

A close-up, high-contrast photograph of a microscope's objective lenses and eyepiece, rendered in a monochromatic blue color. The lenses are positioned diagonally across the frame, with some showing technical markings like '10/0.25' and '160/0.17'.

Contemporary *in vitro* Toxicology Assessment of a Novel Herbal Heated Product (HHP) Compared to Cigarette Smoke

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Role of Heated Products for Tobacco Harm Reduction



THR

Tobacco Harm Reduction (THR) Initiative

Encourage adult smokers, who would otherwise continue to smoke combustible tobacco products like cigarettes, to switch to scientifically substantiated alternatives with reduced risk profile



Smokeless Products

Smokeless Products

Encourage adult smokers, who would otherwise continue to smoke, to switch to scientifically-substantiated alternatives with a reduced risk profile



HTP

Heated Tobacco Products (HTP)

Tobacco rod is heated rather than combusted, which reduces the toxicant content of the aerosol *

* Comparison with smoke from a scientific standard reference cigarette (approximately 9mg of tar) in terms of the average of the 9 harmful components the World Health Organization recommends to reduce in cigarette smoke. Exclusively for discussion purposes. Should not be copied or shared without prior consent. Not intended as promotional materials

Herbal Heated Products: a new product category with potential to support THR



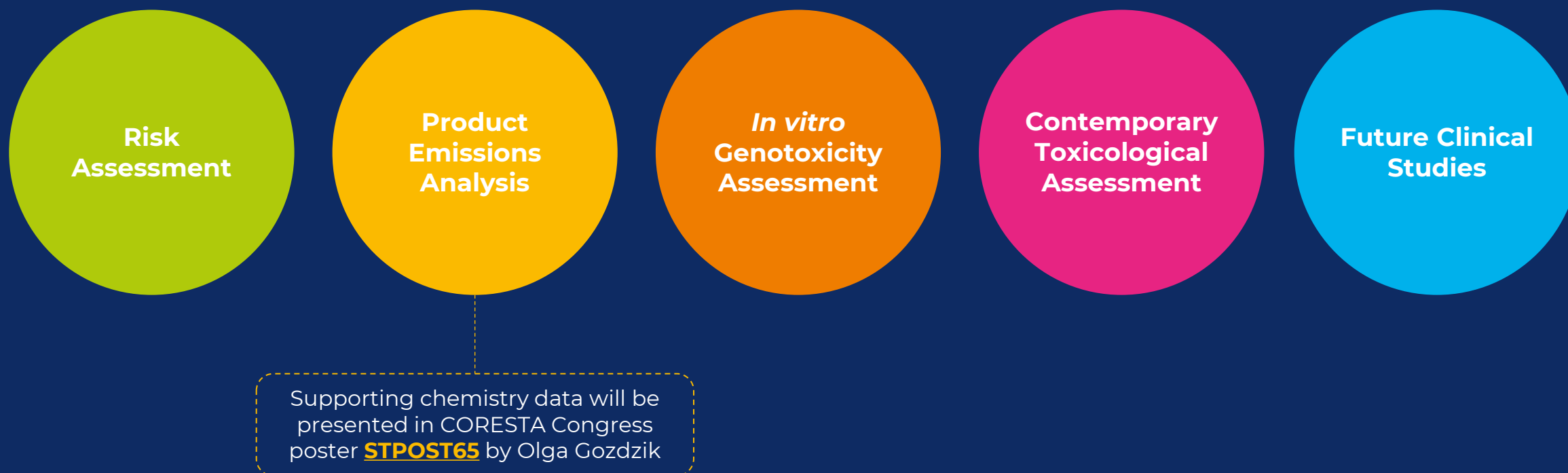
HHP

Herbal Heated Products (HHPs)

- Tobacco-free, contains herbal material (a novel rooibos substrate) with added nicotine and flavourings

Herbal Heated Products: a new product category with potential to support THR

Integrated Testing Approach



Herbal Heated Products: a new product category with potential to support THR

Integrated Testing Approach

Risk
Assessment

Product
Emissions
Analysis

In vitro
Genotoxicity
Assessment

Contemporary
Toxicological
Assessment

Future Clinical
Studies

Contemporary Toxicological Assessment Testing Approach

Test Products

Combustible

1R6F Reference



Device

glo™ Hyper X2



Herbal Heated Product (HHP)

Veo™ Scarlet Click



Veo™ Sunset Click



Test Matrices

Aqueous Aerosol Extract



AqE

Whole Aerosol

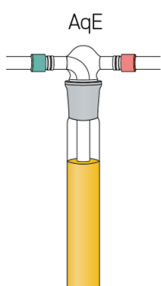


WA

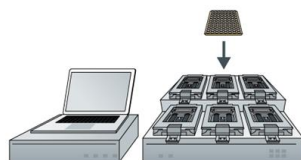
Assays

2D

Test Matrix

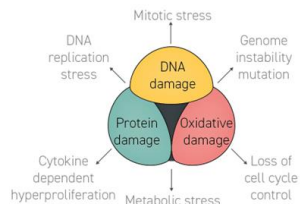


Endpoints assessed



RTCA
(cytotoxicity)

Normalised Cell Index * Hour (%)



ToxTracker™
(genotoxicity)

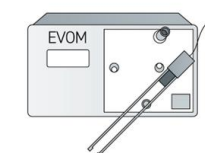
GFP Induction (fold)

