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Analysis of Tire Anti-Oxidant 6PPD- Quinone by LC-MSMS

14th Annual SABCS Conference: Sept 2024
Louis Wagner



Analysis of Tire Anti-Oxidant 6PPD-quinone by LC-MSMS

- 6PPD and 6PPD-quinone (What are they?).
- Discovery timeline.
- Environmental impact.
- Emerging guidelines.
- Analysis by LC-MS/MS (Liquid Chromatography- Tandem Mass Spectrometry).

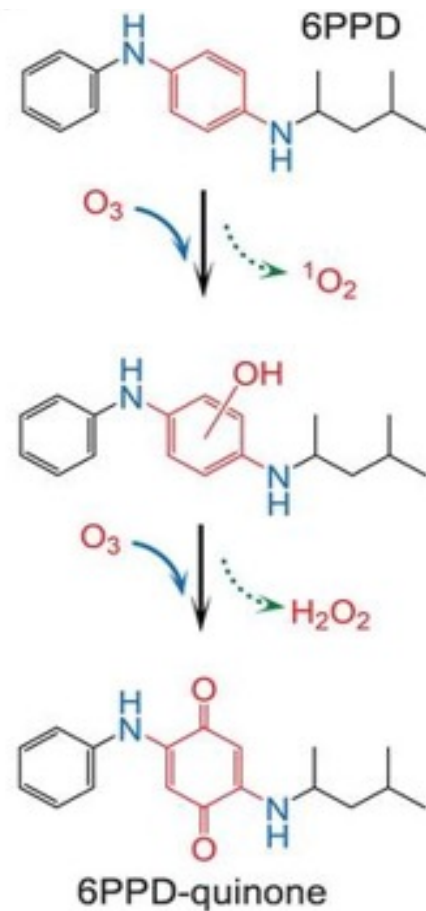


6PPD and 6PPD-quinone

- 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine).
- Tire additive to prevent degradation.
- 6PPD reacts with ozone at road/tire surface to create 6PPD-quinone.
- 6PPD-quinone is extremely toxic to coho salmon.

