



Environment Testing
America

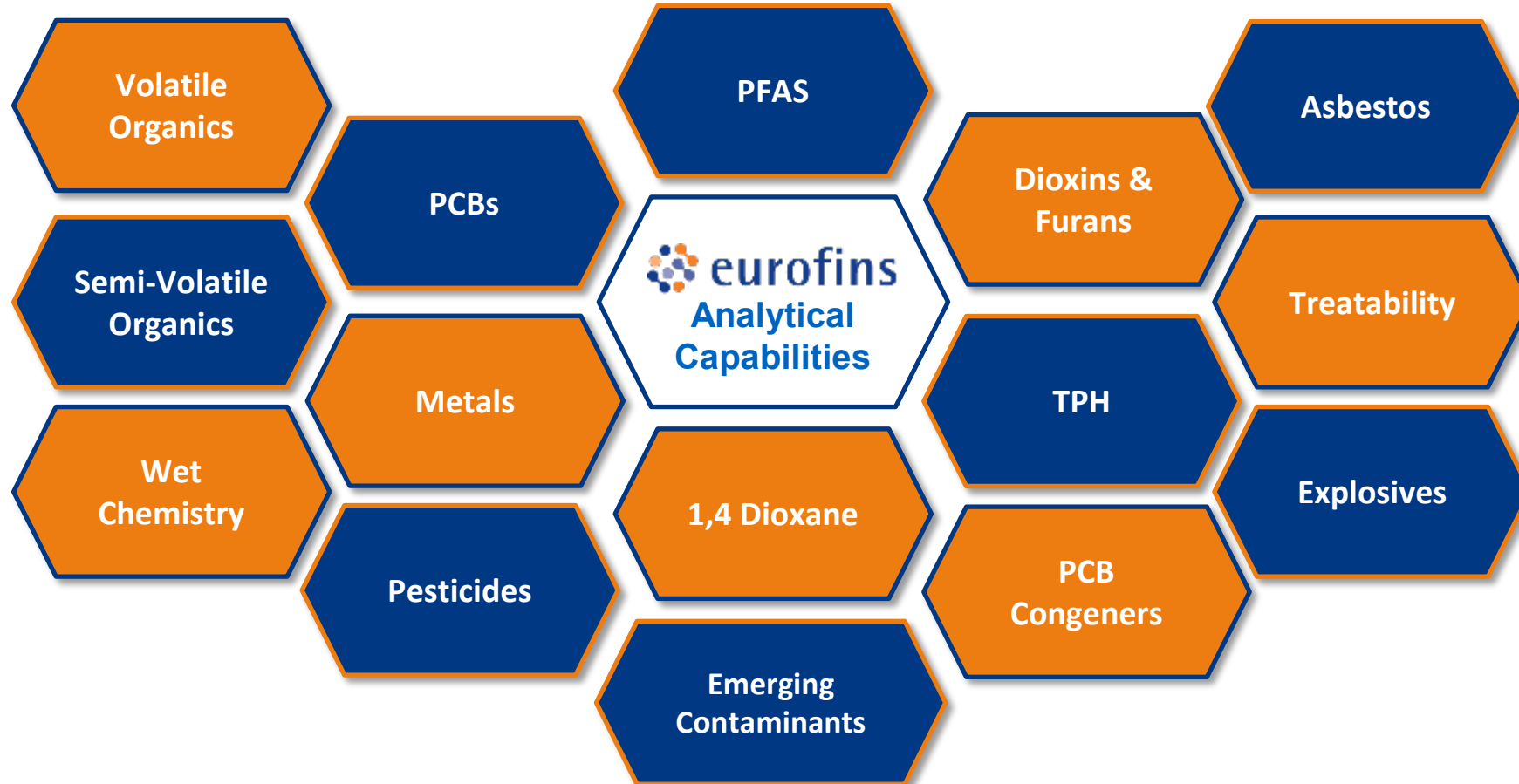
Specialty Services:

Method Development of 6PPD-quinone Analysis



Andrew Patterson,
Technical Director
**Eurofins Environment
Testing America**

Environment Testing Capabilities



Why Are We Here?



Science

REPORTS

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10.1126/science.abd6951 (2020).

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

Zhenyu Tian^{1,2}, Haoqi Zhao³, Katherine T. Peter^{1,2}, Melissa Gonzalez^{1,2}, Jill Wetzel⁴, Christopher Wu^{1,2}, Ximin Hu³, Jasmine Prat⁴, Emma Mudrock⁴, Rachel Hettinger^{1,2}, Allan E. Cortina^{1,2}, Rajshree Ghosh Biswas⁵, Flávio Vinicius Crizóstomo Kock⁵, Ronald Soong⁵, Amy Jenne⁵, Bowen Du⁶, Fan Hou³, Huan He³, Rachel Lundeen^{1,2}, Alicia Gilbreath⁷, Rebecca Sutton⁷, Nathaniel L. Scholz⁸, Jay W. Davis⁹, Michael C. Dodd³, Andre Simpson⁵, Jenifer K. McIntyre⁴, Edward P. Kolodziej^{1,2,3*}

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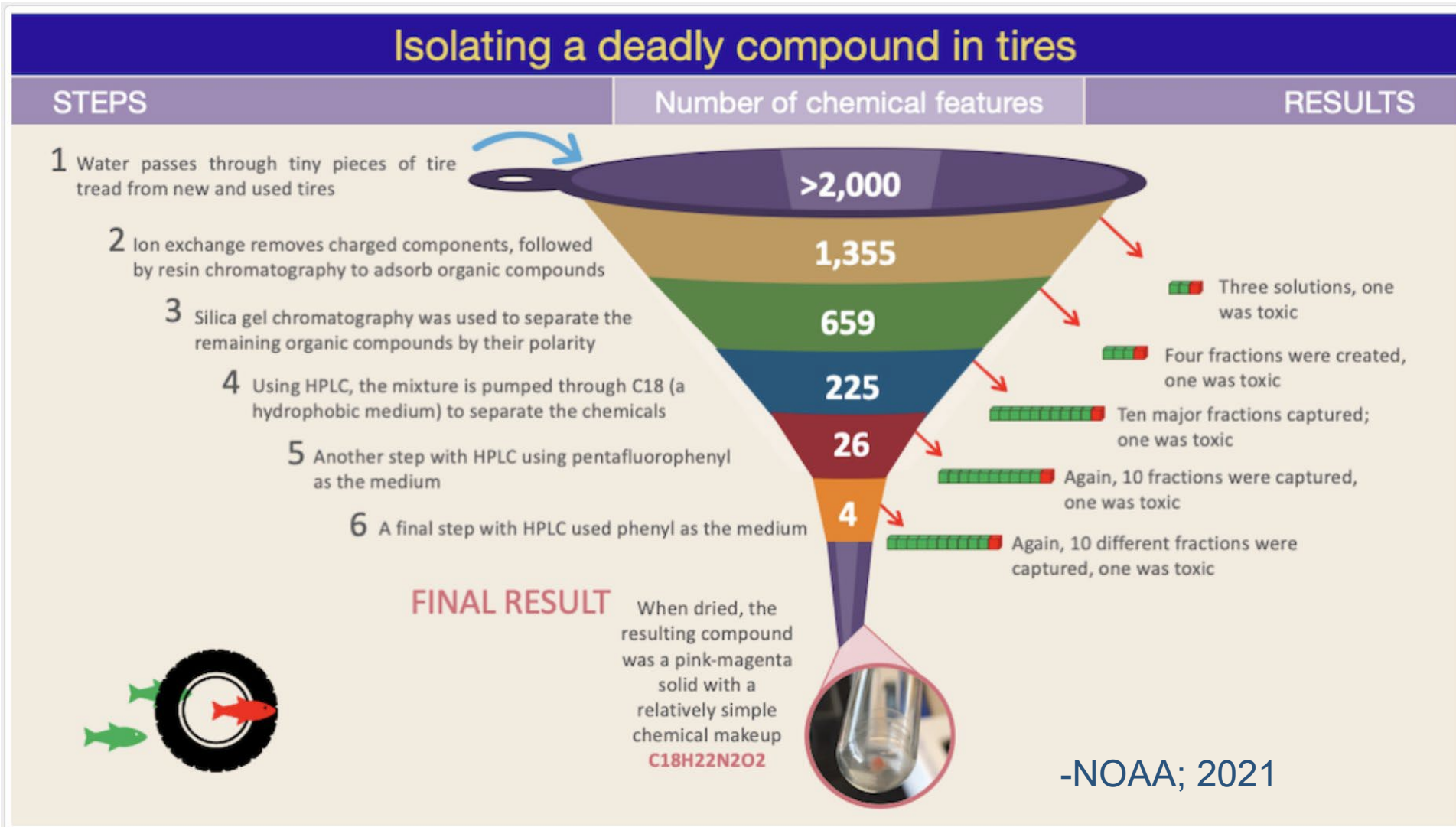
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In U.S. Pacific Northwest coho salmon (*Oncorhynchus kisutch*), stormwater exposure annually causes unexplained acute mortality when adult salmon migrate to urban creeks to reproduce. By investigating this phenomenon, we identified a highly toxic quinone 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-impacted creeks of the U.S. West Coast indicated widespread occurrence of 6PPD-quinone (<0.3-19 µg/L) at toxic concentrations (LC₅₀ of 0.8 ± 0.16 µg/L). These results reveal unanticipated risks of 6PPD antioxidants to an aquatic species and imply toxicological relevance for dissipated tire rubber residues.



