April 2nd, 2025

From Post-it to Promulgation: Method Development Pathways of PFAS and 6PPD-quinone (and beyond)

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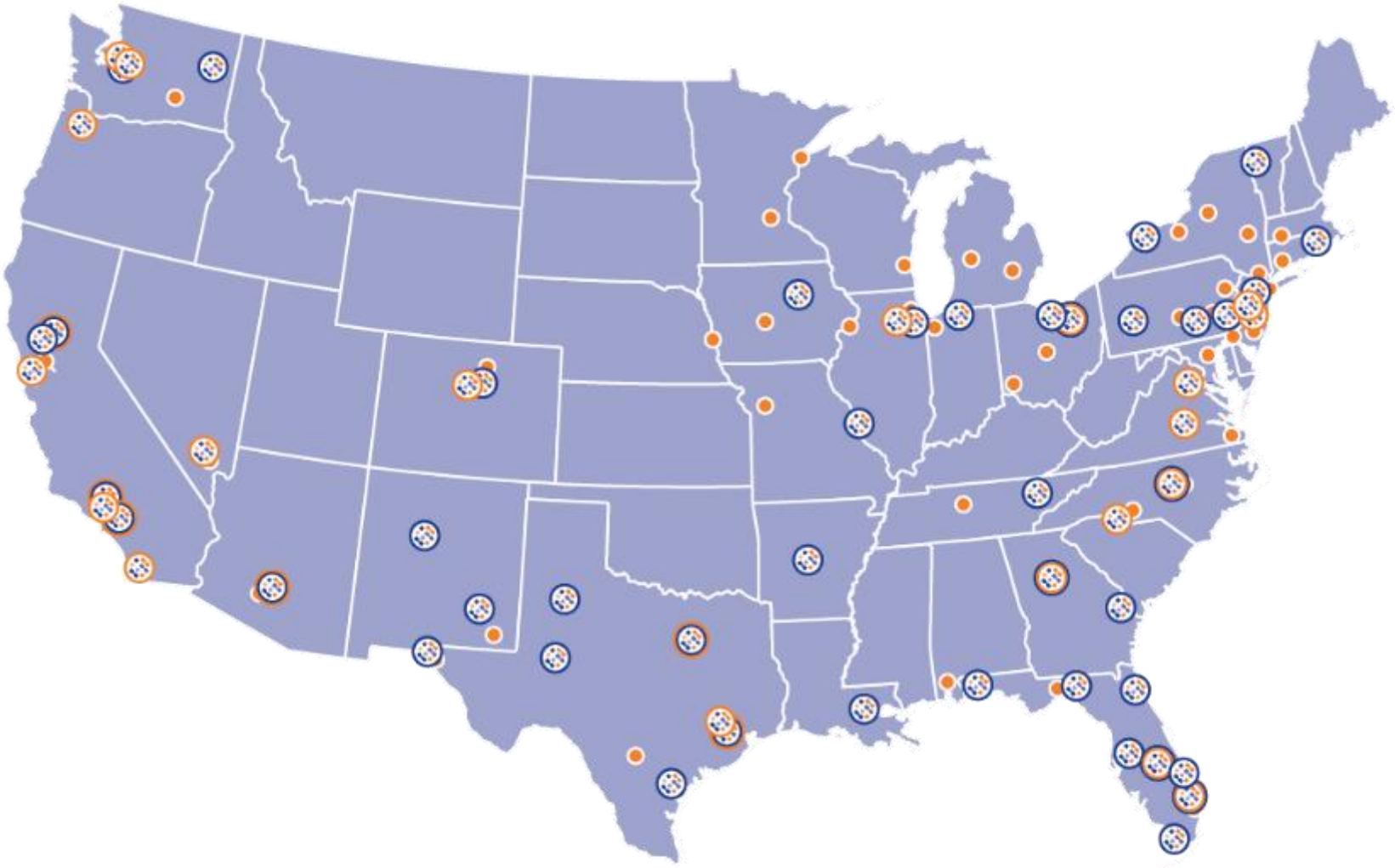

67
Laboratory Locations

~3,000
Staff (2023)

5.5M
Samples
(2023 actual)

