National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Pro-inflammatory responses to PM0.25 from airport and urban traffic emissions at submerge cell culture condition: A comparison with air-liquid interface (ALI) culture

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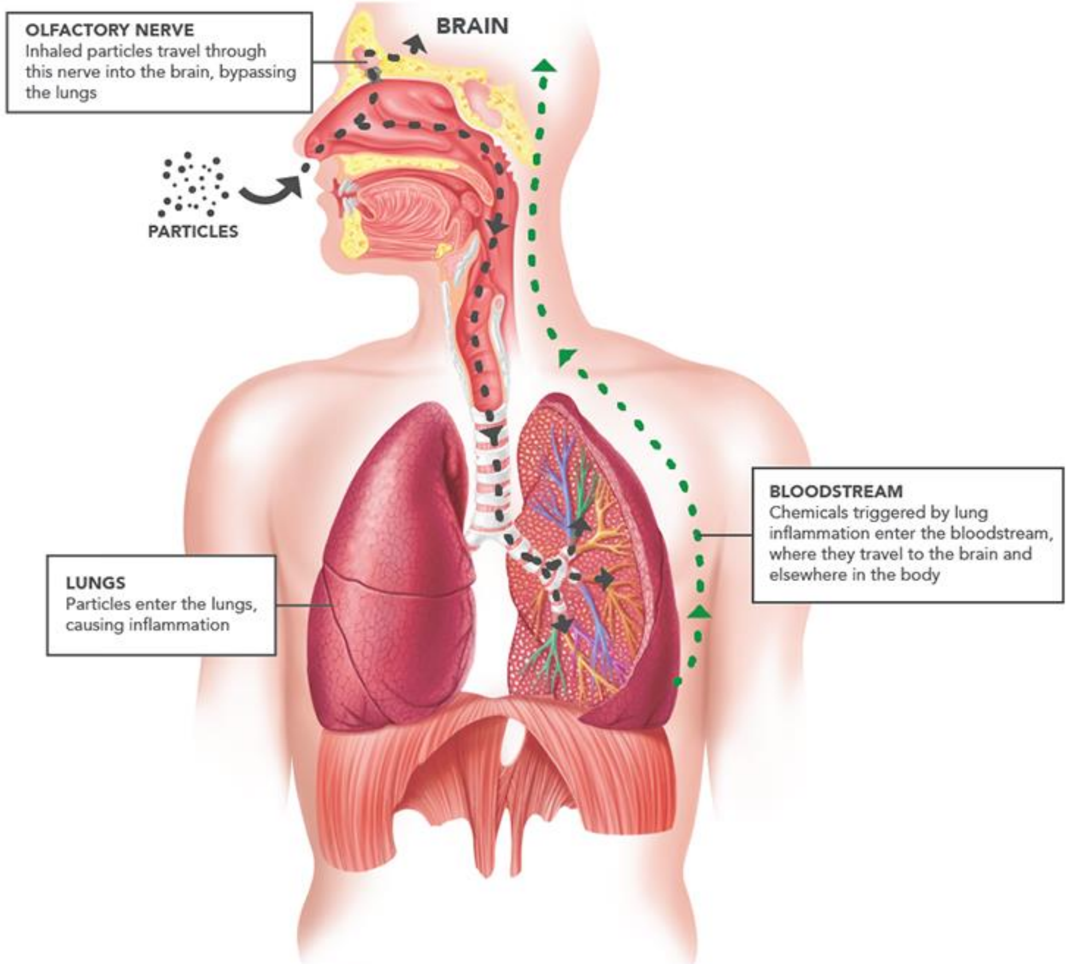
RIVM and Utrecht University in Netherlands

Contents

- The cytotoxicity of aviation and urban traffic PMs under submerge exposure

- Air-liquid interface (ALI) exposure and exposure systems

Inhalation exposure



The adverse effects of inhalation exposure

Humans are constantly exposed to various substances ranging from industrial gases, traffic emissions to cigarette smoke.

The adverse effects: Chronic respiratory disease and lung disease; Neurotoxicity

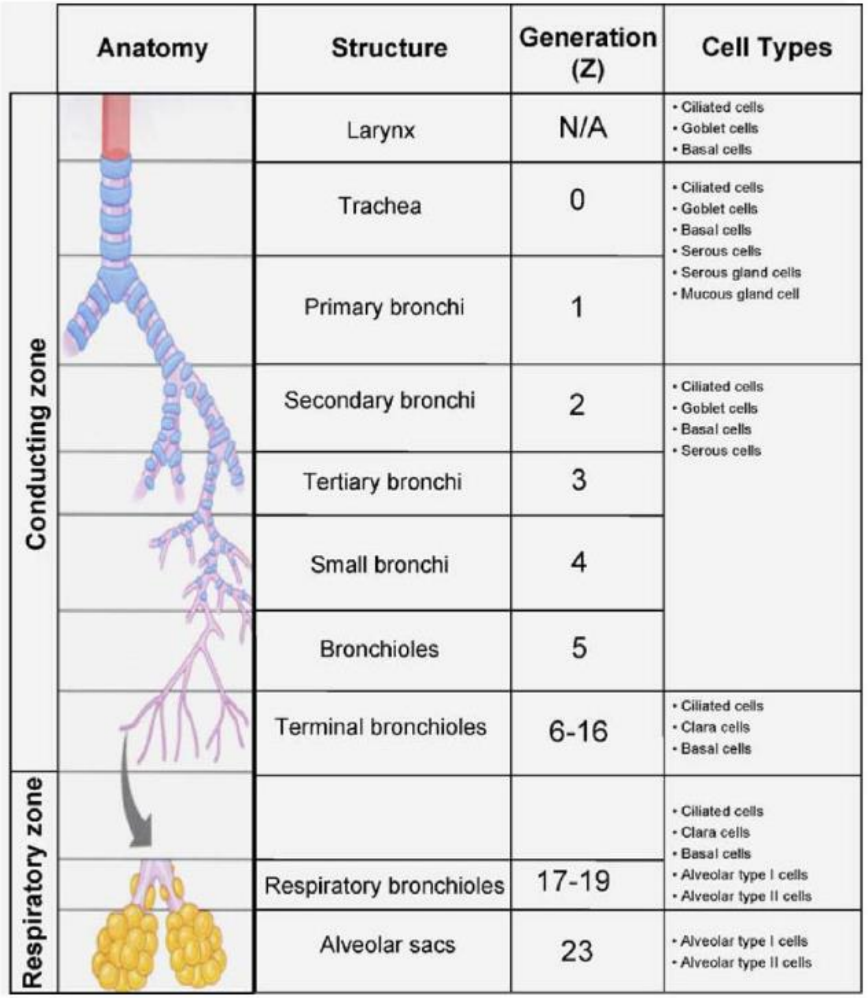
Study the inhalation exposure

Two ways: Epidemiology and Toxicology

Toxicology is traditionally based on in vivo experiments

There is an increasing request to use in vitro models

Cell model:
16HBE (human bronchial epithelial) cell easy to culture and grow fast



