented devices for their effectiveness with 6PPD-q treatment.*
- 4. Assess if there are differences between the reported values of 6PPD-q between two different laboratories: King County Environmental Lab and Manchester Environmental Lab. As laboratory methods are still being developed, it is crucial to understand what might contribute to differences in how laboratories analyze samples.*

All in all, this project is expected to improve understanding of 6PPD-q levels in runoff, assess if existing and already implemented stormwater technologies can filter this emergent contaminant, and will help improve field sampling protocols to ensure that samples intended for 6PPD-q testing remain viable and reliable.

2 Stormwater Action Monitoring (SAM) Program – WA Ecology

- 3 This program has two ongoing 6PPD projects funded by the 2022-2023 proviso granted to WA**
- 4 Ecology’s Water Quality Program.**

- One project in partnership with Washington State University, “Longevity of Bioretention Depths for Preventing Acute Toxicity from Urban Stormwater Runoff,” seeks to determine the efficacy of bioretention media over time. This study is ongoing with preliminary, unpublished results indicating that bioretention media is an effective stormwater management tool for 6PPD-q through a minimum of 11 water years as shown by the survival rates of the experiment’s coho fry.
- Another project in partnership with the City of Redmond seeks to study the benefits of street sweeping in receiving water conditions, and this long-term project has, as of 2022, parameters for 6PPD-q added to its scope of study.
- Seattle Public Utilities, in collaboration with Herrera Environmental Consultants, is seeking to understand the potential for reducing 6PPD-q loads with street sweeping of arterial roads in the City of Seattle. Researchers will investigate possible correlations between 6ppd loading and other stormwater contaminants, and will characterize the seasonality, particle size distribution, and land use differences in street sweeping loads. This project intends to provide evidence of the effectiveness of street sweeping as a source control SCM. Results from the project will inform municipal street sweeping programs in determining the timing, frequency, and location of street sweeping for effective reduction of 6ppd and other stormwater contaminants.
- King County, in partnership with Whatcom County, is seeking to test HPBSM at a full-scale stormwater facility. This study will build upon King County’s HPBSM column bench study ([link to above section on King County’s HPBSM](#)) to test and monitor how HPBSM performs in the field. The HPBSM will replace a set of underperforming bioretention swale cells installed in 2007 in a neighborhood in Bellingham.
- Finally, the City of Tacoma, in partnership with the University of Washington-Tacoma, Herrera Environmental Consultants, and Aspect Consulting are monitoring stormwater outfalls for contaminants of emerging concern (CECs), including 6PPD-q, at 15 sites in the Puget Sound region. This information will be used to predict the magnitude of CEC export in stormwater from different land uses across the region. The dataset will be available for permittees and others to use in pollutant modeling and management efforts.

Floating Treatment Wetland/Biomedial Modules to Reduce Stormwater Contaminants with a Focus on 6PPD-q – University of Washington:

Funded by King County, this research project has used Floating Treatment Wetlands (FTWs) as a vehicle for getting a tested **biomedial mix** into the effected waterbody at the point of stormwater entry ([Seebacher et al. 2023](#)). Using a FTW/biomedial module planted with wetland species and a biomedial mix within and underneath, a mesocosm experiment resulted in 79% 6PPD q reduction (255 ng/L to 53.5 ng/L) and 100% survival of juvenile coho after a 24-hour exposure period with no symptoms of 6PPD q toxicity. This potential SCM would be considered a **treatment facility** and is not intended to take the place of other SCMs but could be utilized at those sites where other SCMs are not feasible and/or in addition to other SCMs to increase the efficacy in the field.

Self-Contained Portable Treatment System – US Department of Transportation

USDOT awarded two grants via the 2023 DOT SBIR solicitation 23-FH1 for “Addressing Stormwater Runoff with a Self-Contained Portable Treatment System.” The awardees will work “...to develop a mechanism to remove suspended and dissolved pollutants and other emerging

stormwater pollutants from highway construction and urban stormwater runoff sources... The proposed technology will be developed for deployment at highway stormwater outfalls to provide treatment of pollutants just prior to discharging stormwater into receiving water bodies.” The system may address stormwater "hotspots" of highly contaminated runoff or areas that generate pollutants of high concern such as 6PPD-q.

NCHRP Research Project 25-61: On-Bridge Stormwater Treatment Practices

This project aims to develop “1) on-bridge stormwater treatment applications with a focus on retrofitting existing bridges utilizing proven stormwater treatment practices and 2) guidelines for selection (e.g., by media and materials), design, placement, and maintenance of the developed on-bridge stormwater treatment applications.” The study includes 6PPD-q as one of the analytes. Publication is anticipated in 2024.

CASE STUDY: Mobile Biofiltration Unit – Nisqually Indian Tribe and Cedar Grove Compost

A pilot project by the Nisqually Tribe and partners has been testing a mobile biofiltration unit, meant to filter harmful materials from stormwater at Ohop Creek in WA. It collects stormwater from over 13,000 square feet of roadway and filters it through layers of compost-based filtration media to remove toxic compounds. It also has an external polishing layer to remove phosphorous before the water is discharged to the wetlands near the creek. They first operated the unit in Summer 2022 and preliminary results from 3 rain events showed high effectiveness, reducing the 6PPD-q levels low enough to not be acutely toxic to coho (greater than 90% reduction in 6PPD-q concentrations). They are looking to conduct more sampling in the future with the goal of getting the system approved for use in projects across Washington.

Opportunities for Stormwater Retrofit - Washington Department of Transportation (WSDOT)

Washington State is focusing on the importance and urgency of treating stormwater including 6PPD-q) and other pollutants of concern, as demonstrated by the Washington State Legislature’s intent to provide \$500 million over 16 years for Washington State Department of Transportation (WSDOT) stormwater retrofits on existing transportation infrastructure, with an emphasis on green infrastructure retrofits. WSDOT is working on a GIS-based stormwater retrofit prioritization update to address priority focus areas identified in the associated legislative budget proviso, including salmon recovery and ecosystem health, reducing pollution, addressing health disparities, and cost

effectiveness. The prioritization update is being developed with input from tribes, federal, state, and local governments, scientists, and others and includes consideration of environmental justice principles and tribal treaty rights. WSDOT is involved in other work to mitigate the effects of 6PPD-q exposure, including efforts to advance science, improve our stormwater features inventory, invite and streamline partnerships, and workforce development in the field of green stormwater infrastructure. One example of where these concepts come together is the Urban Stormwater Partnership - I-5 Ship-Canal Bridge Pilot (Seattle). This pilot project leverages partnerships to establish a multijurisdictional stormwater facility that not only treats high volumes of 6PPD-q and other pollutants, but will also incorporate community identified needs such as public access, park-like features, educational signage, and art. The project also provides opportunities to research the effectiveness of 6PPD-q treatment.

[Transportation Pooled Fund Study #1600: Stormwater Management to Address Highway Runoff Toxicity Due to 6PPD-q from Tire Rubber](#)

In 2023, State Departments of Transportation (DOTs), including WSDOT, CalTrans, ODOT, PennDOT, Alaska DOT, and Nevada DOT funded a “[Transportation Pooled Fund](#)” to study roadside treatment options. The stated objectives of this research are “...to equip DOTs with a targeted approach for effectively managing 6PPD-q in highway runoff by 1) developing methods/criteria for identifying locations that need focused treatment, 2) developing a better understanding of the fate and transport of 6PPD and 6PPD-q, 3) developing cost-effective design guidance for stormwater treatment and management techniques with a focus on reducing the effects of 6PPD-q on receiving waters, and 4) evaluating the degree of 6PPD-q release from pavement products made from recycled tire materials. This research will also assist with providing regulatory agencies with a better understanding of DOT management options including the feasibility, limitations, and effectiveness of treatment methods.” (The National Academies of Sciences, Engineering, and Medicine 2023)

[Mitigating 6PPD-q in Stormwater – US Environmental Protection Agency](#)

EPA’s Office of Research and Development is conducting research related to mitigation of 6PPD-quinone in stormwater to address data gaps, including work on tire wear emissions, fate and transport, and management solutions to mitigate stormwater contamination. Research projects include work on developing and enhancing tools that integrate effective urban green infrastructure programs and assessment techniques for multiple ecosystem services (e.g., the integrated benefit of green infrastructure programs for air quality with improved stormwater management), case studies of green infrastructure solutions to reduce contaminated urban stormwater runoff impacts using the [Visualizing Ecosystem Land Management Assessments \(VELMA\) model](#), and investigation of the fate and transport of tire wear particles from source areas to urban stormwater treatment systems including strategies to reduce transport of tire wear particles in stormwater. EPA is also developing a [Clean Water Act 1600 series analytical method](#) for detection of 6PPD-quinone in surface and stormwater (US EPA 2024) and “...developing draft screening values for 6PPD-quinone and 6PPD in water that are protective of sensitive salmon and other aquatic life” (US EPA 2024). Analytical methods and screening values could be used in conjunction with National Pollutant Discharge Elimination System (NPDES) permit conditions such as stormwater control measures to mitigate effects of 6PPD-quinone.

