**In vitro assessment of a novel prototype e-cigarette**


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**Introduction**

- E-cigarette (e-cig) use has grown significantly over the last decade [1–2] and there is growing consensus that e-cigs hold great potential for reducing the harm associated with cigarette smoking [3] and 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] and 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

**Methods**

**Exposure regimes**

- 3R4F cigarette: Health Canada Intense [9] - 55 mL puff volume, 2 sec puff duration, 30 sec puff interval and 100% blocking of the filter ventilation
- Prototype e-cig: CORESTA recommended method 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
- Cytotoxicity was measured by the Neutral Red Uptake (NRU) assay as described in Azzopardi et al. 2016 [6]

**Oxidative stress**

- NCI-H292 cells were exposed to 0-100% aqueous extracts (AqE) prepared from 3R4F or the prototype e-cig as described in Taylor et al. 2016 [7]
- Glutathione ratio and cell viability were measured using ApoLive-Glo® and ONE-Glo™ Luciferase assay system kits (Promega) [7]

**Endothelial cell migration (scratch) assay**

- Artificial wounds were created in monolayers of human umbilical vein endothelial cells (HUVEC) as described in Taylor et al. 2017 [8]
- Cells were incubated with 0-40% 3R4F or 0-100% prototype e-cig AqE
- Wound repair was assessed over 22 hours using image analysis [8]

**Nicotine measurement**

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

**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 and 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 and 4). Responses were comparable to the vehicle control

**Endothelial cell migration (scratch) assay**

- 3R4F AqE doses greater than 10% inhibited endothelial cell migration with complete inhibition at doses above 25% AqE (Figure 5)
- The prototype e-cig AqE, even at the 100% dose, did not inhibit cell migration. Responses observed were comparable to the media control (Figure 5)

**Conclusions**

- 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**


![Figure 1. NRU determined cell viability in NCI-H292 cells after 1 hour exposure. Cytotoxicity as a % sham (air control) is presented against aerosol dilution. 3R4F n = 4 and prototype e-cig n = 3 experiments.](image1)

![Figure 2. NRU determined cell viability of NCI-H292 cells after 1 hour exposure to a range of 3R4F and prototype e-cig aerosols. Cytotoxicity as % sham (air control) is presented against media nicotine (ng/ml). 3R4F n=4 and prototype e-cig n=3 experiments.](image2)

![Figure 3. GSH:GSSG ratio in NCI-H292 cells following the exposure to increasing concentrations of 3R4F and prototype e-cig AqE. n = 3 experiments.](image3)

![Figure 4. Cell viability in NCI-H292 cells following the exposure to increasing concentrations of 3R4F and prototype e-cig AqE. n = 3 experiments.](image4)

![Figure 5. Relative wound density following the exposure to media (M) and increasing concentrations of 3R4F and prototype e-cig. n = 3 experiments.](image5)
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E-cigarettes have rapidly increased in popularity over the last decade and there is a growing consensus that e-cigarettes hold great potential for reducing the risk associated with cigarette smoking.

In this study, the responses of a novel prototype e-cigarette and scientific reference cigarette (3R4F) in cytotoxicity, oxidative stress and endothelial cell migration endpoints were assessed using the neutral red uptake (NRU), glutathione ratio and wound healing assays, respectively. Exposure matrices were whole aerosol and aqueous aerosol extracts (AqE) generated using Health Canada Intense (HCI) regime for cigarettes or CRM81 regime for e-cigarettes. Nicotine was measured in all exposure matrices.

3R4F whole aerosol induced a concentration dependent increase in cytotoxicity, whereas the prototype e-cigarette resulted in responses comparable to the air control. 3R4F AqE reduced the glutathione ratio at doses >6.25% indicative of oxidative stress and induced cytotoxicity at doses >12.5%. Exposure to the prototype e-cigarette AqE, even at 100% did not affect glutathione ratio or cell viability, aligned to control responses. 3R4F AqE doses >10% inhibited endothelial cell migration with complete inhibition at doses >25% AqE. Prototype e-cigarette AqE, even at the 100% dose, did not inhibit cell migration, equivalent to the control responses.

In all assays, prototype e-cigarette exposure resulted in reduced responses when compared to 3R4F. These data add to the growing weight of evidence that e-cigarettes offer substantially reduced toxicant exposure when compared to conventional cigarettes. Further pre-clinical and clinical assessments are required to fully understand the risk reduction potential of e-cigarettes at individual and population levels.

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