



UNIVERSITY PARIS 7 – DENIS DIDEROT

**BIOLOGICAL EFFECTS
OF FINE PM in PARIS**

Francelyne Marano(LCTC)

Epidemiological studies suggest that particulate matter is implicated in:

➤ Increased mortality

- Cardiopulmonary mortality**
- Lung cancer**

➤ Increased morbidity

- Hospital admissions for asthma and cardiovascular diseases**
- Respiratory symptoms**

➤ **Asthma ↗**

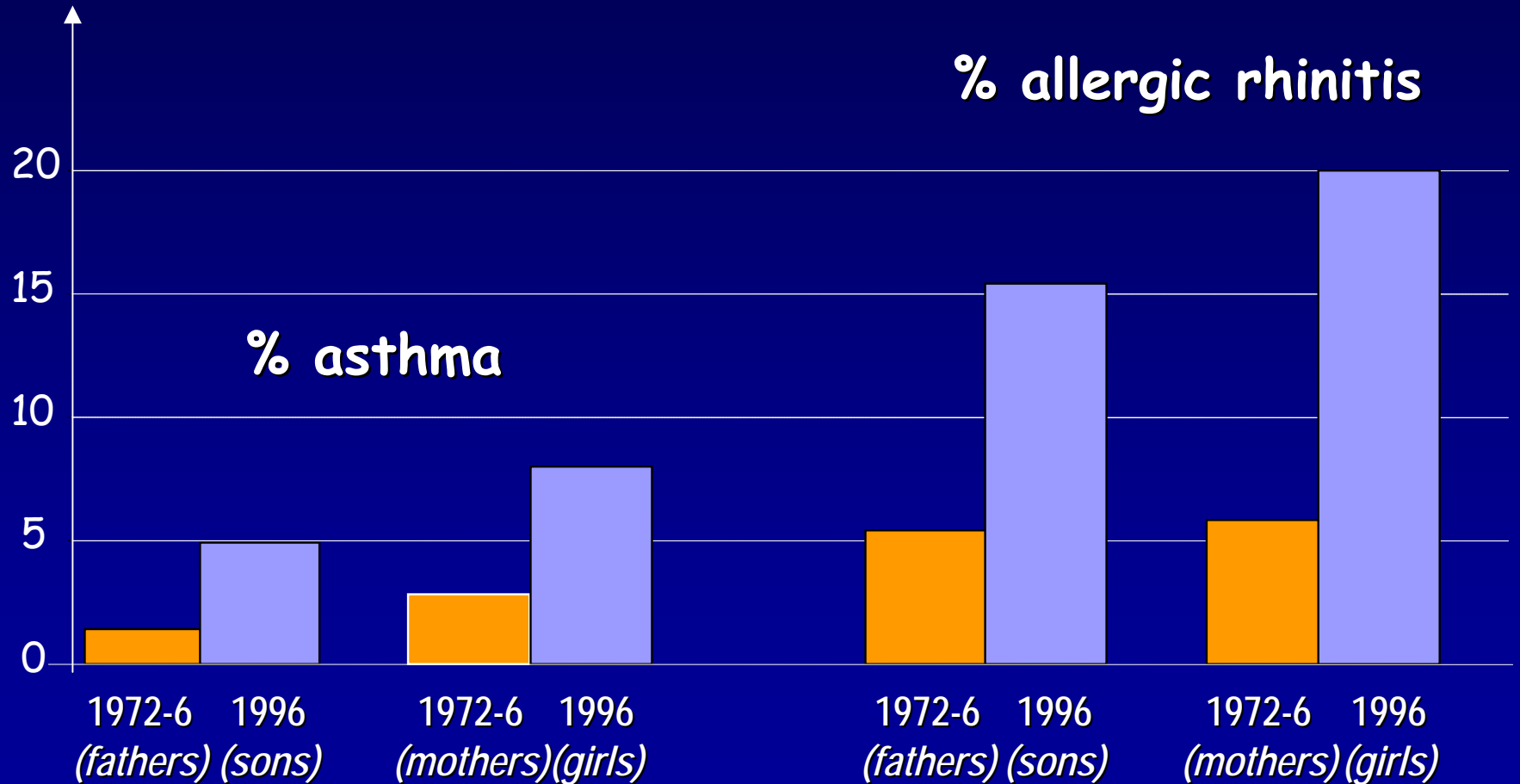
- **Children: 10 %**
Adults: 5.8 %

➤ **Chronic obstructive pulmonary disease (COPD) ↗**

- **6 Millions in France**

Intergenerational 20 year trends in the prevalence of asthma and hay fever

% Scotland
(45-54 years)



Short-term increases in particulate pollution and respiratory morbidity

➤ current explanations / hypotheses:

inhaled particles cause a pulmonary inflammation with systemic release of cytokines

Short-term increases in particulate pollution and respiratory morbidity

- **experimental toxicology:**
 - **which size of particles?**
 - **which constituents of the particles?**
 - **by which mechanisms?**

Sampling of particles

High volume sampler(PM2.5 head)

