1. INTRODUCTION

Combustible tobacco products like cigarettes are known to cause serious disease in smokers, including lung cancer, heart disease and emphysema. A range of next generation products (NGPs), which do not involve combustion, are commercially available and there is a growing belief that NGPs may be a less harmful alternative to combustible tobacco products. The aim of the study was to compare the chemical and in vitro toxicological activity of NGP aerosols to that of cigarette smoke. Products investigated were the Kentucky reference cigarette (3R4F), a tobacco heating product (THP), a hybrid product (HTY) and a myblu™ e-cigarette (Tobacco Flavour 16%). Smoke/aerosol was produced using Health Canada Intense method for 3R4F and THP and Coresta Recommended Method Nº81 for HYB and myblu™. Product smoke/aerosols were tested in established regulatory in vitro toxicity assays.

2. MATERIALS

2.1 Test Cigarettes
- Kentucky 3R4F Reference Cigarette
- Commercially available tobacco heating product (THP), German market
- Commercially available hybrid product (HTY), Romanian market
- E-vapour product: myblu™ device and pod (1.6% w/w nicotine; tobacco flavour), UK market

NGP product formats are shown in Figure 1

2.2 Smoke and Aerosol Generation

Test product aerosol/smoke was generated using the following regimes (Table 1).

Aerosolisation Formats for NGP

<table>
<thead>
<tr>
<th>Format</th>
<th>Full-vol.</th>
<th>Full-duration</th>
<th>Full-intervals</th>
<th>inhalation</th>
<th>Ball</th>
<th>Bell</th>
<th>Ball</th>
<th>Bell in vitro toxicology</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F</td>
<td>Health Canada Intense™</td>
<td>80</td>
<td>36</td>
<td>Yes</td>
<td>Ball</td>
<td>Linear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>THP</td>
<td>Health Canada Intense™</td>
<td>80</td>
<td>36</td>
<td>N/A</td>
<td>Square (passing)</td>
<td>Linear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HYB</td>
<td>myblu™, 3 blocks, each 50 puffs</td>
<td>80</td>
<td>36</td>
<td>N/A</td>
<td>Square</td>
<td>Linear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myblu™</td>
<td>myblu™, 3 blocks, each 50 puffs</td>
<td>80</td>
<td>36</td>
<td>N/A</td>
<td>Square</td>
<td>Linear</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *One method with 1°/2° polarisation, emission orientation was carried out using ball, square puff profiles and rotary streaming machines

2.3 In vitro Toxicology

The following regulatory in vitro toxicology assays were performed: Neutral red uptake (NRU) for cytotoxicity in BEAS-2B cells, following standard assay protocols in accordance with ISO 17025; Salmomella typhimurium reverse mutation assay (Ames test) for mutagenicity in TA98 and TA100 in compliance with OECD test Guideline 471; and in vitro micronucleus (VM) with V79 (3hrs + 9hrs) for genotoxicity in compliance with OECD test Guideline 487. Cells were exposed to smoke or aerosol at the air liquid interface using the internal smoking machine 'smoke aerosol exposure in vitro system' (SASEVIS) (Burghart Tabaktechnik, Wedel, Germany) for NRU and VM and using the smoking machine RM1 (Burghart Instruments, Wedel, Germany) for the Ames assay.

2.6 Data and statistical analysis

All data and statistical analysis were conducted using Microsoft Excel and GraphPad Prism. Statistically significant differences between samples were calculated using ANOVA with poshoc Dunnett test. All differences were considered statistically significant with a p-value ≤ 0.05.

3. RESULTS

3.1 Smoke and Vapour Characterisation

Cambridge Filter Pads

Particulate phase emissions of 5x 3R4F sticks compared to (A) 5x THP sticks (10 puffs per stick), (B) one block of 50 puffs for HYB and (C) one block of 50 puffs for myblu™ (Figure 1).

3.2 In vitro Toxicity

Cytotoxicity (NRU)

All NGP aerosols demonstrated marked cytotoxicity reductions compared to cigarette smoke on a per puff basis (Figure 6). myblu™ displayed low levels of cytotoxicity compared to the other test articles. The puff specific cytotoxicity can be described as: 3R4F > HYB > myblu™ > THP.

Mutagenicity (Ames)

3R4F smoke was highly mutagenic in the Ames test. In TA100+S9, 3R4F smoke showed marked mutagenicity, which was reduced in THP (approximately 2 fold) (Figure 3). In TA98 (data not shown), only 3R4F smoke produced a positive, mutagenic response. Neither HYB or myblu™ aerosol produced a significant number of revertants, up to 300 puffs in the presence of S9 mix compared to ambient air (Figure 5).

