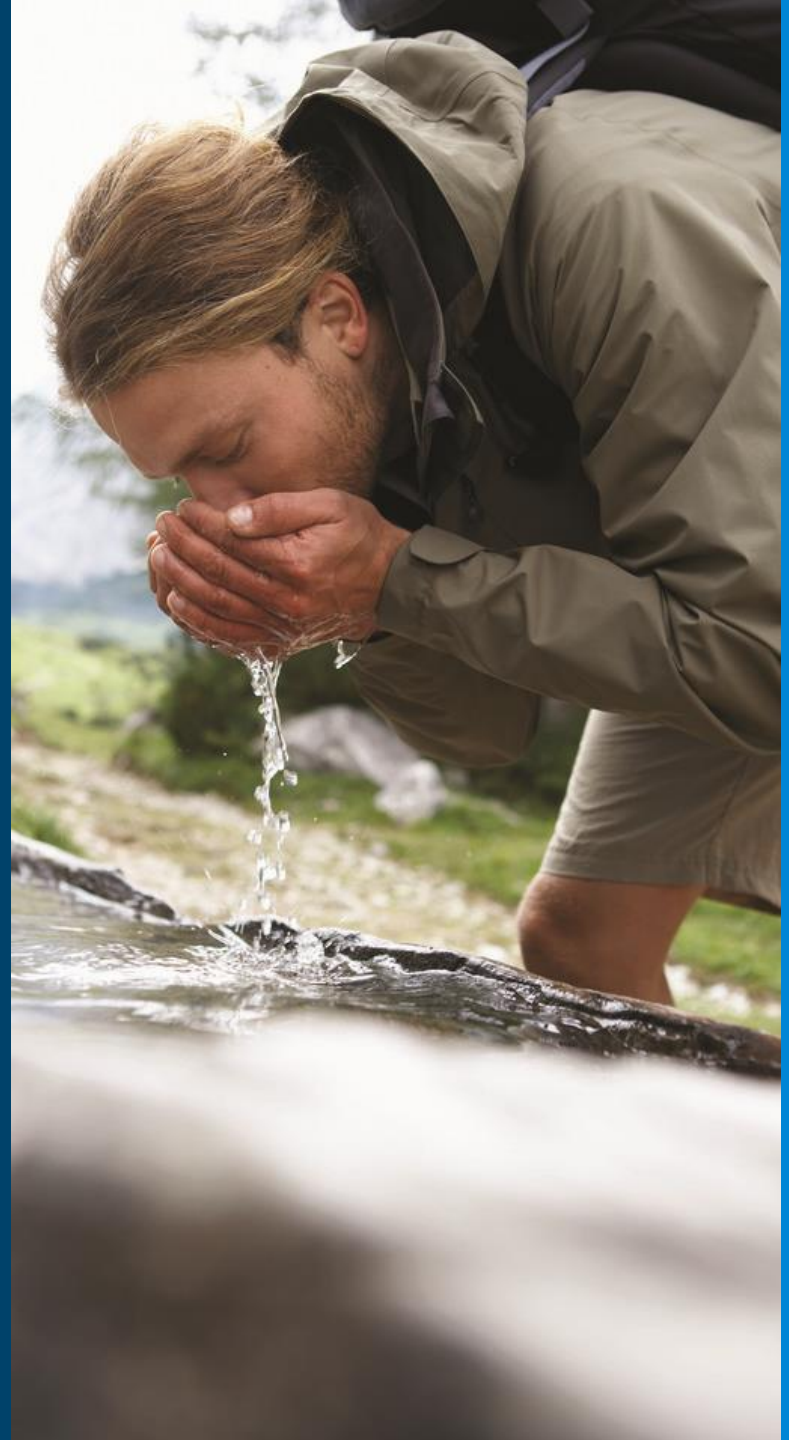
Is this the Next Emerging Contaminant? Quantification of Toxic Tire Degradant 6PPD-Quinone in Surface Waters

NEMC 2022

Tarun Anumol, Ph.D.
Director, Global Environment & Food Markets
Agilent Technologies Inc.



Agilent
Trusted Answers

Acute Toxicity to Coho Salmon observed in North-West US River Streams

Urban Runoff Mortality Syndrome (URMS) responsible for salmon deaths

RESEARCH ARTICLE

Recurrent Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams

Nathaniel L. Scholz, Mark S. Myers, Sarah G. McCarthy, Jana S. Labenia, Jenifer K. McIntyre, Gina M. Ylitalo, Linda D. Rhodes, Cathy A. Laetz, Carla M. Stehr, Barbara L. French, Bill McMillan, Dean Wilson, Laura Reed, [...], Tracy K. Collier

Creek	Year	N*	% Wild**	% Pre-Spawn Mortality Wild	% Pre-Spawn Mortality Total
Longfellow	2002	57	4	100	86
	2003	18	28	20	67
	2004	9	89	88	89
	2005	75	72	72	72
	2006	4	75	100	100
	2007	41	10	75	73
	2008	12	0	n.a	67
	2009	44	0	n.a.	79
Piper's	2006	9	78	100	100
Des Moines	2004	30	33	60	63
Fortson (non-urban)	2002	114	100	0.9	0.9

*Sample size reflects female coho of known spawning condition, with no signs of predation.
**Presumed wild origin based on presence of adipose fin and absence of a coded wire tag.
doi:10.1371/journal.pone.0028013.t002

Urban stormwater runoff responsible for coho salmon deaths.

Acute toxicity and high mortality shown usually within 2-24 hours.

Effect observed since 2002.

Causant not a pathogen or regulated chemicals.

Scholz NL, Myers MS, McCarthy SG, et al. Recurrent die-offs of adult coho salmon returning to spawn in Puget Sound lowland urban streams. PLoS One. 2011;6(12):e28013. doi:10.1371/journal.pone.0028013

Chemical causant of Coho Mortality Identified

6PPD-Quinone, ozonation byproduct of Tire degradant (6PPD) responsible

RESEARCH

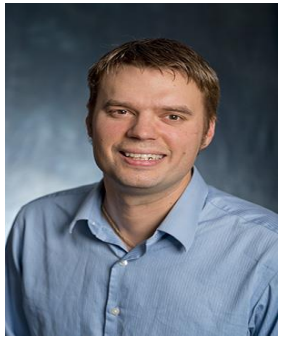
ECOTOXICOLOGY

A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon

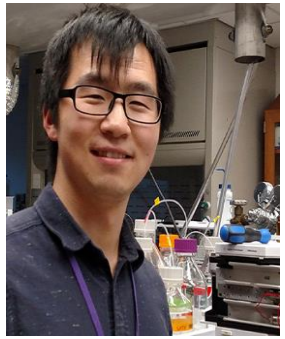
Zhenyu Tian[1,2], Haoqi Zhao[3], Katherine T. Peter[1,2], Melissa Gonzalez[1,2], Jill Wetzel[4], Christopher Wu[1,2], Ximin Hu[3], Jasmine Prat[4], Emma Mudrock[4], Rachel Hettinger[1,2], Allan E. Cortina[1,2], Rajshree Ghosh Biswas[5], Flávio Vinicius Crizóstomo Kock[5], Ronald Soong[5], Amy Jenne[5], Bowen Du[6], Fan Hou[3], Huan He[3], Rachel Lundeen[1,2], Alicia Gilbreath[7], Rebecca Sutton[7], Nathaniel L. Scholz[8], Jay W. Davis[9], Michael C. Dodd[3], Andre Simpson[5], Jenifer K. McIntyre[4], Edward P. Kolodziej[1,2,3]*

In U.S. Pacific Northwest coho salmon (*Oncorhynchus kisutch*), stormwater exposure annually causes unexplained acute mortality when adult salmon migrate to urban creeks to reproduce. By investigating this phenomenon, we identified a highly toxic quinone transformation product of *N*-(1,3-dimethylbutyl)-*N*′-phenyl-p-phenylenediamine (6PPD), a globally ubiquitous tire rubber antioxidant. Retrospective analysis of representative roadway runoff and stormwater-affected creeks of the U.S. West Coast indicated widespread occurrence of 6PPD-quinone (<0.3 to 19 micrograms per liter) at toxic concentrations (median lethal concentration of 0.8 ± 0.16 micrograms per liter). These results reveal unanticipated risks of 6PPD antioxidants to an aquatic species and imply toxicological relevance for dissipated tire rubber residues.

(Tian et al., 2021, Science)



Edward Kolodziej



Zhenyu Tian

LOTS OF HARDWORK !!!

