Size partitioning of airborne particles to compare their proinflammatory effect in airway epithelial cells.

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In our countries, the particulate pollution is currently monitored and a concentration threshold is established for PM10 that should not overcome an annual mean of forty 40 µg/m^3. However the urban aerosol is for nearly 80% composed of fine and ultrafine particles.

There are growing consistent data underlying the role of fine and ultrafine particles in health effects. Epidemiological studies have shown a stronger association between respiratory effects and exposure to ultrafine particles compared with fine and coarse PM. Experimental *in vivo* studies have shown that a short term exposure produce an pulmonary inflammation that can lead to cardiovascular effects

The small size of particles favours their deep penetration in the respiratory tract as well as their deposition especially for ultrafine particles because their alveolar clearance achieved by macrophages is disturbed. Consequently they are retained in the lung. The biological effects induced by particles results from their surface reactivity that increases as the particle size decreases as well as the presence of different compounds such as metals, organic compounds and endotoxins.

When particles in inhaled air reach the airways, they can be endocyted by macrophages and epithelial cells, the main target cells of particles that react to particles stress by producing inflammatory mediators such as GM-CSF, TNFα, IL1, IL-8. These cytokines will recruit inflammatory cells triggering an inflammation in the airways that is the major short term effect of particle exposure described by *in vivo* and *in vitro* studies.

The aim of the present study was to investigate which size-fraction of the urban particulate matter is the most relevant regarding to the biological effect considering the proinflammatory response of airway epithelial cells *in vitro*.

The biological effects were investigated on a human bronchial epithelial cell line. The secretion of the cytokine GM-CSF was chosen as an effect biomarker of the exposure according to the current knowledge of the proinflammatory effect of particles. Numerous studies have shown that metals, organic compounds and the surface properties of particles contribute to the production of reactive oxygen species that trigger signalling pathways leading to the expression of oxidative stress-sensitive genes among which those of proinflammatory cytokines.

Particles were sampled on the roof of a building in the south of Paris with 4 thirteen stage low pressure Dekati impactors running in parallel. 11 sampling campaigns have been carried out, one half in winter and the other in summer. The granulometric mass distributions obtained for the 11 campaigns revealed that the quantity of particles was higher in winter than in summer and that the Parisian aerosol exhibits 2 modes (accumulation and coarse) discriminated by a threshold at 1µm. A third mode corresponding to ultrafine particles and centered on 60 nm appears on some distributions especially in summer underlying their importance because they are characterized by a low mass.

For biological studies, particles were collected on polycarbonate filters which allow an efficient recovering of particles after 3 brief sonications directly in the culture medium without inducing alterations of the filter. Particles from the same size-fraction are gathered in a final volume of six hundred 600µL of culture medium.

From the obtained particle suspensions 2 treatment strategies were used either at isomasse or at isovolume in order to respect the proportion of the different fractions in the sampled air volume: the same volume of particle suspensions was applied on cells whatever the considered fraction. It means that cells exposed to the fine fraction will encounter more particles since this fraction is the most predominant in the Paris aerosol.

This strategy was first assessed in a preliminary study performed only with the two finest fractions. For the isovolume exposure, the GM-CSF secretion increases when the volume of particle suspension increases and is more important for fine particles whereas for an isomasse exposure the GM-CSF release is similar for ultrafine particles and fine particles. From this preliminary study we concluded that the GM-CSF secretion is a quite sensitive biomarker since it can be measured from a particle sampling running for 48h, it is induced from exposure concentrations of 1µg/cm^2 and it increases with the dose (Ramgolam et al., 2008).

The results obtained for the 11 samples being pooled for an isomass exposure it appears that whatever the concentration, the GM-CSF release decreases as the particle size increases. For an isovolume exposure, two representative experiments reveal the higher effect of the fine fraction.

Endotoxins are components of bacteria wall that have a high inflammatory potential. They can be present on particles and have been described to be preferentially associated to the coarse fraction. The use of a recombinant endotoxin neutralizing protein in presence of the different size fractions allows to demonstrate that endotoxins are not involved in the GM-CSF release induced by ultrafine particles but contribute to the one induced by fine and intermodal PM.

The determination of the chemical mass reveals that as the size decreases the content of the carbonaceous species increases. They account for two third of the ultrafine particles mass in summer and more in winter.

The enzymatic activity of the cytochrome p450 1A1 (CYP 1A1) that is specifically induced by polyaromatic hydrocarbons (PAH) and involved in their metabolization was measured to assess PAH bioavailability. The induction of the CYP 1A1 need PAH desorption from particles in order to react with the AhR (aryl hydrocarbon receptor) cytosolic receptor. They both form a complex that translocates into the nucleus, binds to specific sequences of DNA to induce the expression of the CYP 1A1 gene that will be translated in the enzymatic protein responsible for PAH metabolisation. In winter samples the highest enzymatic activity is observed with the fine fraction whereas in summer it is the ultrafine fraction suggesting that the bioavailability and/or the pattern of PAH is different according to the seasons.

Main conclusions:

For an isovolume exposure fine PM1-0.1 exhibits a higher effect as expected due to their high proportion in the aerosol

For an isomass exposure, both the ultrafine and fine 1-.01 fractions are mainly involved in the GM-CSF release by bronchial epithelial cells. Due to their high content in organic compounds, such compounds could be responsible for this effect.

We observed differences according to the seasons for PAH bioavailability that didn' t appear in GM-CSF release.

Endotoxins don't seem to be involved in the ultrafine particles effect whereas they could contribute to the effect of the other size-fraction

The PM1 better describe the background urban pollution in Paris than PM2.5

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Ramgolam K, Chevaillier S., Marano F., Baeza-Squiban A., Martinon L. Proinflammatory effect of fine and ultrafine particulate matter using size-resolved urban aerosols from Paris. 2008, Chemosphere, in press.

