The Use of MucilAir™ Reconstituted Human Upper Airway Epithelium with Whole Aerosol Exposure to Screen the Biological Impact of Heated Product (HP) Emissions compared to Conventional Cigarette Smoke

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CORESTA PSPT 2025
STPOST62

Introduction

The commercial availability of established reconstituted human lung epithelium models has increased the physiological relevance of *in vitro* inhalation toxicology assessment. This, coupled with whole aerosol generating systems capable of producing aerosol from heated and vapour products, provides the most realistic *in vitro* approximation of consumer exposure to date. This allows for the efficient comparison of the effects of product iterations on lung function (measured by cilia number and activity, and barrier function) and cytotoxicity (measured by MTT).

Methodology

Test Products

Table 1: Test Products used in this study. FMC = Factory Made Cigarette; HTP = Heated Tobacco Product; HHP = Heated Herbal product.

Product	Product type	Rod Substrate	Capsule	Device
1R6F	FMC	Tobacco	No	N/A
Product A	HTP	Tobacco	Yes	glo A
Product B	HTP	Tobacco	No	glo B
Product C	HHP	Rooibos	Yes	glo A
Product D	HHP	Rooibos	Yes	glo B

MucilAir™ Whole Aerosol Exposure

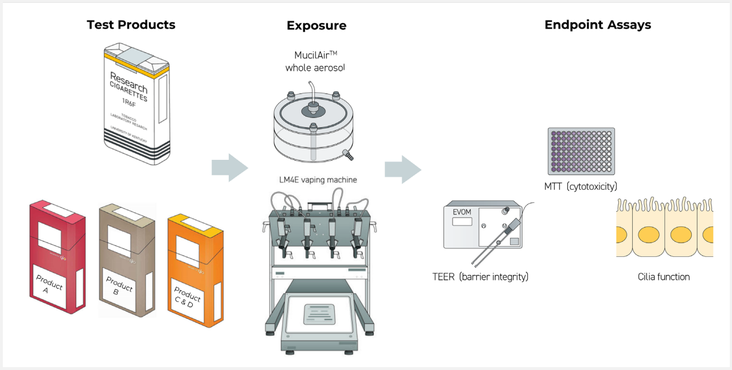

Figure 1: Experimental workflow used in this study. Test products were conditioned prior to testing.[1,3] MucilAir™ were loaded into BAT chamber and products aerosol generated with LM4E vaping robot. Aerosol was generated in accordance with ISO recommendations.[2,3] Following exposure MucilAir™ were given 24-hour recovery before the measurement of cilia function (by SAVA), TEER and MTT.

Cell culture

MucilAir™ tissues (Epithelix, Switzerland), pooled donor (MP0012) of nasal origin were used for these experiments and cultured in accordance with manufacturer's instructions.

Whole Aerosol

Pre-conditioning of products, and generation of aerosol was conducted in accordance with ISO recommendations, for 1R6F[1,2] and HP products.[1,3] The LM4E (Körber Technologies, Germany) was used to aerosolise the products with adaptations for *in vitro* use as described.[4] Post exposure, basal media was collected for nicotine quantification,[5] and MucilAir™ were recovered for 24-hours, prior to the assessment of endpoints as previously described.[6]

Results

In vitro cytotoxicity (MTT)

The cytotoxicity of the test products is shown in Figure 2. As shown below the substrate, capsule and heating profile had effects on the cytotoxicity of the test product in MucilAir™. Demonstrating this approach can differentiate between products within and across product categories.

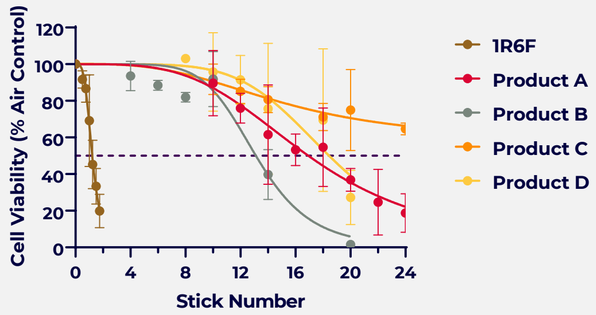

Figure 2: Cytotoxicity of 1R6F reference cigarettes and four HP variants in MucilAir™ measured by MTT. MucilAir™ were exposed up to 24 consecutive sticks to assess full toxicity. Triplicate inserts were exposed in each experimental occasion.

Table 2: Non-linear curve fit and calculated IC_{50} values for products tested within this study.

Product	IC_{50} (sticks)	IC_{50} (nicotine µg/ml)	R^2
1R6F	1.2	13.60	0.89
Product A	16.9	93.32	0.82
Product B	13.1	82.36	0.91
Product C	34.2	199.10	0.68
Product D	18.4	107.40	0.52

Additional Endpoint data

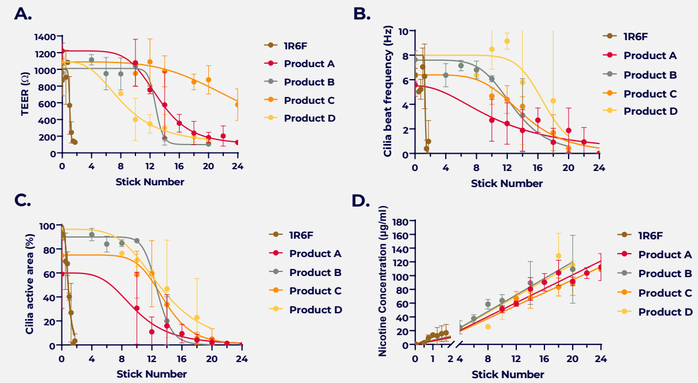

Figure 3: Supporting data collected from cytotoxicity experiments. Following 24-hour recovery Transepithelial Electrical Resistance (TEER) in (A), cilia beat frequency (B) and cilia active area (C) and was measured by SAVA prior to MTT. Basal media from the exposure chamber was collected and assessed for nicotine concentration by LC-MS (D).

Finally, the cytotoxicity data are presented as a function of captured nicotine concentration, as shown in Figure 4.

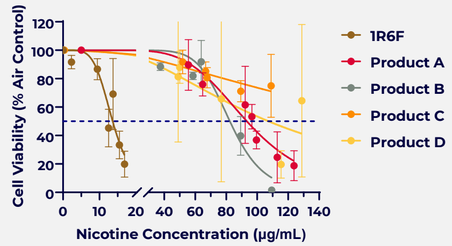

Figure 4: Cytotoxicity of 1R6F reference cigarettes and four HP variants in MucilAir ™ measured by MTT. MucilAir™ were exposed up to 24 consecutive sticks to assess full toxicity. Triplicate inserts were exposed in each experimental occasion.

Conclusion

The data demonstrate the sensitivity of the *in vitro* model system and its ability to identify response differences between HPs and combustible tobacco products, as well as between various iterations of HPs.

Alongside emissions analysis, such assays assist in understanding the biological effects of product emissions and, in turn, may support decision-making across each phase of the product development cycle and wider innovation process.

References

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5. ADAMSON, J., LI, X., CUI, H., THORNE, D., XIE, F. & GACA, M. D. 2017. Nicotine Quantification In Vitro: A Consistent Dosimetry Marker for e-Cigarette Aerosol and Cigarette Smoke Generation. Applied In Vitro Toxicology, 3, 14-27.
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