P 1362

COMPARISON OF TWO *IN VITRO* MODELS TO ASSESS THE IMPACT OF ENVIRONMENTAL POLLUTANT EXPOSURES ON RESPIRATORY CELLS

Charles Persoz[1], Christopher Leleu[2], Sophie Achard[1], Isabelle Momas[1], Nathalie Seta[1]

[1]*Laboratoire de Santé Publique et Environnement, EA 4064. Université Paris Descartes, Paris, France*
[2]*Laboratoire de Parasitologie-Mycologie, EA 3520, Université Paris-Diderot, Pars , France*

Purpose

In environmental toxicology *in vitro* studies of adverse effects of inhaled pollutants come up against difficulties to generate atmospheres charged with gaseous chemicals and to expose cultured cells directly to these atmospheres.

The aim of this study was to compare two *in vitro* approaches, liquid-liquid and air-liquid, to assess the impact of formaldehyde (FA) exposure on the inflammatory response of respiratory tract cells

MATERIALS AND METHODS

Human alveolar epithelial cell line, A549 (ATCC, USA), was exposed at the Liquid-Liquid or Air-Liquid interface to various concentrations of FA

Liquid-Liquid Exposure

Human epithelial cells seeding on 96-wells culture plate in Ham F12 medium (5 % FCS)
Alveolar cells (**A549**, ATCC, USA) 8.10^3 cells/well

Cell exposure to FA

Concentrations of interest of FA (**25 to 800 µM**) were obtained by dilution of liquid FA (Sigma Aldrich) into the culture medium

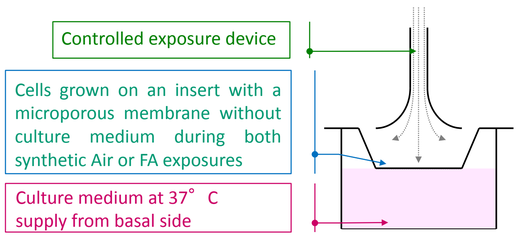
Cells were exposed to FA during 24h

At the end of this period biological activity was assessed

Air-Liquid Exposure

(Persoz *et al.*, 2010, *Toxicol Lett*)

Human epithelial cells seeding on insert in Ham F12 medium (5 % FCS)
Alveolar cells (**A549**, ATCC, USA) 2.10^4 cell/insert



Generation and control of tested atmospheres

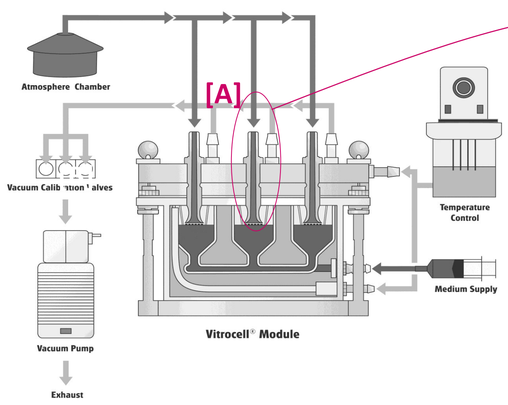
FA atmospheres were generated from liquid FA (Sigma Aldrich) vaporized in a glass generation chamber

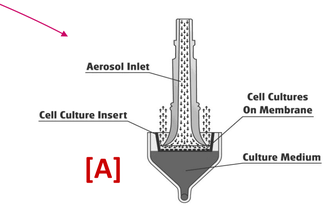
Concentrations tested were from **25 to 200 µg/m³**

Control of generated concentration of FA were made using passive samplers (Radiello ®) and a GC/MS analysis

Vitrocell exposure system ®

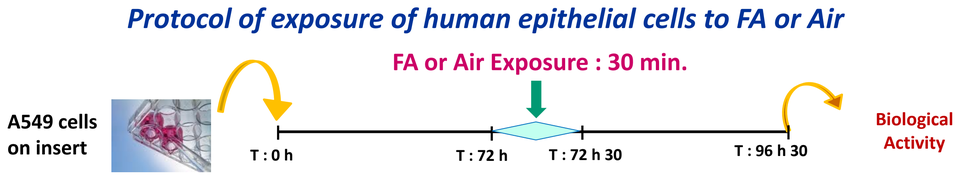
Cells were exposed in a Vitrocell module at the air-liquid interface to a dynamic and continu air flow (5 mL/min).





Air-liquid exposure is performed using a trumpet device, allowing a direct contact between cells and test atmosphere [A]

Protocol of exposure of human epithelial cells to FA or Air



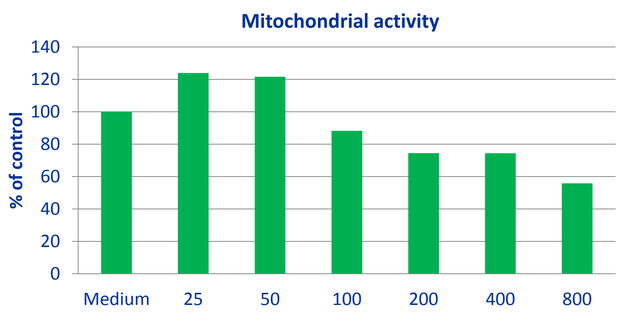
Biological activities assessed for the two in vitro models

Cellular Viability : Metabolic activity using the XTT assay

Inflammatory response : IL-8 and MCP-1 productions in the cellular supernatant by ELISA method

