Identification and analysis of 6PPD-quinone: the coho salmon toxicant

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+ Various Collaborators

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Other collaborators:
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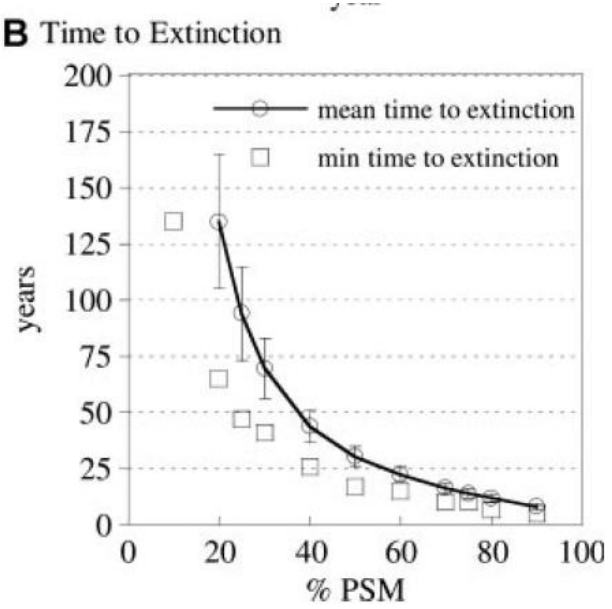
Fall Stormwater kills spawning coho salmon: "Urban Runoff Mortality Syndrome" (URMS)

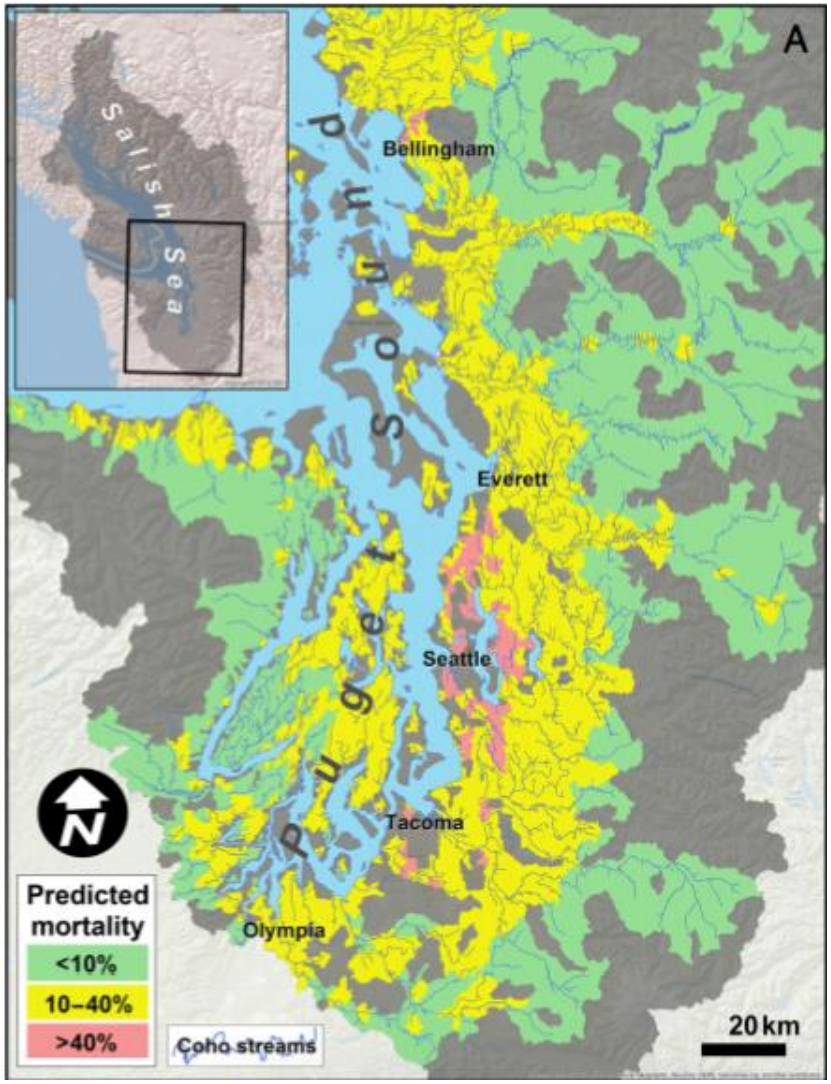


Coho mortality at Miller Creek, Oct 30th, 2018

- PNW, every year after fall storms (Oct- Nov)
- Symptoms: losing equilibrium, gaping, circling
- Killed in hours, many died before spawning
- Species specific