SIZE PARTITIONING OF AIRBORNE PARTICLES TO COMPARE THEIR PRO-INFLAMMATORY EFFECT IN AIRWAY EPITHELIAL CELLS

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Photo J.J. Poirault

Urban background aerosol

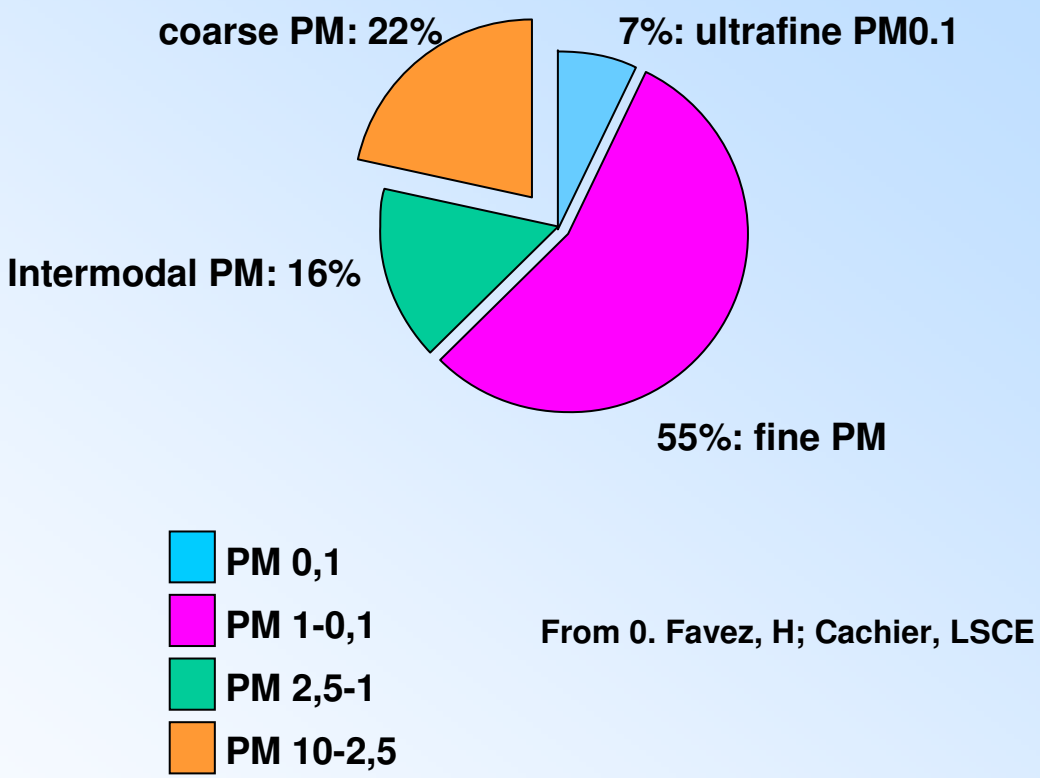
PM_{10} (particulate matter with an aerodynamic diameter ≤10 µm)

↳**40 µg/m³**
annual mean

$PM_{2.5}$
Fine PM

$PM_{0.1}$
Ultrafine PM

Relative mass size distribution in Paris background aerosol



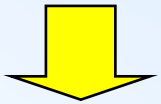
From 0. Favez, H; Cachier, LSCE

Fine and Ultrafine PM health effects

Epidemiology

(Ibald-Mulli, 2002, Stolzel, 2006, Dominici, 2006)

Animal or human exposure to CAPs (concentrated ambient Particles)



Pulmonary inflammation

(Smith 2003, Ghio, 2003, Dick 2003)

Particles characteristics involved in their biological effects

Size

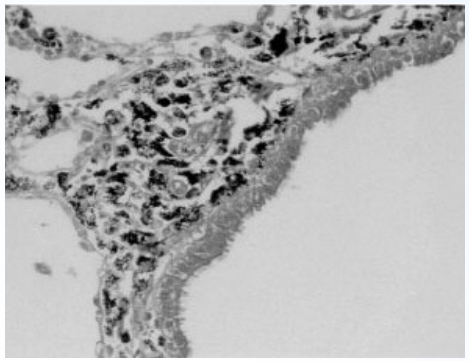
- Penetration
- Deposition
- Epuration

surface reactivity

Composition

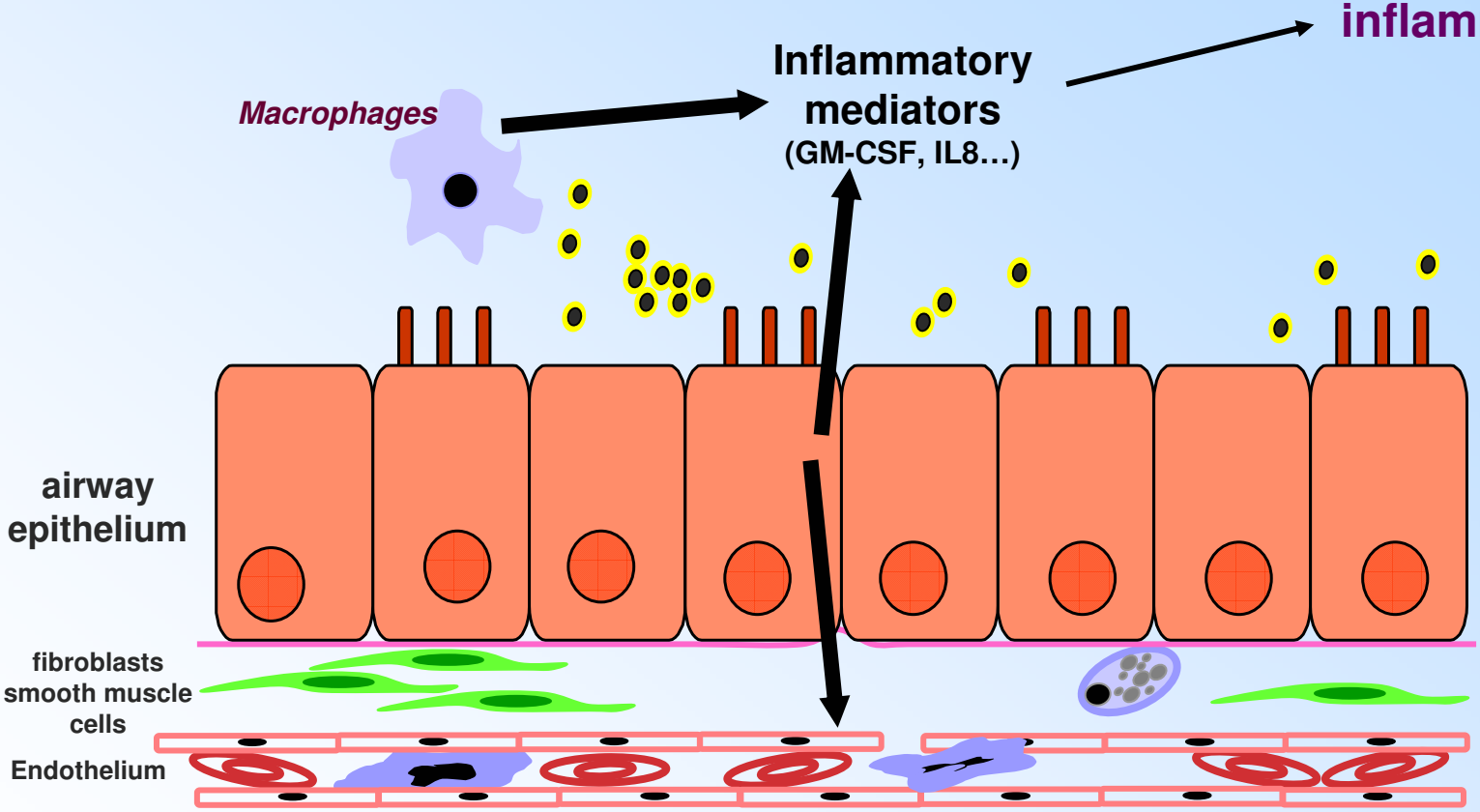
- Metals
- Organic compounds
- Endotoxins