Vitry-sur-Seine

Urban background



Porte d'Auteuil

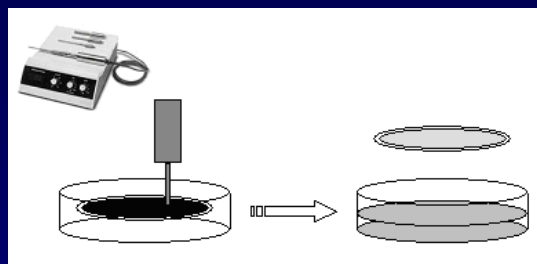
Kerbside station



Particles recovery method



Sampling



**Sonication
in water**



Lyophilization



weighing

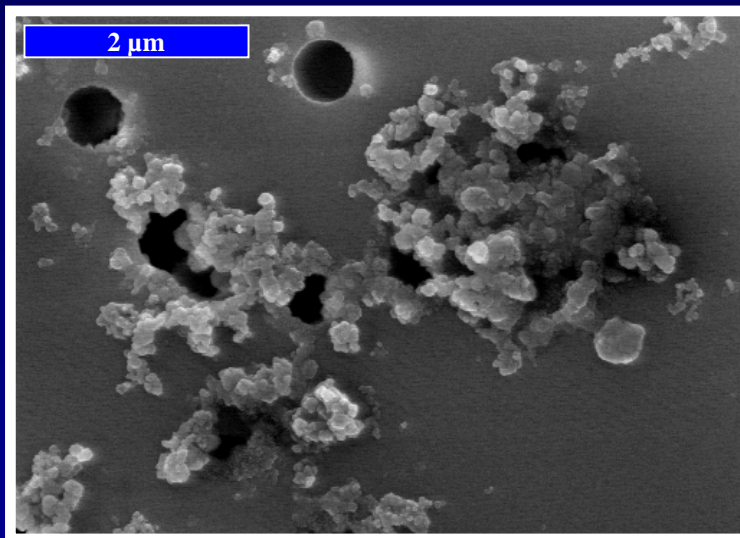


**Sonication
in DPL**

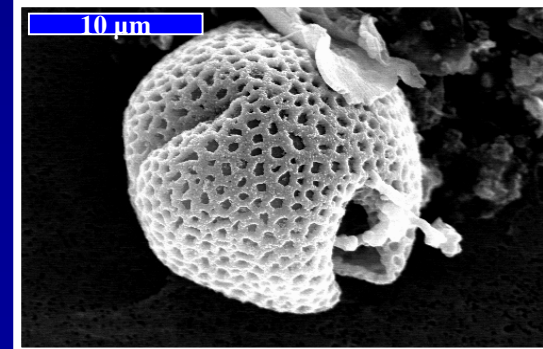
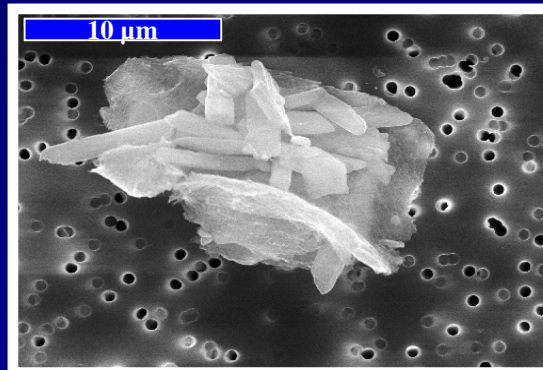
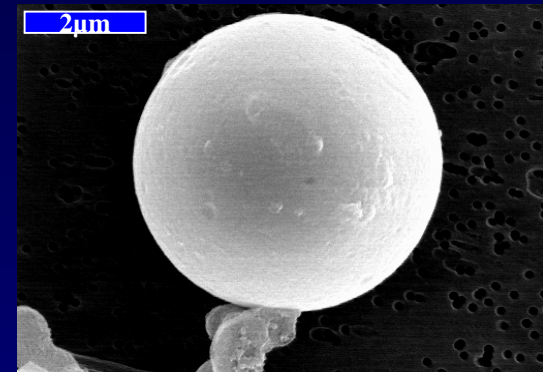
**Stock solution
2 mg/mL**

PM collected in Paris

Porte d'Auteuil Kerbside station

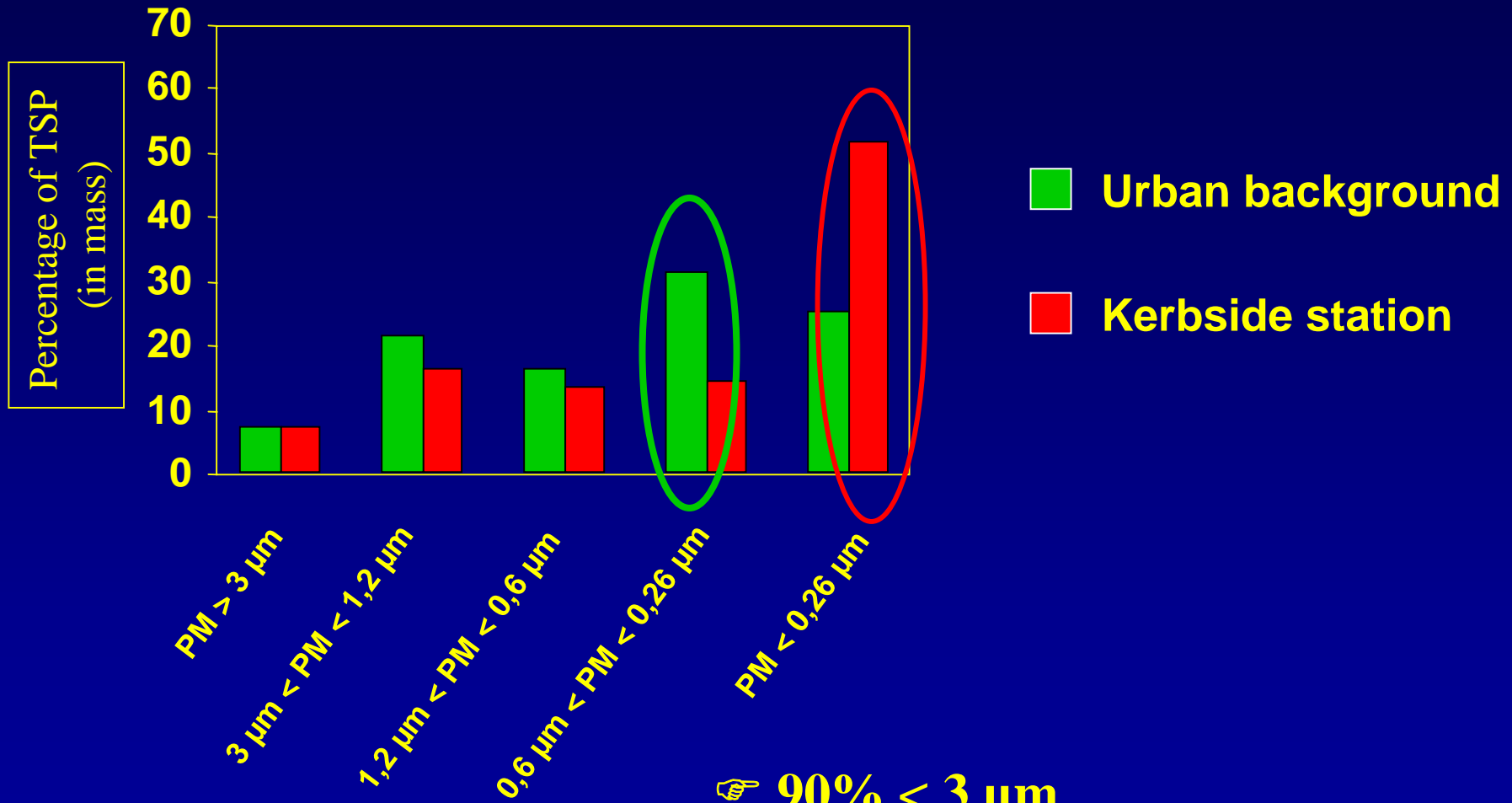


Soot represent about
~80% of PM



Granulometric analysis

Cascade impactor



☞ 90% < 3 μm

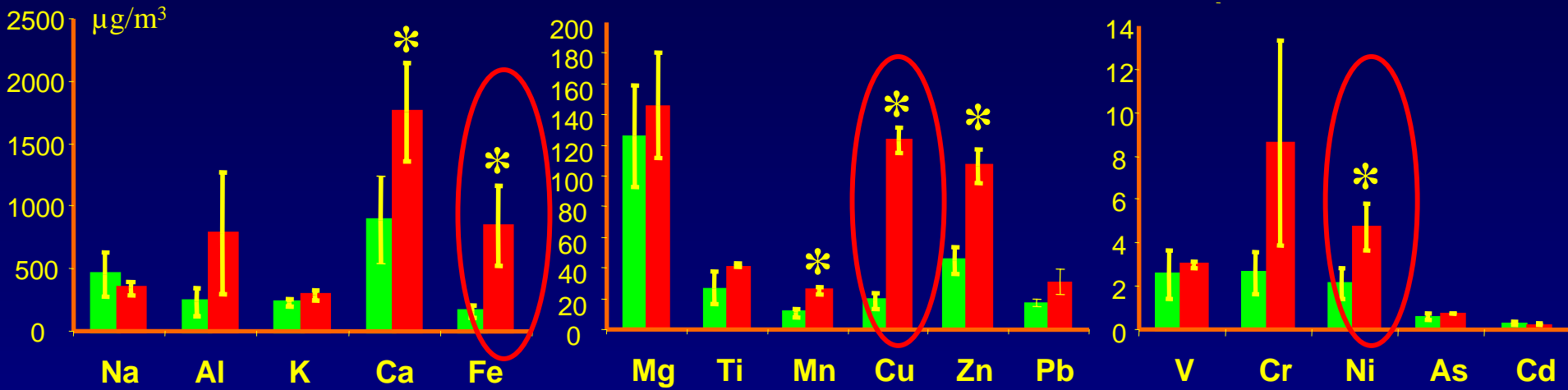
☞ Kerbside \rightarrow 50% (< 0,26 μm)

