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US EPA CSS-HERA Board of Scientific Counselors Chemical Safety Subcommittee Meeting

US EPA CSS-HERA BOSC Meeting – February 2-5, 2021

CSS Session 1D Slides

The work presented within represents US EPA Office of Research and Development research activities. Material includes both peer reviewed, published results and work-in-progress research. Please do not cite or quote slides.

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Development and Harmonization of Organotypic/Co-Culture Models and Assays to Improve Throughput and *In Vivo* Relevance in Inhaled Chemical Testing

2021 CSS BoSC
2/3/2021

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Conflict of Interest and Disclaimer

- No conflicts of interest
- The information presented here does not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

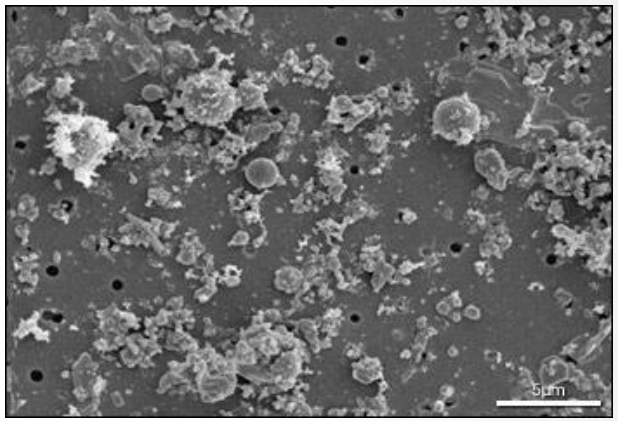
In Vitro Approaches for Inhaled Materials Testing and Research

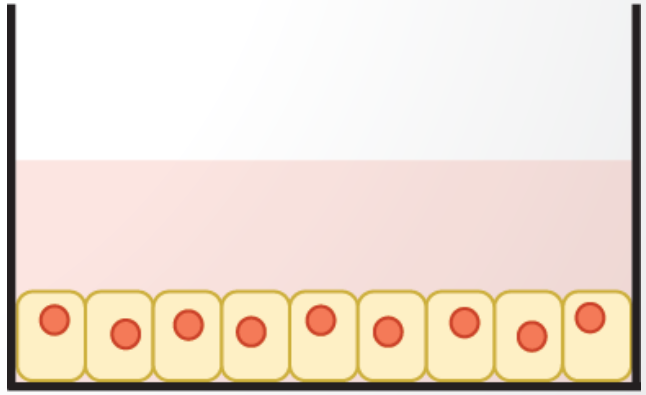
- *In vitro* models are required for testing the thousands of inhalable materials
- The lung is a complex organ with over 40 cell types, but nearly all *in vitro* models include only a single cell type
- Individual inhaled materials impact distinct cell types and physiological functions differently
  - These effects cannot be represented by current systems
- There is a lack of consensus on methods, parameters, thresholds, and reporting standards for models, assays, and exposures
- Fit-for-purpose models and assays are needed for accurate, reliable, and defensible inhalation toxicity testing
- *In vivo* human data from environmental inhaled materials (*e.g.*, ozone, acrolein, particulate matter) are invaluable in guiding the development of lung models and allowing validation for acceptance

Case Study Using A Data-Rich Inhaled Material

- *In vitro* assessment at 24hr
  - Not cytotoxic
  - No change in bronchial epithelial barrier permeability
  - Marginal changes in stress-responsive gene expression (RNA) at 24 hours
- *In vivo* human exposures
  - Acute and chronic lung disease
    - Airway inflammation
    - Susceptibility to infection
    - Asthma
    - COPD
  - Cardiovascular morbidity and mortality
    - MI/stroke/arrythmia
  - Attributed to 4-10 million deaths per year worldwide

Fine Particulate Matter ($PM_{2.5}$)





So, Why Didn't It Work?

- Traditional *in vitro* inhalation models do not represent *in vivo* biology and dosimetry
  - Models/endpoints are often not "fit-for-purpose"
  - *e.g.*, bronchial epithelial cells are not representative of lung microvascular endothelial cells, *et cetera*
- Lack of time course data and limited scope of endpoints resulted in failure to identify adverse effects
  - Looking in the wrong place at the wrong time
- Few *in vitro* inhalation models are representative of individuals most likely to experience adverse effects (*i.e.*, susceptible populations)

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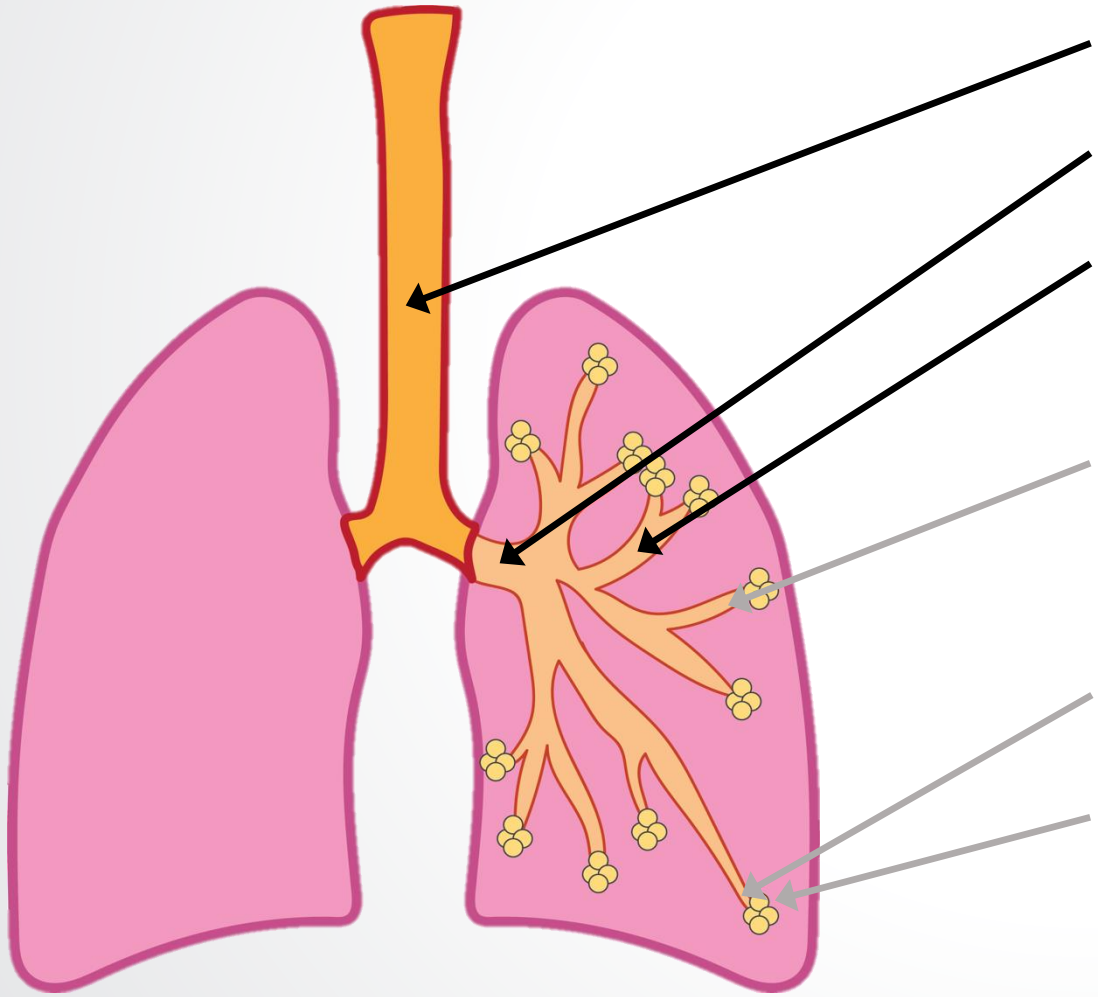
Lung Structure and Function

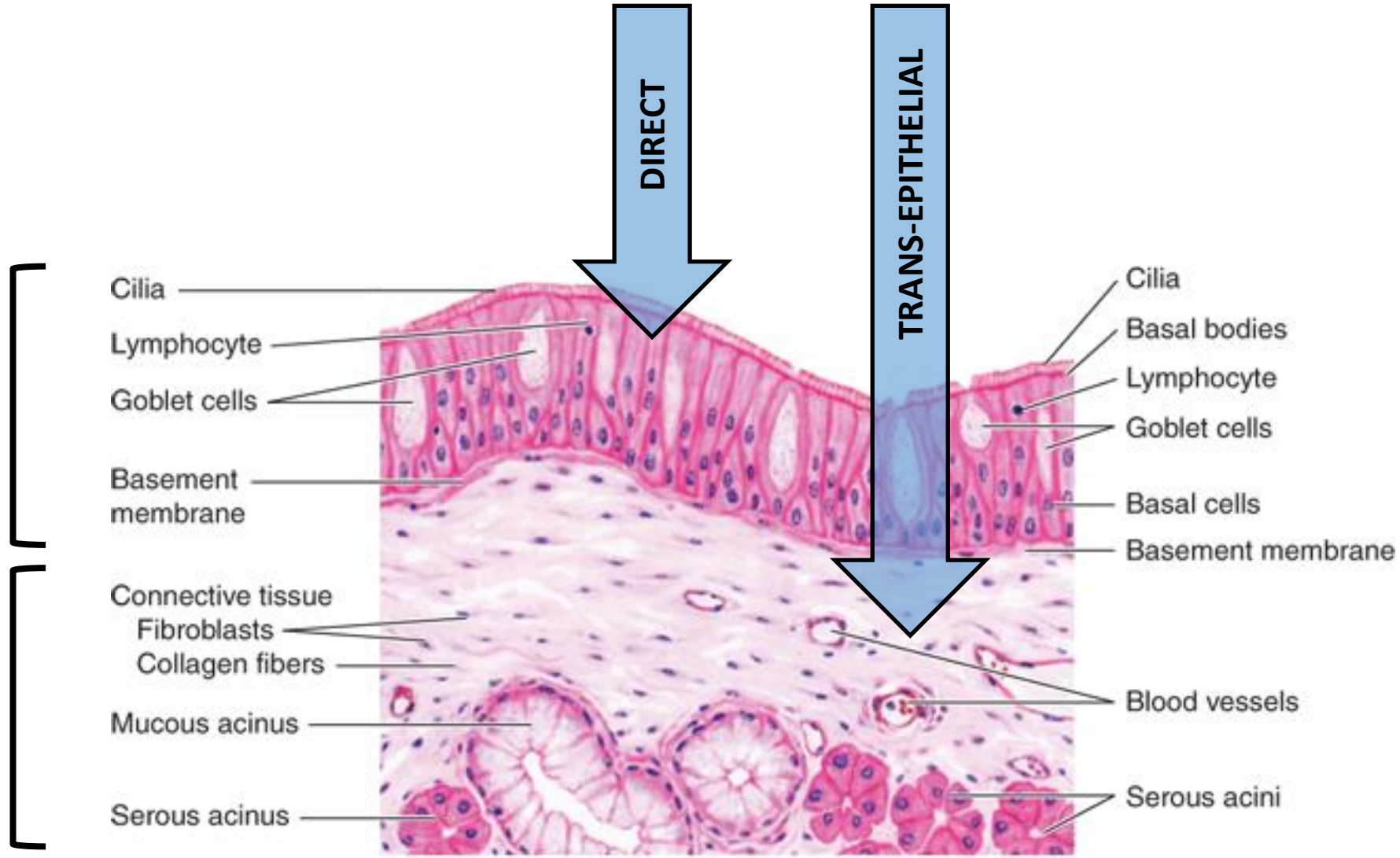


Trachea **Bronchi** **Bronchioles**	**Large conducting airways** **"large airways"**
Terminal bronchioles	Small conducting airways "small airways"
Respiratory bronchioles Alveoli	Pulmonary airways

Bronchial Airway Tissue and Dosimetry

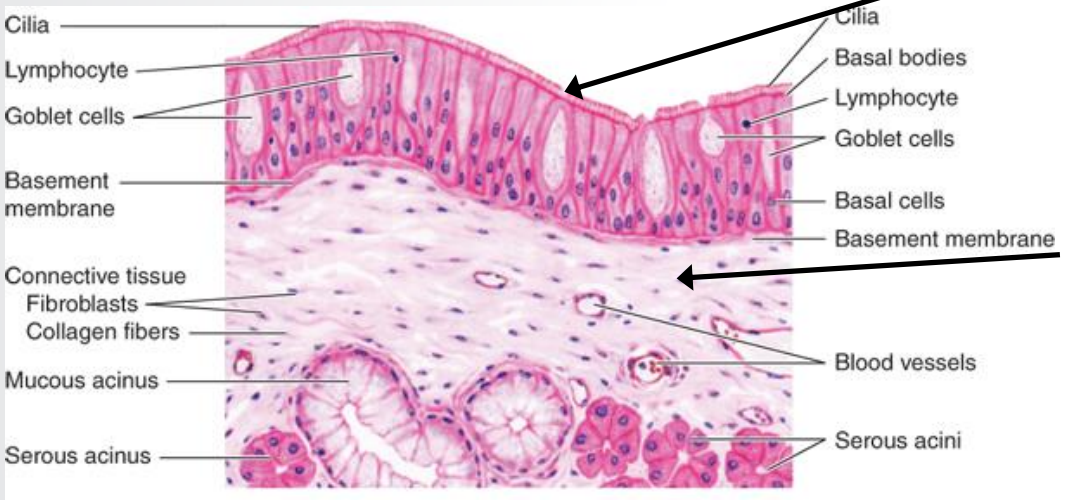
Epithelial barrier

Stroma



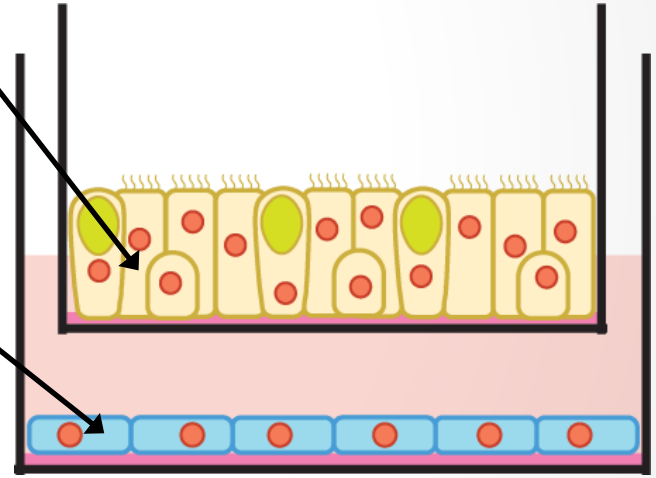
Adapted from Lab Chip, 2014, 14, 3349-3358

Trans-Epithelial Exposure Model



Bronchial epithelium

Bronchial stroma



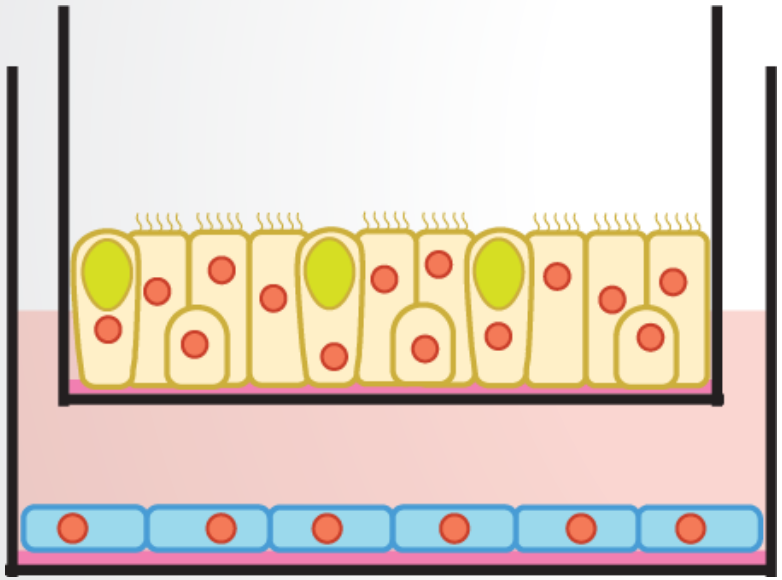
Direct Exposure

Trans-Epithelial Exposure

Faber *et. al.*, (2020) Toxicological Sciences **177(1):**140-155

Trans-Epithelial Exposure Model (TEEM)

TEEM-Mark 2



- Research/testing use
  - Effects on bronchial epithelium
  - Effects on bronchial microenvironment
  - Parallel analysis
  - Suitable for many endpoints
  - Hybrid primary/cell line-based model
- Mark 1 (cell lines)
  - Very low cost (~$4 per well)
  - Set-up to assay time = 2 days
- Mark 2 (primary cells)
  - Low cost (<$10 per well)
  - Set-up to assay time = 24+ days
- Key findings:
  - Trans-epithelial exposure effects on fibroblasts are similar between Mark 1 and Mark 2 models
  - Bronchial epithelial cells are minimally responsive to exposures and may not be the primary targets/mediators of exposure effects
  - Mark 1 model is representative of bronchial epithelial barrier function

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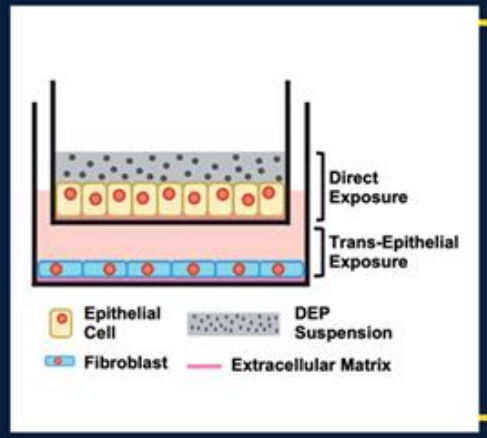

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Advance Access Publication Date: 11 June 2020
Research Article

Exposure Effects Beyond the Epithelial Barrier: Transepithelial Induction of Oxidative Stress by Diesel Exhaust Particulates in Lung Fibroblasts in an Organotypic Human Airway Model

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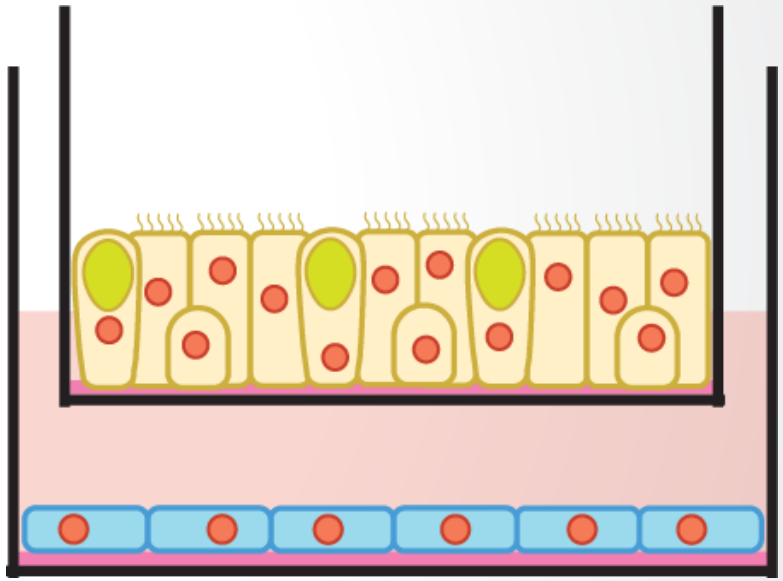
ABSTRACT

In vitro bronchial epithelial monoculture models have been pivotal in defining the adverse effects of inhaled toxicant exposures; however, they are only representative of one cellular compartment and may not accurately reflect the effects of exposures on other cell types. Lung fibroblasts exist immediately beneath the bronchial epithelial barrier and play a central role in lung structure and function, as well as disease development and progression. We tested the hypothesis that *in vitro* exposure of a human bronchial epithelial cell barrier to the model oxidant diesel exhaust particulates caused transepithelial oxidative stress in the underlying lung fibroblasts using a human bronchial epithelial cell and lung fibroblast coculture model. We observed that diesel exhaust particulates caused transepithelial oxidative stress in underlying lung fibroblasts as indicated by intracellular accumulation of the reactive oxygen species hydrogen peroxide, oxidation of the cellular antioxidant glutathione, activation of NRF2, and induction of oxidative stress-responsive genes. Further, targeted antioxidant treatment of lung fibroblasts partially mitigated the oxidative stress response gene expression in adjacent human bronchial epithelial cells during diesel exhaust particulate exposure. This indicates that exposure-induced oxidative stress in the airway extends beyond the bronchial epithelial barrier and that lung fibroblasts are both a target and a mediator of the adverse effects of inhaled chemical exposures despite being separated from the inhaled material by an epithelial barrier. These findings illustrate the value of coculture models and suggest that transepithelial exposure effects should be considered in inhalation toxicology research and testing.