Retention *(Churg, 2000)*



Biological effects of PM

Short-term effects



remodeling

Long-term effects

Boland et al, *AJP,1999, 2000*
Baulig et al., *EST, 2004*

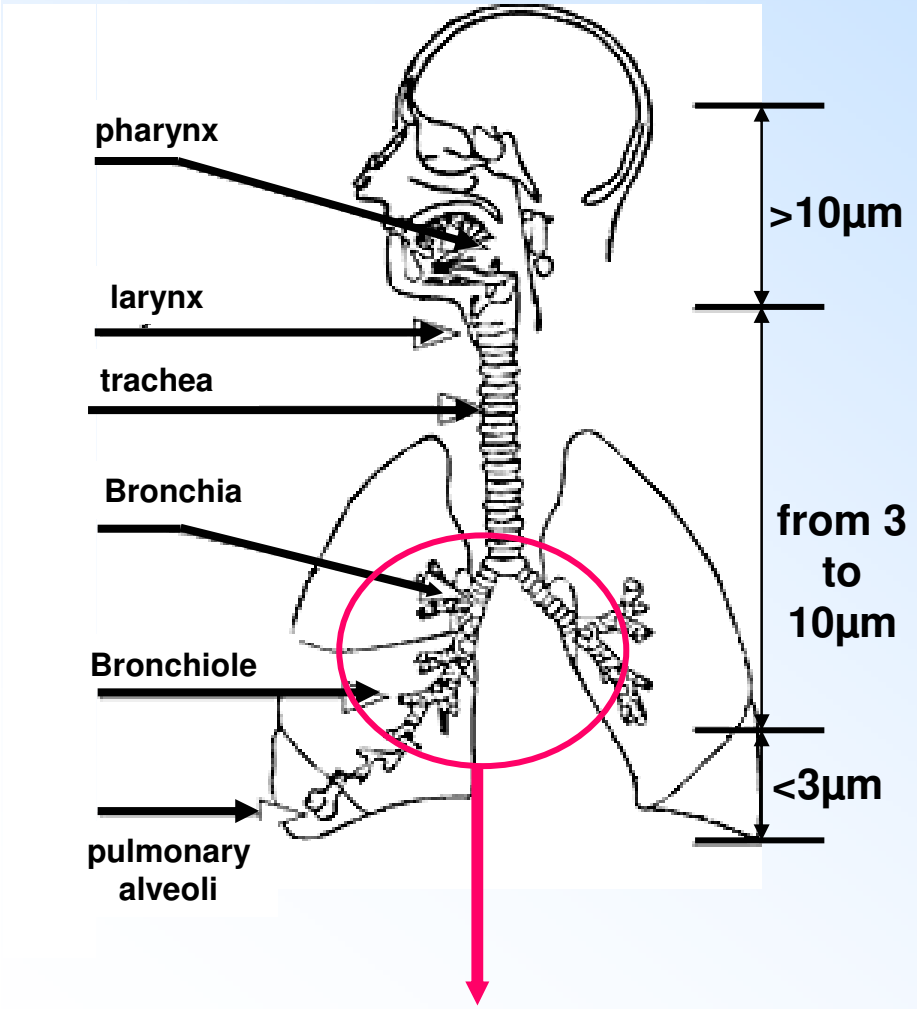
Blanchet et al, *AJRCMB, 2004*
Rumelhard et al, *Tox Lett., 2007*
Rumelhard et al., *Eur Respir J, 2007*

Aim of the study

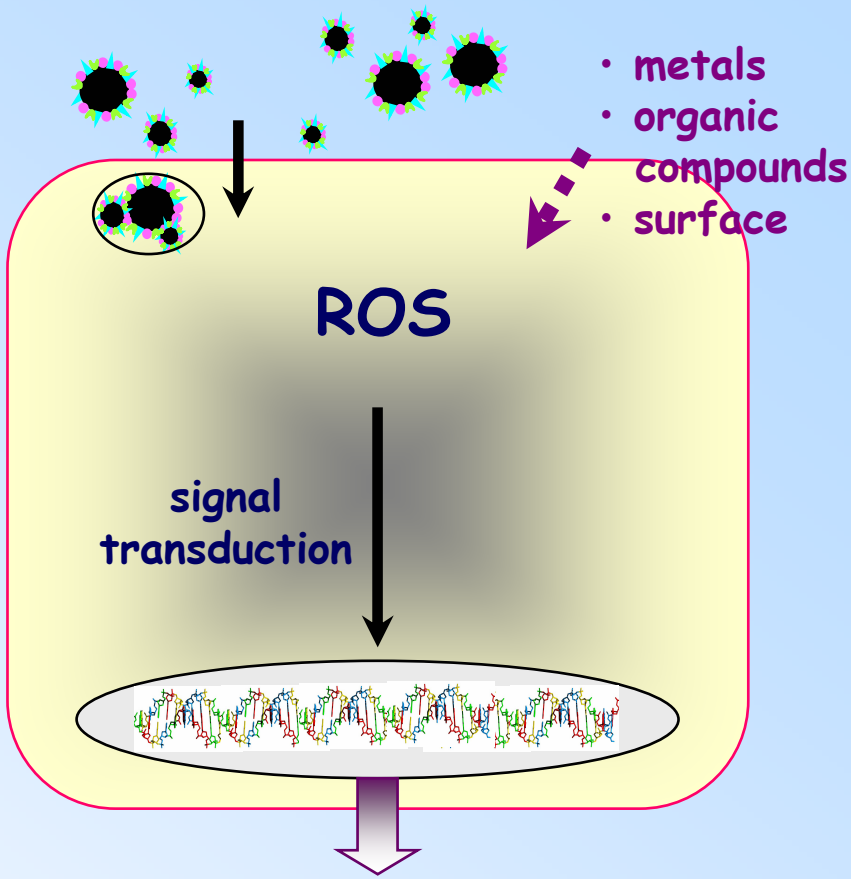
Which size-fraction
from the background urban aerosol
has the most important short term biological effect ?

[10-2.5μm]	Coarse PM
[2.5-1μm]	Intermodal PM
[1-0.1μm]	Fine PM
[0.1-0.03μm]	UltraFine PM

