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

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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 evolving 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 (Ames 48) and Vitrocell® 24/48 (VC24/48)) 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 24/48) were measured for both Ames 48 and VC 24/48 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 overall with the theoretical composition of the formulation. For the VC 24/48, the average osmolarity of the buffer in the wells, in which aerosol was collected, increased linearly from 274 to 676 mOsmol/kg H_2O 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.

INTRODUCTION

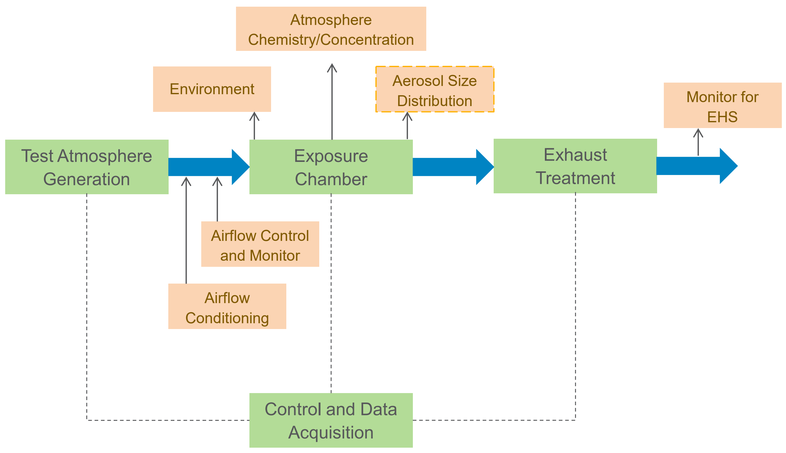
WHY (Why do this?)

- **Fit for purpose characterization of ALI *in vitro* exposure systems**
  - Standardized procedures adopted from OECD Guidance Document on Inhalation Toxicity Studies No. 39

WHAT (What has been done?)

- Monodisperse solid particle deposition in Vitrocell 24/48
- Monodisperse solid particle deposition in Vitrocell Ames 48
- Computational fluid dynamics modeling
- Qualification of VC 1/7 puffing machine
- E-vapor Aerosol Deposition in Vitrocell 24/48 and Ames 48

ALI EXPOSURE SYSTEMS



EXAMPLE ALI SYSTEMS

Aerosol Generation

Vitrocell® VC1/7 (7 individual pumps)

Exposure Systems

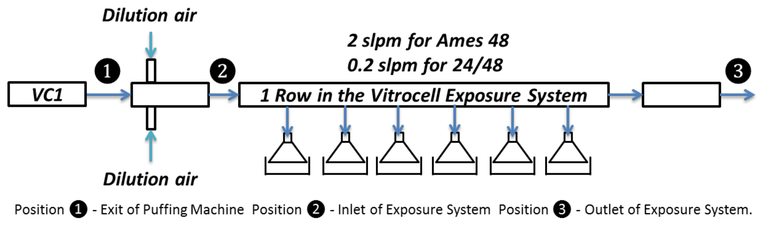
Vitrocell® 24/48

Vitrocell® Ames 48

Pictures from Vitrocell® website: https://www.vitrocell.com/

METHOD

Schematics of Exposure Systems



Position ❶ - Exit of Puffing Machine Position ❷ - Inlet of Exposure System Position ❸ - Outlet of Exposure System.

Operation Conditions

- Trumpet flow for Ames 48: 10 ml/min (unless specified)
- Horn flow for 24/48: 2 ml/min
- Each row was considered independent with its own VC1 pump for aerosol generation.

Test Material: E-vapor Testing Cartridge

- Formulation (by weight): 4% Nicotine, 15% Water, 24.3% Propylene Glycol (PG), 56.7% Glycerin
- Cig-a-like cartridge: Coil resistance 3.5 Ohms
- Puffing regimen: 55 ml/5 s, every 30 s; Square puff profile

Study Design

Qualification of VC1/7 Puffing Machine
- Do 7 units of VC1 function properly?
- Are generated aerosol comparable to the cartridge aerosols?

Aerosol Deposition on Exposure System Sections
- Where do aerosols deposit in the whole system (aerosol loss)?

Aerosol Delivery in the Exposure Inserts
- How much aerosol can be delivered to ALI (effective dose)?

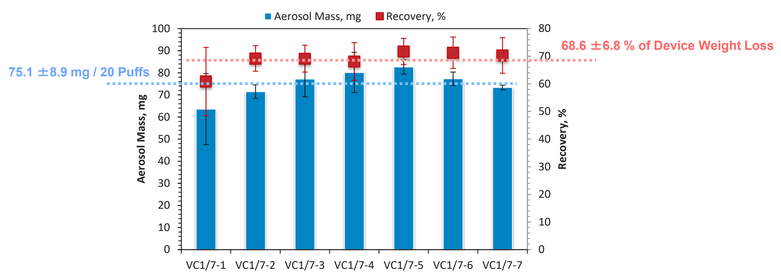
Revised Delivering Regime to Increase Aerosol Delivery
- Can we deliver more?