Spromberg et al., *Integr. Environ. Assess. Manag.*, **2011**

Coho mortality syndrome is linked to urbanization and traffic intensity



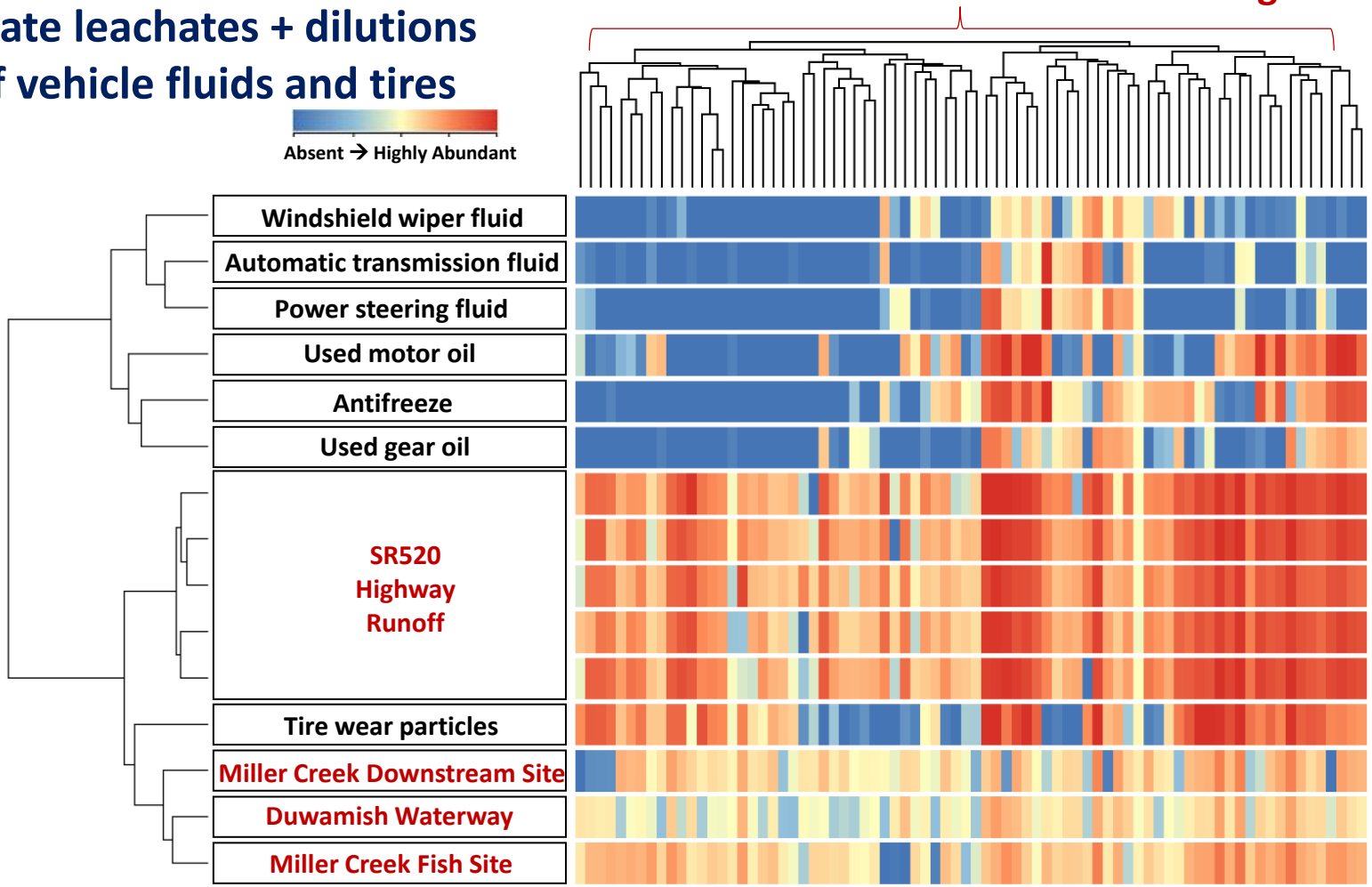
- "Urbanization gradient" predicts coho mortality risk
- Road runoff kills coho, same symptoms as in creeks
- Toxicant(s) currently **unknown**; likely related to road/cars

Feist et al. *Ecol. Appl.* **2017** (NOAA & WSU study)

