Abstract # 5141/P242

Fit-for-purpose Characterization of Air-Liquid-Interface (ALI) Exposure System for a Heated Tobacco Product

Amit Gupta[1], Janae Newswanger[1], Cassady Griffin[1], Alena Amelung[1], Ed Psurny[1], Xuefei Cao[2], Reagan Mcrae[2], Jingjie Zhang[2] and K. Monica Lee[2]
[1] Battelle Memorial Institute, Columbus, OH, USA, [2] Altria Client Services LLC, Richmond, VA, USA

INTRODUCTION

Continuous development and implementation of in vitro test methods are driven by the need for faster and more human-relevant health risk assessment across toxicology communities, including research on tobacco and nicotine products. For standard in vitro testing of inhalable tobacco products, test materials are typically exposed via liquid dosing of smoke or aerosol condensate, which is not ideally suitable for inhalable product testing. Herein, we conducted a fit-for-purpose characterization of an ALI exposure system using whole- aerosol produced from a commercial heated tobacco product.

Three goals were set for this study: 1) producing continuous and stable aerosols from a commercial heated tobacco product (HTP), 2) widening the range of target concentrations that can be tested in a single experiment and 3) selective characterization of aerosols in the exposure system, including real-time monitoring of aerosol concentrations, aerosol particle size measurements and analytical measurements of nicotine and glycerol collected in liquid solvent traps.

METHODS

A modified ISO 20778 puffing regimen (55 mL puff volume, 2 s puff duration, 30 s puff interval, bell-shaped puff profile, no vent blocking) was used to produce aerosols from a commercially available HTP product. An optimized staggered loading pattern was developed to load twelve HTPs on a linear smoking machine (LM24E, Koerber Instruments GmbH, Hamburg, Germany). As shown in Figure-1, a custom-designed mixing bulb installed at the output of the smoking machine allowed proper mixing of the aerosols by introducing humidified dilution air flow (target ~80% relative humidity [RH]). A real-time aerosol monitor (RAM; Casella MD Pro, MA) was used to measure the temporal variability of aerosol concentrations in the mixing bulb.

Mixed aerosol was then introduced into a VITROCELL® 24/48 ALI exposure system (VITROCELL Systems GmbH, Waldkirch, Germany). By using the VITROCELL® dilution manifold (trumpet inlet flow set to 2 mL/min), seven target concentrations were achieved, in addition to a filtered air control. Siphons were connected at the end of each row to widen the range of exposure concentrations (low concentration targeted as ~2% of the highest concentration) that can be tested in a single experiment. A second RAM was installed to monitor the stability of the aerosol concentrations at each concentration group. An automatic multiport rotation valve was installed at the inlet of the RAM that enabled monitoring of multiple locations using a single RAM. Aerosols were collected during the exposure in one well containing liquid solvent trap (PBS, Sigma Aldrich) for each concentration group and quantified for nicotine and glycerol. Aerosol particle size was measured in each row using a Mercer cascade impactor (Model M, In-Tox Products, NM) and an Optical Particle Sizer (Model 3330, TSI Inc, MN).

