Evaluating Toxicity Profiles of Cigarette Smoke and ENDS Aerosols in a MucilAir™ Tissue Model

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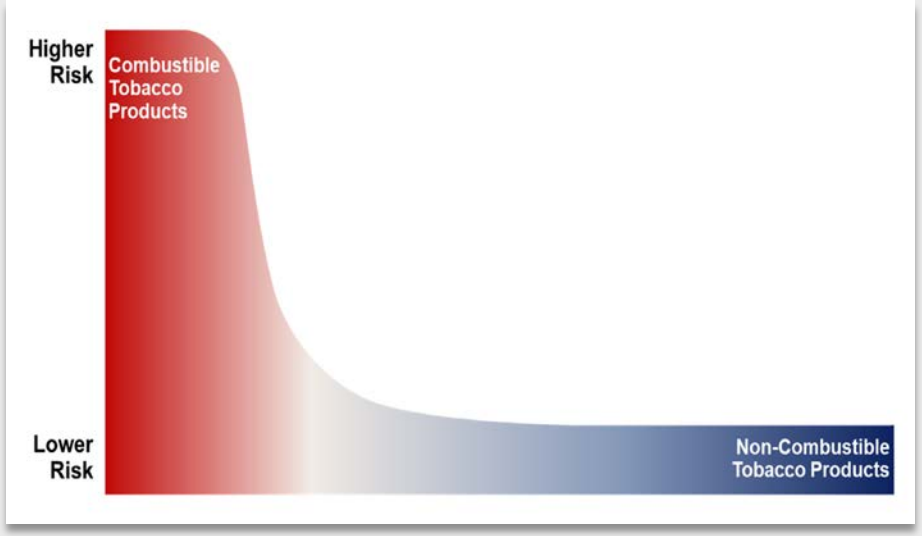
Agenda

- Introduction
- Study Objective
- Methodology
- Results
- Conclusion

Introduction

- Electronic Nicotine Delivery Systems (ENDS) can offer a reduced-harm alternative for nicotine delivery to adult smokers
- ENDS contain significantly fewer harmful and potentially harmful constituents (HPHCs) than cigarette smoke

The Risk Cliff



The Relative Risks of Tobacco Products | FDA

Why Is It Important for Adults Who Smoke to Understand the Relative Risks of Tobacco Products?

Many people who use tobacco products have misperceptions about the varying risks of tobacco products, which may prevent them from switching to a lower-risk alternative. Adults who smoke who fully switch from cigarettes to a lower-risk alternative can generally reduce their health risk and exposure to toxic and cancer-causing chemicals.

Study Objective

TO COMPARE

the toxicological effects of ENDS aerosols to reference cigarette (1R6F) smoke

TO EVALUATE

the acute toxicity and its reversibility using the MucilAir™ tissue model

VS.

Methodology

TESTED PRODUCTS & EXPOSURE REGIMEN

1R6F smoke

(ISO 20778)

- 64 min
- 10 L/min → 1 L/min

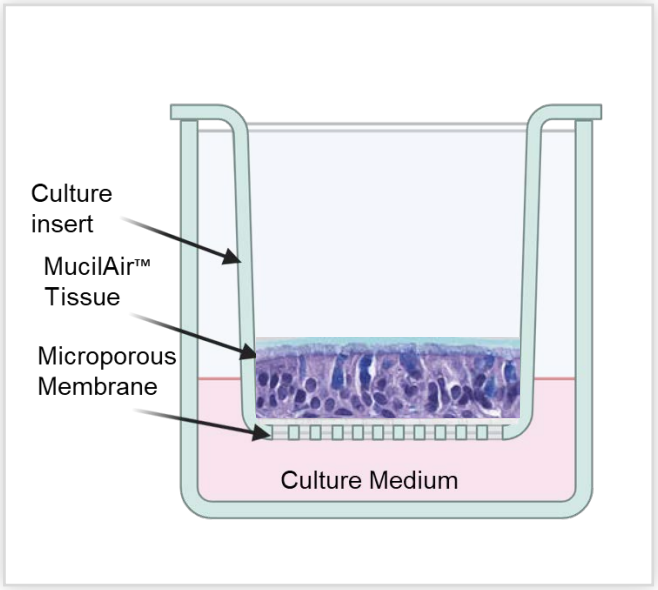
ENDS aerosols

(ISO 20768)

