

# Oxidative properties of particles and biological effects

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## Hypothesis:

Oxidant activity of particles is a unifying factor in their ability to cause pulmonary (and systemic) effects

# Sources of ROS/RNS from particle inhalation

## **ACELLULAR**

**Particle Characteristics:**

**Shape, Size, solubility,**

**Carrier function (LPS, PAH)**

**Surface chemistry and activity**

## **CELLULAR**

**Induction of ROS/RNS by target cells (primary)**

**Induction of signaling and/or toxicity causing**

**Lung inflammation and secondary ROS/RNS**

# Particles on today's menu

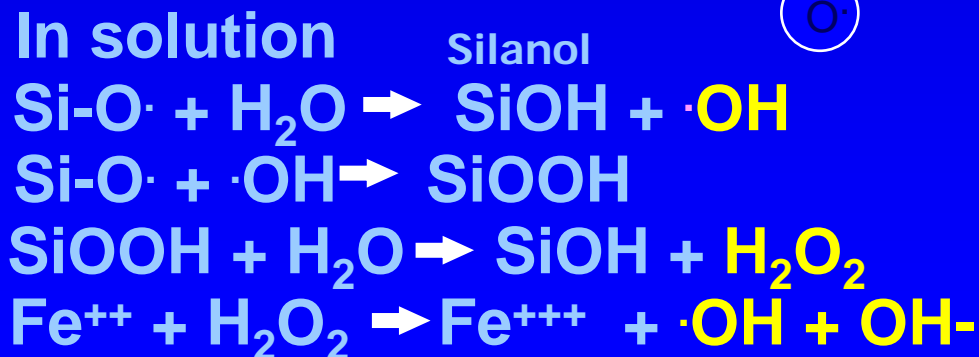
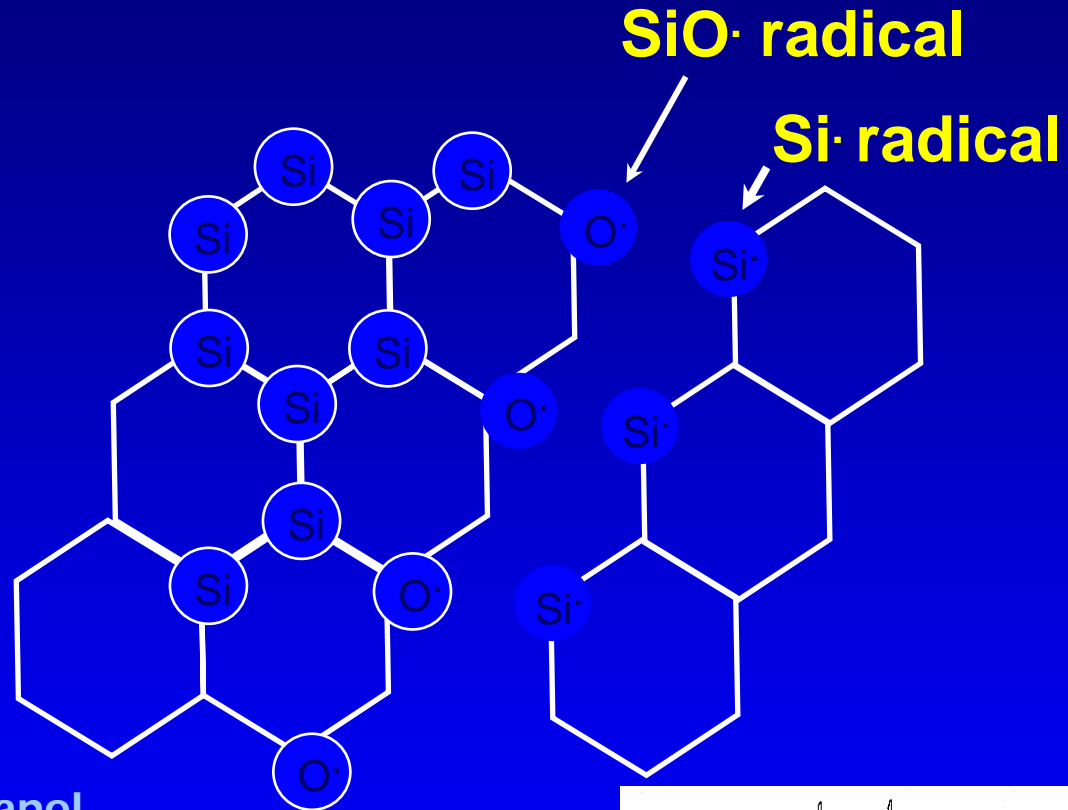
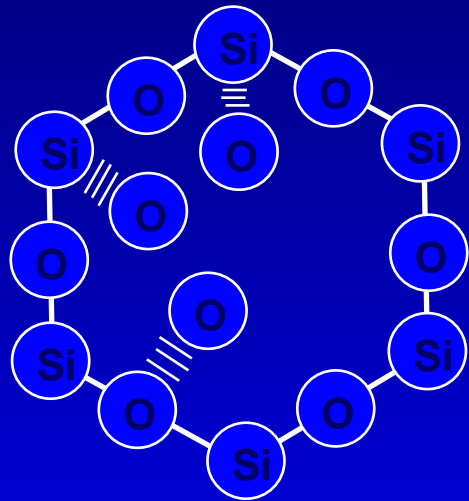
## Quartz

