Evaluation of in vitro air-liquid interface (ALI) airway culture and exposure system technologies in the DTT: A proof-of-concept study with 2,3-pentanedione

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ICCVAM Public Forum (May 20, 2024)

National Institutes of Health • U.S. Department of Health and Human Services

National Institute of Environmental Health Sciences
Division of Translational Toxicology

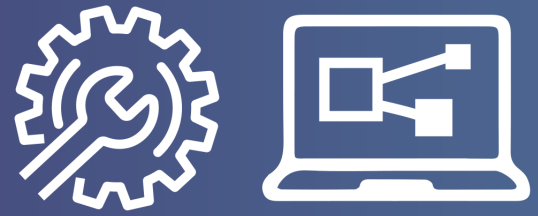
DTT Strategic Areas of Focus

Exposure-based Research Programs

Health Effects Innovation Programs

Responsive Research Programs

Strengthening Capabilities Programs



Collaboratively address public health challenges

Generate trusted scientific information to support decision-making

Develop and apply innovative tools and strategies

National Institute of Environmental Health Sciences
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DTT Strategic Areas of Focus (10 Programs)

Exposure-Based Research Programs

- Combined Exposures and Mixtures
- Consumer Products and Therapeutics
- Occupational and Inhalation Exposures

Responsive Research Programs

- Emerging Contaminants and Issues of Concern
- Safe and Sustainable Alternatives

Collaboratively address public health challenges

Generate trusted scientific information to support decision-making

Develop and apply innovative tools and strategies

Health Effects Innovation Programs

- Carcinogenicity
- Cardiovascular
- Developmental Neurotoxicity

Strengthening Capabilities Programs

- Novel Tools and Approaches
- Scientific Cyberinfrastructure

National Institute of Environmental Health Sciences
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OIE Program Management Team (PMT)

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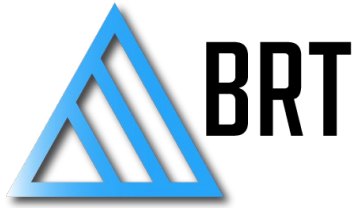
OIE Program Partners & Stakeholders



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OIE Program Objectives

Hazard characterization of inhalation exposures is critical to creating a safe living/working environment and reducing disease burden.

1 **Assess Health Hazards of Airborne or Occupational Substances**

2 **Expand Capabilities for Predicting Adverse Health Effects**

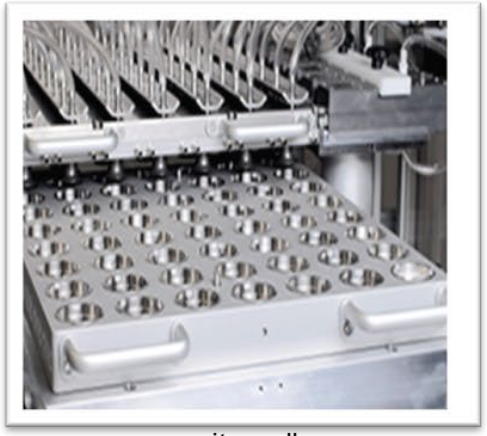
3 **Enhance the Translational Relevance of Experimental Models**

OIE Program Objective 2

2 The OIE Program aims to expand capabilities for predicting adverse health effects

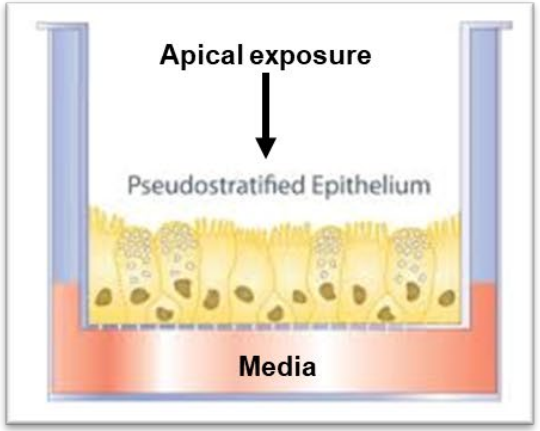
- Currently evaluating novel/alternative technologies (i.e., in vitro airway models and lung microphysiological systems) to investigate human-relevant inhalation (respiratory) toxicity

Air-liquid interface (ALI) airway cultures



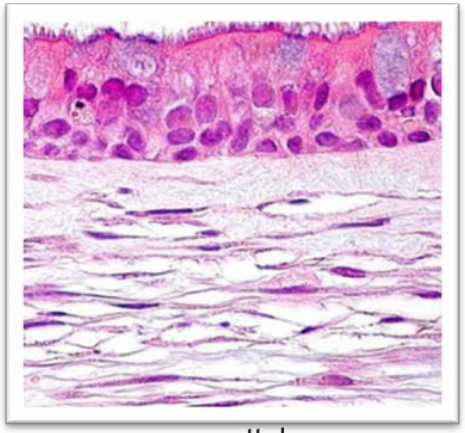
www.vitrocell.com

Exposure to vapors, gases, aerosols, or particles (↑ doses and throughput)



www.atcc.org

Human-derived primary airway cells from tracheobronchial, bronchial, or alveolar compartment (testing of multiple donors and rodent cells also possible)



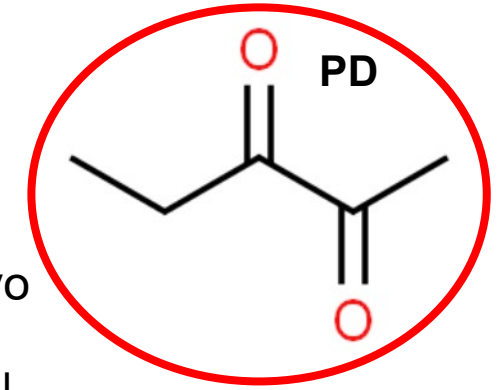
www.mattek.com

Replicates cell types and architecture of the human airways (and rodents in vivo)

