

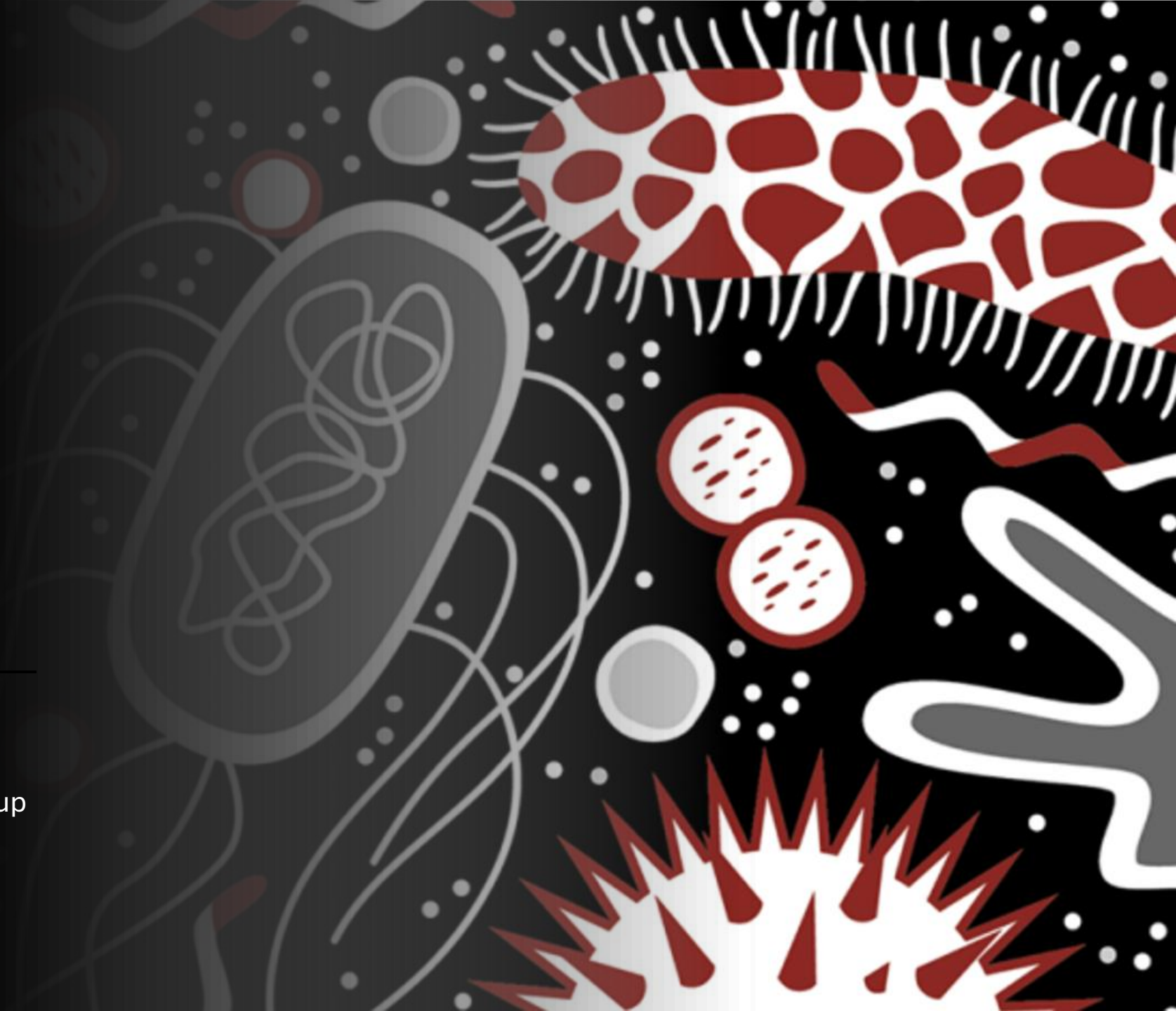
The urothelium, exposed: Exploring how environmental exposures impact bladder health

Vanessa Hale, MAT, DVM, PhD

Ohio State University

CAIRIBU Urobiome Research Interest Group
Summer Meeting

July 30, 2026



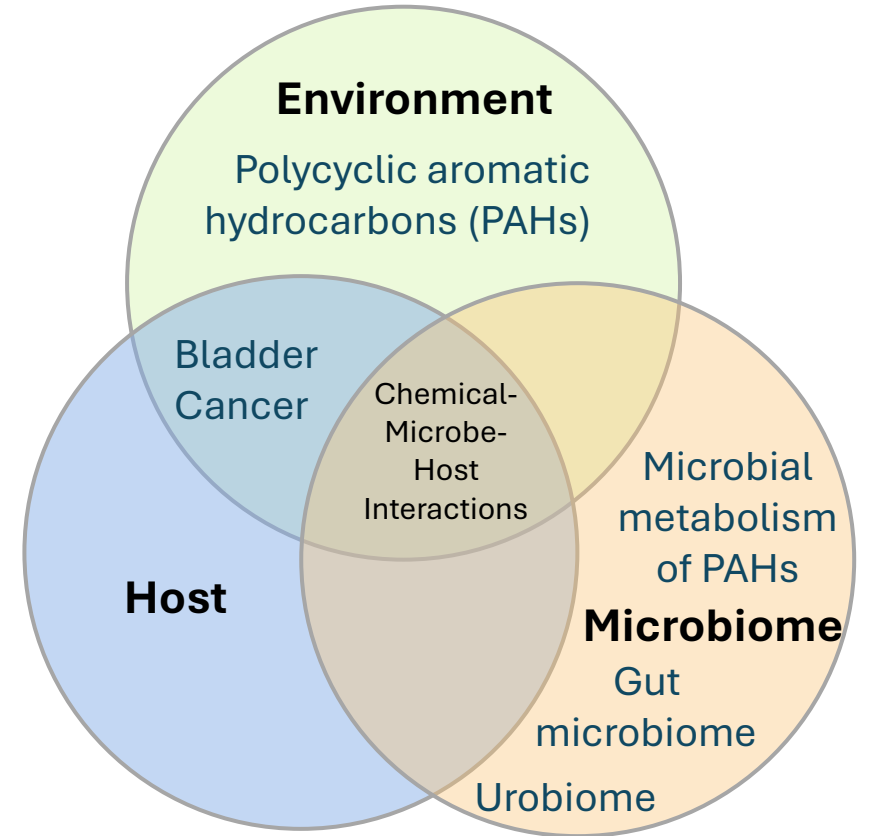
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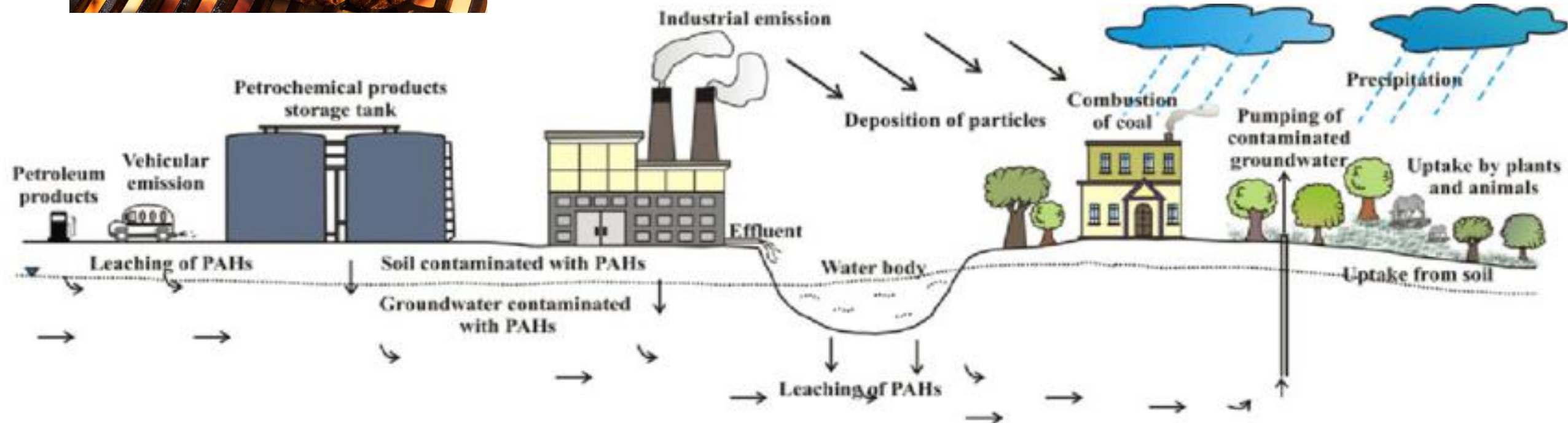