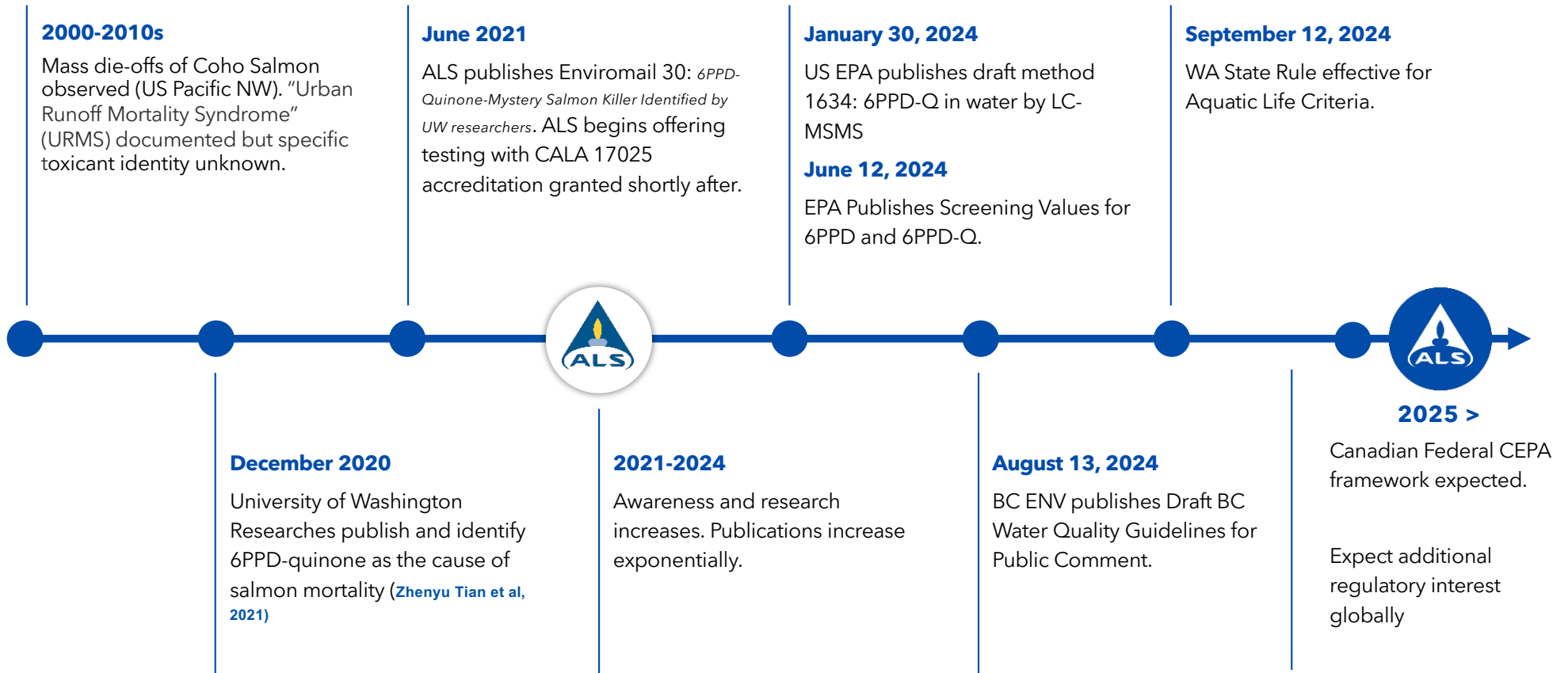


Photo Courtesy of Rodney Hsu (Fishingwithrod.com)



Zhenyu Tian et al. ,A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science* 371,185-189(2021)

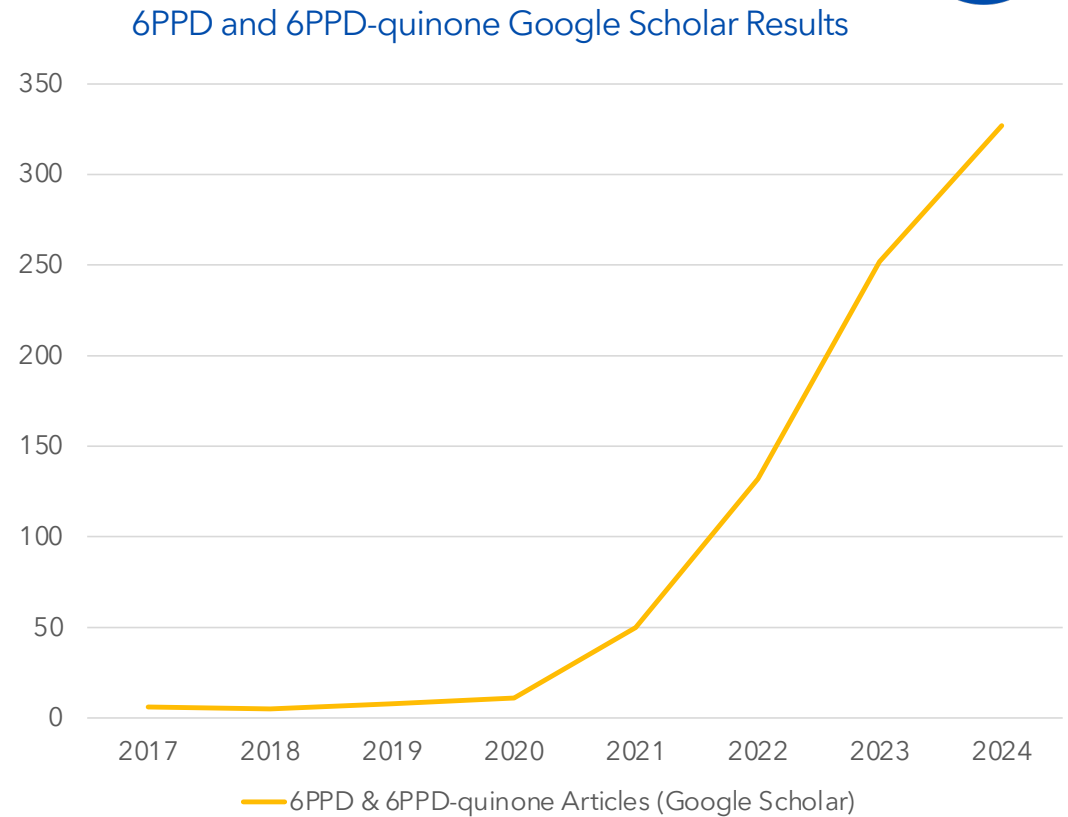
6PPD-Quinone Timeline



Research Trends of 6PPD/6PPD-quinone



- Since 2020, there has been a rapid increase in research into 6PPD and 6PPD-quinone.
- 2024 is not over yet. Emerging as a contaminant of concern globally.