Chemical analysis

Metals (ICP-MS)

Urban background

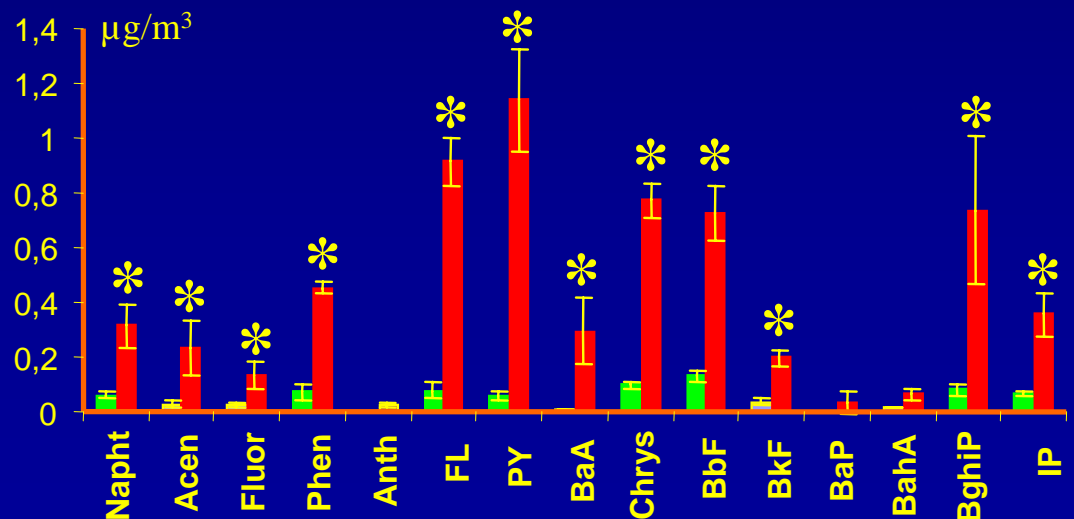
Kerbside station



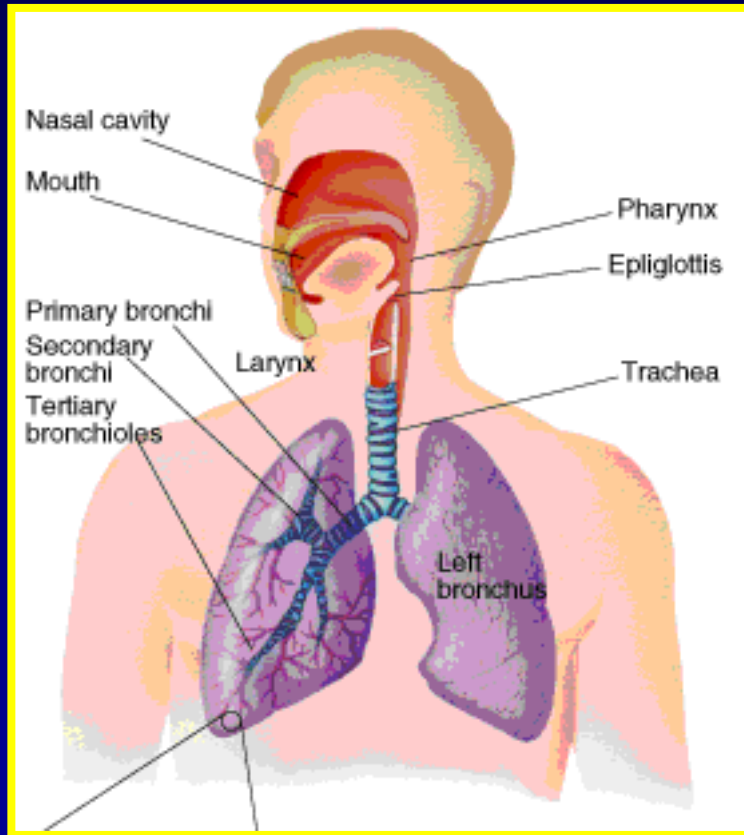
PAHs (HPLC)

