1. INTRODUCTION

When the National Academies of Sciences published "Toxicity Testing in 21st Century: A Vision and a Strategy" a new toxicological paradigm was created, focusing on the use of human derived cells lines/tissues and the disruption of key cellular pathways (NRC, 2007). In keeping with these principles, Imperial Brands has sought current assays for harm reduction assessment of our products, with toxicological framework shown in Figure 1.

The study explored the potential use of in vitro assays utilizing human primary cells. The three in vitro platforms studied were High Content Screening (HCS); the use of primary human cells (BioMap®); and a 3D reconstituted human lung model exposed to e-liquid aerosol. To determine the suitability of each test system, either base e-liquid or e-cigarette aerosol were used.

Figure 1: In vitro testing framework for harm reduction assessment

• HCS was conducted with primary human NHBE cells exposed to neat e-liquids for up to 24 hours, using fluorescent staining to study a range of cell health markers at the same time.

• The BioMap® Diversity PLUS product consisted of 12 primary human cell-based systems designed to model different tissues and disease in vitro (see Table 2). Cells were stimulated with a combination of biological proprietary factors, (e.g. cytokines, growth factors, mediators, etc.) to recreate the multi-component signalling networks associated with disease states.

• Human 3D lung cells (EpiAirway®; MatTek Corp.) were exposed to e-liquid aerosol and a reference cigarette (3R4F) to study cellular effects using a wide range of cellular markers.

2. HIGH CONTENT SCREENING OF E-LIQUIDS

Table 1: MEC that significantly crossed vehicle control threshold (%)

<table>
<thead>
<tr>
<th>Component</th>
<th>BE 1.2%</th>
<th>BE 2.4%</th>
<th>BE 3.6%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BE 1.2%</td>
<td>56.4%</td>
<td>61.4%</td>
<td>66.4%</td>
</tr>
<tr>
<td>BE 2.4%</td>
<td>66.4%</td>
<td>71.4%</td>
<td>76.4%</td>
</tr>
<tr>
<td>BE 3.6%</td>
<td>76.4%</td>
<td>81.4%</td>
<td>86.4%</td>
</tr>
</tbody>
</table>

Methodology:
BioMap® Plus Panel utilizes 12 human primary cell based systems to simulate various key cellular pathways within the human body (Table 2).

Tested base e-liquids (BL) all contained 50:50 propylene glycol (PG) and vegetable glycerine (VG), with varying nicotine content: 0% (BL1), 2.4% (BL2) and 4.5% (BL3). All e-liquids were tested at eight concentrations (0.031-4%). Solutions were added directly to the cell media.

Results:
• BL with different nicotine concentrations added to cell media at concentrations above 0.5% led to a characteristic fingerprint of biomarkers and dose response relationship.

Increasing concentrations of nicotine led to an exaggeration of the fingerprint profile in selected cell panels, above that for base e-liquid itself.

• The cell systems which produced the most notable response was the BT and BE3C cell systems.

• The BioMap Plus Panel is a useful screening tool to give an indication of where to focus further research and is not diagnostic of disease.

3. EFFECTS OF INCREASING CONCENTRATIONS OF NICOTINE IN BASE E-LIQUIDS ON THE BIOMAP®

Figure 2: The effects of base liquid, ±2.4% and 4.5% nicotine concentrations added to BioMap at 1% concentration

Figure 3: Tissue viability

Figure 4: TEER

Figure 5: Oxidative Stress Response

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4. USE OF 3D RECONSTRUCTED LUNG MODELS EXPOSED TO E-CIGARETTE AEROSOL AND CIGARETTE SMOKE AT AIR-LIQUID INTERFACE

Methodology:
E-cigarette aerosol generated using the CORESTA Recommended Method N°81 (55mL/3s/30s; square wave puff profile). Conventional cigarette smoke was generated using the Health Canada Intense method (55s/2s/2s/30s).

Morphological evaluation conducted using H&E staining and EpiAirway® tissue viability was assessed 24 hours after exposure using the MTT assay (MatTek Corp.). Cell membrane integrity was assessed via measurement of the tight junctions using trans-epithelial electrical resistance (TEER). The concentration of 8-isoprostane in conditioned media was measured using a competitive ELISA kit.

Results:
• E-cigarette aerosol up to the highest dose of 400 puffs did not alter tissue viability or barrier function compared to matched air control.

• 8-Isoprostane (a biomarker of oxidative stress and antioxidant deficiency) results demonstrated no oxidative stress responses in samples exposed to either base e-liquid with 2.4% nicotine under the conditions of test.

• CS significant decreased tissue viability and barrier function and caused a significant increase in oxidative stress response.

5. CONCLUSIONS

• Neat e-liquids and aerosol at physiologically relevant concentrations did not generally elicit a toxicological response in the range of in vitro assays tested. Our findings are consistent with other researchers.

• The in vitro assays explored here are a quick screening tool for assessing the toxicity of e-liquids and add to a Weight of Evidence (WoE) approach.

• We believe these in vitro assays have the potential to greatly contribute to our current knowledge of e-liquid ingredients and aerosols and should form part of a larger weight of evidence approach including clinical studies for the assessment of this category of products.

REFERENCES


Guasch-Gonzalez et al. (2017) Epi in vitro Toxicol 3:1); 45-55