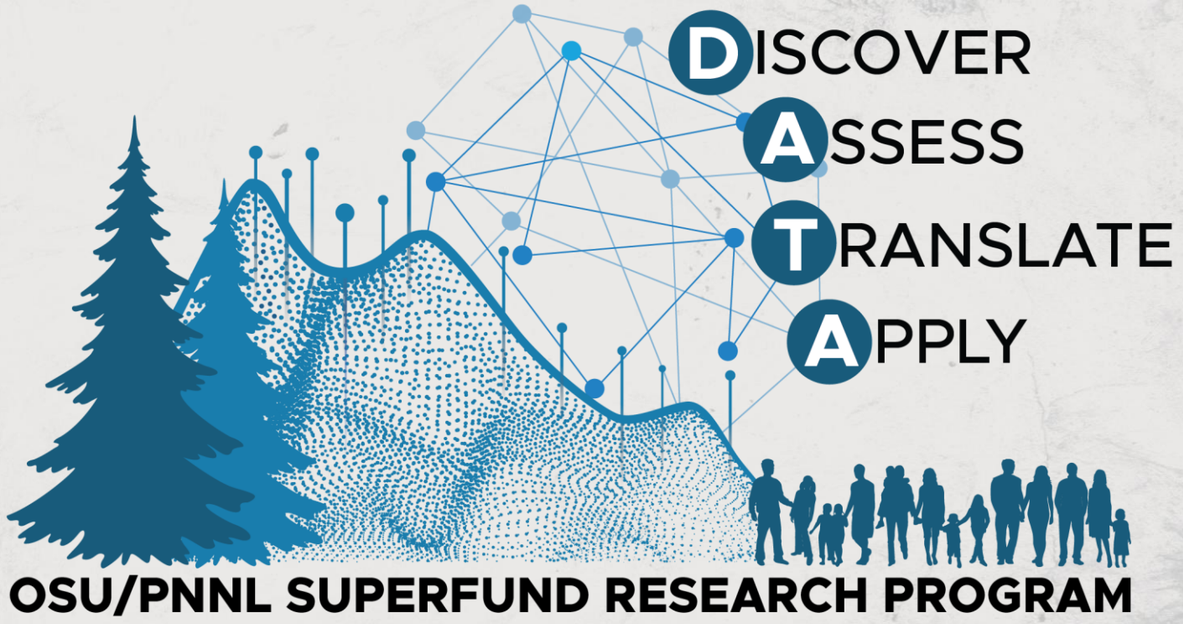
Linking PAH Exposure to Health Outcomes Using a Primary Human *In Vitro* Respiratory Model



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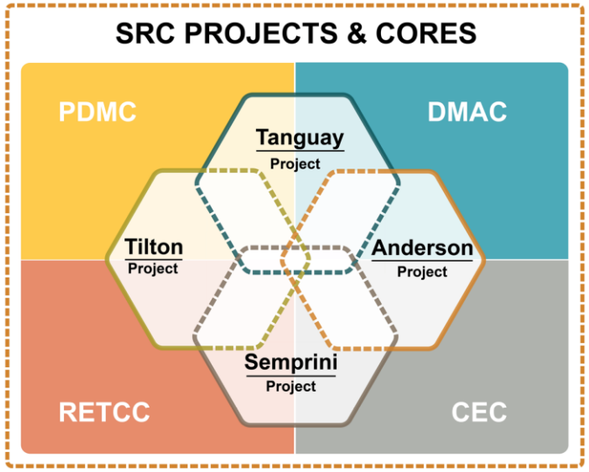
Oregon State University

NIEHS SRP Risk e-Learning Webinar Series
From Cells to Solutions – Session II
January 16, 2026

OSU/PNNL SRP

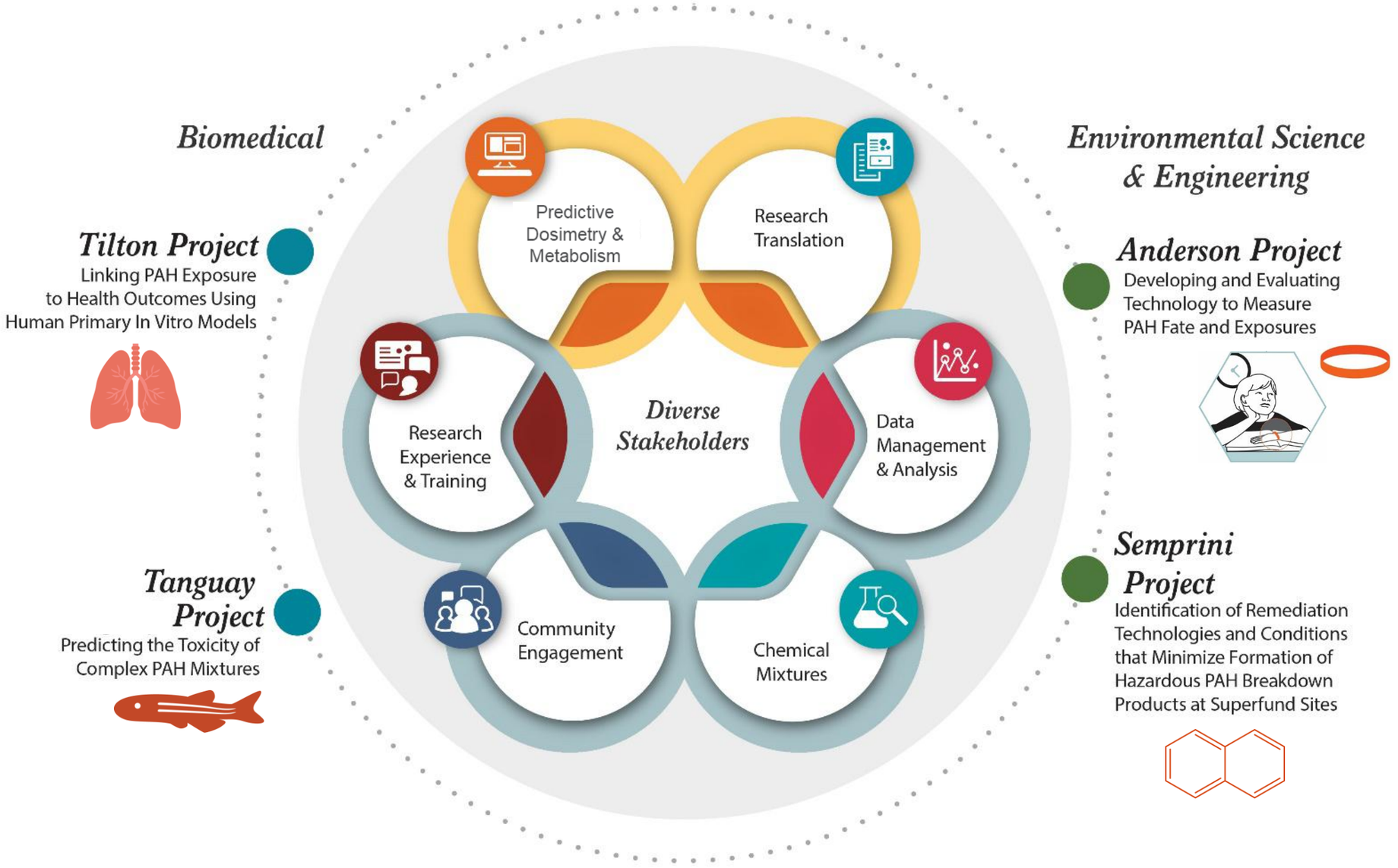
Center for the Science, Technology and Emerging Health Risks of PAHs



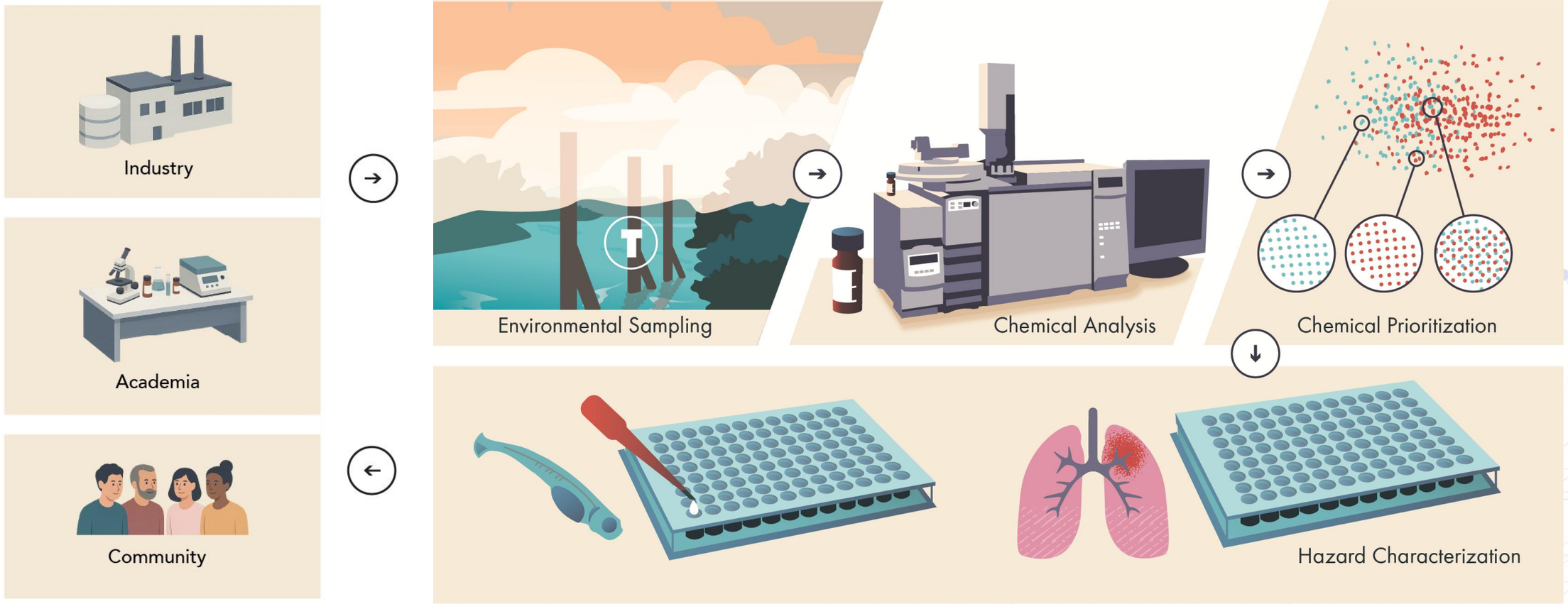
Identification, measurement, and toxicity of PAHs
The mission of the Oregon State University Superfund Research Center is to identify Polycyclic Aromatic Hydrocarbons (PAHs) in the environment, to characterize their toxicity, and to determine what levels of those chemicals in the environment are safe for human health.

Center for the Science, Technology, and Emerging Health Risks of PAHs

OSU/PNNL SRP



Center for the Science, Technology, and Emerging Health Risks of PAHs

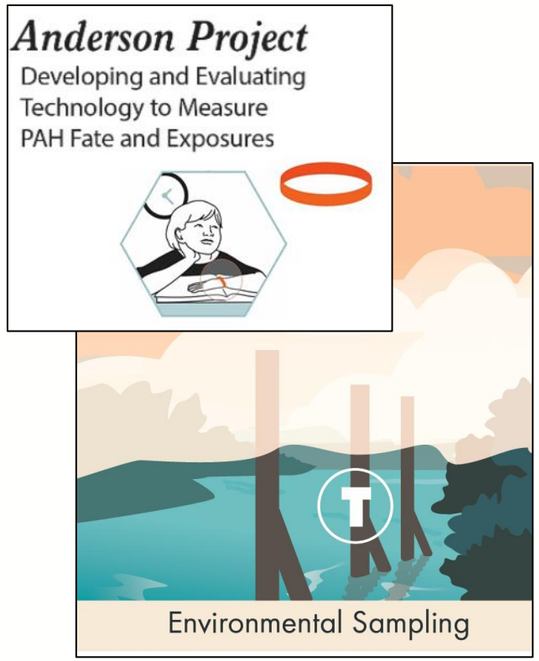


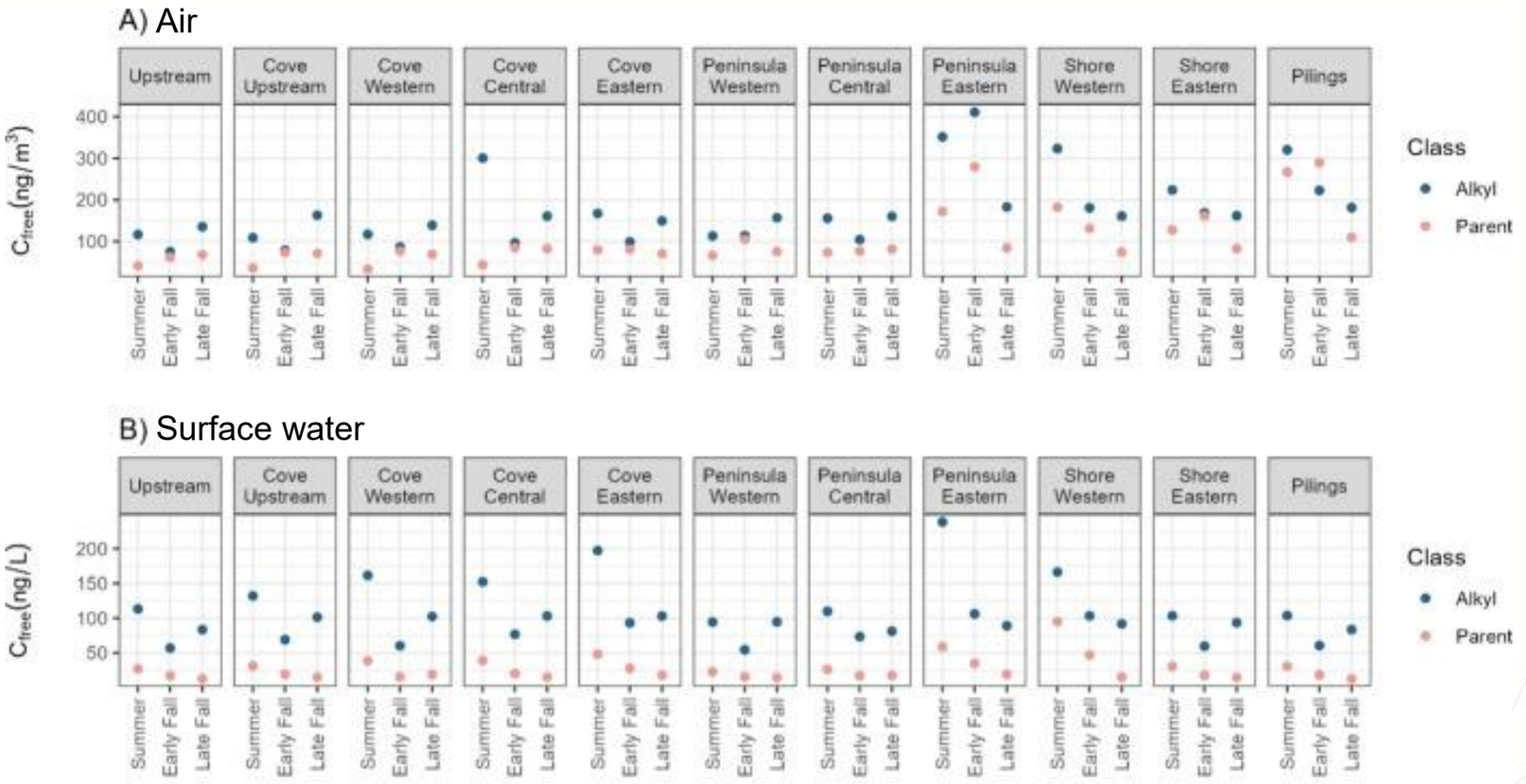
Rivrera et al. (2022) Toxics. 10(11):651.