Zone	Anatomy	Structure	Generation (Z)	Cell Types
Conducting zone		Larynx	N/A	• Ciliated cells • Goblet cells • Basal cells
		Trachea	0	• Ciliated cells • Goblet cells • Basal cells • Serous cells • Serous gland cells • Mucous gland cell
		Primary bronchi	1	
		Secondary bronchi	2	• Ciliated cells • Goblet cells • Basal cells • Serous cells
		Tertiary bronchi	3	
		Small bronchi	4	
		Bronchioles	5	
		Terminal bronchioles	6-16	• Ciliated cells • Clara cells • Basal cells
Respiratory zone				• Ciliated cells • Clara cells • Basal cells • Alveolar type I cells • Alveolar type II cells
		Respiratory bronchioles	17-19	
		Alveolar sacs	23	• Alveolar type I cells • Alveolar type II cells

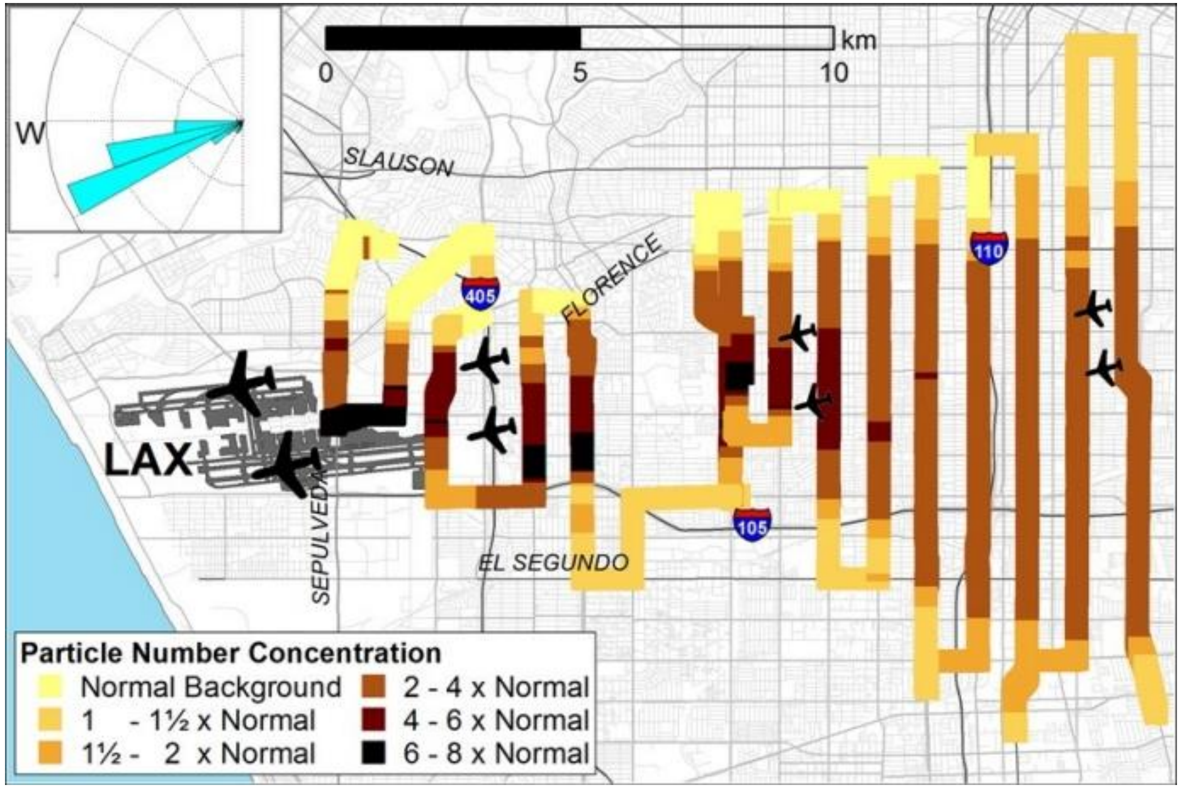
Particles Deposition

Gas exchange

Structures of the respiratory tract (BéruBé et al 2010)

Background:

Aviation industry and airport traffic increase fast in recent years;
Many large airports are located near urban area, which may have a significant impact on our environment and health.



