Juvenile Coho Salmon Mortality Induced by Roadway Runoff During Spring Storm Events

Marlee Lyn Brown

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Committee:

Edward P. Kolodziej

Jessica R. Ray

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Abstract

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Marlee Lyn Brown

Chair of the Supervisory Committee:

Edward P. Kolodziej

Department of Civil and Environmental Engineering

Water quality-linked acute mortality has been well documented in adult coho salmon (*Oncorhynchus kisutch*)—often known as "Urban Runoff Mortality Syndrome" (URMS)—in Pacific Northwest creeks receiving roadway runoff during or shortly after storm events. URMS is primarily caused by exposure to an ozone transformation product, 2-((4-Methylpentan-2yl)amino)-5-(phenylamino)cyclohexa-2,5-diene-1,4-dione (6PPD-quinone), that forms on all vehicle tires. Most research to date has focused on the high mortality rates of returning adult coho salmon spawners after fall rainstorms. Juvenile coho salmon are also sensitive to 6PPDQ, yet limited information exists concerning mortality risks to earlier life stages, especially during spring storms, when coho are hatching from spawning gravels. In laboratory studies, 6PPDQ exposure at concentrations <100 ng/L induce high rates of mortality in juveniles; these concentrations are similar to those reported in urban or roadway impacted watersheds during the

spring. To assess the incidence of URMS in post-hatch juvenile coho salmon in springtime rearing habitats, we conducted a paired water quality-ecotoxicology study at a small field laboratory facility located at Miller Creek—a runoff-impacted watershed in Burien and Normandy Park, WA, USA. Juvenile coho (N=720) were exposed to either creek water or groundwater (N=120 per treatment per storm) across three spring storm events. Water quality and mortality endpoints were compared to groundwater-exposed controls. During the spring storm exposures, 6PPDQ concentrations reached 73-110 ng/L, which exceeded reported LC_{50} values (41-95 ng/L). Over the 24–73-hour storm exposure period, ~80% of Miller Creek-exposed juvenile salmon died with no mortality in groundwater controls. Other PPDs, transformation products and vehicle-derived chemical contaminants exhibited similar spikes in concentration and chemodynamics that were correlated to precipitation and increases in creek discharge volume. These results indicate a previously unquantified impediment to coho salmon recovery via a significant mortality risk to juvenile life stages of coho salmon that complements welldescribed risks to adult spawners across both spawning, rearing, and migratory habitats throughout their range.

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1. Introduction

1.1 Urban Runoff Water Pollution

A major, and often unmanaged, source of environmental pollution is urban runoff.[1,2] Urban runoff results from the horizontal flow of water over and through impervious surfaces such as roads and parking lots, followed by environmental discharge. The volume of runoff flow generated is because water lacks the ability to percolate into underlying layers, resulting in rapid and uncontrolled flows into receiving waters.[3] Another source of surface runoff is soil becoming fully saturated during a storm event, limiting the potential for infiltration, creating new hydrologic flow paths, and therefore generating volumes of overland flow.[4] Whenever water flows across saturated soil or impenetrable surfaces that contain chemical contaminants, a wide array of soluble chemical contaminants are mobilized and transported into receiving waters.[5,6] Roadway runoff therefore provides new and transient transport capabilities for chemical contaminants; unless the water is stored or treated, these non-point source pollutants will end up in aquatic habitats that may contain sensitive species.[7] Many contaminants occurring in roadway runoff originates from vehicular transport, as well as processes like atmospheric deposition and other human activities on or near roadways.[3] Urban runoff is therefore a complex mixture that contains many types and classes of synthetic organic contaminants, metals and inorganics, and various metals, salts, and chemical contaminants, this mixture has proven to be toxic to aquatic

organisms.[8,9,10] Urban runoff is now considered a major driving force for water quality degradation in many receiving waters, especially those in urban areas or are roadway-impacted.[5]

1.2 Tire Wear Particle Constituents in Roadway Runoff

Vehicle tire rubber was first identified in road dust in the 1960s.[11,12] Roadway runoff is the primary transport pathway for tire wear particles (TWPs) and tire rubber-derived chemicals from roadways into runoff-impacted receiving waters, indicating that understanding the fate and transport of tire rubber particles and associated chemicals is important to understanding the impacts of roadway runoff on water quality.[13,14] TWPs are generated from motor vehicles driving, accelerating, braking or turning due to the abrasion between the tires and the surface of the road.[14] The transformation product of the tire additive 6PPD, 6PPDQ, found in roadway runoff has been identified as the leading toxicant in inducing coho salmon mortality.[15]

By 1980, annual TWP generation was estimated at ~500,000 metric tons, including substantial generation of airborne particulates as well as larger particles that are deposited in roadside soils and could be transported by runoff.[16] In the United States, 1,120,000 tons of TWP emissions were estimated to be produced annually by 2010, including contributions from passenger cars, trucks, buses and heavy trucks.[17] In the European Union, an estimated 1,327,000 tons were produced annually (2018 data), and in Germany, an estimated value of 133,000 tons of TWPs were produced annually, with 11,000 tons reaching surface waters.[17]

In recent years, vehicle tire rubber has been linked to the generation of microplastics (MPs) and microrubbers (MRs), which has motivated substantial research into its environmental fate and associated implications.[17,18] Tire wear and tire rubber abrasion is now considered a

major source of environmental microplastics,[18,19] including by artificial turf containing micronized rubber (MR) generated from waste tires.[20,21] Tires are a main source for highly mobile microplastics in the environment and have been estimated to release between 3.0 and 5.5 kg/y per capita.[22] In stormwater samples collected in Gothenburg, Germany, a densely populated city, the number of microplastic particles ≥100 um was up to 3 particle/L, and for particle sizes of ≥ 20 um, up to 5900 particles/L was reported, confirming that high traffic areas generate substantial quantities of microplastics derived from TWPs.[23] Although tire wear emissions are considered microplastics, reviews have been published addressing the analytical chemistry gap that reveals the disadvantages in lacking a distinguished way to characterize tire wear particles from microplastics in size, origin, physiochemical properties, environmental half-lives and aging effects.[17,24,25,26]

The emerging data that TWPs are widely deposited along roadways and within roadway runoff, including runoff-impacted habitats, has raised concerns for associated environmental pollution and roadway runoff impacts to water quality. In the 1980s, heavy metals such as Cd, Cu, Pb and Zn derived from tire rubber have been detected in roadside dust and soils along major highways.[27] Benzothiazoles, which are antioxidants used in tire manufacturing, have been used as a biomarker in estuarine sediments to indicate the presence of roadway runoff.[28] During the 1990s, many rubber-derived compounds have been reported in road dust, tire tread debris and other vehicle residues.[29] The environmental fate and transport of benzothiazole, 2- hydroxy benzothiazole, and 2-(4-morpholino)benzothiazole and other benzothiazoles derived from TWP were investigated suggesting inputs of these contaminants from crumb rubber material in modified asphalt should not be harmful because they are water soluble, volatile and can be

microbially degraded.[30] This uncertainty in rubber derived compounds impacts on water quality allowed for a push in continued research.

Over time, TWP fate and processes in environmental systems were further characterized, including those that affect the chemical composition of TWPs and associated microplastics. For example, atmospheric oxidation is known to be a key transformation process for TWPs.[31] Between 2000 and 2010, studies identifying the environmental implications of chemical classes found in tire emissions were focused on chemicals such as PAHs,[32,33] heavy metals with a primary focus on zinc[34-40] and benzothiazoles.[34,35]

Between 2010 to present further characterization of pollutants found in road dust and stormwater runoff containing tire related emissions and heavy metals were conducted, indicating that trace metals such as copper, lead, zinc and platinum group elements (PGES) are largely found in road dust at levels 100 times greater than those found in background levels.[36] Zinc, copper, nickel and cadmium can occur in their dissolved phases, extending the need to understand the partitioning behavior of these metals.[37] Tire and break wear can contribute up to 55% by mass to total non-exhaust-traffic-related particulate matter emissions in urban environments.[38] Exhaust traffic related particulate can release in the environment after incomplete fuel combustion and volatilization whereas non-exhaust related particulate matter is deposited on to road surfaces from tires and brakes[39] and through abrasion and resuspension.[40] Exhaust and non-exhaust emissions are estimated to contribute almost equally to total traffic related emissions.[39] Brake wear particles (BWPs) were identified as one of the main sources of non-exhaust emissions.[41] High concentrations of metals in runoff seasonally vary showing higher concentrations in colder seasons and correlating to fine particle suggesting weather treatment

such as deicing causes sedimentation increasing removal rates.[42] Elevated levels of microrubbers and microplastics are a direct result of increased traffic emissions and which indicate more populated areas.[43] Non exhaust emissions transported through stormwater as smaller finer particles are mobile and tend to carry immobile contaminants with them acting as a transportor.[44] Numerous studies have detected TWPs and related chemicals in urban creeks impacted by roadway runoff.[6,13,15,45,46] TWPs and related chemicals and transformation products have now been identified in soil, air, water, biota and fine particulate matter.[54-56]

Detecting tire rubber derived chemicals and metals in receiving waters and environmental systems raises obvious questions about their toxicity to organisms. The first tire leaching toxicity experiment observing the effects on two fish species determined that there were no detrimental effects on pinfish or black sea bass due to tire leaching and recommended artificial reefs were constructed out of tires moving forward.[47] Runoff and tire leachate experiments indicated toxic effects on aquatic organisms after experiments using whole scrap tires submerged in water showed that the leachate was toxic to certain biota: Rainbow Trout (*Salmon gairderi*),[58-60] larval sheepshead minnows (*Cyprinodon variegatus*)[48,49] and grass shrimp (*Palaemonetes pugio*).[48] By examining tire reefs in canals, zinc concentrations leached from tires did not solely induce toxicity in the environment indicating it is not the main toxicant in the system [50] because metals on their own do not produce coho mortality.[51] However, in laboratory studies high concentrations of zinc were observed to be toxic but tires submerged in water are unlikely to leach high enough levels of zinc to induce the same acute or chronic toxic effects.[50] Exposure to metals on their own do not produce coho mortality. The effects of roadway runoff within freshwater systems effected the surrounding sediment and biota with elevated concentrations of contaminants detected in both matrices resulting in a functional change in the benthic macroinvertebrate

community.[52,53] One example was proven during a tire leachate toxicity assessment, indicating summer tires were more toxic than winter to *Daphnia magna,* showing the different seasonal effects of roadway runoff.[54] The toxicity identification evaluation indicated nonpolar organic compounds were the source,[55] tire additives increased the toxicity of tires significantly[56] and zinc and organic compounds were the main sources of toxicity but the effects were reduced from sequential leaching.[57] Toxicity effects on crustaceans, fish and human cell lines were conducted inducing oxidative stress[71-73] increasing cell mortality and DNA damage[74-76] and having tetrogenic potential on *Xenopus laevis.*[58] An expansion on toxicity studies was conducted using alga, daphnid and fish to continue assessing the risk from tire wear leachate suggesting organic compounds, are mainly responsible for toxicity [59] and suggesting zinc and aniline as two likely toxicant candidates.[60] Observations of ingestion of tire wear particles in freshwater benthic macroinvertebrates indicated no adverse effects,[61] different from freshwater *Hyalella Azteca* which TWP exposure elicits both short- and long-term toxicity at higher concentrations (500-2000 particles/mL).[62] This evidence indicates that aquatic organisms unintentionally consume TWPs and therefore, this exposure route can lead to acute and long term toxicity risks in organisms.[62,63]

1.3 Initial Studies confirming URMS

As urban area populations grow, the ecological impact on urban streams also grows, reflecting the increase in nutrients, metals, pesticides and other contaminants loadings to receiving waters. The hydrology of urban creeks is frequently highly altered due to the growing proportion of impervious surfaces within the watershed. These changes result in declines to the ecological richness and diversity in these stream habitats.[64] Such ecological degradation in

stream health is known as “urban stream syndrome”.[65] Urban stream syndrome presents as volume discharge spikes on a hydrograph, higher concentrations of nutrients and contaminants, altered channel morphology, and reduced biotic richness and diversity, with increased dominance of pollution- and habitat-tolerant species.[65] To restore urban streams, clearly identifying the sources of degradation along with the range of ecological stressors the stream faces is imperative. Evaluating chronically altered stream flows and associated water quality can provide insight into how urban development correlates to a decline in the biological condition of the stream.[66]

In the Pacific Northwest, one example of urban stream syndrome is reflected by the declining salmon populations due to the deterioration of stream biological conditions. In the 1990s, creek restoration projects focused on the physical habitat of small urban creeks around the Seattle area were implemented. This included creating green space, removing culverts and other barriers affecting fish, addition of large woody debris and spawning gravels and the replacement of noxious weeds with native plants.[67] After restorations were completed, post-project field surveys observing the restoration effectiveness in these Seattle-area watersheds focused on the health and abundance of coho (*Oncorhynchus kisutch*), Chinook (*O. tshawytscha*) and chum (*O. keta*) salmon in these watersheds.[67] Correlated with the incidence of rain events, these monitoring surveys began to observe unusual behaviors and outcomes for adult coho spawners returning to these habitats, characterized by die off rates up to 90% in some watersheds.[67] The adult coho showed signs of erratic surface swimming, gaping, fin splaying and loss or orientation and equilibrium and died within hours to days of observed symptoms.

Following the initial urban stream syndrome observations showing high rates of egg retention in female carcasses (>90%), additional surveys were conducted to address the severity of risk for adult coho and mortality rates, compare mortality rates between urban creeks and

nonurban creeks, and monitor the water quality during mortality events. Daily surveys were taken from one urban stream (Longfellow Creek) and one non-urban stream (Fortson Creek), showing premature morality rates of returning adult coho salmon between 20-100% in Longfellow each fall and <1% in Fortson.[67] Coho salmon exposed to untreated roadway runoff in a controlled experiment exhibited mortality rates of 100% after becoming symptomatic a few hours before.[51] Coho salmon, an indicator species for water quality degradation, face high mortality rates induced during rainy seasons after returning to their natal watersheds. This rainfall-induced mortality phenomena became known as "urban runoff mortality syndrome" or "URMS". This phenomenon was later linked to stormwater runoff and tire associated contaminants mobilized
from road surfaces. [51,87-90] It was only recently the ubiquitous tire derived contaminant inducing coho salmon mortality was identified as N-(1,3-Dimethylbutyl)-N'phenyl-p-phenylenediamine quinone (6PPDQ).[15]

1.4 Tire Rubber and 6PPD-Quinone

Beginning with observations of acute mortality in urban creeks of the Pacific Northwest, stormwater runoff had been documented to have lethal and sublethal effects on adult coho returning to small stream habitats to spawn.[68] In 2020, the rubber-derived chemical compound 6PPD-quinone (6PPDQ) was identified as the primary toxicant for coho salmon inducing urban runoff morality syndrome. 6PPDQ is a transformation product of N-(1,3-Dimethylbutyl)-N'phenyl-p-phenylenediamine (6PPD) that forms when 6PPD is ozonated in the gas phase.[69] 6PPD is an antioxidant and anti-ozonate used as an anti-degradant in vehicle rubbers to prevent rubber degradation by exposure to ozone and oxygen.[93-95] 6PPDQ forms from 6PPD on tire

rubber surfaces. Samples collected from tire surfaces showed detections of 6PPDQ, (Department of Toxic Substances Control, 2022) as did samples of road and parking lot dust along with the inside of tires.[71]

Using a commercial 6PPDQ standard, the LC_{50} value for juvenile coho salmon was estimated at 95 ng/L[72] and an LC_{50} of 41 ng/L was reported for early life juvenile coho salmon.[73] Several species such as sockeye salmon (*Oncorhynchus nerka*), chinook salmon (*Oncorhynchus tshawytscha*), atlantic salmon (*Salmon Salar*), brown trout (*Salmo trutta*), arctic char (*Salvelinus alpinus*), southern dolly varden (*Salvelinus curilus*), and cherry salmon (*Oncorhynchus masou masou*) are not sensitive to 6PPDQ exposure, with LC_{50} values of 3500 ng/L to >82100 ng/L.[74] More sensitive are rainbow trout/steelhead (*Oncorhynchus mykiss*) with reported LC_{50} values of 470 ng/L for feeding fry,[75] 640 ng/L,[76] 1000 ng/L.[77,78] Brook trout (*Salvelinus fontinalis*) and white-spotted char (*Salvelinus leucomaenis pluvius*), both slightly less sensitive to 6PPDQ than coho have reported LC_{50} values of 590[77] and 510 ng/L[79] respectively.

Due to its global growing concern, the EPA has released an Acute Life Screening value for 6PPD-quinone in freshwater of 11 ng/L, as of 2024.[80] This value is meant to protect the multiple freshwater fish species which range in their sensitivity to 6PPDQ, by limiting exposure to the given concentration to no longer than one hour and can only be exceeded once in a threeyear time span.[80]

The above toxicity and screening values can be compared to environmental concentrations of 6PPDQ to understand potential environmental risk in various aquatic habitats. 6PPD along with 6PPDQ originates from tires and tire wear particles (TWPs) generated from simple actions such as road travel, braking, turning and accelerating. 6PPDQ is ubiquitous in

roadway-impacted environments because 6PPD comprises 0.4-2.0% by mass of all vehicle tire rubber,[81] and TWPs enter aquatic systems through stormwater runoff and atmospheric deposition.[15,82,109] Average concentrations in surface water in King County have been reported as 90 ng/L with a maximum concentration detected in stormwater as 1300 ng/L.[98,110]

6PPDQ detections have been reported in different matrices such as air, sediment, dust, soil, snow and snowmelt, roadway runoff and wastewater in treatment plants.[14] For example, 6PPDQ was detected in 57% of Canadian stormwater (mean concentration of 600 ng/L) and 90% of roadway snowmelt (mean concentration range of 80-370 ng/L) samples.[108] In the Puget Sound region, roadway runoff samples had detections of 6PPDQ ranging from 20-200 ng/L and were detected in 100% of the samples.[98] Stormwater samples collected from a runoff impacted tributary in Australia had a max concentration of 88 ng/L and a mass load of 3 g/storm for 6PPDQ.[111] In Hong Kong, China, 6PPDQ was detected in 100% of roadside soil samples, runoff samples and air particles at median concentrations of 234 ng/g, 1200 ng/L and 1.18 pg/m^3 respectively.[54] Samples from Beijing, China had a median concentration of 1362 ng/g in roadside soil.[112]

The physiochemical properties of 6PPDQ helps to explain its incidence and transport in environmental systems and receiving waters. An experimental value for the aqueous water solubility was reported as 38 ± 10 ug/L at 20°C,[113] indicating that no solubility limitations exist for the occurrence of 6PPDQ in water at ng/L concentrations. An octanol-water partitioning coefficient (log Kow) for 6PPDQ of 4.3 was measured,[113] indicating that 6PPDQ has a moderate preference to adsorb to organic matter, soils, and sediments. This Kow value suggests that 6PPDQ would absorb to organic matter and treatment media in a biofiltration system that

contains lots of surfaces and organic matter phases; a strategy that was successfully used to treat stormwater runoff and prevent lethal and sublethal effects on coho salmon.[64]

1.5 Other PPDs + TPs

6PPDQ is formed from 6PPD by reacting with atmospheric ozone. In the 19th century, average concentrations of ozone in the troposphere at ground level were ~10 ppbv.[114] Since this time there has been a steady increase in ground level concentrations due to anthropogenic activities, leading to 20-45 ppbv concentrations in the Northern Hemisphere.[115] During poor air quality events, 200-400 ppbv concentrations are possible.[116] These high ozone concentrations would destabilize tire rubbers in locations highly polluted with ozone, where ozone reactivity toward double bonds in the rubber would induce cracking in the tire. PPD antiozonants protect rubber by donating an electron to ozone during oxidation and therefore scavenge reactive ozone molecules.[117]

6PPD is not the only PPD antioxidant used in rubbers or detected in the environment. Nisopropyl-N'-phenyl-o-phenylenediame (IPPD) is also widely used in rubber tires,[118] and other frequently used PPDs include N,N′-di-2-naphthyl-p-phenylenediamine (DNPD), N,N′-diphenylp-phenylenediamine (DPPD), N-phenyl-N′-cyclohexyl-p-phenylenediamine (CPPD), N,N′-(1,4dimethylpentyl)-p-phenylenediamine (77PD), and 7PPD (N-(1,4-dimethylpentyl)-N′-phenyl-pphenylenediamine).[119,120] Various PPDs, along with PPDQs similar to 6PPDQ, have been detected in water,[108,109,121,122] sediment,[123,124] dust,[97,125-128] atmospheric particulate matter,[54-

[56,129] food samples[130] and human urine.[131]

In relative terms, 6PPD and 6PPDQ have been detected in road dust at concentrations of 45 - 1175 ng/g in Tokyo, Japan.[132] Roadside soil samples, runoff water and air particles collected in Hong Kong, China had total detections of PPDs including IPPD, 6PPD, CPPD, DTPD, DPPD, ranging from 36.3 - 847 ng/g, 40-3000 ng/L, 1.75 - 9.41 pg/m^3, respectively.[54] In Guangzhou, China, total PPDs, including 6PPD, 77PD, DNPD, DPPD, IPPD, and CPPD, were present at concentrations ranging from 20.3-543 ng/g in road dust, 35.8-1481 ng/g parking lot dust, 56.6 - 604 ng/g vehicle dust and <LOQ- 207 ng/g in house dust samples.[97] PPDs and their quinone derivatives were detected across three locations in China in fine particulate matter. In samples collected in Guangzhou ∑PPDs : IPPD, CPPD, 6PPD, 7PPD, 77PD, DPPD, DTPD and DNPD ranged from 98.0 - 13,200 $P.M_{2.5}$ with a median of 3,220 $P.M_{2.5}$, ∑PPD-Qs : IPPD-Q, CPPD-Q, 6PPD-Q, 77PD-Q, DPPD-Q, DTPD-Q ranged from 3.61- 4,490 $P.M_{2.5}$ with a median of 1830 $P.M_{2.5}$. In the same study roadside sample concentrations collected for total PPDs were (10.9 - 14,200 $P.M_{2.5}$ and a median value of 7990 $P.M_{2.5}$) and total PPD-Qs (52.5 - 12,400 $P.M_{2.5}$, median 6,300 $P.M_{2.5}$). Samples collected in Taiyuan, China total PPDs (3.48-8,630 $P.M_{2.5}$, median 1150 $P.M_{2.5}$) and total PPD-Qs (5.59-8,480 $P.M_{2.5}$, median 6300 $P.M_{2.5}$). Roadside soils had a greater median concentration than the two sample sites collected further from the road.[129]

1.6 Stormwater Tracers: Benzothiazoles, HMMM and DPG

In addition to 6PPD, 6PPDQ, and related PPD compounds, many other vehicle related compounds are commonly found in road stormwater runoff. Rubber derived chemicals are compounds used in rubber products which prevent degradation, corrasion and oxidation, among other purposes such as vulcanization accelerators.[14] Tire constituents are not always disclosed but

common organic compounds found in tire manufacturing are: Hexa-(methoxymethyl- melamine (HMMM), used as a tire bonding agent, [133] vulcanization accelerators such as 1,3diphenylguanidine (DPG) and N-cyclohexyl-1,3-benzothiazole-2-amine (NCBA). [14,111] These additional rubber additives such as the bicyclic amine, DPG, HMMM, NCBA and Benzotriazole (BTR) and Benzothiazole (BTH) derivatives are found within roadway runoff and runoffimpacted waters, sometimes at high concentrations after rainfall.[90,134] In an urban tributary in Australia, DPG concentrations were 13- 1079 ng/L [111] which were similar to surface water concentrations in the US ranging between 5-540 ng/L [5] and in Canada at 160-760 ng/L.[109]

HMMM is another compound widely used in tire manufacturing,[52] enters surface waters through surface water runoff [53] and has been suggested as a potential tracer for TWPs.[135-137] Concentrations of HMMM in surface waters during a rain event in Ontario Canada exceeded 1000 ng/L[52] which is higher than those collected off a German highway with concentrations reaching 660 ng/L.[136] HMMM concentrations in a small creek ranged from 1-200 ng/L [6,90] and 0.5- 6900 ng/L in surface water across 44 sample sites in Yangtze River Delta, China.[138]

N-cyclohexyl-1,3-benzothiazole-2-amine (NCBA) also has been suggested as a possible TWP indicator for tire debris in roadway runoff.[41] NCBA dissolved and particulate concentrations in runoff ranged from 22-508 ng/L.[41]

Benzotriazole (BTR) and benzothiazole (BTH) derivatives are corrosion inhibitors such as Benzotriazole (1-H-BTR), 5-methyl-1-H-benzotriazole (5-Me-1-H-BTR), 2-aminobenothiazole (2-N2-BTH), 2-hydroxy-benzothiazole (2-OH-BTH) and 2-(4morpholinyl)benzothiazole (2-Mo-BTH). In a study looking at tire dust, BTR and 2-OH-BT made up between 56%-89% of all BTR and BTH with 2-OH-BT being the most abundant having

a 100% detection frequency.[124] Concentrations of BTH and BTR ranged from 5-540 ng/L in Brisbane Creek in Australia, similar to surface waters in the Pearl River Delta, China where BTH derivatives ranged from 213-1082 ng/L and BTR 112-1279 ng/L.[139]

These additional compounds emphasize there are many contaminants that are reported consistently in roadway runoff inducing environmental pollution.