HRMS: Compare the mortality signature to different vehicle-related sources

Create leachates + dilutions of vehicle fluids and tires

Each column = 1 of the features in the signature

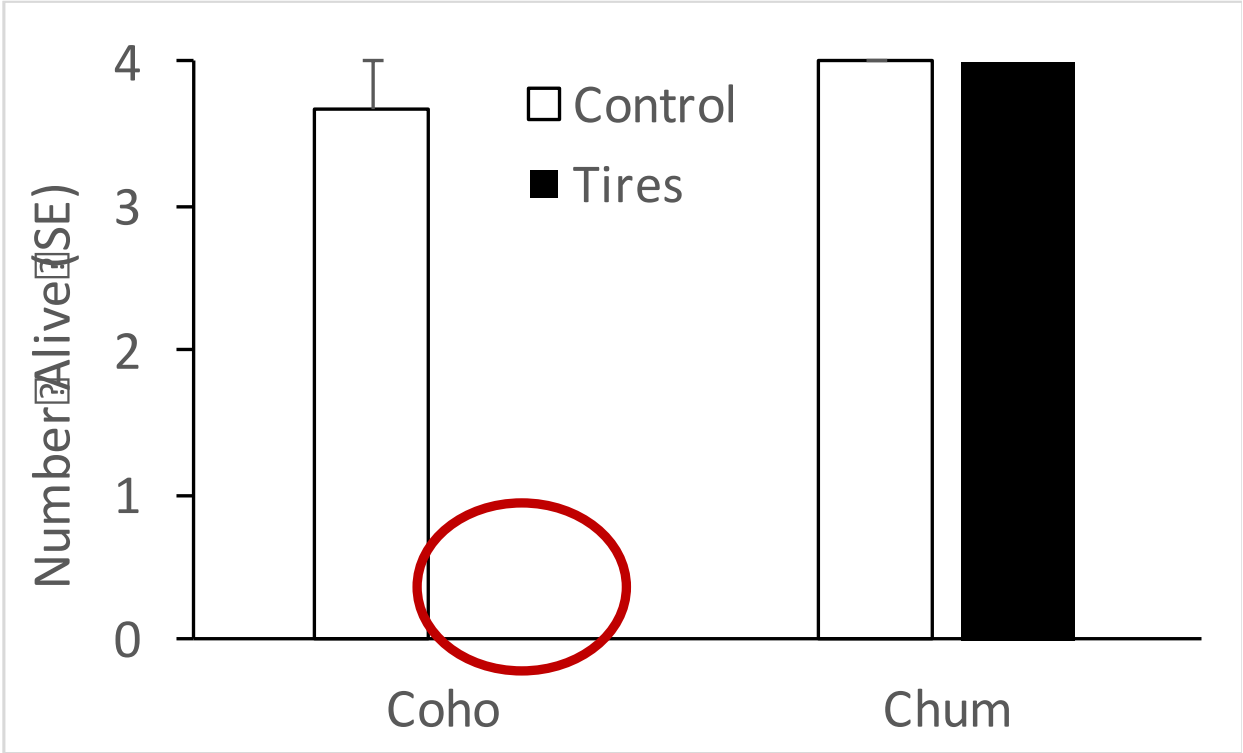


Tire wear particle leachates cluster with waters from coho mortality events

Peter et al, *ES&T,* 2018

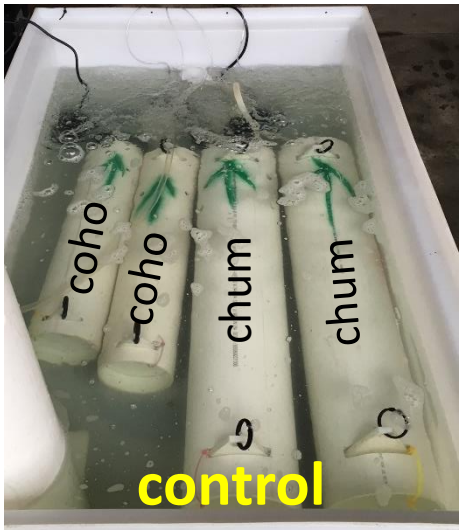
Tire wear particle leachate kills coho

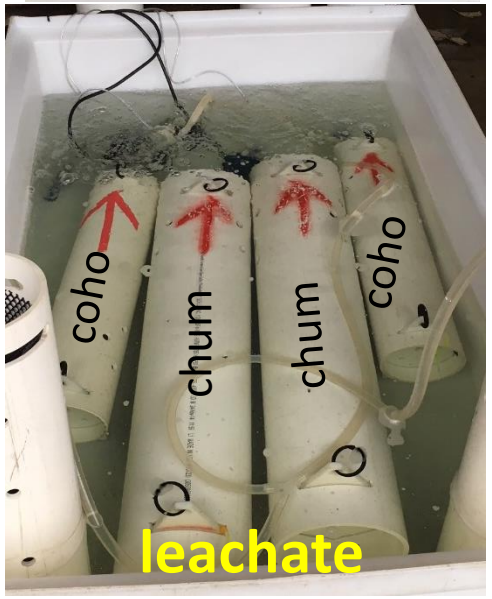
~320 mg/L tire rubber (HRMS: more like ~200 mg/L)
Leaching: 24 h at 8 ºC
Expose fish up to 24 h, Repeated 4X (64 fish total)



16/16 exposed coho died, 16/16 exposed chum lived

What are the lethal toxicant(s) in tire ??