Biological endpoint



human bronchial epithelial cell line
16 HBE

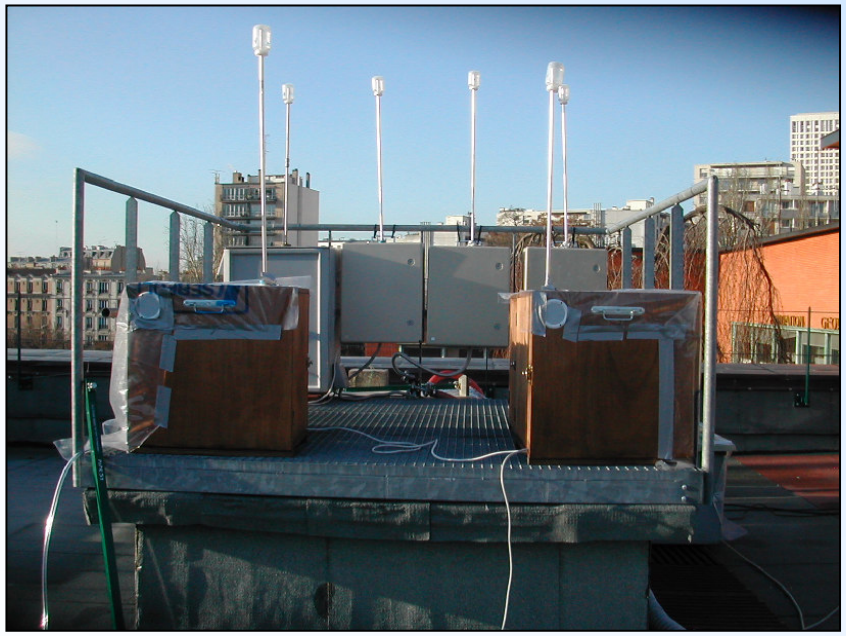


GM-CSF release
pro-inflammatory response

Attraction and survival of inflammatory cells

Particle sampling 11 campaigns (winter/summer)

Roof of LEPI building (Paris 13)



Sampling station

☛ **Background urban pollution (Airparif)**

low pressure Dekati Impactor

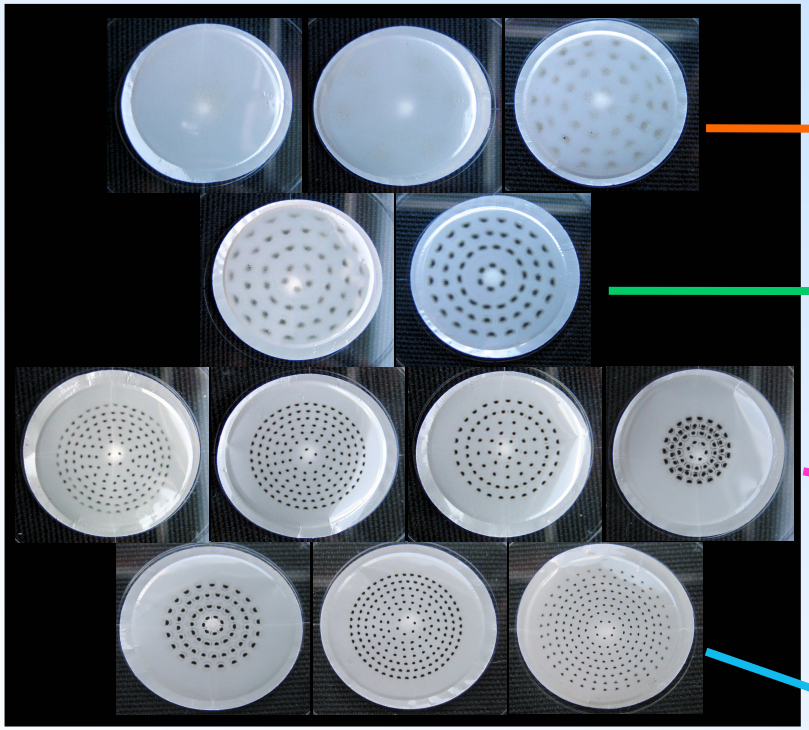
13 stages

$D_{50\,\%}$ (µm)

	≈ 10,5 µm	
PM [10-2,5]	≈ 6,7 µm	coarse fraction between 10 and 2.5 µm
	≈ 4 µm	
	≈ 2,5 µm	
PM [2,5-1]	≈ 1,65 µm	intermodal fraction between 2.5 and 1 µm
	≈ 1 µm	
PM [1-0,1]	≈ 0,65 µm	fine fraction betwen 1 and 0.1 µm
	≈ 0,4 µm	
	≈ 0,25 µm	
	≈ 0,17 µm	
PM 0,1	≈ 0,1 µm	ultrafine fraction < 0.1 µm
	≈ 0,06 µm	
	≈ 0,03 µm	

Particle recovering

12 polycarbonate filters (pore 0.2 µm)
divided in 4 size-fractions



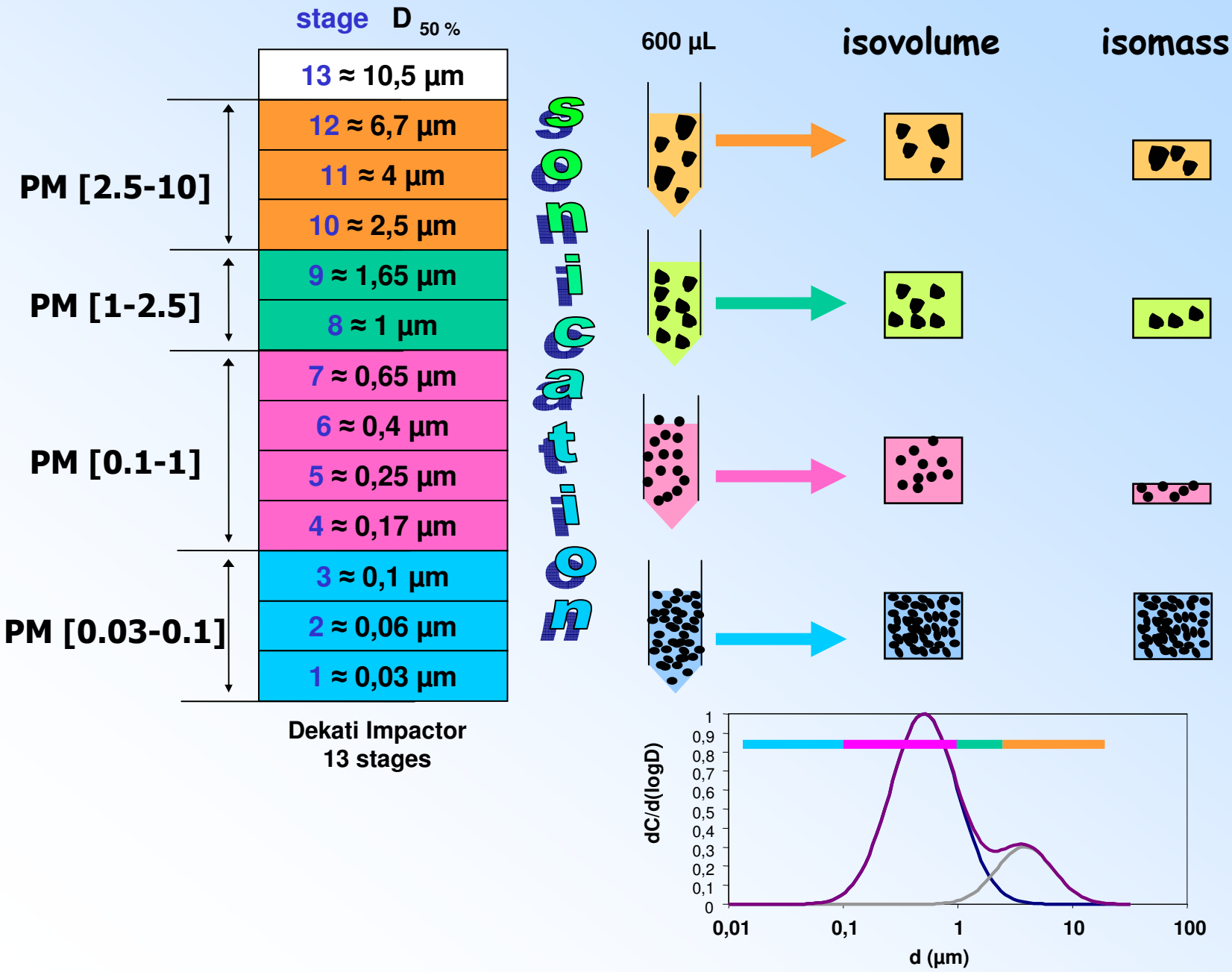
stage	$D_{50\,\%}$	
13	≈ 10,5 µm	
12	≈ 6,7 µm	PM [10-2.5]
11	≈ 4 µm	
10	≈ 2,5 µm	
9	≈ 1,65 µm	PM [2.5-1]
8	≈ 1 µm	
7	≈ 0,65 µm	PM [1-0.1]
6	≈ 0,4 µm	
5	≈ 0,25 µm	
4	≈ 0,17 µm	
3	≈ 0,1 µm	PM [0.1-0.03]
2	≈ 0,06 µm	
1	≈ 0,03 µm	