550+
Accreditations & Certifications

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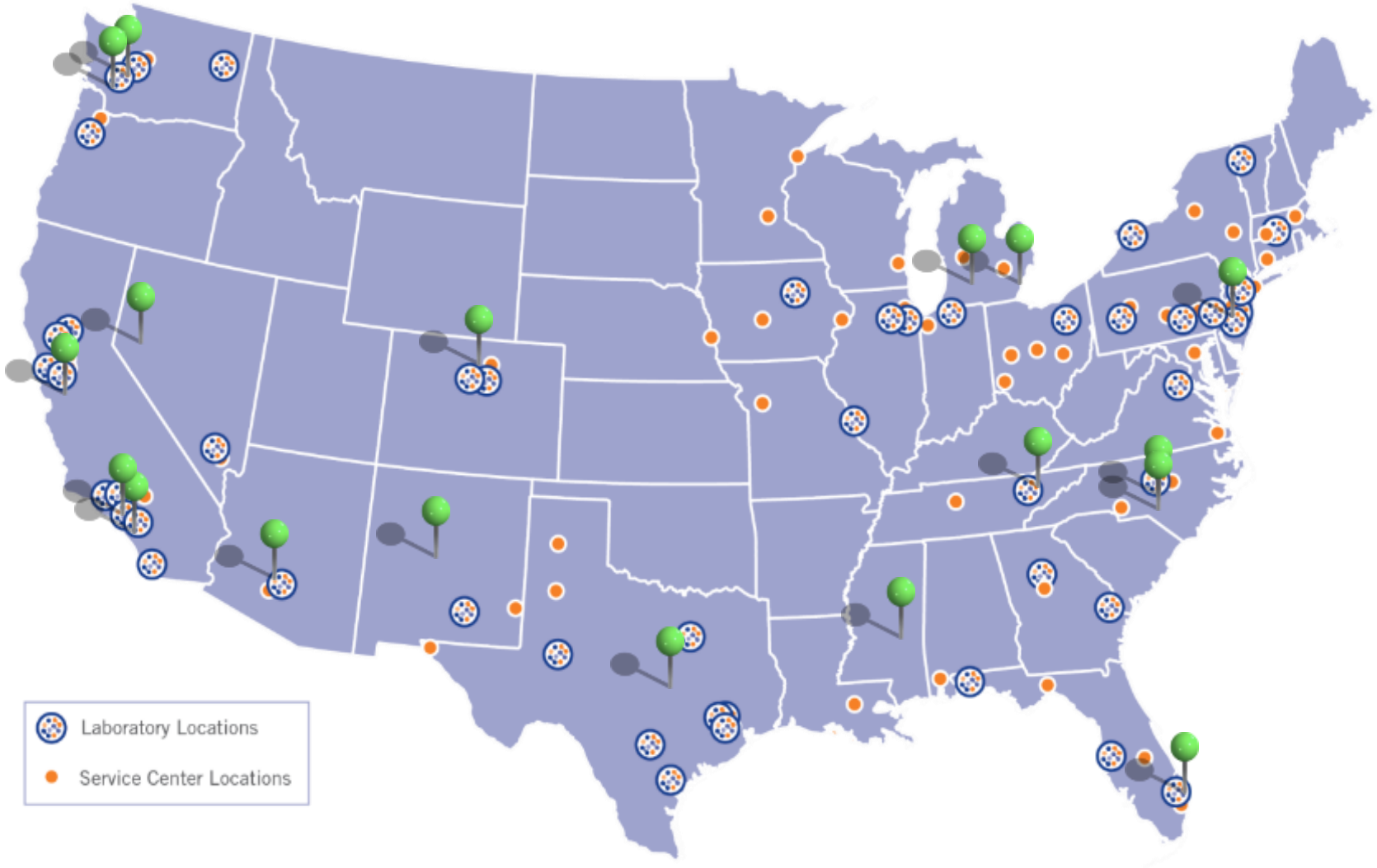
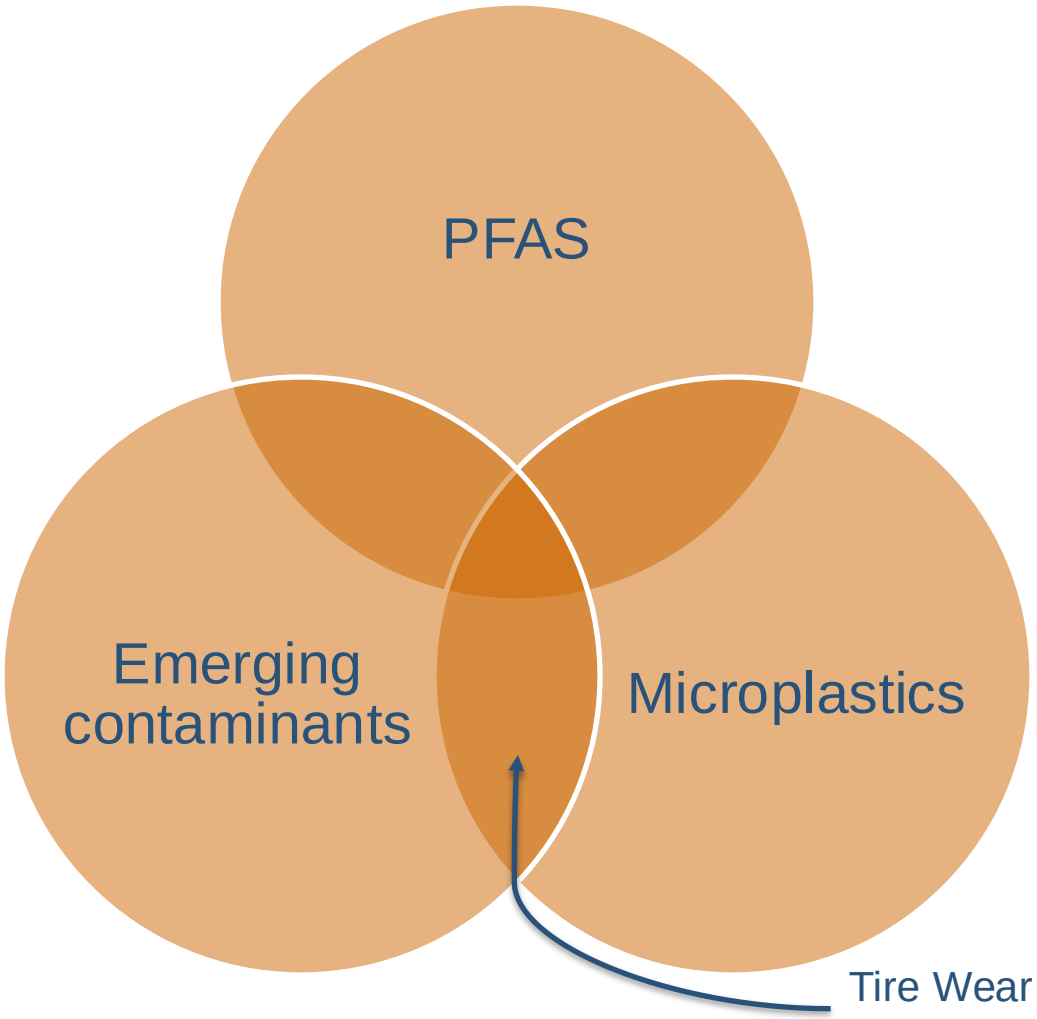