1.7 Coho Salmon and 6PPDQ

In the Pacific Northwest and along the western North American coast, the occurrence of the highly toxic chemical 6PPDQ in habitats of sensitive species like coho salmon creates a substantial risk of acute mortality events in watersheds that are highly impacted by roadway runoff. Coho salmon life cycles are anadromous, meaning they are born and rear in freshwater, migrate to oceans to grow to adulthood, then from the ocean to creeks and rivers to spawn **(Figure 1.1).** Adult coho salmon return to their native watersheds in fall and winter (OctoberDecember) where they lay eggs in a gravel nest (redds) for incubation during the winter months. In early spring the eggs hatch and the alevin life stage begins. Alevin remain in the gravel attached to their egg yolk for several weeks (35-45 days) before "buttoning up" and emerging as fry during the spring. This stage is extremely temperature dependent, the warmer the water, the faster they "button up". They continue to stay in the freshwater ecosystem for up to a year to rear as juveniles. After the juveniles have entered their next life stage, smolts, they migrate from their freshwater system downstream to ocean and estuaries the following spring. Coho spend anywhere from 18 - 24 months in the ocean before returning to their natal stream as adults where they will complete the spawning process and die. After salmon spawn and die their

carcasses act as a source of nutrients in the water column, increasing nitrogen and phosphorous levels and creating a more nutrient rich environment for the newly hatched.

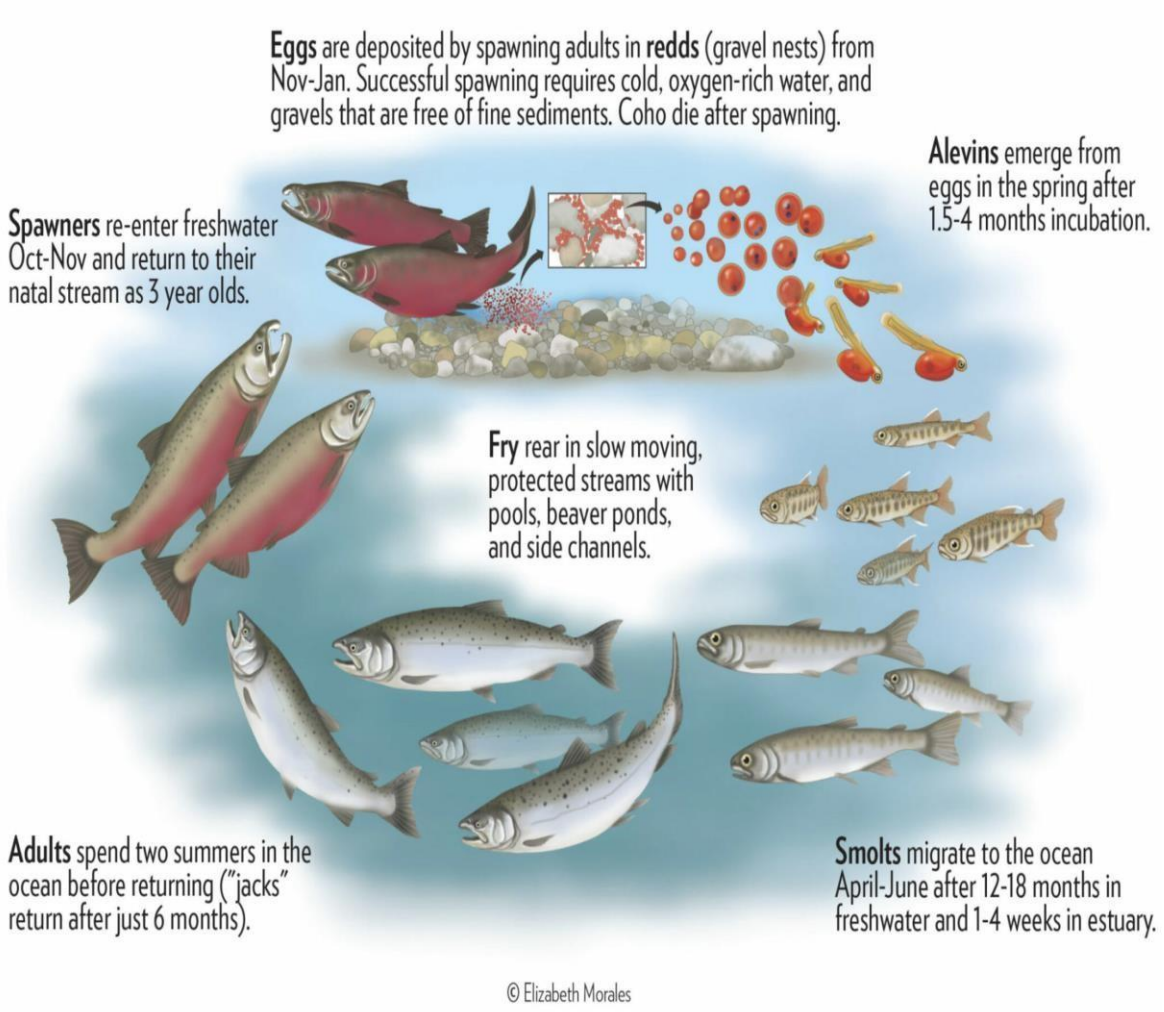

Figure 1.1. Coho Salmon Life Cyle.[140]

Mortality rates of returning adult coho salmon have been documented since the early 2000s causing a drastic decrease in population. Older juvenile coho laboratory studies have been conducted in the recent years identifying LC_{50} values of 95 ng/L and younger as 41 ng/L. Newly feeding juvenile sensitivity to 6PPDQ is greater than fish older than 1 year, resulting in a lower LC_{50}.[99] As of now there is little information regarding embryo and early juvenile coho life stages when exposed to spring storm rain events.

Juvenile coho and chinook salmon were both exposed to 6PPDQ in laboratory work at concentrations of 104.7 ng/L and 307 ng/L respectively. These concentrations resulted in survival rates of 7.1% and 61.4%, indicating coho salmon are at a far greatest risk than other salmonid species. [99] Coho sensitivity observed in another laboratory study in the pacific northwest compared sub yearling and larger (1+) juvenile coho by exposing both to untreated stormwater runoff. During three storm events juveniles showed high mortality rates of (92-100%) where they began dying (2 – 4 h) after exposure.[88]

1.8 Hypothesis and Study Objectives

Notably, both juvenile and adult migrations of coho salmon often occur during rain events when the coho salmon are exposed to higher flows in the streams. Also, coho salmon inhabit smaller rivers and tributaries that are too small for larger salmon species such as chinook, including in small "urban" creeks such as Miller Creek where the water should be cold and clean. These characteristics of extended use of small, dilution limited freshwater habitats for long time periods of time make coho salmon particularly susceptible to impacts from water quality degradation during rain events. While URMS associated mortality in adult coho salmon has been well documented, the possible impacts on alevin, fry, and juvenile fish during spring rain events have not been investigated, although it is clear that 6PPDQ concentrations can be high during spring rain events in small, dilution limited receiving waters, the mortality rates of these early life stages have been unaddressed. To address this research gap, we evaluated mortality of juvenile life stages in the field through a paired water quality and ecotoxicology analysis to observe survival rates when exposed to spring storm events. We hypothesize that poor water quality impaired by roadway runoff/6PPDQ generates high enough concentrations of the

contaminant to induce moderate to extensive acute mortality in juvenile life stages of coho salmon under field conditions.

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2. Roadway Runoff Induced Mortality in Juvenile Coho Salmon During Spring Storm Events

2.0 Publication and contribution statement

One publication was incorporated for this section, and Marlee Brown, Nathan Ivy, Jen McIntyre, John Hansen, Justin Greer and Edward Kolodziej worked on the research and development effort. For Brown et al. 2024, MB designed and conducted the water quality experiment, processed the samples, analyzed experimental data, and wrote the manuscript draft.

2.1 Introduction

With increasing urbanization, salmon-bearing watersheds across the Pacific Coast of North America can be heavily impacted by roadway runoff during storm events, introducing a complex mixture of contaminants into sensitive aquatic ecosystems.[1,2,3,4] Adult coho salmon (*Oncorhynchus kisutch*) returning to lowland creeks subject to urbanization now have a welldocumented history of stormwater-linked acute mortality events, known as Urban Runoff Mortality Syndrome (URMS), [5] previously "coho pre-spawn mortality" [6] or "urban stream syndrome".[1,7,8] URMS observations of symptomatic or dead coho salmon were first systematically documented in "restored" urban creeks of the Pacific Northwest starting in the 1990s.[6,9] In highly impacted watersheds, annual mortality rates for adult coho salmon often exceed 90% from fall storm exposures.[10] Such rates of loss of adult spawners prior to successful reproduction would result in localized extinctions within the most impacted populations over the next century.[6,10,11]

URMS was first linked to tire rubber leachate,[12] and subsequently, the primary causal toxicant responsible for URMS was identified as 6PPD-quinone (6PPDQ).[13,14] 6PPDQ forms from the anti-ozonant 6PPD commonly used in rubber to prevent ozone damage.[15] All vehicle tires globally contain 0.4-2.0% of 6PPD by mass (~40-100 g per tire).[16,17] Atmospheric ozone reactions with 6PPD continually generate 6PPDQ as an oxidation product at air-rubber surfaces.[18] 6PPDQ forms at yields of ~1% from the reaction between 6PPD and ozone.[13,19]

The environmental impacts of the extensive roadway systems of human society are defined in part by vehicle tire use, especially as tire wear particles are continually and abundantly generated from tires by roadway abrasion. 6PPDQ is easily leached from water-exposed rubber surfaces and therefore is almost ubiquitously detected in roadway environments and roadwayimpacted systems, including water, dust, air, roadside soil, sediments and biota.[20,21] In and receiving waters, 6PPDQ concentrations are reported globally with concentrations up to ~200 ng/L in Canada,[22] 88 ng/L in Australia,[23] 140 ng/L in Norway,[24] and up to 2400 ng/L in roadway runoff in Hong Kong.[25] In smaller receiving waters typical of coho spawning and rearing habitats, 6PPDQ levels have been reported up to 450 ng/L in the Pacific Northwest,[26] although peak event concentrations of 20-200 ng/L are more typical for small runoff-impacted urban creeks.[22,27] Runoff-derived contaminant concentrations in receiving waters are dynamic, with peak concentrations typically aligning with maximum discharge and lasting only a few hours.[28]

Juvenile coho salmon fry typically emerge from streambed gravel nests (“redds”) throughout late winter and early spring, coinciding with seasonal storm events that potentially introduce 6PPDQ into rearing habitats. Coho juveniles then spend roughly one year inhabiting

freshwater systems, often including low flow headwaters and small tributaries before migrating through estuaries to the ocean. Median lethal concentrations (LC_{50}) for juvenile coho salmon range from 41-95 ng/L across life stages.[27,29-31] Median lethal concentrations are frequently observed in smaller receiving waters that are moderately to highly affected by roadway runoff and also lack substantial dilution capacity. As a result of coho lethality when exposed to 6PPDQ, the United States Environmental Protection Agency released an Acute Aquatic Life Screening value of 11 ng/L for 6PPDQ.[32] Importantly, we have detected similarly high concentrations of 6PPDQ in coho rearing habitats during spring storm events as we have observed in fall rainstorms affecting adult coho spawners, implying that poor water quality—evidenced by high 6PPDQ concentrations—is a year-round phenomenon in roadway impacted systems.[12]

To the best of our knowledge, systematic observations of URMS events in juveniles have not been conducted, possibly because juvenile fish are small, remain hidden in gravels and protective habitat features, are quickly eaten when weakened, and are generally difficult to observe. However, anecdotal observations of storm-associated mortality in juvenile coho salmon in spring storms have been reported in British Columbia (personal communication, Tim Kulchyski, Cowichan Tribe) and on Miller Creek (personal communication, Iris Kemp, King County). Together, these water quality and anecdotal mortality observations suggest that URMS may pose substantial undocumented risks to the large numbers of juvenile coho present throughout rearing and migratory habitats. Smaller fish, juveniles, are more sensitive because they are smaller in size and developmentally vulnerable when subjected to 6PPDQ and other roadway contaminant exposures during spring periods.[29]

Coho salmon are an economically, ecologically and culturally important species of fish.[33] Additionally, coho salmon are an indicator species reflecting ecological function and health

across coastal watersheds of North America, a role which is threatened by widespread roadway runoff impacts to critical spawning and rearing habitats.[33,34] This study investigated URMS and 6PPDQ risks to juvenile coho salmon health in a representative rearing habitat during spring storm events. To assess URMS risks to juvenile coho salmon, we conducted a paired water quality-ecotoxicology study at a small runoff-impacted creek in Washington State where substantial URMS has been documented for returning adult salmon during the fall.[12] Over three spring storms (April-June 2024), juvenile coho were exposed to either runoff-impacted creek water or groundwater controls. 6PPDQ concentrations were measured using liquid chromatography-tandem mass spectrometry and various endpoints related to survival of juvenile coho were monitored across these exposure periods.

2.2 Materials and Methods

2.2.1 Chemicals. PPD transformation products and other stormwater contaminant stocks were prepared as described previously.[35] PPD stocks were prepared in methanol from analytical standards and stored in airtight bottles in bags at 4 °C. The prepared stocks along with the calibration standards were stored at -20 °C and replaced every 2 months to limit instability effects. LCMS grade Methanol was purchased from Fisher Scientific. Deionized water (18 MΩcm) was generated by a Milli-Q Ultrapure Water System. A complete list of chemicals is provided in the supporting information (Table S1).

2.2.2 Site and Sampling. Miller Creek is a small residential, commercial and urbanizing watershed (~2070 hectares) with multiple inputs of treated and untreated roadway runoff that results in high rates of URMS in adult coho salmon annually.[12,28] Paired exposure studies were conducted in a small field laboratory facility adjacent to Miller Creek, located in Normandy Park,

WA, USA. (47.442236, -122.326659). There is no wastewater discharge in Miller Creek, and therefore water quality is driven by roadway runoff and other urban stormwater inputs during storms. The modified Mediterranean climate of Western Washington typically has nearly dry, warm summers followed by frequent low-moderate intensity precipitation throughout the October-May period.

Figure 2.1. Current field Laboratory set up at Miller Creek for real time exposure of targeted storm events. (a) Parallel raceways with flow through groundwater in raceway A and miller creek water in raceway B. (b) Exposure study tank set up including four fish tanks per raceway per storm in each raceway. (c) Image of the field laboratory located along Miller Creek. (d) Image of

the ISCO set up in between the creek and field laboratory. All photos of sampling site setup were taken at different times.

Water samples were collected from Miller Creek ~20 m from the field laboratory and ~1 m upstream of the pump supplying creek water to the field laboratory using an automated water sampler (Teledyne ISCO 6712, Lincoln, NE USA) as shown in (Figure 2.1D). Twenty-four 350 mL glass bottles were used to collect 2-3 h time-dependent composite samples throughout storms. Compositing generally consisted of 40-160 mL aliquots sampled every 15-30 minutes. Sampling began in baseflow periods prior to storms and continued through the storm hydrograph until all bottles were filled. Three baseflow events (dry periods between storms without measurable rainfall) were sampled with an antecedent dry period (ADP) greater than 2 days on April 16 (6 d ADP), May 14 (6 d ADP), and June 26, 2024 (8 d ADP). Three storms used for fish exposures initiated by forecasted precipitation were sampled on April 25 (3 d ADP), May 21 (2 d ADP), and June 2, 2024 (2 d ADP). The first two storms generated 12 composite water samples over 26 hr. The third storm was longer and generated 24 composite samples over 73 hr. For groundwater controls, collected from the influent plumbing into raceway A, 1 L grab samples were collected before and after storms (N=4) from the exposure tank feed. Similar creek controls (1 L grab sample) were collected before and after each storm and baseflow sampling event (N=13) for pre storm baseline values. Concentrations detected in groundwater control samples of 6PPDQ were > 0.2 ng/L.

2.2.3 Hydrological data. Hydrology data for flow and to calculate cumulative discharge and contaminant mass loads were collected from an automated gage located within 0.6 km of the field laboratory facility (stream gauge 42A; 47.44548, -122.35196; King County Hydrologic

Monitoring Program) and study site. Precipitation data was collected from the nearest automated station reported by Weather Underground (Rabbit Hill - KWASEATT2555, Burien, WA 47.46700, -122.33400) and located 2.7 km away. Targeted storms were selected based on a rainfall forecast of > 6 mm and ADP > 2 days.

2.2.4 Fish Exposures. Juvenile coho salmon were acquired from Soos Creek Hatchery (Washington State Department of Fish & Wildlife) prior to each of three targeted storm events. Fish were age 0+ mass: 2.6 g ± 0.8 g (mean ± SEM), length 62.4 mm ± 5.7 mm (mean ± SEM). Thirty fish were placed in each of 8 glass aquaria containing 32 L of well water. Each aquarium was perforated with a mesh-covered outlet (12.7 mm diameter) enabling water to flow-through at 70 L/h ± 5L/h, a rate and fish loading density that are well below guidance for flow-through exposures. [36] The 8 aquaria were divided among two fiberglass raceways (Figure 2.1B) that were supplied with flow-through creek water to standardize temperatures across treatments. Upon arrival, fish were acclimated for a minimum of 24 hours in respective creek or groundwater and subsequently fed commercial pellets (2.5% body weight) every other day. Daily care included removal of uneaten food and feces, monitoring for clinical signs and survival, and measuring water temperature, dissolved oxygen, pH and conductivity.

Just before storms, inflow waters to the aquaria of one raceway were switched from well water to flow-through water pumped directly from Miller Creek. Fish were not fed the day of a storm pulse and food was withheld for the duration of the storm exposure (5-6 days), to minimize potential confounding factors. Fish were monitored for survival, signs of stress (flashing, erratic swimming, color change, lethargy) and symptoms of URMS (surface gaping, surface swimming, loss of equilibrium) during exposure periods. Dead fish were promptly removed and recorded. Moribund fish (laying on the bottom of the tank, unresponsive) were removed and euthanized by

a lethal dose of MS-222 (500 ng/L buffered to pH 7). Lengths and weights were recorded for mortalities and euthanized survivors at the end of each storm exposure. Animal care and euthanasia methods were approved by the Washington State
University Institutional Animal Care and Use Committee.

2.2.5 Statistical Analyses. Differences in survival among for the three storm events were visualized by Kaplan-Meier survival curves in R (version 4.4.3) using the functions Surv(), survfit(), and ggsurvplot() from the *survival* and *survminer* packages with interval-censored survival times (every 24 h). Survival curves were compared statistically using the log-rank test function survdiff() followed by pairwise comparison using the function pairwise_survdiff() with a false discovery rate adjustment.

2.2.6 Contaminant analysis. Water samples were transported to the Center for Urban Waters (Tacoma, WA) on ice and processed within 24 h. Samples were combined for composites and split into duplicates. Samples (200 mL) were solid phase extracted using Oasis HLB cartridges (200 mg, 6 mL; Waters Corp., Milford, MA, USA) filled with 2.5 g pre-cleaned, 40µm glass beads (3M FA400; Thermo Fisher Scientific, Waltham, MA, USA). Cartridges were preconditioned with 10 mL methanol, 25 mL deionized water at a flow rate of 10 mL/min. Samples were spiked with 6PPDQ-d5 (50 uL of 100 µg/L) before SPE and extracted at 5-10 mL/min. Cartridges were then rinsed with 10 mL deionized water, nitrogen dried for 15 minutes and eluted with 10 mL methanol. Eluates were blown down (N_2 gas) to <1 mL, volumized with methanol and transferred into autosampler vials. Samples were stored at -20 °C until analysis.

6PPDQ and select other stormwater contaminants, including various PPDs and other 6PPD transformation products, were quantified on an Agilent 1290 Infinity II HPLC system coupled with a 6495D triple quadrupole MS/MS instrument (LC-MS/MS) using an expanded

version of the liquid chromatography-triple quadrupole mass spectrometry method reported.[35] Multiple reaction monitoring (dMRM) with 2-3 transition ions was used to quantify and confirm analytes. Instrument parameters, calibration range, internal standards, and method detection/quantification limits are provided in Supporting Information (Tables S2-S4).

2.2.7 Quality Assurance and Quality Control. QA/QC sample preparation for baseflow and targeted storm events consisted of method blanks, field blanks, deionized water spikes, and matrix spikes (Table S5). Field blanks (N = 6) consisted of 1 L of Milli-Q water pumped through the ISCO and processed identically; method blanks (N = 6) consisted of 200 mL Milli-Q water extractions. Due to a sample carryover event, 6PPDQ was detected in one method blank at <4 ng/L and one field blank at 0.6 ng/L. PPDs, TPs and other analyte detections are reported in (Table S6). Matrix spikes (N= 12) consisted of spiking roadway contaminants into creek water at either 125 or 375 ng/L concentrations. Before and after grab samples of targeted storms consisting of groundwater and creek water sample detections are reported in (Table S7-S8). 6PPDQ recovery in matrix spikes was 99 ± 4.1%, 6PPDQ recovery in DI water spikes (N=7, 125 ng/L) was 97 ± 2.7%. Laboratory control spike and recovery in DI water detections are reported in (Table S9). Spike and recoveries for other analytes in matrix spikes are provided in (Table S10). Blanks and spike recovery samples were analyzed in duplicate while DI water spikes were in triplicate; results of replicate analysis for 6PPDQ indicated <10% coefficient of variation.

