Oral Toxicity Evaluation of Tobacco Products in a Buccal Epithelial-Fibroblast Co-culture Tissue Model

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Agenda

- Introduction
- Objective & Experimental Design
- Results
- Conclusions

Tobacco Harm Reduction Framework

CONSTITUENT REDUCTION

Product Design and Control

Chemical and Physical Characterization

THE PRODUCT

- Chemistry Manufacturing and Controls
- Product Stability
- Chemical Characterization

INDIVIDUAL RISK REDUCTION

Toxicology and Risk Assessment

Studies in Adult Human Subjects

EXPOSURE and HEALTH RISK

- Toxicology & Risk Assessment
- Health Risk assessment (Absolute and Relative)
- Human Studies
- Human Factors Assessment

POPULATION HARM REDUCTION

Perception and Behavior Assessment

Risks and Benefits to Health of the Population

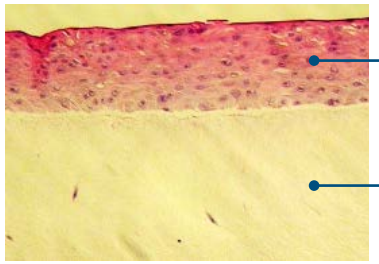
IMPACT on the POPULATION

- Risk Perceptions (Absolute and Relative)
- Impact of Product on Users
- Impact on Non-Users
- Overall Impact on the Population
- Environmental Assessment

Study Objective

OBJECTIVE: Develop an in vitro oral testing platform with enhanced biological relevance for evaluating and differentiating the toxicity of tobacco products