6.6 Remediation

Alternative chemicals and mitigation are the first line of preventative defenses against 6PPD-q in the environment. Remediation follows as a secondary tier of treatment strategies and as a long-term solution to treat 6PPD-q that is not captured by SCMs. The fate and transport of 6PPD-q in the environment is not well understood. While 6PPD-q appears to have a short half-life in stormwater and in the aquatic environment ([Section 3: Properties of 6PPD and 6PPD-q](#)), the propensity of 6PPD-q to sorb to organic matter in soil and sediments remains to be determined (See also [Section 4.2: Soil](#) and [Section 4.3: Sediment](#)). Additionally, remediation of any site requires cleanup levels to determine if remedial action is necessary and how to select the appropriate remedial action to reach the cleanup levels. These cleanup levels vary depending on the affected media and likely exposure pathway of the receptor. See the [Section 6.7 \(Policies,](#)

Regulations, and Laws) for how these cleanup levels are determined by regulatory agencies. The purpose of this section is to provide an overview of what data and information is needed to identify future remedial strategies.

6.6.1 Inputs Needed in the Remedy Selection Process

A widely used framework for identifying a preferred remedy is the **EPA Remedial Investigation/Feasibility Study (RI/FS)** process; most States have similar processes, albeit often with different terminology for certain steps or documents. The following tables describe the steps within the RI/FS process and the current status of applying the remedial process to 6PPD-q given the limited data on fate and transport and toxicity for the compound.

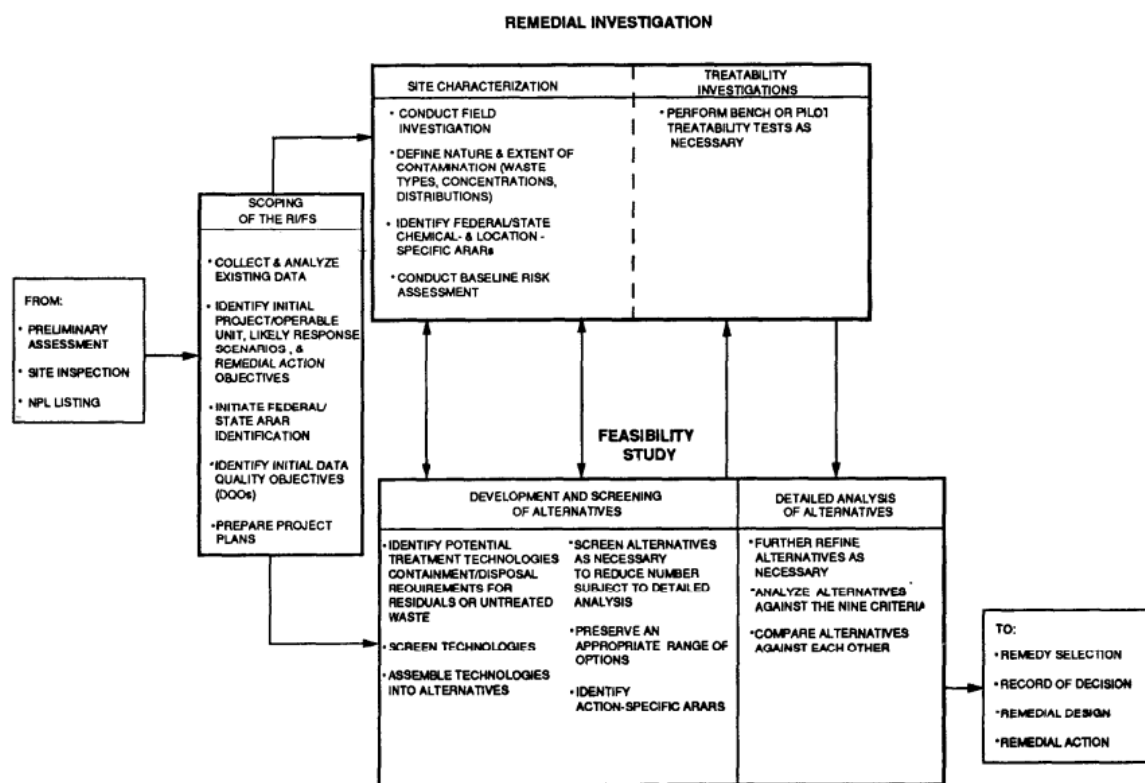


Figure 6-3. Phased RI/FS Process

Source: EPA, 1988 (RI/FS Guidance)

6.6.1.1 Remedial Investigation Phase

Scoping

Step	Current Status and Notes
Collect and analyze existing data	Only limited data is currently available in many areas (See also: Section 4: Occurrence, Fate and Transport of 6PPD and 6PPD-q)

Identify initial project/operable unit, likely response scenarios, and remedial action objectives	Project area may be identified, based on limited data; likely response scenarios may be difficult to identify; remedial action objectives may be difficult to identify, given the data gaps currently associated with human and ecological toxicity
State/Federal ARAR (Applicable or Relevant and Appropriate Requirements) identification	State or Federal ARARs include media-specific and land use-related cleanup criteria (e.g., maximum contaminant levels (MCLs), environmental screening criteria (ELCs), or similar), which have yet to be developed for 6PPD/6PPDq for either human or ecological receptors.
Establish data quality objectives (DQOs)	DQOs may be difficult to establish, given the numerous data gaps e.g. toxicity, mobility, persistence, etc.
Prepare project plans	See Site Characterization and Treatability Investigations, below

1

2 **6.6.1.1.1 Site Characterization**

Step	Current Status and Notes
Conduct field investigation	Laboratory analysis of water, soil, sediment, and particulate samples is currently possible, however sampling methods are not yet standardized for 6PPD-q. See assessment section for additional information.
Define nature and extent of contamination	Currently possible. See assessment section for additional information.
Identify Federal/State chemical- and location-specific ARARs	State or Federal ARARs include media-specific and land use-related cleanup criteria (e.g., MCLs, ECLs, or similar criteria), which have yet to be developed for 6PPD/6PPDq for either human or ecological receptors.
Conduct Baseline Risk Assessment	A Baseline Risk Assessment may be difficult to perform due to the data gaps in toxicity, mobility, persistence, etc.

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1 6.6.1.1.2 Treatability Investigations

Step	Current Status and Notes
Perform bench or pilot treatability studies as necessary	Treatability studies will need to be conducted for all remediation technologies under consideration in order to evaluate their efficacy and cost-effectiveness relative to TWP, 6PPD, and/or 6PPDq; current and future research related to SCMs may be informative. [Links to: Microplastics Guidance, Table 6-3; ITRC Sediment Remediation Guidance; new table by Lisbet re. SCM research].

2 6.6.1.2 Feasibility Study Phase

3 6.6.1.2.1 Development and Screening of Alternatives

Step	Current Status and Notes
Identify potential treatment technologies and containment/disposal requirements for residuals or untreated waste	This step is contingent upon the results of treatability studies performed as part of the Remedial Investigation.
Screen technologies	Technologies may be screened out due to excessive cost, absence of full-scale treatability studies, unavailability of equipment, or other factors.
Assemble technologies into Remedial Action Alternatives	Multiple technologies may need to be assembled (e.g., as a treatment train) to achieve applicable cleanup criteria.
Screen alternatives to reduce the number subject to detailed analysis	A typical range of alternatives evaluated at this step in the process is 5 to 10.
Identify action-specific ARARs	State or Federal ARARs may include laws or regulations pertaining to the management of remediation waste, land disposal restrictions, or other remedial actions.

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1 6.6.1.2.2 Detailed Analysis of Alternatives

Step	Current Status and Notes
<p>Analyze alternatives against the Nine Criteria (other, but similar, sets of criteria will likely be applicable to non-Superfund remediation projects).</p> <p>The nine criteria below are part of the National Contingency Plan (40CFR300.430(e)(9)):</p> <p><u>Threshold Criteria</u></p> <ol style="list-style-type: none"> 1. Overall protection of human health and the environment 2. Compliance with ARARs (applicable or relevant and appropriate standards) <p><u>Primary Balancing Criteria</u></p> <ol style="list-style-type: none"> 3. Long-term effectiveness and permanence 4. Reduction of toxicity, mobility or volume 5. Short-term effectiveness 6. Implementability 7. Cost <p><u>Modifying Criteria</u></p> <ol style="list-style-type: none"> 8. State acceptance 9. Community acceptance 	<p>Evaluation of remedial action alternatives against the evaluation criteria, whether the nine criteria applicable to the Comprehensive Environmental Response, Composition, and Liability Act (CERCLA) process or other sets of Federal or State criteria, is dependent upon data and information that is generally not yet available but are areas of active or future research and development, as described above for the preceding steps. For example, the development of Federal and State ARARs, specifically media- and location-specific cleanup levels, is necessarily dependent upon the outcomes of current and future research examining toxicity (in humans and other organisms) and fate and transport of TWP, 6PPD, and 6PPDq in the environment. In addition, significant research, including bench-scale and pilot-scale studies, are needed to identify and develop treatment technologies or systems that are effective for these constituents.</p> <p>Evaluation of the community acceptance criterion may include community outreach efforts, including to environmental justice communities.</p>
<p>Analyze alternatives against other sets of criteria, e.g., State sustainability criteria.</p>	<p>Evaluation of remedial action alternatives against State sustainability criteria is contingent in part on information specific to the technologies being evaluated, e.g., quantity of greenhouse gases that will be emitted while implementing any given alternative.</p>

2 6.6.2 Remedial Action Technologies: Recent Research

3 Current research efforts are generally focused on SCMs. However, some of the technologies
4 currently under development may eventually translate into remediation technologies. For
5 example, a filtration medium deployed as part of a stormwater management and treatment
6 program may be packed into a treatment vessel for groundwater remediation or can be used to
7 treat wastewater generated during sediment dewatering. The **SCM section** describes current SCM
8 technologies under research, including bioretention and infiltration basins.