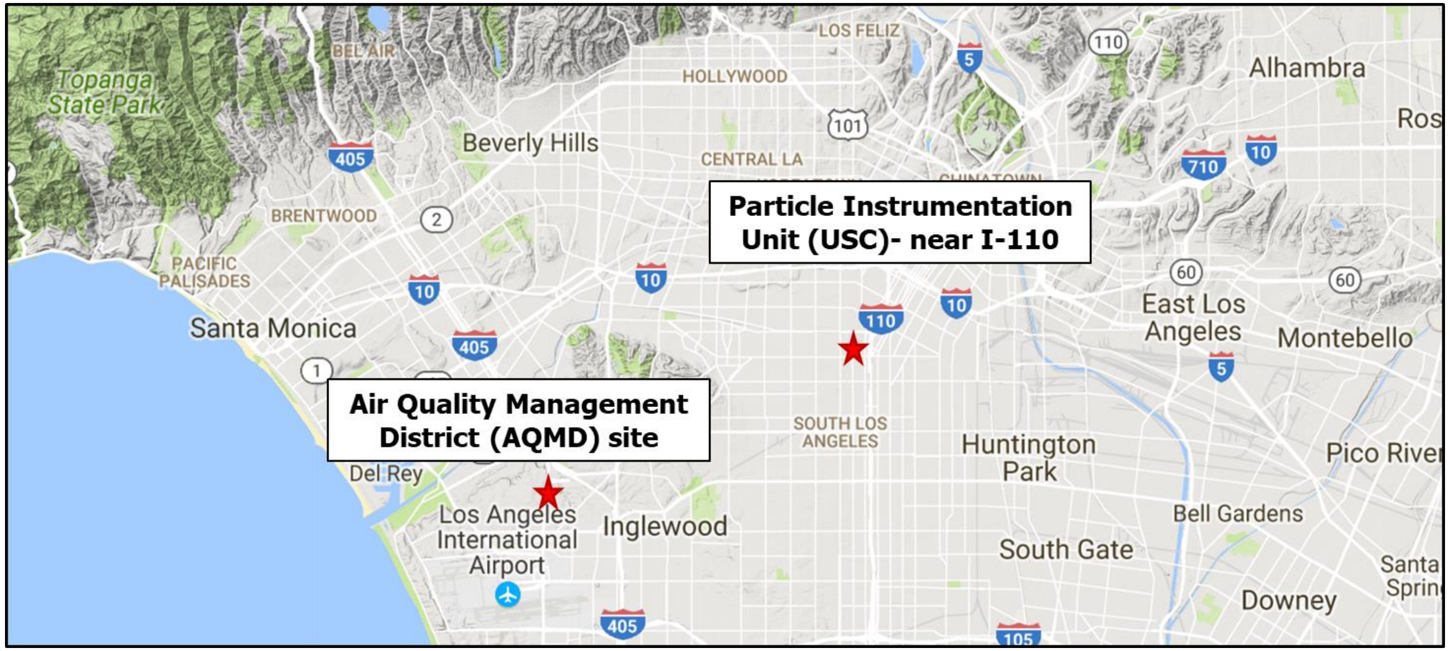
Los Angeles International Airport (LAX) *Hudda et al Environ Sci Technol. 2014*

Few information on sources to airport PM emissions and cytotoxicity of airport PMs

Exposure materials and method

Materials:

- **$PM_{0.25}$ samples** (5 LAX, 5 USC, 1 turbine and 1 diesel samples)
- 5 airport samples from Los Angeles International Airport (LAX).
- 5 urban traffic samples from a freeway around University of Southern California (USC)
- 1 turbine and 1 diesel samples from turbine and diesel engine

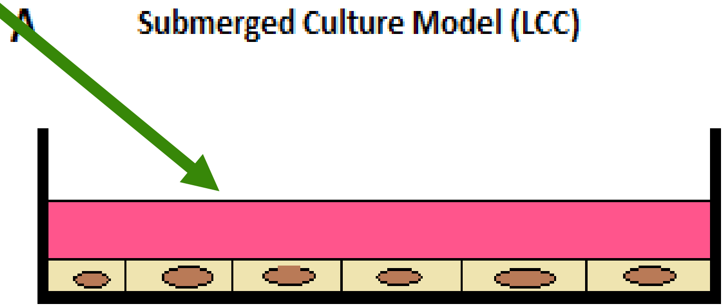


Sampling locations

Submerged exposure:

- Particles were extracted from filters, the suspension is added in culture medium, and exposed to cells for 4 hs.

  After 4 hs, medium was refreshed for 20 hs recovery

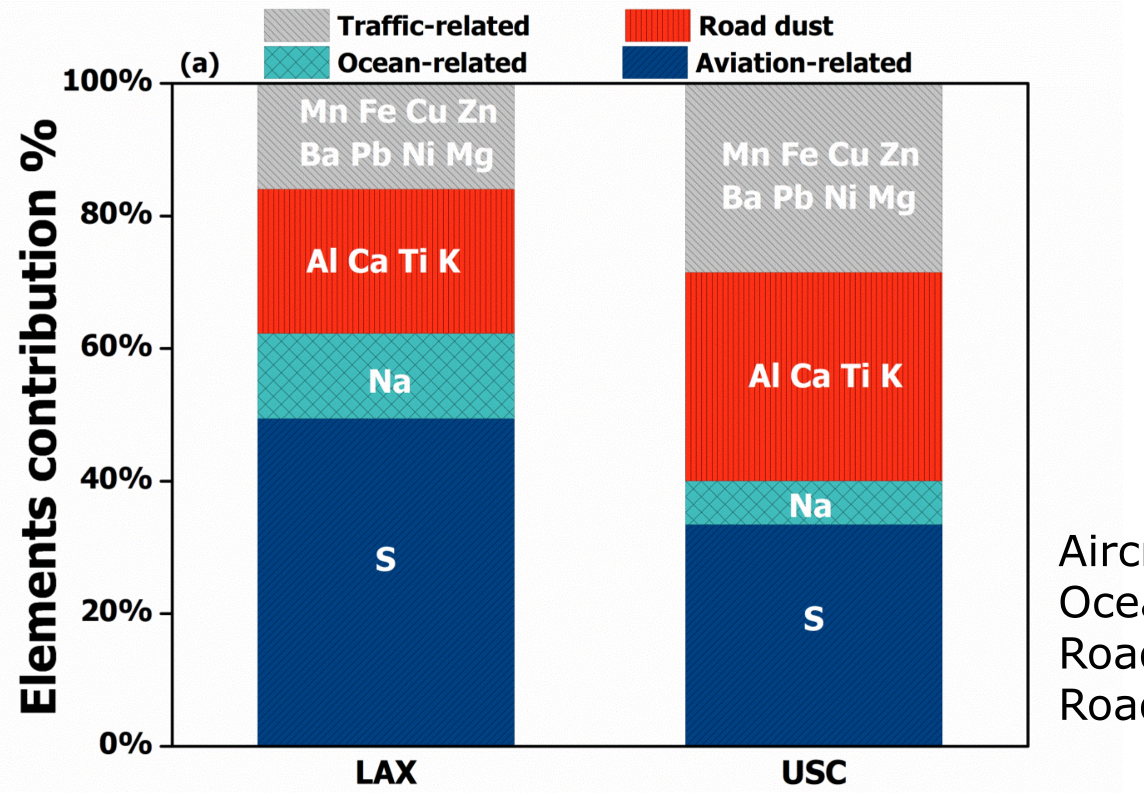


Measurements

1. Measure elemental composition and oxidative potential of PM samples

2. In vitro tests including cell viability, ROS activity and inflammatory responses after exposure

Results – Elemental contributions



Elemental contributions at LAX and USC

Aircraft emission = S
Ocean spray = Na
Road traffic emissions = Mn, Fe, Cu, Zn, Ba, Pb, Ni, Mg
Road/ Soil dust: Al, K, Ca, Ti

