# Evaluation of Water Quality Impacts on Coho Salmon

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Nina Zhao, Ximin Hu, Mike Dodd, Jen McIntyre
+ lots of collaboration with U. Toronto, WSU-Puyallup,
NOAA-NMFS, U.S. Fish and Wildlife

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# Acute Toxicity: "Urban Runoff Mortality Syndrome"

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

Nathaniel L. Scholz[1]*, Mark S. Myers[1], Sarah G. McCarthy[2], Jana S. Labenia[1], Jenifer K. McIntyre[1], Gina M. Ylitalo[1], Linda D. Rhodes[1], Cathy A. Laetz[1], Carla M. Stehr[1], Barbara L. French[1], Bill McMillan[3], Dean Wilson[2], Laura Reed[4], Katherine D. Lynch[4], Steve Damm[5], Jay W. Davis[5], Tracy K. Collier[1]

Coho pre-spawn mortality (PSM) is widespread and recurrent in urban streams

Longfellow Creek 2003

Des Moines Creek 2004

Longfellow Creek 2005

Coho PSM rates measured in Seattle-area urban streams have ranged from 40 – 90% of the total run (2002-2009)

**-Urban stormwater runoff kills coho salmon in 2-24 hrs:**

**"Urban Runoff Mortality Syndrome", or URMS**

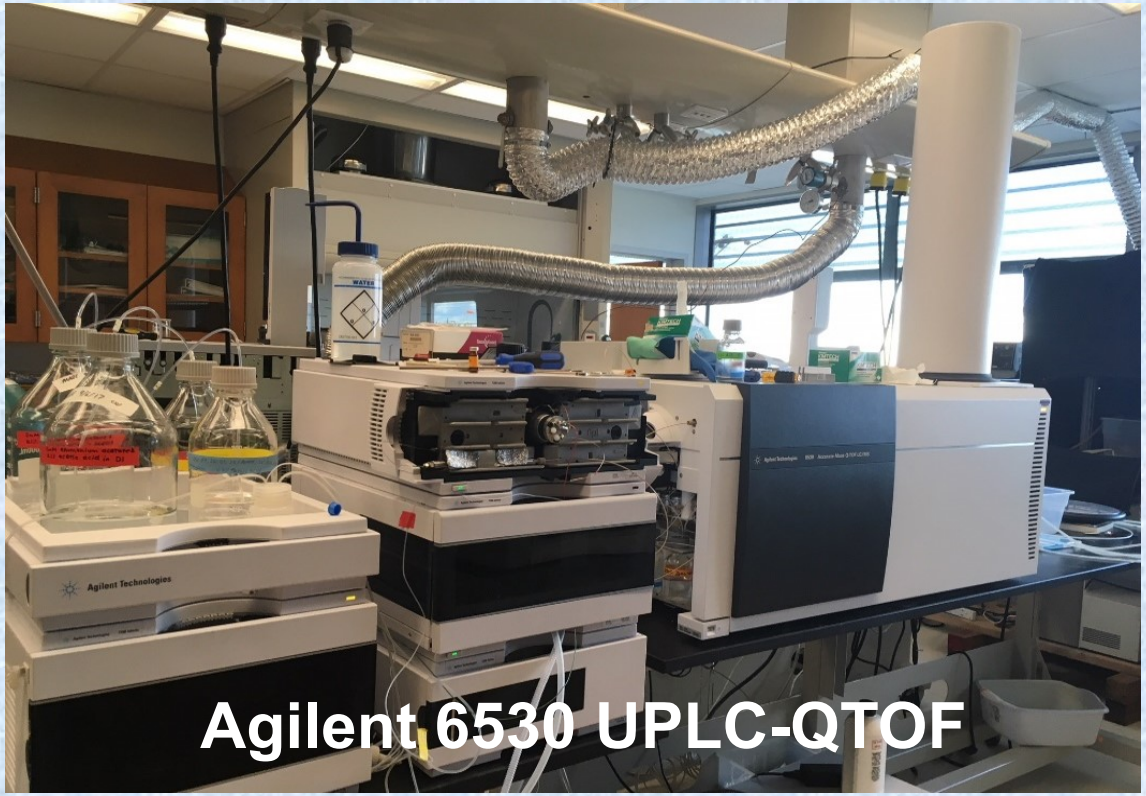
**-URMS cause unknown: not pathogens, metals, pesticides, PAHs, ammonia, basic water quality parameters, etc.**

***-Coho more sensitive, no mortality in cutthroat trout or chum salmon***

*Scholz et al. 2011*

# CUW Approach: High Resolution Mass Spectrometry

**Which chemicals and sources are most important?**




Agilent 6530 UPLC-QTOF

## High Resolution Mass Spectrometry (HRMS):

-Identify novel compounds, holistic screening

-Detect it "all", then figure out what's there.

-More unbiased, fewer assumptions about what is going on

**Coupled together HRMS and toxicology exposures in an "Effects Directed Analysis" framework**

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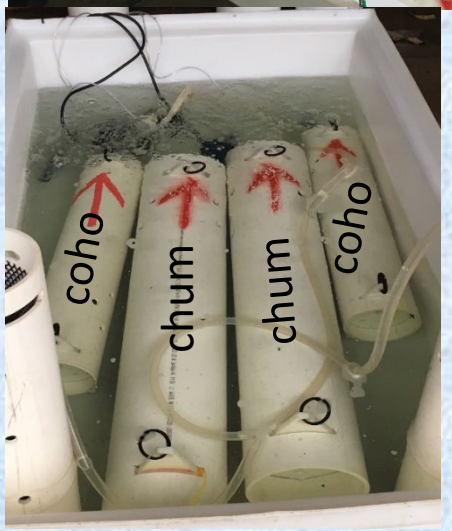
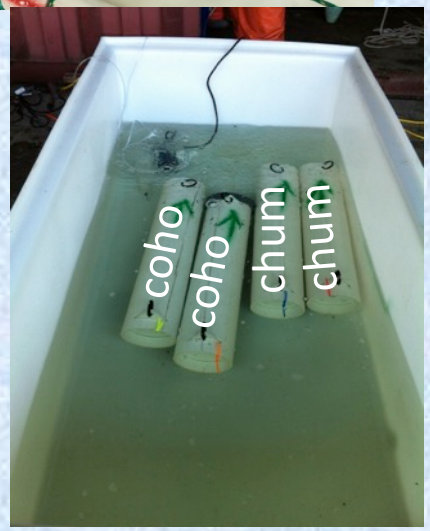
# 1) Ecotoxicology Studies