- Toxic, active surface
- Relatively pure single substance (> 95 %)
- Insoluble
- Associated to fibrosis and lung cancer
- A model particle

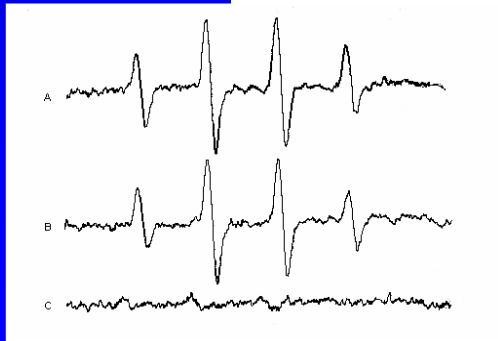
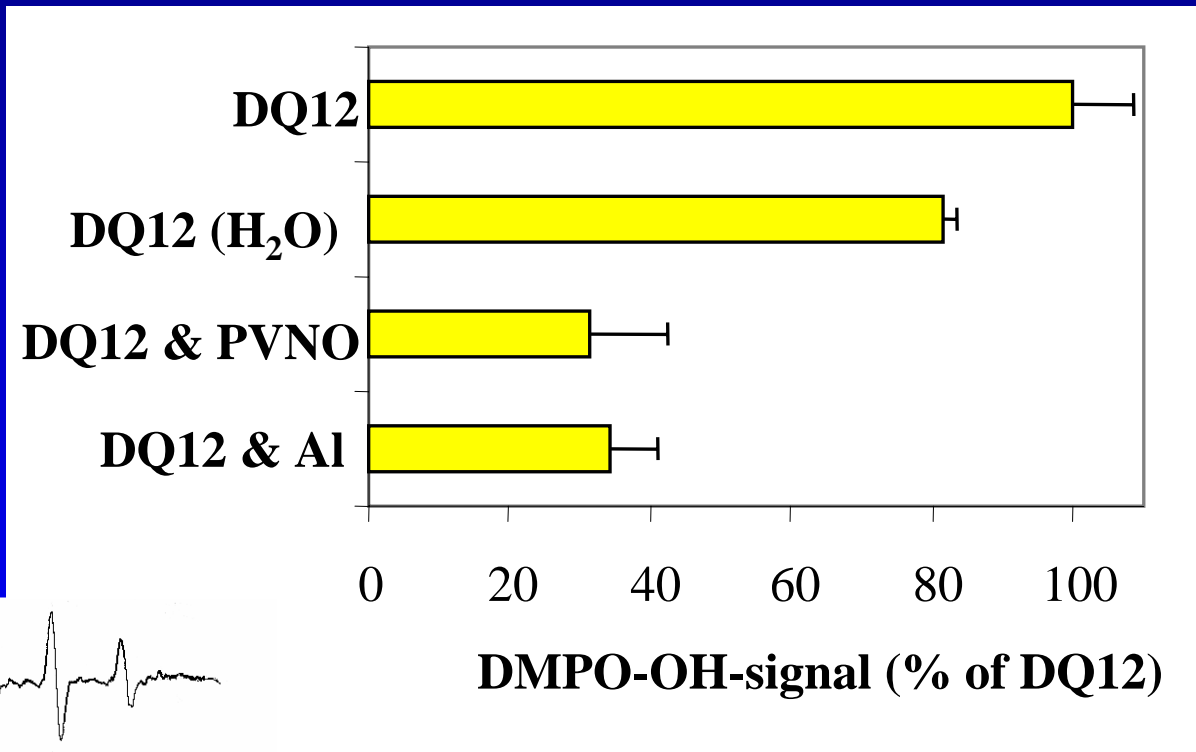
## Particulate matter (PM)

- Low toxic
- Complex and variable composition
- Partly soluble (< 30 %)
- Associated to worsening respiratory diseases, cardiovascular insults, and lung cancer