Exposure Schematic

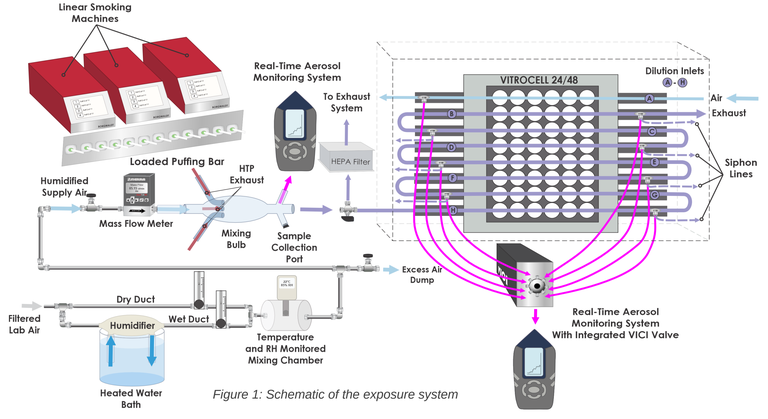

Figure 1: Schematic of the exposure system

RESULTS

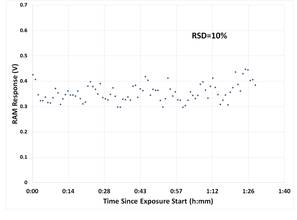

Figure 2: Temporal variability of the aerosol concentration in the mixing bulb

	Calculated Concentration (mg/m³) for Each Valve Position								Replicate Average Concentration	Replicate SD	Replicate RSD
Replicate #	1	2	3	4	5	6	7	8	mg/m³		
1	0.185	0.179	0.186	0.174	0.226	0.212	0.186	0.188	0.192	0.018	9%
2	0.187	0.168	0.213	0.225	0.208	0.182	0.186	0.188	0.195	0.019	10%
3	0.195	0.201	0.167	0.187	0.187	0.165	0.201	0.197	0.187	0.014	8%
4	0.168	0.166	0.181	0.177	0.198	0.185	0.151	0.157	0.173	0.015	9%
5	0.267	0.243	0.194	0.219	0.219	0.211	0.211	0.219	0.221	0.023	11%
6	0.181	0.191	0.239	0.256	0.238	0.207	0.198	0.178	0.211	0.030	14%
7	0.180	0.182	0.166	0.186	0.212	0.189	0.185	0.187	0.186	0.013	7%
Mean	0.195	0.190	0.192	0.202	0.211	0.193	0.188	0.188			
SD	0.033	0.027	0.026	0.030	0.017	0.018	0.019	0.019			
RSD	17%	14%	14%	15%	8%	9%	10%	10%			
Grand Mean	0.195										
Grand SD	0.024										
Grand RSD	12.3%										
% Difference from Grand Mean	0%	-2%	-1%	4%	8%	-1%	-3%	-4%			

Table 1: Port equivalency measurements

- Aerosol concentration in the mixing bulb was temporally stable (RSD < 15%)
- During the setup phase, port equivalency measurements were collected. These measurements showed no bias associated with the multiport rotation valve.

Sample Location	Dilution / Siphon Flow (LPM)	Theoretical Anticipated Concentration[1] (% of Dose 1)	Measured RAM Response (% of Dose 1)	Nicotine Mass in Liquid Trap (ug)	Glycerol Mass in Liquid Trap (ug)	Nicotine Concentration (% of Dose 1)	Glycerol Concentration (% of Dose 1)
VC Row H	0 / 1	100%	100%	53.0	257	100%	100%
VC Row G	1 / 1	52%	43%	34.4	171	65%	67%
VC Row F	1 / 1	27%	17%	16.3	85.8	31%	33%
VC Row E	1 / 1	14%	9%	9.87	45.5	19%	18%
VC Row D	1 / 1	8%	5%	6.75	30.0	13%	12%
VC Row C	1 / 1	4%	2%	3.30	36.1	6%	14%
VC Row B	1 / 1	2%	1%	1.58	18.1	3%	7%

[1] Theoretical calculations based on combination of dilution and siphon flows in each row

Table 2: Measured RAM response and analytical measurements compared to theoretical estimates

- Able to achieve a wide range of concentration in an experiment (based on RAM response; the high dose and low dose has ~100 to 1 ratio)
- The normalized RAM response from each row was consistent with theoretically anticipated values based on dilution and siphon flows

Aerosol Particle Size via Mercer style Impactors and Optical Particle Sizer

	Mercer, M-series Impactor		Optical Particle Sizer (OPS)	
Sampling Location	MMAD[a] (µm)	GSD	Average MMAD (µm) ± s[b]	GSD
Mixing Bulb	1.0	1.4	1.3 ± 0.01	1.3
VC Row H	0.9	1.4	1.2 ± 0.06	1.3
VC Row G	-	-	1.3 ± 0.02	1.3
VC Row F	-	-	1.3 ± 0.01	1.3
VC Row E	-	-	1.2 ± 0.03	1.4
VC Row D	-	-	1.1 ± 0.02	1.4
VC Row C	-	-	0.9 ± 0.01	1.5
VC Row B	1.0	1.4	0.8 ± 0.02	1.5
VC Row A	-	-	0.4 ± 0.09	1.7
Room Air (7-3-10)	-	-	4.8 ± 1.93	3.2
Field Blank	-	-	0.0 ± 0.00	0.0

[a] Mass Median Aerodynamic Diameter
[b] N = 3, s = Standard deviation

Table 3: Aerosol particle size measurements

- Nicotine and glycerol concentrations measured in liquid traps showed similar trend with decreasing values with decreasing target concentration
- Aerosol particle size as measured with cascade impactor and OPS were consistent with each other. MMAD and GSD were within acceptable range (MMAD<2.0 µm, GSD<3.0)

CONCLUSIONS

- Fit-for-purpose methods were developed to generate stable and consistent aerosols from a commercial HTP product in an ALI exposure system.
- A well-controlled, wide dynamic range of exposure concentrations monitored in real-time using a single RAM were achieved. This allows designing dose range finding studies with a wide range of exposure concentrations and more precise monitoring and control of aerosol concentrations during the exposures.
- Further work needs to be done to assess homogeneity of deposited dose in the transwells within each row. The dose range finding studies need to be conducted with airway cell cultures to define a range of target concentrations for subsequent cell exposure studies.

Altria Science

This project is sponsored by Altria Client Service LLC

BATTELLE