Polycyclic Aromatic Hydrocarbons (PAHs)

- Planar compounds of benzene rings
- Occurring naturally in coal, creosote, etc.
- Formed during incomplete combustion
- Connected with many adverse health effects
  - Carcinogenicity, Immunotoxicity, Reproductive/Developmental
  - Inhalation is a primary route of exposure

Prevalence of Alkylated PAHs

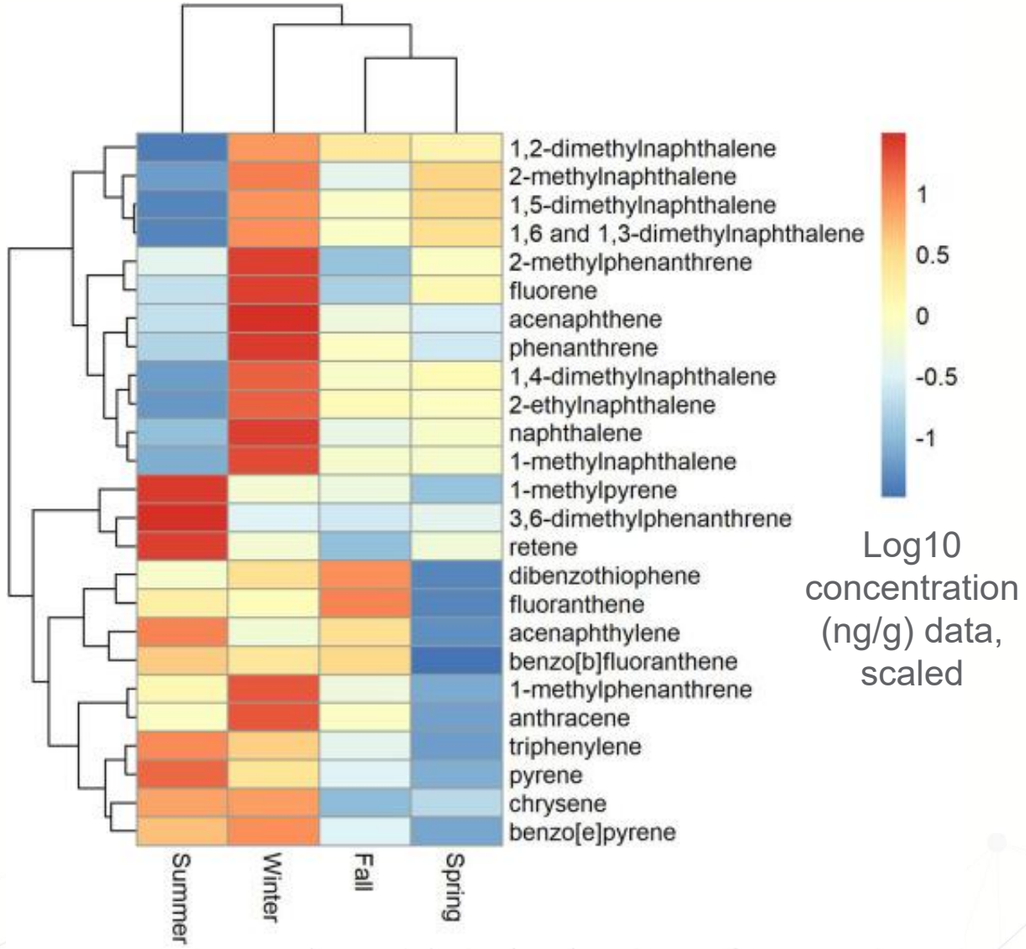


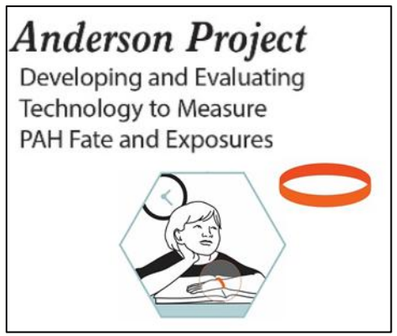


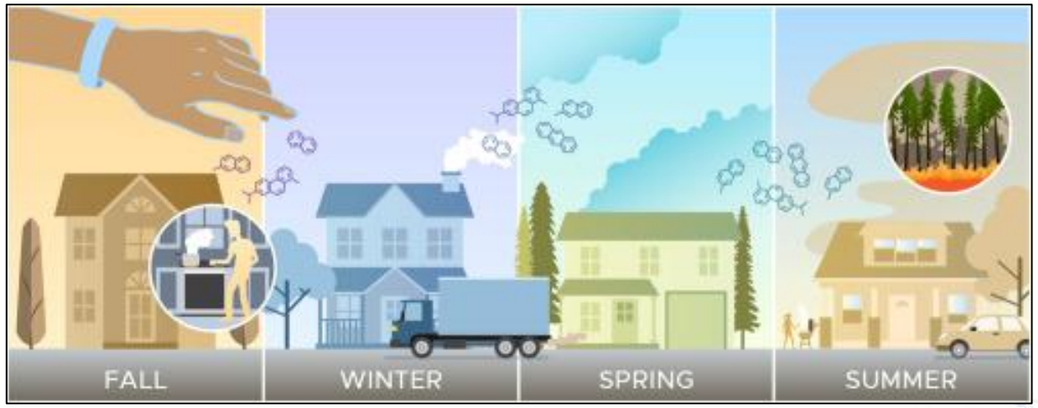
- Passive sampling devices were deployed in the air and water at a Brownsfield creosote site
- In all cases, the alkylated PAHs were more abundant than unsubstituted PAHs

Moran et al. (2024) ACS ES T Water. 5(1):146-155.

Prevalence of Alkylated PAHs







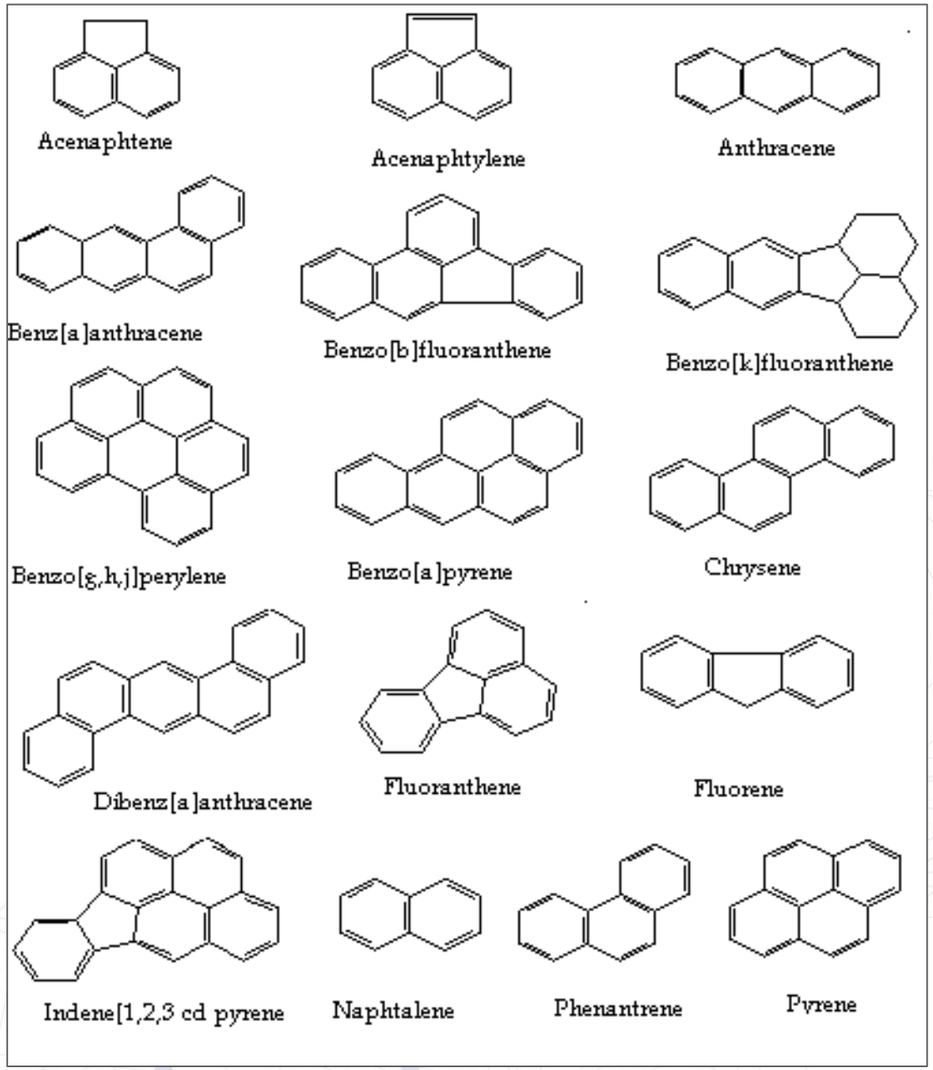
- Quantitative data on personal exposure to polycyclic aromatic hydrocarbons (PAHs) in 162 silicone wristbands
- Several 2-3 ring alkylated PAHs were measured in high abundance depending on season

Bonner et al. (2026) Environ Poll. 390:127549.

Polycyclic aromatic hydrocarbons (PAHs): Chemical characteristics

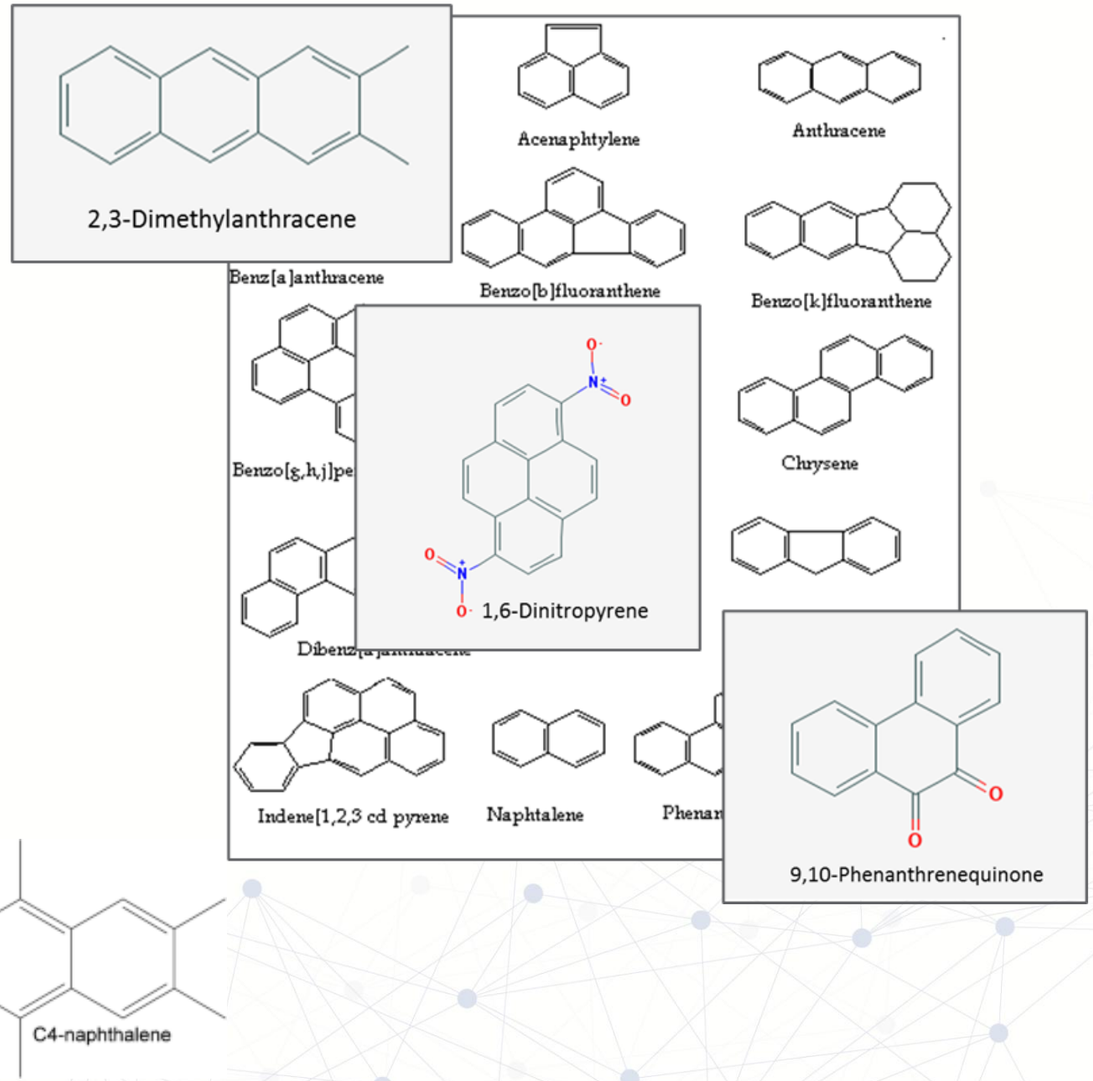
16 priority EPA PAHs (ATSDR, 2005)

- Toxicity
- Potential for human exposure
- Frequency of occurrence at hazardous waste sites
- Available information
- Include probable and known human carcinogens

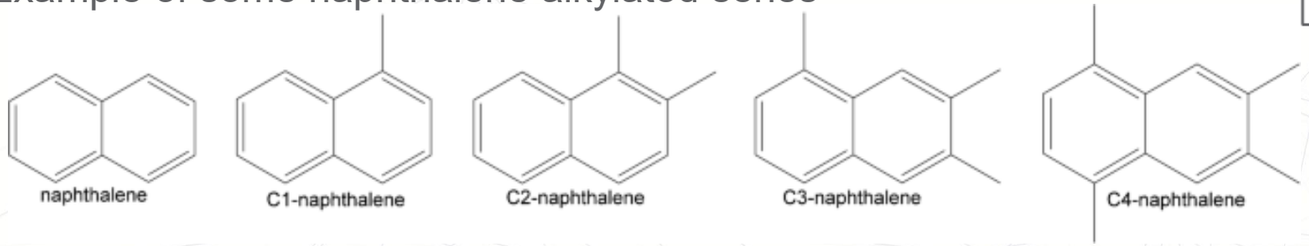


Polycyclic aromatic hydrocarbons (PAHs): Chemical characteristics

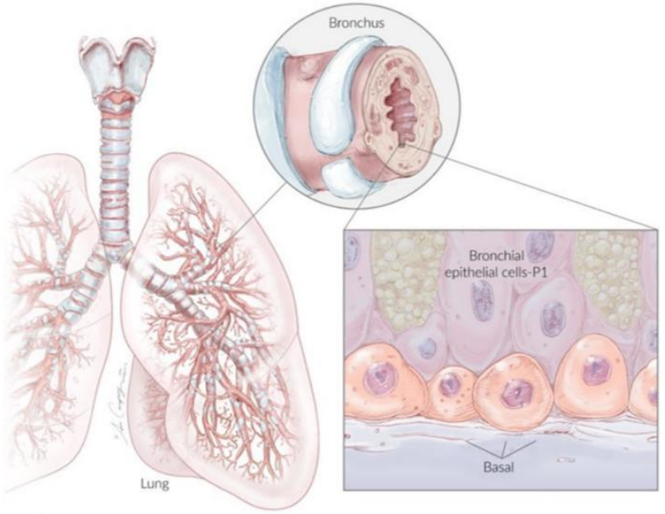
- Broader class of polycyclic aromatic compounds
  - >1500 chemicals total
  - Diverse structural features
  - Includes both unsubstituted and substituted forms
  - O-, N-, S-, CH3-
  - Little data available on sources, exposure, toxicity, mechanisms

