CENTER FOR URBAN WATERS
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Subject: 6PPD in Stormwater Technical Memo - Characterizing 6PPD and 6PPD-Quinone in Stormwater

In August 2021, Dr. Ed Kolodziej at the Center for Urban Waters (CUW) began discussion with representatives of the Washington State Department of Ecology (Ecology) to produce technical memorandums describing the current state of knowledge and various data gaps around 6PPD-quinone. These memos would summarize current knowledge around the formation, characteristics, and fate of 6PPD-quinone, which is currently believed to be the "primary causal toxicant" for regional observations of coho mortality. The memos also would research gaps with respect to 6PPD-quinone research, management, and impacts. 6PPD-quinone is a newly discovered (by CUW and WSU researchers) environmental transformation product of the industrial antioxidant "6PPD", a compound that is used as an antioxidant and antiozonant in all vehicle tire rubbers globally to the best of our knowledge. Given its high lethality, consideration of the occurrence, fate, and transport of 6PPD-quinone is critical to understanding options for its management and control. This memo is expected to help summarize certain aspects of the current state of knowledge around 6PPD-quinone chemical properties and characteristics.

The Center for Urban Waters subsequently agreed to develop a technical memorandum that would include the following:

"CENTER will produce a technical memorandum or similar white paper on the evaluation of the chemical properties of 6PPD and 6PPD-quinone, known and estimated to date, such as Koc, Kow, Kd, sorption isotherms, solubility, and specific gravity.

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Discuss and compare the results provided by EPA's EPI Suite for both compounds and describe verification work of these modeled results through laboratory analysis or other techniques. Discuss or recommend key parameters for Ecology to focus on for an evaluation of capture, containment, and treatment approaches and BMPs for stormwater. Summarize key properties that impact fate and transport of these compounds in surface or stormwater, included the mechanism(s) for capture and removal. If known, describe a surrogate compound with more data available for fate and transport that would have a similar fate and transport in stormwater systems."

We note that as a newly discovered compound, substantial and pervasive data gaps exist around 6PPD-quinone occurrence, chemical properties, environmental fate, transport, and toxicological effects across various endpoints. Additionally, little existing knowledge exists surrounding chemical characteristics and properties of quinones as a class of environmental pollutants. Therefore, at the current time, substantially more unknowns exist relative to knowns for 6PPD-quinone. While certain properties and aspects of fate and transport may be predicted computationally, studies to develop data derived parameters from direct observation have largely not been reported by CUW or any other research group to date. In contrast, 6PPD has been used for decades, so substantially more information is known concerning it. However, despite that long history of usage and the substantial industrial production of this compound, there still exist some considerable data gaps regarding 6PPD characteristics and fate, particularly with respect to its mass balance, formation of oxidative transformation products, and the toxicological relevance of such transformation products against acute and chronic endpoints.

1. <u>Predicted chemical properties of 6PPD and 6PPD-quinone</u>

Select predicted chemical properties of 6PPD and 6PPD-quinone derived from literature reports are summarized in **Table 1** below. We also note that a recent report by the California Department of Toxic Substances Control (DTSC) provides an excellent summary of 6PPD physicochemical properties, environmental fate, and current knowledge regarding fate/transport,[1] as do older OECD and OSPAR Commission reports from the European Union.[2,3] Reports generated by EPA EPI-Suite are provided as Appendices A and B.

Table 1. Predicted or estimated chemical properties of 6PPD and 6PPD-quinone. Note that many of these for 6PPD-quinone are computational predictions derived from software platforms.

Property	6PPD	6PPD-quinone	Reference
Molecular Formula	$C_{18}H_{24}N_2$	$C_{18}H_{22}N_2O_2$	--
logK_{ow}	4.68, 4.91, 5.6	3.98, 3.24, 4.1, (5-5.5*)	EPI-Suite[4], Marvin[5] and XLogP3[6], (Tian et al. 2021)[7]
logK_{oc}	4.36, 4.04	3.94	EPI-Suite[4], EPA CompTox[8]
Solubility in water	2.84 mg/L (25 °C), 1 mg/L (20 °C)	51.34 mg/L (25 °C), 67 ± 5 µg/L (pH 8, 23 °C)	6PPD: EPI-Suite[4], OECD[3] 6PPD-quinone: EPI-Suite[4], Hiki et al. 2021[9]
Vapor pressure	6.85×10^{-3} Pa	6.57×10^{-6} Pa	EPI-Suite[4]

*range of observed LC retention times

Both 6PPD and 6PPD-quinone are moderately non-polar compounds, indicating aspects of both dissolved phase and particle/solid associated behaviors are likely important to their fate. Estimates for logK_{ow} support that 6PPD-quinone is more polar than 6PPD, which is expected for an oxidized transformation product. For 6PPD-quinone, logK_{ow} values estimated by available prediction algorithms are lower than that initially estimated by Tian et al. (2021).[7] The estimate provided by Tian et al. (2021) relied on a previously developed linear regression between liquid chromatography-based retention time and logK_{ow} for a group of 260 chemical standards.[10] This instrument-specific estimate of relative polarity is subject to greater estimation error relative to algorithm-based estimates based upon structural identity. However, both estimate types provide a similar overall comparison, indicating the greater polarity of 6PPD-quinone relative to 6PPD. In general, this would imply more potential for dissolved phase environmental transport for 6PPD-quinone relative to 6PPD.