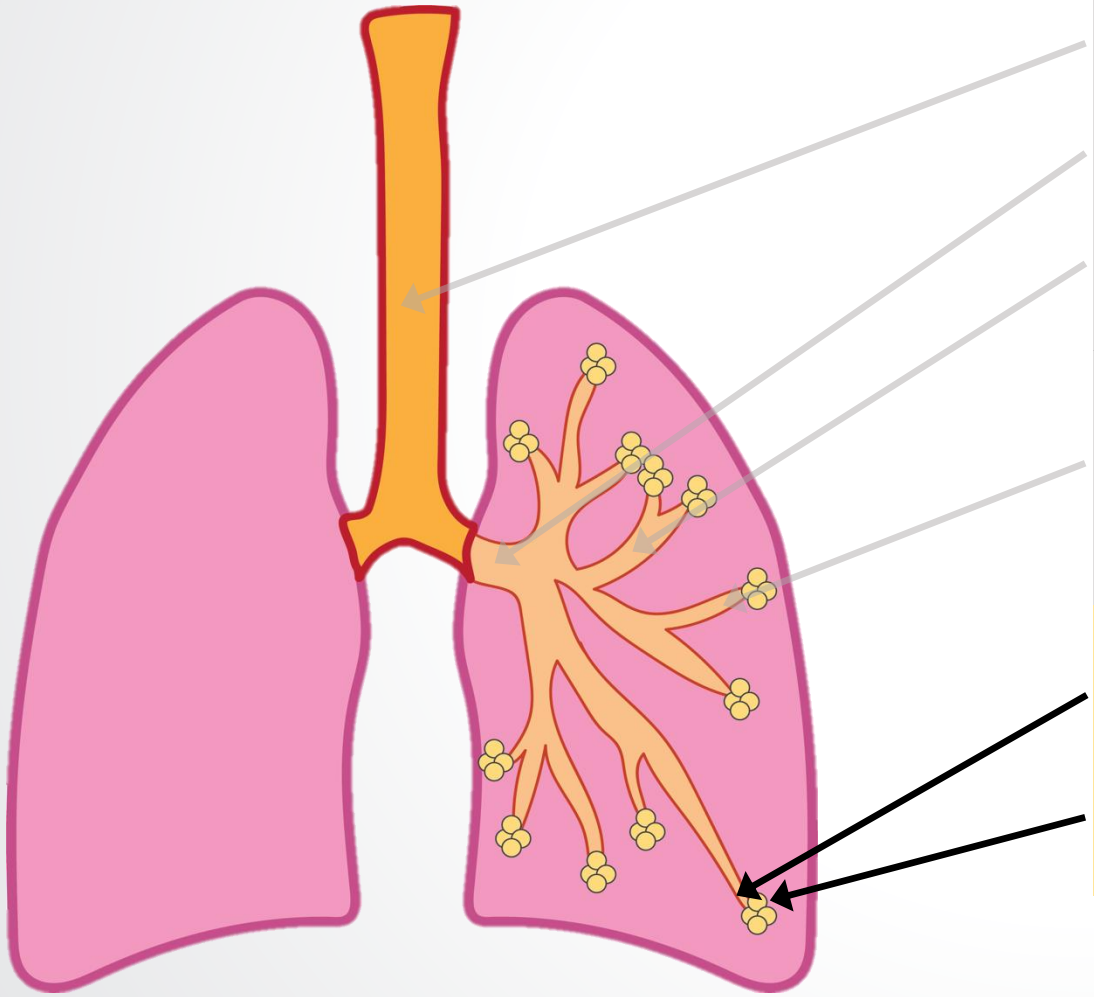
Key words: *in vitro*; coculture; oxidative stress; lung; fibroblast; transepithelial; epithelial.

Moving Forward

- TEEM Mark 2 model
- Assess culture longevity, key parameter values, and expected variability over time (differentiated + up to 90 days) for subsequent sub-chronic exposures
  - Barrier permeability
  - Histology/immunofluorescent staining
  - Viability
  - Redox potential
  - Ciliary beat frequency
  - Marker gene expression
  - Mucus production
  - Metabolism
- Inter-donor and inter-experimental variability using a group of donors
  - Power calculations to strengthen future studies

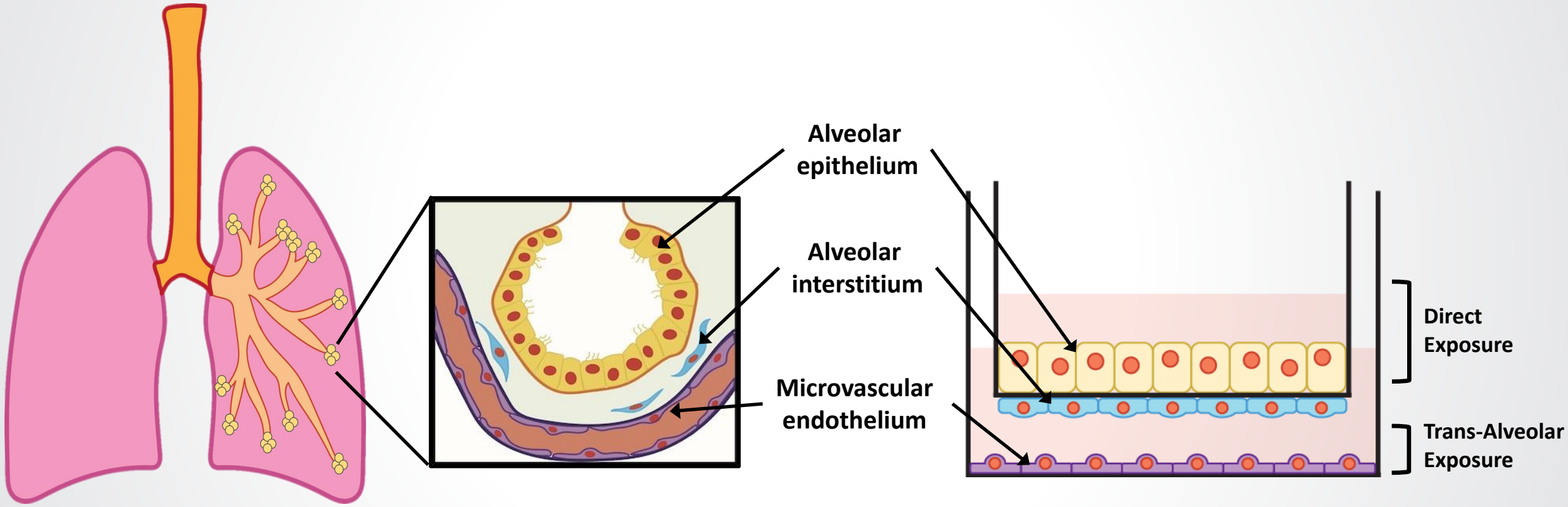


Lung Structure and Function in *In Vitro* Testing

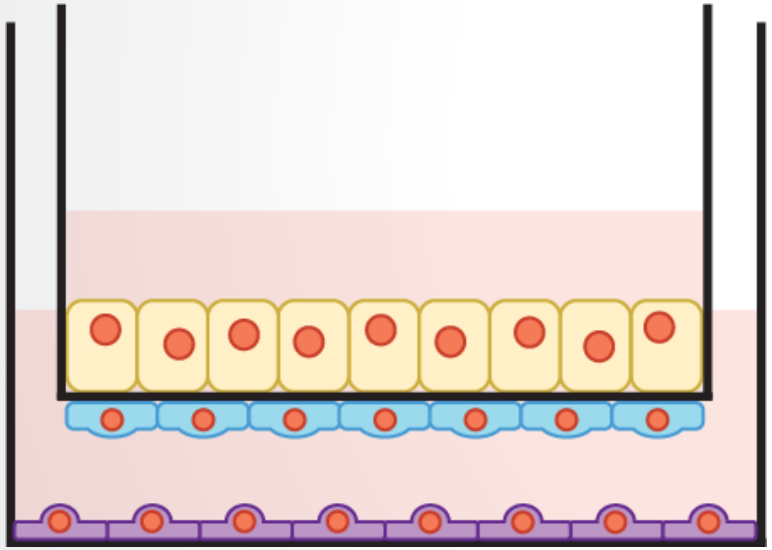


Trachea Bronchi Bronchioles	Large conducting airways "large airways"
Terminal bronchioles	Small conducting airways "small airways"
Respiratory bronchioles **Alveoli**	**Pulmonary airways**

Alveolar Region

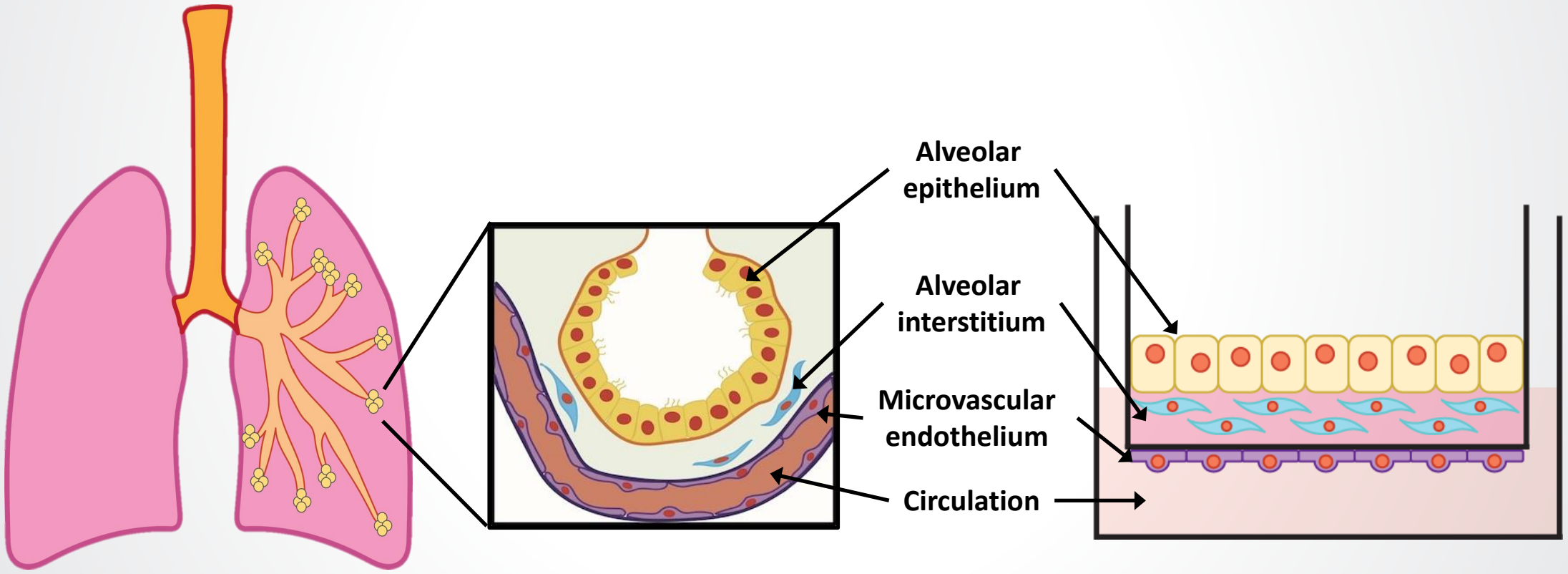


Alveolar Capillary Region Exposure (ACRE) Model – Mark 1

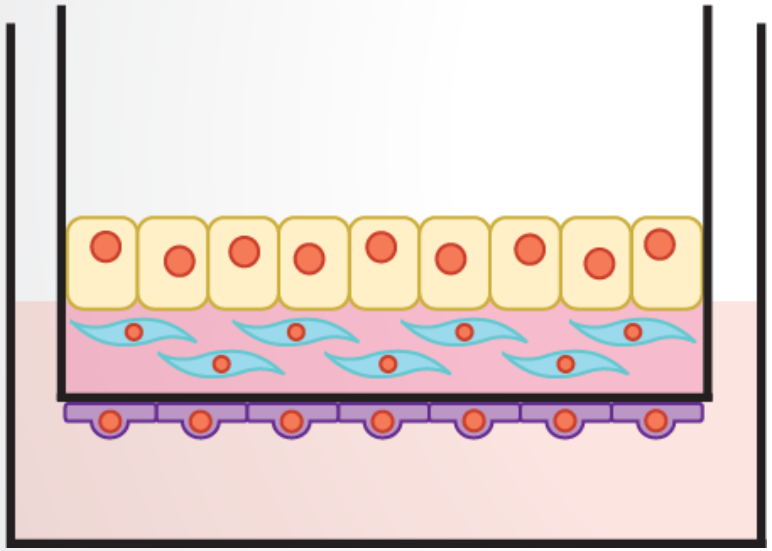


- Indirect co-culture model
  - Submerged – completed
  - Air-liquid interface – in progress
  - Incorporation of immune cells – under development
- Research/testing use
  - Effects on alveolar compartment
  - Effects on lung microvasculature
- Cell line or hybrid model
  - NCI-H441
  - IMR90/pHLF
  - HULEC/pLMVEC
- Cost per sample: ~$4 (cell lines)
- Set-up to assay = 5 days
- Key findings to date:
  - Trans-alveolar exposures to diesel exhaust particulates cause effects that reflect *in vivo* human outcomes

Alveolar Region



Alveolar Capillary Region Exposure (ACRE) Model – Mark 2



- Currently under development
  - Submerged and air-liquid interface
  - Planned incorporation of immune cells
- Research/testing use
  - Effects exposure on pulmonary barrier
  - Release of mediators/metabolites into the circulation
  - Bioavailability of inhaled materials
- Acute or repeated/sub-chronic/chronic exposure scenarios
- Cell line or hybrid model
  - NCI-H441
  - IMR90/pHLF
  - HULEC/pLMVEC
- Cost per sample: ~$5
- Set-up to assay = 4-5 days (expected)

Summary

- Bronchial epithelial cell models provide valuable information but are not representative of other aspects of the structure and function of a complex tissue
- Fit-for-purpose multi-cellular models are necessary for accurate, reliable, and defensible inhalation toxicity testing and computational model development
- Human data from environmental inhaled materials is invaluable in lung model development and validation
- Bronchial epithelial/stromal co-culture model indicates that trans-epithelial exposure effects on the stroma may exceed direct effects in the epithelium
- Outcomes in ACRE-Mark 1 model align with *in vivo* human data
- ACRE-Mark 2 model for alveolar-vascular permeability and trans-alveolar bioavailability is in progress
- Incorporation of immune cells into all models is in development
- Protocols/methods are designed to be accessible, cost-effective, and compatible with high throughput assays, and will be made publicly available

Questions:

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An Approach Using NAMs for the Evaluation of Inhalation Toxicity in OCSPP Chemical Registrations

Mark Higuchi, PhD

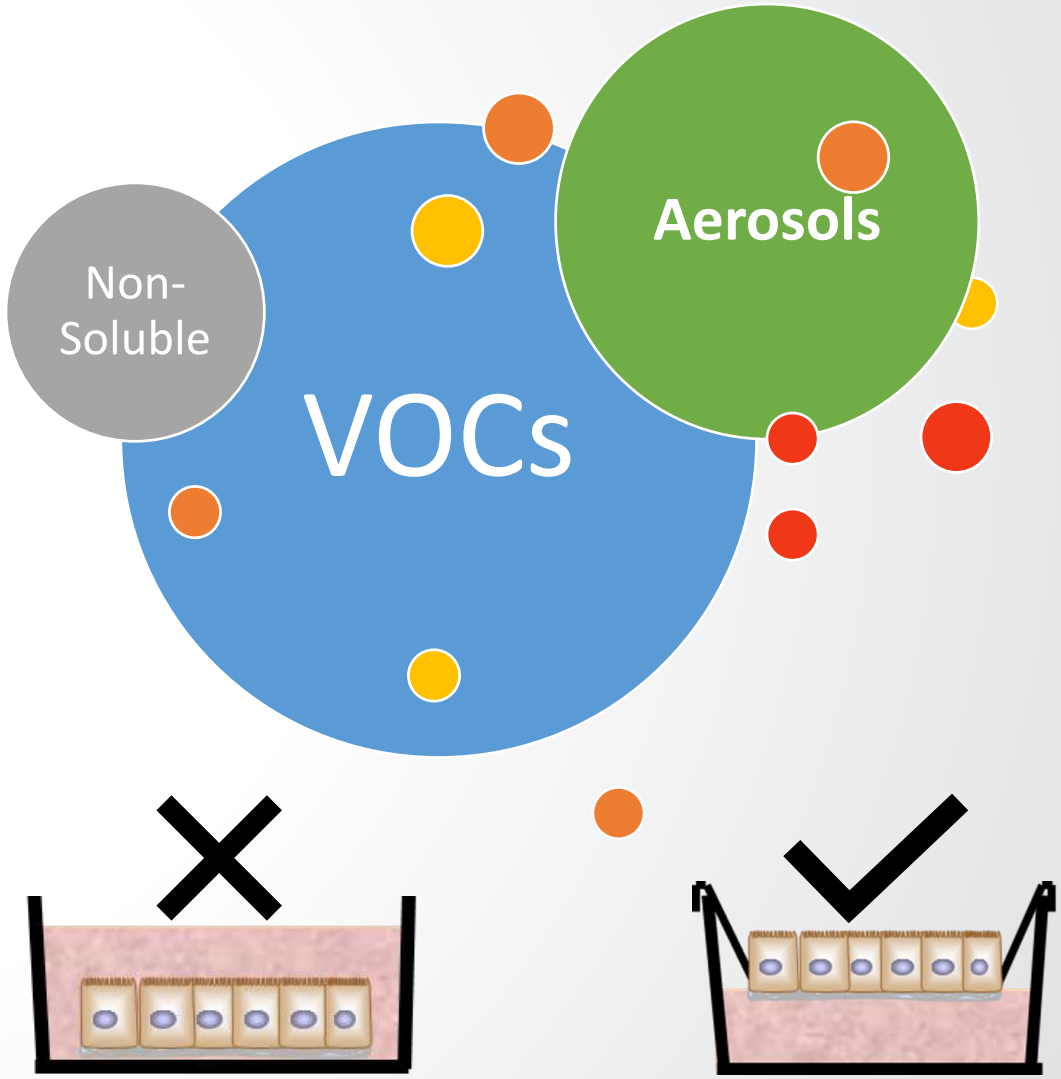
ORD/CPHEA/PHITD/ITFB

2/2/2021

Challenges to traditional *in vitro* exposure methodology

Problem: VOCs and Aerosols are incompatible with traditional *in vitro* testing methods.