6.6.3 Remedial Action Technologies Development and Evaluation: Additional Resources

Several existing ITRC guidance documents may provide additional information and remedy evaluation frameworks.

- [Microplastics \(2023\)](#) – May provide a framework for evaluation of approaches that may be effective for sample collection and analysis and in capturing TWP tire wear particles, given that both microplastics and TWPs are both physical particles and have overlapping size ranges. (ITRC 2023)
- [Stormwater Best Management Practices Performance Evaluation \(2018\)](#) - This guidance provides details on post-construction stormwater SCM performance evaluation. The guidance is based on SCM lifecycle processes from selection to long-term maintenance. (“Stormwater Best Management Practices Performance Evaluation,” n.d.)
- [Remedy Selection for Contaminated Sediments \(2014\)](#) - This guidance document is intended to assist decision-makers in identifying which contaminated **sediment** remedial technology is most favorable for a site based upon an evaluation of site-specific physical, **sediment**, contaminant, and land and waterway use characteristics. It should be noted that any such evaluation must consider demonstrated effectiveness relative to TWPs, 6PPD, and/or 6PPDq. (ITRC 2014)
- Other ITRC guidance documents may provide helpful tools such as frameworks for monitored natural attenuation that may be adapted to monitoring 6PPDq in surface water, for the optimization of groundwater pump and treat systems or evaluation of in-situ groundwater treatment technologies, and other steps in the evaluation of remediation processes. These documents are available on the [ITRC website](#).

6.7 Policies, Regulations, and Laws

The purpose of this section is to describe regulations, policies, and laws that might be considered by decision-makers when addressing 6PPD and 6PPD-q. This section also provides several examples of how regulations, policies, and laws have been leveraged to take action on 6PPD. It is important to note that this section does not provide policy recommendations.

For many environmental laws in the U.S., such as those included below, carrying out pollution prevention, remediation, permitting, regulation, oversight, and enforcement tasks can vary. For some laws, like the Clean Water Act, permitting of pollution point sources largely is delegated to states (with exceptions like in Massachusetts and New Hampshire where the EPA maintains permitting jurisdiction), enforcement is all-hands-on-deck (including through community-brought actions), regulation development is federal, and science informing these decisions can be done through efforts led locally like the National Estuary Program. Across these statutes, there are different federal agencies involved, and different public processes required for taking action. The discussion below uses many federal-level laws to narrow the discussion of legal programs that might affect the ultimate management of 6PPD, 6PPD-q, and tires, and is not meant to definitively speak to any given entity’s jurisdiction over an issue or decision.

1

TRIBAL TREATY RIGHTS: Supreme Law of the Land

Treaties between the United States government and Tribal Nations are considered the “supreme law of the land” and should be interpreted as having the same legal status as federal statutes ([US Department of the Interior 2021](#)). Therefore, Treaty Rights expressly stated in Treaties must be recognized by state governments, and the federal government must uphold its Treaty obligations through federal actions.

*For Tribal Nations in Washington State, the right to fish is an inherent right that has been affirmed through Treaty Rights and reaffirmed through court case decisions. By causing mortality in salmon, 6PPD and 6PPD-quinone directly threaten Tribal Treaty Rights. Salmon are significant to Tribal Nations for cultural practices, food sovereignty, community health, traditional knowledge, their way of life, and their identity as Salmon People ([Columbia River Inter-Tribal Fish Commission, 2021](#)). The Boldt Decision (also known as *United States v. Washington*, 1974) established that the Tribes are entitled to 50% of the fishing catch in their usual and accustomed fishing grounds (U&As) within WA State; additionally, the decision requires that the Tribes and WA State co-manage fisheries together ([UW Gallagher Law Library, 2023](#)). Because of this co-management status, the Tribes are important decision-makers in WA State. Over 50 additional court filings between 1974 – 2021 have expanded upon the Boldt Decision and have continued to reaffirm Tribal Treaty Rights.*

Washington State’s Centennial Accord is an example of how WA State holds government-to-government relation between Tribes and Washington State ([WA State Governor’s Office of Indian Affairs](#)). In addition to the protocols defined in the Centennial Accord, there are several best practices that state governments can take when engaging and consulting Tribal Nations to honor Tribal Treaty Rights. Foremost, it is essential to recognize that each Tribal Nation holds unique customs, cultures, and governance systems. Characterizing the opinions and decisions of one or some Tribal Nations as that of all Tribes can lead to harmful consequences. Creating systems that support the co-production of knowledge can lead to the representation of multiple knowledge systems, which enrich decision making processes ([University of Colorado Boulder](#)). It is also essential to honor Tribal Data Sovereignty, which is the right of Tribal Nations to collect and own their own data and to consent to the use of that data by other parties ([American Indian Health Commission for Washington State](#)). Washington State agencies utilize the 30-60-90 day rule, where significant agency actions that will require attendance from Tribal partners are notified 90 days prior to the event, and then also re-notified 60 and 30 days before the event. Many state agencies also employ a Tribal Liaison, who often is a member of a Tribal Nation themselves. As a best practice, staff should work closely with their Tribal Liaison to determine the level of consultation and engagement appropriate for state actions.

For more information about the significance of 6PPD to Tribal Treaty Rights, see [Section 1.3.3. Tribal Nations](#).

2

6.7.1 Toxic Substances Control Act (TSCA)

The following text is excerpted and summarized from the USEPA (2023) website: [Chemicals under the Toxic Substances Control Act \(TSCA\)](#). In summary, this act, as amended by the Chemical Safety for the 21st Century Act, requires USEPA to evaluate “...potential risks from new and existing chemicals and acts to address any unreasonable risks chemicals may have on human health and the environment”. This requirement applies to new chemicals made in the US or imported to the US. If an alternative chemical is identified that is not “new” but is an existing chemical substance or mixture that would be used in a new way, it would be regulated under TSCA Section 5(a)(1). Under this section, EPA could determine that the use of a chemical substance is a “significant new use” after considering all relevant factors. Such a finding would lead to a submission requirement of a “significant new use notice” to EPA, obligating EPA to assess risks that may be associated with the significant new use (USEPA 2014).

Under TSCA Section 5(f) Actions: Protection Against Unreasonable Risk, “...if EPA determines that a new chemical or significant new use presents unreasonable risk of injury to health or the environment without consideration of cost or other nonrisk factors, including an unreasonable risk to a potentially exposed subpopulation under the conditions of use, EPA may (1) limit the amount manufactured/processed/distributed in commerce or impose other restrictions on the substance via an immediately effective proposed rule under section 6 of TSCA, or (2) issue an order to prohibit or limit the manufacture, processing or distribution in commerce to take effect on the expiration of the applicable review period.” (USEPA 2014)

On November 2, 2023, the EPA approved a petition under section 21 of the TSCA that was filed on behalf of the Puyallup, Port Gamble S'Klallam, and Yurok tribes of Indians. The petition asked the agency to create rules that forbade the production, use, distribution, and processing of 6PPD. In approving the petition, the EPA stated that it:

“...plans to take action to address the risk to the environment presented by 6PPD, and the degradant 6PPD-q through an advance notice of proposed rulemaking under TSCA section 6. EPA also plans to propose a rule under section 8(d) of TSCA to require manufacturers (including importers) of 6PPD to report certain lists and copies of unpublished health and safety studies to EPA.” (USEPA 2024)

6.7.2 [Clean Air Act \(CAA\)](#)

There are no specific provisions in the CAA directly regulating 6PPD/6PPD-q or tire wear. Because a portion of tire wear is emitted as **PM_{2.5} and PM₁₀**, some regulatory programs related to motor vehicle emissions and ambient air concentrations of these pollutants will include tire wear particles. For example, implementation of the PM_{2.5} and PM₁₀ National Ambient Air Quality Standards (NAAQS) will consider the contribution of tire wear particles to ambient pollutant concentrations. Under the **PM NAAQS**, states with areas that do not meet the PM NAAQS concentration levels (that is, “nonattainment areas”) are required to develop **State Implementation Plans (SIPs)** that include the measures that will be implemented to reduce PM concentrations and attain the NAAQS.

