Characterization of an Air-Liquid-Interface (ALI) in vitro Exposure System (VITROCELL® 24/48) Using a Prototype e-Vapor Product

Altria Client Services

Jingjie Zhang[1], Michael J. Oldham[1]*, Utkarsh Doshi[1], I. Gene Gilman[2], Russell L. Wolz[2], Pavel Kosachevsky[2], Bonnie Coffa[1]*, Willie J. McKinney[1]*, K. Monica Lee[1]

[1]Altria Client Services LLC, Richmond, VA, USA; [2]Enthalpy Analytical, Richmond, VA

SOT 58th Annual Meeting & ToxExpo, March 10 - 14, 2019, Baltimore, Maryland, USA

Abstract 1866
Poster P249

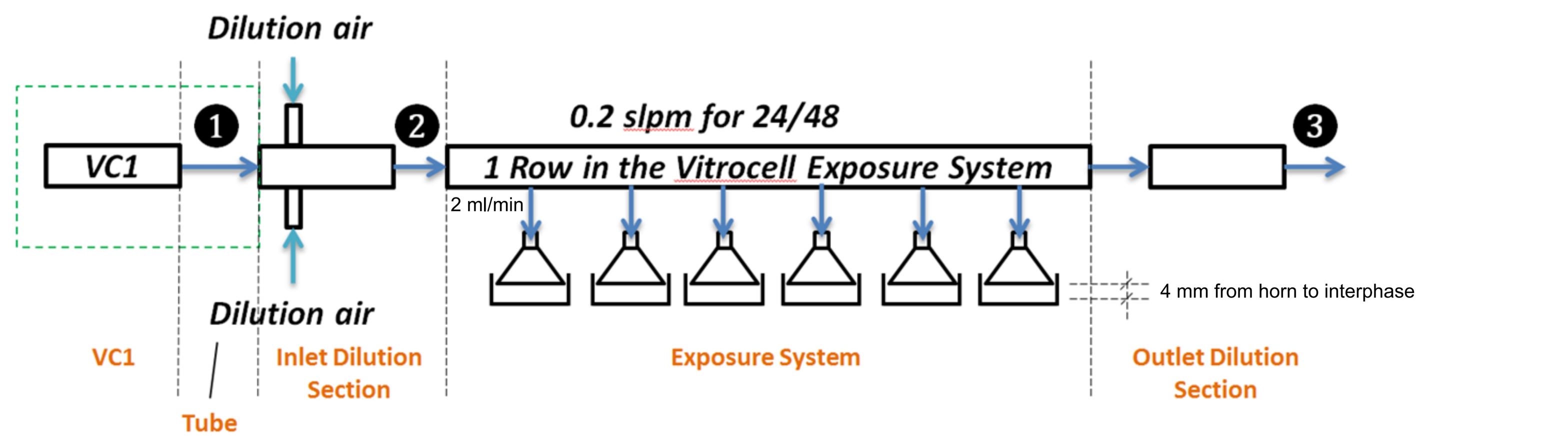
This poster may be accessed at www.altria.com/ALCS-Science

Abstract

Direct delivery of aerosol or vapor to cells at ALI allows more relevant exposure for in vitro toxicological evaluation of inhalable chemicals. In this study, the aerosol delivery was determined in a commercially available ALI in vitro exposure system (VITROCELL® VC1/7 (VC1/7) puffing machine and VITROCELL® 24/48 (VC 24/48)) using a prototype e-vapor product (a cig-a-like cartridge with a prototype e-liquid comprising propylene glycol, glycerin, nicotine, and water). The e-vapor product, with a fully-charged battery, was puffed using a 55 ml puff over 5 seconds, with a 30 second inter puff period (unless specified), by a VC1/7 puffing machine. As specified by the manufacturer, e-vapor aerosol was pulled into the VC1/7 puffing machine and then pushed into the exposure system over 8 seconds. The default air flowrate was 0.2 slpm (standard liter per minute) and the horn flowrate was 2 ml/min for all the exposure inserts in the VC 24/48. Aerosol size distribution was measured at the exit of the puffing machine, the inlet and the outlet of the exposure system. Aerosol mass deposition on all system sections were gravimetrically measured following 400 puffs of e-vapor aerosol (100 puffs/cartridge). The percent aerosol mass deposition was calculated as measured mass on each section divided by the total cartridge weight loss. The results showed ~30% aerosol loss in the aerosol transportation path (VC1/7 and tubing) prior to entry into the VC 24/48, with an average of 0.56% delivered to the 6 exposure inserts in a row in total. To minimize the aerosol loss and consequently increase the aerosol deposition in the inserts, a revised delivering method was developed by shortening the aerosol transportation path, leading to ~ 90% aerosol delivery to the VC 24/48 and ~2.02% delivered to all 6 exposure inserts in a row.