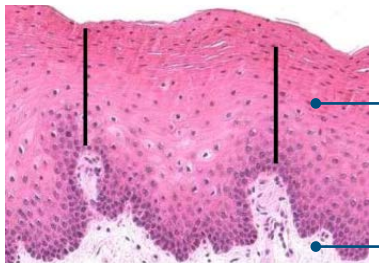
ORL-300 FT



Buccal epithelium

Fibroblasts/ Lamina Propria

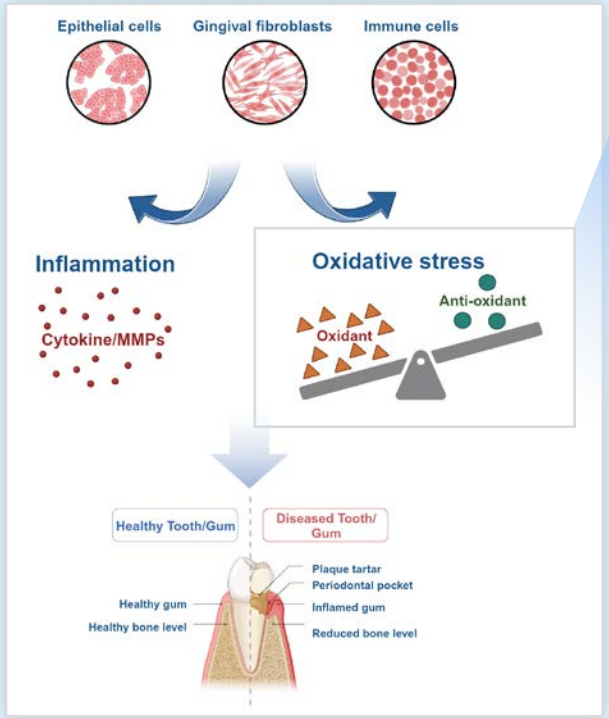
Human Explant

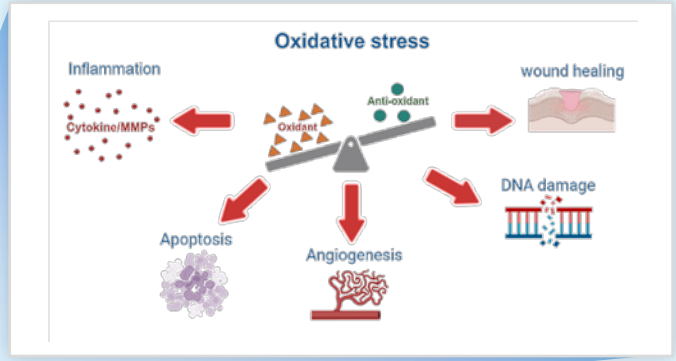


Buccal epithelium

Fibroblasts/ Lamina Propria

Klausner, M. et al. *Toxicology in Vitro* 21, 938-949 (2007)





ENDPOINTS

- Oxidative stress
- Inflammatory responses
- Cytotoxicity
- DNA damage
- Morphological changes

Experimental Design

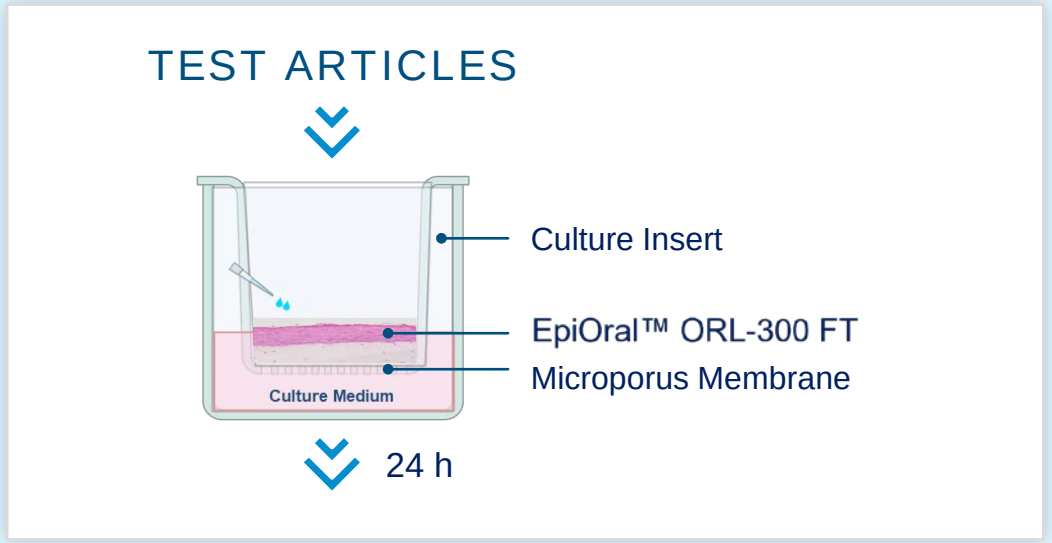
TEST ARTICLES (TA)

- CRP1.1 (CORESTA snus reference)
- CRP2.1 (CORESTA moist snus reference)
- Market NP (cinnamon-flavor, 3 mg)
- 1R6F reference tobacco condensate in ethanol

TEST CONCENTRATIONS

- Oral products: 20%, 40%, 60%, 80%, 100% (v/v) complete artificial saliva (CAS) extract
- 1R6F: 1.5%, 4%, 6%, 8%, 10% (v/v) ethanol condensate

