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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25 October 2024

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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



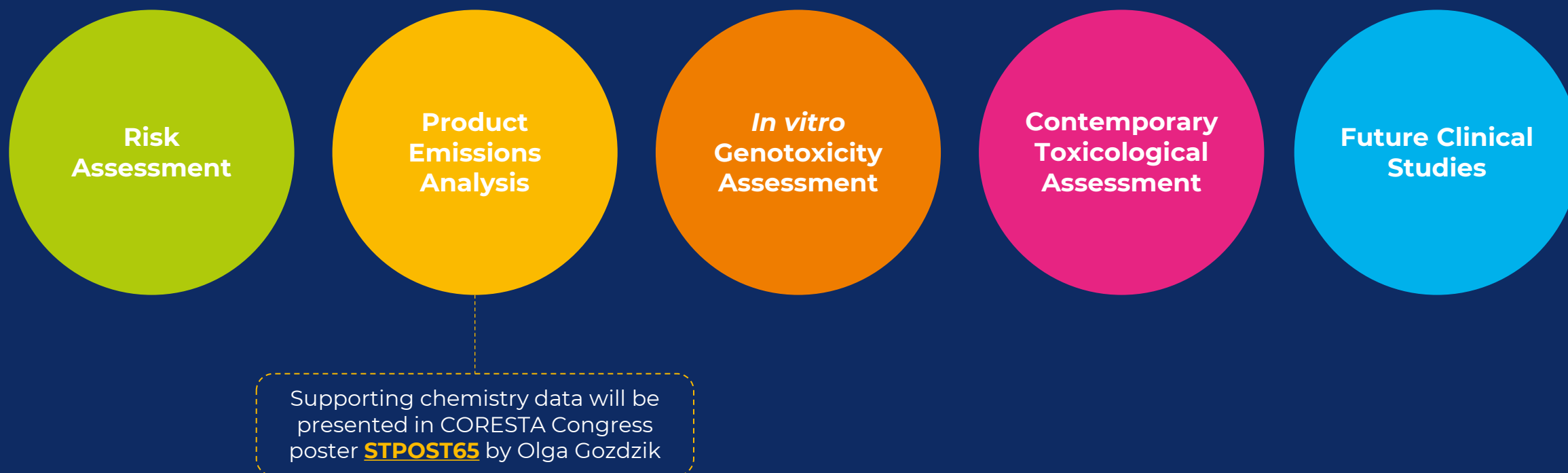
**HHP**

### **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

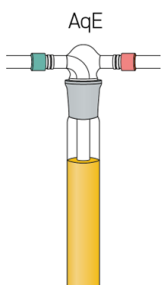


WA

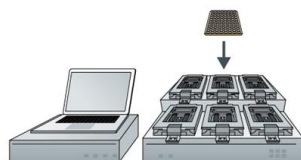
## Assays

2D

Test Matrix

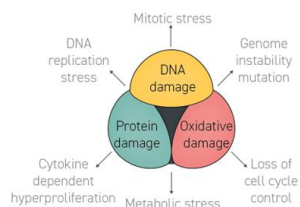


Endpoints assessed



**RTCA**  
(cytotoxicity)

Normalised Cell Index \* Hour (%)



**ToxTracker™**  
(genotoxicity)

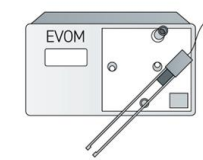
GFP Induction (fold)

3D

Test Matrix

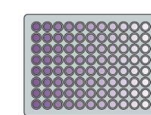


Endpoints assessed



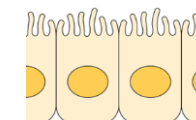
**TEER**  
(barrier integrity)

TEER ( $\Omega$ )



**MTT**  
(cytotoxicity)

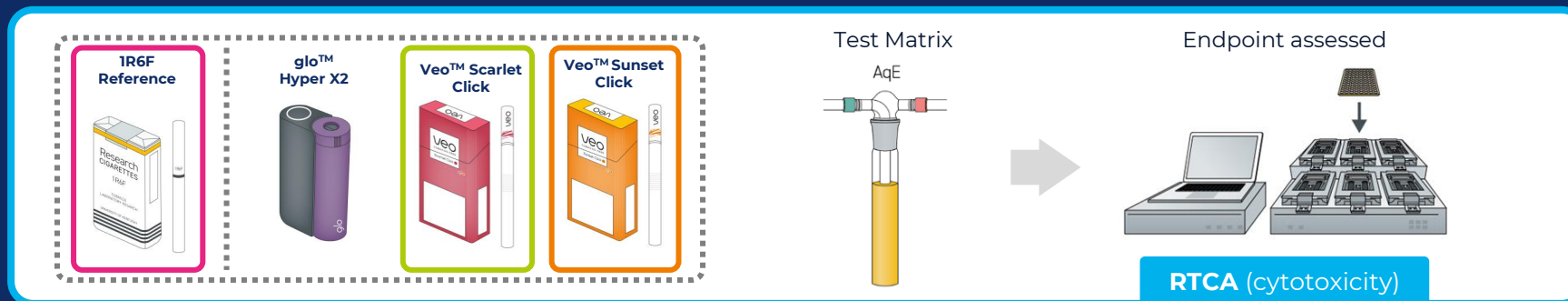
Cell Viability  
(% Air Control)



