Reparatory Toxicity of Cigarette Smoke and ENDS Aerosols in MucilAir™ Airway Model

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Callout
ENDS aerosols were substantially less toxic than cigarette smoke.

INTRODUCTION

- **The Continuum of Risk concept recognizes that not all nicotine-containing products carry the same level of harm, positioning ENDS as a potentially reduced-harm alternative for adult smokers, given their significantly lower levels of harmful and potentially harmful constituents (HPHCs), compared to combustible cigarette smoke**
- **The CTP/FDA[1] notes that many smokers misunderstand the relative risks of tobacco products, which can discourage the use of lower-risk alternatives like ENDS. Public health efforts, aimed at harm reduction, depend on accurate risk communication supported by scientific data**
- **Our study builds on that premise and contributes scientific data to the growing evidence supporting the risk continuum and the potential for ENDS products to serve as reduced harm alternatives among adults who smoke by comparing the toxicological profiles of cigarette smoke and ENDS aerosols in a physiologically relevant airway model**

AIMS

- **To evaluate the reduced harm potential of aerosols from a commercial ENDS product in comparison to 1R6F Reference cigarette smoke**
- **To assess both acute effects and the reversibility of damage after a 7-day recovery period using MucilAir™ Bronchial tissue model paired with Vitrocell® exposure systems**

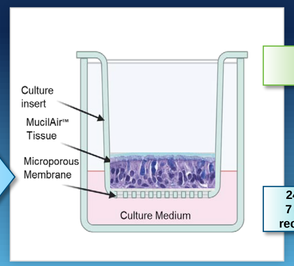
METHODS

TESTED PRODUCTS & EXPOSURE REGIMEN

1R6F smoke
(ISO 20778)
- 64 min
- 10 → 1 L/min

ENDS aerosols
(ISO 20768)
- 240 min, 6 → 0.25 L/min
- 180 min, undiluted

EXPOSURE SYSTEM



Exposure

24 h & 7 days recovery

- MucilAir™ Bronchial tissue
- Vitrocell® HTP2.0+ 24-well module
- Vitrocell® VC10® smoking robot

AEROSOL CHARACTERIZATION & DOSIMETRY ASSESSMENT

1R6F: nicotine, carbonyls
ENDS: nicotine, carbonyls

BIOLOGICAL ASSESSMENT (N = 3)

Tissue:
- **Cytotoxicity:** WST-8
- **Tissue integrity:** TEER
- **Ciliary Function:** AA & CBF
- **Mucociliary clearance***
- **Histology:** H&E*
- **Immunohistochemistry***

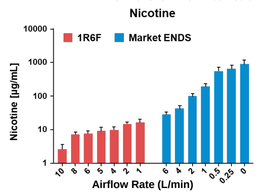
Supernatant:
- **Cytotoxicity:** LDH
- **Oxidative Stress:** 8-Isoprostane
- **Inflammation:** Cytokines

AA=cilia active area; CBF=cilia beat frequency; H&E=Hematoxylin and Eosin staining; LDH=Lactate Dehydrogenase Assay; TEER=transepithelial electrical resistance. *Not presented here. Pending analysis.

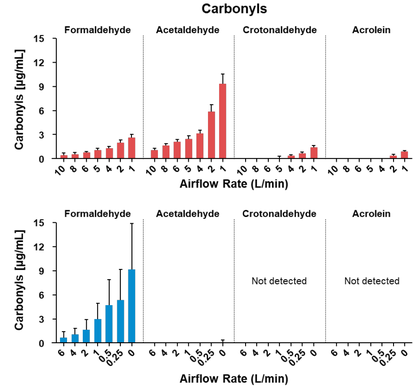
Reference:
1. The Relative Risks of Tobacco Products | FDA.

RESULTS

Aerosol Characterization



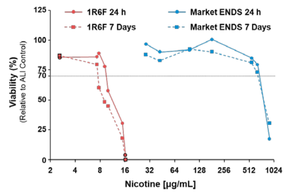
- **Delivered nicotine:**
  1R6F: 2.6 – 16.3 µg/mL
  ENDS: 28.8 – 909.0 µg/mL



- **Carbonyls profile:**
  1R6F: detected formaldehyde, acetaldehyde, crotonaldehyde, acrolein
  ENDS: detected formaldehyde and acetaldehyde*

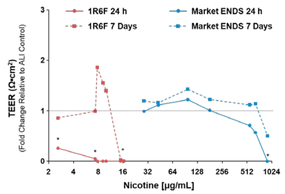
*Acetaldehyde was detected in just one out of three experiments, only at the highest concentration.