**Tire Rubber Leachate**

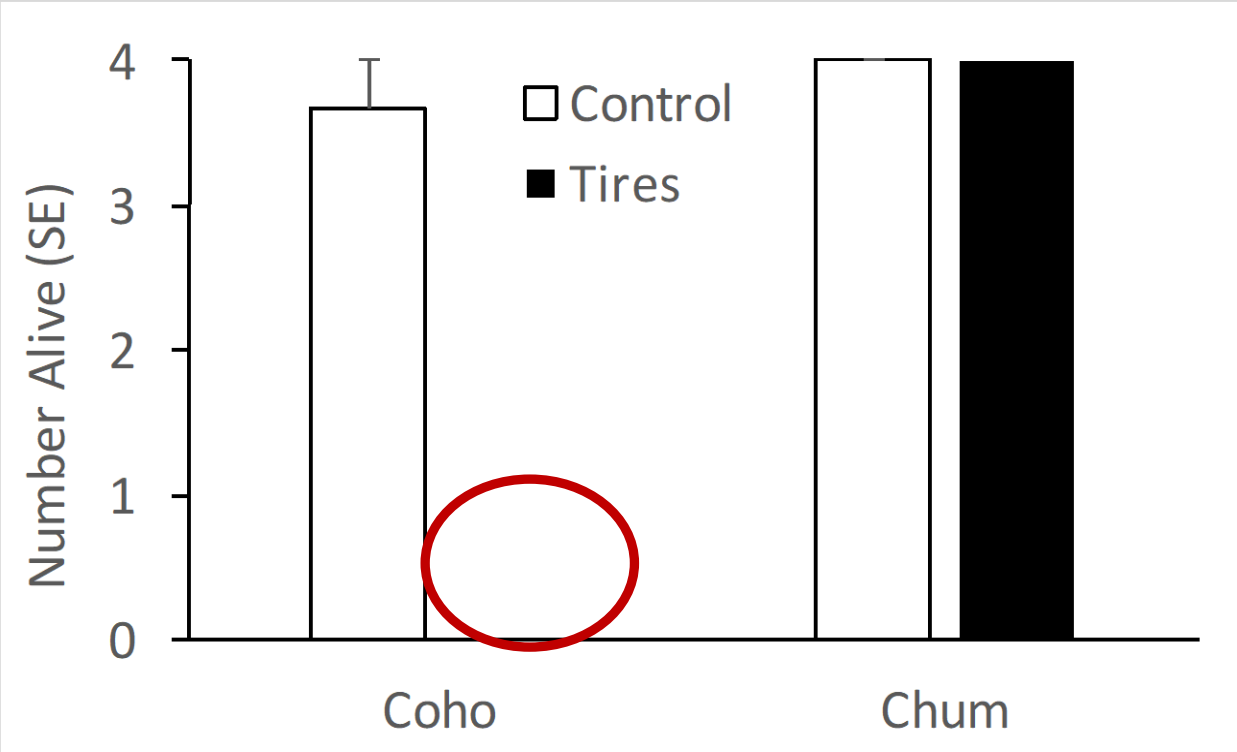


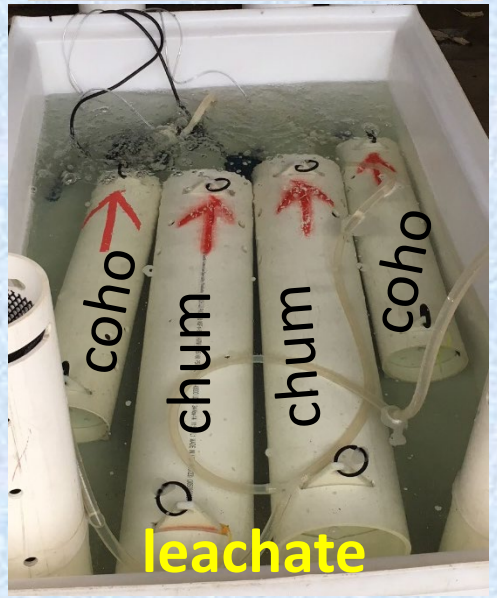

Jen McIntyre (WSU-Puyallup) and NOAA studies

# 1) Tire Leachate is Lethally Toxic to Adult Coho

**~320 mg/L tire rubber (HRMS: more like 100 mg/L)**
**Leaching: 24 h at 8-10 ºC**
**Exposure: 24 h**
**Repeated 4X (64 fish total)**