1. Airplane emission was the major contributor to airport PMs, following by road dust and traffic emissions
2. Urban traffic PMs have multiple comparable contributors including traffic emissions, suspended road dust and atmospheric secondary sulfates.

Results – oxidative potential

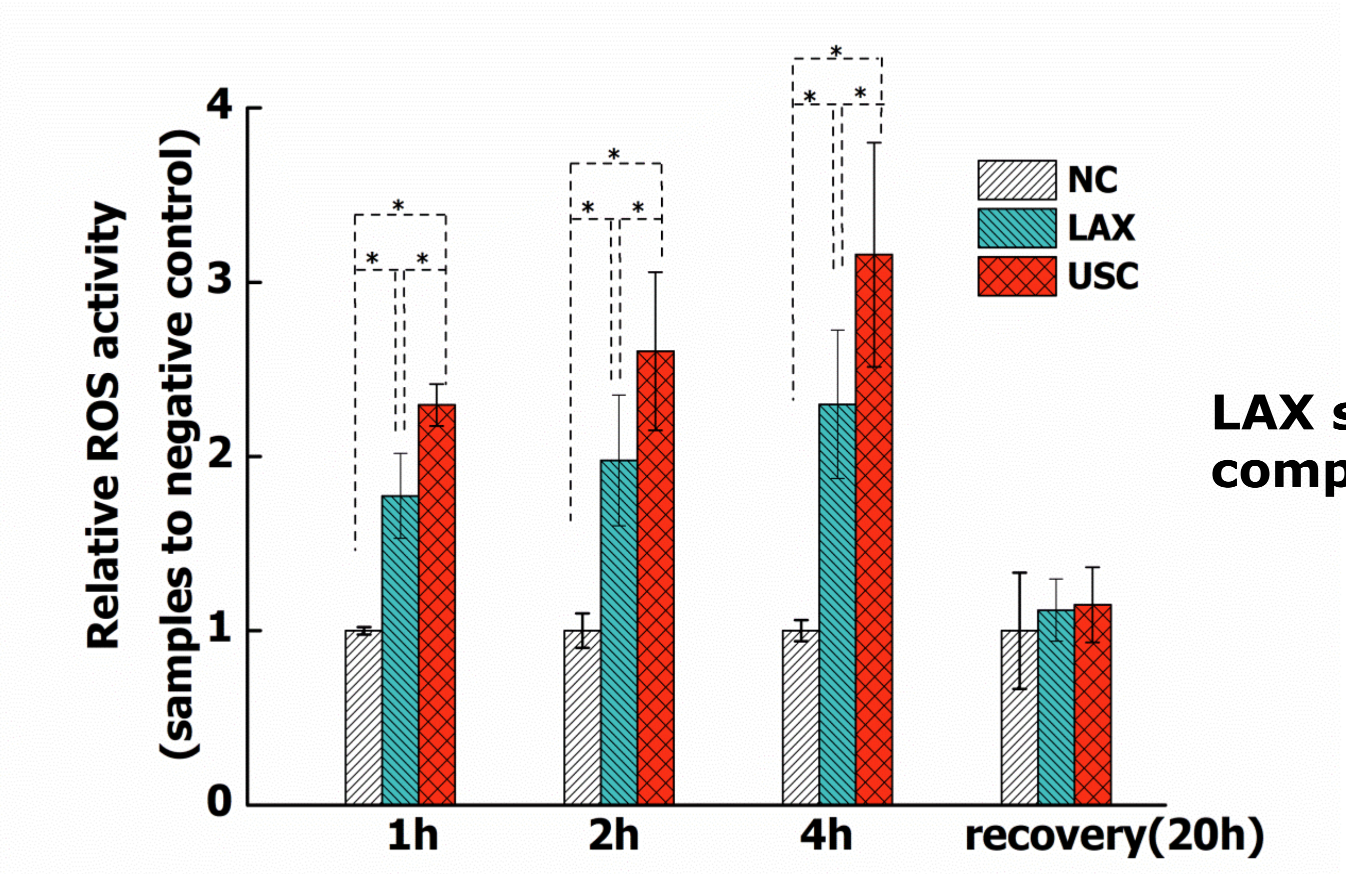
the redox activity induced by heavy metals in particle samples

Geometric mean of oxidative potential (AA and ESR) values.

	AA(nmol AA/s/µg)	ESR(A.U/1000/µg)
LAX (n=5)	0.33±0.10	1.97±0.51
USC (n=5)	1.14±0.18	3.58±0.24
Negative control (dH_2O)	0.08±0.02	0.49±0.02
Positive control (DOFA)	1.36±0.13	11.4±0.37

Compared to USC samples, LAX samples seem less reactive
Lower oxidative potential (lower heavy metals)

