



Ecotox hazards of tire particles

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Acknowledgements



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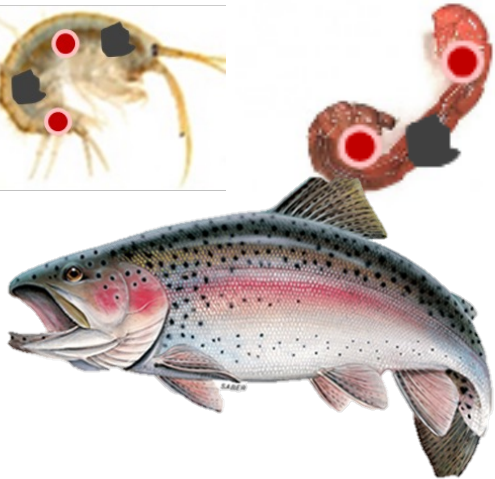
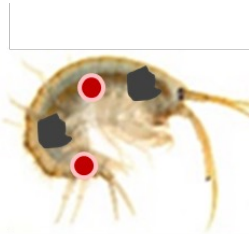
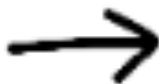
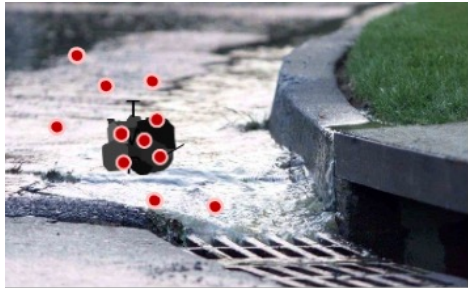
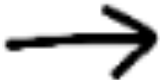
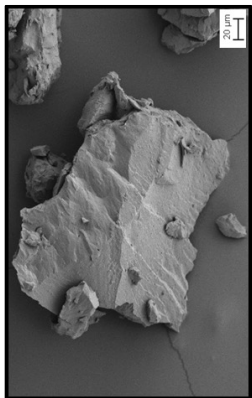
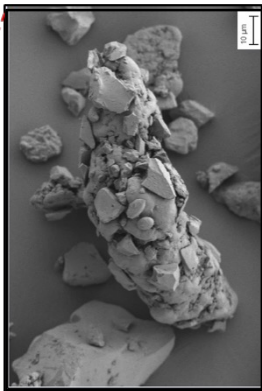
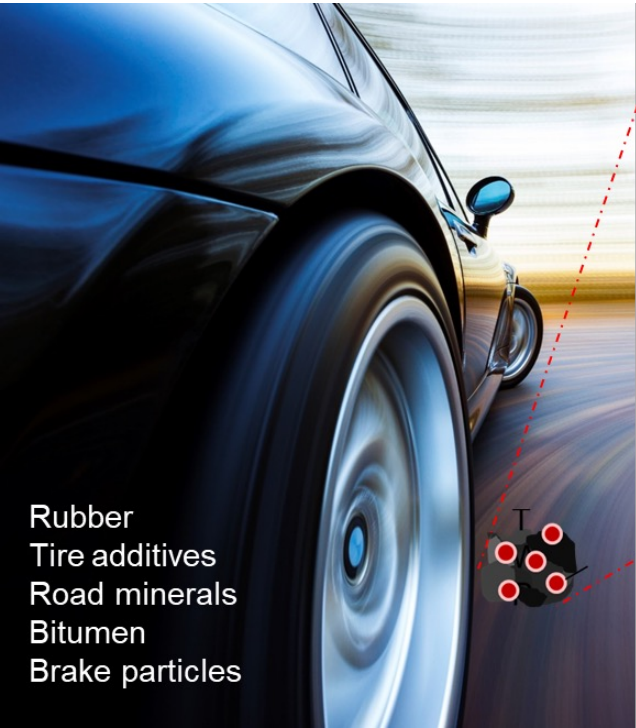
oekotoxzentrum
centre ecotox



Narrowing the scope



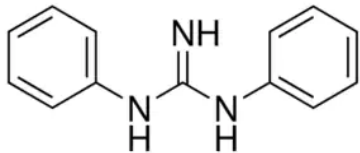
Tire and Road Wear Particles (TRWP)



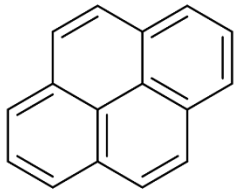
Tire Wear Particles (TWP)

Tire Particles (TP)

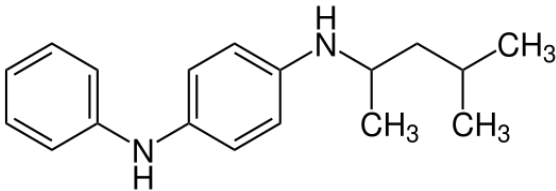
Cryogenically Milled
Tire Tread (CMTT)



1,3-diphenylguanidine
Vulcanization accelerator



pyrene
PAH



6PPD
Antiozonant

Mortality associated with urban runoff

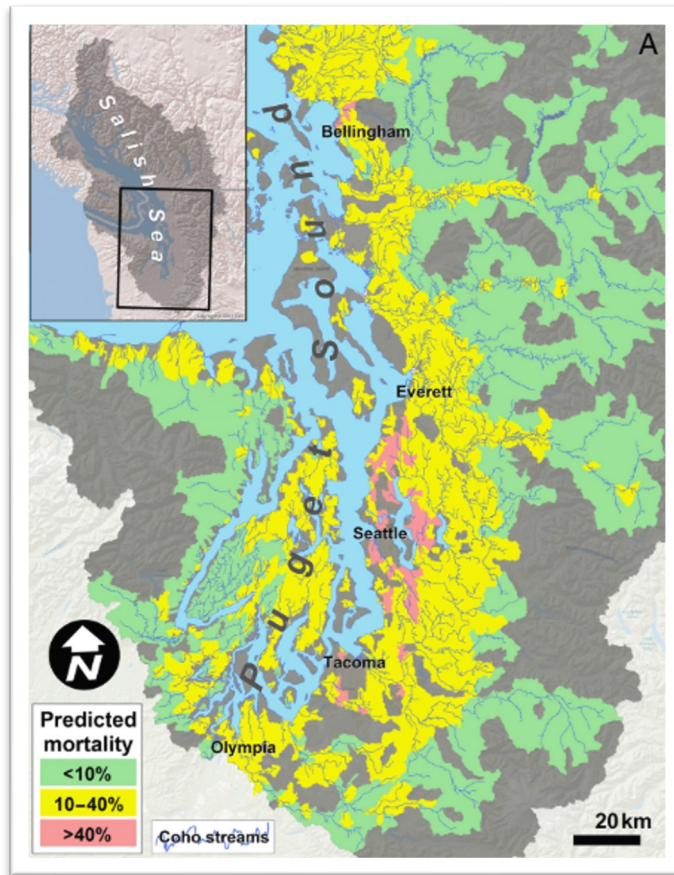


Pre-spawn mortality (PSM)



Urban runoff mortality syndrome

- surface swimming
- gaping
- loss of equilibrium
- pectoral fin splaying

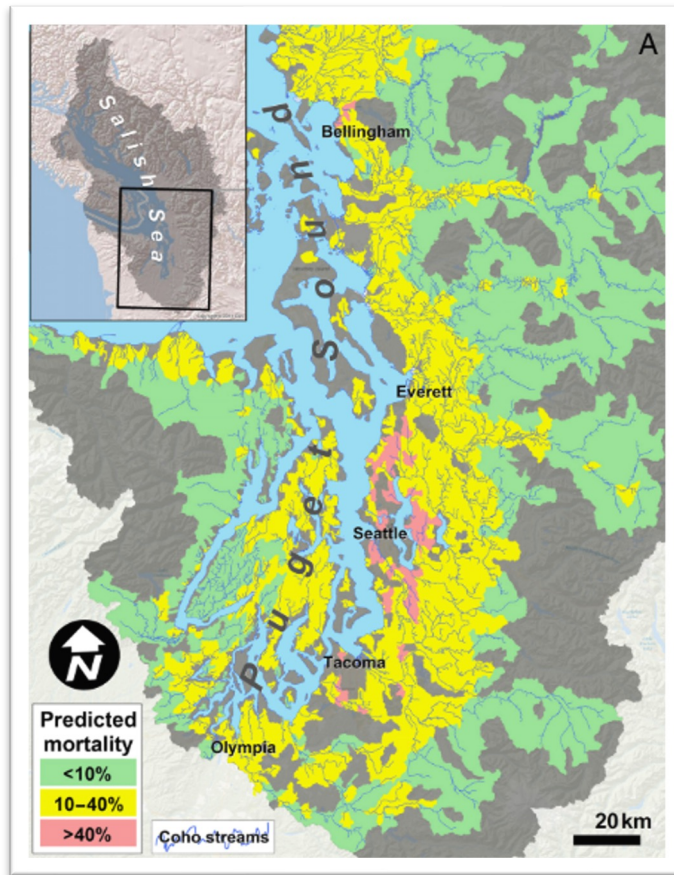


Mortality associated with urban runoff



Early studies ruled out:

- Common physical/chemical parameters
 - Temperature
 - Dissolved oxygen
 - Ammonia
 - pH
- Pathogens
- Common insecticides
- Metals



“The weight of evidence suggests that freshwater-transitional coho are particularly vulnerable to an **as-yet unidentified toxic contaminant** (or contaminant mixture) in urban runoff.”