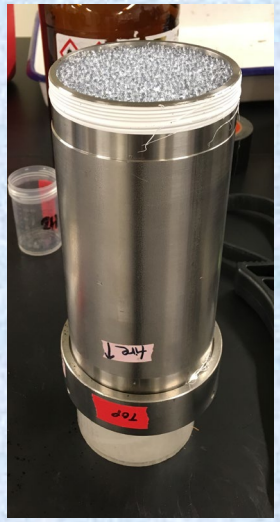

**16 of 16 exposed coho salmon died,**
**16 of 16 exposed chum salmon lived**

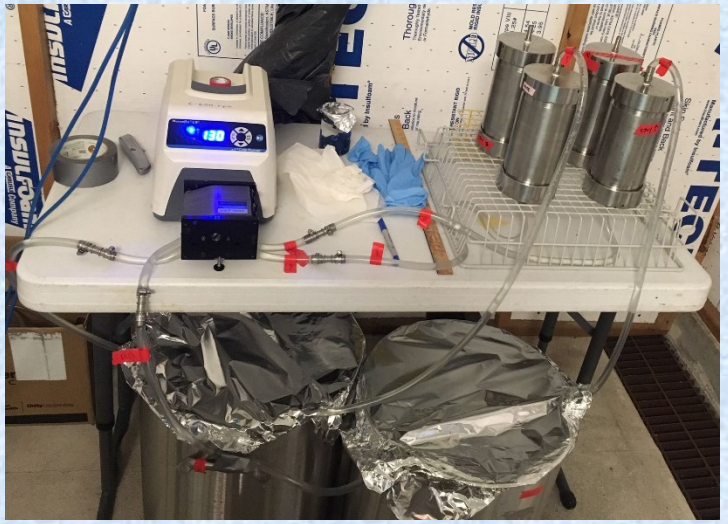



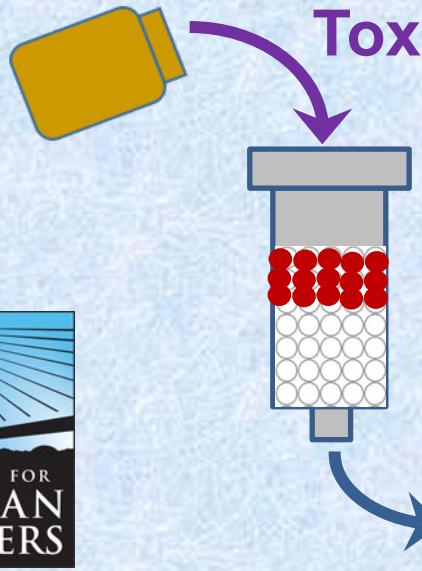

Jen McIntyre (WSU-Puyallup) and NOAA studies

# 2) TIE/EDA Identify Toxicant(s) in Tire Rubbers

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








**What chemicals were removed?**

**Are these still toxic?**

5 juvenile coho in 15-30 L, 24 h

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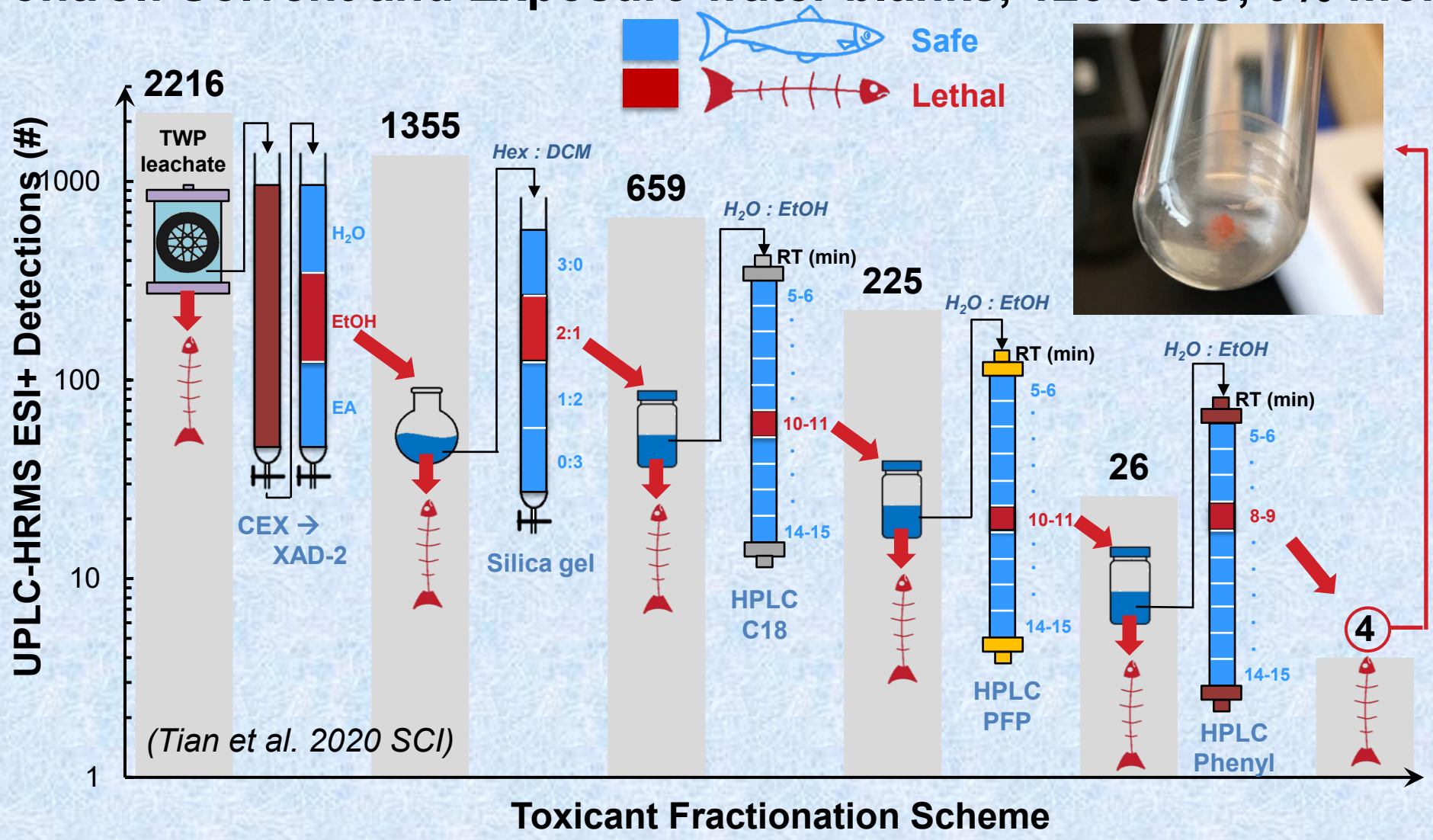
# 2) We Isolated a Single Toxic Fraction..

