Characterization of a Vitrocell VC1 Using Nicotine Dosimetry: An Essential Component Towards Standardised *In Vitro* Aerosol Exposure of Tobacco and Next Generation Nicotine Delivery Products

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ABSTRACT

The US-FDA has regulatory authority over tobacco products, including conventional cigarettes and next generation products (NGPs) such as e-cigarettes and tobacco heating products (THPs). There is a desire by the industry, regulator and animal protection organizations to incorporate non-animal test methods for tobacco product and NGP assessment. When assessing respiratory effects *in vitro*, reliable exposure systems that deliver aerosols to cellular/tissue cultures at the air-liquid interface are needed.

Using nicotine dosimetry, we report the characterisation of a Vitrocell VC1 in our laboratories (IIVS, USA). Nicotine, generated from a 3R4F reference cigarette or NGP (e-cigarette and THP) aerosols at source and the exposure interface (culture media), was assessed using UPLC-MS/MS. This data was compared to published dosimetry data for the same products, generated at a different laboratory (BAT R&D, UK), on different exposure systems (VC10 and Borgwaldt RM20S) to confirm repeatability.

The nicotine content of 3R4F and NGP aerosols at VC1 source generation were established. Results demonstrated no statistical difference between laboratories (IIVS and BAT) (p=0.903) when comparing puff-by-puff nicotine concentrations from the three products. Culture media nicotine assessment demonstrated no significant difference between replicate wells in the exposure module (p=0.855), indicating uniform delivery.

This study demonstrates successful Vitrocell VC1 aerosol generation and delivery across multiple nicotine product categories, as characterised using nicotine as a dosimetry marker. The data suggests the VC1 established in our lab can reproducibly generate and deliver tobacco product and NGP aerosols for future *in vitro* assessment and matches the performance of reported exposure systems.

INTRODUCTION

The adverse health effects associated with traditional combustible cigarettes have been well established and include lung cancer, cardiovascular disease, and emphysema. Efforts to find less harmful alternatives have led to the development of e-cigarettes THPs, oral nicotine products like snus or gum, and medical/pharmaceutical nicotine inhalers. The Tobacco Control Act of 2009 gave the U.S. Food and Drug Administration's Center for Tobacco Products (CTP) regulatory authority over tobacco products in the United States. NGPs must be registered and approved before they come to market either via the Substantial Equivalence (SE) pathway (if a predicate product exists), or a Premarket Tobacco Application (PMTA). A key component of the PMTA process is the assessment of the safety of these products with reliable assays, including non-clinical testing. Keeping to the most current toxicological approaches including the National Research Council's Toxicity Testing in the 21st Century these would include non-animal, human tissue-based in vitro methods. This is not just for ethical concerns, but because the animal models are limited in their ability to accurately assess human health impacts, are expensive and take a long time to conduct. A key component of an *in vitro* testing approach for tobacco products and NGPs is the implementation of an acceptable standardized and reproducible in vitro exposure system that includes the generation of the test matrix.

MATERIALS & METHODS

- Test articles and puffing regimens – Scientific reference cigarettes (3R4F) were tested at 2 smoking regimes, ISO and HCI. An e-cigarette using blended tobacco flavor e-liquid was vaped at the high voltage setting at the CRM81 regime. A THP was tested at a modified HCI regime (Figure 1).
- To characterize VC1 performance, repeatability of aerosol generation was assessed by quantifying nicotine at the aerosol source on a puff-by-puff basis, across all products.
- To characterize repeatability of aerosol delivery to the exposure module, and uniformity of delivery across replicate exposure wells under different exposure conditions, nicotine was quantified in the exposed culture media from 3R4F reference cigarette and e-cigarette.

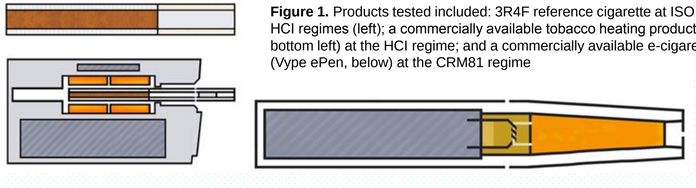

Figure 1. Products tested included: 3R4F reference cigarette at ISO and HCI regimes (left); a commercially available tobacco heating product (glo™, bottom left) at the HCI regime; and a commercially available e-cigarette (Vype ePen, below) at the CRM81 regime

VC 1 EXPOSURE SCHEMATIC

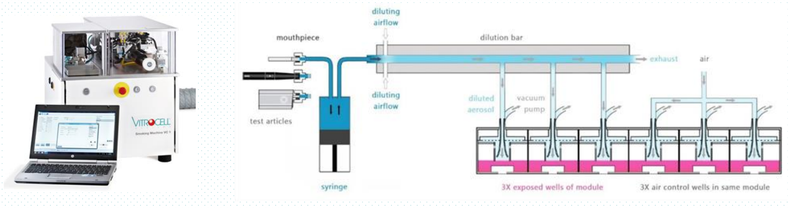

Figure 2. The Vitrocell VC1 exposure system and a schematic cross section including the12/6 exposure module