- 240 min, 6 L/min → 0.25 L/min
- 180 min, undiluted

Exposure

EXPOSURE SYSTEM



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

- **24 h**
- **7 days (recovery)**

AEROSOL CHARACTERIZATION & DOSIMETRY ASSESSMENT

1R6F: nicotine, carbonyls

ENDS: nicotine, carbonyls

BIOLOGICAL ASSESSMENT

(N = 3)

Tissue:

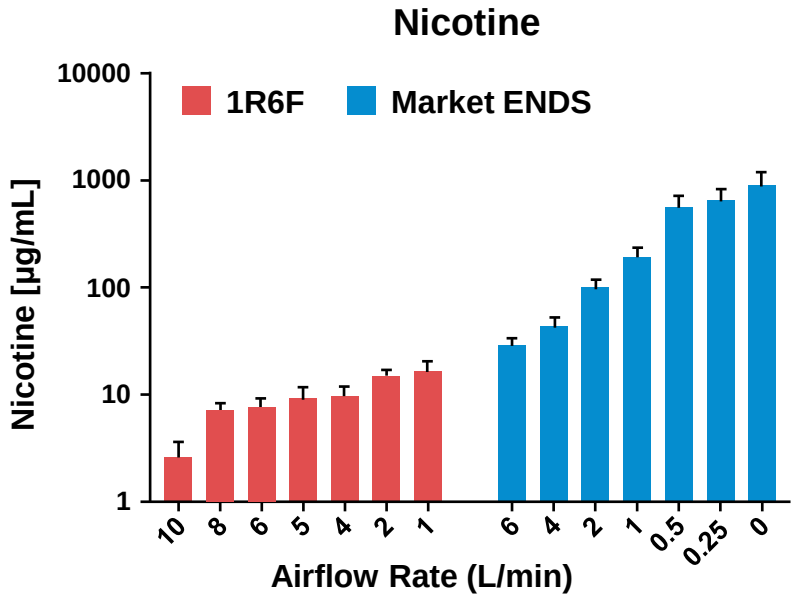
- **Cytotoxicity:** WST-8
- **Tissue integrity:** TEER
- **Ciliary Function:** AA & CBF
- **Mucociliary clearance***
- **Histology:** H&E*
- **Immunohistochemistry***

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.