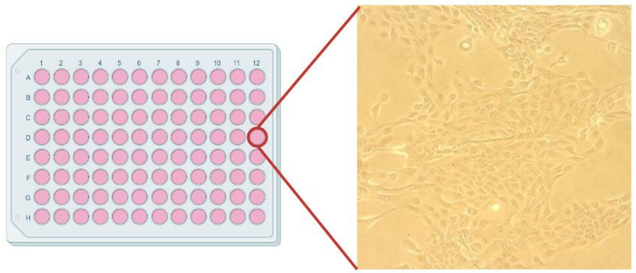


Example of some naphthalene alkylated series

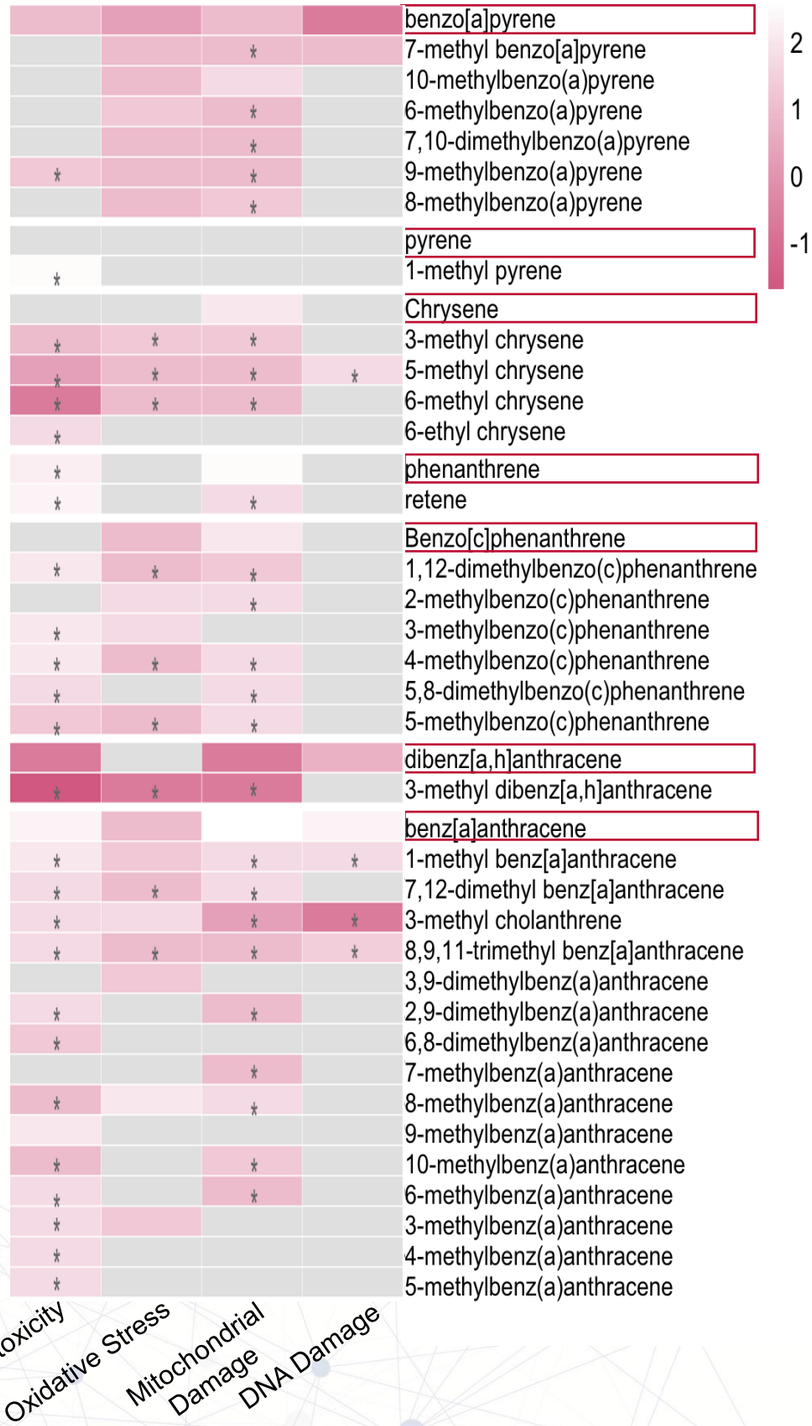


Alkyl-PAH Toxicity





Many alkyl-PAHs cause toxicity at concentrations lower than unsubstituted PAHs across multiple endpoints



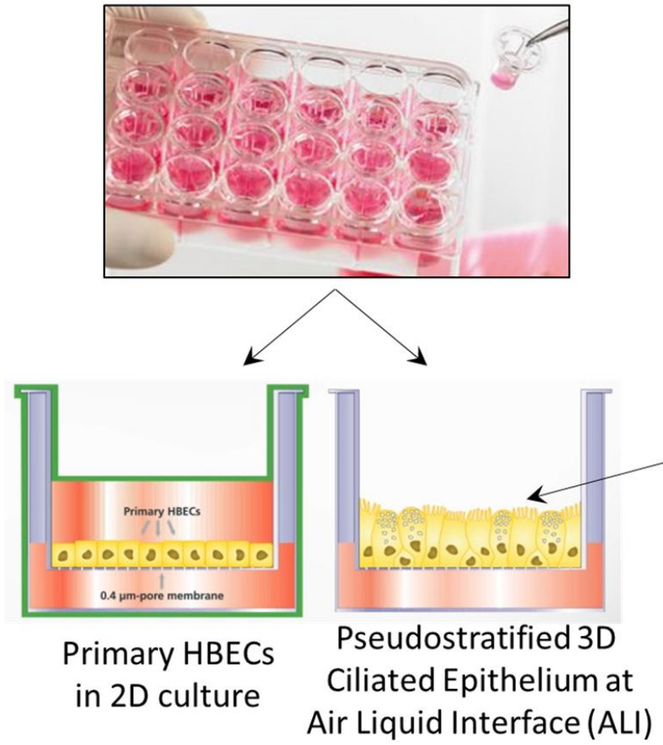
Asterisks (*) indicate that the alkylated PAH caused a statistically significant response at a concentration lower than its corresponding parent compound

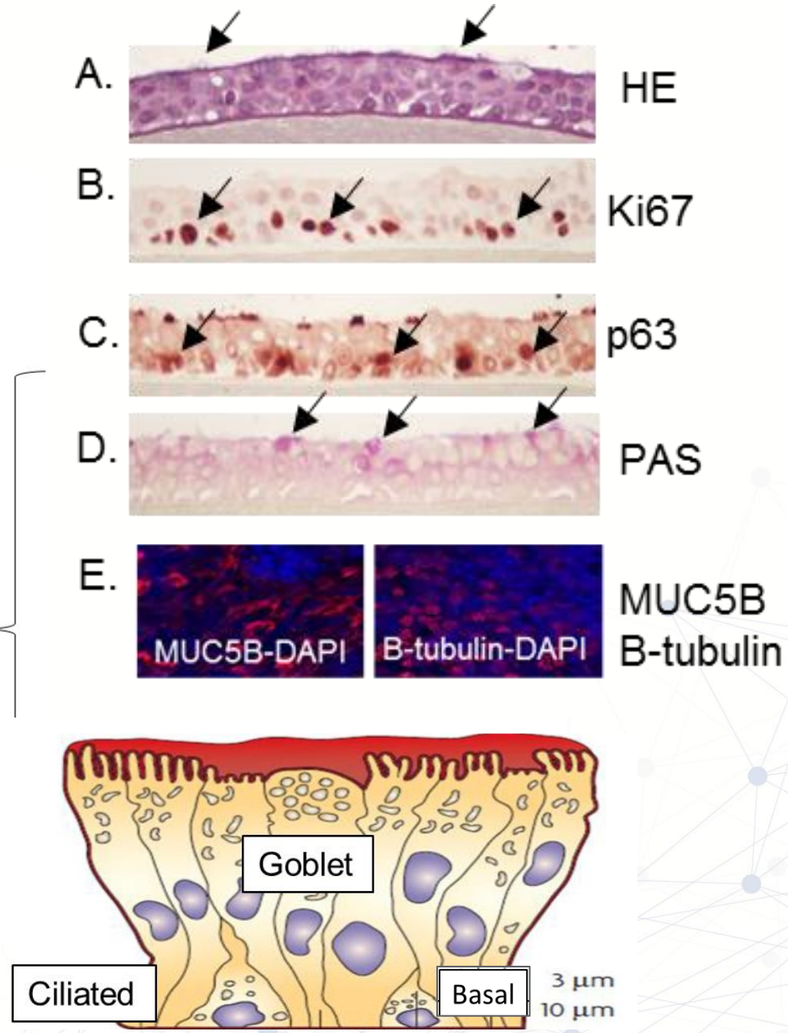
3D Airway Organotypic Culture Model

Air-Liquid Interface
(ALI) Culture Models:

<u>Benefits of model:</u>

- Primary human bronchial epithelial cells (HBECs)
- 3D structure
- Metabolic and mitotic activity
- Multi-cellular communication and cell signaling
- More accurately reflects exposure at tissue interfaces
- Better recapitulates *in vivo* response





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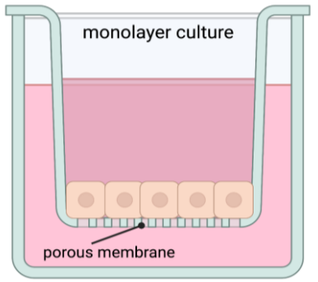
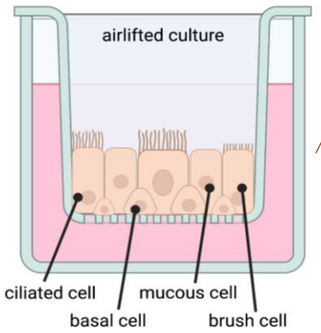
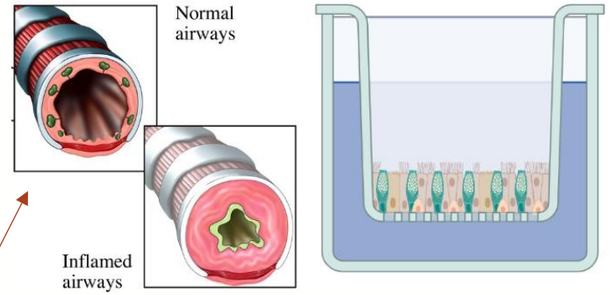
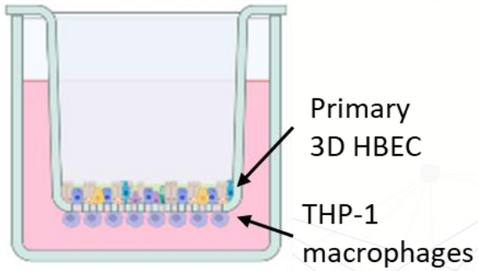




3D Disease Model



Co-Culture with Immune Cells



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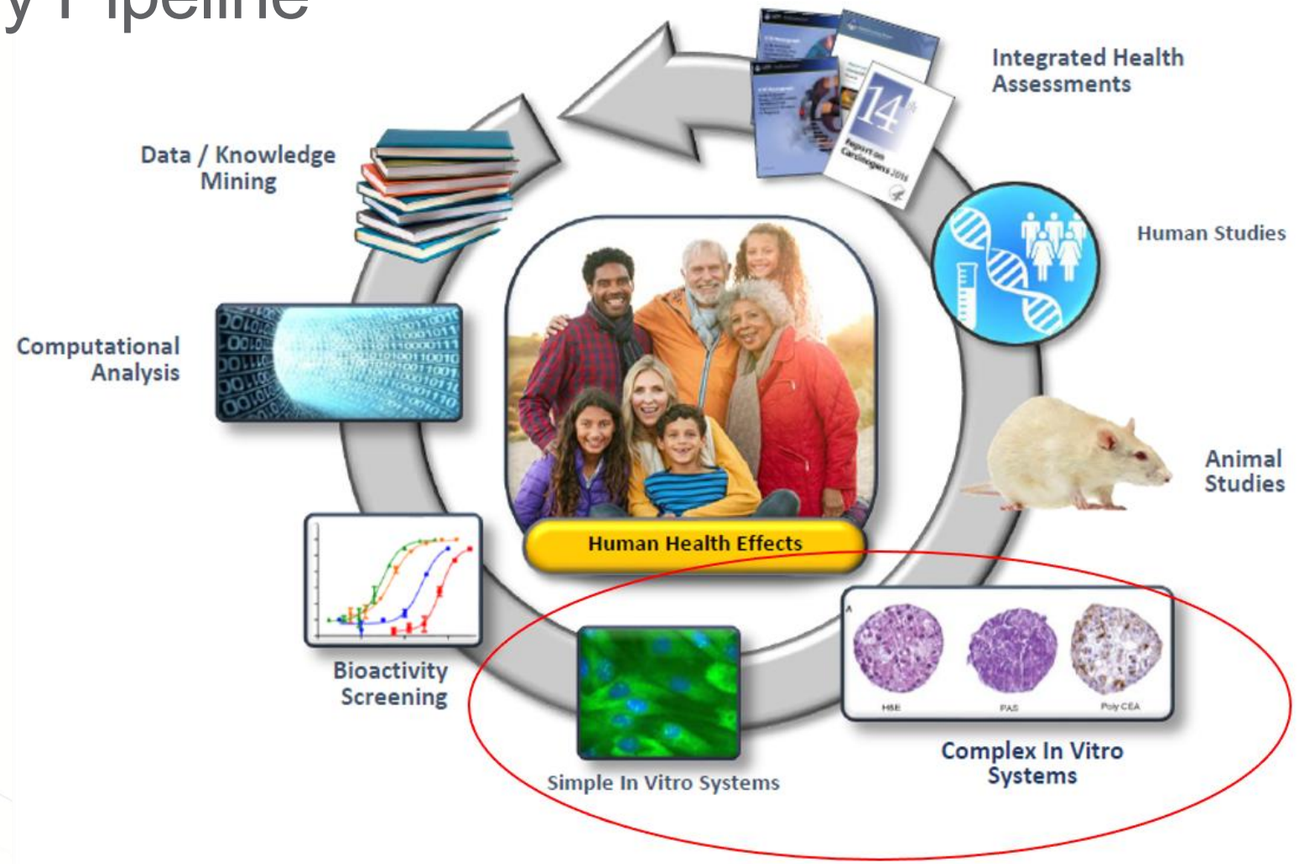
Utilization of New Approach Methodologies (NAMs) in environmental health

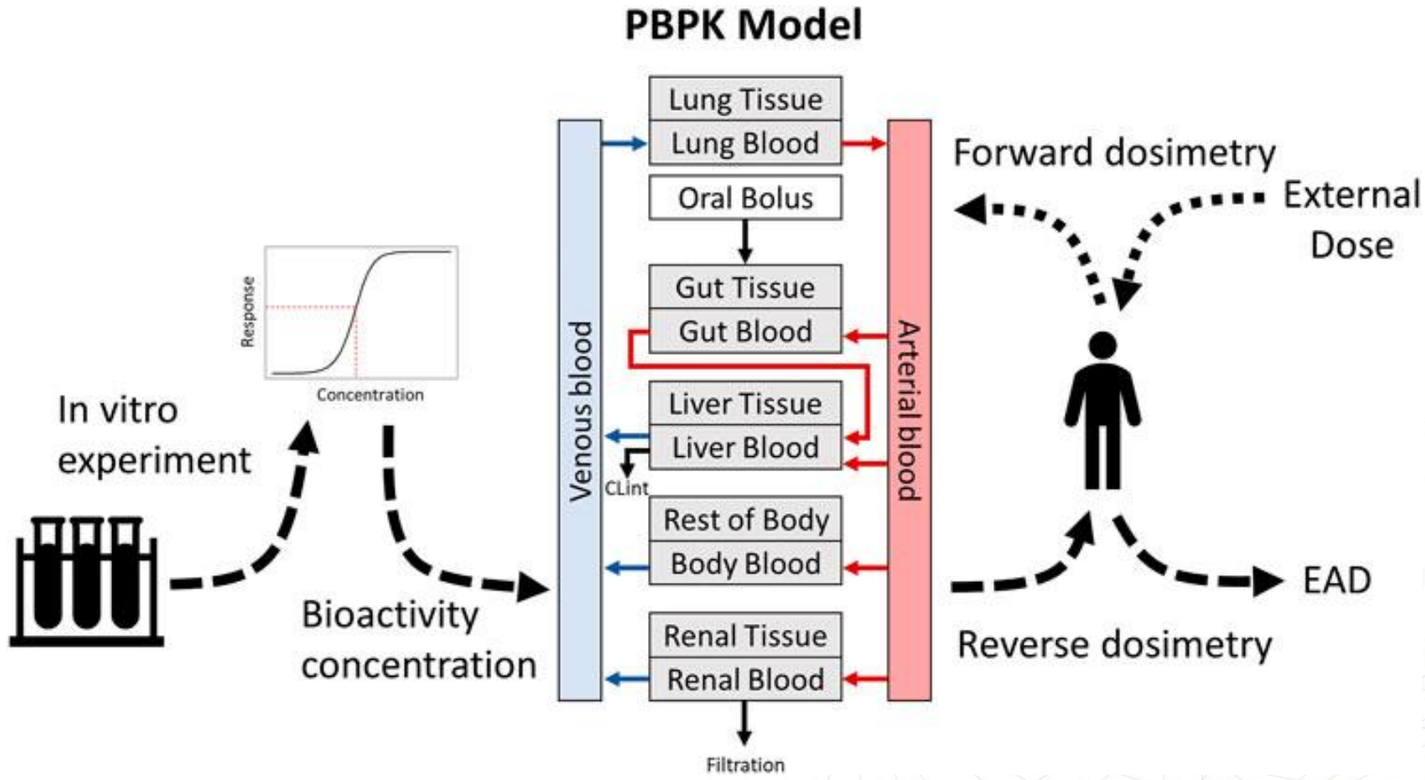
Translational Toxicology Pipeline

- Development of complex in vitro systems
  - Multicellular
  - Metabolically competent
- Replace traditional animal models
- Allow translation/extrapolation to humans
- Improve understanding of key events leading to adverse outcomes in humans
- More predictive of toxicity and disease



Develop a quantitative PBPK/dosimetry model for 3D culture systems

- Develop pharmacokinetic model to extrapolate *in vitro* toxicity to adverse health outcomes *in vivo*
- Understanding role of metabolism in for PAH toxicity in 3D model
- Compare to environmentally-relevant exposure concentrations for assessing risk



Hines et al. 2022. Front. Pharmacol. 13:864742.