Kerbside station
SOF=12%

Urban background
SOF=10%



Particle deposition in lungs



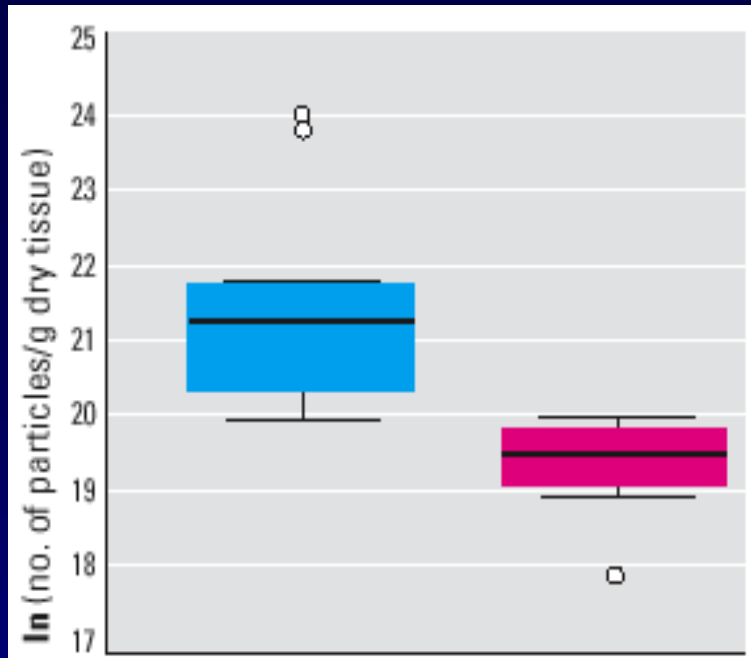
nasopharynx : $> 5\mu\text{m}$

trachea / bronchi : $1 - 5\mu\text{m}$

bronchioles / alveoli : $< 1\mu\text{m}$

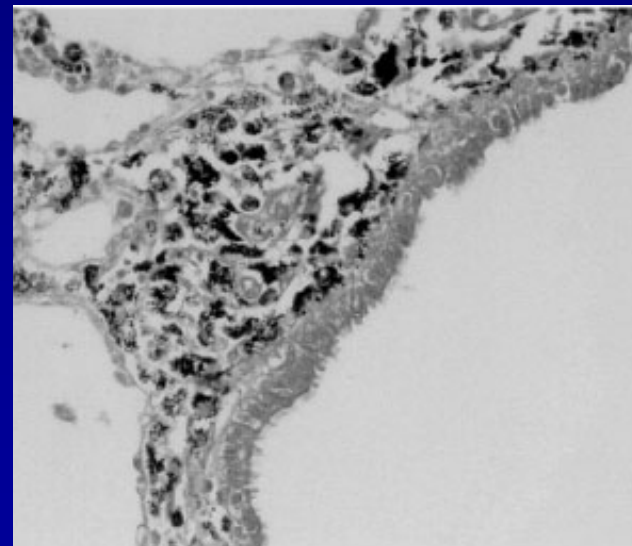
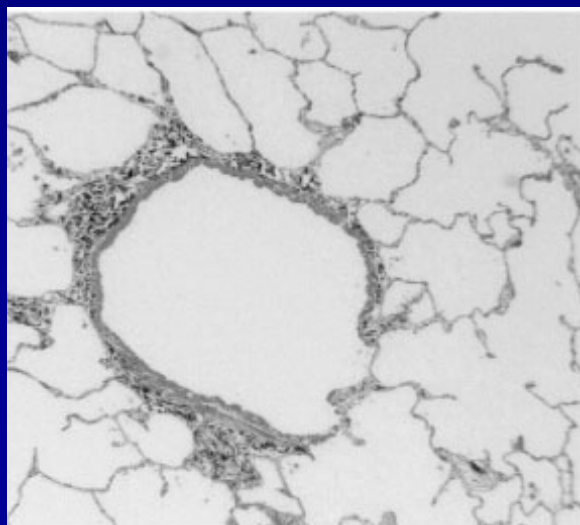
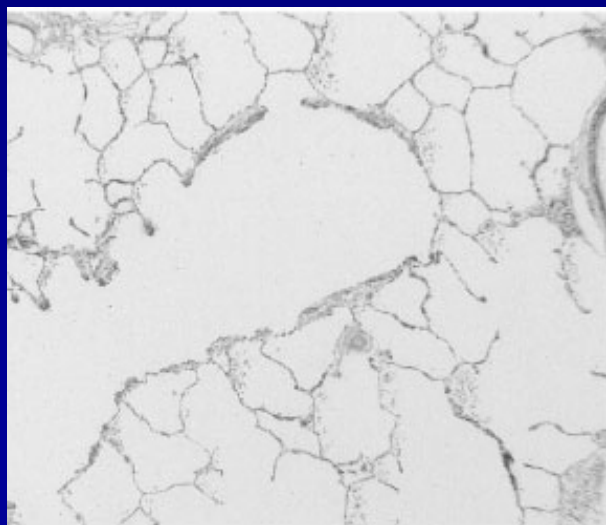
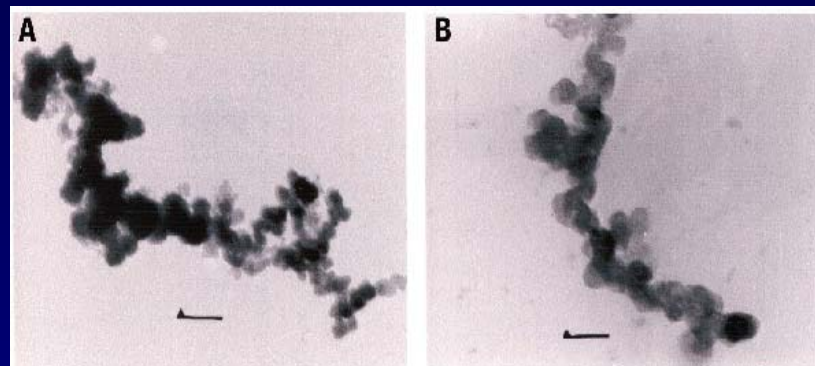
Pulmonary biopersistence of fine particles

(Churg et al. 2003)



Mexico
n=11

Vancouver
n=11



What biological effects of these particles in the lung?

Controlled human exposure to DEP

- Increase in number of inflammatory cells
- Increase of cytokine mRNA in nasal lavages
(*Diaz-Sanchez et al. 1997, Salvi et al. 1999*)

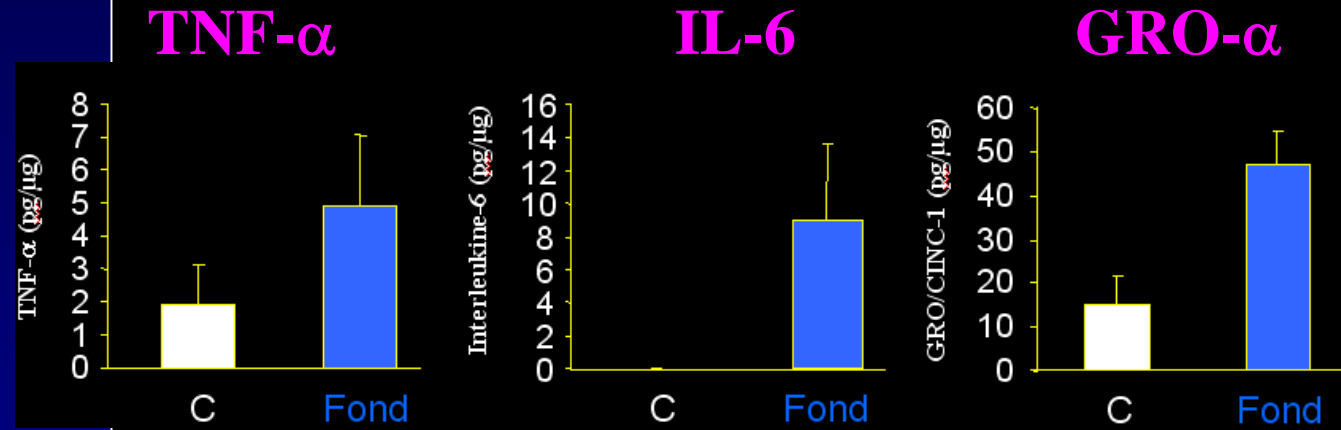
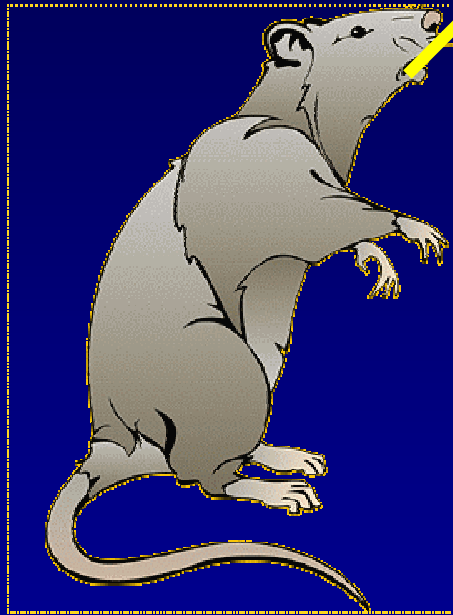
In vitro exposure

- Increase in secretion of proinflammatory cytokines by macrophages and airway epithelial cells
(*Bayram et al. 1998, Boland et al. 1999, Fahy et al. 2000*)

PM Effects?

in vivo Tests

Intratracheal injection of PM



After 24 h, broncho-alvéolar lavage and measure of inflammatory cells and cytokines

Mechanistic studies with PM

- To determine if the respiratory epithelial cells play a role in the inflammatory response.
- To understand the cellular and molecular mechanisms involved in this response.
- To evaluate the relative importance of the organic and aqueous fractions of PM in the response.