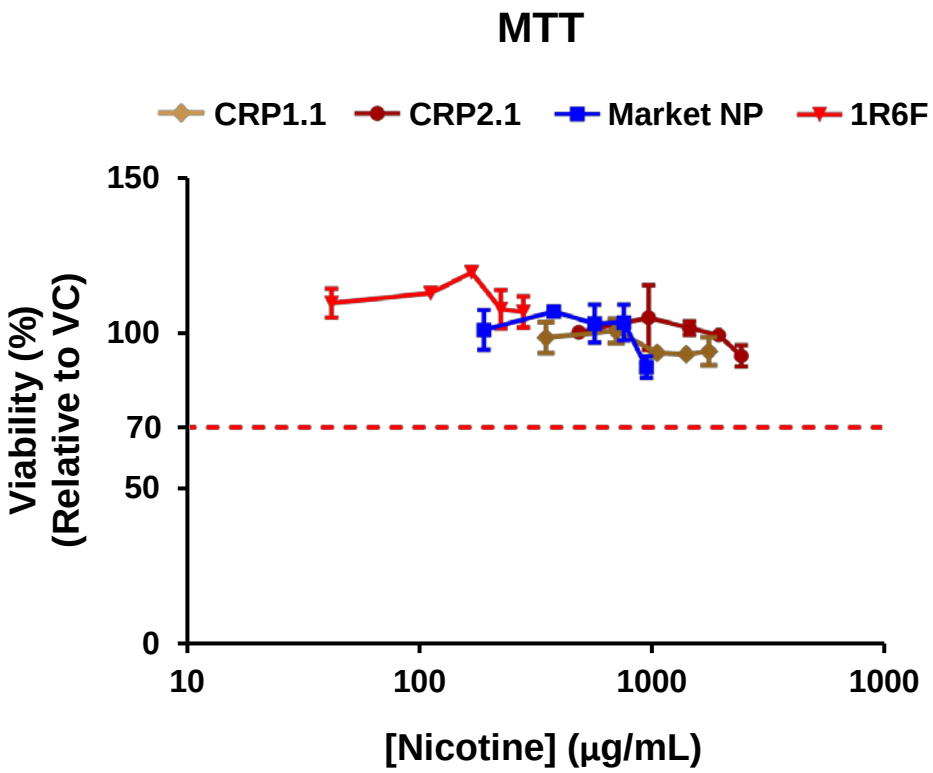
	[Nicotine] (µg/mL ± SD)	
Test Article	**Lowest**	**Highest**
CRP1.1 CAS extract	356 ± 4	1,779 ± 20
CRP2.1 CAS extract	491 ± 2	2,454 ± 10
Market OTDN CAS extract	192 ± 3.2	960 ± 16
1R6F EtOH condensate	42.6 ± 0.11	284 ± 7

