P 2056

# A novel dynamic gas mixing system to assess the human lung cells response to BTXE exposure at low levels

Sophie ACHARD[1], Mathilde DELAVAL[1], Tobias KREBS[2], Isabelle MOMAS[1], Nathalie SETA[1]

[1] Laboratory of Public Health and Environment– EA 4064 – Paris Descartes University, F-75006 Paris.

[2] VITROCELL Systems GmbH, Waldkirch, Germany

## Background

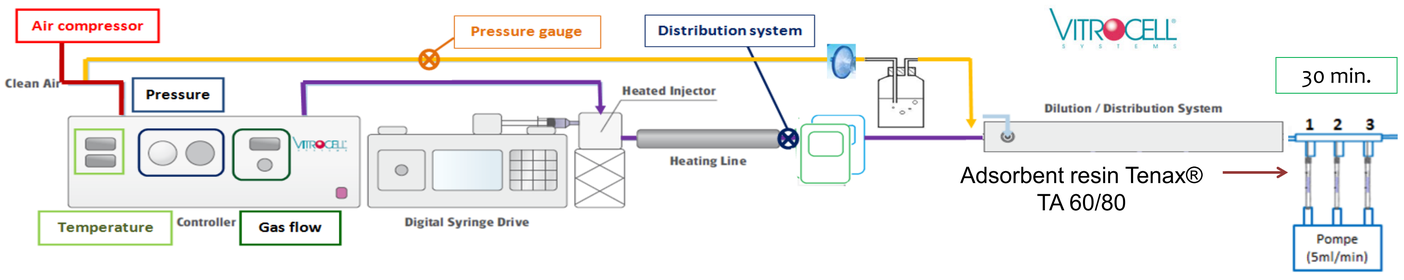
Some indoor air pollutants such as volatil organic compounds (VOCs) seem to contribute to the implementation of airway inflammation which can lead to severe respiratory disorders: rhinitis, bronchitis, asthma. The assessment of the impact of these environmental pollutants, single or in mixtures, using alternative methods, faces several difficulties due to the conditions required to mimic human conditions of exposure. Indeed, the indoor air is a very complex mixture of pollutants at low and variable levels. The generation of such atmospheres for experimental studies is thus difficult.

**Evaluate the impact of low doses of indoor air pollutants on the inflammatory response of human lung epithelial cells. Case of toluene.**

## Purpose

To set up and validate the use of the Vitrocell® Spiking System (VSS), a novel gas mixing system generating low doses of VOCs, coupled to a system of exposure in air/liquid interface, to estimate the VOCs impact on the inflammatory response of airways cells.

## Generation of gaseous atmosphere and analytic control



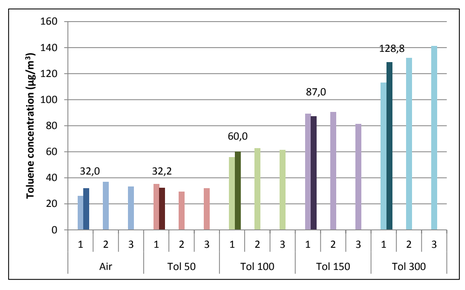

### Generation of gaseous atmosphere :

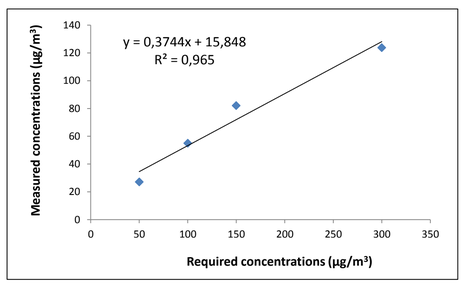
- Pollutant injection in the system thanks to a syringe,
- Pollutant vaporization in function of the pollutant dew point (110°C + 50°C for the toluene),
- Distribution into the system and dilution with humidified and heated air (5L/min, 24°C)

### Measurements of toluene level :

- Active sampling on Adsorbent Tenax® TA 60/80 (Supelco, France) at 5 ml/min,
- Thermal desorption and et gas chromatography/ mass spectrometry (LHVP).