Genotoxicity (IVM)

Both 3R4F smoke and THP aerosol induced reproducible and statistically significant increased in micronuclear frequencies, with 3R4F smoke inducing significant genotoxicity after 1 puff. When comparing micronucleus frequencies over background levels (ECCM2) from HTP aerosol compared to THP, a 30 fold lower genotoxicity was observed for the THP (Figure 6). HYB and myblu™ aerosol, up to 100 puffs, did not induce any statistically significant increases in MN frequency, compared to negative control.

4. CONCLUSIONS

• The regulatory assays described above form part of a core battery of tests, to determine the potential hazard of cigarettes and NGP products.
• As expected, there are clear cytotoxic, mutagenic and genotoxic effects observed for 3R4F smoke. HTP produced some responses but to a much lesser degree.
• There are marked reductions in the emissions and in vitro toxicity of NGP products compared to 3R4F cigarettes.

Society Toxicology MARCH 2019
Kathryn Rudd1, Roman Wieczorek2, Edgar Treles Sticken3, Jutta Pani4, Cle Dethloff5, Matthew Stevenson1
1. Imperial Brands PLC, 121 Winterton Road, Bristol, BS3 2LL, UK
2. Revaenigma Zigarettenfabriken GmbH, An Imperial Brands PLC Company, Albert-Einstein-Ring-7, D-02271, Hamburg, Germany

Visit our science website: www.imperialbrandsScience.com

This work was supported by Imperial Brands plc. Imperial Brands plc is the manufacturer of the myblu™ product used in this study.

REFERENCES


3. RESULTS

3.1 Smoke and Vapour Characterisation

Cambridge Filter Pads

Particulate phase emissions of 5x 3R4F sticks compared to (A) 5x THP sticks (10 puffs per stick), (B) one block of 50 puffs for HYB and (C) one block of 50 puffs for myblu™ (Figure 1).

3.2 In vitro Toxicity

Cytotoxicity (NRU)

All NGP aerosols demonstrated marked cytotoxicity reductions compared to cigarette smoke on a per puff basis (Figure 6). myblu™ displayed low levels of cytotoxicity compared to the other test articles. The puff specific cytotoxicity can be described as: 3R4F > HYB > myblu™ > THP.

Mutagenicity (Ames)

3R4F smoke was highly mutagenic in the Ames test. In TA100+S9, 3R4F smoke showed marked mutagenicity, which was reduced in THP (approximately 2 fold) (Figure 3). In TA98 (data not shown), only 3R4F smoke produced a positive, mutagenic response. Neither HYB or myblu™ aerosol produced a significant number of revertants, up to 300 puffs in the presence of S9 mix compared to ambient air (Figure 5).

Genotoxicity (IVM)

Both 3R4F smoke and THP aerosol induced reproducible and statistically significant increased in micronuclear frequencies, with 3R4F smoke inducing significant genotoxicity after 1 puff. When comparing micronucleus frequencies over background levels (ECCM2) from HTP aerosol compared to THP, a 30 fold lower genotoxicity was observed for the THP (Figure 6). HYB and myblu™ aerosol, up to 100 puffs, did not induce any statistically significant increases in MN frequency, compared to negative control.

4. CONCLUSIONS

• The regulatory assays described above form part of a core battery of tests, to determine the potential hazard of cigarettes and NGP products.
• As expected, there are clear cytotoxic, mutagenic and genotoxic effects observed for 3R4F smoke. HTP produced some responses but to a much lesser degree.
• There are marked reductions in the emissions and in vitro toxicity of NGP products compared to 3R4F cigarettes.

Society Toxicology MARCH 2019
Kathryn Rudd1, Roman Wieczorek2, Edgar Treles Sticken3, Jutta Pani4, Cle Dethloff5, Matthew Stevenson1
1. Imperial Brands PLC, 121 Winterton Road, Bristol, BS3 2LL, UK
2. Revaenigma Zigarettenfabriken GmbH, An Imperial Brands PLC Company, Albert-Einstein-Ring-7, D-02271, Hamburg, Germany

Visit our science website: www.imperialbrandsScience.com

This work was supported by Imperial Brands plc. Imperial Brands plc is the manufacturer of the myblu™ product used in this study.

REFERENCES