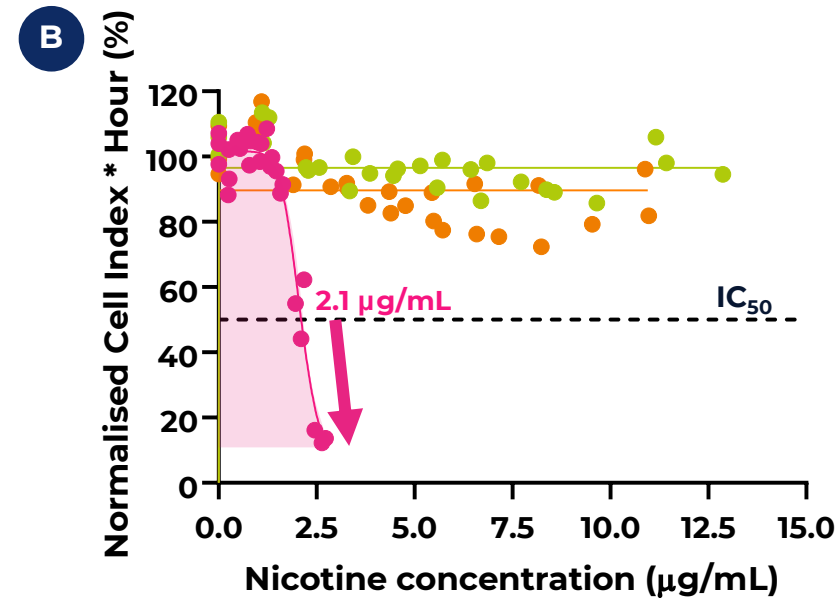
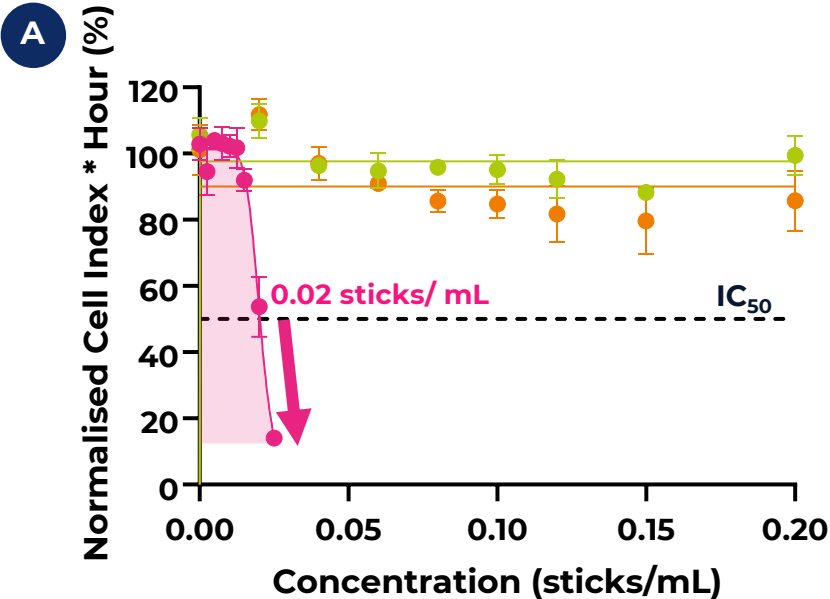
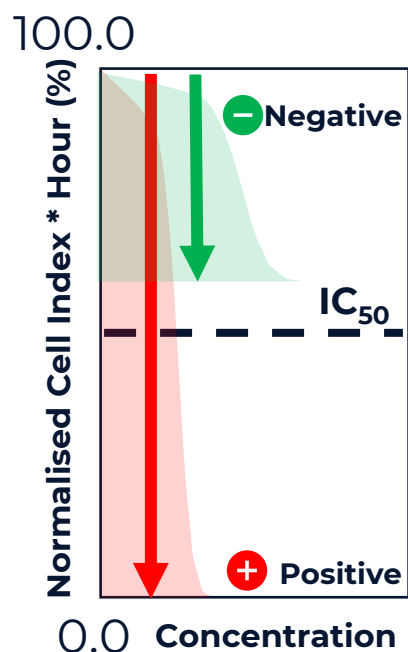
**Cilia function**