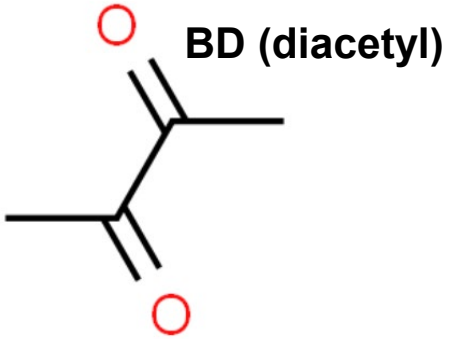
Proof-of-concept study testing 2,3-pentanedione (ALI model)

BACKGROUND

- 2,3-pentandione (PD) is a major and highly volatile component of artificial butter flavorings
  - Replacement for 2,3-butanedione (BD) which causes obliterative bronchiolitis (OB) (bronchiolitis obliterans) in exposed-workers ("Popcorn Lung")



- PD and BD are structurally similar (α-diketones) and exhibit similar inhalation toxicity in vivo
  - Inhalation exposure to vapors causes airway toxicity in rats and mice including adverse bronchial and bronchiolar effects (OB-like fibrotic lesions in rats) [e.g., Morgan et al. 2016 and TOX-98]



- There are in vitro human ALI airway toxicity data for BD (using tracheobronchial epithelial (TBE) cell-derived cultures) to help elucidate the mechanisms of airway injury and fibrosis [e.g., Gwinn et al. 2017 and McGraw et al. 2020]
  - Very limited data for PD (only one published study – Zaccone et al. 2015)
  - Would expect similar in vitro toxicological effects for PD

Proof-of-concept study testing 2,3-pentanedione (ALI model)

PROJECT-SPECIFIC OBJECTIVES

1. Characterization/optimization of a VITROCELL 48 2.0 Plus exposure system

   - Method development/validation (perform initial testing of the exposure system set-up and vapor generation with PD)

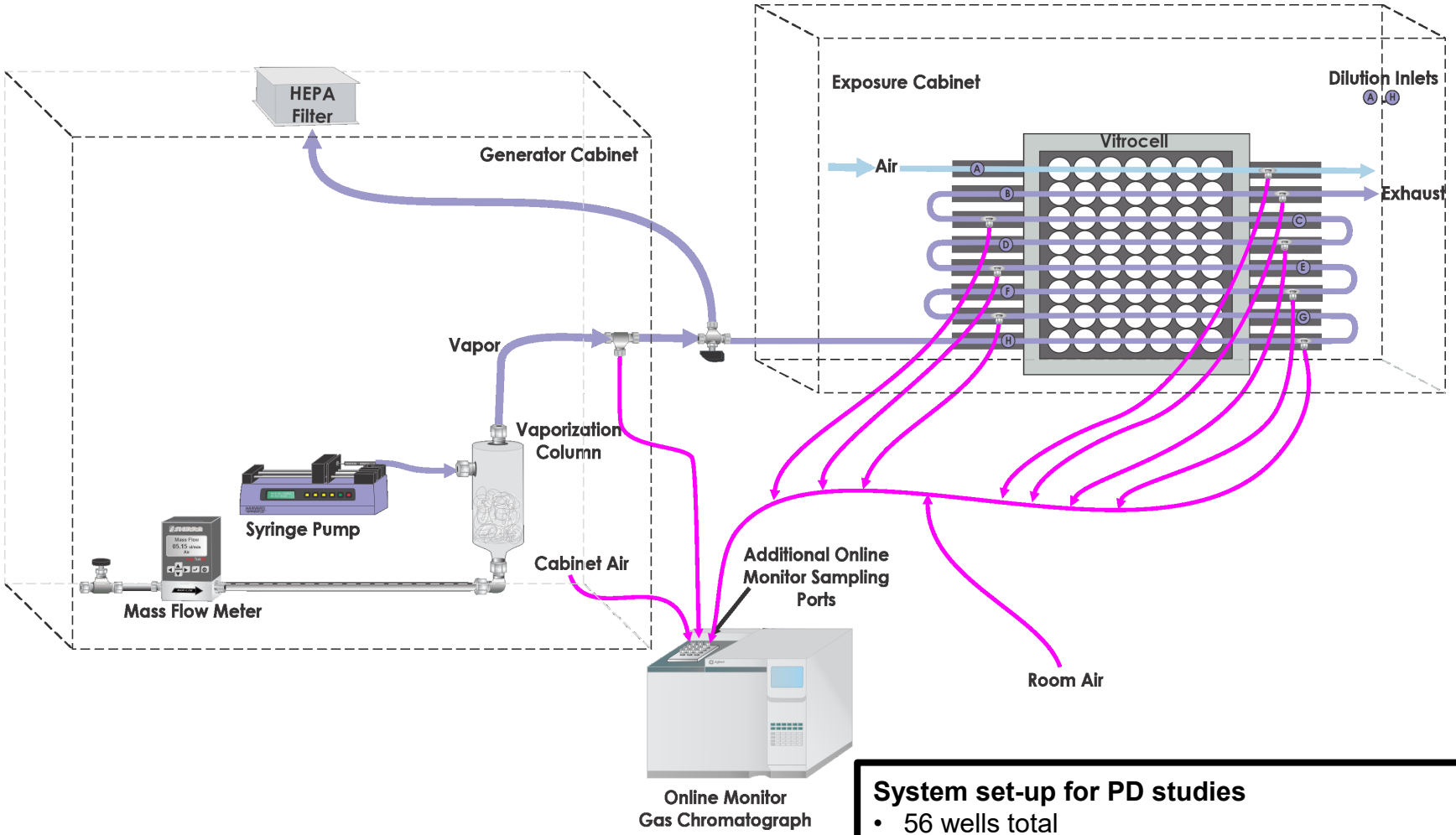
2. Expose human and rat ALI cultures (TBE cell-derived) to evaluate PD-induced airway toxicity in vitro (and between species)