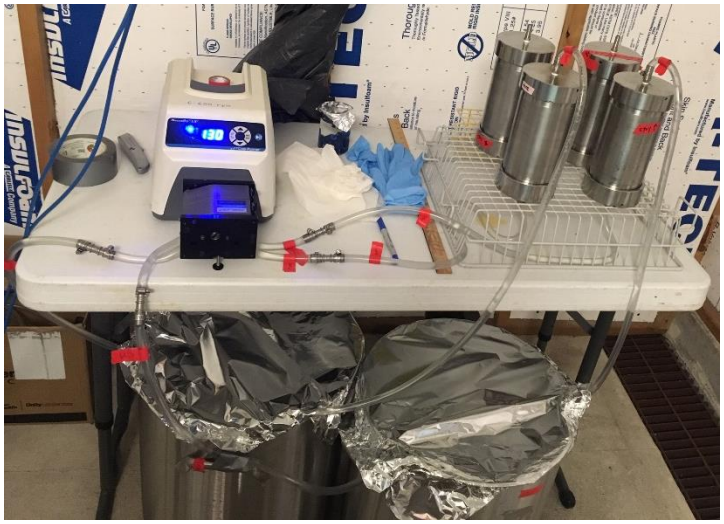




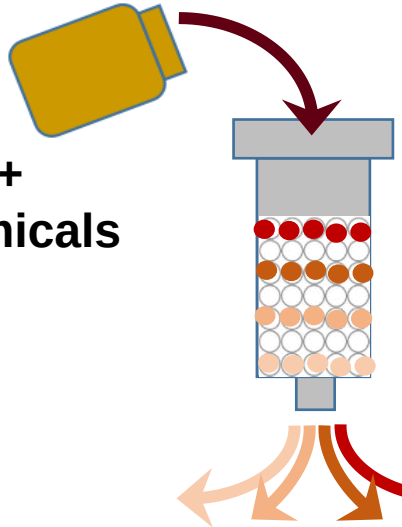
Jen McIntyre WSU/NOAA/USFWS studies

Identify the toxicant(s): tire leachate fractionation

Leach tire particles into water



Fractionate tire leachate & expose juvenile coho



2000+ chemicals

Which part is toxic?

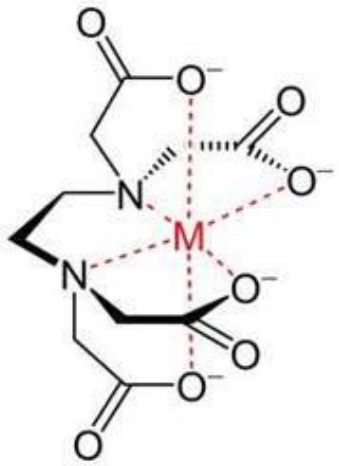
Toxic fraction go to HRMS for identification

Initial fractionations told us:

1. Sand filtration

Still toxic

2. Add EDTA



EDTA deactivates metals in solution

Still toxic

3. Cation exchange

Still toxic

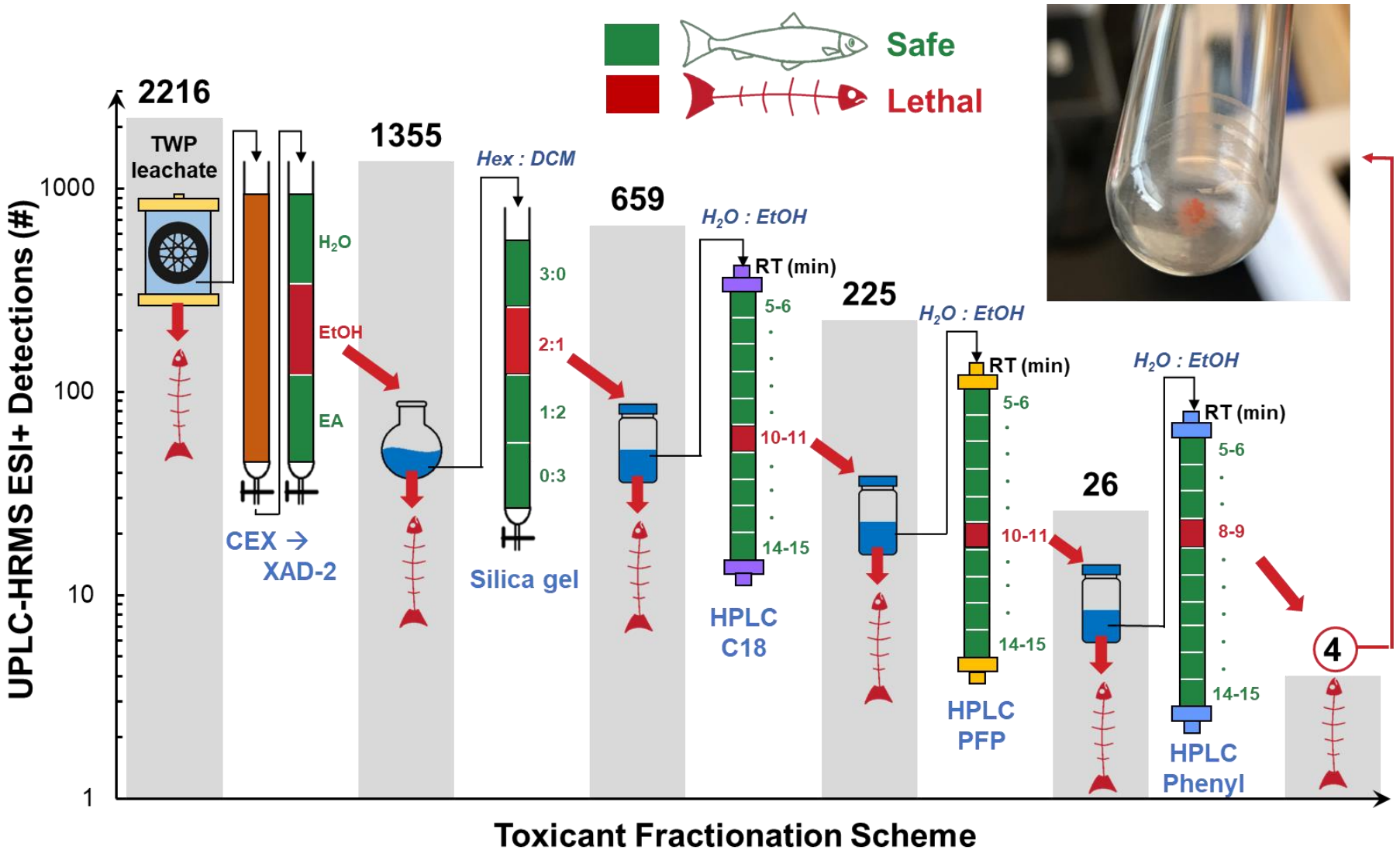
The toxicant is:

1 - Dissolved (not particles)

2 - Organic (not a metal)

3 - Not a cation (not positively charged)

Fractionation of tire wear particle leachate

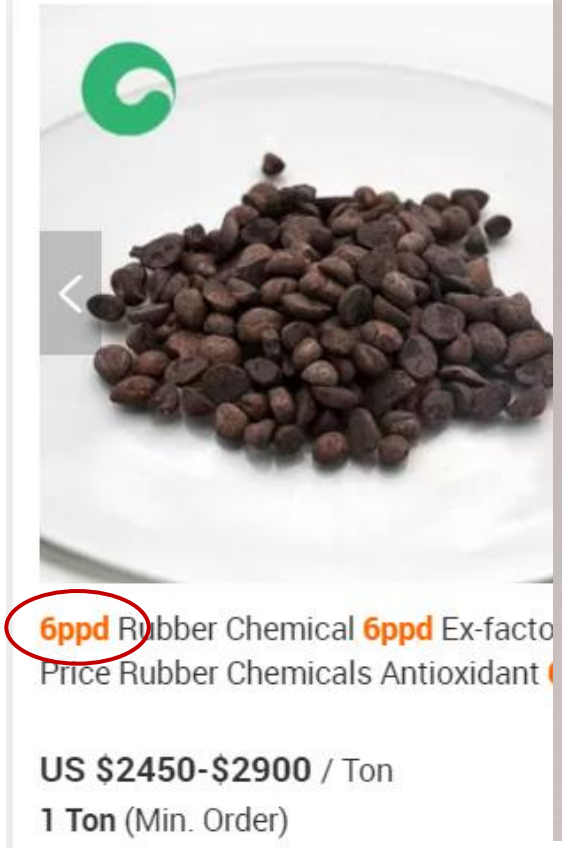


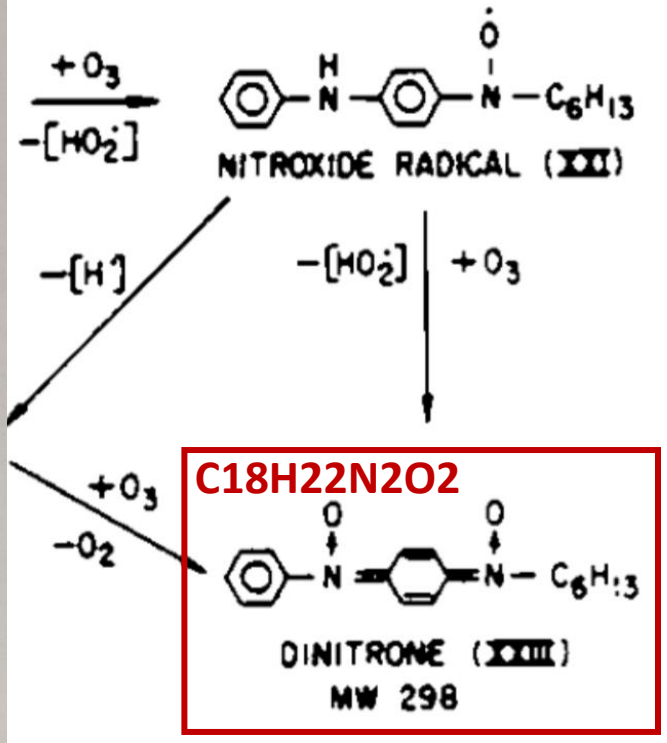
Purified pink-magenta solid (C18H22N2O2) acutely lethal to coho in hours

Tian et al., *Science*, **2020**

Linking the toxicant to industrial chemical

- **$C_{18}H_{22}N_2O_2$** NOT found in literature/database about tire rubber chemicals: "**True Unknown**"
- Assuming transf… changed H and O but same C ar… **$_{24}N_2$** ("**6PPD**")



