**Endothelial cell migration (scratch) assay**

- Artificial wounds were created in monolayers of human umbilical vein endothelial cells (HUVEC) as described previously [6 & 8].

**Products**

- Cigarettes: 3R4F Kentucky reference cigarette
- Prototype e-cig: produced by BAT R&D with an e-liquid containing 5 mg/mL nicotine

**Methods**

- **Exposure regimes**
  - 3R4F cigarette: Health Canada Intense [9] - 55 mL puff volume, 2 sec puff duration, 30 sec puff interval & 100% blocking of the filter ventilation
  - Prototype e-cig: CORESTA, No. 81 [10] - 55 mL puff volume, 3 sec puff duration, 30 sec puff interval

**In vitro analysis**

**Cytotoxicity**

- Human bronchial epithelial cells (NCI-H292) were exposed to 1:20-10,000 v/v dilution of 3R4F or 1:2-100 v/v dilution of prototype e-cig aerosol for 1 hour.
- The Neutral Red Uptake (NRU) assay measured cytotoxicity as detailed in Azzopardi et al. 2016 [6].

**Oxidative stress**

- 3R4F AqE doses greater than 10% inhibited endothelial cell migration with complete inhibition at doses above 25% AqE (Figure 5)

**Results**

**Cytotoxicity**

- 3R4F smoke induced a concentration dependent decrease in cell viability (Figure 1).
- The prototype e-cig aerosol exposure resulted in responses comparable to sham air control (Figure 1).
- In the case of 3R4F, increasing levels of nicotine in the media correlated with higher exposures, which reduced cell viability (Figure 2).
- Although the cells exposed to the prototype e-cig had similar to higher levels of nicotine, this had no effect on cell viability (Figure 2).

**Oxidative stress**

- 3R4F AqE lowered the glutathione ratio at doses greater than 6.25% (Figure 3) indicative of oxidative stress & induced cytotoxicity at doses greater than 12.5% (Figure 4).
- Exposure to the prototype e-cig AqE, even at 100%, did not affect the glutathione ratio or level of cell viability (Figures 3 & 4). Responses were comparable to the vehicle control.

**Introduction**

- E-cigarette (e-cig) use has grown significantly over the last decade [1–2] & there is growing consensus that e-cigs hold great potential for reducing the harm associated with cigarette smoking [3] & therefore should be promoted as a smoking substitute [4].
- Recent studies have shown that e-cig aerosols have significant reductions in constituents when compared to cigarette smoke [5] & reduced toxicity in vitro [6-8].
- This study assessed the in vitro assessment of a novel prototype e-cig in 4 biological assays.

**Products**

- Cigarettes: 3R4F Kentucky reference cigarette
- Prototype e-cig: produced by BAT R&D with an e-liquid containing 5 mg/mL nicotine

**Results**

**Cytotoxicity**

- Deposited nicotine was measured in basal exposure media & AqE to quantify the exact exposure dose as described previously [6 & 8].

**Oxidative stress**

- 3R4F AqE lowered the glutathione ratio at doses greater than 6.25% (Figure 3) indicative of oxidative stress & induced cytotoxicity at doses greater than 12.5% (Figure 4).
- Exposure to the prototype e-cig AqE, even at 100%, did not affect the glutathione ratio or level of cell viability (Figures 3 & 4). Responses were comparable to the vehicle control.

**Conclusion**

- The prototype e-cig did not induce cytotoxicity, oxidative stress or effect wound healing.
- These studies indicate that the prototype e-cig has the potential to reduce risk of disease when compared to cigarette smoking.

**References**

10. CORESTA, No. 81 - 55 mL puff volume, 3 sec puff duration, 30 sec puff interval