# The silica surface is (inter)active:

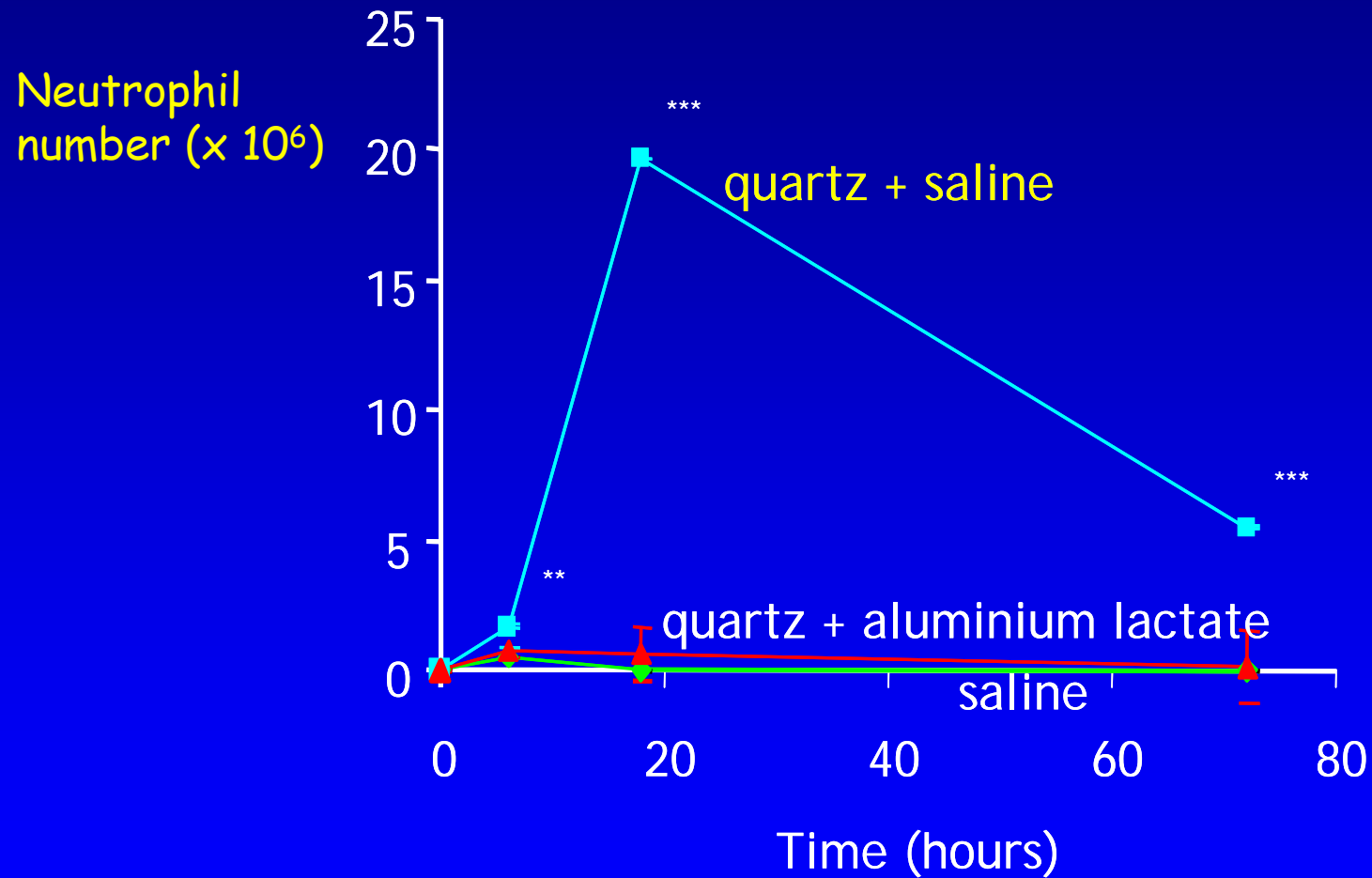


# Effects of surface-coatings of quartz on OH-radical formation (ESR)

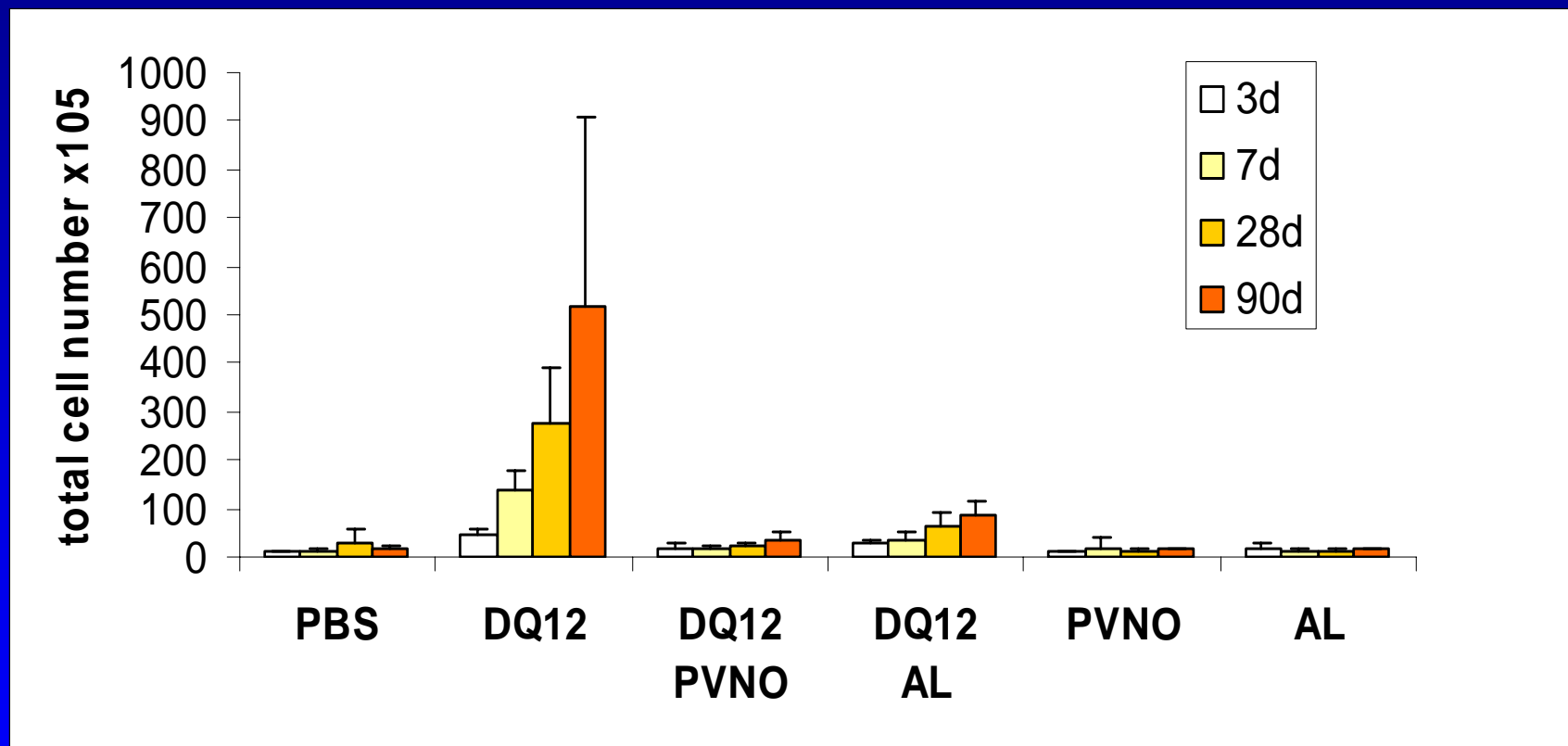


DMPO-OH signal

# Neutrophil number after acute instillation of quartz and AL-coated