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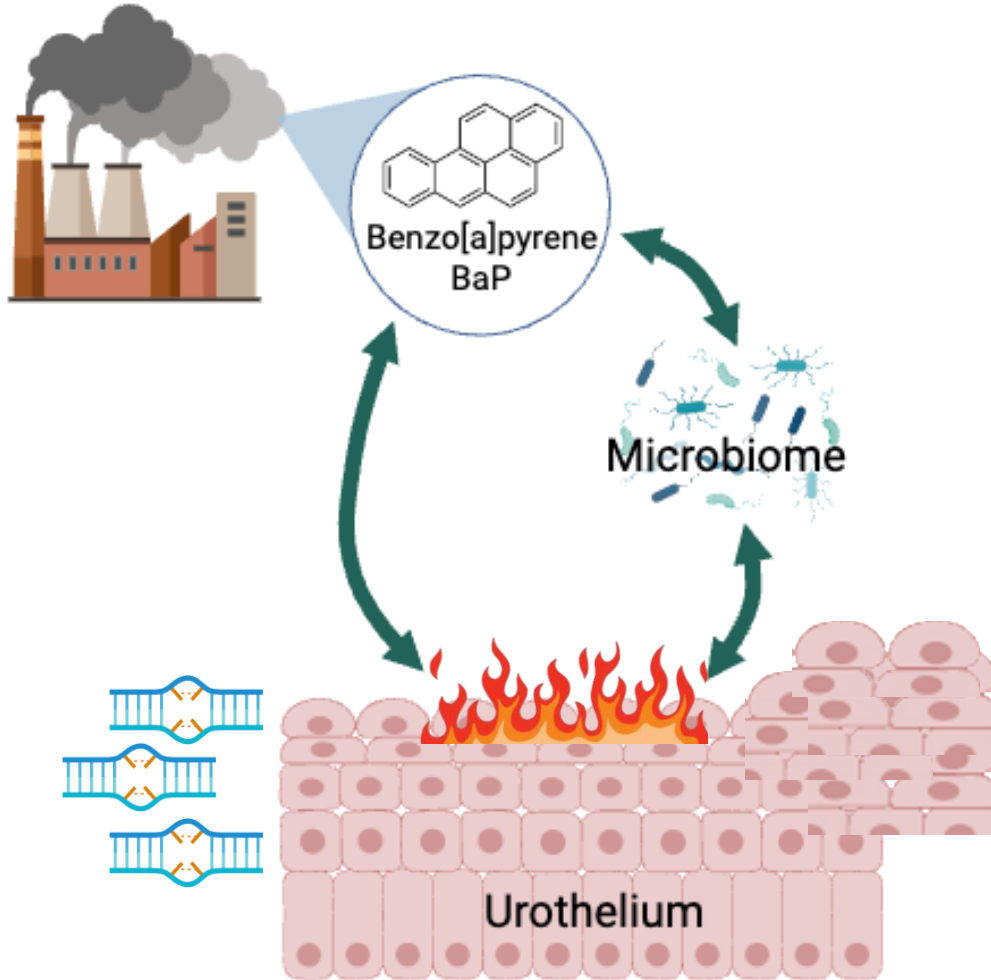
Polycyclic Aromatic Hydrocarbons



- Associated with multiple cancers – skin, lung, bladder, GI
- Renal excretion
- Air pollution is also associated with increased UTI risk



Conceptual Framework

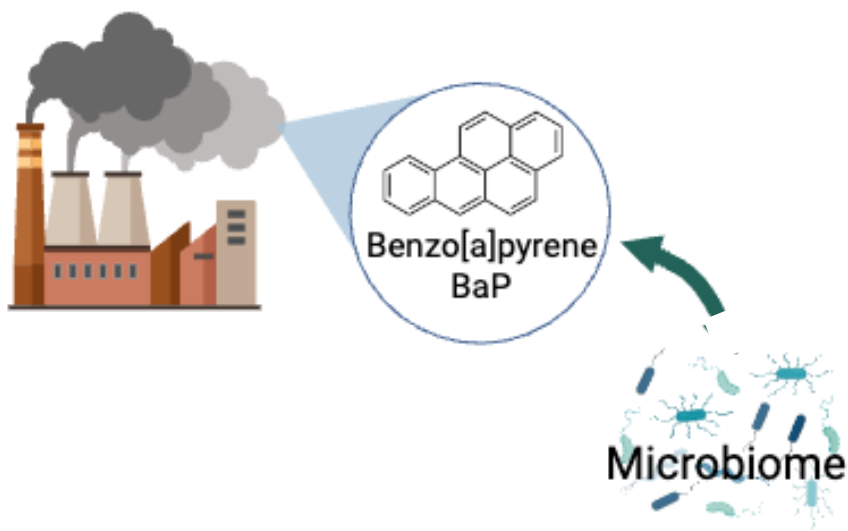


- Benzo[a]pyrene (BaP) is one of the most common and most toxic Polycyclic Aromatic Hydrocarbons

How does BaP exposure affect the urothelium?

DNA Adducts ✓
Inflammation ?
Proliferation ?

Spoiler Alert: Proliferation

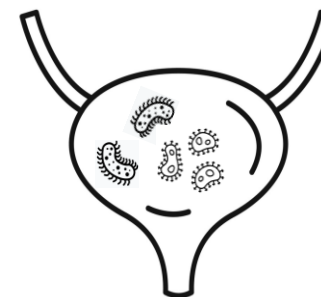


Can urine microbes metabolize BaP?

Based on 16S rRNA sequencing: *Enterococcus*, *Acinetobacter*, *Micrococcus*, and *Ralstonia* - all potential PAH degraders - were associated with bladder cancer

Global Meta-analysis of Urine Microbiome: Colonization of Polycyclic Aromatic Hydrocarbon-degrading Bacteria Among Bladder Cancer Patients