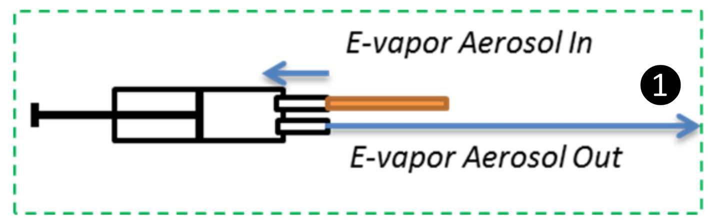
With the revised delivering method, nicotine deposition, pH, and osmolarity in the exposure inserts were measured after 50, 100, 200, or 400 puffs of e-vapor aerosol, or 400 puffs of air, was collected in PBS in the inserts. Results showed that 1) nicotine increased linearly with the puff number; 2) pH was within 7.5 – 7.6 for up to 400 puffs of aerosol and in the air control group; and 3) osmolarity of the buffer increased linearly with the puff number. The aerosol delivery characterized in this study laid the foundation for the ALI in vitro toxicity assessment of e-vapor products with the VITROCELL® 24/48.

Experimental Setup

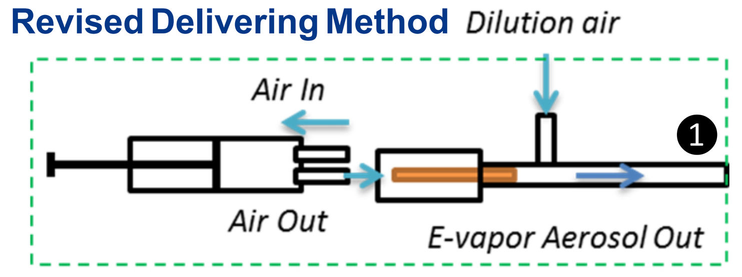


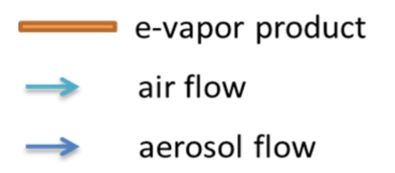
Position ❶ - Exit of Puffing Machine; Position ❷ - Inlet of Exposure System; Position ❸ - Outlet of Exposure System

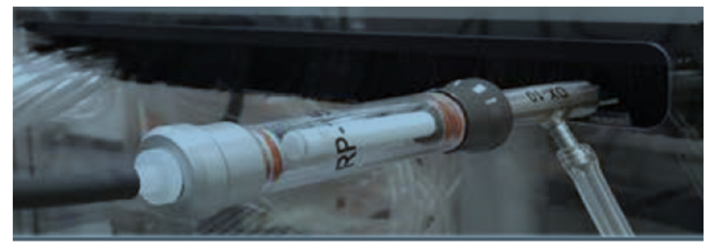
Regular Delivering Method



Revised Delivering Method







(Schematics of the experimental setup adapted from Zhang et al., 2018)

Aerosol Size Distribution

Aerosol Size Distribution was measured with a cascade impactor (MSP 135-8) (Oldham et al., 2018) at Positions 1, 2, and 3 for the regular delivering method. The first 3 puffs of each cartridge were collected for 3 cartridges (N = 3). Assuming a lognormal distribution, the mass median aerodynamic diameter (MMAD, µm) and the geometric standard deviation (GSD) were obtained by using a linear data reduction scheme (O'Shaughnessy and Raabe, 2003). The data are presented as Mean ± SD (standard deviation).