Dekati Impactor
13 stages

Each filter : 3 sonications of 5sec

Each PM-size fraction in 600 µL culture medium

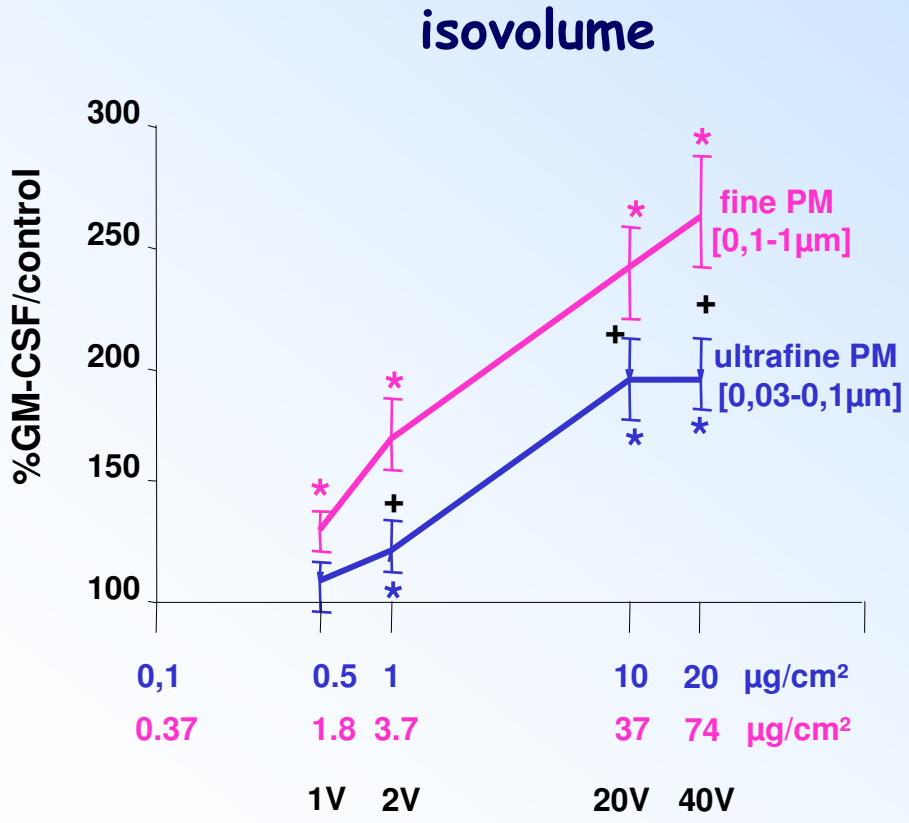
16 HBE cells treatment strategy

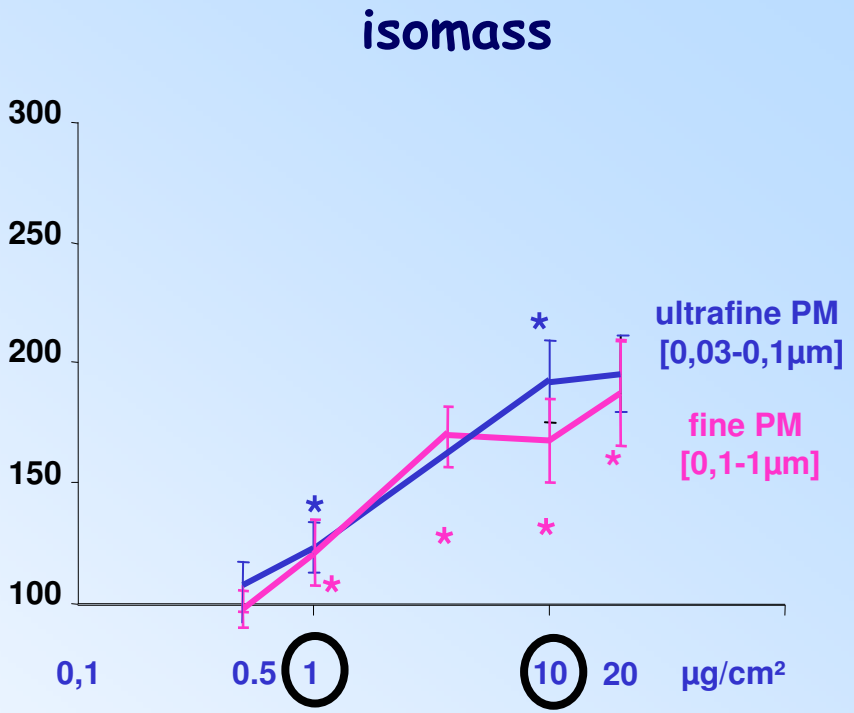


GM-CSF secretion: feasability study

48h particle sampling

Bronchial cell exposure: 24h





* Different from control

