Introduction

Tobacco heating products (THPs) represent a subset of the next-generation nicotine and tobacco product category, in which tobacco is heated at temperatures of less than 350°C instead of burning (800°C), having the potential to significantly reduce cigarette smoke toxicants. THPs hold great potential for reducing the harm associated with tobacco use, but this needs to be scientifically proven.

Objective

To characterise the aerosol emissions and assess the biological impact of the novel THP, THP1.0 (commercially known as glo™) (Figure 1), comparing results to a reference 3R4F cigarette.

Methods

Assessment of emissions

The emissions of toxicants in THP1.0 aerosol were compared with those from a reference 3R4F cigarette under a machine-puffing regimen of 55 mL puff volume, 2 s puff duration and 30 s puff interval. 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 tobacco breakdown products. Overall, 22 different analytical techniques were used to quantify the emissions of 126 analytes in 3R4F mainstream smoke and THP1.0 emissions, as described in Forster et al., 2017.

In vitro assessment

Using the same puffing regimen as described above, two different test matrices were generated for in vitro assessment.

Total Particulate Matter (TPM): Approximately 150 mg of TPM was collected on 44 mm Cambridge filter pads (Whatman, UK). DMDS (Sigma-Aldrich, UK) was used to elute the TPM from the pads to a stock concentration of 24 mg/mL.

Whole aerosol (WA): A Vitrotec VC10® smoking robot (Vitrotec Systems, Germany) was used to generate whole aerosols for the Ames assay, as previously described3. A Borgwald RM205® exposure system was used for the cytotoxic assay, as detailed previously4.

Ames bacterial reverse mutation assay

TPM exposures were conducted to the principles of OECD 471, using five S. typhimurium strains: TA98, TA100, TA10153, TA1053 and TA1012, 2 metabolic activation (S9). For product WA exposures, the Ames assay was employed with S. typhimurium tester strains: TA98, TA100, TA1053, TA97 and TA102 using a modified methodology as previously described5.

Mouse lymphoma assay (MLA)

TPM was assessed following OECD 490, ≥ 50 with short 3 h exposures and longer 24 h – 60 h exposures5.

Neutral red uptake (NRU) cytotoxicity assay

TPM cytotoxicity was assessed using BALB/c 3T3 mouse fibroblasts1. WA cytotoxicity was assessed using human bronchial epithelial cells (H292) exposed at the air-liquid interface (ALI) for 1 h at dilutions of 1:200 – 1:10,000 for 3R4F and 1:2- 1:20 for THP1.0 (aerosol:air; v:v)4.

Bhas cell transformation assay

The potential of TPM from the products to induce tumour development was evaluated using the Bhas 42 cell transformation assay, promoter protocol6. TPM was tested at various concentrations up to a maximum concentration of 48 µg/mL.

Luciferase-based reporter gene assay to assess oxidative stress

Antioxidant response element (ARE) transcriptional activation in stably transfected H292 cells were assessed after 6 to 24 h treatment5.

Multiparametric analysis using high-content screening (HCS) approaches

The Cellomics ArrayScan V10 platform was used to assess 10 endpoints in normal human bronchial epithelial cells (NHBEs) after 4 or 24 h exposures, as previously described6.

Results

Quantification of FDA priority toxicants

Toxicant levels in the emissions from THP1.0 were significantly lower than those from 3R4F (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>THP1.0</th>
<th>3R4F</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Histidine</td>
<td>µg</td>
<td>108</td>
<td>781.0 (SD 0.53)</td>
</tr>
<tr>
<td>Aspartate</td>
<td>µg</td>
<td>2390</td>
<td>95.0</td>
</tr>
<tr>
<td>Acetone</td>
<td>µg</td>
<td>157</td>
<td>2.22</td>
</tr>
<tr>
<td>Benzene</td>
<td>µg</td>
<td>17.6</td>
<td>95.0</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>ng</td>
<td>12.9</td>
<td>9.34</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>mg</td>
<td>32.0</td>
<td>95.0</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>µg</td>
<td>15.10</td>
<td>95.0</td>
</tr>
<tr>
<td>4-(N-Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)</td>
<td>ng</td>
<td>281</td>
<td>6.61</td>
</tr>
<tr>
<td>Nitrosocarcinogen</td>
<td>ng</td>
<td>263</td>
<td>24.7</td>
</tr>
</tbody>
</table>

Average 95.0

THP1.0 was negative for both toxicology tests using a HCS approach. In all two endpoints 3R4F was positive at both timepoints tested, Table 3. THP1.0 was negative for each HCS endpoint, apart from activation of the antioxidant response element (ARE), where there was a moderate response at both the 4 and 24 h timepoints. However, the data showed a significantly higher response to TPM generated from 3R4F than from THP1.0 at both timepoints tested, Figure 3.

Conclusions

• Toxican levels in THP1.0 emissions were significantly reduced across all chemical classes compared to 3R4F reference cigarette
• Across all the in vitro techniques employed a clear positive response was observed with 3R4F cigarette smoke particulate matter and whole aerosol
• THP1.0 particulate matter and whole aerosol showed little or no activity in any of the in vitro assays at doses equivalent or higher than 3R4F reference cigarette
• The data generated add to growing evidence that suggests THPs may provide a less risky alternative to traditional cigarettes, however further studies investigating the longer term effects on consumers is required to substantiate disease relevant risk reduction

References


www.bat-science.com

Correspondence: damien.breheny@bat.com

The data for 126 chemicals including the extended FDA, Health Canada and WHO list can be found in Forster et al., 2017.