In some circumstances, a Federal Implementation Plan (FIP) may be developed by EPA to help states or Tribes attain and/or maintain the NAAQS. Thus, for 6PPD or 6PPD-q to be controlled through CAA regulatory programs, they would have to be considered as a part of tire wear PM emissions that contribute to overall ambient PM concentrations. Since tire wear is only one of many sources that contribute to ambient PM concentrations, the use of CAA programs to control emissions of 6PPD/6PPD-q may be limited. 6PPD and 6PPD-q may also be subject to regulation through the CAA's air toxics or permitting programs, though data gaps exist as to whether or to what extent these chemicals persist in air emissions after, for example, combustion for fuel.

6.7.3 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)

CERCLA "...established prohibitions and requirements concerning closed and abandoned hazardous waste sites, provided for liability of persons responsible for releases of hazardous waste at these sites, and established a trust fund to provide for cleanup when no responsible party could be identified. The law authorizes two kinds of response actions: short term removals to address releases or threatened releases requiring prompt response and long-term remedial response actions ... at sites listed on EPA's National Priorities List" (USEPA 2022). At this time, neither 6PPD-q, tire wear particles, nor waste tire material have been identified as "hazardous waste." As was the case for PFAS-family chemicals, in the event 6PPD-q is indeed identified as hazardous waste, CERCLA sites may be reexamined or reopened, new sites proposed, or new remedial actions considered.

6.7.4 **Resource Conservation and Recovery Act (RCRA)** & Waste Management

As described by USEPA, RCRA establishes the framework for the proper management of hazardous and non-hazardous solid waste. Under this framework, "...[n]on-hazardous solid waste is regulated under Subtitle D of RCRA. Regulations established under Subtitle D ban open dumping of waste and set minimum federal criteria for the operation of municipal waste and industrial waste landfills, including design criteria, location restrictions, financial assurance, corrective action (cleanup), and closure requirement. States play a lead role in implementing these regulations and may set more stringent requirements. In absence of an approved state program, the federal requirements must be met by waste facilities."(OLEM US EPA 2015)

Presently, data gaps exist as to the level to which 6PPD-q is a leachate of concern in landfills, but this would be one area where RCRA would be leveraged with respect to waste that contains 6PPD (as well as rules governing wastewater management and groundwater discharges from solid waste facilities). As with PFAS management at these sites, hazard assessments may need to be conducted to determine how, whether, and to what extent 6PPD, 6PPD-q, and tires or their by-products would be managed under these programs. Similarly, such assessments may be conducted concerning waste transport, storage, and non-landfill disposal – again, for both tires and tire by-products that contain 6PPD. Jurisdictions may also be working to address illegal dumping of tires, generally for policy reasons that predate the discovery of 6PPD-q; efforts that could lead to 6PPD-q remediation. Lastly, RCRA and other solid waste programs may affect the handling and disposal of filtration media and systems used as treatment for 6PPD, 6PPD-q, and TWP.

State recycling and circular tire economy programs may also consider inclusion of tires in materials that could be inserted into waste-diversions streams or be added to curbside collection

programs. Similarly, waste management of street sweeper decant solids and liquids, and tracking of TWP waste post-secondary uses or disposal options may be regulated through RCRA programs or state and local initiatives. These systems are generally different locality-by-locality, even though tire disposal and reuse processing networks operate regionally or nationally.

6.7.5 Endangered Species Act (ESA)

“Under Section 7(a)(1) of the **Endangered Species Act**, federal agencies are directed to implement programs for the conservation of threatened and endangered species... Under Section 7(a)(2) federal agencies must consult with NOAA Fisheries when any project or action they take might affect an **ESA-listed marine species** or designated **critical habitat**” (Fisheries 2024b). In addition, “Federal agencies must consult with United States Fish and Wildlife Service (Service) when any project or action they authorize, fund, or carry out may affect a listed species or designated critical habitat” (“ESA Section 7 Consultation | U.S. Fish & Wildlife Service” 1998). The relevant agency(ies) for consultation will depend on the location of the activity and potentially affected species. Projects/actions that commonly require consultation include large construction activities and permitting programs.

As described by NOAA, “[t]he process usually begins as informal consultation. The federal agency proposing the project or action approaches NOAA Fisheries [and/or USFWS] in the early stages of project planning and requests consultation. Discussions between [the] agencies include which ESA-listed species might occur in the proposed action area and what effect the proposed action might have on those species and/or designated critical habitat. ...[I]f it appears that the agency’s action might affect a listed species or designated critical habitat, that agency prepares a biological assessment to determine the project’s effect on an ESA-listed species or its critical habitat. A biological assessment includes details on potential effects to a species or its designated critical habitat... A biological opinion usually also includes conservation recommendations that further the recovery of ESA-listed species[,]...reasonable and prudent measures as needed to minimize any harmful effects, and may require monitoring and reporting to ensure that the project or action is implemented as described” (Fisheries 2024b).

Overall, the ESA protects against both mortality for listed species and also against harm or “takes” to individuals or species. As summarized by NOAA (2023b), “[u]nder section 9 of the ESA, it is illegal to import, export, or take endangered species for any purpose, including commercial activity. The term ‘take’ means to harass, hunt, shoot, capture, trap, kill, collect, wound, harm, or pursue an ESA-listed species, or attempt any of these activities”.

Section 9 of the ESA prohibits “take” of endangered species (16 U.S.C. § 1538(a)(1)(B)) and makes it unlawful for any person to “harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect” an individual of a protected species (16 U.S.C. § 1532(19)). Mitigation and management measures, therefore, may be structured to protect species in myriad ways, from protecting characteristics of a species’ habitat and food, or protecting against impacts that affect reproductive potential.

In November 2023, two fishing groups, the Institute for Fisheries Resources (IFR) and the Pacific Coast Federation of Fishermen’s Associations (PCFFA), filed a lawsuit against 13 U.S.-based tire manufacturers under Section 9 of the Endangered Species Act (Earthjustice 2023). Attorneys for the fishing groups cite to NOAA’s rules regarding threatened salmon and steelhead

as stating that “discharges or dumping of toxic chemicals or other pollutants (e.g., sewage, oil, gasoline) into waters or riparian areas supporting listed [entities]” may violate the Section 9 take prohibition. (Earthjustice 2023)

6.7.6 Magnuson-Stevens Fishery Conservation and Management Act (MSA)

The MSA is the primary law governing marine fisheries management in U.S. federal waters (NOAA Fisheries 2023a). One of its objectives is “...protecting habitat that fish need to spawn, breed, feed, and grow to maturity” (NOAA Fisheries 2023a). In 1996, Congress revised MSA and “...established new requirements for fishery management councils to identify and describe **Essential Fish Habitat**, and to protect, conserve, and enhance EFH for the benefit of fisheries. A 2002 update to EFH regulations allowed fishery management councils to designate **Habitat Areas of Particular Concern**, specific areas within EFH that have extremely important ecological functions and/or are especially vulnerable to degradation... [The Sustainable Fisheries Act also] established a federal **EFH consultation** process that advises federal agencies to avoid, minimize, mitigate, or otherwise offset adverse effects on EFH” (NOAA Fisheries 2023c).

6PPD and 6PPD-q may affect fisheries management in at least two ways. First, through the establishment of fisheries quotas themselves. Under the MSA, fishery management councils work through a host of processes to develop, establish, monitor and adjust catch quotas. Part of this process includes long-running scientific assessments of stock sizes and sustainability over time – reviews which, especially for fish like salmon with significant connections to inland waterways, generally include assessments of spawning stock biomass, trends in population abundance and range, and threats (such as stormwater-driven mortality). As more research is finalized about population-level impacts to 6PPD-q-affected species, fisheries managers may look to apply that emerging data to MSA decisions about catch quotas. Second, as noted above, EFH decisions, and consultations conducted pursuant thereto, may lead to some development of new (or changes to existing) EFH designations to account for 6PPD-q discharges into the ecosystems that MSA-governed fisheries depend upon, or which are vulnerable to degradation. Identification of 6PPD-q hot spots has been a work in progress for NOAA, work which may inform EFH decision making (NOAA Fisheries 2024).

6.7.7 Source Water Protection under the Safe Drinking Water Act (SDWA)

Under the Safe Drinking Water Act (SDWA), states conduct assessments of their sources of drinking water (USEPA 2004). The 1996 SDWA amendments added Source Water Assessment Programs, stating that “...[e]very state must conduct an assessment of its sources of drinking water (rivers, lakes, reservoirs, springs, and ground water wells) to identify significant potential sources of contamination and to determine how susceptible the sources are to these threats” (USEPA 2004). Given the connection between tires, TWP, and 6PPD-q, operators of drinking water systems with untreated stormwater discharges (especially from roads and bridges) may be interested in research into how 6PPD and 6PPD-q are impacted by drinking water treatment technologies (See, for example, New York City’s filtration avoidance determination (New York State Dept of Health 2022). Ultimately, whether and to what extent human health is impacted by 6PPD or 6PPD-q from drinking water will also bear on this issue.

