3377. Optimizing air-liquid interface respiratory NAM models for acute exposure scenarios to support regulatory screening and safety assessment

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Introduction

- New approach methodologies (NAMs) are increasingly at the forefront of inhalation toxicity assessment, supporting regulatory safety evaluation. In alignment with the FDA Modernization Act 2.0 and its April 2025 roadmap to replace, reduce and refine animal studies, advanced human-relevant approaches – such as organ-on-a-chip systems, 3D tissue models, computational modeling and *in vitro* assays – are being encouraged for investigational new drug (IND) applications and beyond.
- Creating aerosols for inhalation studies requires specialized systems tailored to the physicochemical properties of the test article. For gases, dynamic dilution and continuous-flow exposure systems are commonly used to deliver controlled concentrations under physiologically relevant conditions. Complex mixtures often employ whole-mixture generation chambers or nebulization systems that maintain chemical integrity while helping to ensure reproducible particle size distribution. Nanoparticles typically require high-precision aerosolizers such as electrospray or dry powder dispersers, which prevent agglomeration and allow control over particle size and number concentration. For fibers, rotating brush generators or fluidized bed systems are used to suspend fibers in air without altering their morphology, supporting accurate representation of inhalation exposure scenarios. These technologies are designed to deliver aerosols to *in vitro* platforms, supporting mechanistic studies and regulatory screening under NAM frameworks.

Methods

- MucilAir™ tissues (donor MD067001) from Epithelix Sarl, Switzerland were cultured following manufacturer's guidelines.
- Ethylene Oxide (EtO) (0.65%, 6500 ppm), Nitric Oxide (NO) (0.2%, 2000 ppm) and Sulphur Hexafluoride (SF6) (10%, 100,000 ppm), were purchased from BOC (Linde PLC, Pullach, Germany).
- Controls: Air-liquid interface (ALI) control for treatment was exposed to 0.2L/min flowing air. Triton X-100 at 1% treated basolaterally used as a positive control.
- Gaseous exposure: Vitrocell® 24/4 exposure modules. Test article cylinders were set to 1 Bar before being regulated at 0.1 L/min for each dilution bar. Test article was then diluted with different flow rates of flowing. A vacuum rate of 5 mL/min was used for all exposures. Exposure duration was 60 min.

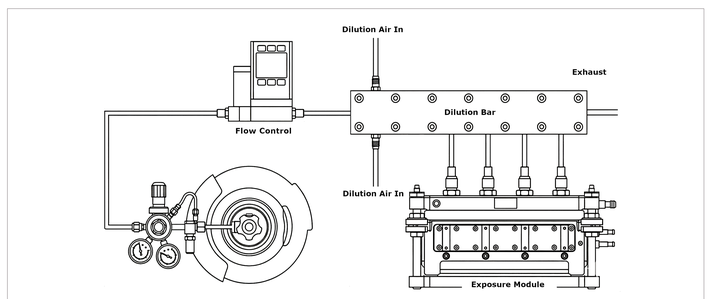

Figure 1. Exposure schematic (drawn by author Adam Seymour with GIMP software).

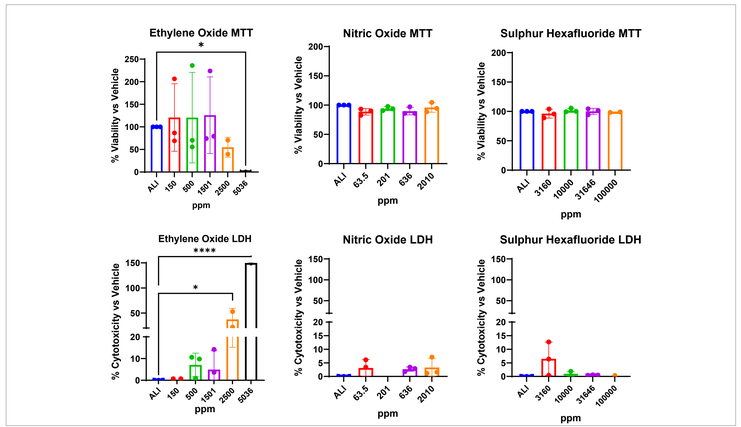

Figure 2. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] results as a %Viability of ALI, for each dose, for all 3 test articles. Lactate dehydrogenase (LDH) release presented as %Cytotoxicity against ALI, each dose, for all 3 test articles. Noting statistical significance between doses. Statistical difference was noted for EtO between 2500 and 5036 ppm against ALI control (Two-way ANOVA with multiple comparisons. * P < 0.05, **** P < 0.0001).