Results – Aerosol Characterization



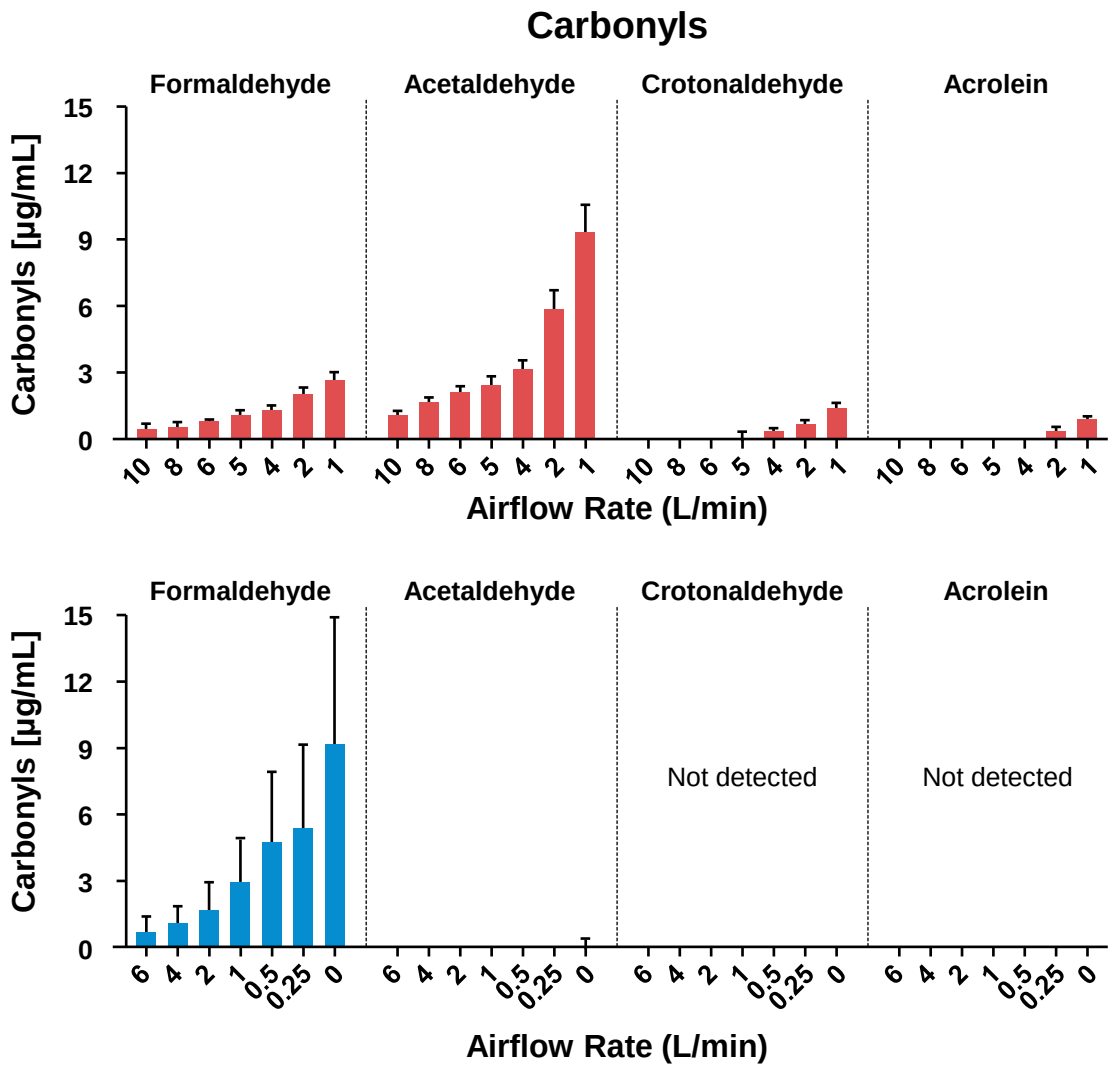
Delivered nicotine:

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

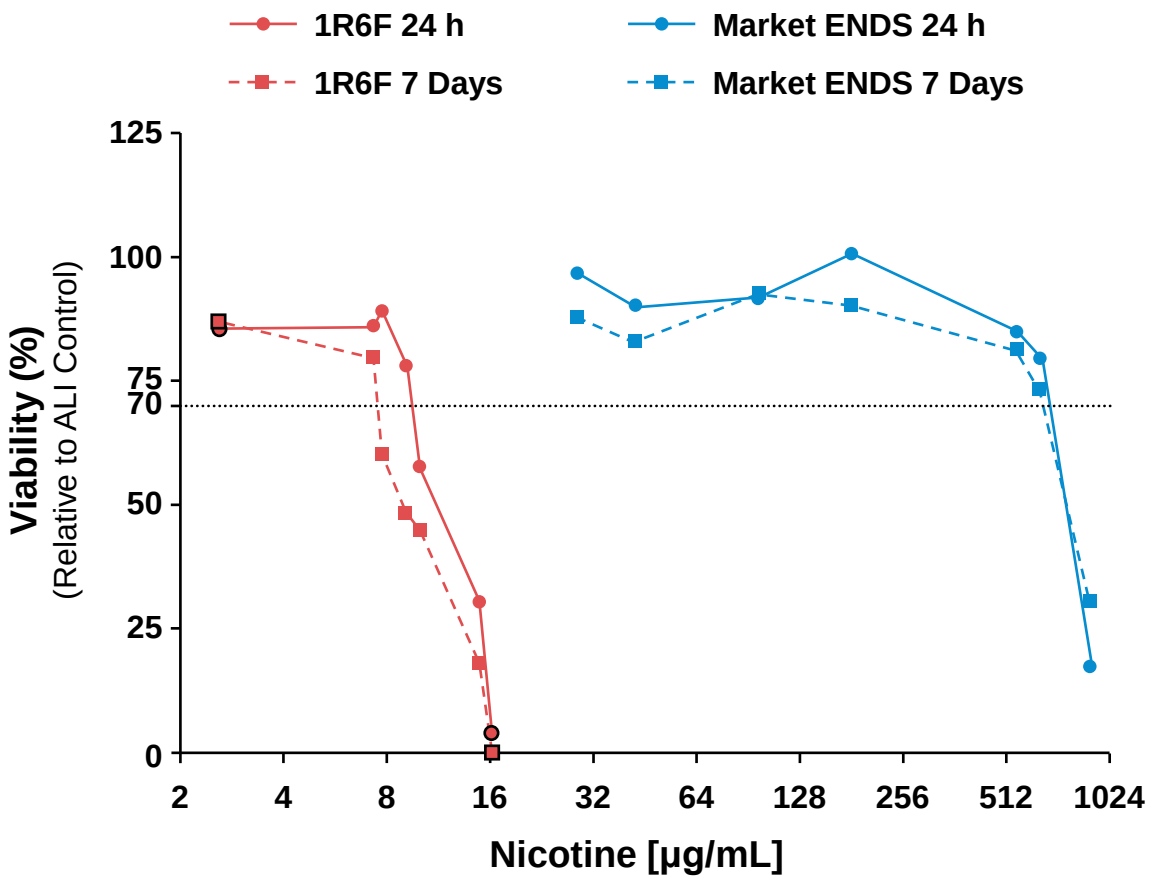
Carbonyls profile:

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

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



Results – Cytotoxicity (WST-8)