The estimated logK_{oc} values for 6PPD and 6PPD-quinone indicate a slightly higher likelihood of sorption to organic carbon for 6PPD, which would tend to reduce its mobility and increase treatment system performance for 6PPD in systems where equilibrium sorption dominates removal. A recent study by Huang et al. (2021) reported 6PPD and 6PPD-quinone on roadside dust and in-vehicle dust at 6PPD-quinone:6PPD concentration ratios of 0.1 – 8.5, although the relative contribution of 6PPD transformation to 6PPD-quinone on the dust surface versus separate sorption of the two compounds to dust remains uncertain.[11]

6PPD is poorly soluble in water and is known to be quite unstable in water due to rapid hydrolysis.[2,9] This instability is not surprising: as a redox-sensitive antioxidant, 6PPD has an inherent instability and propensity for reaction, especially when considering the widespread abundance of oxidant species in aqueous (and other) environmental systems. Reported timescales of reaction for 6PPD in aqueous systems can be as low as 1.9 h (the OSPAR-reported half-life of 6PPD in microbially active surface waters), with instability reportedly dependent on pH, ionic strength, heavy metals, and dissolved oxygen concentrations.[2] Acid-base speciation of 6PPD (based on an estimated pK_a of ~6-7 for the most basic amine in 6PPD) will yield both protonated (occurrence as a cation) and neutral species under typical circumneutral environmental conditions. As an ionizable chemical, this characteristic would tend to increase 6PPD solubility at neutral to lower pH values relative to that expected for neutral conditions. Overall, in our experience, accurate and reproducible 6PPD quantification in water is highly uncertain and challenging, and would require development of rapid, specialized methods and expertise. 6PPD is primarily expected to be sorbed to soil/dust, with negligible amounts in air, given its low vapor pressure. Despite its low vapor pressure, recent reports indicate a substantial quantity of both 6PPD and 6PPD-quinone present in house dusts and atmospheric PM2.5 particulates,[11,12] indicating a relatively high potential for human exposures via atmospheric aerosol pathways.

Although 6PPD-quinone is predicted by EPI-Suite to be much more soluble in water than 6PPD (51.34 mg/L),[4] Hiki et al. (2021) recently reported a 6PPD-quinone water solubility of 67 ± 5 µg/L in dechlorinated tap water (pH 8, 23 ºC).[9] Our preliminary observations at CUW laboratories also indicate low values for aqueous solubility and higher than expected propensities for impaired dissolution and formation of solid phases or sorption to various solid surfaces. Although 6PPD-quinone is expected to be neutral across the environmental pH spectrum, the impact of ionic strength, pH, oxidants, and other water chemistry parameters on 6PPD-quinone solubility and related fate/transport behaviors require further investigation. Like 6PPD, negligible amounts of 6PPD-quinone would be expected to volatilize. However, the expected distribution and partitioning rates of 6PPD-quinone between soil/dust, sediment, and water phases under various environmental conditions requires further investigation and is currently unknown.

2. Key properties to evaluate with respect to informing 6PPD-quinone fate, transport, and treatment in stormwater

There are several key parameters that should be evaluated to inform both analytical methods and environmental occurrence, fate, transport, and treatment of 6PPD-quinone. These are summarized in **Table 2** and described further below.

Table 2. Key parameters to evaluate for 6PPD and 6PPD-quinone.

Parameter [unit]	Additional Detail	Utility
Aqueous solubility [ng/L]	Evaluate dependency on environmental variables and constituents (e.g., pH, temperature, ionic strength, oxidants/reductants, dissolved organic carbon, etc.)	Fundamental to accurate detection
Half-life [months]		Informs environmental persistence
Reaction rate constant, k [s^{-1}]	Evaluate with respect to range of environmental and engineered oxidants (e.g., ozone, metals, chlorine, etc.) and possible transformation processes (photolysis, hydrolysis, biotransformation, redox reactions)	Informs environmental persistence, treatment approaches
Formation rate constants (6PPD to 6PPD-quinone), k [s^{-1}]	Evaluate for pure compound in air and water	Provides fundamental understanding of reactivity
Mass load in tire rubber [ng/g]	Evaluate across various tire rubber matrices, such as whole tires, skid marks, tire and road wear particles, recycled rubber products, etc. Many such parameters would be surface area normalized	Informs expected contaminant loads, release rates, and dominant sources
Diffusivity in tire rubber, D [m^2/s]		
Formation rate constant (6PPD to 6PPD-quinone) in tire rubber, k [s^{-1}]		
Leaching rates from tire rubber [ng / (g-h)]		
Partitioning coefficients (e.g., K_{aw}, $K_{w\text{-}sed}$, etc.)	Evaluate partitioning with respect to air, water, soil, sediment, rubber, and biological tissue	Informs environmental transport and fate, important for modeling efforts
Sorption coefficients (K_d, K_{oc})	Evaluate for stormwater treatment media (e.g., soil, compost, high performance BSM, etc.)	Inform BMP design, efficacy, maintenance, and longevity
Sorption rate [min^{-1}]		

In particular, understanding 6PPD-quinone aqueous solubility and stability in water, including the impact of environmental variables (e.g., pH, temperature, ionic strength, oxidants/reductants, and dissolved organic carbon) is critical fundamental knowledge relevant to

accurate detection and subsequent environmental persistence. While we do not currently anticipate 6PPD-quinone to be a very long-lived pollutant (half-lives of days to months are probably expected in most environmental systems), little to nothing is known regarding its stability and reactivity with respect to various environmental constituents.

We also note that 6PPD and 6PPD-quinone are redox active and redox-sensitive chemicals (an inherent attribute of antioxidants), a characteristic that likely strongly contributes to their potential to induce toxic adverse effects in organisms and has substantial implications for environmental fate and reactivity. Experiments specifically designed to probe abiotic redox-sensitive behaviors and characteristics will be needed to understand the potential for oxidative polymerization, complexation, and addition type reaction endpoints. We also note that a relatively limited base of knowledge exists for quinone environmental pollutants relative to other pollutant structures, indicating some potential for unexpected or poorly documented fate outcomes relative to expectations for environmental pollutants. Therefore, a more limited series of analogous pollutants is available to fully define or predict aspects of environmental fate and instability related to redox-sensitive behaviors. Evaluating reactivity with respect to environmental oxidants (e.g., in air, in water, and on environmental surfaces) and engineered oxidants (e.g., chlorine, advanced oxidation processes, etc.) is needed as an aspect of understanding 6PPD and 6PPD-quinone environmental fate.