3D

Test Matrix

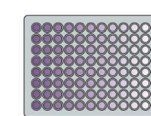


Endpoints assessed



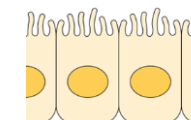
TEER
(barrier integrity)

TEER (Ω)



MTT
(cytotoxicity)

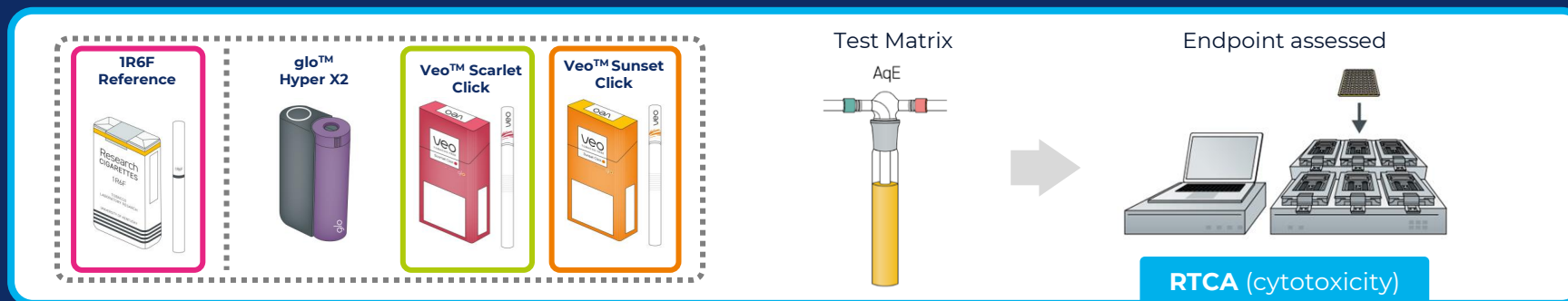
Cell Viability
(% Air Control)



Cilia function

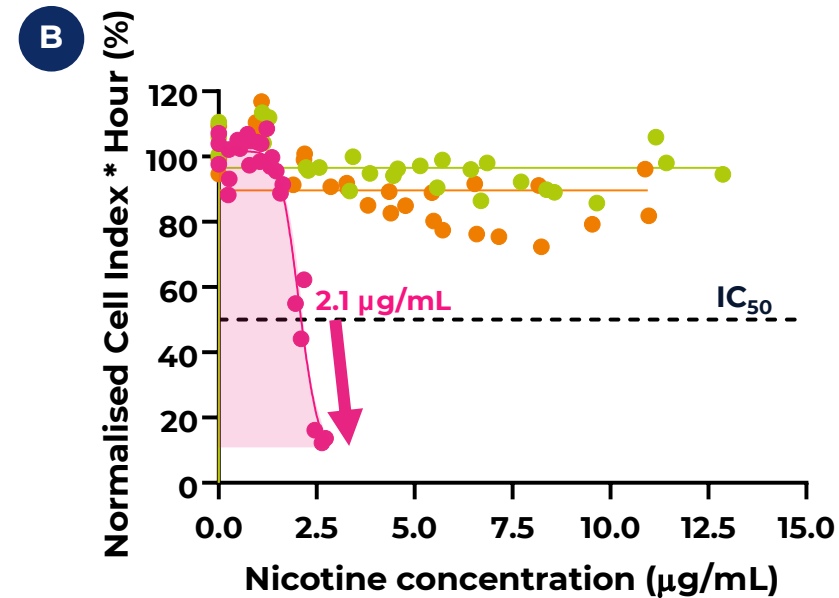
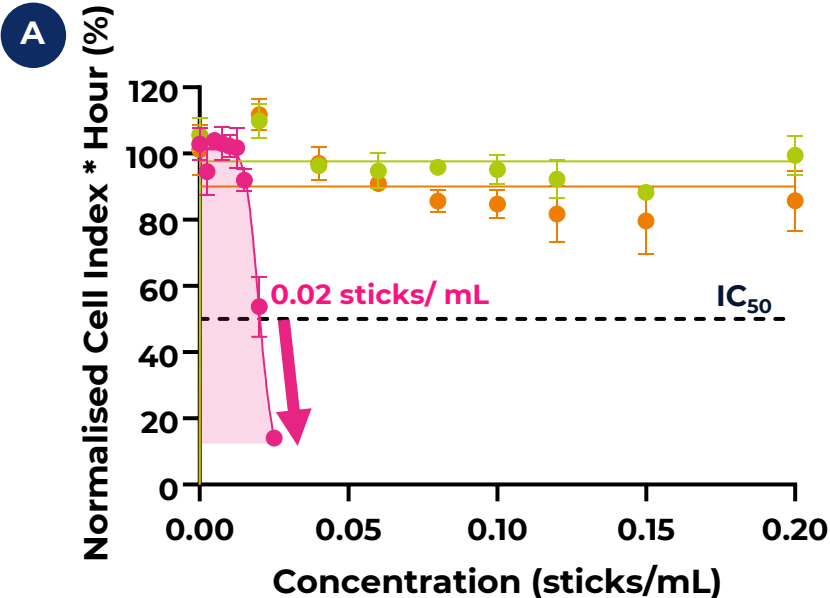
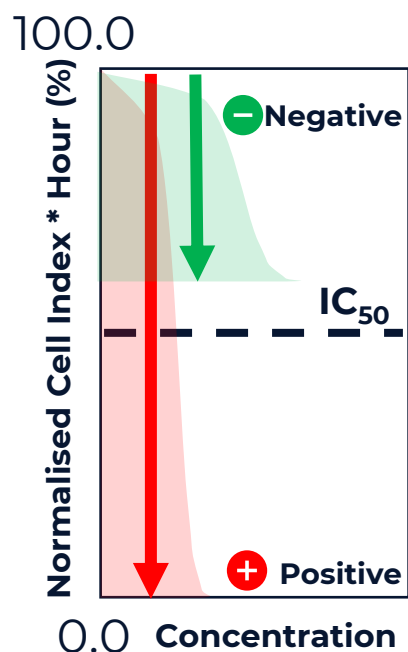
Cilia Active Area (%)
Cilia Beat Frequency (Hz)