Bukavina et al.
European Urology Oncology, 2023



Potentially

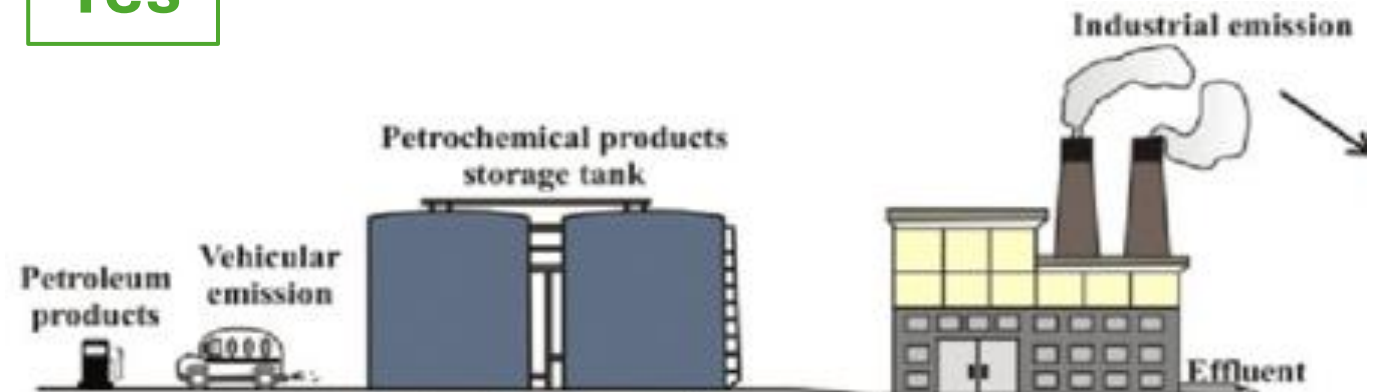
Evaluating urine volume and host depletion methods to enable genome-resolved metagenomics of the urobiome

Zachary J. Lewis^{1,2}, Angela Scott¹, Christopher Madden¹, Dean Vik^{2,3}, Ahmed A. Zayed^{4,5}, Garrett J. Smith^{2,3}, Sheryl S. Justice⁶, Adam Rudinsky⁷, Jessica Hokamp⁸ and Vanessa L. Hale^{1,2*}

Microbiome 2025

**Can we find
polycyclic aromatic
hydrocarbon
metabolizing genes
in human urine
microbes?**

Yes



Cataloging the phylogenetic diversity of human bladder bacterial isolates

[Jingjie Du](#), [Mark Khemmani](#), [Thomas Halverson](#), [Adriana Ene](#), [Roberto Limeira](#), [Lana Tinawi](#), [Baylie R. Hochstedler-Kramer](#), [Melline Fontes Noronha](#), [Catherine Putonti](#) & [Alan J. Wolfe](#)

[Genome Biology](#)

2024

We examined ~1800 publicly available urine bacterial isolates



Garrett Smith

 | Genomics and Proteomics

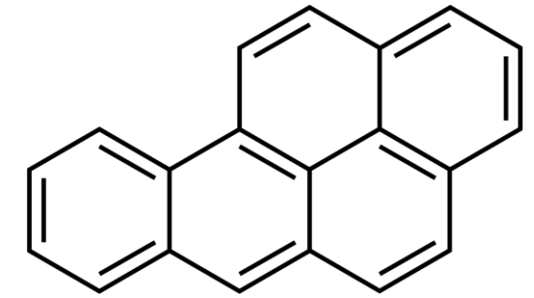
Genetic and functional enrichments associated with *Enterococcus faecalis* isolated from the urinary tract

Authors: [Belle M. Sharon](#), [Amanda P. Arute](#), [Amber Nguyen](#), [Suman Tiwari](#), [Sri Snehita Reddy Bonthu](#), [Neha V. Hulyalkar](#), [Michael L. Neugent](#), [Dennise Palacios Araya](#), [Nicholas A. Dillon](#), [Philippe E. Zimmern](#) , [Kelli L. Palmer](#) , [Nicole J. De Nisco](#) 

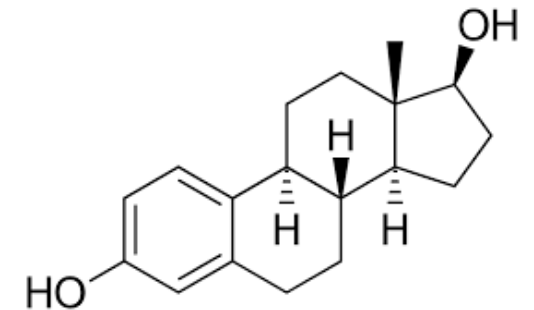
2023

Environmental chemical and steroid metabolism genes present in human urine microbes

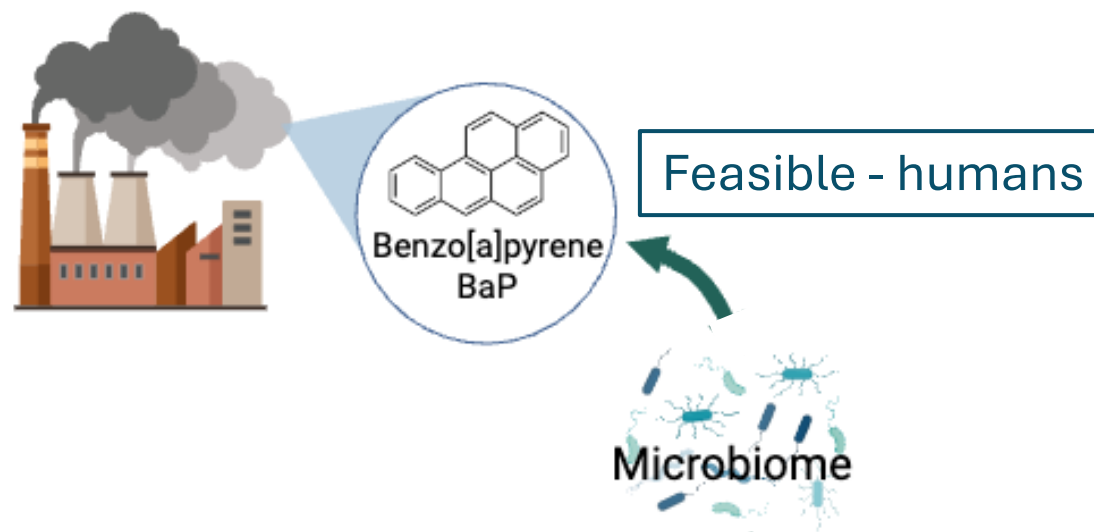
- Polycyclic aromatic hydrocarbon (PAH) metabolism genes present in ~122 (of ~1800) urine microbial isolates
 - nah and phd genes were present, but full pathways for degradation were not present
 - Co-metabolism between microbes or microbes / host is also feasible
 - Databases for microbial PAH metabolism are based on soil bacteria and it is possible urine microbes metabolize PAHs via different pathways
- Long chain alkane metabolism was present in ~380 isolates
- Steroid metabolism was present in ~1600 isolates
 - Bacterial CYP genes are promiscuous and could feasibly degrade PAHs
 - Urine microbial isolates derived from males had significantly greater number of genes for steroid metabolism