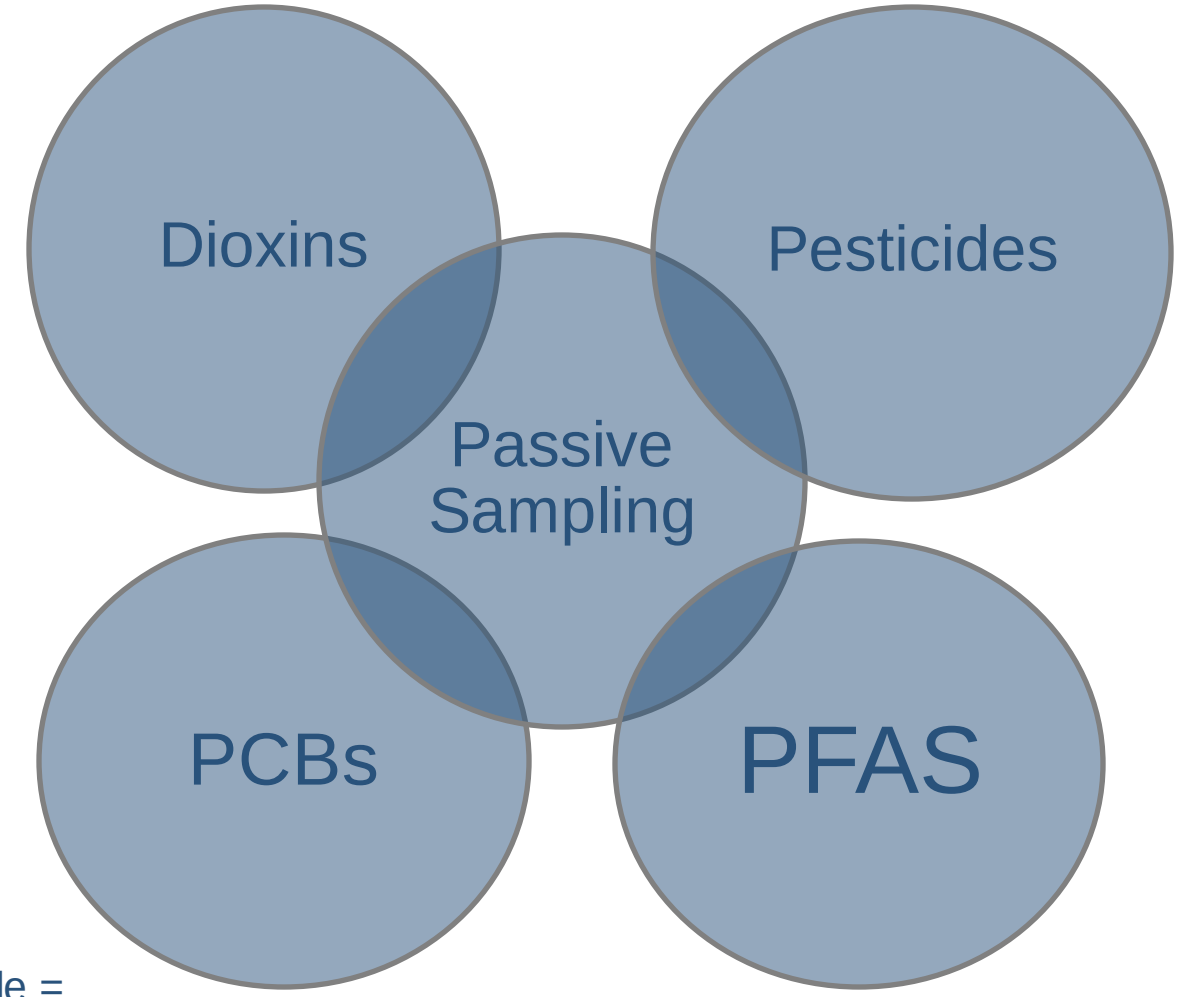
Blur The Lines: Academia + Industry



