Characterization of a Vitrocell VC1 Using Nicotine Dosimetry: An Essential Component Towards Standardised In Vitro Aerosol Exposure 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, regulators and animal protection organizations to incorporate non-animal test methods for tobacco products and NGP assessment. When assessing respiratory effects in vitro, reliable exposure systems that deliver aerosols to cell/eukaryote cultures at the conditions as needed. Using nicotine dosimetry, we report the characterization of a Vitrocell VC1 in our laboratories (IVS, USA). Nicotine, generated from a 3PM reference cigarette, was tested in 4 chamber size aerosol and THP aerosols at source and the exposure interface (culture media), was assessed using UPLC-SRM. This data was compared to published literature for the same products, generated at the lab and regulatory level. The nicotine content of 3PM and NGP aerosols at VC1 source generation were established. Results demonstrated no statistical difference between laboratories (IVS and BAT) (p=0.92) when comparing puff-by-puff nicotine concentration in the three products. Culture media nicotine measurement demonstrated no significant difference with replicate wells in the exposure module (p=0.85), indicating uniform delivery. This study demonstrates successful Vitrocell VC1 aerosol generation and delivery across multiple nicotine product categories, as characterized using nicotine as a dosimetry marker. The data suggests the VC1 established in our lab is robustly 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, which are nicotine products like an e-pen, or medical/international 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 (PTA); a key component of the PTRA process is the assessment of the safety of these products with reliable, repeatable, and consistent testing. Keeping to the most current toxicological approaches including the National Research Council’s Toxicity Testing in the 21st Century: a Vision for Animals in Vitro methods. This is not just for ethical reasons because the animal models are limited in their ability to accurately assess human health impacts, are expensive and take a long time. A key component in 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 • Science reference cigarettes (3PMF) were tested at 2 smoking regimens, ISO and HCI. An inhaled nicotine dose 4 mg/ml nicotine e-liquid was vaped at the high voltage setting at the CRCM1 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 3PMF reference cigarette and e-cigarette.

RESULTS

Figure 1. Products tested included: 3PMF reference cigarette at ISO and HCI regimes (left), a commercially available tobacco heating product (THP, bottom left) at the HCI regime, and a commercially available e-cigarette (Type A, below) at CRCM1 regime.

Vehicle exposure 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 3PMF reference cigarette and e-cigarette.

RESULTS – PUFF-BY-PUFF NICOTINE CONCENTRATIONS

1. Tobacco 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 replicate well positions

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