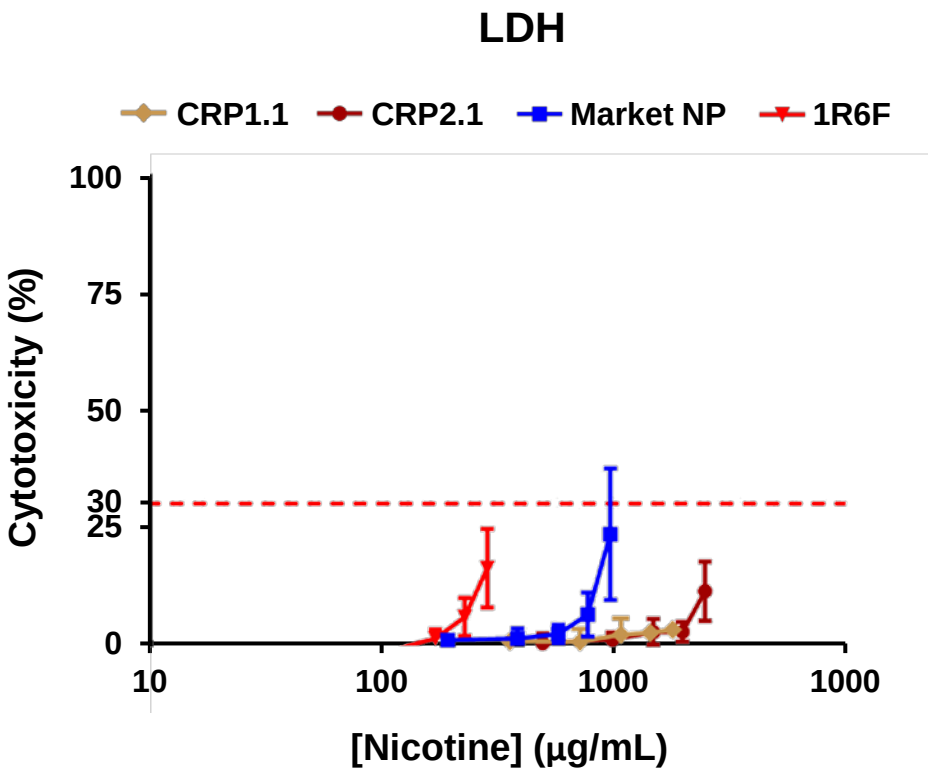


ENDPOINTS

- **Oxidative stress:** 8-isoprostane
- **Inflammatory responses:** cytokine secretion
- **Cytotoxicity:** MTT, LDH
- **DNA damage:** γH2Ax
- **Morphology:** H&E, Ki67, fibronectin, MMP secretion

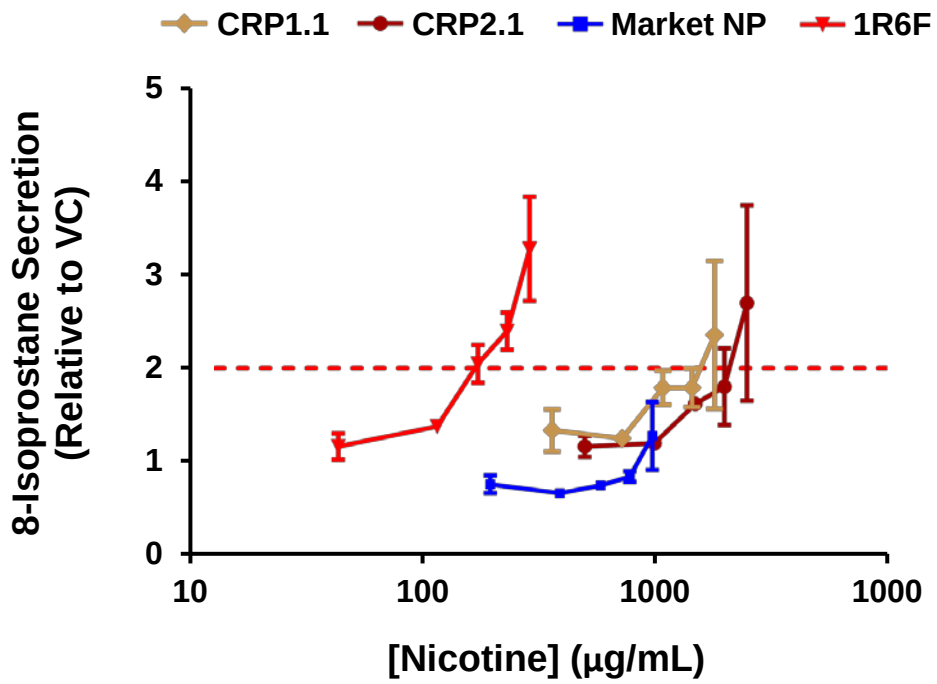
Results – Cytotoxicity





CONCLUSION: None of the TAs is cytotoxic based on both MTT and LDH assays

Results – Oxidative Stress by 8-Isoprostane



CONCLUSIONS:

- CRP1.1, CRP2.1, and 1R6F significantly increased 8-isoprostane secretion
- The Market NP moderately increased 8-isoprostane secretion, but less than 2-fold