- Partner with academics to truly help them
- Gain insight to what is possible, what is coming
- Coauthor publications that will be seen by industry
- Help secure grant opportunities through in-kind contributions
- Seek and hire innovative candidates! (with permission)

New Endeavors Merge With Existing





Method Development Considerations

Hold Time

SPE Chemistry

EXTRACTION

Bottle composition

Wash Step(s)

Sequential Sonication

Eluent Choice

Carryover tests

ANALYSIS

Column Chemistry & Dimensions

Injection volume

Calibration range

~~PFAS~~ Current Analytical Approaches

AQUEOUS

Weigh sample, then add isotopes → Perform Solid Phase Extraction (SPE) → Elute SPE and add additional isotopes → Introduce to instrument for analysis

SOLIDS

Weigh sample, then add isotopes → Add basic methanol, shake 30 mins, centrifuge 10 mins & decant → Repeat methanol shake & spin down 2x more, combine extracts → Perform Solid Phase Extraction (SPE) → Elute SPE and add additional isotopes → Introduce to instrument for analysis

TISSUES

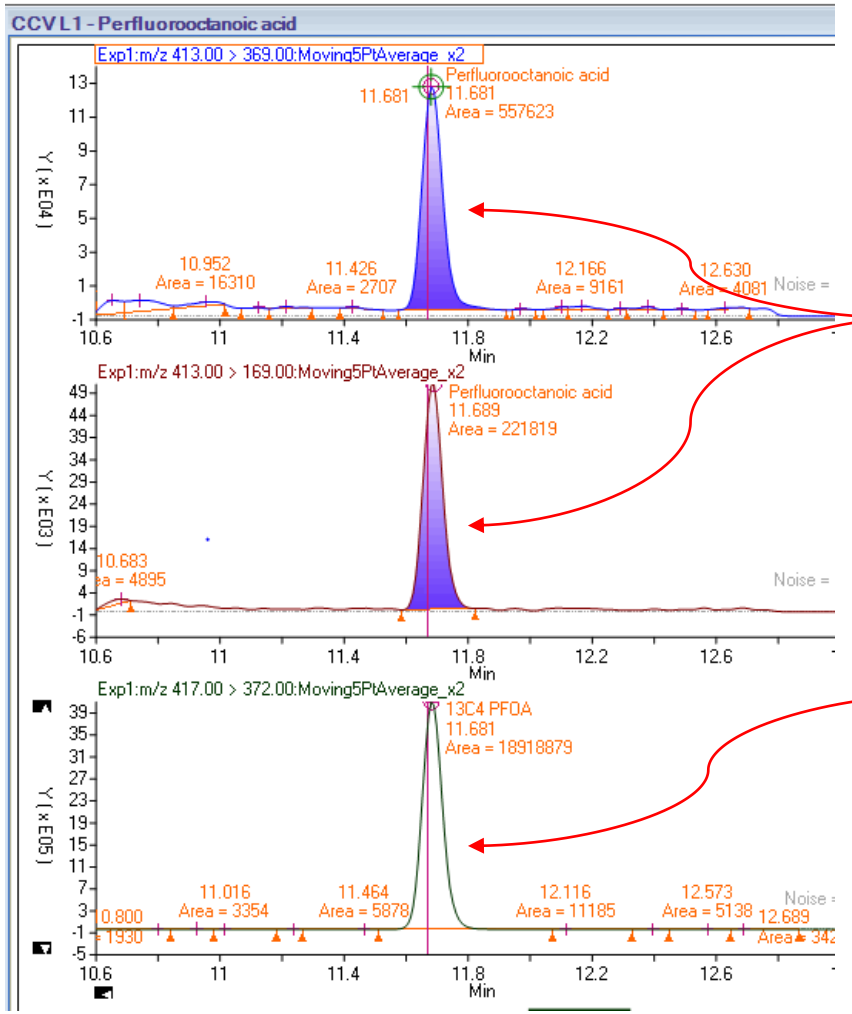
Weigh sample, then add isotopes → Add basic methanol, slowly shake for 16 hours, centrifuge & decant → Add acetonitrile, sonicate for 30 mins, centrifuge & decant → Add basic methanol sonicate & briefly shake centrifuge & decant, combine all extracts → Perform Solid Phase Extraction (SPE) → Elute SPE and add additional isotopes → Introduce to instrument for analysis

~~PFAS~~ Analytical Tool Belt

- Isotopes & Isomers
- LC-MS/MS
- GC-MS/MS
- HRMS
- TOP Assay
- CIC-TOF
- Thinking Caps

All Analyses Benefit From Isotopes

- Isotope analogues have same retention time as target compound
- Data are loss-corrected using isotopes
- Adds confidence to data
- Mitigates false negatives and false positives
- Not radioactive!



Target Compound

Isotope of Target compound

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6PPD-quinone: One Compound, Loads of Interest

6PPD-Q: Much Progress in <5 Years

Science

REPORTS

Cite as: Z. Tian *et al.*, *Science* 10.1126/science.abd6951 (2020).

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

La pollution des pneus de voiture tue le saumon sur la côte ouest des États-Unis

Home › EcoNews Report › Are Toxic Tires Killing Salmon?

EcoNews Report

re Toxic Tires Killing Salmon?

side News Source December 12, 2020

Researchers in the Journal Science report that a chemical in car tires, 6PPD quinone (pronounced "kwih known"), is a likely coho salmon killer. The chemical, used as a preservative in tires, was found to kill juvenile salmon in lab tests that replicated concentrations often found in nature. Warner Chabot, executive director of the San Francisco Estuary Institute, joins Gang Green to talk about the research and what California can do to protect its endangered salmon.

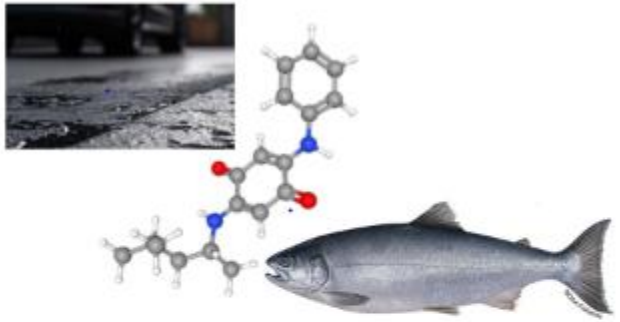
EPA United States Environmental Protection Agency

Office of Water

www.epa.gov December 2023

DRAFT Method 1634

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



Tire Soup – Finding the Toxic Constituents

- 7PPD?
- 8PPD?
- Other PPDs?
- HMMM?