2.2.8 Runoff and Mass Loads. To create hydrographs and pollutographs, normalized cumulative precipitation values were calculated (Accumulation, in mm Eq. 1). Normalized cumulative runoff volumes were calculated from archived discharge values (Q, in m^3/s; Eq. 2); calculations began from the point when observed discharge values increased. Normalized

contaminant mass loads (Eq. 3) were calculated using measured contaminant concentrations (C_t; in ng/L) over the sampled period. Mass load calculations and curves match those reported by Peter.[28]

$$\text{Normalized cumulative precipitation volume } (in\ \%) = \frac{(Accumulation_t - Accumulation_{t0})}{(Accumulation_{t,final} - Accumulation_{t0})} * 100 \quad (1)$$

$$\text{Normalized cumulative runoff volume } (in\ \%) = \frac{\sum_{t0}^{tfinal} (Q_t - Q_{min})\Delta t}{\sum_{t0} (Q_t - Q_{min})(t_{final} - t0)} * 100 \quad (2)$$

$$\text{Normalized contaminant cumulative mass load } (in\ \%) = \sum_{t0}^{final} \frac{(C_t)(\sum Q_{tt}t0 - CQ_tQ_{min}t\Delta t)(t_{final} - t_0)}{} * 100 \quad (3)$$

2.3 Results and Discussion

2.3.1 Hydrology. Between April 1, 2024, and June 30, 2024, 16 precipitation events occurred in Miller Creek where flows at least doubled from baseflow conditions; most of these storms were low intensity and lasted only 1-3 h. Baseflow discharge in Miller Creek ranged from 0.05-0.1 m^3/s. Antecedent dry periods prior to the three study storms were 3, 2, and 2 days for Storms 1-3, respectively. Storm events required a predicted rainfall >0.2" and an antecedent dry period >48 hours to be considered for a targeted storm event. Pre-storm baseflows in this rainfall dependent watershed are generally linked to the length of antecedent dry periods, with shorter antecedent dry periods generating higher baseflows. The antecedent storm on April 21, 2024, had notably high rainfall of 19.6 mm, however the next two antecedent storms preceding the targeted

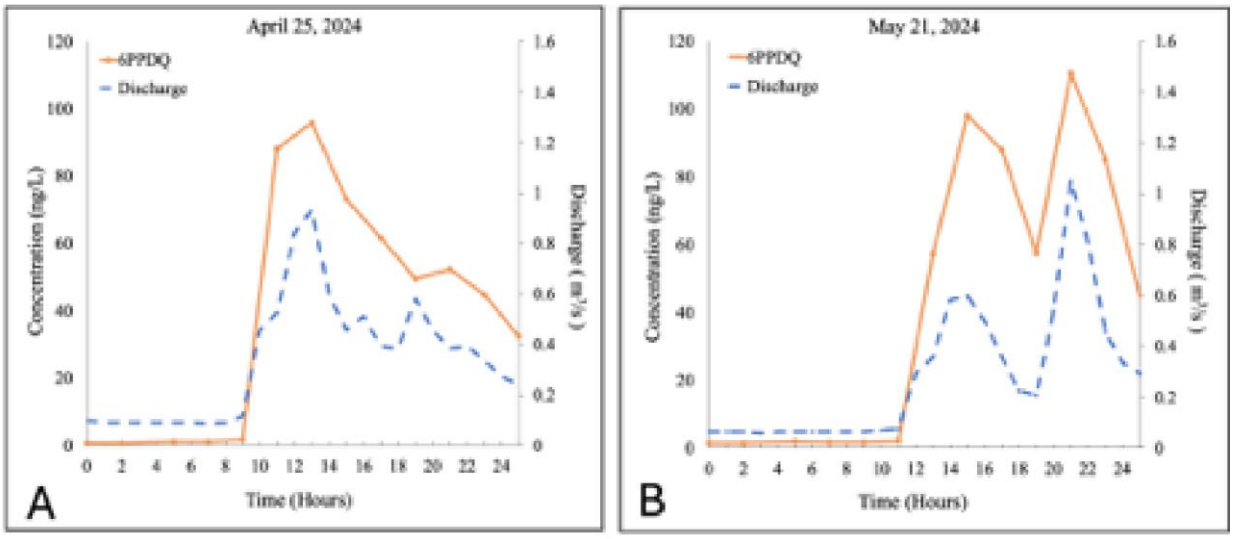
storms were small (0.3-8.1 mm), low intensity events lasting only 1-2 hours and with only <0.5 m^3/s changes to creek discharge.

Targeted storm 1 (April 25, 20.8 mm precipitation, 12.7 mm/h maximum intensity), storm 2 (May 21, 17.3 mm precipitation, 9.4 mm/h maximum intensity), and storm 3 (June 2, 22.9 mm precipitation, 11.9 mm/h maximum intensity) increased flows to 0.93, 1.1, and 1.4 m^3/s, respectively. These flows were about 10-fold higher than their preceding baseflows and the study storms were the three largest storms during this period. Storms 1 and 2 reflected relatively common spring storm events in size and duration; the June 2 storm (i.e., Storm 3), with two major pulses of precipitation and 2 days of substantially higher flows, represented a somewhat unusually large and long storm for a late spring-early summer event.

2.3.2 Water Quality. In baseflow conditions between three targeted storms, 6PPDQ concentrations in Miller Creek averaged 1.1 ng/L, 1.0 ng/L and 1.8 ng/L, respectively. Baseflow concentrations for other analytes are provided in (Table S11-S19). Complementary samples taken before and after baseflow and storm events (N=13) were collected and processed in duplicates. In 10 of 13 samples before and after grab samples, 6PPDQ was <4.0 ng/L, two after storm grabs and one before baseflow grab had concentrations greater than 15 ng/L. 6PPDQ was not detected in any groundwater control samples.

During the three targeted storms, 2-3 h composite samples were collected with automated samplers over 26-73 hours producing 12-24 samples. Because concentrations of roadwayderived contaminants in small watersheds can vary over time scales of minutes, these composites likely underestimate peak 6PPDQ concentrations in the creek but are reflective of biological exposure dynamics. [22,28,37] 6PPDQ was detected in 98% of Miller Creek storm samples (N=48). 6PPDQ concentrations rapidly rose as streamflow rose, with peak concentrations in composites

reaching 96, 110, and 73 ng/L ($\bar{x}$ = 93 ng/L) for the three targeted storms, respectively (Figure 2.2A-2.2C). Peak composite 6PPDQ concentrations tended to occur within 2-4 hours after precipitation began as stream discharge rose, but for the second targeted storm event with two separate waves of precipitation, the peak 6PPDQ concentration occurred 8-9 hours later and was concurrent with the second peak in the hydrograph. After 6PPDQ concentrations and hydrographs peaked, 6PPDQ concentrations slowly declined to 10-40 ng/L, although not dropping below the <10 ng/L levels typical of baseflow within the sampled time frame after rainfall ended. 6PPDQ concentrations exceeded the 11 ng/L and 12 ng/L EPA and WA ECOL Aquatic Life Screening criteria [32,38] for >16, 14, and 27 h for Storms 1-3 respectively. These values also exceeded the 41 ng/L LC_{50} value of [29] for 14, 14, and 15 h respectively, and exceeded the 80 ng/L LC_{50} value of [30] for 4, 8, and 0 h, respectively. Concentrations of PPDs are provided in (Table S20-S22), 6PPDQ and other transformation products are provided in (Table S23-S25) and other vehicle related chemicals are reported in (Table S26-S28). The data does not conclude a correlation between storm intensity and 6PPDQ concentration, specifically.



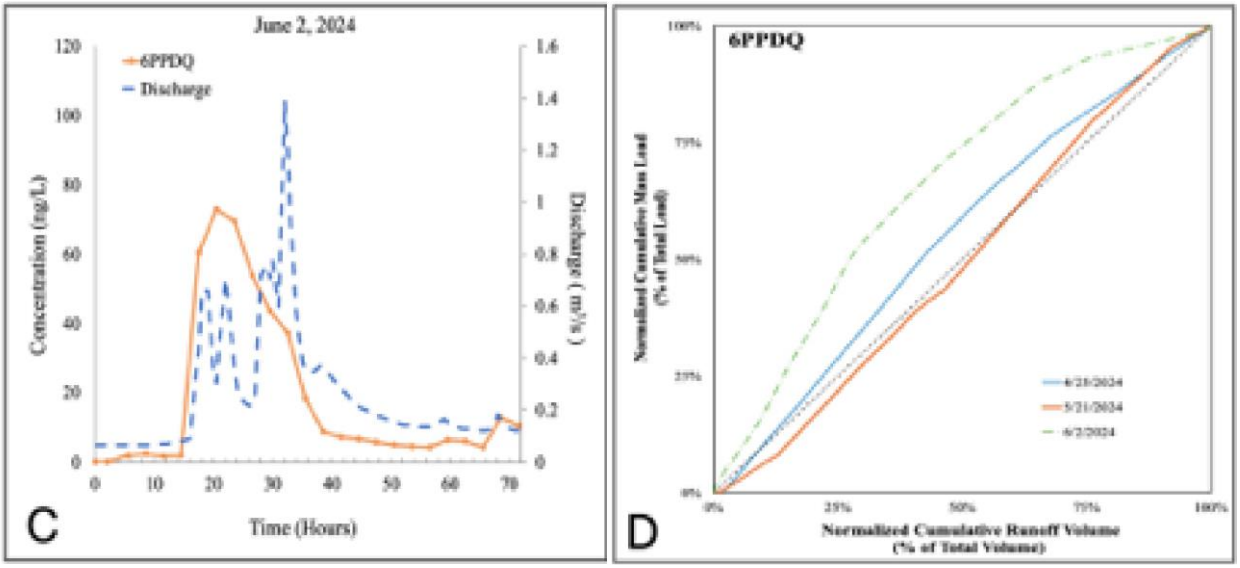


Figure 2.2. Pollutograph characteristics in Miller Creek during three targeted storm events in April, May and June 2024 as (A) 6PPDQ concentrations during storm 1 (B) 6PPDQ concentrations in storm 2 (C) 6PPDQ concentrations in storm 3 and (D) normalized cumulative mass load as (% of total load) plotted against cumulative runoff volume as (% of total volume). In panel d the mass load to runoff volume curve (dashed line) is represented as a 1:1 showing an uniform rate of mass transport into miller creek across each targeted storm event.

Other studies have reported similar 6PPDQ concentrations and dynamics,[22,23,39] especially for smaller urbanizing watersheds similar to Miller Creek. In hourly composites, 6PPDQ was present up to concentrations of 79-164 ng/L, with similar temporal dynamics, across three small runoff-impacted creeks in British Columbia, Canada.[22] 6PPDQ reached 88 ng/L in a roadway runoff impacted tributary (160-1800 m downstream of two major roadways) of the Brisbane River in Australia.[23] Samples collected across the United States from stormwaterimpacted receiving waters detected 6PPDQ in 57% stormwater and 45% urban impacted samples at concentrations of 2-290 ng/L.[39] In the Great Lakes region, 6PPDQ was present in 80% of roadway-impacted tributary samples at concentrations up to 82 ng/L.[40] Notably, many of these

reported concentrations in receiving waters were near or exceeded LC_{50} values for sensitive species where some mortality would be expected in exposed populations.

Contaminant mass and transport dynamics depend heavily on weather and watershed characteristics such as size, shape, chemical properties, temperature, land use, hydraulic alteration, and receiving water volumes available to support dilution.[22] 6PPDQ normalized cumulative mass loads against cumulative volume for each storm are shown in (Figure 2.2D). Surprisingly, despite the ~50% variation in peak concentration (73 to 110 ng/L), the estimated mass loads (1900-2100 mg) of 6PPDQ were similar (within ~10%) for each of the three storms.

Mass loads for all contaminants are reported in (Table 2.1). In a preceding study conducted across seventeen 2020-2023 storms in Miller Creek, 6PPDQ mass loads ranged from 78-2700 mg/storm, with an average load of 980 mg and median load of 760±770 mg.[41] By load, the three targeted spring storms of this study were among the larger mass transport events yet observed for Miller Creek.[28]

Table 2.1. Observed concentration range, peak concentrations and estimated mass loads for the targeted stormwater derived contaminants. Total concentrations, peaks and mass loads for PPDs, PPDQs, 6PPD TPs and vehicle related contaminants juvenile coho were exposed to across three spring storm events (April 25, May 20, and June 2, 2024) in Miller Creek. Concentration values for PPD parents are considered semi-quantifiable due to their instability. Detection frequency is reported across three storms (N=48), the concentration range, peak and mass loads are per duplicate composite samples (N=48).

Contaminant		Detection Frequency	Concentration Range (ng/L)	Peak Storm Concentrations (ng/L)	Mass Loads per Storm (g)
PPDs	6PPD	75%	ND – 360	52 - 360	1.3 - 5.3
	IPPD	19%	ND – 4.9	3.6 - 4.9	ND - 0.09
	7PPD	2%	ND – 0.6	ND - 0.6	ND - 0.04
Σ PPDS	DPPD	40%	ND – 120	8.9 - 120	0.2 - 1.2
	DTPD	42%	ND – 83	17 - 83	0.5 - 1.4
	DNP	0%	ND	ND	ND
			ND - 570		**ND - 8.0**
PPDQs/TPs	6PPDQ	98%	ND – 110	73 - 110	1.9 - 2.1
Σ PPDQs/TPs	IPPDQ	0%	ND	ND	ND
	7PPDQ	0%	ND	ND	ND
	DPPDQ	0%	ND	ND	ND
	DTPDQ	15%	ND – 52	ND - 52	ND - 1.2
	1,3-DMBA 4-OH-DPA	90%	ND - 2700	340 - 2700	11 - 44
	4-ADPA	81%	ND - 140	85 - 140	1.7 - 2.7
	4-DPA	40%	ND – 470	ND - 470	ND - 7.6
	4-NDPA	71%	ND – 510	170 - 510	3.5 - 9.1
		71%	ND - 20	7.0 - 20	1.50 - 2.7
			ND – 4000		**ND - 67**

Vehicle Related Contaminants	1,3-Diphenylguanidine	100%	50 - 1300	950 - 1300	23 - 29
	HMMM	100%	24 - 1200	750 - 1200	18 - 25
	NCBA	71%	ND – 18	14 - 18	0.3 - 0.4
Σ VRCs	1-H-BTR	100%	10 - 550	280 - 550	5.2 - 9.9
	5-Me-1-H-BTR	100%	30 - 1700	970 - 1700	19 - 28
	2-NH2-BTH	98%	0.7 - 91	87 - 91	1.6 - 2.5
	2-OH-BTH	100%	34 - 240	210 - 240	4.6 - 7.1
	2-Mo-BTH	94%	1.5 - 40	15 - 40	0.3 - 0.5
			ND - 5100		**72 - 102**

While we have now developed a storm sampling data set for Miller Creek, we have not yet identified predictable relationships between storm conditions, contaminant sources, transport mechanisms, and concentrations or mass loads.[41] 6PPDQ dynamics across these storm events were most consistent with middle flush and mixed flush characteristics with continuing and increasing mass transport throughout the storms (Figure 2.2D). In the April and May storm events, cumulative 6PPDQ mass loads either somewhat exceeded, or nearly mirrored, cumulative runoff volumes, with middle or mixed flush dynamics over the storms. The larger and longer June 2 storm was more typical of a middle flush dynamic.[17,42] This watershed was again seemingly defined by transport-limited contaminant mass dynamics for 6PPDQ and roadwayderived contaminants more generally. In these situations, continued precipitation results in continued and even growing 6PPDQ mass transport relative to streamflow as hydraulic connectivity and mass transport pathways are established that move contaminant mass to the receiving water. [43] Notably, no clear evidence of dilution with continued precipitation or streamflow is apparent in this data, indicating that even in late spring, after dozens of fall-

winterspring storm events had occurred, substantial 6PPDQ mass remains available for environmental transport within this watershed. While some roadway-impacted watersheds do not exhibit such transport-limited behavior, it is likely that the more "urban" or "urbanizing" watersheds, or the more highly trafficked locations, have accumulated over time substantial reservoirs of roadway and vehicle-derived contaminants that are difficult to deplete during many precipitation-induced transport events.[22]

While not a primary focus of this manuscript, concentration trends and dynamics for other roadway and vehicle derived contaminants analyzed concurrently with 6PPDQ were broadly consistent with those reported here for 6PPDQ, and with prior studies focused on Miller Creek contaminants.[12,28] Pollutographs and normalized cumulative mass loads and runoff volume for PPDs (Figures S1-S4), 6PPD transformation products (Figures S5-S8), and for other vehicle related compounds (Figure S9-S12) were derived, and were also broadly similar to results for 6PPDQ and other runoff-derived contaminants. Observed concentration and mass dynamics across the roadway-derived contaminants tended to reflect and support observations reported above for 6PPDQ, with increasing concentrations and mass transport with discharge and time. Many roadway and tire-derived contaminants, including various other PPDs, PPD-quinones, and transformation products of 6PPD, are co-present with 6PPDQ and often reflect similar chemodynamics. In particular, the similar middle flush and transport-limited dynamics in Miller Creek emphasize the common source, abundant mass, and similar chemical characteristics and structures of these contaminant classes.

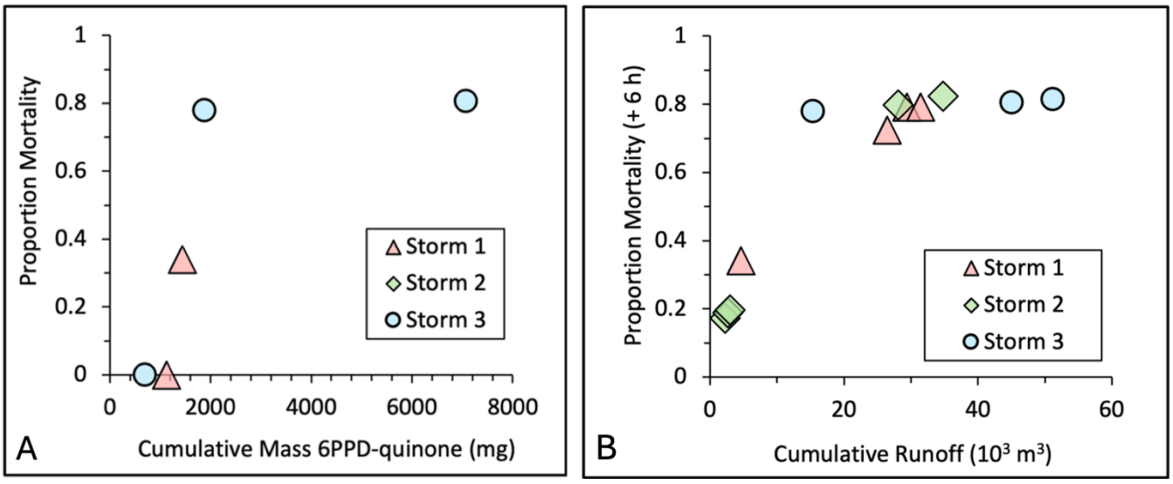
Hexamethoxymethylmelamine (HMMM), a chemical found in tire manufacturing, also occurs in roadway runoff impacted waters and tends to exhibit hydrological profiles similar to

6PPDQ. [44] In Miller Creek, concentrations ranged from 24 -1200 ng/L, similar to previous reports of HMMM in surface waters. [44,45] Diphenylguanidine (DPG) is another widely-detected and abundant (up to 1000s of ng/L in surface waters)[17,46,47] rubber compound used in tire manufacturing. Miller Creek concentrations ranged from 50 – 1300 ng/L comparable to previous detections. [12,35] Though we consider PPD parent concentrations semi-quantitative due to their instability, 6PPD concentrations (52 – 360 ng/L across the storms) largely mirrored those for 6PPDQ and spiked 2-4 hours after the rain started. (Tables S20 – S22).

Mass loads for these contaminants ranged as low as 0.004 g/storm and as high as 5.3 g/storm for PPDs with a detection frequency of 0-75% in all storm samples. Transformation products ranged from 1.2 to 44 g/storm, detection frequency 0-98% and other vehicle related chemicals ranged across 0.4 to 29 g/storm, detection frequency 71-100% across the three storms (Table 2.1). Again, noting that storm sampling stopped before contaminant concentrations had returned to baseflow values as we were mainly targeting the peak, total PPD mass loads were at least 8 g/event, PPD transformation products were at least 67 g/event, and other vehicle-derived chemicals were at least 100 g/event (Table 2.1). Of these, 6PPDQ represented only 3.2% of the total detected PPD transformation product load mobilized across each event.

2.3.3 Coho survival. For control groups receiving groundwater, no mortality was observed during any storm event. In contrast, high rates of mortality occurred for juvenile coho salmon directly exposed to water from Miller Creek during storm events. Researchers were present at the onset of clinical symptoms (e.g., surface swimming, gaping) for two of the three storms (Storm 1 and 3). For both, the onset of symptoms occurred 13-15 h after precipitation commenced, 7-9 hours after increased (>15%) stream discharge, and 3-5 h after the peak

composite concentration (95.8 ng/L for Storm 1 and 78.4 ng/L for Storm 3). Regardless of storm duration and intensity, the average cumulative mortality across all three storms was quite similar (79-81%), with a mean mortality of 80%. Daily mortality rates varied across the three storms (chisq(2) = 122, p <0.001), with Storm 2 showing a significantly different pattern (p <0.001) of more protracted mortality across time (Figure 2.3). This was a result of two pulses of precipitation within the same several-day storm window. The first pulse on May 18 produced just 5 mm of rainfall that resulted in 20% mortality by May 21. A second pulse on May 21 produced an additional 20 mm and an additional 60% mortality by May 23. Across storms, mortality increased with estimated mass of 6PPDQ mobilized into the stream (Figure 2.3A) up to approximately 1800 mg. Across storms, mortality increases as cumulative runoff (Figure 2.3B) and cumulative precipitation (Figure 2.3C) also increase showing there is a correlation.



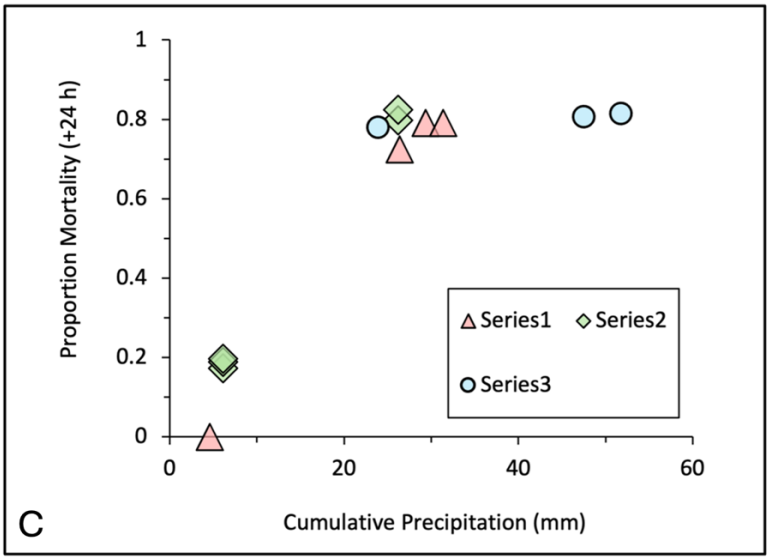


Figure 2.3. Survival of juvenile coho for three targeted storm events. A) Average daily survival across three storm events as a function of cumulative mass of 6PDPQ. B) Average daily survival as a function of cumulative runoff in the preceding 6 h. C) Average daily survival as a function of cumulative precipitation in the preceding 24 h. No mortality occurred in fish concurrently exposed to groundwater.