Benzo[a]pyrene



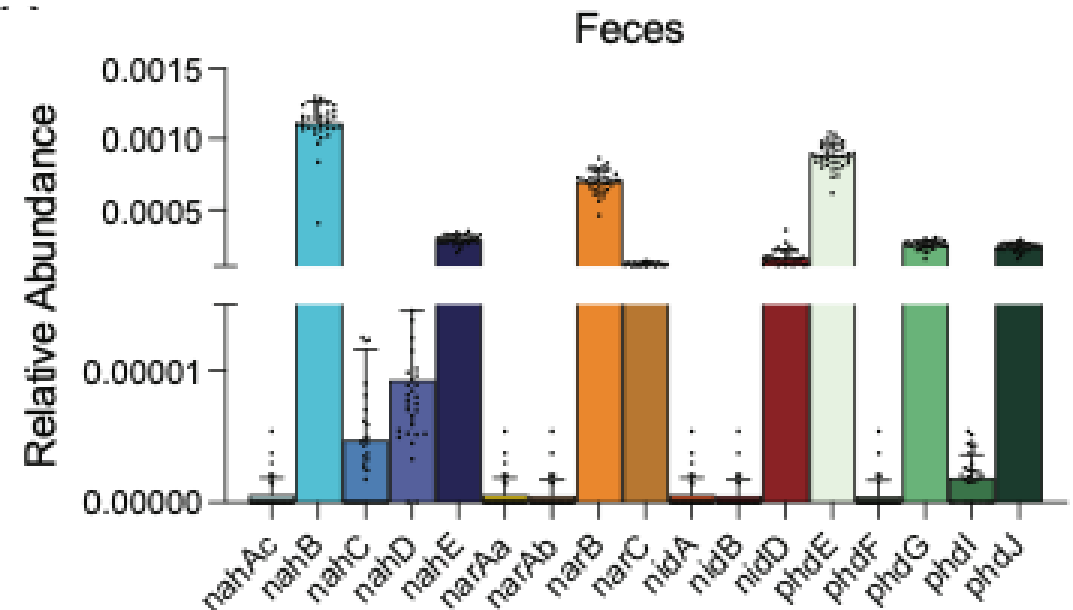
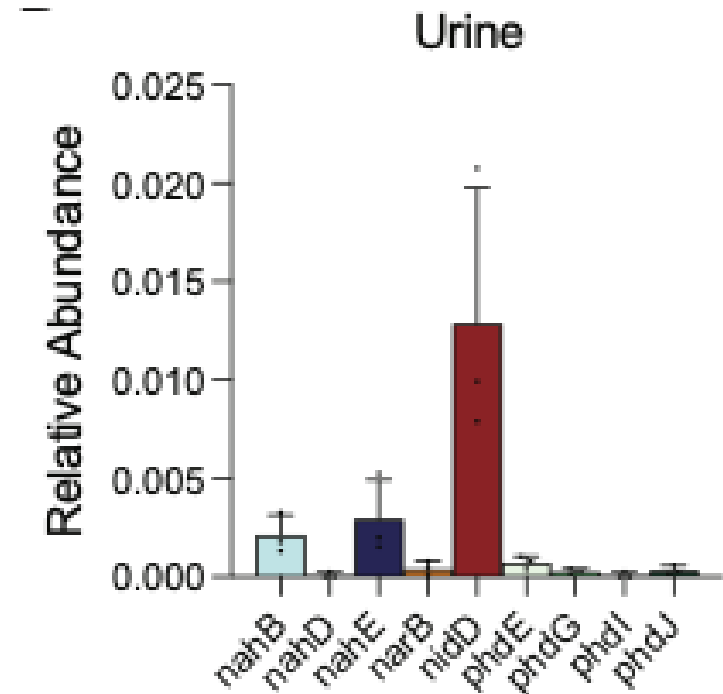
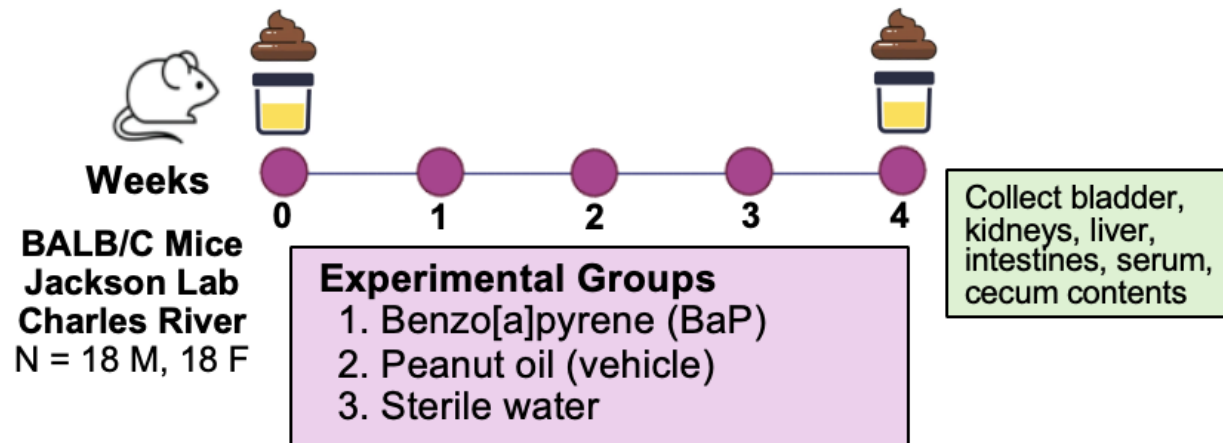
Estrogen

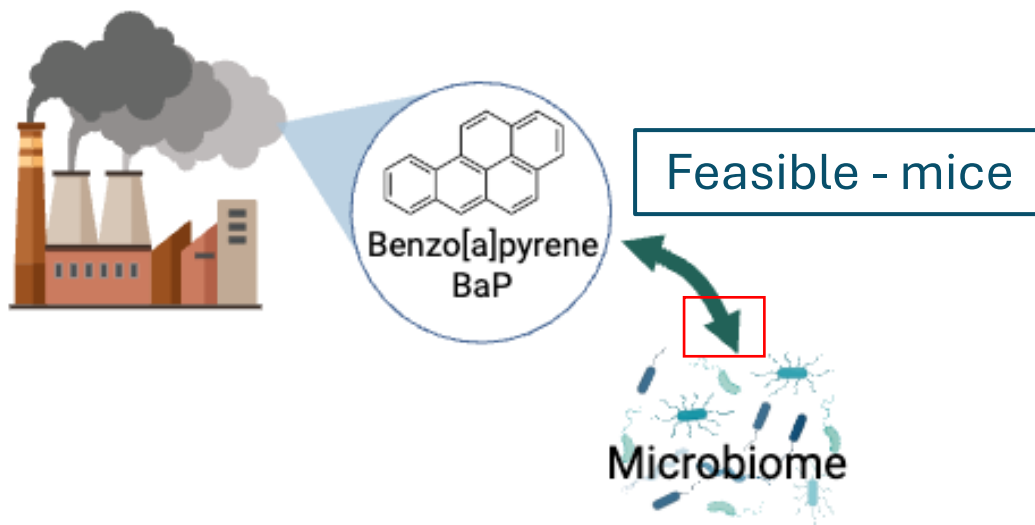


**Can urine microbes
metabolize BaP?**

in mice?

Polycyclic aromatic hydrocarbon metabolism genes present in mouse urine and gut microbes





**Can urine microbes
metabolize BaP?**

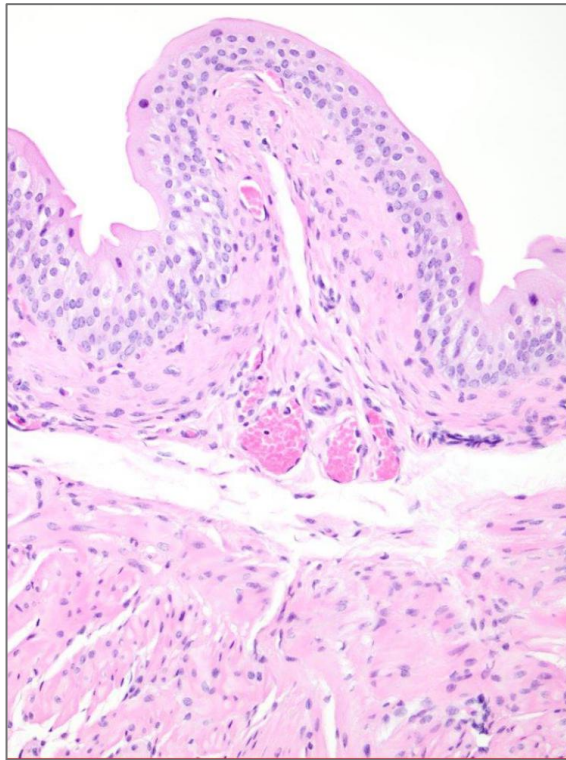
in mice?

