In Vitro Cytotoxicity Assessment of Whole Aerosol/Smoke Generated from Heated Tobacco Products and Combustible Cigarettes in the EpiAirway Tissue Model

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Abstract

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay measures the metabolic activity of the cell and is therefore an indicator of cell survival/viability. Cigarette smoke and other types of tobacco product emissions are known to contain respiratory irritants. Assessment of MTT cytotoxicity using a 3D culture human airway tissue model (e.g., EpiAirway) following exposure to smoke or aerosols from tobacco products is a useful tool for distinguishing the impact of exposure during smoking or vaping.

This study exposed EpiAirway™ tissues to whole aerosol/smoke generated from four styles of a glo™ Heated Tobacco Product (HTP), a market comparator HTP, two marketed combustible cigarettes (CC), and the 1R6F Reference cigarette. A Vitrocell® VC10® robot was used to generate whole aerosol/smoke using the Health Canada Intense (HCI) or a modified HCI (market comparator HTP only) smoking regimen. Whole aerosol (WA) / smoke (WS) was diluted with clean air at increasing air flows (L/minute) to achieve the delivered exposure dose range. The exposure conditions were 5.0 - 0 L/min (undiluted) for the HTP and 10 – 0.5 L/min for the combustible cigarettes. Liquid traps containing PBS within the exposure module allowed aerosol/smoke dosimetry via nicotine quantification. Cell survival/viability was assessed with the MTT assay.

Whole smoke generated from each of the two market CCs was cytotoxic at the 6 L/min dilution and below, as measured by the MTT assay with calculated IC_{50} values of 29.59 and 26.95 µg nicotine/mL. Mainstream aerosol from the HTP test articles induced observed cytotoxicity in the MTT assay at the 1 L/min dilution and below. The calculated IC_{50} values for the HTP test articles (ranging from 154.15 - 195.40 µg nicotine/mL) were significantly higher than the CCs, with almost 5-fold higher IC_{50} levels. These results demonstrate that HTP aerosol is less cytotoxic than CC smoke.

Materials and Methods

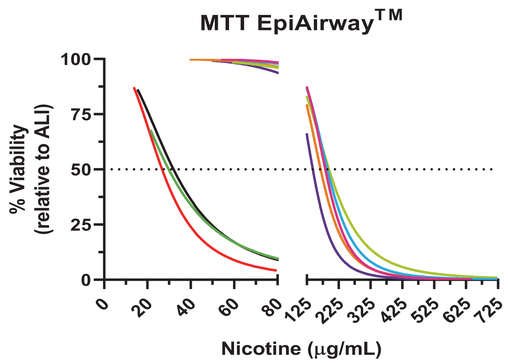
3D Tissue Model: The 3-dimensional EpiAirway™ tissues were obtained from MatTek, Inc. Tissues were maintained at the air-liquid interface according to the manufacturer's guidelines.

Test Articles: Two market comparators CC; Reference test article: 1R6F Kentucky Reference Cigarette; One market comparator HTP; and four variants of glo™ neo test articles.

Negative and Positive Controls: Control air-liquid interface (ALI) treatments (airflow 0.2 L/min) and incubator controls were included in all experiments. Negative controls were blank Transwells™ (with no tissue) in appropriate media to control for MTT staining of the Transwells™.

Results

Figure 1: Percent (%) cell viability compared to the concentration of nicotine measured in the dosimetry exposure well



LEGEND:

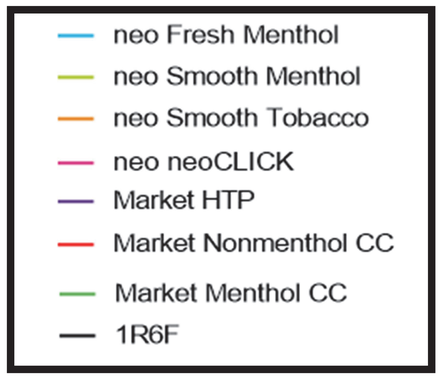


Figure 2: LDH absorbance (signal of cell death) compared to the concentration of nicotine measured in the dosimetry exposure well

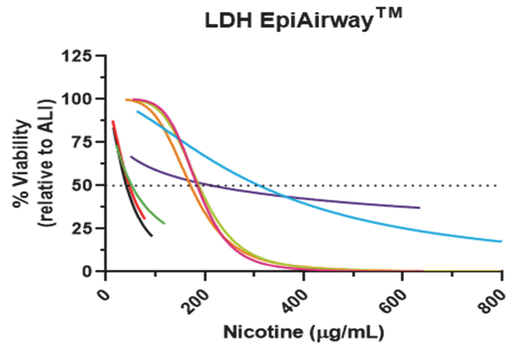


Table 1:. MTT and LDH IC_{50} Table

Test Article	MTT IC_{50} Nicotine (µg/mL)	LDH IC_{50} Nicotine (µg/mL)
neo neoCLICK	195.40	184.8
neo Fresh Menthol	187.97	310.2
neo Smooth Menthol	185.10	187.7
neo Smooth Tobacco	176.90 (n=2)	187.2
Market HTP	154.15 (n=2)	160.4
Market Menthol CC	29.59	52.16
Market Nonmenthol CC	26.95	47.09
Reference CC Product 1R6F	33.10	41.36