6.7.8 Clean Water Act (CWA)

Permit types that regulate stormwater discharges from three potential sources: municipal separate storm sewer systems (MS4s), combined sewer outfall permits, and construction and industrial activities (USEPA 2023). To reduce conveyance of pollutants into adjacent surface water, operators of these sources may need a NPDES permit before they can release stormwater to a receiving water body. Most states have been delegated NPDES permitting authority from EPA, though EPA continues to write permits for federal facilities, certain tribal facilities, and for permittees in states that do not have delegated authority. The EPA and states also have a residual designation authority to write permits for facilities or discharges that aren't covered by more traditional permits.

Beyond stormwater permits, many other aspects of the CWA apply to 6PPD and 6PPD-q management. For example, the CWA establishes the rules for establishing water quality criteria (numeric and narrative) that are affected by what is already known about these pollutants. Based on those criteria, the CWA also controls how waterbodies are assessed as to their level of attainment of water quality standards, and, if warranted, lays out the processes through which total maximum daily loads (TMDLs) or other watershed plans (see, e.g., CWA Section 204) are developed to address pollution. Further, the CWA includes many geographic area programs – from the Puget Sound to Lake Champlain – where 6PPD-q-affected fish species live. These programs, which include the National Estuaries, often drive research into emerging issues like 6PPD, but also into the practicalities of pollution management such as local methods for source control or stormwater treatment technologies.

6.7.9 Municipal Separate Storm Sewer Systems (MS4s)

“Polluted stormwater runoff is commonly transported through municipal separate storm sewer systems (MS4s), and then often discharged, untreated, into local water bodies. An MS4 is a conveyance or system of conveyances that is:

- owned by a state, city, town, village, or other public entity that discharges to waters of the U.S.,
- designed or used to collect or convey stormwater (e.g., storm drains, pipes, ditches),
- not a combined sewer, and
- not part of a sewage treatment plant, or publicly owned treatment works (POTW).

To prevent harmful pollutants from being washed or dumped into MS4s, certain operators are required to obtain NPDES permits and develop stormwater management programs (SWMPs). The SWMP describes the stormwater control practices that will be implemented consistent with permit requirements to minimize the discharge of pollutants from the sewer system” (USEPA 2015a).

Where industrial and construction stormwater permits are traditionally understood to be point sources that tend to apply to discrete parcels and can require that runoff be monitored closely with results reported on with consistency, MS4 permits tend to apply to larger municipal landscapes or transportation networks, and municipal stormwater tends to be considered a nonpoint source of pollutants until a community grows to the point where it must be covered by an MS4 permit administered by the EPA or an authorized state regulatory program. Historical

development has traditionally only considered stormwater management approaches that rely on flood control, or peak flow storage, and have generally not targeted pollutant reduction or sought to mitigate problems associated with changes in hydrology and hydraulics (USEPA 2023). USEPA (2023) states that “Population growth and development and redevelopment of urban/urbanized areas are major contributors to the level of pollutants in runoff as well as the volume and rate of runoff from impervious surfaces. Together, they can cause changes in hydrology and water quality that result in habitat modification and loss, increased flooding, decreased aquatic biological diversity, and increased sedimentation and erosion.”

Because of the scope of MS4 permits, and the age of infrastructure in place by the time an MS4 permit has been issued or is required, not every discharge can be monitored or contained, and not every parcel of land under control of the permitted entity (to say nothing of privately owned lands within the permitted entity’s jurisdiction) can be retrofitted to contain pollutants. MS4 permits differ from other “point source” NPDES stormwater permits that are written with technology-based and water quality-based effluent limits necessary to comply with applicable water quality standards. “Operators of regulated MS4s are required to design their programs to reduce the discharge of pollutants to the “maximum extent practicable”, protect water quality, and satisfy the appropriate water quality requirements of the Clean Water Act” (USEPA 2000).

“Permits for discharges from municipal storm sewers...shall require controls to reduce the discharge of pollutants to the maximum extent practicable, including management practices, control techniques and system, design and engineering methods, and such other provision as the Administrator or the State determines appropriate for the control of such pollutants.” (House of Representatives 2011)

For this reason, though MS4 permits are a potentially useful tool for instituting 6PPD-q controls, an iterative approach is necessary. MS4 permits are largely focused on developing and implementing a suite of SCMs (sometimes known as BMPs), structural and non-structural, that reduce the discharge of pollutants from the MS4 to the maximum extent practicable. Structural SCMs may include things like pervious pavement, rain gardens, and green roofs, whereas non-structural SCM may include awareness campaigns aimed at reducing homeowner use of pesticides, street sweeping, and inspection and maintenance schedules for structural SCMs. MS4-permitted entities “...are required to develop, implement, and enforce a stormwater management program (SWMP). The focus of the SWMP is to describe how the MS4 will reduce the discharge of pollutants from its storm sewer system and addresses various program areas, including Construction Site Runoff Control, Illicit Discharge Detection and Elimination, Pollution Prevention/Good Housekeeping, Post-Construction Runoff Control, Public Education and Outreach, Public Involvement/Participation, Program Effectiveness, and Total Maximum Daily Loads” (USEPA 2015a). Not all these program areas can be related to 6PPD-q, but the most directly applicable is Post-Construction Runoff Control. Pollution Prevention/Good Housekeeping and Education and Outreach goals can also be especially helpful in managing 6PPD-q.

MS4 owners/operators are required by permit to include a program that reduces pollutants from new development and redevelopment projects in the SWMP, and to manage stormwater through retrofits of existing developed areas. Such programs institute a jurisdiction-wide requirement to incorporate long term stormwater control measures into a development project, which is far more

cost-effective than allowing unregulated development and then retrofitting to deal with pollutants after the fact. Technical standards for post-construction site performance may be imposed through an MS4 permit, including setting area thresholds for requiring treatment and flow controls, infiltration requirements, design storm standards, prioritization of the installation of certain structural SCMs, or requirements to include certain design elements such as soil media designed for water quality improvement, or other methods.

The Pollution Prevention/Good Housekeeping portion of an MS4 permit is also relevant in establishing pollution reduction practices like street sweeping, which may take place jurisdiction-wide, or be targeted to the most relevant areas.

All of these areas of policy and regulation focus, which exist under current MS4 permits, are applicable to the problem of 6PPD-q. MS4 permits may also direct jurisdictions to place emphasis on particular pollutant removal practices, such as by requiring closer scrutiny for projects over a certain square footage, or for road projects with anticipated average daily traffic over a given threshold, or projects with a certain level of proximity to sensitive waters. The EPA has published a Compendium of Transportation MS4 guidance that may be highly relevant to the application of MS4 permitting to the problem of 6PPD, and Washington's report, "6PPD in Road Runoff: Assessment and Mitigation Strategies," also offers information on specific SCMs and research that may also be applicable and useful to consider in MS4 permit writing.

For some MS4 permits, consideration of endangered species has been included as a coverage prerequisite. This is the case in New Hampshire and Massachusetts for example, where NPDES permitting is managed by the EPA and permits are written by the EPA Region 1 water program. For both states' MS4 General Permit, which took effect in 2021, the EPA has added an Appendix that includes steps required for discharges that may affect species listed as endangered or threatened. For those permits, which cover hundreds of municipalities in both states, EPA's requirement reads:

"When listed species are present, permit coverage is only available if EPA determines, or the applicant determines and EPA concurs, that the discharge or discharge related activities will have "no affect" on the listed species or critical habitat, or the applicant or EPA determines that the discharge or discharge related activities are "not likely to adversely affect" listed species or critical habitat and formal or informal consultation with the Services has been concluded and results in written concurrence by the Services that the discharge is "not likely to adversely affect" an endangered or threatened species or critical habitat."

6.7.10 Combined Sewer Outfalls (CSO):

In many older cities, where sewer systems were built to combine runoff and sewage in the same system, management of 6PPD and 6PPD-q – and other pollutants found largely in stormwater – would look slightly different than through MS4 permits. First, CSOs and the overflow events that lead to discharges into receiving waterways are usually included as elements of a wastewater treatment plant's NPDES permit, not in a stormwater general permit. As such, the rules and regulations governing the discharges are subject to slightly different legal standards (and sometimes different water quality requirements) as MS4 permits.

Second, as NPDES-permit-covered discharges, many CSO communities and sewersheds are subject to Long Term Control Plans (LTCPs) and enforcement orders directing permittees as they bring significant long-term infrastructure systems online to minimize overflows. As a relatively newly discovered chemical, jurisdictions' LTCPs or infrastructure projects may not have been designed or crafted with 6PPD and 6PPD-q control in mind. Most of these programs, aim to reduce the volume and/or frequency of overflows through CSOs – a goal which may have the unintended consequence of reductions in 6PPD-q or TWP discharges.