Linking toxic waters to tire wear particles

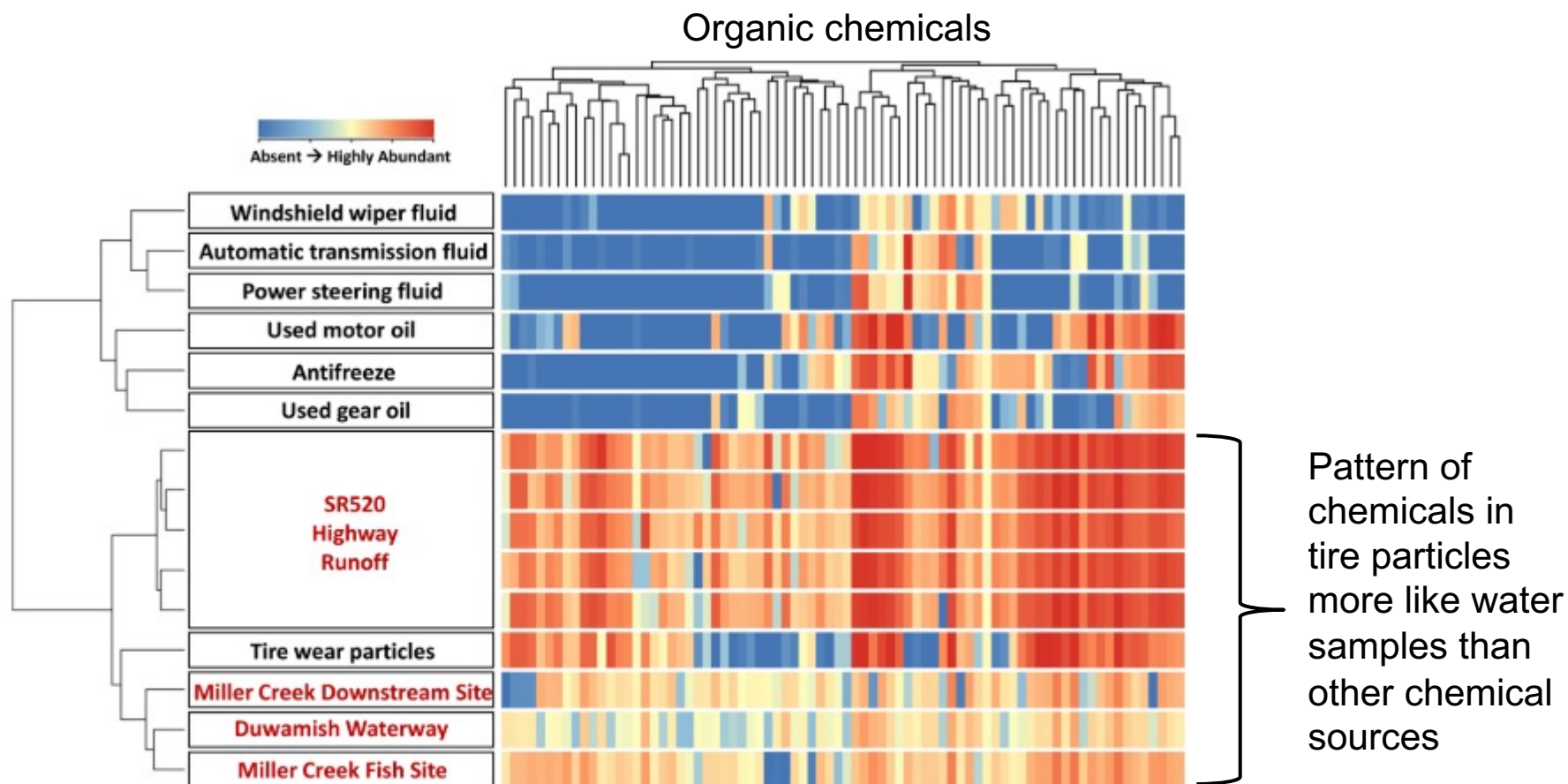
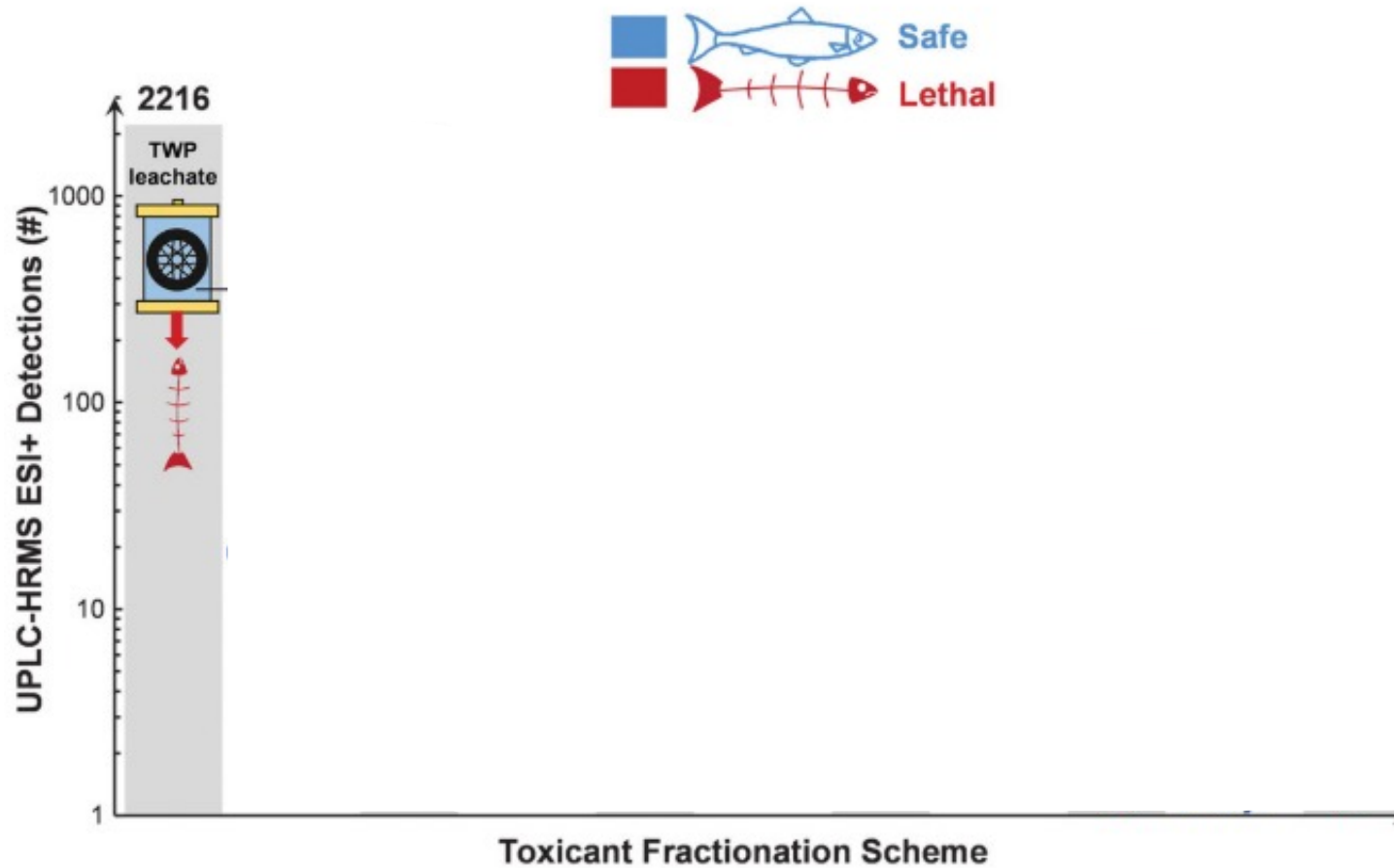


Figure: Hierarchical clustering of high-resolution mass spectrometry (HRMS) data

Fractionation



Most abundant candidate:

[M+H]⁺: 299.1752

RT: 11.0 min

Formula:



Linking unknown to 6PPD



MECHANISMS OF OZONATION OF N-(1,3-DIMETHYLBUTYL)-N'-PHENYL-*p*- PHENYLENEDIAMINE*

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INTRODUCTION

TABLE I

COMPOSITIONS OF OZONE-HPPD REACTION PRODUCTS

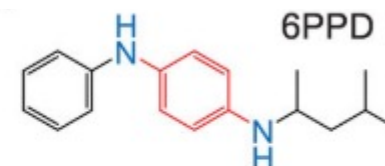
Measured mass ^a	Atomic composition	Calculated mass ^b
184.0997 ^c	C ₁₂ H ₁₂ N ₂	184.1000
198.0793 ^c	C ₁₂ H ₁₀ N ₂ O	198.0793
214.0742 ^c	C ₁₂ H ₁₀ N ₂ O ₂	214.0742
268.1579 ^c	C ₁₇ H ₂₀ N ₂ O	268.1576
268.1944 ^c	C ₁₈ H ₂₄ N ₂	268.1939
211.1235	C ₁₄ H ₁₅ N ₂	211.1235
282.1734 ^c	C ₁₈ H ₂₂ N ₂ O	282.1732
225.1023	C ₁₄ H ₁₃ N ₂ O	225.1028
296.1889 ^c	C ₁₉ H ₂₄ N ₂ O	296.1888
298.1688 ^c	C ₁₈ H ₂₂ N ₂ O 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 ₄ O	546.3358
503.2819	C ₃₃ H ₃₅ N ₄ O	503.2811
489.2654	C ₃₂ H ₃₃ N ₄ O	489.2654

^a Measured by peak matching in EI mode (Finnigan MAT 311A, $M/\Delta M \sim 10000$).

^b Based on given atomic composition.

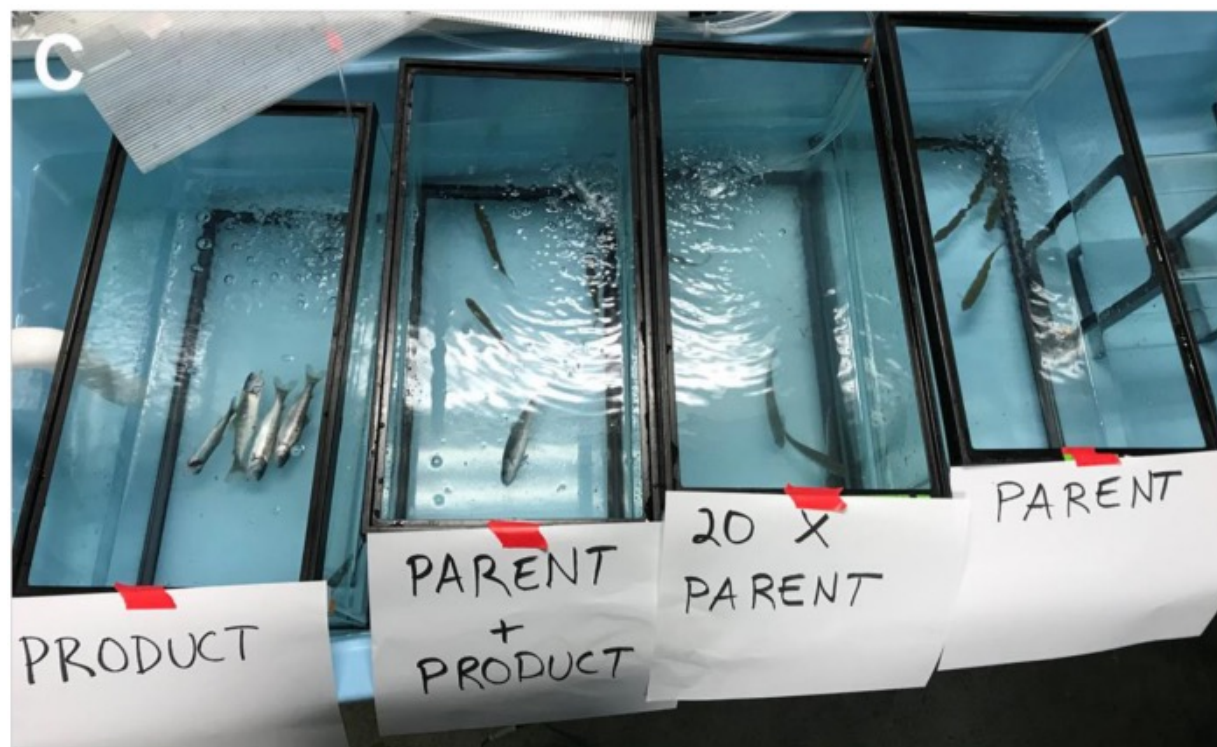
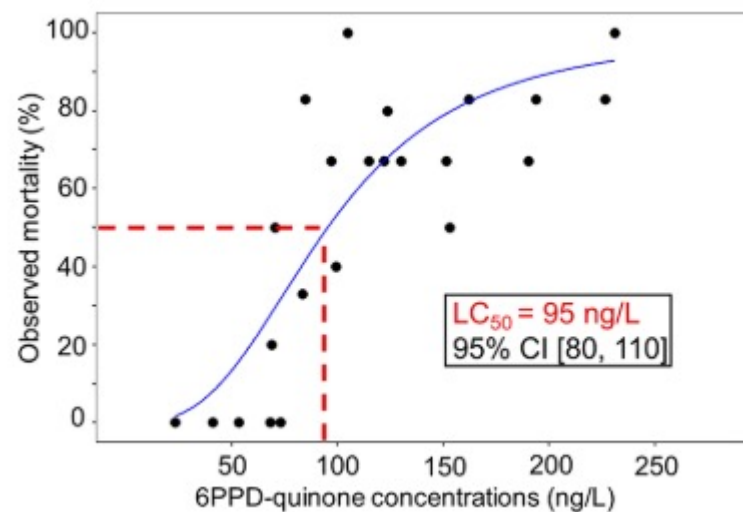
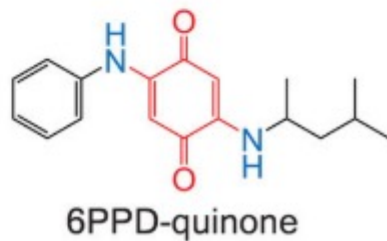
^c Molecular ion. (Other ions given are fragment ions.)

