Application of the Capillary Aerosol Generator (CAG) to Generate Aerosols for E-liquid Preclinical Inhalation Studies

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Abstract

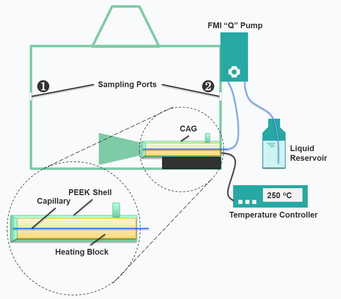
Electronic nicotine delivery systems (ENDS) have gained popularity with various types of products in the commercial marketplace. While most ingredients used in e-liquids are generally recognized as safe for oral consumption, their safety upon inhalation exposure is not yet known. In this study, we evaluated the suitability of a capillary aerosol generator (CAG) to generate condensation aerosol from e-liquids for preclinical inhalation safety studies. A base formulation (BF) containing propylene glycol (PG), glycerin, water and nicotine and a flavored formulation (Mix) containing the base and flavor ingredients were used to generate aerosol atmospheres for nose-only inhalation exposures. Aerosol samples were collected with Cambridge filter pads and/or impingers at the nose ports and characterized for aerosol mass, major ingredients (PG, glycerin, and nicotine), selected carbonyls (e.g., acetaldehyde, acrolein, and formaldehyde), particle size distribution (by cascade impaction), and exposure port uniformity (gravimetric) during exposures. Results were compared to a published inhalation study (Lee et al. 2018) in which commercial cig-a-like ENDS products filled with the same formulations (BF and Mix) were puffed (55 ml puff volume, 5-second puff duration, and 30-second inter-puff interval) using rotary smoking machines to generate an aerosol exposure atmosphere for mice. The CAG consistently delivered respirable aerosols for rodents (the mass median aerodynamic diameter of 1.0 – 1.3 µm and the geometric standard deviation of 1.4 – 1.7) with aerosol mass concentrations ranging from ~250 µg/L to ~5500 µg/L at the nose port. The CAG aerosols contained similar composition of PG, glycerin and nicotine at the nose ports as the ENDS product aerosols from the same formulation. When detected above the limit of quantitation, the levels of formaldehyde and acrolein were slightly higher while acetaldehyde was slightly lower in the CAG aerosol compared to the ENDS product aerosol, but overall the levels of the carbonyls in both the CAG and product aerosols were of a similar order of magnitude. In summary, the results support that the CAG is a pragmatic option to generate stable and comparable e-vapor aerosols to study preclinical inhalation toxicity of ENDS ingredients.

Introduction

Capillary Aerosol Generator (CAG)

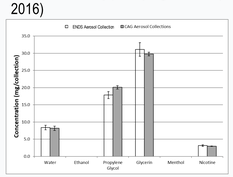
The CAG can generate respirable aerosols from humectant-based e-liquid formulations. (Werley et al. 2011; Zhang et al., 2017)

- A 1-channel CAG consists of a heated capillary tube attached via tubing to a reservoir containing the e-liquid.
- The e-liquid is directed to the capillary tube where it is heated to its boiling point or above, producing a vapor jet at the outlet of the tube.
- The air surrounding the capillary tube is cooler, allowing rapid condensation and generation of a fine particulate aerosol.



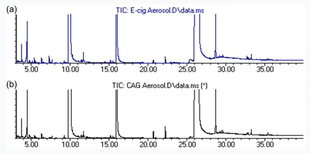
Schematic of a bench-top CAG (Zhang et al. 2017)

Characteristics of CAG Aerosols and Comparison to a Prototype (cig-a-like) ENDS product (Werley et al. 2016)

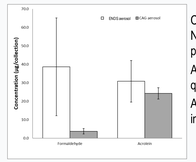


Major chemical analytes in aerosols (mean ± 1 SD; N=3) from a prototype ENDS product and CAG.

A statistically significant difference was observed only in PG ($p < 0.05$)



Chemical "fingerprint" using GC-MS for an identical formulation using (a) a prototype ENDS product and (b) CAG.



Comparison of aerosol carbonyls (mean ± 1SD; N=3) using UPLC-UV for a prototype ENDS product and CAG.

All values for acetaldehyde were below the limit of quantification (137 µg/collection).