Additionally, limited data are currently available regarding the total load/lifetime of 6PPD within tire rubbers (including recycled rubber products), the diffusivity of 6PPD within tire rubber, transformation rates from 6PPD to 6PPD-quinone within tire rubber matrices or at tire rubber surfaces, and the rates at which 6PPD and 6PPD-quinone leach from tire rubber surfaces and move through phase boundaries. These parameters are relevant across whole tires, rubber embedded in roads (such as in skid marks), crumb rubbers and other recycled rubber materials, and dispersed tire and road wear particles (TRWP, which are heteroaggregates of tire rubbers and road minerals). Determining whether one or multiple of these matrices is a dominant source of 6PPD-quinone mass loads in stormwater or impacted receiving waters will be important for informing on-road and road-adjacent BMPs and source control efforts (e.g., street sweeping). This aspect of fate essentially involves the need to understand the broader mass balance of 6PPD in tires (e.g., formation and yields of key transformation products) and its potential to move (along with 6PPD-quinone) across interfacial boundaries into air, water, soil,

and biological tissue. Without such information, leaching rates and time scales of pollution regeneration (for example, how long does it take for roadway runoff to re-pollute itself during or after storm events) cannot be accurately determined. It is also currently unclear if 6PPD and 6PPD-quinone are transporting within the environment and into receiving waters as dissolved chemicals, or whether transport of the residual rubber phases or of dusts/soils/aerosols with sorbed 6PPD and 6PPD-quinone, represent the primary transport vectors for these chemical pollutants.

To inform treatment system performance with respect to 6PPD-quinone removal from the dissolved phase, we anticipate that sorption characteristics and timescales are especially important parameters. In particular, many aspects of passive green stormwater infrastructure (GSI) like bioretention, bioswales, and bioinfiltration systems rely upon sorptive sequestration for initial pollutant removal. Therefore, the sorption capacity and K_d/K_{oc} values (e.g., developing sorption isotherms to understand mass or area normalized maximum mass removals, as well as evaluating possible desorption from treatment media) and sorption kinetics (e.g., partitioning rates) of various matrices that are anticipated to be important within stormwater treatment systems/BMPs (e.g., compost, soil, GAC, biochar, synthetic media) should be evaluated. These parameters are needed to help define treatment system sizes and flowrates (e.g., hydraulic retention times). In addition, evaluating 6PPD-quinone partitioning between aqueous and particulate phases (including tire wear particles and soil/road dust/sediments, of varying size fractions and with varied organic carbon content) will also inform best management practices with respect to management approaches focused on removal of suspended solids (and potentially rubber particulates) from stormwater (e.g., street sweeping, settling, etc.) relative to treatment of the dissolved phase.

BMP efficacy, maintenance needs, and longevity will also be impacted by the fate of captured 6PPD-quinone. For example, research is needed to determine whether 6PPD-quinone sorbed to treatment system media is further transformed and/or may be re-exported as hydrologic conditions vary (including wet/dry cycles) or over time as sorption capacity is exceeded. We might also expect that over the long term, microbial processes are ultimately responsible for mineralization of 6PPD and 6PPD-quinone that are captured in treatment systems and removed from the mobile phase. Rates and mechanisms of microbial biotransformation, including their extension to operating treatment systems and BMPs, would also need characterization. Given

previous observations of tire-derived chemical concentrations and mass loads in receiving waters that indicate the existence of semi-infinite sources in urban watersheds, it will be important to understand the relative role of dissolved phase 6PPD-quinone in stormwater runoff vs. 6PPD-quinone that is released from in-place sediments/tire rubber deposits (e.g., on road surfaces, in dusts/sediments adjacent to roads, or in stormwater treatment or conveyance systems). BMPs might need to consider both rubber particulate removal and dissolved phase 6PPD-quinone removal to be fully protective, which may include multiple barrier or dual component conceptual approaches to treatment.

3. Surrogate compounds for 6PPD-quinone in stormwater systems

To date, there is no known or hypothesized surrogate compound that would be reasonably anticipated to accurately represent 6PPD-quinone fate and transport in stormwater systems. We anticipate that identification of surrogate compounds from within existing databases of "standard" stormwater pollutants may be especially difficult and unlikely because few commonly monitored stormwater pollutants have similar chemical characteristics or functional groups. For removal of residual rubber phases and particulates, TSS potentially might be correlated to the transport of bulk rubber microplastics as one source of 6PPD-quinone, although further investigation of the size distribution and densities of tire rubber particles is needed to assess this potential. As another common component of tire rubbers, zinc may also offer some correlative potential for 6PPDS-quinone, although it is uncommon that organic contaminants and metals exhibit similar environmental fate and transport outcomes. Additionally, methods for direct tire rubber particle quantification (i.e., by pyrolysis GC-MS) require further refinement because of inherent uncertainties in tire rubber chemical composition and relative amounts of natural and synthetic rubbers.[13] However, 6PPD-quinone contains nitrogen and oxygen groups that are atypical of stormwater contaminants subject to routine monitoring efforts. There is little reason to believe that metals, nutrients, turbidity, basic water quality parameters, and even other organics like PAHs might serve as accurate surrogates for 6PPD-quinone, given both its redox sensitivity and chemical composition.

Looking at existing data for emerging organic contaminants, Peter et al. (2018) previously developed a chemical fingerprint for the coho salmon urban runoff mortality syndrome (URMS) by isolating co-occurring contaminants in laboratory and field waters that

were known to induce acute mortality.[14] This chemical fingerprint contained several tire-derived compounds, including 1,3-diphenylguanidine (DPG), hexa(methoxymethyl)melamine (HMMM) and other related MMM family members, and 1,3-dicyclohexylurea. Accordingly, the presence of these chemicals is generally indicative of tire rubber impacts on water quality. However, relationships between the relative abundance and fate/transport behavior of these contaminants and that of 6PPD-quinone remain largely undefined at the current time.