Lattimer et al. 1983.

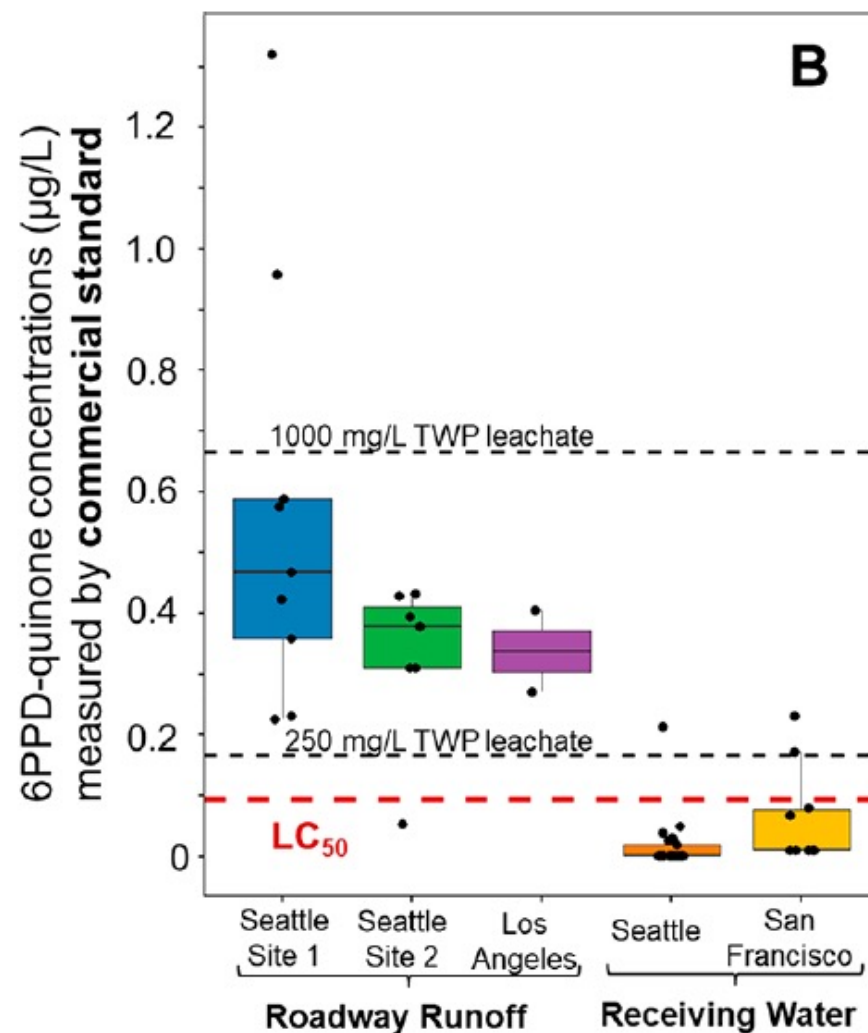
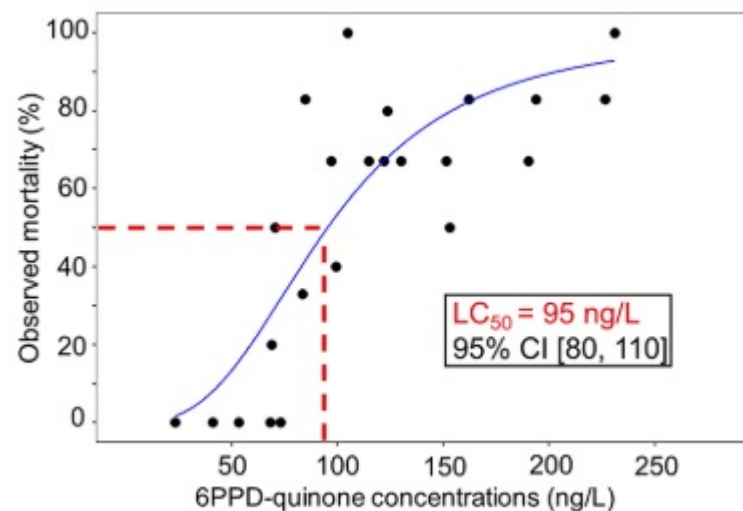
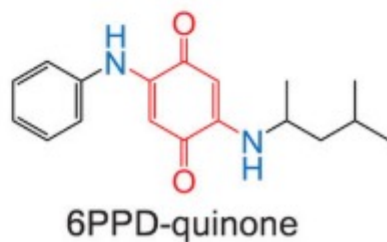


Tian et al. 2021. Science.

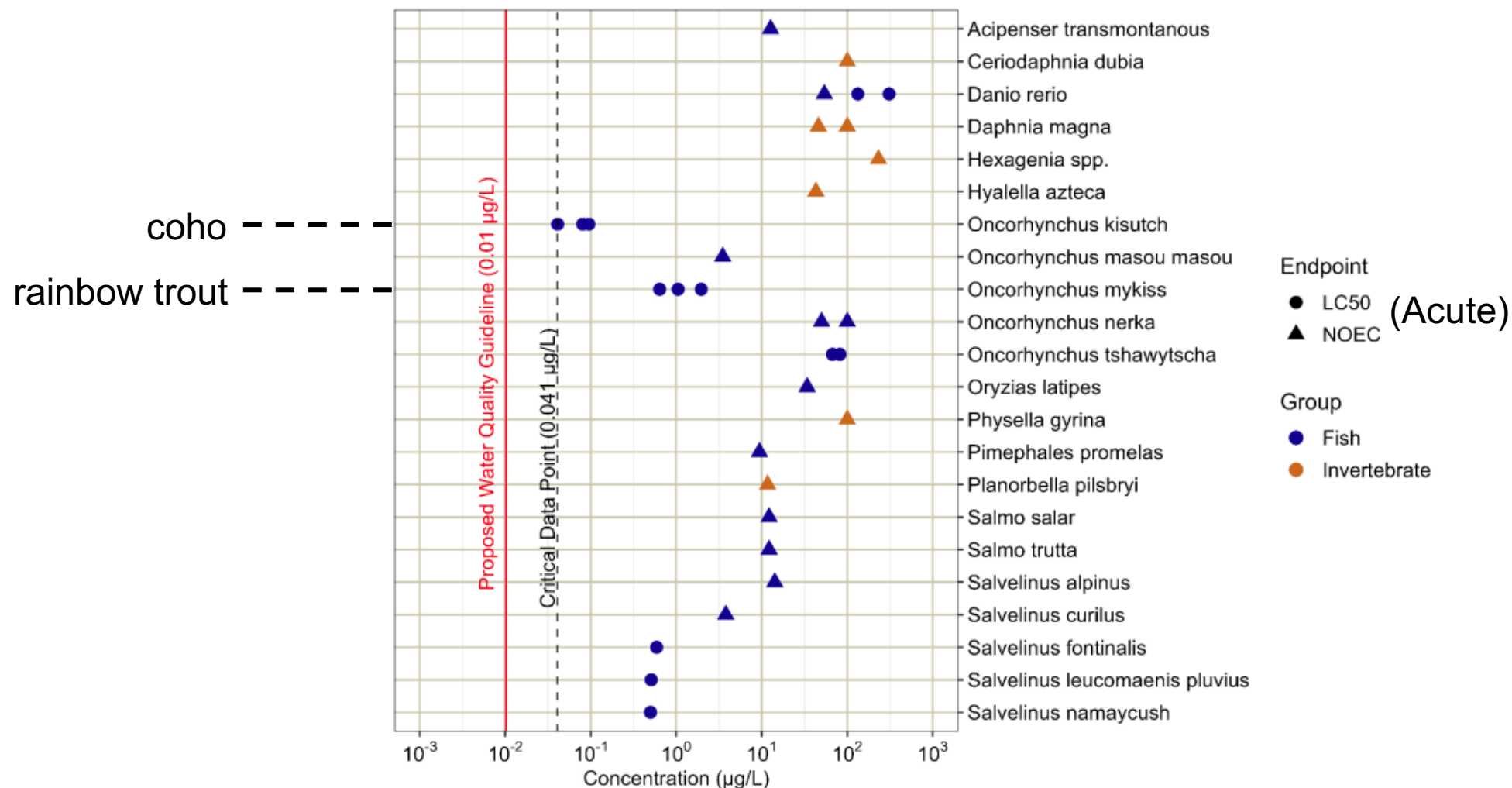
6PPDQ reproduces coho mortality



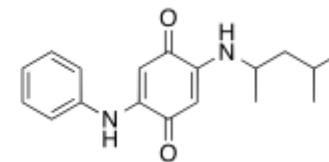
Estimated environmental concentrations



Proposed water quality guideline: British Columbia



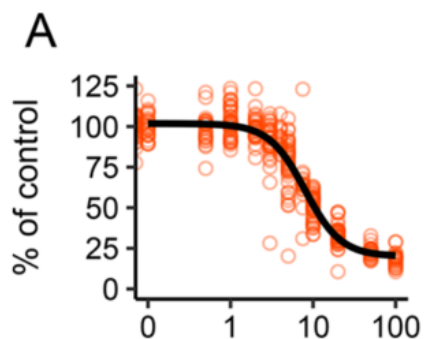
In vitro toxicity corresponds to in vivo



