**Introduction**

Electronic cigarettes (e-cigarettes) represent a subset of the next-generation nicotine and tobacco product category, which have the potential to expose the user to significantly lower levels of toxins than those present in cigarette smoke. Hence, e-cigarettes hold great potential for reducing the harm associated with tobacco use, but this needs to be scientifically proven.

The aim of this study was to characterise the aerosol emissions and assess the biological impact of a novel e-cigarette, that has a unique aerosolisation technology (Figure 1). The results from this prototype e-cigarette device were compared to those obtained from a scientific reference 3R4F cigarette.

**Materials and Methods**

**Aerosol generation**

Products were puffed according to the regimens detailed below (Table 1).

<table>
<thead>
<tr>
<th>Product</th>
<th>Full Regimen</th>
<th>Full Volume (µL)</th>
<th>Puff interval (s)</th>
<th>Puff duration (s)</th>
<th>Puff Profile Vent blocking</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F reference cigarette</td>
<td>15</td>
<td>30</td>
<td>2</td>
<td>Bell</td>
<td>100%</td>
</tr>
<tr>
<td>Novel e-cigarette</td>
<td>15</td>
<td>30</td>
<td>2</td>
<td>Bell</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Test matrix preparation**

Using the puffing regimens as described above, three different test matrices were generated for in vitro assessment:

- **Total Particulate Matter (TPM)**: trapped on a Cambridge filter pad and eluted at 24 h after treatment with TPM.
- **Whole aerosol (WA)**: cells directly exposed to aerosol at the air-liquid interface.
- **Total Particulate Matter (TPM)**: trapped on a Cambridge filter pad and eluted at 24 h after treatment with TPM.

**Neutral red uptake (NRU) cytotoxicity assay**

Neutral red uptake (NRU) cytotoxicity assay was performed by treating H292 cells with TPM (Figure 2a). The novel e-cigarette induced significantly less cytotoxicity at up to 1,000 puffs (Figure 2a), and at comparable and higher levels of nicotine delivered to the cells (Figure 2b).

**High content screening and ARE assay**

3R4F was positive in 6 endpoints assessed using a HCS approach (Table 2). 3R4F TPM caused activation of the antioxidant response element (ARE) at both timepoints assessed (Figure 3). In contrast, the novel prototype e-cigarette was negative for each HCS endpoint, and was negative in the ARE assay.

**Endothelial wound healing (scratch) assay**

3R4F inhibited wound repair in a HUVEC monolayer, in a dose-dependent manner. AqE from the novel e-cigarette did not significantly affect wound repair, up to the maximum concentration tested (100% AqE; Figure 4).

**Conclusions**

- The novel e-cigarette showed little or no activity in any of the in vitro assays where it was assessed.
- Activity was significantly less than from 3R4F reference cigarette across all studies.
- The results show that this novel e-cigarette has the potential to be a reduced risk product compared to cigarette smoking, though further longer-term studies would be required to substantiate this potential.

**References**


**Results**

WA cytotoxicity assessment

WA cytotoxicity assessment demonstrated that 3R4F produced a concentration-related decrease in cell viability, resulting in complete cytotoxicity after just 7 puffs of undiluted aerosol (Figure 2a). The novel e-cigarette induced significantly less cytotoxicity at up to 1,000 puffs (Figure 2a), and at comparable and higher levels of nicotine delivered to the cells (Figure 2b).

**Figure 1.** Schematic drawing of prototype e-cigarette. a) e-liquid; b) distiller technology; c) electronics and battery; d) device body.

**Figure 2.** Neutral red uptake determined cell cytotoxicity of H292 cells following exposure to aerosols from a novel e-cigarette and 3R4F reference combustible cigarette. Cell viability is expressed as a function of (a) puff number, and (b) nicotine levels measured in the media following exposure. Data are mean ± S.D. (n=3).

**Figure 3.** Activation of the H292-ARE-Luc2P RGA following exposure to TPM from 3R4F and a novel e-cigarette. Data shown are mean fold changes in response normalized to the vehicle control (0.83% DMSO). ARE activation (a) and corresponding cell viability (b) following 6 h exposure to 3R4F and e-cigarette. Data are mean ± S.D. (n=6).

**Figure 4.** Wound healing rates (mean % increase in relative wound density) in HUVEC monolayers during 24 h treatment with AqE from 3R4F cigarette (4 puffs/20 mL) and prototype e-cigarette (24 puffs/20 mL). Data are mean ± S.D. (n=3).

**Table 1.** Puffing regimens for aerosol generation

**Table 2.** High content screening assay data

Values are the minimum required TPM concentration (µg/mL) to elicit a ≥ 1.5 fold increase (↑) or a ≤ 0.5 fold decrease (↓) in assay signal from the 0.5% DMSO vehicle control or a 30% decrease (↓).
In vitro Assessment of a Novel E-Cigarette – Comparison with a Combustible Cigarette

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Abstract

Electronic cigarettes (e-cigarettes) have rapidly risen in popularity in recent years. E-cigarette users have played a central role in driving fast-paced development of devices and e-liquids. This has led to an increasing need to assess the biological impact associated with the use of e-cigarettes. We have proposed a framework for the assessment of next generation products, including e-cigarettes, which includes preclinical, clinical and population studies. In the current study we have assessed the biological effects of a novel prototype e-cigarette, using a battery of in vitro test methods which form part of our preclinical assessment phase. E-cigarette responses were compared with those elicited by a 3R4F reference cigarette.

Aerosols from both products were assessed as various matrices; total particulate matter (TPM), whole aerosol (WA), and aqueous aerosol extracts (AqE). These aerosols were generated using the Health Canada Intense regime, and modified CORESTA regime for the cigarette and e-cigarette, respectively. Biological endpoints included cytotoxicity (neutral red uptake viability assay), oxidative stress (antioxidant response element (ARE) activation in lung epithelial reporter cells) and endothelial cell migration (wound healing). In addition, a high content screening (HCS) approach was employed to assess 10 different toxicity endpoints such as DNA damage and intracellular glutathione content.

The prototype e-cigarette induced little or no activity across all assays, in comparison to 3R4F aerosol responses, which were almost exclusively positive. E-cigarette WA induced cytotoxicity in H292 cells, but only at much higher doses than 3R4F WA. In the ARE reporter assay, 3R4F TPM induced oxidative stress at both 6 hr and 24 hr timepoints, while e-cigarette TPM was negative. HCS assays were also assessed at two timepoints (4 hr and 24 hr). 3R4F TPM was positive in all but two of the ten endpoints assessed, while e-cigarette TPM was negative in all ten. 3R4F AqE exhibited a concentration-related response in the endothelial wound healing assay; in contrast, exposure to the e-cigarette AqE did not do so.

When taken together as a whole, results from these in vitro assays suggest the novel prototype e-cigarette shows the potential of risk reduction compared with traditional cigarettes. Further understanding of the risk reduction of these novel products can be gained through complementary studies at individual and population levels.

Key Words

Electronic cigarette; cigarette smoke; high content screening; cytotoxicity; oxidative stress