Oral Irritation Assessment of Electronic Liquids using an In-Vitro Oral Testing Model

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ABSTRACT

While data are still being collected and analyzed, there were at least 1,500 accidental electronic liquid (e-liquid) exposures reported as of 2013. Deaths have occurred as a result of ingestion of e-liquid with the effects being attributed primarily to nicotine.

The Food and Drug Administration (FDA) sought to regulate e-liquid through the Tobacco Control Act passed in 2009. In 2014, the FDA issued its “Deeming” proposals for public comments, which covered e-liquid manufacturing; the Final Rule giving the FDA authority to regulate e-liquid was released on May 7th, 2016. This study investigated the oral irritation of 3 different formulations of e-liquid using an in-vitro time course assay in the reconstructed tissue model EpiOral™ (MatTek Corporation, Ashland, MA, USA). All products were from the same manufacturer, contained 1.2% nicotine and differed only in their flavoring.

The e-liquid was tested neat in duplicate tissue. Tissue viability was measured using the vital dye MTT at 15 minutes, 1, 2, 4, 6, and 12 hours, respectively. Tissue irritation was determined by measuring the exposure time which reduced the tissue viability by 50% were calculated and used to rank-order the irritation potential of the products. The ET50 values were 4.9, 4.1, and >16 hrs, respectively, for the 3 products tested. The results of this study highlight the applicability of the EpiOral™ model in evaluating the oral irritation potential of a variety of e-liquid formulations.

Further work will be performed with the cultured medium to analyze the inflammatory profile of e-liquid via cytokine analysis.

INTRODUCTION

In general, e-liquids are composed of glycerin, propylene glycol, water, and nicotine. The ratio of the constituents of e-liquids can vary from product to product. In addition, e-liquids can be “flavored.” This flavoring process can result in toxicity dependent on the formulation and chemicals responsible for the flavoring. The simplicity in creating e-liquids allows for a wide range of potential formulations with unknown toxicity.

The majority of exposure to e-liquids, whether accidental or intentional, involves exposure to the undiluted formulation. The goals of this study were two-fold. The first was to determine if e-liquids could impart any irritation in an in-vitro oral model. The second was to determine if the flavoring played any role in oral irritation associated with e-liquids.

MATERIALS & METHODS

- Test Materials: three commercially available e-liquid products (A-C) were purchased and dosed topically. All 3 products had the same concentration of nicotine and differed only in their flavorings.
- Test System: MatTek “EpiOral™ Tissue Model (SRL-200).”
- Endpoint: Tissue viability (%): time to toxicity (ET50) values were calculated for each product. Exposures were conducted over 2 different trials to assess reproducibility. Tissues were dosed with 100 μL in duplicate.
- Assay Controls: Negative control: sterile, deionized water
- Positive control (P.C.): 1% Triton X-100
- Exposure Times: The time points for trial 1 were 0, 0.5, 1, 2, 4, and 16 hrs
- Data analysis: Results were tabulated and analyzed using Microsoft Excel and GraphPad Prism.

RESULTS – ET50 VALUES

<table>
<thead>
<tr>
<th>E-liquid</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.9</td>
<td>4.1</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>8.4</td>
</tr>
<tr>
<td>C</td>
<td>&gt;16</td>
<td>10.6</td>
</tr>
<tr>
<td>P.C.</td>
<td>1.43</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Figure 1. Commercially available e-liquids and vaping devices.

Figure 2. A schematic cross section of a ‘Vape Pen’. E-liquid is placed in the tank/chamber, the atomizer generates heat for the vaporization of the e-liquid that users inhale through the mouth piece.

Figure 3. The EpiOral™ reconstructed tissue model. Courtesy of MatTek Corporation, www.mattek.com

Figure 4. EpiOral™ e-liquid trial 1 data.

Figure 5. EpiOral™ e-liquid trial 2 data.

CONCLUSIONS

1. Our data show that e-liquids induce various levels of oral irritation in the in-vitro oral model based on human reconstructed tissues (EpiOral™). The test system was sensitive enough to capture three levels of irritation potential for products A, B, and C (Figure 6).

2. The results indicate that flavoring plays an important role in imparting irritation as the only ingredient different between the products.

FUTURE DIRECTIONS

1. Medium samples will be analyzed for cytokine expression for better understanding of pathways involved in the response of oral tissues to e-liquids.

2. The contribution of nicotine to the toxicity will be investigated by using products with the same flavorings and different concentrations of nicotine (dose response assessment).

3. Additional e-liquid products with a wider range of flavoring will be tested to determine the range of irritation potential that could be expected.