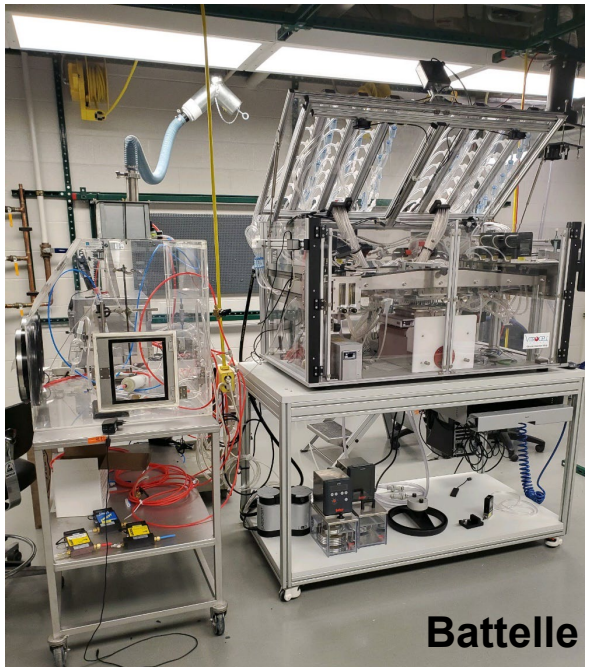
   - **Compare rat in vivo – rat in vitro – human in vitro**
   - Feasibility study (troubleshooting and optimization)
   - Definitive study

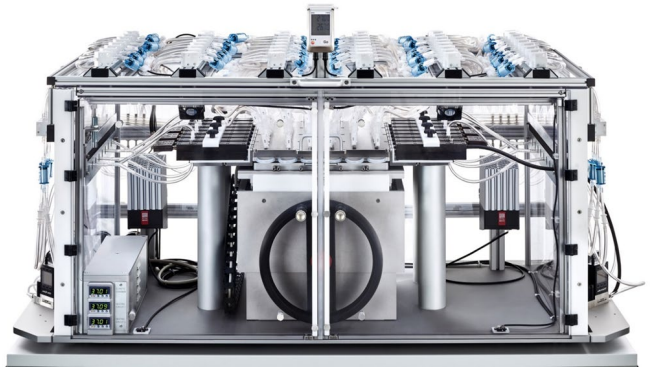
Rationale for test article selection:

- PD has been well-characterized in vivo and is relatively “straightforward” to work with from a chemistry perspective (generation of stable vapor atmospheres).
- There are currently very little in vitro human ALI airway toxicity data for PD, but there have been multiple studies conducted with BD (can use as a “guide” for study design and anticipated findings).

VITROCELL 48 2.0 Plus exposure system







System set-up for PD studies

- 56 wells total

(8 concentrations x 7 replicates per concentration)

- Dilution manifold can be used for dose spacing

PD definitive study

Pre-Exposure Sample Collection

Exposure Start

6-hr Exposure

Timepoint1: Post-Exposure Sample Collection (0 hr)

Timepoint 2: Post-Exposure Sample Collection (18 hr)

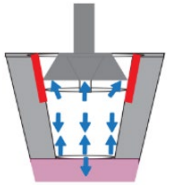
0, 40, 70, 100, 130, 160, 200, or 240* ppm PD

Apical rinse and basolateral culture media samples and tissues (including lysates)

Human (day 22) and rat (day 16) ALI airway cultures

- MatTek (EpiAirway)
- Single donor
- Wistar strain

Normal TBE cell-derived



***Excessive cytotoxicity/tissue destruction were observed at higher exposure concentrations (>240 ppm) in the feasibility study.**

End-points

- External (apical and media samples) and internal (tissue lysates) PD concentrations by gas chromatography-mass spectrometry (GC-MS)
- Airway injury/cytotoxicity/cell viability (TEER*; LDH and AK assays)
- Airway injury/histopathology (H&E and periodic acid-Schiff (PAS) staining)
- Secreted biomarkers (multiplex assays including IL-8 and AREG)
- IHC (e.g., ubiquitin and E-cadherin)

*TEER also measured pre-exposure

Toxicity end-points selected are relevant to in vivo rat (BD and PD) and in vitro human ALI (BD) airway findings and AOP 280.