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

# 2D Testing Approach: RTCA Cytotoxicity



## Response for Cytotoxicity



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

\* Dashed line is the IC<sub>50</sub> - Half maximal inhibitory concentration

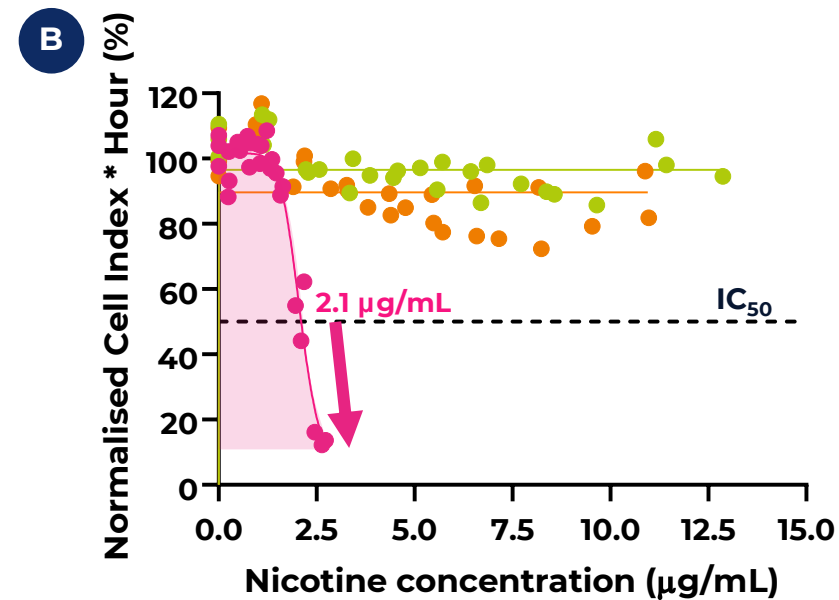
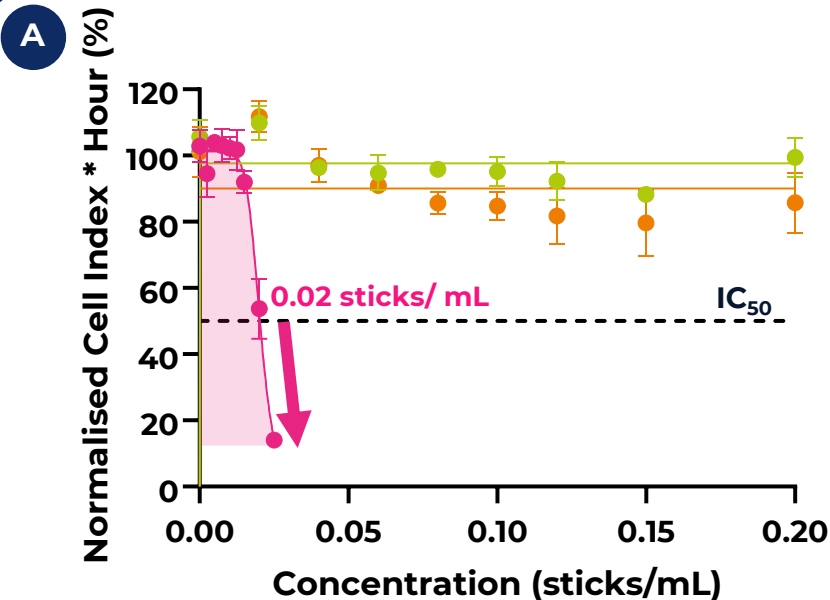
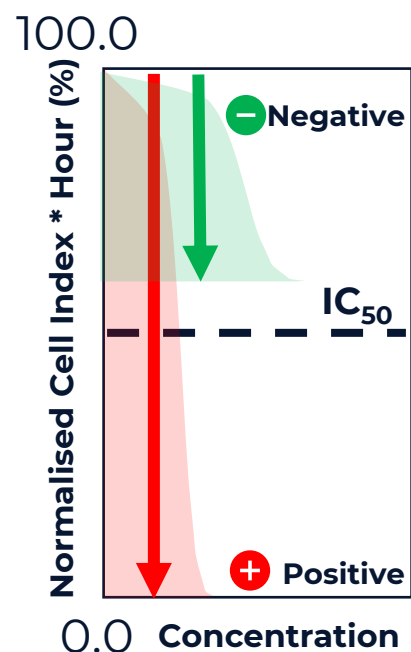
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# 2D Testing Approach: RTCA Cytotoxicity