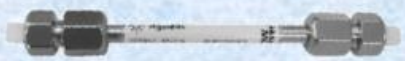
Analytical Methods and HRMS Workflow

Sample Processing:

Water samples

Oasis HLB + micro glass beads

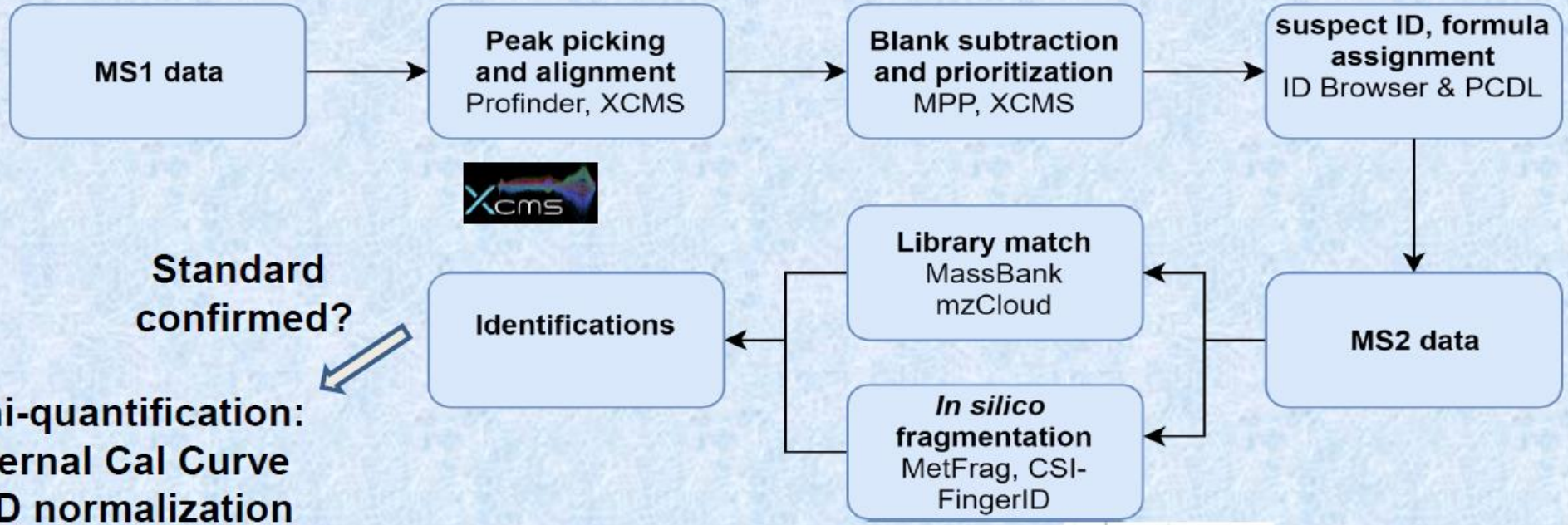
(or solvent extracts)



Agilent Zorbax Eclipse Plus RRHD 1.8 µm

Analyze on UHPLC-qToF-HRMS
Agilent 1290 UHPLC
Agilent 6530 QTOF (ESI +/-)

Data Analysis:



CENTER FOR URBAN WATERS

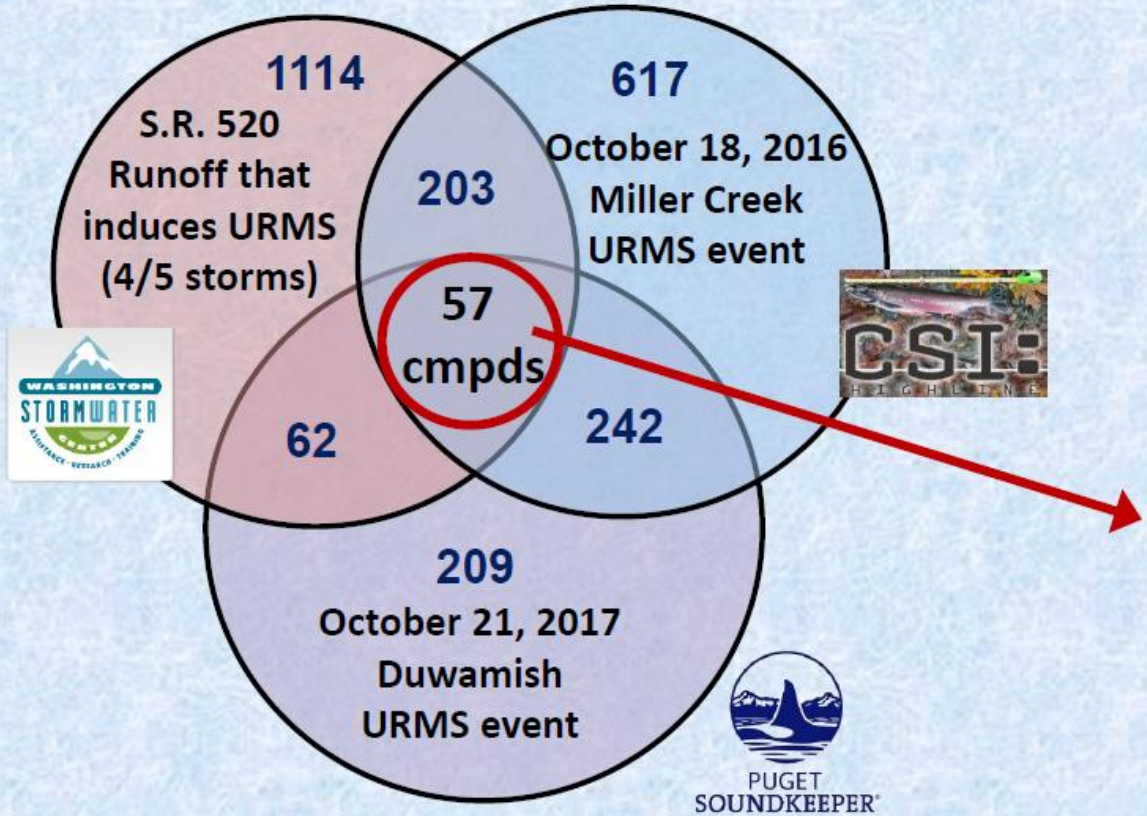
Slide Credit: Edward Kolodziej

MassBank m/z CLOUD MetFrag

(Peter et al. 2018)
(Tian et al. 2021)

1) "Toxicant Signature" of Coho Mortality

Concept: Find a "toxicant signature" in all waters where coho died. What chemicals were always present?



Can compare "new" waters to the waters that we know induced URMS

-Track chemical sources in creeks
-Track signature in treatment systems
-Provide targets for identification

Blue = # chemicals detected

CENTER FOR URBAN WATERS

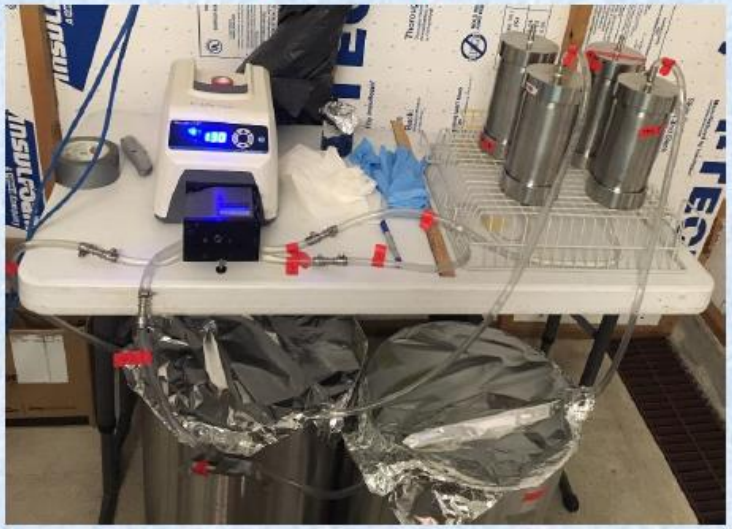
Slide Credit: Edward Kolodziej

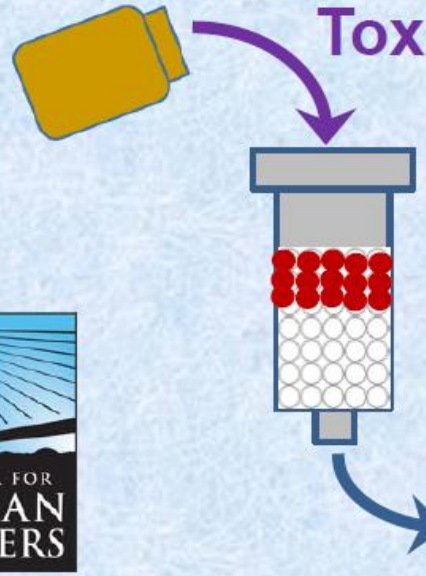
(Peter et al. 2018 ES&T)

