The chemical and mutagenic assessment of an electronic cigarette

Introduction

Cigarette smoking is a cause of many human diseases including cardiovascular disease, lung disease and cancer. The use of novel tobacco and nicotine products with reduced yields of toxicants compared to cigarettes, in particular electronic or e-cigarettes, holds great potential for reducing the harm associated with cigarette smoking. Currently, in the UK, several public health agencies have advocated a potential role for novel nicotine products in tobacco harm reduction, as they deliver nicotine in a cleaner form than cigarette smoke (1-2).

Objective

In this study we explored the levels of toxicants in the aerosol of a commercial, e-cigarette, Vype ePen, and compare it’s mutagenic potential through in vitro assessment relative to smoke from a scientific reference cigarette (3R4F).

Methods

Table 1. Aerosol generation regimens

<table>
<thead>
<tr>
<th>Product</th>
<th>Puff Regimen</th>
<th>Puff Volume (mL)</th>
<th>Puff Frequency (secs)</th>
<th>Puff Duration (secs)</th>
<th>Puff Profile</th>
<th>Vent blocking</th>
<th>Coil pre-activation (secs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F</td>
<td>HCl¹</td>
<td>55</td>
<td>30</td>
<td>3</td>
<td>Bell</td>
<td>100 %</td>
<td>N/A</td>
</tr>
<tr>
<td>Vype ePen</td>
<td>CRM²</td>
<td>55</td>
<td>30</td>
<td>3</td>
<td>Square</td>
<td>N/A</td>
<td>0</td>
</tr>
</tbody>
</table>

¹ = HCl T-115 (3); ² = CRM N° 81 (4); N/A = metric not applicable

Assessment of emissions

The emissions of toxicants in Vype ePen aerosol were compared with those from 3R4F cigarettes under machine-puffing regimes detailed in table 1. The list of toxicants measured included those proposed by Health Canada, the WHO Study Group on Tobacco Product Regulation (ToReg), the US Food and Drug Administration and possible thermal breakdown products. Overall, an analyte in 3R4F mainstream smoke and Vype ePen emissions were assayed (5-6).

Generation of test matrices

Two different test matrices were used for in vitro assessments: total particulate matter (TPM) and whole aerosol (WA). These were produced according to the puffing regimes detailed in Table 1.

TPM: Approximately 150 mg of TPM were collected on 44 mm Cambridge filter pads (Whatman, Maidstone, UK). DMSO (Sigma-Aldrich, UK) was used to elute the TPM from the pads to a stock concentration of 24 mg/mL. The extracts were stored in single-use volumes at -80°C until required.

WA: a Vitrocell Smoking Robot VC10 (Vitrocell Systems, Waldkirch, Germany) was used as previously described (7-8).

Ames bacterial reverse mutation assay

TPM exposures were conducted according to the principles of OECD 471, however utilising only S. typhimurium strain TA98+S9. For WA exposures, the Ames assay was modified as previously described (7).

YH2AX genotoxicity

Human bronchial epithelial cells (BEAS-2B) were cultured as previously described (6). YH2AX staining intensity was determined using a Cellomics Arrayscan VTI platform combined with the Target Activation Bioapplication software (8).

Bhas cell transformation assay

The potential of TPM to induce tumour development was evaluated using the Bhas 42 cell transformation assay, promoter protocol as previously described (9). TPM was tested at various concentrations up to a maximum concentration of 48 μg/mL.

Results

Assessment of emissions

- Toxicant levels in the emissions from Vype ePen were significantly lower than those from 3R4F (Table 2). The data for all analytes measured including the extended FDA, Health Canada and WHO list can be found in Margham et al., 2016 (5).

Table 2. The comparison of untargeted emissions in 3R4F and Vype ePen

<table>
<thead>
<tr>
<th>Product</th>
<th>Tobacco present</th>
<th>Aerosol formation mechanism</th>
<th>Number of compounds in aerosol</th>
<th>Typical number of toxicant types</th>
<th>Untargeted emissions</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F</td>
<td>Yes</td>
<td>Tobacco combustion &amp; pyrolysis</td>
<td>&gt; 7,000</td>
<td>100 – 150</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Vype ePen</td>
<td>No</td>
<td>Vaporisation of e-liquid</td>
<td>10 – 100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ames bacterial reverse mutation assay

- Vype ePen TPM or WA did not induce mutagenicity in the TA98 bacterial strain (Figure 2), while 3R4F induced a dose-related response.

YH2AX genotoxicity

- Vype ePen WA did not induce DNA damage (Figure 3), while 3R4F induced a dose-related response in BEAS-2B cells.

Bhas cell transformation assay

- Vype ePen TPM did not induce foci (Figure 4) whereas 3R4F induced a dose-related response in fibroblasts.

Conclusions

- Toxicant levels in Vype ePen aerosol were significantly reduced compared with cigarette smoke
- Vype ePen showed no activity in any of the in vitro assays assessed
- These studies indicate that Vype ePen may be a safer alternative to cigarette smoking

References

The chemical and mutagenic assessment of an electronic cigarette

David Thorne, Damien Breheny, Ian Crooks, Frazer Lowe, Jason Adamson, Kevin McAdam, Christopher Proctor and Marianna Gaca
British American Tobacco, R&D Centre, Southampton SO15 8TL

Cigarette smoking is a cause of many human diseases including cardiovascular disease, lung disease and cancer. The use of novel tobacco and nicotine products with reduced yields of toxicants compared to cigarettes, and in particular electronic or e-cigarettes, holds great potential for reducing the harms associated with cigarette smoking. Currently in the UK, several public health agencies have advocated a potential role for novel nicotine products in tobacco harm reduction, as they deliver nicotine in a cleaner form than cigarette smoke.

A key underlying mechanism for smoking related diseases such as cancer, from long term exposure to smoke toxicants has been proposed as DNA damage. In this paper we explore the levels of toxicants in the aerosol of a test e-cigarette (EC) and compare it’s mutagenic potential through an in vitro assessment relative to smoke from a scientific reference cigarette (3R4F).

We tested EC and 3R4F to measure levels of over one hundred constituents inclusive of the Harmful and Potentially Harmful Constituents. The in vitro tests included the globally recognised Ames test to determine mutagenic potential; the quantification of DNA damage in human lung cells by assessing phosphorylation of H2AX and the Bhas promotion assay as an in vitro surrogate for detecting tumour promoters.

The chemical analysis showed the absence or substantial reduction in constituents in the aerosol of EC relative to 3R4F smoke. Furthermore, data from these assays indicated that the biological response to EC aerosol was significantly lower relative to 3R4F smoke. EC was non-mutagenic (even after extreme testing) in the Ames assay using strains TA98 and TA 100 with metabolic activation (S9), however 3R4F was mutagenic. Increasing concentrations of 3R4F smoke demonstrated a dose response in DNA damage-induction, in contrast, EC did not induce DNA damage. 3R4F was positive in the Bhas promotion assay at concentrations ≥ 6 µg/mL, while EC did not have any in vitro cancer promoter activity.

The chemical and in vitro techniques employed were able to distinguish responses between EC and 3R4F. The data generated add to growing evidence that suggests that e-cigarettes may provide a safer alternative to traditional cigarettes, with the potential to contribute significantly to tobacco harm reduction at both the individual and population levels.