Tire Sleuthing

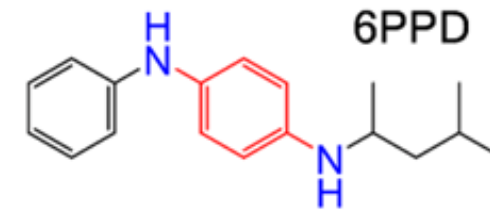




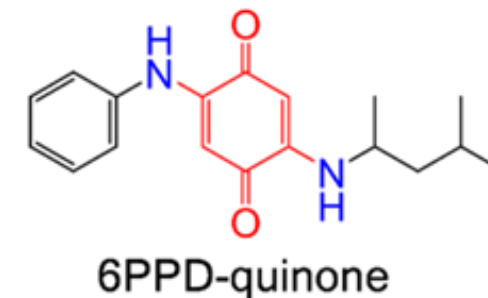
6PPD ≠ 6PPD-Q



- PPD compounds added to tires to achieve safety standards
- 6PPD and other PPDs are added to tire components during manufacturing up to percent level composition and can vary by tire component
- 6PPD-quinone is not added to tires and only forms in the process of protecting the tire



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Message From The Top: We Will Support 6PPD-Q



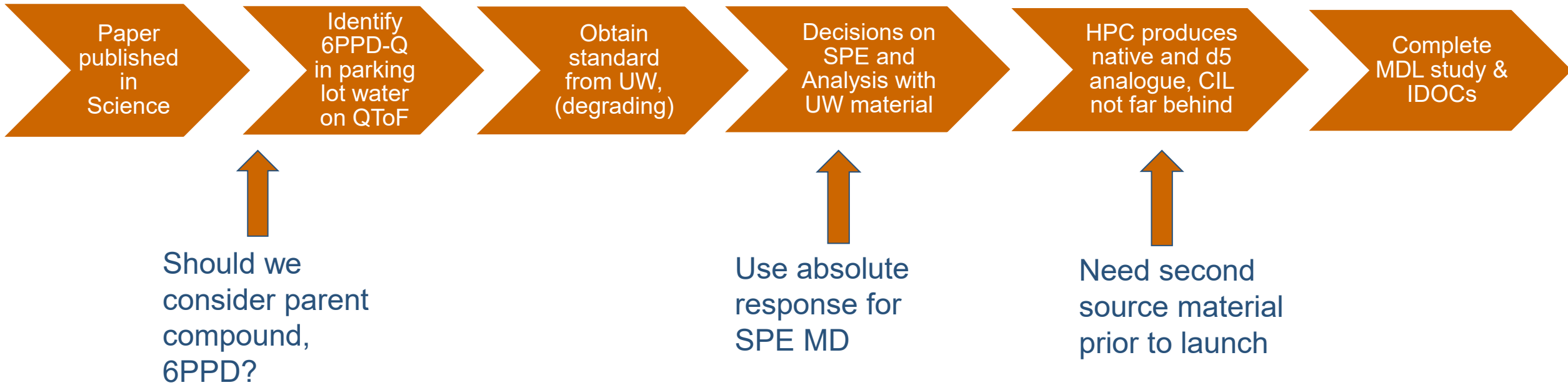
Method Must:

- Be geared towards storm water and complex matrices
- Have an RL orders of magnitude lower than LD50
- Incorporate isotope dilution quantitation

Method Should:

- Be flexible enough to increase extraction volume (and decrease RL)
- Take about the same amount of time as PFAS, use existing infrastructure
- Consider “green” and sustainable development choices

6PPD-Q Timeline



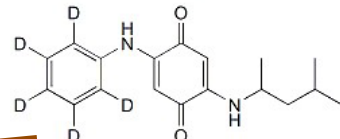
Snapshot: How is the Analysis Performed?



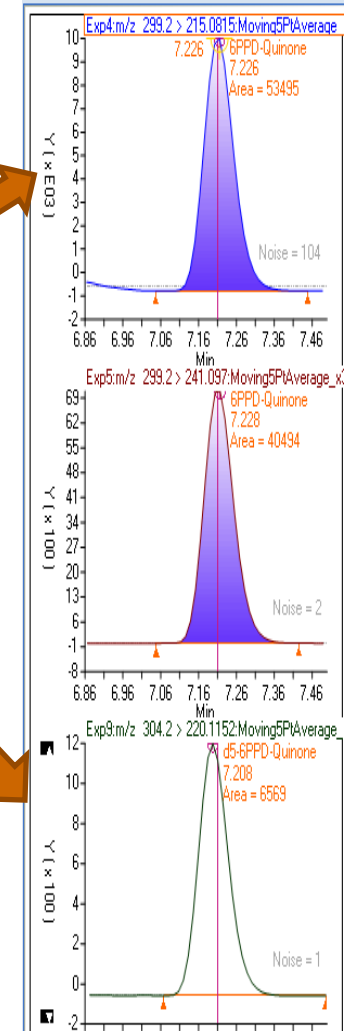
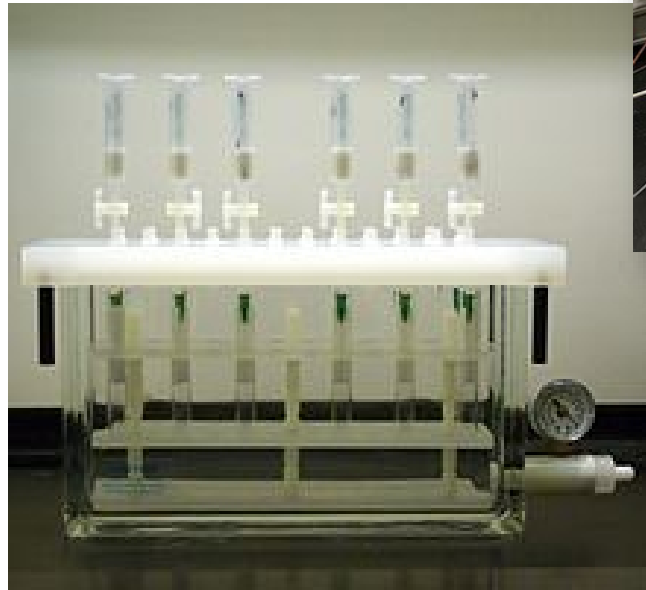
Your Sample



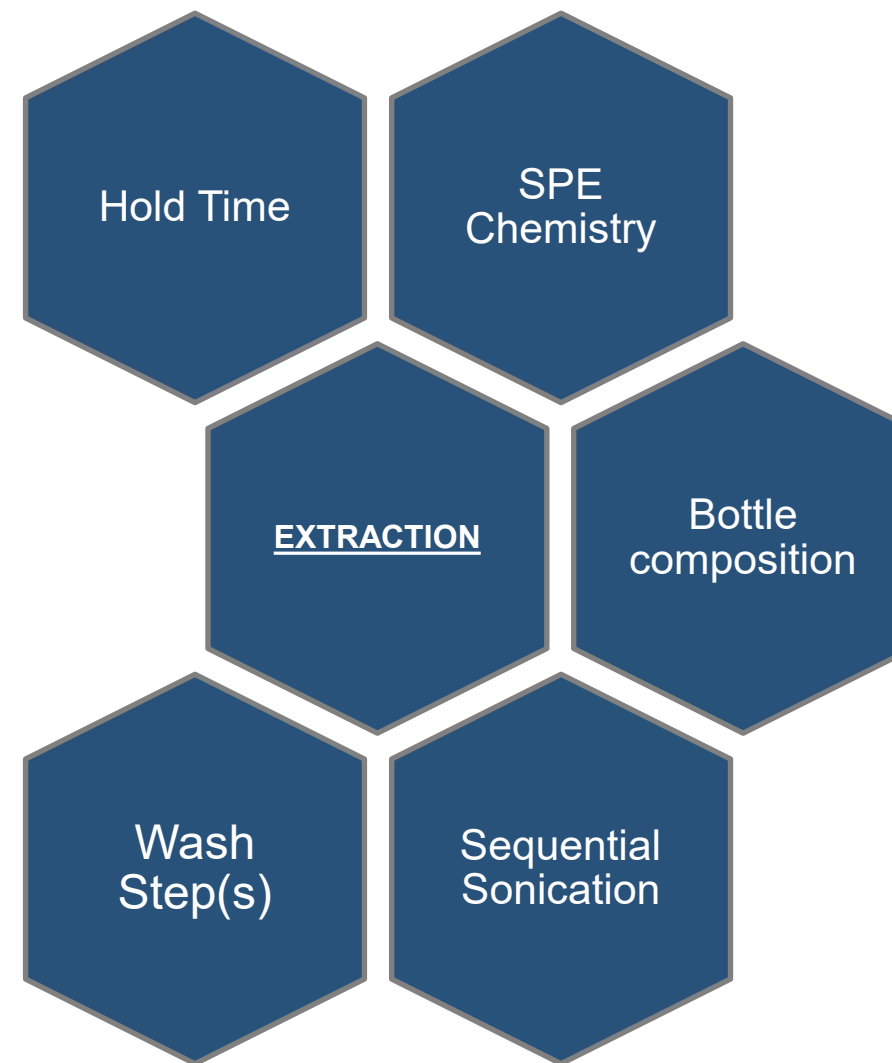
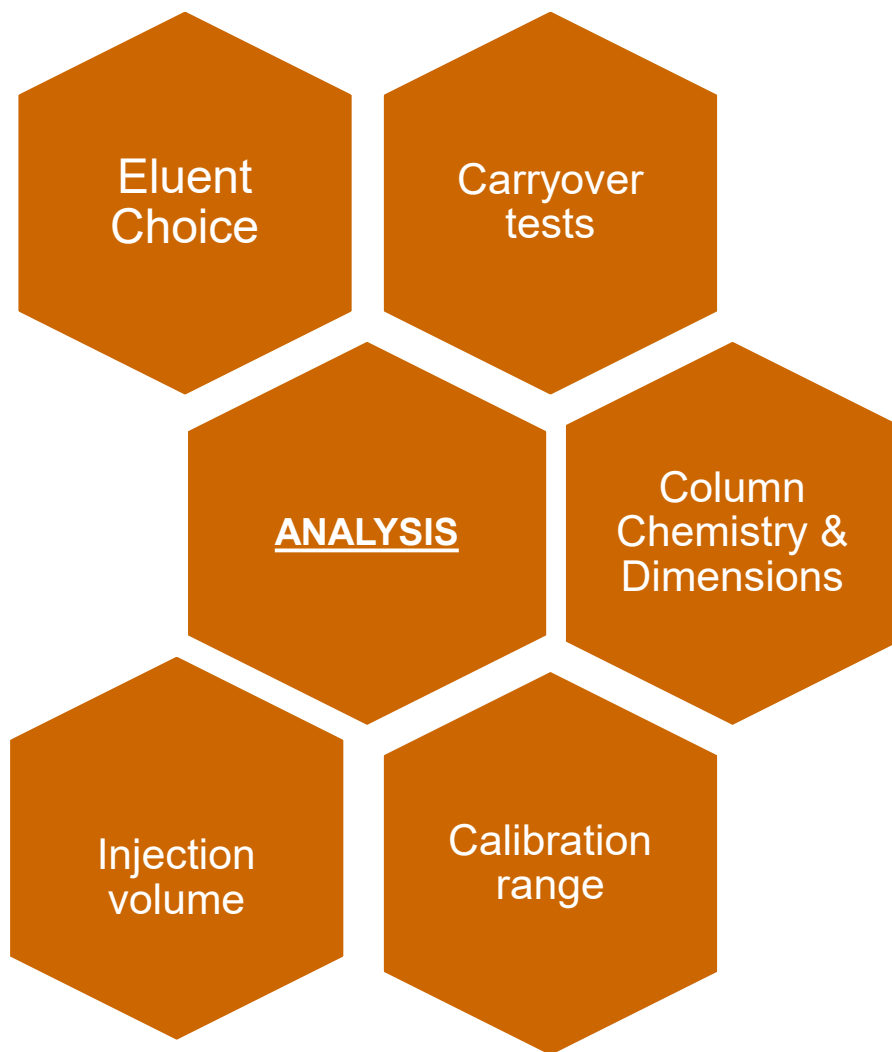
250mL
HDPE