quartz

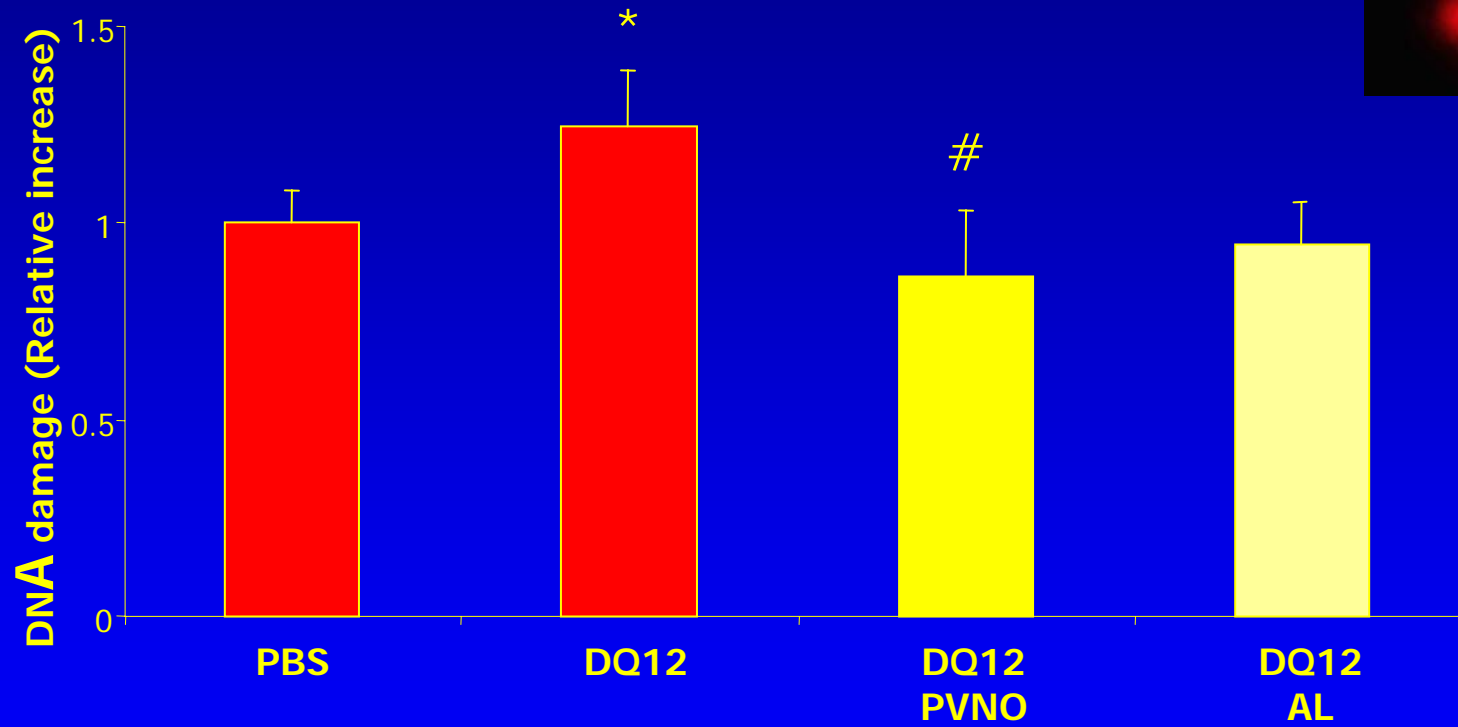


## Surface modification also inhibits chronic inflammation



Albrecht et al (2004), in press

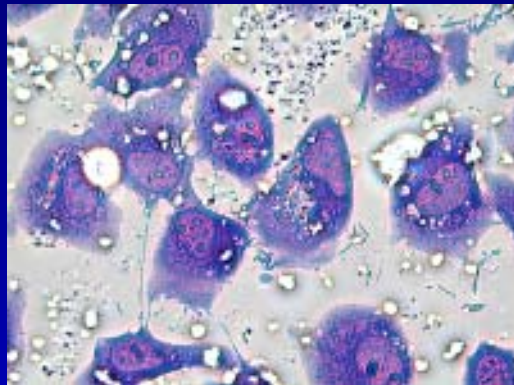
# Acute in vivo DNA damage in isolated epithelial cells is reduced by surface modification



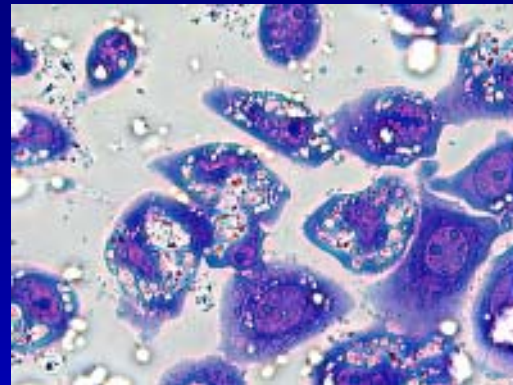