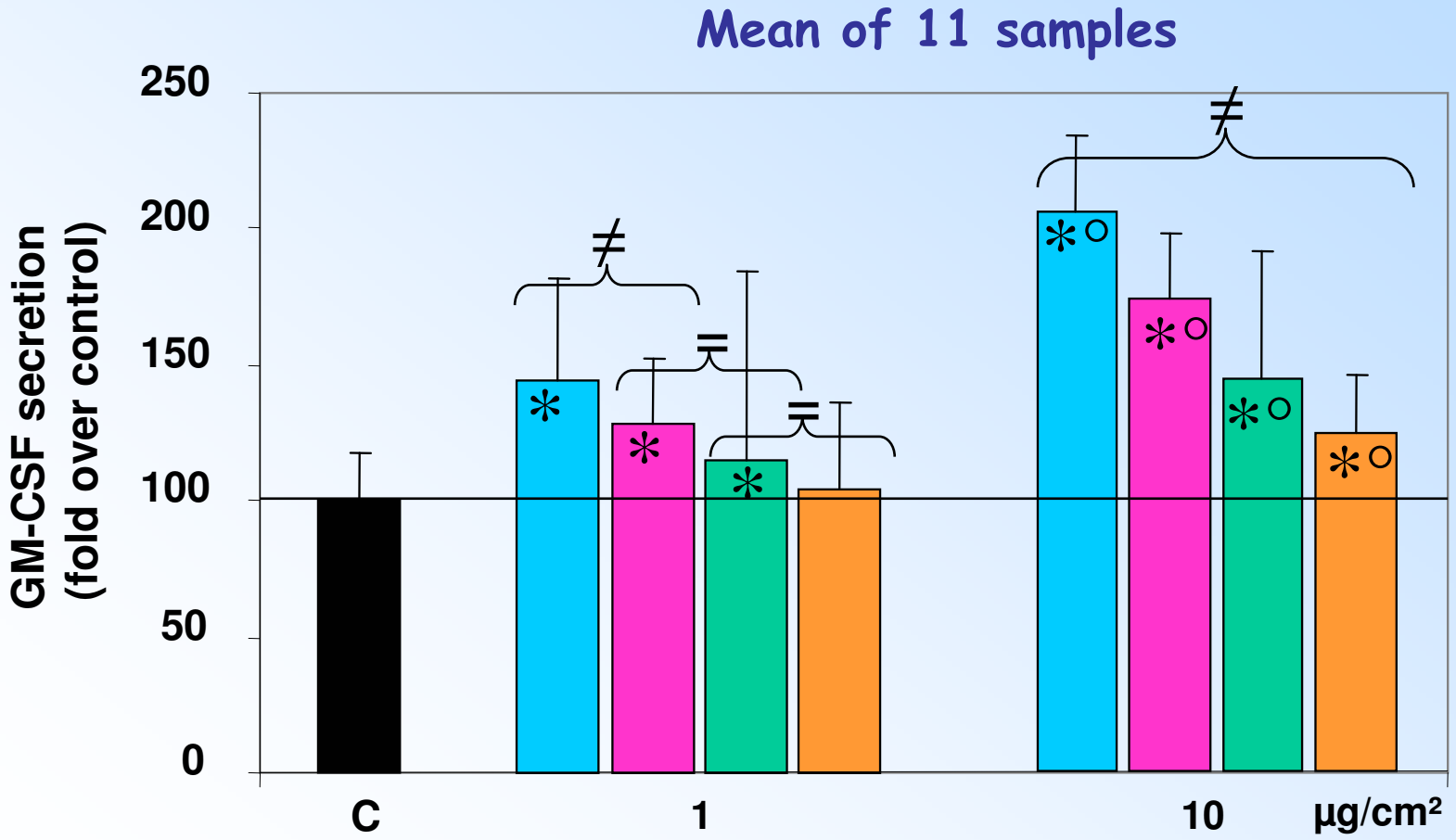
\+ Difference between PUF and PF

Ramgolam et al., *Chemosphere, 2008*

GM-CSF release:

Isomass exposure

ultrafine PM [0,03-0,1µm]
fine PM [0,1-1µm]
intermodal PM [1-2,5µm]
coarse PM [2,5-10µm]



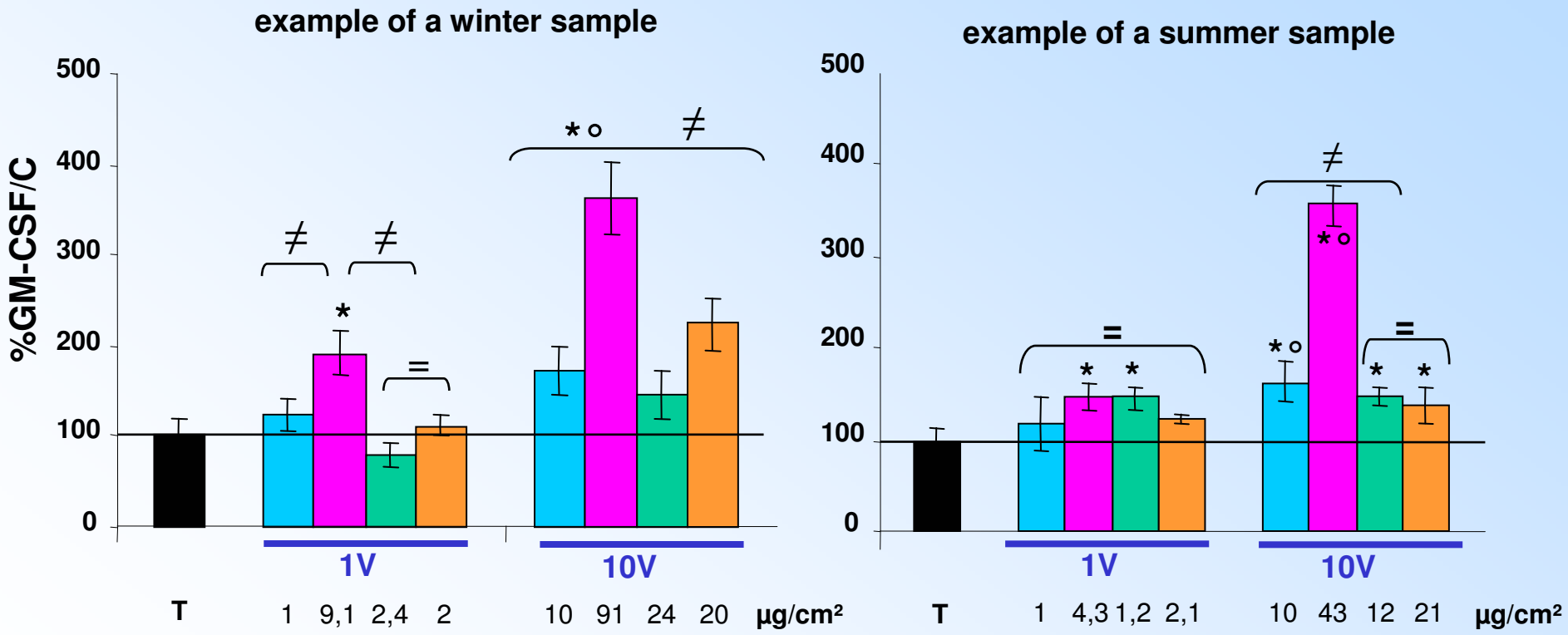
*: different from control °difference between the 2 assessed concentrations