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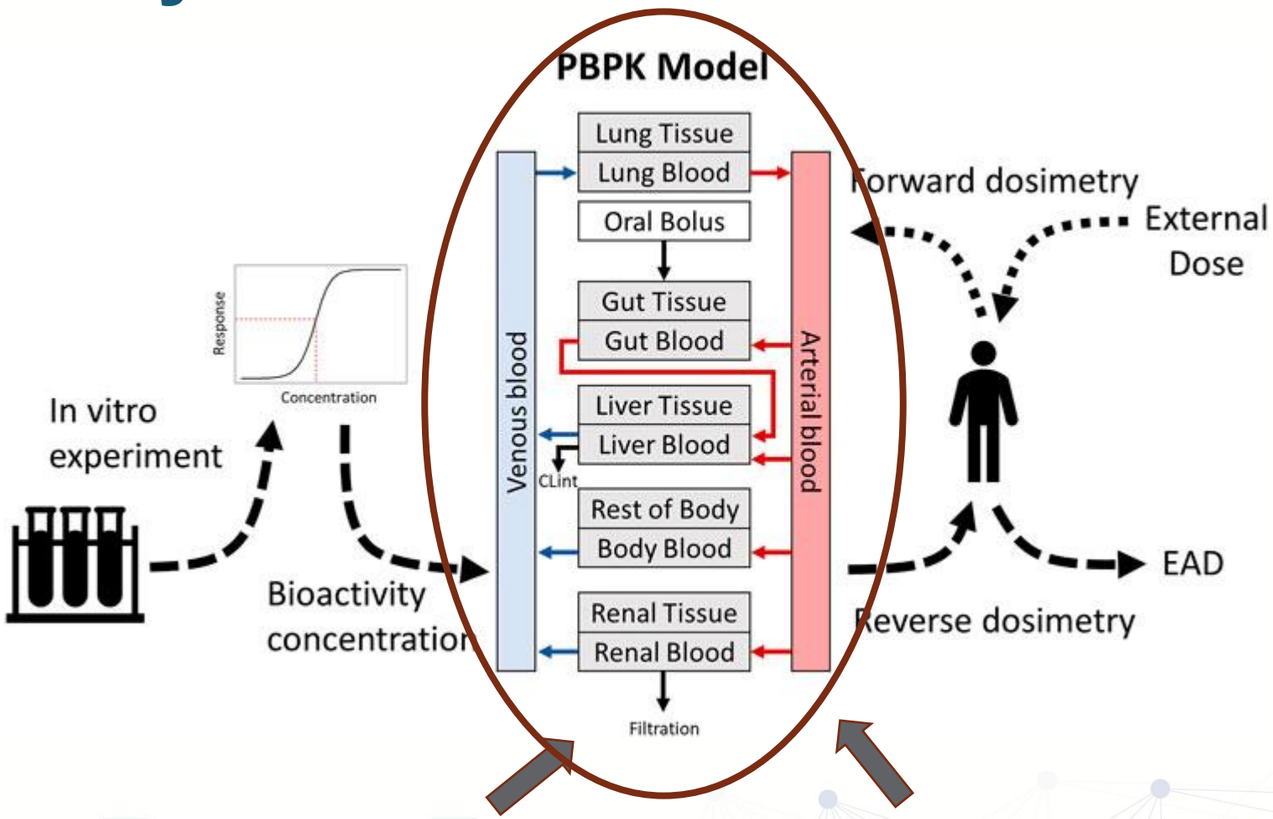
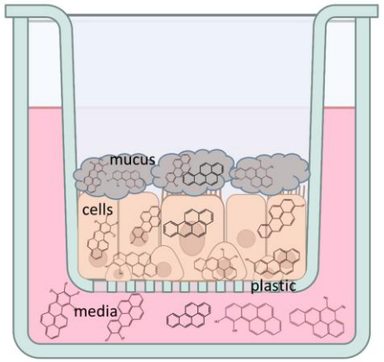
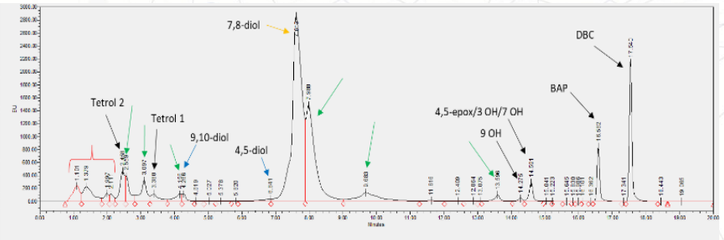




PAH metabolism in 3D model



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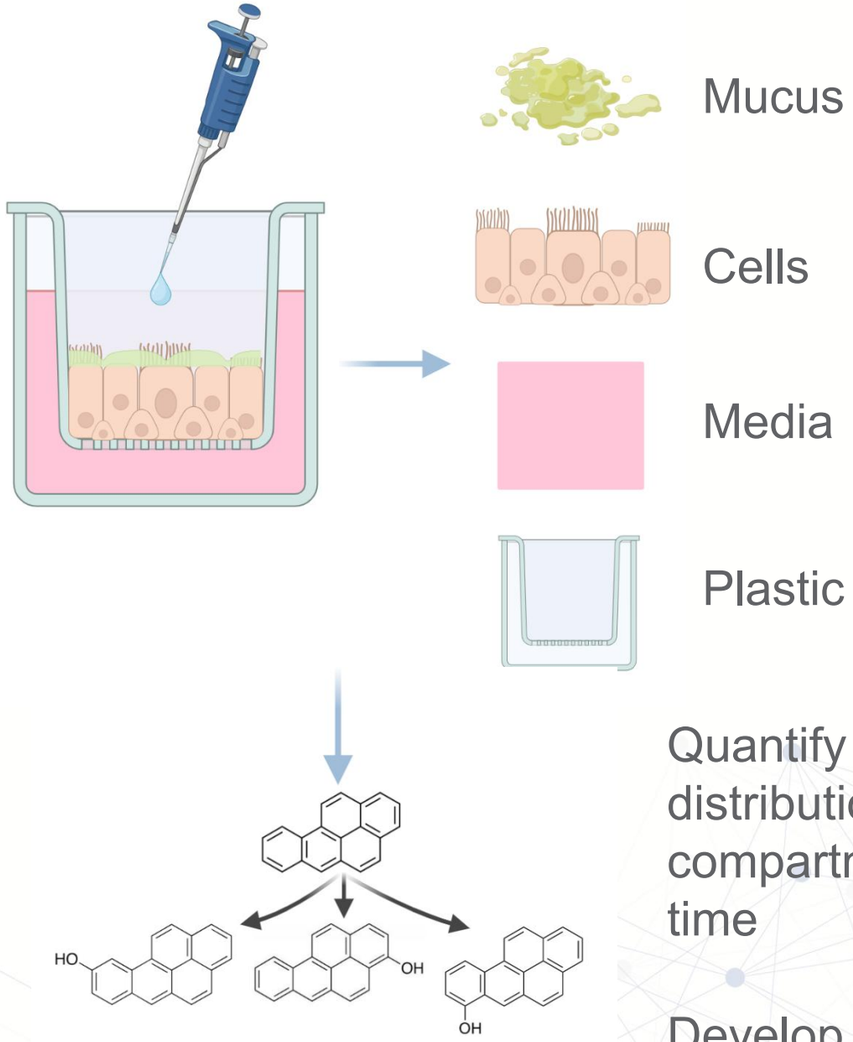

Quantify PAH metabolism and distribution into different 3D model compartments across dose and time

Develop parameters for dosimetry model

Develop a quantitative PBPK/dosimetry model for 3D culture systems

- Develop pharmacokinetic model to extrapolate *in vitro* toxicity to adverse health outcomes *in vivo*
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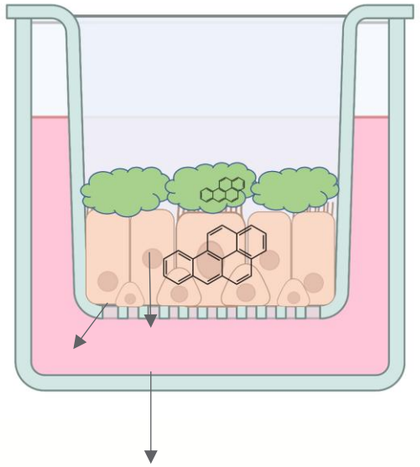
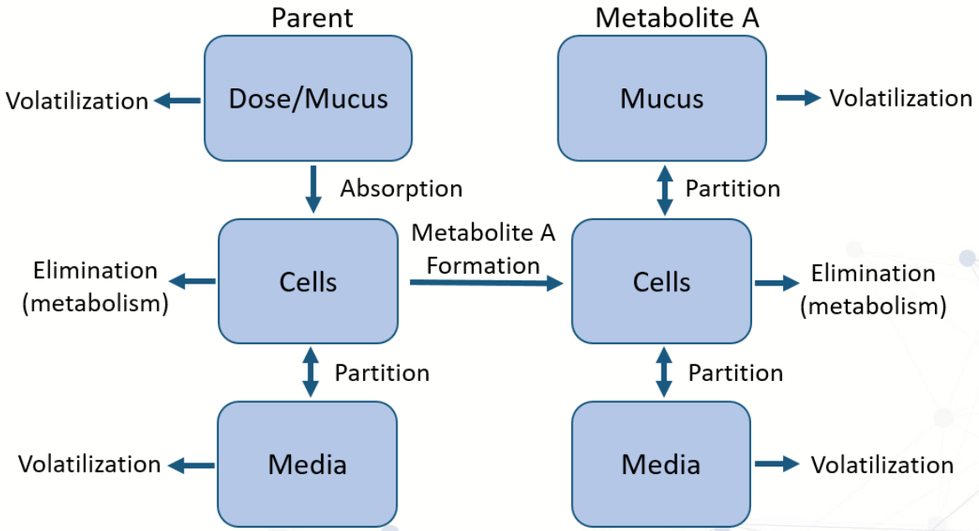
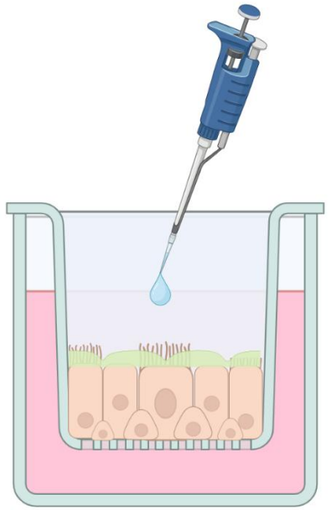
PBPK Model Building

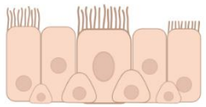




Disposition of BAP in 3D model

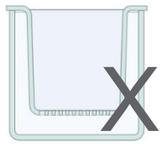


Mucus

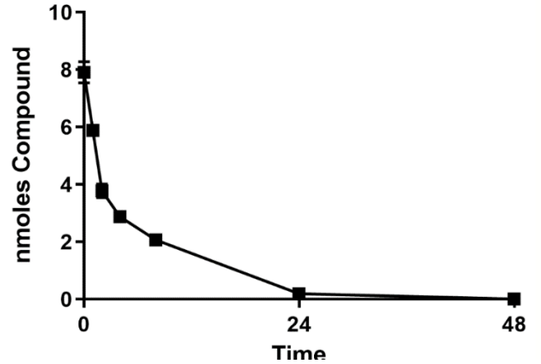


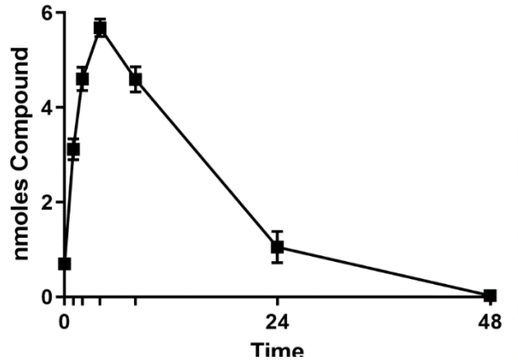
Cells

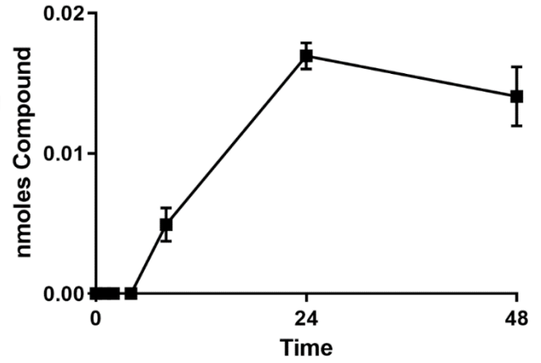
Media



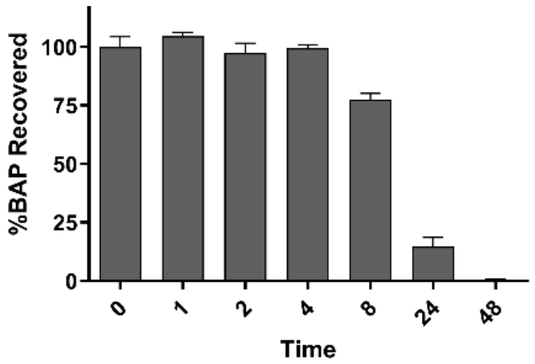
Plastic







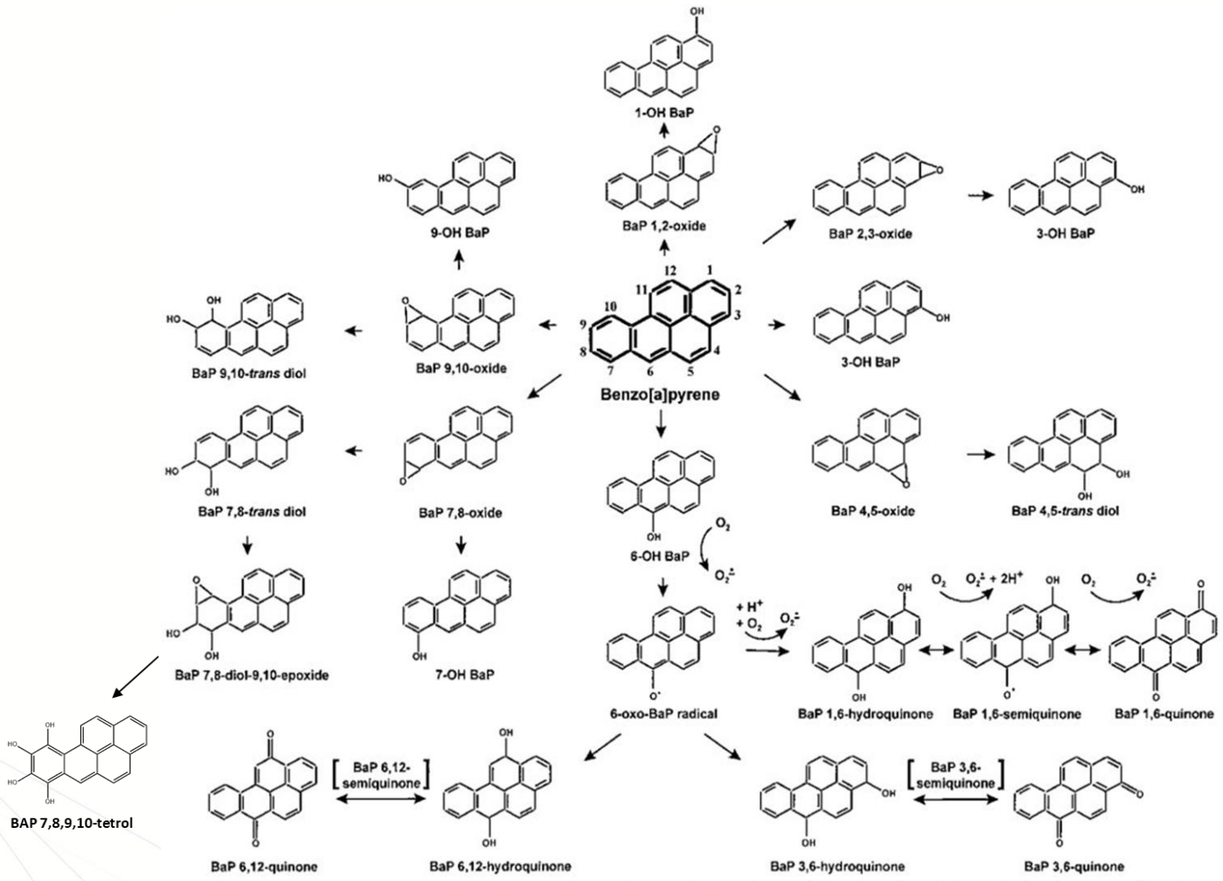
Total recovery of BAP:



Can we observe PAH metabolite formation?

