A Novel Framework to Form Sufficiently Similar Mixtures

RASS/Mixtures Joint Webinar
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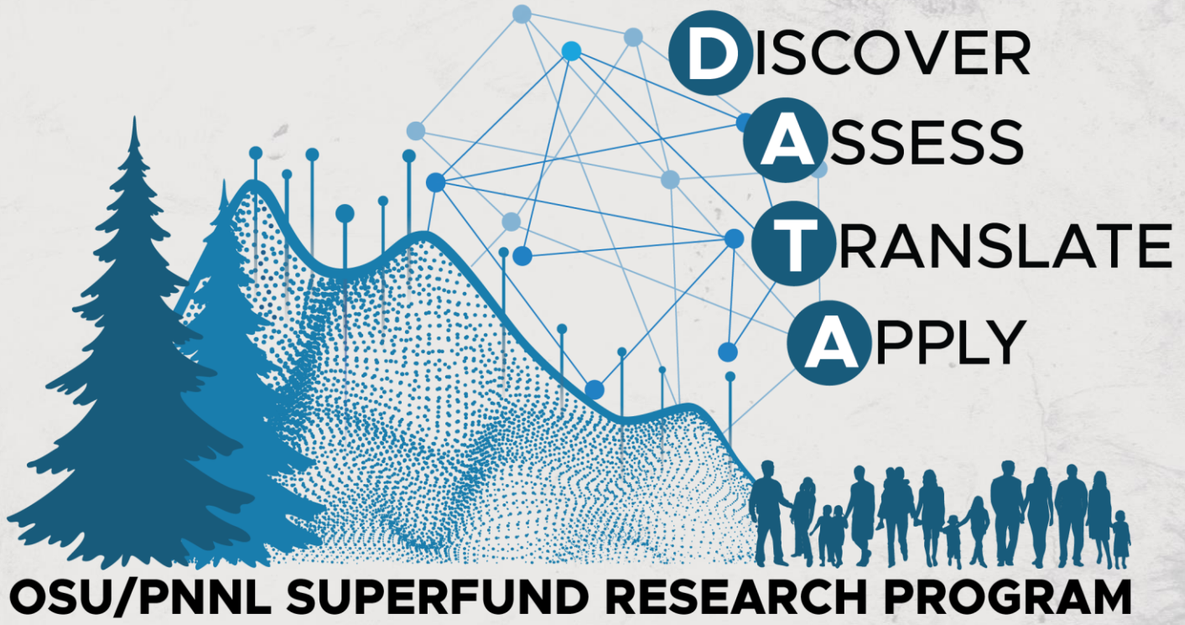