### Results:



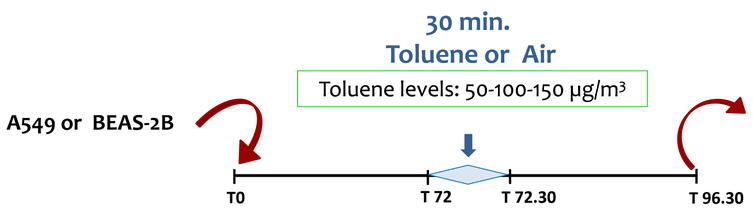

- Good distribution of atmosphere at the three ways of toluene release in the exposure chamber
- Good correlation between required and measured toluene concentrations
- Average efficiency of 55%

## Cells exposure to toluene

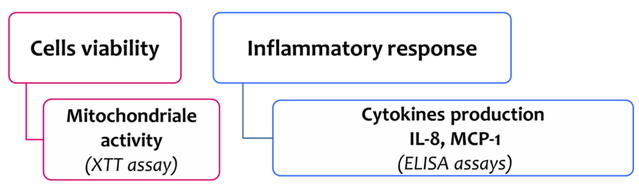
*Cell lines and culture conditions*

- Human alveolar epithelial **A549** cells ($2.10^4$ cells/insert) in Ham's F12 with 5% FCS
- Human bronchial epithelial **Beas-2B** cells ($5.10^4$ cells/insert) in Ham's F12 with 5% FCS

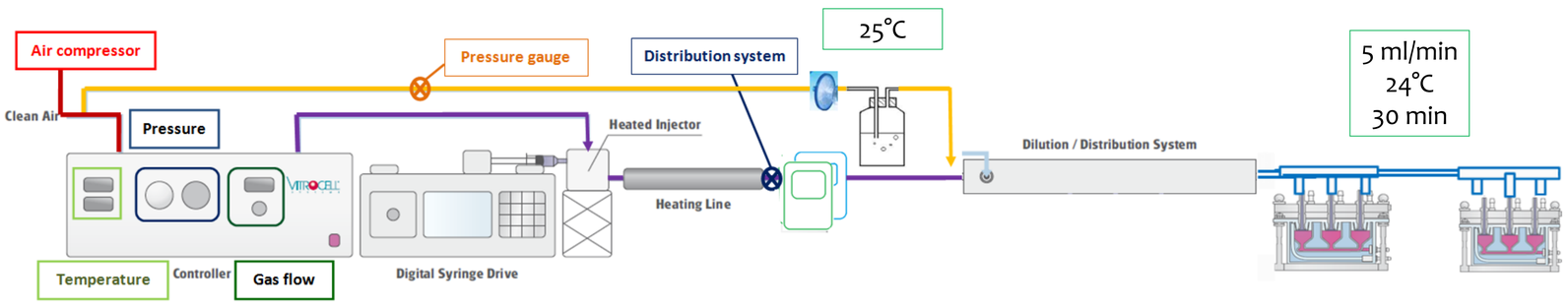
*Exposure protocol to toluene or air*




*Biological activity assessment*



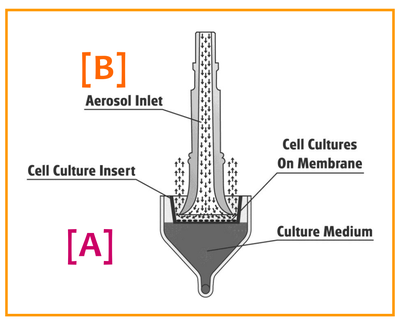

*Generation system (VSS)*




*Exposure system*

*Air-liquid interface exposure*

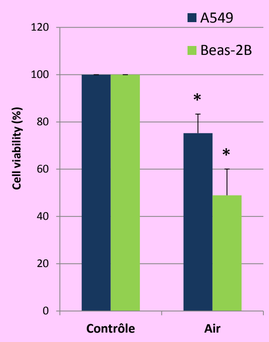
After atmosphere generations, cells into the atmosphere chamber [A] are exposed on their apical side to pollutant or air, delivered through a trumpet device [B].