Near complete toxicity curve for 1R6F samples was observed, whereas for HHP samples an IC<sub>50</sub> calculation was not possible

## Response for Cytotoxicity

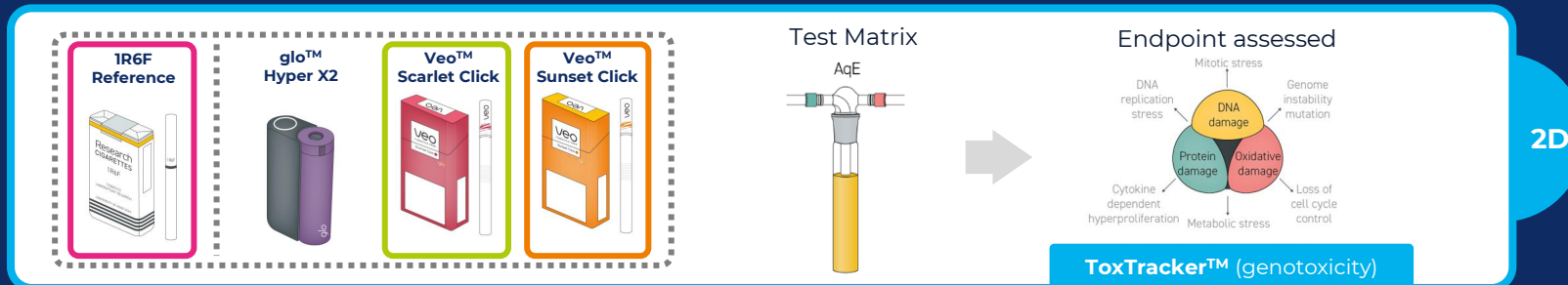


—●— 1R6F      —●— Veo™ Scarlet Click      —●— Veo™ Sunset Click

\* Dashed line is the IC<sub>50</sub> - Half maximal inhibitory concentration

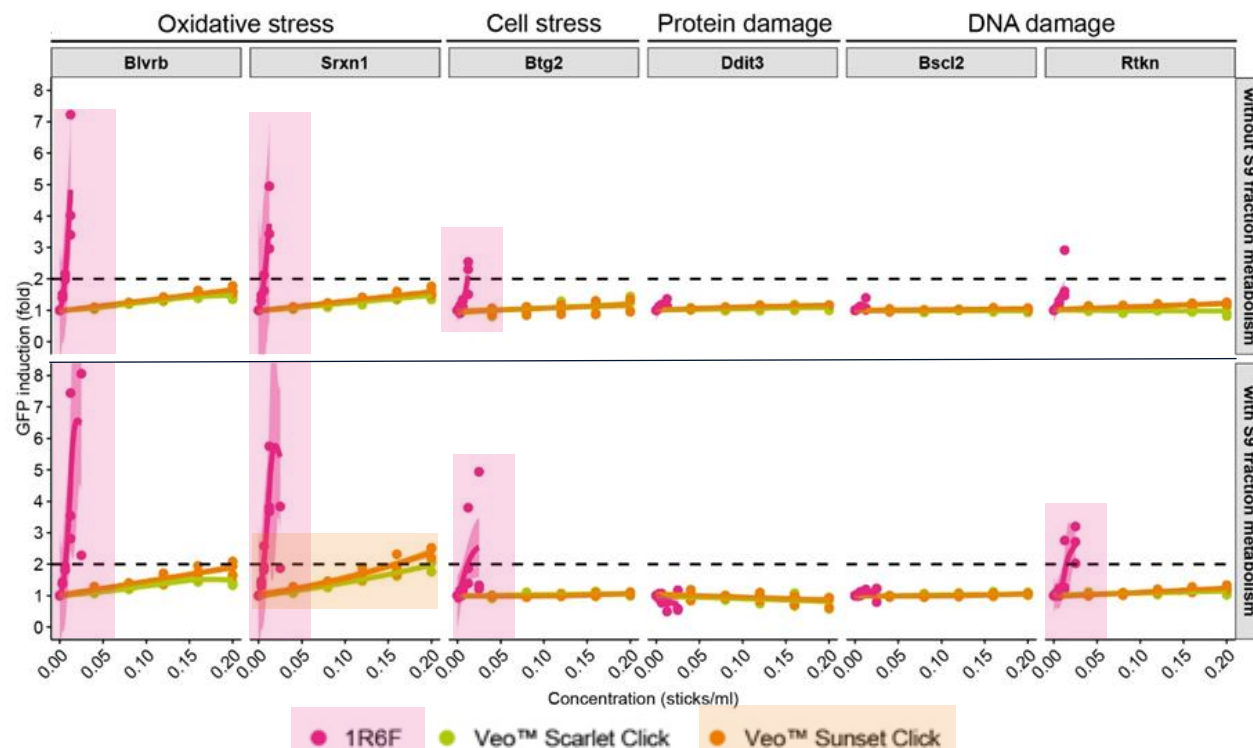
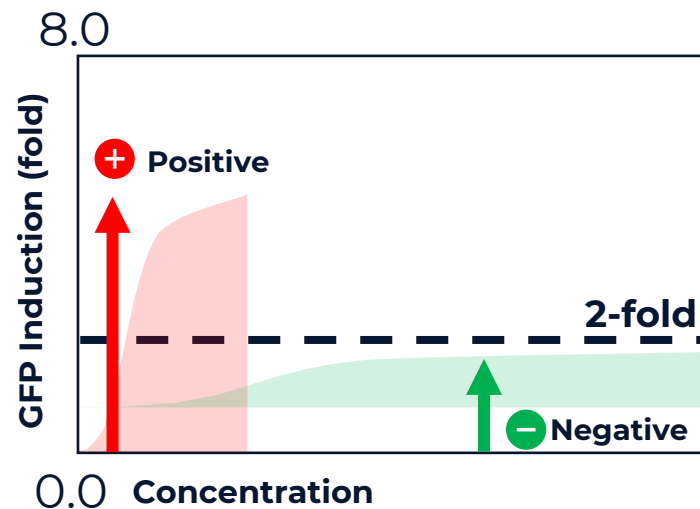
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# 2D Testing Approach: ToxTracker™ Genotoxicity