INHALED AIR

Particles

Allergens

**bronchial
epithelium**



**INFLAMMATORY
RESPONSE**



CYTOKINES

GM-CSF

immune cell activation



ALLERGIC RESPONSE - IgE PRODUCTION

The particules for biological studies on human bronchial cells in culture

- *Atmospheric Particules :*

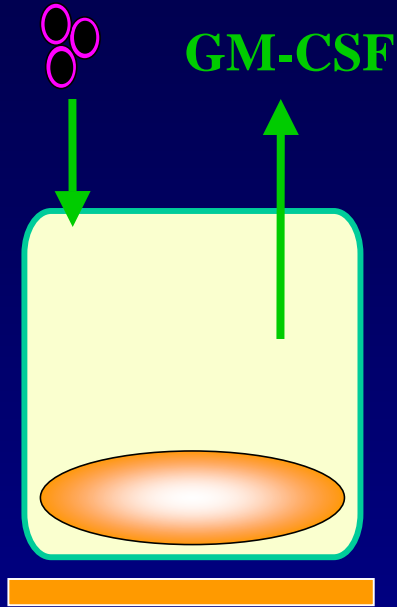
PM_{2,5} sampled in Paris

- *Diesel Particules :*

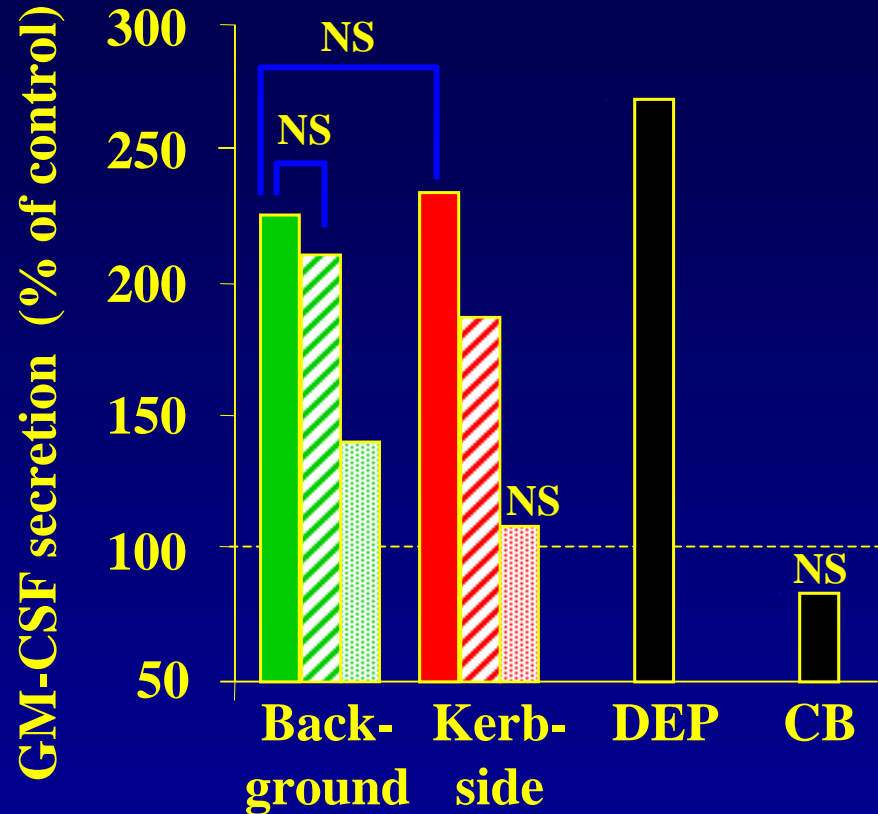
SRM 1650a (NIST)

Organic and aqueous extracts

Secretion of GM-CSF



Treatment
10 $\mu\text{g}/\text{cm}^2$ – 24h



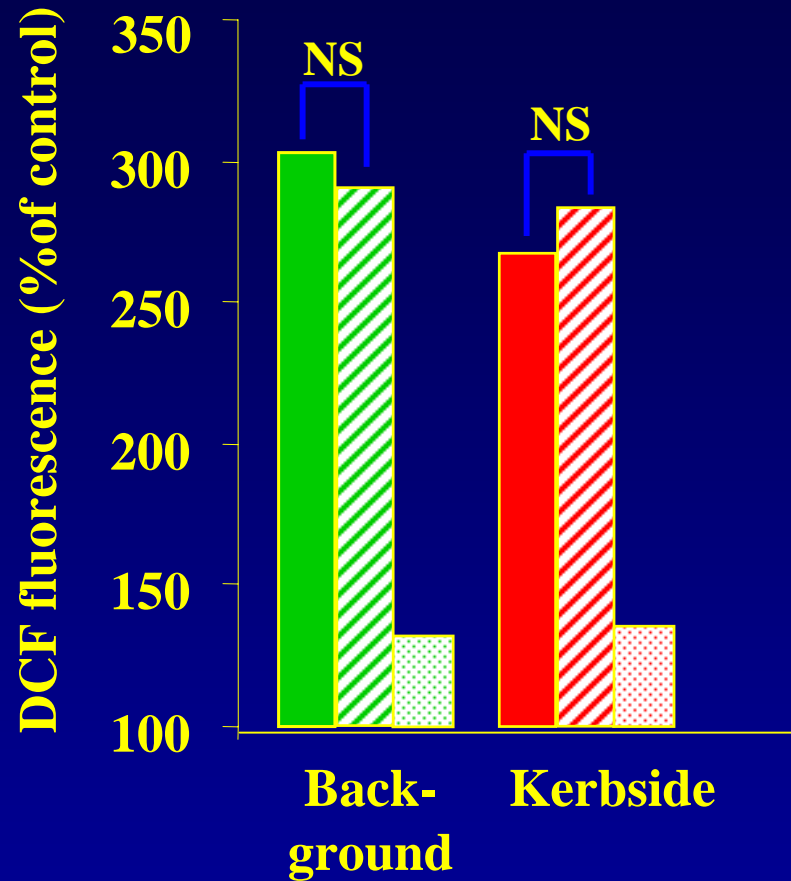
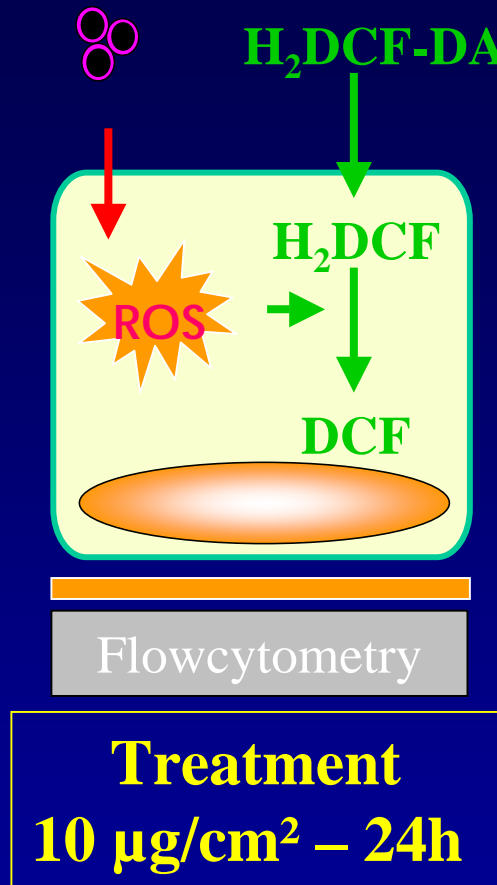
NS : not statistically different