Stable isotope of
6PPD-quinone



Method Development Considerations



SPE Approach- Lemons to Lemonade

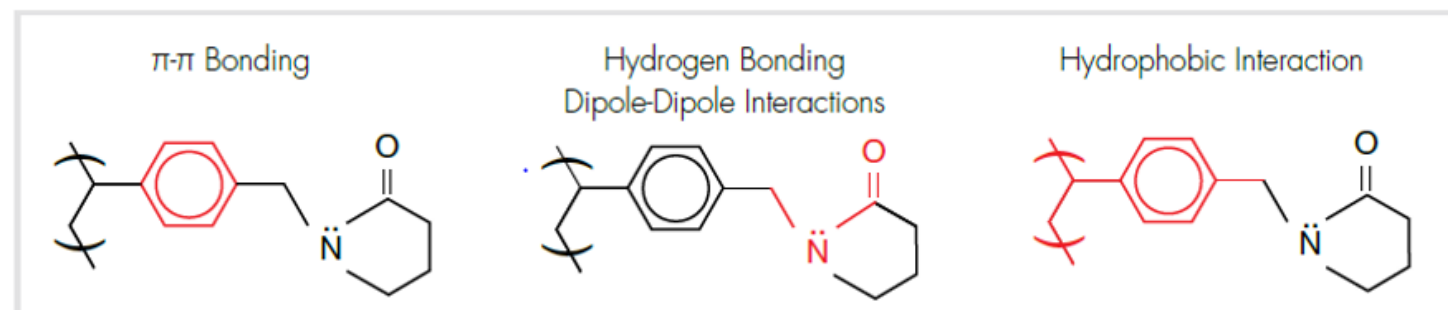


- Matrices likely to contain particulates
- Amines usually “sticky”
- A strong wash will remove bulk interferences
- Elute & adjust up to FV
 - Saves time and gasses w/o blow-down
 - Less error at larger volumes
- 100% organic composition for injection possible

STEP	SPEC
Cartridge:	200mg, 6mL Phenomenex Strata-X
Size:	100um
Condition:	5mL ACN
Equilibrate:	10mL H2O
Load:	10-15mL/min
Wash:	5mL 1:1 MeOH:H2O
Dry:	5 min, 10-15inch Hg, 5 min
Elute:	2x 5mL ACN



Strata-X (33µm) and Strata-XL (100µm)



Alternative to: Agilent® (formerly Varian) Bond Elut Plexa® and Waters® Oasis® HLB

SPE Wash Optimization



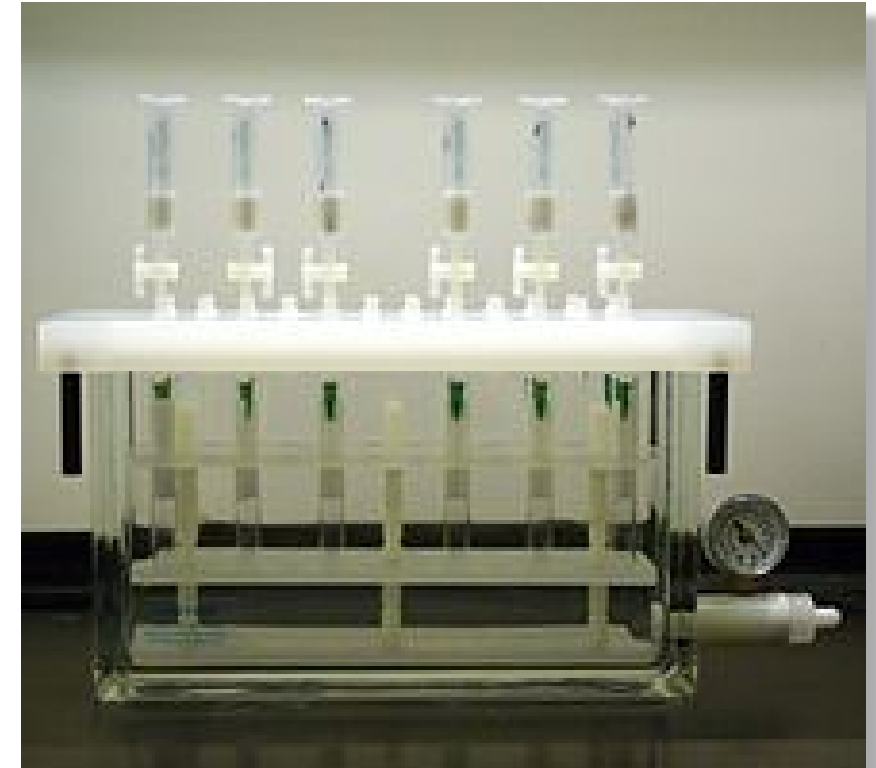
-A strong wash step preserves the instrument source and promotes column longevity

SPE wash step, Trial-A

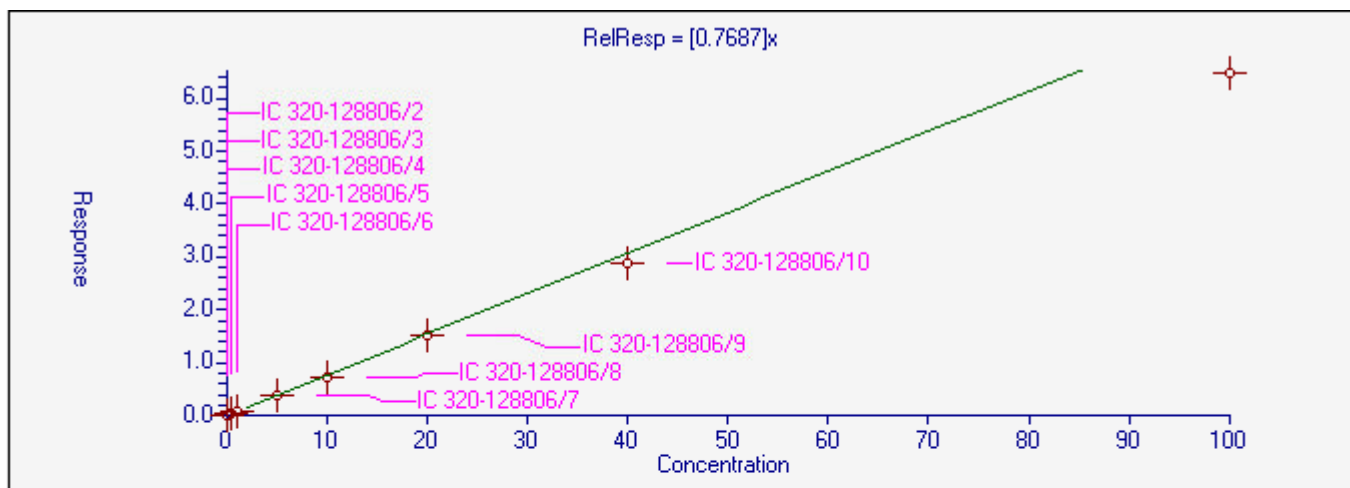
- 10% increments of MeOH
 - 0%-60% MeOH = 0% recovery?