- 3D cells exposed to BAP 0-48 hrs total hours
  - BAP 10 nmoles
  - Collect media, mucus, and cell fractions
- Ethyl acetate extraction on media, mucus, and cell fractions
  - DBC used as internal standard
- Identify parent and metabolites by UPLC
  - Standards for 14 BAP metabolites
  - Fluorescence detection

BAP Metabolism

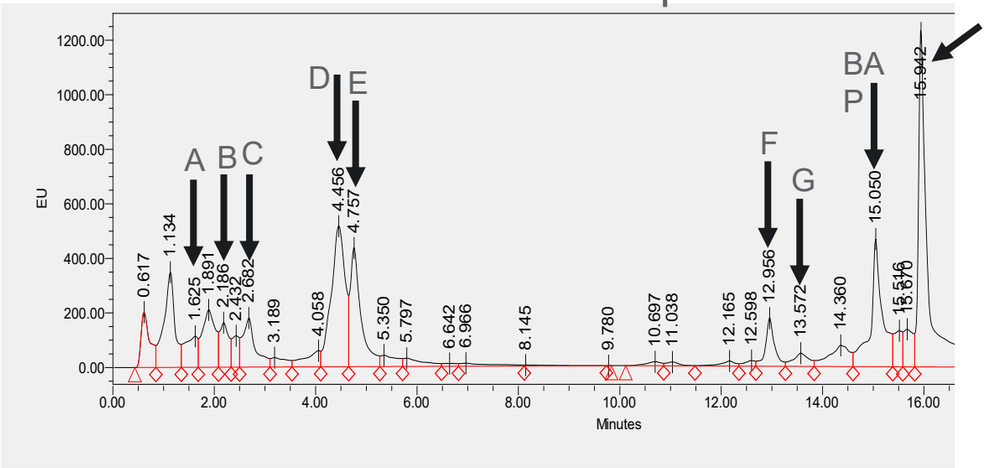


Standards

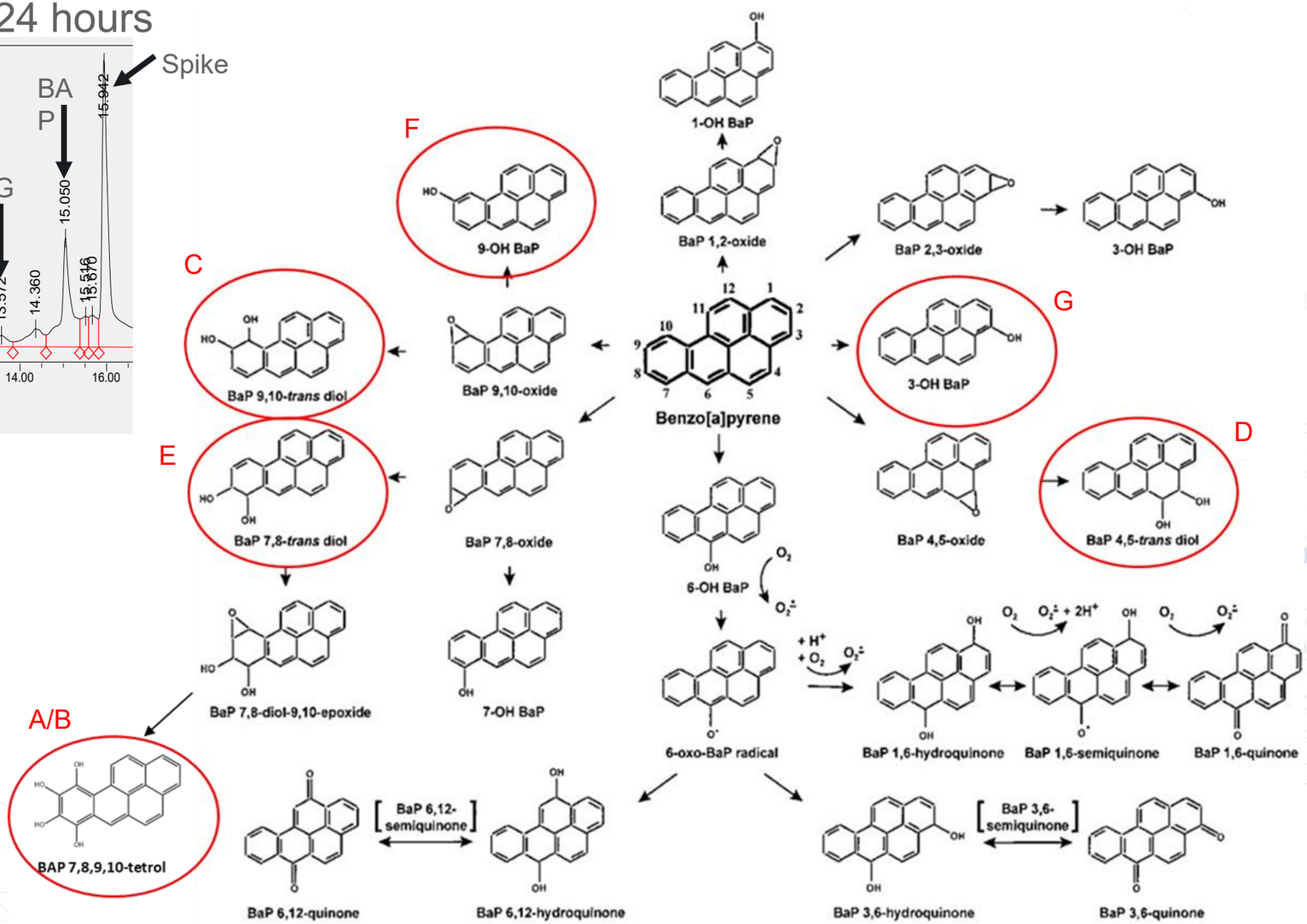
Metabolite	Steps from Parent
4,5-epoxide	1
6-OH	1
3-OH	1,2
1-OH	2
7-OH	2
9-OH	2
4,5-diol	2
7,8-diol	2
9,10-diol	2
7,8-quinone	3
3,6-quinone	4
6,12-quinone	4
tetrol	4
1,6-quinone	5

Formation of metabolites

BAP Treated Media Sample at 24 hours

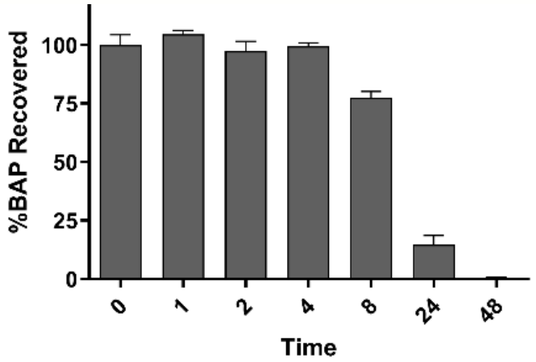


Identified metabolite

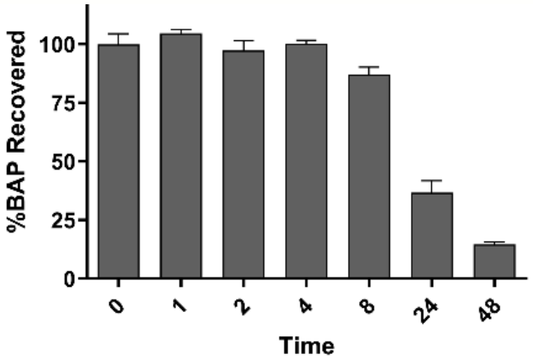


Formation of metabolites

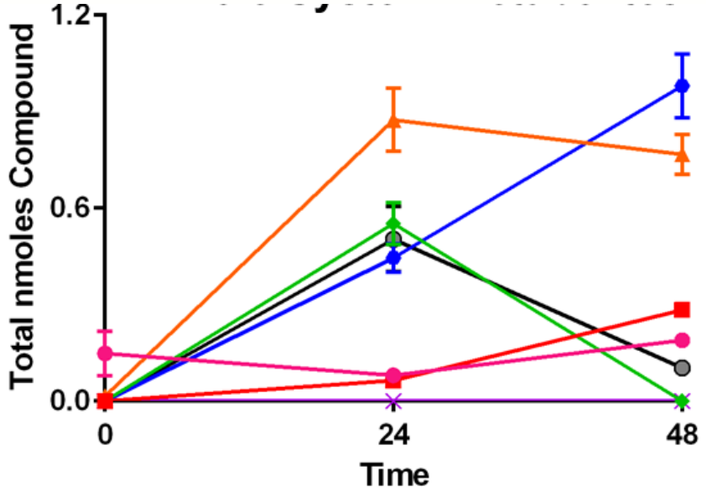
Recovery of BAP only:



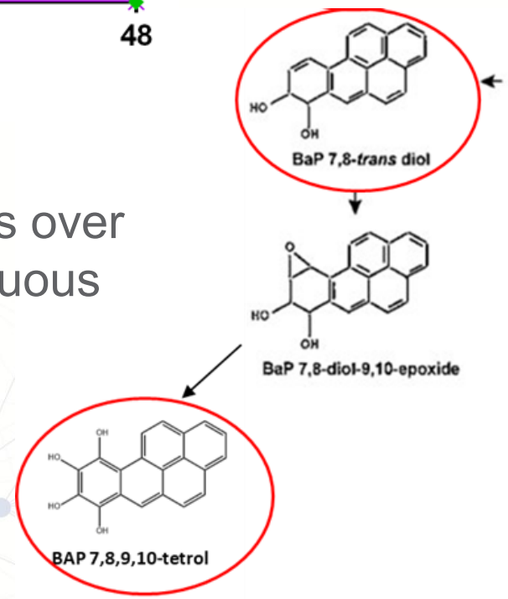
Recovery of BAP + Metabolites:



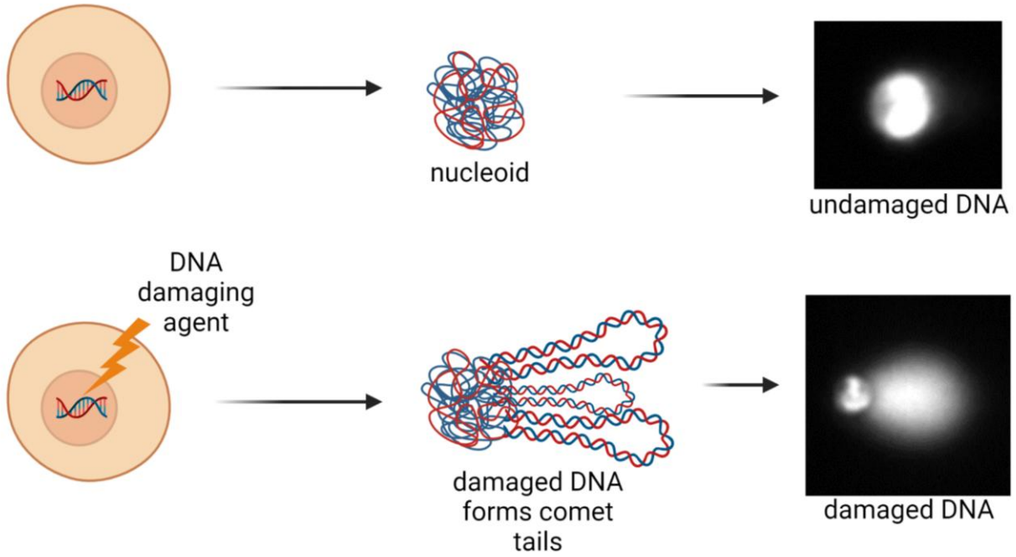
Combined total BAP metabolites



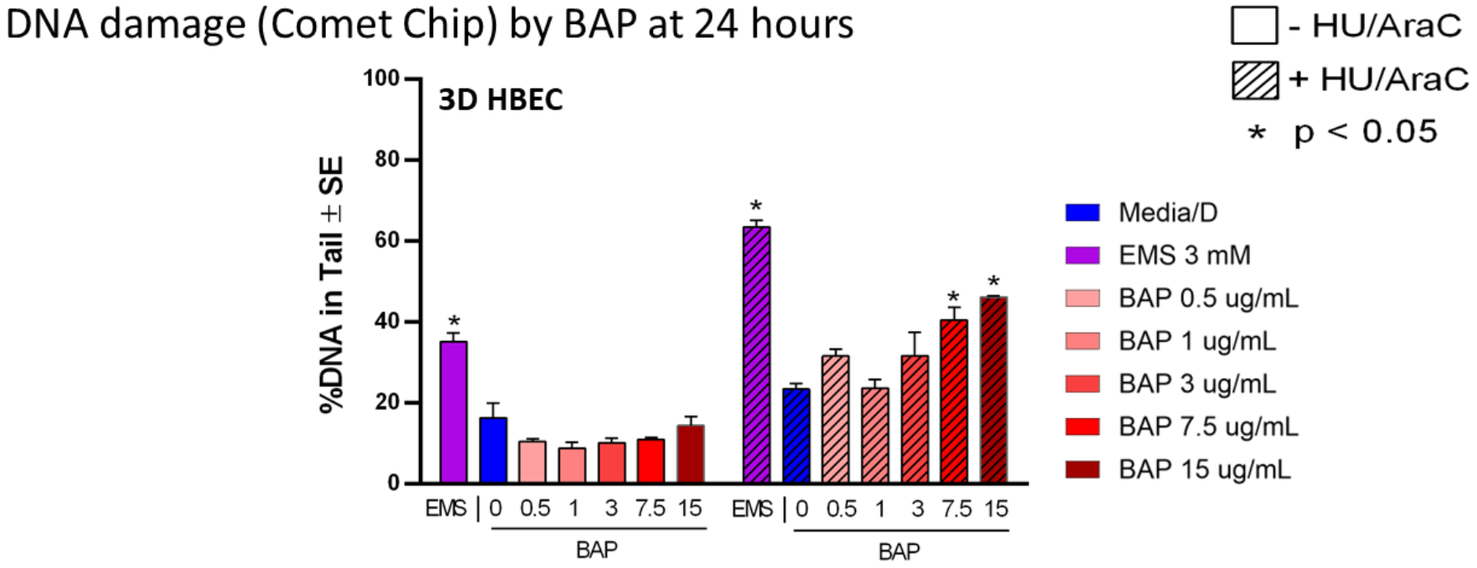
Increase in 7,8-diol and tetrols over time show potential for continuous formation of BPDE



DNA Damage Mediated by PAHs in 3D HBEC

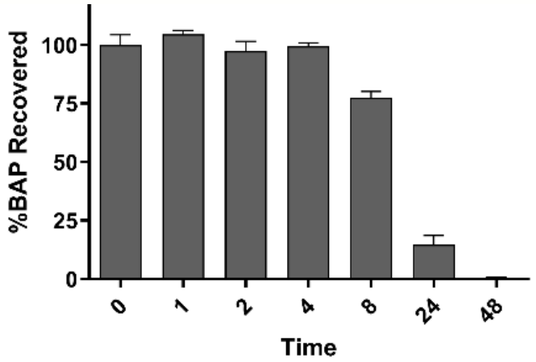


- Detect single strand breaks by PAHs
- Trapping agents halt NER leaving SSBs
  - Hydroxyurea (HU)
  - 1-β-D-arabinofuranosyl cytosine (AraC)