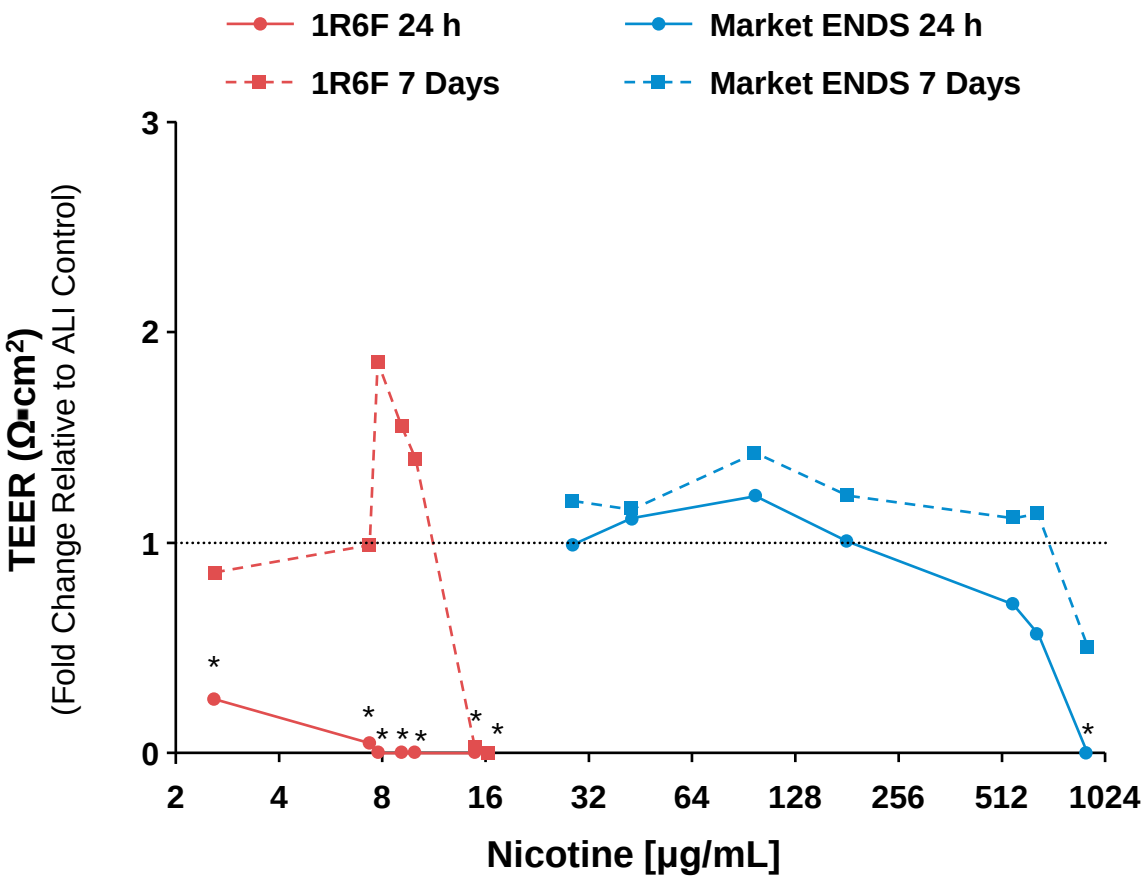


AT BOTH 24 H AND 7 DAYS TIME POINTS, 1R6F **exhibited significantly higher toxicity compared to** ENDS

No differences in recovery were observed for either test product

* Statistical Analysis: Dose-response curves were fitted using a 4PL model in GraphPad Prism (v10.4).

Results – Tissue Integrity (TEER)



AT 24 H

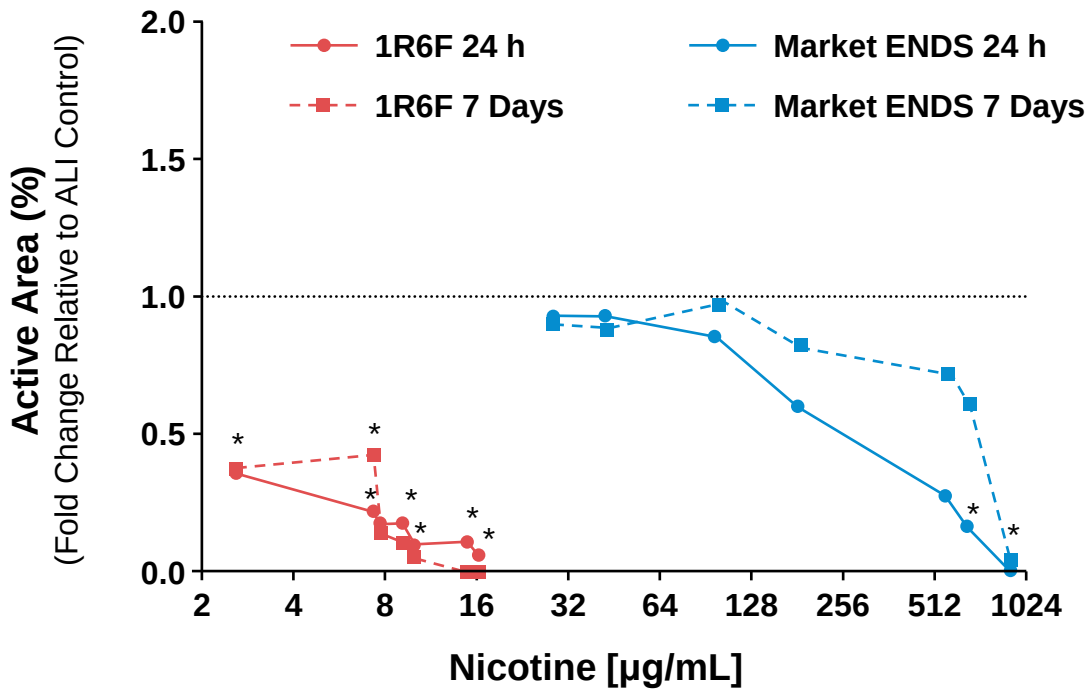
- 1R6F: **near-complete disruption of tissue integrity** (except the lowest concentration)
- ENDS: **minimal disruption** (except the top concentration)