**+Control: TWP leachate, 27 exposures, 135 coho, 98.5% mortality**

**-Control: Solvent and Exposure water blanks, 125 coho, 0% mortality**



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# 2) Final Toxic Fraction: What Was $C_{18}H_{22}N_2O_2$??

**$C_{18}H_{22}N_2O_2$** NOT found in literature/databases for environment or tire rubber chemicals "**True Unknown**"

-Assumed transformation product, held C and N constant.. Looked for matches.

→ **$C_{18}H_{24}N_2$** ("**6PPD**") in EPA Crumb Rubber report

Lattimer et al., 1983
*Rubber. Chem. Technol.*

**$C_{18}H_{22}N_2O_2$**
**"dinitrone"**

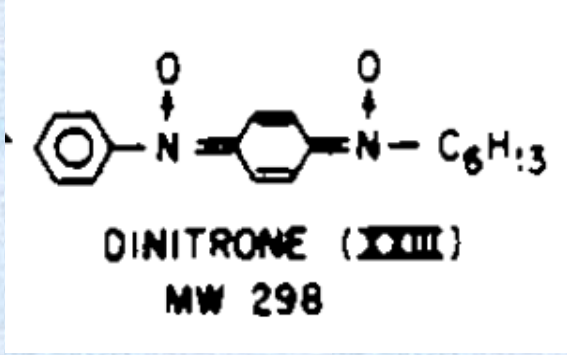


TABLE I

COMPOSITIONS OF OZONE-HPPD REACTION PRODUCTS

| Measured mass[a] | Atomic composition | Calculated mass[b] |
|---|---|---|
| 184.0997[c] | $C_{12}H_{12}N_2$ | 184.1000 |
| 198.0793[c] | $C_{12}H_{10}N_2O$ | 198.0793 |
| 214.0742[c] | $C_{12}H_{10}N_2O_2$ | 214.0742 |
| 268.1579[c] | $C_{17}H_{20}N_2O$ | 268.1576 |
| 268.1944[c] | $C_{18}H_{24}N_2$ | 268.1939 |
| 211.1235 | $C_{14}H_{15}N_2$ | 211.1235 |
| 282.1734[c] | $C_{18}H_{22}N_2O$ | 282.1732 |
| 225.1023 | $C_{14}H_{13}N_2O$ | 225.1028 |
| 296.1889[c] | $C_{19}H_{24}N_2O$ | 296.1888 |
| 298.1688[c] | $C_{18}H_{22}N_2O_2$ | 298.1681 |
| 534.3716[c] | $C_{36}H_{46}N_4$ | 534.3722 |
| 477.3011 | $C_{32}H_{37}N_4$ | 477.3018 |
| 546.3356[c] | $C_{36}H_{42}N_4O$ | 546.3358 |
| 503.2819 | $C_{33}H_{35}N_4O$ | 503.2811 |
| 489.2654 | $C_{32}H_{33}N_4O$ | 489.2654 |

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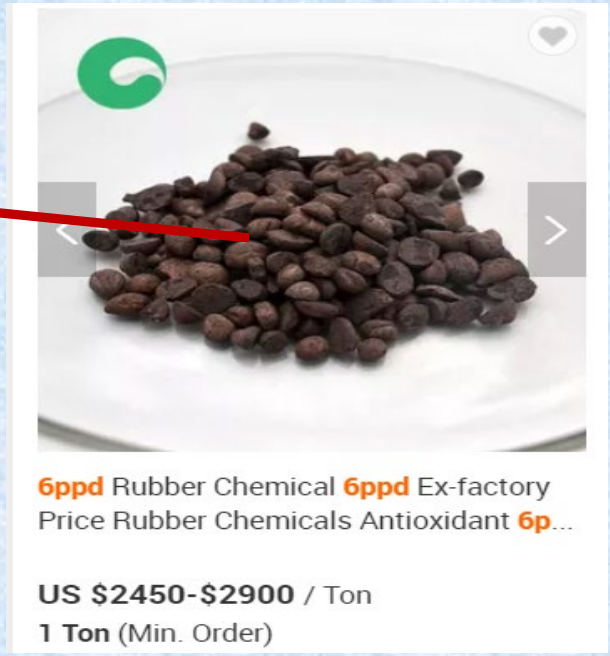
# 3) 6PPD Ozonation