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Outline

1. Background on Polycyclic Aromatic Hydrocarbons (PAHs) and Current Mixture Safety Assessment Approaches

2. Mixture Formation and Hazard Characterization

3. Study Conclusions, Future Directions, and Limitations

Why we focus on PAHs

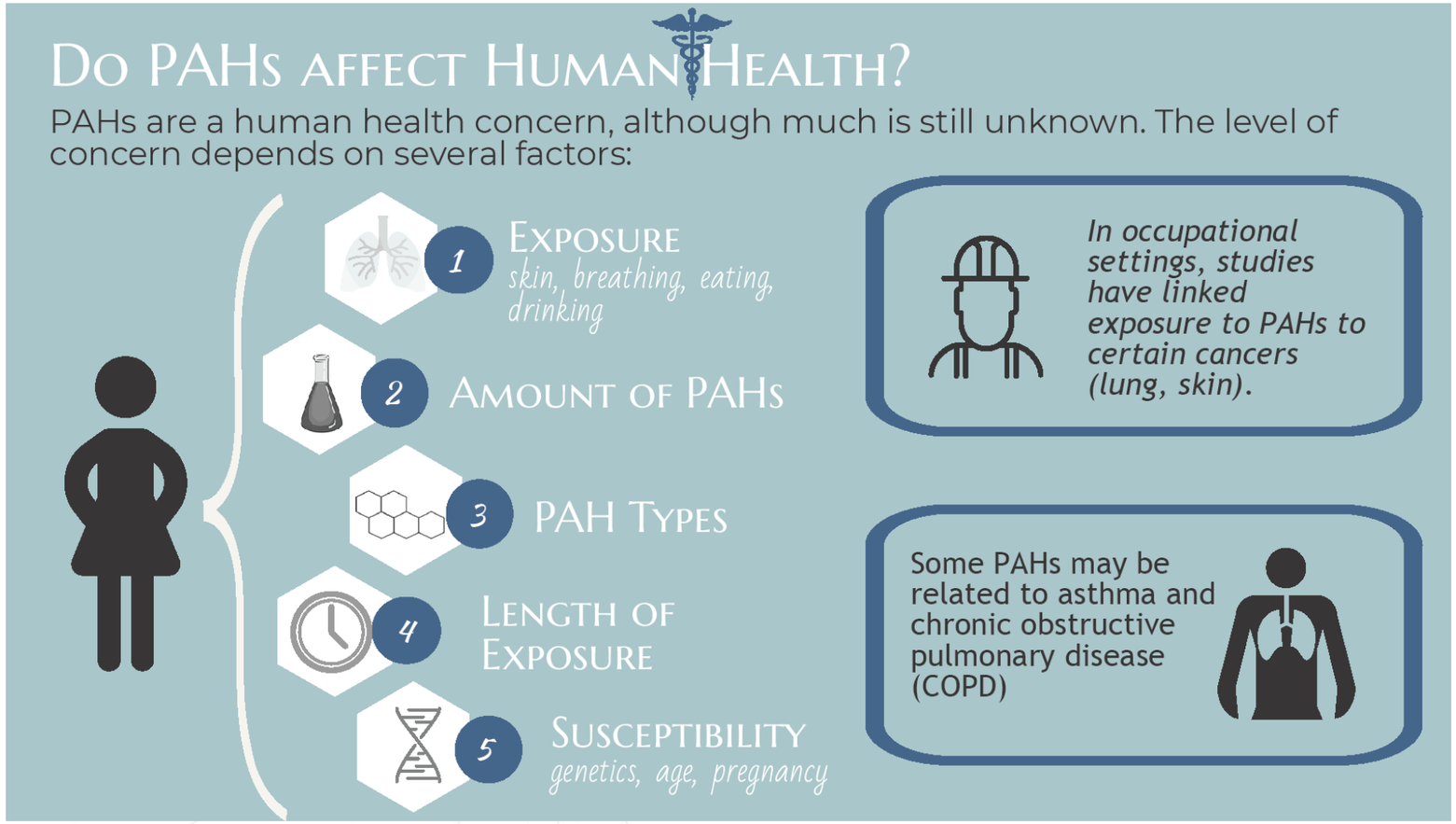
Found in over 40% of Superfund sites (1995)
https://www.atsdr.cdc.gov/toxprofiles/tp69.pdf

Ubiquitous in the environment
(wildfires, industrial pollution, car exhaust, cereal or grains)

Known health effects
(irritation, respiratory and cardiovascular disease, neurological and developmental disorders, adverse perinatal outcomes, and elevated cancer risk)

Slide Provided by Dr. Diana Rohlman

Influences on Health Effects from PAH Exposure



- Focus primarily on carcinogenic PAHs
- Less known about non-carcinogenic PAHs
- Need for toxicity information on less widely studied PAHs