SPE Wash Step, Trial-B

- 10% increments of MeOH
 - 60% = 0%
 - 70% = 0%
 - 80% = 8-10%
 - 90% 15-20%
 - 100% = 72-100%



Initial Calibration-Extended Curve Example



Calibration Standard Levels							
Level	Used	Amount	Area	ISArea	%Error	ReadBackAmount	Error
IC 320-128806/2	✓	0.025	1105	51181	12.35	0.02809	
IC 320-128806/3	✓	0.05	2081	53590	1.04	0.05052	
IC 320-128806/4	✓	0.1	4033	48332	8.56	0.10856	
IC 320-128806/5	✓	0.5	20951	51857	5.12	0.5256	
IC 320-128806/6	✓	1	41070	50079	6.69	1.06691	
IC 320-128806/7	✓	5	178485	49070	5.36	4.73199	
IC 320-128806/8	✓	10	341822	46370	4.10	9.59005	
IC 320-128806/9	✓	20	696795	46082	1.64	19.67125	
IC 320-128806/10	✓	40	1247588	43556	6.84	37.2633	
IC 320-128806/11	✓	100	2572954	39757	15.81	84.19311	

Analyte	Standard Level - Concentration as ng/mL						
	L1	L2	L3	L4*	L5	L6	L7
6PPD-Q	0.025	0.05	0.1	0.5	1	5	10
d5-6PPD-Q (IDA)	1	1	1	1	1	1	1

* The L4 concentration is typically used as the CCV.

Note: Additional calibration points above L7 may be added to enhance the calibration range.

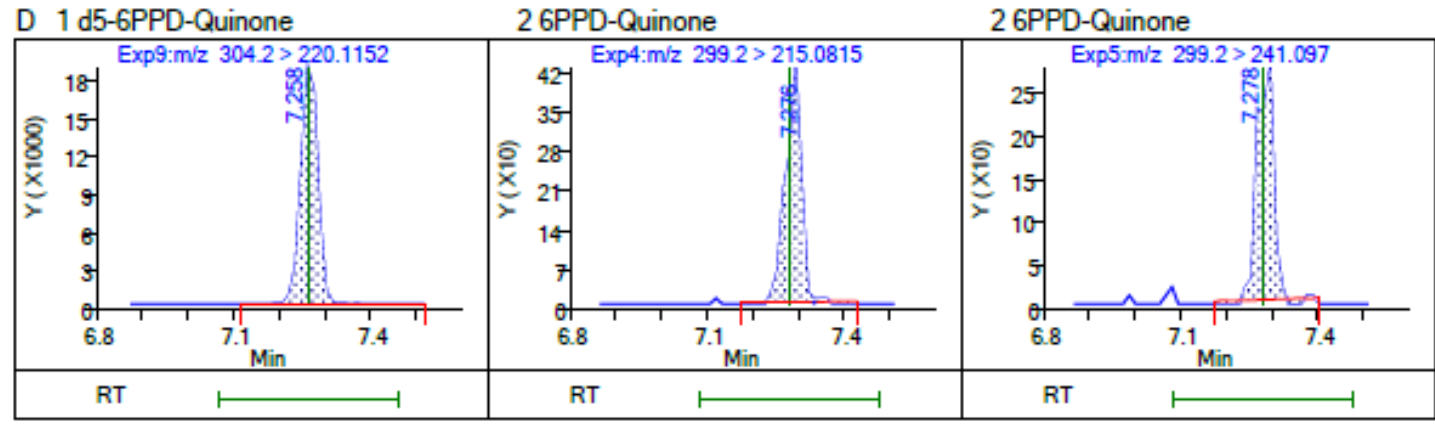
- Extended curve useful for product samples
- Aqueous Range from 2-4000ppt (ng/L)
- Solid Range from 0.25-1000ppb (ng/g)

Average response, 70-130 all points, <30% RSD (This curve at 8.5%)

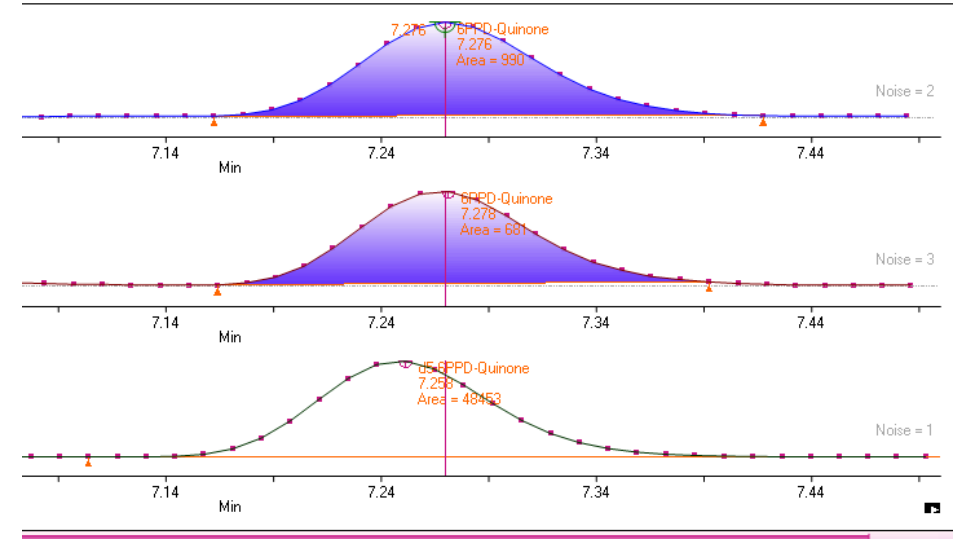


Chromatogram Examples

Injection Date: 26-Jan-2022 15:09:48 Instrument ID: A19
 Lims ID: CCV L1
 Client ID:
 Operator ID: A19 ALS Bottle#: 52 Worklist Smp#: 1
 Injection Vol: 50.0 ul Dil. Factor: 1.0000
 Method: 6PQ_A19 Limit Group: LC LCMS_Novel ICAL



CS1 (L1)

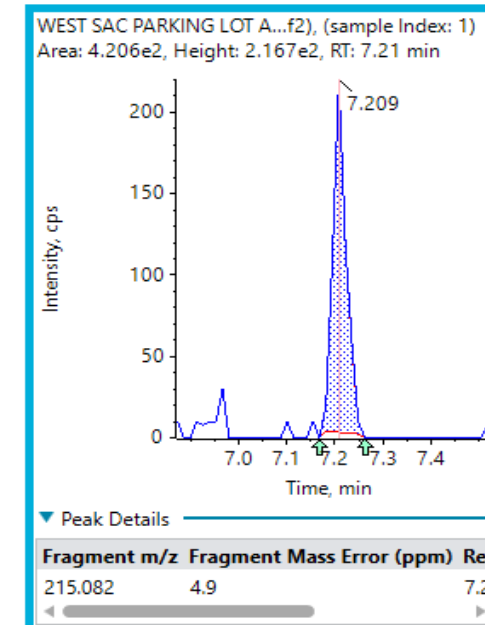


Zoomed for Scans Across Peak

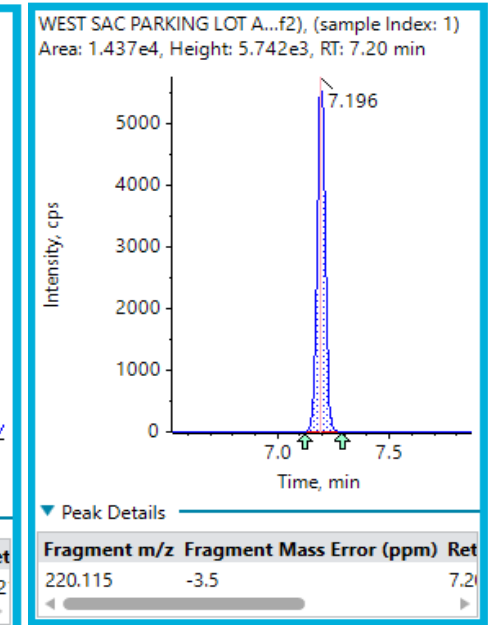
LC Conditions



Column (Column temp = 45°C)	Phenomenex Kinetex C18 3.5 μm, 3.6 mm x 100mm				
Mobile Phase Composition	A = 0.2% Formic acid in Water B = Acetonitrile				
Gradient Program	Time	%A	%B	Curve	Flow Rate mL/min.
	0	90	10	0	0.60
	1.0	90	10	0	0.60
	3.0	45	55	0	0.60
	6.0	1	99	0	0.60
	8.0	1	99	0	0.60
	8.50	90	10	0	0.60
	9.0	90	10	0	0.60
	Maximum Pressure limit = 7,500 psi				
Injection Size	50 μL (fixed amount throughout the sequence)				
Run Time	10.0 minutes				



Planter box soil



Planter box soil-
Internal standard

- 50uL Injection allows for no concentration step
- Increasing to ~1:1 Aq:Organic removes interferences and improves peak shape
- 100mm column more forgiving than 50mm

Tried:

- 50x3 XB C18
- 50x4.6 XB C18
- 50x3 Phenyl-Hexyl

Analytical Sequence



A typical run sequence for 6PPD-Q would be as follows:

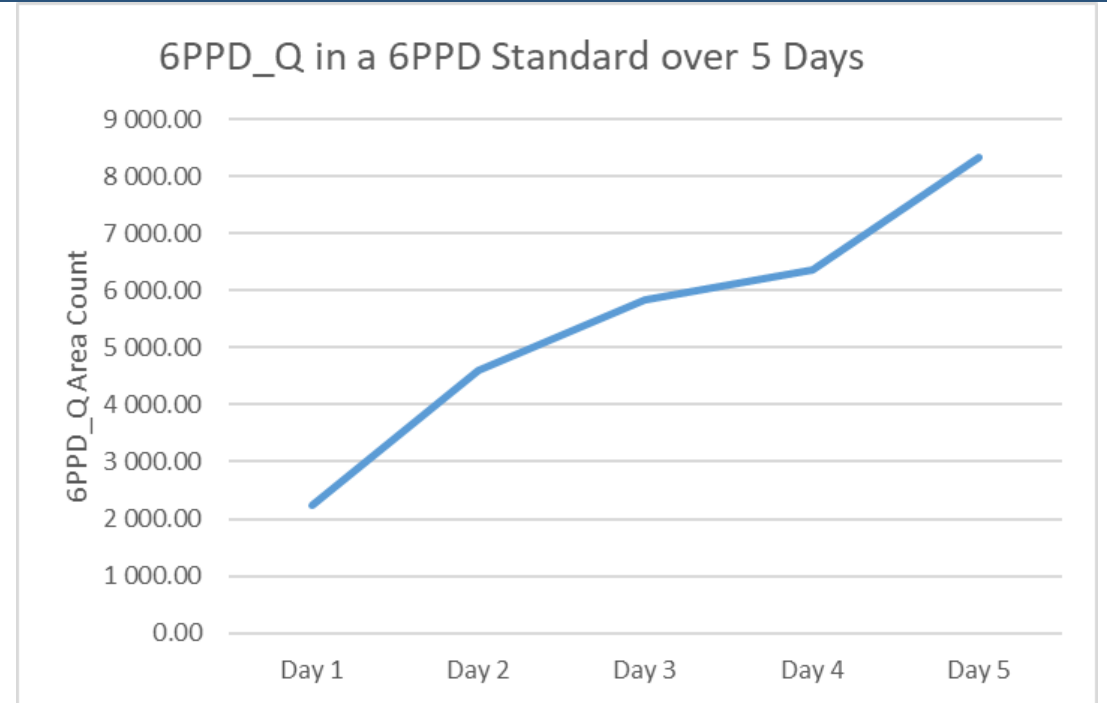
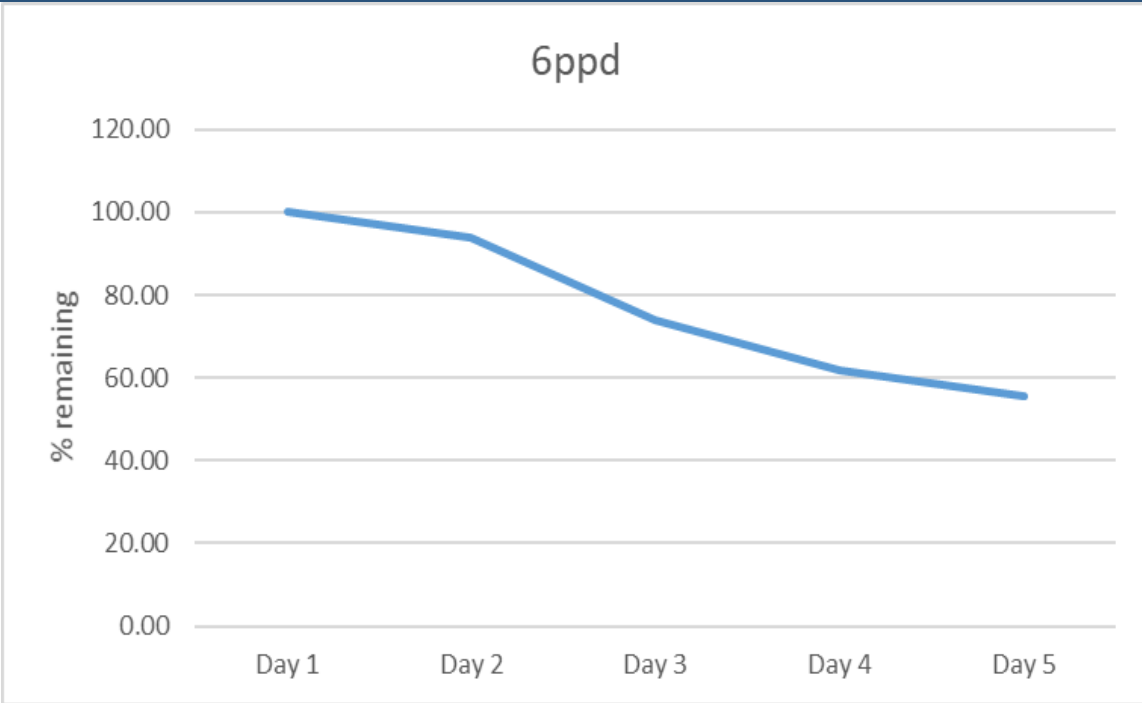
1. Primer (A number of primers until the instrument is stable)
2. Blank
3. Calibration Curve (A minimum of 5 calibration levels)
4. Blank + IDA
5. ICV
6. MB
7. LCS
8. Sample 1
9. Sample 1 MS
10. Sample 1 MSD
11. Sample 2
12. etc. (up to 10 field samples between CCVs).
13. CCV mid-level

Or:

4. CCVL at the reporting limit
5. CCV mid-level
6. MB
7. LCS
8. Sample 1
9. Sample 1 MS
10. Sample 1 MSD
11. Sample 2
12. etc. (up to 10 field samples between CCVs).
13. CCV mid-level

#	Lab ID	Date-Time
1	CCV L1	Feb-10-22 10:26
2	CCV L5	Feb-10-22 10:36
3	MB 320-564452/1-A	Feb-10-22 10:47
4	320-83943-A-7-A MDLV	Feb-10-22 10:58
5	320-83943-A-8-A LOQV	Feb-10-22 11:10
6	MB 320-564454/1-A	Feb-10-22 11:21
7	320-83943-A-9-A MDLV	Feb-10-22 11:32
8	320-83943-A-10-A LOQV	Feb-10-22 11:42
9	RB	Feb-10-22 11:53
10	RB	Feb-10-22 12:04
11	CCV L5	Feb-10-22 12:14

Parent Compound (6PPD)



- 6PPD Standard in Methanol, in the dark, at 4C.
- New vials injected each day

- Purging with Nitrogen did not slow degradation
- pH changes did not slow degradation
- 6PPD and 6PPD-q measured in same injection

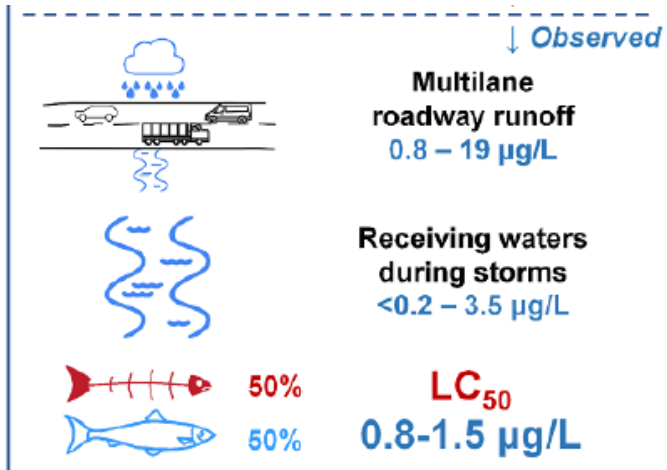
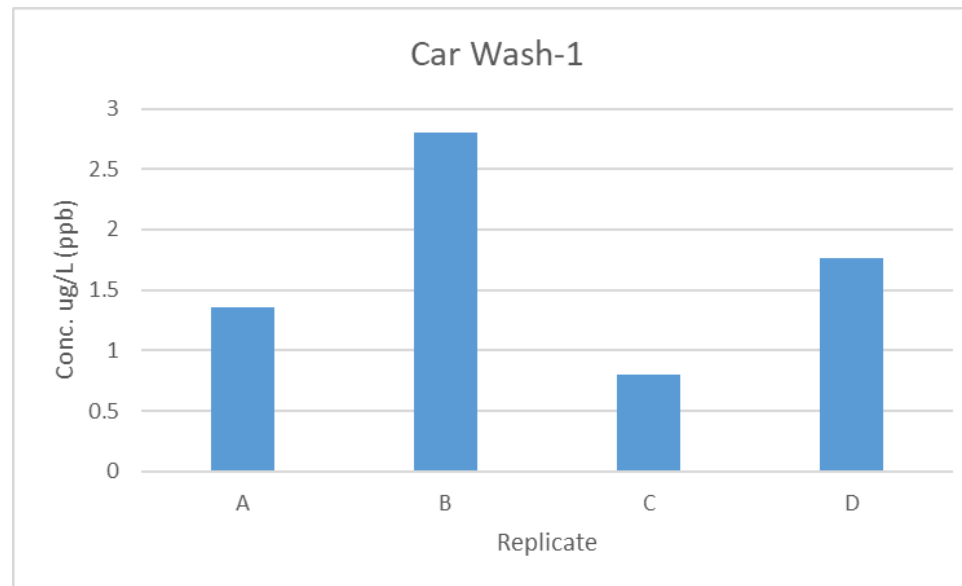
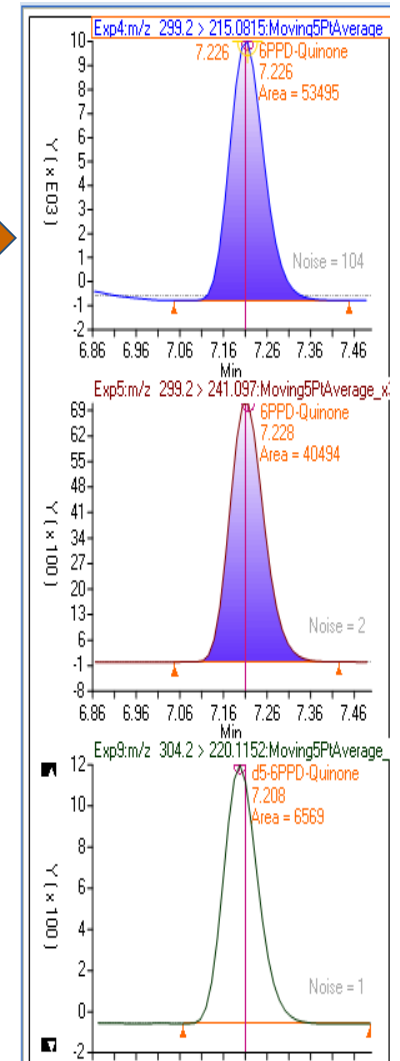
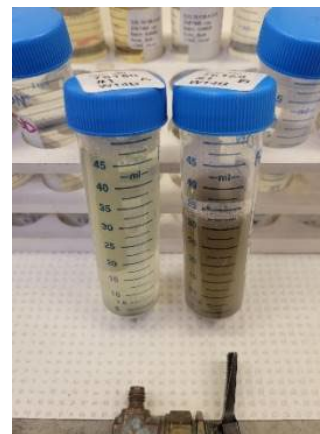