**Does BaP affect the gut
microbiome and
urobiome?**

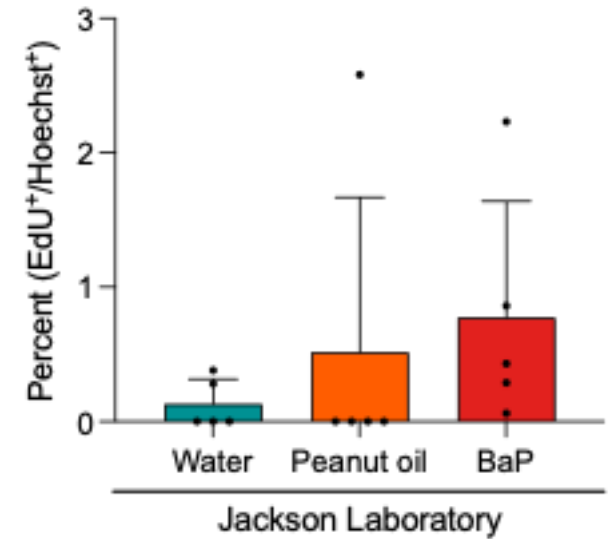
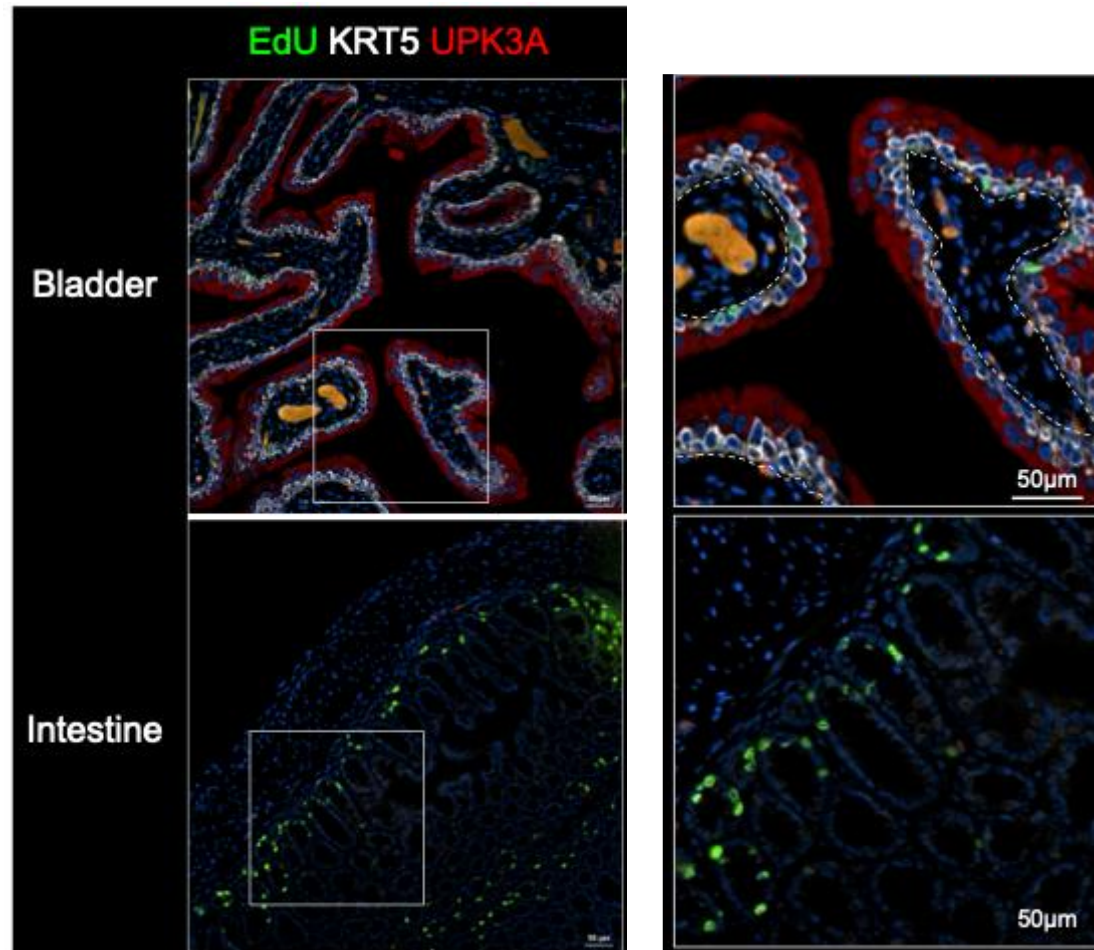
See Dr. Audra
Crouch's Poster #8

**How does BaP affect
the urothelium?**

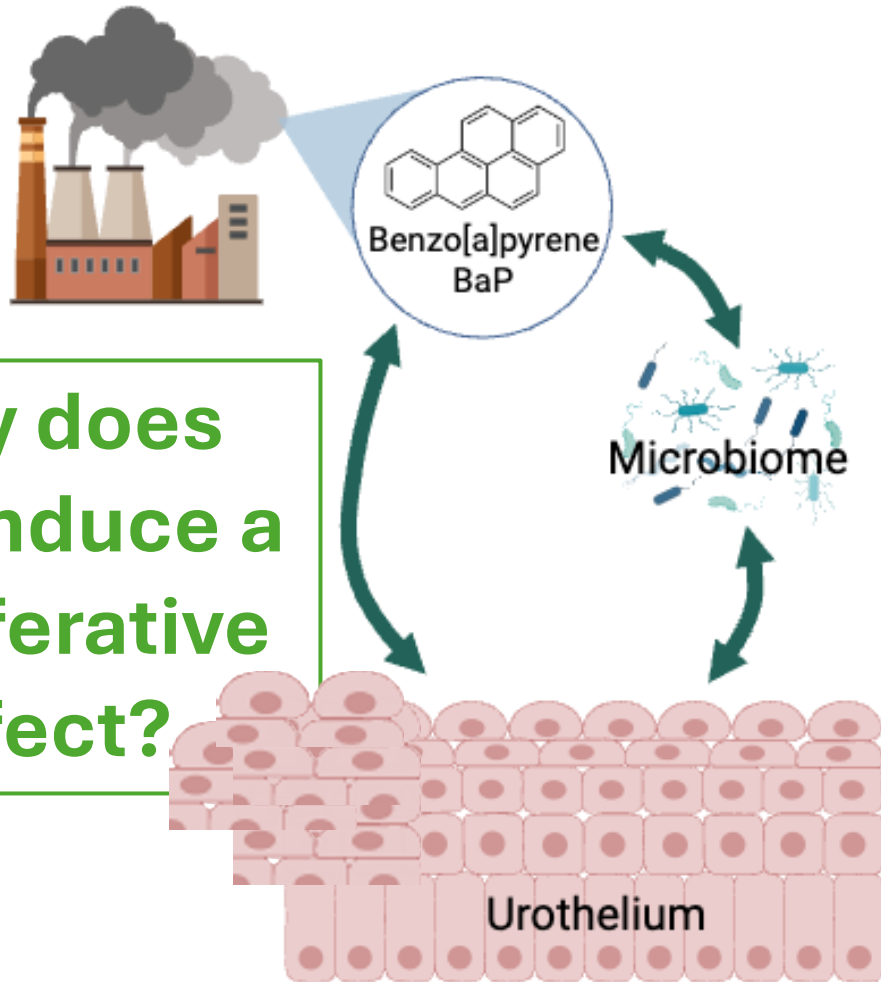
Urothelial proliferation but no inflammation in bladders of BaP-exposed mice



Bladder of BaP
Exposed Mouse



EDU: Epithelial proliferation
KRT5: Basal cells
UPK3A: Superficial cells
Hoechst: Nuclei



Why does
BaP induce a
proliferative
effect?

How does BaP affect
the urothelium?