3) TIE/EDA Identify Toxicant(s) in Tire Rubbers

Leach tire particles into water, then fractionate & expose juvenile coho





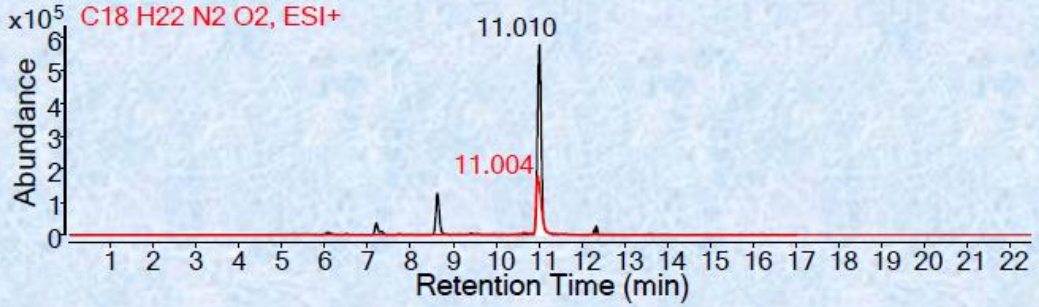
5 juvenile coho in 15-30 L, 24 h

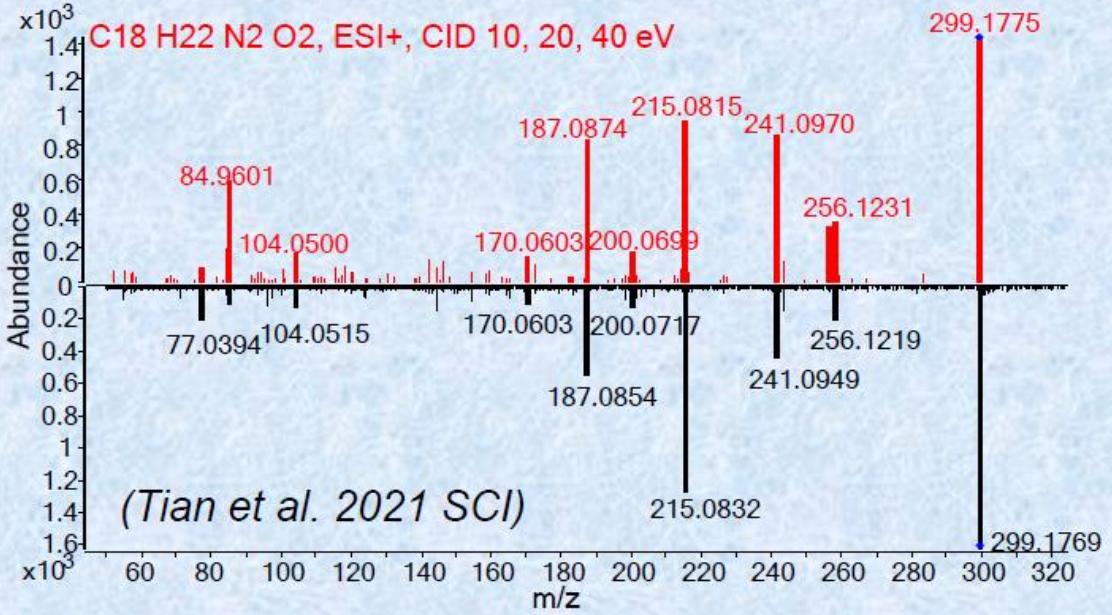
CENTER FOR URBAN WATERS

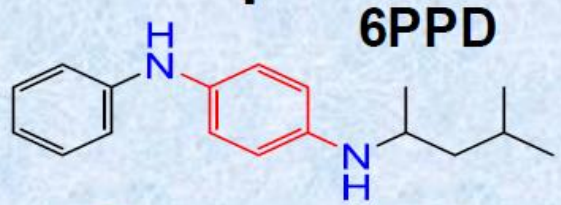
3) Purified $C_{18}H_{22}N_2O_2$ from TWP Leachate and Ozonation

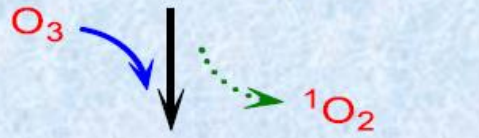
-Andre Simpson, U. Toronto NMR Analysis:
Identical structures, O_3 synthesized ~98% pure

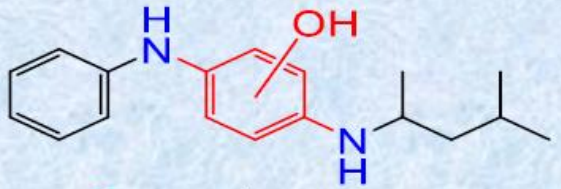
Tire leachate | 6PPD ozonation

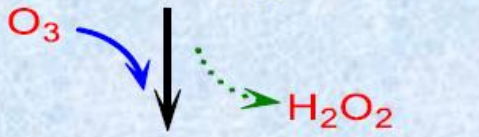


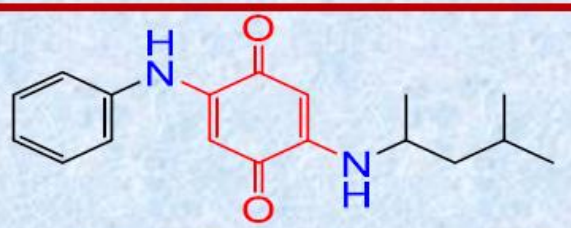












"6PPD-quinone"

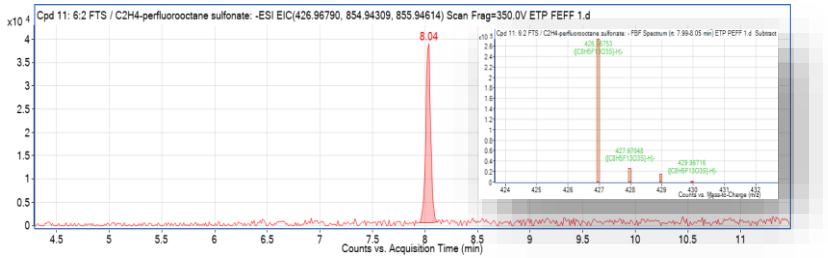
CENTER FOR URBAN WATERS

Identifying new contaminants can be very time consuming but fruitful

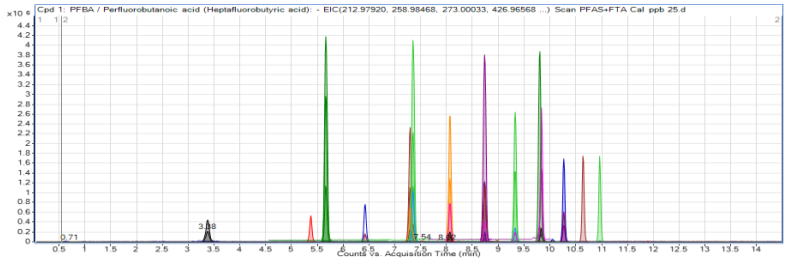
The larger monitoring community needs to take this and deliver targeted methods

Known knowns
Expanded targeted list

Known unknowns
Expanded database of contaminants



Continually refining targeted method



GOAL: Develop a targeted, fast, reliable, robust, transferable method for routine monitoring

Unknown unknowns
Identifying new compounds to add to database

Analytes of Interest

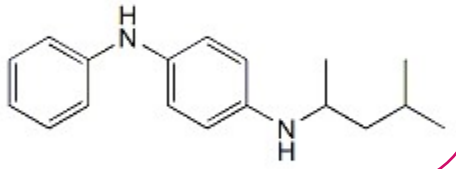
Optimized mass transitions & compound parameters

6PPD

MW: 268.2

Product Ions:

- 184.1
- 185.1
- 107.0



Rapidly degrades in water.

