Development of the *in vitro* micronucleus test for exposure to whole aerosol from cigarettes and heated tobacco products

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Introduction

In the genotoxic assessment of tobacco products, traditional approaches involve capturing the two phases of the aerosol, such as pad-collected total particulate matter (TPM) and/or gas-vapour phase (GVP) in solvents and applied to cell cultures. This approach may under-represent the interactions of the whole aerosol (WA). The exposure of cells to WA at the air-liquid interface (ALI) mitigates the need for this fractionation.

The aim of this study was to investigate the potential of WA from a reference cigarette (1R6F) and a Heated Tobacco Product (HTP) to induce micronuclei in cells exposed at the ALI.

Methodology

Test Articles

The 1R6F reference cigarette and a HTP were used in this study. A schematic of the HTP consumable and heating device are shown in Figure 1. Analytical chemistry demonstrates HTPs emit lower levels of toxicants per stick (Table 1)

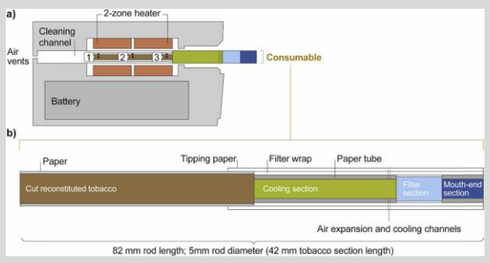

Figure 1. Schematic representation of a HTP

Toxicant	1R6F	HTP
TSNAs (ng/stick)	836.0	73.71
Acrolein (µg/stick)	157.0	2.22
Crotonaldehyde (µg/stick)	42	0.6
Ethylene oxide (µg/stick)	19.3	BDL
Naphthalene (ng/stick)	994	2.2
NDMA (ng/stick)	14.2	BDL

Table 1. Examples of selected emissions from 1R6F and a HTP

Aerosol Generation

- The Health Canada Intense (HCI; 55 mL puff volume, taken over 2 seconds, every 30 seconds, 100% vent blocking) regime was used for 1R6F
- The HTP was puffed using the same regime, but the ventilation holes were not blocked
- The aerosols generated on the Vitrocell VC10 were diluted with fresh air (diluting airflow) to achieve the required aerosol dilution (Figure 2)
- Diluting airflows of 2 and 6 L/min were trialled with 1R6F, but only 6 L/min was used for the HTP
- The aerosol was then passed over the cells in Transwells™ at a vacuum rate of 5 mL/min (Figure 3)
- The deposited nicotine in each Transwell™ was measured

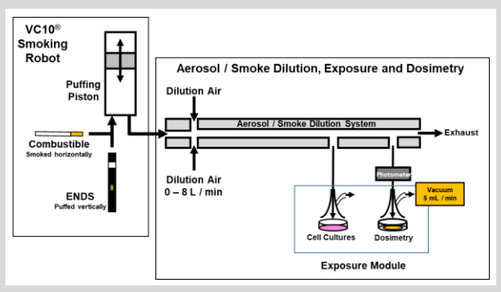

Figure 2. Schematic representation of the aerosol generation, dilution and cell exposure.

In vitro micronucleus assay

- V79 cells were seeded on 24 mm Transwells™ at 6 x10^4 cells/mL
- Transwells™ incubated for 24 h to achieve approximately 50% confluency
- Prior to aerosol exposures, the apical media was removed from the surface of the cells, and the Transwells™ placed in the Vitrocell exposure modules and exposed for the required number of puffs
- Following exposure, Transwells™ were incubated for 24 h and analysed for micronuclei induction using Litron MicroFlow kits and cytotoxicity using RPD
- Air was used as the negative control and the positive control was MMC

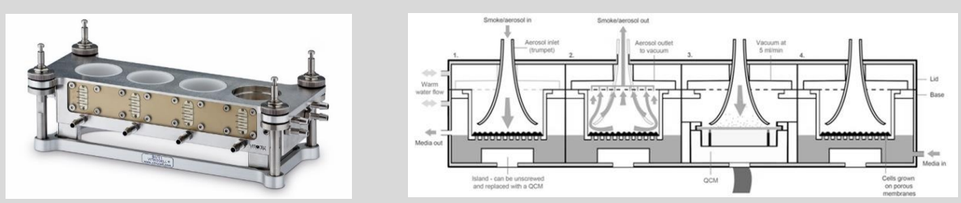

Figure 3. An example of a Vitrocell exposure module.