Beyond these legal and planning differences, management of stormwater in a CSO sewershed may look very similar to stormwater management through MS4 permits, including SCM deployment, low impact development, flow control, source control, education and outreach, and public involvement in planning processes. When a CSO is not overflowing, absent critical failures along the way, the system's combined stormwater/wastewater mixture makes it to a treatment facility; at the moment there are data gaps as to whether and to what extent 6PPD, 6PPD-q, or TWPs are removed through wastewater treatment processes before ultimate discharge as the facility's effluent. Lastly, permittees with treatment plants that take stormwater combined with sewage may also release 6PPD, 6PPD-q, and TWPs through emergency facility overflows and bypass systems.

6.7.11 Industrial, Commercial, and Construction Stormwater

Beyond MS4 areas and CSO sewersheds, the CWA also governs, through both state and federal permits, regulations, oversight and enforcement, discharges of stormwater from industrial and commercial sites and construction operations. EPA nationally issues the **Construction General Permit** and the **Multi-sector General Permit** for industrial and commercial sites which apply where EPA is the permitting authority, and states (and some Tribes) issue their own versions of those permits (which can include requirements above and beyond those of the EPA) which apply locally.

Construction stormwater permits can contain conditions that can support mitigating 6PPD-q conveyance into local surface waters, for example, conditions related to pollution prevention practices (e.g., tire wash stations and wash water capture), materials management, and inspections. These permits apply beyond new construction or redevelopment parcels in ways that connect directly to 6PPD-q, such as required bridge maintenance and safety (a process which involves cleaning the bridges by power washing debris away from decks, trusses, girders and all other aspects of a bridge, potentially leading to discharge of TWPs or 6PPD-q into waterways under the bridge). Industrial/commercial stormwater permits can contain conditions that can support mitigating 6PPD-q conveyance into local surface waters, for example, on-site stormwater control measures like good housekeeping and maintenance, or even actual treatment of stormwater prior to discharge. Both permits can also include sector-specific conditions, and facilities can always opt to (or be directed to) be covered by a permit individually tailored to the stormwater situation at a specific facility.

For 6PPD, 6PPD-q, and TWP management, there are many similarities to how MS4 permittees may look to address this pollution – including establishment of SCMs designed to address tires and TWPs (e.g., storage on-site of tires, handling of sweeping solids and fluids). Construction permits in some states are enforced both by the permitting agency and by MS4 permittees. Industrial and commercial permits, however, are largely solely overseen by the state permitting

authority and only apply to specifically listed facilities carrying out specific industrial operations. These lists of covered activities and industries may not fully encapsulate all sites with potential to discharge 6PPD, 6PPD-q, or TWPs into receiving waters. Permitting agencies may already be reviewing the scope of these permits to ensure that both the requirements of the permit (e.g., SCMs) and the facilities covered by these permits adequately address the risks of 6PPD and 6PPD-q.

6.7.12 Residual Designations

Finally, under the CWA, the EPA has authority (USEPA 2015) to require permits for water pollution discharges that are not covered by existing permitting programs, called “residual designation authority” (RDA). This issue may arise where, for example, there are discharges (or categories of discharges) from a site (or many sites) not covered by an industrial/commercial general permit system, MS4 permit, wastewater permit (covering CSOs), or construction permit. EPA, to make an RDA decision, must determine that:

“...the discharges contribute to a violation of water quality standards, are a significant contributor of pollutant to federally protected surface waters, or controls are needed for the discharge based on waste load allocations that are part of “total maximum daily loads” (TMDLs) that address the pollutant(s) of concern. ... In addition, designation can be requested by petition.” (USEPA 2016)

In New England, EPA’s Region 1 office granted a petition to use the CWA’s residual designation authority (USEPA 2018) and plans to develop permits to control pollutants in stormwater from privately-owned and unregulated sources throughout three watersheds in Eastern Massachusetts.

6.8 State Actions on 6PPD and 6PPD-q

California’s Department of Toxic Substances Control began regulating 6PPD in motor vehicles tires through the Safer Consumer Products Program on October 1, 2023. These regulations require tire manufacturers to conduct and alternatives analysis. In their report, the tire manufacturers will analyze the hazards and adverse environmental impacts of potential alternatives to 6PPD, as well as evaluate the benefits and tradeoffs of replacing 6PPD. This process leverages the technical expertise of the tire manufacturers and enables them to meet vehicle safety and consumer product safety requirements, while providing a rigorous, transparent, and scientific framework to evaluate and compare potential alternatives to 6PPD. The tire manufacturers’ initial screening of potential alternatives is due on March 29, 2024. This will be followed by a more in-depth analysis of several promising alternatives. The final report will be due in mid-2025, though extensions may be requested under the regulations.

Washington State is developing a statewide action plan, funding research to fill in data gaps, assessing other potential tire anti-degradants, and developing specific data requirements and standards to assess the hazards of the alternatives. “Technical Memo: Assessment of Potential Hazards of 6PPD and Alternatives” (Washington State Department of Ecology 2021) provides an overview of known toxicological hazards of chemicals that are or have been used as anti-degradants in tires. Washington State has developed hazard criteria to define “safer” when looking at alternatives to 6PPD (see Ssection 6.2.3). The [Safer Products for Washington](#) program, which aims to reduce toxic chemicals in consumer products, identified 6PPD as a priority chemical. Washington and California supported the “Collaborative Innovation Forum: Functional Substitutes to 6PPD in Tires”

- 1 (Sustainable Chemistry Catalyst 2023) to develop a road map for identifying safer alternatives to
- 2 6PPD.

7 INFORMATION GAPS AND RESEARCH NEEDS

6PPD-quinone (6PPD-q) was discovered in 2020. Prior to the publication of Tian et al. (2021), the chemical was not known to exist. Thus, there are more data gaps than data points for the chemical. Since its discovery, research has rapidly ramped up on environmental occurrences, sampling protocols, mitigation strategies, and toxicity to aquatic species. Information on toxicity to humans and other terrestrial species is limited.

6PPD toxicity is better characterized than 6PPD-q since it is a high production volume chemical (global production estimated at 130,000 metric tonnes per year in 2001 (OECD 2004)) that has been used in tires since the 1960's. However, the publication of Tian et al. (2021) sparked new interest on analytical methods, occurrences, fate, and transport of both 6PPD-q and its parent compound, 6PPD.

Many institutions are funding and conducting research to address remaining questions on 6PPD and 6PPD-q. These include Federal agencies, most prominently the U.S. EPA, USGS, and NOAA. State agencies, Tribes, academia, and citizen science groups are also helping to fill the research needs.

In the table below, we highlight key information gaps that could help to characterize the risks associated with 6PPD-q or its parent compound, 6PPD, and how best to mitigate those risks. Our list is not intended to be comprehensive, and many questions are embedded within the information gaps identified below. The type and breadth of data needed for decision making depend on the institution and their intended action. The available data on the concern posed by 6PPD-q may be sufficient for an agency to act. For example, [Washington State](#) and [California](#) have promulgated regulations on the parent compound, 6PPD.

Example Knowledge Gap / Research Need	Why it Matters
TOXICITY	
The toxicity mechanism of 6PPD-q: Genetically close relationships are not a predictor of the sensitivity of species.	Need sensitive species predictions to help clarify the requirements of a replacement chemical for use in tires.
Toxicity to algae, aquatic plants, and terrestrial organisms: To date, one study has specifically evaluated the toxicity of 6PPD-q in algae (Wu et al 2023).	Need to know if 6PPD-q toxicity has wider ecological impacts which would have implications for solutions.
Latent and sub-lethal impacts to both acutely affected and tolerant species and whether exposure to 6PPD-q make species more sensitive to effects from exposure to other contaminants.	Need to know if 6PPD-q has long term and cumulative toxicity to a broader suite of species.

Example Knowledge Gap / Research Need	Why it Matters
Is 6PPD-q toxic to humans and through which exposure routes? Few studies are available which are relevant to potential human health effects of 6PPD-q exposure (J. Zhang et al. 2024; He, Gu, and Wang 2023; Zhao, Thomas, et al. 2023; L. Fang et al. 2023)).	If 6PPD-q is toxic to humans, there will be wide implications.
EXPOSURE	
Evaluate human exposure routes (for example, inhalation, dermal contact with TRWP, consumption of fish or other foods exposed to 6PPD-q, etc.) and the potential human health effects of 6PPD-q exposure. Du et al. (2022) reported concentrations of 6PPD and 6PPD-q in human urine samples which provides biomonitoring results that indicate environmental exposure can occur.	If 6PPD-q is toxic to humans, understanding health effects and how humans are exposed will be necessary.
Do the main human exposure pathways indicate potential disproportionate impacts on vulnerable populations and overburdened communities?	Communities near roadways are disproportionately lower-income and non-white, making the potential impacts of airborne 6PPD and 6PPD-q on these communities a notable environmental justice (EJ) concern (Rowangould 2013; Tian and Barzyk 2023).
FATE and TRANSPORT	
What are the different ways 6PPD-q reaches waterways, including leaching rates from TRWP?	Informs understanding of exposure routes and managing sources of 6PPD-q to aid preventative measures.
What factors influence the formation of 6PPD-q in tires and TRWP in the environment (for example, only in air with ozone, in water, via other oxidants, etc.)?	
What factors (chemical, physical, biological) influence the fate, transport, distribution, and persistence of 6PPD-q in the environment and the bioaccumulation potential in species?	
What are other sources of 6PPD-q in the environment (for example, tire disposal and re-use, crumb rubber materials and related uses, rubber-modified asphalt, etc...)?	