- Over 30% of the TSCA chemical inventory are volatile or insoluble
- Require biologically relevant air-liquid interface (ALI) exposures to mimic human exposures
- *Currently lack information on these compounds to support risk assessment*

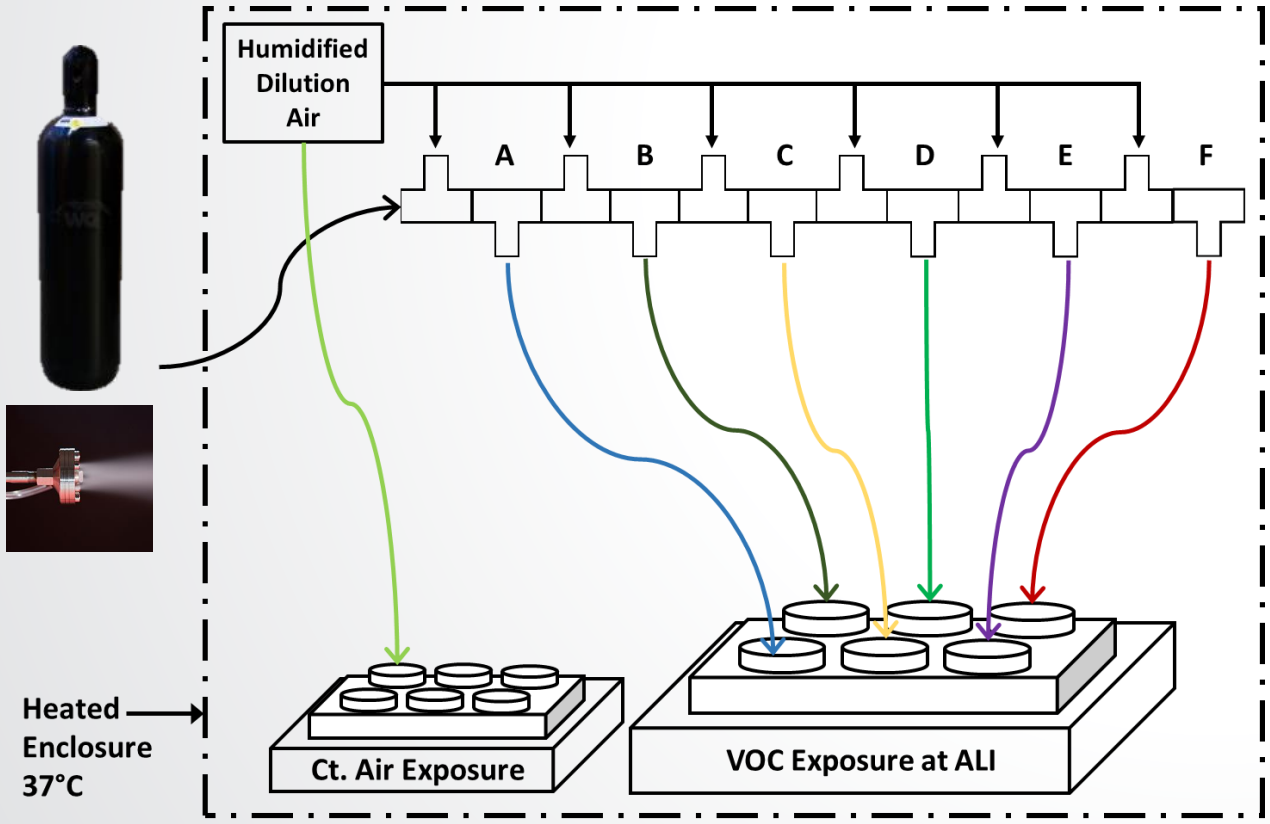


In vivo vs. *in vitro* Inhalation Studies

	***In vivo* Inhalation Studies**	**Traditional *in vitro* studies**	**NAMs for *in vitro* Inhalation Studies**
Eligible Compounds	Aerosols and VOCs	Soluble compounds only	Aerosols and VOCs
Dosing Methods	Known concentration over a certain time (C*t)	Direct dosing	Known concentration over a certain time (C*t)
Realistic Exposure?	Yes, whole animal with intact airway	No, submerged culture	Yes, airway cells cultured at ALI
Dosimetry	Lacks analytical animal dosimetry	Known concentration applied to media	Lacks analytical cell dosimetry
Conditions	Control temp and RH for animal	Submerged culture in incubator	Control temp and RH for ALI cultures
Eligible Endpoints	Portal-of-entry and systemic effects	Only assesses cellular effects	Portal-of-entry and cellular effects
Repeated Dosing Possible?	Yes	Limited	Limited

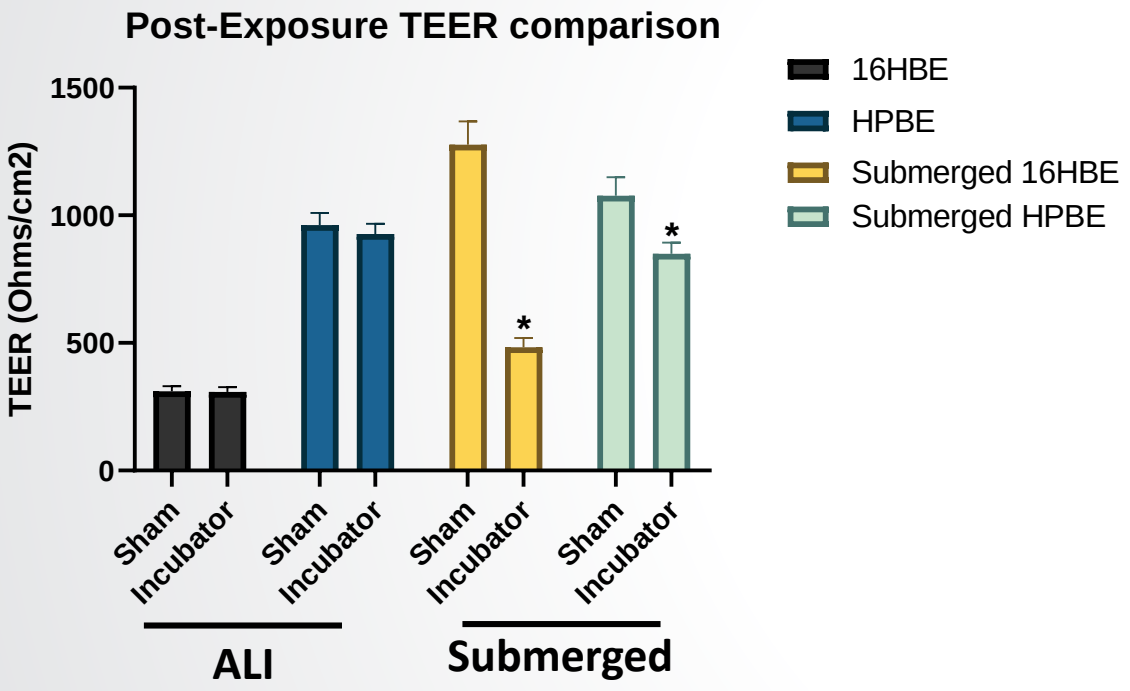
EPA Cell Culture Exposure System (CCES)

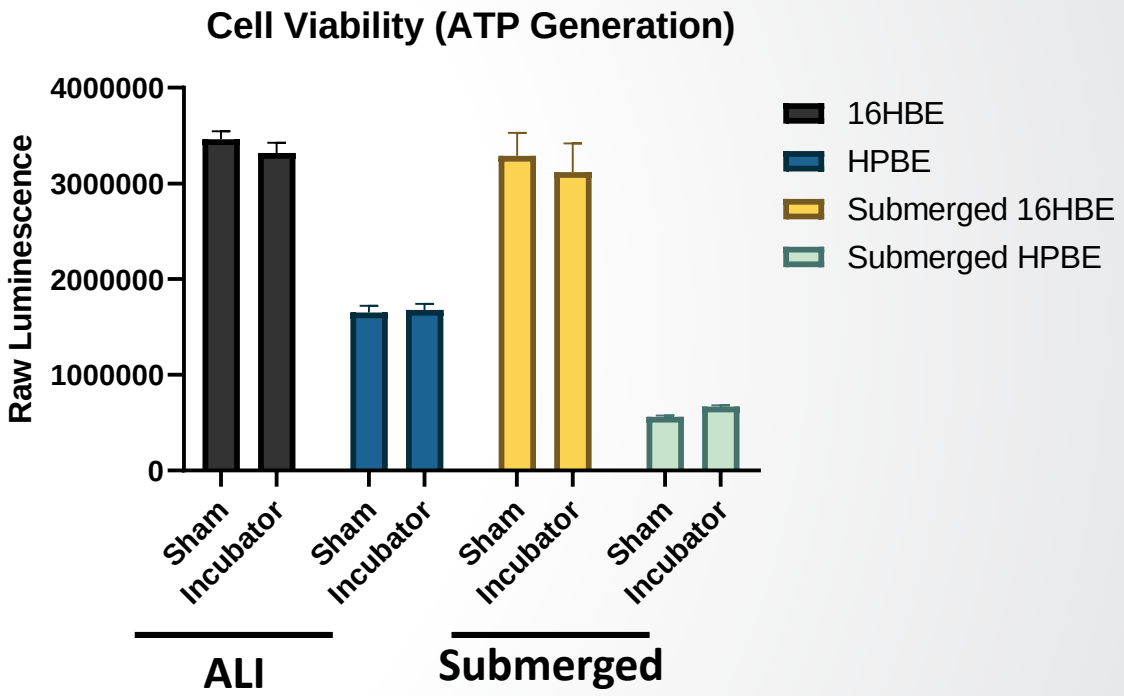
To address this need, we developed a novel system: the EPA's **Cell Culture Exposure Systems (CCES)** permits the exposure of mammalian lung cells at **air-liquid interface (ALI).**



- Delivers VOCs to advanced ALI airway models to produce a biologically realistic inhalation exposure
- Medium-throughput testing strategy
- Unlike other ALI exposure technologies, the CCES maintains ideal temperature and humidity conditions for cells at ALI

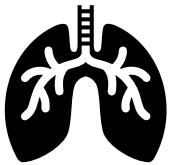
CCES Provides Superior Exposure Conditions





- Immortalized and human primary bronchial epithelial cells do not experience any negative impacts during a 2h exposure to clean, humidified air (Sham)
- TEER, the most sensitive endpoint, shows significant differences between controls during submerged exposures

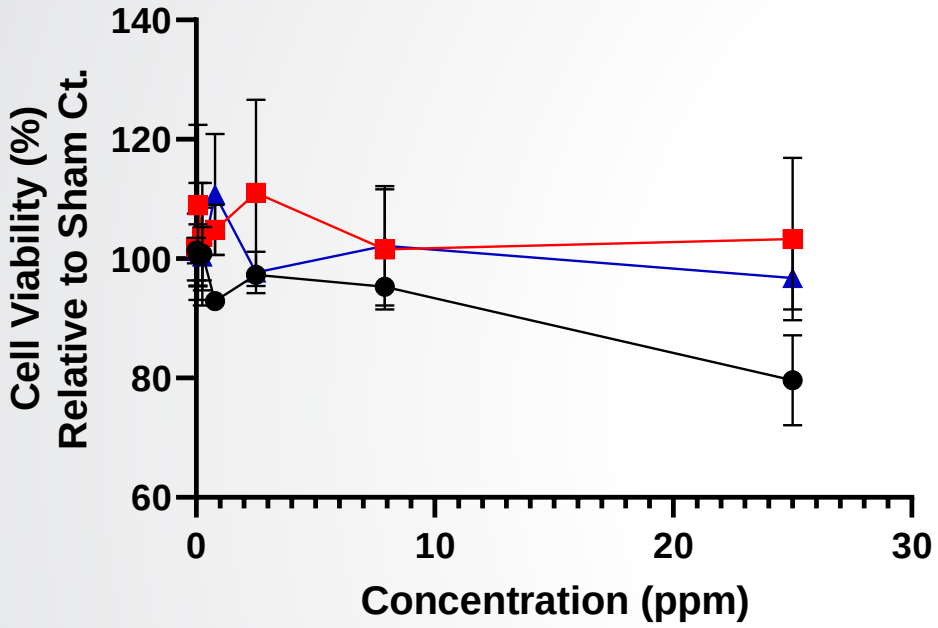
Pilot and Proof-of-Concept Studies

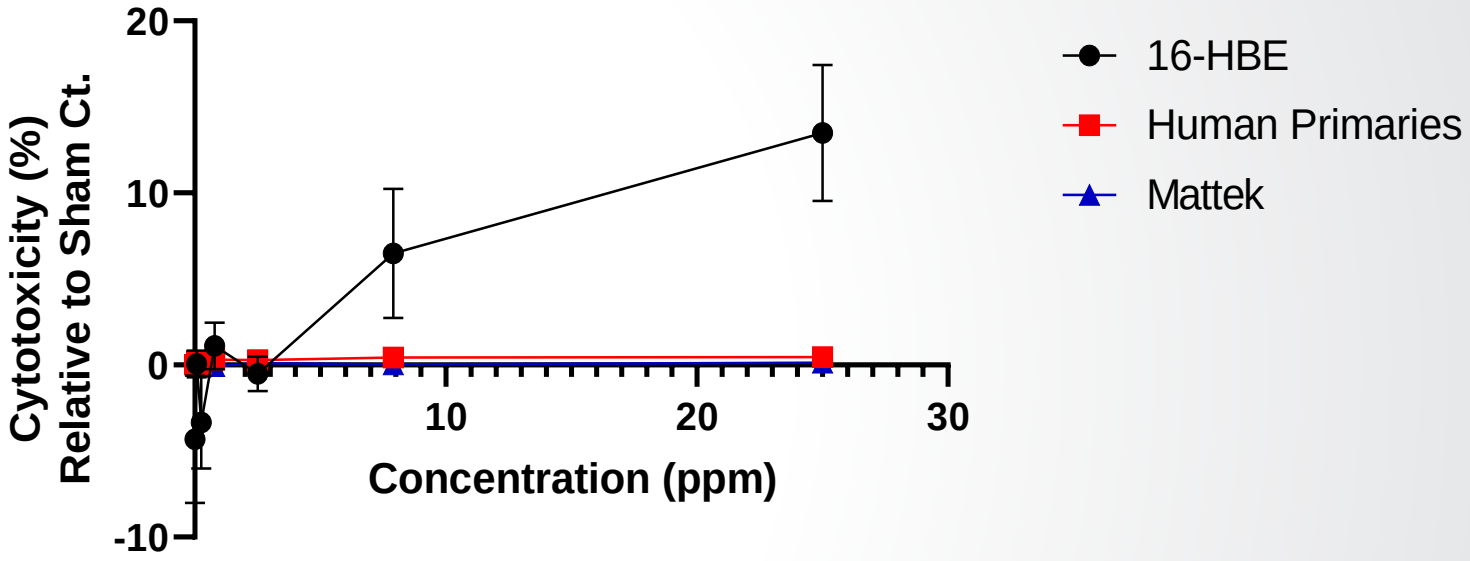


Pilot Study Overview	
Cell Types at ALI	Primary Human Bronchial Epithelial Cells* BEAS-2B cells
Chemicals Tested	1,3-Butadiene Acetaldehyde Carbon Tetrachloride* Acrolein Trichloroethylene* Dichloromethane* Formaldehyde 1-Bromopropane* *Tested in both B2B and HBECs
Exposure Regimen	• 2h exposure, endpoints collected 4h later • 6 concentrations, sham + incubator controls • Temp and RH monitored
Assay Formats	• TempO-Seq • Cytotoxicity [LDH Release, Cell Titer Glo]

Proof-of-Concept Study Overview	
Cell Types at ALI	Primary Human Bronchial Epithelial Cells **16HBE cells** **Mattek Epi-Airway cells**
Chemicals Tested	Naphthalene 1,3-Dichloropropene Chloropicrin Methylisothiocyanate Zinc pyrithione* Metribuzin* Didecyldimethyl ammonium chloride* Tetramethrin* 2-phenylphenol* Indoxacarb* Naled* Azoxystrobin* Oxamyl* ***aerosol exposure necessary**
Exposure Regimen	• 2h exposure, endpoints collected 4h later • 6 concentrations, sham + incubator controls • Temp and RH monitored
Assay Formats	• TempO-Seq • Cytotoxicity [LDH Release, Cell Titer Glo] • **Trans Epithelial Resistance (TEER)** • **Inflammatory response [ELISA for IL-6 and IL-8]**

Proof-of-Concept: 1,3-Dichloropropene



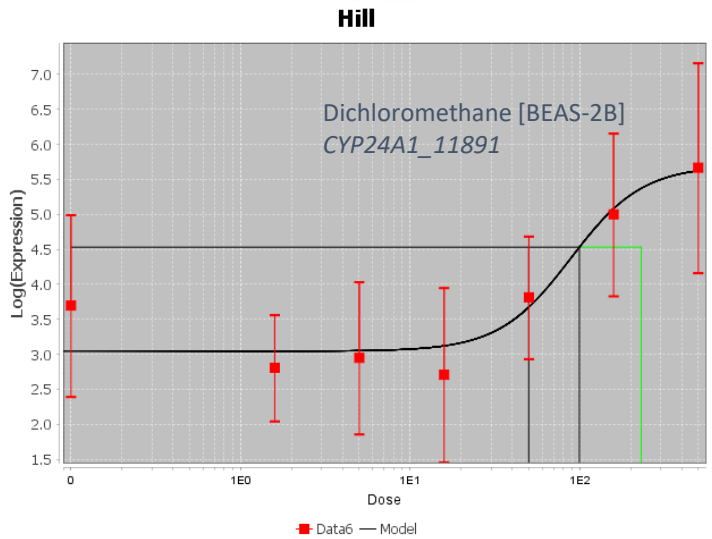


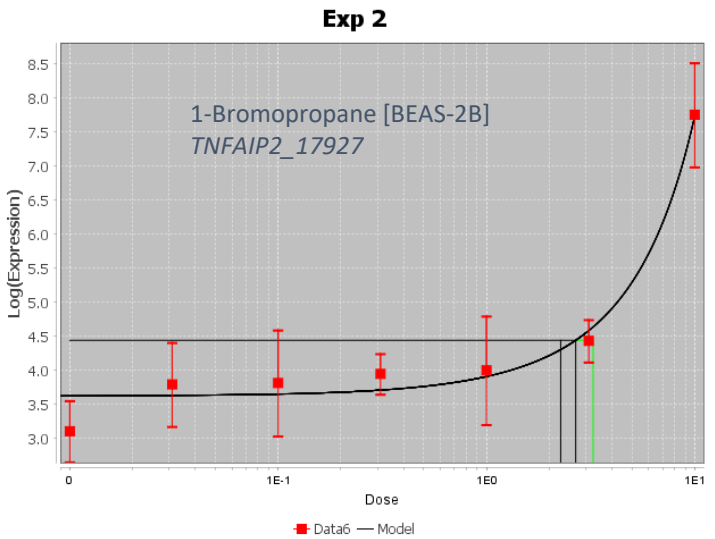
Technical Replicates	• Viability, n=2; Cytotoxicity, n=4
Biological Replicates	• Conducted over three days, n=3
Exposure Regimen	• 6 concentrations, Sham exposure control, incubator control • Several sub-cytotoxic doses are included

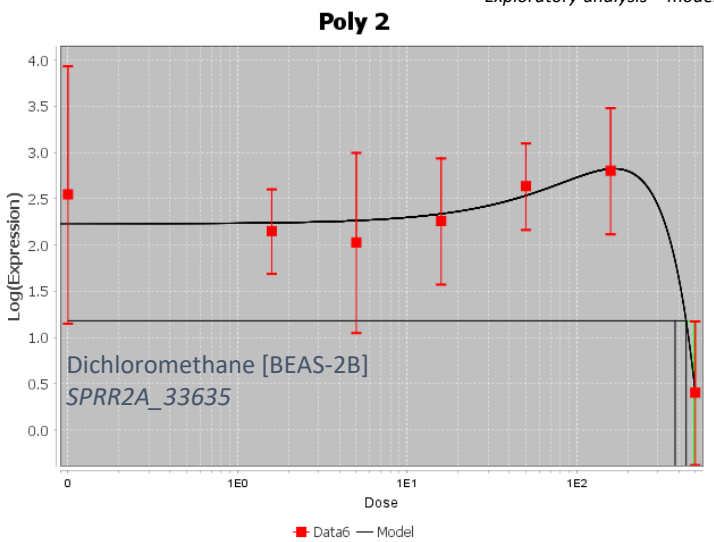
VOC Benchmark Dose Modeling

- Data generated by High-Throughput Transcriptomics (HTTr) shows promise for quantitative human health risk assessment
- BMD analysis is the current standard for submerged high-throughput chemical screening
  - We are uniquely suited to provide missing BMD values for VOCs