Environment Testing
America

REAL WORLD EXAMPLES

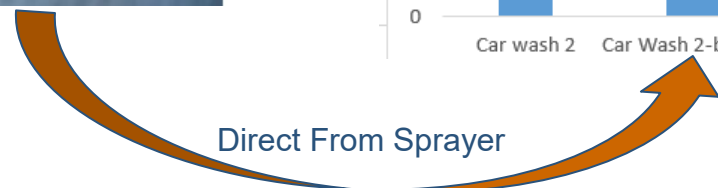
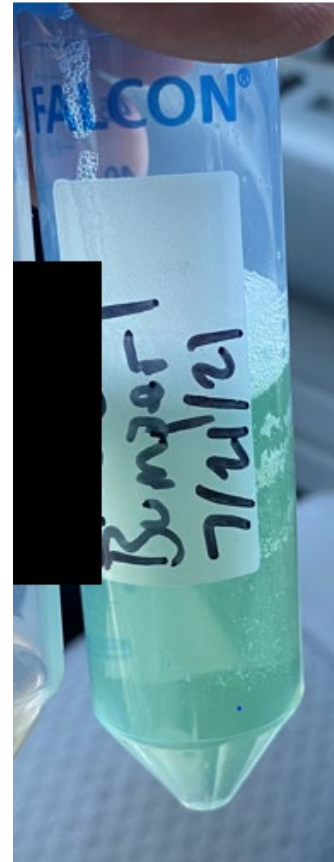
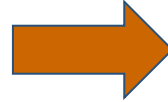


Artificial Rain: Car Washes

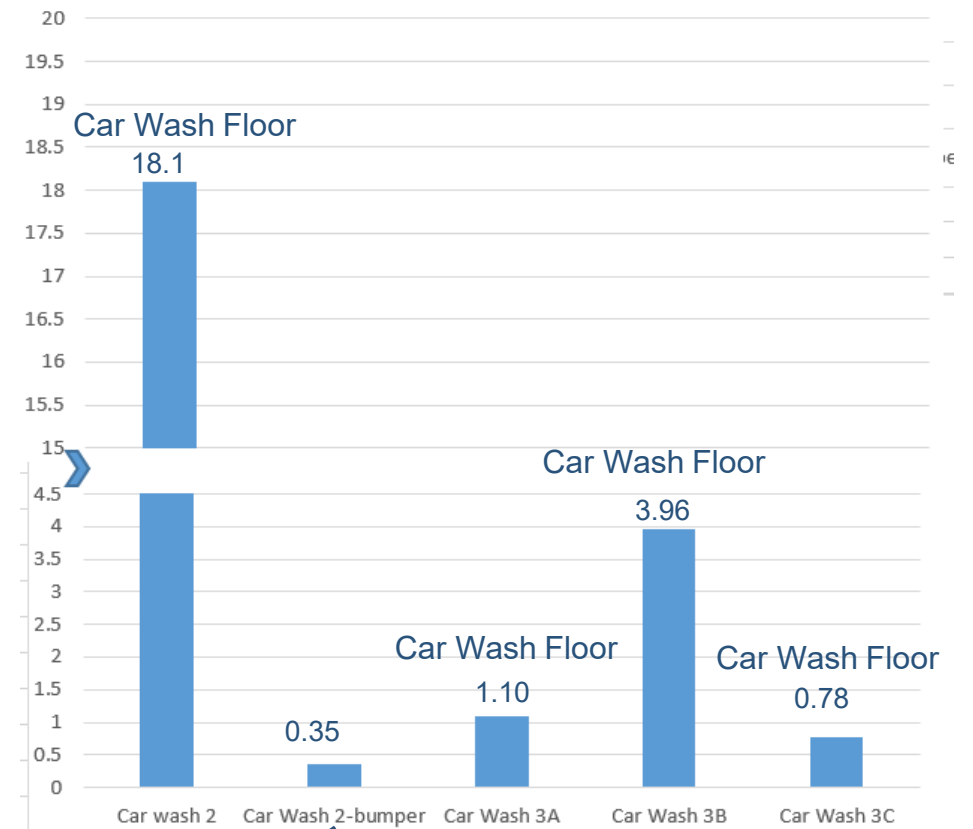


-University of Washington; Z. Tian *et al.*

Car Washes 2.0



Additional Car Washes, ug/L (ppb)

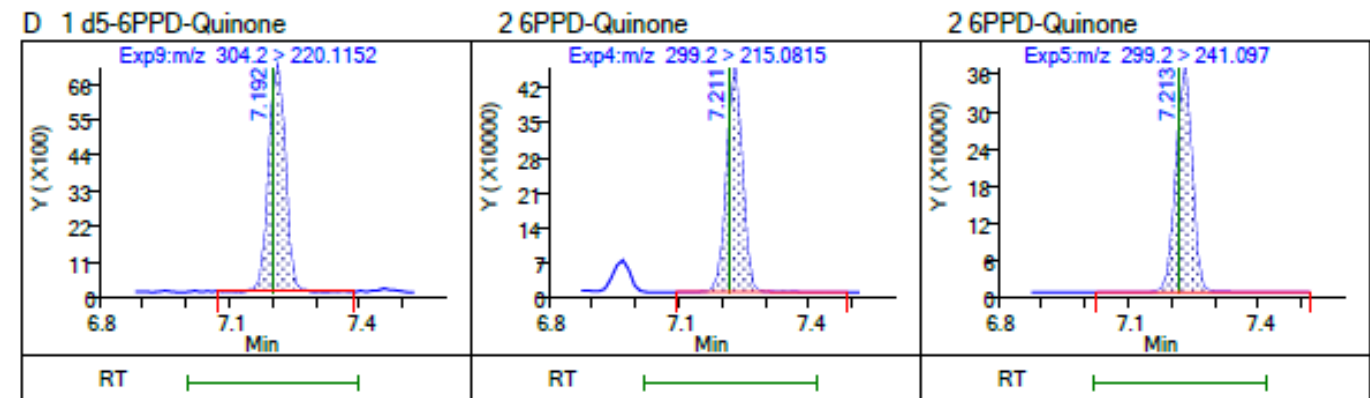


- HDPE bottles taped to bumper
- Containers open for the entire car wash
- Isotope dilution is loss corrected data