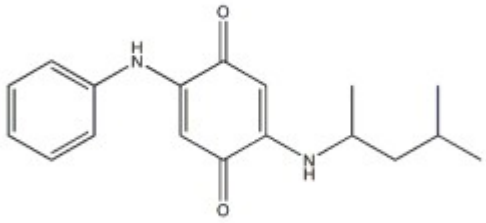
Poor chromatographic performance due to the presence of multiple charge states in solution

6PPD-quinone

MW: 298.2

Product Ions:

- 215.1
- 187.1
- 241.1

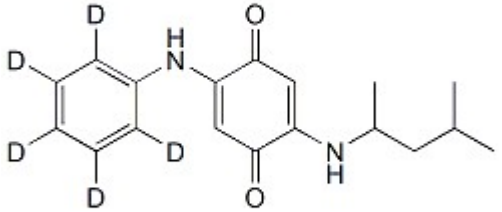


D_5-6PPD-quinone

MW: 303.2

Product Ions:

- 220.1
- 192.1
- 246.1



Compound Name	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Ret. Time (min)	Fragmentor (V)	Collision Energy (V)	Cell Accelerator Voltage	Polarity
6PPD-Quinone	299.2	241.1	3.52	105	32	4	Positive
6PPD-Quinone	299.2	215.1	3.52	105	16	5	Positive
6PPD-Quinone	299.2	187.1	3.52	105	32	5	Positive
D_5-6PPD-Quinone	304.2	246.1	3.49	110	36	4	Positive
D_5-6PPD-Quinone	304.2	220.1	3.49	110	20	4	Positive
D_5-6PPD-Quinone	304.2	192.1	3.49	110	36	5	Positive

Develop a fast, reliable LC/TQ method for analysis of 6PPD-Quinone in Water

LC/MS Optimized Conditions

Parameter	Value
LC	Agilent 1290 Infinity II LC: Agilent 1290 Infinity II multisampler (G7167B), Agilent 1290 Infinity II high-speed pump (G7120A), and Agilent 1290 Infinity II multicolumn thermostat (G7116B)
Analytical Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 x 50 mm, 1.9 µm (p/n 699675-902)
Column Temperature	40 °C
Injection Volume	5 µL
Mobile Phase	A) 1 mM ammonium fluoride in water (HPLC grade) B) acetonitrile (HPLC grade)
Flow Rate	0.6 mL/min
Gradient	Time (min) % B 0.0 30 0.5 30 4.7 70 4.8 100
Stop Time	5.7 min
Post Time	1.5 min

Total run time: 7.2 min

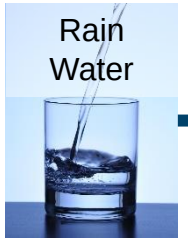
Compound transitions and Mass Spectrometer Parameters optimized automatically through MassHunter Optimizer

Agilent 1290 Infinity II LC +
6470 Triple quadrupole LC/MS

Parameter	Value
MS	Agilent 6470 triple quadrupole LC/MS with Agilent Jet Stream ESI source (G6470B)
Source Parameters	
Gas Temperature	300 °C
Gas Flow	10 L/min
Nebulizer	40 psi
Sheath Gas Temperature	375 °C
Sheath Gas Flow	11 L/min
Capillary Voltage (Neg)	2,500 V
Nozzle Voltage (Neg)	0 V
Delta EMV (+)	400

Sample Preparation

Goal was to develop simple protocol that would lead to fast turnaround



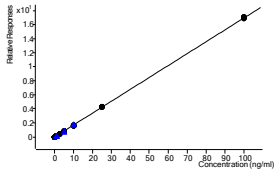
5-10 mL Sample in PP vials

Centrifugation for 5 min @ 13,000 rpm

+ SUR

Aliquot 1 mL sample into LC/MS vials

Calibration standards (CAL) in 100% H2O



LC/TQ

Upto 12 samples could be prepared for analysis on LC/MS in Approximately 10 min

Method Performance & Validation

Abundance & Retention Time Repeatability

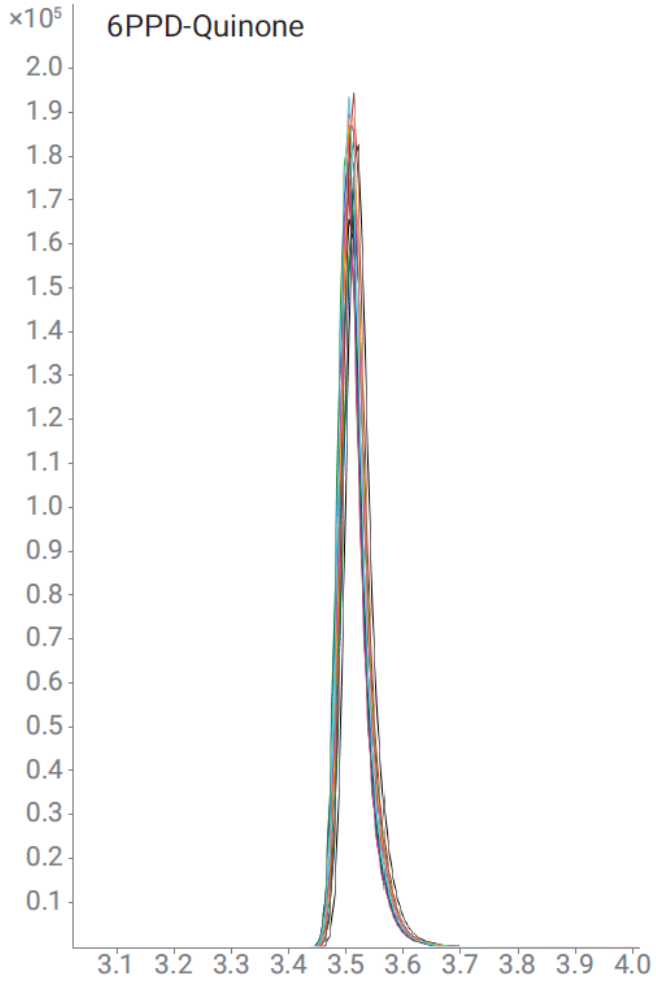
Stability test performed uninterrupted over 3 days.

To demonstrate repeatability & stability of analysis, a mid-level calibrator (3.1 ng/mL) was injected over the course of 67 hours interspersed with 172 samples & QC injections.

The figure shows an overlay of 17 injections

Abundance RSD: <4%
RT RSD: <0.1%

A calibration range of 0.02 – 50 µg/L in water was created. All calibration curves during the study had R^2>0.99 with a minimum of six points

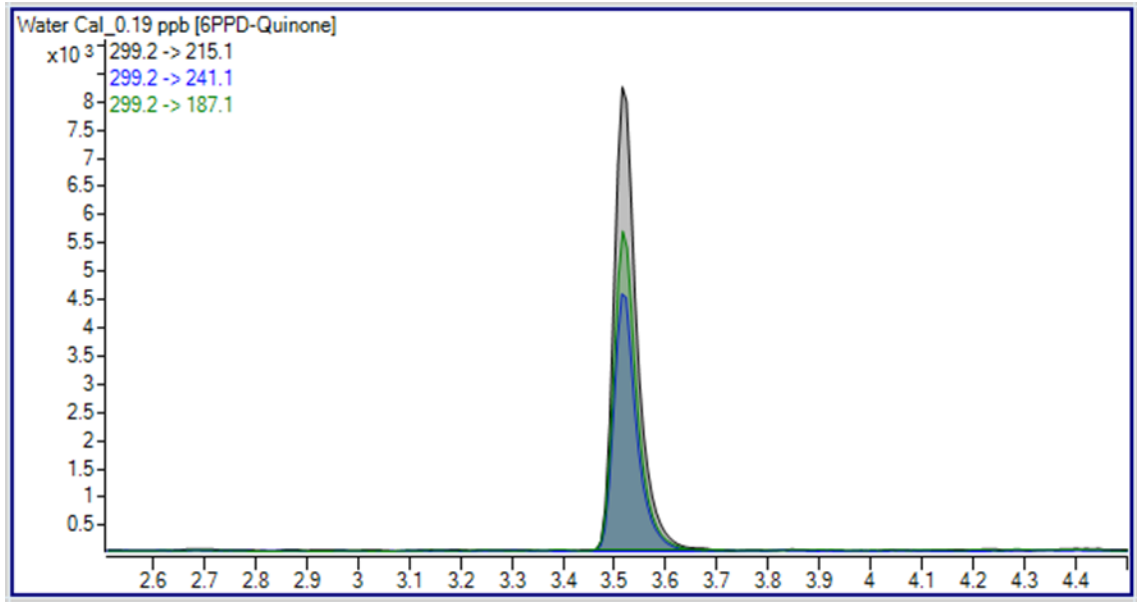


Precision, Accuracy & Recovery

Recovery (%) was assessed by spiking five replicates at 5 µg/L in HPLC Grade & Surface Water