[a] *Exploratory analysis – modeling criteria not finalized*



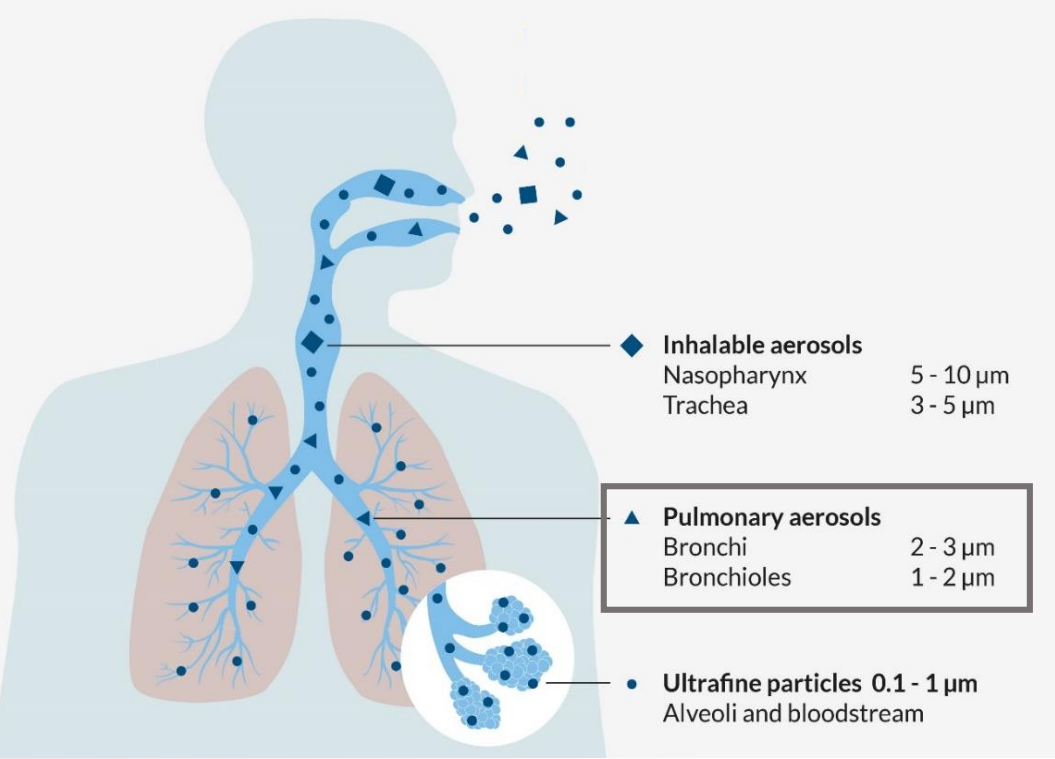




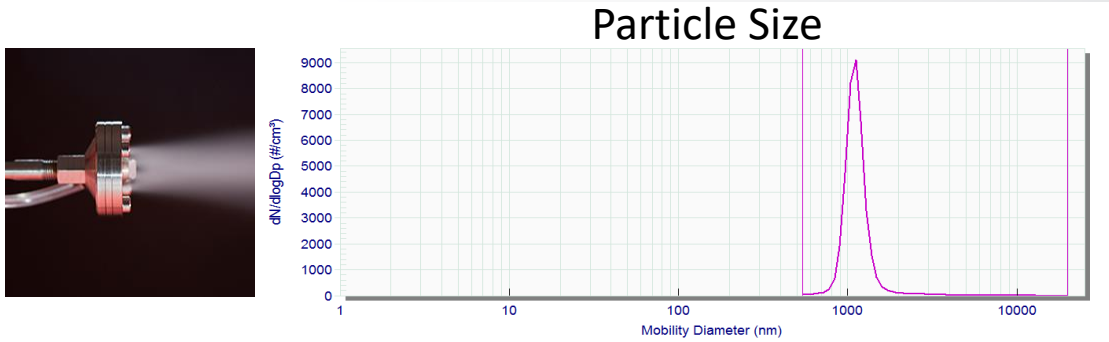
Comparison of *in vitro* to *in vivo* exposure studies

	in vitro		*in vivo*		
Chemical Name	**BEAS-2B BMD (ppm)**	**HPBE BMD (ppm)**	**Representative LOAEL (ppm)**	**Representative NOAEL (ppm)**	**TLV (ppm)**
Acrolein	**0.586**	**--**	0.4	NR	0.1ppm
1-Bromopropane	**2.246**	**N/A**	125	250	0.1ppm
Formaldehyde	**N/A**	**--**	404	152	0.3ppm
1,3-Butadiene	**13.979**	**--**	625	8000, 200	10ppm
Carbon Tetrachloride	**9.563**	**N/A**	10	5, 1	10ppm
Acetaldehyde	**N/A**	**--**	2	1	25ppm
Trichloroethylene	**44.842**	**28.148**	25, 2.6	50, 5.2	50ppm
Dichloromethane	**142.127**	**226.73**	500-1000	200	100ppm

Next Steps: New Priority Compounds Must Be Generated as Aerosols for ALI Exposures



→ Must be generated and delivered as 1-2 µm particles; possess different transport mechanisms than VOCs.



$$m_p \frac{d\vec{v_p}}{dt} = -f_{ric}(\vec{v_p} - \vec{v_f}) + m_p\vec{g} + \vec{F(t)}$$

aerosols

VOCs

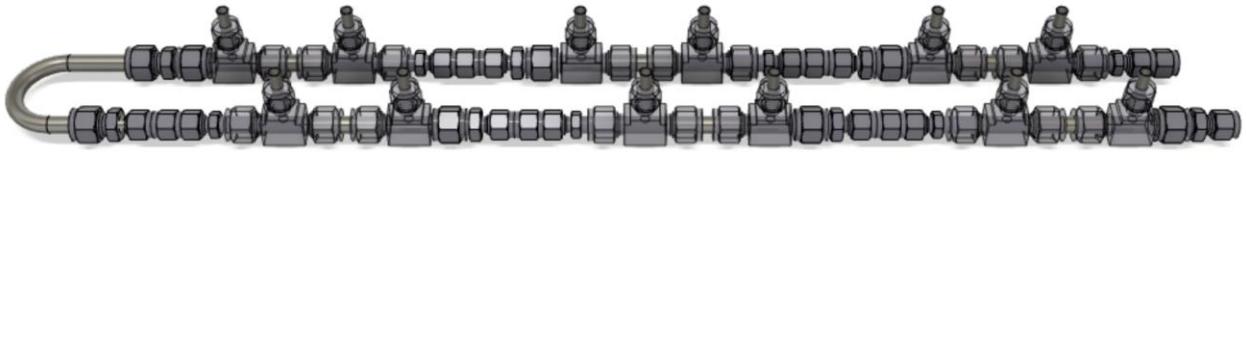
Langevin Equation for Transport

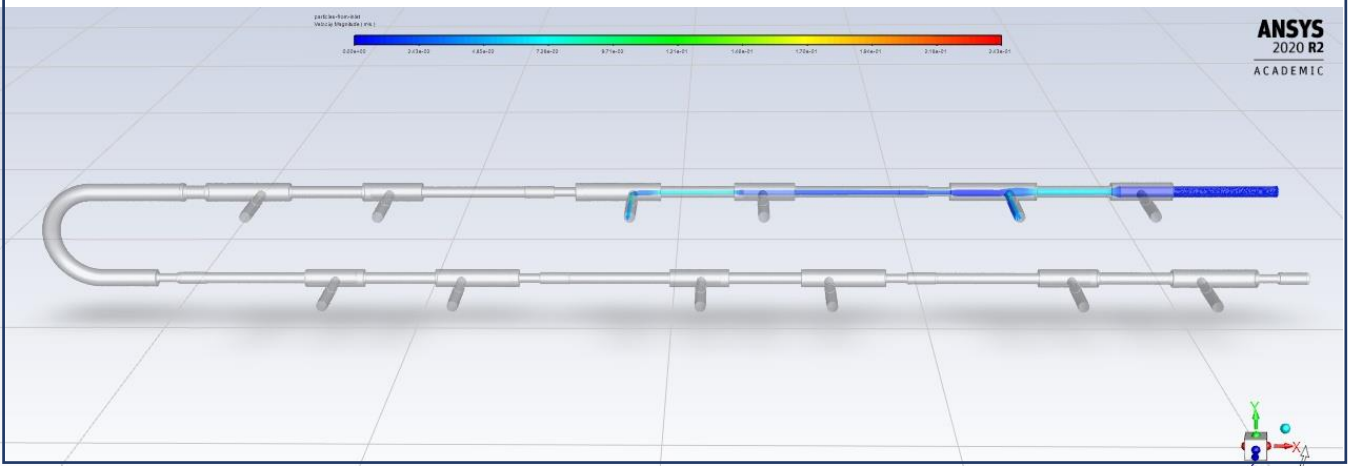
- Particle Acceleration (≥0.5 µm)
- Gravitation forces (>0.5 µm)
- Diffusion (<0.5 µm)

CAD and CFD Modeling Guide Development of Aerosol-Specific CCES

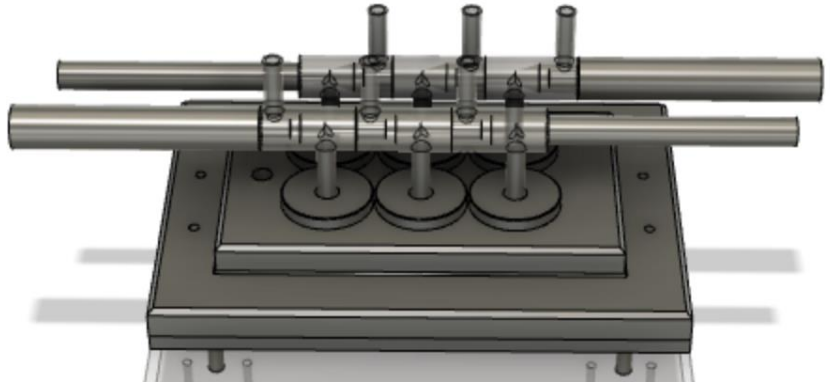
- Computer Aided Design + Computational Fluid Dynamics allow virtual testing of CCES:

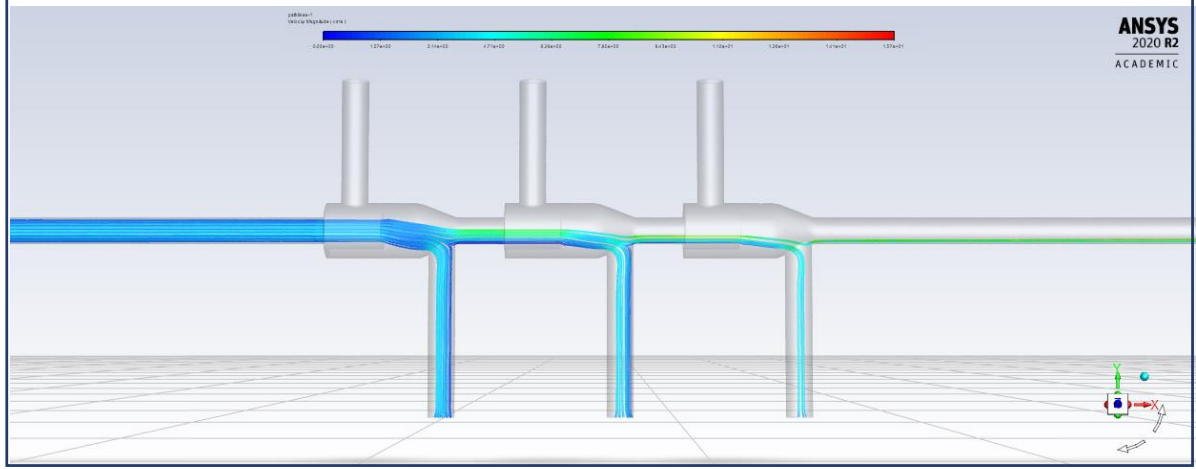
Generation 1: Original VOC Manifold





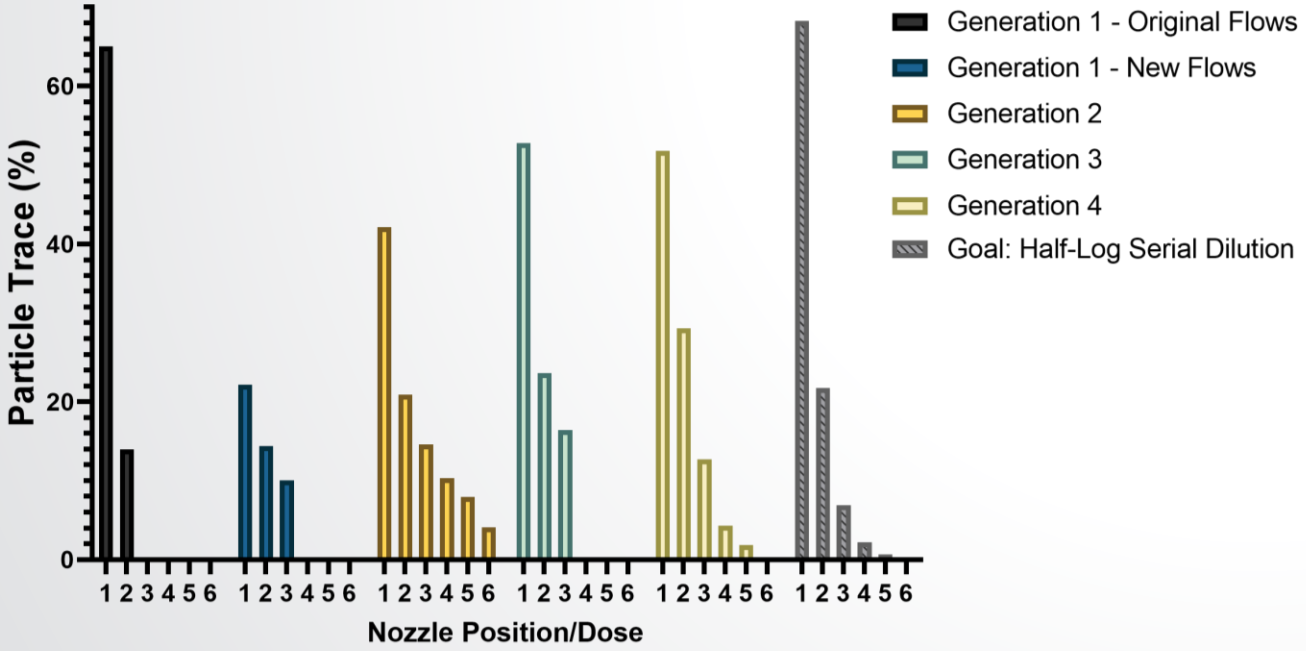
Generation 4: Aerosol Dilution Manifold

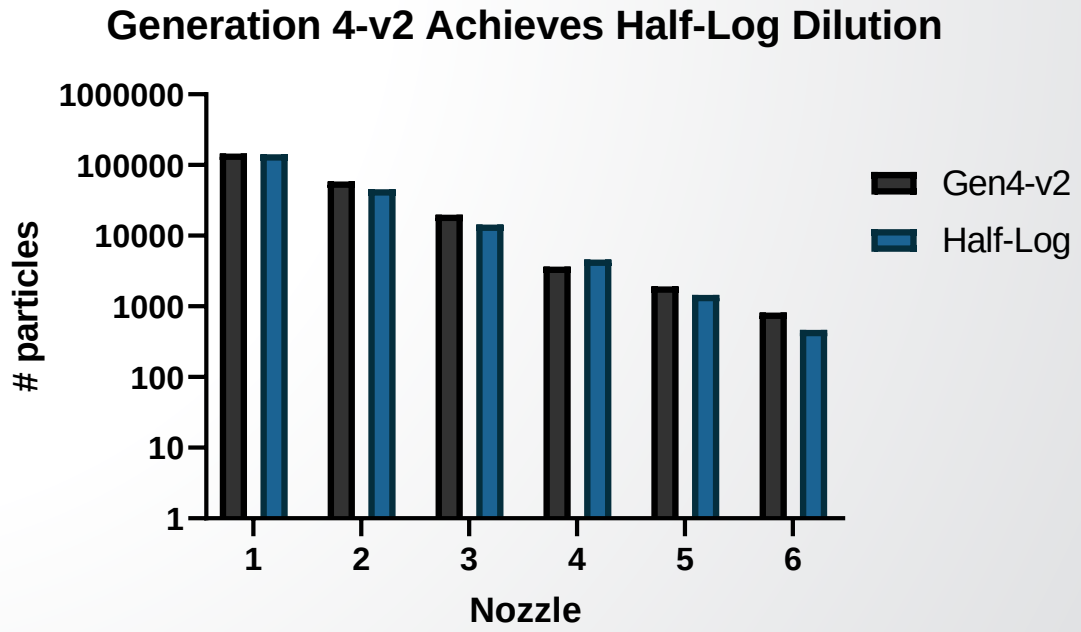




CAD/CFD Increase Testing Efficiency

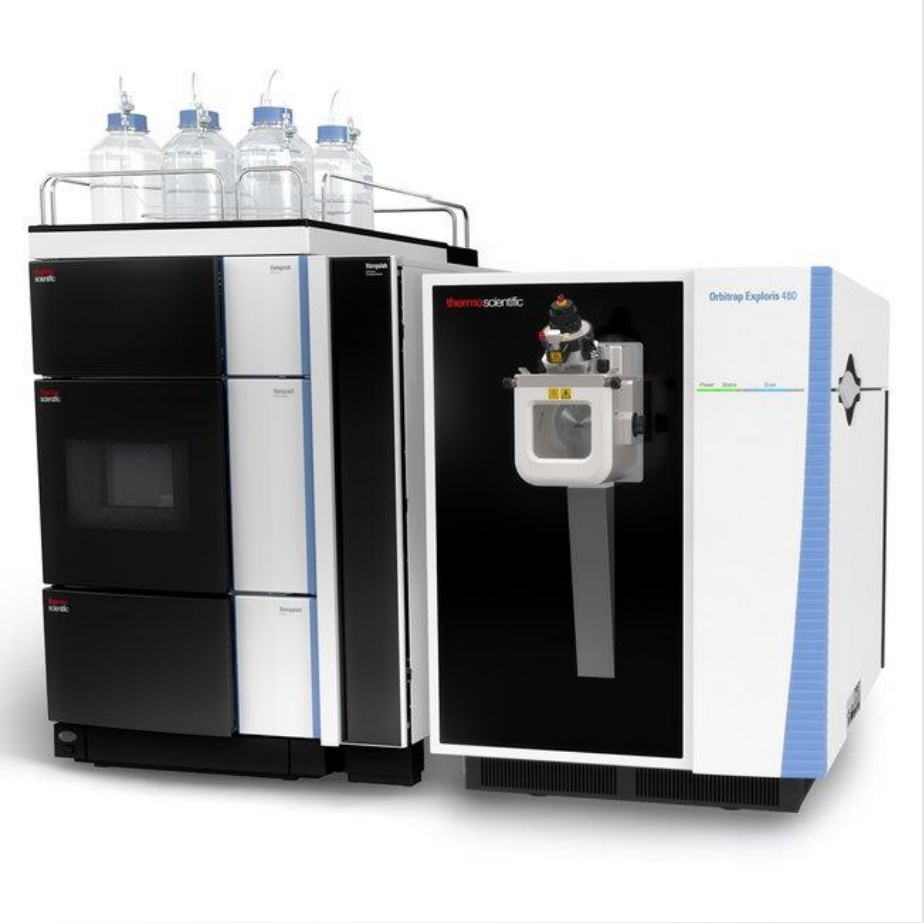
- Time and cost-effective method to develop aerosol-specific dilution and delivery systems
  - 70+ geometry and flow combinations tested in 3 months
- Each aerosol may require CFD testing to optimize flow parameters for each chemical exposure (account for range of particle diameters and densities)





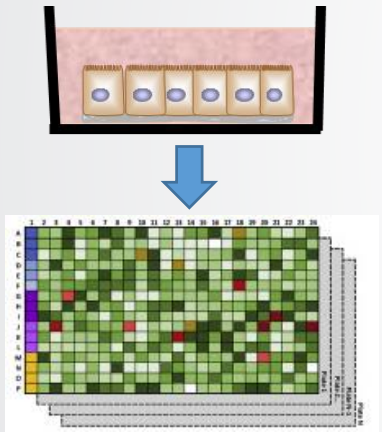
Next Steps: Quantitative Dosimetry

- Inhalation studies currently rely on [C]*t and lack analytical dosimetry
- ITFB received CE-funding for High-Resolution Hybrid Orbitrap Mass Spectrometer
  - Fg-level sensitivity with sub-ppm specificity
- High sensitivity/specificity vital to quantify toxicant deposition, cellular uptake, and biomarkers of internal exposure

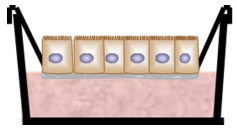


Alignment with Program Office Needs

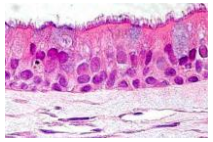
Submerged monoculture



- ALI human airway models with appropriate inhalation exposures aims to reduce our reliance on animal testing
- Strategy will provide missing risk assessment data for VOCs/aerosols that cannot be tested with submerged methods