RESULTS

Liquid-Liquid Exposure



Cellular Viability

- At low doses (25 and 50 µM), beneficial effect of FA on cellular viability (Hormesis effect?)
- Decrease of 30% of the viability for FA concentrations from 100µM to 800µM

Low toxicity of FA in the conditions of Liquid-Liquid exposure

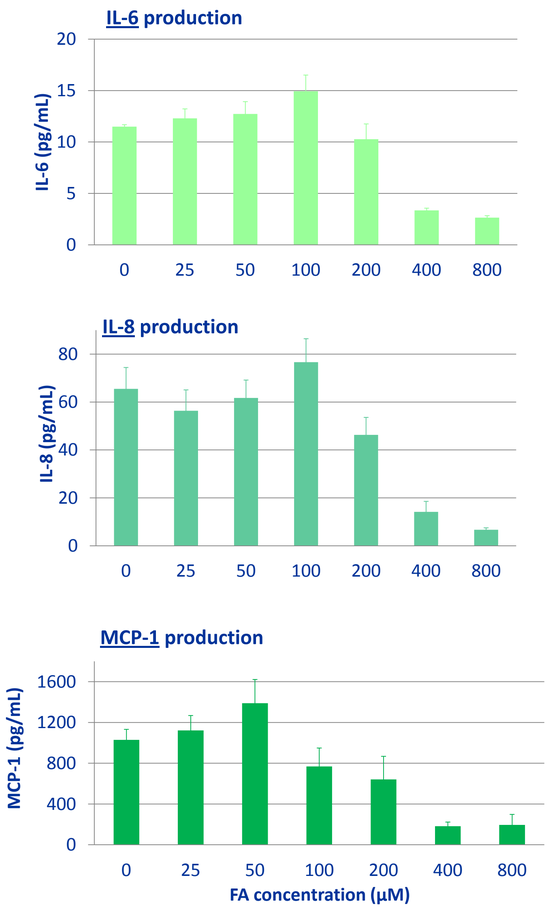
Inflammatory response - Preliminary study

Control of the capacity of A549 cells to produce cytokines (IL-6, IL-8 and MCP-1) after 24h of TNFα–treatment at 2 ng/mL (positive control) in Liquid-Liquid conditions.

	Cytokine production in pg/ml		
	IL-6	IL-8	MCP-1
Control cells	7,1	57,4	422,9
Positive Control	27,9	434,9	1041,9

- Cytokine productions were quantifiable in supernatants of A549 maintained in culture without treatment (Control cells)
- After TNFα treatment, cytokine productions compared to the Control cells were significantly increased

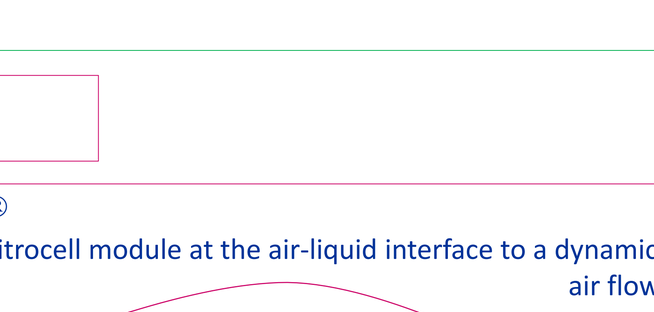
A549 cells were able to react to a stress in term of inflammatory response



Inflammatory response

- Slight increase of the IL-6 and IL-8 productions for respectively 100 µM and 50µM
- Significant decrease of cytokine productions beyond these doses

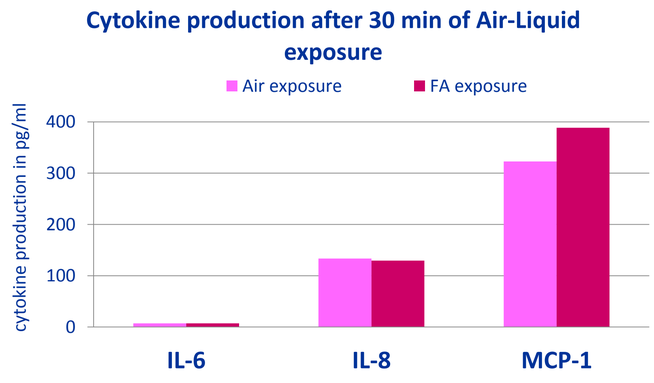
Air Liquid Exposure



Cellular Viability

- Decrease of cellular viability of 20% since the lowest dose (25 µg/m³)
- Significant toxicity of FA for the higher dose (200 µg/m³)

Decrease of A549 viability in a dose-dependent manner in the conditions of Air-Liquid exposure



	Cytokine production in pg/ml		
	IL-6	IL-8	MCP-1
Air exposure	7,3	133,5	322,8
FA exposure	7,2	129,6	388,6

Inflammatory response

No modulation of cytokine production after 30 min of FA exposure

CONCLUSION

The liquid-liquid exposure model is classically used for toxicological studies because it is easy to perform. But the results obtained, when we consider gaseous pollutants, do not allow extrapolation to human situations because, the conditions of exposure are not physiological and the tested concentrations are not transposable with the environmental pollutant levels.

Despite the absence of modulation of the inflammatory response following the exposure to FA in air-liquid conditions this Vitrocell system is adapted and very promising for the evaluation of the impact of the atmospheric pollutants at low dose on the respiratory health (see posters P1361 and P2056)