n=60 for each size fraction and 216 for the control

GM-CSF release

Isovolume exposure

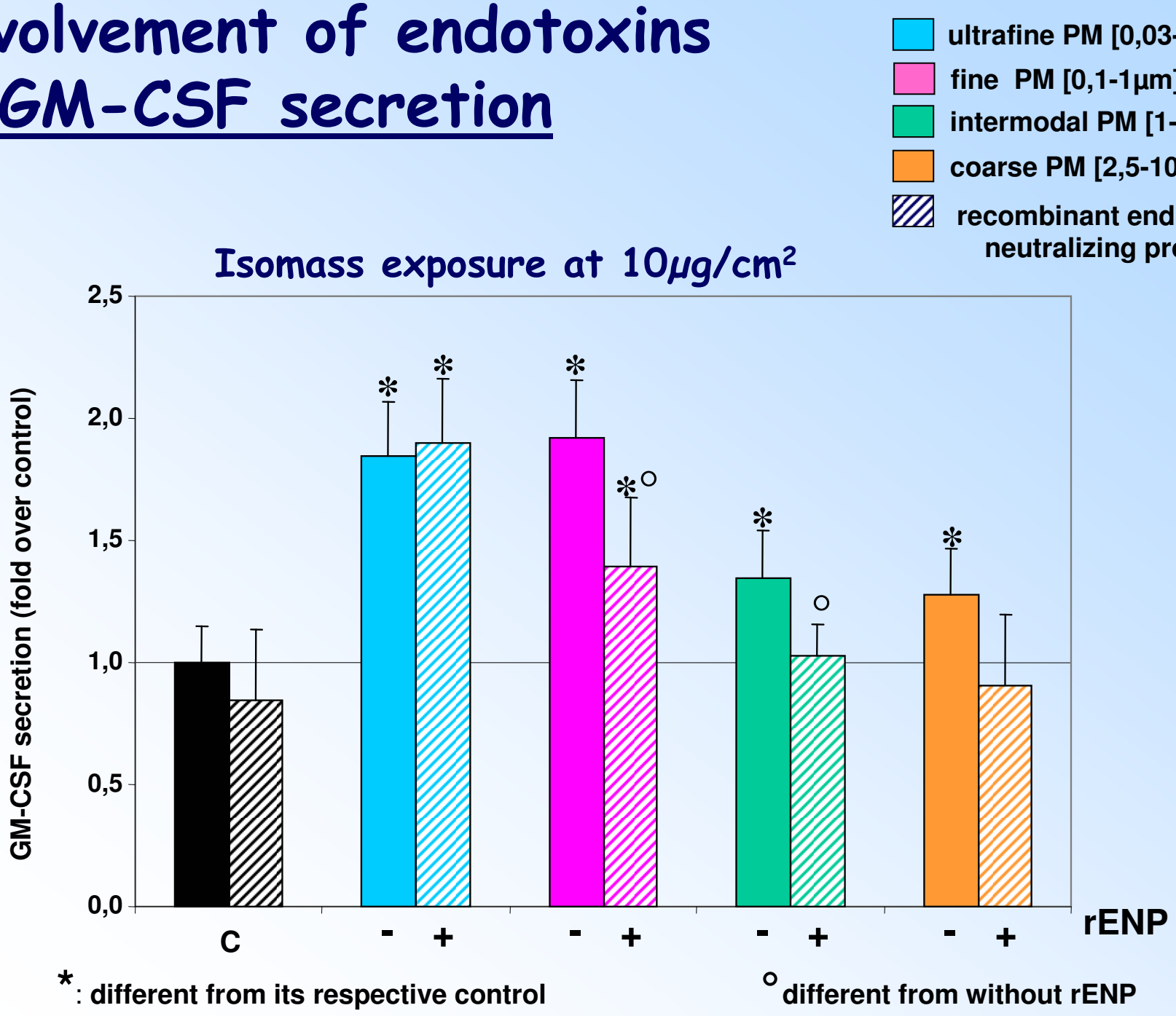
ultrafine PM [0,03-0,1µm]
fine PM [0,1-1µm]
intermodal PM [1-2,5µm]
coarse PM [2,5-10µm]



n=6 for each size fraction and 30 for the control

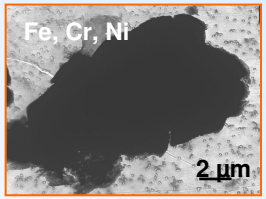
*: different from control °difference between the 2 assessed concentrations

Involvement of endotoxins in GM-CSF secretion



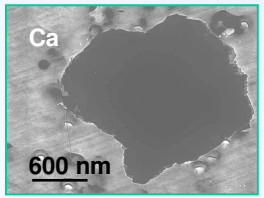
Size-segregated chemical compositions:

Coarse PM [10-2,5]

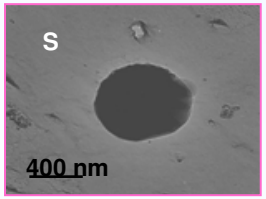


The chemical composition of each fraction have been identified

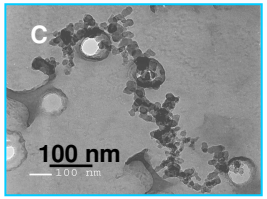
Intermodal PM [2,5-1]



Fine PM [1-0,1]



Ultrafine PM 0,1



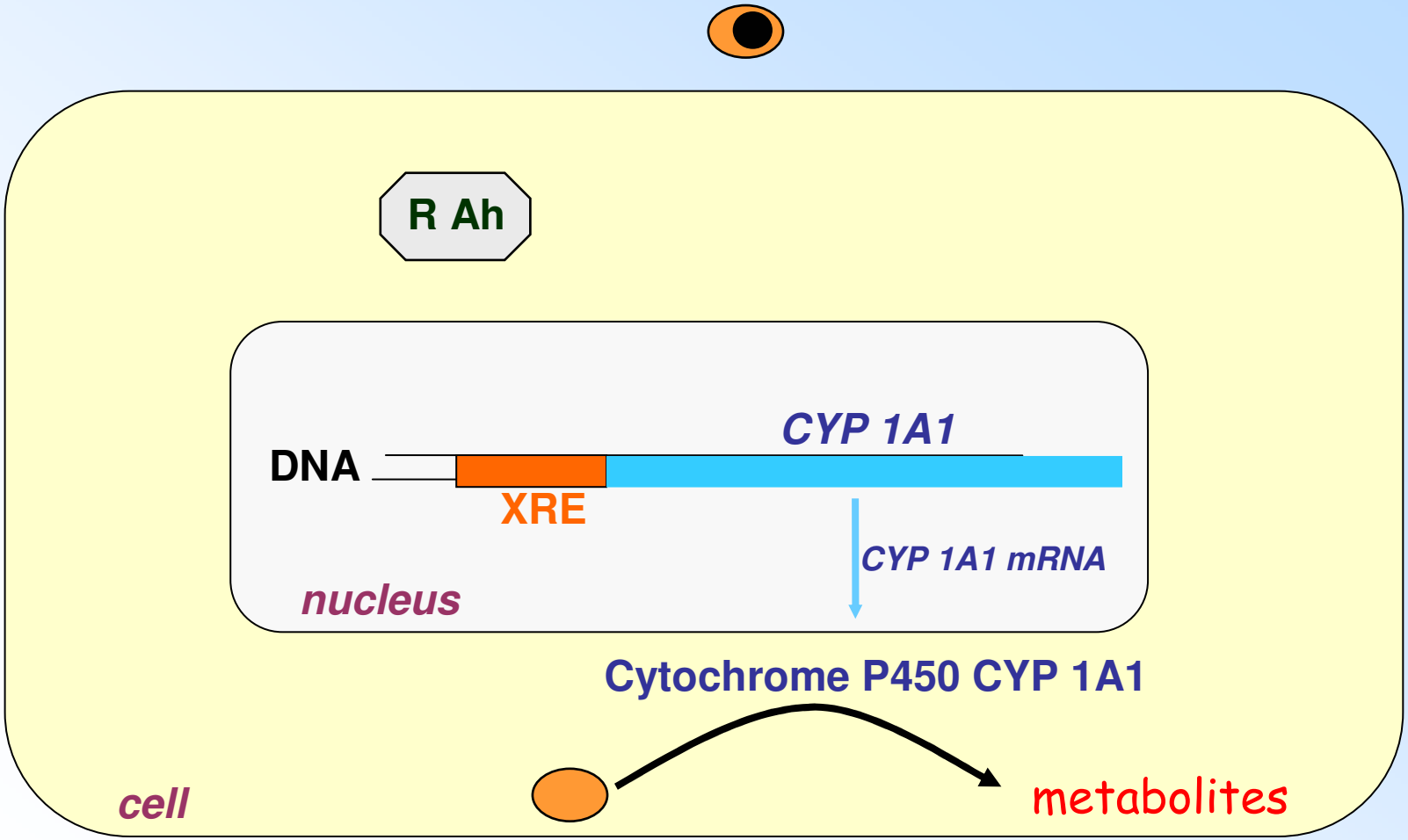
The different fractions exhibit a clear chemical differentiation :