2D Testing Approach: RTCA Cytotoxicity



2D

Response for Cytotoxicity



—●— 1R6F —●— Veo™ Scarlet Click —●— Veo™ Sunset Click

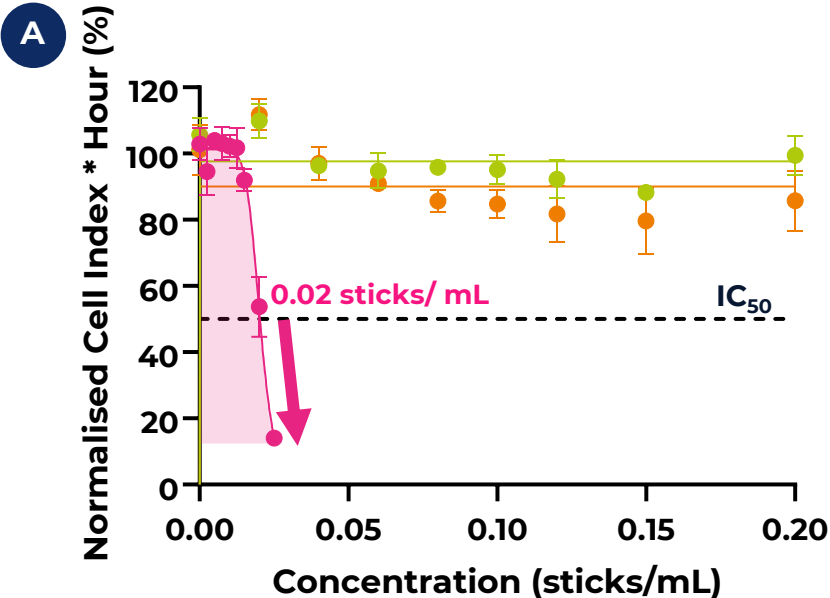
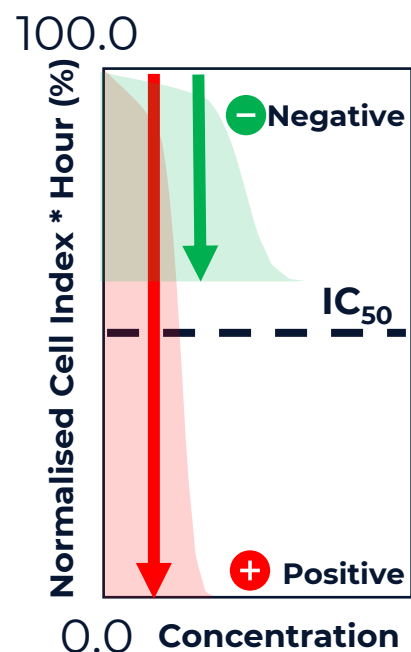
* Dashed line is the IC₅₀ - Half maximal inhibitory concentration

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2D Testing Approach: RTCA Cytotoxicity

Near complete toxicity curve for 1R6F samples was observed, whereas for HHP samples an IC_{50} calculation was not possible