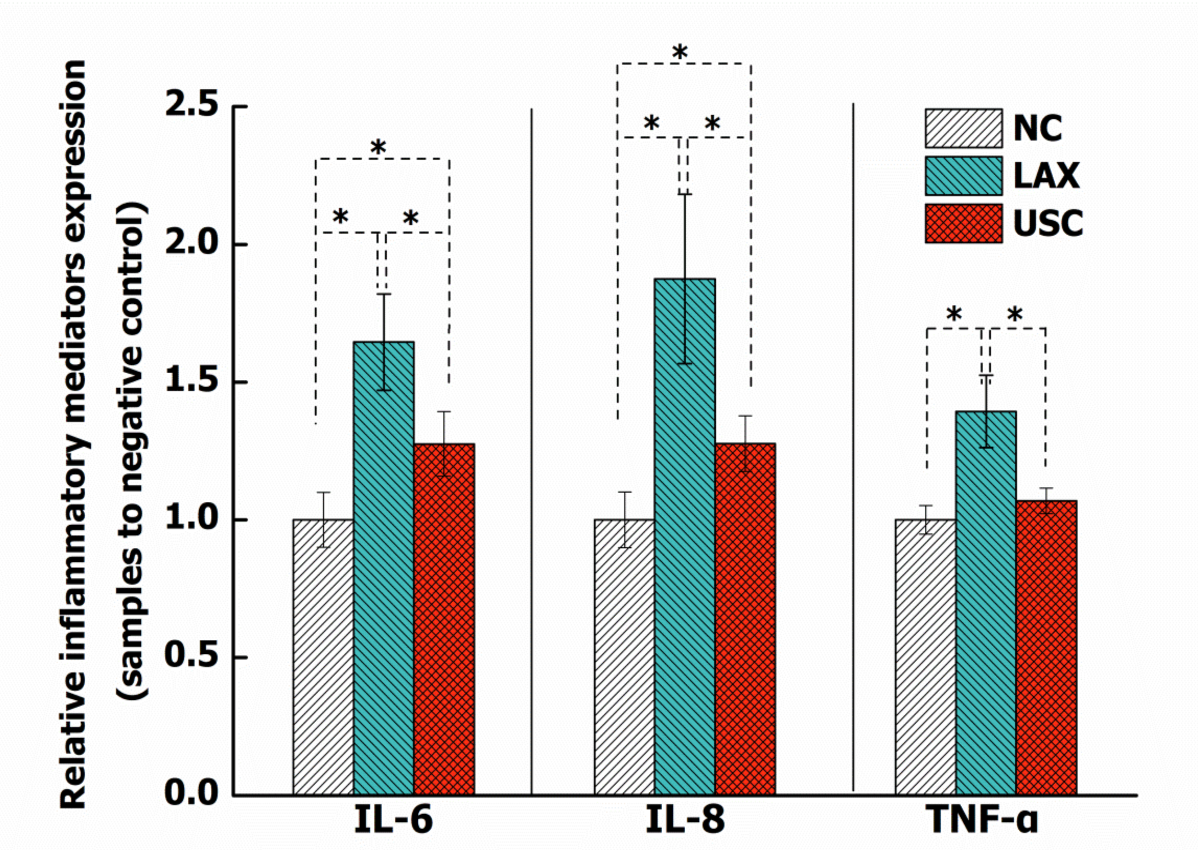
Results- intracellular ROS generation



LAX samples induced lower ROS generation compared to USC

Average ROS activity after 1, 2, and 4 h exposure with 10 µg/mL PMs and 20 h recovery

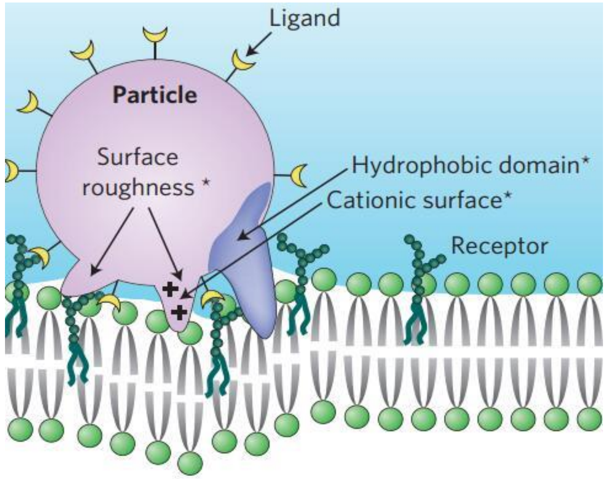
Results- inflammatory responses



IL-6, IL-8, and TNF-α expression after 20 h recovery

LAX samples induced higher level of inflammation during 20 h recovery

Transloacted particles in cells play an important role in inflammatory responses during 20 h recovery



Translocation of particle into cells
(Andre Nel et al; Nature Material. 2009)

Size distribution:
Mean Particles diameter **(LAX)≈ 20 nm**
Mean Particles diameter **(USC)≈ 35 nm**

Conclusions

- **Airplane emission was the major contributor to airport PMs; Urban traffic PMs have multiple contributors.**
- **LAX samples seem less reactive compared to downtown USC**
  - Lower oxidative potential and ROS generation
- **LAX samples were more potent in inducing inflammation**

Airport PMs showed similar toxic properties to the urban traffic PMs

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Pro-inflammatory responses to $PM_{0.25}$ from airport and urban traffic emissions

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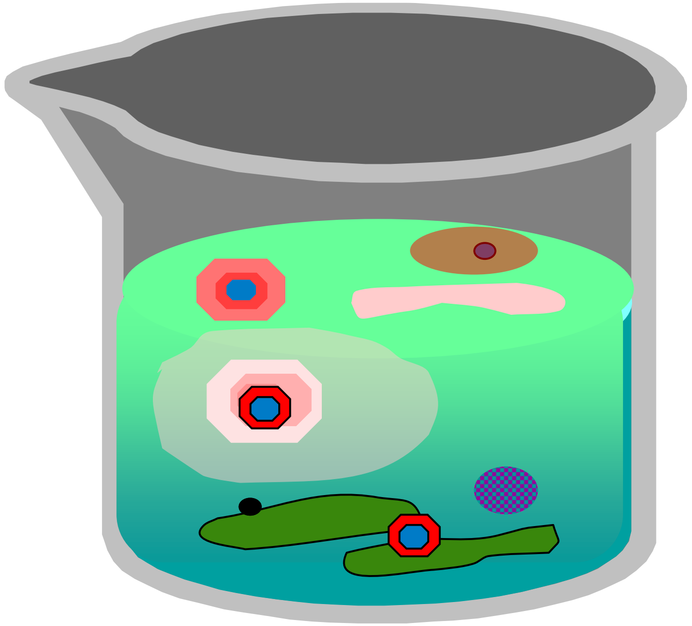
Improvements