Impact: Species-Specific Toxicity of 6PPD-Q to Aquatic Life

- Highest Toxicity to date observed in Coho Salmon
- Species-Specific Response
- Important consideration: Laboratory Toxicity Studies
 - Rainbow Trout is a common test organism.
 - Shows acute toxicity, however not as severe as Coho.

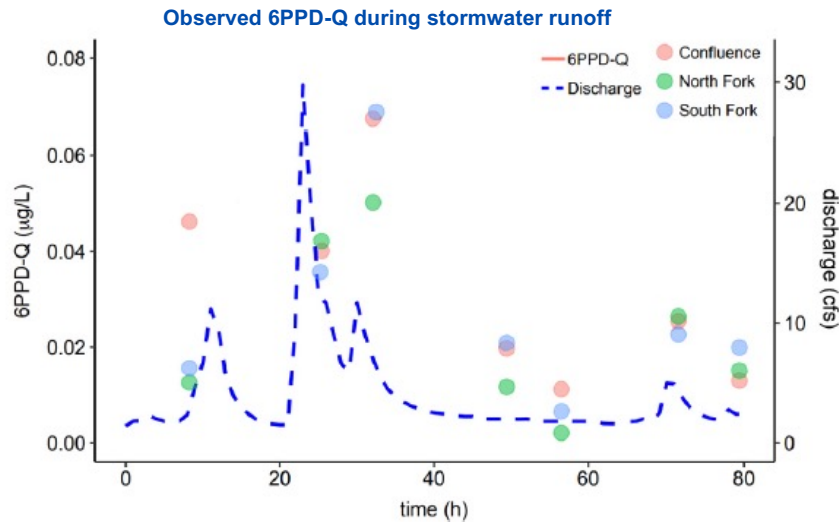
Half lethal concentrations LC50 of 6PPD-Q across different species				Toxicity level	
Species	LC50 (µg/L)	Test duration	Reference		
Coho salmon (<i>Oncorhynchus kisutch</i>)	0.041	24 h	(Lo <i>et al.</i> , 2023)	higher	
	0.0804	24 h	(Greer <i>et al.</i> , 2023b)		
	0.095	24 h	(Tian <i>et al.</i> , 2022)		
White-spotted char (<i>Salvelinus leucomaenis pluvius</i>)	0.51	24 h	(Hiki and Yamamoto, 2022b)		
Brook trout (<i>Salvelinus fontinalis</i>)	0.59	24 h	(Brinkmann <i>et al.</i> , 2022)		
Rainbow trout (<i>Oncorhynchus mykiss</i>)	1.0	96 h	(Brinkmann <i>et al.</i> , 2022)		
	0.9	24 h	(Liao <i>et al.</i> , 2023)		
	1.96	24 h	(Brinkmann <i>et al.</i> , 2022)		
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	> 80	24 h	(Greer <i>et al.</i> , 2023b)		lower
	> 67.3	24 h	(Lo <i>et al.</i> , 2023)		
Zebrafish (<i>Danio rerio</i>)	308.67	24 h	(Varshney <i>et al.</i> , 2022)		
	132.92	96 h	(Varshney <i>et al.</i> , 2022)		
	16.31 µg/(kg soil)	28 d	(Xu <i>et al.</i> , 2023a)		
<i>Folsomia candida</i>	> 100	4.5 d	(Hua <i>et al.</i> , 2023a)		
Sockeye salmon (<i>Oncorhynchus nerka</i>)	No death at 50	24 h	(Greer <i>et al.</i> , 2023b)		
Atlantic salmon (<i>Salmo salar</i>)	No death at 12.2	48 h	(Foldvik <i>et al.</i> , 2022)		
Brown trout (<i>Salmo trutta</i>)	No death at 12.2	48 h	(Foldvik <i>et al.</i> , 2022)		
Arctic char (<i>Salvelinus alpinus</i>)	No death at 14.2	96 h	(Brinkmann <i>et al.</i> , 2022)		
White sturgeon (<i>Acipenser transmontanus</i>)	No death at 12.7	96 h	(Brinkmann <i>et al.</i> , 2022)		
Southern dolly varden (<i>Salvelinus curilus</i>)	No death at 3.8	48 h	(Hiki and Yamamoto, 2022b)		
Cherry salmon (<i>Oncorhynchus masou masou</i>)	No death at 3.5	48 h	(Hiki and Yamamoto, 2022b)		

