MEASURING THE EFFICIENCY IN TERMS OF CAPTURING THE AEROSOL FROM RRPs AND GRAVIMETRIC VALIDATION OF CETI5 - ZEPHYR PROTOTYPE FLOW SPLIT

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ABSTRACT

Targeted toxicity of air toxicants within the upper or lower respiratory tract is dependent on numerous factors, but most importantly the physical and chemical character of the chemical agent, site-specific tissue dosimetry and sensitivity, and host-dependent factors. Knowledge of these factors is critical in interpreting the toxicology effects. In addition, species and/or cell strain differences must be considered in the interpretation of data to be used in a risk assessment. In naturally occurring diseases, the respiratory response to toxicant-induced injury is often nonspecific, so it is important to consider the gross distribution of respiratory lesions, and to use detailed history and ancillary test results in conjunction with histological or cytotox evaluation to determine potential risks and toxicological effects.

For this nonspecific answer and the continuous reactions on the organic systems, a continuous exposure is required for the evaluation of inhalation toxicology. Otherwise, the air-rest period, without the test article to be studied, could affect to the toxicology profile and the results could be non-conclusive and don't mimic the forced environmental conditions.

The **CETI5** is an entry level Continuous Aerosol Generator (CAG) puffing/vaping machine intended for use in in vitro and in vivo toxicology applications, primarily for toxicologists, R & D, product stewardship, university or medical research. The **CETI5** consists of five individual puff engines which have their exhaust valves connected into a single outlet port. The puff engines can sample volumes at 35ml or 55ml and run in a synchronised manner to provide a continuous aerosol. The machine is set up to run in a synchronised manner through the configuration screen. It allows fine tuning of the timing of the exhaust profile to eliminate spikes/gaps in the airflow. This is achieved by changing the overlap time between exhaust cycles.

In this article, the **CETI5** is coupled to a **Zephyr** Air Liquid Interface (ALI) chamber that provides an excellent flow separation onto the cell membrane cultures making up to 7 different cell lines or TEER cell confluency with the same aerosol concentration.

INTRODUCTION

Inhalation toxicology plays a crucial role in assessing the potential risks associated with exposure to various substances, ensuring the wellbeing of individuals and safeguarding the environment.
In vivo research has been a cornerstone of scientific discovery for centuries. However, as our understanding of biological systems has grown, so has the recognition of the limitations and ethical concerns associated with in vivo experimentation. In response, in vitro research, conducted within the sterile confines of a laboratory, has emerged as a viable and often superior alternative.

The ability to cultivate and study cells in a controlled environment has significantly contributed to our understanding of cell biology, disease mechanisms, and drug development. Traditionally, cells are grown submerged in a liquid medium, but more recently, the use of Air-Liquid Interface (ALI) cultures has gained popularity. We will explore the advantages of ALI exposure compared to submerged cell cultures, highlighting the scientific insights that can be gained from this innovative approach.

ALI exposure involves the cultivation of cells in a way that allows them to be exposed to both air and a liquid medium. ALI exposure is often achieved by placing the cells on a porous support, which separates the cells from the liquid medium while allowing them to access to oxygen and nutrients from the surrounding air. This setup creates a unique environment for the cells, resembling conditions found in vivo, such as the lung alveoli or the intestinal epithelium.

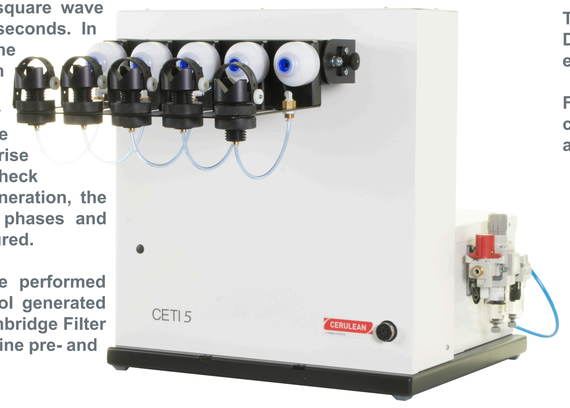
The principal objectives of these tests were to evaluate the flow split pattern in the **Zephyr** ALI chamber prototype and its feasibility coupled with **CETI5** aerosol generator. The **CETI5** robustness generation was also evaluated by CFP capture before ALI exposure.
The efficiency of the sham group's filters placed on 2 chamber positions was also studied during the test with an RRP.

MATERIAL AND METHODS

Test item aerosol generation and characterisation.