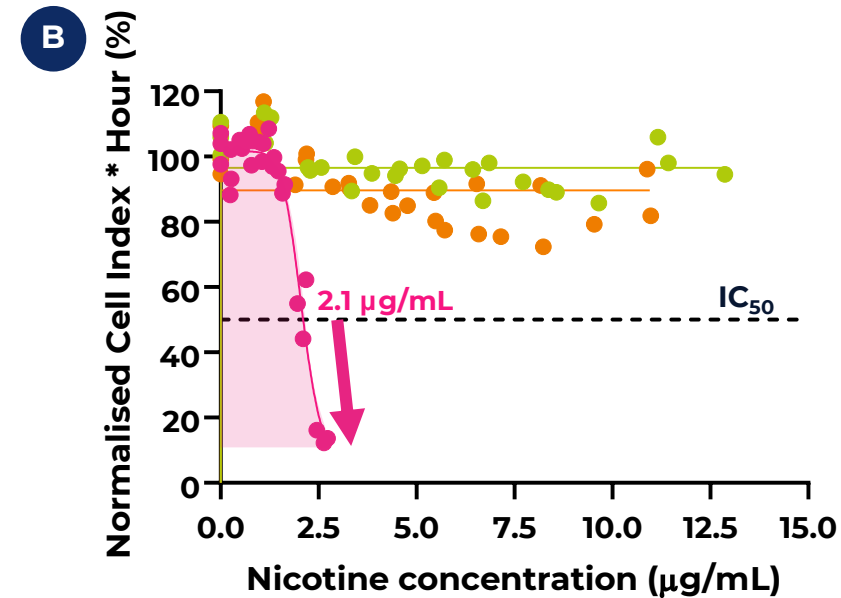
Response for Cytotoxicity



1R6F

Veo™ Scarlet Click

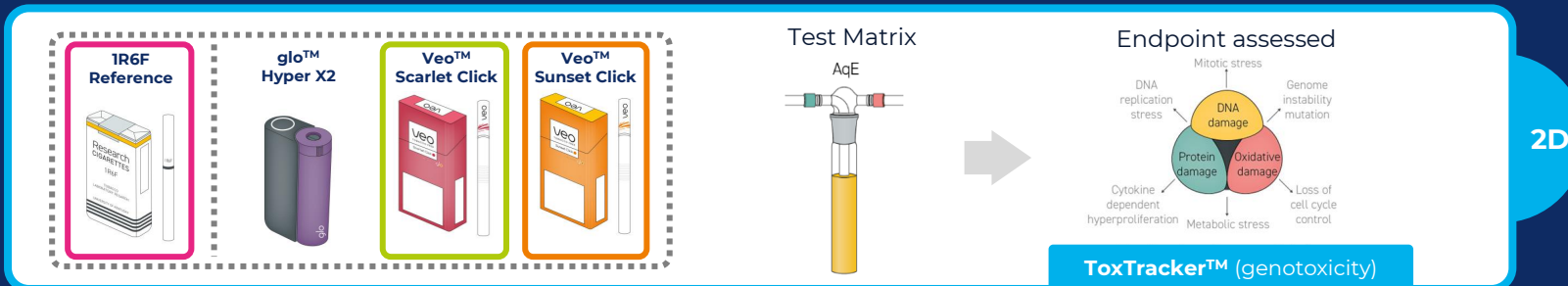
Veo™ Sunset Click



* Dashed line is the IC_{50} - Half maximal inhibitory concentration

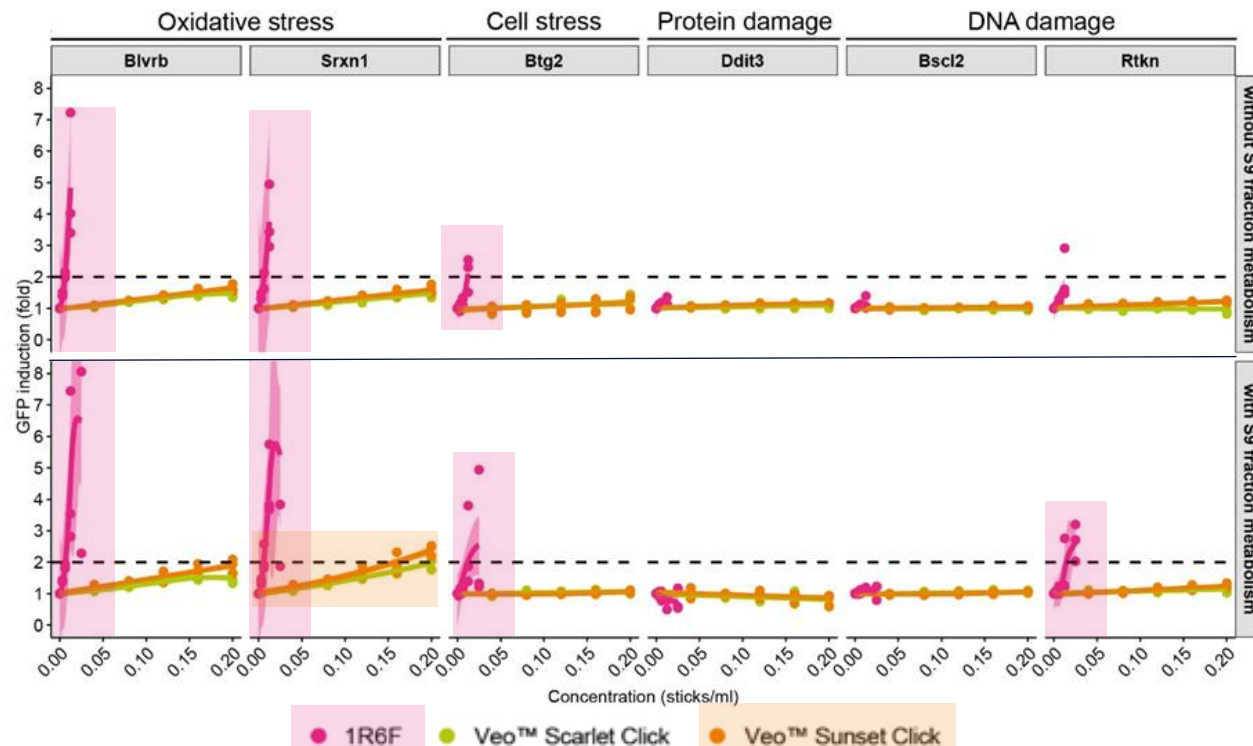
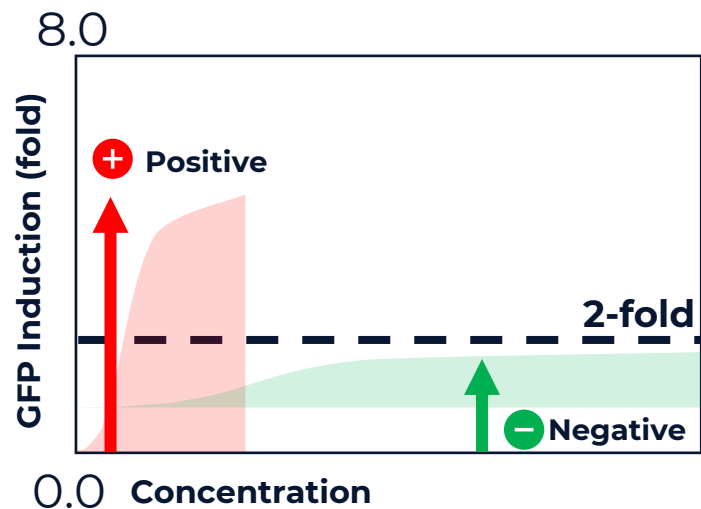
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2D Testing Approach: ToxTracker™ Genotoxicity