From Alaska to California and throughout much of the Pacific Northwest USA and British Columbia, coho salmon spend up to 18 months in freshwater streams and creeks including their time as spawning adults and from fertilization to smoltification. Previous research detailed the fatal impacts of fall storms on adult spawning populations and recent studies have demonstrated that coho salmon and coastal cutthroat trout fry are extremely sensitive to 6PPDQ with 24-hour static LC_{50} estimates of 38-41 ng/L.[29,48] Currently coho salmon, coastal cutthroat trout, rainbow trout, steelhead salmon (Shankar unpublished), brook trout and lake trout are all sensitive to 6PPDQ at environmentally measurable levels. Still, the direct mechanism(s) leading to death are unknown. Suggested modes of action for 6PPDQ include vascular permeability

leading to compromised blood brain barrier[49] which could trigger pathological/neuroinflammatory responses as well as potential bio-transformed, toxic 6PPDQ metabolites that may interfere with cellular respiration.[49,50,51]Additional sublethal effects have been noted for susceptible species including yolk sac edema, blood pooling, reduced eye size, delayed growth, reduced swimming performance, irregular hematocrit and/or spinal abnormalities.[52]

2.3.4 Environmental Implications

Historically, Miller Creek has supported several salmonid species including coastal cutthroat trout, coho salmon, and chum salmon but in 2025 the coho salmon and likely coastal cutthroat trout populations are depleted due to habit degradation. During the last several decades, a recurrent and widespread mortality syndrome linked to stormwater has been documented in coho salmon in the Pacific Northwest.[6,11] Initial observations were limited to adult spawning salmon which would have significant impacts on wild coho populations as well as conservation efforts in watersheds adjacent to heavy vehicle traffic.[10] Based on emerging water quality data around 6PPDQ occurrence, coho mortalities are not a simple first flush or early fall storm phenomenon, but rather one that is characterized by year-round potential exposure risks that last throughout all aspects of fresh water residency for coho salmon.[11] The potential effects of storm water runoff on juvenile coho salmon prior to outmigration are difficult to quantify due to their small size and cryptic behaviors. Therefore, the potential loss of earlier life stages including juvenile development due to the mortality syndrome reflects a possible barrier to recovery for these populations.

Here, we observed composite, maximum concentrations for 6PPDQ during three spring storms that were at or above reported LC_{50} values for similarly staged juvenile coho salmon (LC_{50} 80 ng/L [CI: 63-98])[30] and far exceeding coho fry LC_{50} (41 ng/L CI 34-48)[29] with concurrent observations of 79–81% juvenile coho salmon mortality per storm. These data indicate the potential for widespread and extensive mortality events in runoff-impacted rearing and migration habitats for juvenile coho salmon. Juvenile coho salmon often spend a year or more in freshwater habitat before outmigration, maximizing the probability that they will encounter one or more storms in dilution-limited yet traffic-impacted watersheds that result in extensive water-quality linked mortality. It is likely that roadway-derived 6PPDQ exposures are changing coho salmon abundance and population structure in watersheds with busy traffic across the west coast of North America and, potentially, the Great Lakes. State, federal and tribal hatcheries release an average of 26M coho salmon annually (2019-2023) in Washington State alone. The potential loss of significant numbers of juvenile hatchery fish after release in water quality impaired habitats is both expensive and inefficient. Hundreds of thousands of fry are released annually into small creeks like Miller Creek by community groups focused on conservation efforts. At the other end of their life cycle, approximately 700K adult wild and hatchery reared coho salmon migrate back to spawning grounds annually in Washington State, almost all of which pass by roadways or roadway-impacted locations at some point during their migrations.

During coho salmon juvenile residency in streams and creeks near busy roadways, our data suggests significant losses from precipitation events >5 mm. This further compounds the already low (16%) chance of salmon successfully transitioning from alevin to smolt stages in non-fragmented habitats.[53] Considering losses to predation and other natural fates of juveniles in

freshwater and sub-adults in ocean habitats, it may be unreasonable to expect that populations of coho resistant to 6PPDQ would evolve in impacted habitats. Nonetheless, there are anecdotal observations of coho that survive the entire rearing year in highly runoff-impacted watersheds. The possibility of genetic selection among surviving coho or resistance conferred by sub-lethal exposure should be explored in future studies. Finally, pulsed exposures of developing coho embryos to collected runoff [54] or 6PPD-quinone[49] resulted in sublethal impairments to growth and morphometrics. The integrated effects of real-time runoff exposure throughout development should be investigated to understand the assess life cycle impacts for fish developing in impacted systems.

Roadway runoff treatment and source control is needed to protect water quality in roadway adjacent habitats, especially those with sensitive species and ecosystems. However, given the widespread nature and substantial volumes of roadway runoff, and expensive costs of treatment, mitigation of 6PPDQ concentrations in habitats of sensitive species is not practical as the only approach. Reformulation of tire rubber chemical compositions to reduce or eliminate all chemicals with toxic attributes is both a cost-effective and technically efficient strategy to protect aquatic ecosystems and enhance salmonid health. There remains a need for continued assessment of occurrence, concentration, and mass dynamics of tire rubber and vehicle-derived contaminants in watersheds highly affected by roadway runoff, even those far from urban centers, especially as the role of transient water quality degradation in ecosystem health becomes increasingly betterdefined.

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Supporting Information

Table S1. Chemical standards with CAS numbers, molecular formulas, vendor source and internal standards.

Compound	Abbreviation	CAS Number	Molecular Formula	Source	ISTD used
p-phenylenediamine (PPD) antioxidants					
N-(1,3-dimethylbutyl)-N'-phenyl-pphenylenediamine	6PPD	793-24-8	C18H24N2	Usolf Chemicals	6PPDQ-d_5
N-isopropyl-N'-phenyl-1,4-phenylenediamine	IPPD	101-72-4	C15H18N2	Richest Group Ltd	6PPDQ-d_5
N-(1,4-dimethylpentyl)-N'-phenyl-pphenylendiamine	7PPD	3801-01-4	C19H26N2	Richest Group Ltd	6PPDQ-d_5
N,N'-diphenyl-p-phenylenediamine	DPPD	74-31-7	C18H16N2	Richest Group Ltd	6PPDQ-d_5
N,N'-ditolyl-p-phenylenediamine	DTPD	68953-84-4	C20H20N2	Richest Group Ltd	6PPDQ-d_5
N,N'-di-2-naphthyl-p-phenylenediamine	DNP	93-46-9	C26H20N2	Richest Group Ltd	6PPDQ-d_5
N,N'-dicyclohexyl-p-phenylenediamine	CCPD	4175-38-6	C18H28N2		6PPDQ-d_5
PPD transformation products					
4-nitrosodiphenylamine	4-DPA	156-10-5	C12H10N2O	ChemService Inc	6PPDQ-d_5
1,3-dimethylbutylamine	1,3-DMBA	108-09-8	C6H15N	Sigma-Aldrich	6PPDQ-d_5
4-aminodiphenylamine	4-ADPA	101-54-2	C12H12N2	Sigma-Aldrich	6PPDQ-d_5
4-nitrodiphenylamine	4-NDPA	836-30-6	C12H10N2O2	Sigma-Aldrich	6PPDQ-d_5

4-hydroxydiphenylamine	4OH DPA	122-37-2	C12H11NO	Fisher Scientific	6PPDQ-d_5
6PPDQ	6PPDQ	2754428-18-5	C18H22N2O2	HPC Standards Inc	6PPDQ-d_5

IPPDQ	IPPDQ	68054-73-9	C15H16N2O2	Lab synthesized	6PPDQ-d_5
7PPDQ	7PPDQ	2894124-00-4	C19H24N2O2	Lab synthesized	6PPDQ-d_5
DPPDQ	DPPDQ	3421-08-7	C18H14N2O2	LGS standards	6PPDQ-d_5
DTPDQ	DTPDQ	252950-56-4	C20H18N2O2	Lab synthesized	6PPDQ-d_5
Vehicle-Related chemicals					
1,3-diphenylguanidine	DPG	102-06-7	C13H13N3	Sigma-Aldrich	DPU-d_{10}
hexa-(methoxymethyl)melamine	HMMM	3089-11-0	C15H30N6O6	Combi-Blocks ltd	Prometon-d_3
N-cyclohexyl-1,3-benzothiazole-2-amine	NCBA	28291-75-0	C13H16N2S	Enamine	5-Me-BTR-d_6
benzotriazole	1-H-BTR	95-14-7	C6H5N3	Sigma-Aldrich	5-Me-BTR-d_6
5-methyl-1-H-benzotriazole	5-Me-1-H-BTR	136-85-6	C7H7N3	Sigma-Aldrich	5-Me-BTR-d_6
2-amino-benzothiazole	2-NH2-BTH	136- 95-8	$C_7H_6N_2S$	Sigma-Aldrich	5-Me-BTR-d_6
2-hydroxy-benzothiazole	2-OH-BTH	934-34-9	C_7H_5NOS	Sigma-Aldrich	5-Me-BTR-d_6
2-(4-morpholinyl)benzothiazole	2-Mo-BTH	4225-26-7	C11H12N2OS	Sigma-Aldrich	5-Me-BTR-d_6
Internal Standards					

6PPDQ-d_5	6PPDQ-d_5	2750119-14-1	NA	HPC Standards Inc (Atlanta, GA, USA)	NA
13C6-6PPDQ	13C6-6PPDQ	NA	NA	Cambridge Isotopes	NA
N,N'-diphenylguanidine-d10	DPG-d10	NA	NA	LGC Standards	NA
N-cyclohexyl-13C6-1,3-benzothiazole-2-amine	NCBA-13C6	NA	NA	LGC Standards	NA
Diphenyl-d10-urea	DPU-d10	108009-46-7	NA	Toronto Research Chemicals	NA

Prometon-d3	Prometon-d3	1219803-43-6	NA	CDN Isotopes	NA
5-methyl-1-H-Benzotriazole-d_6	5-Me-BTR-d_6	1246820-65-4	NA	Toronto Research Chemicals	NA

Table S2. Instrument parameters for analyte quantification using LC – MS/MS.

Parameter	**Value**
Gas Temp	280 °C
Gas Flow	14 L/min
Sheath Gas Temp	400 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	2600(+) V / 3500(-)V
Nozzle Voltage	200 V (+) / 1000 V (-)
Nebulizer	40 psi
Fragmentor Voltage	166 V

Table S3. Instrumental parameters for detection of analytes.

Analyte	**RT (min)**	**Precursor ion (m/z)**	**Quantifier Ion (m/z) Collision Energy (V)**	**Qualifier Ion (m/z) Collision Energy (V)**	**Second Qualifier Ion (m/z) Collision Energy (V)**	**ESI Mode**
6PPD	4.2	269.2	184.0 (20)	107.1 (50)	93.1 (40)	Positive
7PPD	4.7	283.2	184.0 (25)	107.1 (60)	-	Positive
IPPD	3.3	227.1	184.0 (15)	107.0 (50)	-	Positive

DPPD	6.4	260.1	183.0 (35)	167.0 (40)	156.0 (40)	Positive
DTPD	7.5	288.2	197.0 (35)	182.0 (35)	167.0 (50)	Positive

DNP	8.2	360.2	233.0 (40)	217.0 (50)	115.1 (90)	Positive
4-DPA	4.5	199.0	181.0 (25)	169.0 (20)	128.0 (50)	Positive
1,3-DMBA	2.1	102.1	85.1 (5)	57.2 (10)	-	Positive
4-ADPA	2.9	185.1	167.0 (23)	108.0 (23)	93.1 (23)	Positive
4-NDPA	5.34	215.0	198.0 (15)	168.0 (25)	-	Positive
4OH DPA	3.8	186.0	109.0 (25)	92.0 (20)	-	Positive
6PPDQ	6.4	299.2	215.1 (14)	187.1 (26)	-	Positive
IPPDQ	4.72	257.1	215.0 (15)	200.0 (20)	187.0 (25)	Positive
7PPDQ	7	313.2	215.0 (15)	187.0 (30)	-	Positive
DPPDQ	5.4	293.1	263.1 (20)	77 (50)	-	Positive
DTPDQ	6.2	319.2	301.0 (25)	212.0 (20)	184.0 (25)	Positive
DPG	2.9	212.1	119.1 (20)	94.1 (15)	-	Positive
HMMM	4.13	391.0	207.1 (15)	177.1 (25)	-	Positive
NCBA	4.4	233.1	151.1 (20)	55.1 (30)	-	Positive

DCU	5	225.1	143.1 (15)	100.1 (10)	-	Positive
1-H-BTR	2.97	120.0	92.1 (15)	65.1 (20)	-	Positive
5-Me-1-H-BTR	3.35	134.0	79.0 (20)	77.0 (25)	-	Positive
UV-234	10.2	448.2	370.2 (20)	119.1 (35)	-	Positive
UV-326	10.2	316.1	260.0 (17)	107.0 (5)	-	Positive
2-NH2-BTH	2.6	151.1	124.1 (20)	109.0 (25)	-	Positive
2-OH-BTH	3.56	152.0	123.9 (20)	92.1 (20)	-	Positive
2,4-MoBT	4.2	221.1	177.1 (20)	150.0 (25)	-	Positive
6PPDQ-d_5	6.4	304.2	220.1 (14)	192.1 (26)	-	Positive
13C6-6PPDQ	6.4	305.1	193.2 (26)	247 (14)	-	Positive
DPU-d10	4.1	223.0	99.3 (18)	81.2 (28)	-	Positive
Prometon-d3	3.6	229.0	187.0 (18)	145.0 (20)	-	Positive
5-methyl-1-H-Benzotriazole-d_6	3.35	140.0	85.0 (20)	81.0 (28)	-	Positive

Table S4. Summary of prepared calibration curve ranges, method detection limits, method quantification limits and internal standards used for Analytes.

Analyte	MDL (ng/L)	MQL (ng/L)	Calibration range (ng/L)	ISTD Used
6PPD	1.6	4	0.025-100	6PPDQ-d5
7PPD	2.1	5.5	0.025-100	6PPDQ-d5
IPPD	0.94	3.3	0.025-100	6PPDQ-d5
DPPD	1.8	6.1	0.025-100	6PPDQ-d5
DTPD	1.2	4.3	0.025-100	6PPDQ-d5
DNP	0.09	0.3	0.025-100	6PPDQ-d5
4-DPA	1	3.5	0.025-100	6PPDQ-d5
1,3-DMBA	4.1	14	0.025-100	6PPDQ-d5
4-ADPA	0.55	1.9	0.025-100	6PPDQ-d5
4-NDPA	1.1	3.9	0.025-100	6PPDQ-d5
4OH DPA	1.4	4.8	0.025-100	6PPDQ-d5
6PPDQ	0.3	0.9	0.025-100	6PPDQ-d5
IPPDQ	0.93	3.2	0.025-100	6PPDQ-d5
7PPDQ	0.99	3.4	0.025-100	6PPDQ-d5
DTPDQ	1.2	4	0.025-100	6PPDQ-d5

DPG	0.87	3.01	0.1-100	DPU-d10
HMMM	0.57	1.95	0.1-100	Prometon-d3
NCBA	0.94	3.25	0.05-100	5-Me-BTR-d_6
1-H-BTR	2.2	7.6	2.0-200	5-Me-BTR-d_6
5-Me-1-BTR	0.45	1.6	1.0-200	5-Me-BTR-d_6
2-NH2-BTH	0.53	1.8	1.0-200	5-Me-BTR-d_6
2-OH-BTH	8	28	1.0-200	5-Me-BTR-d_6
2,4-MoBT	1.3	4.5	0.2-100	5-Me-BTR-d_6

Table S5. QAQC samples for each storm and baseflow event including Method Blanks, Field Blanks, Laboratory control spikes (LCS) and Matrix spikes along with the expected spiked concentrations.

Sample Event	**Event Type**	**Method Blanks**	**Field Blanks**	**LCS**	**Matrix Spikes (Low conc)**	**Matrix Spikes (High conc)**
4/16/2024	Baseflow	N=2	N=2	375 ng/L; N=3	125 ng/L; N=2	375 ng/L; N=2
4/25/2024	Storm	N=2	N=2	125 ng/L; N=3	125 ng/L; N=2	375 ng/L; N=2
5/14/2024	Baseflow	N=2	N=2	125 ng/L; N=3	125 ng/L; N=2	375 ng/L; N=2

5/20/2024	Storm	N=2	N=2	125 ng/L; N=3	125 ng/L; N=2	375 ng/L; N=2
6/1/2024; 6/3/2024	Storm	N=2	N=2	125 ng/L; N=3	125 ng/L; N=2	375 ng/L; N=2
6/25/2024	Baseflow	N=2	N=2	125 ng/L; N=3	125 ng/L; N=2	375 ng/L; N=2

Table S6. Concentrations (ng/L) detected in Method and Field Blanks.

Analyte	4/16/2024		4/26/2024		5/14/2024		5/21/2024		6/2/2024		6/5/2024	6/26/2024	
	Method Blank	Field Blank	Method Blank	Field Blank	Method Blank	Field Blank	Method Blank	Field Blank	Method Blank	Field Blank	Method Blank	Method Blank	Field Blank
6PPD	*	*	1.9	1.6	*	*	*	*	*	*	*	*	*
7PPD	*	*	9.4	8.8	*	4.9	*	*	*	*	*	*	*
IPPD	*	*	1.5	1.4	*	*	*	*	*	*	*	*	*
DPPD	13	4.5	10	10	*	*	*	*	*	*	*	*	*
DTPD	*	3.3	*	8.9	*	*	*	*	*	*	*	*	*
4-DPA	*	*	*	*	*	2.6	*	*	*	*	*	*	*

1,3-DMBA	*	14.5	35.5	11	*	*	2.9	31	*	*	*	*	84
4-ADPA	*	*	*	*	*	13	*	3.0	*	*	*	*	*
4-NDPA	*	*	*	*	*	42	*	*	*	*	*	*	*
4OH DPA	*	*	7.4	6.9	*	*	7.3	9.1	*	3.0	*	*	*
6PPDQ	*	*	*	*	*	0.6	*	*	*	*	*	3.7	*
IPPDQ	*	*	*	*	*	*	*	*	*	*	*	*	*
7PPDQ	*	*	*	*	*	*	*	*	*	*	*	*	*
DTPDQ	*	*	*	*	*	*	*	*	*	*	*	*	*
DPG	*	1.7	4.6	*	*	2.1	*	3.3	-	-	*	*	*
HMMM	0.8	1.8	1.3	1.2	*	2.3	*	1.1	-	-	*	*	6.6
NCBA	*	*	*	*	*	*	*	*	*	*	*	*	*
Benzotriazole	16.7	*	*	*	*	*	*	2.3	-	*	*	*	*
5-Me-1-H-BTR	3.4	4.6	*	*	*	*	*	*	-	*	*	*	*
2-NH2-BTH	1.3	2.5	*	*	*	*	*	*	-	*	*	*	*
2-OH-BTH	11	19	12	9.4	*	8.0	2.4	32	25	21	5.1	9.4	27
2,4-MoBT	*	*	*	*	*	*	*	*	-	*	*	*	*

[*] Concentrations are below method detection limit.

[-] Values were not quantified.

Table S7. Concentrations (ng/L) of analytes detected in groundwater grab samples collected from the well feed before and after storms 5/20/2024 and 6/1/2024.

Analyte	**5/20/2024**		**6/1/2024**	
	Before	**After**	**Before**	**After**
	Concentrations (ng/L)			

6PPD	*	*	*	*
IPPD	1.5	1.5	*	*
7PPD	*	9.4	*	*
DPPD	*	*	*	*
DTPD	*	9.8	*	*
1,3-DMBA	17	15	7.3	13
4-ADPA	4.8	*	*	*
4-NDPA	*	*	*	*
4OH DPA	9.3	7.5	3.8	2.5
6PPDQ	*	*	*	*
7PPDQ	*	*	*	*
1,3-diphenylguanidine	1.8	1.4	1.9	2.6
HMMM	18	11	15	13

[*] Represents values less and MDL.

Table S8. Concentrations (ng/L) of analytes detected in Miller Creek grab samples before and after baseflow and storm sampling events

	4/16/2024		4/26/2024		5/14/2024		5/21/2024		6/2/2024		6/5/2024	6/26/2024	
Analyte	Before Grab	After Grab	Before Grab	After Grab	Before Grab	After Grab	Before Grab	After Grab	Before Grab	After Grab	After Grab	Before Grab	After Grab
6PPD	*	*	1.9	19	2.9	*	*	5.5	*	4.0	*	*	*
7PPD	3.3	*	9.0	11	4.8	*	*	*	*	*	*	*	*
IPPD	*	*	1.5	1.7	2.3	*	*	*	*	*	*	*	*
DPPD	*	9.0	10	11	*	*	*	*	*	*	*	*	*

DTPD	7.5	7.1	10	16	18	*	*	*	*	*	*	*	*
1,3-DMBA	*	*	*	22	*	*	1.2	2.1	*	14	*	*	*
4-ADPA	36	21	40	680	46	130	36	73	53	190	57	40	380
4-NDPA	1.0	0.7	*	*	8.2	*	*	*	*	*	*	*	*
4OH DPA	*	*	*	3.9	0.3	0.2	*	*	*	*	*	*	*
4OH DPA	*	*	7.1	13	*	*	3.9	4.1	*	3.8	*	*	*
6PPDQ	1.0	1.2	1.4	20	25	1.6	1.4	3.3	2.9	15	3.9	1.4	1.0
IPPDQ	*	*	*	*	*	*	*	*	*	*	*	*	*
7PPDQ	*	*	*	1.4	*	*	*	*	*	*	*	*	*
DTPDQ	*	*	*	*	*	*	*	*	*	*	*	*	*
DPG	40	27	32	420	76	72	70	120	-	-	85	29	29
HMMM	37	30	15	-	56	32	46	150	-	-	120	12	11
NCBA	*	*	*	6.2	*	*	*	1.3	*	3.9	1.0	*	*
Benzotriazole	35	32	17	130	42	26	10	33	35	90	49	25	25
5-Me-1-H-BTR	72	86	41	310	57	64	31	78	62	230	87	52	51
2-NH2-BTH	5.1	5.3	*	21	*	6.0	1.8	7.3	3.9	27	6.9	6.9	6.1
2-OH-BTH	15	16	7.3	65	19	15	14	34	21	65	17	20	20
2,4-MoBT	*	*	*	7.0	*	*	*	1.9	*	6.0	1.6	1.3	1.3

[*] Concentrations that are below the method detection limit.

Table S9. Laboratory Control Spike (LCS) recovery values for each analyte over seven separate days.