Figure 3: Schematic representation of WA exposures. A Vitrocell® VC10® robot generated and delivered aerosols to the Mammalian 6/48 aerosol dilution and exposure system, with up to 7 concurrent doses plus a clean air control. The dosimetry module allowed the capture and quantification of deposited aerosol constituents (nicotine, glycerol, and carbonyls).

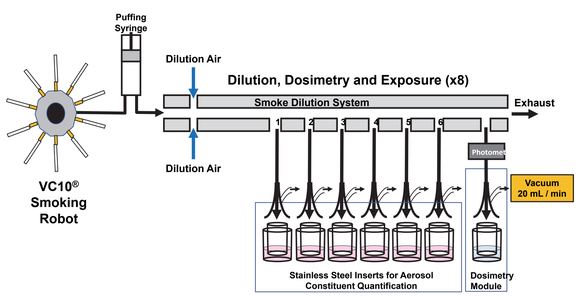
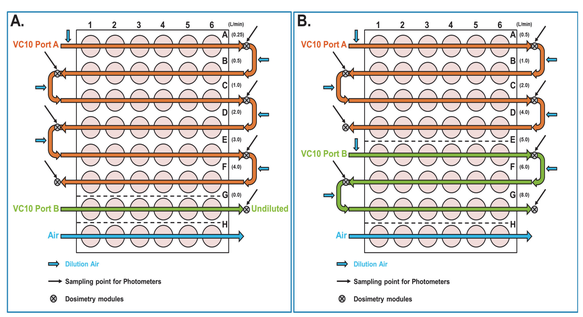


Figure adapted, with modifications, from Keyser et. al. (2019) *Toxicology Reports*, 6, 1281-1288.

Figure 4: Mammalian 6/48 WA exposure module set up for HTP (A) and combustible cigarette (B) exposures. HTP WA was serially diluted through rows A – F (0.25 to 4.0 L/min dilution airflows) or undiluted (0 L/min) in row G. For combustible cigarettes, WA was serially diluted through rows A – D (0.5 to 4.0 L/min) and rows E – G (5.0 to 8.0 L/min). Row H (A & B) was used for air controls.



Materials and Methods

Positive control exposures consisted of exposures with Triton X-100, Heptanal, Heptyl Butyrate, Formaldehyde, and Olive Oil (vehicle).

Whole Aerosol/Smoke Exposures: An initial range-finder and three main exposure experiments were conducted for all test articles. The exposure conditions were 5, 4, 3, 2, 1, 0.5, and 0 L/min diluting air for the HTP test articles and 10, 8, 6, 4, 2, 1, and 0.5 L/min diluting air for the market comparator CC. The duration of exposures was approximately 120 and 68 minutes, respectively.

MTT Analysis: The MTT assay was performed according to the manufacturer's instructions (MatTek corporation, cat. # MTT-100). The reaction is quantified by measuring the absorbance of the purple formazan solution at 570 nm.

LDH Analysis: Culture medium from each control and exposure dose was collected after the 24-hour post-exposure period and analyzed using the LDH Cytotoxicity Detection Kit (Clontech Cat# 630117/Takara Cat# MK401) following the manufacturer's protocol recommendation.

Nicotine Determination: Samples from the PBS dosimetry trap were analyzed using a LC-MS/MS. The linear range of the method was 0.08 to 50 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were 0.026 and 0.08 µg/mL, respectively.

Statistical Analysis: Normalized nicotine concentration to induce a 50% reduction in cell viability (IC_{50}) was calculated using a 4-PL model (SAS).

Summary and Conclusions

- Whole Aerosol generated from the four glo HTP and the market comparator HTP test articles induced a cytotoxic response in the EpiAirway™ tissue model using the MTT assay at the 1 L/min airflow and below relating to approximately 150-250 µg/mL nicotine. Whole Smoke generated from the market combustible and 1R6F test articles induced a cytotoxic response at the 6 L/min airflow and below relating to approximately 26-32 µg/mL.
- IC_{50} values for all HTP test articles were generally ~ 5-fold higher than the CC IC_{50}s, indicating it would take 5x the level of exposure to HTPs to attain a similar level of respiratory (relevant) toxicity as 1x exposure for CCs.

References

1. Health Canada Test Method, T-115. Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31
2. ISO 3308 (2012). Routine analytical cigarette-smoking machine - Definitions and standard conditions (5th edition)
3. ISO 3402 (1999). Tobacco and tobacco products - Atmosphere for conditioning and testing (4th edition)