## Results

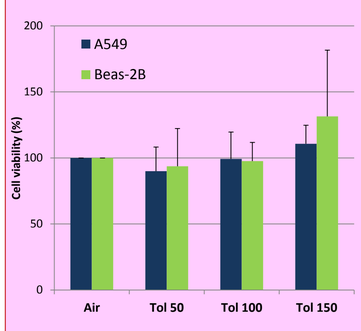
*Cell viability*

A549 and Beas-2B cell viability (n=3), 24h after 30 min **air exposure**




**Statistically significant effect** on alveolar and bronchial cell viability due to **air** exposure.

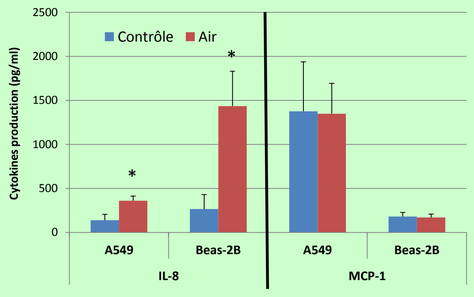
A549 and Beas-2B cell viability (n=3),24h after 30 min **toluene exposure**




**No effect** on cell viability whatever **toluene** level exposure.

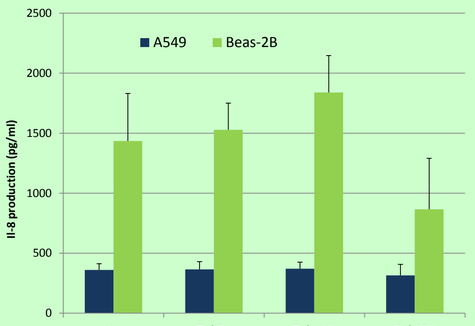
*Inflammatory response*

A549 and Beas-2B cell viability (n=3), 24h after 30 min **air exposure**



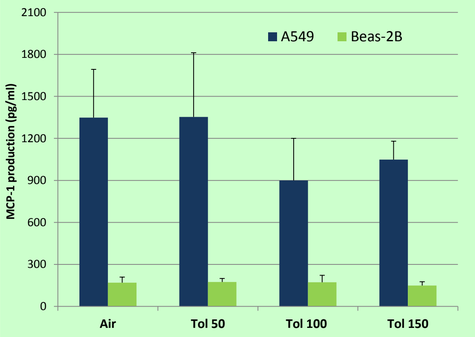

**Significant increase** of Il-8 **production** by both cell lines after **air exposure**
**No difference** of **MCP-1 production** by both cell lines after **air exposure**

A549 and Beas-2B Il-8 production (n=3), 24h after 30 min **toluene exposure**




**No difference in Il-8 production** after **toluene exposure** whatever the cell line

A549 and Beas-2B MCP-1 production (n=3), 24h after 30 min **toluene exposure**




**No difference in MCP-1 production** after **toluene exposure** whatever the cell line

## Conclusion

- **Generation of gaseous atmosphere and analytic control:**

**Our results indicate that generation of controlled toluene atmosphere at environmental levels using the VSS is feasible, simple and adapted to air/liquid cell exposure. Further testing pollutant generation for reproducibility and reliable dose-response establishment is in progress.**

- **Pulmonary cells inflammatory response at air or toluene exposure**

**Alveolar and bronchial cells inflammatory response to air exposure is modulated with increase Il-8 level. These results confirm the respiratory cells capacity to respond to a stress. However, after toluene exposure, no significant variation of cytokines production occurs in good agreement with the reported results by Persoz *et al.* (2010, Toxicology Letters) after formaldehyde exposure.**