Use toxicity end-points to model Key Events in AOP

[AOP 280: "α-Diketone-Induced Bronchiolitis Obliterans"]

https://aopwiki.org/aops/280

MIE 1584: Interaction of α-diketones with arginine residues

→ **AO 1588:** Bronchiolitis obliterans

KE 1585: Proteasomal dysfunction
Ubiquitin immunohistochemistry (IHC)

KE 1586: Airway epithelial injury
TEER and cytotoxicity/cell viability assays, histopathology

KE 149: Increase, inflammation
Multiplex assays for biomarkers

Limitation: No recruited inflammatory/immune cells in standard ALI airway cultures

KE 1457: Increase, Epithelial Mesenchymal Transition
E-cadherin IHC

KE 1587: Fibroproliferative airway lesions

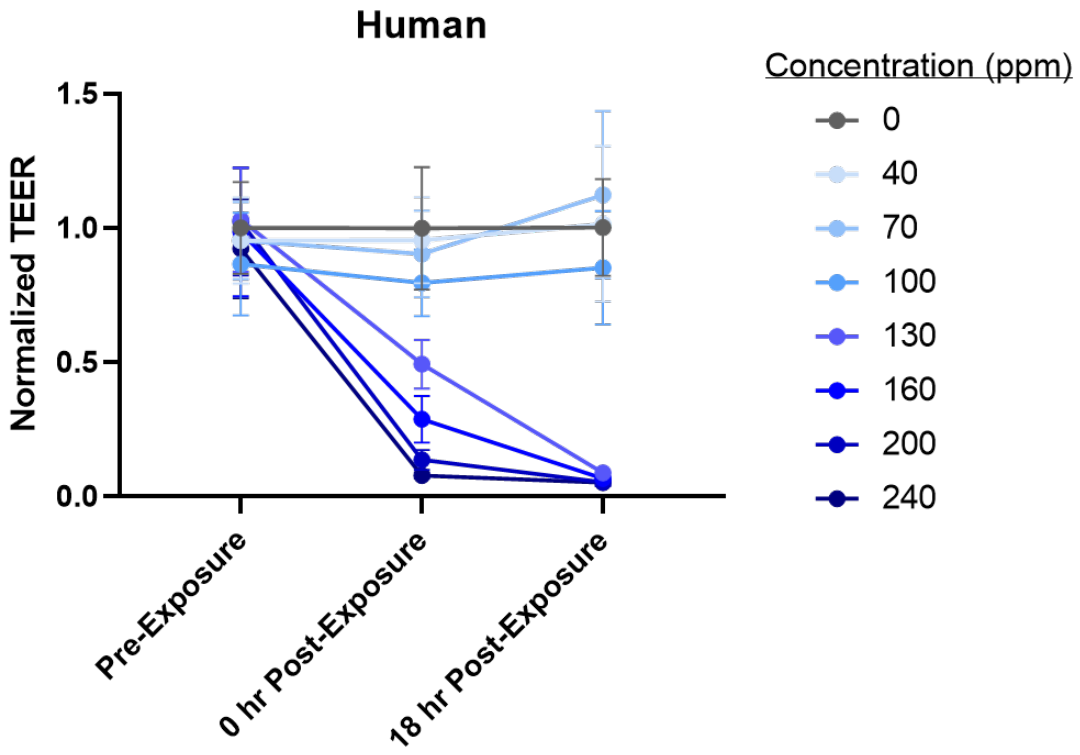
Limitation: Challenge to model aberrant repair, re-modeling, and fibrosis in standard ALI airway cultures

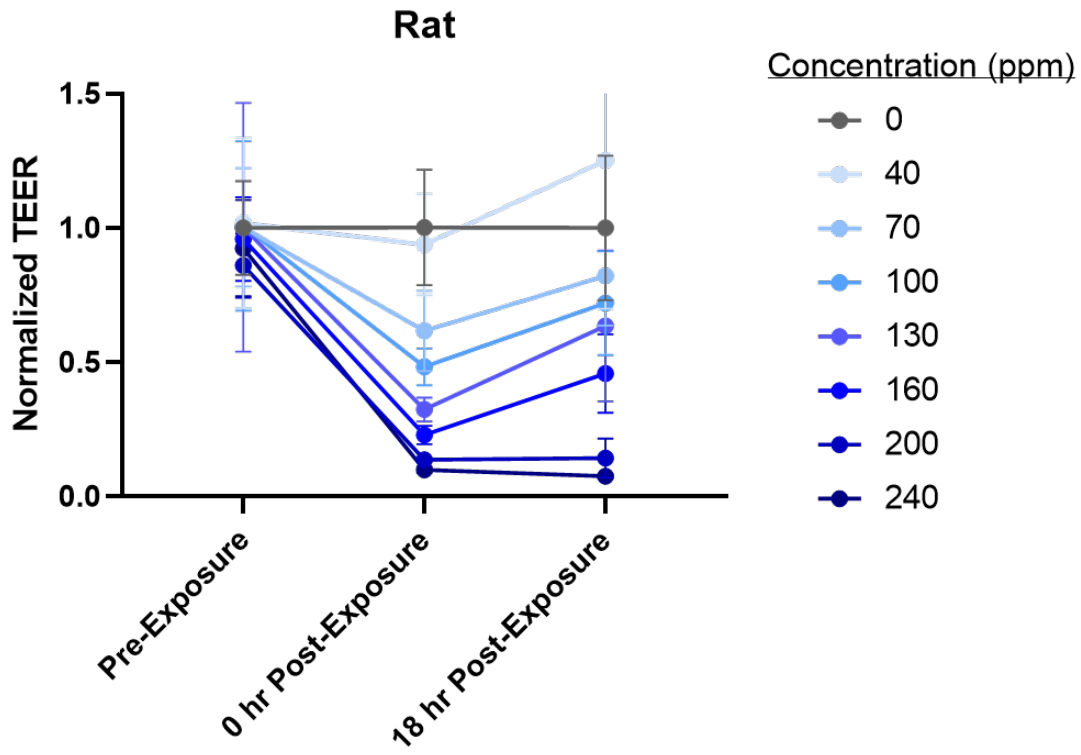
PD vapor exposure data summaries

	Human		Rat	
Target Concentration (ppm)	Mean Concentration (ppm)	Percent Target ± RSD	Mean Concentration (ppm)	Percent Target ± RSD
Exposure Chamber	<LOD	NA	<LOD	NA
Filtered Air	<LOD	NA	<LOD	NA
40	40.2 ± 0.6	101 ± 2	40.8 ± 1.3	102 ± 3
70	69.5 ± 1.0	99 ± 1	72.6 ± 7.9	104 ± 11
100	99.5 ± 1.3	100 ± 1	103 ± 6.2	103 ± 6
130	131 ± 1.6	101 ± 1	133 ± 8.6	102 ± 6
160	162 ± 2.8	101 ± 2	164 ± 5.9	102 ± 4
200	203 ± 2.8	101 ± 1	203 ± 6.5	102 ± 3
240	242 ± 3.5	101 ± 1	241 ± 12.2	100 ± 5