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

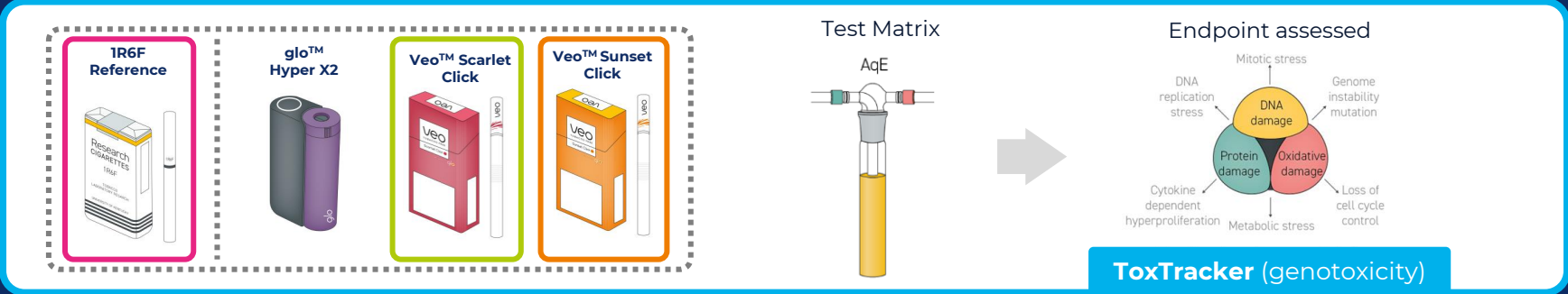
## Response for ToxTracker™



\* Dash line is the 2-fold threshold

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# 2D Testing Approach: ToxTracker™ Genotoxicity



2D

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 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