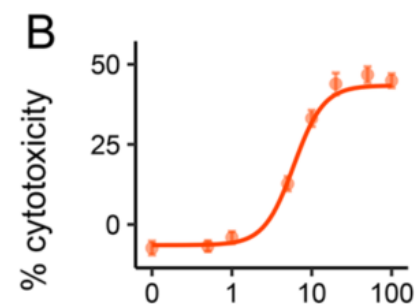
Cell lines: coho salmon embryo



metabolic activity



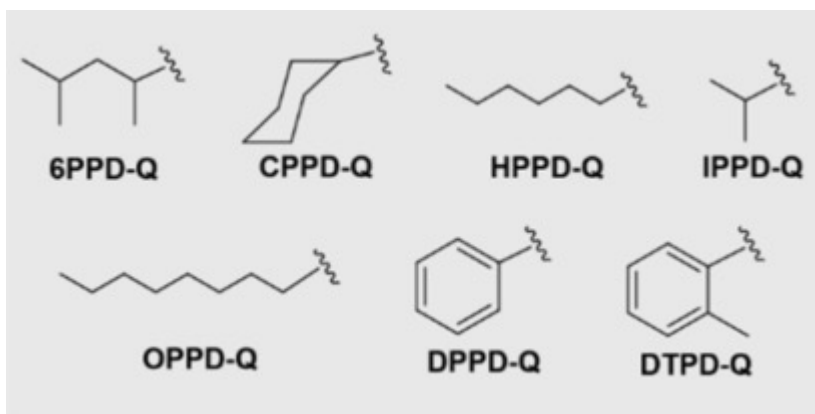
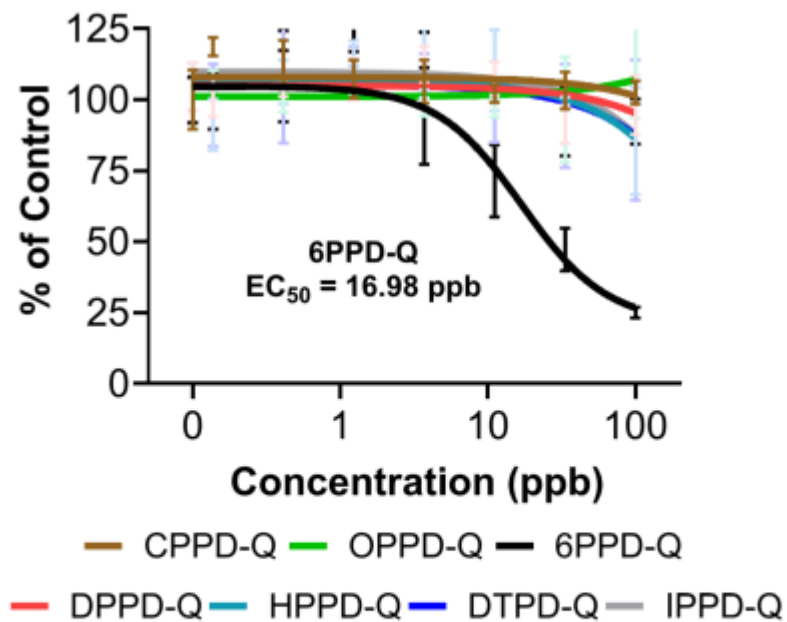
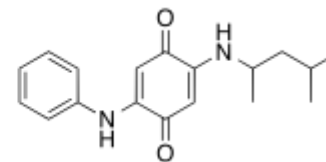
cytotoxicity



6PPD-quinone concentration (µg/L)

Structure specific toxicity

coho salmon embryo cell line



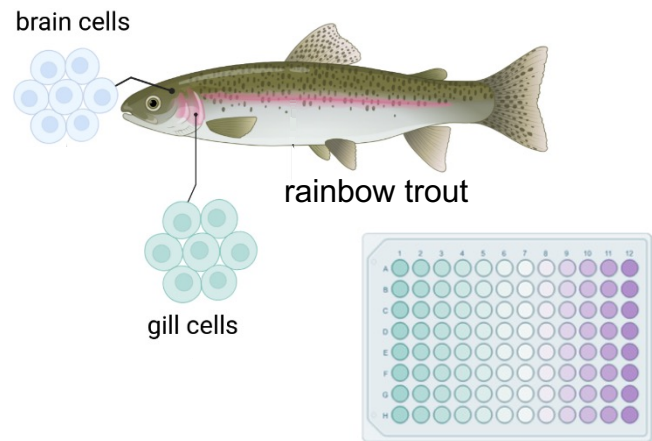
Tissue specific toxicity



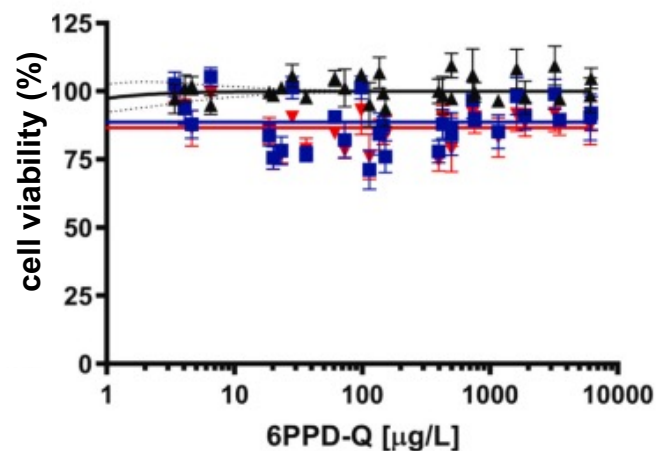
Marie Pigneres



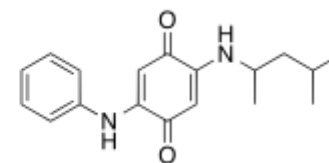
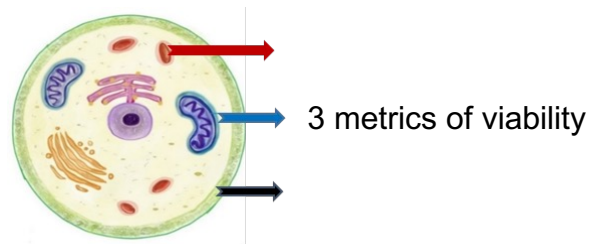
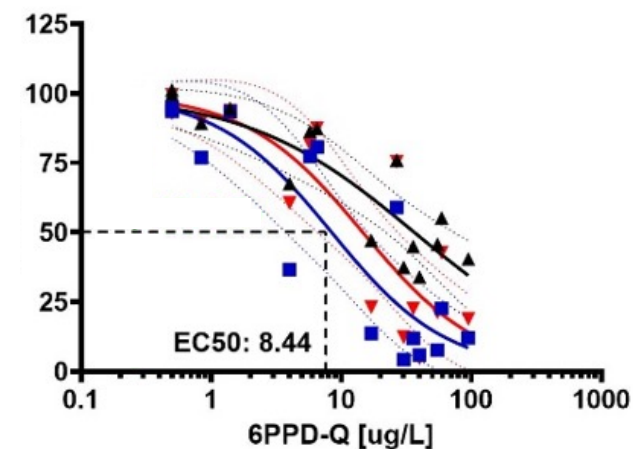
William Dudefoi

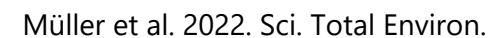
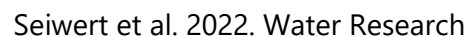


Gill cells



Brain cells





Many other effects – not linked to 6PPDQ



Tire associated compound	<i>in vitro</i> biological endpoints:		
	Estrogenicity	Genotoxicity (-S9)	Bacterial Toxicity
6PPD	Yes*	No	Yes
6PPD-Q	No	No	No
2-Mercaptobenzothiazole	No	No	Yes
2-(Methylthio)benzothiazole	No	No	Yes
2-2'-Dithiobisbenzothiazole	Yes*	No	Yes
Benzothiazole	No	No	Yes
2-Aminobenzothiazole	No	No	Yes
2-Hydroxybenzothiazole	No	No	Yes
Aniline	No	No	Equivocal [†]
1,3-Diphenylguanidine	No	Yes	No
Cyclohexylamine	No	Yes	No

Biofilm is a sink for tire particles

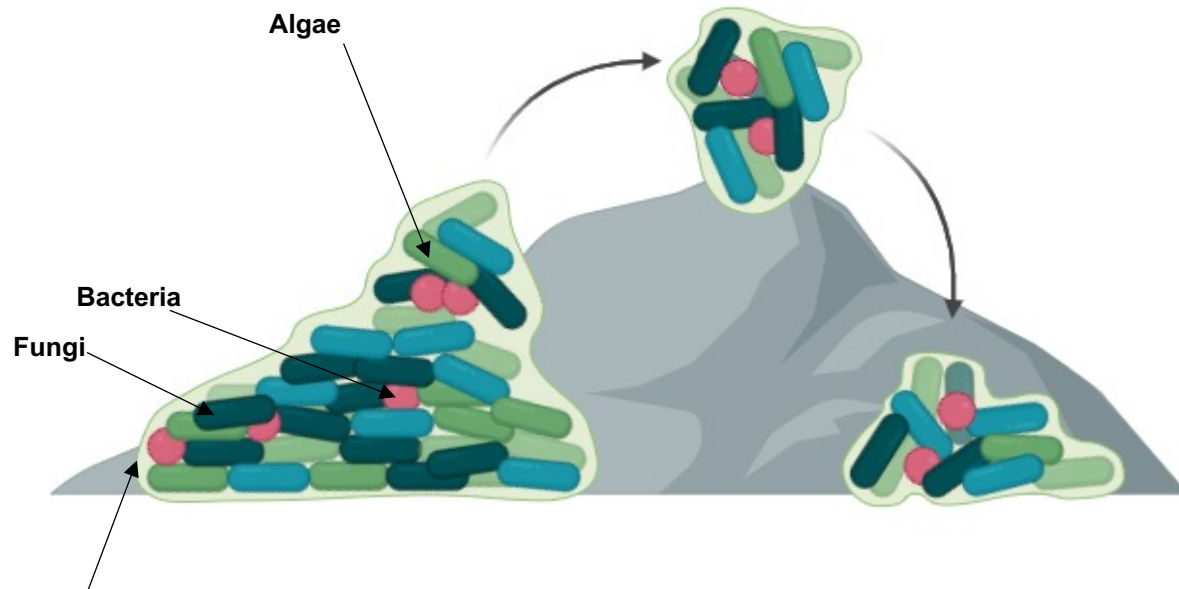
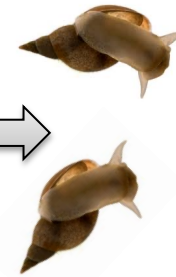
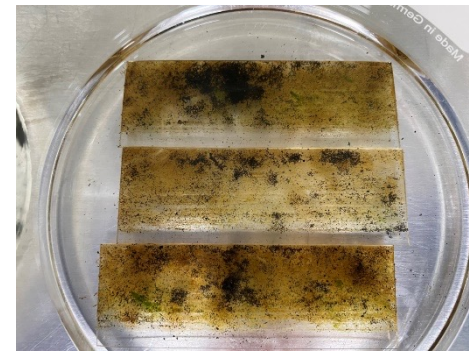