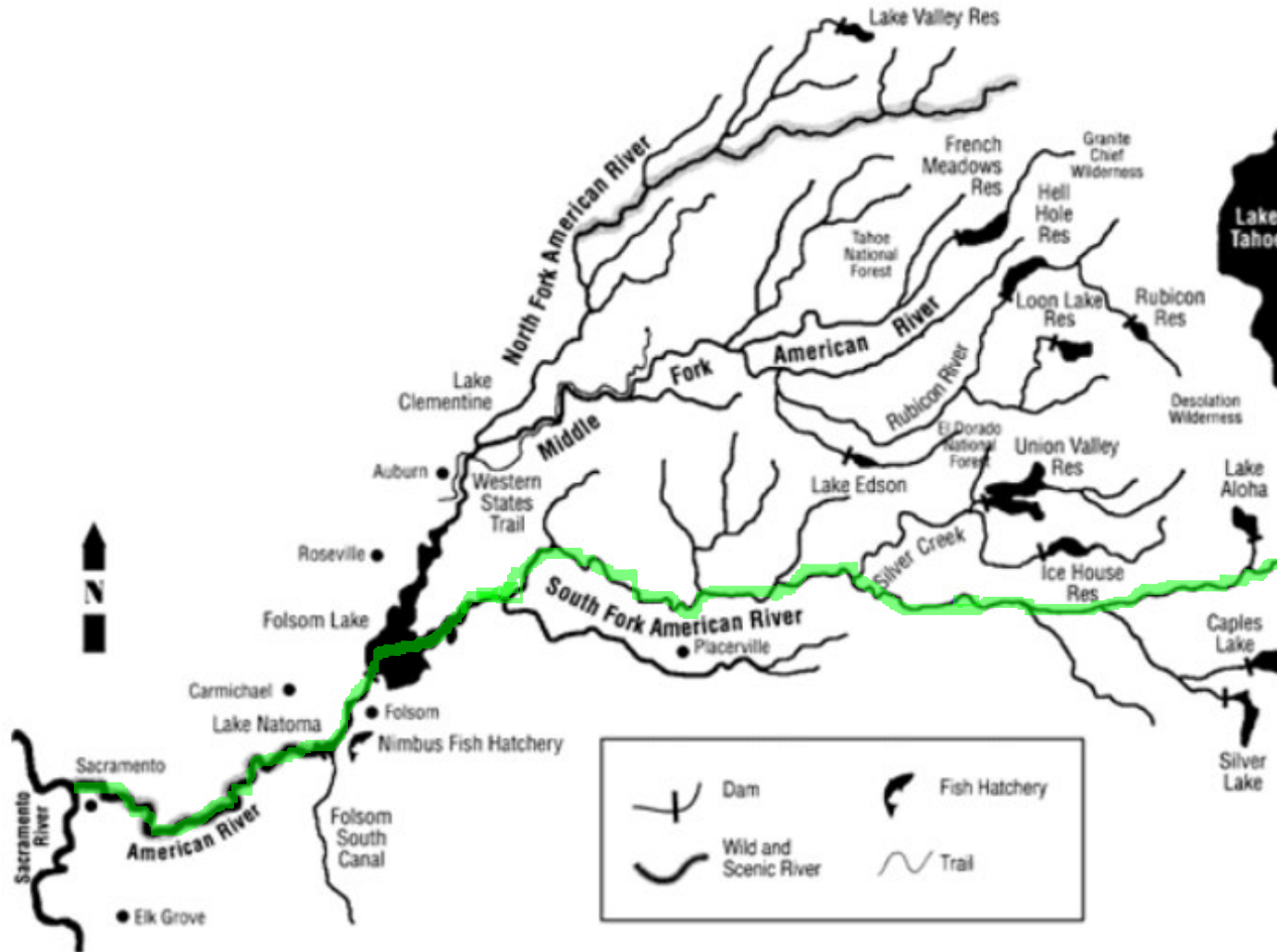
Analytical Approach



Sample	Conc. (ng/g)
Method Blank	Non Detect (RL: 0.25)
Used Wiper 1	374
Used Wiper 2	136
New Wiper 1	14.2



American River Watershed- South Fork

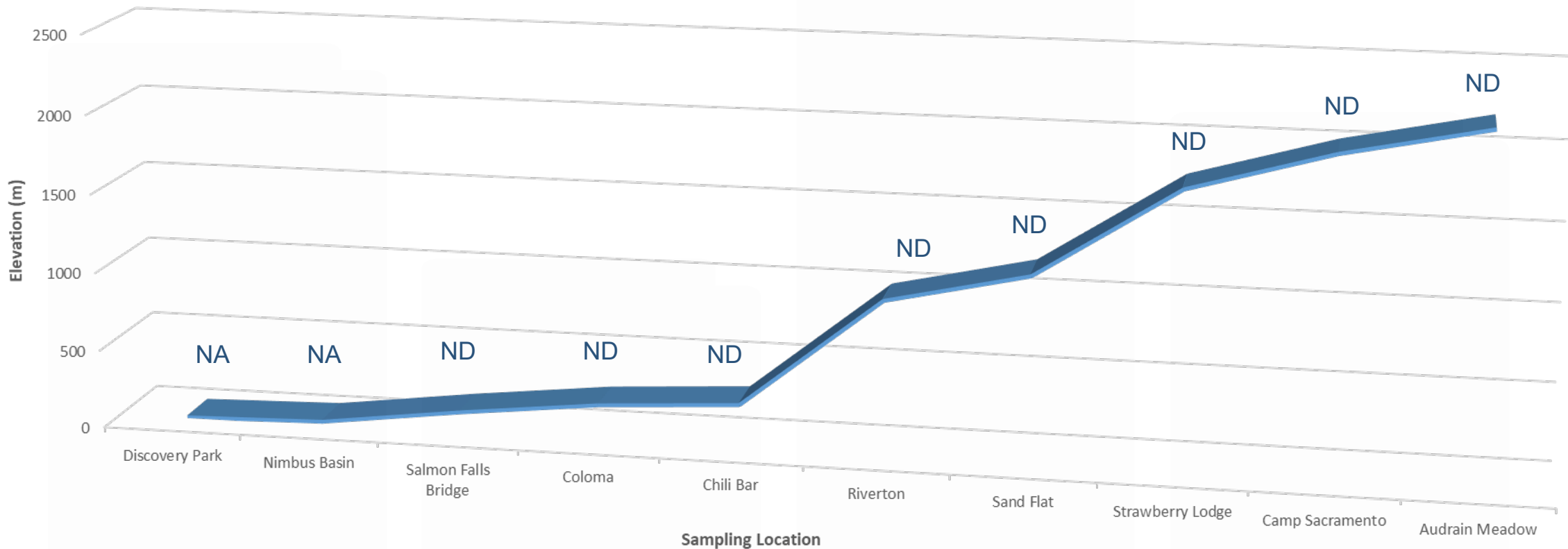


- 90 miles long
- 850 square miles of drainage
- Headwaters at crest of the Sierra Nevada
- Mouth is at the Sacramento River
- ~60K cars/month over Echo Summit (Elevation 7300')

Dry Conditions Sampling: Oct-15-2021



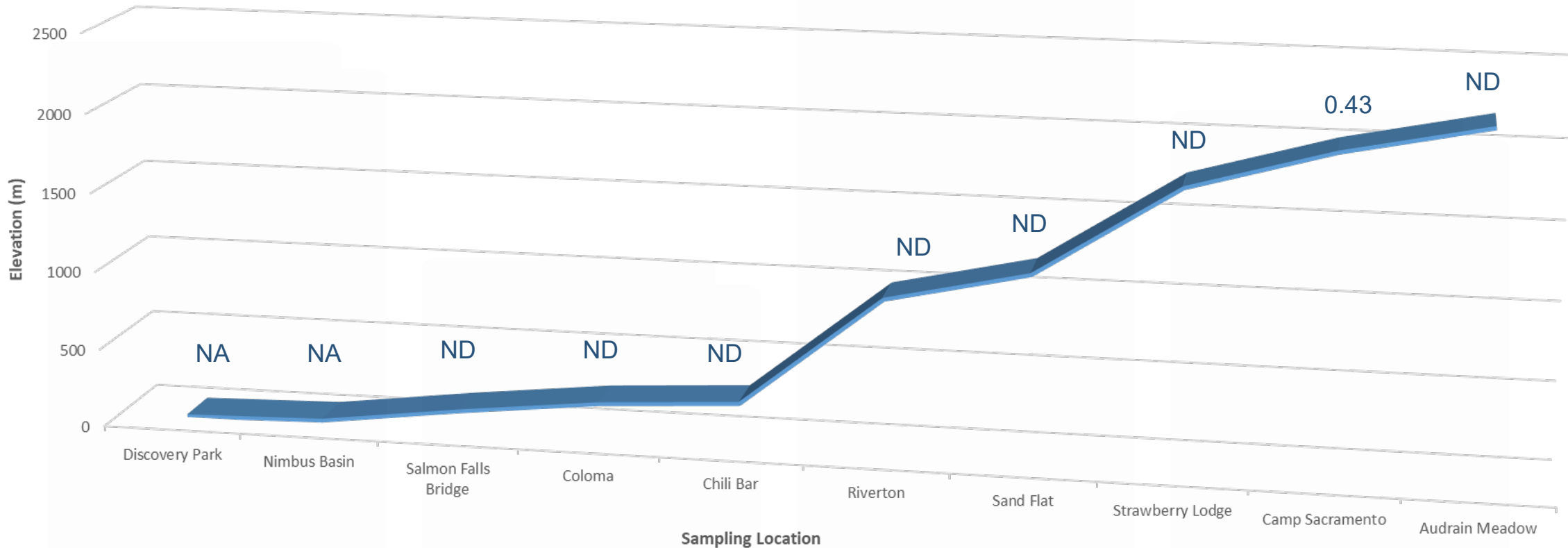
Aqueous Detections (ng/L, RL = 2ppt)



Dry Conditions Sampling: Oct-15-2021



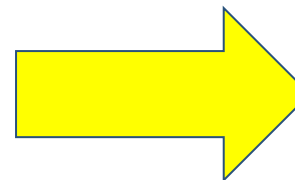
Solids Detections (ng/g, RL = 0.25ppb)



QA/QC Data: Pre-Storm



AQUEOUS	pre storm	NS % Rec	IS %rec	RL (ng/L)
Audrain Meadow	0		58	1.7
Camp Sacramento	0		55	1.7
Strawberry Lodge	0		48	1.7
Sand Flat	0		51	1.6
Riverton	0		46	1.7
Chili Bar	0		49	1.7
Coloma	0		47	1.7
Salmon Falls Bridge	NA		NA	ND
Nimbus Basin	NA		NA	ND
Discovery Park	NA		NA	ND
MB1	0	NA		2
LCS1	NA	108	50	NA
LCSD1	NA	98	41	NA