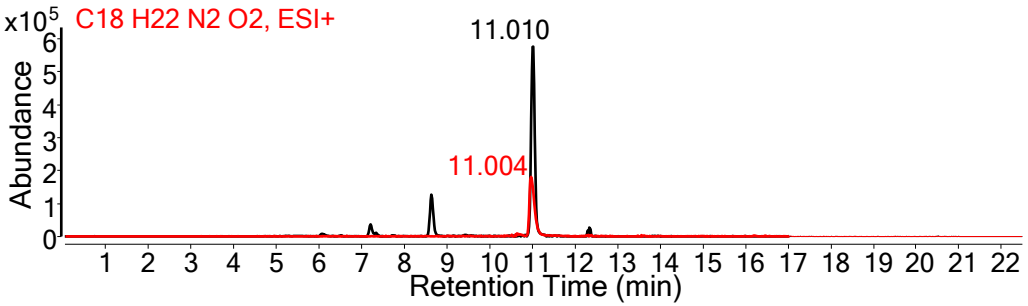


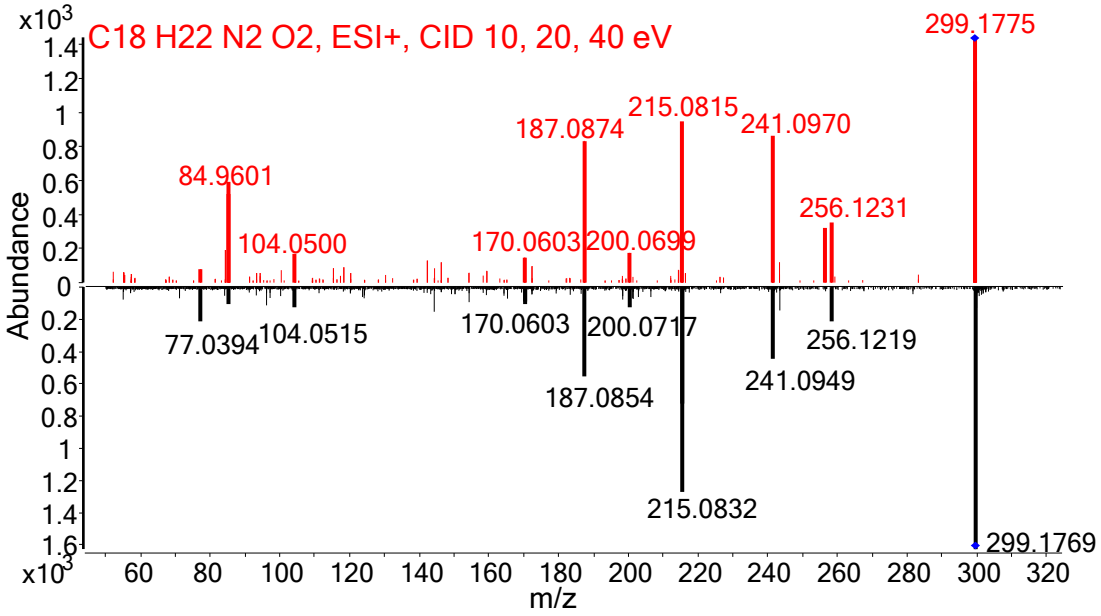
t al., *Rubber. Chem. Technol.*, **1983**