i Data shown are capped at **75% cytotoxicity**

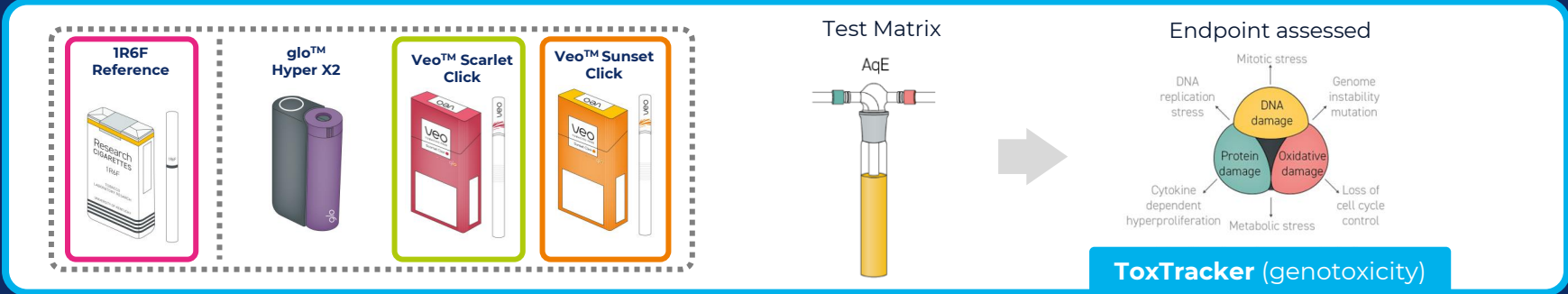
Response for ToxTracker™



* Dash line is the 2-fold threshold

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2D Testing Approach: ToxTracker™ Genotoxicity



ToxTracker™ Endpoint												
Test Products	Oxidative stress				Cell stress		Protein damage		DNA damage			
	Blvr1		Srxn1		Btg2		Didt3		Bsc12		Rtkn	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1R6F cigarette	Red	Red	Red	Red	Red	Red	Green	Green	Green	Green	Yellow	Red
Veo™ Scarlet Click	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Veo™ Sunset Click	Green	Green	Green	Red/White	Green	Green	Green	Green	Green	Green	Green	Green

**Negative**
Average GFP fold change <2

**Equivocal**
Average GFP fold change ≈2

**Positive**
Average GFP fold change >2
Equal potency to 1R6F

**Positive**
Average GFP fold change >2
Less potent than 1R6F

2D Testing Approach: ToxTracker™ Genotoxicity

- HHP variants showed a markedly reduced response compared to 1R6F
- One of the HHP variants showed a positive oxidative stress response (Srxn1, Nrf2-dependent) which was less potent than 1R6F

Test Products	ToxTracker™ Endpoint											
	Oxidative stress				Cell stress		Protein damage		DNA damage			
	Blvrbl		Srxn1		Btg2		Didt3		Bsc12		Rtkn	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1R6F cigarette	Red	Red	Red	Red	Red	Red	Green	Green	Green	Green	Yellow	Red
Veo™ Scarlet Click	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Veo™ Sunset Click	Green	Green	Green	Red/White Stripes	Green	Green	Green	Green	Green	Green	Green	Green