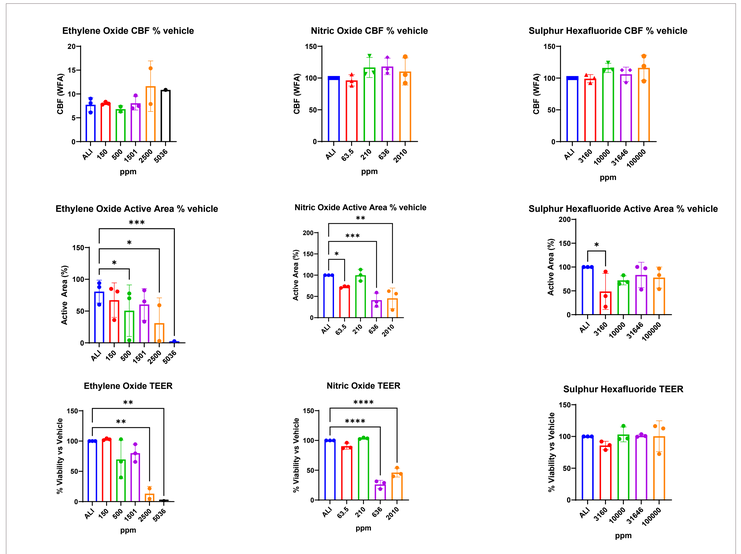

Figure 3. Barrier integrity and cilia beat frequency (CBF)/active area (AA) measurements exposure EtO, NO and SF6. Barrier integrity following exposure was measured by transepithelial electrical resistance (TEER). Mean ± SEM. CBF/AA measurements were performed using the Sisson-Ammons Video Analysis (SAVA) system and normalized to ALI control. Two-way ANOVA with multiple comparisons. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

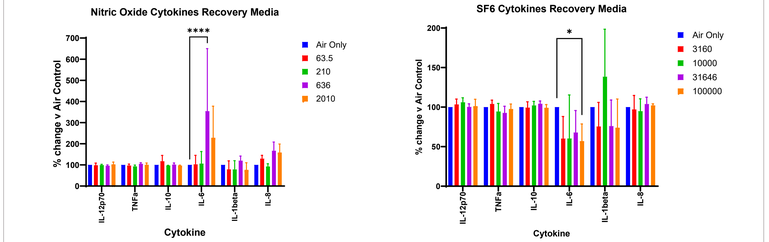

Figure 4. Cytokine release was measured in the 24-hour recovery media following exposure aerosol and normalized to the ALI control. Increases in IL-6 and IL-1β were for NO with significance for IL-6. Decrease was observed for IL-6 with SF6 with significance. Statistics run with two-way ANOVA with multiple comparisons. * P < 0.05. **** P <0.0001).

Results

- Exposure of MucilAir™ to EtO, NO and SF6 aerosols resulted in cytotoxicity for EtO only at highest two concentrations. No cytotoxicity was observed for NO or SF6 with either MTT or LDH assays.
- TEER values were decreased in a concentration related fashion for both EtO and NO and therefore indicating decreased membrane integrity with increasing concentration. No tight junction decrease was observed for SF6, although some variability was observed at the highest concentration. The results for EtO and NO did not correlate with a reduction in CBF, instead an increase was observed for EtO (although not significant) and no increase observed for NO or SF6.
- Increased release of various pro-inflammatory cytokines was observed for NO, IL-6 and IL-1β. A decrease was observed for SF6 for IL-6. These cytokines have been shown to be involved in the NLRP3 inflammasome pathway. Cytokine analysis is being conducted for EtO.

Conclusions

- MucilAir™ tissues can be used alongside the MTT and LDH assays to expose to various gaseous test articles, results can be used to differentiate statistically between doses.
- Furthermore, additional endpoints can be included to further elucidate mechanistic endpoints.
- MucilAir™ tissues are appropriate to be used to support regulatory screening and safety assessment.

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