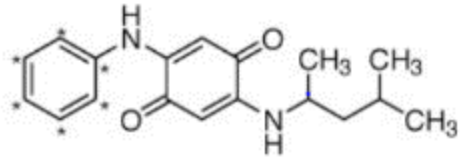
-NOAA; 2021

6PPD-Q Development Pathway

- Started by characterizing parking lot water on LC-QToF
- Obtain 6PPD standard, begin SPE evaluation
- Try different LC column phases and conditions
- Discover that ACN is best option for 6PPD-Q rinsing (“sticky stuff”)
- Begin stability studies
- Obtain small amount of authentic 6PPQ-Q
- Refine SPE and Analytical
- Pivot to QQQ
- HPC launches 6PPD-q and d5-6PPD-Q standards
- CIL synthesizes 13C6 and 13C12 6PPD-Q
- Begin validations
- Offer method to the public

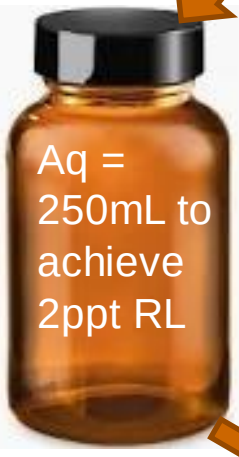
How is the Analysis Currently Performed?

Solids: 0.25ng/g RL

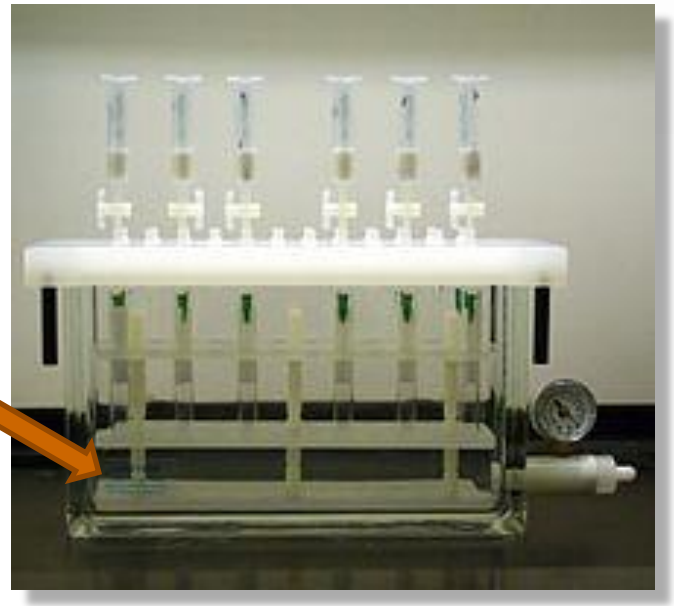


EIS: 13C6-6PPD-Q

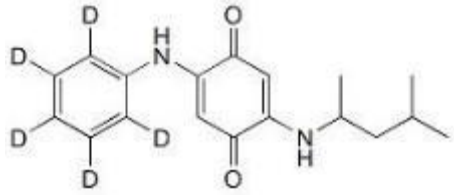
Serial Sonication

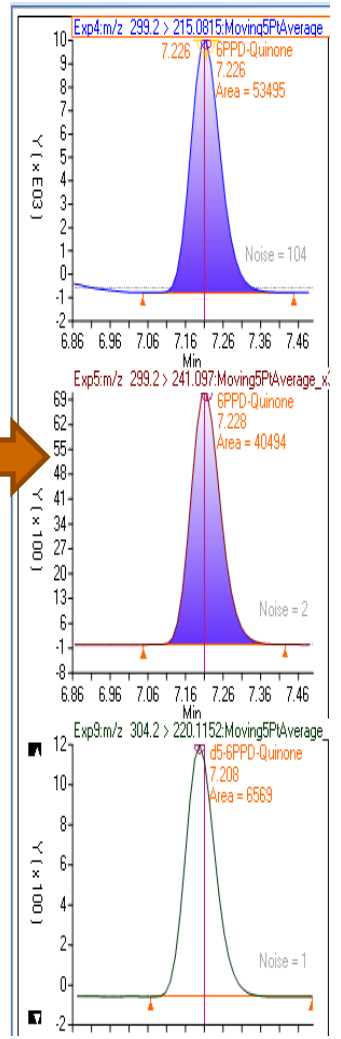


Aq = 250mL to achieve 2ppt RL



NIS d5-6PPD-Q





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PFAS: Still Forever Yours

A Brief History of PFAS Analysis

Utilizes Internal/ External Std. Quantitation and unique techniques

Utilizes Isotope Dilution and other consistent techniques

2000 — User-defined method for all matrices

2008 — EPA 537.1 for DW

2017 — DoD QSM Table B-15 for NPW & Solids

2019 — EPA 533 for DW

2021 — EPA OTM-45 for SV PFAS in Source Air

2021 — EPA 8327 for NPW

2022-2023 — EPA Draft 1633 for NPW & Solids

2024 — OTM-50 for volatile PFAS in air, OTM-45 revision

2024 — 1633A

How Would AI Validate Additional PFAS?

