Abstract ID: 579

Aerosol Exposure at the Air-Liquid Interface of Human Lung Organotypic Cultures using VITROCELL™ Continuous Flow Inhalation System

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1 INTRODUCTION

In vitro organotypic 3D cell models of regions of the respiratory tract such as MucilAir™ and EpiAirway™ are widely used to predict toxicity in the lung (OECD, 2022). These upper airway (nasal/tracheal) models have morphology and functions mirroring the human tracheo-bronchial epithelium, including basal cells (roles in repair), goblet cells which secrete mucus, and ciliated cells which beat and sweep away mucus/particles. Exposure to potential toxic compounds at the air-liquid interface can be performed by either liquid or aerosol exposure; liquid exposure is simple and direct but not physiologically relevant. Therefore, the objective of this study was to compare the toxicity of SDS by both direct liquid and aerosol exposure when applied at the same dose (µg/cm2). SDS was selected because it is used as a positive control for direct cellular toxicity in *in vitro* safety assessment studies and therefore may also be useful if applied as an aerosol.

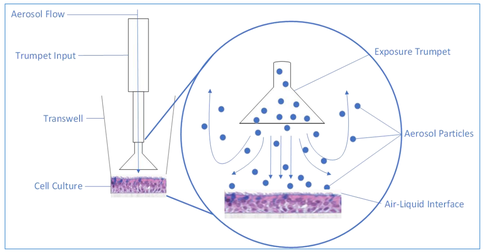


Figure demonstrates the delivery of an aerosol at the air-liquid interface of the lung organotypic culture. The aerosol is delivered through a 'trumpet' at an airflow of 5 mL/min; the aerosol is 'pulled' through the system under vacuum.

2 METHODS

MucilAir™ units (pooled donors) were obtained from Epithelix Sàrl. SDS was obtained from Sigma-Aldrich, Dorset, UK.

Direct SDS Application: SDS was applied as an aerosol to MucilAir™ tissues at two dose levels, with an aerosol exposure time of 25 min. Aqueous SDS was applied directly to additional MucilAir™ 3D organoids at the same nominal dose levels. Following aerosol exposure, tissues were incubated at 37ºC in a 5% CO2 atmosphere for ca 24 hours.

SDS Aerosol Generation: Aerosols were generated using a vibrating mesh nebuliser (Aerogen Solo). 2.5-3.0L/min of air was delivered to the primary flow and 5.0mL/min was extracted from this flow for exposure to the tissues. Sampling: The aerosol attributes and depositions were monitored using Glass Fibre Filters, Transwell Filters and Cascade Impactors.

Analysis: Following 24 h incubation, tissues were analysed under a digital microscope to examine cilia beat frequency (CBF). MucilAir™ monolayer integrity was determined by measurement of trans-epithelial electrical resistance (TEER). Tissues were processed histopathology. Spent culture media was assessed for lactate dehydrogenase (LDH) release.

3 VITROCELL EQUIPMENT

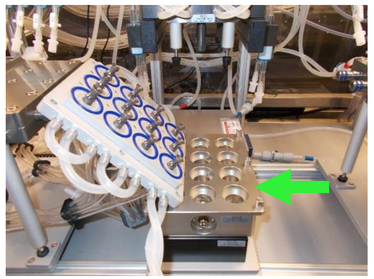


Figure shows exposure block (green arrow) in which 3D cultures are placed and metal trumpets for aerosol delivery.

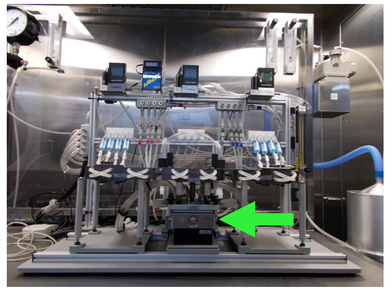


Figure shows the location of the sealed exposure block in context of the aerosol generating system.

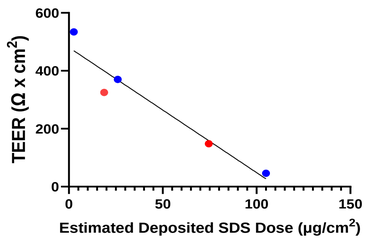
4 RESULTS

SDS Aerosol Deposited Mass

Group	Expected Result from Liquid Application	Targeted Deposited Dose (MucilAir™) µg/cm²	Estimated Deposited Dose (MucilAir™) µg/cm²	Compared with Target
SDS Mid Dose	Moderate Toxicity	26.2	18.8	28% less than targeted
SDS High Dose	Severe Toxicity	104.8	74.5	29% less than targeted

Targeted deposited doses of aerosol SDS were based on known amounts to cause moderate or severe toxicity when applied in liquid formulation. Estimated deposited aerosol doses of SDS were approx. 30% lower than targeted.

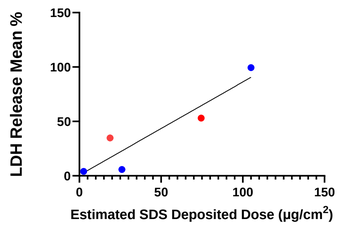
TEER: Group Mean (Liquid and Aerosol) vs Dose



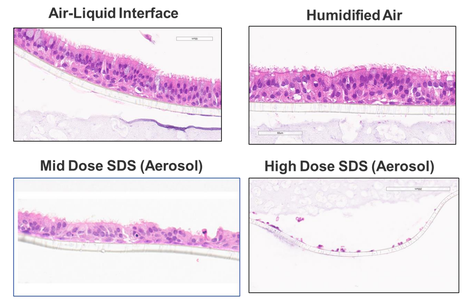
The extent of SDS-induced TEER reduction was broadly similar between liquid and aerosol when correlated with applied dose (P<0.01, Pearson Correlation).

4 RESULTS

LDH Release: Group Mean (Liquid and Aerosol) vs Dose



LDH release following SDS application by liquid application versus aerosol application was boardy similar when correlated with applied dose (P<0.05, Pearson Correlation).



Lung organotypic cultures were scored for cilia loss, epithelial thinning, necrosis, intercellular separation and extensive necrosis. Humidified air controls were similar to the air-liquid interface controls. Mid Dose SDS was associated with epithelial thinning, some cilia loss and cell separation. High Dose SDS damaged the epithelial layer such that it sloughed-off the membrane during processing.

5 CONCLUSION

Aerosol application of Sodium Dodecyl Sulfate (SDS) elicited a broadly similar toxicity when compared with liquid application based on TEER and LDH results (when corrected for total deposited mass). However, aerosol exposure at the air-liquid interface negatively impacted ciliary beat frequency (data not shown) and further studies are underway to refine exposure without affecting CBF.

REF: OECD Case Study on the use of an Integrated Approach for Testing and Assessment (IATA) for New Approach Methodology (NAM) for Refining Inhalation Risk Assessment from Point of Contact Toxicity of the Pesticide, Chlorothalonil. No. 367, 2022.

The development work described here will be used to develop relevant context of use applications based on the ICCVAM framework.

REF: Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies, March 2024 (figure has been adapted).