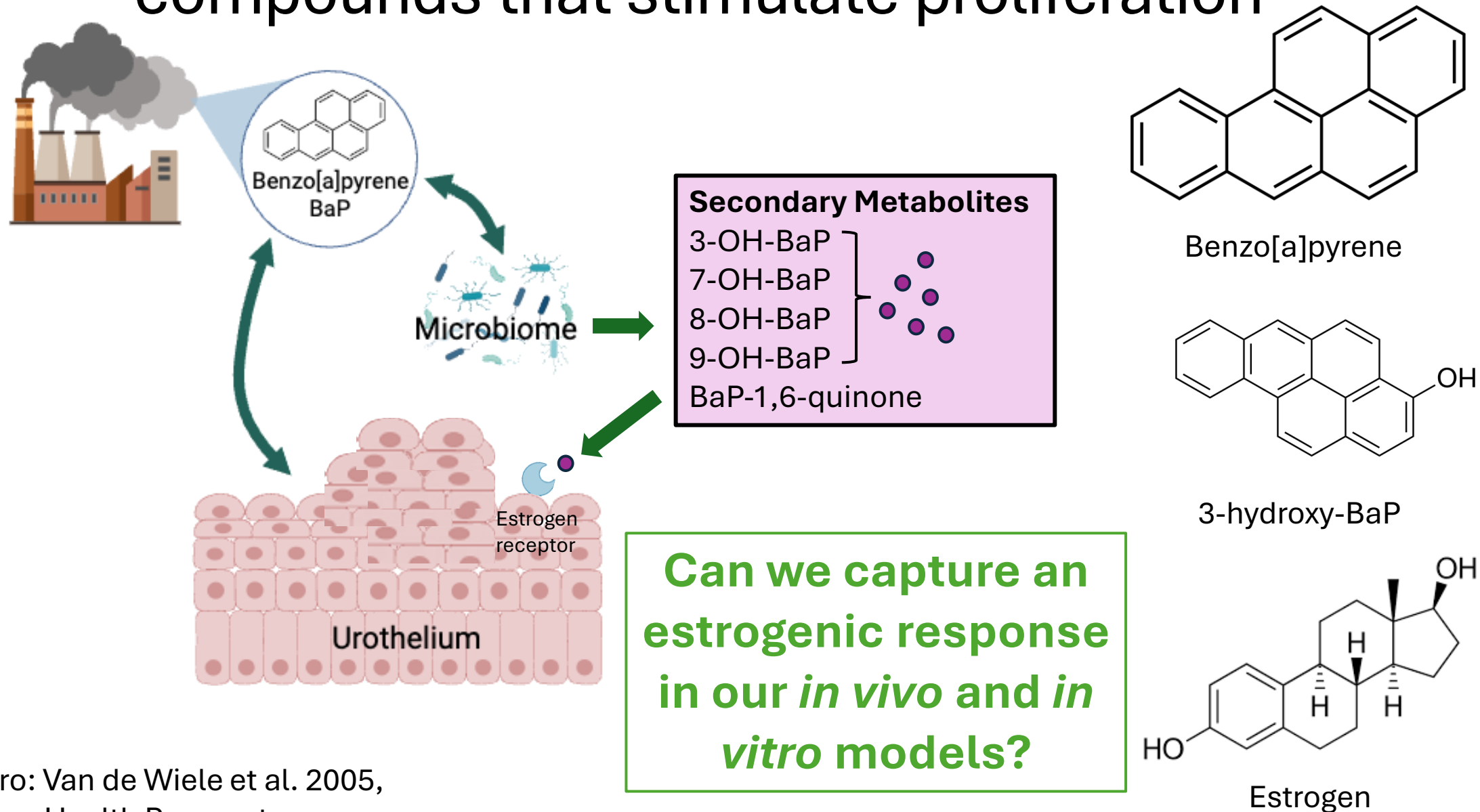
Inflammation **X**
Proliferation **✓**

Can we recapitulate
this BaP-exposure-
induced proliferative
effect *in vitro*?

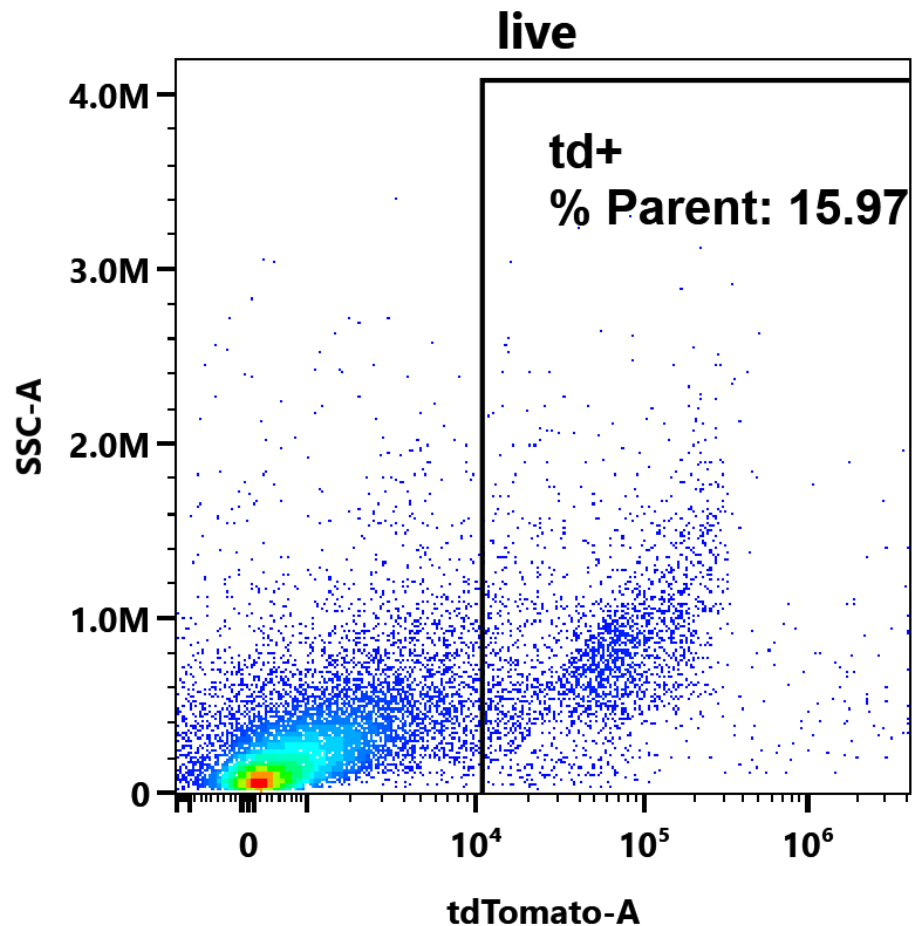
Yes!