Confirmation and structure elucidation

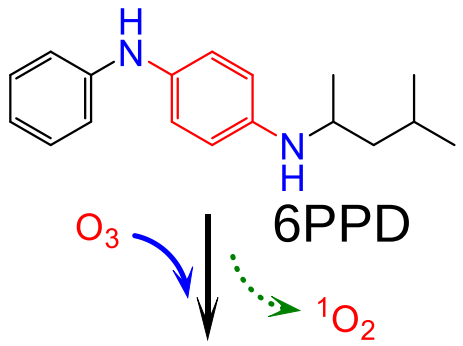
HRMS & NMR: same compound

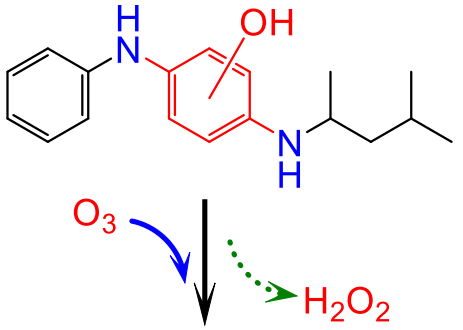
Tire leachate | 6PPD ozonation

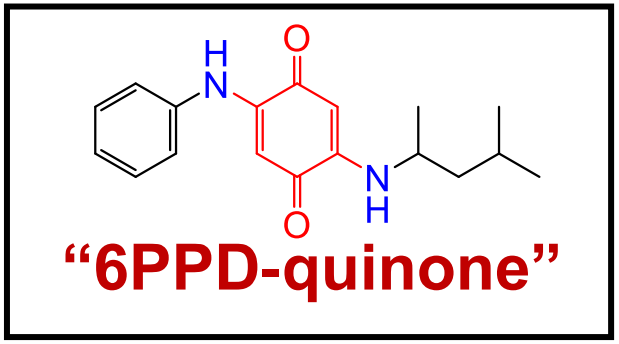




NMR: identify structure







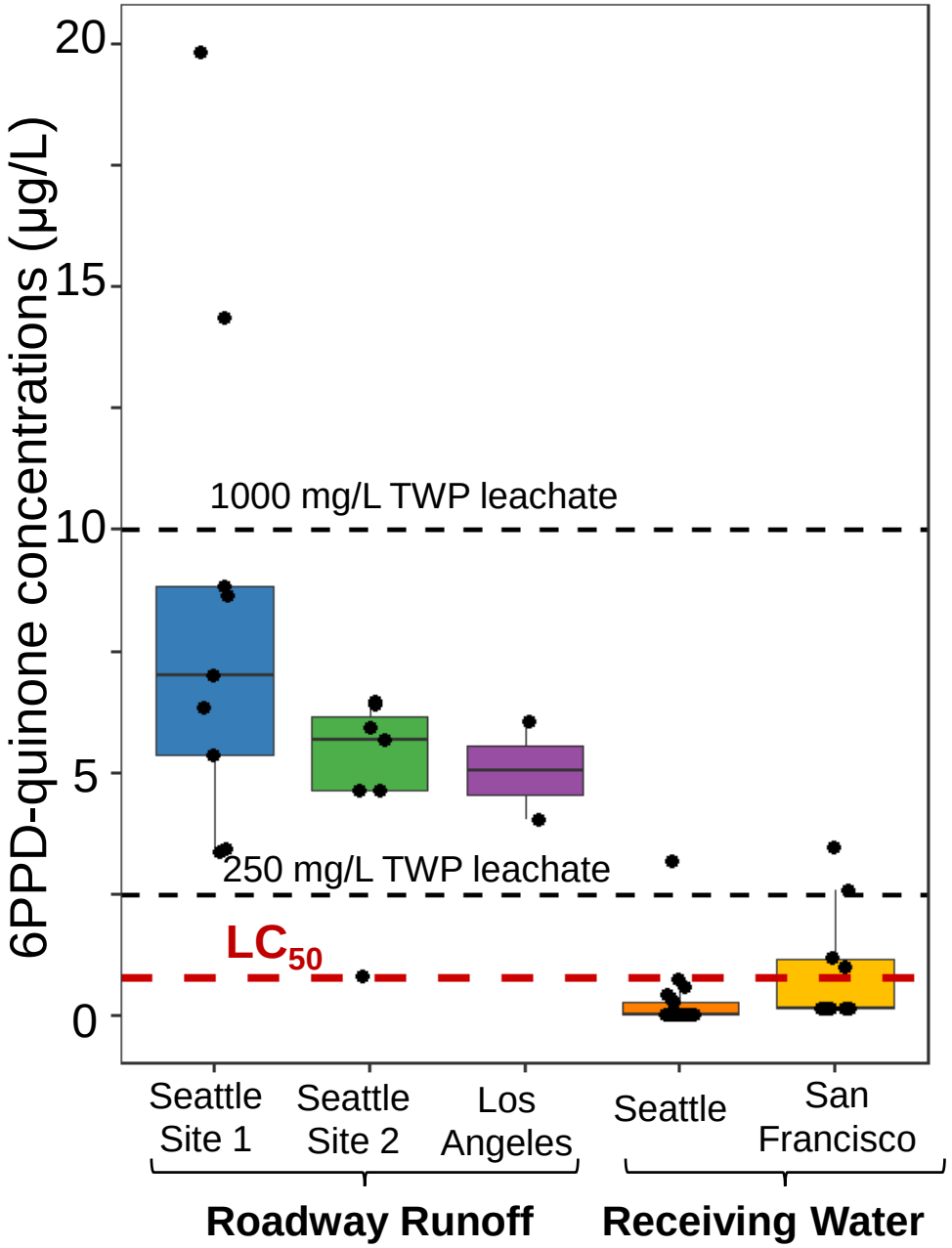
Tian et al., *Science*, **2020**

Field URMS
(Longfellow Creek, 2014)

Lab exposure with purified 6PPD-quinone

Same symptoms before death

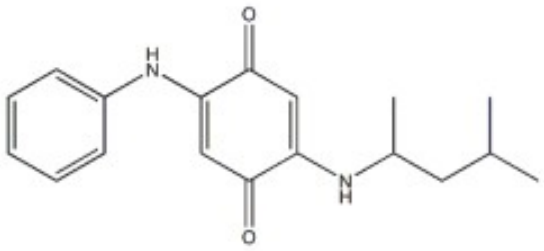
Retrospective analysis: environmental detection



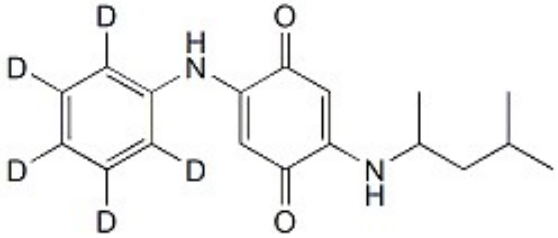
- Detected in 18/18 road runoff, all above LC50 for coho
- Detected in 6/7 creek water samples related to URMS, concentration near or above LC50
- Widespread in US west coast (Seattle, Los Angeles, San Francisco

Tian et al., *Science*, **2020**

New progress: commercial standard and ISTD



6PPD-quinone
C18H22N2O2
m/z 299



D5-6PPD-quinone
C18H17D5N2O2
m/z 304

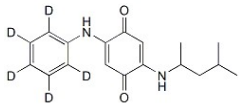
Item number	688151
Formula	C18H17D5N2O2
Molecular weight	303,41 g/mol
Quantity	1 ml
Concentration	100 µg/ml
Solvent	Acetonitrile
Storage conditions	4 °C
CoA / SDS	Retrieve CoA Retrieve SDS / MSDS Show Safety Data Sheet online

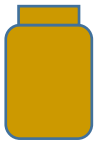
Accurate quantification based on isotope-labeled internal standard

6PPD-Q quantification method (ISTD)

Sample Processing

D5-6PPD-Q
5 ng





Water samples (200 mL)