**-Used industrial grade (96%) 6PPD, protocol built from Lattimer et al. 1983, worked with Mike Dodd (UW CEE)**

**500 mL/min, 20 min, 6.9% (v/v) $O_{3(g)}$**


**Ozonation Products form..**

**6PPD**




*(Tian et al. 2020 SCI)*

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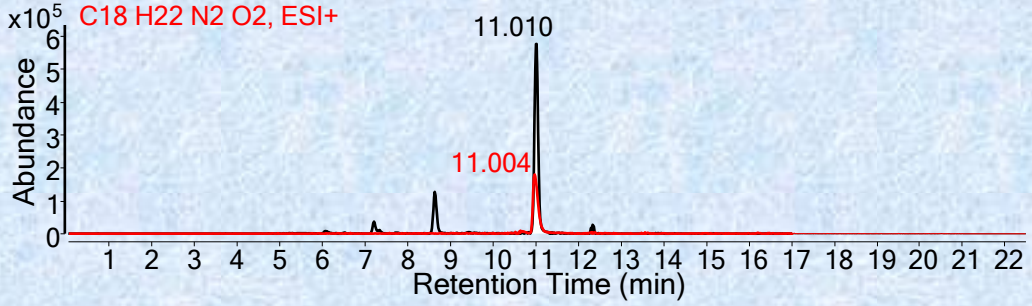
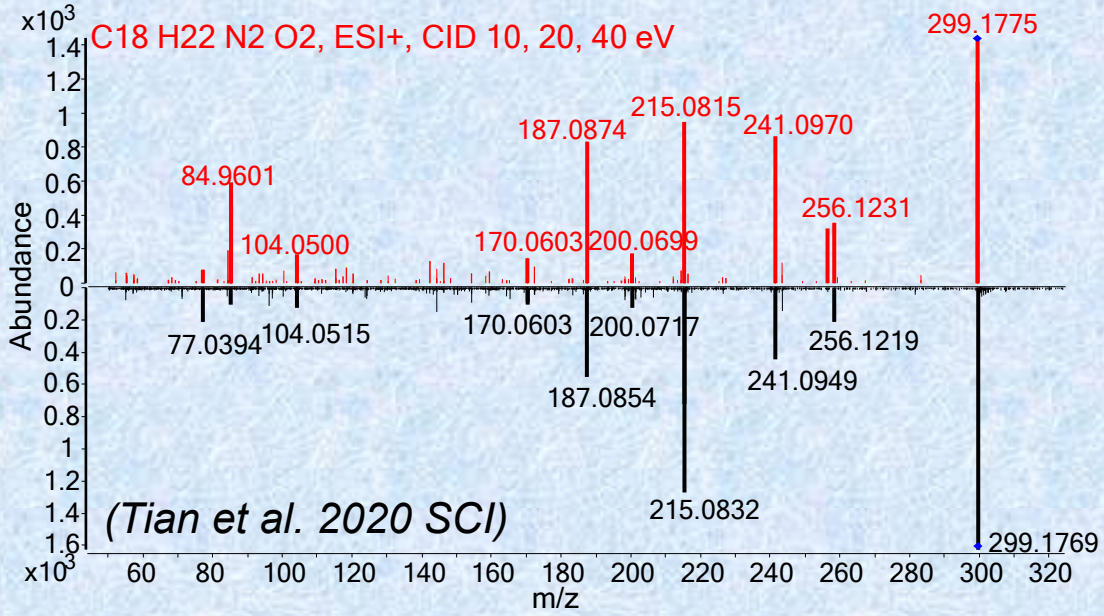
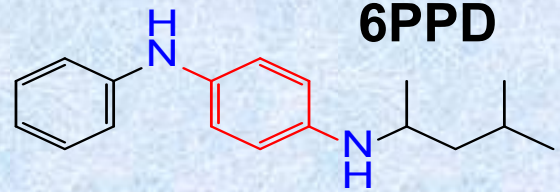
# 3) Purified $C_{18}H_{22}N_2O_2$ from TWP Leachate and Ozonation