-predominance of dust in coarse and intermodal fractions

-predominance of carbonaceous particles in the finest fractions

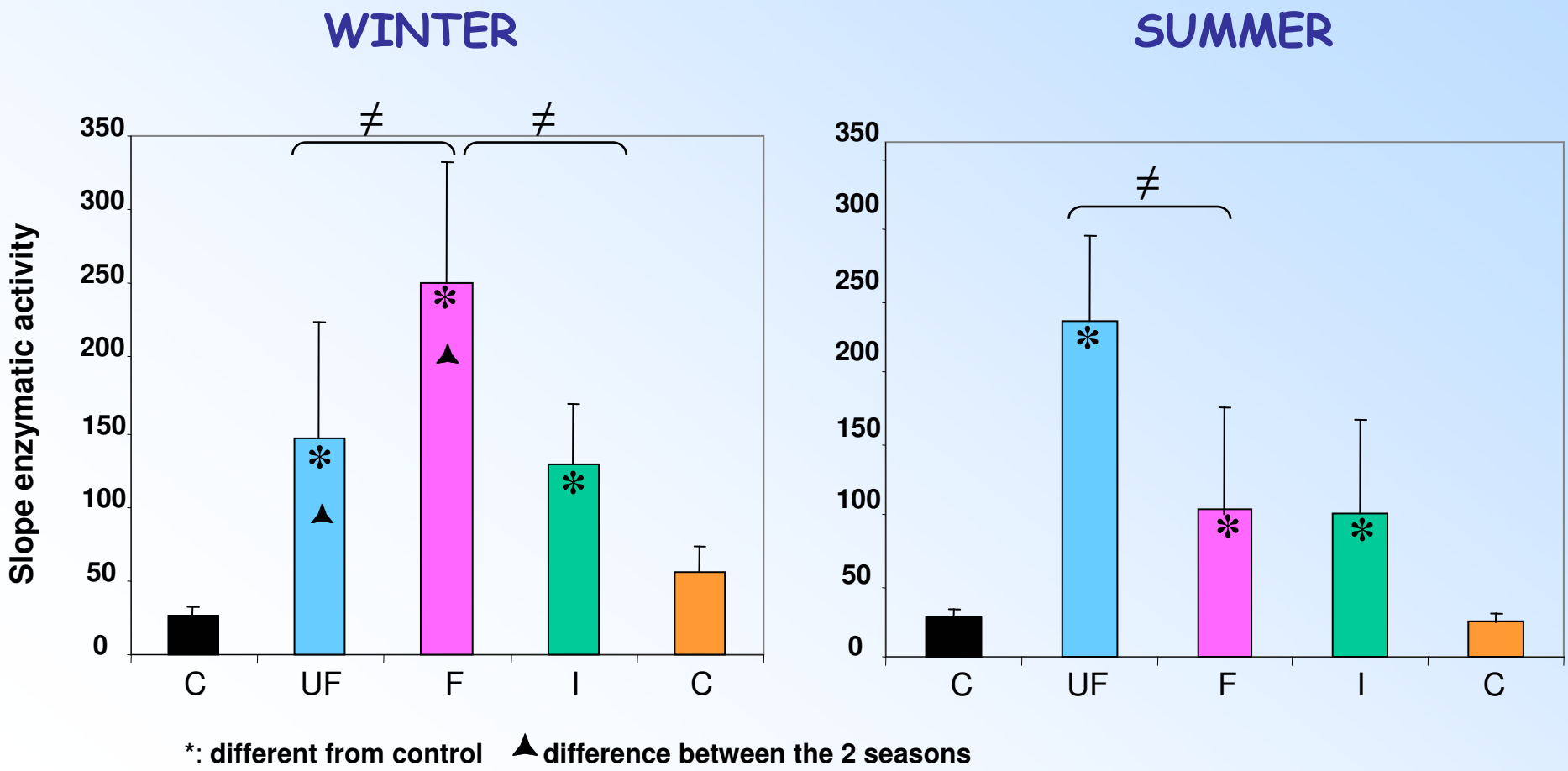
-the ultrafine fraction is enriched in organic compounds

CYP 1A1 induction by PAH: biomarker of PAH bioavailability



Cytochrome P450 1A1 activity: biomarker of PAH bioavailability

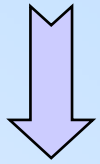
Isomass exposure at 10µg/cm² for 24 h



Conclusion

➔ **Isovolume exposure:**
high contribution of the **PM 1-0.1** fraction

➔ **Isomass exposure**:
high contribution of both the **PM 0.1-0.03**
PM 1-0.1 fraction



Organic compounds

➔ The **PM 0.1-0.03** -induced GM-CSF secretion is not driven by endotoxins

➔ In Paris **PM 1** better describes background urban pollution

PUFFIN

Particules
Ultra**F**ines
et **FIN**es

Primequal action funded by

MEDAD: Ministery of Ecology and of Sustainable Development

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Laboratoire Interuniversitaire des Systèmes Atmosphériques
Metals, ATEM

Laboratoire d'Étude des Particules Inhalées
sampling

Laboratoire d'Hygiène de la Ville de Paris
PAH

Laboratoire des Sciences du Climat et de l'Environnement, CEA/CNRS
C, ions, mass

PUFFIN

Particules
Ultra**F**ines
et **FIN**es

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