Oasis HLB + micro glass beads

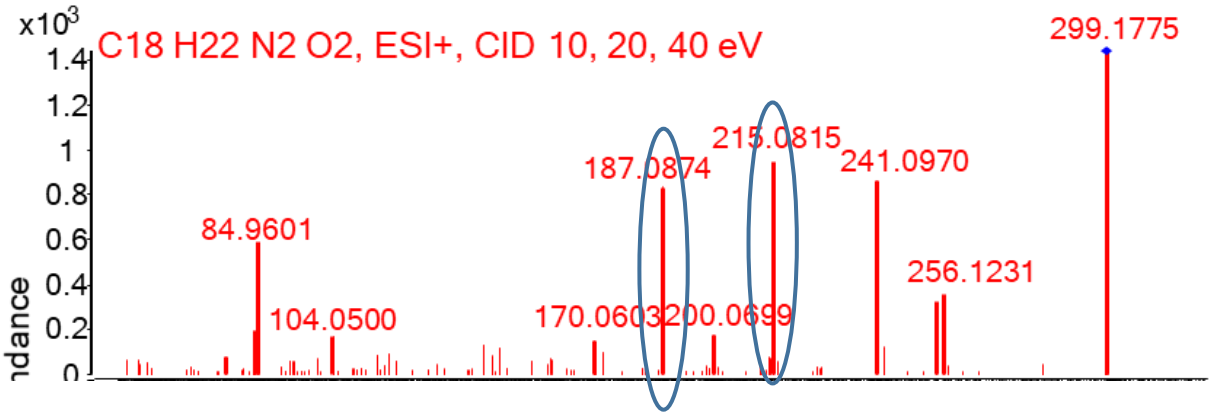
C18 Poroshell HPH 2.7 µm

Analyze on HPLC-QqQ

Agilent 6460

ESI (+)

MRM by HPLC-QqQ

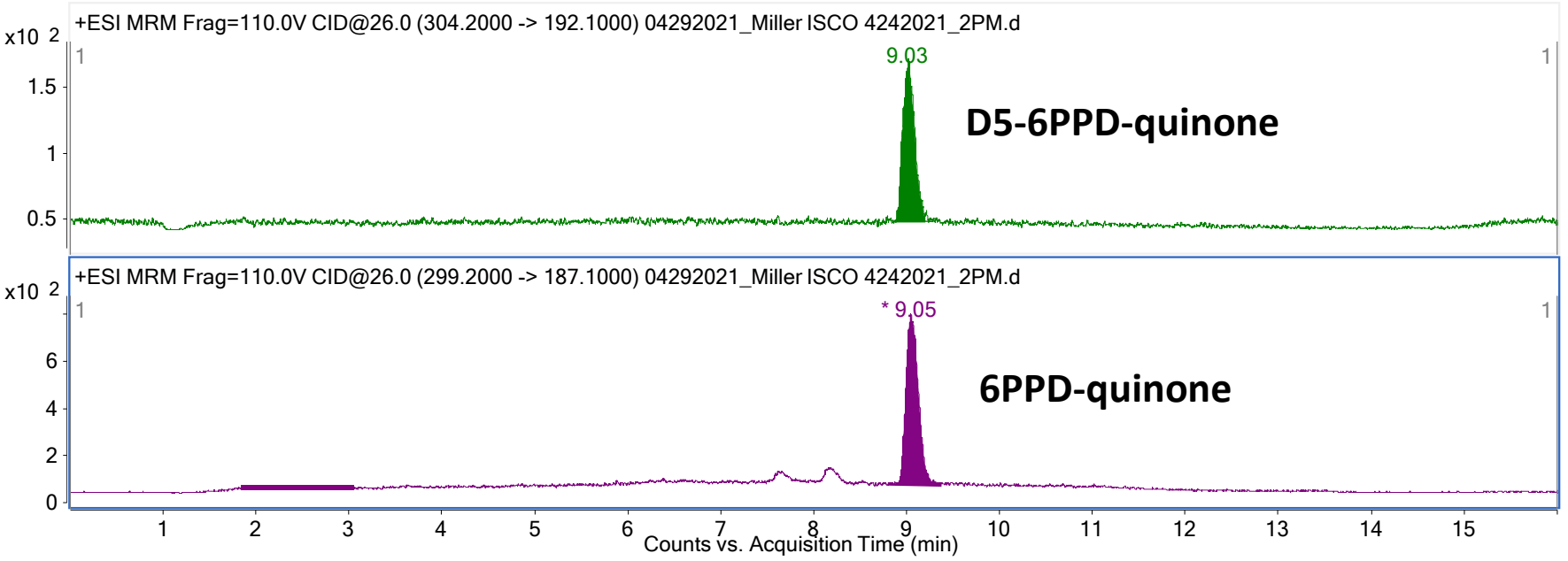


Qual: 299 -> 215

Quan: 299 -> 187

Method performance

- D5-6PPD-Q recovery: 54-82%; 6PPD-Q spike recovery: 85-109%
- Instrumental sensitivity: LOD ~0.01 ng/mL, LOQ ~0.03 ng/mL
- Method performance
  - Creek baseflow: LOD ~0.8 ng/L, LOQ ~2.5 ng/L
  - Creek stormwater and road runoff: LOD 1.5-2.0 ng/L, LOQ 5.0-6.5 ng/L
  - Matrix suppression >> analyte loss



Thanks for your attentions!
Any questions?

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