Cytotoxicity (WST-8)



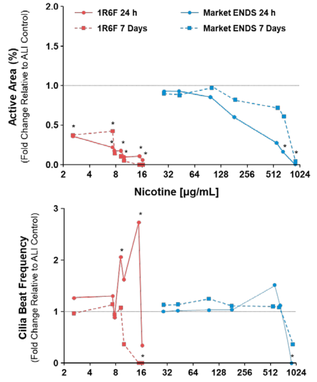
- **AT BOTH 24 H AND 7 DAYS** TIME POINTS,
  1R6F **exhibited significantly higher toxicity compared to** ENDS
- **No differences in recovery were observed between 24h and 7-day timepoints for either test product**

Tissue Integrity (TEER)



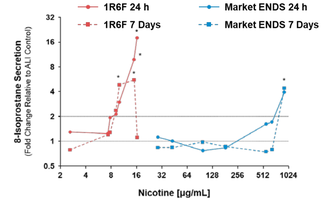
- **AT 24 H**
  1R6F: **near-complete disruption of tissue integrity** (except the lowest concentration)
  ENDS: **minimal disruption** (except the highest concentration)
- **AT 7 DAYS OF RECOVERY**
  1R6F: **recovery** (except the two highest concentrations)
  ENDS: **full overall recovery** (except the highest concentration)

Ciliary Function (%AA and CBF)



- **AT 24 H**
  1R6F: **severe ciliary disruption,** compensatory ciliary hyperkinesia (mid-concentrations)
  ENDS: **partial ciliary disruption,** reduction in %AA and CBF at the three highest concentrations
- **AT 7 DAYS OF RECOVERY**
  1R6F: **persistent, exacerbated ciliary disruption**
  ENDS: **near-complete recovery,** except the highest concentration

Oxidative Stress (8-Isoprostane)



- **AT 24 H**
  1R6F: **significant oxidative stress (OS) via 8-Isoptostane > 2-fold increase at highest 4 concentrations**
  ENDS: **OS > 2-fold increase at the highest concentration**
- **AT 7 DAYS OF RECOVERY**
  1R6F: **OS > 2-fold increase at intermediate concentrations,** then decreased at the highest concentration
  ENDS: **near complete return to baseline OS levels,** except at the highest concentration

Toxicity Potency Comparison Between 1R6F and ENDS

Endpoint	24 h	7 days
Cytotoxicity	~ 70-fold	~ 200-fold
Tissue Integrity	~ 350-fold	~ 60-fold
Active Area	~ 240-fold	~ 340-fold
Ciliary Function	~ 50-fold	~ 60-fold
Oxidative Stress	~ 100-fold	~ 100-fold
IL-1α	<LOQ	<LOQ
IL-6	ns	ns
MCP-1	~ 10-fold	~ 60-fold
VEGF	~ 100-fold	~ 60-fold

Statistical Analysis: One-way ANOVA (GraphPad Prism v10.4), Dunnett's multiple comparison test, $p<0.05$. The lowest tested concentration of 1R6F and ENDS that showed a statistically significant effect compared to the concurrent ALI control was determined and used to calculate the fold difference in ENDS potency relative to 1R6F at each time point.

LOQ - Limit of Quantitation; ns – not significantly different compared to VC.

CONCLUSIONS

- 1R6F is more toxic than ENDS, with more severe and persistent effects on viability, tissue integrity, oxidative stress, and ciliary function
- ENDS was less toxic, demonstrated overall better recovery over time, across all endpoints evaluated
- The reduction in the toxicity profile of ENDS align with their substantially lower levels of HPHCs

This study provides strong in vitro evidence supporting ENDS **as a less harmful alternative to combustible cigarettes**

Highlights the potential for ENDS to offer a reduced-harm alternative for adult smokers

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