Table Source Y.Jiang et al. Environment International 187 (2024) 108677



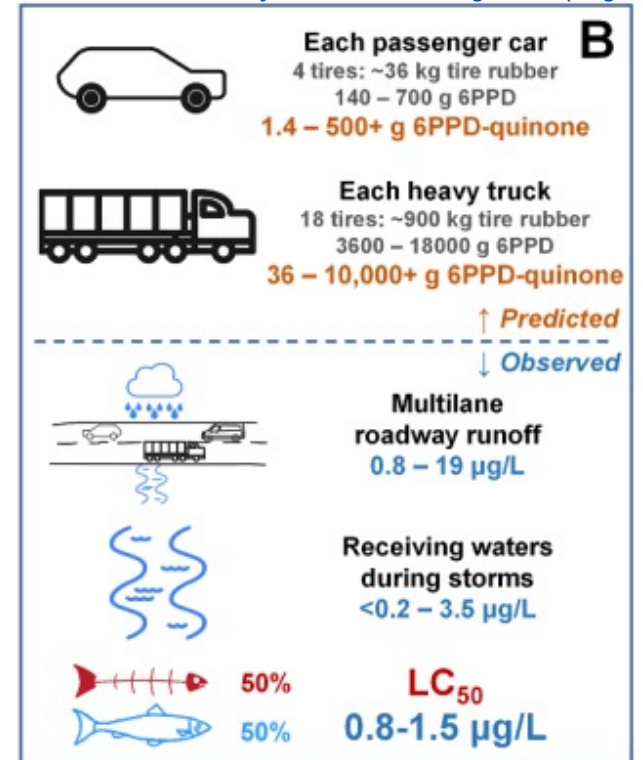
Impact: Observed 6PPD-Q Environmental Concentrations

- Passenger cars + trucks contain a lot of tire rubber.
- Driven by roadway/urban runoff events - Rainfall
- Roadways and receiving waters contain levels of 6PPD-Q toxic to Coho Salmon. Additional surveys in literature worldwide can reach hundreds of ug/L 6PPD-Q .



R.F. Lane et al., Tire-derived contaminants 6PPD and 6PPD-Q: Analysis, sample handling, and reconnaissance of United States stream exposures. Chemosphere 363 (2024) 142830

Observed 6PPD-Q in roadway runoff and receiving waters (Original Paper)



Zhenyu Tian et al., A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. Science 371,185-189(2021)



6PPD and 6PPD-Q Guidelines (US EPA).

- EPA has published "Screening Values" for 6PPD and 6PPD-Quinone in Freshwater (June 12, 2024)

https://www.regulations.gov/document/EPA_FRDOC_0001-30852

- 6PPD-Q screening value set at 11 ng/L (ppt)
- "Consider Screening values preliminary levels that do not yet impose legally binding requirements on the regulated community."

TABLE 1—RECOMMENDED AQUATIC LIFE ACUTE SCREENING VALUES FOR FRESHWATER (ng/L)

	6PPD	6PPD-q
	8,900	11
Duration	1 hour Not to be exceeded more than once in three years on average	
Frequency		



6PPD-Q Aquatic Life Criteria (WA State).

- WA State has published its update to the Aquatic Life Toxics Criteria (August 12, 2024). Rule effective September 12, 2024

<https://ecology.wa.gov/regulations-permits/laws-rules-rulemaking/rulemaking/wac-173-201a-aquatic-life-toxics-criteria>

- Level set at 0.012 ug/L (12 ng/L).

2.4.9 6PPD-quinone (freshwater acute)

Baseline

State

- Freshwater Acute: None

Federal

- Freshwater Acute: None

Adopted

- Freshwater Acute: 0.012 ug/L

Expected Impact

Freshwater acute criterion-based limits for 6PPD-quinone are new criteria introduced by this rulemaking, and not otherwise in the baseline. Future permits for new facilities would also need to establish limits under this rule compared to no 6PPD-quinone criteria in the baseline.

Permit holders impacted by these are assumed to already be monitoring monthly. Sampling costs in this case would be negligible, however they would still need to test for the new criteria. If general permit holders exceed effluent limits, they would be required to undertake progressive levels of corrective actions. Similarly, individual permit holders that exceed effluent limits would be required to take corrective action, however the specific actions required would likely differ by permit.