Analyte	Recovery (%)						
	LCS						
	4/16/2024	4/26/2024	5/14/2024	5/21/2024	6/2/2024	6/5/2024	6/26/2024
6PPD	68±6.7	91±9.0	82±16	100±15	65±3.4	55±2.0	29±15
7PPD	45±2.3	59±9.4	42±4.0	57±12	48±11	35±11	16±12
IPPD	101±12	113±6.1	118±33	130±11	74±3.1	66±0.6	39±19
DPPD	72±14	60±10	52±8.1	66±3.5	83±6.8	43±22	40±12
DTPD	68±10	65±11	49±3.0	79±13	66±4.1	40±7.8	38±5.2
1,3-DMBA	120±25	131±8.4	169±22	135±2.8	109±2.1	103±4.7	112±13
4-ADPA	60±7	56±20	105±5.8	84±22	51±12	33±30	184±18
4-NDPA	72±24	170±42	261±25	177±26	94±5.5	76±5.5	27±0.5
4OH DPA	169±44	146±17	118±24	155±32	195±23	116±25	114±17
4OH DPA	136±24	145±27	210±20	124±17	134±15	118±21	148±41
6PPDQ	101±2.9	101±2.3	86±2.1	101±4.5	95±3.2	91±0.5	103±3.6
IPPDQ	130±21	127±6.7	^	^	^	^	^
7PPDQ	76±3.5	85±3.6	^	^	^	^	^
DTPDQ	89±13	94±10	^	^	^	^	^
DPG	140±66	77±3.8	79±3.4	78±2.4	58±5.6	78±2.8	72±8.6
HMMM	195±90	104±8.4	107±2	85±5.2	99±2.5	89±10	129±4.5

NCBA	150±68	66±5.4	54±0.37	40±8.4	56±2.6	53±12	66±4.4
DCU	204±81	140±23	129±1.5	128±12	105±6.4	117±9.4	109±2.7
Benzotriazole	127±47	85±7.9	81±3.6	57±10	53±3.0	58±16	84±0.9
5-Me-1-H-BTR	144±68	79±3.3	76±1.3	55±8.5	70±2.9	65±13	81±1.8
2-NH2-BTH	152±77	86±5	79±1.4	56±8.9	69±1.3	71±8.8	89±5.2
2-OH-BTH	93±10	91±1.6	74±2.8	75±8.1	130±10	113±24	90±0.6
2,4-MoBT	144±70	78±3.3	74±1.8	43±6.5	44±3.5	52±8.2	80±7.7

[^] Analyte recovery was excluded due to use of incorrect spiking solution used to prepare sample

Table S10. Spike and Recovery (Matrix spike, low and high) for each analyte for seven separate days.

Analyte	Recovery (%)						Recovery (%)						
	Before grab S&R "Low"						After grab S&R "high"						
	4/16/2024	4/26/2024	5/14/2024	5/21/2024	6/2/2024	6/26/2024	4/16/2024	4/26/2024	5/14/2024	5/21/2024	6/2/2024	6/5/2024	6/26/2024
6PPD	17±0.3	9±0.6	+	16±2.3	3±0.8	13±1.1	68±0.8	138±18	56±6.1	101±6.4	31±7.5	43±0.8	37±2.1
7PPD	11±0.02	4±0.2	+	13±1.0	2±1.0	11±1.3	49±1.7	85±12	28±12	61±7.4	27±8.6	40±1.9	34±1.4
IPPD	8.4±0.1	4±0.3	+	7±0.9	3±0.1	8±0.9	78±4.1	112±13	46±18	89±2.3	30±8.2	49±2.0	25±1.3
DPPD	67±5.5	47±1.4	+	30±43	29±16	0±0	64±3.7	114±12	48±22	69±2.8	76±17	45±10	30±42
DTPD	66±1.6	77±5.8	+	76±6.7	22±9.6	37±4.0	61±3.2	136±14	62±35	78±1.6	48±11	36±5.2	50±3.1
1,3-DMBA	0.6±0.3	1.0±0.1	+	2±0.8	2.2±0.4	2.3±0.1	51±1.9	51±2.4	79±31	72±5.2	15±1.7	44±3.3	20±2.0
4-ADPA	114±37	183±23	+	189±11	63±48	170±28	71±0.4	12±3.4	159±105	157±36	61±22	82±4.2	60±49
4-NDPA	5.7±0.04	42±2.2	+	75±0.8	9.1±0.7	13±1.8	32±3.6	23±1.3	37±13	35±6.6	29±4.9	53±3.4	36±2.2
4OH DPA	105±14	81±0.7	+	85±0.9	70±11	79±4.5	80±3.1	54±5.8	61±2.0	74±6.0	52±3.5	65±0.4	89±8.3
4OH DPA	84±16	80±0.9	+	116±2.2	66±12	112±11	40±0.7	77±6.2	90±38	72±7.5	54±13	54±4.9	129±12
6PPDQ	98±6.1	95±2.8	+	92±1.1	104±4.6	97±7.3	90±3.9	95±6.9	103±2.6	101±1.7	101±3.4	99±0.9	114±7.9
IPPDQ	81±13	64±0.3	+	^	^	^	71±1.3	54±4.5	81±36	^	^	^	^
7PPDQ	74±8.3	86±8.9	+	^	^	^	71±0.7	96±8.6	74±23	^	^	^	^
DTPDQ	69±12	94±4.7	+	^	^	^	83±2.6	79±5.7	68±15	^	^	^	^
DPG	123±19	173±5.2	+	141±3.5	106±11	129±12	156±12	227±10	179±26	197±8.0	183±23	154±7.1	156±9.1
HMMM	134±5.5	143±6.0	+	118±1.5	89±2.4	147±12	161±1.4	131±3.0	130±4.9	109±1.6	90±6.9	93±6.6	165±8.9
NCBA	115±9.4	73±5.0	+	26.8±0.3	87±12	73±5	115±4.8	102±3.5	68±18	51±4.9	73±0.3	47±6.7	89±9.1
DCU	166±5.7	224±14	+	177±3.0	157±11	156±16	167±5.2	228±5.2	239±39	242±16	206±17	181±7.0	177±9.1
Benzotriazole	108±18	115±2.6	+	35±0.9	91±4.5	102±93	116±1.3	116±2.2	103±2.8	61±4.1	92±1.8	73±9.8	120±9.7

5-Me-1-H-BTR	91±8.0	113±3.0	+	30±0.7	89±7.4	85±8.4	100±1.4	100±0.9	82±1.3	52±4.6	88±0.5	59±9.2	101±8.2
2-NH2-BTH	128±1.4	118±4.7	+	51±0.2	170±2.6	120±10	146±5.0	147±0.5	119±3.5	101±7.4	208±4.7	100±13	140±12
2-OH-BTH	112±76	47±6.5	+	34±1.3	81±0.5	61±1.7	-	44±0.1	36±0.8	47±3.5	50±5.9	35±4.1	66±4.3
2,4-MoBT	103±11	69±6.2	+	23.6±0.7	74±0.5	77±6.1	108±0.02	83±2.5	69±1.9	42±2.9	73±0.3	40±4.6	85±9.6

[^] Analyte recovery was excluded due to use of incorrect spiking solution used to prepare sample.

[+] May 14, 2024 sample was lost during laboratory processing.

Table S11. Concentrations (ng/L) for PPDs per sample for the 4/16/2024 baseflow sampling event.

Sample #	Midpoint	6PPD	IPPD	7PPD	DPPD	DTPD	DNP
1	4/16/2024 17:15	*	*	*	12	7.3	*
2	4/16/2024 20:15	*	*	*	4.1	7.1	*
3	4/16/2024 22:15	9.5	0.2	*	130	7.5	*
4	4/16/2024 12:15	*	*	*	4.3	7.2	*
5	4/17/2024 2:15	*	*	*	*	7.0	*
6	4/17/2024 4:15	*	*	*	12	7.0	*
7	4/17/2024 6:15	*	*	*	3.9	3.7	*
8	4/17/2024 8:15	8.6	0.2	*	9.9	7.7	*
9	4/17/2024 10:15	*	*	*	9.5	7.4	*
10	4/17/2024 0:15	*	*	*	4.3	9.9	*

11	4/17/2024 14:15	6.8	*	*	*	7.1	*
12	4/17/2024 16:15	*	*	*	7.9	7.1	*

[*] Concentrations that are below the method detection limit.

Table S12. Concentrations (ng/L) for PPDs per sample for the 5/14/2024 baseflow sampling event.

Sample #	Midpoint	6PPD	IPPD	7PPD	DPPD	DTPD	DNP
1	5/14/2024 22:00	*	*	*	*	*	*
2	5/15/2024 1:00	*	*	*	*	*	*
3	5/15/2024 3:00	*	*	*	*	*	*
4	5/15/2024 5:00	*	*	*	*	*	*
5	5/15/2024 7:00	*	*	*	*	*	*
6	5/15/2024 9:00	*	*	*	*	*	*
7	5/15/2024 11:00	*	*	*	*	*	*

[*] Concentrations that are below the method detection limit.

Table S13. Concentrations (ng/L) for PPDs per sample for the 6/25/2024 baseflow sampling event.

Sample #	Midpoint	6PPD	IPPD	7PPD	DPPD	DTPD	DNP
1	6/25/2024 13:30	*	*	*	*	*	*
2	6/25/2024 16:30	*	*	*	*	*	*
3	6/25/2024 18:30	1.6	*	*	*	*	*
4	6/25/2024 19:30	1.6	*	*	*	*	*
5	6/25/2024 22:30	1.6	*	*	*	*	*
6	6/25/2024 12:30	1.6	*	*	*	*	*
7	6/26/2024 2:30	1.7	*	*	0.2	*	*
8	6/26/2024 4:30	1.6	*	*	*	*	*
9	6/26/2024 6:30	1.7	*	*	*	*	*
10	6/26/2024 8:30	1.7	*	*	*	*	*
11	6/26/2024 10:30	1.6	*	*	*	*	*
12	6/26/2024 12:30	1.	*	*	*	*	*

[*] Concentrations that are below the method detection limit.

Table S14. Concentrations (ng/L) for PPDQs and TPs per sample for the 4/16/2024 baseflow sampling event.

Sample #	Midpoint	4-DPA	1,3-DMBA	4-ADPA	4-NDPA	4OH DPA	6PPDQ	IPPDQ	7PPDQ	DTPDQ
1	4/16/2024 17:15	*	45	1.5	0.7	*	1.0	*	*	*
2	4/16/2024 20:15	*	28	1.0	*	*	0.8	*	*	*
3	4/16/2024 22:15	*	63	1.6	*	*	1.2	*	*	*
4	4/16/2024 12:15	*	24	0.6	*	*	0.8	*	0.1	*
5	4/17/2024 2:15	*	23	0.8	*	*	0.7	*	*	*
6	4/17/2024 4:15	*	18	0.6	*	*	0.7	*	*	*
7	4/17/2024 6:15	*	30	0.7	*	*	0.7	*	*	*
8	4/17/2024 8:15	0.5	72	1.6	*	*	1.9	*	*	*
9	4/17/2024 10:15	*	25	1.1	*	*	1.2	*	*	*
10	4/17/2024 0:15	*	24	0.9	0.7	*	2.3	*	*	*

11	4/17/2024 14:15	0.5	62	0.9	*	*	1.0	*	*	*
12	4/17/2024 16:15	0.3	29	0.8	*	*	0.8	*	*	*

[*] Concentrations that are below the method detection limit.

Table S15 Concentrations (ng/L) for PPDQs and TPs per sample for the 5/14/2024 baseflow sampling event.

Sample #	Midpoint	4-DPA	1,3-DMBA	4-ADPA	4-NDPA	4OH DPA	6PPDQ	IPPDQ	7PPDQ	DTPDQ
1	5/14/2024 22:00	*	73	*	0.5	8.1	0.6	*	0.2	*
2	5/15/2024 1:00	*	40	*	0.8	7.4	1.2	*	*	*
3	5/15/2024 3:00	*	48	*	0.5	7.4	1.1	*	*	*
4	5/15/2024 5:00	0.8	47	*	4.9	7.6	1.2	*	4.5	*
5	5/15/2024 7:00	*	42	*	0.5	7.4	1.1	*	*	*
6	5/15/2024 9:00	*	55	*	0.4	7.4	1.2	*	*	*
7	5/15/2024 11:00	*	79	*	0.5	7.9	0.9	*	*	*

[*] Concentrations that are below the method detection limit.

Table S16. Concentrations (ng/L) for PPDQs and TPs per sample for the 6/25/2024 baseflow sampling event.

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Sample #	Midpoint	4-DPA	1,3-DMBA	4-ADPA	4-NDPA	4OH DPA	6PPDQ	IPPDQ	7PPDQ	DTPDQ
1	6/25/2024 13:30	*	3200	*	*	2.6	1.7	*	*	*
2	6/25/2024 16:30	*	160	*	*	2.6	1.0	*	*	*
3	6/25/2024 18:30	*	540	*	*	2.5	1.1	*	*	*
4	6/25/2024 19:30	*	160	*	*	2.5	1.0	*	*	*
5	6/25/2024 22:30	*	580	*	*	2.5	0.8	*	*	*
6	6/25/2024 12:30	*	230	*	*	2.5	1.0	*	*	*
7	6/26/2024 2:30	*	1100	*	*	2.5	9.4	*	*	*
8	6/26/2024 4:30	*	270	*	*	2.4	0.9	*	*	*
9	6/26/2024 6:30	*	250	*	*	2.5	0.8	*	*	*
10	6/26/2024 8:30	*	610	*	*	2.2	0.9	*	*	*
11	6/26/2024 10:30	*	340	*	*	2.2	1.3	*	*	*

12	6/26/2024 12:30	*	320	*	*	2.3	1.1	*	*	*

[*] Concentrations that are below the method detection limit.

Table S17 Concentrations (ng/L) for vehicle related chemicals per sample for the 4/16/2024 baseflow sampling event.

Sample #	Midpoint	1,3diphenylguanidine	HMMM	N-cyclohexyl-1.3benzothiazole-2amine	Benzotriazole	5-methyl-1-H-benzotriazole	2-aminobenzothiazole	2-hydroxy benzothiazole	2-(4-morpholinyl)-benzothiazole
1	4/16/2024 17:15	35	38	*	24	62	5.7	120	5.4
2	4/16/2024 20:15	27	38	*	35	66	5.1	40	2.6
3	4/16/2024 22:15	45	41	*	32	69	5.5	44	2.6
4	4/16/2024 12:15	31	37	*	28	62	5.1	35	2.2
5	4/17/2024 2:15	26	37	*	32	71	5.1	37	2.2
6	4/17/2024 4:15	28	35	*	23	71	5.1	37	2.4
7	4/17/2024 6:15	28	34	*	34	67	5.0	33	2.0

.

8	4/17/2024 8:15	81	36	*	33	80	5.8	39	2.5
9	4/17/2024 10:15	29	38	*	24	69	5.3	39	2.9
10	4/17/2024 0:15	33	41	*	23	62	5.4	51	4.3
11	4/17/2024 14:15	41	36	*	30	69	5.4	49	3.5
12	4/17/2024 16:15	28	33	*	30	65	5.1	40	4.5

[*] Concentrations that are below the method detection limit.

Table S18. Concentrations (ng/L) for vehicle related chemicals per sample for the 5/14/2024 baseflow sampling event.

Sample #	**Midpoint**	**1,3diphenylguanidine**	**HMMM**	**N-cyclohexyl-1.3benzothiazole-2amine**	**Benzotriazole**	**5-methyl-1-H-benzotriazole**	**2-aminobenzothiazole**	**2-hydroxybenzothiazole**	**2-(4-morpholi benzothi**
1	5/14/2024 22:00	54	44	3.7	22	60	6.3	78	4.8

2	5/15/2024 1:00	43	36	3.8	23	63	6.1	34	2.3
3	5/15/2024 3:00	47	37	3.8	26	64	6.6	34	2.4
4	5/15/2024 5:00	46	35	3.8	24	63	6.5	32	2.3
5	5/15/2024 7:00	42	35	3.8	25	60	5.6	30	2.2
6	5/15/2024 9:00	49	37	4.1	25	63	6.2	34	2.5
7	5/15/2024 11:00	54	38	3.7	27	60	6.3	48	3.4

[*] Concentrations that are below the method detection limit.

Table S19 Concentrations (ng/L) for vehicle related chemicals per sample for the 6/25/2024 baseflow sampling event.

Sample #	Midpoint	1,3diphenylguanidine	HMMM	N-cyclohexyl-1.3benzothiazole-2amine	Benzotriazole	5-methyl-1-H-benzotriazole	2-aminobenzothiazole	2-hydroxy benzothiazole	2-(4-morpholinyl)-benzothiazole
1	6/25/2024 13:30	31	22	*	22	43	8.1	32	3.0
2	6/25/2024 16:30	27	17	*	22	44	6.5	38	2.4

.

3	6/25/2024 18:30	27	17	3.8	22	46	7.2	41	2.5
4	6/25/2024 19:30	34	18	3.9	25	53	7.4	40	2.4
5	6/25/2024 22:30	36	17	3.8	26	53	7.1	40	2.2
6	6/25/2024 12:30	36	19	1.9	29	56	7.6	40	1.9
7	6/26/2024 2:30	34	20	3.9	27	52	6.6	34	1.9
8	6/26/2024 4:30	32	15	3.7	24	49	6.3	30	1.7
9	6/26/2024 6:30	28	13	3.7	22	45	6.5	30	1.5
10	6/26/2024 8:30	33	16	3.8	27	51	7.4	35	1.6
11	6/26/2024 10:30	31	17	3.8	27	52	6.6	33	1.8
12	6/26/2024 12:30	31	15	3.8	25	50	7.2	32	1.8

[*] Concentrations that are below the method detection limit.

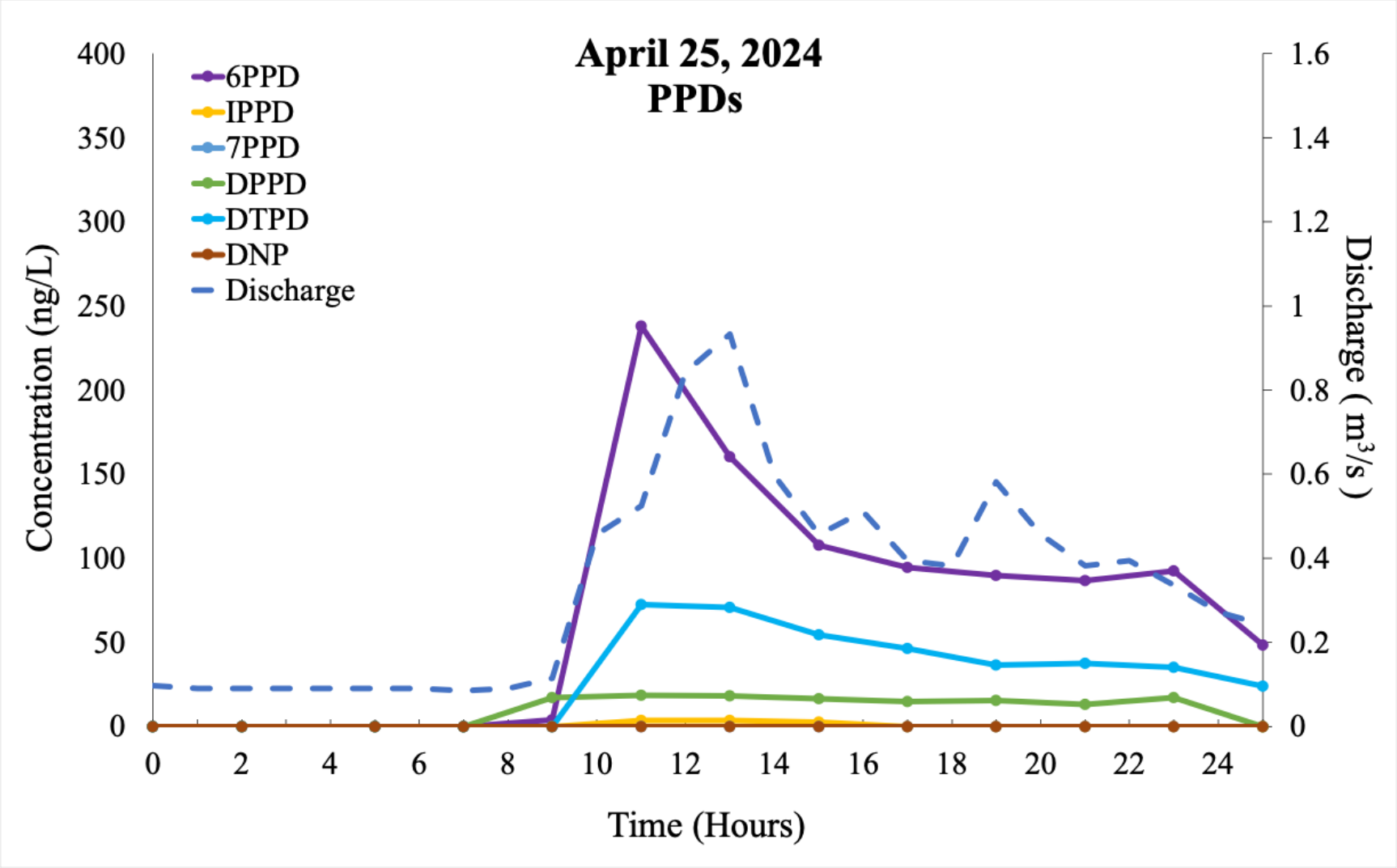

Figure S1. Creek samples were collected on April 25, 2024. PPD concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

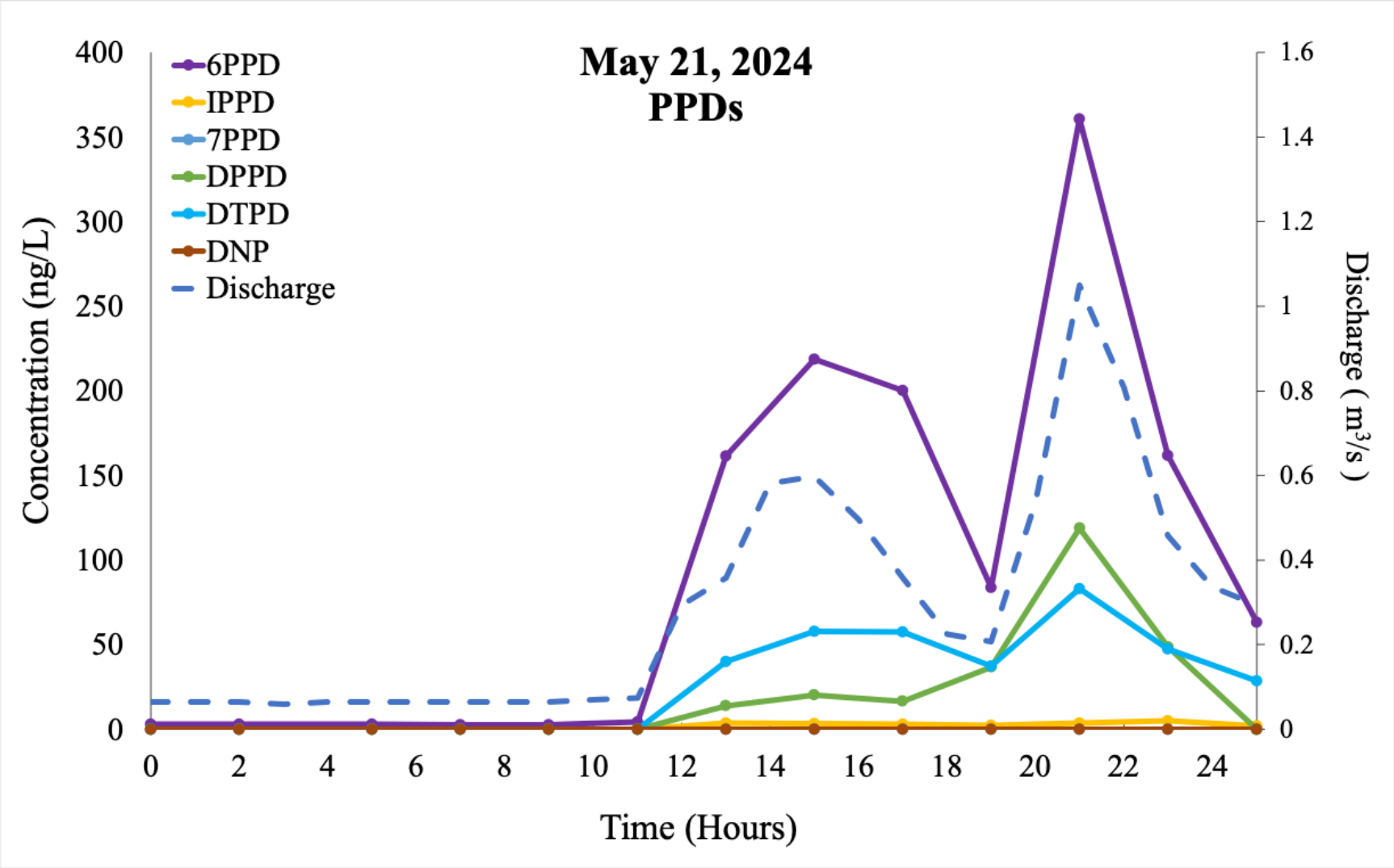

Figure S2. Creek samples were collected on May 21, 2024. PPD concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