Precision (%RSD) was assessed by spiking five replicates at 0.2 µg/L in HPLC Grade & Surface Water

6PPD-Quinone	Recovery	%RSD
HPLC Water	113.5	3%
Stream (Surface) Water	112.6	1%



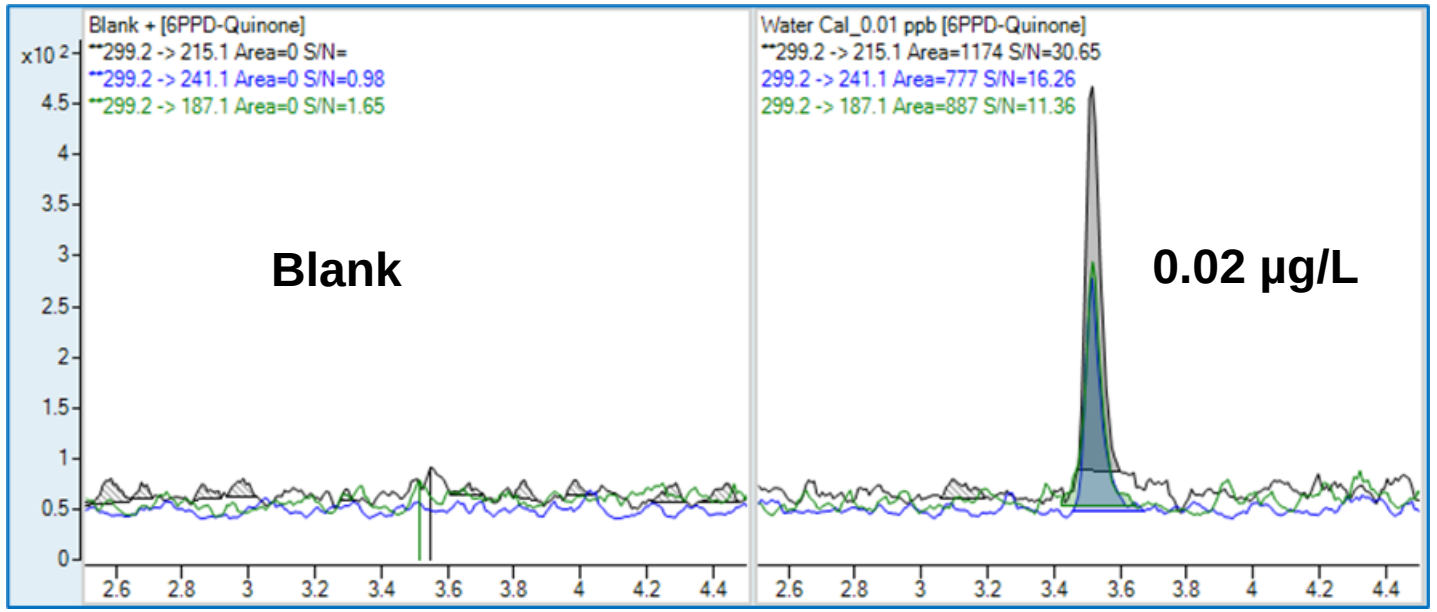
6PPD-quinone Chromatography at 0.2 ng/mL

Blank & Lowest Concentration Method Reporting Limit (LCMRL)

No blank interference observed and LCMRL >20 times lower than LD50

USEPA LCMRL (Lowest Concentration Minimum Reporting Level)

- lowest concentration for which the future recovery is predicted to fall between 50% and 150% with high confidence (99%)
- replicate spikes in HPLC grade water
- 4 replicates at each of 7 concentrations
- Spiking range from 0.02 ng/mL to 3 ng/mL



Calculated LCMRL for 6PPD-Quinine

0.023 µg/L

Median LD_{50}: 0.8 µg/L

Holding Time Study

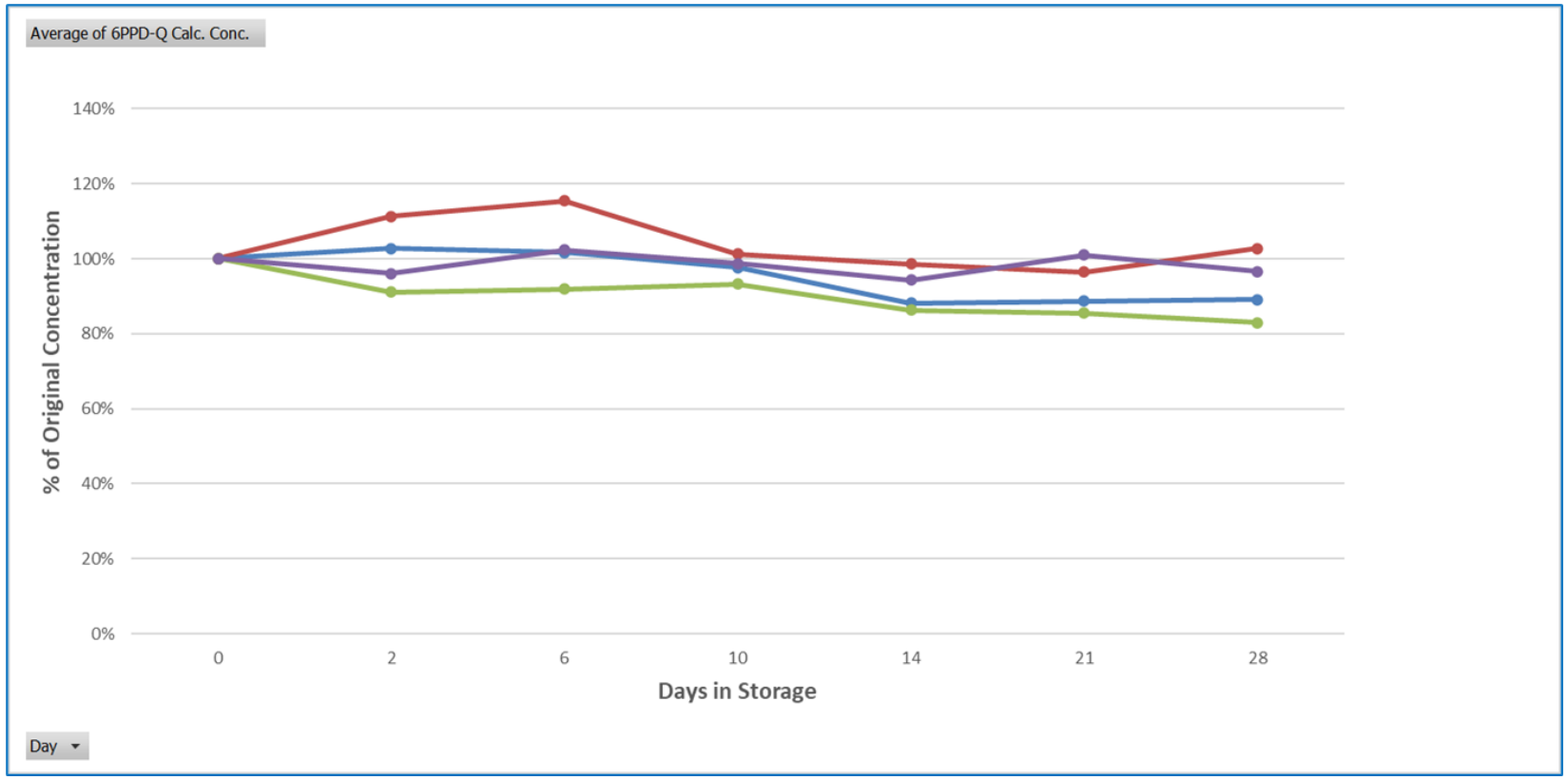
Samples with 6PPD-Quinone stable for upto 28 days when stored cold

4 sets of trials were done

Storage in Glass vials (4ºC)

Storage in Glass vials (-20ºC)
Storage in PP vials (4ºC)
Storage in PP vials (-20ºC)

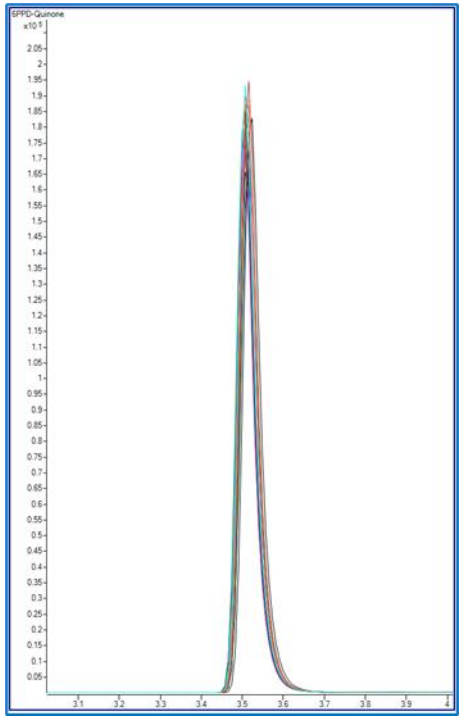
Concentrations of 6PPD-Quinone were within 80-120% at all data points collected.

