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 1,300 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-liquids was released on May 5th, 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 flavorings.
The e-liquids were tested neat in duplicate tissues. Tissue viability was measured using the vital dye MTT at 15 minutes, 30 minutes, 1 hour, 2 hours, and 16 hours. The ET_{50} values (representing the exposure time which reduces the tissue viability by 50%) were calculated and used to rank-order the irritation potential of the products. The ET_{50} values were 4.9h, 6.0h, and >16h, 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 culture medium to analyze the inflammatory profile of e-liquids via cytokine analysis.

INTRODUCTION

In general, e-liquids are composed of glycerin, propylene glycol, water, and nicotine. The ratios 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, involve exposures 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 flavorings played any role in oral irritation associated with e-liquids.

MATERIALS & METHODS

- **Test Materials:** three commercially available e-liquid products (A-B-C) were purchased and dosed topically. All 3 products had the same concentration of nicotine and differed only in their flavorings.
- **The Test System:** MatTek™EpiOral™ Tissue Model (ORL-200).
- **Endpoint:** Tissue viability (%): time to toxicity (ET_{50}) 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: 0.25, 0.5, 1, 2, and 16 hrs.
  The time points for trial 2 were: 1, 2, 4, 8, and 16 hrs.
- **Data analysis:** Results were tabulated and analyzed using Microsoft Excel and GraphPad Prism.

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

VAPE PEN SCHEMATIC

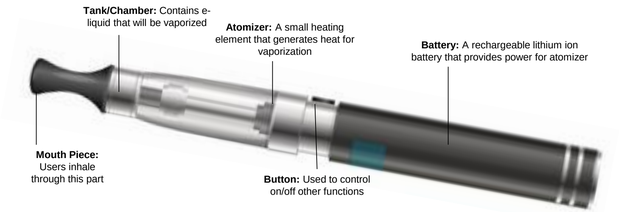

Figure 2. A schematic cross section of a Vape Pen. E-liquid is placed in the tank/chamber, the atomizer generates heat for the vaporization of the e-liquid that users inhale through the mouth piece.

MATTEK EPIORAL™ TISSUE MODEL

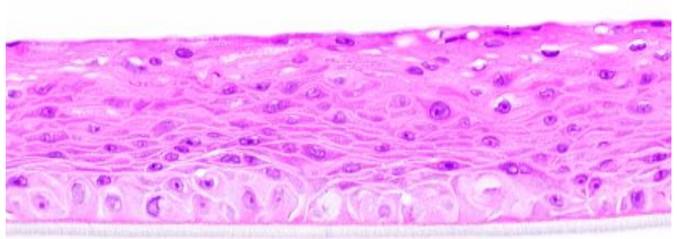

Figure 3. The EpiOral™ reconstructed tissue model. Courtesy of MatTek Corporation, www.mattek.com

RESULTS – ET_{50} VALUES

	ET_{50} (hrs)	
E-liquid	Trial 1	Trial 2
A	4.9	4.1
B	6	8.4
C	>16	10.6
P.C.	1.43	1.39

Table 1. Calculated ET_{50} values. Exposure were conducted over 2 different trials with 2 different tissue lots.

TISSUE VIABILITY RESPONSE CURVES – ET_{50} VALUES

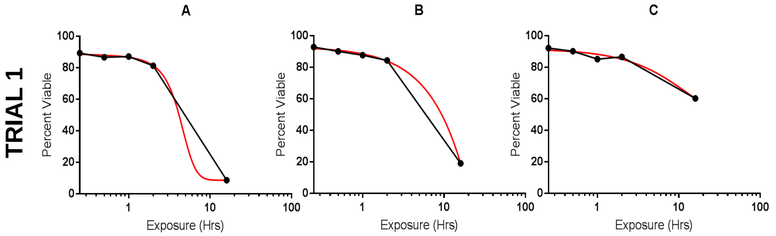

FIGURE 4. EpiOral™ e-liquid trial 1 data.

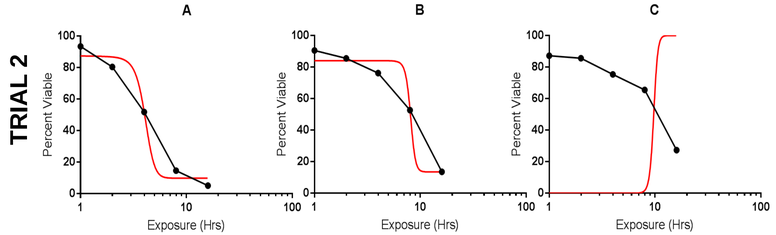

FIGURE 5. EpiOral™ e-liquid trial 2 data.

CONCLUSIONS

1. **Our data show that e-liquids induce various levels of acute 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 tested.**
3. **Reproducibility of test system's performance (inter-lot) is crucial for safety testing as demonstrated also by our results (Table 1).**

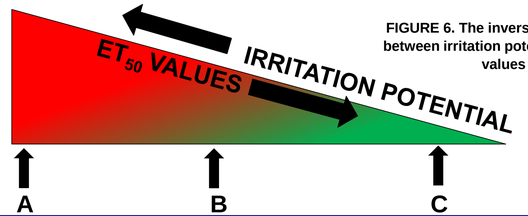

FIGURE 6. The inverse relationship between irritation potential and ET_{50} values

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 can be expected.**