See Dr. Audra Crouch's Poster #8
(HBLAK cells & a transwell system)

Hypothesis: BaP is metabolized into estrogenic compounds that stimulate proliferation



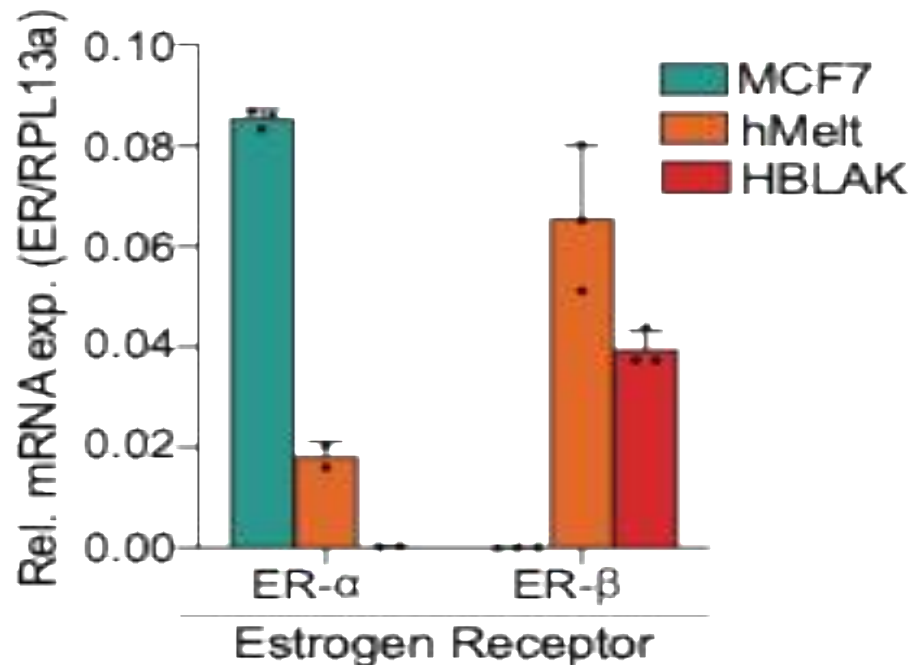
***In vivo: Do mouse
bladders contain
estrogen receptors (ER)?***



- In “tdTomato” mice engineered for fluorescent expression of the ER β gene, bladders were removed and dissociated into a single cell suspension and assess via flow cytometry.
- ~16% of the bladder cells (excluding endothelial cells and lymphocytes) expressed ER β

YES

***In vitro: Do HBLAK
urothelial cells contain
estrogen receptors?***



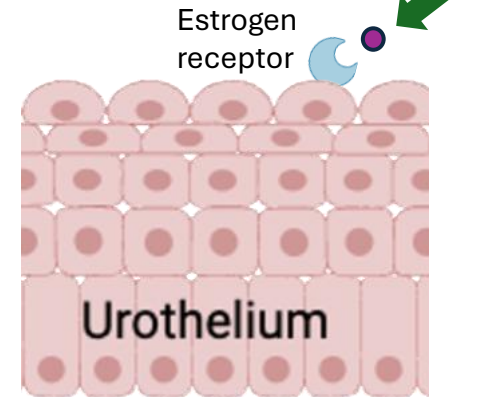
- ERβ but not ERα expression was observed in HBLAK urothelial cells (RT-qPCR)
- Controls: MCF7 breast cell line - contains ERα but not ERβ
- hMelt melanocyte cell line engineered to express exogenous ERβ
- Also confirmed ERβ in HBLAK cells via Western blot

YES

In vitro: Are BaP secondary metabolites estrogenic?

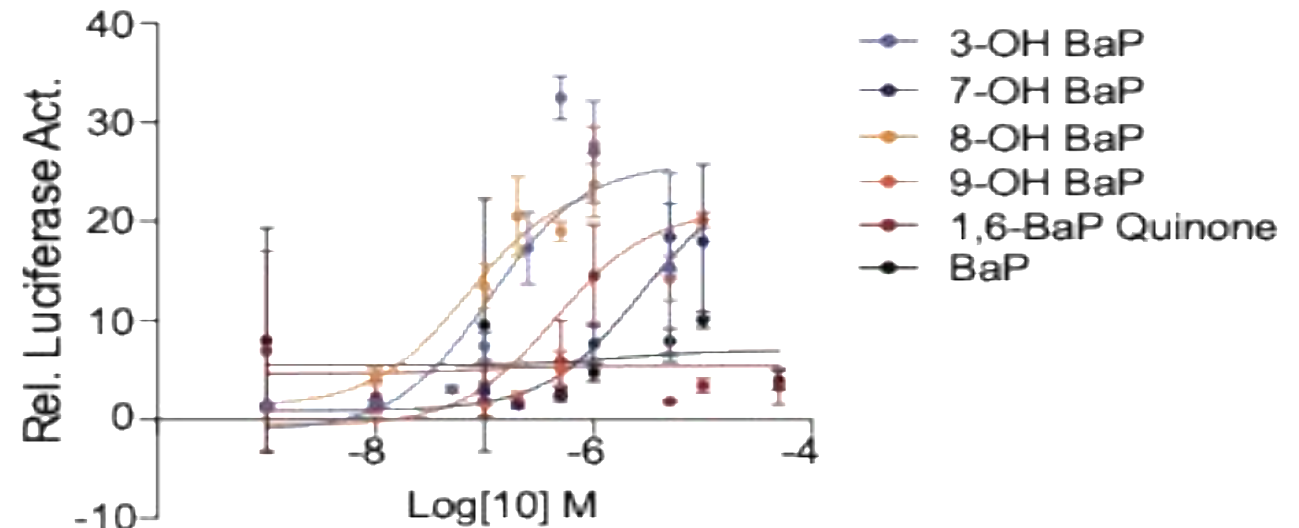
- Using HEK293 human kidney cells engineered to contain ER β , we assessed ER stimulation by each metabolite (measured by luciferase activity)
- Mono-hydroxylated BaP metabolites (3-, 7-, 8-, and 9-OH BaP) were all strongly estrogenic while BaP and 1,6-BaP quinone were not