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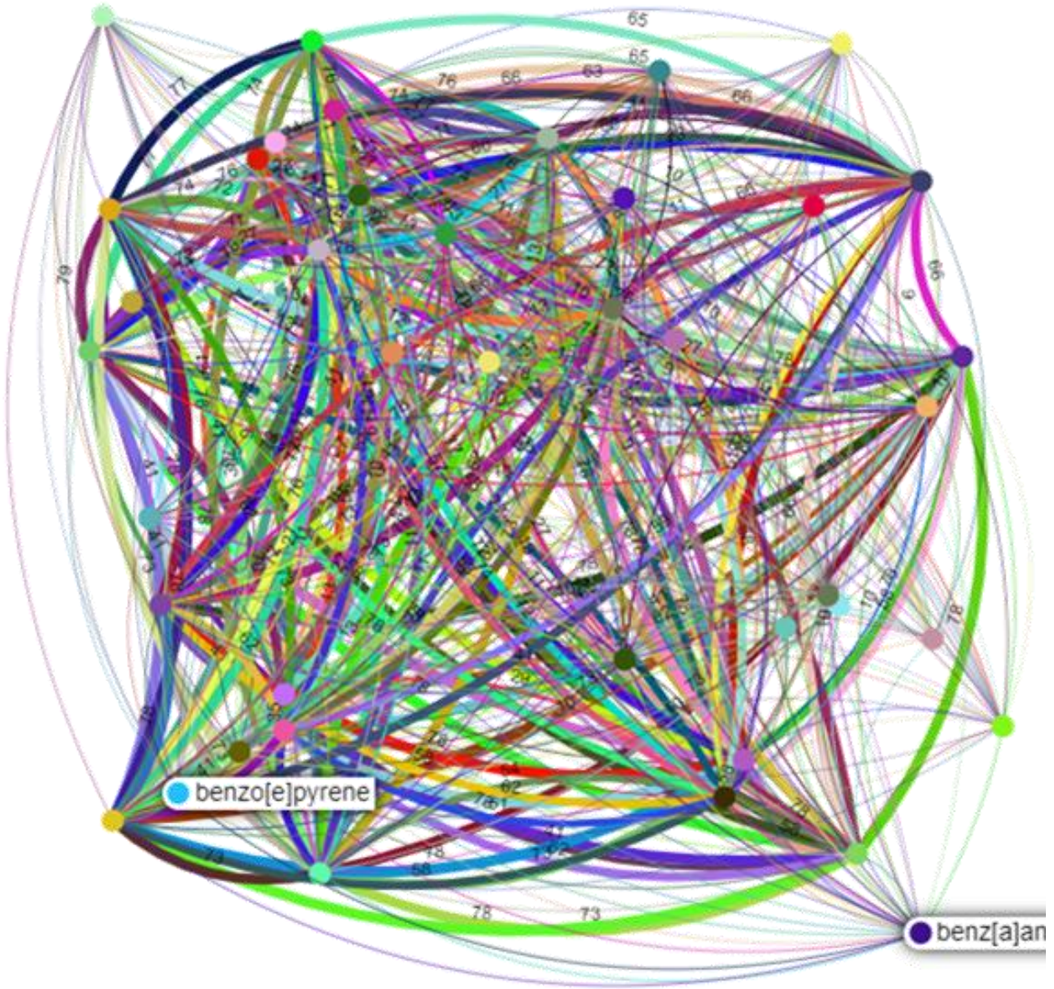
Complexity of Environmental Mixtures

- Combination of 65 PAHs in our analytical method
- Infinite number of combinations of chemicals in our environment
- Need to simplify environmental mixtures for toxicity testing



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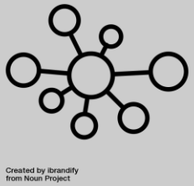
Current Approaches to Mixtures Safety Assessment

COMPONENT BASED	WHOLE MIXTURE	REPRESENTATIVE MIXTURE
Use individual chemical toxicities to predict mixture toxicity	Conduct toxicity tests with the entire mixture	Create a mixture representative of whole mixture. Maintains environmental ratios.
• Assumes additivity • Requires a comprehensive database of toxicity values	• Difficult to parse out drivers of toxicity • Infinite number of potential mixtures	• Contains chemicals of interest • Abundance? Frequency? Toxicity?

Objectives

- Investigate approaches to form mixtures from an environmental sample



- Determine which mixture captures the response of the mixture of interest
- Identify mixtures with high hazard potential to human health

Project Workflow

1-2	3-4	5	6a-6b
Collect Environmental Sample	Process Sample	Identify Chemicals in Sample	Collect Toxicity Information Form Mixtures and Test for Toxicity