# 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

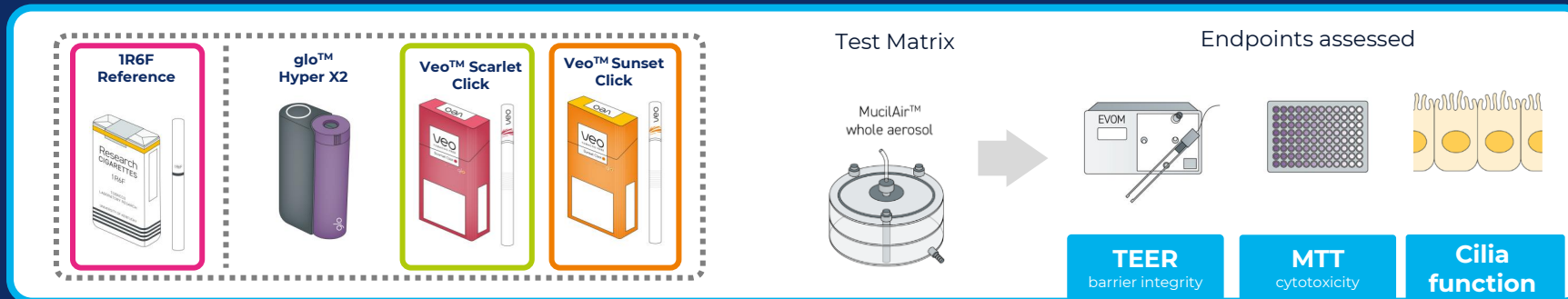


**Positive**  
Average GFP fold change >2  
Equal potency to 1R6F

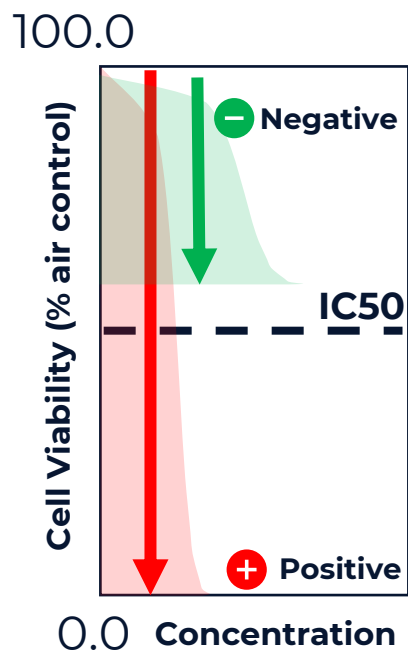


**Positive**  
Average GFP fold change >2  
Less potent than 1R6F

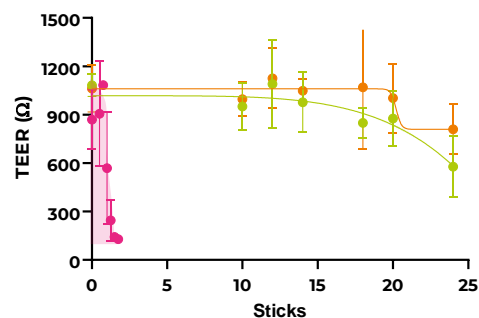
# 3D Testing Approach: MucilAir™ WA



## Response for Cytotoxicity

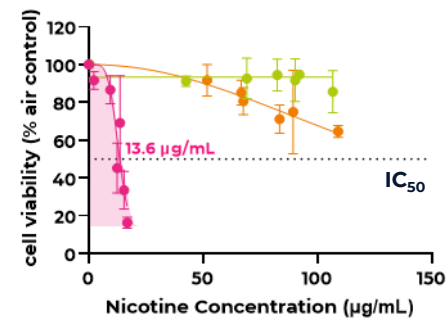
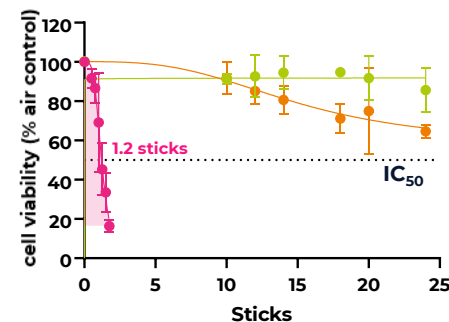


## TEER (barrier integrity)

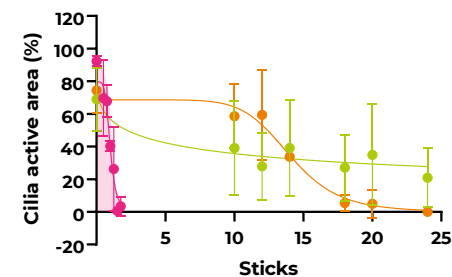
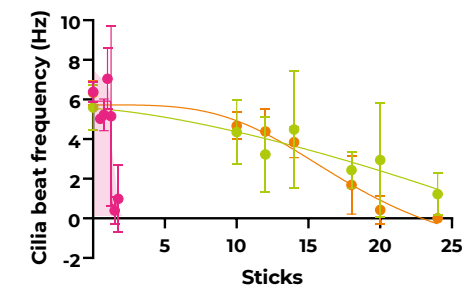