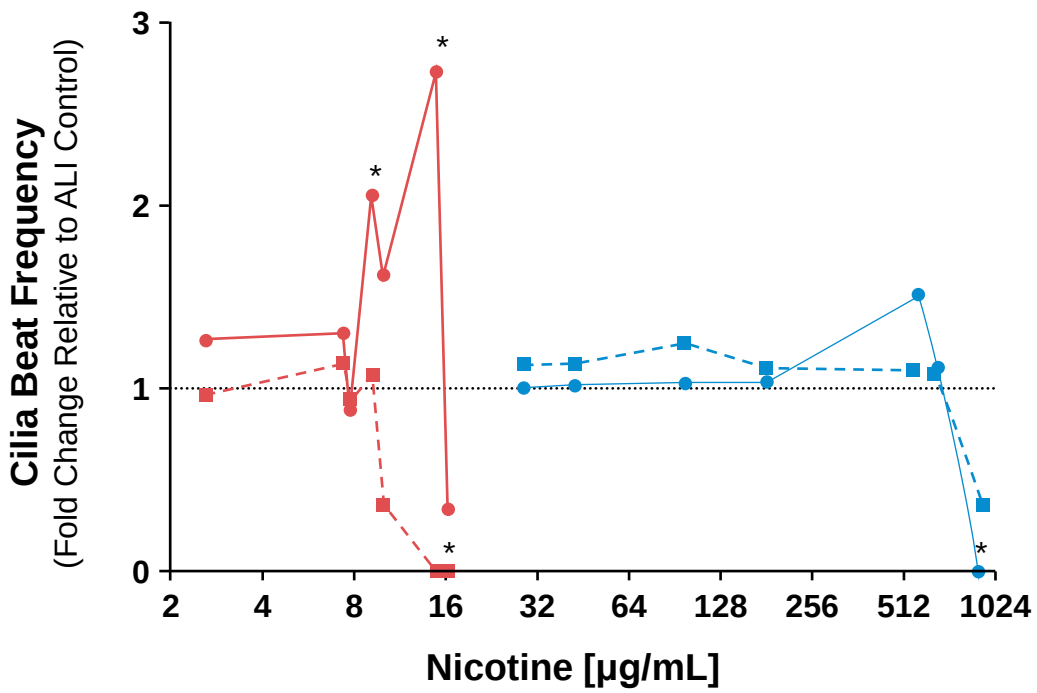
AT 7 DAYS OF RECOVERY

- 1R6F: **recovery** (except the two highest concentrations)
- ENDS: **full overall recovery** (except the highest concentration)

* Statistical Analysis: One-way ANOVA (GraphPad Prism v10.4), Dunnett's multiple comparison test, $p<0.05$..

Results – Ciliary Function (%AA and CBF)