Example Knowledge Gap / Research Need	Why it Matters
BEST MANAGEMENT PRACTICES, SOLUTIONS, and ALTERNATIVES	
Are stormwater control measures (SCM) such as street sweeping, catchment/management, and permeable pavement effective at preventing 6PPD-q exposure?	Informs measures that intercept, prevent, and manage pathways to exposures from 6PPD-q in waterways.
What policies may reduce 6PPD-q exposures (for example, road design, stormwater permitting)?	
How does the fate and transport of TRWP in air impact 6PPD-q concentrations in stormwater? How might this influence stormwater control measures?	
Are there safer alternatives to 6PPD (within the PPD chemical family or non-PPD alternatives) that still provide protection to tires and ensure tire durability & safety and what information is needed to inform any alternatives assessment?	Informs long-term replacement of 6PPD and removal of 6PPD-q from pathways that lead to exposure.

7.1 Ecological Toxicity and Effects of 6PPD-quinone

7.1.1 Acute Toxicity

Since its discovery, researchers have pursued testing 6PPD-quinone's acute toxicity on aquatic species, including fish, invertebrates, and algae. Research continues to assess the toxicity of 6PPD-q in species from different trophic levels and inhabiting different aquatic regions (benthic versus pelagic, riparian versus lakes, marine/estuary versus freshwater). Acute toxicity to 6PPD-q at environmentally relevant concentrations has not been documented outside the salmonids [note: 2.8 µg/L is the highest reported surface water detection, (Johannessen et al. 2022)]. Because of the ubiquity of 6PPD and thus expected ubiquity 6PPD-q, identifying other sensitive species, which may represent different trophic levels, will help to broaden the understanding of potential ecological impacts of these chemicals. Little information is available on the toxicity of these chemicals to terrestrial species (for example, amphibians, reptiles, and birds) except for *C. elegans*, a soil-dwelling roundworm (Hua and Wang 2023b; 2023a; Hua et al. 2023; 2022)

Predicting 6PPD-q toxicity to a given species is difficult because research has shown that closely related salmonids have varying responses to exposure. Further research into the mode of toxicity will help explain species sensitivity.

7.1.2 Sublethal Effects

Due to the acute effects observed in coho salmon, most research to date has focused on understanding short term effects from exposure to 6PPD-q and identifying additional sensitive aquatic species. Research on sublethal impacts to species is an emerging field of study. If

sublethal effects occur, they may impact the survival of wild populations, which could be especially concerning in species of commercial and cultural importance, such as Chinook salmon. These effects could occur to any organism, at any trophic level – whether they occur, remains a major question. A related question is whether exposure to 6PPD-quinone can make species more susceptible to effects from pathogens or exposure to other contaminants, especially since 6PPD-q may promote permeability in blood vessels (Blair, Barlow, and McIntyre 2021; Greer et al. 2023).

7.1.3 Other Uncertainties

Other significant knowledge gaps relate to ecological effects. For example, does 6PPD or 6PPD-q affect microbial diversity? Microbial communities are important for nutrient cycling, which could have broad ramifications on the ecosystem. Other data gaps relate to bioaccumulation and potential exposure through the food web. Can 6PPD-q move through the food web? Does it bioaccumulate or is it rapidly broken down or excreted from organisms? The ability of plants and algae to take up 6PPD-q in environmental conditions, as opposed to hydroponic conditions (in the lab) is still unknown. It remains unclear whether animals that eat plants can be exposed to 6PPD-q through their diet. We know that aquatic organisms are exposed to 6PPD-q during rain or runoff events, but we don't know how long it remains in their bodies.

7.2 Human Toxicity, Effects, and Exposure to 6PPD-quinone

7.2.1 Human Toxicity and Hazard Traits

Few studies are available that can provide information about the potential human health effects of 6PPD-quinone exposure. As of January 2024, there are just 4 *in vivo* mouse studies on 6PPD-q (J. Zhang et al. 2024; L. Fang et al. 2023; He, Gu, and Wang 2023; Zhao, Thomas, et al. 2023). While these studies have started to provide us with information about the potential target organs and exposure-potential hazard traits (for example, transplacental transfer) of 6PPD-q, none of these studies involve long-term exposures that assess the health of multiple organs and organ systems. Two of the studies use oral gavage to expose the mice to 6PPD-q (Zhao, Thomas, et al. 2023; L. Fang et al. 2023). This is a relevant exposure pathway if humans are exposed primarily from our food and incidental ingestion.

Inhalation may also be a relevant route of exposure (Zhang et al. 2022) and inhalation toxicity studies have not yet been conducted. Modeled toxicity data can be used to help address data gaps, but U.S. EPA's Cheminformatics Hazard Module shows that the information on toxicity endpoints has low certainty (U.S. EPA 2022). Some studies have suggested that both chemicals may cause oxidative stress (Wang et al. 2022) as a mode of toxicity; however, that needs to be confirmed by laboratory experiments.

Exposure-potential hazard traits such as bioaccumulation and transplacental transfer provide information on potential human exposure but do not offer information health impacts. Bioaccumulation is still uncertain. Modeling predicts that 6PPD-q has the potential to bioaccumulate; however, laboratory studies in mammals are lacking, as are thorough studies on our food sources.

7.2.2 Human Exposure

6PPD-q has been reported in human urine (Du et al. 2022), serum (J. Zhang et al. 2024), and cerebrospinal fluid (J. Fang et al. 2024) in China. These studies have yet to be repeated in the U.S. where traffic patterns and urban planning are different. In addition, modeling particulate inhalation and studies of indoor dust (Wang et al. 2022; Y.-J. Zhang et al. 2022; Zhu et al. 2024) indicate that human exposures to 6PPD-q occur; however, the sources and routes of exposure have yet to be identified and characterized.

- **Diet.** To what extent does our food contain 6PPD-q? We don't know the extent of exposure to crops, aquatic life (shellfish and fish), domesticated animals, and game. Nor do we fully know the uptake potential from environmental media, kinetics, or persistence off 6PPD-q in their tissues. If the food contains the chemical, does cooking alter these levels? These questions are especially important for subsistence harvesters of fish and other aquatic life, for example Tribal Nations that rely on fish (Norgaard 2005) and shellfish (Kuhnlein and Humphries 2017) in their diet.
- **Drinking water.** 6PPD and 6PPD-quinone could potentially be in source water, finished water as it leaves the drinking water treatment plant, or potentially contaminated at the point of use (Marques dos Santos and Snyder 2023). At this time, there are no studies of 6PPD or 6PPD-q in US drinking water. Questions remain about the efficacy of removal of particle-bound or dissolved 6PPD and 6PPD-quinone in DWTP treatment processes and media.
- **Recreational water.** While the human exposure potential for 6PPD-q in sediment is unknown, sediment disturbance due to human activities such as wading and swimming can resuspend sediment particles into the water column (For additional discussion of exposure during recreational activities, see [Section 2.4.5.2](#)), making them available for dermal contact or accidental ingestion.
- **Traffic proximity.** Further research is needed on the levels of exposure in different sectors of the population from near-road air and dust. Are there potential health impacts from exposure to 6PPD-q from the air?
- **Air particulates.** More investigation is needed to determine potential human exposure to 6PPD and 6PPD-q due to ambient particulate matter (PM).
- **Indoor air.** Ambient PM, TRWP tracked into the home, and products containing 6PPD may contribute to dust in the indoor environment. Does 6PPD-q form in indoor air? At what rate?
- **Contact with- or use of- recycled rubber products.** Some rubber and recycled rubber products are used in the home and may contribute to exposure. In addition, recycled tires may be in institutional settings such as crumb rubber infill on artificial turf fields or as aggregated chip surfaces at playgrounds. Because playing fields involve activities that may increase exposure potential such as increased breathing rate, repeatedly falling, and sweating on skin surface and young children on playgrounds have unpredictable behavior that increases direct and indirect contact and potential exposure to these tire-derived end-of-life products. These exposure pathways are likely to be different than a manufactured consumer product.
- **Other manufactured products.** Products used in the home may contribute to indoor dust, but we do not know the entire universe of products that contain 6PPD. One study suggested that 6PPD may be used in electronics, for example dark colored cords (Liang et al. 2022). 6PPD or 6PPD-q have been reported in dust samples from electronic-waste

facilities (Liang et al. 2022; Wu, Venier, and Hites 2020; Z. Zhang et al. 2024) and one in dust from solid waste facilities (Y.-J. Zhang et al. 2022). Whether consumer electronics or e-waste expose people in the US to 6PPD or 6PPD-q is uncharacterized.