Johannessen et al. (2021) examined concentrations of DPG and 6PPD-quinone during storm events in a river in Toronto, Canada, finding that larger storm events (more precipitation) correlated to higher observed mass loads in the river.[15] This data reflected trends observed by Peter et al. (2020) for tire-derived chemicals (including DPG, but not 6PPD-quinone) in Miller Creek in Burien, WA, USA.[16] During a single storm event, Johannessen et al. (2021) observed both first-flush and middle-flush dynamics for DPG (i.e., mass loads rapidly entering the receiving water early in the storm event, followed by sustained loading with increasing runoff volumes during the hydrograph peak), but primarily observed middle-flush dynamics for 6PPD-quinone.[15] To date, this is the only available side-by-side data describing fate and transport behavior of 6PPD-quinone with respect to any other tire-derived or vehicle-derived contaminants. Additional sampling is necessary to evaluate whether the observations by Johannessen et al. (2021) are repeatable, and to evaluate the behavior of 6PPD-quinone relative to other tire-derived contaminants or pollutants that are regularly monitored in stormwater.
We here note one additional observation from our unpublished data: One additional detection from the chemical fingerprint reported by Peter et al. (2018) is notable: a compound initially reported as an *m/z* 333.2212 adduct was later determined by CUW to be a compound with a formula of *m/z* 275.1741 ($C_{16}H_{22}N_2O_2$). This compound (currently denoted TP 274) was recently detected as a 6PPD transformation product during laboratory-scale ozonation of both pure 6PPD and TWP, and was observed by retrospective analyses to be relatively abundant within roadway runoff samples (>10-fold higher peak area than 6PPD-quinone).[17] Thus, in retrospect, inclusion of TP 274 in the coho mortality chemical signature provided a direct link to tire rubber and 6PPD transformation products, thus indicating the primary chemical source of the coho mortality syndrome. Notably, detection of TP 274 in the 2019 EPA crumb rubber report[18] and by Klöckner et al.[19] in tire rubbers and road dust suggest its potential value as a chemical indicator for tire rubber and roadway runoff, as it can be both abundant and detectable by mass spectrometry even

in cases where 6PPD-quinone itself is not easily identifiable within bulk data due to matrix suppression. However, its utility as a direct and analytically viable surrogate chemical for the subsequent fate/transport of 6PPD-quinone requires additional investigation.

References

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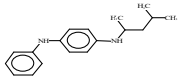
Appendix A. EPA EPI-Suite Report for 6PPD

CAS Number: 793-24-8
SMILES : N(c(ccc(Nc(cccc1)c1)c2)c2)C(CC(C)C)C
CHEM : 1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N -phenyl-
MOL FOR: C18 H24 N2
MOL WT : 268.41

---------- EPI SUMMARY (v4.11) ----------

Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------



Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 4.68
Log Kow (Exper. database match) = 4.47
Exper. Ref: SAKURATANI,Y ET AL. (2007)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 369.67 (Adapted Stein & Brown method)
Melting Pt (deg C): 121.50 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 4.93E-006 (Modified Grain method)
VP (Pa, 25 deg C) : 0.000658 (Modified Grain method)
Subcooled liquid VP: 4.49E-005 mm Hg (25 deg C, Mod-Grain method)
: 0.00598 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 2.841
log Kow used: 4.47 (expkow database)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 2.8262 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Neutral Organics

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 3.36E-009 atm-m3/mole (3.41E-004 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 6.129E-007 atm-m3/mole (6.210E-002 Pa-m3/mole)
VP: 4.93E-006 mm Hg (source: MPBPVP)
WS: 2.84 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 4.47 (exp database)
Log Kaw used: -6.862 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 11.332
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.2804
Biowin2 (Non-Linear Model) : 0.0564
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.3581 (weeks-months)
Biowin4 (Primary Survey Model) : 3.2486 (weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : -0.1043
Biowin6 (MITI Non-Linear Model): 0.0069
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.9047
Ready Biodegradability Prediction: NO

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Hydrocarbon Biodegradation (BioHCwin v1.01):
    Structure incompatible with current estimation method!

 Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
  Vapor pressure (liquid/subcooled):  0.00599 Pa (4.49E-005 mm Hg)
  Log Koa (Koawin est  ): 11.332
   Kp (particle/gas partition coef. (m3/ug)):
       Mackay model           :  0.000501
       Octanol/air (Koa) model:  0.0527
   Fraction sorbed to airborne particulates (phi):
       Junge-Pankow model     :  0.0178
       Mackay model           :  0.0385
       Octanol/air (Koa) model:  0.808

 Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
   Hydroxyl Radicals Reaction:
      OVERALL OH Rate Constant = 226.4928 E-12 cm3/molecule-sec
      Half-Life =     0.047 Days (12-hr day; 1.5E6 OH/cm3)
      Half-Life =     0.567 Hrs
   Ozone Reaction:
      No Ozone Reaction Estimation
   Fraction sorbed to airborne particulates (phi):
      0.0282 (Junge-Pankow, Mackay avg)
      0.808 (Koa method)
    Note: the sorbed fraction may be resistant to atmospheric oxidation

 Soil Adsorption Coefficient (KOCWIN v2.00):
      Koc    :  2.305E+004  L/kg (MCI method)
      Log Koc:  4.363       (MCI method)
      Koc    :  2151  L/kg (Kow method)
      Log Koc:  3.333       (Kow method)

 Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
    Rate constants can NOT be estimated for this structure!

 Bioaccumulation Estimates (BCFBAF v3.01):
   Log BCF from regression-based method = 2.616 (BCF = 413.3 L/kg wet-wt)
   Log Biotransformation Half-life (HL) = -0.1137 days (HL = 0.7697 days)
   Log BCF Arnot-Gobas method (upper trophic) = 2.468 (BCF = 293.8)
   Log BAF Arnot-Gobas method (upper trophic) = 2.468 (BAF = 294)
       log Kow used: 4.47 (expkow database)

 Volatilization from Water:
    Henry LC:  3.36E-009 atm-m3/mole  (estimated by Bond SAR Method)
    Half-Life from Model River: 2.855E+005  hours   (1.19E+004 days)
    Half-Life from Model Lake : 3.114E+006  hours   (1.298E+005 days)

 Removal In Wastewater Treatment:
    Total removal:              54.44  percent
    Total biodegradation:        0.51  percent
    Total sludge adsorption:    53.93  percent
    Total to Air:                0.00  percent
      (using 10000 hr Bio P,A,S)

 Level III Fugacity Model: (MCI Method)
           Mass Amount    Half-Life    Emissions
            (percent)        (hr)       (kg/hr)
   Air       0.0146          1.13         1000
   Water     10.8            900          1000
   Soil      75              1.8e+003     1000
   Sediment  14.2            8.1e+003     0
     Persistence Time: 1.71e+003 hr

 Level III Fugacity Model: (MCI Method with Water percents)
           Mass Amount    Half-Life    Emissions
            (percent)        (hr)       (kg/hr)
   Air       0.0146          1.13         1000
   Water     10.8            900          1000
```