Sara Gonçalves



Interaction with particles:

Biofilm and EPS help particles to settle out of water column
Accumulate particles and chemicals



EPS – exopolymeric substances

Direct effects:

Photosynthesis disruption (Liu et al. 2024)

Alteration in community structure (Ding et al. 2022)

Invertebrates can ingest tire particles

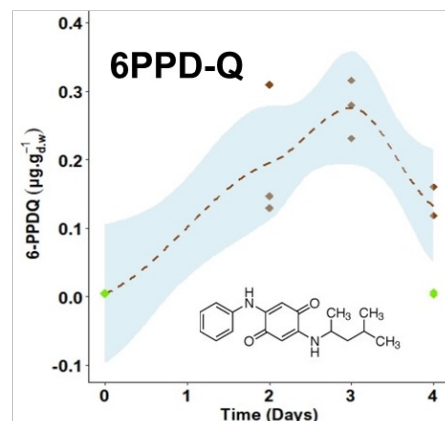
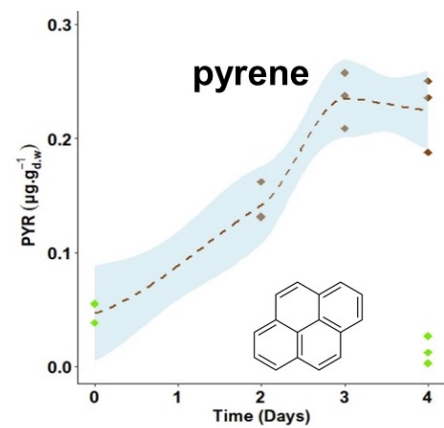


Thibault Masset



Khan et al. 2019

Interaction with particles:
Ingesting particles and chemicals
Accumulate tire associated chemicals



Masset et al. In preparation.

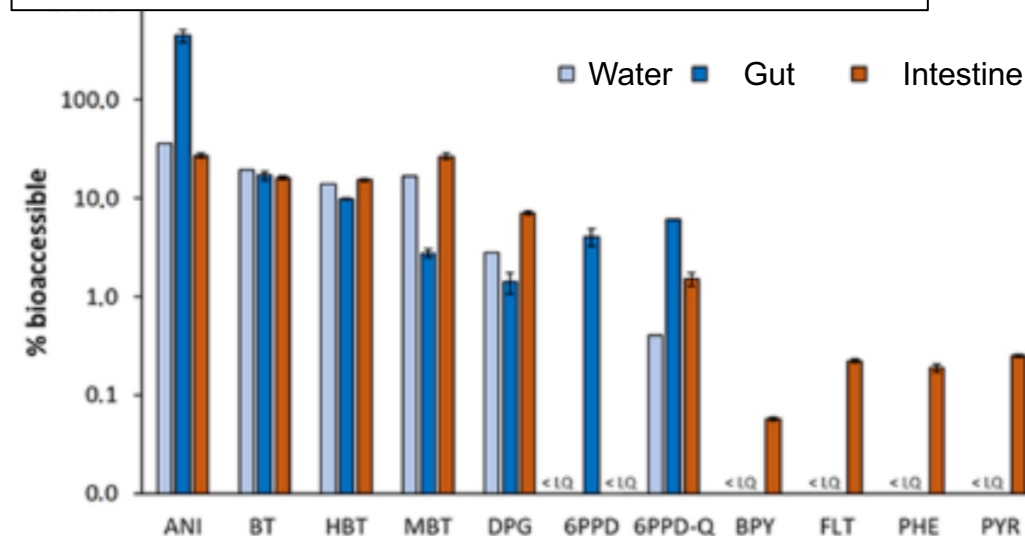
Direct effects

Mortality (Roubeau Doumont et al. 2023)

Impaired reproduction (Cunningham et al. 2024)

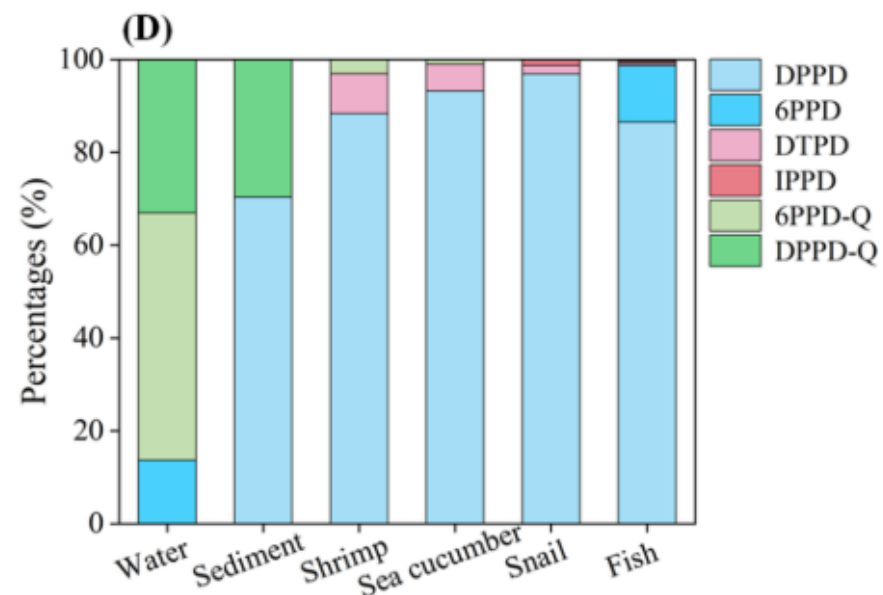
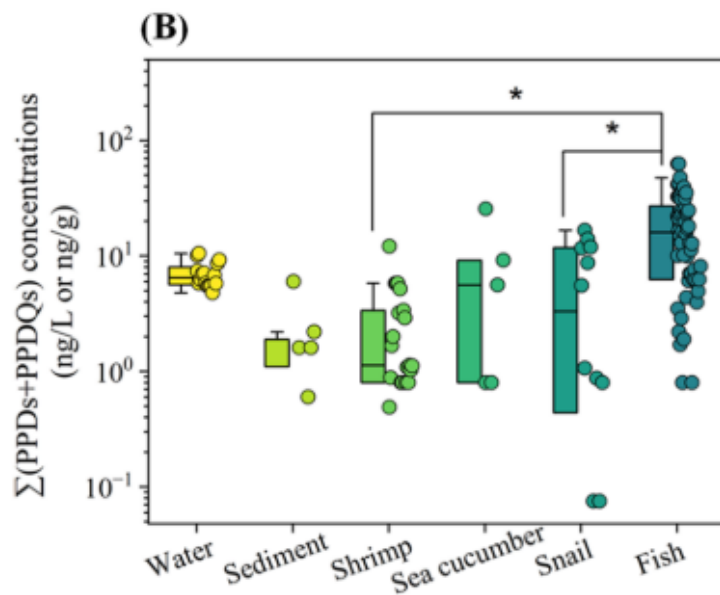
Delayed development (Moreira et al. 2023)

Chemicals can become bioaccessible in simulated fish digestion



Masset et al. 2022. Environ. Sci. Technol.

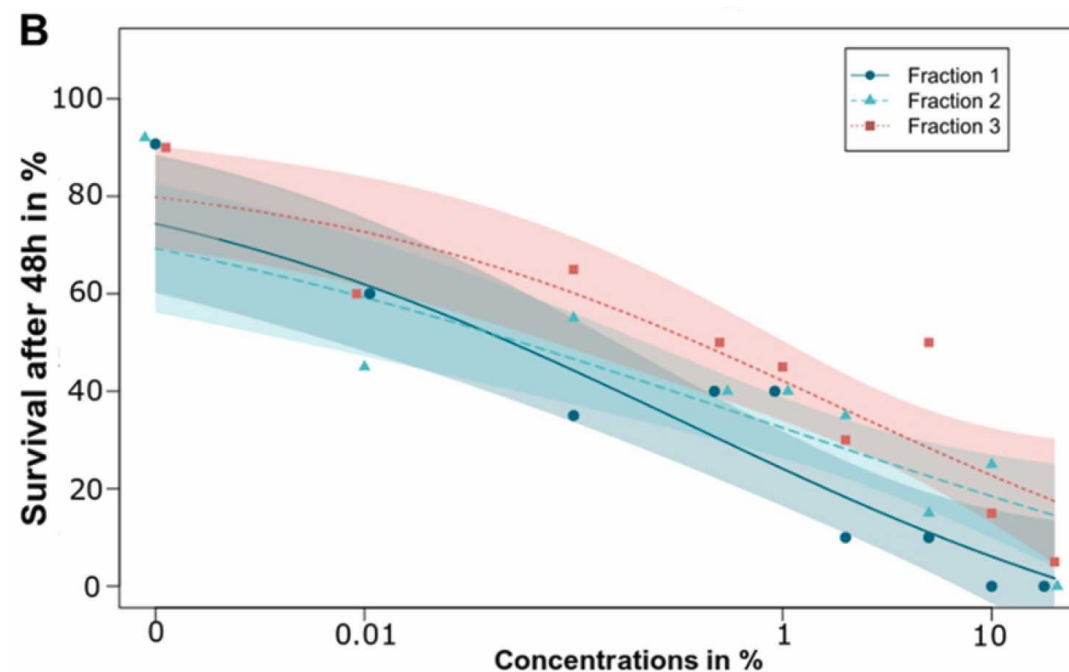
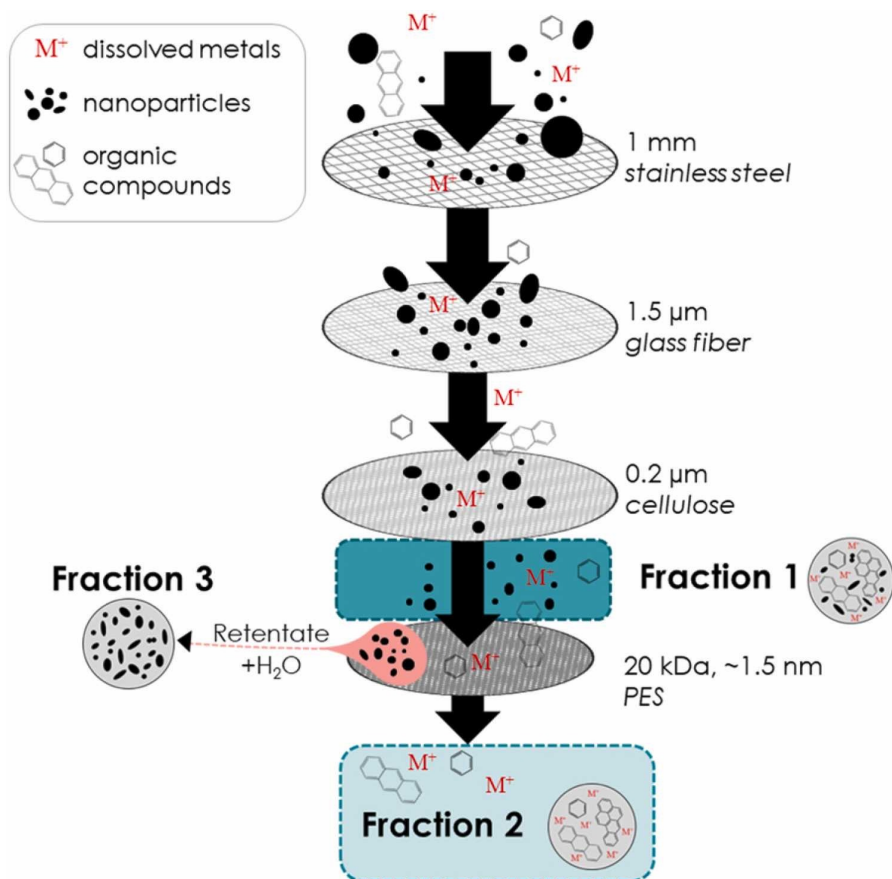
Trophic transfer



