Characterization of an Air-Liquid-Interface (ALI) 
*In Vitro* Exposure System for E-Vapor Product

**ABSTRACT**

Direct delivery of aerosol or vapor to the apical surface of cells (ALI) allows clinically relevant exposure for in vitro toxicological evaluation of inhalable chemicals. However, dose assessment in the ALI exposure system remains a challenge, especially for evoking aerosols such as e-vapor. In this study, we quantitatively characterized the aerosol delivery in commercially available ALI in vitro exposure systems (Vitrocell® Ames 48 and Vitrocell® 24/48 (VC2448)) for e-vapor applications. A cig-a-like test cartridge filled with a prototype e-liquid containing propylene glycol, glycerin, nicotine, and water was used to generate e-vapor aerosols using a Vitrocell® 1/7 puffing machine. Aerosol size distribution, mass deposition, and effective delivery to the exposure inserts (i.e., the petri dish in the Ames 48 or wells in the VC 2448) were measured for both Ames 48 and VC 2448 systems with the regular aerosol delivering method per manufacturer’s instructions. Results showed that 1) the mass median aerodynamic diameter of the delivered aerosol was below 1.5 µm with the geometric standard deviation between 1.8 to 2.1 as measured with a cascade impactor; 2) aerosol delivery in the exposure inserts increased linearly with the puff number; and 3) there was about 30% loss of aerosol mass in the aerosol transportation path prior to entry into the exposure system. To minimize aerosol loss, we revised the aerosol delivering method by shortening the transportation path and showed that 1) the aerosol loss prior to the exposure system was reduced to ~10%; 2) aerosol delivery to the exposure inserts was increased up to 3 fold compared to that of the regular delivery method. For both systems, the aerosol composition of nicotine, propylene glycol, and glycerin were comparable with the theoretical composition of the formulation. For the VC 2448, the average moisture content of the buffer in the wells, in which aerosol was collected, increased linearly from 274 to 676 mOsm/kg H2O in the air control and the 400 puffs exposure group, respectively. The methods developed in this study can be applied to standardize the ALI aerosol characterization of e-vapor products.

**METHOD**

**RESULTS**

**Aerosol Mass Deposition (%) on System Exposure Sections**

<table>
<thead>
<tr>
<th>Aerosol Mass Deposition (%)</th>
<th>Ames 48</th>
<th>Regular Delivering Regime (Trumpet Flow 2 min)</th>
<th>Revised Delivering Regime (Trumpet Flow 2 min)</th>
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**Aerosol Delivery in the Exposure Inserts**

**Nicotine, PG, Glycerin Delivery in the Exposure Inserts**

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**REFERENCES**