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ChatGPT

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how would you validate adding new PFAS to EPA 1633?

Validating the addition of new PFAS (per- and polyfluoroalkyl substances) to the **EPA 1633** method (which is the "*Standard Method for the Determination of Per- and Polyfluoroalkyl Substances in Water by High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS)*") involves several key steps to ensure the new substances are detected accurately and reliably. Here's a broad outline of the process:

How Would AI Validate Additional PFAS?

1. **Identify the New PFAS Compounds**
2. **Literature Review and Data Collection**
3. **Select Appropriate Analytical Parameters**
4. **Method Development and Optimization**
5. **Quality Control and Validation Testing**
6. **Interlaboratory Testing and Peer Review**
7. **Update EPA 1633 Method Documentation**
8. **Regulatory and Stakeholder Communication**
9. **Continuous Monitoring and Reassessment**

- Continue to monitor the performance of the method and, if necessary, make adjustments based on feedback from users or emerging research on PFAS. As the scientific understanding of PFAS evolves, the method may need further refinement.

1633A Comparison

1633A	537M
NPW, Soil, Biosolid, Leachate, Tissue	ALL MATRICES
40 compounds	Up to 100 compounds (lab specific)
SPE WAX	SPE WAX
Hold Time: 28/90 days	Hold Time: 28/28 days
Branched/Linear Isomers -YES	Branched/Linear Isomers -YES
LCMSMS with confirmation ion	LCMSMS - with confirmation ion
Isotope Dilution	Isotope Dilution
Recovery Correction - YES	Recovery Correction – YES
RLs: 2-20ppt	RLs: 2ppt - 20ppt

1633A Compared to 537M

Compounds Included in EPA 1633A

Perfluorobutanoic acid (PFBA)
Perfluoropentanoic acid (PFPeA)
Perfluorohexanoic acid (PFHxA)
Perfluoroheptanoic acid (PFHpA)
Perfluorooctanoic acid (PFOA)
Perfluorononanoic acid (PFNA)
Perfluorodecanoic acid (PFDA)
Perfluoroundecanoic acid (PFUnA)
Perfluorododecanoic acid (PFDoA)
Perfluorotridecanoic acid (PFTriA)
Perfluorotetradecanoic acid (PFTeA)
Perfluorobutanesulfonic acid (PFBS)
Perfluoropentanesulfonic acid (PFPeS)
Perfluorohexanesulfonic acid (PFHxS)
Perfluoroheptanesulfonic Acid (PFHpS)
Perfluorooctanesulfonic acid (PFOS)
Perfluorononanesulfonic acid (PFNS)
Perfluorodecanesulfonic acid (PFDS)
Perfluorododecanesulfonic acid (PFDoS)
Perfluorooctanesulfonamide (FOSA)
NEtFOSA
NMeFOSA
NMeFOSAA
NEtFOSAA
NMeFOSE
NEtFOSE
4:2 FTS
6:2 FTS
8:2 FTS
9Cl-PF3ONS
11Cl-PF3OUdS
DONA
HFPO-DA (GenX)
3:3 FTCA
5:3 FTCA
7:3 FTCA
NFDHA
PFMBA
PFMPA
PFEESA

Target Compounds Not Part of EPA 1633A

10:2 FTS
6:2 FTCA
8:2 FTCA
10:2 FTCA
6:2 FTUCA
8:2 FTUCA
10:2 FTUCA
PFECHS
PFPrS
PFPrA
PFMOAA
PFECAG
PFO4DA
PFO3OA
PFO2HxA
R-EVE
NVHOS
Hydro-EVE Acid
Perfluoro-n-octadecanoic acid (PFODA)
Perfluoro-n-hexadecanoic acid (PFHxDA)
EVE Acid
PFO5DA
PMPA
PEPA
MTP
PS Acid
Hydro-PS Acid
R-PSDA
Hydrolyzed PSDA
R-PSDCA
6:2 diPAP
8:2 diPAP
6:2/8:2 diPAP
10:2 diPAP
10:2 FTOH (RL=1ug/L)
8:2 FTOH (RL=1ug/L)
7:2 FTOH (RL=1ug/L)
6:2 FTOH (RL=1ug/L)
4:2 FTOH (RL=1ug/L)

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Chemical Shadows of Humans: Compounds in Wastewater

Wastewater Intrusion – A Reemerging Issue

- Cotinine
- Acetaminophen
- Paraxanthine
- Atenolol
- Caffeine
- Metoprolol
- Diphenhydramine
- Carbamazepine