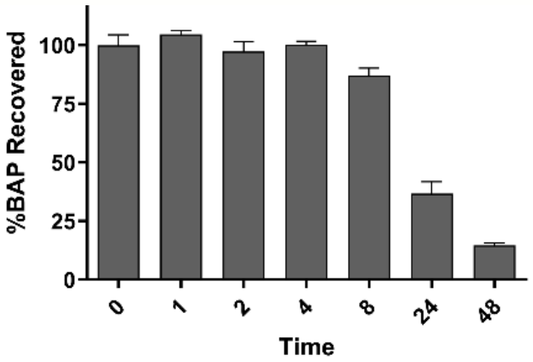


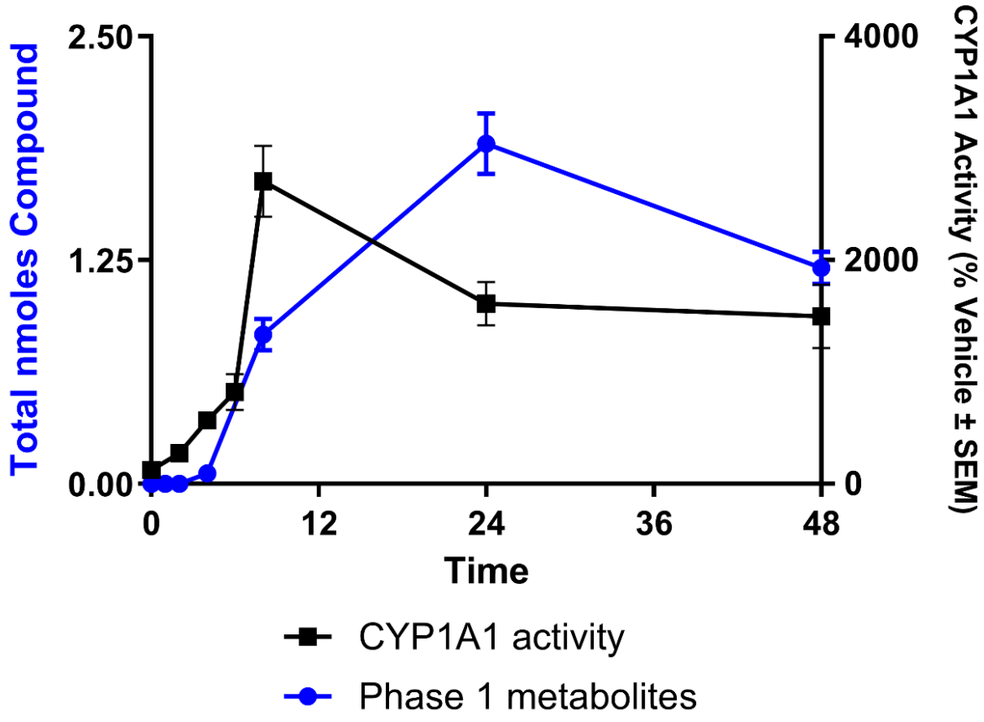
Formation of metabolites

Recovery of BAP only:



Recovery of BAP + Metabolites:





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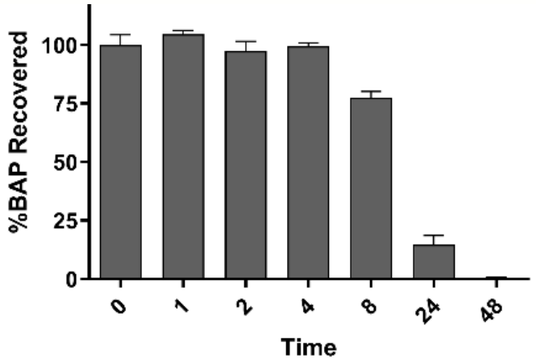
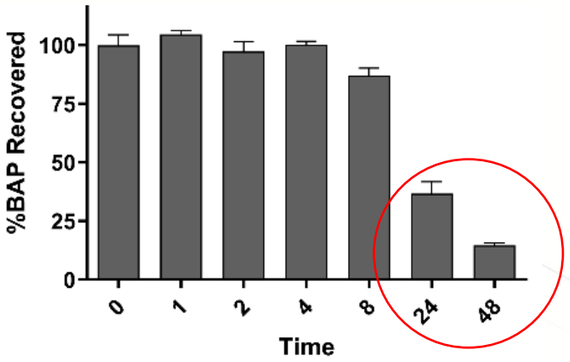
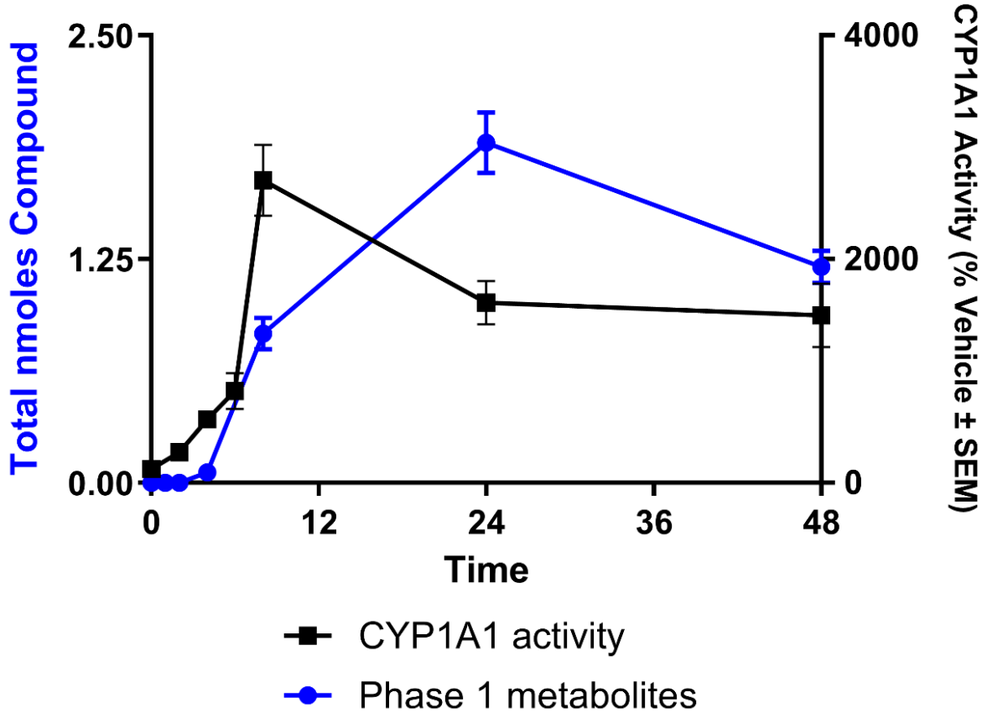


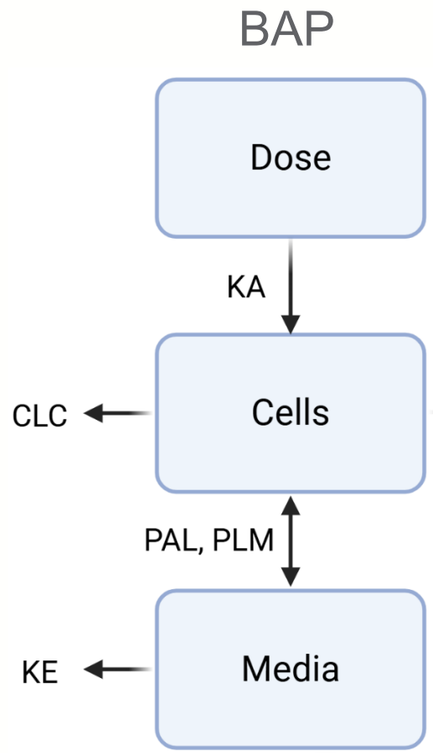
Recovery of BAP + Metabolites:

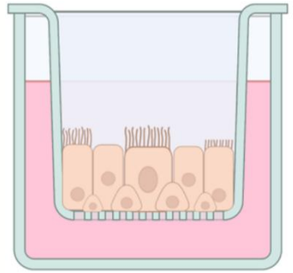




Building a Dosimetry Model in ALI HBEC

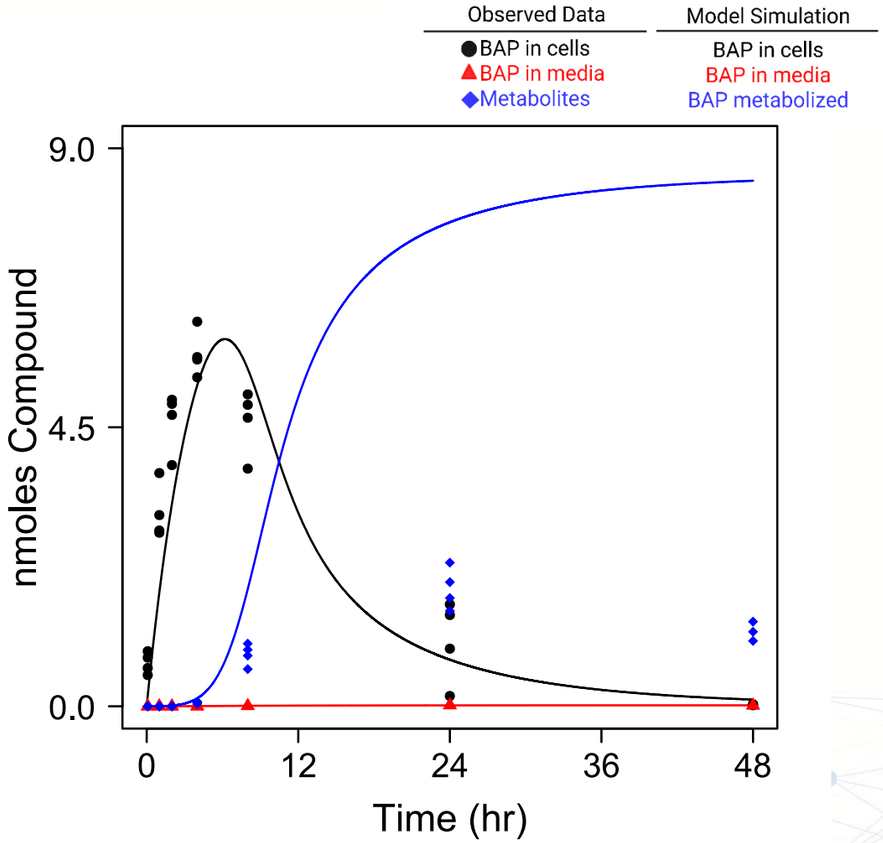
- Describe BAP ADME
  - Absorption into cells (KA)
  - Permeation through cells (PAL)
  - Partitioning into media (PLM)
  - Elimination
    - Metabolism in cells (CLC)
    - Volatilization from media (KE)





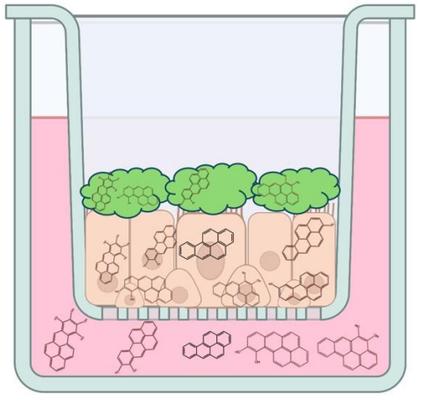
Building a Dosimetry Model in ALI HBEC

- Good fit for BAP in media
  - Very little makes it to media
- Good fit for BAP in cells
- Overestimate metabolism after 8 hours
  - Data represents a minimum amount of metabolism
  - Metabolites begin to appear at 4 hours

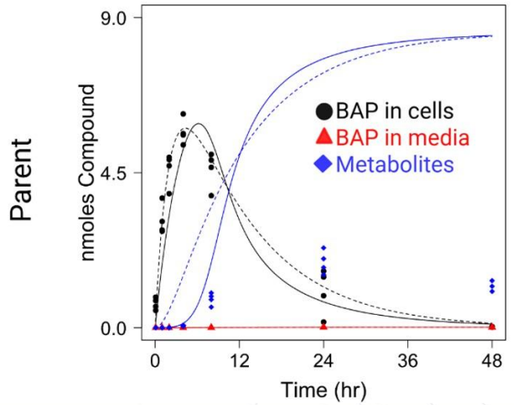


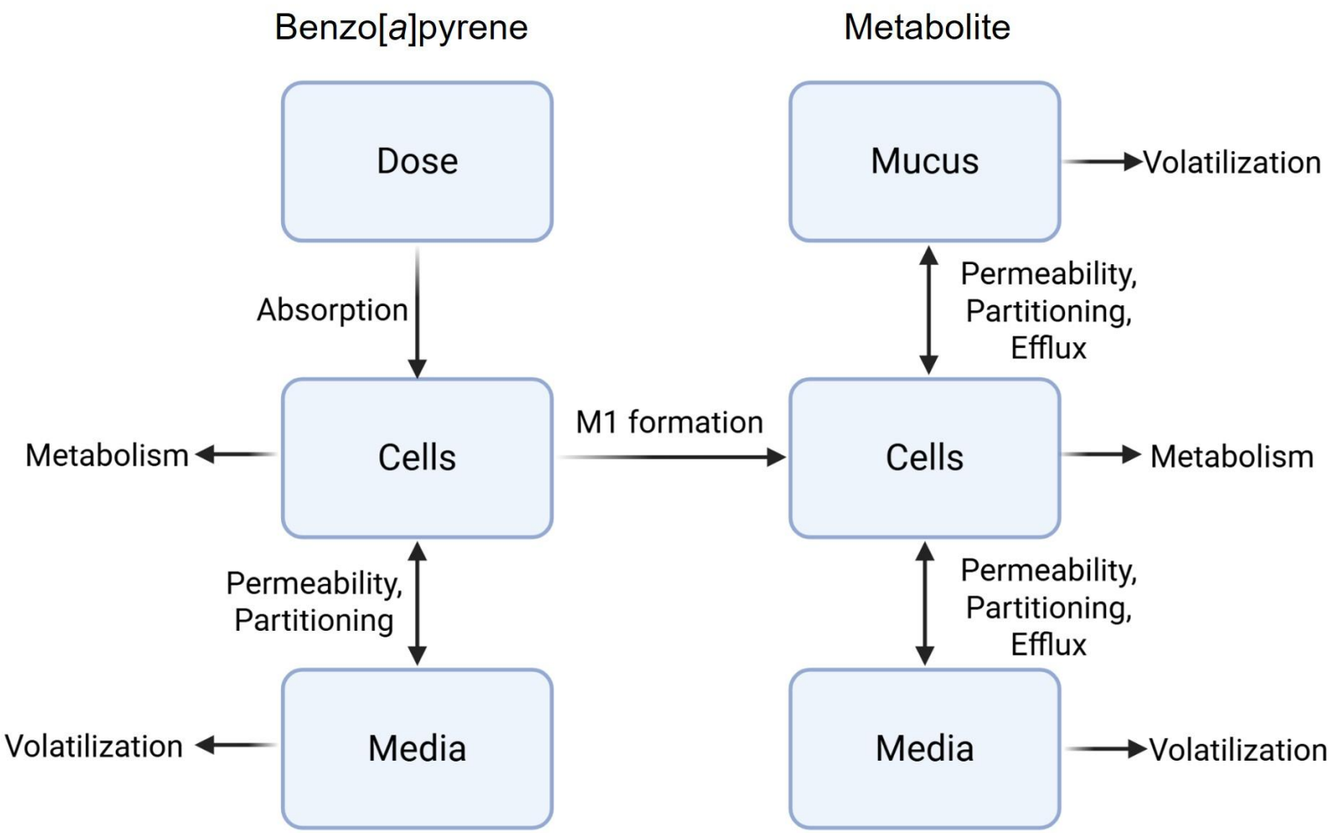
Building a Dosimetry Model in ALI HBEC

HBEC-ALI Culture Model:



PBPK Model Output:

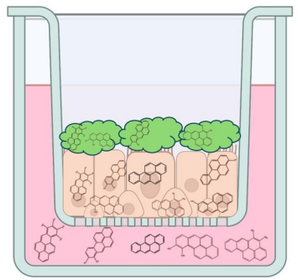




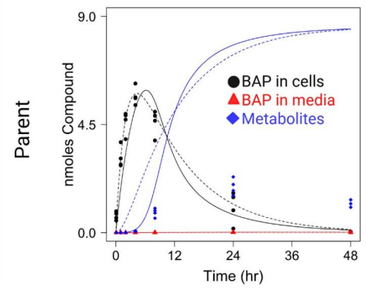
Building a Dosimetry Model in ALI HBEC

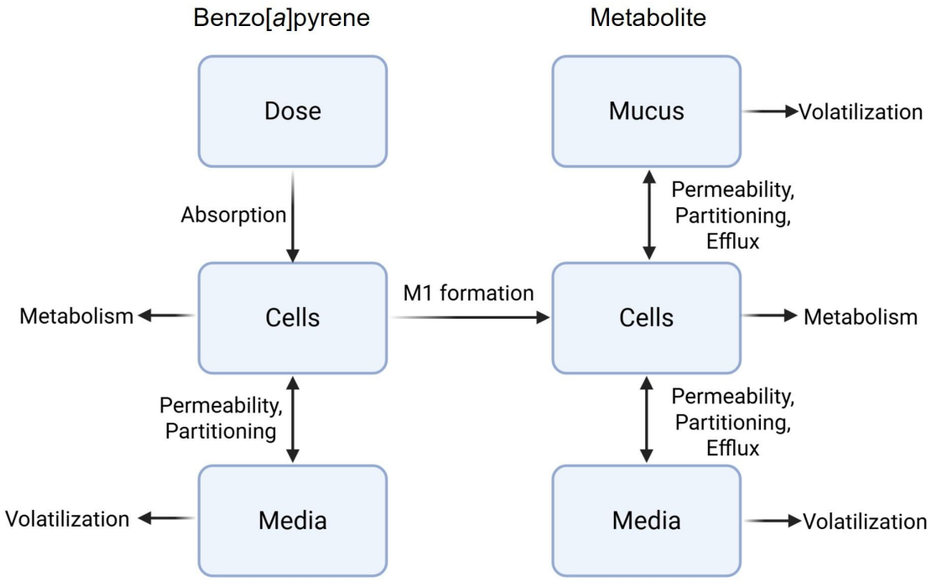
- Develop a PBPK model for quantitative extrapolation of toxicity across models
- Establish a better understanding of metabolic competency of an organotypic airway model
- Improves application for predictive toxicology
- Future studies will assess dosimetry for other PAHs and apply to IVIVE for translation of toxicity

HBEC-ALI Culture Model:



PBPK Model Output:

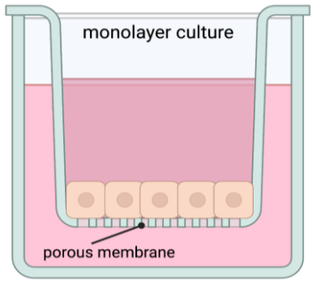


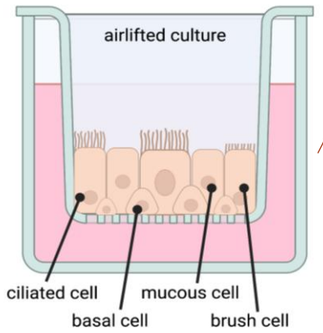


Overall: These studies support the use of ALI-HBECs as a competent NAM for PAH toxicity assessment and for predicting toxicity in humans.

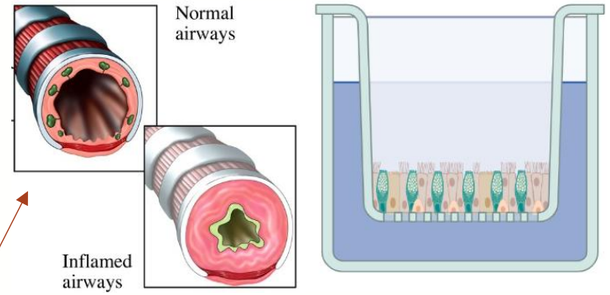
3D Airway Organotypic Culture Models

Air-Liquid Interface (ALI) Culture Models:

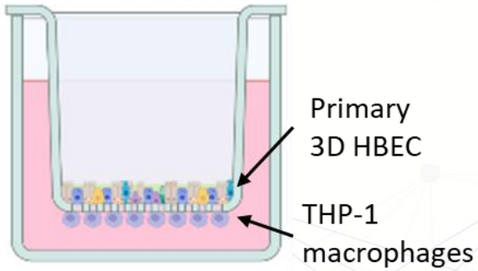




3D Disease Model



Co-Culture with Immune Cells

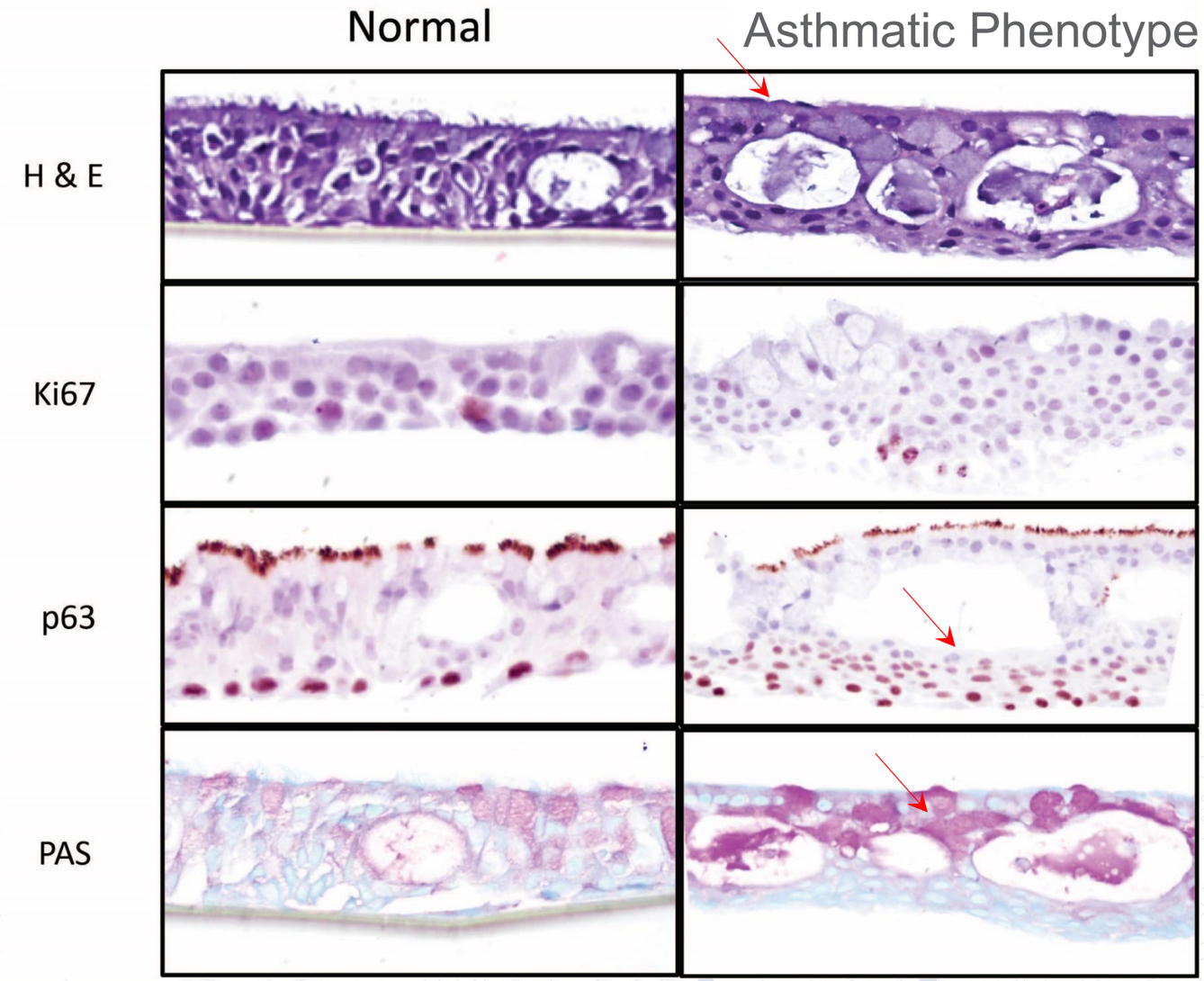


Benefits of model:

- Primary human bronchial epithelial cells (HBECs)
- 3D structure
- Metabolic and mitotic activity
- Multi-cellular communication and cell signaling
- More accurately reflects exposure at tissue interfaces
- Better recapitulates *in vivo* response

3D Airway Organotypic Culture Model

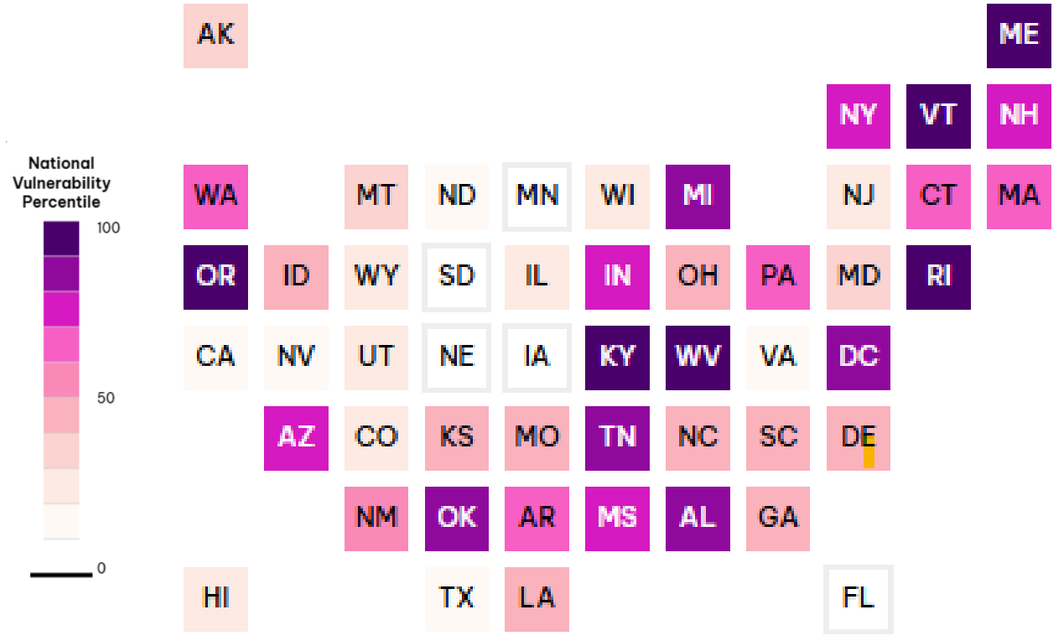
- <u>Benefits of model:</u>
- Development of disease models (e.g. Asthmatic phenotype)
- Pathophysiology similar to allergic asthma
  - Mucociliary dysfunction
  - Airway remodeling
  - Mucus hypersecretion
  - Loss of barrier integrity



Valdez et al. (2024) Frontiers in Toxicol. 6:1287863.

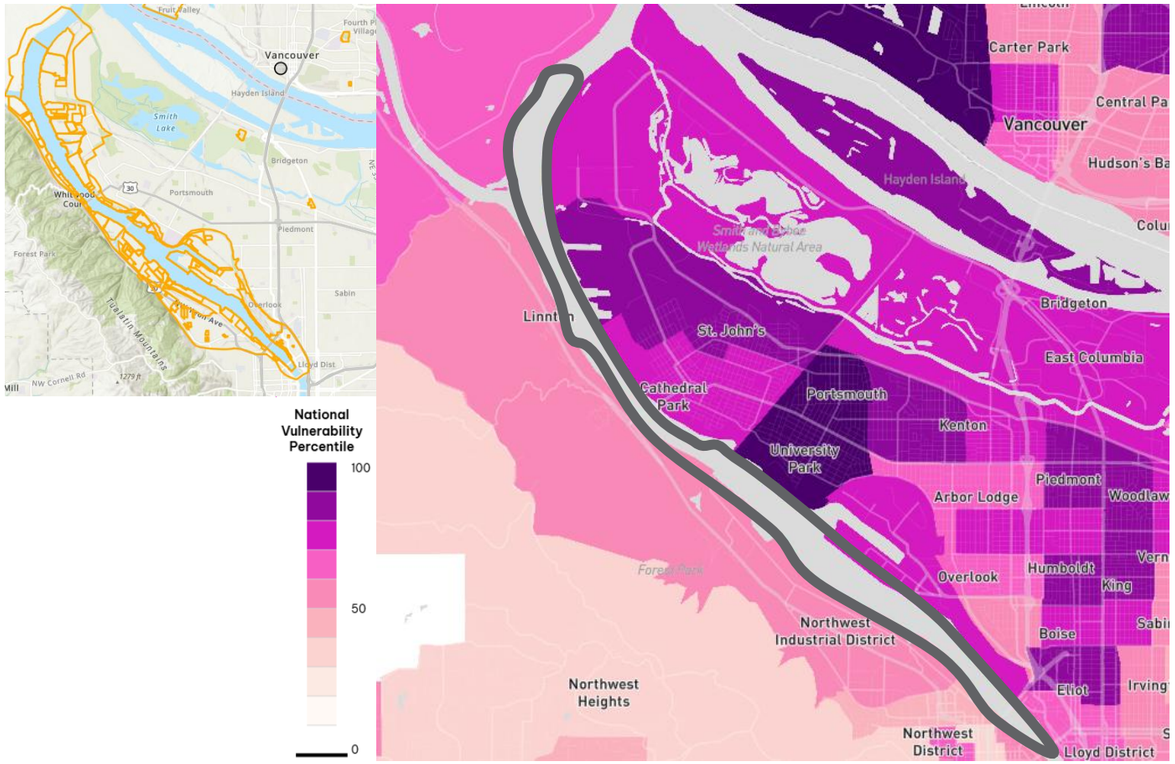
National Asthma Rates

- Climate Vulnerability Index allows us to visualize rates of adult asthma
  - 2021 data from CDC
  - Coloring shows National Vulnerability Percentile
  - Percentile is scaled where 100th percentile is the highest rate of asthma
- Oregon ranks 5th out of 51 states/districts
  - High vulnerability
- State rankings based on the median value of all census tract data within the state



Higher rates of asthmatic adults surrounding an exposure source suggests a need to better understand how combined stressors contribute to toxicity

- National asthma rate: ~8%
- Oregon ranks 5th out of 51 states/districts
  - High vulnerability
- Total asthma rate in Oregon: 11.2%
  - Children & Adults
- Areas surrounding the Portland Harbor Superfund site generally rank between 57th and 94th percentile
  - Adult asthma rate of 9-13%

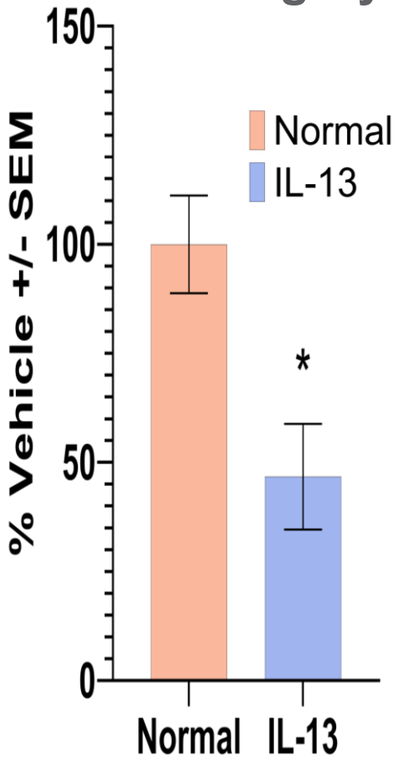


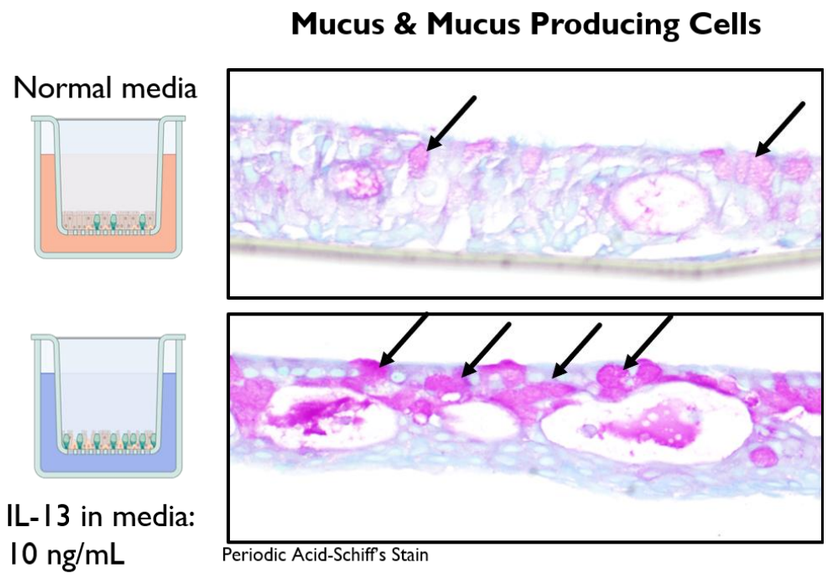
Climate Vulnerability Index; Center for Disease Control and Prevention