- 1R6F
- Veo™ Scarlet Click
- Veo™ Sunset Click

## MTT (cytotoxicity)



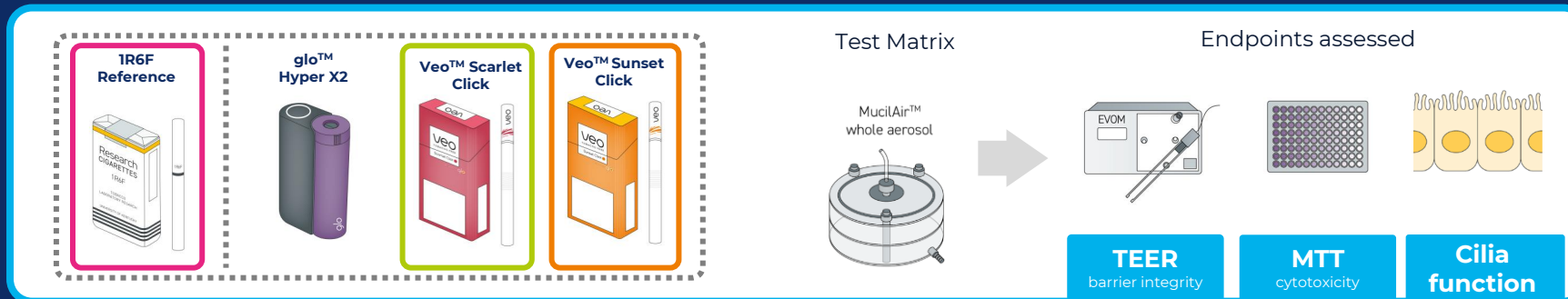
## Cilia function



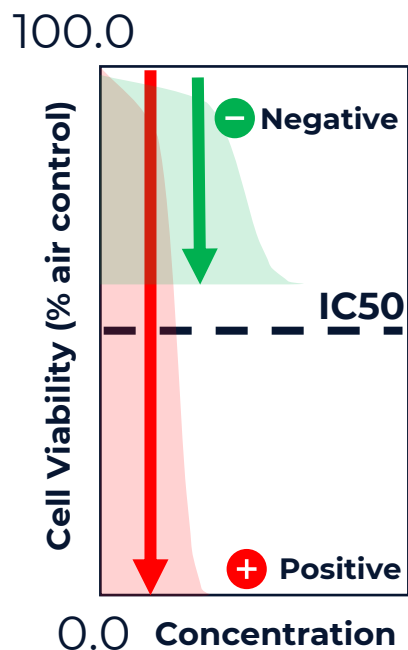
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\* Dashed line is the IC<sub>50</sub> - Half maximal inhibitory concentration

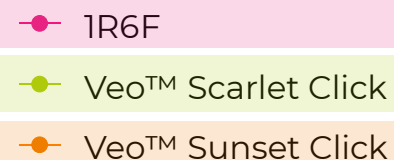
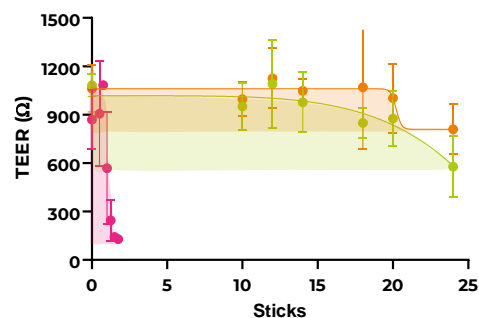
# 3D Testing Approach: MucilAir™ WA



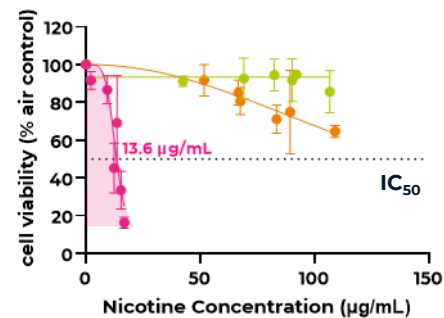
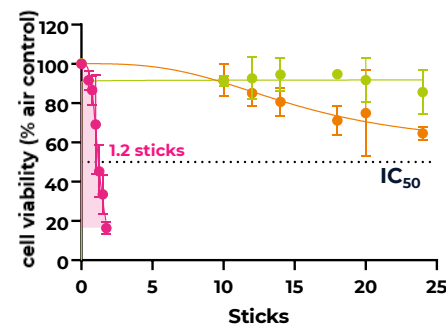
## Response for Cytotoxicity