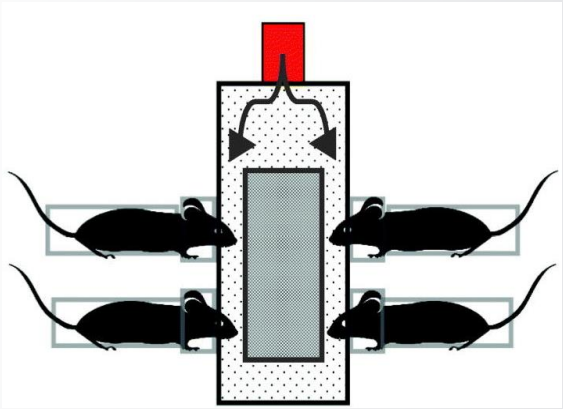


2D Monoculture



3D Organotypic

In vivo Inhalation Studies



High Throughput
Lower Complexity

High Cost
Human Relevance
Low Throughput

Conclusions and Future Research

- Novel exposure approach transects traditional *in vitro* submerged dosing and *in vivo* inhalation exposures
- Support Program Office(s) risk assessors by providing NAMs to directly test chemicals of interest in similar fashion to *in vivo* inhalation exposures
- Provide data from HTTr analysis to be used by ToxCast for SAR, IVIVE, etc
- Aim to develop NAMs for analytical dosimetry in cell cultures to translate to *in vivo* inhalation studies

Acknowledgements

Thank you to ORD, CPHEA, and CCTE collaborators:

Engineers, Toxicologists, and Data Scientists

- Adam Speen
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- Joseph Bundy
- Rusty Thomas
- Maureen Gwinn

Adam Speen, PhD

- HTTr, BMD Analysis

Jessica R. Murray, PhD

- CAD/CFD Modeling, Analytical Dosimetry

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U.S. EPA ORD, CSS Research Program
Board of Scientific Counselors Subcommittee
NAMS Research and Development, Session D: System-specific Models and Approaches
February 2, 2021

Neurovascular Unit Modeling and Blood Brain Barrier Function

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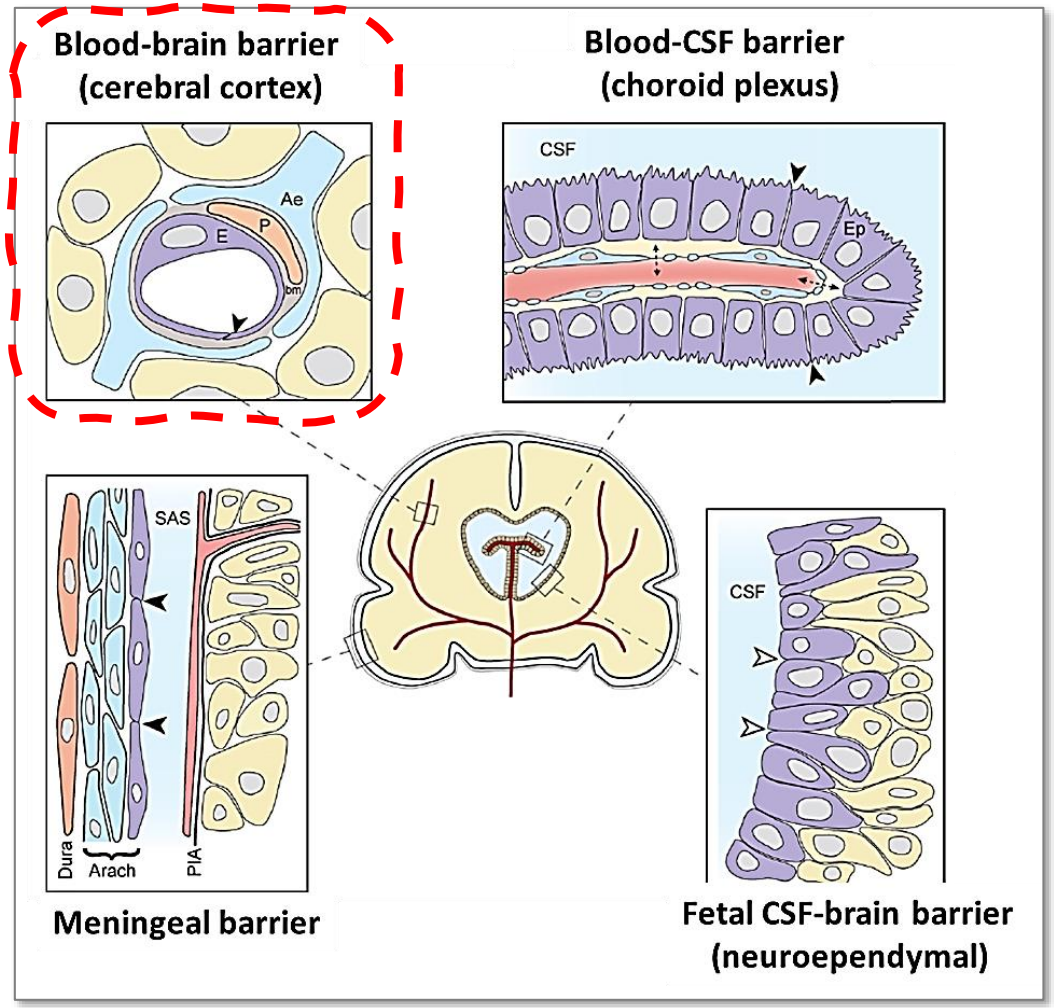
DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

Fetal Brain Barriers

The Neurovascular Unit (NVU) is a relatively recent concept describing the relationship between neuronal and vascular compartments, particularly for two key processes:



Ek et al. 2012. *NeuroToxicology*

- main driver of functional hyperemia, matching local blood supply to neuronal demand via glutamate (stimulates release of vasoactive signals from astrocytes and pericytes).

- development and regulation of the cerebral blood-brain barrier (BBB) that is fundamental as a selective transport barrier to maintain an optimal environment for brain function.

BBB pathophysiology

- Evidence linking BBB dysfunction with prenatal/antenatal pathophysiological states:

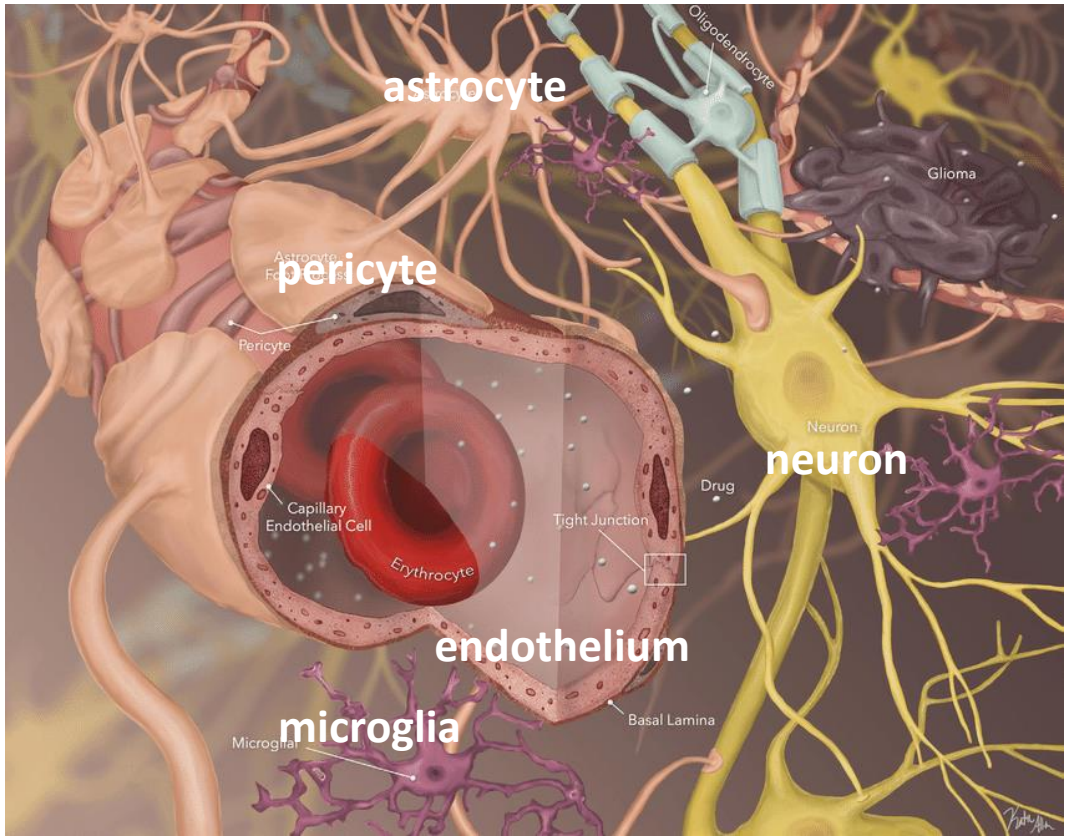
Vogel (2016) Science

  - defective brain transport of leptin (obesity)
  - reduced CNS insulin (baroreceptor deficiency in pregnancy)
  - microglial activation and neuroinflammation (Zika-microcephaly, FIRS)
  - GLUT1 deficiency syndrome (epilepsy, learning disabilities)
  - SL75A (LAT1) dysfunction (autism)
  - SL16A2 (MCT8) deficiency (altered thyroid delivery and neurological impairment)
  - DNT – hypoxia, metal toxicity, pesticide toxicity, …

- OECD Test No. 424: Neurotoxicity Study in Rodents – does not directly evaluate BBB function but can be influenced by a breakdown in the function in the various cell types.

- We know that chemicals interact with the BBB, but to what extent do chemicals of interest disrupt its development and function?

BBB microvasculature: *late fetal to adult lifestages*



Researchgate.net

<u>Endothelial cells</u>: continuous tight junctions, no fenestrations, limited transcytosis.

<u>Pericytes</u>: produce a basement membrane continuous with that produced by the endothelial microvasculature.

<u>Astrocytes</u>: processes (end-feet) interact directly with the basement membrane; appear after formation of the BBB.

<u>Microglia</u>: resident macrophages of the brain, are of hematopoietic origin in the early embryonic yolk sac.

- Microglia orchestrate neurovascular patterning through local signaling; however, when activated they can invoke a local neuroinflammatory response.

BBB phylogeny

DOI: 10.1002/bdr2.1180

REVIEW ARTICLE

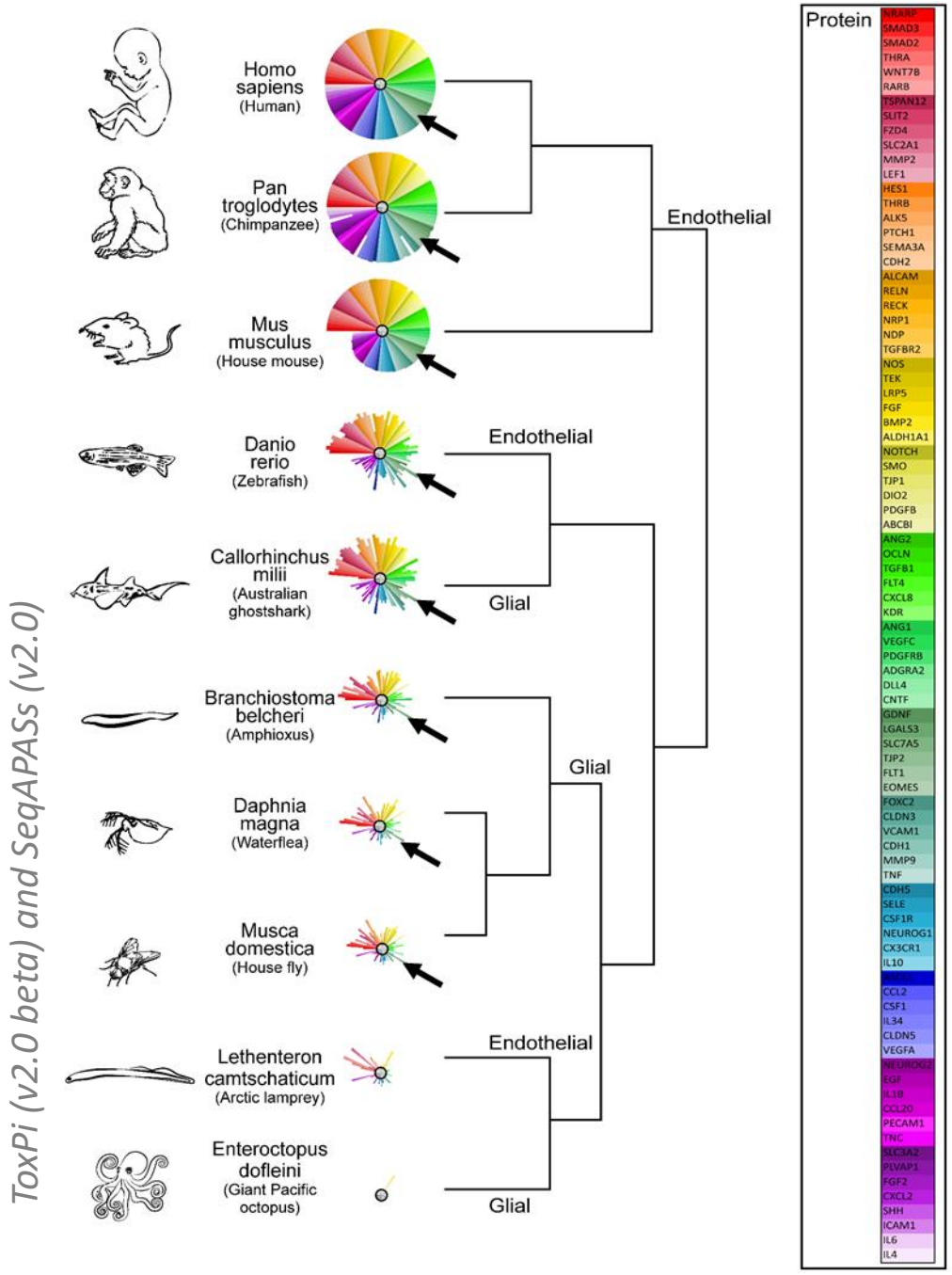
WILEY Birth Defects Research

Blood-brain barrier development: Systems modeling and predictive toxicology

Katerine S. Saili[1] | Todd J. Zurlinden[1] | Andrew J. Schwab[2] | Aymeric Silvin[3] | Nancy C. Baker[4] | E. Sidney Hunter III[2] | Florent Ginhoux[3] | Thomas B. Knudsen[1]

Key BBB transporters are conserved

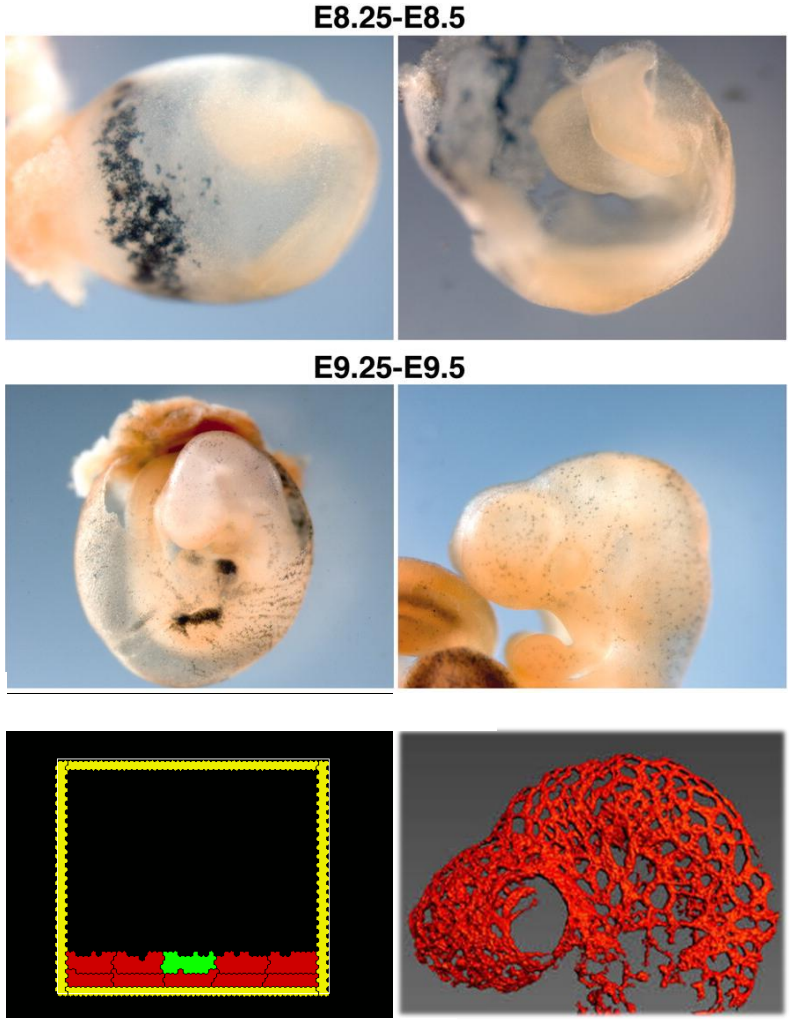
Species	GLUT1	P-gly	SLC7A5
Human	100	100	100
Chimpanzee	99.7	99.5	99.6
House mouse	97.3	87.1	81
Zebrafish	81.3	64.8	77.7
Australian ghostshark	82.7	65.7	72.7
Amphioxus	38	54.5	61.6
Waterflea	46.1	48.5	45.4
House fly	50	41.5	48.5
Arctic lamprey	64.4	---	---
Giant Pacific octopus	---	---	---



