Dose-response evaluation of lung injury in human reconstructed lung epithelium in vitro models following acute propylene oxide exposure

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Introduction

- Validated new approach methods (NAMs) are needed to support timely, defensible risk assessments for personnel in extreme, complex operational environments.
- In vitro lung models are essential for warfighter health protection because they enable reliable, rapid evaluation of complex, varied and mixed, and potentially high-risk inhalation exposures, to support development of risk mitigation strategies.
- A standardized protocol has not been formalized for in vitro respiratory toxicity methods, though these models have been demonstrated to be promising tools for identifying respiratory irritants, and are being investigated for use in respiratory sensitization and chronic disease progression[1,2,3].
- In this study, multiple lung injury key events were assessed in an established reconstructed human airway in vitro model (EpiAirway™) with gas exposures at the air liquid in interface in a VitroCell® exposure system.
- In vitro dose-response profiles were compared to in vivo data to evaluate the utility of the in vitro model for testing reactive gases in support of health risk assessments for novel chemicals.

Methods

Cell Culture: EpiAirway™ human bronchial epithelial cells were grown on porous inserts to create a differentiated, multi-layered culture that mimics the lining of the human airway. This is known as an air-liquid interface (ALI) culture.

Culture Optimization: A preliminary study first determined the optimal age for these cell cultures, concluding that use up to 28 days after differentiation yielded the most stable and reliable results.

Exposure System: A custom-built delivery and measurement system was developed and validated for use with the VitroCell 24/48 exposure system (Figure 1). This system was designed to deliver propylene oxide (PropOx) vapor at stable and precise concentrations directly to the cells.

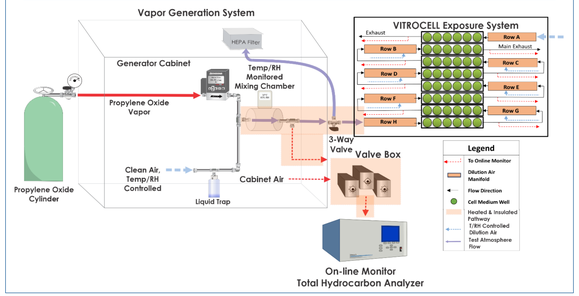
Rangefinding Studies:

- **Exposure**:
  - Study 1: 16, 31, 63, 125, 250, 500, 1000 ppm PropOx vapor for 4 hours (hrs)
  - Study 2: 500, 700, 900, 1000, 1100, 1300, 1500 ppm PropOx vapor for 4 hrs
- **Endpoints:**
  - **Barrier Integrity:** Trans-epithelial electrical resistance (TEER)
  - **Cell Morphology:** Phase contrast microscopy (10x) to assess cell shape, mucus production, loss of cilia, histopathology (hematoxylin and eosin [H&E] staining)
  - **Viability:** Adenylate kinase

Definitive Dose-response Study:

- **Exposure**: Cell cultures were exposed to 350, 750, and 1250 ppm PropOx vapor for 4 hrs exposure
- **Endpoints:**
  - **Barrier Integrity:** Trans-epithelial electrical resistance (TEER)
  - **Cell Morphology:** Phase contrast microscopy (10x) to assess cell shape, mucus production, loss of cilia, histopathology (H&E)
  - **Viability:** Adenylate kinase
  - **Mechanistic Biomarkers: I**nflammation (cytokines), oxidative stress (GSH/GSSG ratio)

Figure 1. Exposure System. The exposure system contained the test article vapor generation system, the VITROCELL® exposure chamber, and a real-time test article concentration monitoring system (on-line monitor). During exposure, tissues were housed within the exposure chamber of the VITROCELL® system.



Results: Exposures

Table 1. PropOx Concentration and Environmental Condition Stability for Rangefinding #1 and Definitive Study. Mean concentrations at each exposure level were within 2% of the respective target. Rangefinding #2 had similar precision/stability of PropOx and environmental conditions (data not shown).