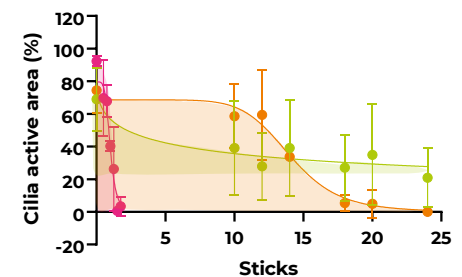
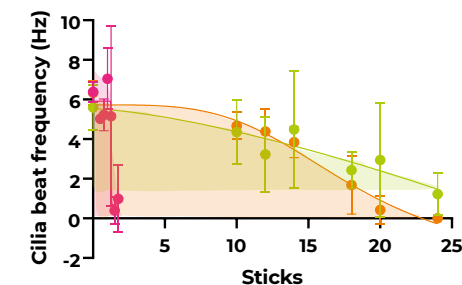
## TEER (barrier integrity)



## MTT (cytotoxicity)



## Cilia function



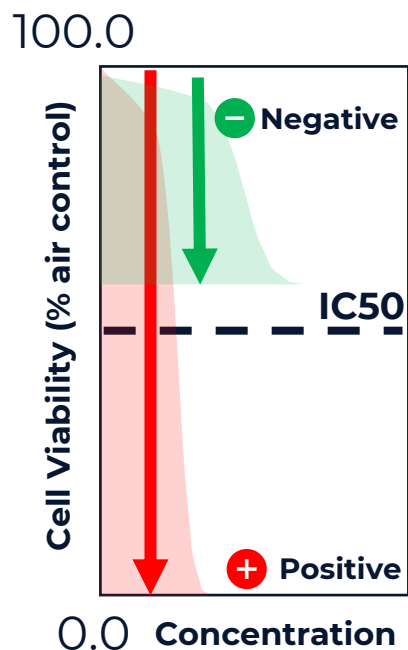
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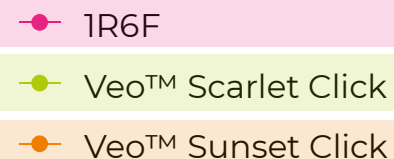
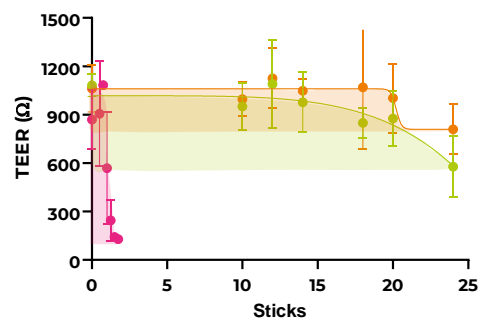
# 3D Testing Approach: MucilAir™ WA

- 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

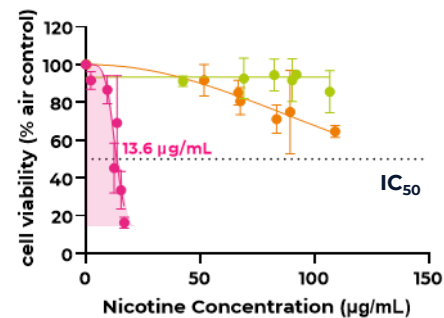
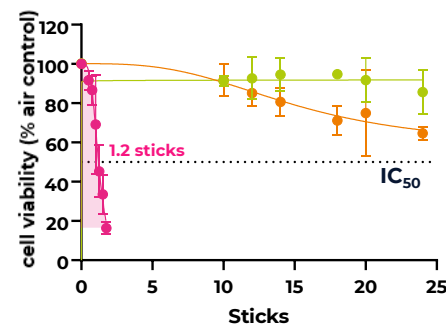
## Response for Cytotoxicity



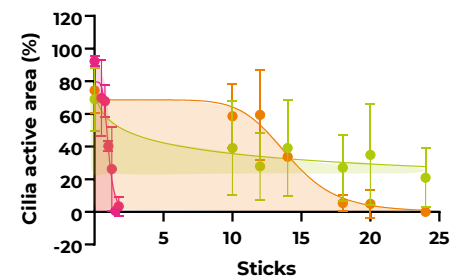
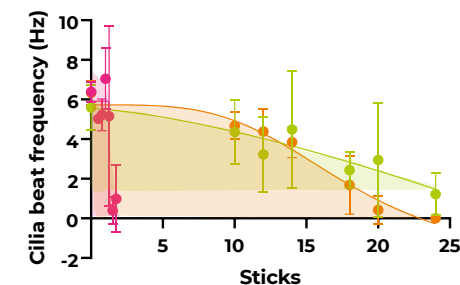
## TEER (barrier integrity)



## MTT (cytotoxicity)



## Cilia function



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\* Dashed line is the IC<sub>50</sub> - Half maximal inhibitory concentration

# 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?

Correspondence: [emma\\_bishop@bat.com](mailto:emma_bishop@bat.com) and [stela\\_bozhilova1@bat.com](mailto:stela_bozhilova1@bat.com)