Mean ± SD shown
LOD = 2.09 ppm

PD vapor-induced alterations in transepithelial electrical resistance (TEER)





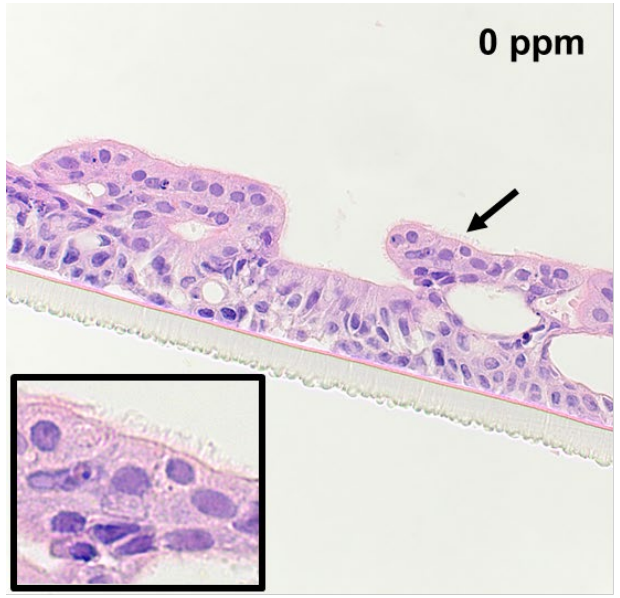
Mean ± SD shown
(n = 12 biological replicates per concentration for Pre- and 0 hr and n = 6 for 18 hr)

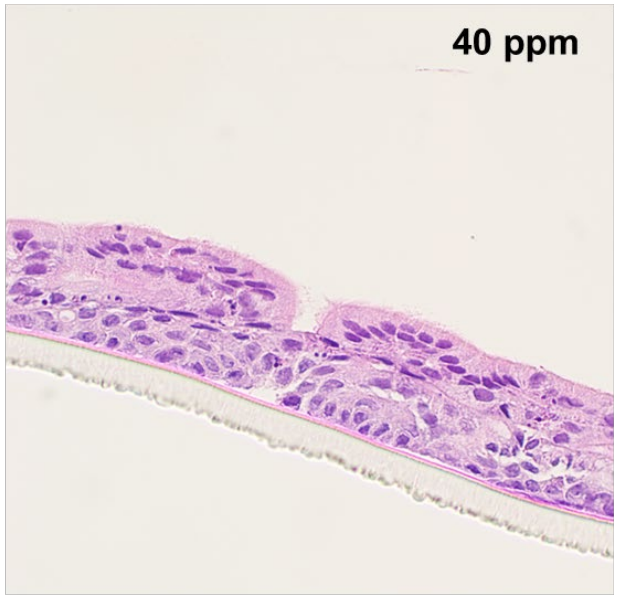
PD vapor-induced histopathological effects (human, 0 hr)

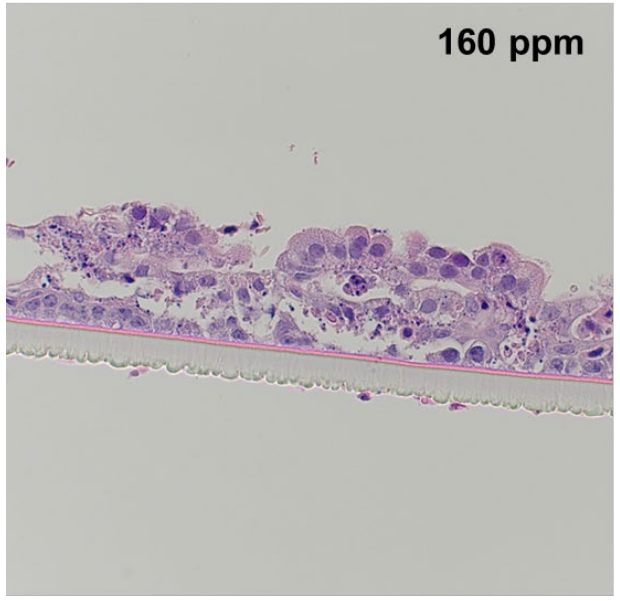
Concentration (ppm)	Cell layers	Degeneration & necrosis	Loss of cilia	% Cells denuded (focal areas)
0	5 to 7	0	-	0
40	5 to 7	0	-	0
70	5 to 7	0/1	-	0
100	5 to 7	1	-	0
130	4 to 6	2	Y	0
160	3 to 5	2	Y	0
200	1 to 5	3	Y	0
240	0 to 4	3	Y	5 to 10