Rangefinding #1 Exposure Summary			Dilution Air Parameters	
Target Conc. (ppm)	Mean Conc. (ppm)[a]	%Target ± RSD	Temp. (°C) (Mean ± SD)	% RH (Mean ± SD)
0	<LOD	NA	35.9 ± 1.9	85 ± 3
16	16.2 ± 0.3	102 ± 2		
31	31.6 ± 0.5	102 ± 1		
63	63.9 ± 0.7	101 ± 1		
125	127 ± 1.6	102 ± 1		
250	254 ± 2.1	102 ± 1		
500	505 ± 2.5	101 ± 0		
1000	1010 ± 4.0	101 ± 0		

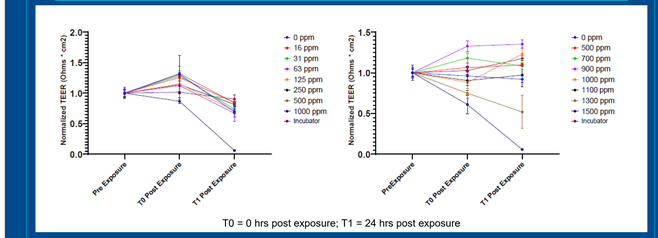
[a]Data shown are mean ± standard deviation (SD), n=8
[b]Limit of detection = 1.10 ppm
RSD = Relative standard deviation

Definitive Study Exposure Summary			Dilution Air Parameters	
Target Conc. (ppm)	Mean Conc. (ppm)[a]	%Target ± RSD	Temp. (°C) (Mean ± SD)	% RH (Mean ± SD)
0	<LOD[b]	NA	36.2 ± 0.2	86 ± 0[c]
350	354 ± 3.3	101 ± 1		
750	756 ± 4.1	101 ± 1		
1250	1250 ± 7.0	100 ± 1		

[a]Data shown are mean ± standard deviation (SD), n=11
[b]Limit of detection = 1.10 ppm
[c]Standard deviation reported as 0, actual value was >0.0 and <0.5
RSD = Relative standard deviation

Results: Rangefinding Studies

Figure 2. TEER Measurements in Rangefinding #1 (left) and #2 (right). TEER measurements decreased slightly in controls over time (blue lines), but PropOx decreased TEER significantly and in a dose-dependent manner at both post-exposure time-points, indicating a rapid and sustained decrease in barrier integrity at higher doses.



T0 = 0 hrs post exposure; T1 = 24 hrs post exposure

Figure 3. Adenylate kinase (AK) Measurements in Rangefinding #1 (left) and #2 (right). AK measurements increased in a dose-dependent manner.

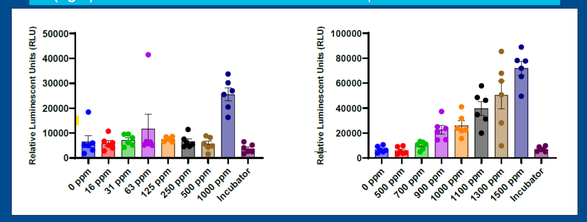
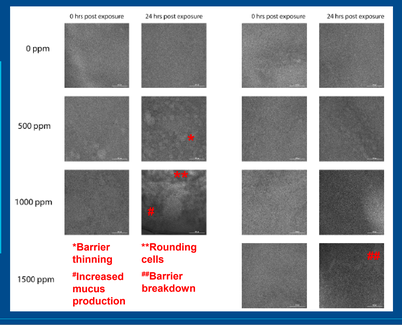


Figure 4. Phase contrast images (10x) in Rangefinding #1 (left) and #2 (right) immediately after (0 hrs) and 24 hrs post exposure. Increased barrier stress was observed, including increased mucus production, cell rounding, and cell loss at higher concentrations of PropOx.



Results: Definitive Dose-Response Study

Figure 5. TEER (left) and AK responses (right) at 24 hrs post-exposure to PropOx in EpiAirway™ reconstructed human airway models

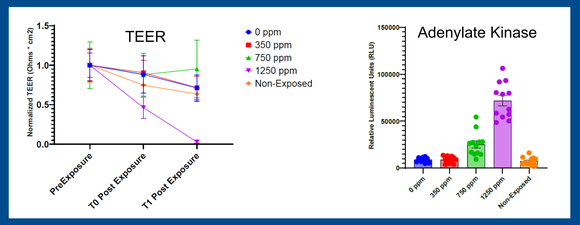


Figure 6. Select cytokine expression of the apical and basal compartment 24 hours following PropOx exposure. Dose-dependent changes were observed in multiple markers of inflammation on both the apical (top) and basal (bottom) side of the EpiAirway tissue.