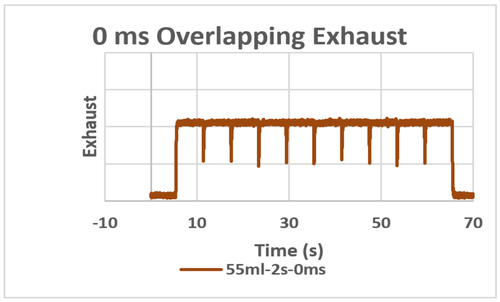
A **CETI5** instrument was set up with five equivalent RRPs which were then vaped using a regime based on CRM81 i.e., a 55 mL square wave puff over 3 seconds, every 30 seconds. In this gravimetric experiment, the aerosol was not diluted. For sham exposure, filtered aerosol was provided to the exposure chamber by placing a filter just before the cell insert. In order to characterise the test atmosphere and to check the reproducibility of aerosol generation, the experiment was divided in two phases and several determinands were measured.

In phase 1, sampling runs were performed in triplicate, with the total aerosol generated measured by weighing CFPs (Cambridge Filter Pads) positioned in the sampling line pre- and post vaping.

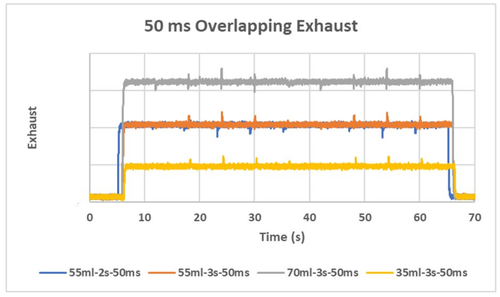


***CETI5** (CAG) Constant Aerosol Generator*

The generation of the aerosol was produced continuously using the five puff engines technology of **CETI5.**



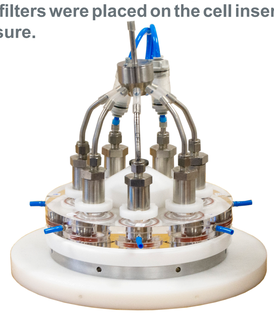
The diagram shows an exhaust profile where there is no overlap (0 ms) between the exhausts of the 5 puff engines.



The diagram shows an exhaust profile where there is an overlap between the exhausts of the 5 puff engines.

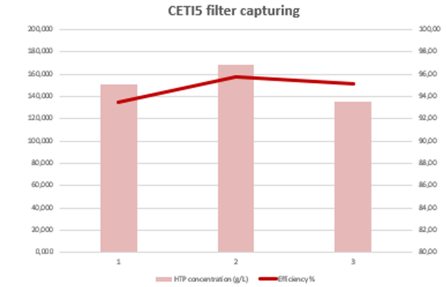
The effect of adding an overlap time of 50 ms for different puffing regimes can be seen.
Dips/spikes are reduced, and the five puff engines exhaust in a synchronised manner, such that the exhaust appears as a near continuous flow, a feature that is highly desirable for exposure studies.

For phase 2, two different tests were carried out passing the aerosol from **CETI5** to the **Zephyr** ALI chamber. Customised glass fibre filters were placed on the cell inserts to determine the concentration achieved, mimicking a cell exposure.

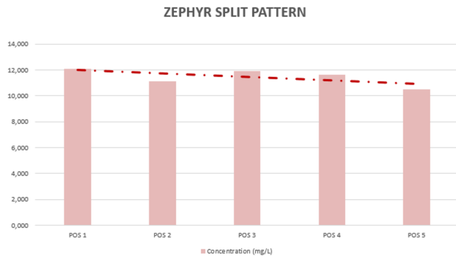


RESULTS

<u>Phase 1:</u> **CETI5** generated a mean concentration equal to 151.39mg/L through the five channels with a CV=10.9% and with an efficiency of 95.01% versus the nominal concentration.



<u>Phase 2:</u> After 2 exposures from **CETI5** to **Zephyr** ALI chamber a mean concentration of 5 test item ports was 11.46mg/L with a CV=5.64%.
The 2 SHAM positions were a values of 0mg/L of particle concentration.



CONCLUSIONS

- The five puff engines of the **CETI5** demonstrated a generator profile across time inter and intra assays with a 95.01% efficiency.
- The flow pattern across five ports of **Zephyr** was determined as a split with a CV equal to 5.64%.
- Control group filter efficiency was >99% without weighable particles deposited.

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