Results

Diluting airflow optimisation

- With 1R6F WA, the 2 L/min diluting airflow was too cytotoxic, and no analysable concentrations were obtained
- The 6 L/min diluting airflow provided the optimal cytotoxic response (Figure. 4)

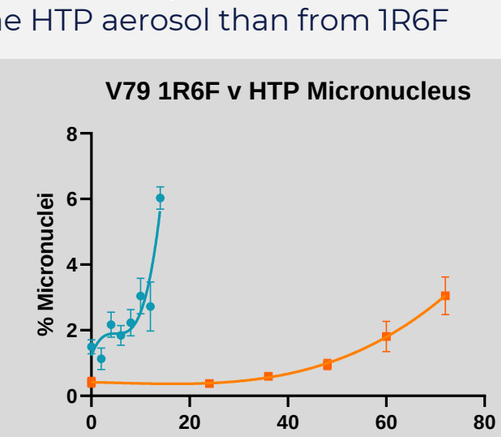

Figure 4. Cytotoxicity of 1R6F at two diluting airflows to V79 cells. 2 L/min: 6 L/min:

Micronuclei Induction

- The positive control, MMC, induced dose related increases in micronuclei that were considered positive responses (data not shown)
- 1R6F and the HTP aerosols induced dose related decreases in cell viability to 50-60%, and also induced dose related increases in micronuclei relative to the vehicle (air) control (Figure 5), but:
  - The HTP aerosol was 3 times more concentrated than 1R6F
  - No micronuclei were induced when HTP aerosol was tested at the same number of puffs as 1R6F
  - A significantly higher number of puffs was needed to induce a response from the HTP aerosol than from 1R6F

Figure 5. Cytotoxicity and genotoxicity of aerosol from 1R6F and the HTP. 1R6F: HTP: Limits of cytotoxicity:

- When the dose was expressed as deposited nicotine, reduced genotoxicity of the HTP was still observed (Figure 6)

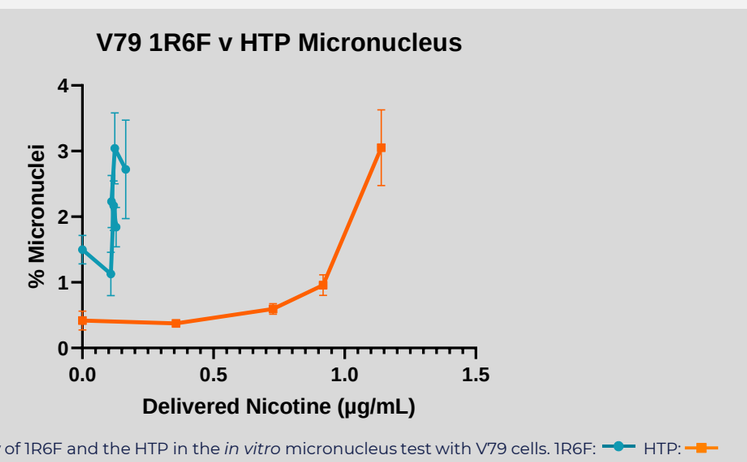

Figure 6. Genotoxicity of 1R6F and the HTP in the *in vitro* micronucleus test with V79 cells. 1R6F: HTP:

Conclusion

It is feasible to adapt submerged cell cultures to air-liquid interface exposures, a more biologically relevant approach than using separate assays for particulate and gas phase fractions, for gases and whole aerosols

The HTP was less genotoxic than 1R6F cigarette, on a puff-by-puff basis, and when the delivered dose was expressed as units of nicotine

This further supports the role of HTPs in the tobacco harm reduction agenda

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