Draft BC Water Quality Guidelines – 6PPD-Q



- Posted for public comment August 13, 2024

<https://www2.gov.bc.ca/gov/content/environment/air-land-water/water/water-quality/water-quality-guidelines/draft-water-quality-guidelines>

Table ES.1. Proposed water quality guideline

Designated use	WQG for 6PPD-quinone ($\mu\text{g/L}$)	
	Long-term chronic WQG	Short-term acute WQG
Freshwater aquatic life	--	0.01

6PPD & 6PPD-Q Impacts - Canada/Federal and Beyond



- Canadian Federal Government evaluation of 6PPD & 6PPD-Q under CEPA (Canadian Environmental Protection Act).
 - April 30, 2024 request for evaluation granted.
 - Possible regulatory framework by June 2025??
- EU is moving towards (Dec 2023 policy outlook published) restricting 6PPD use in tires under the “REACH” regulation. (Registration, Evaluation, Authorization, and restriction of Chemicals).
- Expect water quality guidelines to reach other jurisdictions as additional sensitive species are identified and chronic effects studied.



Photo Courtesy of Rodney Hsu (Fishingwithrod.com)



Laboratory Analysis of 6PPD-quinone by LC-MS/MS

- Analysis by LC-MS/MS (Liquid Chromatography- Tandem Mass Spectrometry)
 - “LC triple quad” or “LC-QQQ”
- Implementation of LC-MS/MS in environmental labs exploding over past 5 years.
- Driven by many different emerging contaminants of concern.
 - PFAS!!! (Hot topic for another day)
- Many Instrument Manufacturers
 - Shimadzu, Agilent, Thermo, PerkinElmer, Waters, AB Sciex.
 - Instrumentation mature and well-established

ALS has an extensive network of LC-MS/MS globally and throughout Canada. Canadian capabilities are present in Waterloo, Vancouver and Calgary.



Laboratory Analysis by LC-MS/MS



Pros



Extremely sensitive: ppt to ppq level detection.

Extremely selective: Able to detect analyte of interest in complex mixtures.

Widespread use of Isotope Dilution methods which can correct for many matrix effects and extraction biases.

Cons



Very expensive (~\$750k CAD per instrument) Hardware.

Standards costly (\$\$), isotopically labelled (\$\$\$), Custom Synthesis (\$\$\$\$\$).

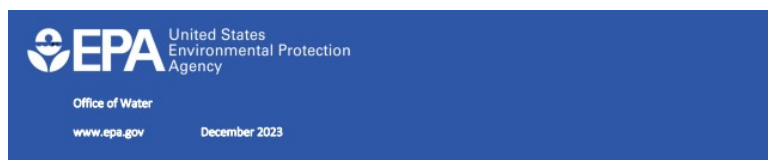
High complexity of analysis, high skill levels req'd.

Cost of laboratory analysis is very high!!!

EPA 1634: 6PPD-quinone by LC-MSMS

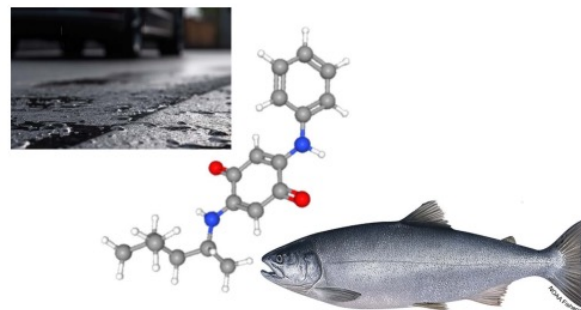


- Test Method Summary:
 - Reference Method: EPA 1634
 - Performance Based Method
 - Solid Phase Extraction (SPE) is used to clean-up and concentrate water samples.
 - Analysis by LC-MS/MS.
 - Uses 2 isotopically labelled internal standards (IS).
 - Extracted IS corrects for matrix / recovery / instrumental effects.
 - Non-extracted IS is added prior to analysis to monitor instrument performance.