particles

Organic extract

Aqueous extract

Measure of cellular ROS

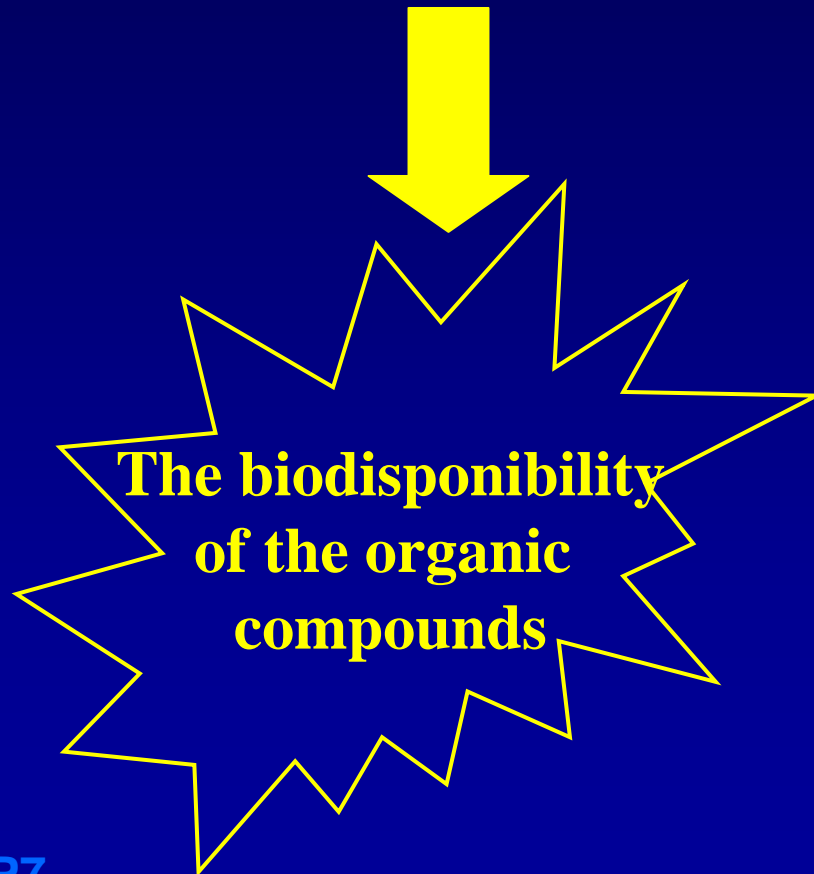


■ Particles

▨ Organic extract

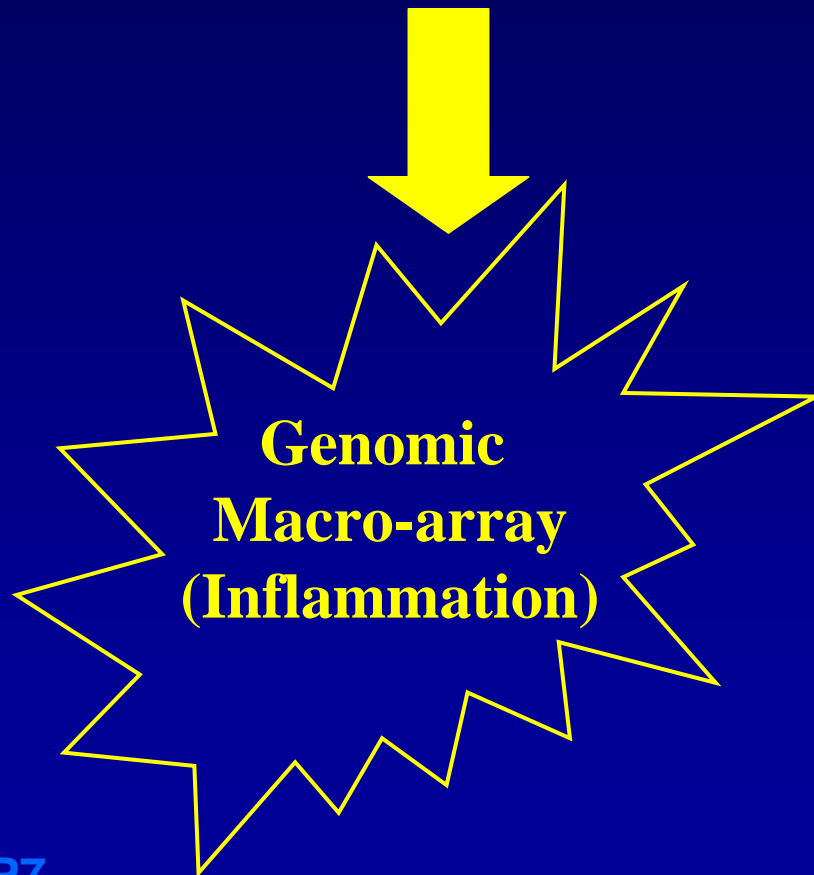
▤ Aqueous extract

- **Importance of organic compounds for the production of ROS and GM-CSF**
- **What is the origin of oxidative stress ?**



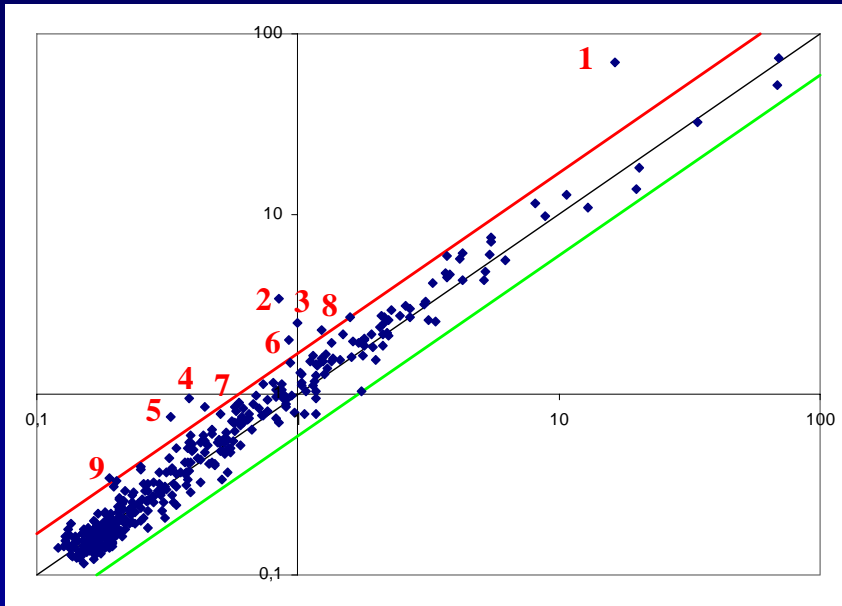
METABOLISATION

- **Investigate the mechanism of action of particles**
- **Establishment of new biomarkers**



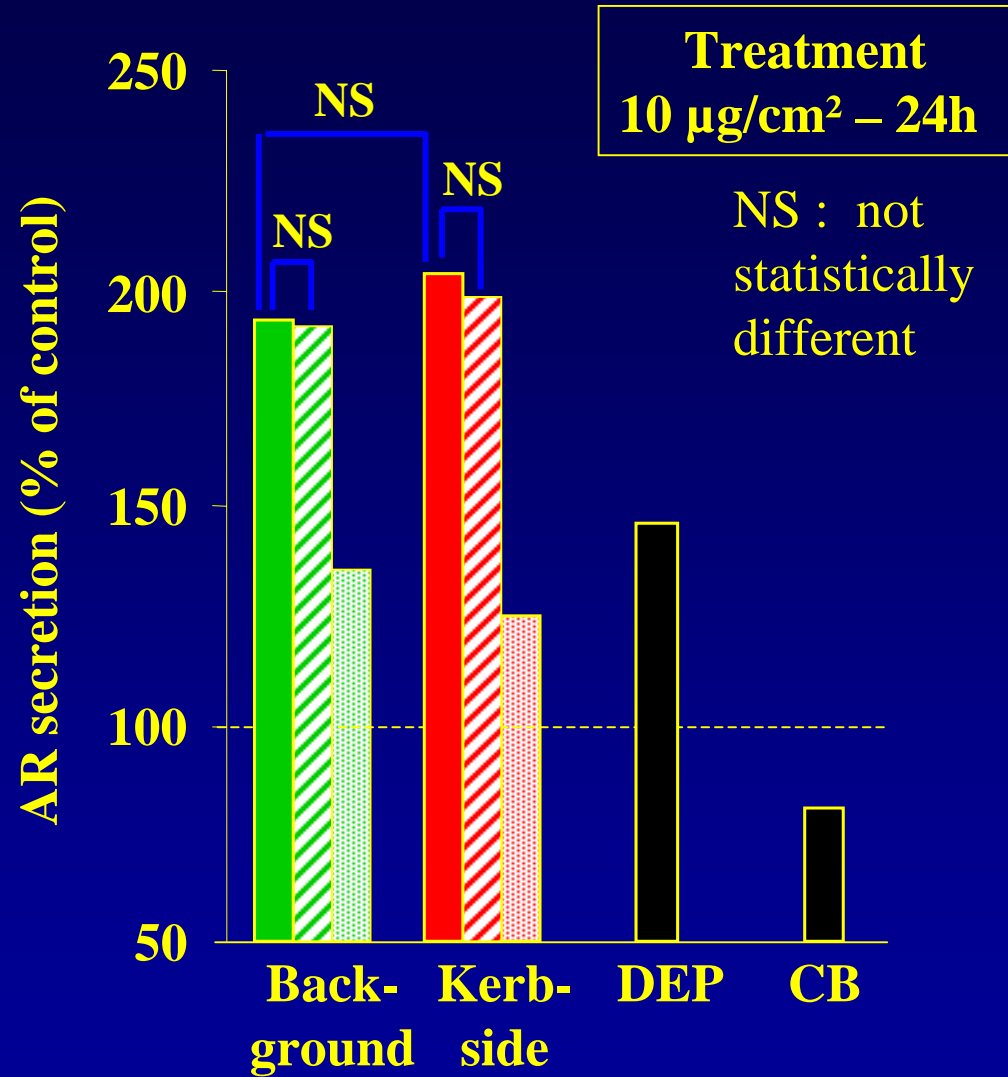
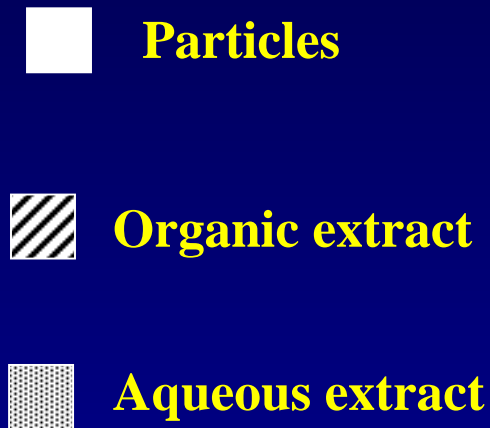
Macro-Array