\* $p < 0.05$  vs. PBS  
# $p < 0.05$  vs. DQ12

Knaapen et al (2002) Carcinogenesis

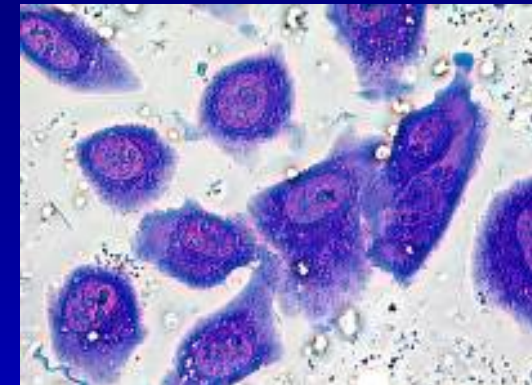
Quartz



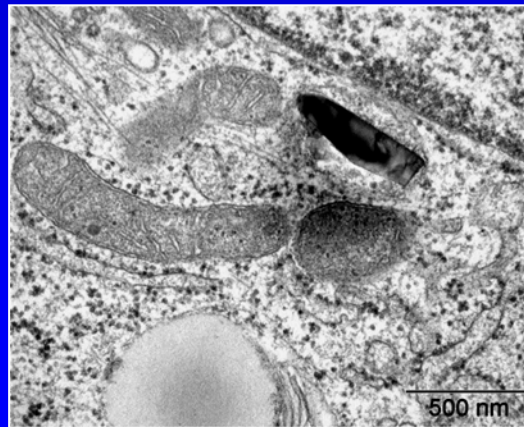
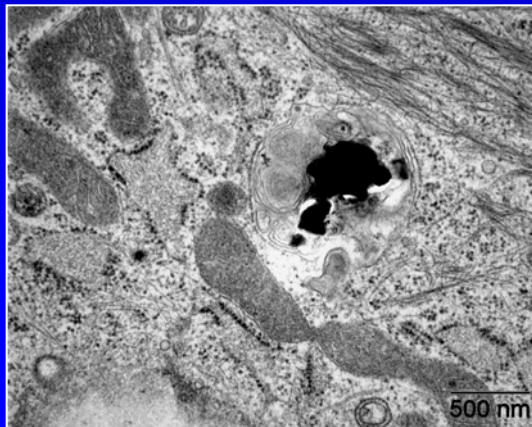
Quartz -Al(0.1%)