Negative
Average GFP fold change <2



Equivocal
Average GFP fold change ≈2

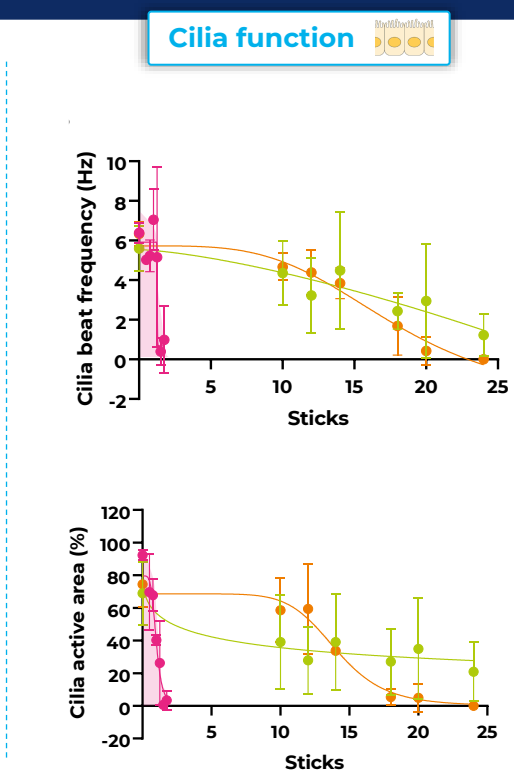
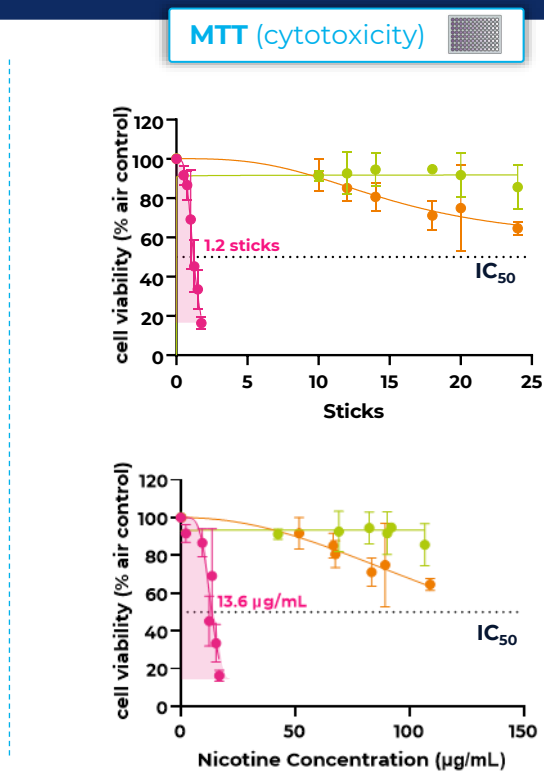
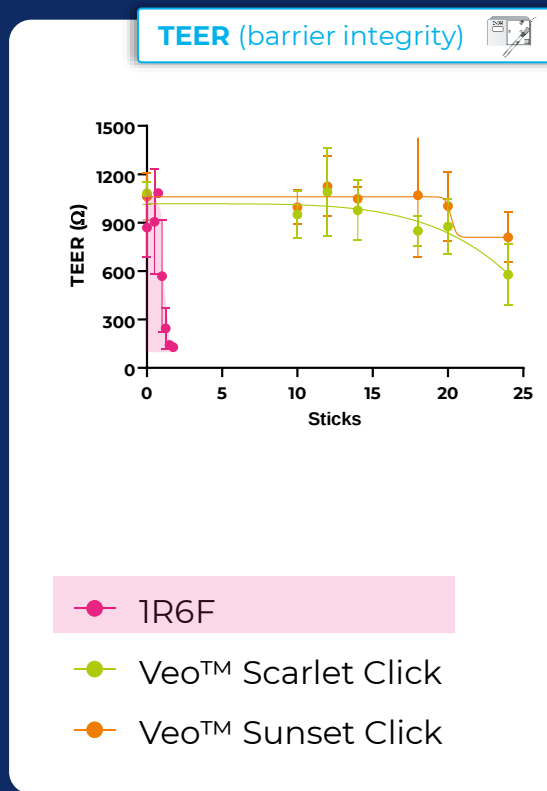
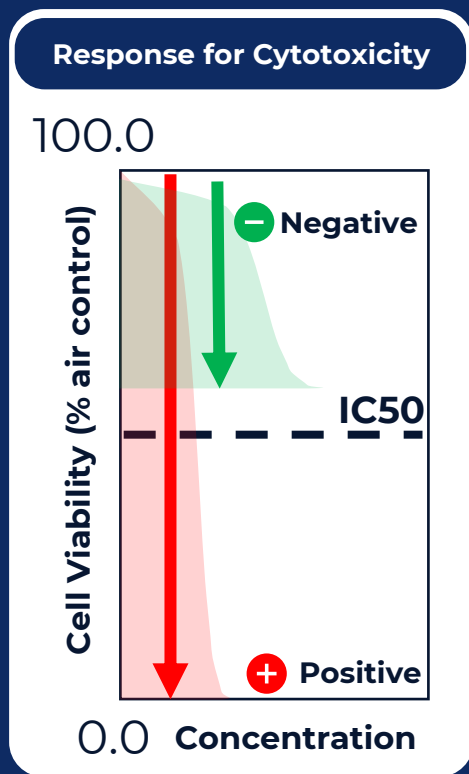
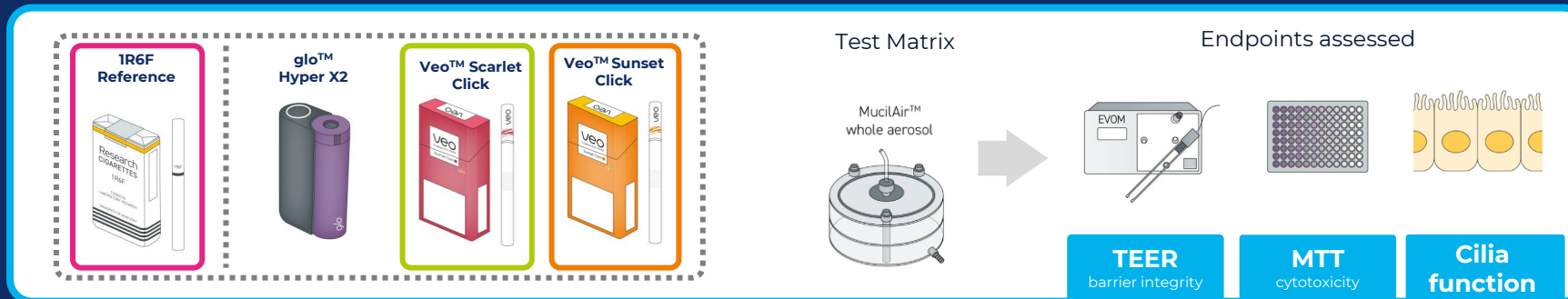