DRAFT Method 1634

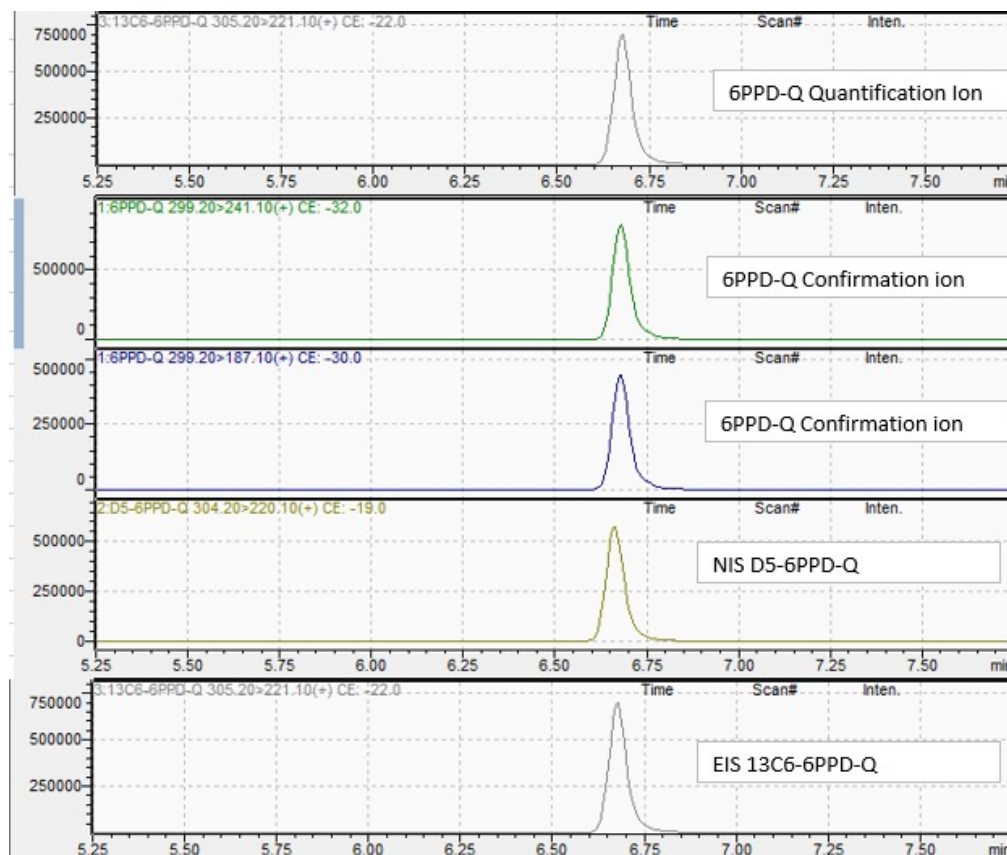
Determination of 6PPD-Quinone in Aqueous Matrices Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)



LC-MS/MS Chromatogram Example



6PPD-Q Mass Transitions	
Quantification Ion Mass	299.2 -> 241.1
Confirmation Ion Mass	299.2 -> 215.1
Confirmation Ion Mass	299.2 -> 187.1
EIS ¹³ C ₆ -6PPD-Q	305.2 -> 221.1
NIS D ₅ -6PPD-Q	304.2 -> 220.1





ALS Reporting Limits and Sampling Requirements

Currently 17025 accredited in ALS Waterloo (CALA).

CALA Accreditation submission occurring late Sept 2024 for ALS Vancouver.

ALS 6PPD Quinone Reporting Limits and Sampling Details.		
	<i>Routine</i>	<i>Trace</i>
Limit of Reporting (LOR)	0.002 µg/L	0.0002 µg/L
<i>Sampling Details</i>		
Test Method	LC-MSMS	
ALS Test Code	E744	E744-L
Sample Container	2x 100mL Amber glass teflon lined	
Preservation	<6°C	
Recommended Holding Time	14 days (EPA 1634)	



Future Outlook for 6PPD & 6PPD-Q??

- Regulatory landscape for 6PPD-Q is evolving rapidly.
- Environmental Testing for 6PPD?
 - Very unstable (half-life measured in hours)
 - ALS is currently performing stability studies with various preservatives.
- What about environmental impacts of other tire additives and potential alternatives??
 - Many other similar compounds.
 - 6PPD, 7PPD, DPPD, IPPD, CPPD, DTPD, DNPD and their quinone derivatives???
- Bioaccumulation / Chronic effects? Mammalian toxicity?
- What about testing in other matrices?
 - Soils and Tissues?
- Mitigation/treatment strategies for runoff?
- When will 6PPD be replaced as tire additive?



THANK YOU

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Questions?