A statistically significant difference was observed in formaldehyde ($p < 0.05$).

Reference

Lee et al. 2018. Inhalation Toxicology 30 (13-14): 53-567
OECD 2018. OECD TG 413.
Werley et al. 2011. Toxicology 287 (1-3): 76-90
Werley et al. 2016. Aerosol Sci. & Technol. 50 (12): 1284 – 1293
Zhang et al. 2017. 71st TSRC, Bonita Springs, FL, USA

Material and Method

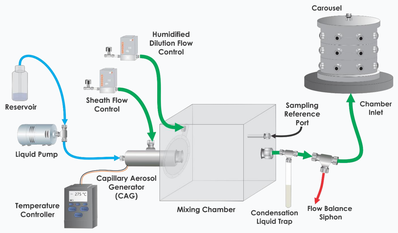
Device and Operation Condition

1-channel CAG: a CAG with a single stainless-steel capillary heated at 275 °C

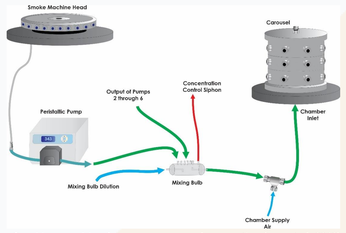
E-liquid

Base (BF): PG, glycerin, water, nicotine (4% by weight); **Mix**: PG, glycerin, water, nicotine (4% by weight), and flavors

Schematic of Nose-Only Inhalation Exposure System



CAG for e-vapor aerosol generation



Smoking machine for e-vapor aerosol generation (Lee et al. 2018)

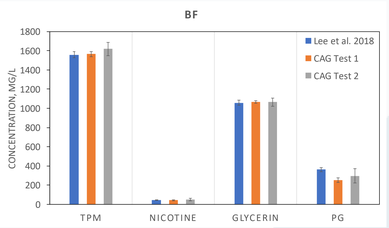
Characterization of e-vapor aerosols at the nose port

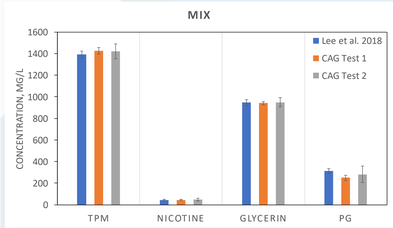
Parameters	Sampling for Analysis	Test Mix Target	Test Mix Observed [1]	BF Target	BF Observed [1]
Aerosol Mass (AM), µg/L	Cambridge filter; gravimetric	1400	1410	1550	1583
Nicotine, µg/L	Cambridge filter with two sorbent tubes	44	43.7	44	43.1
Glycerin, µg/L	Cambridge filter with two sorbent tubes	NA	946	NA	1058
Propylene glycol, µg/L	Cambridge filter with two sorbent tubes	NA	311	NA	361
Nicotine/Total Analytes (%)	NA	4.7	3.4	4.3	2.9
Glycerin/Total Analytes (%)	NA	68.3	72.7	69.3	72.4
PG/Total Analytes (%)	NA	27	23.9	26.4	24.7
Selected Carbonyls	Cambridge filter with bubblers	NA	See results	NA	See results
Particle Size Distribution MMAD (µm)	Cascade impactor	≤ 1.3			
AM Temporal Stability	Real-time aerosol monitor (RAM)	%RSD ≤ 15%			
AM Spatial Stability	AM and flowrate measured from 4 ports per tier from top, middle and bottom tiers	%CV < 7%			
Nose-Port Temperature (°C)	HOBO External Temp/RH Data Logger	22 ± 2			
Supply Air Relative Humidity (%)	HOBO External Temp/RH Data Logger	55 ± 15			

[1] Observed values are as reported in Lee et al. (2018) in which a cartridge-based, cig-a-like ENDS device is puffed with a rotary smoking machine utilizing the CRM81 puffing regimen (3 second 55 mL puff every 30 seconds) with a near square wave shape profile.
NA = Not applied; %RSD = percent relative standard deviation; %CV = percent coefficient of variability

Results: AM and Major Ingredients

The CAG aerosols contained similar compositions of PG, glycerin and nicotine at the nose ports as the ENDS aerosols from the same formulation.