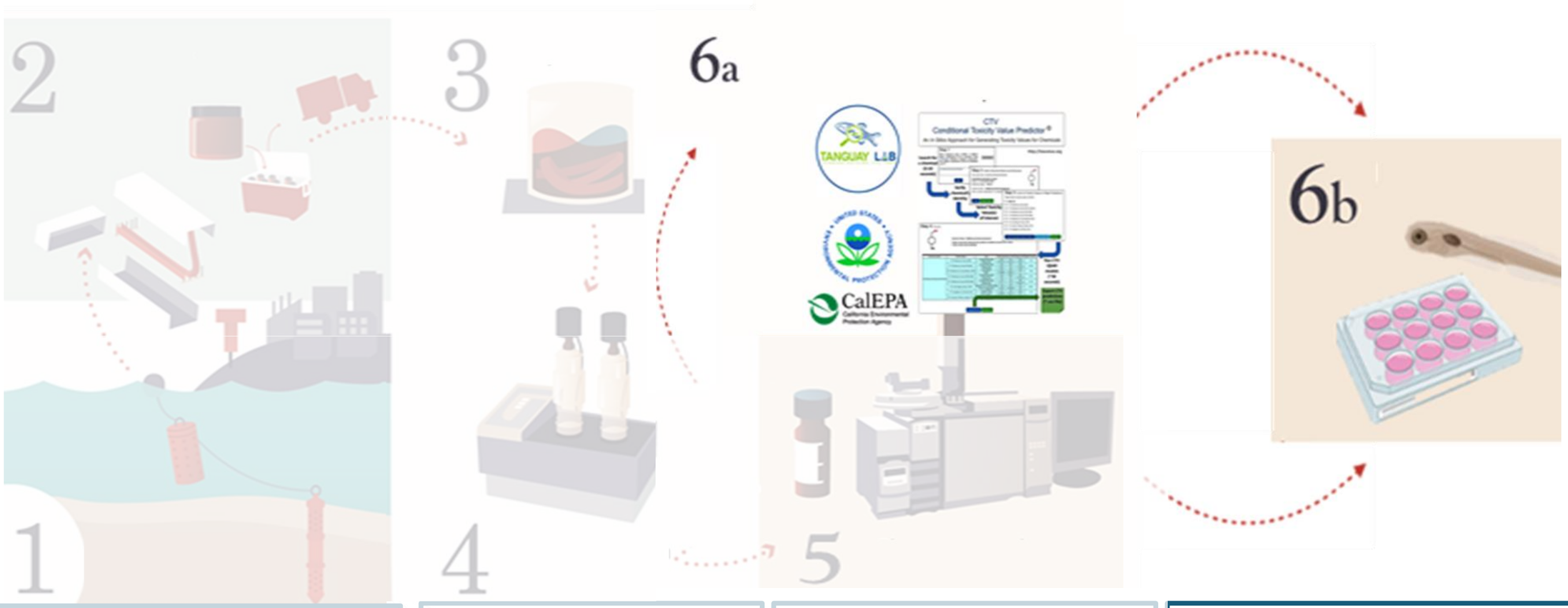
1-4: Sampling Site Details and Chemical Characterization

- Legacy creosote site impacted by wildfire smoke
- Air sampling occurred using stationary low-density polyethylene passive air samplers
- Targeted approach due to existing knowledge of PAHs in creosote and wildfire smoke

5: Sample Analysis

- Traditionally, ~20 PAHs analyzed
- Currently, our GC/MS-MS method contains 65 PAHs
- 32 PAHs identified in our samplers

Project Workflow



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Collect Environmental Sample	Process Sample	Identify Chemicals in Sample	Collect Toxicity Information Form Mixtures and Test for Toxicity

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Different Types of Mixture Formation

Abundance

- Most abundant chemicals from environmental sampling

Toxicity

- Prioritize chemicals based on toxicity information

Weighted-Toxicity

- Toxicity information is weighted based on environmental concentrations

6a: Collection of Toxicity Values for Toxicity-based Mixtures

- Collected both cancer and non-cancer toxicity values
- Individual chemicals in PAH method screened in zebrafish and BMC values calculated
- Publicly available toxicity values gathered from federal and state databases
- QSAR model was used for chemicals lacking toxicity values (Wignall 2018)
- Not all chemicals had the same type of toxicity information

IARC Classification – International Agency for Research on Cancer Classification

TEF – Toxic Equivalency Factor

RfD/C – Reference Dose/Concentration

OSF – Oral Slope Factor

IUR – Inhalation Unit Risk

BMC – Benchmark Concentration

6a: Chemical Prioritization for Toxicity-based Mixtures

- Toxicity values were sorted based on relative hazard and given a rank
- Weighted Tox Mix (W-Tox Mix), toxicity values first multiplied by chemical concentrations
- Average rank for each chemical was calculated and top 7 chosen
- Mixtures were formed using environmentally relevant ratios

Equation 1:

$$Proportion\ of\ Total = \frac{Mass\ Fraction\ of\ Chemical}{Mass\ of\ Mixture}$$