```
    water     (10.4)
    biota     (0.0154)
    suspended sediment (0.36)
  Soil      75              1.8e+003     1000
  Sediment  14.2            8.1e+003     0
    Persistence Time: 1.71e+003 hr

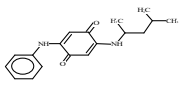
Level III Fugacity Model: (EQC Default)
          Mass Amount    Half-Life    Emissions
           (percent)        (hr)       (kg/hr)
  Air       0.0155          1.13         1000
  Water     11.9            900          1000
    water     (11.7)
    biota     (0.0172)
    suspended sediment (0.212)
  Soil      79.7            1.8e+003     1000
  Sediment  8.34            8.1e+003     0
    Persistence Time: 1.61e+003 hr
```

Appendix B. EPA EPI-Suite Report for 6PPD-quinone

CAS Number:
SMILES : CC(C)CC(C)NC1=CC(=O)C(=CC1(=O))Nc2ccccc2
CHEM :
MOL FOR: C18 H22 N2 O2
MOL WT : 298.39

---------- EPI SUMMARY (v4.11) ----------

Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------
Vapor Pressure (mm Hg) : ------
Water Solubility (mg/L): ------
Henry LC (atm-m3/mole) : ------



Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 3.98

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 430.19 (Adapted Stein & Brown method)
Melting Pt (deg C): 169.18 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 4.93E-008 (Modified Grain method)
VP (Pa, 25 deg C) : 6.57E-006 (Modified Grain method)
Subcooled liquid VP: 1.52E-006 mm Hg (25 deg C, Mod-Grain method)
: 0.000202 Pa (25 deg C, Mod-Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 51.34
log Kow used: 3.98 (estimated)
no-melting pt equation used

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 1317.4 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Aliphatic Amines
Quinones

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 1.12E-013 atm-m3/mole (1.14E-008 Pa-m3/mole)
Group Method: Incomplete
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 3.770E-010 atm-m3/mole (3.820E-005 Pa-m3/mole)
VP: 4.93E-008 mm Hg (source: MPBPVP)
WS: 51.3 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 3.98 (KowWin est)
Log Kaw used: -11.339 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate): 15.319
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.6673
Biowin2 (Non-Linear Model) : 0.2437
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 2.4063 (weeks-months)
Biowin4 (Primary Survey Model) : 3.3126 (days-weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.0470
Biowin6 (MITI Non-Linear Model): 0.0117
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.9987
Ready Biodegradability Prediction: NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

```
   Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
 Vapor pressure (liquid/subcooled):  0.000203 Pa (1.52E-006 mm Hg)
 Log Koa (Koawin est  ): 15.319
  Kp (particle/gas partition coef. (m3/ug)):
      Mackay model           :  0.0148
      Octanol/air (Koa) model:  512
  Fraction sorbed to airborne particulates (phi):
      Junge-Pankow model     :  0.348
      Mackay model           :  0.542
      Octanol/air (Koa) model:  1

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
  Hydroxyl Radicals Reaction:
     OVERALL OH Rate Constant = 144.9250 E-12 cm3/molecule-sec
     Half-Life =     0.074 Days (12-hr day; 1.5E6 OH/cm3)
     Half-Life =     0.886 Hrs
  Ozone Reaction:
     OVERALL Ozone Rate Constant =     0.350000 E-17 cm3/molecule-sec
     Half-Life =     3.274 Days (at 7E11 mol/cm3)
     Half-Life =     78.583 Hrs
  Fraction sorbed to airborne particulates (phi):
     0.445 (Junge-Pankow, Mackay avg)
     1 (Koa method)
   Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
     Koc    :  8481  L/kg (MCI method)
     Log Koc:  3.928       (MCI method)
     Koc    :  8697  L/kg (Kow method)
     Log Koc:  3.939       (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
   Rate constants can NOT be estimated for this structure!

Bioaccumulation Estimates (BCFBAF v3.01):
  Log BCF from regression-based method = 2.291 (BCF = 195.4 L/kg wet-wt)
  Log Biotransformation Half-life (HL) = -0.4467 days (HL = 0.3575 days)
  Log BCF Arnot-Gobas method (upper trophic) = 2.120 (BCF = 131.9)
  Log BAF Arnot-Gobas method (upper trophic) = 2.120 (BAF = 131.9)
      log Kow used: 3.98 (estimated)

Volatilization from Water:
   Henry LC:  1.12E-013 atm-m3/mole  (estimated by Bond SAR Method)
   Half-Life from Model River:  9.03E+009  hours   (3.763E+008 days)
   Half-Life from Model Lake : 9.851E+010  hours   (4.105E+009 days)

Removal In Wastewater Treatment:
   Total removal:              29.16  percent
   Total biodegradation:        0.31  percent
   Total sludge adsorption:    28.84  percent
   Total to Air:                0.00  percent
     (using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)
          Mass Amount    Half-Life    Emissions
           (percent)        (hr)       (kg/hr)
  Air       1.33e-006       1.73         1000
  Water     10.4            900          1000
  Soil      84.4            1.8e+003     1000
  Sediment  5.13            8.1e+003     0
    Persistence Time: 1.94e+003 hr