Particle vs leaching chemicals



Liu et al. 2023



Even “depleted” particles were toxic to daphnia magna



Take home messages

- 6PPDQ is a clear example of risk from tire-associated chemicals to aquatic environment
- Many potential hazards beyond 6PPDQ
- Organisms interact with TWP
 - Some associated chemicals can be transferred between trophic levels
- Hazards from tire particles mostly attributed to leachable chemicals
 - Particle effects should not be discounted



Thanks for your attention

References 1



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Solutions! Not just problems...



McIntyre et al. 2015. Chemosphere

Exposure (h)	Mortality Adult coho		
	Unexposed	Unfiltered	Filtered
4	0% (0/4)	100% (4/4)	0% (0/4)
24	0% (0/4)	100% (4/4)	0% (0/4)
24	0% (0/4)	100% (4/4)	0% (0/4)
24	0% (0/4)	100% (4/4)	0% (0/4)
24	0% (0/4)	100% (4/4)	0% (0/4)

Spromberg et al. 2016. J. Applied Ecology

Bioretention treatment (soil/sediment filtration) is successful in preventing coho mortality



ANTHROPOGENIC POLYMER PARTICULATES

Man-made particles of high molecular weight chains formed either by the addition or condensation of numerous sub-units (monomers).



THERMOPLASTIC

Ability to soften when heated and harden when cooled.

PLASTICS

Containing a high molecular weight polymer, which at some stage can be shaped by flow. Elastomers are excluded from this definition, as they cannot be 'shaped by flow'.

PLASTIC DEBRIS / MICROPLASTICS

To gain a common ground for defining and interpreting the vast array of plastic debris a few recent papers discuss different criteria, possible pitfalls and future mitigation strategies; Based on these, the following traits that are relevant for describing plastic debris are; chemical composition, origin (primary or secondary), solubility, size, shape (morphology), colour and additives.

THERMOSET

Irreversibly hardened by curing, creating crosslinks in the polymer.



ELASTOMERS

The ISO defines elastomers as macromolecular materials, which after deformation can return rapidly to its initial dimensions.

NATURAL ELASTOMERS

Latex rubber, naturally occurring cis-1,4-polyisoprene, from *Hevea brasiliensis* in both liquid and cured form.

SYNTHETIC ELASTOMERS

Encompasses all elastomers that are produced synthetically (examples: styrene, butadiene and also synthetic cis-1,4-polyisoprene).

MICRORUBBER

Umbrella term for all micronized rubber particles.

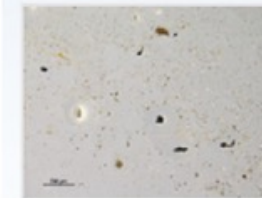
TWP

Tire wear particles abraded from the tread of tires. Most likely form to be generated for laboratory experiments by manual abrasion or by simulation. Unlikely to be sampled from the environment.



TRWP

Tire-road-wear particles, abraded from the tread of tires when driving on a road. Rubber particles found in combination with other car emissions and road dust. Most likely form to be sampled from the environment.



ELT

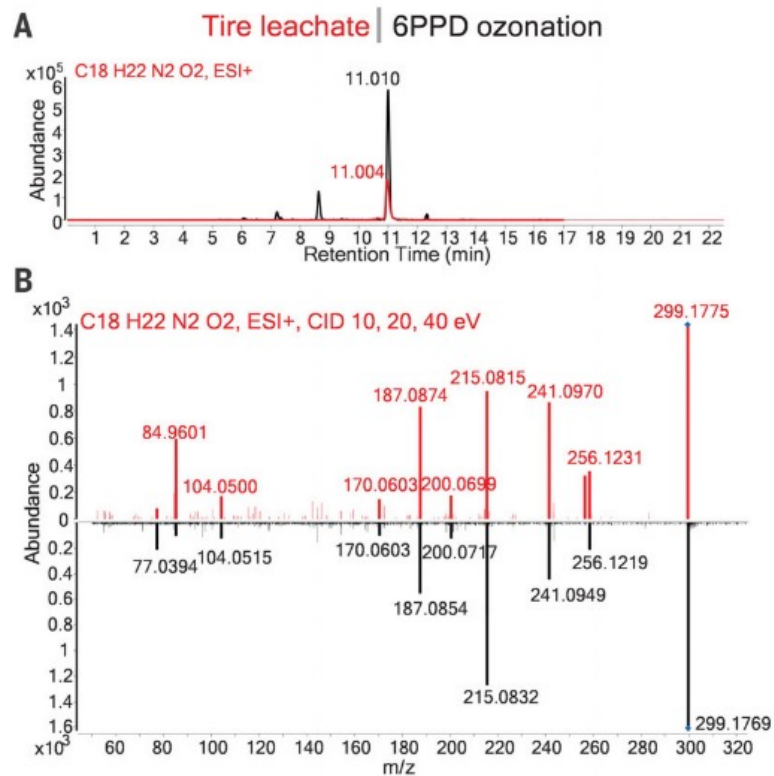
End-of-life-tires; Worn tires repurposed for alternative applications for example artificial turfs.

CRUMB RUBBER

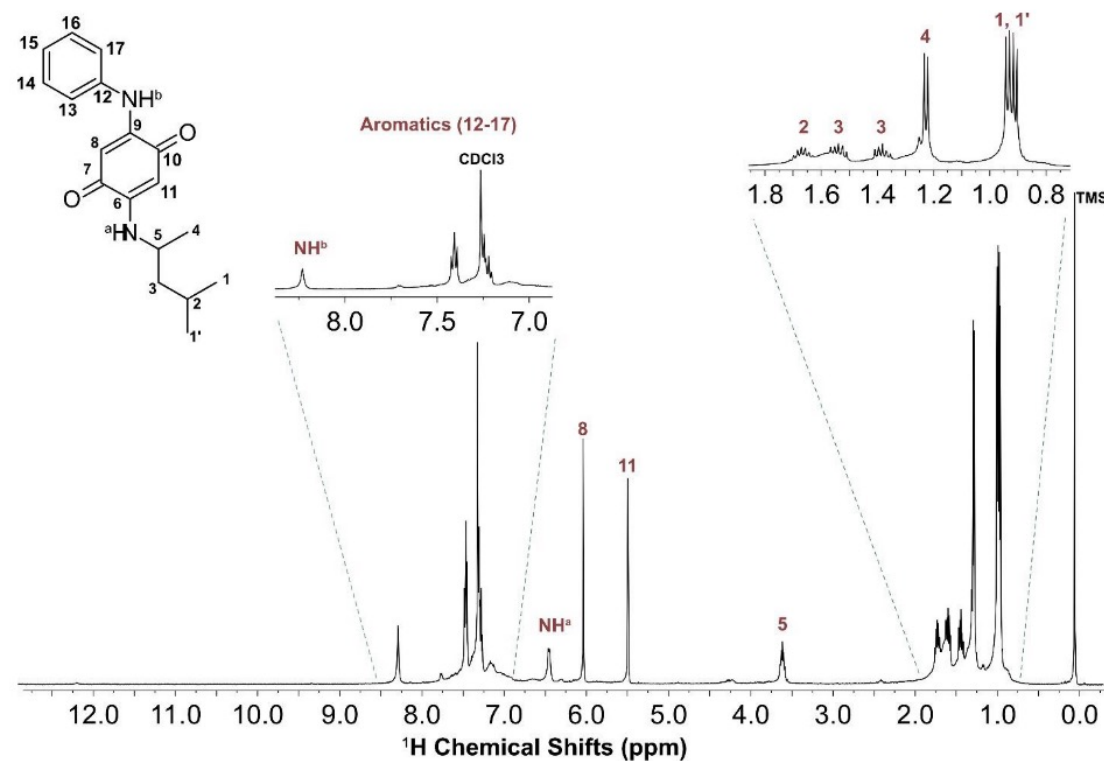
Described by ISO as "elastomers in particulate form" Divided into size by ISO: Chip rubber (10-50 mm), Granulated rubber (1-10 mm), Ground vulcanized rubber (0.1 - 1 mm) and Fine powdered rubber (≤ 0.1 mm).