Quartz-PVNO



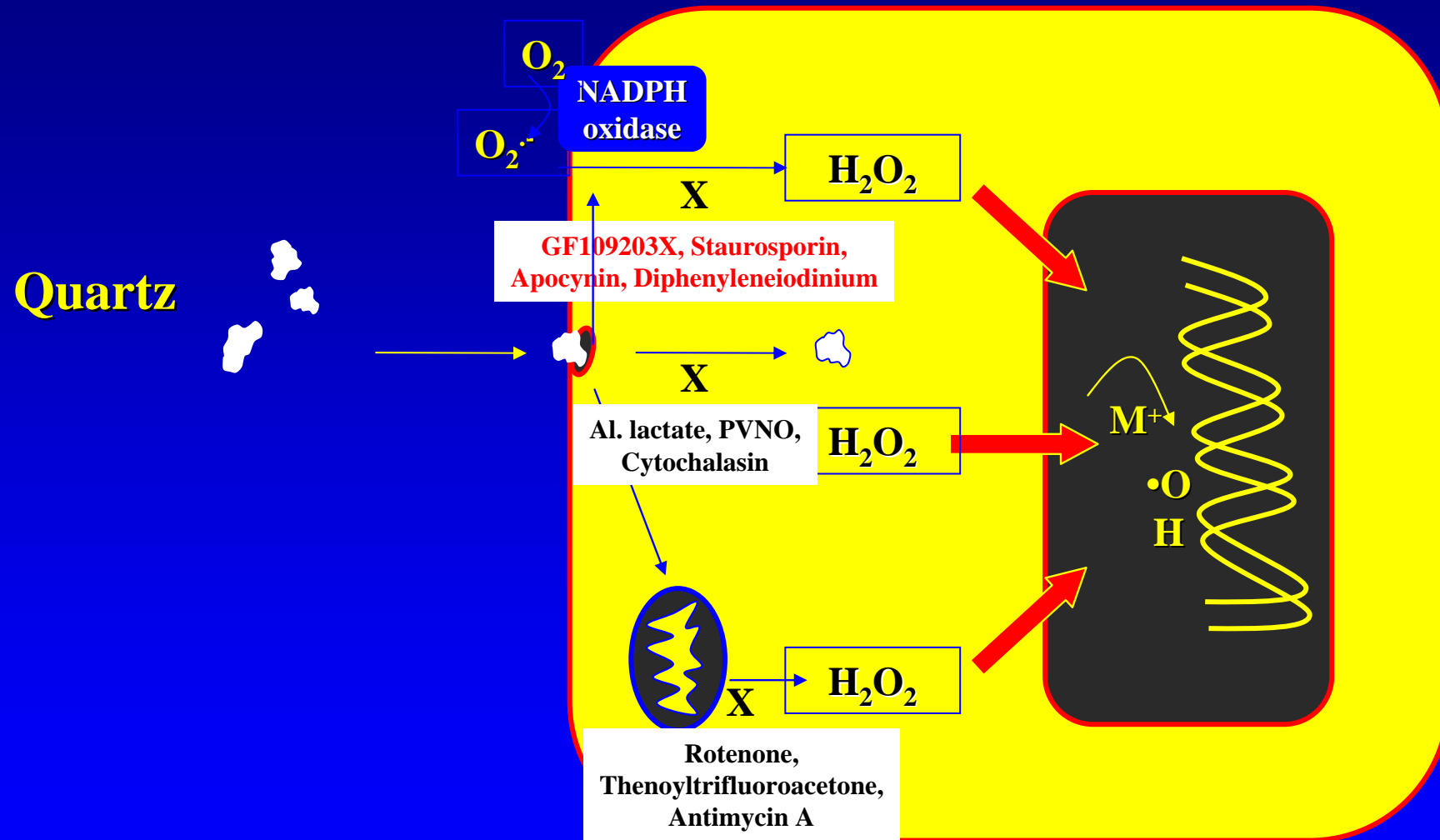
**Surface modification affects endocytosis in epithelial cells  
But not intracellular distribution.**



**DQ12 quartz particles  
do not appear in the  
the nucleus (2h - 48h)**

Data courtesy: Doris Hohr, Li Hui, Roel Schins

# How can quartz induce oxidative DNA damage in the nucleus?



Courtesy: Li Hui, Roel Schins

lung epithelial cell

Quartz can induce a number of effects that are mediated by or produce ROS.