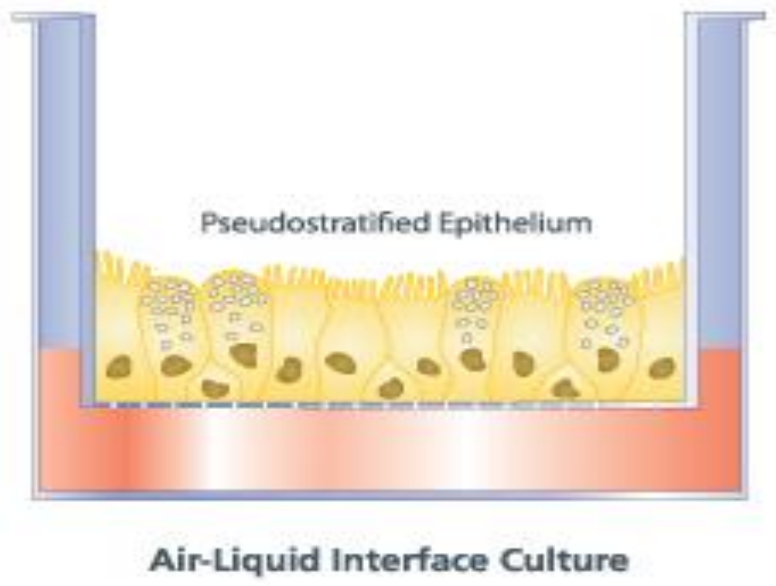
Submerged exposure
Deposition mass?

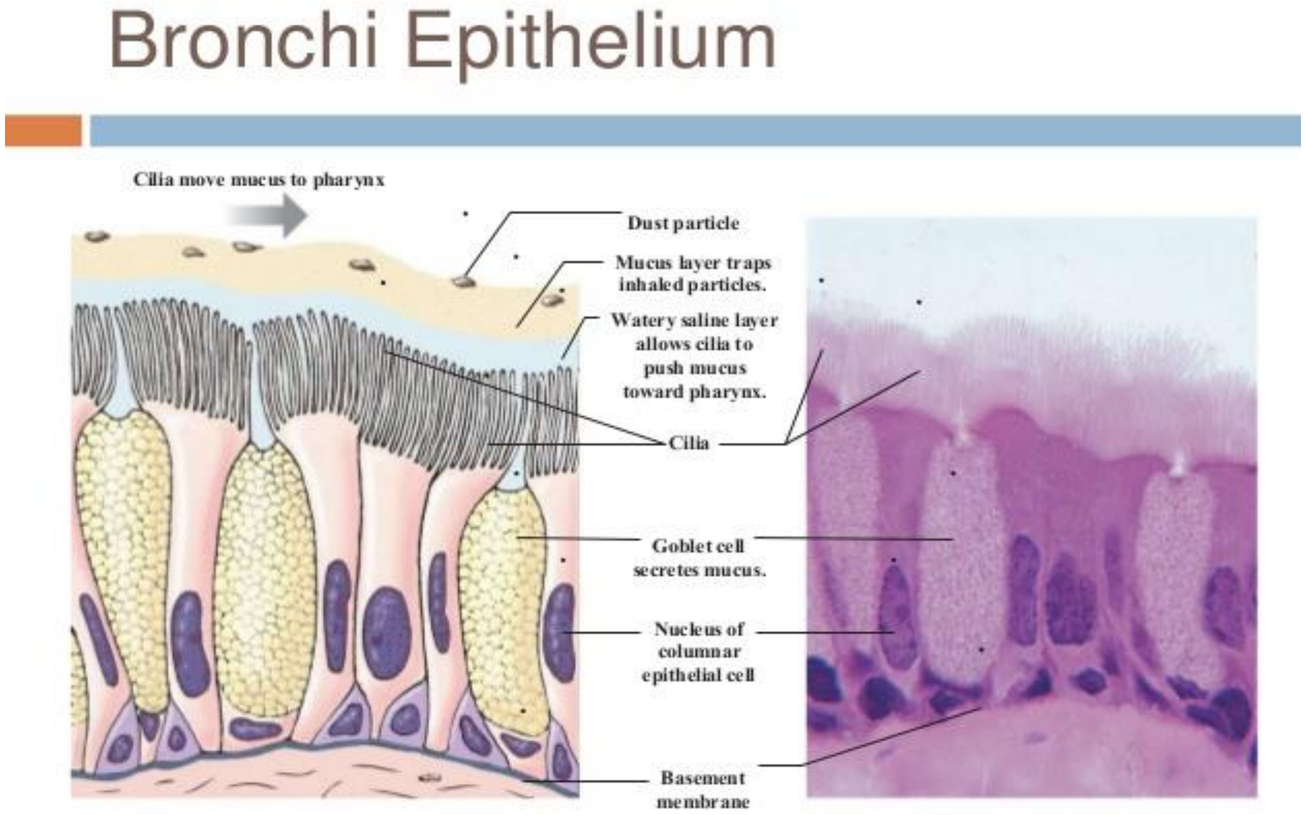
Conventional Submerged exposure



Shortcomings:
1.Deposited particles remains unknown
2.Characteristics of particles can be altered
3. Can not be used for gases and aerosol real-time exposure

Air-Liquid Interface culture



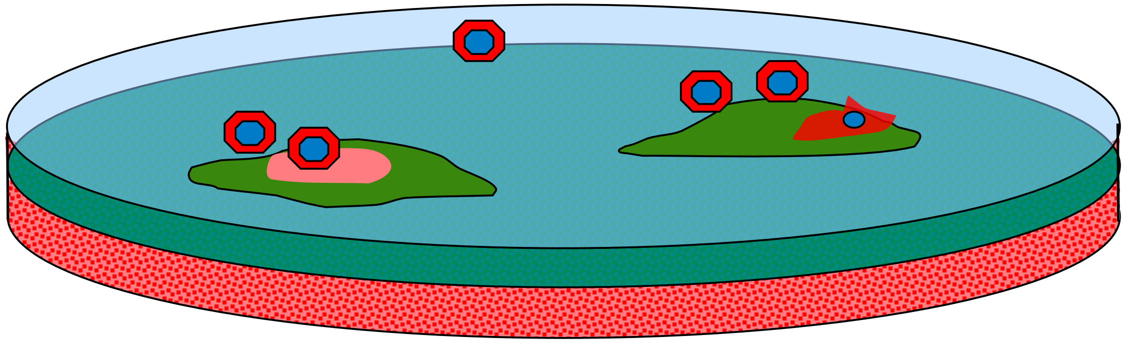


ALI culture: Cells are cultured on the apical membrane of insert without covered medium. Culture medium is added to the basolateral side.