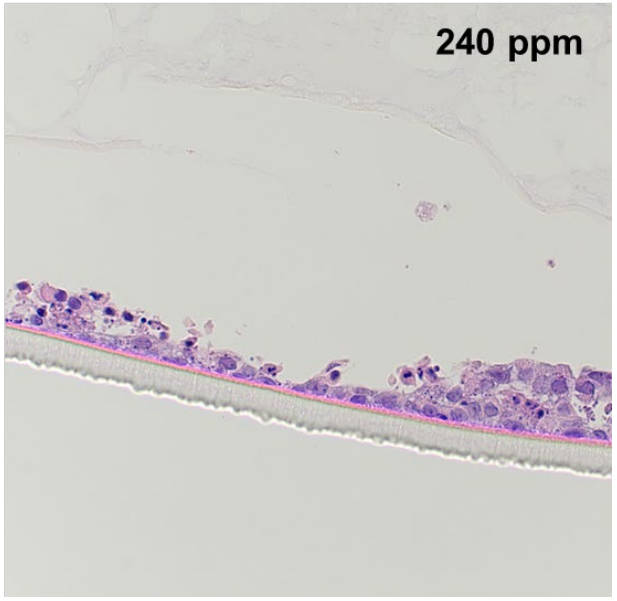
Severity scoring (for degeneration & necrosis)
0 = within normal limits; 1 = minimal (< 5%); 2 = mild (5-10%); 3 = moderate (11-25%); 4 = marked (>25%)

Single donor (ID 9831)









PD vapor-induced histopathological effects (rat, 0 hr)

Concentration (ppm)	Cell layers	Degeneration & necrosis	Loss of cilia	% Cells denuded (focal areas)
0	3 to 5	0	-	0
40	3 to 5	0	-	0
70	3 to 5	0/1	-	0
100	3 to 5	1	-	0
130	0 to 4	2	Y	0
160	0 to 4	2	Y	< 10
200	0 to 3	3	Y	< 10
240	0 to 3	3	Y	40 to 60

Results summary

- **↑ PD in basolateral culture media samples (at 0 hr) with increasing exposure concentration (low levels in apical samples and lysates) [*data not shown*]**
  - 12-27% (human) and 10-15% (rat) of theoretical max deposition
  - Fate of PD within cells (e.g., metabolites)?
- **↓ TEER**
  - ≥130 ppm (0 and 18 hr) in human; ≥70 ppm (0 and 18 hr with some recovery at 70-160 ppm) in rat
- **↑ Cytotoxicity measurements (at 18 hr only) [*data not shown*]**
  - LDH and AK release (above 2-fold vs. 0 ppm): ≥130 ppm in human; ≥200 ppm in rat
- **↑ Histopathologic effects (degeneration & necrosis, loss of cilia, and denudation)**
  - ≥130 ppm (0 and 18 hr) in human; ≥130 ppm (0 hr) and ≥100 ppm (18 hr) in rat
  - Histopathologic damage more severe for rat at higher exposure concentrations, especially at 18 hr

Additional toxicity end-points (in-progress)
- Secreted biomarkers (e.g., IL-8 and AREG)
- Histopathology (PAS staining; ubiquitin and E-cadherin IHC)

Conclusions

- **Exposure of human and rat ALI airway cultures to PD (6 hr) induced concentration-dependent changes in toxicological parameters relevant to in vivo rat (BD and PD) and in vitro human ALI (BD) airway findings as well as some key events in AOP 280.**
  - Modeling inflammatory/immune cell recruitment and fibrosis using ALI culture approaches are challenges
    - Airway epithelial injury is thought to be an initiator of bronchial/bronchiolar fibrosis
- **Based on the results of this proof-of-concept study with PD, this VITROCELL exposure system/ALI airway model has the potential to be used to investigate human-relevant inhalation (respiratory) toxicity in vitro.**
  - Applications may include the following:
    - Providing screening level assessments to help predict the adverse airway/lung effects of inhaled substances
    - Selecting (prioritizing) compounds for further toxicity testing
    - Performing compound-specific mechanistic evaluations
    - Use for regulatory agency decision-making purposes???

Further optimization of the human and rat ALI models needed
- Age of cultures
- Donors and strains; cell types
- Use for transcriptomics

Future considerations

- **The OIE Program (DTT) recently released a "Request for Information (RFI) on the Use of In Vitro Lung Models in Inhalation Toxicology Research with Potential Application to Regulatory Decision-Making"**
  - NOT-ES-24-007 (FR)
  - https://www.niehs.nih.gov/research/atniehs/dtt/strategic-plan/exposure/occupational/rfi-lung-models
  - All feedback and comments electronically – in any file format – by **June 7, 2024**

- **Inter-lab reproducibility study**
  - NICEATM coordination of multiple ICCVAM labs testing 2,3-pentanedione using their ALI airway models and exposure systems
    - Compare and contrast output data from multiple toxicity end-points

Thank you!