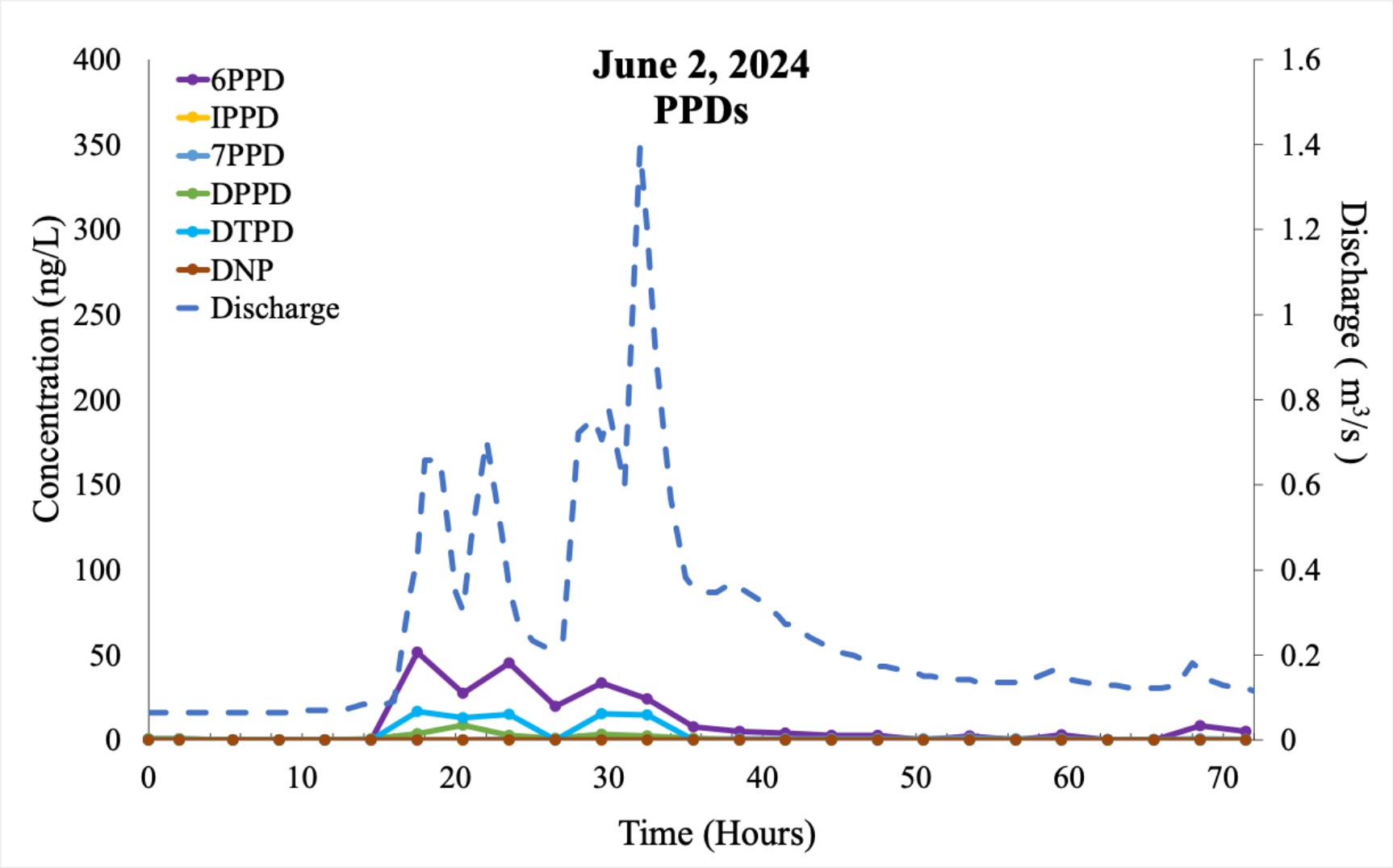

Figure S3. Creek samples were collected on June 1 through June 4, 2024. PPD concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

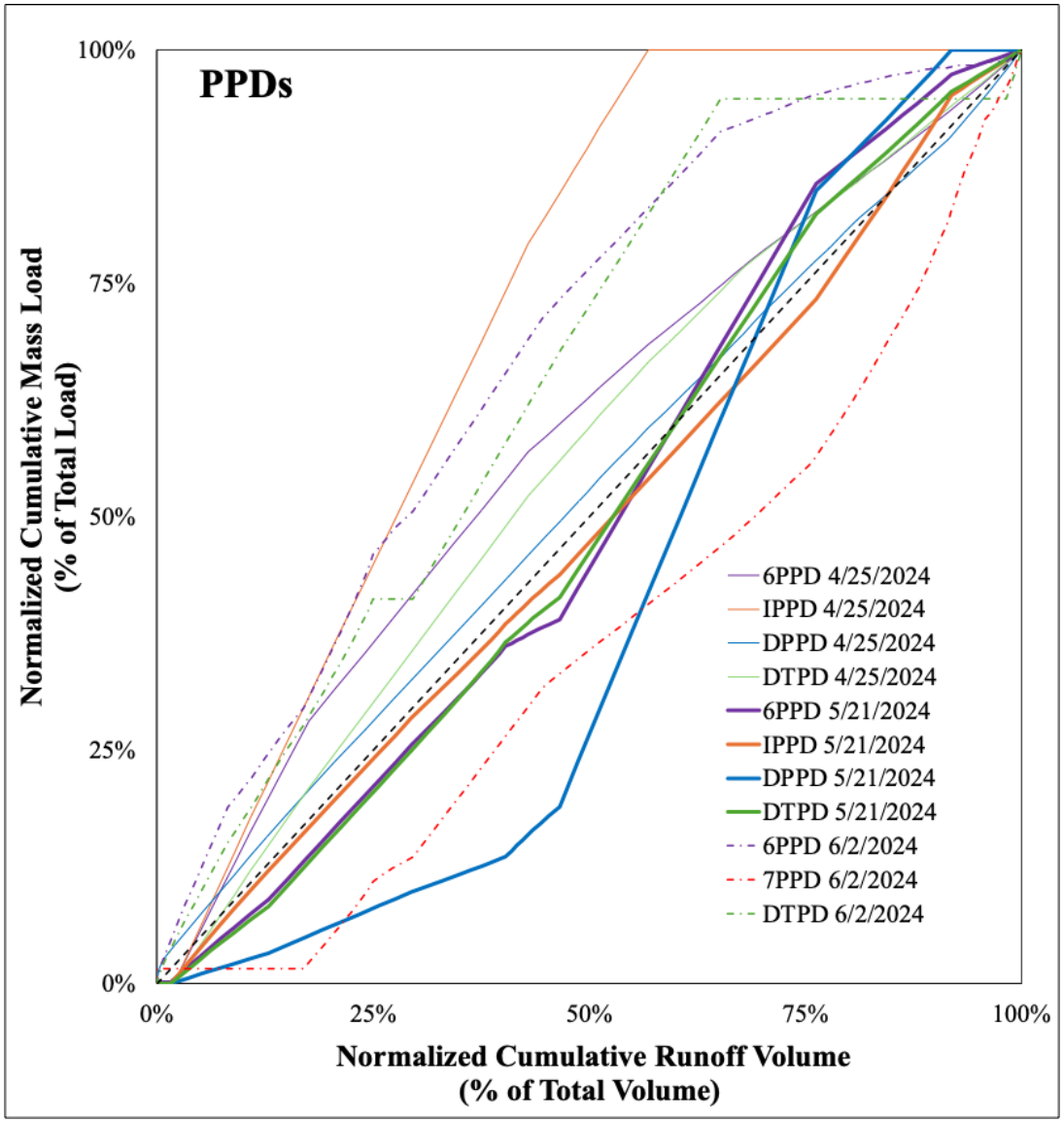

Figure S4. Normalized cumulative volume and normalized cumulative mass load for each PPD across three storm events.

Table S20. Concentrations (ng/L) for 6 PPDs per sample for the 4/25/2024 storm.

Sample #	Midpoint	6PPD	IPPD	7PPD	DPPD	DTPD	DNP
1	4/25/2024 2:00	*	*	*	*	*	*
2	4/25/2024 5:00	*	*	*	*	*	*
3	4/25/2024 7:00	*	*	*	*	*	*
4	4/25/2024 9:00	4.1	*	*	17	*	*
5	4/25/2024 11:00	240	3.5	*	18	72	*
6	4/25/2024 13:00	160	3.6	*	18	71	*
7	4/25/2024 15:00	110	2.6	*	17	54	*
8	4/25/2024 17:00	95	*	*	15	47	*
9	4/25/2024 19:00	90	*	*	16	37	*
10	4/25/2024 21:00	87	*	*	13	38	*
11	4/25/2024 23:00	93	*	*	17	35	*
12	4/26/2024 1:00	48	*	*	*	24	*

[*] Concentrations that are below the method detection limit.

Table S21. Concentrations (ng/L) for 6 PPDs per sample for the 5/21/2024 storm.

Sample #	Midpoint	6PPD	IPPD	7PPD	DPPD	DTPD	DNP
1	5/21/2024 0:00	2.9	*	*	*	*	*
2	5/21/2024 3:00	2.9	*	*	*	*	*

3	5/21/2024 5:00	2.8	*	*	*	*	*
4	5/21/2024 7:00	2.7	*	*	*	*	*
5	5/21/2024 9:00	4.5	*	*	*	*	*
6	5/21/2024 11:00	160	3.6	*	14	40	*
7	5/21/2024 13:00	220	3.5	*	20	58	*
8	5/21/2024 15:00	200	3.1	*	17	57	*
9	5/21/2024 17:00	84	2.5	*	37	37	*
10	5/21/2024 19:00	360	3.7	*	120	83	*
11	5/21/2024 21:00	160	4.9	*	49	47	*
12	5/21/2024 23:00	63	1.9	*	*	29	*

[*] Concentrations that are below the method detection limit.

Table S22. Concentrations (ng/L) for PPDs per sample for the 6/1/2024-6/4/2024 storm.

Sample #	Midpoint	6PPD	IPPD	7PPD	DPPD	DTPD	DNP
1	6/1/2024 23:00	*	*	0.6	0.7	*	*
2	6/2/2024 2:30	*	*	*	*	*	*

3	6/2/2024 5:30	*	*	*	*	*	*
4	6/2/2024 8:30	*	*	*	*	*	*
5	6/2/2024 11:30	*	*	*	0.6	*	*
6	6/2/2024 14:30	52	*	*	3.7	17	*
7	6/2/2024 17:30	27	*	*	8.9	13	*
8	6/2/2024 20:30	45	*	0.7	2.8	15	*
9	6/2/2024 23:30	20	*	0.3	1.2	*	*
10	6/3/2024 2:30	34	*	0.8	3.4	15	*
11	6/3/2024 5:30	24	*	0.5	2.3	15	*
12	6/3/2024 8:30	7.9	*	0.5	0.9	*	*
13	6/3/2024 12:30	5.0	*	0.8	*	*	*
14	6/3/2024 15:30	4.0	*	0.8	*	*	*
15	6/3/2024 18:30	2.6	*	0.7	*	*	*
16	6/3/2024 21:30	2.6	*	0.8	*	*	*
17	6/4/2024 0:30	*	*	0.7	*	*	*
18	6/4/2024 3:30	2.5	*	0.9	*	*	*
19	6/4/2024 6:30	*	*	0.8	*	*	*
20	6/4/2024 9:30	3.1	*	0.8	*	*	*

21	6/4/2024 12:30	*	*	0.3	*	*	*
22	6/4/2024 15:30	*	*	0.5	*	*	*
23	6/4/2024 18:30	8.4	*	0.7	*	*	*
24	6/4/2024 21:30	5.0	*	0.5	*	*	*

[*] Concentrations that are below the method detection limit.

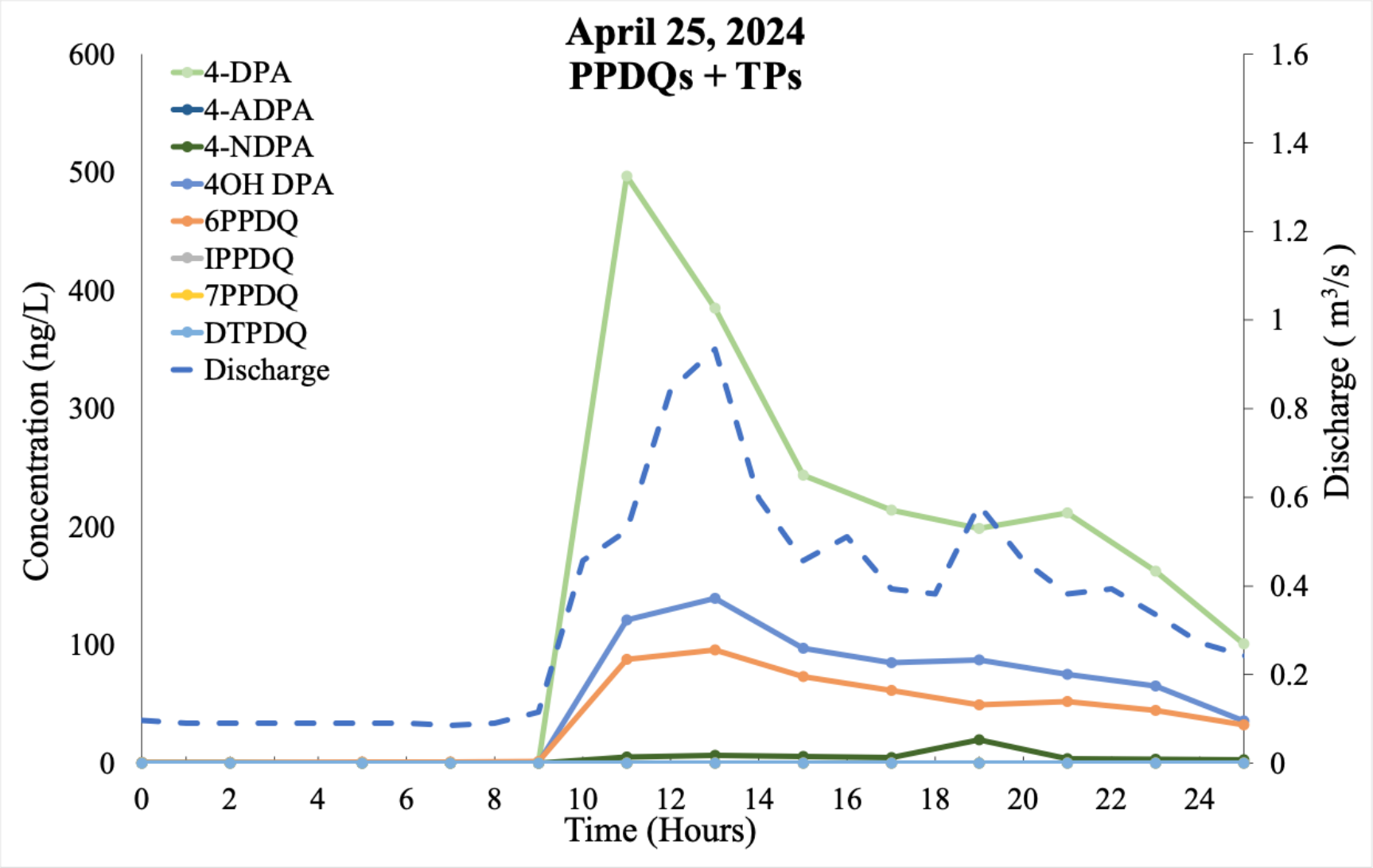

Figure S5. Creek samples were collected on April 25, 2024. PPD Transformation product concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

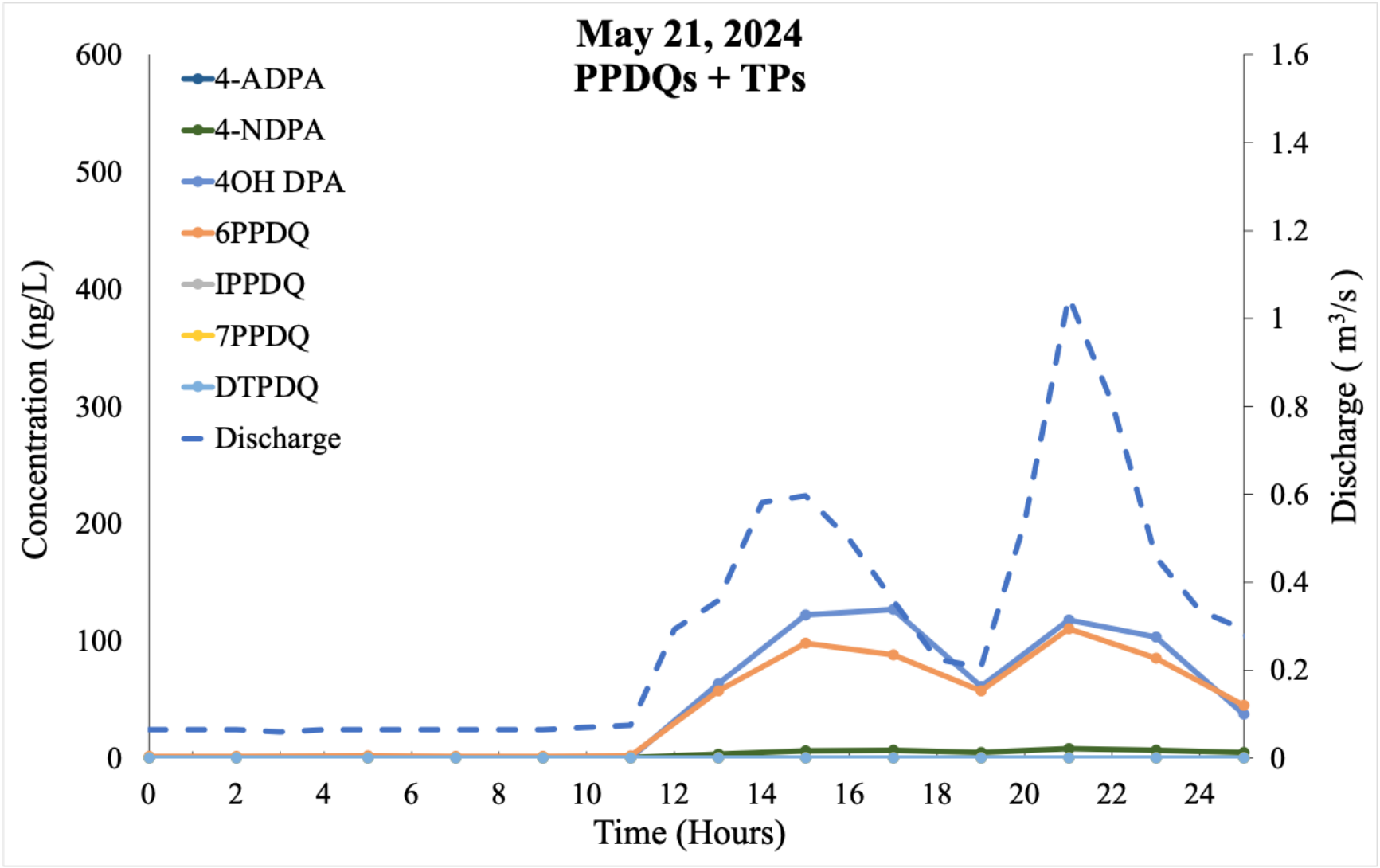

Figure S6. Creek samples were collected on May 21, 2024. PPD Transformation product concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

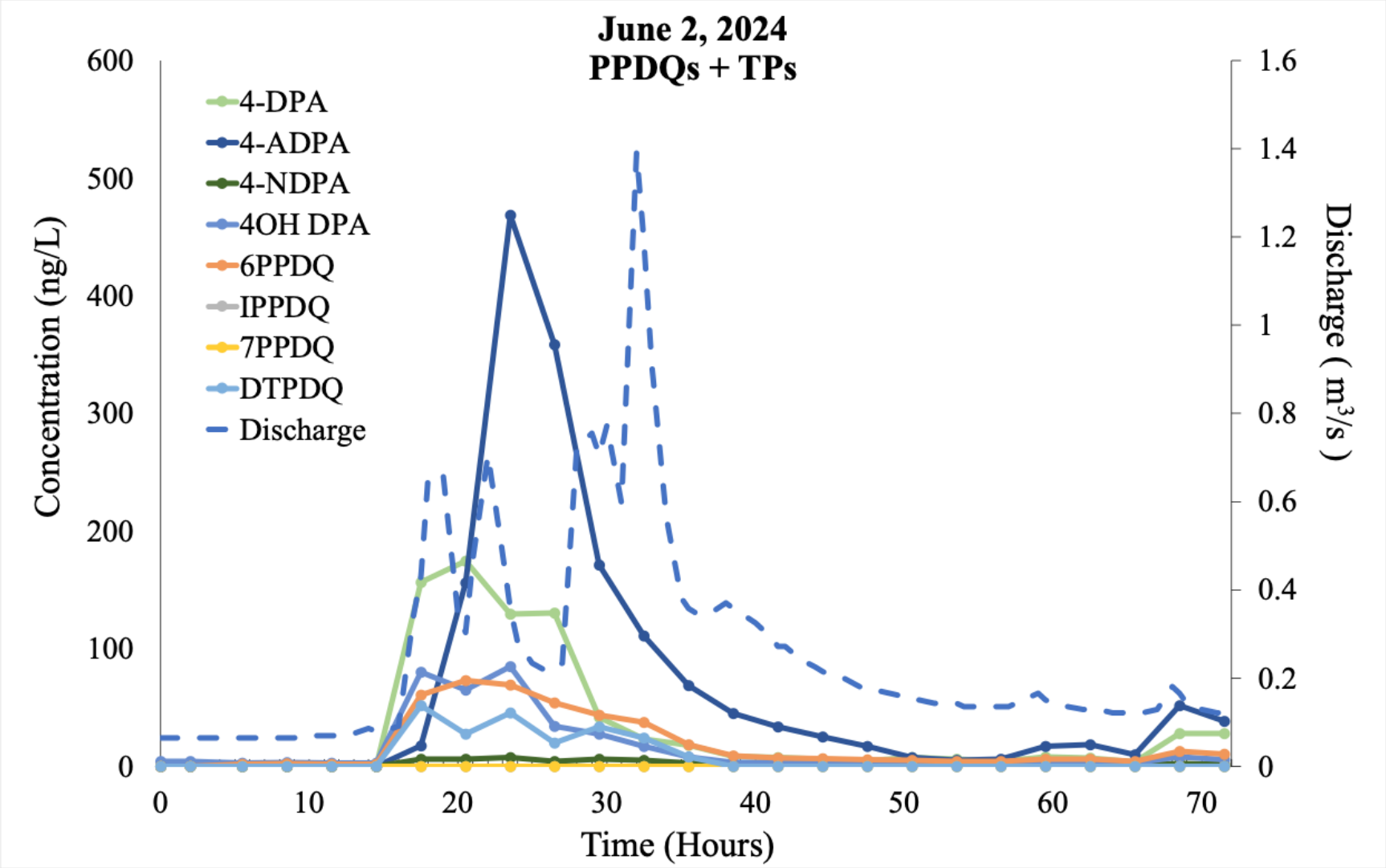

Figure S7. Creek samples were collected on June 1 through June 4, 2024. PPD Transformation product concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