The setup simulates the human airway conditions and stimulates the cell differentiation (mucus release and cilia formation)

Air-Liquid Interface Exposure

ALI Exposure

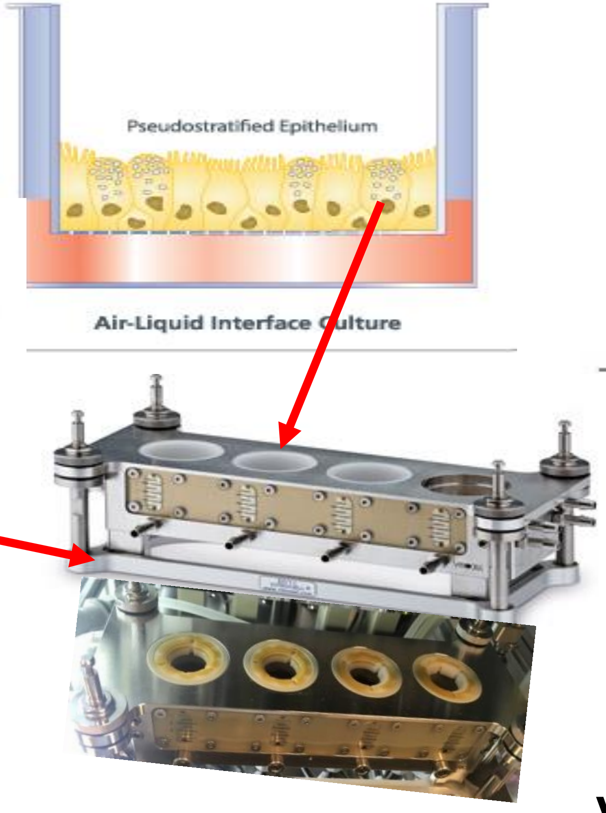


1. Real-time exposure
2. No loss
3. Realistic

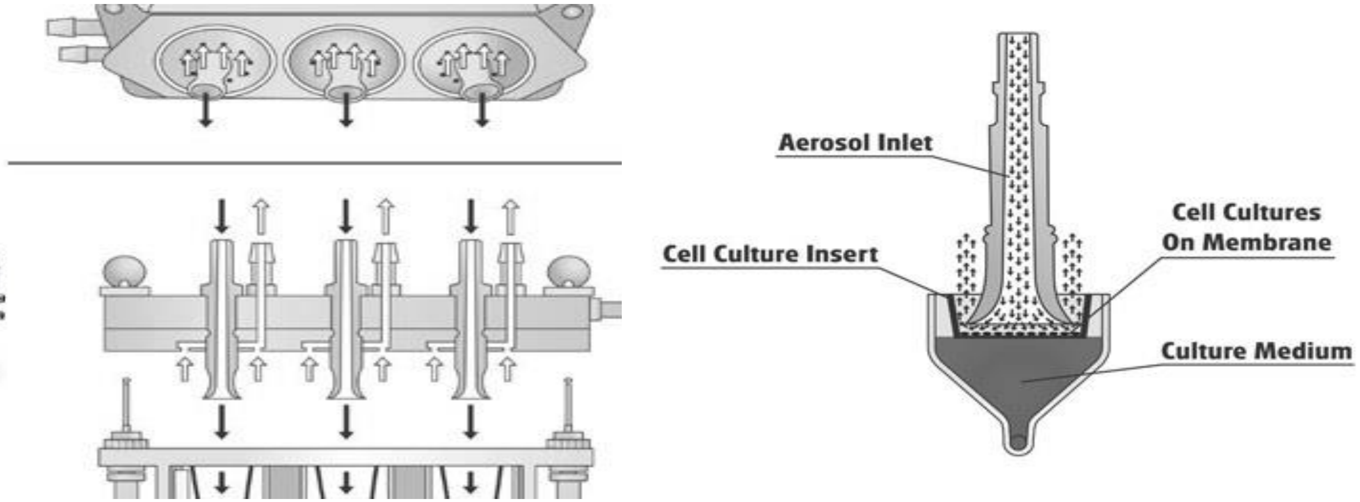
ALI exposure systems

VITROCELL® automated exposure station

Control **Exposure Cabinet**



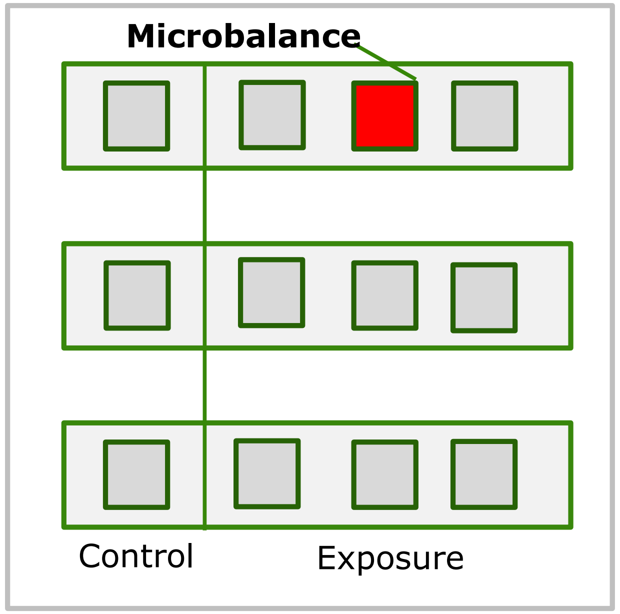
Module

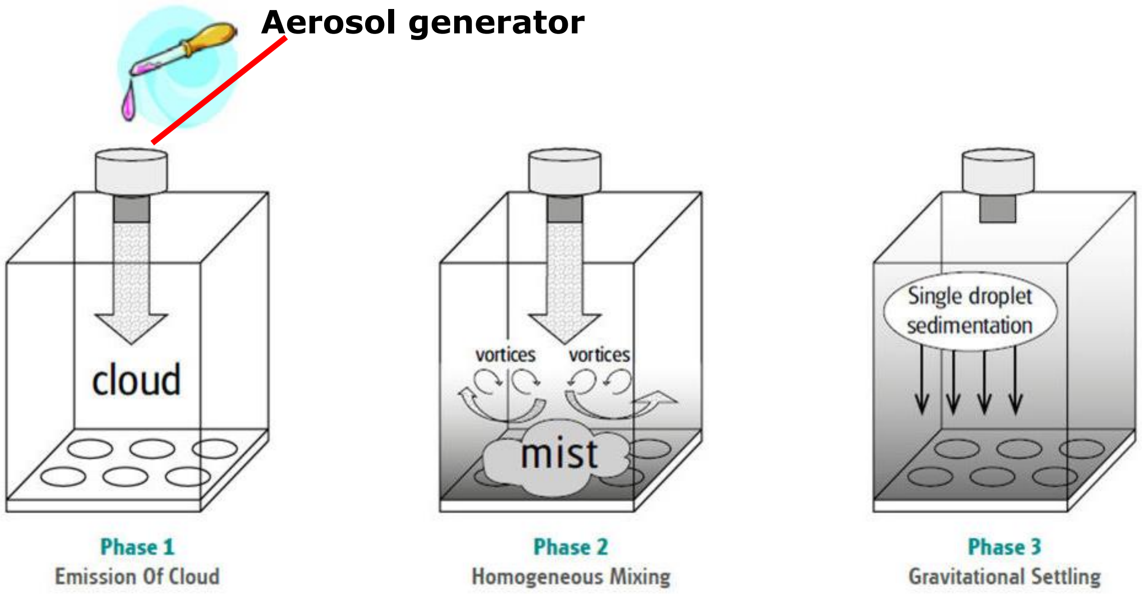


Various materials transferred into exposure cabinet and passed over cells by continuous air flow

ALI exposure systems

VITROCELL® Cloud exposure system





Droplet sedimentation mechanism

Comparison – exposure systems

Advantages
1. Different materials exposures
2. More realistic (continuous exposure)

Disadvantages
1. Airflow might be harmful to cells
2. Less cells can be selected
