Method Development to Establish and Characterize an Air Liquid Interface (ALI) Exposure System Using Perfluorooctanoic Acid (PFOA) Liquid Aerosol

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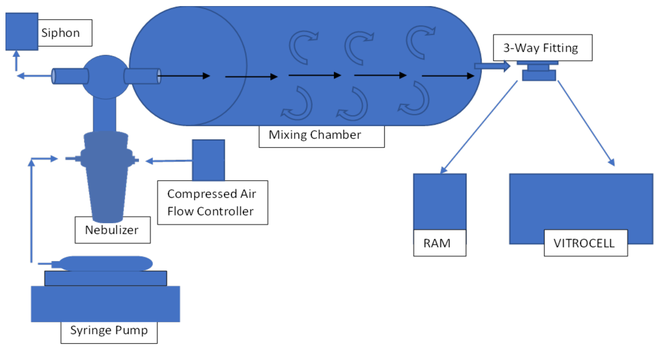
INTRODUCTION

There has been an accelerating trend to shift from traditional in vivo inhalation toxicology studies to the development of alternate animal models (e.g., in vitro inhalation exposure systems). A major caveat in using such in vitro inhalation systems is that the controlled delivery and accurate characterization of exposure atmosphere is extremely challenging. The goal of this study was to establish consistent, reproducible exposures using a liquid aerosol and collect preliminary biological data following exposure of 3D normal human bronchial epithelial (NHBE) cell cultures (EpiAirway, MatTek, Ashland, MA). A VITROCELL® 24/48 exposure system (VITROCELL Systems GmbH, Germany), was used to conduct air liquid interface (ALI) exposures of cell cultures. Perfluorooctanoic acid (PFOA) was used as a representative test compound for producing liquid aerosol. Methods were developed to generate and characterize inhalation exposure atmosphere in ALI exposure system.

TECHNICAL APPROACH

PFOA was formulated in 0.9% saline solution adjusted to pH 6 to 8 at a concentration of 10 mg/mL. To generate exposure atmosphere, an air-jet based commercially available nebulizer (Mini-HEART Continuous Hi-Flo Nebulizer; Westmed, AZ) was used to generate aerosol from the liquid formulation. Compressed air was used to dilute the aerosol at the output of the nebulizer and carry it to the delivery manifold. A real time aerosol monitor (RAM; Casella, Bedford, UK) was used to monitor the temporal variability of the aerosol atmosphere. Humidified dilution air was added at the inlet of each row of the VITROCELL to dilute the atmosphere to the target concentration. The aerosol particle size was measured using Mercer cascade impactor (MCI-G series, Intox Products, NM) and optical particle sizer (Model 3330, TSI Inc, MN) from numerous sampling locations in the exposure system. The NHBE cell cultures were exposed to PFOA aerosol for 6-hour duration. Deposited dose on cell cultures was determined using LC-MS/MS by measuring amount of PFOA collected in the liquid trap (0.8 mL volume) placed in one of the inserts in each dose group. The barrier integrity of the cells was evaluated using trans epithelial electric resistance (TEER) both prior to and after the exposures.

EXPOSURE SCHEMATIC



RESULTS

Deposited Dose Measurements

Sampling Location	PFOA Deposited within 6 hours (Day 1)	Measured Deposit Normalized to Row 1 (Day 1)	PFOA Deposited within 6 hours (Day 2)	Measured Deposit Normalized to Row 1 (Day 2)	Theoretical Deposit Normalized to Row 1 (for both days)
	(ug)	(%)	(ug)	(%)	(%)
Dose Group 1	10.1	100%	11.4	100%	100%
Dose Group 2	7.9	78%	7.6	67%	81%
Dose Group 3	6.0	60%	7.4	65%	68%
Dose Group 4	6.1	60%	5.8	51%	59%
Dose Group 5	4.8	48%	4.5	40%	52%
Dose Group 6	5.2	51%	3.5	31%	42%
Dose Group 7	2.3	23%	2.0	18%	32%

Analytically determined deposited amount of PFOA in each dose group was consistent with theoretical estimate

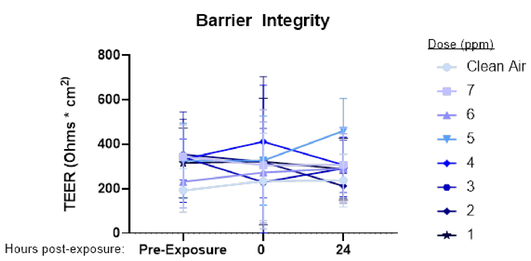
Aerosol Particle Size Measurements

Aerosol Particle Size Measurements				
Sampling Location	Cascade Impactor		Optical Particle Sizer	
	MMAD (µm)	GSD	MMAD (µm)	GSD
Aerosol Generator	1.7	2.5	1.7	1.9
Vitrocell Row 1	1.4	2.1	1.6	1.9
Vitrocell Row 3	-	-	1.6	2.0
Vitrocell Row 5	-	-	1.5	1.9
Vitrocell Row 7	1.4	2.1	1.6	2.0

The aerosol particle size measurements using both instruments were consistent with each other and within the respirable size range; mass median aerodynamic diameter (MMAD) ranged from 1.4 to 1.7 µm with geometric standard deviation (GSD) ranging from 1.9 to 2.0 across all measurements.

TEER Measurements

Barrier integrity measurements did not indicate significant toxicity; TEER values before and after exposure were consistent with each other.



CONCLUSIONS

In summary, methods were developed to establish in vitro ALI exposures using liquid aerosol. Future experiments may focus on elucidating mechanisms of toxicity using longer or repeated exposures and more complex cell culture models to recapitulate additional aspects of the biological effects of chronic exposure.