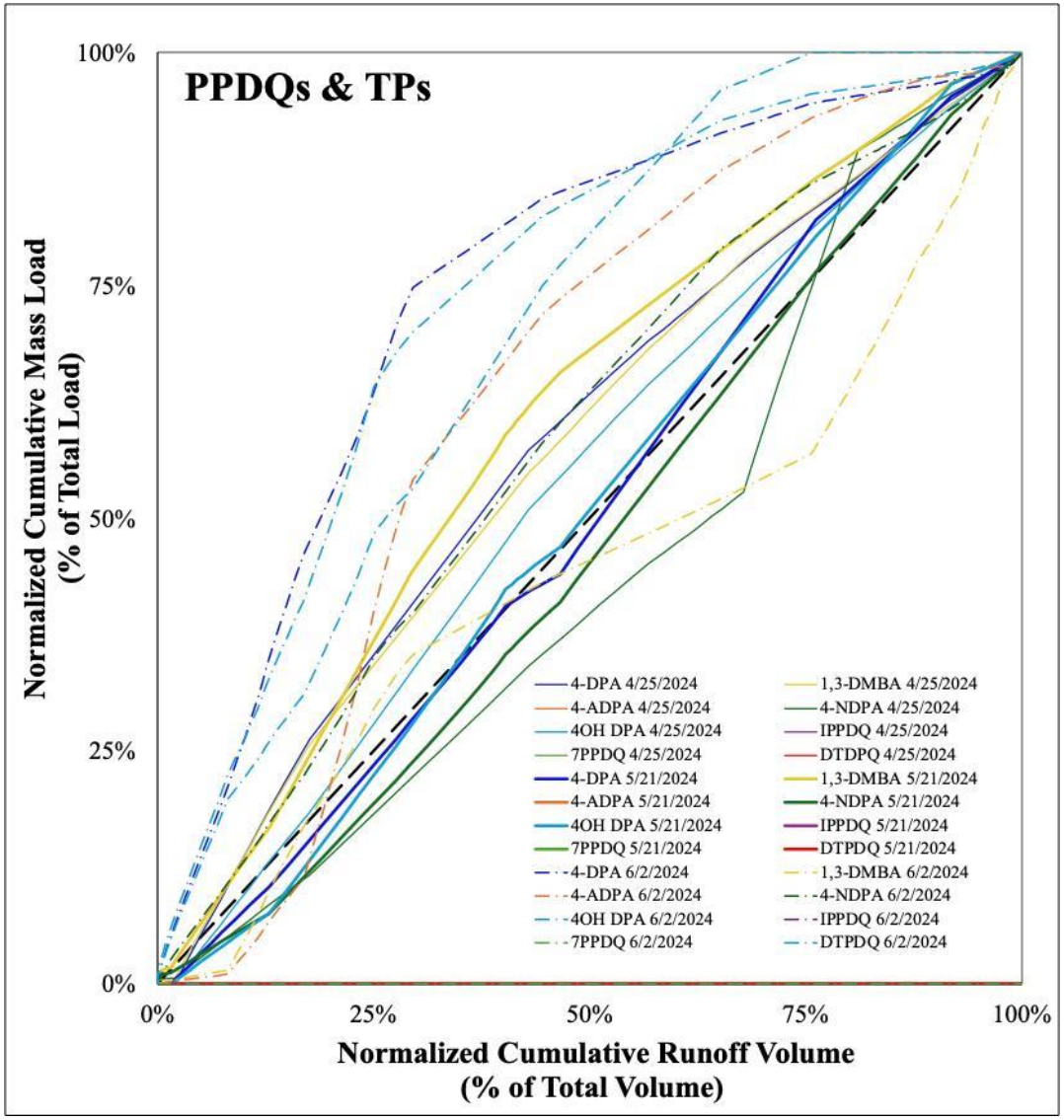

Figure S8. Normalized cumulative volume and normalized cumulative mass load for each transformation product across three storm events.

Table S23. Concentrations (ng/L) for PPDQs and TPs per sample for the 4/25/2024-4/26/2024 storm.

Sample #	Midpoint	4-DPA	1,3-DMBA	4-ADPA	4-NDPA	4OH DPA	6PPDQ	IPPDQ	7PPDQ	DTPDQ
1	4/25/2024 2:00	*	66	*	0.4	*	0.6	*	*	*
2	4/25/2024 5:00	*	44	*	0.3	*	1.1	*	*	*
3	4/25/2024 7:00	*	56	*	0.3	*	1.1	*	*	*
4	4/25/2024 9:00	*	130	*	0.3	*	1.7	*	*	*
5	4/25/2024 11:00	500	2700‹	*	5.2	120	88	*	*	*
6	4/25/2024 13:00	390	2100‹	*	6.9	140	96	*	*	*
7	4/25/2024 15:00	240	1600‹	*	5.7	97	73	*	*	*
8	4/25/2024 17:00	210	1400‹	*	4.8	85	62	*	*	*
9	4/25/2024 19:00	200	1100‹	*	20	87	50	*	*	*
10	4/25/2024 21:00	210	1100‹	*	3.9	75	52	*	*	*
11	4/25/2024 23:00	160	1000‹	*	3.5	65	45	*	*	*
12	4/26/2024 1:00	100	840‹	*	2.7	36	32	*	*	*

[‹]Concentrations (ng/L) were recalculated after 10X dilution.

[*] Concentrations that are below the method detection limit.

Table S24. Concentrations (ng/L) for PPDQs and TPs per sample for the 5/21/2024 storm.

Sample #	Midpoint	4-DPA	1,3-DMBA	4-ADPA	4-NDPA	4OH DPA	6PPDQ	IPPDQ	7PPDQ	DTPDQ

1	5/21/2024 0:00	*	83	*	*	*	1.4	*	*	*
2	5/21/2024 3:00	*	53	*	*	*	1.9	*	*	*
3	5/21/2024 5:00	*	50	*	*	*	1.6	*	*	*
4	5/21/2024 7:00	*	50	*	*	*	1.6	*	*	*
5	5/21/2024 9:00	*	78	*	*	*	2.1	*	*	*
6	5/21/2024 11:00	330	580‹	*	3.4	63	57	*	*	*
7	5/21/2024 13:00	410	770‹	*	6.2	120	98	*	*	*
8	5/21/2024 15:00	400	600‹	*	6.5	130	88	*	*	*
9	5/21/2024 17:00	170	410‹	*	4.8	61	58	*	*	*
10	5/21/2024 19:00	510	340‹	*	8.0	120	110	*	*	*
11	5/21/2024 21:00	320	300‹	*	6.8	100	85	*	*	*
12	5/21/2024 23:00	200	170‹	*	4.7	38	45	*	*	*

[‹]Concentrations (ng/L) were recalculated after 10X dilution.

[*] Concentrations that are below the method detection limit.

Table S25. Concentrations (ng/L) for PPDQs and TPs per sample for the 6/1/2024-6/4/2024 storm

Sample #	Midpoint	4-DPA	1,3-DMBA	4-ADPA	4-NDPA	4OH DPA	6PPDQ	IPPDQ	7PPDQ	DTPDQ
1	6/1/2024 23:00	*	*	*	*	4.3	0.0	*	*	*
2	6/2/2024 2:30	*	*	*	*	2.9	1.9	*	*	*
3	6/2/2024 5:30	*	*	*	*	3.2	2.4	*	*	*
4	6/2/2024 8:30	*	*	*	*	2.7	1.7	*	*	*
5	6/2/2024 11:30	*	*	*	*	2.9	2.0	*	*	*
6	6/2/2024 14:30	160	36‹	18‹	6.2	80	61	*	*	52
7	6/2/2024 17:30	170	340‹	160‹	6.2	65	73	*	*	27
8	6/2/2024 20:30	130	300‹	470	7.3	85	69	*	*	45
9	6/2/2024 23:30	130	220‹	360	4.2	34	54	*	*	20
10	6/3/2024 2:30	42	110‹	170	6.2	27	44	*	*	34
11	6/3/2024 5:30	23	94‹	110	5.3	17	37	*	*	24
12	6/3/2024 8:30	18	87‹	69	3.0	7.8	18	*	*	7.9
13	6/3/2024 12:30	8.4	280	45	2.0	3.4 ¦	8.8	*	*	*
14	6/3/2024 15:30	7.7	270	34	1.9	3.3 ¦	7.2	*	*	*

15	6/3/2024 18:30	6.1	270	25	1.6	3.1 ¦	6.6	*	*	*
16	6/3/2024 21:30	4.6	160‹	17	1.6	3.0 ¦	5.6	*	*	*
17	6/4/2024 0:30	7.3	180	7.6	1.5	2.8 ¦	4.9	*	*	*
18	6/4/2024 3:30	6.0	150	5.1	1.5	2.7 ¦	4.4	*	*	*
19	6/4/2024 6:30	4.0	260	6.3	1.5	2.9 ¦	4.2	*	*	*
20	6/4/2024 9:30	8.0	280	17	1.9	3.7 ¦	6.3	*	*	*
21	6/4/2024 12:30	7.0	130‹	19	1.6	3.2 ¦	5.9	*	*	*
22	6/4/2024 15:30	3.9	250	9.9	1.5	2.	4.2	*	*	*
23	6/4/2024 18:30	28	130‹	52	2.2	8.2	13	*	*	*
24	6/4/2024 21:30	28	130‹	38	2.0	5.8	11	*	*	*

[‹]Concentrations (ng/L) were recalculated after 10X dilution.

[*] Concentrations that are below the method detection limit.

[¦] Concentration between method detection limit and method quantification limit.

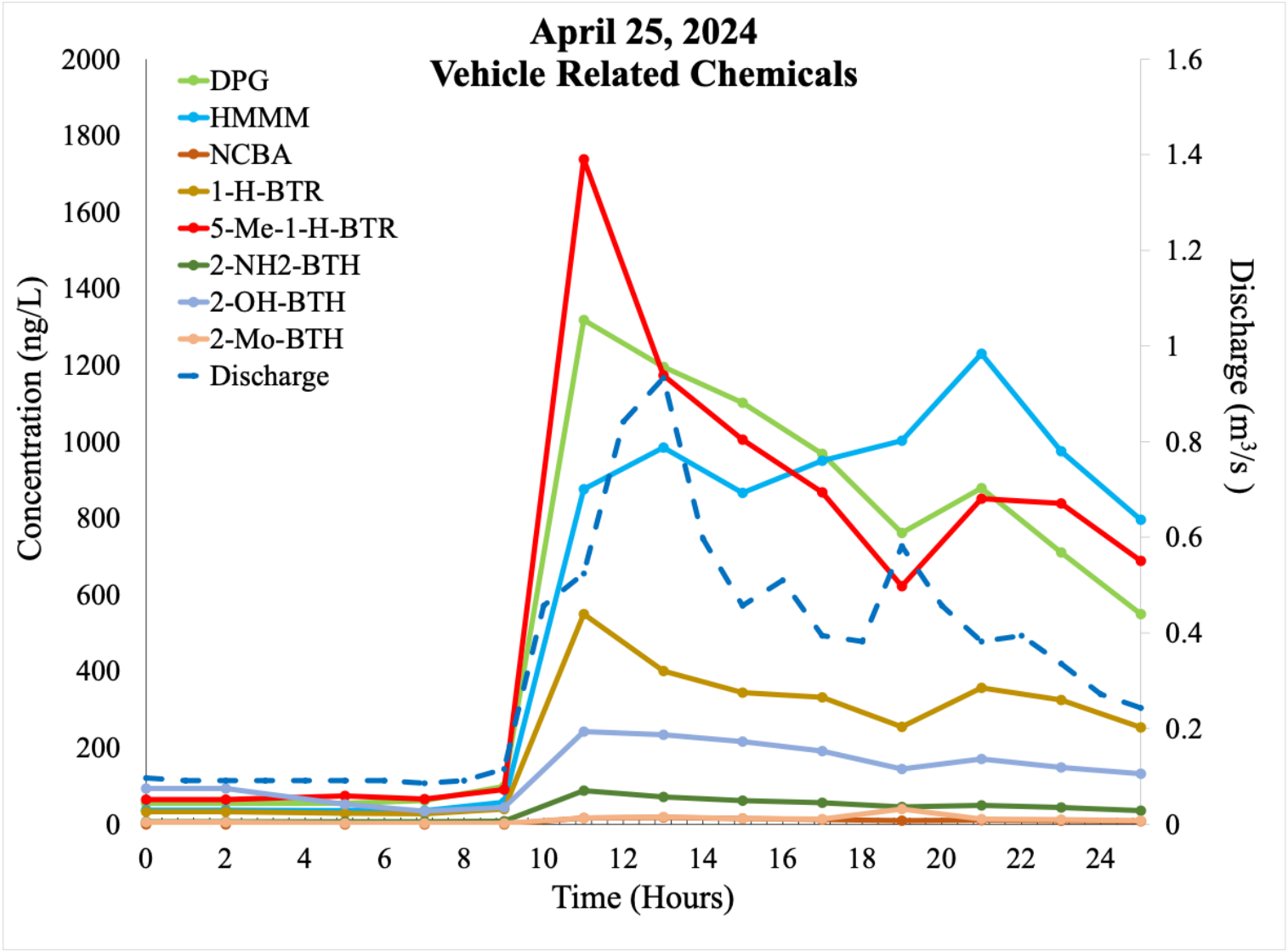

Figure S9. Creek samples were collected on April 25, 2024. Other vehicle related chemical concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

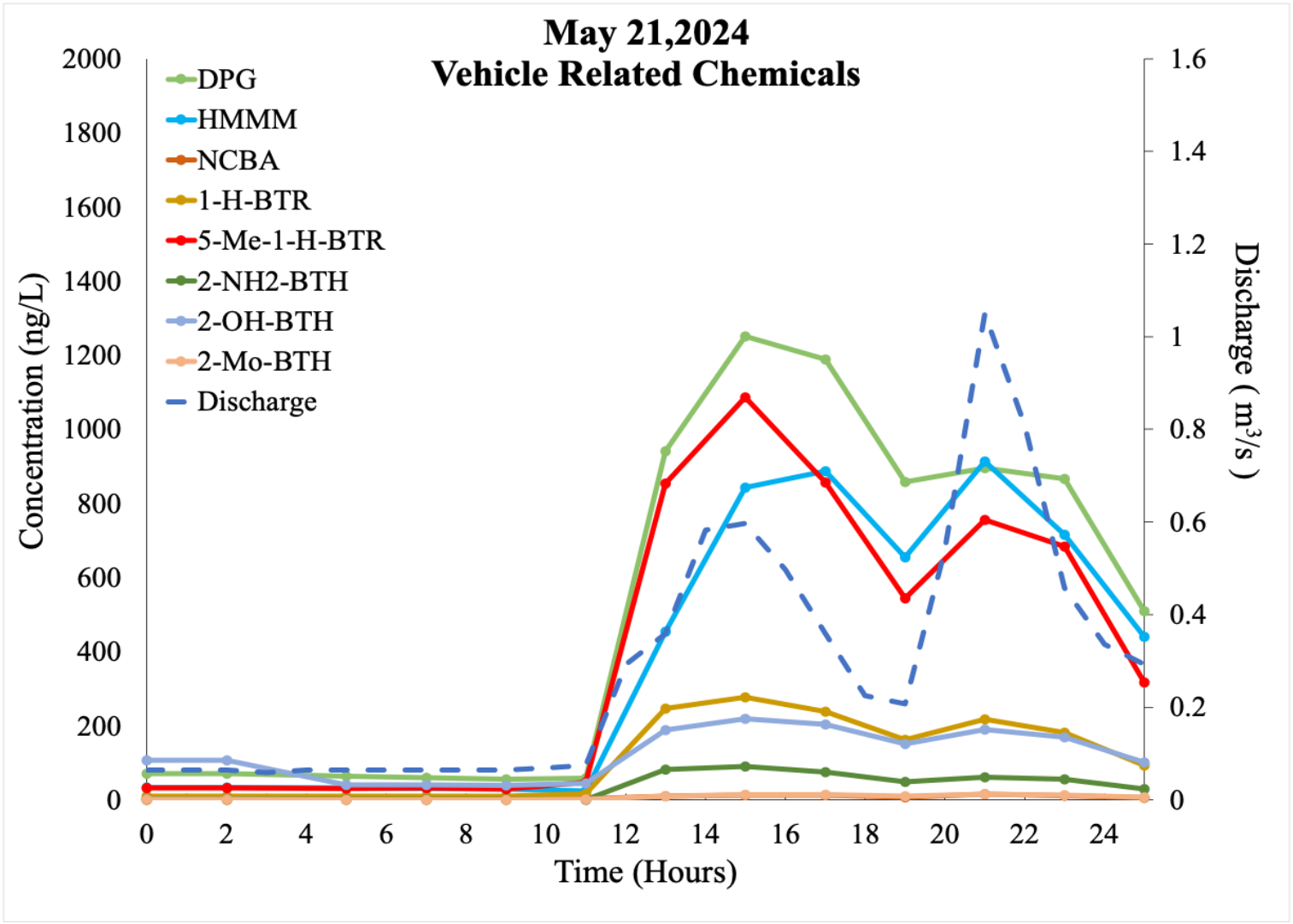

Figure S10. Creek samples were collected on May 21, 2024. Other vehicle related chemical concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

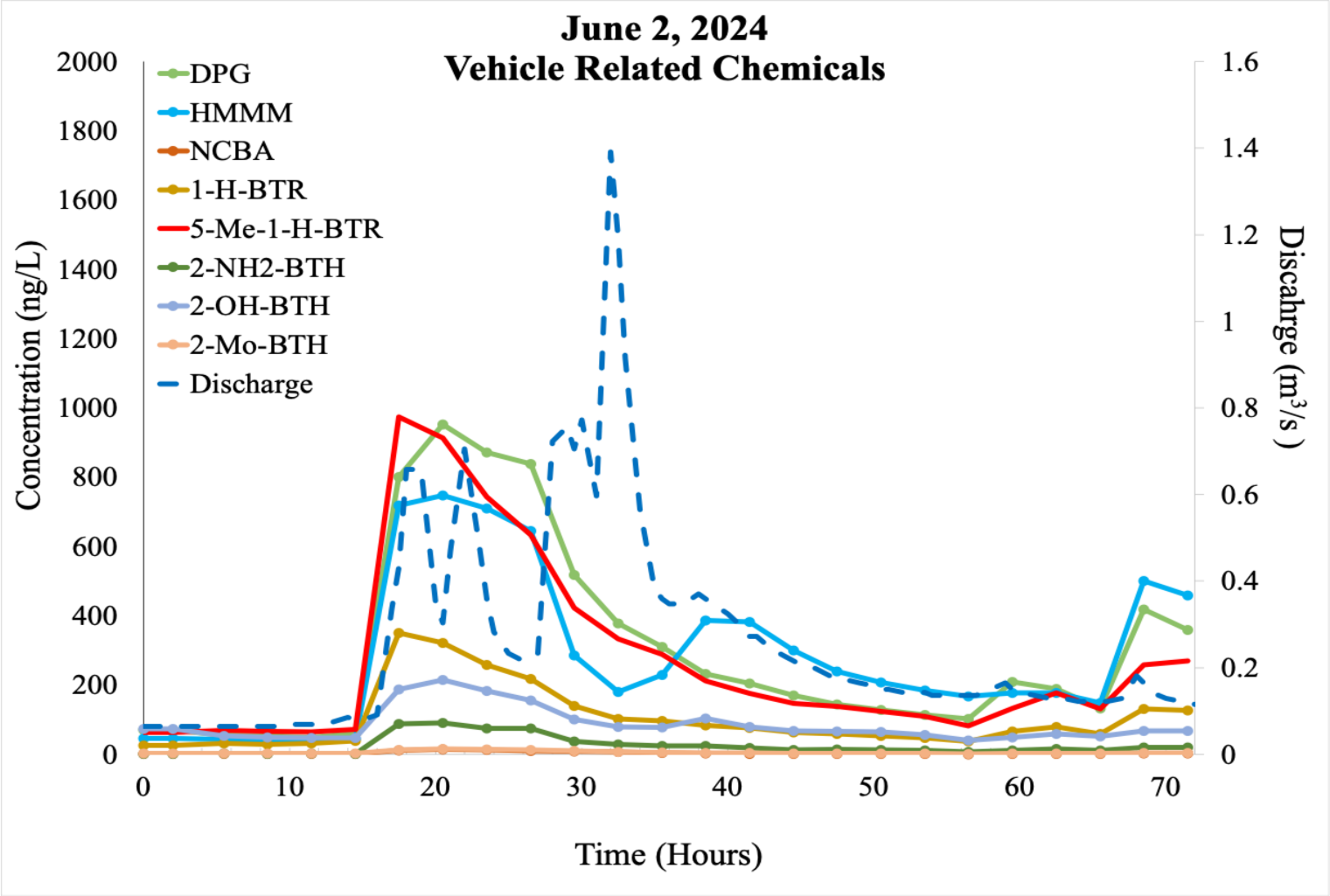

Figure S11. Creek samples were collected on June 1 through June 4, 2024. Other vehicle related chemical concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series.