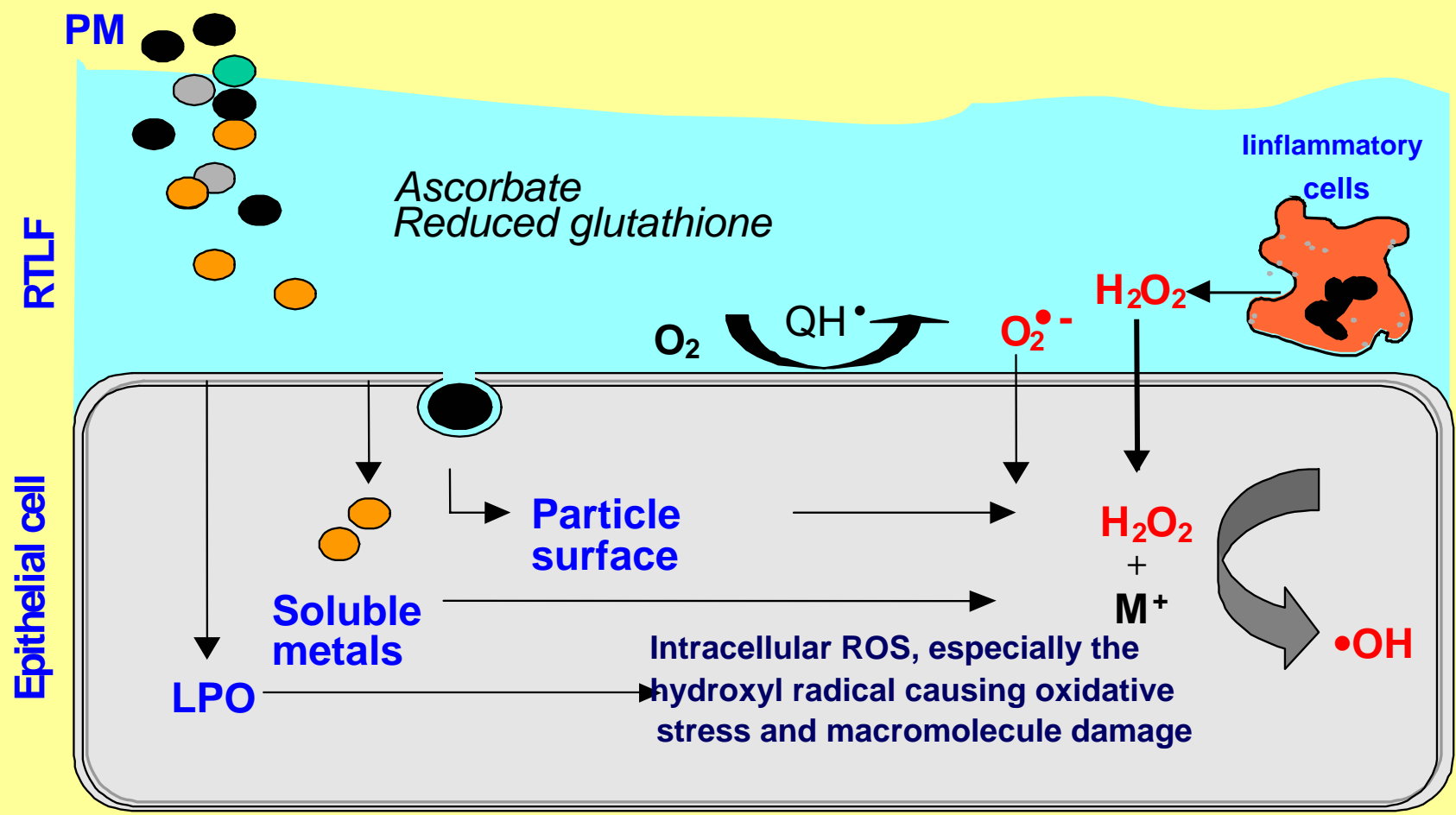
- OH- Radical formation (ESR)
- Production of ROS and MPO by inflammation
- In vitro genotoxicity (Comet, 8-OH-dG)
- In vivo genotoxicity (Comet) related to inflammation
- Acute and chronic activation on NFkB.