Level III Fugacity Model: (MCI Method with Water percents)
          Mass Amount    Half-Life    Emissions
           (percent)        (hr)       (kg/hr)
  Air       1.33e-006       1.73         1000
```

Water	10.4	900	1000
water	(10.3)		
biota	(0.00491)		
suspended sediment	(0.131)		
Soil	84.4	1.8e+003	1000
Sediment	5.13	8.1e+003	0

Persistence Time: 1.94e+003 hr

Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.36e-006	1.73	1000
Water	11	900	1000
water	(10.9)		
biota	(0.0052)		
suspended sediment	(0.064)		
Soil	86.6	1.8e+003	1000
Sediment	2.47	8.1e+003	0

Persistence Time: 1.89e+003 hr

CENTER FOR URBAN WATERS
UNIVERSITY of WASHINGTON | TACOMA

January 13, 2022

To: Brandi Lubliner
Water Quality Program
Washington State Department of Ecology

From: Katherine T. Peter, Edward P. Kolodziej
Center for Urban Waters
University of Washington-Tacoma

Subject: 6PPD Research Assessment Technical Memo – Status and Next Steps for Research on 6PPD-Quinone to Manage Impacts from Stormwater

In August 2021, Dr. Ed Kolodziej at the Center for Urban Waters (CUW) began discussion with representatives of the Washington State Department of Ecology (Ecology) (e.g., Brandi Lubliner) to produce technical memorandums describing the current state of knowledge and various data gaps around 6PPD-quinone. These memos would summarize current knowledge around the formation, characteristics, and fate of 6PPD-quinone, which is currently believed to be the "primary causal toxicant" for regional observations of coho mortality. The memos also would research gaps with respect to 6PPD-quinone research, management, and impacts. 6PPD-quinone is a newly discovered (by CUW and Washington State University (WSU) researchers) environmental transformation product of the industrial antioxidant "6PPD", a compound that is used as an antioxidant and antiozonant in all vehicle tire rubbers globally to the best of our knowledge. Given its high lethality, consideration of the occurrence, fate, and transport of 6PPD-quinone is critical to understanding options for management and control. This memo is expected to help summarize knowledge of and short- and long-term research needs regarding 6PPD-quinone.

The Center for Urban Waters subsequently agreed to develop a technical memorandum that would include the following components:

"Provide a brief background and timeline of UW - Tacoma's involvement on stormwater toxicity, impacts, and stormwater toxicity treatment studies. Describe research questions and data needs to control or limit 6PPD and 6PPD-quinone contamination of stormwater and

surface waters from the known sources of these compounds. Describe research needs or questions to characterize different sources, land uses, treatments, or management options for nonpoint and point source stormwater. Briefly describe active university research on stormwater or 6PPD-quinone, such as treatment BMPs and modelling. Data gaps and future information needs are anticipated to be wide and ranging therefore characterization of needs should be done in the next two, five, and ten year timeframes."

1. Background of UW Tacoma Center for Urban Waters stormwater research

In 2014, Ed Kolodziej joined the faculty at University of Washington (Tacoma/Seattle) and began building a research group focused on water quality characterization at the Center for Urban Waters (CUW). Dr. Kolodziej soon became involved in the on-going collaborative research effort by Washington State University Puyallup Research & Extension Center (WSU), the NOAA Northwest Fisheries Science Center (NOAA), and the U.S. Fish & Wildlife Service (USFWS) to better understand ongoing coho salmon (*Oncorhynchus kisutch*) mortality events linked to urban stormwater exposure (i.e., "urban runoff mortality syndrome" or "URMS"). Initial research efforts at CUW focused on development of advanced analytical methods that leveraged liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) to perform broad-scope screening of organic contaminants in environmental samples, including both water and fish tissues. Relative to targeted analytical methods, LC-HRMS enables non-targeted analyses that do not require *a priori* knowledge of the contaminants of interest, instead allowing researchers to detect as many chemicals as possible (within the limitations of certain sample processing and analytical method choices) and subsequently use advanced data reduction approaches to prioritize chemicals of interest for identification.

These methods were first described in Du et al. (2017), along with optimized water and fish tissue extraction methods.[1] Du et al. (2017) analyzed roadway runoff samples and fish tissue samples collected during controlled exposures of adult coho salmon,[1,2] leading to the identification of several to many contaminants these samples. Notably, acetanilide (a toxic compound used industrially and in rubber vulcanization) was detected in runoff and in runoff-exposed fish gill and liver.[1] Diphenylguanidine, a compound also used in rubber vulcanization, also was widely present, supporting the importance of roadway derived chemicals in affected

receiving waters. However, given the limited number of chemical identifications (from the thousands of total detections) achieved during this initial effort, it was evident that substantial further refinement and prioritization was necessary to isolate the URMS causal toxicant(s).

Beginning in fall 2015, CUW researchers began collaborating with local citizen scientists (Miller-Walker Community Salmon Investigation, Puget Soundkeeper) that conduct daily surveys in regional creeks (Miller Creek, Walker Creek, Longfellow Creek) during the fall storm and salmon spawning season to count live and dead coho and chum salmon, document instances of URMS (identified based on egg retention in female coho), and mark/count redds. Citizen scientists were asked to alert CUW researchers if they observed symptomatic salmon in distress (i.e., actively dying) to allow for water and tissue collection during actual mortality events. Through these collaborations, CUW researchers collected and analyzed paired water and fish tissue samples from field observations of URMS across 2016 – 2020 years. These samples provided a valuable link between laboratory observations that roadway runoff replicated the symptomology of the mortality syndrome[2] and water quality during actual field mortality events.

In particular, these data allowed CUW researchers to develop a chemical "signature" or "fingerprint" for the mortality syndrome that consisted of all chemicals that co-occurred in both laboratory and field water samples that were linked to mortality events.[3] In the absence of a known causal toxicant at the time, the coho mortality chemical signature provided a surrogate chemical metric that could be tracked in other waters, through treatment systems, and used to evaluate potential sources of chemicals linked to URMS. The mortality signature contained 57 chemicals in total, of which 32 were ultimately identified: polyethylene glycols (PEGs), polypropylene glycols (PPGs), octylphenol ethoxylates (OPEOs), bicyclic amines (e.g., diphenylguanidine, dicyclohexylurea), and a family of (methoxymethyl)melamine (MMM; e.g., hexa-MMM, tetra-MMM) compounds.[3] Additionally, based on a concurrent effort between Washington State University-Puyallup (WSUP) and CUW to characterize vehicle-derived contaminant sources to roadway runoff, the occurrence and relative abundance of the coho mortality signature in several complex mixture sources such as motor oil, antifreeze, and tire wear particles was evaluated with respect to the mortality signature in waters linked to coho mortality events.[3] Results indicated the closest chemical similarity between mortality-linked waters and tire tread wear particle (TWP) leachates, providing early evidence of the importance