DTT, NIEHS

- Georgia Roberts (COR)
- Pei-Li Yao (Alternate COR)
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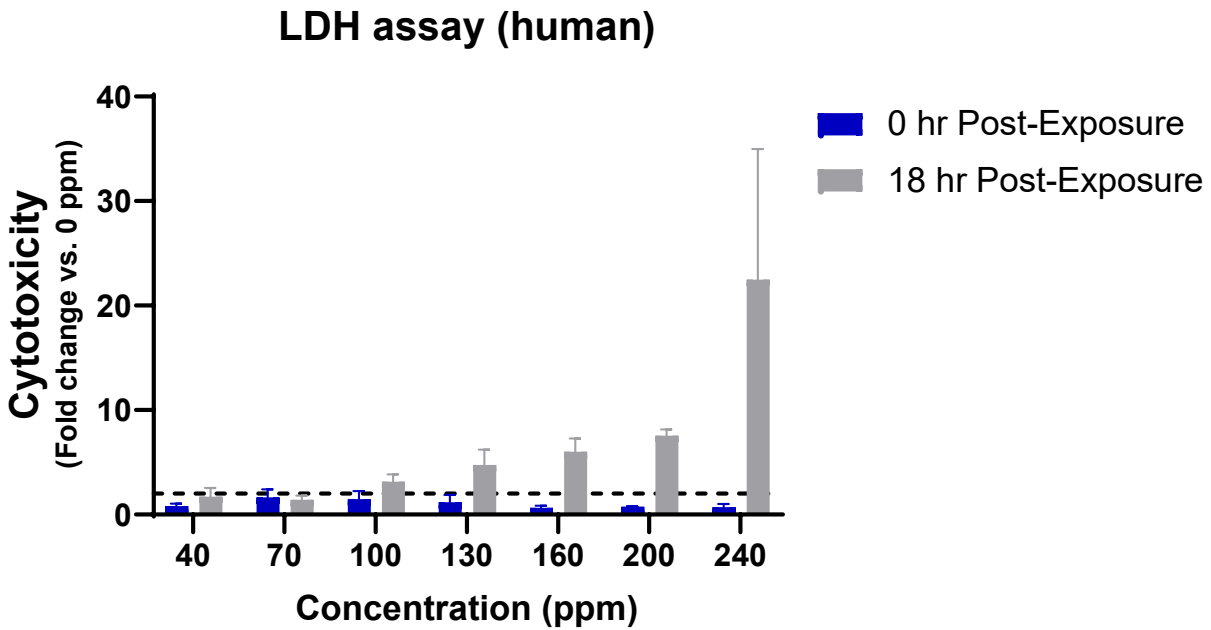
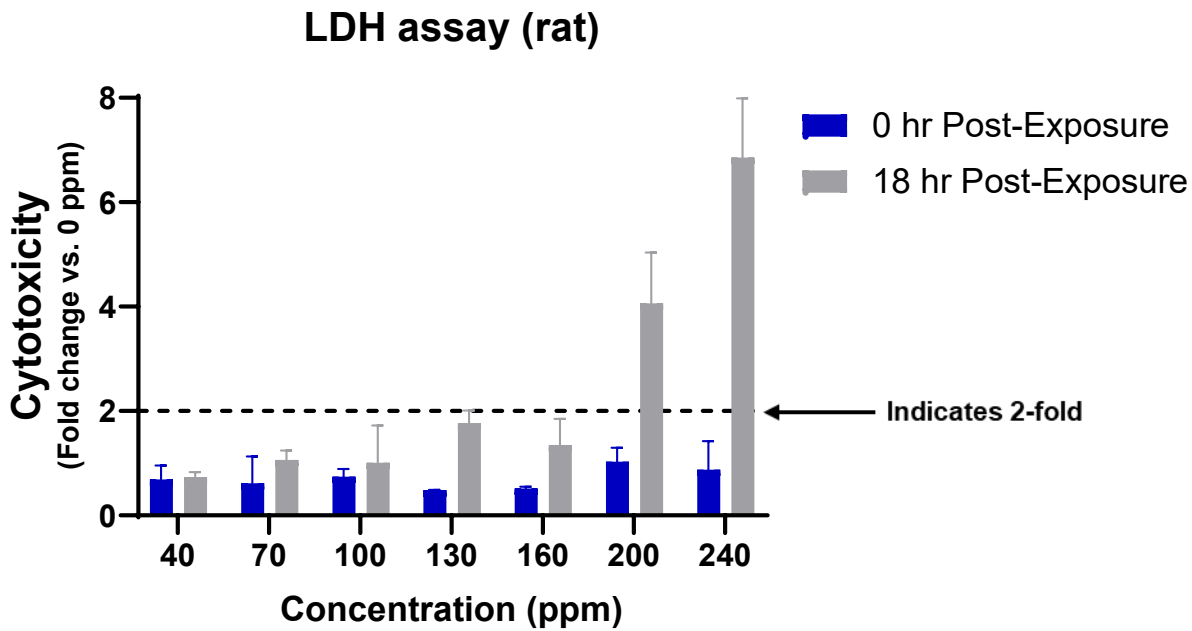
Battelle

- Amit Gupta
- Steph Pearson
- Jamie Richey
- Bob Moyer
- John Shaw
- Sayak Mukherjee
- Anthony Skowronek
- Dawn Fallacara
- Barney Sparrow

Additional (back-up slides) follow

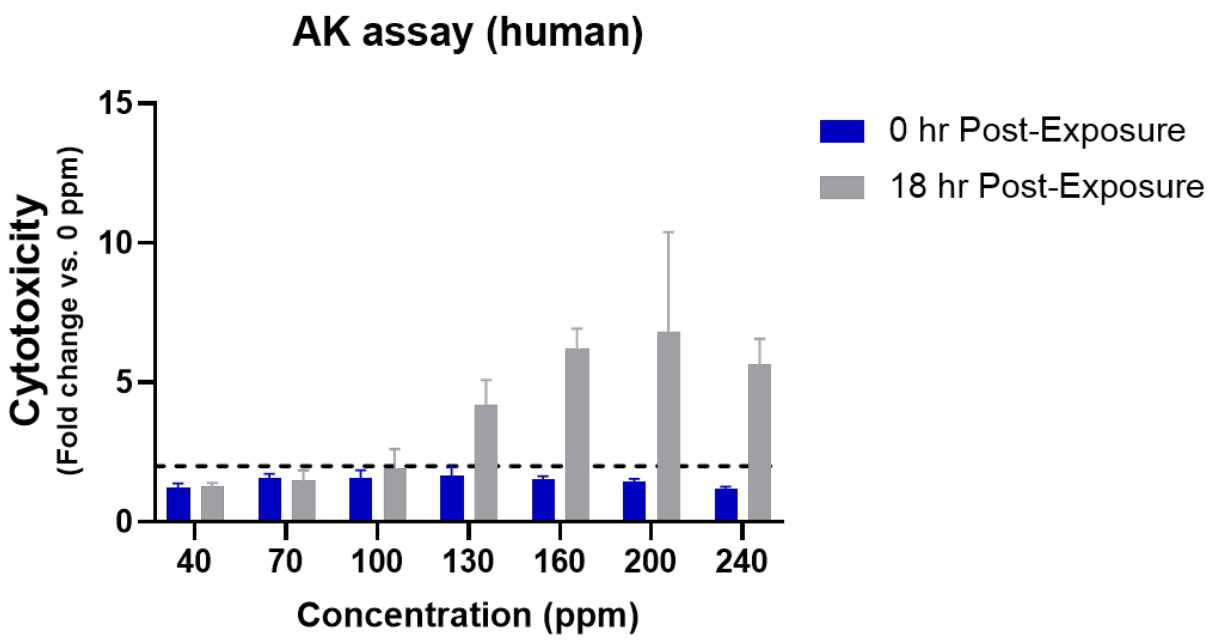
PD vapor-induced lactate dehydrogenase (LDH) activity in apical samples