Positive
Average GFP fold change >2
Equal potency to 1R6F



Positive
Average GFP fold change >2
Less potent than 1R6F

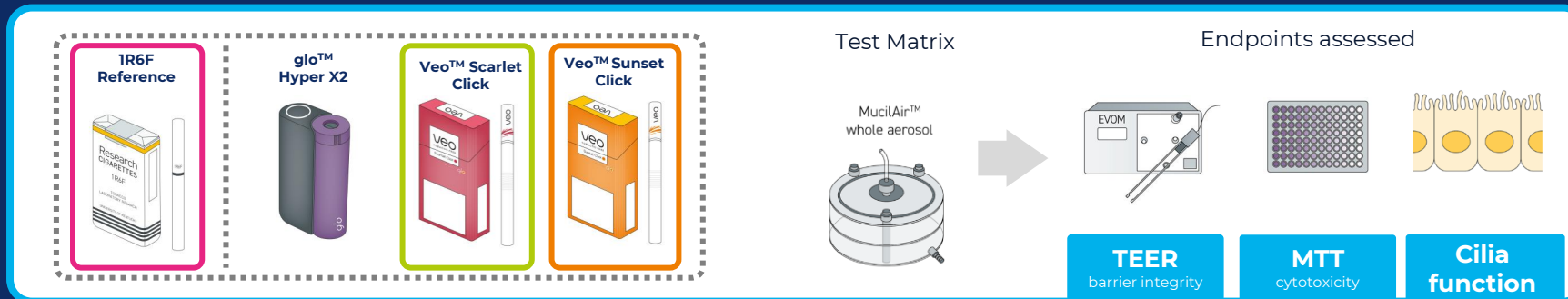
3D Testing Approach: MucilAir™ WA



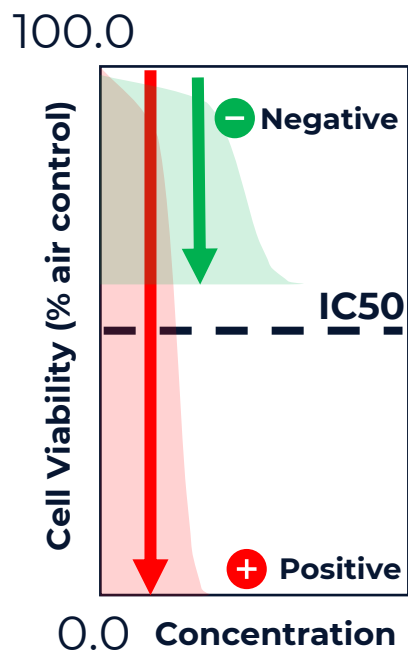
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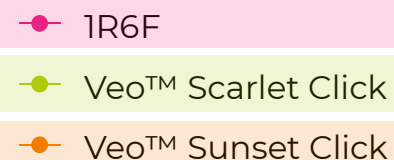
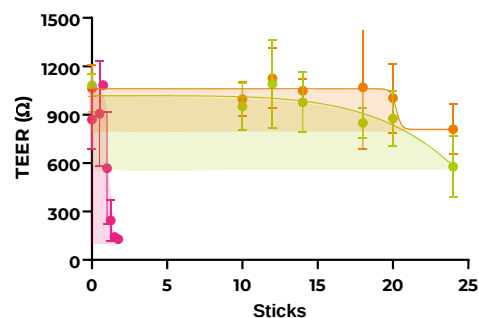
3D Testing Approach: MucilAir™ WA



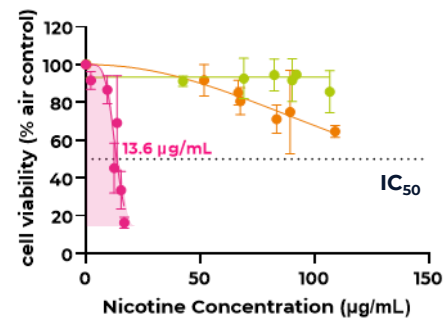
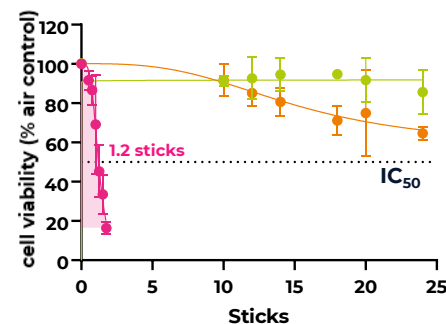
Response for Cytotoxicity