of tire wear particles as a dominant source of contaminants in roadway runoff and as a potential toxicant source.[3] The importance of TWP leachates as a source of the toxicant(s) driving URMS was then confirmed by exposures of juvenile coho salmon and subsequently, concurrent exposures of adult coho and chum salmon to TWP leachate, which replicated the symptomology observed during both field mortality events and laboratory exposures to roadway runoff.[4] These exposures clearly indicated that coho salmon were extremely sensitive to unknown toxicant/s present in TWP leachate.

By late 2017, an extensive effort was underway to isolate the causal toxicant(s) from TWP leachates, using fractionation (i.e., physical/chemical manipulations to separate the thousands of chemicals present in TWP leachate into smaller groups, or fractions, based on their physico-chemical properties) and effects-directed analysis (exposures of juvenile coho salmon to determine which fraction(s) were toxic)). This effort, in close collaboration with WSU, required hundreds of fish and thousands of person-hours to develop and apply new methods and fractionation techniques, conduct exposures, analyze samples, and identify candidate chemical toxicants. Ultimately, in late 2019-early 2020, these collaborative efforts allowed CUW researchers to successfully isolate and identify a single chemical as the primary causal toxicant for URMS – 6PPD-quinone, a newly discovered oxidative transformation product of the ubiquitous tire rubber antiozonant 6PPD.[5] CUW researchers have since built on the discovery of 6PPD-quinone, including development of a targeted analytical method for 6PPD-quinone[6] (currently submitted to Ecology for accreditation) and on-going efforts to understand both fundamental properties and environmental occurrence/dynamics of 6PPD-quinone.

In parallel with research efforts specifically focused on URMS toxicant identification, CUW research projects have also sought to address a range of related questions about stormwater quality, impacts, and treatment. These include studies employing both targeted and non-targeted analytical approaches, including extensive retrospective analysis of archived HRMS data and samples, to examine the efficacy and of stormwater treatment systems, such as bioretention barrels, compost-amended bioswales,[7] and engineered hyporheic zones.[8] Additionally, a targeted LC-MS/MS method was developed for a suite of 39 stormwater tracers[9] (also currently submitted to Ecology for accreditation) that includes several tire-derived organic contaminants as an improved metric of urban stormwater composition which includes representative organic

contaminants. Both targeted and non-targeted analytical methods have been applied over the past several years to understand contaminant occurrence and dynamics in Puget Sound watersheds, with respect to storm hydrographs and land-use characteristics. For example, CUW researchers evaluated contaminant pollutographs (concentration and mass load profiles as a function of time) with respect to the hydrograph in Miller Creek, a representative small urban watershed.[10] Results indicated that emerging organic contaminants (including many tire-derived chemicals) exhibited both "first flush" and "middle flush" dynamics, where concentrations in the receiving water were rapidly elevated (before the peak of the storm hydrograph) and remained elevated through and after the hydrograph peak.[10] These observations pointed to the potential role of "semi-infinite" stagnant contaminant sources in urban watersheds, such as tire wear particle residuals in stormwater conveyance systems or watershed sediments.[10] Such efforts are currently being extended to include 6PPD-quinone and other roadway tracers to better understand linkages between roadway runoff and water quality composition during baseflow and storm events, including identification of key source zones within watersheds.

2. Research questions and data needs to characterize, control, and manage 6PPD and 6PPD-quinone

Research needs are summarized in **Table 1** below, with respect to 2, 5, and 10+ year timeframes.

Table 1. Research needs regarding 6PPD and 6PPD-quinone over the next 0-10+ years.

Research Category	0-2 years	2-5 years	5-10+ years
Analytical Methods	• Develop sample processing methods for 6PPD-quinone analysis in soils, sediments, dusts, aerosols, and biological tissues	• Develop quantitative 6PPD sample processing and analytical methods, including analysis of similar or alternative antioxidants	• High throughput LC-HRMS screening and identification of stormwater contaminants and linkages to key sources
Sources	• Evaluate 6PPD-quinone mass loads across various tire types (passenger car, light truck, commercial truck) and tire components (tread, sidewall, etc.) • Develop representative tire mixture to support source tracking and ecotoxicological studies • Evaluate aqueous leaching rates of 6PPD-quinone from tire rubber (including impact of liquid/solid ratios, environmental variables/constituents, turbulent vs. static flow, etc.)	• Evaluate 6PPD and 6PPD-quinone mass loads and aqueous leaching rates across various types of environmentally relevant rubber deposits, such as whole tires, skid marks, tire and road wear particles, recycled rubber products (e.g., crumb rubber), etc. • Determine 6PPD and 6PPD-quinone diffusivity in tire rubbers (long-term release rate) • Assess potential for non-tire 6PPD and 6PPD-quinone sources (e.g. crumb rubber playing fields, building materials, etc) based on production/manufacturing data • Characterize and report tire rubber chemical composition and linkages to water quality	• Assess 6PPD-quinone and TWP contaminant releases from recycled and scrap tire products • Evaluate long term trends in TWP chemical composition and link such trends to receiving water quality • Perform regional and national comparisons with respect to management and source control efforts • Identify and promote best in class options for passenger and commercial tires; implement purchase of salmon safe tires
Fate & Transport	• Determine 6PPD-quinone solubility (aqueous, organic solvents; impact of environmental variables/constituents) • Evaluate 6PPD-quinone stability and half-life (aqueous, organic	• Identify additional 6PPD transformation pathways (ozonation, hydrolysis, etc.) and resulting transformation products • Evaluate the role of TWP particles in subsequent environmental transport of TWP-derived chemicals	• Identify transformation products of alternative anti-ozonants • Evaluate long term trends on concentrations and mass loads in relation to management and source control efforts

	solvents; impact of environmental variables/constituents)
	• Assess whether other PPDs form PPD-quinones
	• Ozonation kinetics and yields
	  ○ pure 6PPD to 6PPD-quinone
	  ○ 6PPD in tire rubber to 6PPD-quinone
		• Evaluate reactivity (reaction rates, end-products) of 6PPD-quinone (and other 6PPD transformation products) with respect to a range of environmental and engineered oxidants under varied environmental conditions
		• Evaluate 6PPD-quinone partitioning with respect to environmental matrices (water, soil, sediment, air, biological tissues), including persistence and potential for (bio)accumulation
			• Extend fate and transport knowledge from aqueous systems to soils, sediments, and atmospheric aerosol transport pathways.
			• Develop mechanistic and predictive insights for fate and reactivity of PPD industrial chemicals