YES

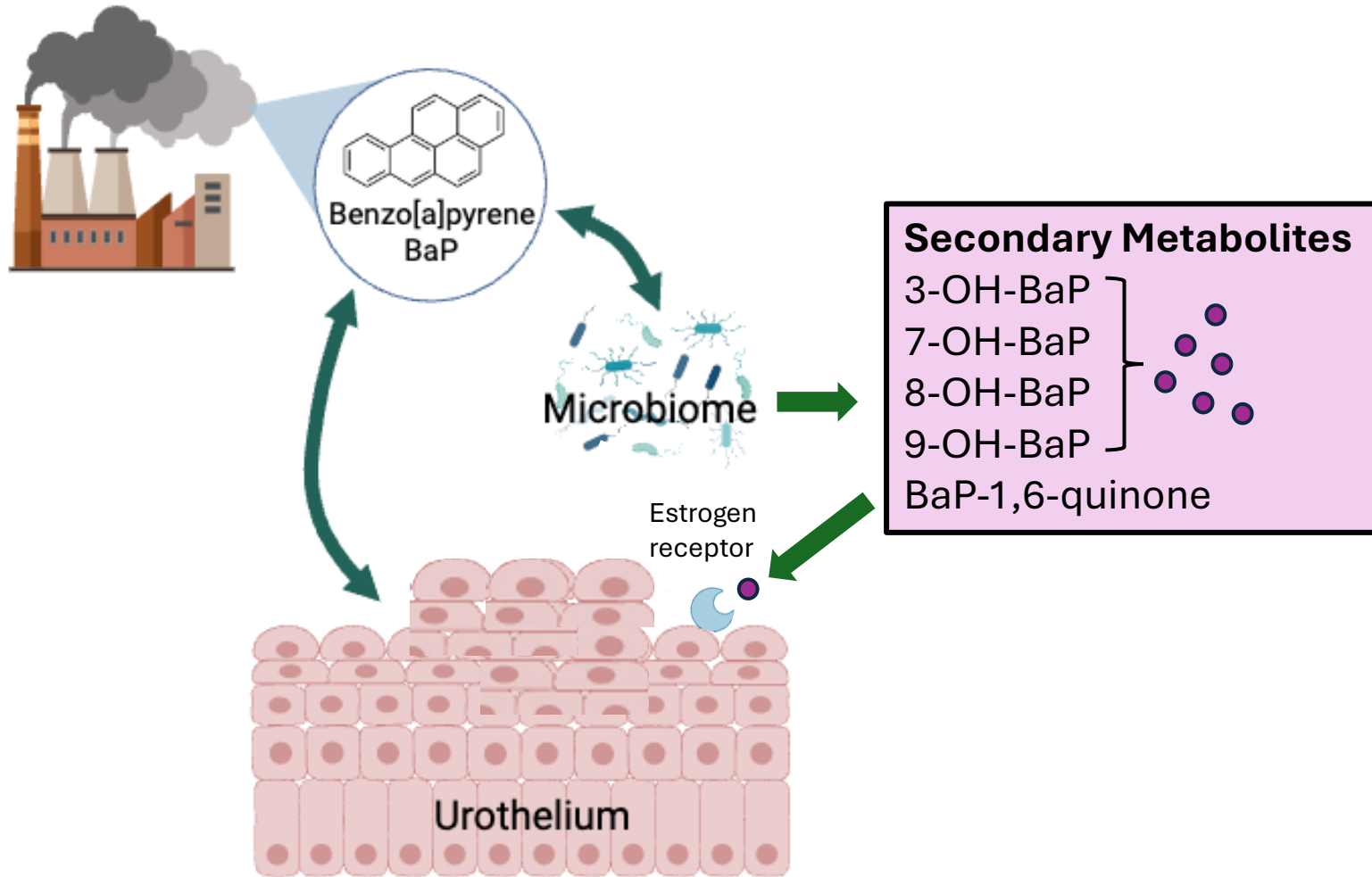


Secondary Metabolites

3-OH-BaP
7-OH-BaP
8-OH-BaP
9-OH-BaP
BaP-1,6-quinone

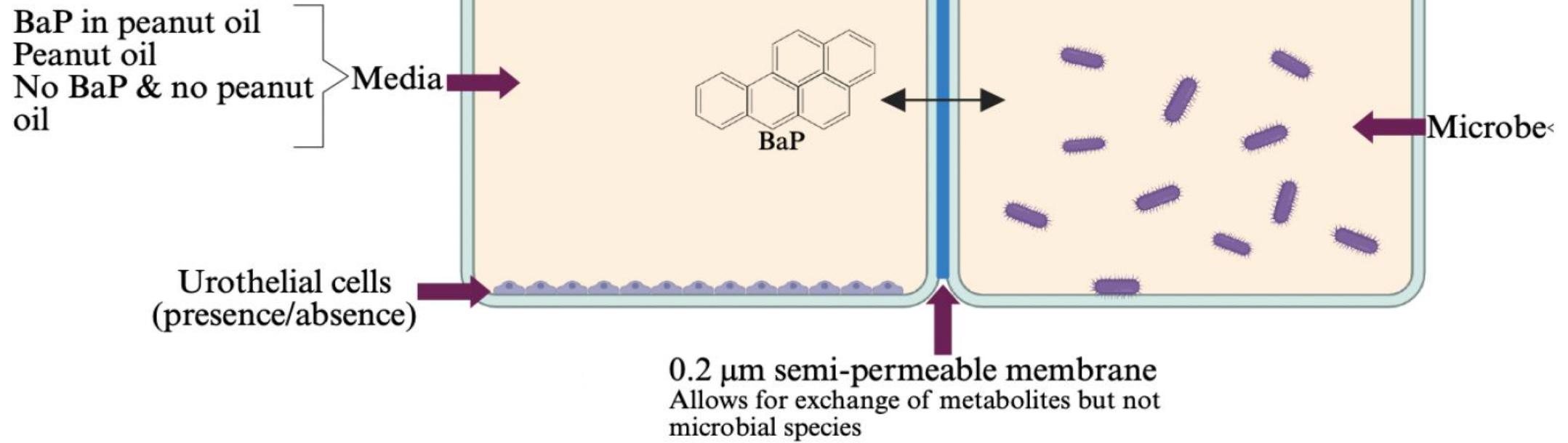


BaP exposure induces urothelial proliferation

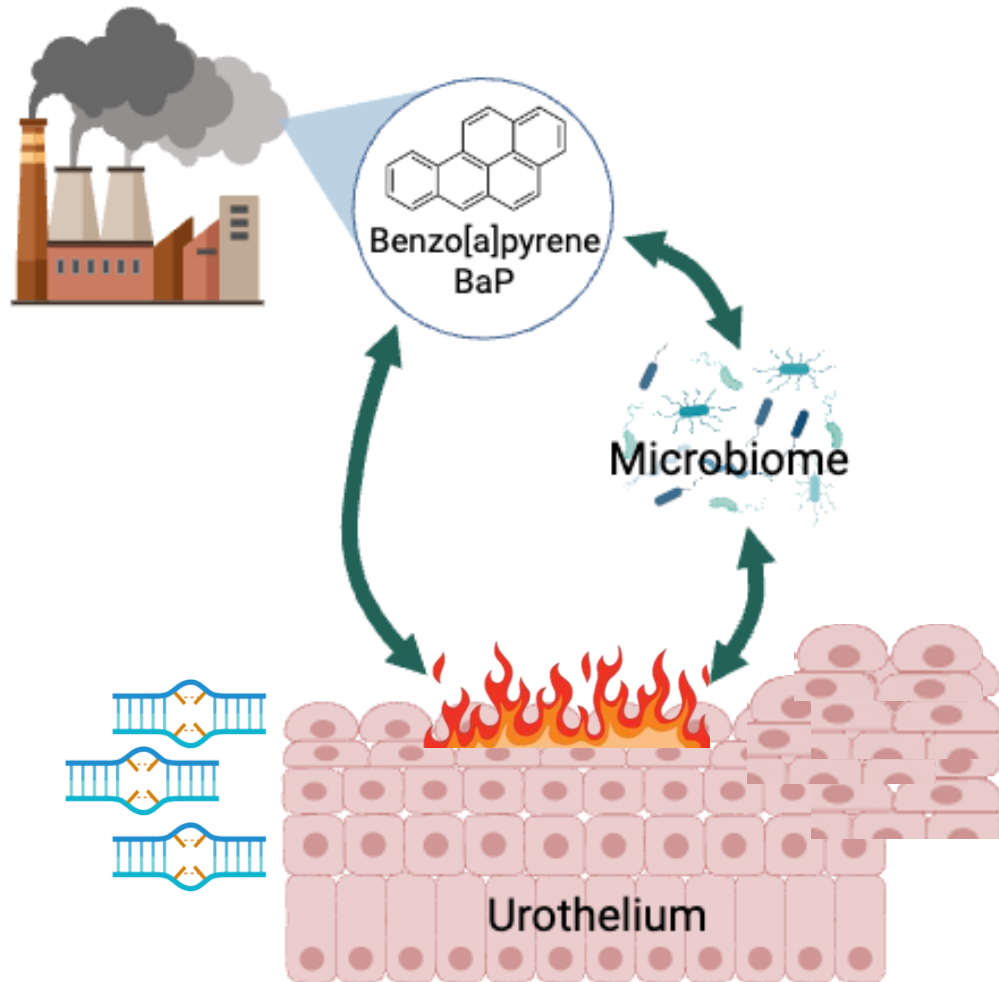


- This response could be linked to the estrogenic activity of secondary BaP metabolites
- To what extent are host and microbes metabolizing or co-metabolizing BaP?
- How are microbiomes impacted by BaP?
- Do differing microbiomes alter proliferation potential?
- What implications does BaP exposure (or other pollutants) have on bladder cancer? UTI risk? Overall bladder health?

Transwell system



Key Take Aways



- Environmental exposures play an important role in bladder health
- Microbial metabolism of these exposures may serve to enhance or mitigate cytotoxic or genotoxic effects of these exposures
 - See: Roje et al 2024, Nature
 - Gut microbes were necessary and sufficient to increase number and severity of bladder tumors in a BBN (chemical)-induced bladder cancer mouse model
- Chemical-microbe-host interactions are likely critical to bladder cancer development and recurrence

Acknowledgements

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