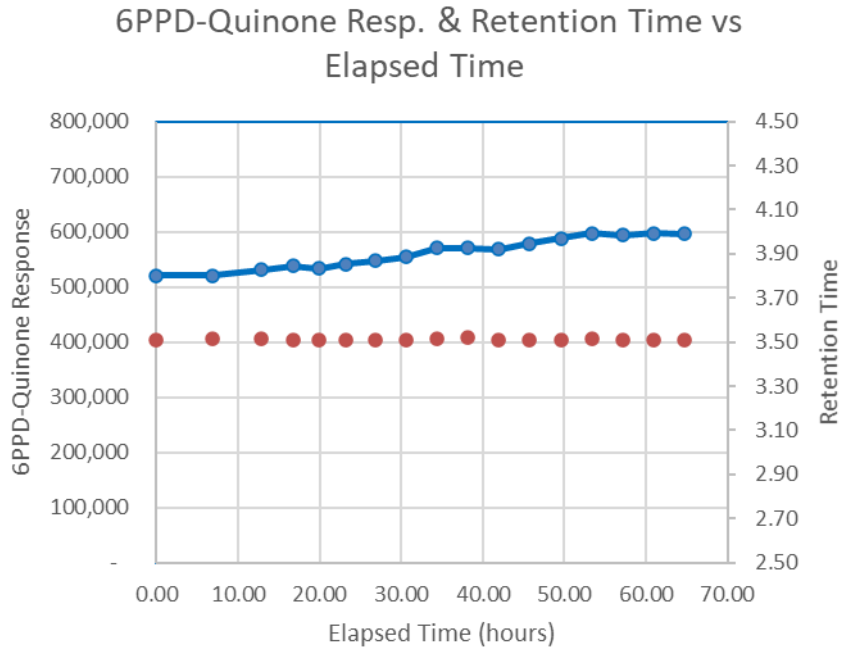


Stability in the Sample

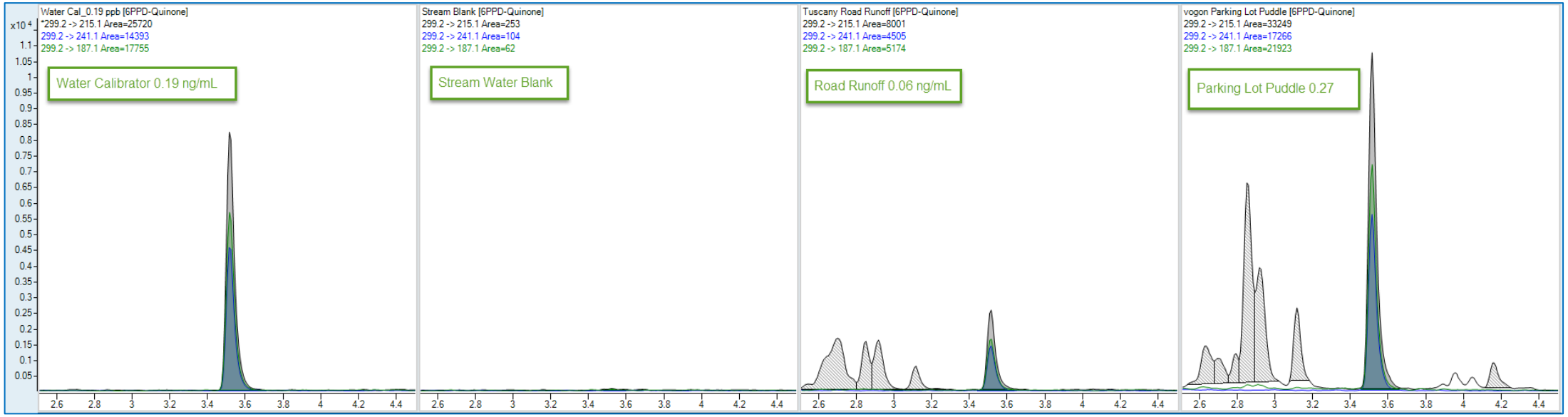
CAUTION: 6PPD in your sample can degrade to 6PPD-Quinone!

- A slight increase in response was noted (blue line) upto 3 days that is likely due to degradation of 6PPD that may have been in the actual sample.
- This issue will not be seen in standards as long as 6PPD is not added into the standard separately.





Surface Water & Stormwater Sample Analysis

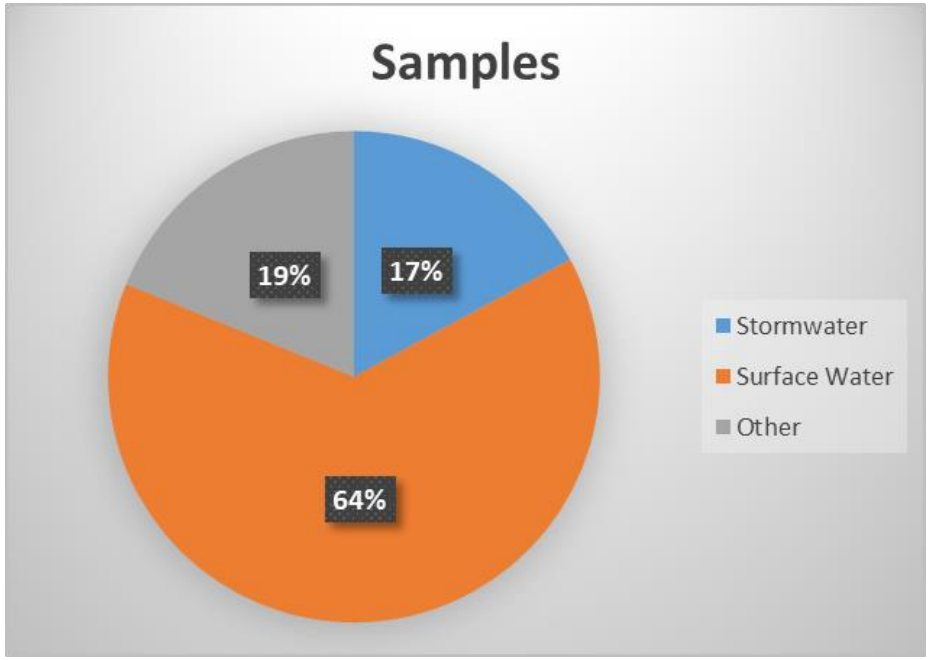


The highest detection for a stormwater runoff sample collected from a residential street during a rain event tested positive for 6PPD-quinone at 0.2 µg/L

The sample collected from a residual puddle in a parking lot tested positive for 6PPD-quinone at 0.27 µg/L