Environmental Occurrence	• Environmental occurrence of 6PPD, 6PPD-quinone, and related transformation products (roadway runoff, surface waters, road dust, soils, sediments) • Evaluate 6PPD-quinone pollutograph behavior to understand contaminant occurrence, transport, and risk profile in receiving waters during stormflow conditions • Initial identification of high risk locations and time periods	• Evaluate relative importance of 6PPD-quinone "transport pathways" (e.g., stormwater outfall pipes vs. overland flow vs. TWP deposits in pipes vs. TWP deposits in road-side soils, detention basins, stagnant waters, freshwater sediments, or stormwater treatment systems) • Evaluate relationship between tire tread wear particle (TWP) concentrations and TSS in stormwater, including 6PPD-quinone loads • Relate occurrence data to land-use parameters (e.g., road types, urbanization levels) to begin predictive modeling and optimize management efforts	• Monitor and evaluate long term trends in receiving water quality, relate to land use and management efforts • Attain predictive capabilities for relative water quality within and across watersheds, and as a function of management and source control efforts
Human & Environmental Health Risk	• Screen 6PPD-quinone as a risk to human health • Evaluate toxicity of 6PPD-quinone to aquatic species other than coho salmon	• Identify pathways for human exposure to 6PPD-quinone and quantify exposures • Validate toxicity mechanism for 6PPD-quinone in coho salmon, translate to in vitro biological screening techniques	• Evaluate potentials for sub-lethal toxicity impacts in other species • Determine whether alternative anti-ozonants are a risk to human and ecological health

	• Identify mechanisms of acute toxicity	• Evaluate potentials for sub-lethal toxicity impacts in coho salmon and other salmonids • Assess toxicity of other PPD-quinones • Evaluate toxicity of alternative anti-ozonants and their transformation products	
Treatment & Management	• Evaluate sorption coefficients, capacities, rates for stormwater treatment media (soil, compost, high performance BSM, etc.) • Develop an approach to prioritize locations (watersheds, land-use types, road types, etc.) and treatment options for 6PPD-quinone management	• Identify and optimize BMPs and treatment systems for 6PPD-quinone removal to protective levels (e.g., < 40-50 ng/L) • Determine hydraulic retention times (i.e., sizing) of stormwater treatment systems needed for 6PPD-quinone removal to protective levels • Evaluate treatment system longevity, including potential for 6PPD-quinone export • Determine treatment system maintenance needs, such as sediment/TWP removal or media replacement • Evaluate efficacy of non-treatment BMPs (e.g., street sweeping) • Develop a method to evaluate and certify BMPs for treatment of 6PPD-quinone (i.e. integrate 6PPD-quinone treatment performance into TAPE program)	• Evaluate long term performance of treatment systems, including effect of various maintenance efforts • Identify and widely implement best in class options for treatment and management efforts

3. Active University Research on Stormwater or 6PPD-Q and alternatives

Researchers at CUW and WSU are currently working to maintain their global leadership on these topics by pursuing several lines of research regarding 6PPD-quinone and the broad role of tire rubber derived chemicals on water quality and impacts on salmonids. First, CUW has developed a targeted LC-MS/MS analytical method based on isotope dilution techniques for 6PPD-quinone quantification in water samples;[6] this analytical method was submitted to Ecology for accreditation in June 2021. This method is currently being applied to quantify 6PPD-quinone concentration in samples collected both by CUW researchers and by other institutions (including from on-going WSU studies examining 6PPD-quinone toxicity). CUW researchers are currently leveraging sample collections occurring for other projects to evaluate 6PPD-quinone occurrence and dynamics in representative regional surface waters and the efficacy of several stormwater treatment systems, although no substantial newly funded projects are currently specifically focused on evaluating environmental occurrence or treatment of 6PPD-quinone in detail. Select samples from previous studies (e.g., compost-amended bioswale influent/effluent samples,[7] roadway runoff,[3,5] regional surface water samples[5,10]) have also been retrospectively analyzed for 6PPD-quinone, although results should be considered semi-quantitative because sample processing methods were not optimized at the time for 6PPD-quinone analysis. Nevertheless, valuable relative comparisons along with reasonably accurate concentration data can typically be derived from these sample types.

Notably, comparisons between a commercial standard for 6PPD-quinone (HPC Standards Inc., available in March 2020) and the CUW in-house standard prepared via purification of tire leachate and/or ozonated 6PPD revealed a substantially higher (~15 fold) peak area response for the commercial standard relative to the in-house standard used for our prior research efforts. This difference was attributed to an unexpected loss of 6PPD-quinone mass in in-house stocks due to previously unobserved instability (e.g. oxidative polymerization) or solubility issues with 6PPD-quinone. To understand possible causes, laboratory studies are on-going to examine 6PPD-quinone solubility in aqueous and solvent systems, stability during sample handling (e.g., stock solution preparation, filtering, storage), sorption to a range of common laboratory materials (e.g., tubing, stoppers, containers), and possible oxidative polymerization or addition reactions typical of similar redox-active compounds. Such efforts are expected to be critical to both analytical

accuracy and key aspects of environmental fate for 6PPD-quinone. While all prior relative comparisons of environmental occurrence and toxicity remain accurate, absolute values for both environmental concentrations and LC50 values will decrease by approximately one order of magnitude. Publication of such results is expected shortly.

Researchers at CUW are also investigating the ozonation of both pure 6PPD phases and 6PPD in tire wear particles through controlled laboratory studies. These efforts include evaluating ozonation reaction rates and yields for 6PPD-quinone from 6PPD, examining the 6PPD to 6PPD-quinone transformation pathway, and identification of other 6PPD transformation products, including evaluation of their environmental occurrence. Similar initial observational efforts are being extended to other PPDs, including with respect to discussions led by State of California management agencies focused on potential alternatives to 6PPD within tire rubbers.

Finally, through the "Clean Cars" effort focused on consumer product safety (led by Craig Manahan Ecology), CUW will quantify the concentration of surface-available 6PPD-quinone and screen for other tire-derived chemicals/transformation products in tire wear particles. The concentration/mass load and variability of available 6PPD-quinone in different types of new and used tires (passenger car, light truck, commercial truck) will be evaluated beginning in 2022. Experiments to characterize the leaching dynamics of 6PPD-quinone from TWPs as a function of time and environmental variables will also be conducted. Additionally, samples from studies conducted by WSU researchers to understand changes in 6PPD-quinone toxicity to coho salmon with respect to various environmental variables (e.g., pH, temperature, ionic strength) will be analyzed at CUW.

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