RESULTS

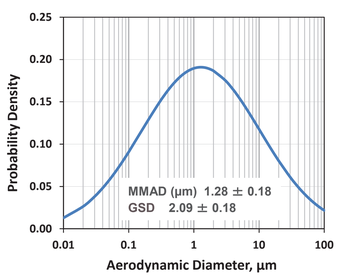
Qualification of VC1/7 Puffing Machine

Aerosol mass (mg) at the exit of VC/1 (Position 1)

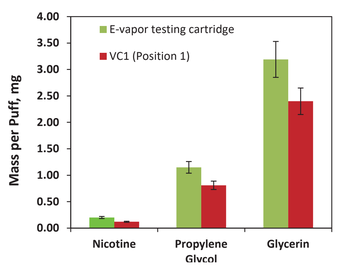
- First 20 puffs from the cartridge were collected with a Cambridge filter pad
- Recovery (%) = $\frac{\text{Aerosol Mass Collected at the Exit of VC1 (mg)}}{\text{E-vapor Cartridge Weight Loss (mg)}} \times 100\%$



Aerosol Size Distribution



Nicotine, PG, Glycerin



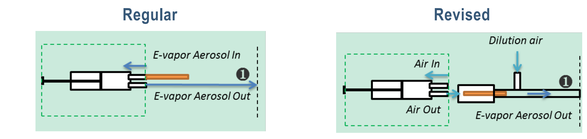
RESULTS

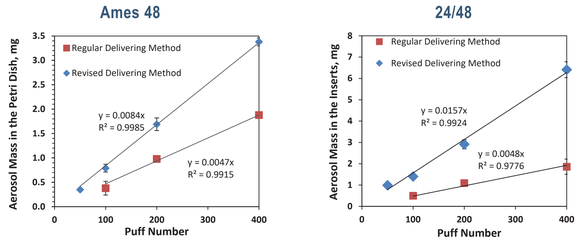
Aerosol Mass Deposition (%) on Exposure System Sections

Ames 48 (Mean ± SD; N = 3) (%)	Regular Delivering Regime (Trumpet Flow 5 ml/min) 400 puffs
Prior to Position 2 (Total)	**33.8**
VC1 Puffing Machine	15.1 ± 2.3
Connecting Tube	12.8 ± 1.0
Inlet Dilution Section	5.9 ± 1.0

24/48 (Mean ± SD; N = 3) (%)	Regular Delivering Regime (Horn Flow 2 ml/min) 400 puffs
Prior to Position 2 (Total)	**30.0**
VC1 Puffing Machine	15.1 ± 0.8
Connecting Tube	12.9 ± 0.4
Inlet Dilution Section	2.0 ± 1.7

Aerosol Delivery in the Exposure Inserts



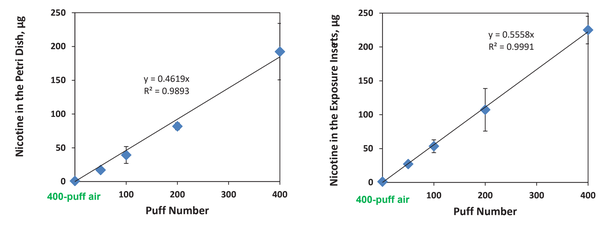


Percent Aerosol Mass Deposition in All 6 Exposure Inserts

(Mean ± SD; N = 3)	Ames 48 (Trumpet flow = 10 ml/min)		24/48 (Horn flow = 2 ml/min)	
Puff number = 400	Regular	Revised	Regular	Revised
Measured Deposition (All 6 Inserts)	0.55% ± 0.01%	1.13% ± 0.05%	0.56% ± 0.10%	2.02% ± 0.07%
Estimated Deposition *	2.2% - 2.8%		1.4% - 3.9%	

* Estimated based on flowrate ratio.

Nicotine, PG, Glycerin Delivery in the Exposure Inserts



(400 puffs) (3 Replicates for Each Measurement)	Ames 48 Nicotine	Ames 48 PG	Ames 48 Glycerin	24/48 Nicotine	24/48 PG	24/48 Glycerin
Overall Average (mg)	0.19	1.54	2.48*	0.16	1.01	2.12
Overall RSD (mg)	0.01	0.02	0.18	0.07	0.05	0.16
Overall % RSD	3.1%	1.3%	7.3%	4.7%	5.0%	7.8%
% of Sum of 3 Constituents (Measured)	4.5%	36.5%	59.0%	4.7%	30.7%	64.5%
% of Sum of 3 Constituents (Formulation)	4.7%	28.6%	66.7%	4.7%	28.6%	66.7%

* The result of one run was below the LOQ.

SUMMARY

For E-Vapor Products:

- Qualified VC1/7 puffing machine
- Established mass delivery curves for dosimetry prediction
- Optimized operating conditions
  - -Revised puffing methods
- Developed methods for determining
  - -Aerosol mass deposition (gravimetric)
  - -Aerosol size distribution (cascade impactor)
  - -Nicotine, PG, Glycerin (aerosol and media)
  - -pH and osmolarity (media)
- The methods developed in this study can be used for other e-vapor products

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