Position 1 (Exit of Puffing Machine)

	MMAD (µm)	GSD
VC1/7 - 1	1.20 ± 0.09	2.13 ± 0.11
VC1/7 - 2	1.29 ± 0.13	2.19 ± 0.12
VC1/7 - 3	1.36 ± 0.05	2.01 ± 0.07
VC1/7 - 4	1.31 ± 0.28	1.99 ± 0.41
VC1/7 - 5	1.15 ± 0.16	2.15 ± 0.05
VC1/7 - 6	1.42 ± 0.34	2.11 ± 0.19
VC1/7 - 7	1.23 ± 0.09	2.05 ± 0.15
Overall Mean	1.28	2.09
Overall SD	0.18	0.18
Overall %RSD	14.4	8.5

Position 2 (Inlet of Exposure System)

	MMAD (µm)	GSD
Mean ± SD	1.12 ± 0.03	1.83 ± 0.06
%RSD	2.8	3.2

Position 3 (Outlet of Exposure System)

	MMAD (µm)	GSD
Mean ± SD	1.46 ± 0.10	1.89 ± 0.27
%RSD	7.0	14.1

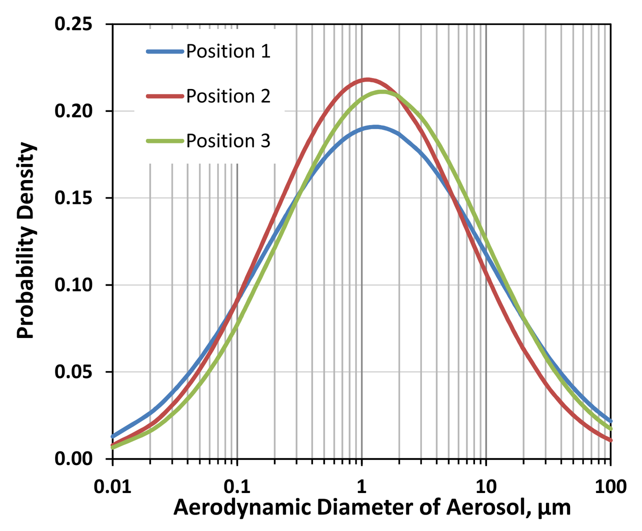


Figure 1. Lognormal Distribution of Aerosol Size Based on Parameters Estimated from the Impactor Measurement

Aerosol Mass Delivery

- **Aerosol Mass per Exposure Insert (mg)** was gravimetrically measured in each of 6 exposure inserts in a row with pre-weighed filter pads and averaged over the 6 wells. The mean of 3 rows with independent aerosol generation are plotted in **Figure 2** with SD.
- The aerosol mass deposited on various parts throughout the system was measured gravimetrically in 3 rows with independent aerosol generation. **Percent Aerosol Mass on All System Sections (%)** = $\left(\frac{\text{Aerosol Mass on Each Section (mg)}}{\text{E-vapor Cartridge Weight Loss (mg)}}\right) \times 100\%$.

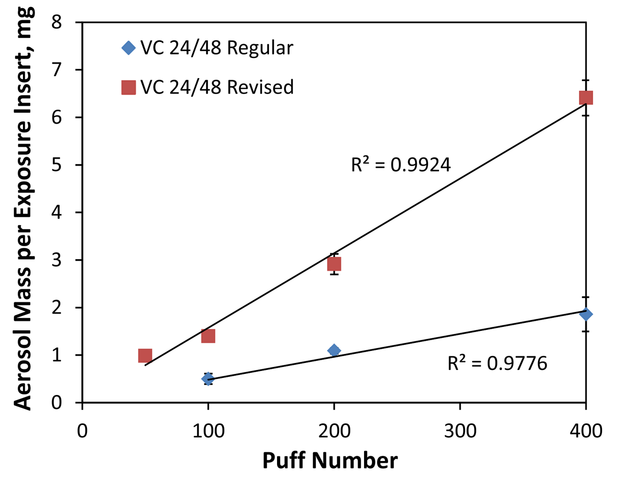


Figure 2. Aerosol Mass Deposition per Exposure Insert (Mean ± SD; Horn Flowrate = 2 ml/min)