CMTT:
Cryogenically milled tire tread

Confirmation

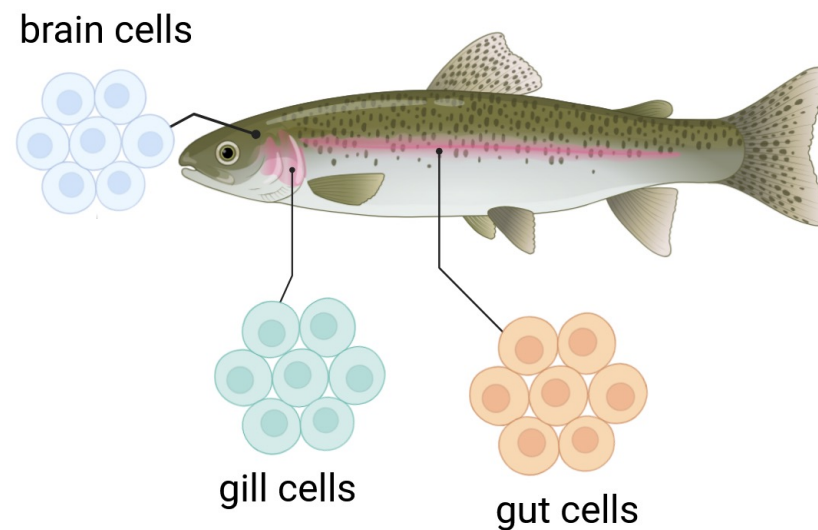


Liquid Chromatography –
High Resolution Mass
Spectrometry



Nuclear Magnetic
Resonance

Fish toxicity testing... in vitro



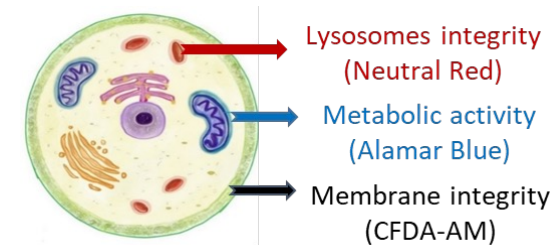
seeding



exposure



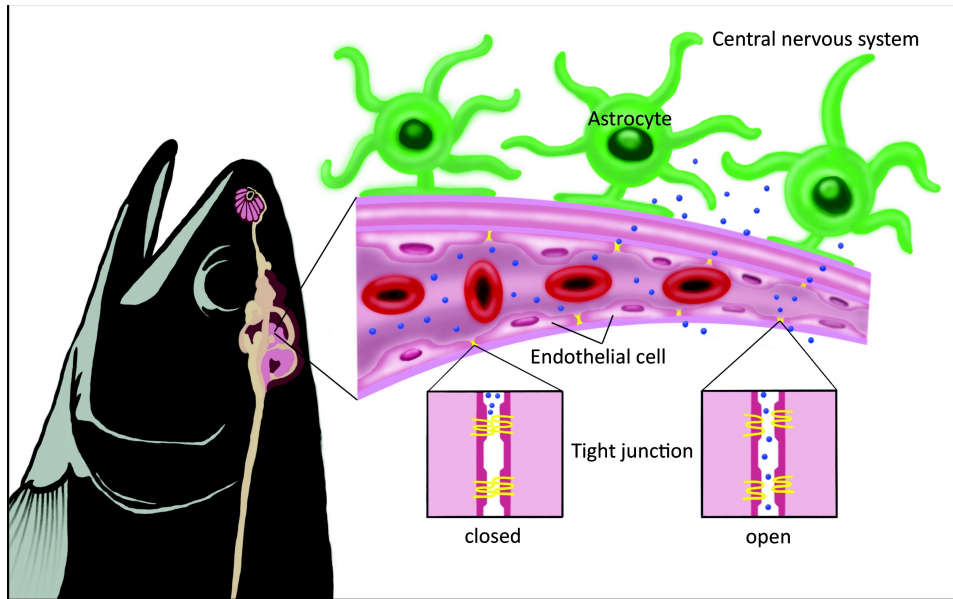
detection



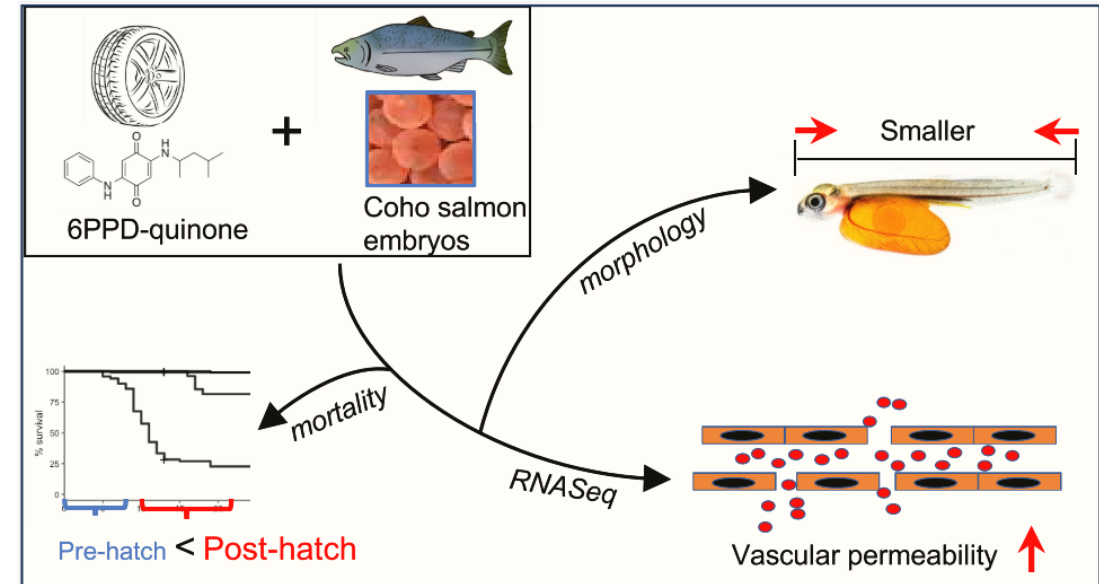
analysis

Neural/cal

Mechanism of toxicity not fully established, but evidence points to nervous system / cardiorespiratory disruption



Blair et al. 2021. Environmental Science & Technology



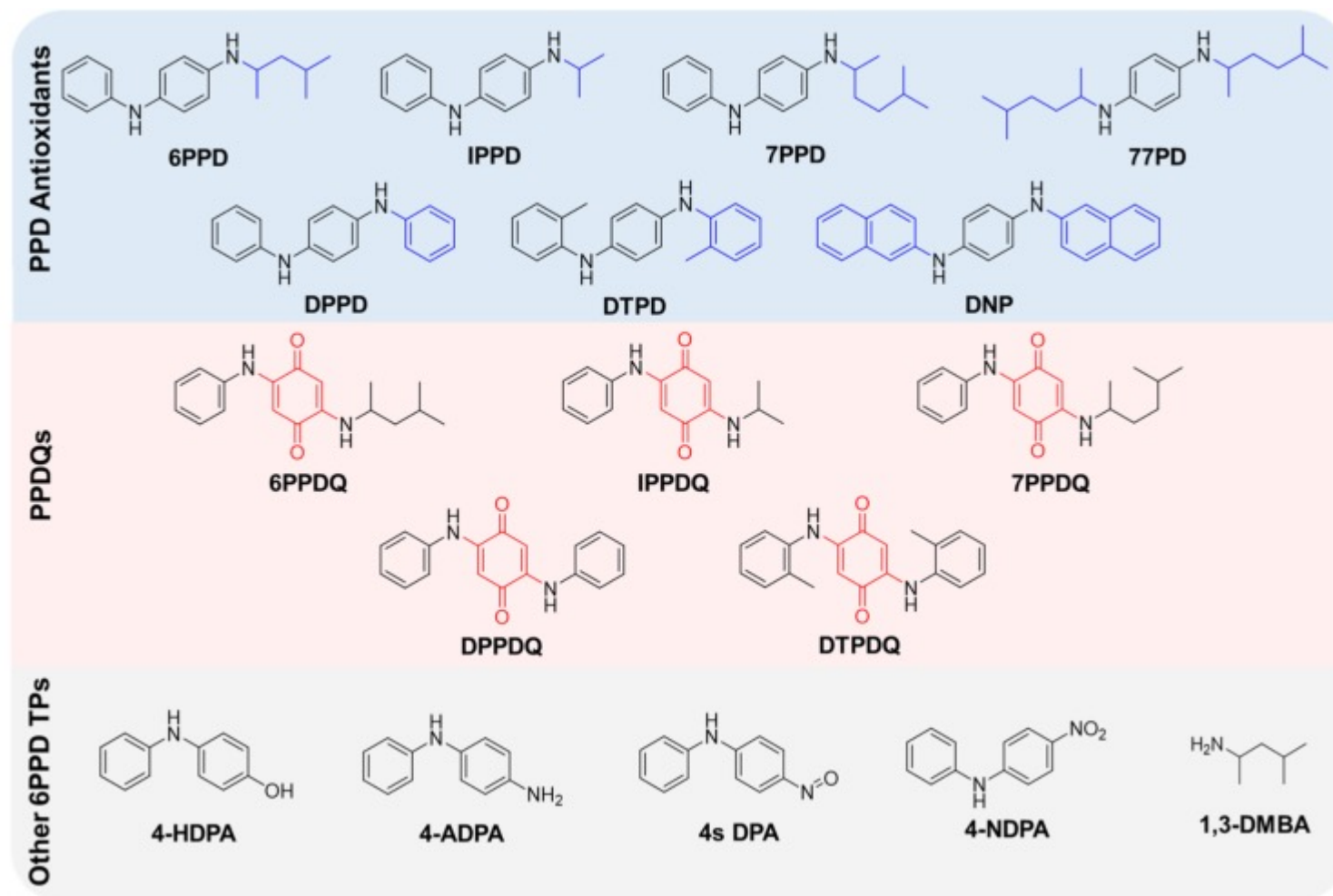
Greer et al. 2023. Environmental Science & Technology



Figure 3. Examples of morphological changes in rainbow trout alevins exposed to 6PPD-Q for 28 days from hatch. (A) Spinal curvature occurring at $1.30 \mu\text{g/L}$. (B) Yolk sac edema occurring at $0.44 \mu\text{g/L}$. (C) Caudal fin pathology occurring at $0.44 \mu\text{g/L}$.