Urban background



$I_{PM}/I_{vehicle}$	Expression		Genes
	Vehicle	PM	
4,28	16.4	70.1	Amphiregulin
3,97	0.8	3.4	IL-1 α
2,54	1.0	2.5	Integrin A2
2,49	0.4	0.9	G-CSF
2,30	0.3	0.7	IL-1 β
2,19	0.9	2.0	Betacellulin
1,94	0.4	0.8	VCAM-1
1,86	1.2	2.3	TNF-R1
1,84	0.2	0.3	IL-8
0,59	1.7	1.0	TIMP-2

Amphiregulin (AR)



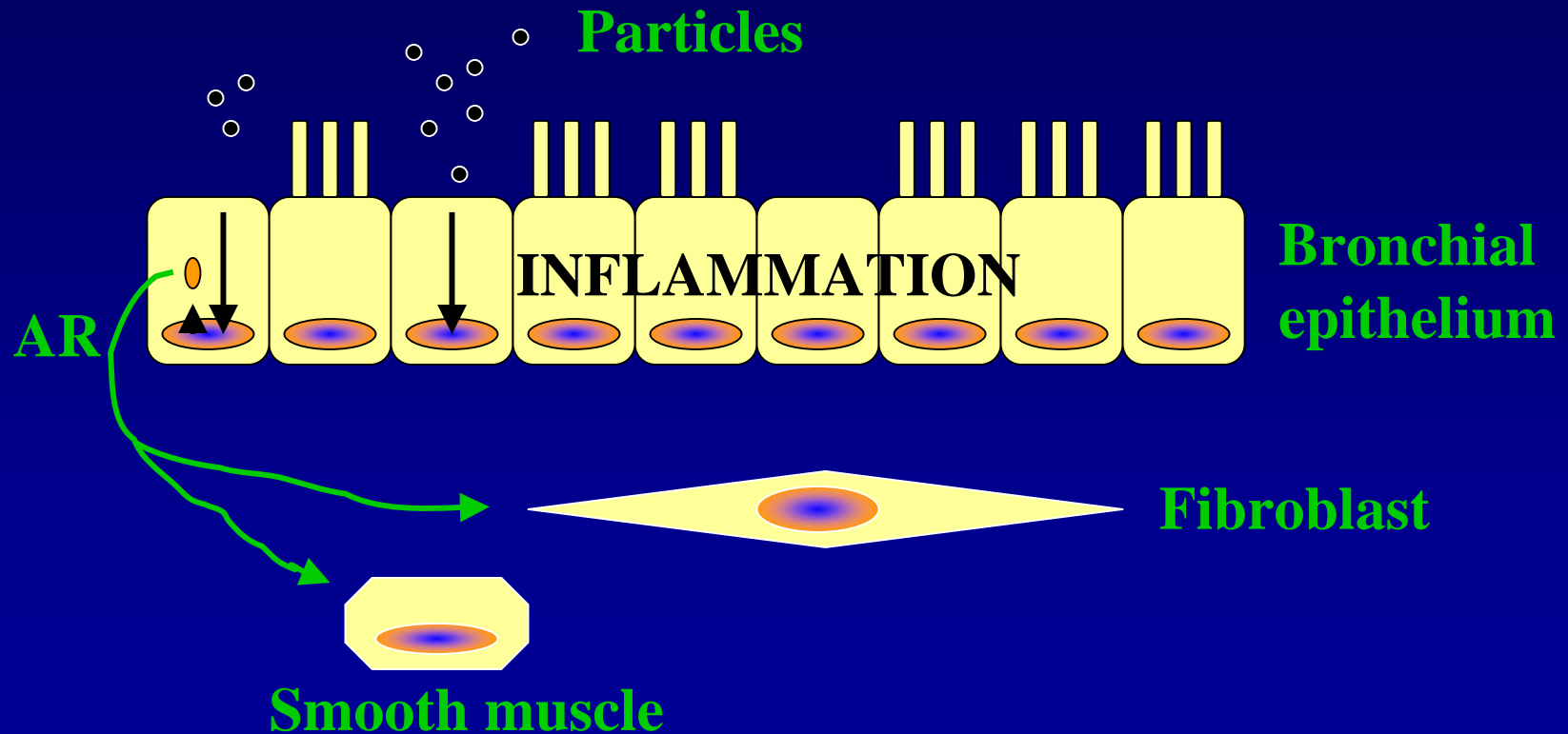
Conclusions (1)

- **Physico-chemical differences between $PM_{2,5}$ depending on their sampling site**
- **Particles → pro-inflammatory response (GM-CSF, ROS)**
- **Importance of organic compounds: Quantity of SOF more important than composition in PAH**
- **No difference in biological effects between $PM_{2,5}$ in spite of chemical differences**