Percent Aerosol Mass Deposition on 24/48 System Sections Prior to Position 2

Horn Flow = 2 ml/min (Mean ± SD; N = 3) (%)	Regular 400 puffs	Revised 400 puffs
Prior to Position 2 (Total)	30.0	10.5
VC1 Puffing Machine	15.1 ± 0.8	NA
Connecting Tube	12.9 ± 0.4	NA
Custom-built Dilution Tube	NA	9.2 ± 1.0
Inlet Dilution Section	2.0 ± 1.7	1.3 ± 0.3
Exposure Inserts (Total)	0.56 ± 0.10	2.02 ± 0.07
Estimated Target [a]	1.4 – 3.9	1.4 – 3.9

[a] The target was estimated based on the flowrate ratio, assuming all aerosol drawn into the trumpet deposit in the petri dish. The total flowrate through the exposure system during puffing was 0.860 L/min, while the total trumpet flowrate for all 6 exposure inserts was 12 mL/min.

Aerosol Chemistry

- **Nicotine, PG, and Glycerin per Exposure Insert (mg)**
  - 50, 100, 200, and 400 puffs of aerosol were collected in the exposure insert containing 150 µL of 0.2 M sodium phosphate buffer. After exposure, the buffer was analyzed for nicotine with a GC-MS method. PG and glycerin were analyzed for 400-puff aerosol only by a GC-FID method.
  - For each measurement, there were 3 replicates; and at least 1 row in the VC 24/48 system served as the concurrent air control with 400 puffs of air. The results are the mean of 3 replicates with independent aerosol generation.
- **Ratio of Constituents (% of Sum of 3 Compounds: Nicotine, PG, and Glycerin)**
  - Mass of each compound was normalized to the sum of mass of the 3 measured compounds (nicotine, PG, and glycerin).
  - The theoretical values were calculated based on the formulation composition (4% nicotine, 24.3% PG, 56.7% glycerin, and 15% water; by weight).
- **pH and Osmolarity** of the buffer was measured after exposure. The results are the mean of 3 replicates with independent aerosol generation.

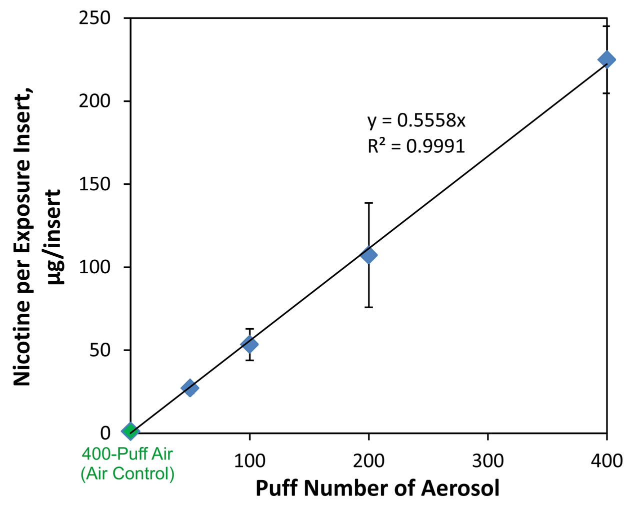


Figure 3. Average Nicotine Deposition in the Insert (Mean ± SD)

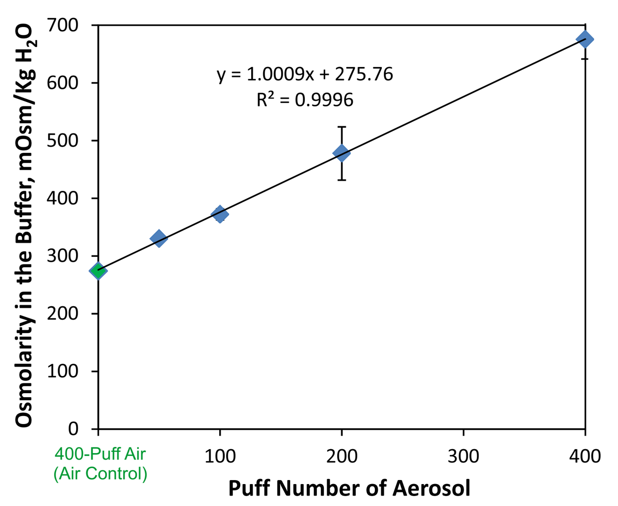


Figure 4. Osmolarity of the Buffer in the Insert (Mean ± SD)