3D respiratory model for asthma

- Normal primary human bronchial epithelial cells (HBEC)
  - Cultured at air-liquid interface to create 3D model
  - Cells differentiate
  - Comparable response to *in vivo* models
  - Can be cultured in presence of interleukin-13 to induce an asthma-like phenotype

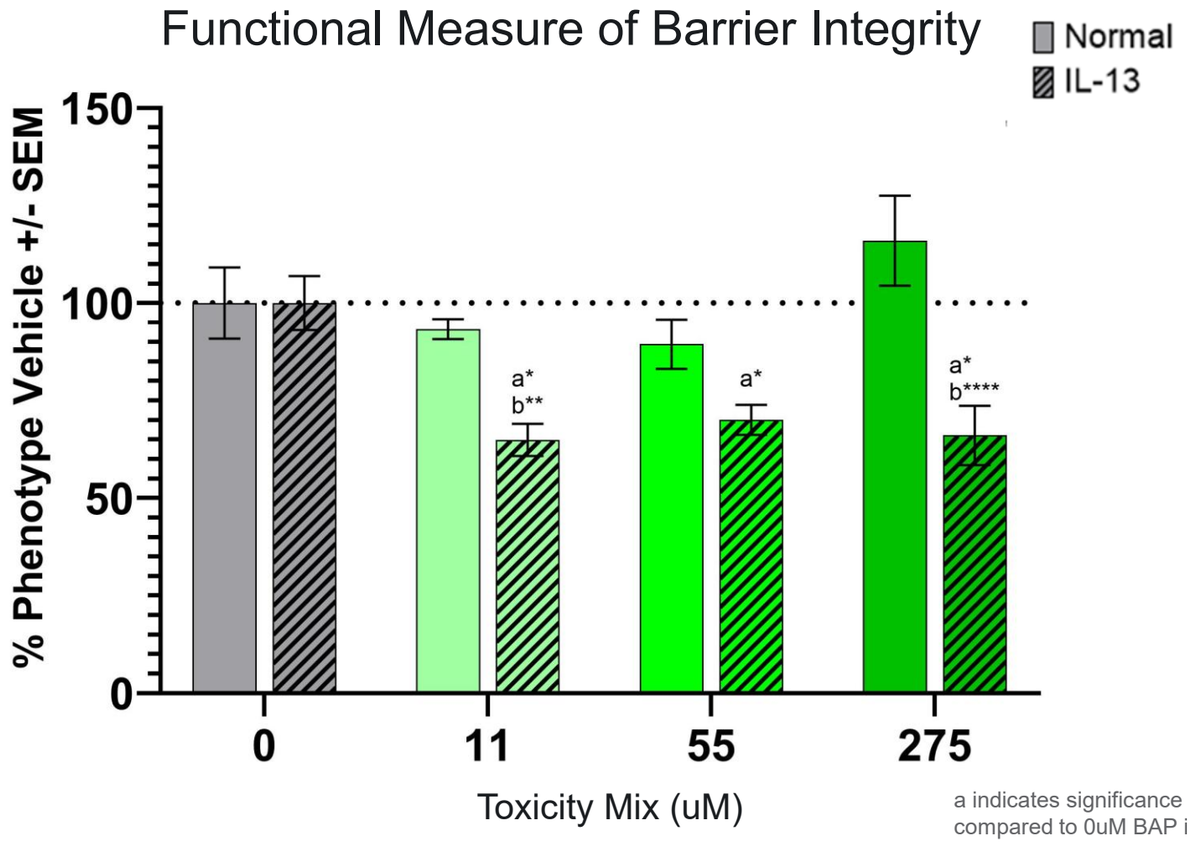
Functional measure of Barrier Integrity





A PAH mixture uniquely exacerbates barrier disruption in the IL-13 phenotype.

- Barrier integrity of airway epithelium maintains homeostasis and protects against environmental factors
- Barrier dysfunction can result in increased risk of chemical uptake and contribute to worsening chronic inflammation
- Toxicity Mix exposure caused further exacerbation of barrier disruption in the IL-13 phenotype, but had no impact in the normal phenotype
- This trend was not observed after exposure to BAP

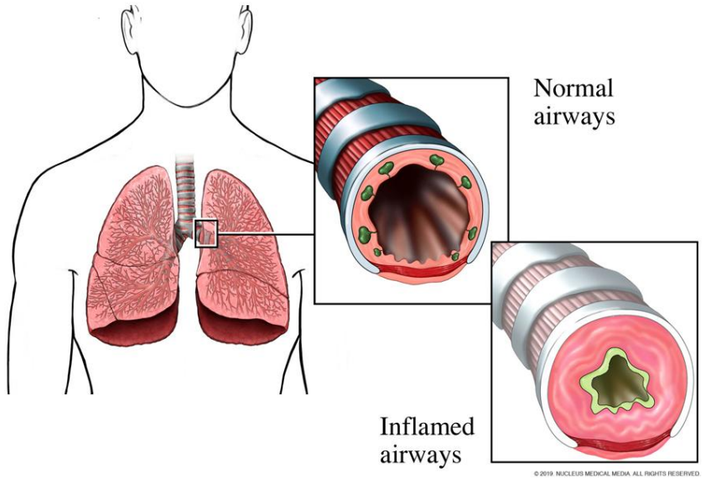


a indicates significance compared to 0uM BAP in same phenotype

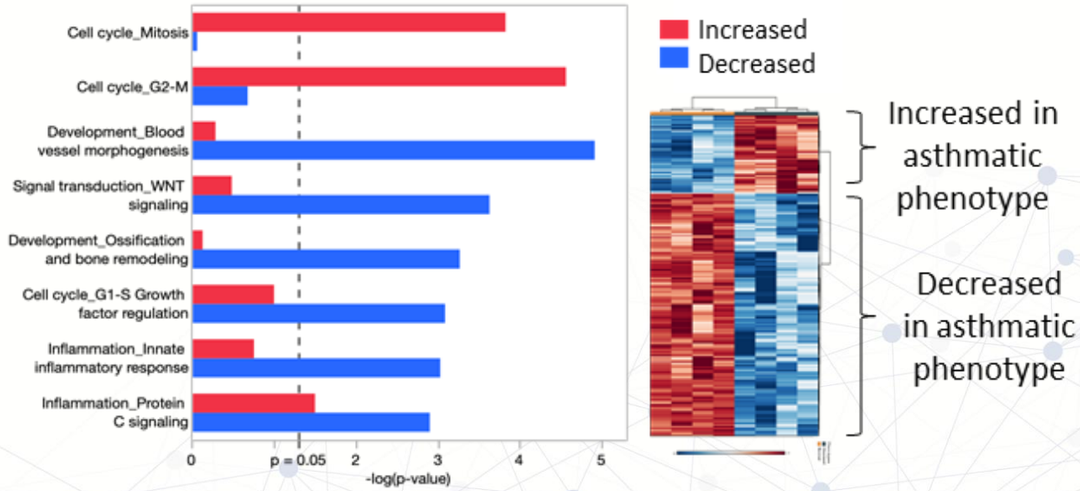
b indicates significance compared to Normal phenotype at same chemical concentration

Evaluating role of combined stressors contributing to toxicity

- Pulmonary inflammation associated with inflammation-based respiratory disease (e.g. asthma) is potential modifier and risk factor for chemical toxicity
  - May respond to chemical toxicity with altered susceptibility
  - Utilize lung cells from diseased donors or expressing diseased phenotype
- Utilize a macrophage-epithelial co-culture to effectively evaluate immune contribution to lung inflammation



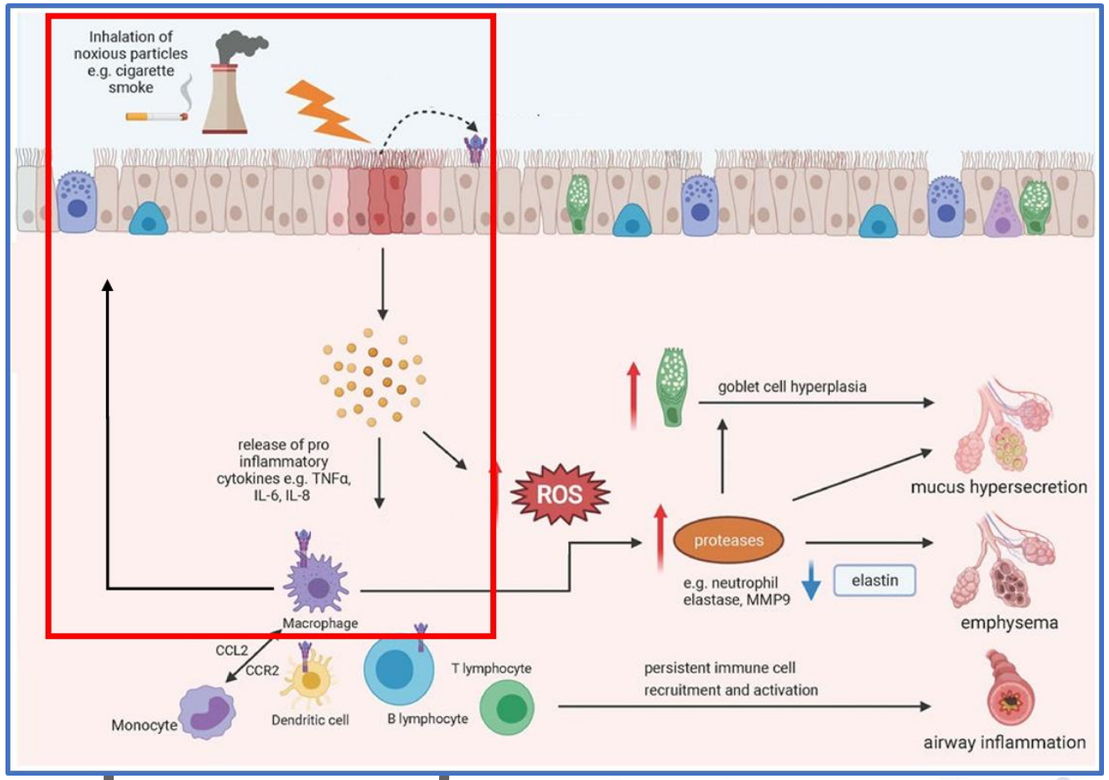
Response to chemical toxicity in normal and asthmatic lung cells

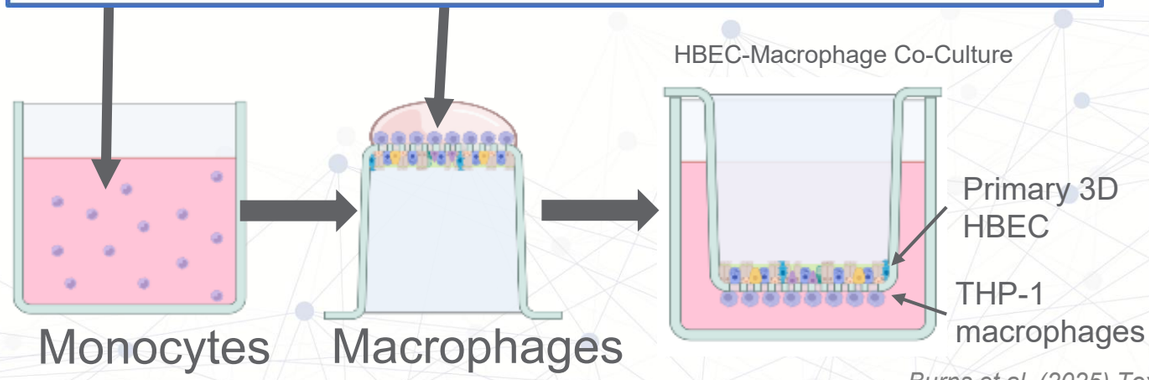


Valdez et al. (2024) Frontiers in Toxicol. 6:1287863.

Utilization of an *in vitro* co-culture model to evaluate PAH mixture toxicity

- Utilize a macrophage-epithelial co-culture to effectively evaluate immune contribution to lung inflammation
- In the respiratory system, induction of airway inflammation is orchestrated through signaling between airway epithelial cells and macrophages.
- Evaluate the combined contribution of airway epithelial-macrophage response to chemicals from wildfire smoke

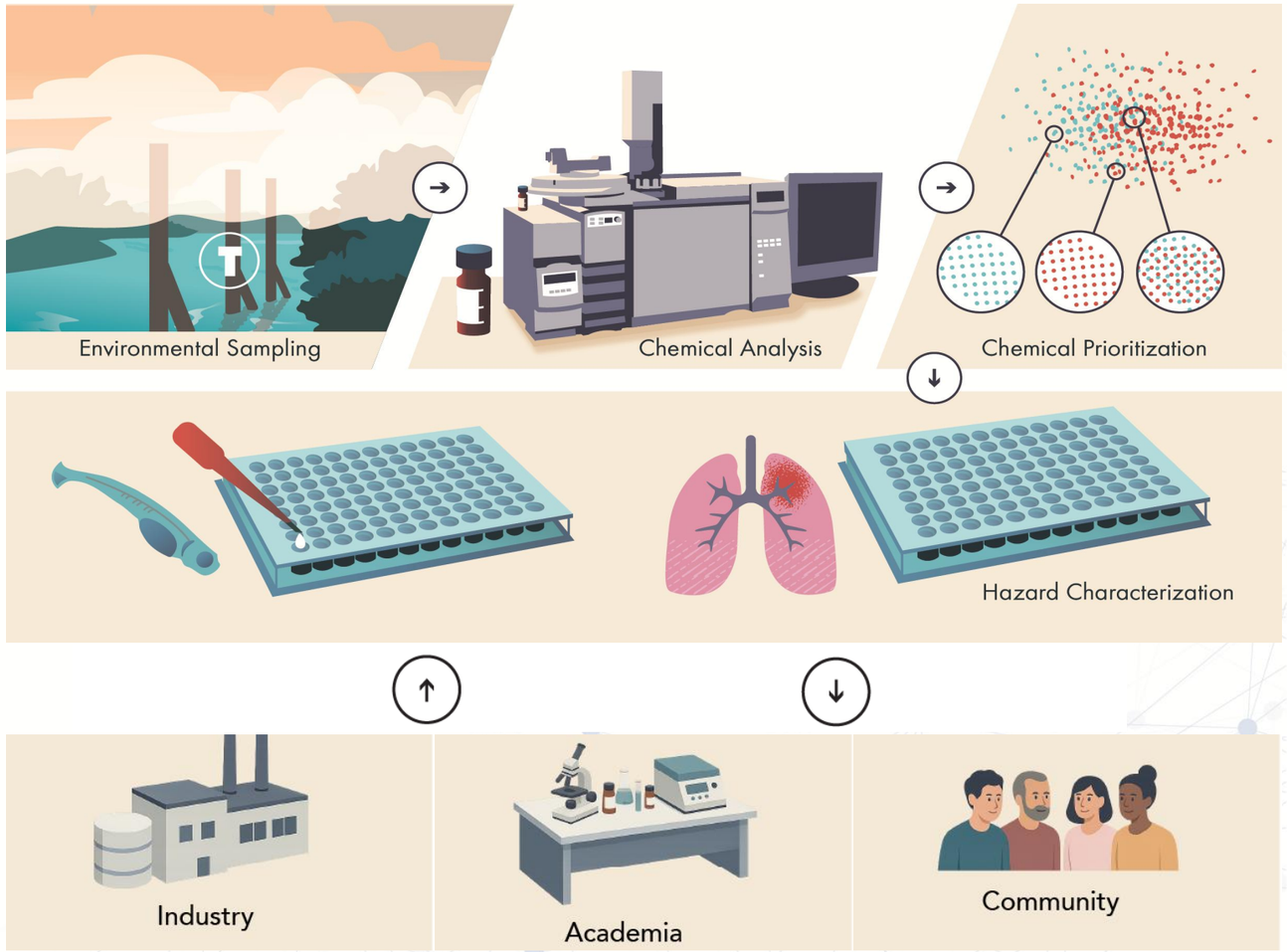




Burns et al. (2025) Toxics. 13(12):1065.

Benefits of 3D *in vitro* models to evaluate PAH toxicity

- Application of integrated approaches for understanding chemical toxicity and prioritizing environmentally-relevant exposures
- Utilization of cell-based NAMs in combination with *in silico* approaches to develop quantitative methods for *in vitro* to *in vivo* translation
- Leveraging human-relevant systems to improve our understanding of toxicity to combined stressors and interindividual variability



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Thank you