- **Occupational exposure.** Workers in tire or rubber manufacturing facilities, tire re-treading facilities, rubber-modified asphalt operations, and road construction crews represent a few of the occupations that may have exposures to 6PPD or 6PPD-q. Research is needed to fill this data gap.

7.2.3 Other Uncertainties

Significant questions remain about the potential impacts to different groups of people. Among these data gaps are:

- Health and cultural implications for tribal communities, particularly in the Pacific Northwest (DTSC 2022)
- Socioeconomic impacts to subsistence and commercial fishers
- Impacts on communities near heavily trafficked roads. Communities near roadways are disproportionately lower-income and non-white, making the potential impacts of airborne 6PPD and 6PPD-q on these communities a notable environmental justice (EJ) concern (Rowangould 2013; N. Tian, Xue, and Barzyk 2013).
- Potential cumulative impacts, especially in overburdened communities

7.3 6PPD-quinone Fate, Transport, Occurrences, and Sources

Many knowledge gaps remain about the physicochemical fate properties of 6PPD and 6PPD-q. These properties could help to elucidate questions surrounding the fate, transport, distribution, and exposure to these chemicals.

7.3.1 How Does 6PPD-q Behave in the Environment?

Several critical questions and knowledge gaps remain on the behavior of 6PPD-q in the environment.

- Half-life in the environment and how that varies with different conditions.
- Fugacity modeling predicts where 6PPD-q will partition in the environment; however, there is not sufficient data to verify the modeling.
- What are other transformation byproducts of 6PPD, in addition to 6PPD-quinone?
- The uptake and distribution of 6PPD-q into terrestrial and aquatic biota and its potential for bioaccumulation require more research.

7.3.2 Fate

Questions remain about the transformation of 6PPD to 6PPD-q. For example, do conditions (for example, temperature, concentration of ozone in the air, humidity) impact the reaction rate of 6PPD into 6PPD-q, and ultimately the mass of 6PPD-q produced? Can 6PPD-q still form in an aqueous solution – either from TRWP in waterways or when ozonation is used to disinfect water that may contain residual 6PPD? Can other oxidants cause 6PPD-q to form? Although the occurrence of 6PPD and 6PPD-q has been measured in various media, the overall distribution of

6PPD and 6PPD-q in the environment is understudied (particularly in the United States), as well as the physical-chemical and environmental conditions that impact the fate, transport, and distribution.

7.3.3 Transport

Many questions remain regarding transport of 6PPD-q, including a full understanding of the pathways leading from 6PPD in tires to 6PPD-q in waterways. TRWP are thought to be the primary carrier of 6PPD-q into waterways, although the transport of the solubilized chemicals directly from the tires into puddles on the road has not yet been characterized. Some of these key questions focus on the transport of TRWP through water and air whereas other transport questions focus more on the chemicals.

7.3.3.1 Movement of TRWP

Key questions about the movement of TRWP include:

- Are TRWPs the primary vector to transport the chemicals through the air? Is either chemical volatile?
- Of the TRWP in the air, what fraction settles directly into waterways versus TRWP that deposit on the road and then are transported into the broader environment? Of the TRWP on the road, what fraction stays there or is removed by other means, like street sweeping.
- What are the size fractions of TRWP containing 6PPD and 6PPD-q and how do these size distributions change under varying tire, vehicle, road, and other environmental conditions?

7.3.3.2 Transport of the Chemicals from TRWP or Other Sources

Questions about the transport of 6PPD and 6PPD-q from TRWP or other sources include:

- Are there soluble or volatile fractions of the chemicals that move separately from TRWP? If so, how do the chemicals move and disperse in the environment?
- What are the leaching rates of the 6PPD or 6PPD-q from TRWP or whole tires? Does 6PPD transform into 6PPD-q when TRWP or whole tires are fully submerged in water?
- How does the outflow from combined sewer systems affect transport of 6PPD-q during average runoff events and during those that cause combined sewer overflows?
- In the combined sewer systems, 6PPD and 6PPD-q are predicted to sorb to biosolids. What happens to those chemicals when the biosolids are applied to urban landscapes, agricultural lands, or cultivated forests? What happens when the biosolids are landfilled?
- Are there particular facilities, such as those that produce rubber, manufacture tires, or retread tires, that emit or release, tire-related particulates, 6PPD, or 6PPD-q?
- After extremely large snow events, snow that has been removed from roads may be dumped into water bodies in cities where that is an option. What contribution do these pulses of dumping have on 6PPD-q levels in nearby waterways?
- Do decant facilities that process the waste collected by street sweeping potentially act as another source of 6PPD or 6PPD-q?

7.3.4 Other Product-related Sources of 6PPD and 6PPD-quinone

As mentioned previously, 6PPD is the primary antidegradant used in tires. Similarly, it can be used directly in other rubber products such as sneaker soles, garden hoses (Zhao, Hu, et al. 2023), and climbing shoes (Sherman et al. 2024). In addition, products made from recycled rubber can contain 6PPD and 6PPD-q (Armada et al. 2023). The majority of states have programs that divert scrap tires from landfills, so the use of recycled tires is extensive. Recycled tires can be used in outdoor products (for example, tire reefs, erosion control along embankments, crumb rubber infill for artificial turf, playground surfaces, tire mulch, and tire aggregate used in civil engineering projects), as well as those used inside buildings (for example, mats, flooring, ramps) (CalRecycle 2023). Understanding if these products act as sources – and the relative importance of those sources – for both ecological and human exposure requires further research.

7.3.5 Occurrences and Potential Exposures

Most of the monitoring studies in media beyond surface water have been conducted in China. A major data gap remains whether those occurrences are similar in the U.S. Studies are needed to investigate the levels of 6PPD and 6PPD-q in relevant media in the U.S. These studies can help provide information on exposures to humans and other biota, and potentially, help track other sources of the chemicals in addition to tires.

7.4 How Effective Are the Proposed Solutions?

7.4.1 Mitigation Strategies

Significant uncertainty surrounds the effectiveness of the various solutions discussed in this document. Some of the proposed mitigation strategies have preliminary or pilot data (Rodgers et al. 2023; Jenifer K. McIntyre et al. 2023; J. K. McIntyre et al. 2014) but have not been deployed on a large scale. Air mitigation strategies are being tested to remove particulate matter emissions near roadways (Baldauf 2016) but effectiveness of removal TRWP is unknown. While strategies like street sweeping are predicted to be effective but testing results are not yet available. Understanding these will be required to select the most efficacious or cost-effective strategies.

- How can stormwater control measures (SCM) be optimized for capture of TRWP? Does the capture of TRWP abate 6PPD-q runoff? SCMs including street sweeping, catchment/management, and permeable pavement require more research to understand their efficacy at mitigating 6PPD-q runoff.
- What SCM designs are most effective from a multi-media perspective? These would mitigate 6PPD-q in stormwater runoff and air pollution.
- How can SCMs be optimized for the available space or the challenges at specific sites?
- Do street sweeping and other sediment-disturbing practices result in a liberation or re-distribution of 6PPD, 6PPD-quinone, and TRWP?

7.4.2 Alternatives to 6PPD

One approach to reduce 6PPD-q in the environment is to decrease the amount of 6PPD in tires or replace it with an alternative antidegradant. The company Emissions Analytics has data

demonstrating that different tires have dramatically different levels of 6PPD (Carey and Lewis 2023); unfortunately, there are no public data to support how little 6PPD could be used in different tires to provide the antidegradant activity. Another important data gap is how much the amount of 6PPD would need to be reduced in tires to mitigate the adverse impacts. Tire manufacturers and USTMA prefer to seek to replace 6PPD with alternatives. At this time, much is unknown about alternatives to 6PPD. It is still unclear what alternatives can provide equivalent or better protection against ozone-damage to tires. Many of these potential alternatives lack comprehensive data on human and ecological toxicity endpoints on the parent compound and the transformation products. The alternatives assessments (AAs) required by California's DTSC or conducted by Washington's Ecology can help address the safety of these alternatives across multiple hazard endpoints. The AAs can also address whether there are other health or environmental trade-offs associated with the adoption of those alternatives. The tire manufacturers initial screening of alternatives will be available on [DTSC's website](#) on March 29, 2024.

7.5 Current Research

The science on 6PPD-q is evolving rapidly and several Federal agencies are engaged in research on the topic.

- [EPA's website on 6PPD-quinone](#) highlights ongoing research, as well as funding opportunities associated with 6PPQ-q.
- [USGS](#) has been leading work on monitoring in surface water and studying toxicity in different fishes.
- [NOAA](#): Northwest Fisheries Science Center first characterized URMS and researchers are actively engaged in understanding 6PPD-q's aquatic toxicity, bioaccumulation, and fate.
- Washington State's Department of Ecology is developing a database of funded research. [The link should be available by the end of external review.]

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