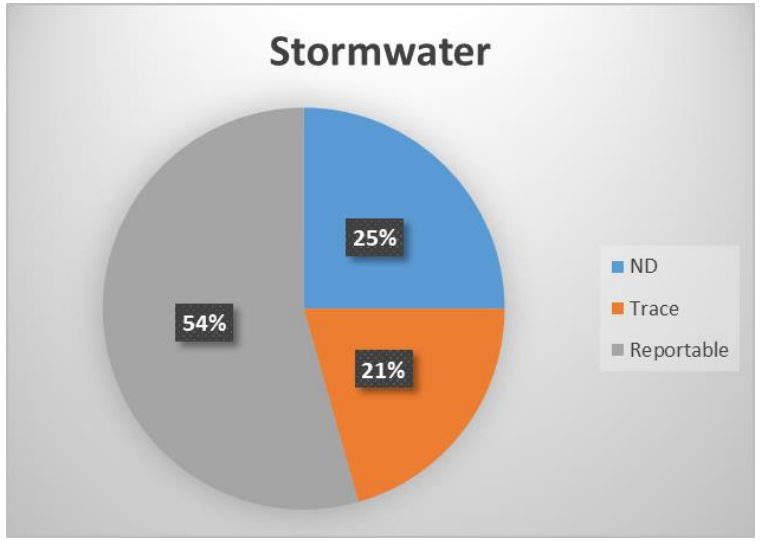
Detection by Water Type

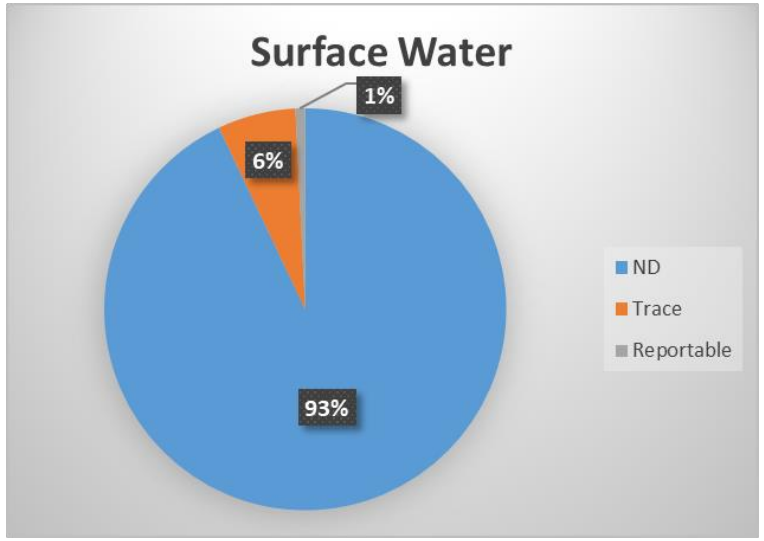
Samples in Southern Canada; all detections lower than median LD50



Sample Type	Samples	ND	Trace	Reportable
Stormwater	68	17	14	37
Surface Water	255	237	16	2
Other	75	74	1	-
Total	398	328	31	39

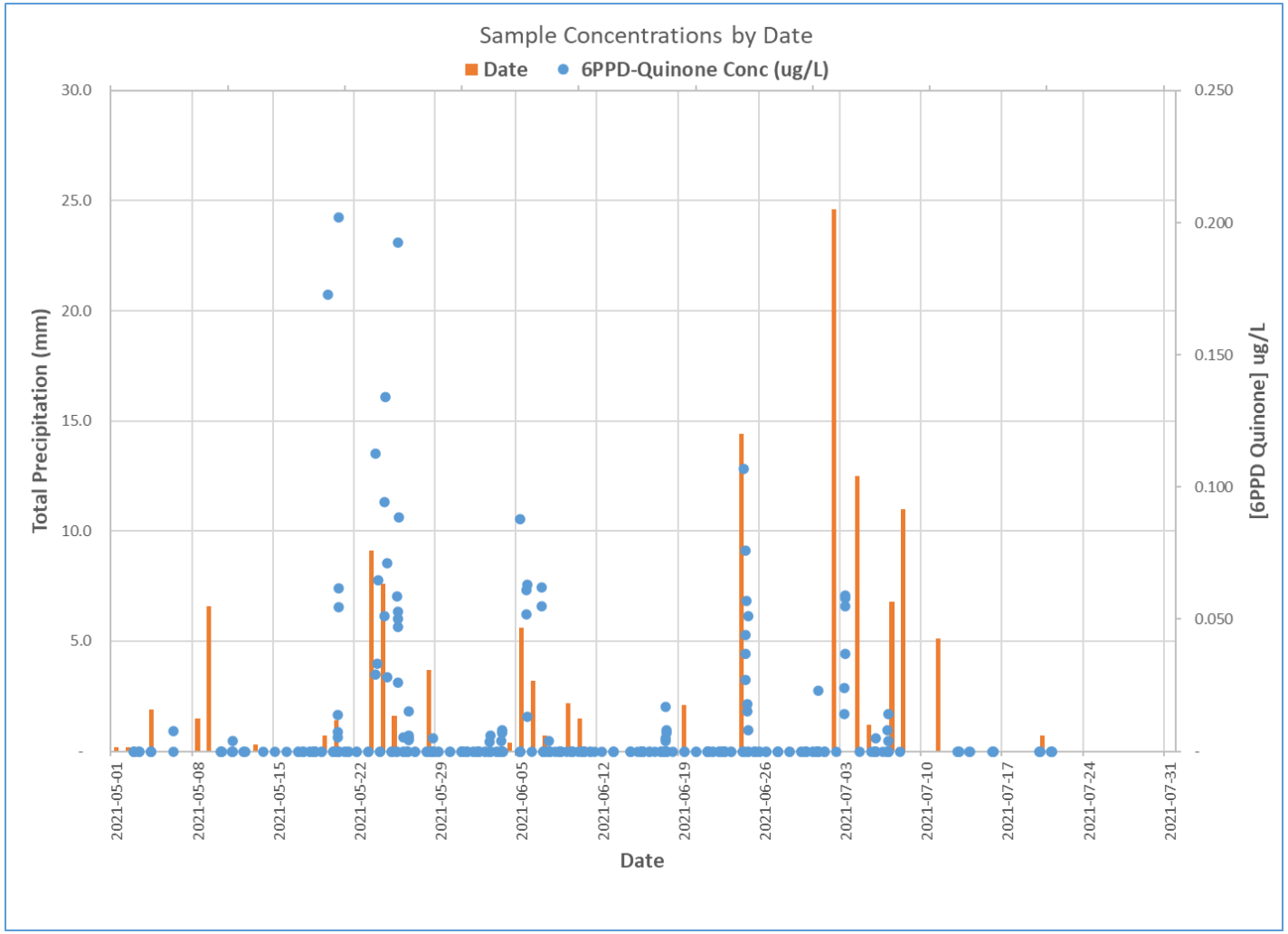
Trace 0.004 – 0.02 µg/L
Reportable >0.02 µg/L
Median Lethal Concentration 0.8 µg/L





Is precipitation an indicator of higher concentration?

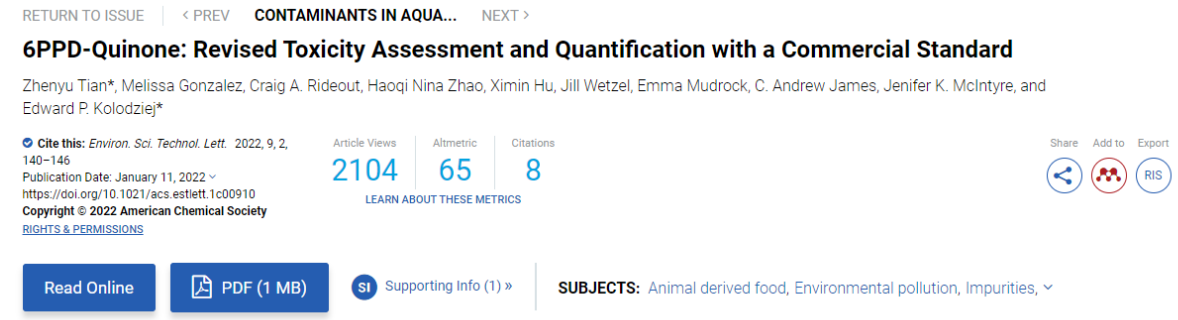
Maybe. In some instances, higher conc. Of 6PPD-Quinone were observed but not always indicating additional sources too.