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

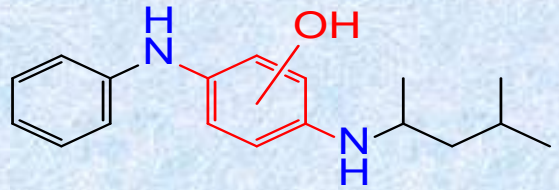
Tire leachate | 6PPD ozonation



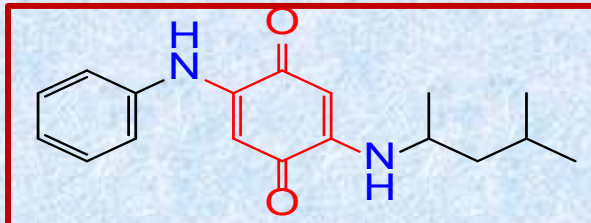









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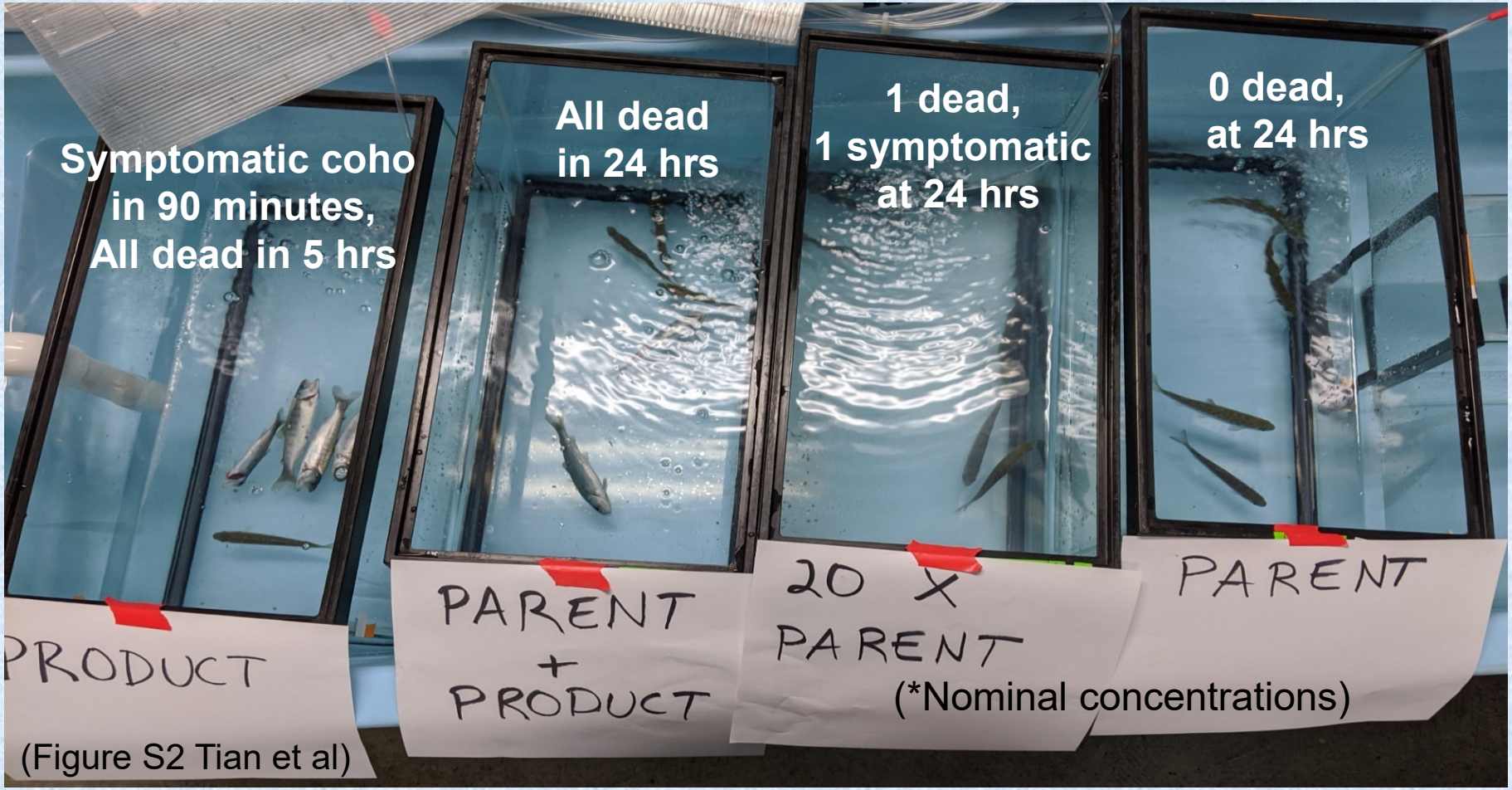
# 3) 6PPD-Quinone Exposure to Juvenile Coho

**~20 ug/L* 6PPD-quinone** | **~20 ug/L* $O_3$ mixture** | **~450 ug/L* 6PPD** | **~30 ug/L* 6PPD**




(Figure S2 Tian et al)

**Products can be more toxic than parent chemicals…**

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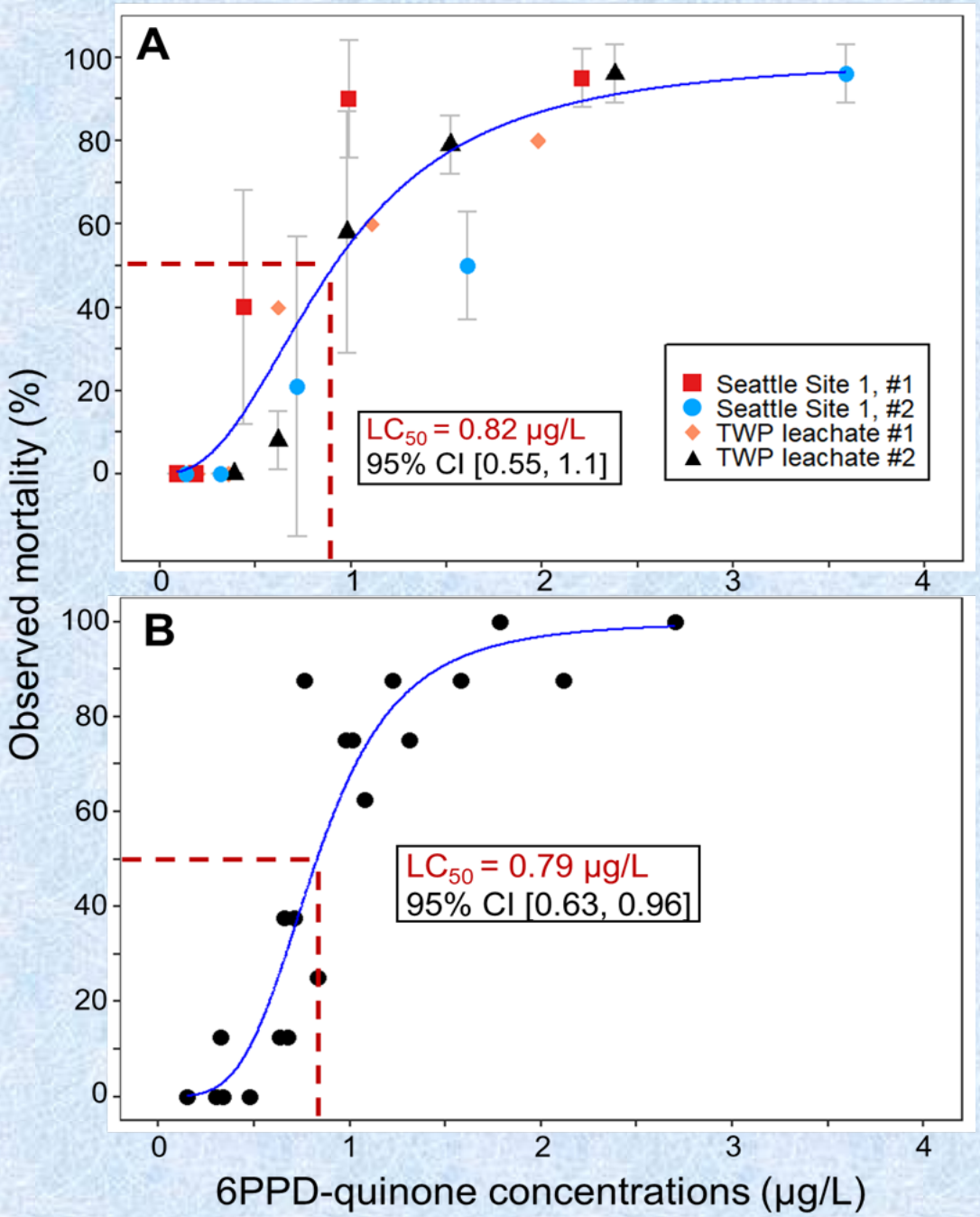
# 6PPD-Quinone Controlled Exposures