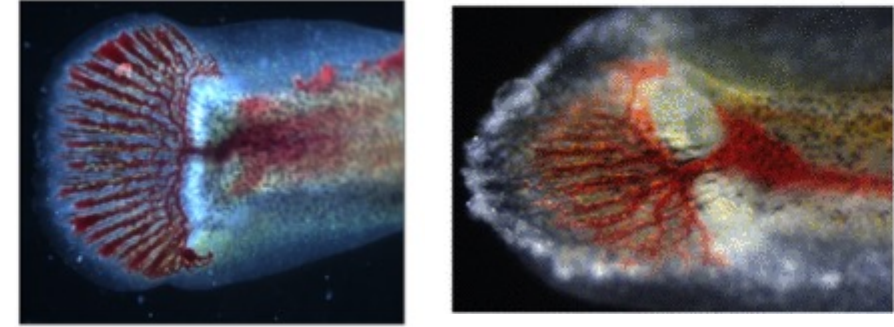
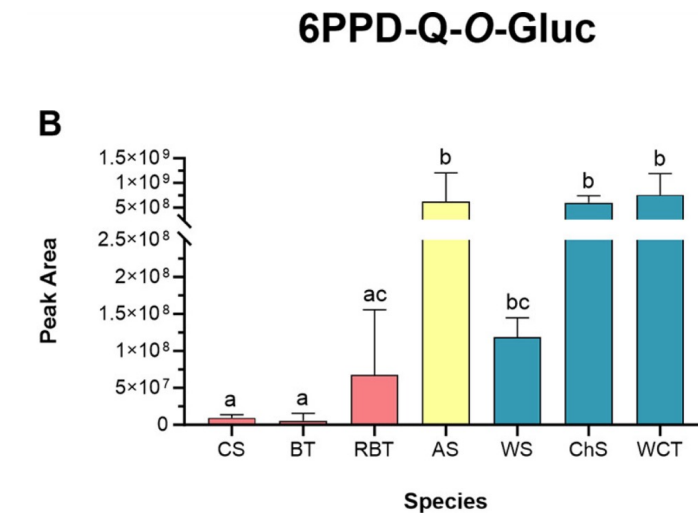
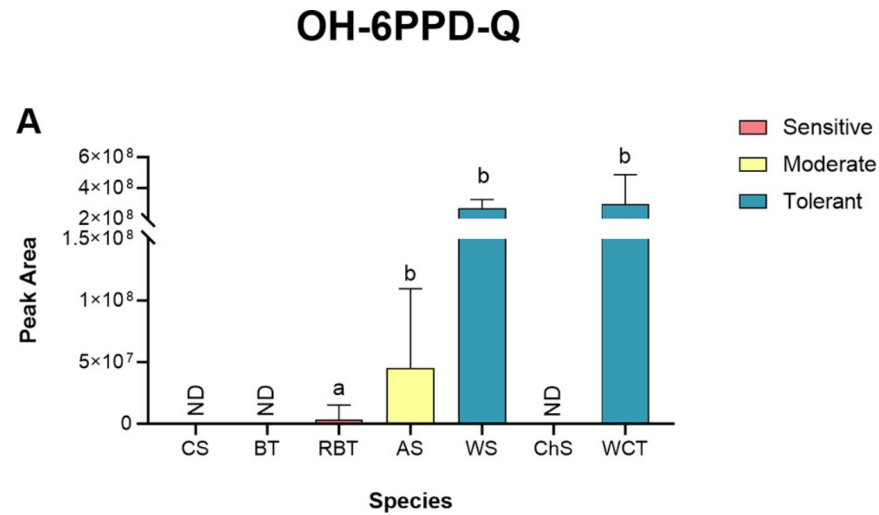
Roberts et al. 2025. Environmental Science & Technology

Family of phenylenediamine antiozonants

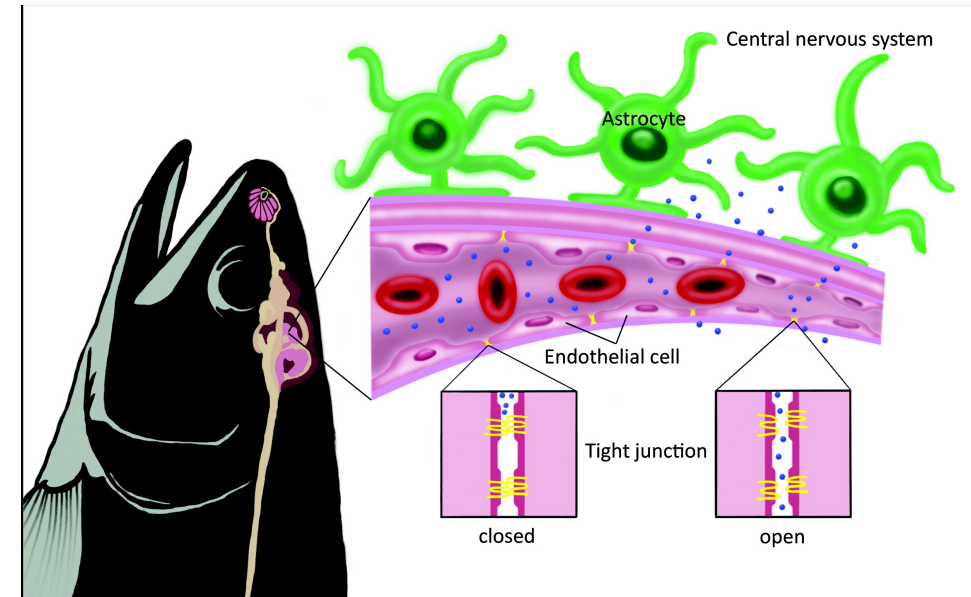


Zhao et al. 2023. J. Applied Ecology

Species sensitivity possibly linked to metabolic capacity



Roberts et al. 2025a &b. Environmental Science & Technology

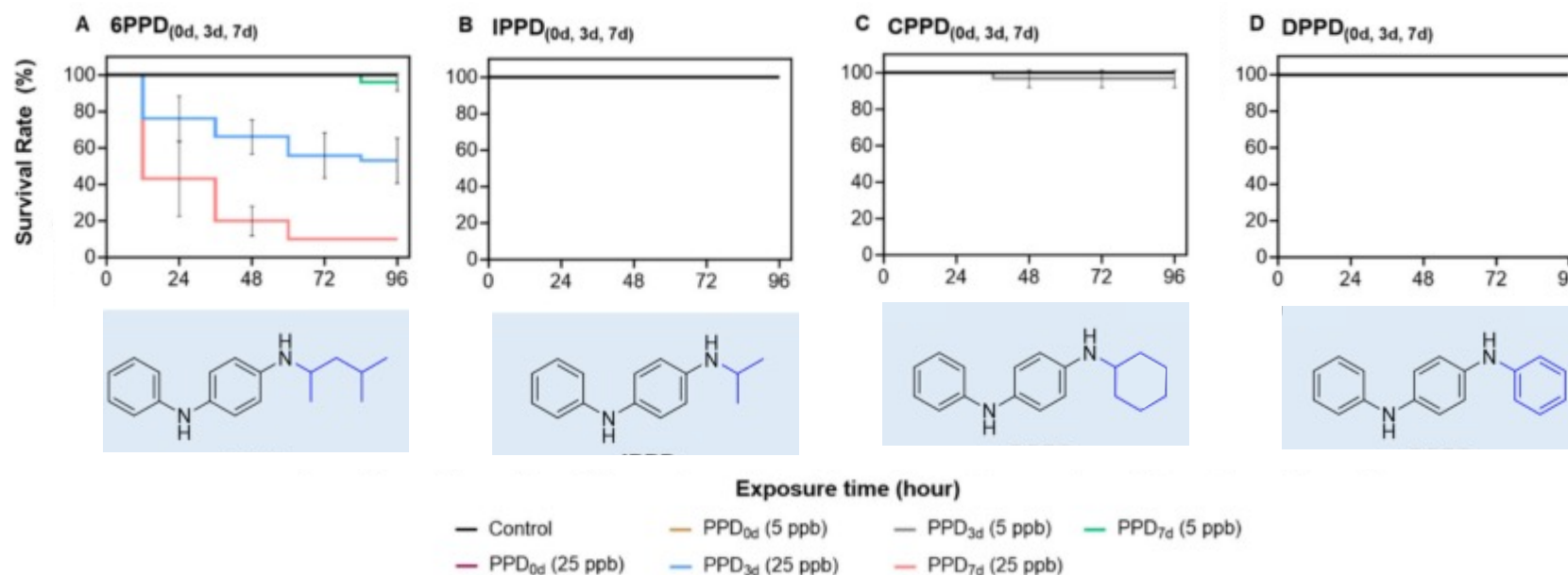


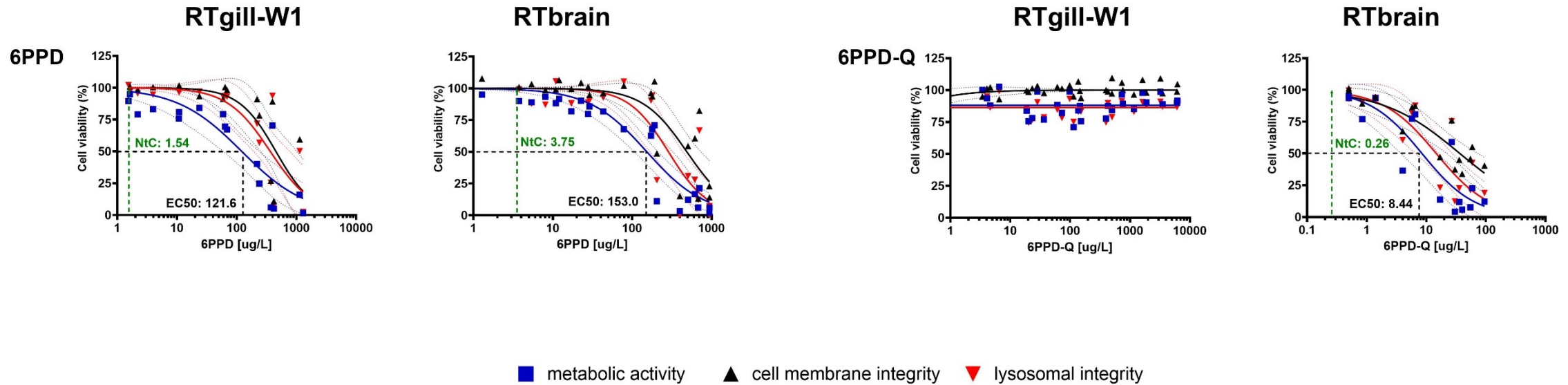
Blair et al. 2021. Environmental Science & Technology

Toxicity is structure specific



96h survival of juvenile rainbow trout exposed to ozonated PPDs





References:

RTgill-W1 data: Dudefoi W, Ferrari BJD, Breider F, Masset T, Leger G, Vermeirssen E, Bergmann AJ, Schirmer K. Evaluation of tire tread particle toxicity to fish using rainbow trout cell lines. Sci Total Environ. 2024 Feb 20;912:168933. doi: 10.1016/j.scitotenv.2023.168933. Epub 2023 Dec 1. PMID: 38042189.

RTbrain data: Pigneres M, Dudefoi W, Schirmer K et al. Manuscript on preparation.



Knowledge gaps

- 6PPDQ mechanism of action
- 6PPDQ chronic toxicity assessment
- Identity of chemicals responsible for other effects
- Evidence of trophic transfer in controlled setting:
Mesocosms
- Attribution of toxic effects: leachates vs particles