Same development pathway as PFAS and 6PPD-Q

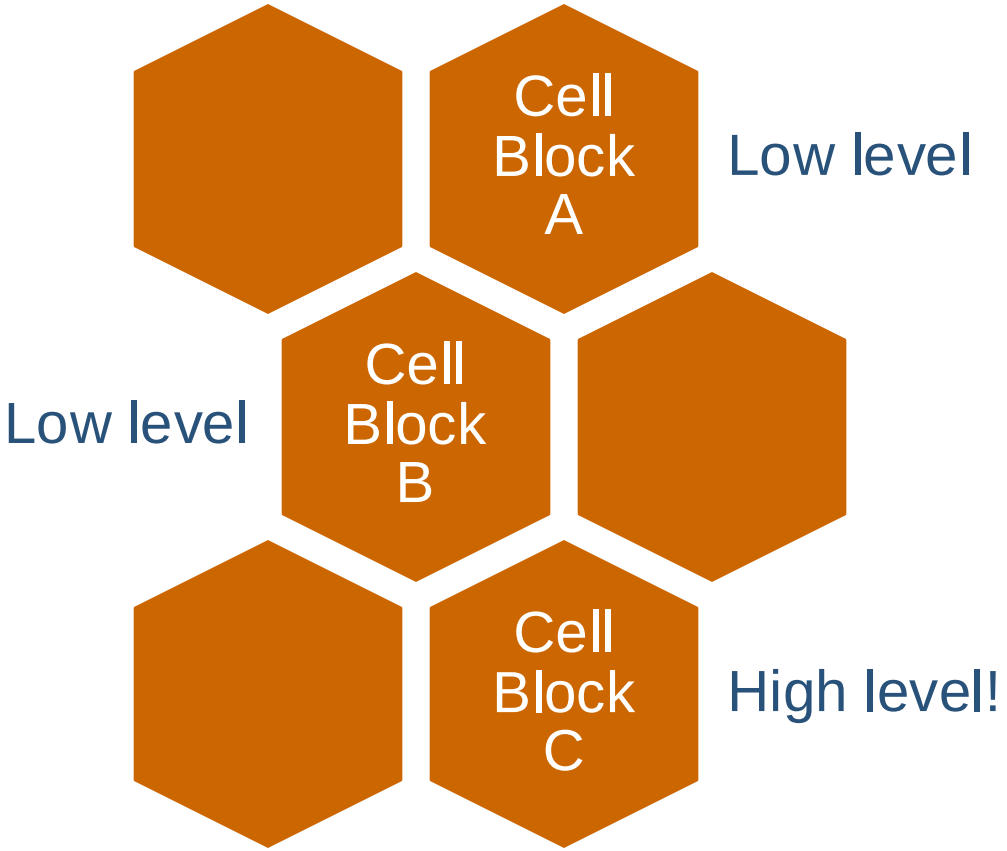
Substances of Abuse

Methamphetamine*
Cocaine*
Heroin*
Phencyclidine
Delta-9-THC
Fentanyl*
Hydrocodone*
Oxycodone*
Buprenorphine

- Xylazine*
- Codeine*
- Naloxone
- Morphine
- Methadone
- JWH Suite
- AM2201
- RCS-4,8
- HU-211

* Analyte available in "short list" substance abuse monitoring panel

Tough to Monitor “In” vs Monitoring “Out”



Now replace “Cell Block” with “Dorm” or “High-Rise Floor” or “Municipality” …

NASEM to Standardize Emerging Contaminant Response

NCHRP 25-72 [Pending]

Methods to Measure Emerging Contaminants in Stormwater

Project Data	
Funds:	$650,000
Contract Time:	36 months
Staff Responsibility:	Mike Brooks

BACKGROUND

Recent data on emerging contaminants has prompted state departments of transportation (DOTs) and water authorities to explore significant investments in stormwater sampling and treatment infrastructure. This proactive approach aims to more fully understand the occurrence and impact of emerging contaminants and the effectiveness of stormwater treatment systems. By gathering this data, decision-makers can offer more accurate, informed strategies for managing stormwater, leading to more efficient design, construction, and maintenance of treatment systems.

Research is needed to determine the methods and materials needed to measure concentrations of emerging contaminants in runoff, stormwater-treatment system effluent, and receiving waters to support cost-effective quantitative threat assessments and treatment decisions.

OBJECTIVE

The objective of this project is to develop recommended practices for state DOTs for assessing sampling methods used to measure emerging contaminants as well as new contaminants identified in the future.

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THANK YOU!