Results – Cytokine Functions

Cytokine	Impact	Implication in Oral Diseases
Pro-Inflammatory		
TNF-α	Tissue destruction, immune activation	Increased in periodontitis, linked to tissue destruction and bone resorption
IL-6	Chronic inflammation, bone resorption	Increased in periodontitis & oral cancer, chronic inflammation
IL-8	Neutrophil recruitment, tissue damage	
Anti-Inflammatory		
IL-10	Anti-inflammatory, protective	Decrease linked to unchecked inflammation in periodontitis, oral lichen planus
Growth Factor		
GM-CSF	Immune cell recruitment	Elevated in oral inflammation
G-CSF	Neutrophil support, mucosal healing	Involved in periodontitis by inducing bone resorption and tissue destruction
VEGF	Angiogenesis in tumors	Increased in oral cancer
Chemokine		
Eotaxin	Eosinophilic inflammation	Involved in oral lichen planus and periodontitis
IP-10	T-cell recruitment, chronic inflammation	Elevated in chronic inflammatory oral conditions

Results – Cytokine Secretion

Cytokine	FOLD CHANGE CRP1.1	CRP2.1	Market NP	1R6F
Pro-Inflammatory				
TNF-α	1.7 – 39.1	1.1 – 49.9	1.4 – 3.5	1.2 – 2.8
IL-6	1.8 – 11.3	1.9 – 14.1	1.8 – 2.9 (4.7)*	2.8 – 4.6 (6.3)*
IL-8	1.2 – 6.5 (7.3)*	1.0 – 8.0	1.1 – 2.9	1.5 – 1.4 (2.2)*
Anti-Inflammatory				
IL-10	1.1 – 2.1	0.9 – 1.9	0.8 – 0.9	0.9 – 0.7
Growth Factor				
GM-CSF	1.3 - 4.9	1.0 – 5.0	1.3 – 3.4	1.0 – 1.1 (1.4)
G-CSF	1.0 – 2.7	1.0 – 3.1	0.9 – 1.5	1.6 – 0.6 (2.6)*
VEGF	1.3 – 2.2	1.3 – 2.5	1.3 – 1.2 (1.6)*	1.1 – 0.7
Chemokine				
Eotaxin	1.3 – 4.4	1.0 – 6.9	0.5 – 2.0	2.0 – 0.7
IP-10	1.0 – 2.4	0.7 – 2.5	0.6 – 0.8	0.8 – 0.5