86 genes (of >400) play a role in BBB formation

Saili et al. 2017, Birth Defects Res

BBB ontogeny

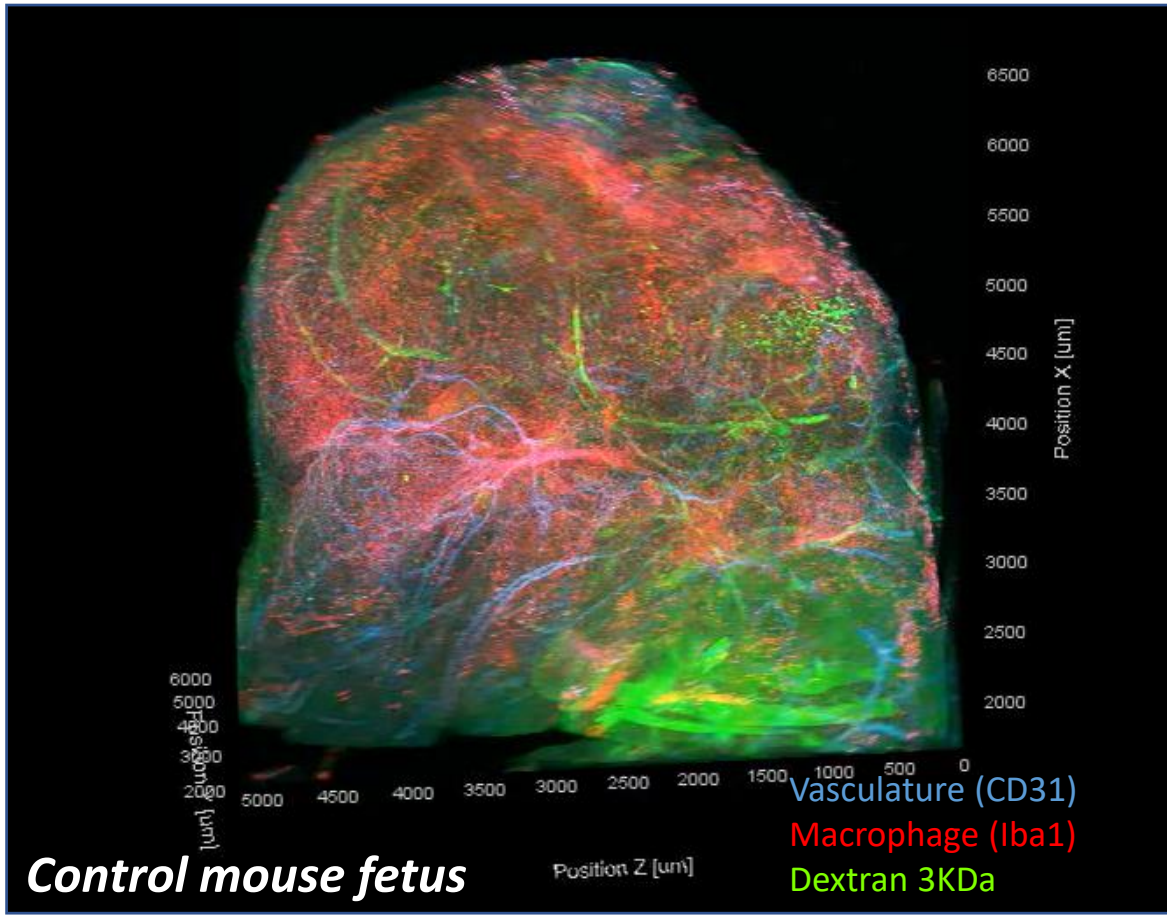


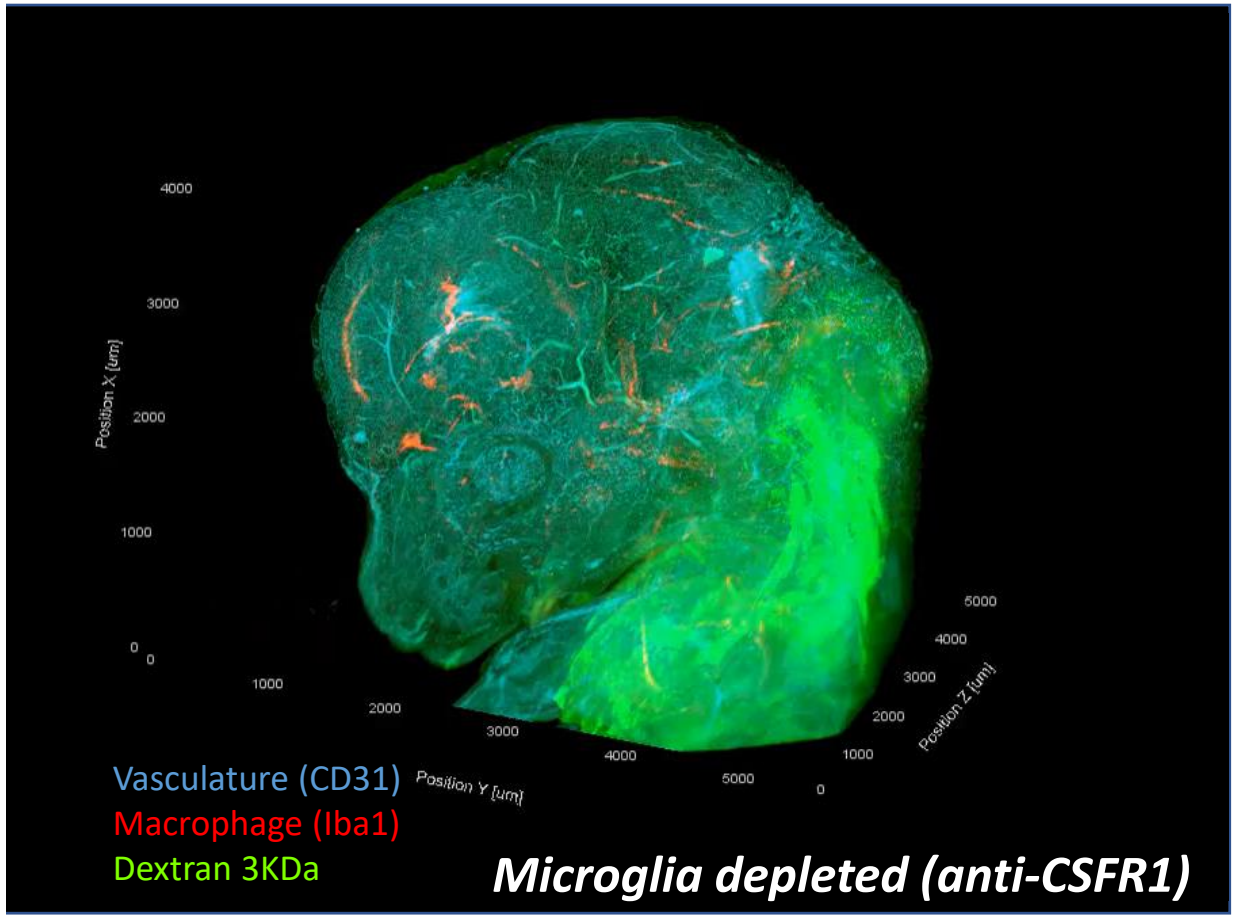
Ginhoux et al 2010, *Science*

- Different components emerge and mature at different stages of prenatal development.
- Commences with angiogenic sprouting from the perineural vascular plexus (PNVP).
- ECs + PCs invade the embryonic neural epithelium on E9-10 (mouse) and GD 26 (human).
- Circulating microglia from the yolk sac colonize the neuroepithelium → resident macrophages of the brain.
- BBB properties (tight junctions, GLUT1) and barrier function (TEER) evident by E11 and increases to birth.

Microglia are required to establish BBB/microvasculature

- promote vascular patterning and BBB barrier development in the embryonic forebrain.

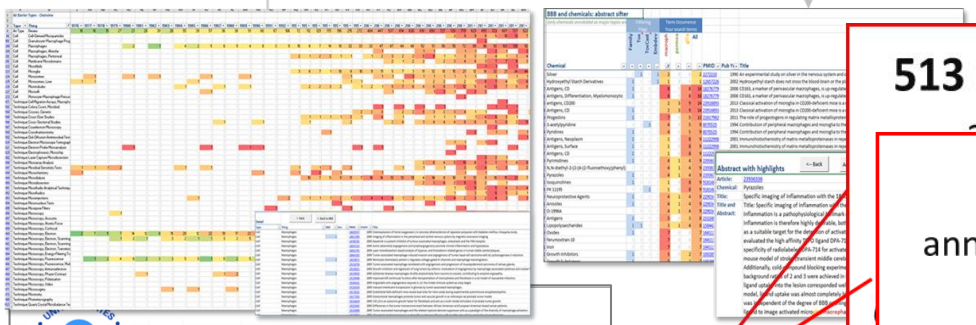




*EPA-A*STAR collaboration with A Silvin, F Ginhoux – A*STAR/SIgN*

Hypothesis: *'microglial sensing' is a key event in BBB developmental toxicity*

BBB Knowledgebase sifter



513 records → main subject (MeSH) on BBB in an article annotated for development and a chemical effect

115 DNT chemicals → main subject (MeSH) in an article annotated for development/embryology AND neurological effects

82 chemicals → 5 with DNT effects on BBB

BPA, 5FU, Pb, paraquat, retinoic acid

Int J Dev Neurosci. 2004 Feb;22(1):31-7.

Mosquito repellent (pyrethroid-based) induced dysfunction of blood-brain barrier permeability in developing brain.

Sinha C[1], Agrawal AK, Islam F, Seth K, Chaturvedi RK, Shukla S, Seth PK.

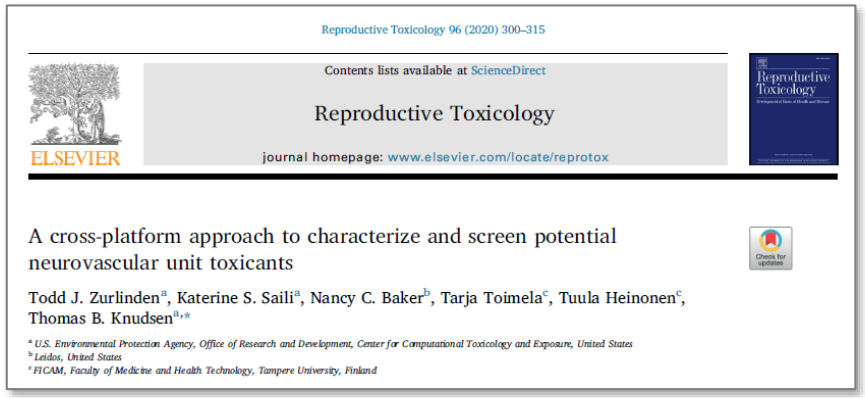
Author information

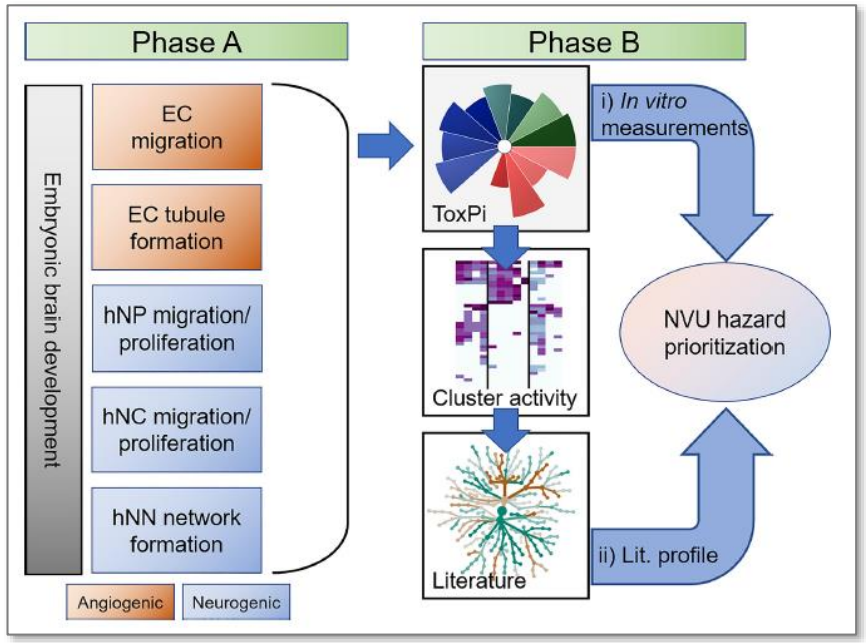
Abstract

Pyrethroid-based mosquito repellents (MR) are commonly used to protect humans against mosquito vector. New born babies and children are often exposed to pyrethroids for long periods by the use of liquid vaporizers. Occupational and experimental studies indicate that pyrethroids can cause clinical, biochemical and neurological changes, and that exposure to pyrethroids during organogenesis and early developmental period is especially harmful. The neurotoxicity caused by MR has aroused concern among public regarding their use. In the present study, the effect of exposure of rat pups during early developmental stages to a pyrethroid-based MR (allethrin, 3.6% w/v, 8h per day through inhalation) on blood-brain barrier (BBB) permeability was investigated. Sodium fluororescein (SF) and Evan's blue (EB) were used as micromolecular and macromolecular tracers, respectively. Exposure during prenatal (gestation days 1-20), postnatal (PND1-30) and perinatal (gestation days 1-20 + PND1-30) periods showed significant increase in the brain uptake index (BUI) of SF by 54% ($P < 0.01$), 70% ($P < 0.01$), 79% ($P < 0.01$), respectively. This increase persisted (68%, $P < 0.01$) even 1 week after withdrawal of exposure (as assessed on PND37). EB did not exhibit significant change in BBB permeability in any of the group. The results suggest that MR inhalation during early prenatal/postnatal/perinatal life may have adverse effects on infants leading to central nervous system (CNS) abnormalities, if a mechanism operates in humans similar to that in rat pups.

development.

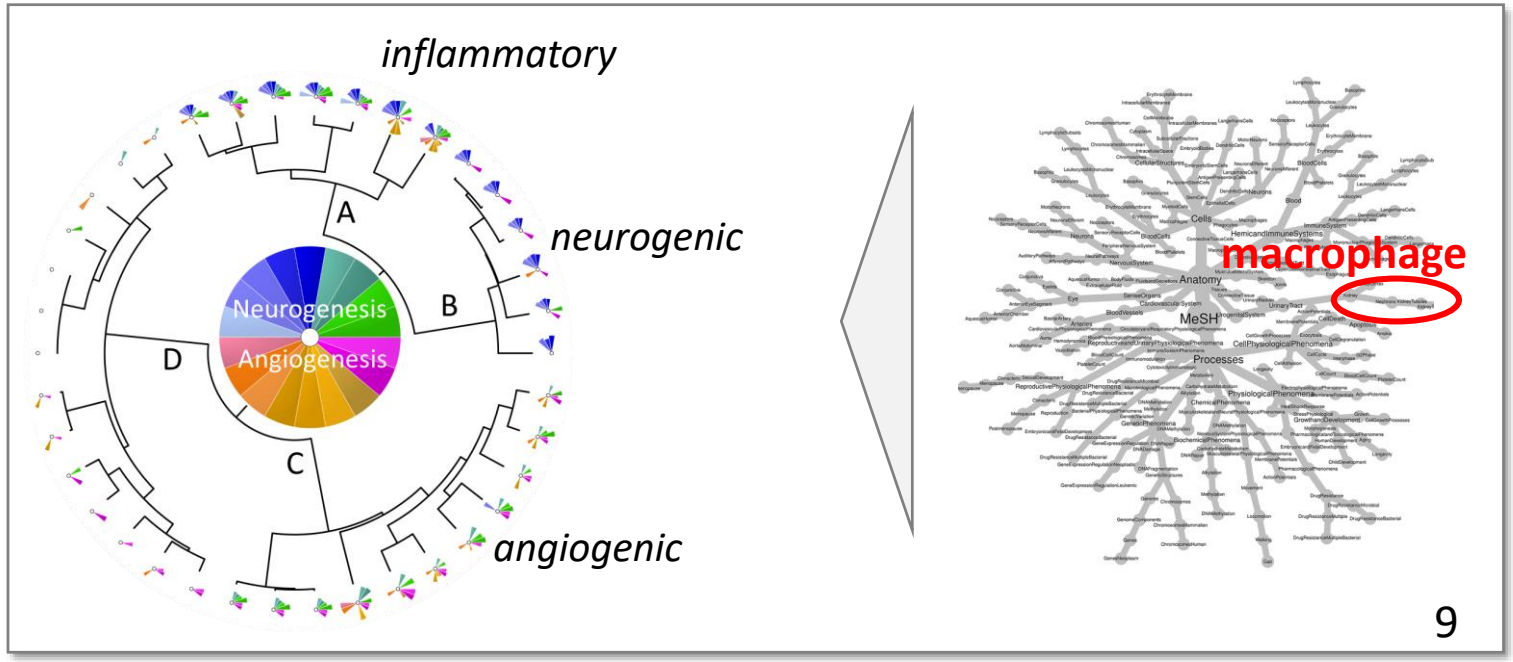
HTS profiling of angiogenic-neurogenic chemical bioactivity





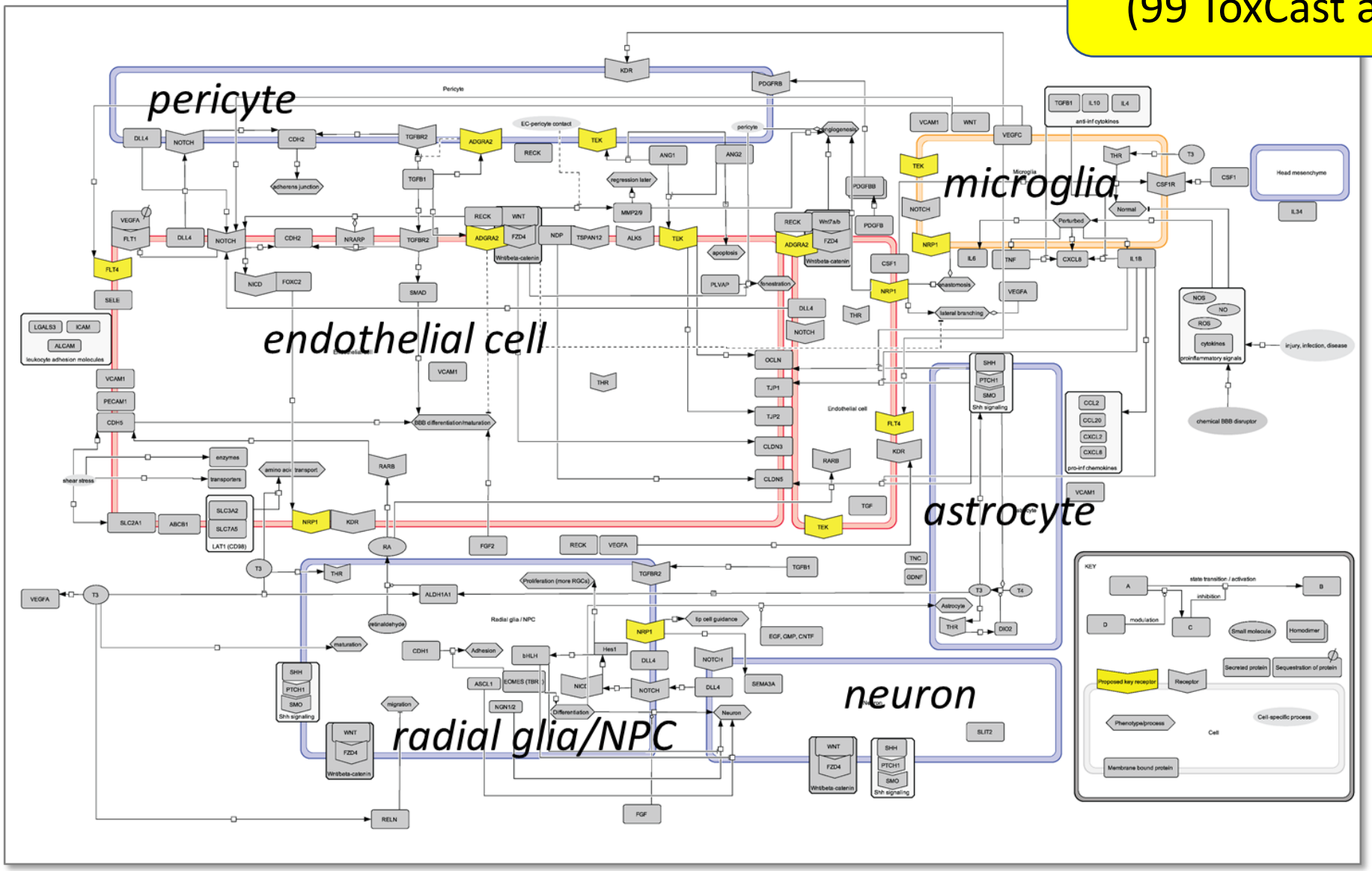
Zurlinden et al. 2020, Reprod Toxicol

- HTS data generated on up to 58 reference chemicals across 18 diverse cell-based angiogenic and neurogenic features.
- ToxPi bioactivity signatures used to train a logistic regression literature model to annotate clusters with PubMed MeSH.
- Chemical-specific pairwise mutual information score predicts NVU developmental hazard potential for advanced modeling.