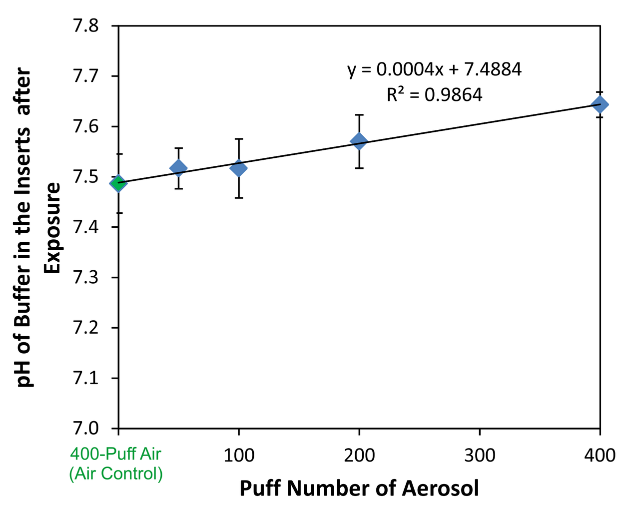


Figure 5. pH of the Buffer in the Insert (Mean ± SD)

Nicotine, PG, and Glycerin Deposited in the Inserts after 400-Puff Aerosol Exposure

(3 Replicates for Each Measurement, N = 3)	Measurement 1 (5-s puff)			Measurement 2[a] (3-s puff)		
	Nicotine	PG	Glycerin	Nicotine	PG	Glycerin
Overall Average (mg/insert)	0.25	1.65	3.69	0.16	1.01	2.12
Overall RSD (mg/insert)	0.02	0.01	0.19	0.07	0.05	0.16
Overall %RSD	0.8%	0.6%	5.2%	4.7%	5.0%	7.8%
Ratio of Constituents (Measured)	4.5%	29.4%	66.0%	4.7%	30.7%	64.5%
Ratio of Constituents (Theoretical)	4.7%	28.6%	66.7%	4.7%	28.6%	66.7%

[a] The puffing regimen for Measurement 2 was 55 ml puff over 3 seconds, with a 30 second inter puff period.

Summary

- The revised delivering method delivered about 2.4-fold more aerosol mass to the exposure insert than the regular delivering method. The revised delivering method reduced aerosol loss in the transportation line prior to the exposure system from ~30% to ~10%.
- For all methods, the aerosol mass deposited in the exposure insert increased linearly with the puff numbers.
- Nicotine, PG, and glycerin were measured with the revised delivering method. Nicotine delivered to the insert increased linearly with the puff number. After normalization (% sum of 3 measured constituents), the composition of the deposited aerosol (measured) was in general comparable with that of the formulation (theoretical).
- Osmolarity increased linearly from 274 mOsmol/kg H_2O in the air control group to 676 mOsmol/kg H_2O for 400-puff aerosol. pH was within 7.5-7.6 for up to 400 puffs of aerosol and in the air control group, and increased slightly with the puff number.
- Aerosol size remained in the respirable range during the transportation as determined based on the impactor measurement.

References

- O'Shaughnessy, P.T. & Raabe, O.G. (2003). A Comparison of Cascade Impactor Data Reduction Methods. Aerosol. Sci. Technol., 37(2):187-200. DOI: 10.1080/02786820300956
- Oldham, M.J., Zhang, J., Rusyniak M.J., et al. (2018). Particle Size Distribution of Selected Electronic Nicotine Delivery System Products. Food Chem. Toxicol., 113:236-240. DOI: 10.1016/j.fct.2018.01.045
- Zhang, J., Oldham, M.J., Gilman, I.G., et al. (October 2018). Characterization of an Air-Liquid-Interface (ALI) in vitro Exposure System (VITROCELL® VC1/7 and Ames 48) Using a Prototype E-vapor Product. Poster presented at CORESTA Congress 2018, Kunming, Yunnan, China.

Financial Disclosure/Conflict of Interest: The authors are employees of Altria Client Services LLC or Enthalpy Analytical.

*Former Altria Client Services employees.