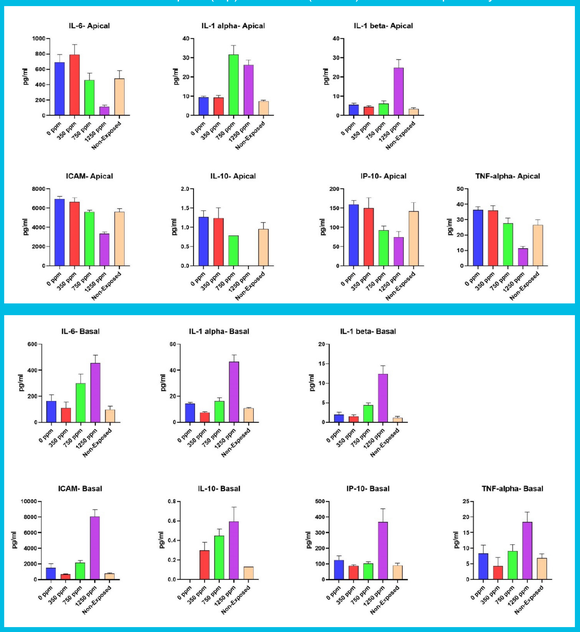
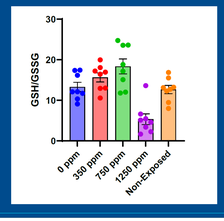


Figure 7. GSH/GSSG ratio in EpiAirway™ tissue following PropOx exposure. The ratio was reduced in the 1250 ppm exposure, indicating antioxidant depletion.



Results: Definitive Dose-Response Study

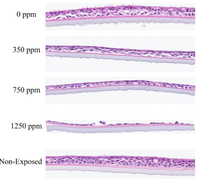


Figure 8. Histology (40x) of EpiAirway™ Tissues. Inserts were fixed and processed for H&E staining (MatTek, Ashland, MA). High PropOx concentrations damage the ALI barrier resulting in cell loss.

Table 2. Benchmark Dose Lower Limit (BMDL) of all Endpoints Measured in the Definitive Dose-Response Study. The U.S. Environmental Protection Agency R package tcplfit2 (v.0.1.9) and BMDS software (v.25.1) were used to model curve fits to the dose-response data. The benchmark dose was defined as one standard deviation from control. In Table 2, darker green indicates a lower BMDL. The lowest adverse BMDLs (350-460 ppm) were derived from increases in adenylate kinase and proinflammatory cytokines IL-1α, IL-1β, and IL-6.

Endpoint	Assay	Timepoint	Location	TCPL BMDL (ppm)	BMDS BMDL (ppm)	Direction
Barrier integrity	TEER	0 hour	Tissue	Inactive	711	Negative
	TEER	24 hour	Tissue	1142	916	Positive
Cytotoxicity	LDH	24 hour	Apical Wash	889	759	Positive
	Adenylate Kinase	24 hour	Apical Wash	440	463	Positive
Oxidative Stress	GSH/GSSG	24 hour	Tissue	Inactive	955	Negative
Inflammation	IL-1α	24 hour	Apical Wash	332	359	Positive
	IL-1α	24 hour	Basal Media	832	652	Positive
	IL-1β	24 hour	Apical Wash	1152	641	Positive
	IL-1β	24 hour	Basal Media	705	451	Positive
	IL-6	24 hour	Apical Wash	Inactive	530	Negative
	IL-6	24 hour	Basal Media	914	444	Positive
	ICAM-1	24 hour	Apical Wash	580	387	Negative
	ICAM-1	24 hour	Basal Media	870	629	Positive
	IP-10	24 hour	Apical Wash	238	348	Negative
	IP-10	24 hour	Basal Media	1146	772	Positive
	TNF-α	24 hour	Apical Wash	670	431	Negative
	TNF-α	24 hour	Basal Media	Inactive	711	Positive

Conclusions

- Rangefinding studies demonstrated dose-response for cytotoxicity and TEER, which were used to set the exposure concentrations for the definitive dose response study to elicit minimal (350 ppm), moderate (750 ppm) and severe (1250 ppm) effects on lung tissue.
- The definitive dose-response study demonstrated dose-dependent effects on key events in the acute lung injury toxicity pathway in the human EpiAirway™ model, including barrier disruption (TEER), cell death, oxidative stress and an array of inflammatory biomarkers.
- BMDLs were calculated for all endpoints; most effects were observed in the 750 and 1250 ppm doses. BMDS provided the more robust BMDL modeling results.
- The most sensitive endpoints were IL-1α, IL-1β, IL-6, and adenylate kinase, with BMDLs of 350-460 ppm. In rats, the reported 50% lethality concentration (LC_{50}) is 3200 ppm[4], with a lowest observable adverse effect level for lung injury in dogs of 2000 ppm[5].
- **These data demonstrate biological fidelity to acute lung irritation and provided a sensitive model for PropOx induced acute lung injury, indicating that this model is a promising alternative to acute in vivo inhalation toxicity studies in a rapid NAM-based testing scheme.**

References

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