**Dilutions of multilane roadway runoff and TWP leachate**
**N = 365 coho, error bars reflect 3X replicates of 8 fish**

**Controlled Exposures:**
**6PPD-quinone ~98% purity**
**Each exposure:**
**N = 80 coho, 2 replicates**
**(160 fish total)**

**6PPD-quinone:**
**"Primary causal toxicant"**

*(Tian et al. 2020 SCI)*

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# Environmental Relevance

**6PPD-quinone formation is expected for any location containing tire rubber residuals, including recycled materials**

**Hazardous Substances Series**

OSPAR Commission
2005 (2006 Update)

## 4-(dimethylbutylamino)diphenylamine (6PPD)

it might evaporate into the atmosphere. It might also (anew) be bound to the rubber matrix. Since 6PPD is a reactive anti-ozonant and antioxidant, 6PPD which reaches the surface of rubber products will be rapidly degraded by ozone, or other photooxidants (*Lattimer et al. 1982, Layer*

- in general - are not extractable with water or organic solvents. The predominant sink of 6PPD is the reaction with ozone, which is the chemical base of the anti-cracking effect of 6PPD (*Lorenz et al. 1985,*

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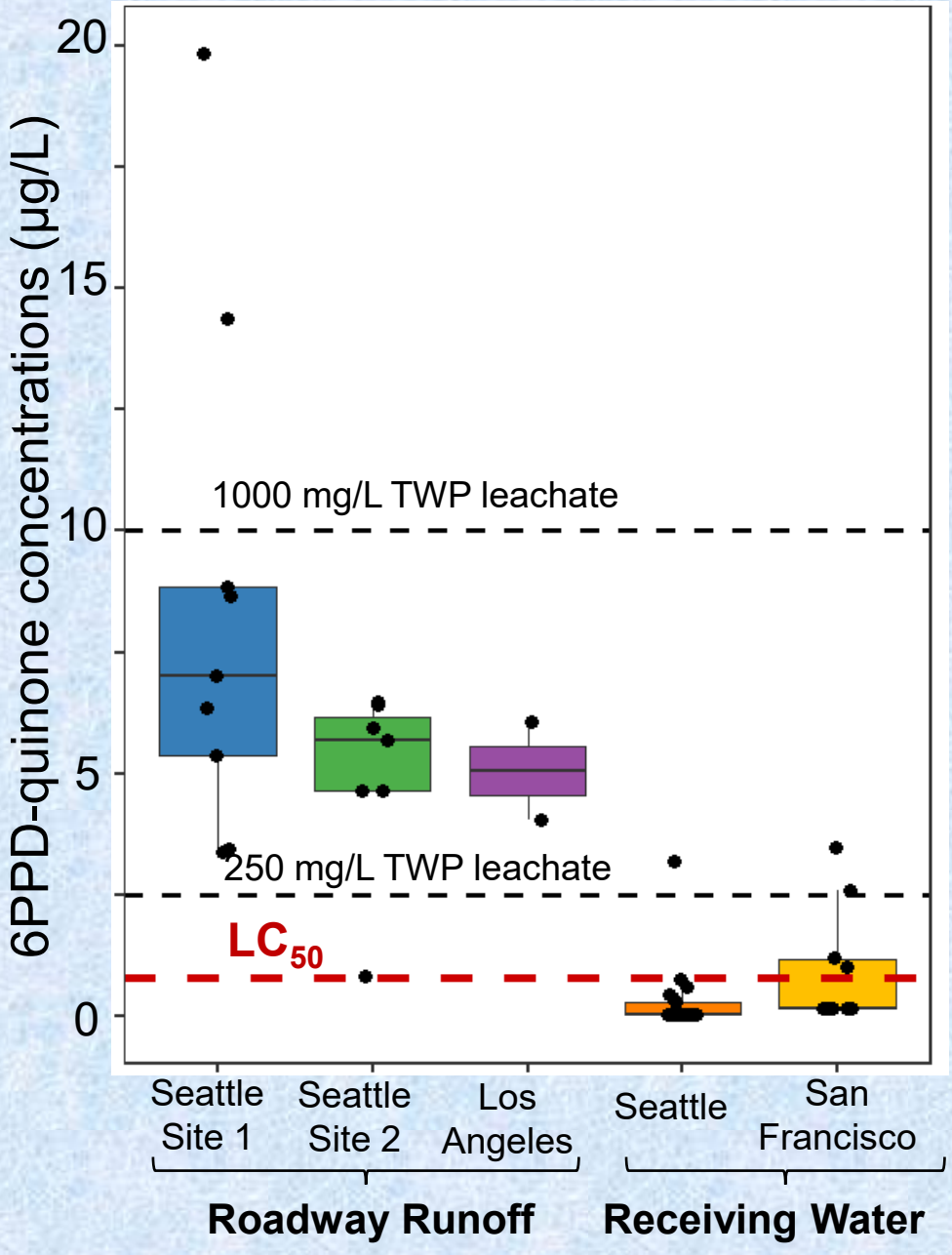
# 4) Environmental Relevance




- **Detected in 18/18 roadway runoff, all above $LC_{50}$**
- **Detected in 6/7 creeks sampled during URMS events, concentrations near or above $LC_{50}$**
- **Detected in Seattle, Los Angeles, San Francisco samples**

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# Field Mortality

October 21, 2017
Lower Duwamish R.
(Puget Soundkeeper)

# 6PPDQ Lab Exposure

February 17, 2020
WSU-Puyallup
(Kolodziej video)

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# Observations

-Tire rubber residuals are sources of complex mixtures of emerging organic contaminants and water quality impairment. Coho salmon are highly sensitive to these mixtures.