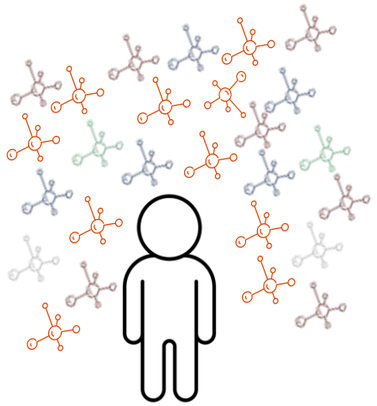
Equation 2:

$$Weighted\ Toxicity\ Value = Proportion\ of\ Total\ * Toxicity\ Value$$

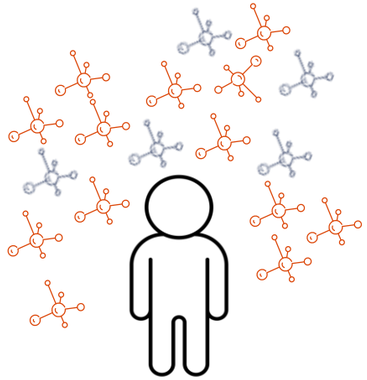
Equation 3:

$$Average\ Rank = \frac{\begin{array}{c} IARC\ Classification\ Rank + TEF\ Rank + \\ IUR\ Rank + RfC\ Rank + \\ OSF\ Rank + RfD\ Rank + \\ Zebrafish\ BMC50\ Rank \end{array}}{Total\ \#\ of\ Rankings}$$

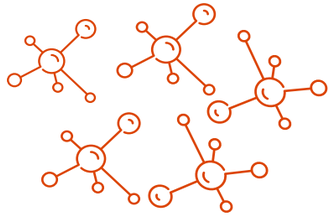
Formation of Simple PAH Mixtures



Creosote site impacted by wildfire smoke (Real-world exposure)

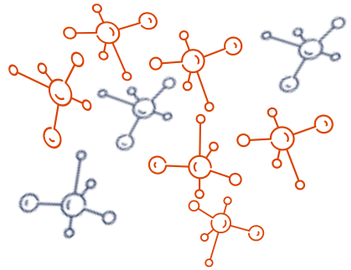


PAHs Identified in Sample (Creosote Fire Mixture) (CF Mix)



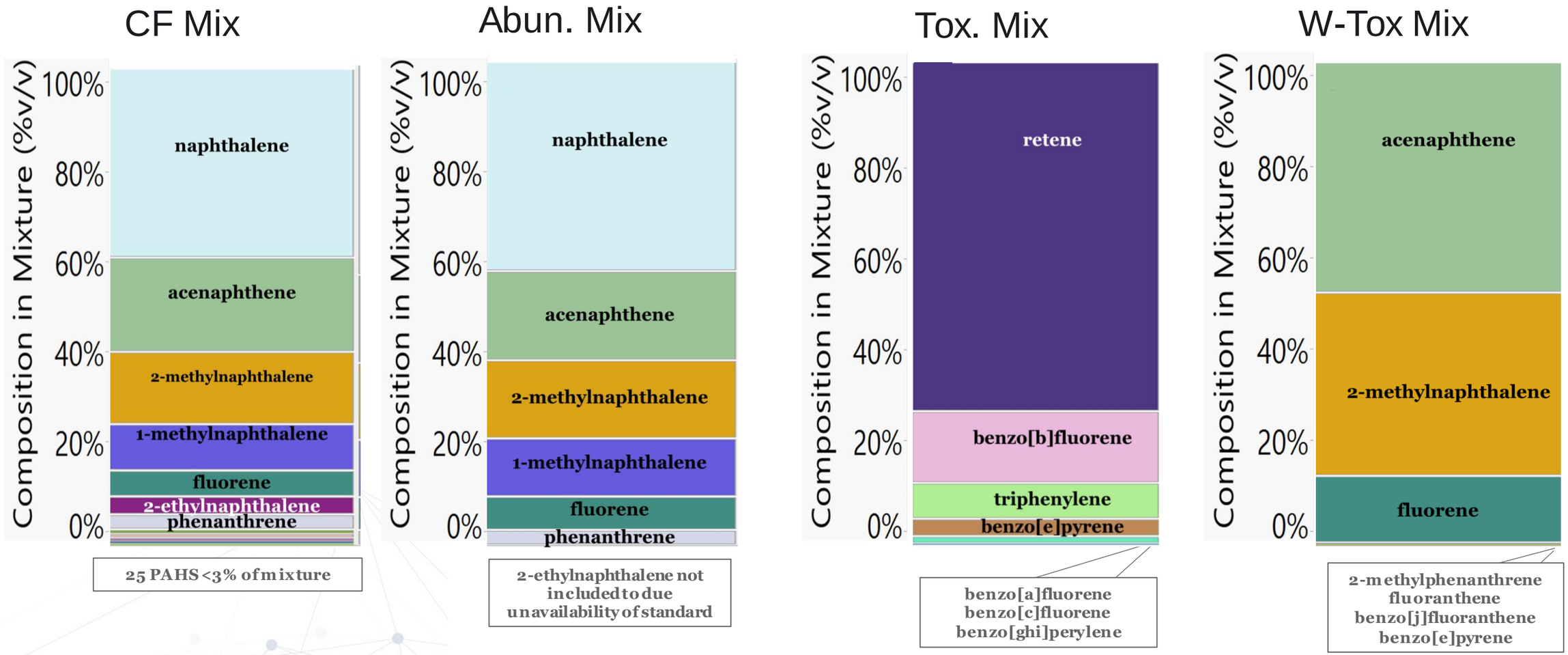
Abundance Mixture (Abun. Mix)