HOT OFF THE PRESSES

LD_{50} for 6PPD-Quinone is much lower than previously calculated

- New LD_{50} for 6PPD-Quinone is expected to be 95 ng/L
- ~8 times lower than previously reported.
- Re-evaluating our samples, **8% of surface water samples measured in this study would be higher than new LD_{50}**



RETURN TO ISSUE | < PREV CONTAMINANTS IN AQUA... NEXT >

6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard

Zhenyu Tian*, Melissa Gonzalez, Craig A. Rideout, Haoqi Nina Zhao, Ximin Hu, Jill Wetzel, Emma Mudrock, C. Andrew James, Jenifer K. McIntyre, and Edward P. Kolodziej*

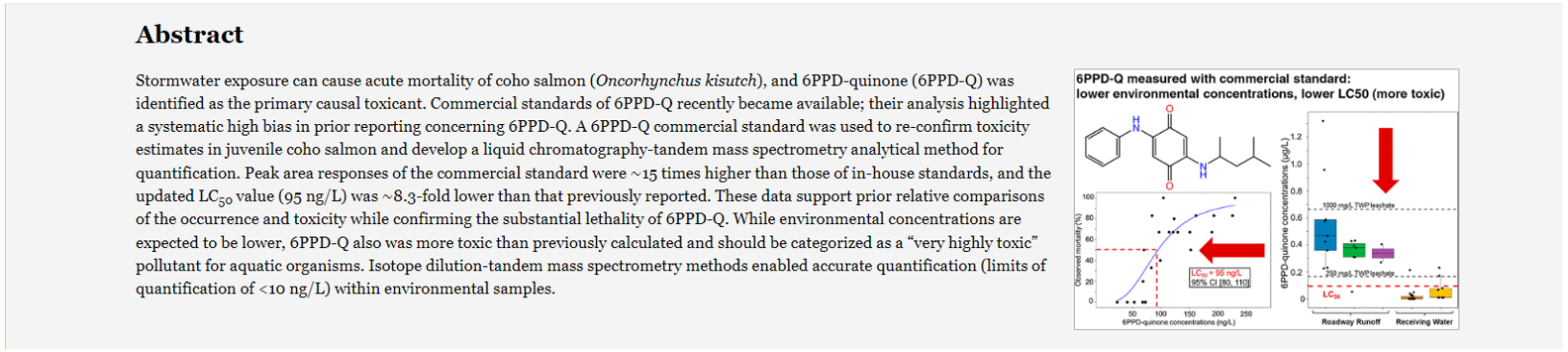
Cite this: *Environ. Sci. Technol. Lett.* 2022, 9, 2, 140−146
Publication Date: January 11, 2022
https://doi.org/10.1021/acs.estlett.1c00910
Copyright © 2022 American Chemical Society
RIGHTS & PERMISSIONS

Article Views 2104 | Altmetric 65 | Citations 8
LEARN ABOUT THESE METRICS

Share | Add to | Export RIS

Read Online | PDF (1 MB) | Supporting Info (1) » | SUBJECTS: Animal derived food, Environmental pollution, Impurities,

Environmental Science & Technology Letters



Abstract

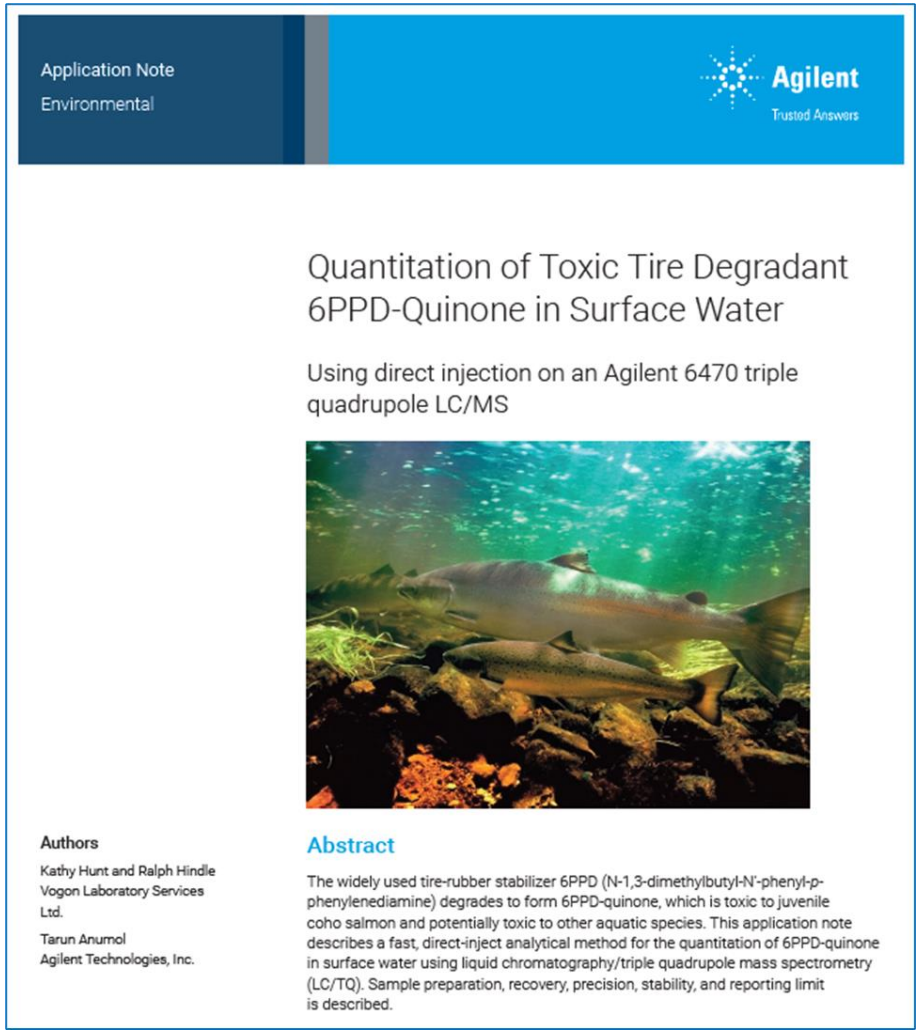
Stormwater exposure can cause acute mortality of coho salmon (*Oncorhynchus kisutch*), and 6PPD-quinone (6PPD-Q) was identified as the primary causal toxicant. Commercial standards of 6PPD-Q recently became available; their analysis highlighted a systematic high bias in prior reporting concerning 6PPD-Q. A 6PPD-Q commercial standard was used to re-confirm toxicity estimates in juvenile coho salmon and develop a liquid chromatography-tandem mass spectrometry analytical method for quantification. Peak area responses of the commercial standard were ~15 times higher than those of in-house standards, and the updated LC_{50} value (95 ng/L) was ~8.3-fold lower than that previously reported. These data support prior relative comparisons of the occurrence and toxicity while confirming the substantial lethality of 6PPD-Q. While environmental concentrations are expected to be lower, 6PPD-Q also was more toxic than previously calculated and should be categorized as a "very highly toxic" pollutant for aquatic organisms. Isotope dilution-tandem mass spectrometry methods enabled accurate quantification (limits of quantification of <10 ng/L) within environmental samples.

6PPD-Quinone: Revised Toxicity Assessment and Quantification with a Commercial Standard
Zhenyu Tian, Melissa Gonzalez, Craig A. Rideout, Haoqi Nina Zhao, Ximin Hu, Jill Wetzel, Emma Mudrock, C. Andrew James, Jenifer K. McIntyre, and Edward P. Kolodziej; Environmental Science & Technology Letters 2022 9 (2), 140-146; DOI: 10.1021/acs.estlett.1c00910

Conclusions

Agilent Application: 5994-3754EN

- A Fast method for the analysis of 6PPD-quinone in water was created and optimized on LC/MS/MS with minimal sample preparation.
- LCMRL of 0.023 ug/L for 6PPD-Quinone.
- Stability, Precision, Recovery, Accuracy & Sensitivity were within acceptable limits.
- 75% of stormwater samples analyzed had some level of 6PPD-Quinone but were lower than median LD_{50} of 0.8 ug/L.
- 8% of samples would be higher than new LD_{50} of 95 ng/L though.


Application Note
Environmental

Agilent
Trusted Answers

Quantitation of Toxic Tire Degradant 6PPD-Quinone in Surface Water

Using direct injection on an Agilent 6470 triple quadrupole LC/MS

Authors

Kathy Hunt and Ralph Hindle
Vogon Laboratory Services Ltd.

Tarun Anumol
Agilent Technologies, Inc.

Abstract

The widely used tire-rubber stabilizer 6PPD (N-1,3-dimethylbutyl-N′-phenyl-*p*-phenylenediamine) degrades to form 6PPD-quinone, which is toxic to juvenile coho salmon and potentially toxic to other aquatic species. This application note describes a fast, direct-inject analytical method for the quantitation of 6PPD-quinone in surface water using liquid chromatography/triple quadrupole mass spectrometry (LC/TQ). Sample preparation, recovery, precision, stability, and reporting limit is described.

Acknowledgements

Ralph Hindle, Vogon Laboratory Services
Kathy Hunt, Vogon Laboratory Services
Edward Kolodziej, University of Washington
Zhenyu Tian, Northeastern University

Contact: tarun.anumol@agilent.com