Results: Carbonyls

- Levels of selected carbonyls in the ENDS and CAG aerosol at the nose port were very low (close to the LOQ) and were in the similar order of magnitude.

Formulation	Test Description	Mean AM Conc. (µg/L)	Mean Measured Analyte Concentration (µg/L) [Range of all replicates throughout study] Acrolein	Acetaldehyde	Formaldehyde	Propionaldehyde	Crotonaldehyde
Mix [a]	Prototype ENDS [b]	1393	0.0064	0.0514	0.0861	0.0099	BLOQ
			[BLOQ - 0.0070]	[0.0314 - 0.0748]	[0.0296 - 0.1578]	[BFB - 0.0132]	[ND - BLOQ]
	CAG	1425	0.0147	0.0319	0.2695	ND	ND
BF [a]	Prototype ENDS [b]	1559	0.0019	0.0169	0.1095	0.0026	BFB
			[BLOQ - 0.0021]	[0.0036 - 0.0310]	[0.0125 - 0.2014]	[BFB - 0.0040]	[ND - BFB]
	CAG	1565	0.0113	0.0103	0.1960	ND	ND
Carbonyl / AM Ratio (%)							
Mix [a]	Prototype ENDS [b]	1393	0.0005%	0.0037%	0.0062%	0.0007%	NA
	CAG	1425	0.0010%	0.0022%	0.0189%	NA	NA
BF [a]	Prototype ENDS [b]	1559	0.0001%	0.0011%	0.0070%	0.0002%	NA
	CAG	1565	0.0007%	0.0007%	0.0125%	NA	NA

a. Concentrations corrected for room field blank background concentrations. b. Reported in Lee et al. 2018.
BLOQ = Blow Limit of Quantitation; ND = Not Detected; BFB = Below Field Blank Background Subtraction; NA = Not Applied;

Results: Particle Size and Uniformity

- The CAG consistently delivered respirable aerosols for rodents.
- The temporal uniformity was monitored and confirmed by the RAM. The spatial uniformity was confirmed by the AM measurement and flow rate measurement at the nose port.

Nose-Port AM Spatial and Temporal Uniformity

Exposure Unit	Total Port Variation (%) [1] Nose Port AM Concentration [2]	Mean Nose Port Flow Rate [3]	Within Tier Variation (%) [1] Nose Port AM Concentration [2,4]	Temporal Variation (%) [5] RAM
BF	0.6%	3.2%	0.9%	3% (N = 34)
Mix	0.5%	2.6%	0.6%	4% (N = 35)

[1] Acceptable Total Port Variation and Within Tier Variation < 7%; [2] Based on AM gravimetric filter data; [3] Based on Kurz Meter measurements; [4] Based on Tier 2 measurements; [5] Acceptable temporal variation ≤ 15%

Nose-Port Particle Size Distribution

BF Mean AM, µg/L	BF MMAD (µm)	BF GSD	Mix Mean AM, µg/L	Mix MMAD (µm)	Mix GSD
6226	1.1	1.6	6492	1.1	1.5
268	1.2	1.6	222	1.1	1.7
5617	1.3	1.5	5982	1.2	1.4
1565	1.2	1.5	1425	1.3	1.5
1154	1.0	1.6	1141	1.2	1.5
1877	1.2	1.5	1628	1.2	1.5
1619	1.3	1.5	1420	1.3	1.6

Acceptable MMAD < 2 µm and GSD < 2 (OECD, 2018). For comparison, Lee et al. (2018) reported MMAD 0.9 µm and GSD 1.6 for BF, and MMAD 1.0 µm and GSD 1.6 for Mix.

Strength and Limitation

- **Strength**: The CAG and the ENDS device are evaluated using the same *in vivo* nose-only exposure setup which allows for a fair comparison.
- **Limitation**: The comparison is based on flavor-free e-liquid formulations. Therefore, the capability of the CAG to deliver flavors in the aerosol is not evaluated in this study.

Conclusion

CAG can consistently deliver a wide range of aerosol atmospheres (~200 to ~6,000 ug/L) that are comparable to e-vapor aerosols and it can be a pragmatic (device-agnostic), fit-for-purpose exposure system to support preclinical inhalation studies of ENDS ingredients and e-liquids.