Toxicity Mixture (Tox Mix)



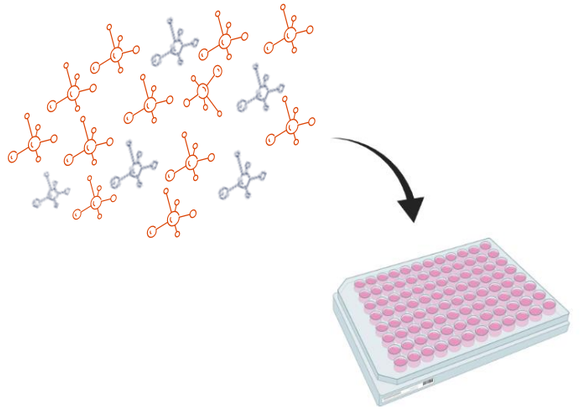
Weighted-Toxicity Mixture (W-Tox Mix)

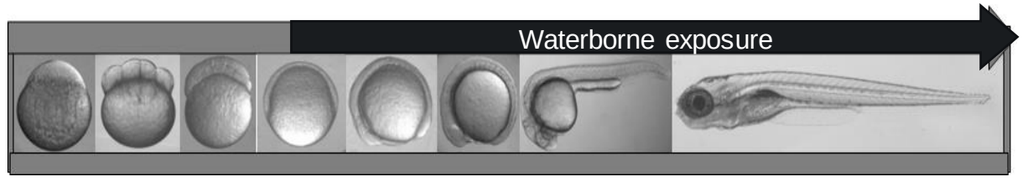
Composition of Simple PAH Mixtures



6b: Hazard Characterization Methods

- Primary normal human bronchial epithelium(NHBE) and dechorionated early lifestage zebrafish used as model organisms
- Range finding experiments conducted to define concentration-range
- In NHBE cell viability and mitochondrial membrane potential (MMP) assessed as indicators of toxicity (n = 6/conc)
- In zebrafish 13 morphological endpoints were assessed (n=12/conc)
- Concentration response curves and EC_{50} values were generated using R: *drc* package



