Use of Ex Vivo Precision-Cut Lung Slices as a Screening Tool for Potential Respiratory Toxicity of E-Liquids

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ABSTRACT

The Family Smoking Prevention and Tobacco Control Act gave the FDA regulatory authority over next generation tobacco products (NGTPs) such as e-liquid products. E-liquid product liquids contain a variety of ingredient combinations that should be assessed for human risk. One human lung relevant testing platform with reasonable throughput is in human precision-cut lung slices (HuPCLS). The purpose of the current study was to assess the safety of three NGTP ingredients (the most common, 1.8% PG in CM, 4% PG in glycol, and 8% PG in glycol-vapor) by using a panel of assays and histological assessment of HuPCLS. Three concepts (0.5%, 0.2%, and 1.2% of propylene glycol (PG) on an Ex-Vapor product concentration) were used in a 21-day exposure period. Exposure effects were evaluated biochemically (WST-6 assay) and histologically (viability assessment of H&E stained slices). Positive control experiments elicited tissue damage as a severe inflammatory response, and there was no effect histologically or via WST-6 viability for prolonged exposure to 0.1% and 0.5% PG in CM. In summary, PG is demonstrated to be a safe ingredient with no adverse effects in a human pulmonary model in an exposed exposure regimen. The observed changes with changes in PG concentration in a HuPCLS platform have potential to serve as a screening tool for risk (and other regulatory use) for modifying potentially relevant, long-term effects following NGTP ingredient exposure.

MATERIALS & METHODS

Test System: HuPCLS (donor was a healthy non-smoker), provided by H. P. Behrsing, Ph.D.

Well-Controlled Animal-Free Models: HuPCLS (NSC 710305, 1.8% PG in CM) were exposed via media to 1.8% PG in CM, 4% PG in glycol, and 8% PG in glycol-vapor. The 4% PG in glycol-vapor group was used as a positive control. All treatment media were tested for osmolality and pH.

RESULTS

RESULTS cont.

Table 1: Average (AVG) viability score (0-4) derived from H&E slides

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8% PG (CM)</td>
<td>3.2 ± 1.1</td>
<td>3.1 ± 1.1</td>
<td>3.3 ± 0.9</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td>4% PG (glycol)</td>
<td>3.2 ± 0.9</td>
<td>3.2 ± 0.9</td>
<td>3.3 ± 1.0</td>
<td>3.4 ± 1.1</td>
</tr>
<tr>
<td>8% PG (glycol-vapor)</td>
<td>3.1 ± 0.8</td>
<td>3.2 ± 0.9</td>
<td>3.3 ± 1.0</td>
<td>3.4 ± 1.1</td>
</tr>
</tbody>
</table>

Table 2: Average (AVG) viability score (0-4) derived from WST-6 slides

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8% PG (CM)</td>
<td>2.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>4% PG (glycol)</td>
<td>2.0 ± 0.0</td>
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<td>8% PG (glycol-vapor)</td>
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</table>

1. Inflame lung tissue and prepare for exposure.
2. Create tissue cones
3. Slice tissue with slicer
4. Collect slices and plate into viable medium.
5. Rotate slices at 1-3 rpm in E-100 medium

VIABILITY & OSMIOLITY

Omology Results:

- All treatment groups agreed with corresponding to the accepted human physiological range (280-285 mOsm).

The reference materials (PG) performed as expected. The high osmolality of the exposure media containing the 1.2% PG was hypothesized to be the cause of the significant loss of WST-8 viability — it is expected that a longer culture would have shown this effect histologically.

CONCLUSIONS

- For 16-day post-exposure, HuPCLS exhibited characteristic retention of overall health and viability, as measured using the WST-8 viability assay, which can serve for longer-term screening and for identifying more relevant ingredients.

- Combining the biochemical and histological assessment of HuPCLS viability allows for a more thorough assessment of treatment impact on human lung tissue, with the capacity for in vivo-like assessment.

- The reference materials (PG) performed as expected. The high osmolality of the exposure media containing the 1.2% PG was hypothesized to be the cause of the significant loss of WST-8 viability — it is expected that a longer culture would have shown this effect histologically.

- Development and Validation of HuPCLS as a cytotoxicity assay require a significant amount of additional replicate experiments, testing the addition of the reference materials, and possibly an expansion of the battery of toxicity endpoints.

REFERENCES