Markedly induced by all four TAs

Moderately upregulated by CRP1.1 and possibly CRP2.1

Upregulated, but mostly by CRP1.1 and CRP2.1

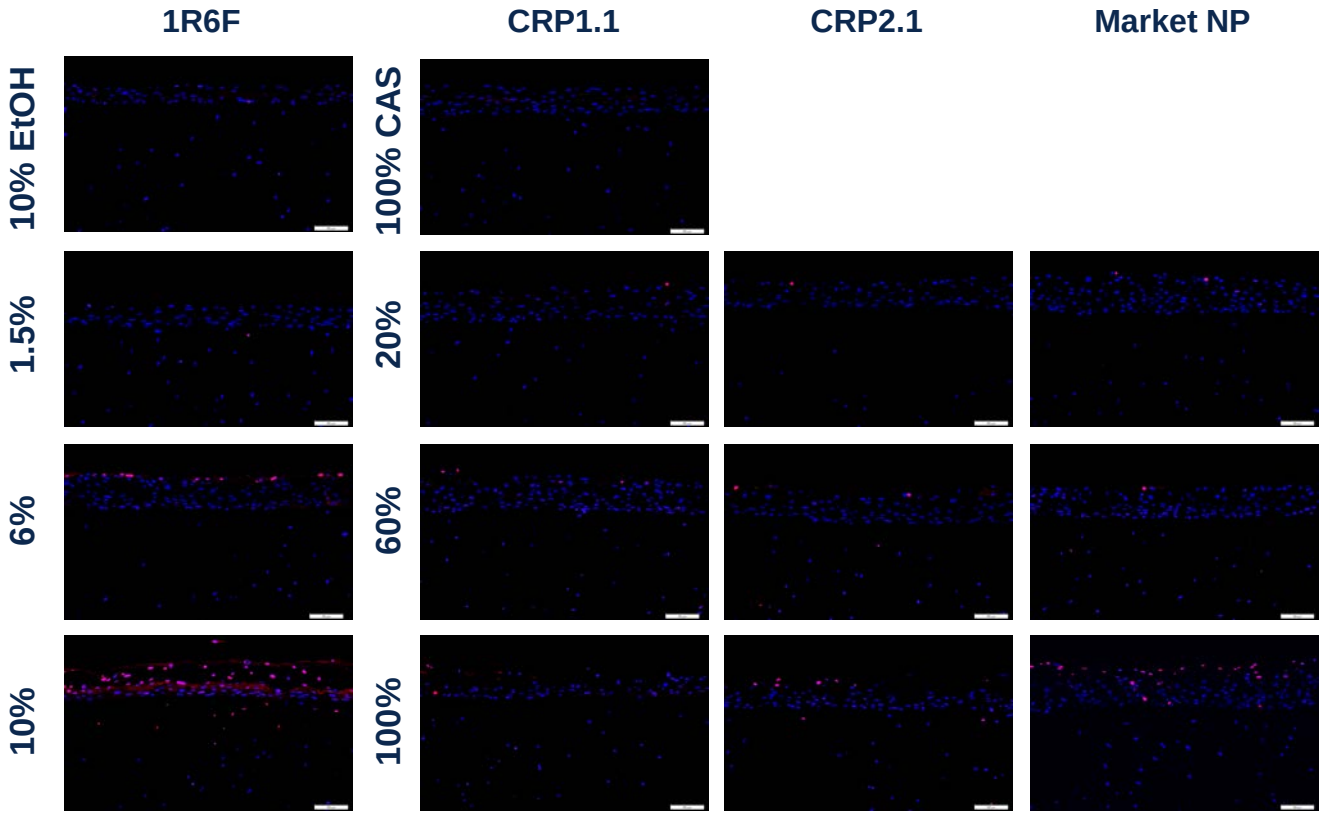
*indicates the peak responses.
Red: Indicates greater than 2-fold increase; Light green: Indicates ~50% decrease.

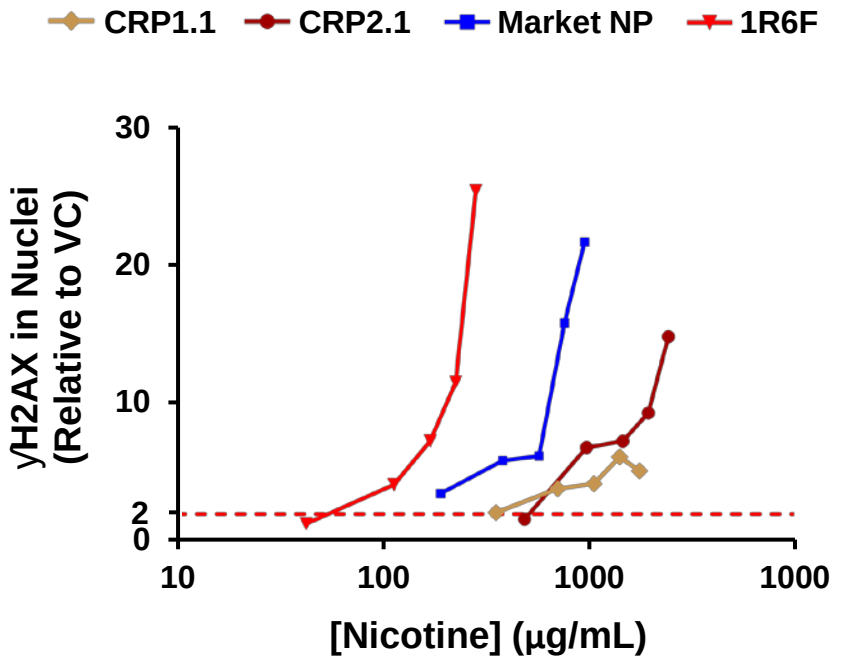
CONCLUSION:

Overall, the data demonstrate clear modulation of cytokine secretion by these TAs

These findings highlight variability in cytokine profiles among the TAs, suggesting distinct inflammatory pathways activated by each TA

Immunohistochemistry (IHC) Assessment – γH2AX

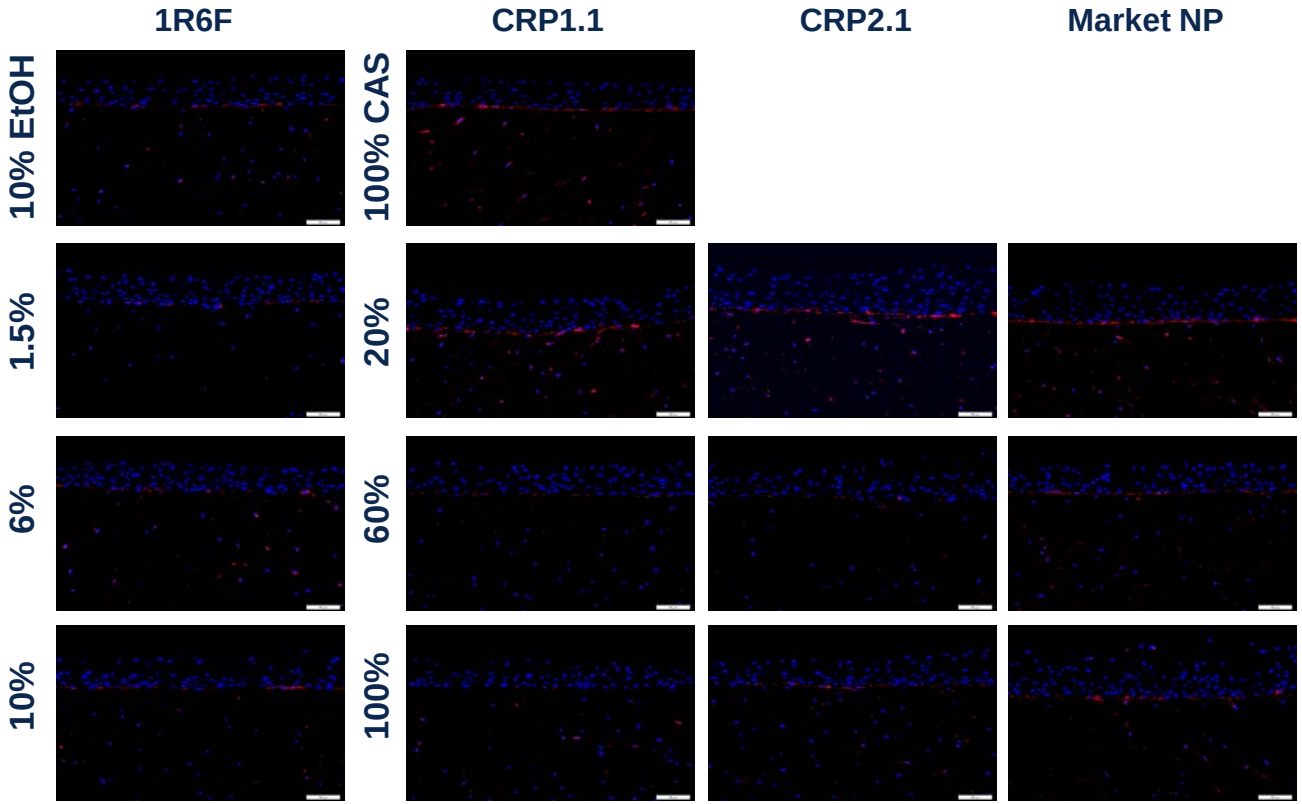


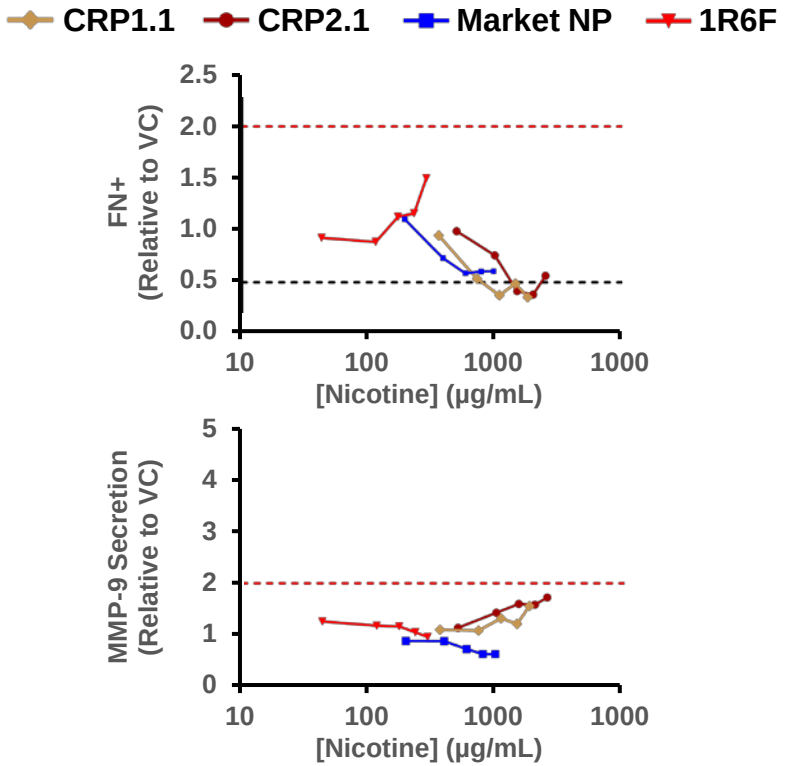


CONCLUSIONS:

- γH2AX staining was present in both epithelium and fibroblast in 1R6F-treated cultures
- All four TAs significantly induced the expression of γH2AX
- The responses between CRP1.1 and CRP2.1 mostly overlapped

IHC Assessment – Extracellular Matrix (ECM) Modulation





CONCLUSIONS

- Fibronectin was primarily expressed in fibroblasts
- 1R6F caused a slight increase in fibronectin expression
- All three oral products reduced fibronectin expression, with CRP1.1 and CRP2.1 decreasing levels by > 50%
- CRP1.1 and CRP2.1 moderately increased MMP-9 secretion

Conclusions

Exhibited similar response profiles

TA	Oxidative stress	Pro-inflammatory	Anti-inflammatory	Growth factor	Chemokine	DNA damage	ECM	
							FN	MMP-9
CRP1.1	↑	↑	↑	↑	↑	↑	↓	↑
CRP2.1	↑	↑	↑	↑	↑	↑	↓	↑
Market NP	↑	↑	—	↑	↑	↑	↓	—
1R6F	↑	↑	—	↑	↑	↑	↑	—

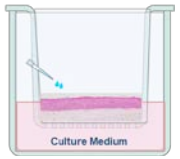
The size of the arrows indicates a magnitude of response (not drawn to scale).

All induced pro-inflammatory cytokines

All triggered DNA damage

- Collectively, the data indicate that CRP1.1 and CRP2.1 may promote more extensive inflammation and tissue remodeling compared to Market NP and 1R6F, which are primarily associated with acute pro-inflammatory and DNA-damaging effects

- The observed increase in FN by 1R6F indicates potential fibrotic changes, while the interplay between FN and MMP-9, as modulated by CRP1.1 and CRP2.1 suggests possible ECM degradation



ORL-300 FT offers an advanced, biologically relevant in vitro platform for the comprehensive assessment of toxicity mechanisms induced by tobacco products