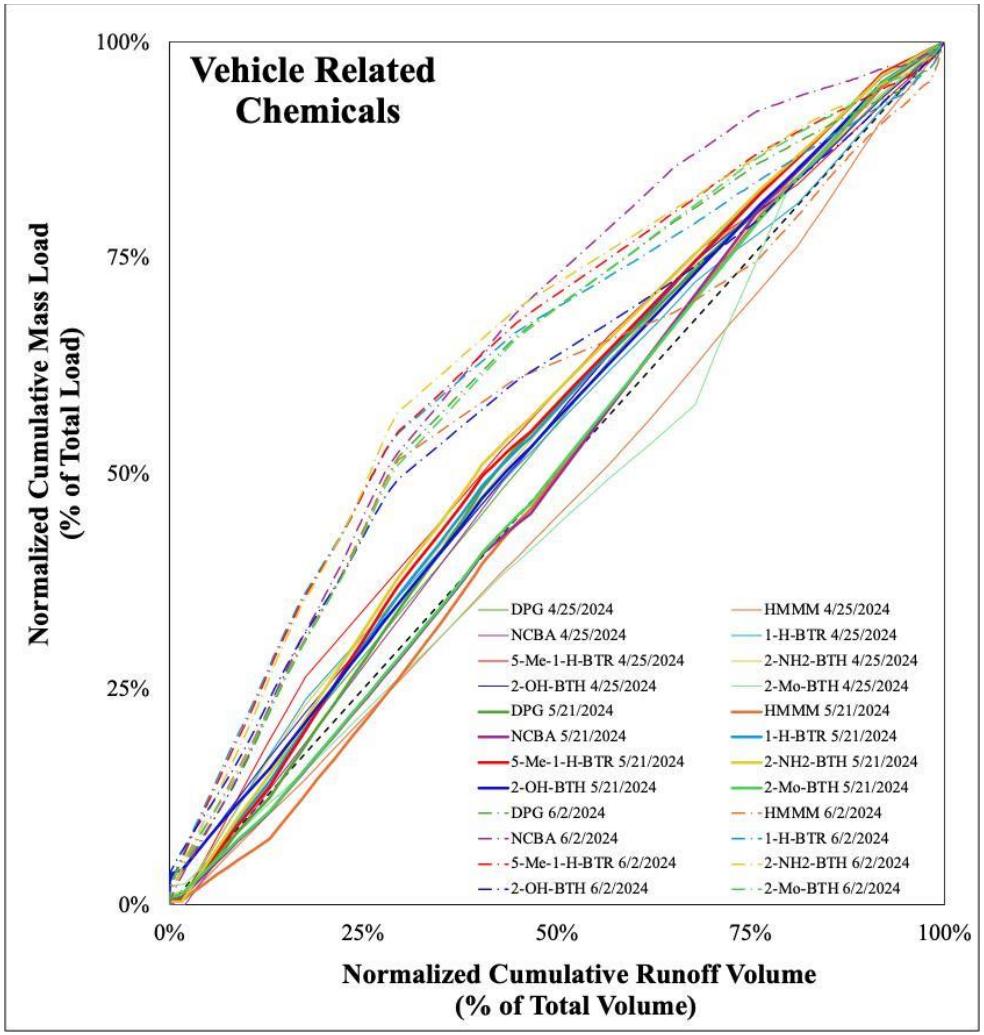

Figure S12. Normalized cumulative volume and normalized cumulative mass load for each vehicle related chemical across three storm events.

Table S26. Concentrations (ng/L) for vehicle related chemicals per sample for the 4/25/2024-4/26/2024 storm.

Sample #	Midpoint	1,3diphenylguanidine	HMMM	N-cyclohexyl-1.3benzothiazole-2amine	Benzotriazole	5-methyl-1-H-benzotriazole	2-aminobenzothiazole	2-hydroxy benzothiazole	2-(4-morpholinyl)-benzothiazole
1	4/25/2024 2:00	55	37	*	33	64	6.7	94	4.6
2	4/25/2024 5:00	55	36	*	29	74	6.5	52	2.5
3	4/25/2024 7:00	61	35	*	27	66	5.9	34	2.3
4	4/25/2024 9:00	99	58	*	39	90	7.6	43	2.9
5	4/25/2024 11:00	1300‹	880‹	17	550	1700‹	87	240	16
6	4/25/2024 13:00	1200‹	990‹	18	400	1200‹	71	230	18
7	4/25/2024 15:00	1100‹	870‹	15	340	1000‹	62	220	16
8	4/25/2024 17:00	970‹	950‹	13	330	870‹	57	190	14
9	4/25/2024 19:00	760‹	1000‹	9.9	260	620‹	45	140	40

10	4/25/2024 21:00	880‹	1200‹	11	360	850‹	50	170	14
11	4/25/2024 23:00	710‹	980‹	9.8	320	840‹	44	150	13
12	4/26/2024 1:00	550‹	800‹	8.1	250	690‹	35	130	10

[‹]Concentrations (ng/L) were recalculated after 10X dilution.

[*] Concentrations that are below the method detection limit.

Table S27. Concentrations (ng/L) for vehicle related chemicals per sample for the 5/21/2024 storm.

Sample #	Midpoint	1,3diphenylguanidine	HMMM	N-cyclohexyl-1.3benzothiazole-2amine	Benzotriazole	5-methyl-1-H-benzotriazole	2-aminobenzothiazole	2-hydroxy benzothiazole	2-(4-morpholinyl)-benzothiazole
1	5/21/2024 0:00	71	33	*	9.8	32	2.7	110	1.5
2	5/21/2024 3:00	64	33	*	10	31	1.9	41	0.9
3	5/21/2024 5:00	60	31	*	11	33	2.1	41	1.0
4	5/21/2024 7:00	56	30	*	9.8	29	1.9	40	0.9
5	5/21/2024 9:00	59	24	3.4	17	48	0.7	44.	1.6
6	5/21/2024 11:00	94‹	450‹	10	250	850	82	190	10
7	5/21/2024 13:00	1300‹	840‹	13	280	1100	91	220	14

8	5/21/2024 15:00	1200‹	890‹	13	240	860	75	200	14
9	5/21/2024 17:00	860‹	660‹	7.7	160	550	49	150	9.9
10	5/21/2024 19:00	900‹	910‹	15	220	760	62	190	15
11	5/21/2024 21:00	870‹	720‹	11	180	680	55	170	13
12	5/21/2024 23:00	510‹	440‹	6.3	93	320	29	100	6.8

[‹] Concentrations (ng/L) were recalculated after 10X dilution.

[*] Concentrations that are below the method detection limit.

Table S28. Concentrations (ng/L) for vehicle related chemicals per sample for the 6/1/2024-6/4/2024 storm

Sample #	**Midpoint**	**1,3diphenylguanidine**	**HMMM**	**N-cyclohexyl-1.3benzothiazole-2amine**	**Benzotriazole**	**5-methyl-1-H- benzotriazole**	**2-aminobenzothiazole**	**2-hydroxy benzothiazole**	**2-(4-morpholinyl)- benzothiazole**
1	6/1/2024 23:00	70	46	*	26	63	1.4	74	2.5
2	6/2/2024 2:30	54	43	*	31	70	2.9	53	2.2

3	6/2/2024 5:30	53	40	*	28	66	*	49	1.9
4	6/2/2024 8:30	49	40	*	31	65	2.8	48	2.1
5	6/2/2024 11:30	61	45	*	39	72	1.1	47	2.1
6	6/2/2024 14:30	800‹	720‹	12	350	970	87	190	13
7	6/2/2024 17:30	950‹	750‹	14	320	910	90	220	16
8	6/2/2024 20:30	870‹	710‹	13	260	740	74	180	14
9	6/2/2024 23:30	830‹	650‹	9.8	220	630	75	160	13
10	6/3/2024 2:30	520‹	290‹	8.1	140	420	37	100	9.2
11	6/3/2024 5:30	380‹	180‹	6.5	100	330	28	79	6.7
12	6/3/2024 8:30	310‹	230‹	4.1	96	28	24	77	5.7
13	6/3/2024 12:30	230	390	2.1	83	210	25	100	4.4

14	6/3/2024 15:30	210	380	1.6	77	180	18	78	3.5
15	6/3/2024 18:30	170	300	1.4	64	150	12	67	3.0
16	6/3/2024 21:30	140	240	1.4	59	140	15	67	3.1
17	6/4/2024 0:30	130	210	1.4	53	120	12	65	2.8
18	6/4/2024 3:30	110	180	1.1	48	110	11	56	2.4
19	6/4/2024 6:30	100	170	*	38	83	6.8	40	1.7
20	6/4/2024 9:30	210	180	1.3	66	130	12	49	2.5
21	6/4/2024 12:30	190	180	1.5	80	180	16	59	3.2
22	6/4/2024 15:30	130	150	1.6	59	130	12	52	2.6
23	6/4/2024 18:30	420	500	2.4	130	260	20	67	3.9

24	6/4/2024 21:30	360	460	2.0	130	27	20	67	3.7

[‹] Concentrations (ng/L) were recalculated after 10X dilution.

[*] Concentration below method detection limit (MDL).

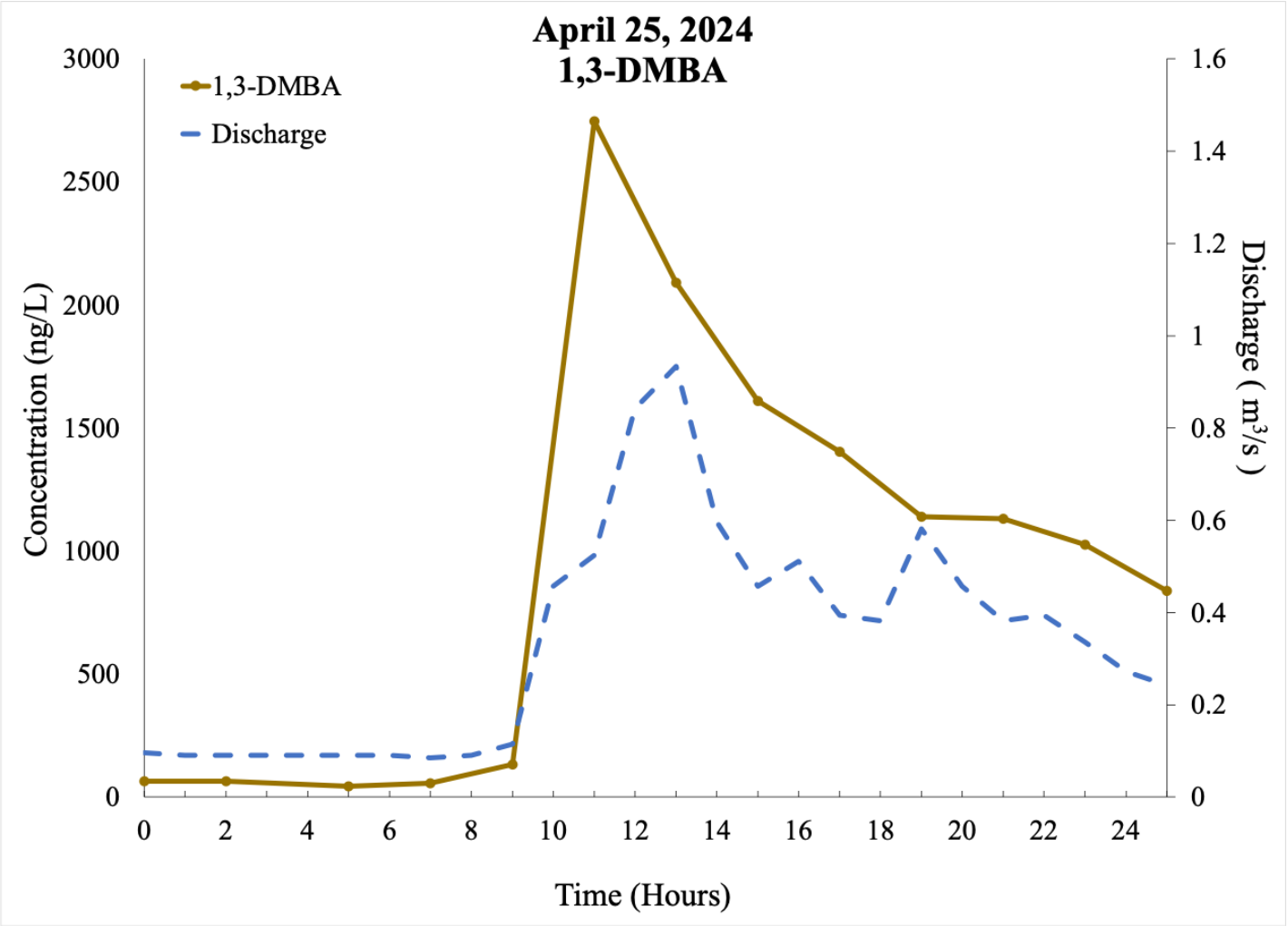

Figure S13. 1,3-DMBA concentraions plotted against discharge volume. 1,3-DMBA concentrations were siginificantly greater than other contamiants and plotted on its own graph. Storm 1, April 25, 2024 sampling event, 1,3-DMBA concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series starting at time point 0 to 26 hours.

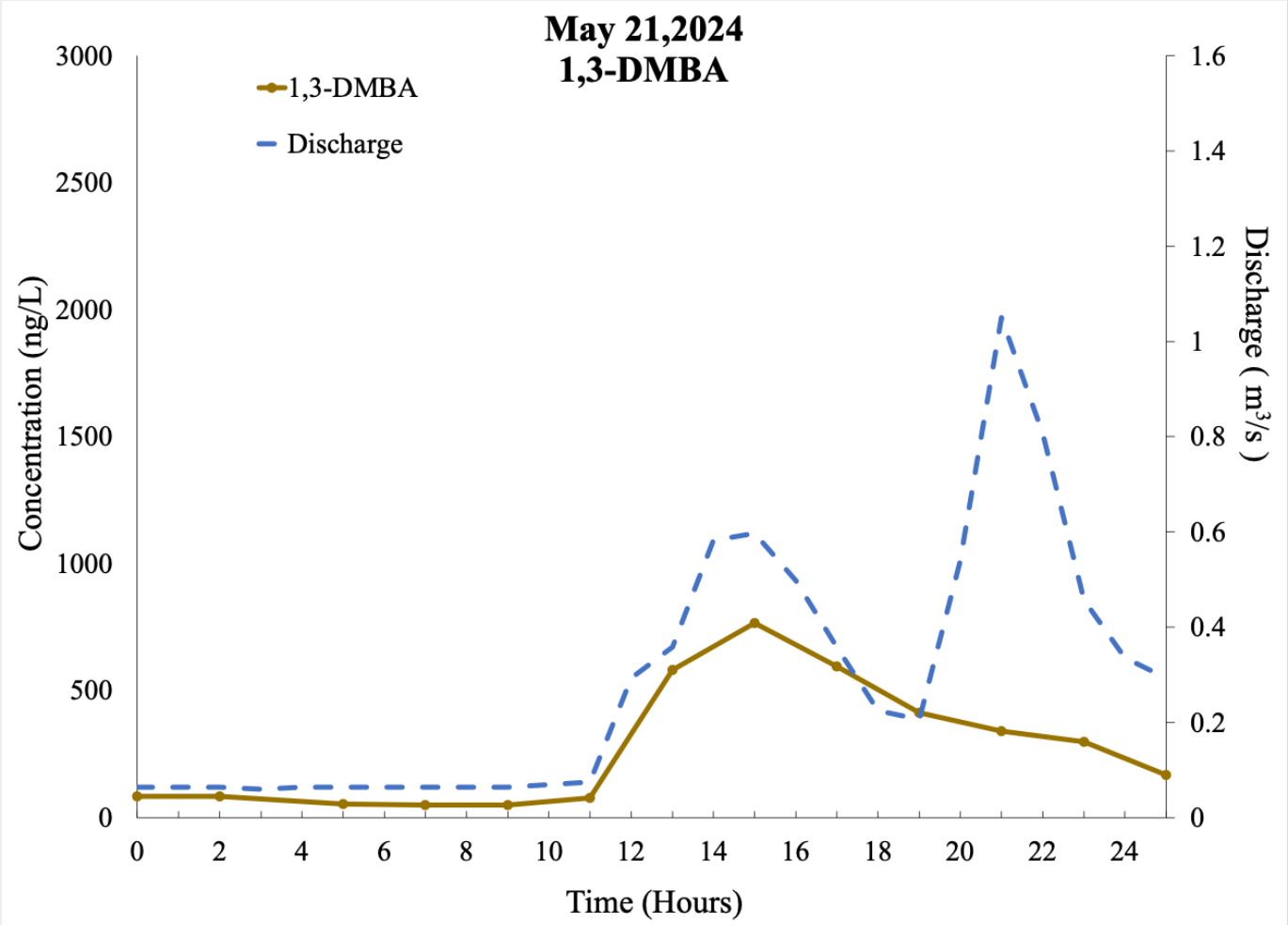

Figure S14. 1,3-DMBA concentraions plotted against discharge volume. 1,3-DMBA concentrations were siginificantly greater than other contamiants and plotted on its own graph. Storm 2, May 21, 2024 sampling event, 1,3-DMBA concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series staring at time point 0 to 26 hours.

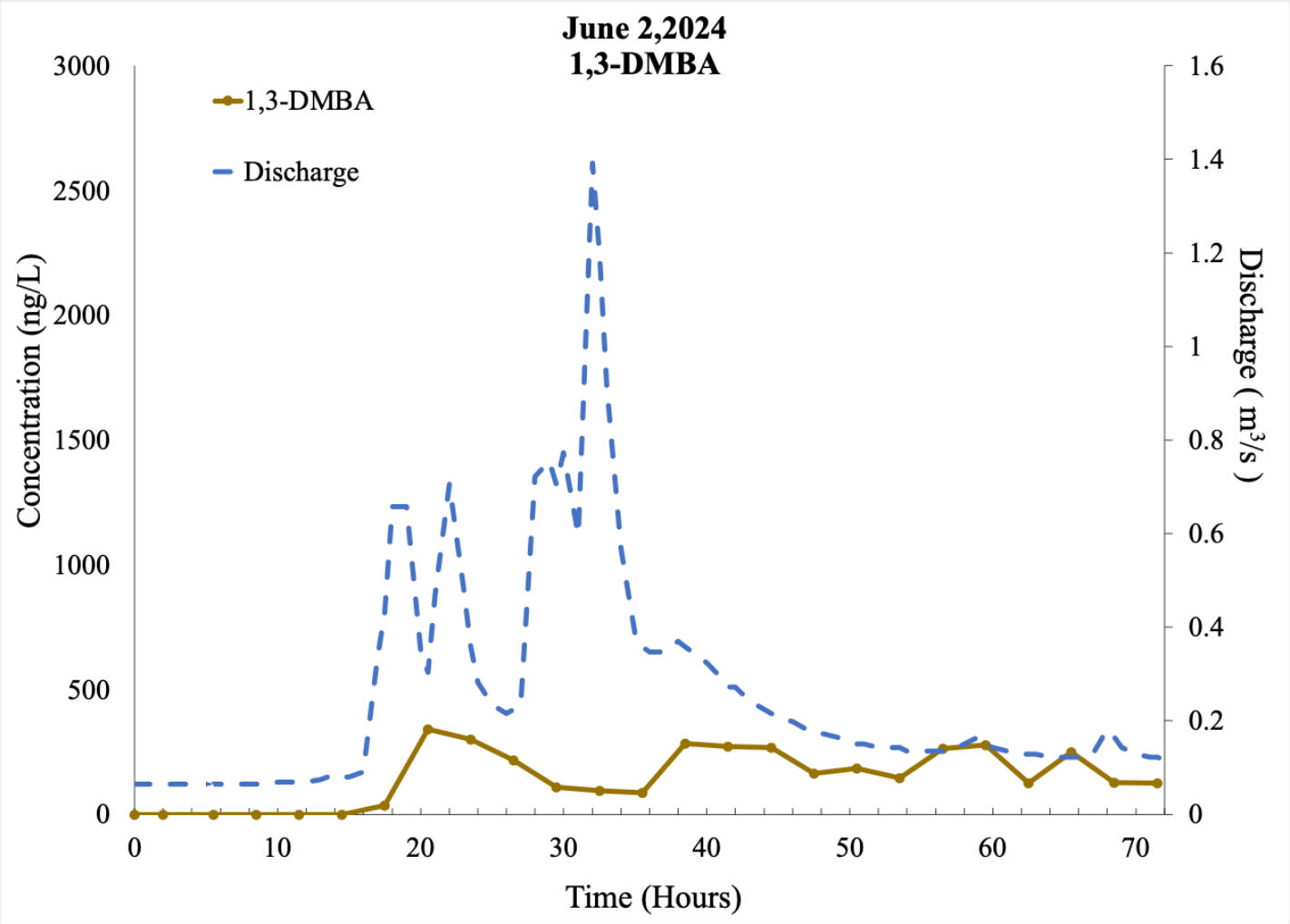

Figure S15. 1,3-DMBA concentraions plotted against discharge volume. 1,3-DMBA concentrations were siginificantly greater than other contamiants and plotted on its own graph. Storm 3, June 1 through June 4, 2024 sampling event, 1,3-DMBA concentrations (ng/L) were quantified and plotted on the left y axis, discharge (m^3/s) plotted on the right y axis, both plotted as a time series starting at time point 0 to 73 hours.

Table S29. Estimated mass load calculations for (24) targeted stormwater derived organic contaminants. Concentration ranges expressed for each contaminant across three targeted storms. Peak detections and peak concentrations expressed per storm and per contaminant for targeted storms events.

Analyte	Storm	Concentrations Detected (ng/L) All Storms	Peak Concentrations (ng/L) per Storm	Mass Load (g/storm)
6PPD	(1) April 26 (2) May 21 (3) June 2	1.6 - 360	240 360 52	3.5 5.3 1300
IPPD	(1) April 26 (2) May 21 (3) June 2	1.4 - 4.9	3.6 4.9 ND	ND ND ND
7PPD	(1) April 26 (2) May 21 (3) June 2	ND - 11	11 ND ND	ND ND 35
DPPD	(1) April 26 (2) May 21 (3) June 2	2.3 – 120	18 120 8.9	0.5 1.2 0.2
DTPD	(1) April 26 (2) May 21 (3) June 2	8.9 - 83	72 83 17	1.4 1.4 0.5
DNP	(1) April 26 (2) May 21 (3) June 2	ND	ND ND ND	ND ND ND
TP198 (4-DPA)	(1) April 26 (2) May 21 (3) June 2	1.2 - 510	500 510 170	7.7 9.1 3.5

TP101 (1,3-DMBA)	(1) April 26 (2) May 21 (3) June 2	ND- 2700	2800 770 340	44 11 11

TP184 (4-ADPA)	(1) April 26 (2) May 21 (3) June 2	ND - 470	ND 470 ND	ND ND 7.6
TP214 (4-NDPA)	(1) April 26 (2) May 21 (3) June 2	1.5 - 20	20 8.0 7.3	0.2 0.2 0.3
4OH DPA	(1) April 26 (2) May 21 (3) June 2	2.7 - 140	140 130 85	2.7 2.4 1.7
6PPDQ	(1) April 26 (2) May 21 (3) June 2	0.6 - 110	96 110 73	1.9 2.1 2.1
IPPDQ	(1) April 26 (2) May 21 (3) June 2	ND	ND ND ND	ND ND ND
7PPDQ	(1) April 26 (2) May 21 (3) June 2	ND - 1.4	1.4 ND ND	ND ND ND
DTPDQ	(1) April 26 (2) May 21 (3) June 2	7.9 - 52	ND ND 52	ND ND 1.2
DPPDQ	(1) April 26 (2) May 21 (3) June 2	ND	ND ND ND	ND ND ND

DPG	(1) April 26 (2) May 21 (3) June 2	1.4 - 1300	1300 130 95	27 2.5 6.9
HMMM	(1) April 26 (2) May 21 (3) June 2	1.1 - 1200	1200 150 75	26 1.9 8.1
NCBA	(1) April 26 (2) May 21 (3) June 2	1.0 - 18	18 15 14	0.4 0.3 0.4

1-H-BTR	(1) April 26 (2) May 21 (3) June 2	2.3 - 550	550 280 350	9.9 5.2 9.5
5-Me-1-H-BTR	(1) April 26 (2) May 21 (3) June 2	29.4 - 1700	1700 1100 970	28 19 27
2-NH2-BTH	(1) April 26 (2) May 21 (3) June 2	0.7 - 91	87 91 90	1.6 1.6 2.5
2-OH-BTH	(1) April 26 (2) May 21 (3) June 2	ND - 240	240 220 220	5.4 4.6 7.1
2-Mo-BTH	(1) April 26 (2) May 21 (3) June 2	1.5-40	40 15 16	0.5 0.3 500

[ND] No detection, concentration below method detection limit (MDL).