BBB systems model for predictive toxicology

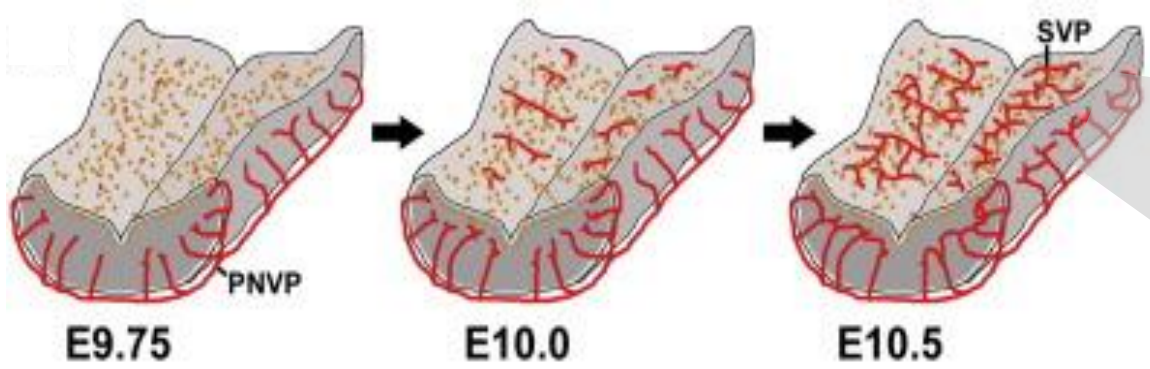
24 molecular targets
(99 ToxCast assays)

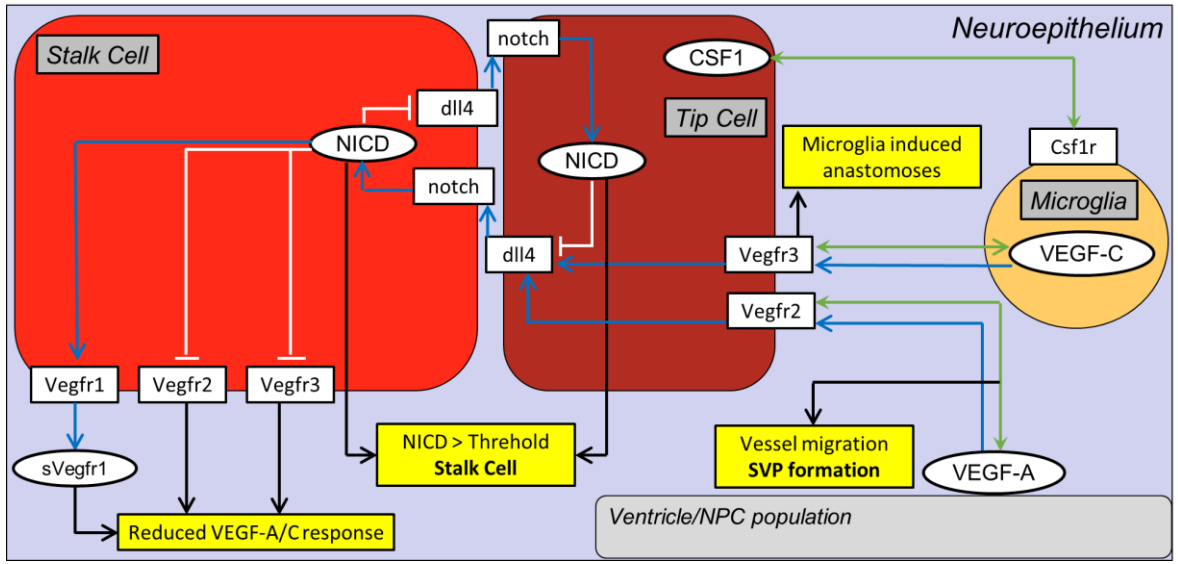


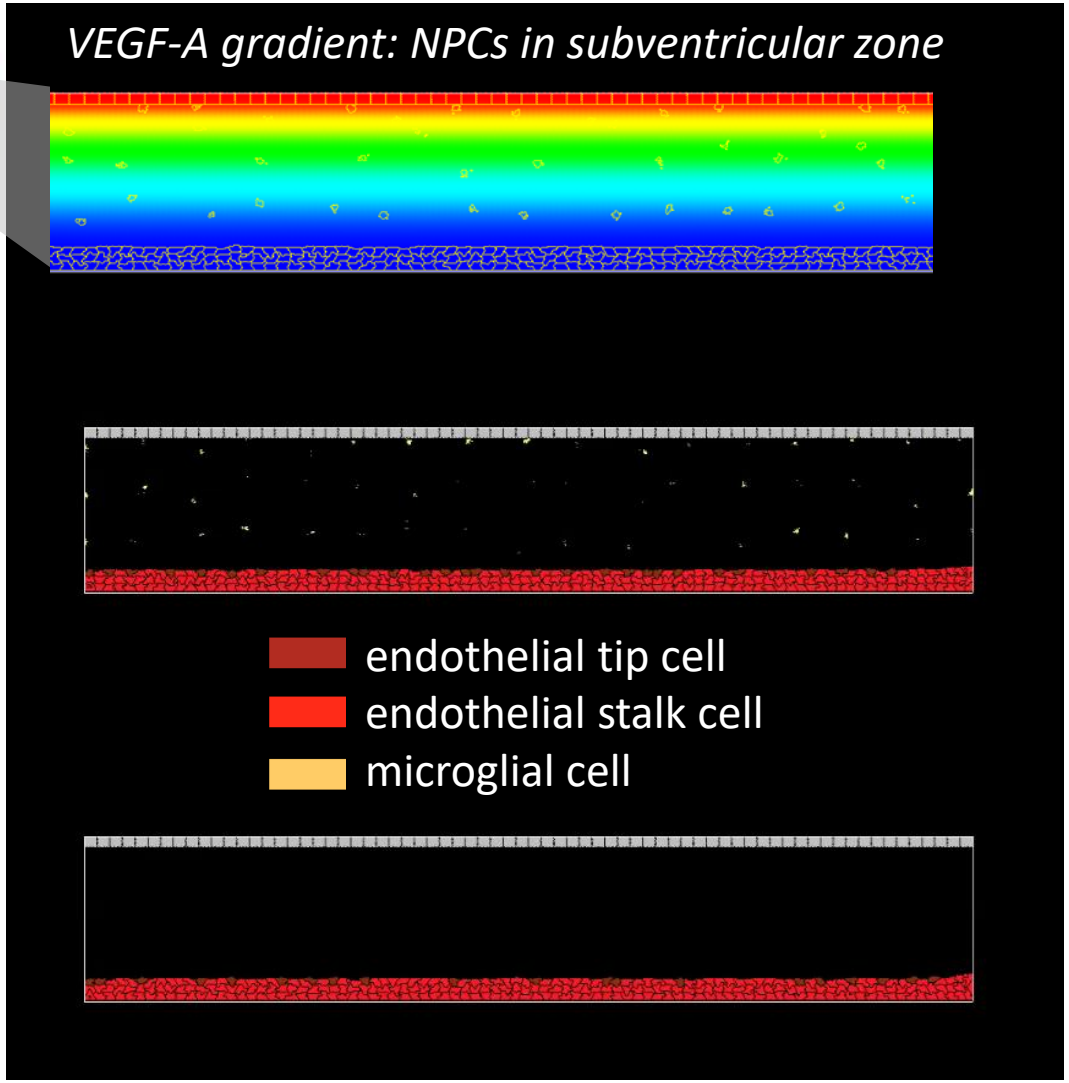
Saili et al. 2017, Birth Defects Res

Advanced Modeling: *neovascularization of the neural tube*

Tata et al. 2015, *Mech Dev*

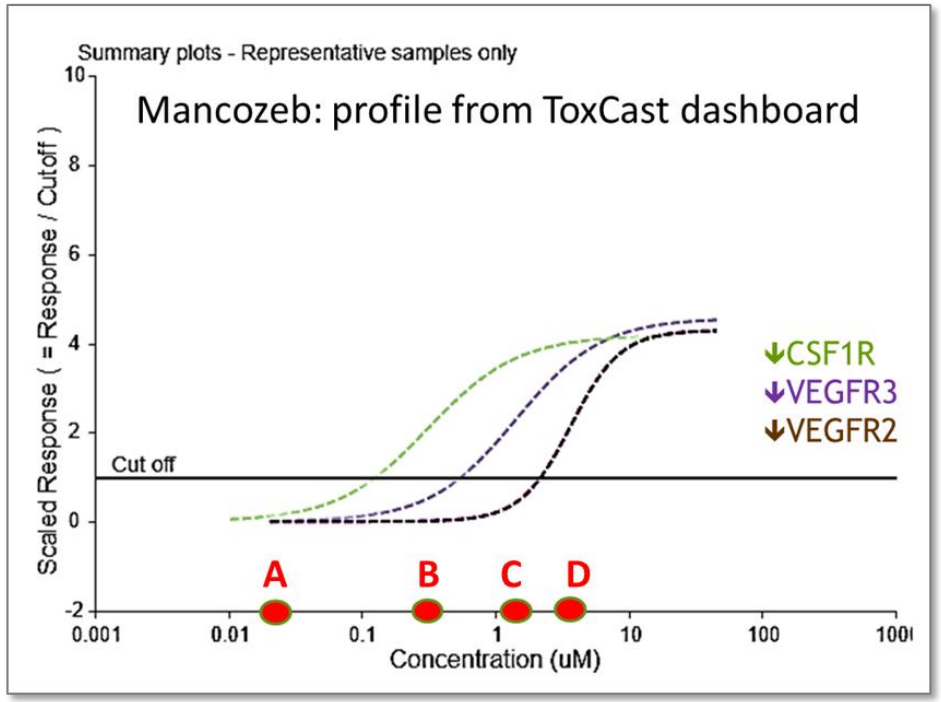


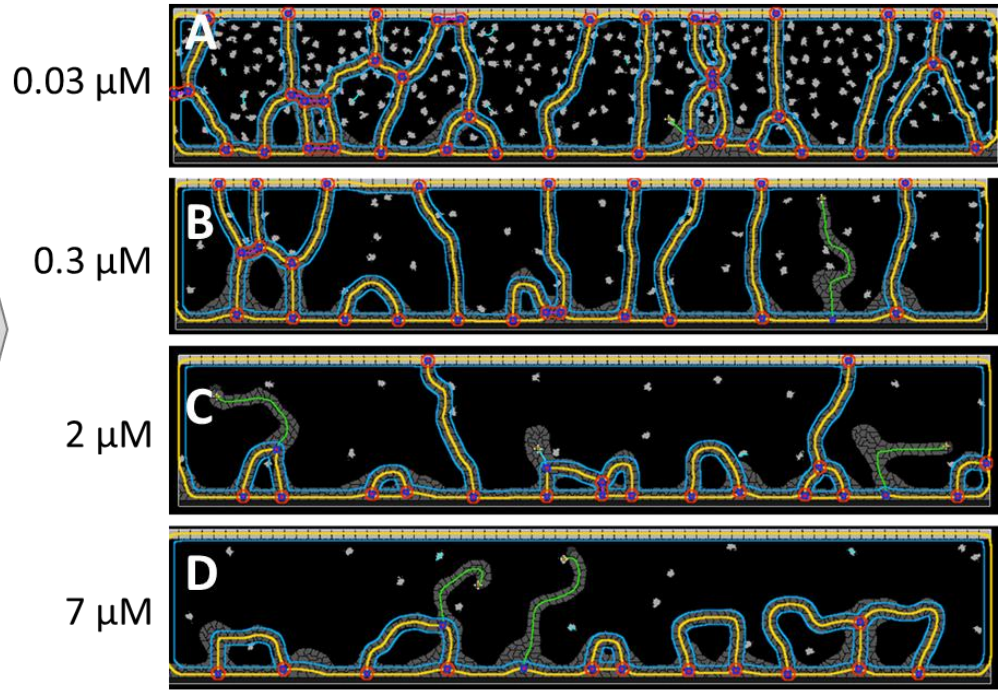


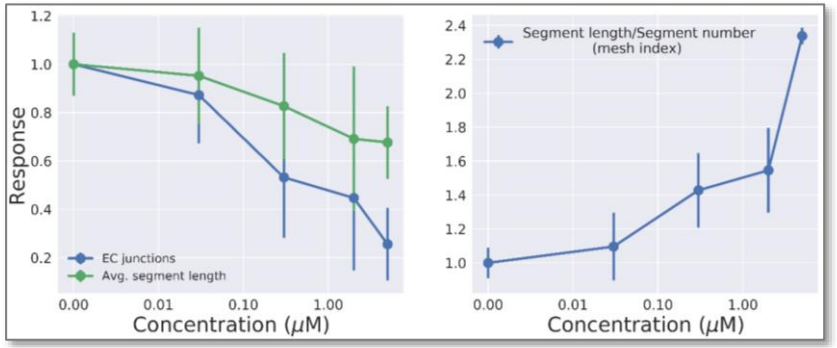


CCTE, work in progress

Executing a simulated concentration-response

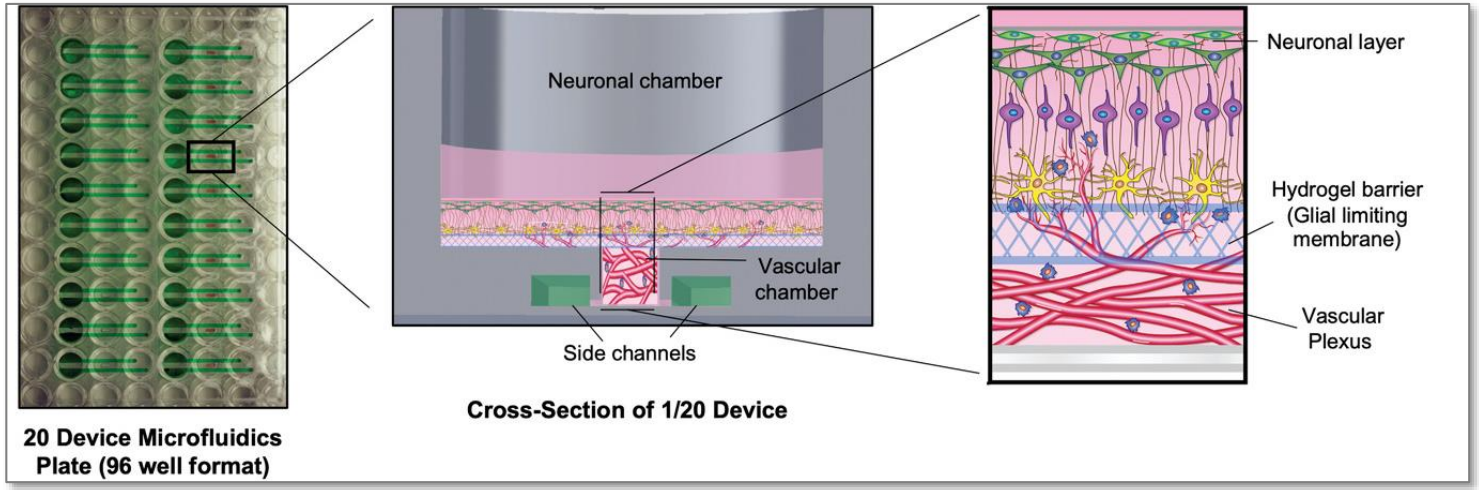






- Progressive bioactivity affects microglial-endothelial interaction (reduced tortuosity → deficiency of SVZ).
- Quantitative microvascular ‘cybermorphs’ predicts an AC50 for Mancozeb disruption at 0.5 µM.

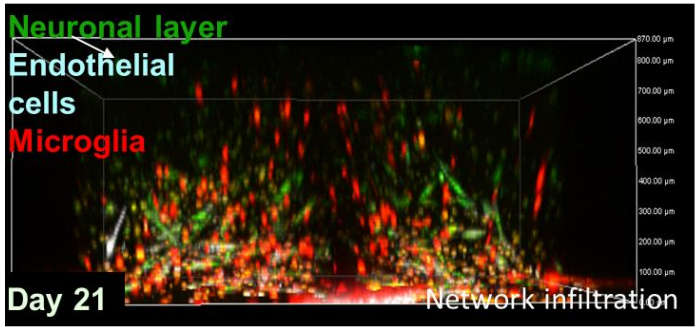
Checking the prediction: *microglial integration in a synthetic microsystem*



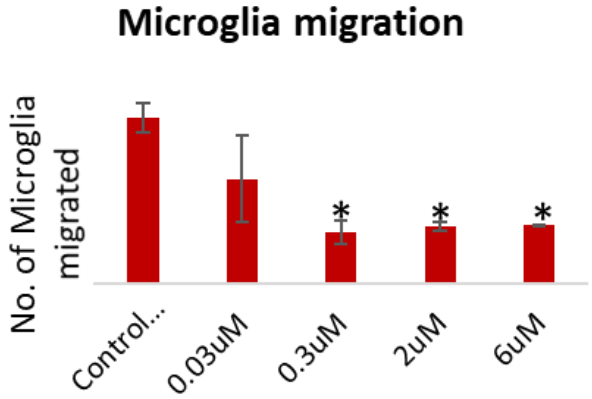
FULL PAPER
www.advhealthmat.de

Engineered Perineural Vascular Plexus for Modeling Developmental Toxicity

Gaurav Kaushik, Kartik Gupta, Victoria Harms, Elizabeth Torr, Jonathan Evans, Hunter J. Johnson, Cheryl Soref, Suehelay Acevedo-Acevedo, Jessica Antosiewicz-Bourget, Daniel Mamott, Peyton Uhl, Brian P. Johnson, Sean P. Palecek, David J. Beebe, James A. Thomson, William T. Daly, and William L. Murphy**



Microglia Migration into Neural Layer

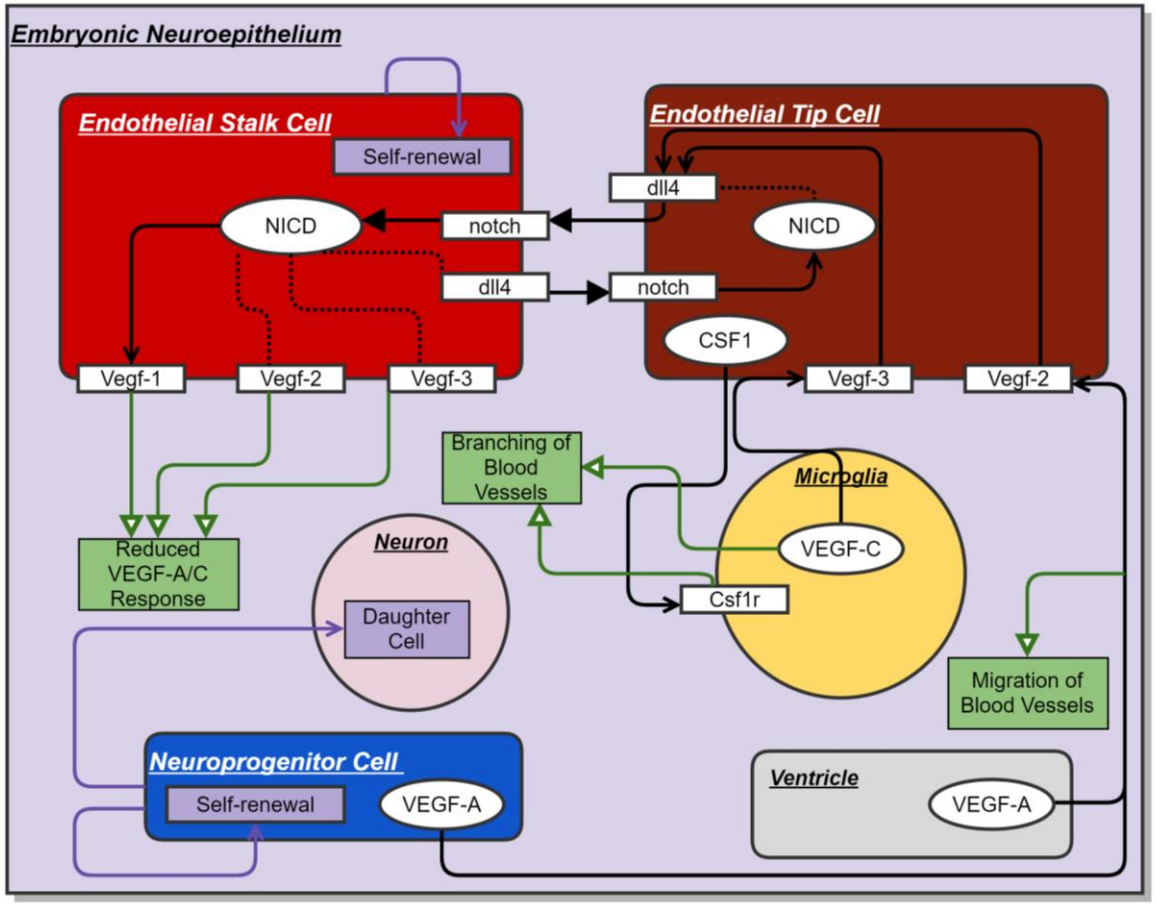


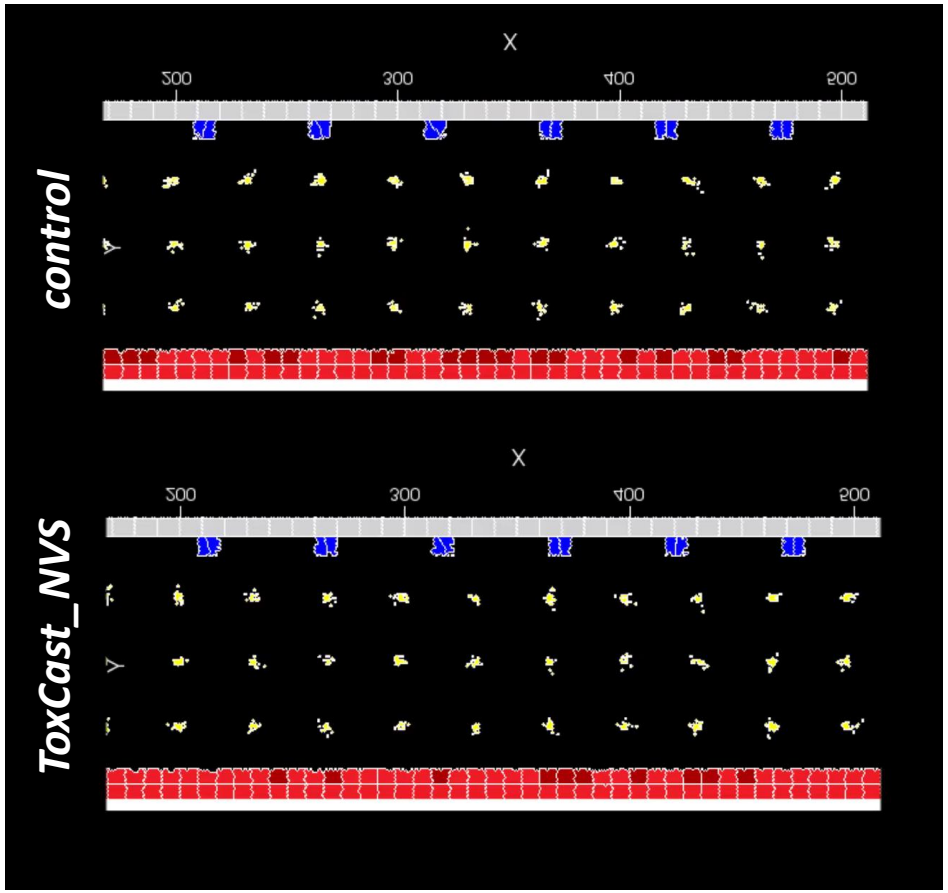
Critical concentration (PoD) for Mancozeb on neural tube vascularization:

- ***predicted*** *by in silico cNVU = 0.5 µM*
- ***observed*** *in organotypic culture = 0.3 µM.*

EPA STAR Center Co-operative grant #835737 , University of Wisconsin (W Murphy)

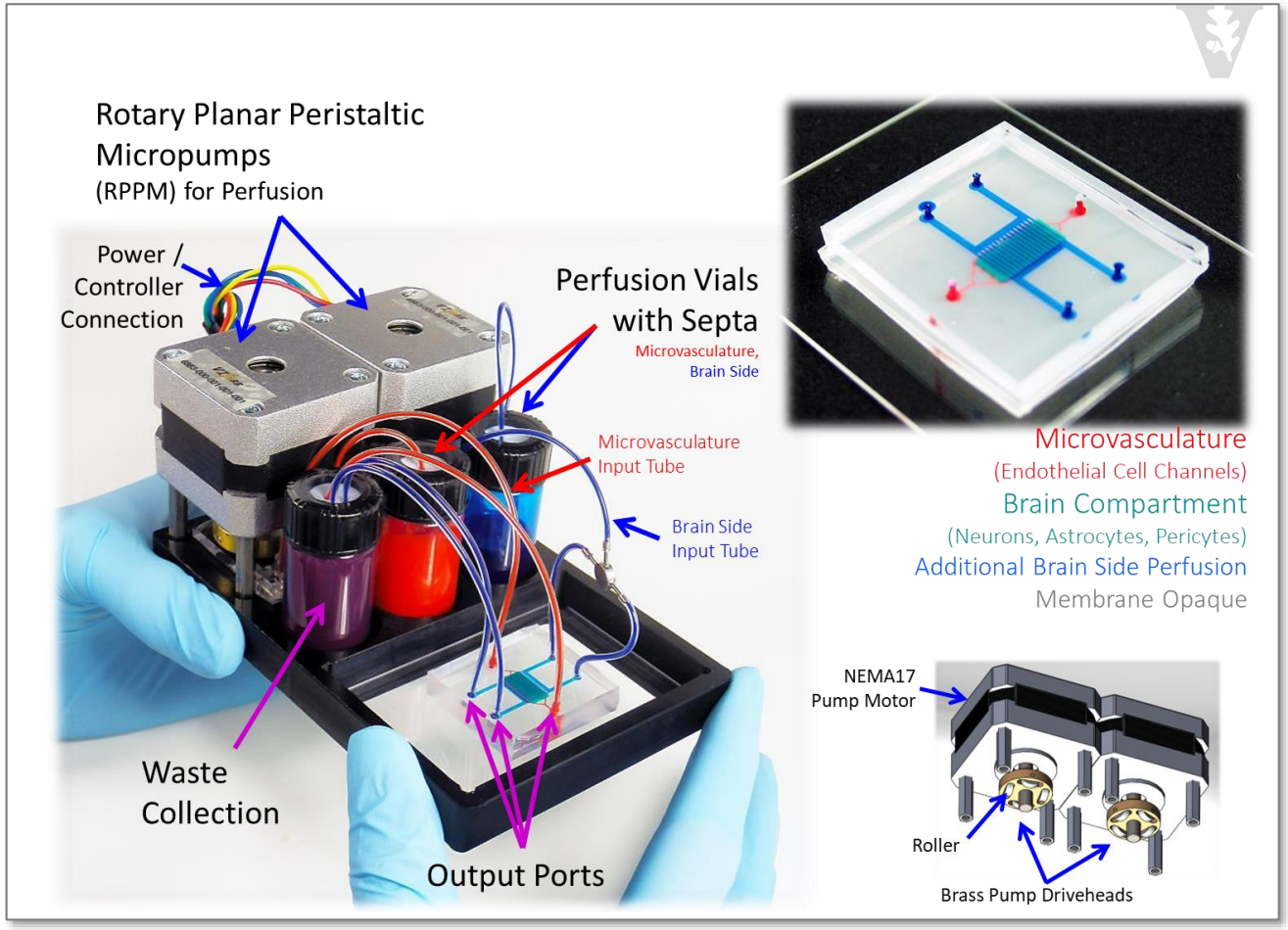
Incorporating the neurogenic domain (preliminary)





O Naphade, work in progress

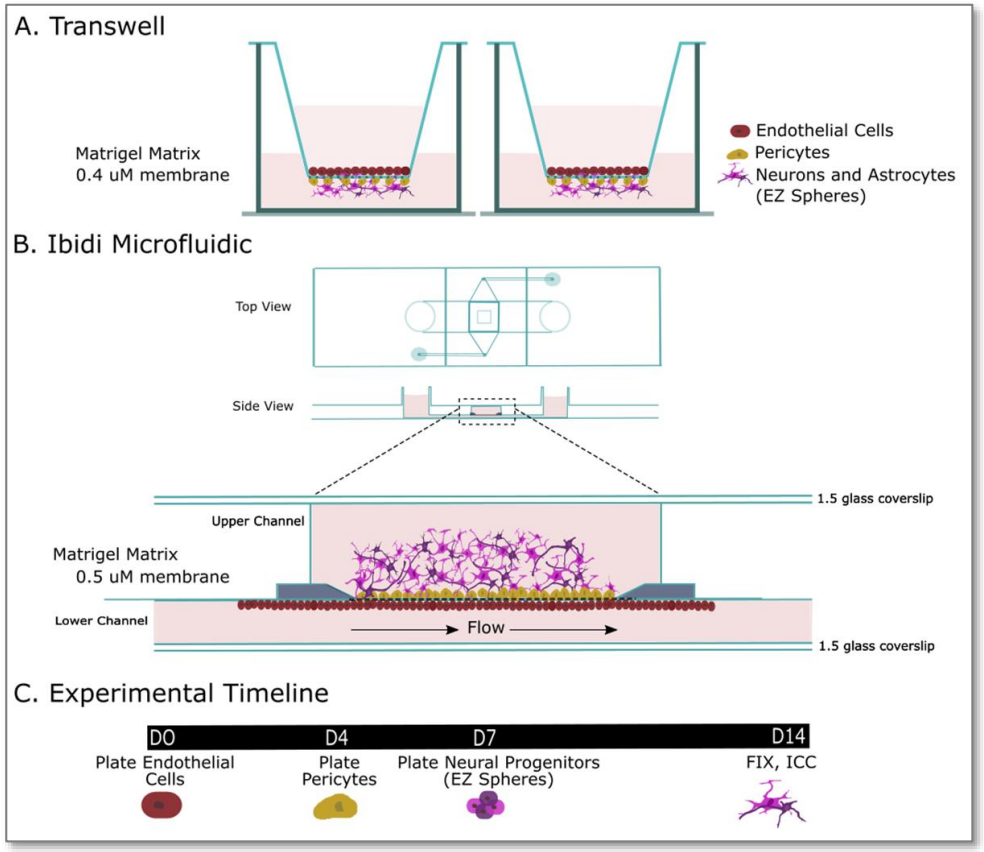
Neurovascular Unit-on-a-Chip Module

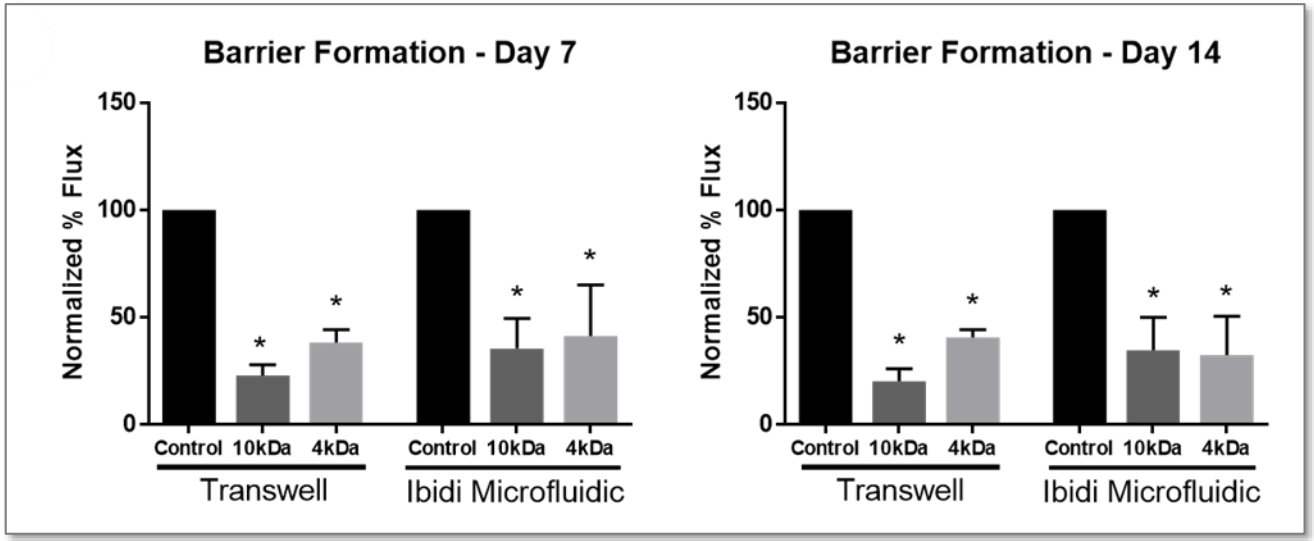


- Human endothelial cells, astrocytes, pericytes, and neurons.
- Microanalyzer for real time data (glucose, lactate, oxygen, pH, and 4 neurotransmitters).
- Testing neuroinflammation (LPS) and neurotoxicity (CPF) pathways.

EPA STAR grant (new), Vanderbilt University, D Cliffel and J Wikswo

Embryonic Human Neurovascular Unit (hNVU): *quantitatively assess the impact of chemical-induced disruption of neural morphogenesis and function.*

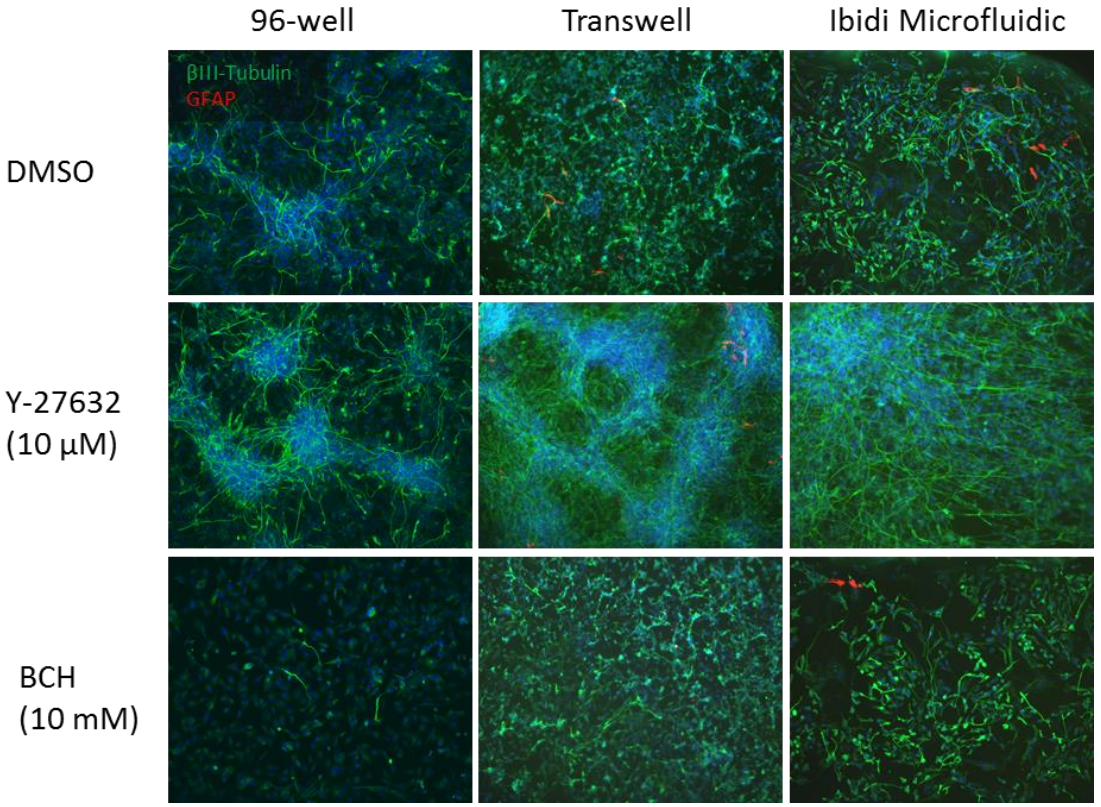


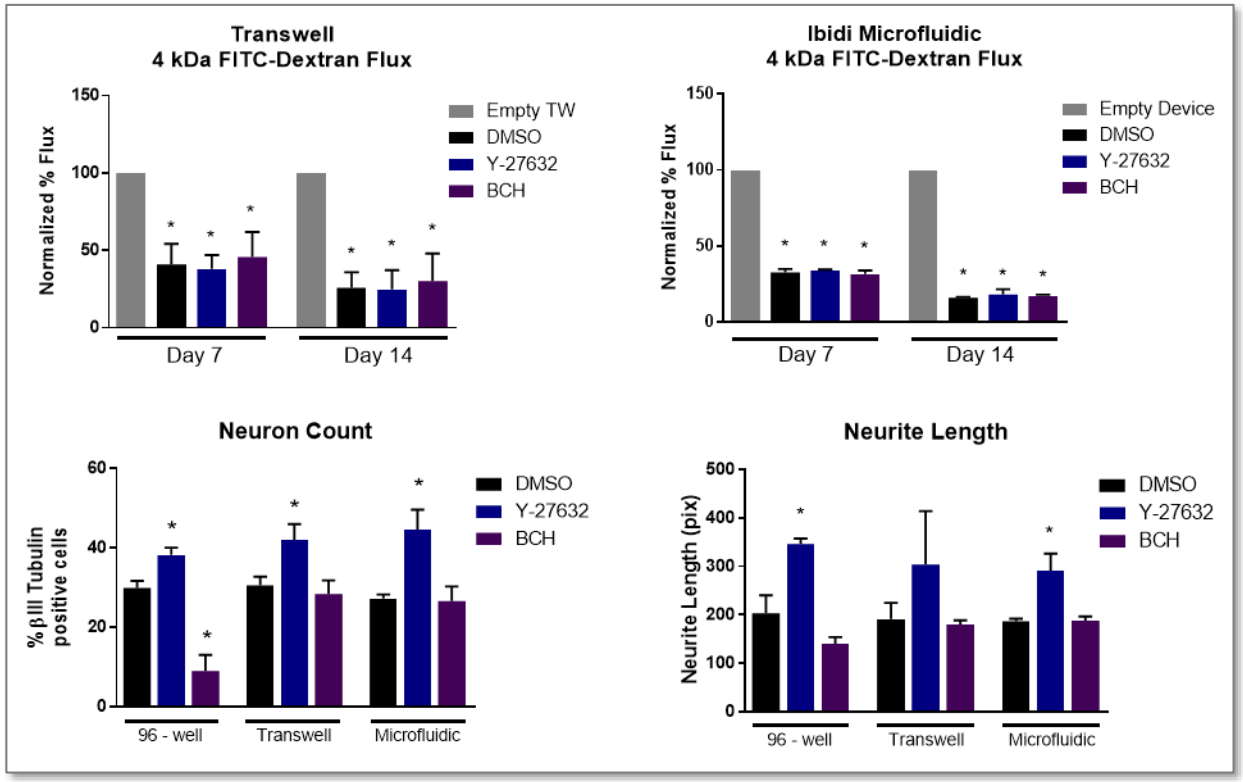


- Impact of the endothelial-pericyte barrier on developmental neurotoxicity,
- Assess chemical effects on barrier function in a human cell-based *in vitro* system(s).

S Hunter, work in progress

Qualification of barrier function in the hNVU

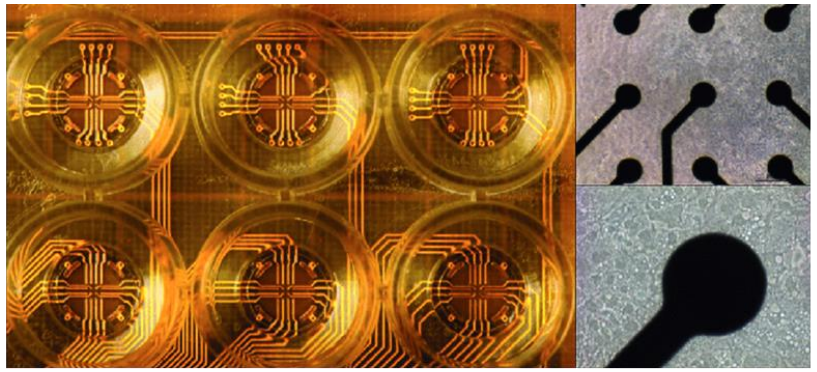


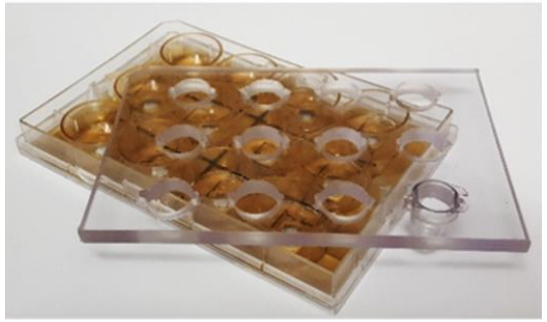


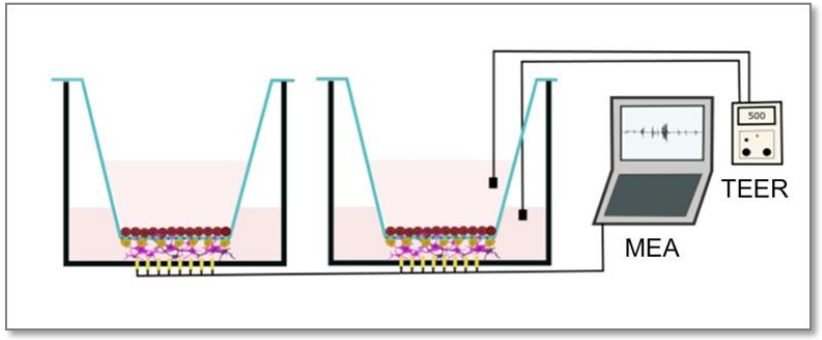
- Y-27632 is a cell permeable rock inhibitor that cross the NVU barrier – increases proliferation and differentiation of human ‘EZ neurosphere’ cells to neural and astrocytic phenotypes (green fluorescence) in all models.

- BCH is a LAT-1 transporter inhibitor that does not cross the barrier; with BCH there are few NPCs cells, little differentiation (bright green neural structure) and almost no red astrocytes. In the MPS devices, proliferation and differentiation are similar to control cultures.

Embryo-fetal NVU Barrier: *application to developmental neurotoxicity*







- Microelectrode array (MEA) assay platform developed in Tim Shafer's lab.
- Monitors rat cortical neuronal network formation and electrochemical activity.
- Used to profile ToxCast chemicals for direct effects on neuronal networks.
- Rat cortical MEA system has been integrated with the transwell hNVU.

T Shafer, S Hunter - work in progress

Summary

- NVU composed of multiple cells types and >400 genes, at least 86 of which play important roles in BBB development and function.
- BBB becomes functional soon after it forms during organogenesis (6-14 weeks in human gestation).
- Development and function is perturbed by multiple pathophysiological conditions and may underlie neurodevelopmental disorders linked to chemical exposure.
- Dynamics of the system modeled *in silico* and *in vivo* focusing on microglial sensing as potential roles in neurodevelopmental toxicity linked to their activation.

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