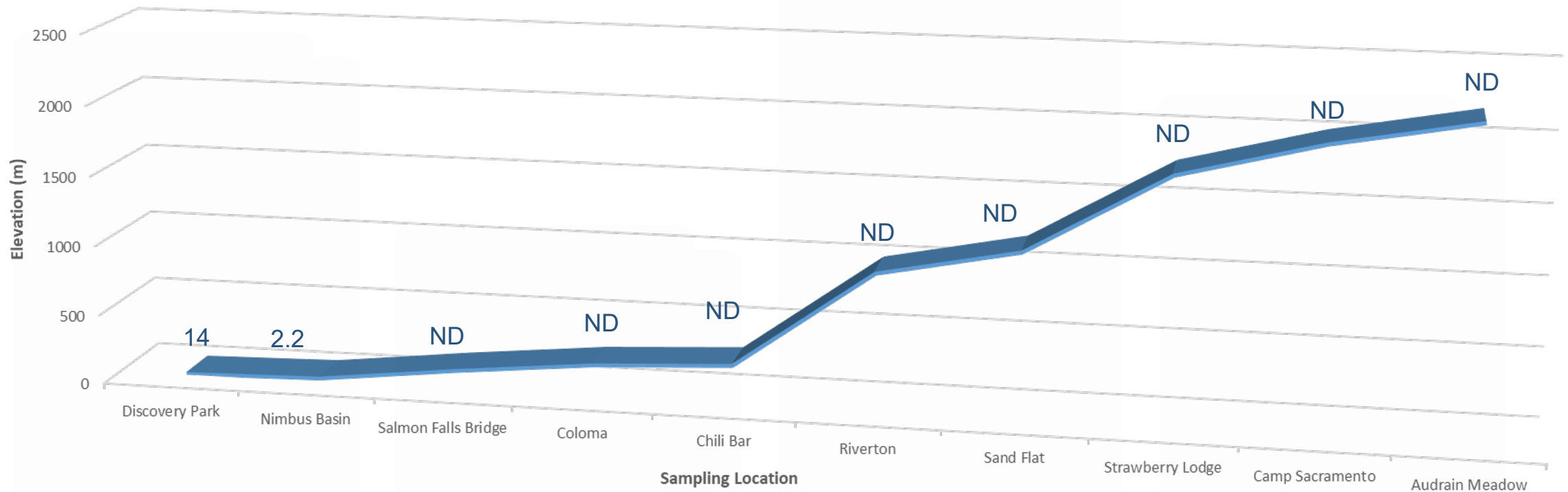


SOLIDS	pre storm	NS %rec	IS %rec	RL (ng/g)
Audrain Meadow	0		45	0.24
Camp Sacramento	0.43		53	0.22
Strawberry Lodge	0		64	0.22
Sand Flat	0		57	0.24
Riverton	0		57	0.23
Chili Bar	0		73	0.21
Coloma	0		77	0.22
Salmon Falls Bridge	0		77	0.21
Nimbus Basin	NA		NA	NA
Discovery Park	NA		NA	NA
MB1	0	NA	86	0.25
LCS1		108	50	
LCSD1		98	41	
MS1-17		97	73	
MSD1-17		104	76	
MS1-16		102	90	
MSD1-16		106	77	

Wet Conditions Sampling: Oct-19/20-2021



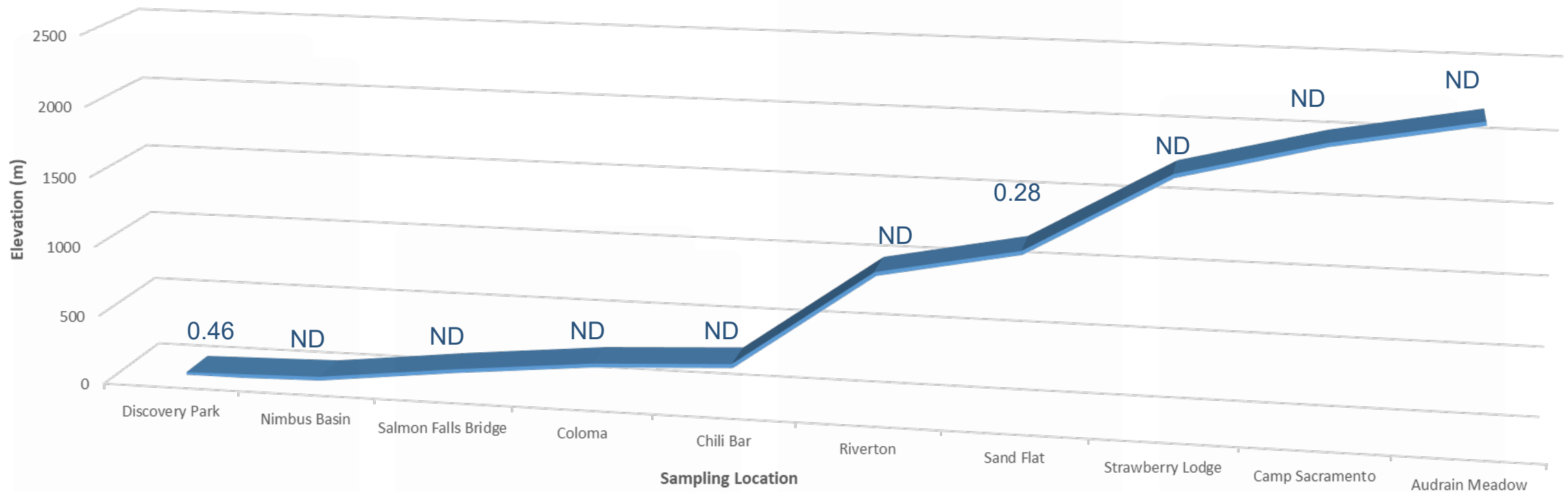
Aqueous Detections (ng/L, RL = 2ppt)



Wet Conditions Sampling: Oct-19/20-2021



Solids Detections (ng/g, RL = 0.25ppb)



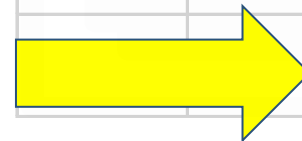
QA/QC Data: Post-Storm



AQUEOUS	post storm	NS %rec	IS %rec	RL (ng/L)
Audrain Meadow	0		71	1.6
Camp Sacramento	0		69	5.9
Strawberry Lodge	0		77	1.6
Sand Flat	0		78	1.7
Riverton	0		79	1.7
Chili Bar	0		90	1.9
Coloma	0		72	1.7
Salmon Falls Bridge	NA		NA	NA
Nimbus Basin	2.2		70	1.7
Discovery Park	14			
MB1	0	NA	79	2
LCS1		101	82	NA
LCSD1		97	94	NA



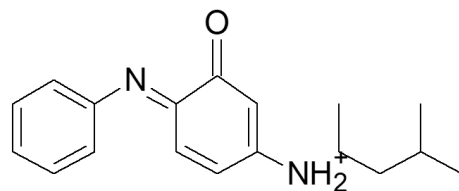
SOLIDS	SOLID	post storm	NS %rec	IS %rec	RL (ng/g)
Audrain Meadow		0		77	0.23
Camp Sacramento		0		106	0.23
Strawberry Lodge		0		86	0.23
Sand Flat		0.28		75	0.22
Riverton		0		90	0.25
Chili Bar		0		97	0.23
Coloma		0		106	0.24
Salmon Falls Bridge		NA		NA	NA
Nimbus Basin		0		107	0.24
Discovery Park		0.46		64	0.23
MB2		0		104	0.25
LCS2		5	98	108	
MS2-2			114	77	
MSD2-2			102	80	



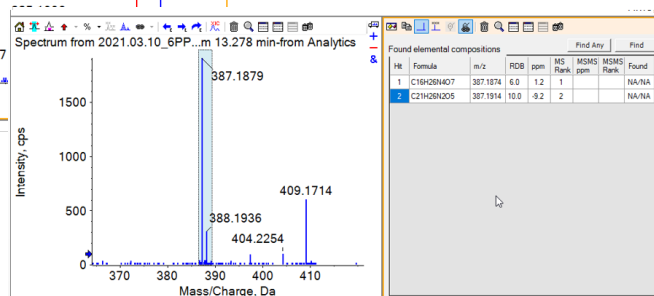
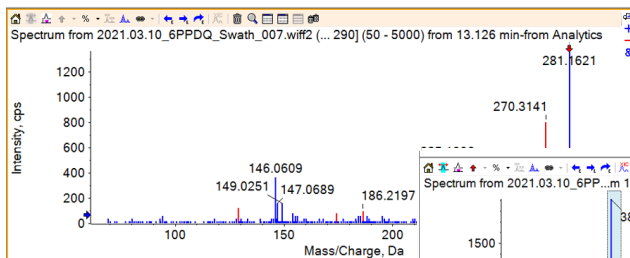
6PPD-Q It's The End As We Know It...Or Is It?



6PPD-Ketone

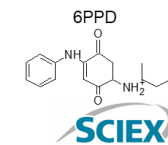
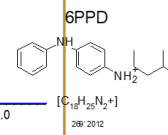
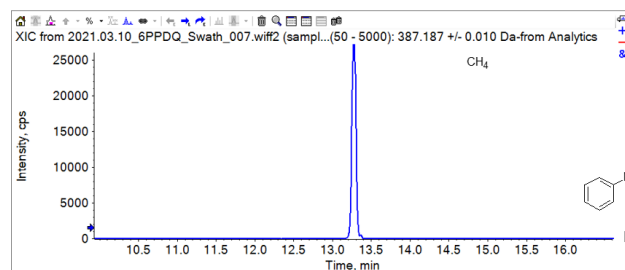
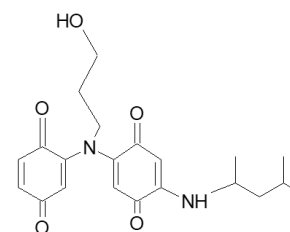


D2: C₁₈H₂₀N₂O
6PPD-Q: C₁₈H₂₂N₂O₂



Unknown: C₂₁H₂₆N₂O₅
6PPD-Q: C₁₈H₂₂N₂O₂

+C₃H₄O₃



-Courtesy Simon C. Roberts, SCIEX

- Isomers?
- Other potentially toxic quinone formation?
- Are there non tire-based point sources?
- Identify and characterize replacement for 6PPD?
- Impact from Microplastics?
- Fate of TWP?

Thank You