AT 24 H

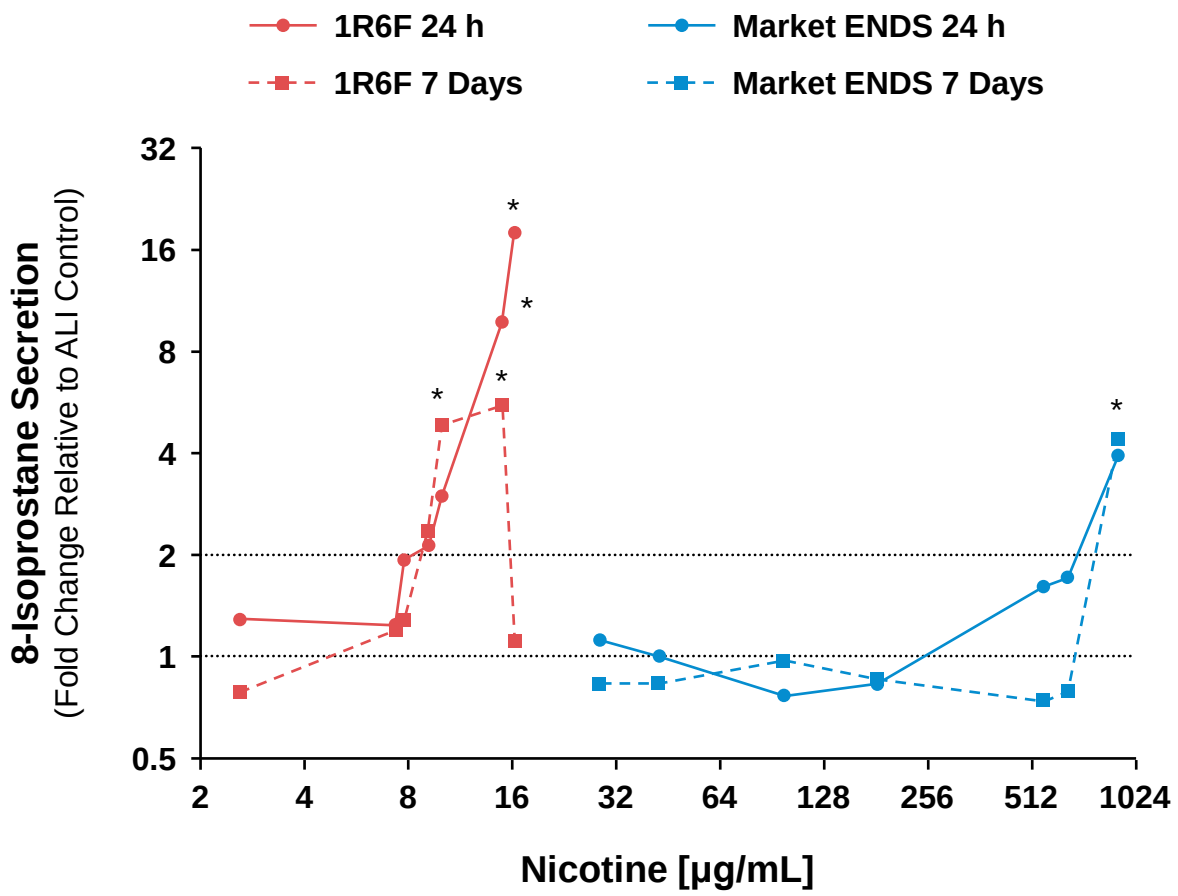
- 1R6F: **severe ciliary disruption**
- ENDS: **partial ciliary disruption**

AT 7 DAYS OF RECOVERY

- 1R6F: **persistent, exacerbated ciliary disruption**
- ENDS: **near-complete recovery,** except the highest concentration

* Statistical Analysis: One-way ANOVA (GraphPad Prism v10.4), Dunnett's multiple comparison test, $p<0.05$.

Results – Oxidative Stress (8-Isoprostane)



AT 24 H

- 1R6F: **significant 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

* Statistical Analysis: One-way ANOVA (GraphPad Prism v10.4), Dunnett's multiple comparison test, $p<0.05$..

Toxicity Potency Comparison Between 1R6F and ENDS

ENDS aerosols are significantly less toxic than 1R6F cigarette smoke

1R6F smoke caused widespread, persistent damage and limited recovery, while ENDS aerosols allowed for substantial and largely complete tissue recovery

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

Statistical Analysis: One-way ANOVA (GraphPad Prism v10.4), Dunnett's multiple comparison test, $p<0.05$..

Conclusion

- 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 aligns 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

THANK YOU!

Questions