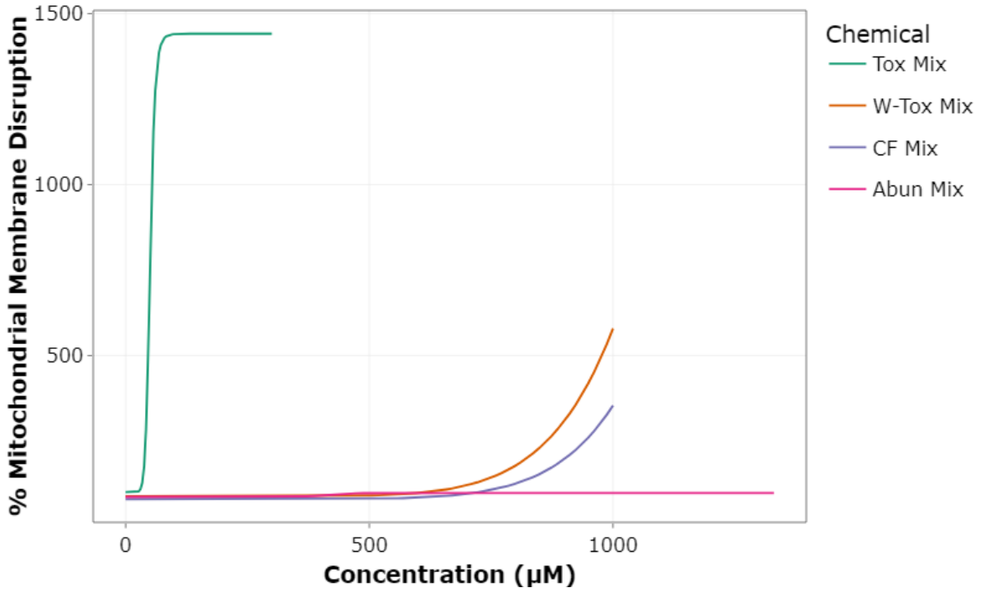


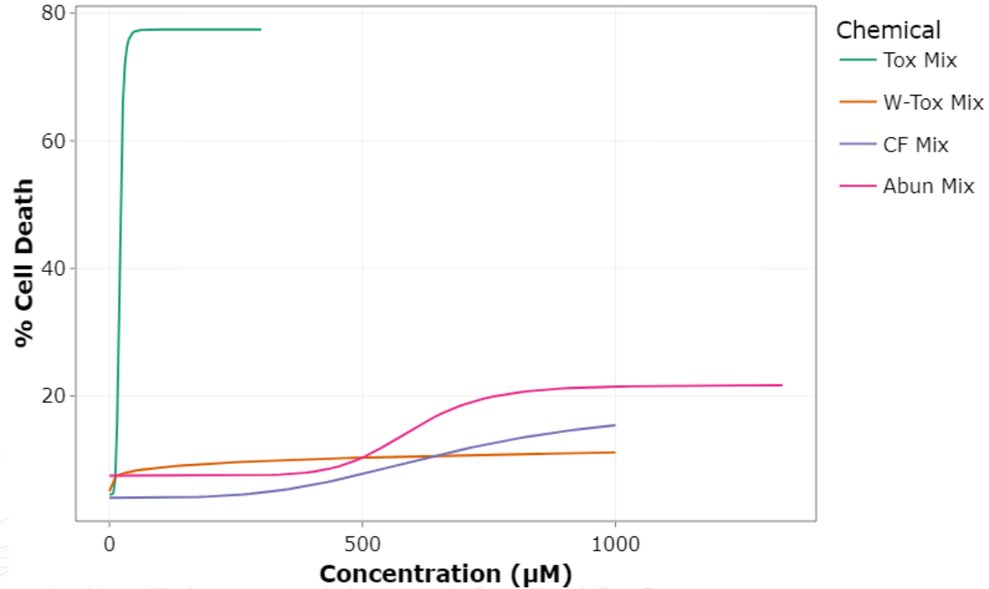
6-8 hpf
Exposure begins

24 hpf
Screen for malformations

120 hpf
Screen for malformations

Hazard Characterization of Simple PAH Mixtures in NHBE

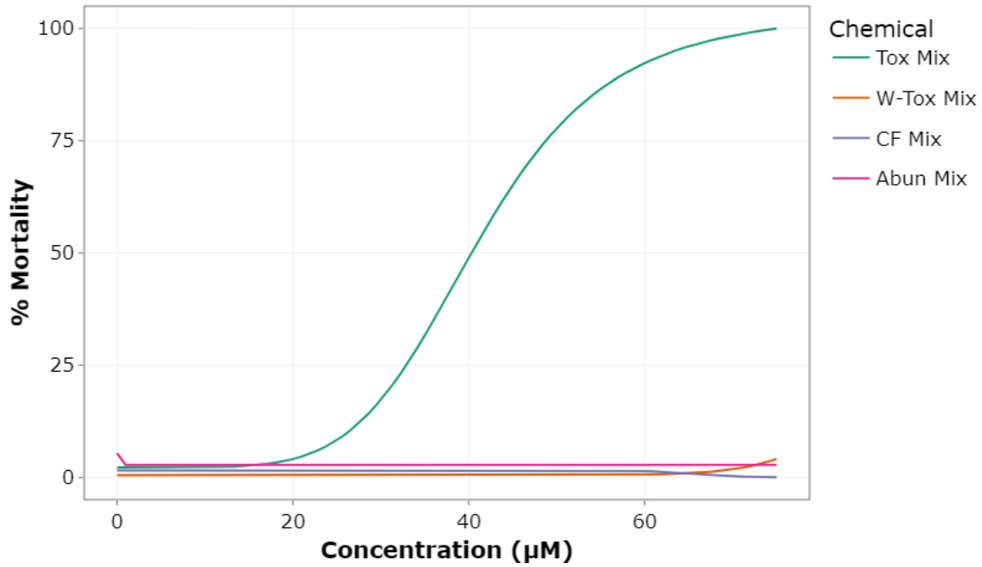


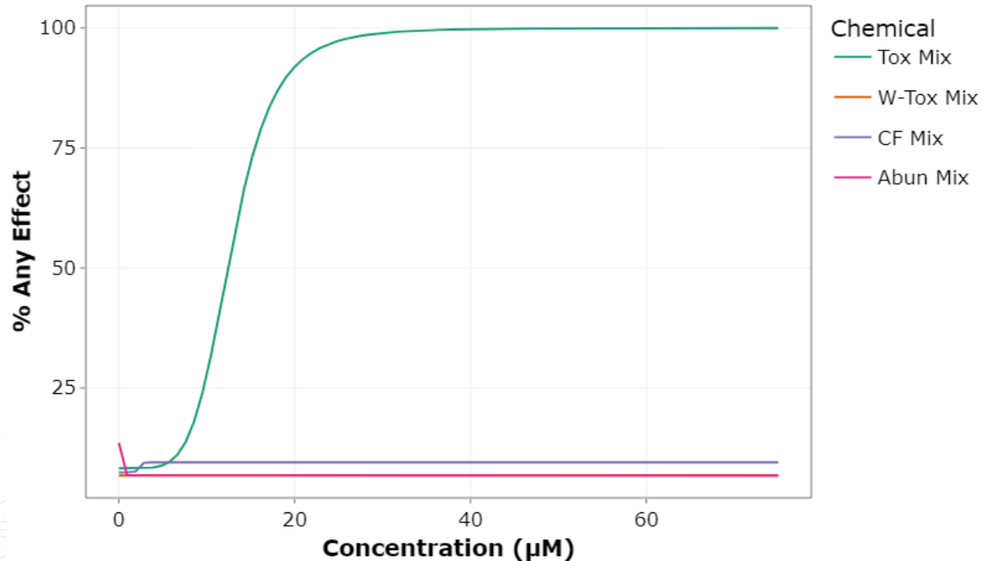


- Tox Mix most hazardous mixture in NHBE
- W-Tox Mix response most similar to CF Mix for MMP

Mixture	EC_{50} (μM)	Endpoint
Abun. Mix	NA	Cell Death
	NA	MMP
Tox. Mix	20	Cell Death
	38	MMP
W-Tox Mix	NA	Cell Death
	1700	MMP
CF Mix	NA	Cell Death
	1750	Cell Death

Hazard Characterization of Simple PAH Mixtures in Zebrafish





- Tox Mix most hazardous mixture
- Results in zebrafish confirm those in NHBE

Mixture	EC_{50} (μM)	Endpoint
Abun. Mix	NA	Any Effect
	NA	Mortality
Tox. Mix	12.8	Any Effect
	41.1	Mortality
W-Tox Mix	NA	Any Effect
	NA	Mortality
CF Mix	NA	Any Effect
	NA	Mortality

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Main Takeaways

Weighted approach most similar response to targeted mixture

Mixture formation should consider both hazard and abundance

Tox Mix components identified as high hazard in both model systems

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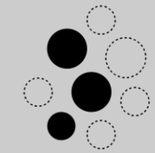
Future Directions

Narrow down concentration range in NHBE for MMP and Cell Viability

Evaluate additional endpoints in NHBE

Further investigate individual components in Tox Mix

Limitations



Created by Strategiemakers
from Noun Project

Chemicals prioritized using a targeted approach

Created by The Icon Z
from Noun Project

Influences of sampling on chemicals identified in sample

Created by Saeful Muslim
from Noun Project

Availability of toxicity data

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Thank you

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