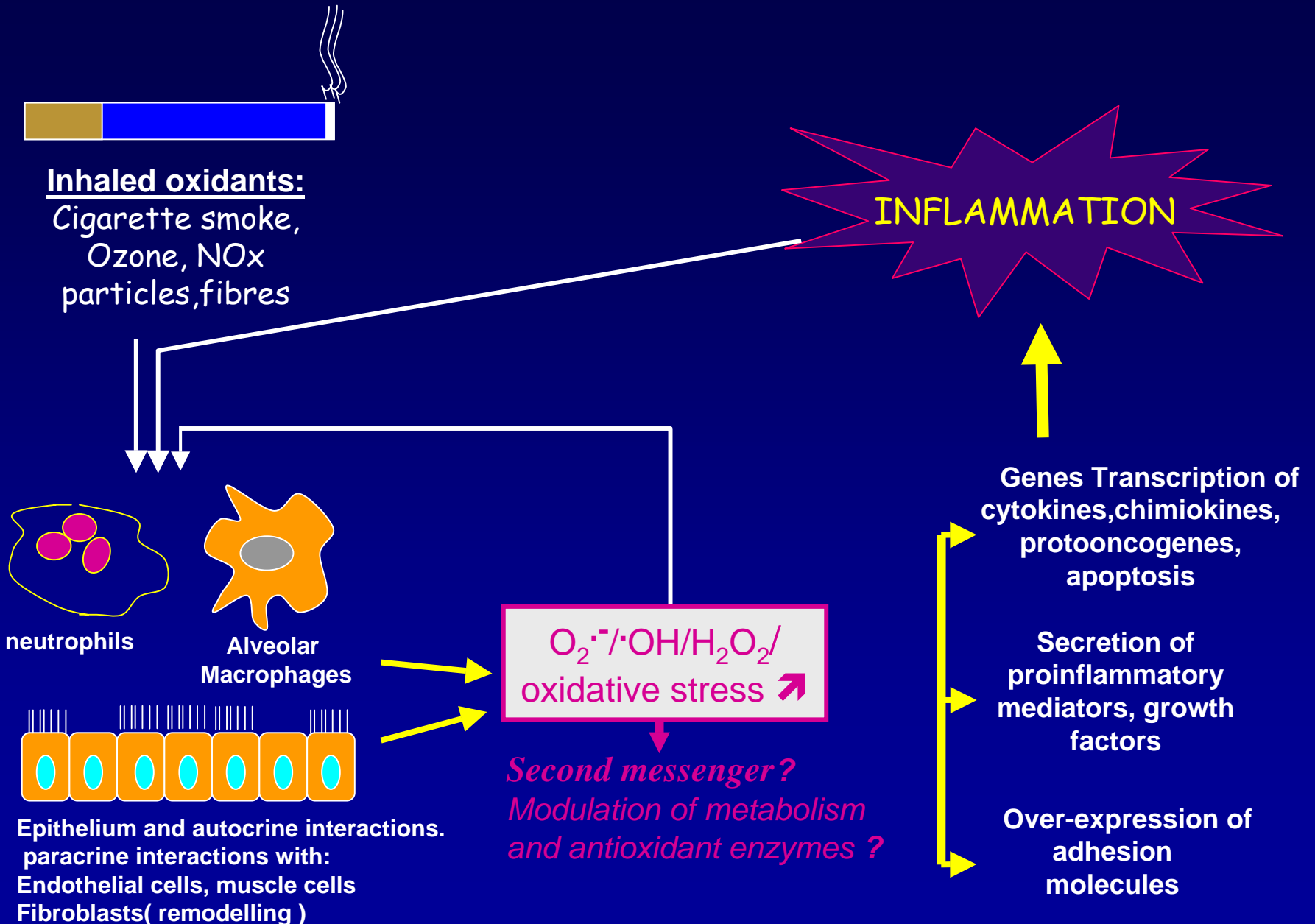
**Biological effects : different organic compounds + metals
→ INTERACTIONS**

Conclusions (2)

- Identification of a new biomarker :Amphireguline (AR)
- AR → role in bronchial remodelling



Interaction of pollutants





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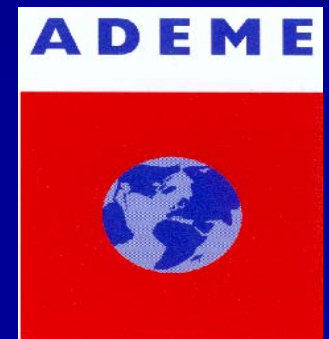
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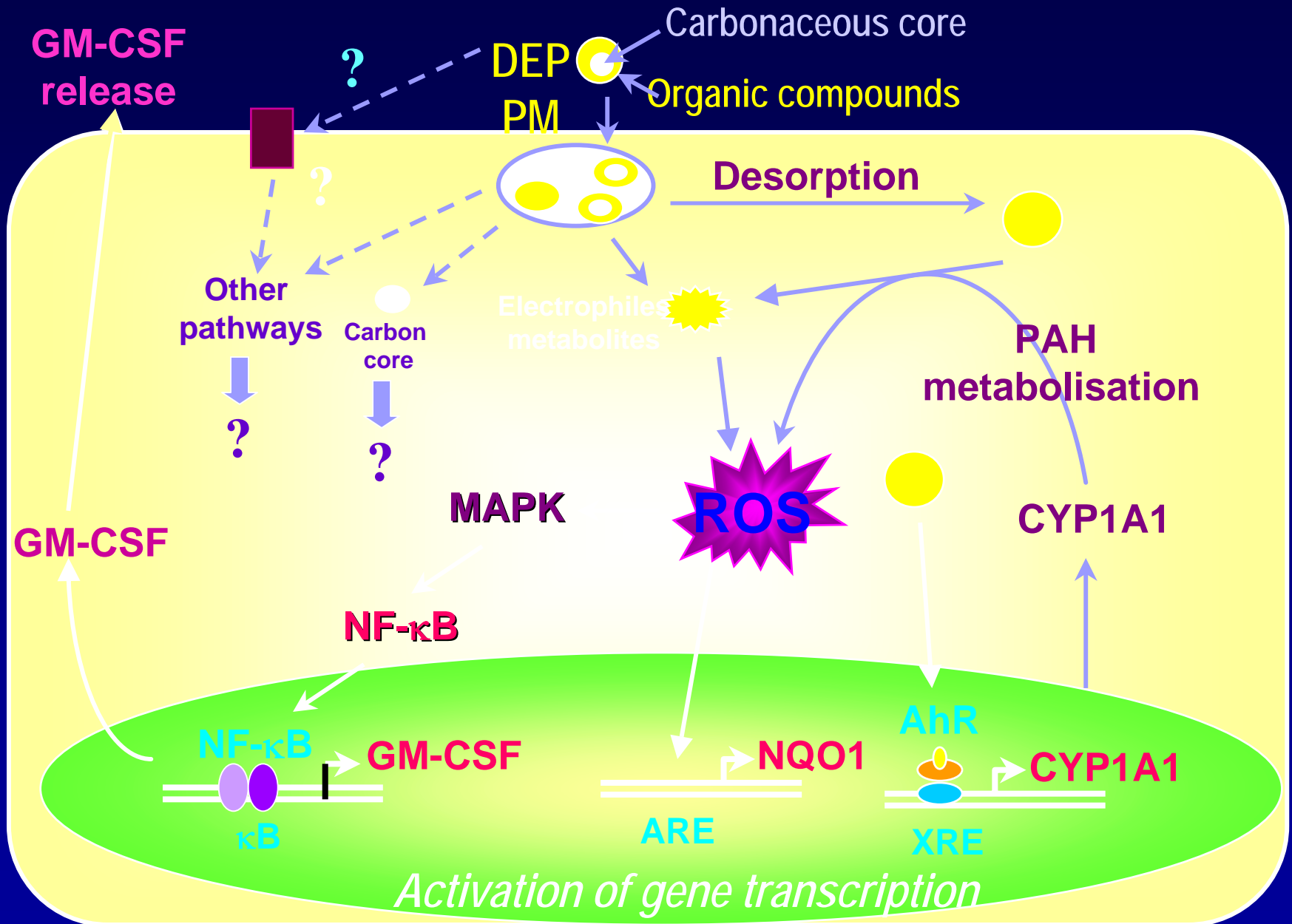
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PRIMEQUAL



DEP and PM-mechanisms of action



Puce à ADN