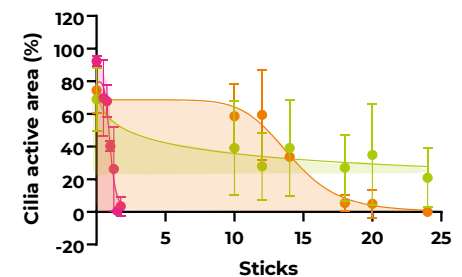
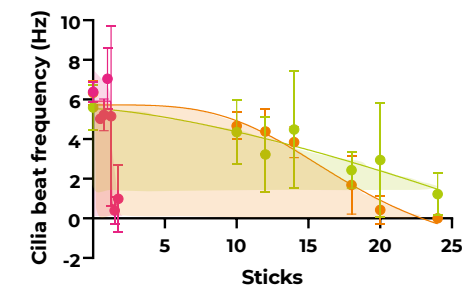
TEER (barrier integrity)



MTT (cytotoxicity)



Cilia function



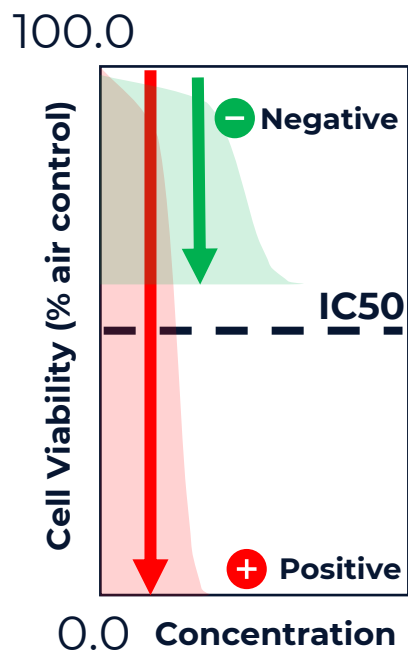
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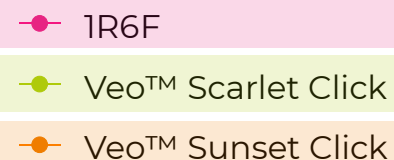
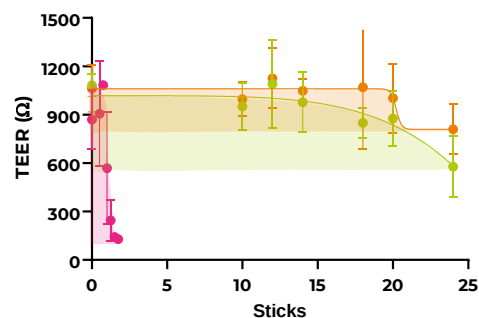
3D Testing Approach: MucilAir™ WA

- 1R6F showed a strong cytotoxic response
- HHP variants showed a markedly reduced response compared to 1R6F
- HHP variants showed some loss of barrier integrity, cilia function and number indicating some pre-cytotoxic changes have occurred

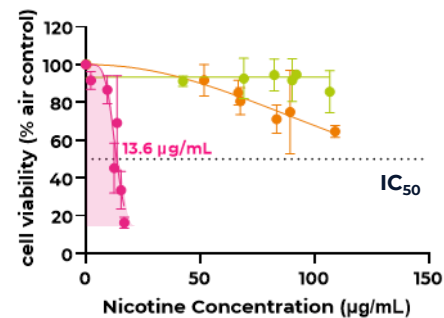
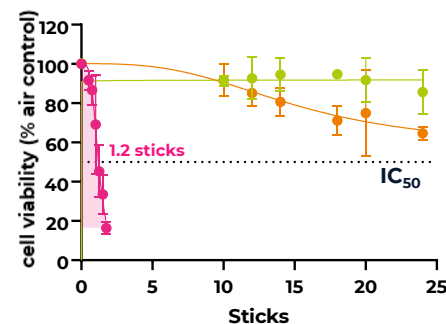
Response for Cytotoxicity



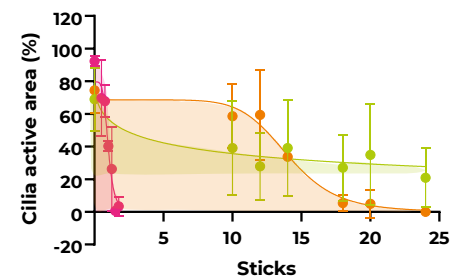
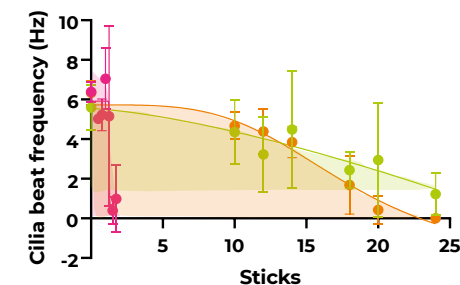
TEER (barrier integrity)



MTT (cytotoxicity)



Cilia function



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Conclusions

- 1**
In all assays conducted HHP variants showed reductions in cytotoxicity and genotoxicity compared to 1R6F research cigarette
- 2**
Independent of aerosol matrix used (aqueous aerosol extract or whole aerosol) HHP variants showed reductions in cytotoxicity and genotoxicity compared to 1R6F research cigarette
- 3**
The data presented here demonstrate the potential of HHPs as a product with a reduced risk profile in comparison to combustible tobacco products



Thank You & Questions?

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