3. Large volume of suspension is required

VITROCELL® automated exposure station

V.S

Advantages
1. User-friendly
2. More cells can be selected
3. Less volume of suspension

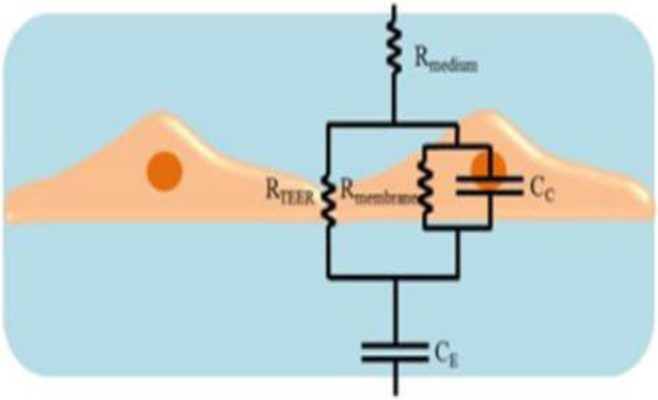
Disadvantages
1. Only for suspension exposure
2. Less realistic (one-time exposure)

VITROCELL® Cloud exposure system

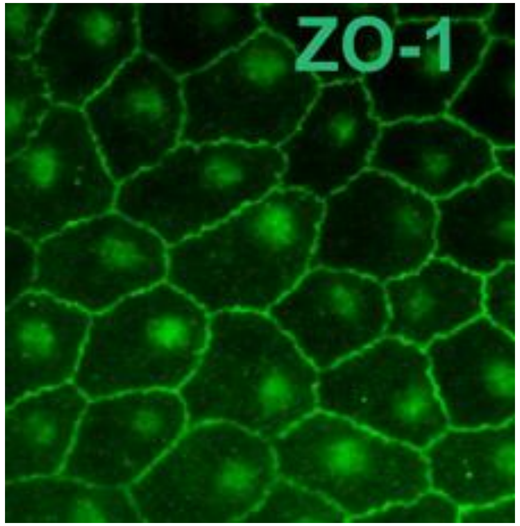
Selection of cell models under ALI conditions

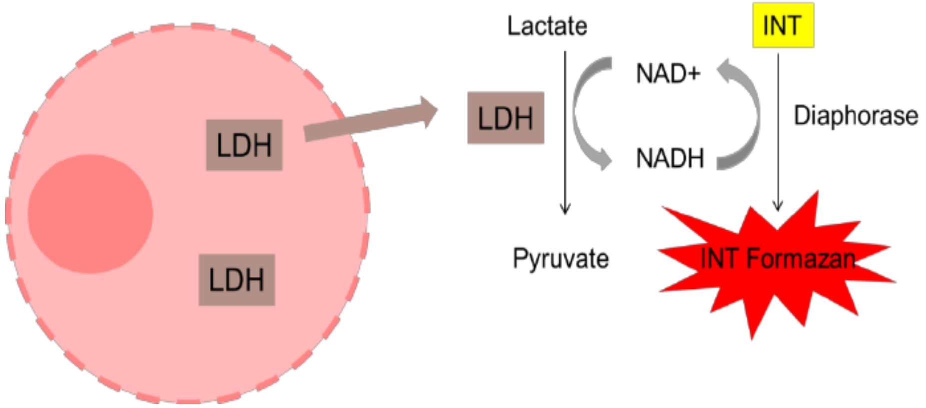
Cells： **16HBE, Calu-3, H292 and BEAS-2B cells**

Tests: the integrity of cell membrane (TEER, ZO-1 protein staining, and LDH leakage)



Transepithelial/transendothelial electrical resistance (TEER)





LDH release

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Next…

Toxicity of UFPs from Amsterdam airport and urban traffic emissions under ALI exposure

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Thank you