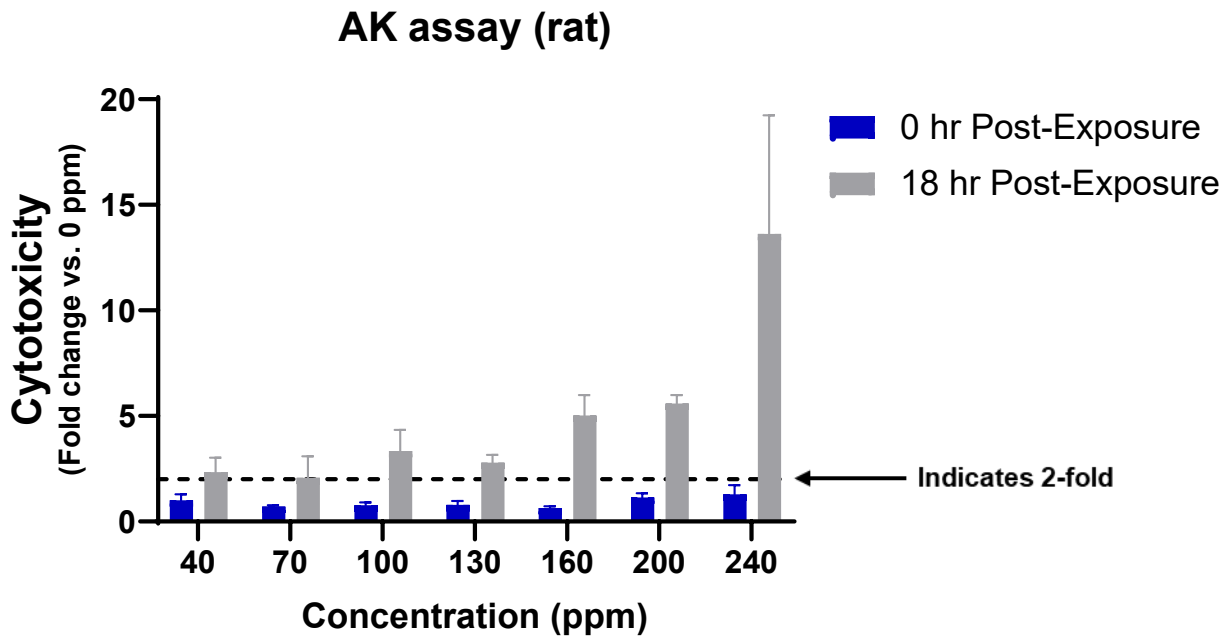




Mean ± SD shown
(n = 3 biological replicates per concentration)

PD vapor-induced adenylate kinase (AK) activity in apical samples





Mean ± SD shown
(n = 3 biological replicates per concentration)

Evaluate additional “direct” cell viability/cytotoxicity assay (non-destructive)

PD vapor-induced histopathological effects (human, 18 hr)

Concentration (ppm)	Cell layers	Degeneration & necrosis	Loss of cilia	% Cells denuded (focal areas)
0	5 to 7	0	-	0
40	5 to 7	0	-	0
70	5 to 7	0	-	0
100	5 to 7	1	-	0
130	4 to 7	1	Y	0
160	2 to 5	2	Y	0
200	0 to 5	3	Y	< 5
240	0 to 3	3	Y	50 to 80

PD vapor-induced histopathological effects (rat, 0 and 18 hr)

0 hr

Concentration (ppm)	Cell layers	Degeneration & necrosis	Loss of cilia	% Cells denuded (focal areas)
0	3 to 5	0	-	0
40	3 to 5	0	-	0
70	3 to 5	0/1	-	0
100	3 to 5	1	-	0
130	0 to 4	2	Y	0
160	0 to 4	2	Y	< 10
200	0 to 3	3	Y	< 10
240	0 to 3	3	Y	40 to 60

18 hr

Concentration (ppm)	Cell layers	Degeneration & necrosis	Loss of cilia	% Cells denuded (focal areas)
0	3 to 5	0	-	0
40	3 to 5	0	-	0
70	3 to 5	0	-	0
100	2 to 4	1	Y	0
130	0 to 4	2	Y	< 10
160	0 to 3	3	Y	60 to 80
200*	0 to 2	3	Y	80 to 90
240*	0 to 1	3	Y	90 to 100

**Membranes ~ completely denuded*