-6PPD: Toxic + designed reactive. Surprised? We should not be surprised when products of toxic compounds also are toxic.

-For California: Coho salmon are telling us to pay special attention when busy roads intersect with sensitive habitats/species.

-Habitat restoration includes chemical habitat (water quality) in addition to physical habitat. Sensitive species restoration will likely teach us new things about chemical habitat quality.

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**The big picture: How can fish and people coexist? What will we need to change in our lives and products to reduce our toxicity? Can we make "salmon safe" tires?**

# Acknowledgements and Thanks To:

## The CUW Research Team!

+

Kathy Peter

Alex, Allan, Zhenyu, Rachel H., Rui, Ting, Nina, Melissa, Rachel L., and Kathy

# Acknowledgements and Thanks To:


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  - NOAA NWFSC – Nat Scholz, James Cameron, Jessica Lundin (and many others)
  - WSU-Puyallup Stormwater Center – Jen McIntyre, John Stark (and many others)
  - Andre Simpson et al. (U. Toronto)
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  - WSDOT – Alex Nguyen, Jana Crawford
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  - EPA-National Estuary Program
  - WA Department of Ecology
  - Miller Walker Community Salmon Investigation, Puget Soundkeeper, Thornton Creek Alliance

# Emcon 2021 Conference

**7th International Conference on Emerging Contaminants**
**Virtual Event, September 13-14, 2021**

**Will have lots of content on roadway runoff, microplastics, PFAS, other emerging contaminants, ecotoxicology**
**Host E.P.K., email koloj@uw.edu**

EmCon

**https://cvent.me/7kvWG9**

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# Questions?

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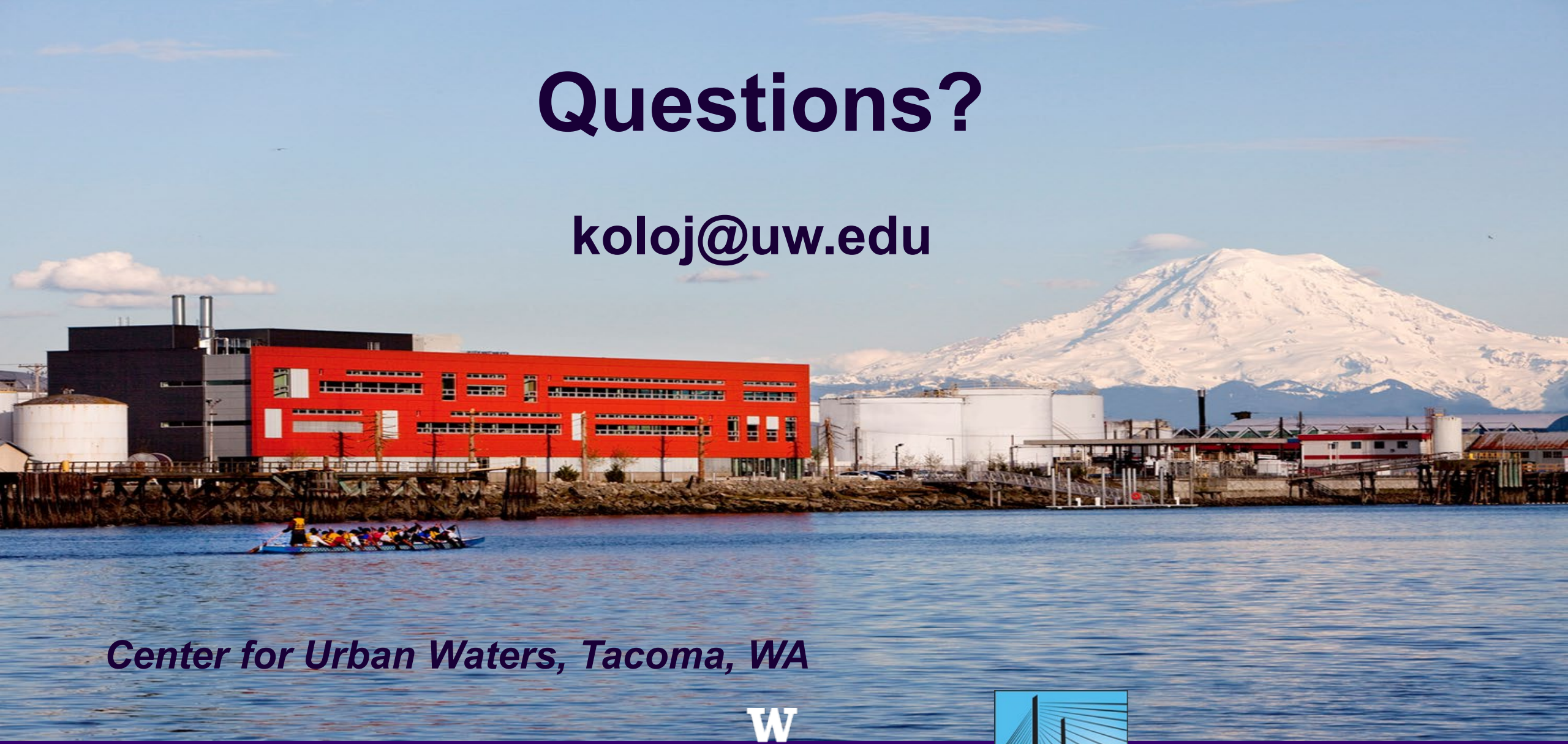

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