TABLES – TECHNICAL SPECIFICATIONS

Table 1. Comparison of the technical specifications between the Vitrocell VC1 and the VC10 in this study

Machine	VC 1 (*US lab*)	VC 10 (*UK lab*)
Serial number	VC 1/051517	VC 10/141209
Dimensions (L × W × H)	0.61 × 0.46 × 0.53 m	1.5 × 0.8 × 0.85 m
Footprint	Bench top (0.3 m^2)	Bench top (1.2 m^2)
Device holder/mouthpieces	Single mouthpiece	Rotary carousel with 10 mouthpieces
Cigarette loading, lighting and removal	Manual	Automated
Dilution systems	1 dilution bar	Up to 4 dilution bars
Exposure module (insert well size/number)	12/6 stainless steel mammalian module	6/4 stainless steel mammalian module

Table 2. Test articles and puffing parameters

Product type	Source	Puffing profile						
		Regime	Vol (ml)	Duration (s)	Interval (s)	Puff wave	Filter vents	Puffs
3R4F reference cigarette	University of Kentucky	ISO	35	2	60	Bell	Open	8
		HCI	55	2	30	Bell	Blocked	10
E-cigarette, Vype ePen (1.8% nicotine)	British American Tobacco	CRM81	55	3	30	Square	N/A	10
THP, glo™ and Neostiks	British American Tobacco	HCIm	55	2	30	Bell	Open	8

RESULTS – PUFF-BY-PUFF NICOTINE CONCENTRATIONS

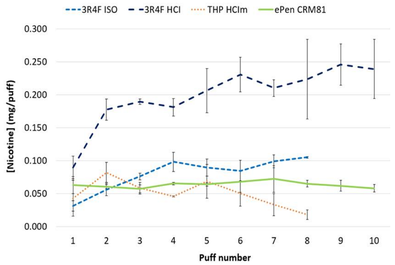

FIGURE 3. Puff-by-puff nicotine concentration profiles of the four products and specific smoking regimes on the VC 1 (n=3)

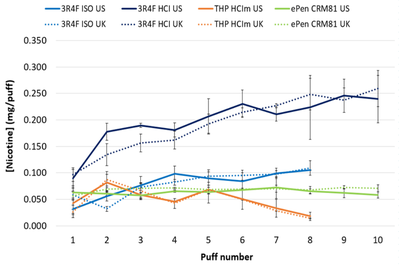

FIGURE 4. Puff-by-puff nicotine concentration inter-laboratory comparisons of the four products and specific smoking regimes between the US lab (IIVS) (solid lines) and the UK lab (BAT R&D) (dotted lines) (n=3)

RESULTS – MEAN PUFF NICOTINE CONCENTRATIONS

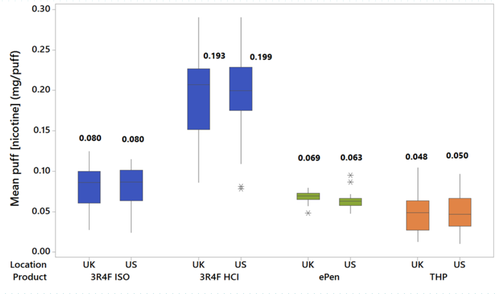

FIGURE 5. Mean puff nicotine concentration inter-laboratory comparisons of the four product/regimes between the US lab (IIVS) and UK lab (BAT R&D). Boxplots display the mean (central line, and values above boxplots), the 25th and 75th percentiles (bottom and top lines of box, respectively), and the 5th and 95th percentiles (bottom and top whiskers, respectively). Asterisks denote single outliers calculated as data points falling outside 1.5 x 25th–75th percentile range (3R4F ISO and THP n=24; 3R4F HCI and e-cigarette n=30). A GLM ANOVA demonstrated there was a significant difference between all products (p=0.000) but there was no statistically significant difference between the laboratories (p=0.903)

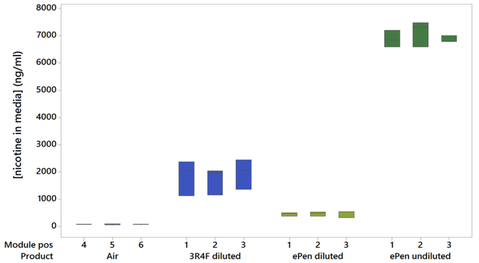

FIGURE 6. Mean nicotine concentration in exposed culture media across three wells of the Vitrocell 12/6 module under various exposure conditions (n=3/module position). A General Linear Model ANOVA (product nested within module position) demonstrated no significant difference between the three replicate exposure wells in the 12/6 module (p=0.855)

CONCLUSIONS

1. **Nicotine is a robust marker for *in vitro* aerosol delivery dosimetry assessment**
2. **Puff-by-puff nicotine content correlated well between prior studies and between labs**
3. **Aerosol delivery to the cellular interface was found to be reproducible across replicate well positions**

ACKNOWLEDGMENTS

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