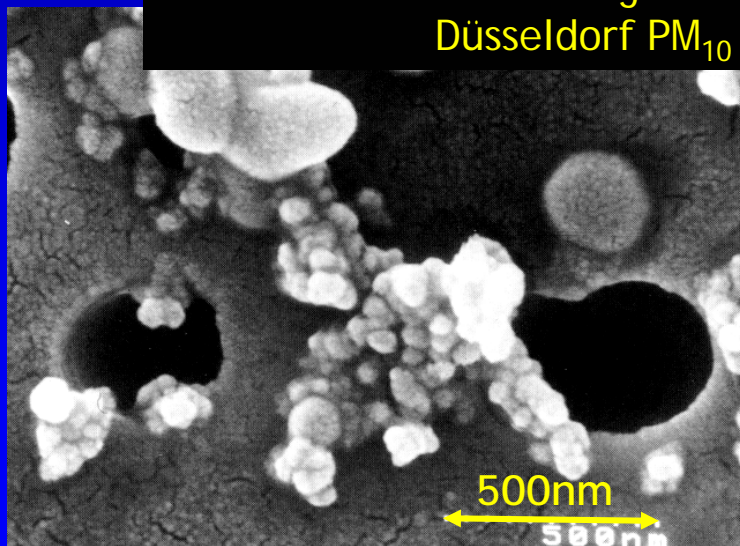
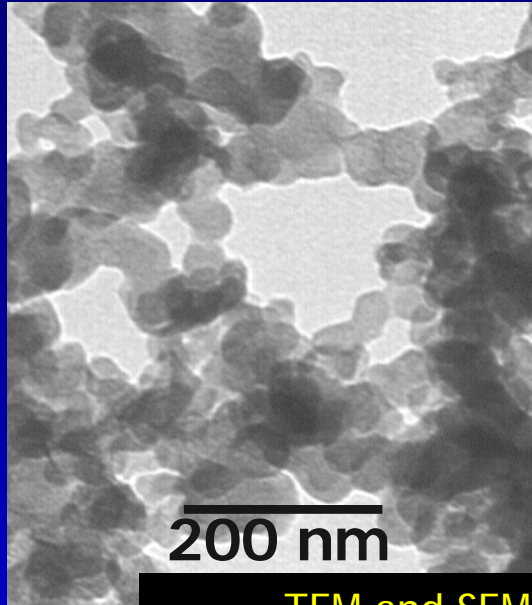
Surface modifications reduce most of the above events by:

- Inhibition of acellular radical production
- Reduction of particle toxicity with effects on inflammation, clearance and secondary genotox
- Different uptake of particles by target cells (AM, Epi)

*Schins et al, 2000,2002a,b; Hohr et al, 2002; Knaapen et al, 2002; Clouter et al, 2001  
Albrecht et al (2002), Cakmak et al, in press; Hohr et al, in preparation; Duffin et al, in prep)*

In analogy: we have elaborated this concept for PM





TEM and SEM images of  
Düsseldorf PM<sub>10</sub>

## Properties and components that contribute to ROS generation by PM:

- Surface area, amount of ultrafines
- Redox active metals (Fe, Cu, V)
- Solubility of metals
- Presence of quinones
- presence of anti-oxidants (plants)

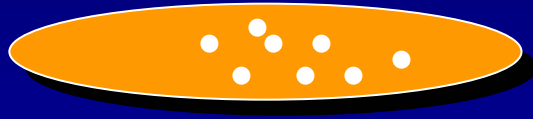


Need for a method that integrates  
These properties



Radical generating (oxidant) capacity  
Measured in the presence of H<sub>2</sub>O<sub>2</sub>

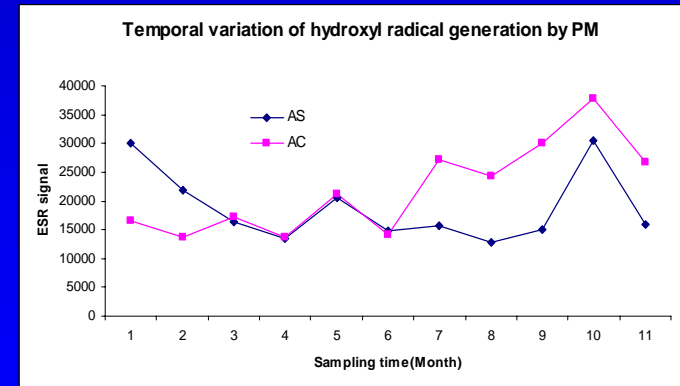
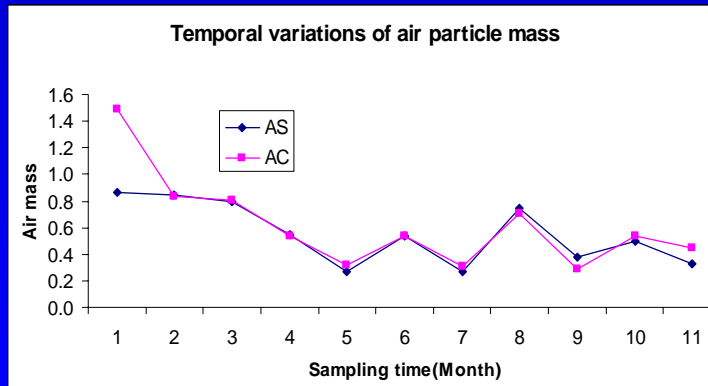
# Teflon filters



Remove plastic ring,  
agitation ( 5 min)  
Sonication (5 min) in water

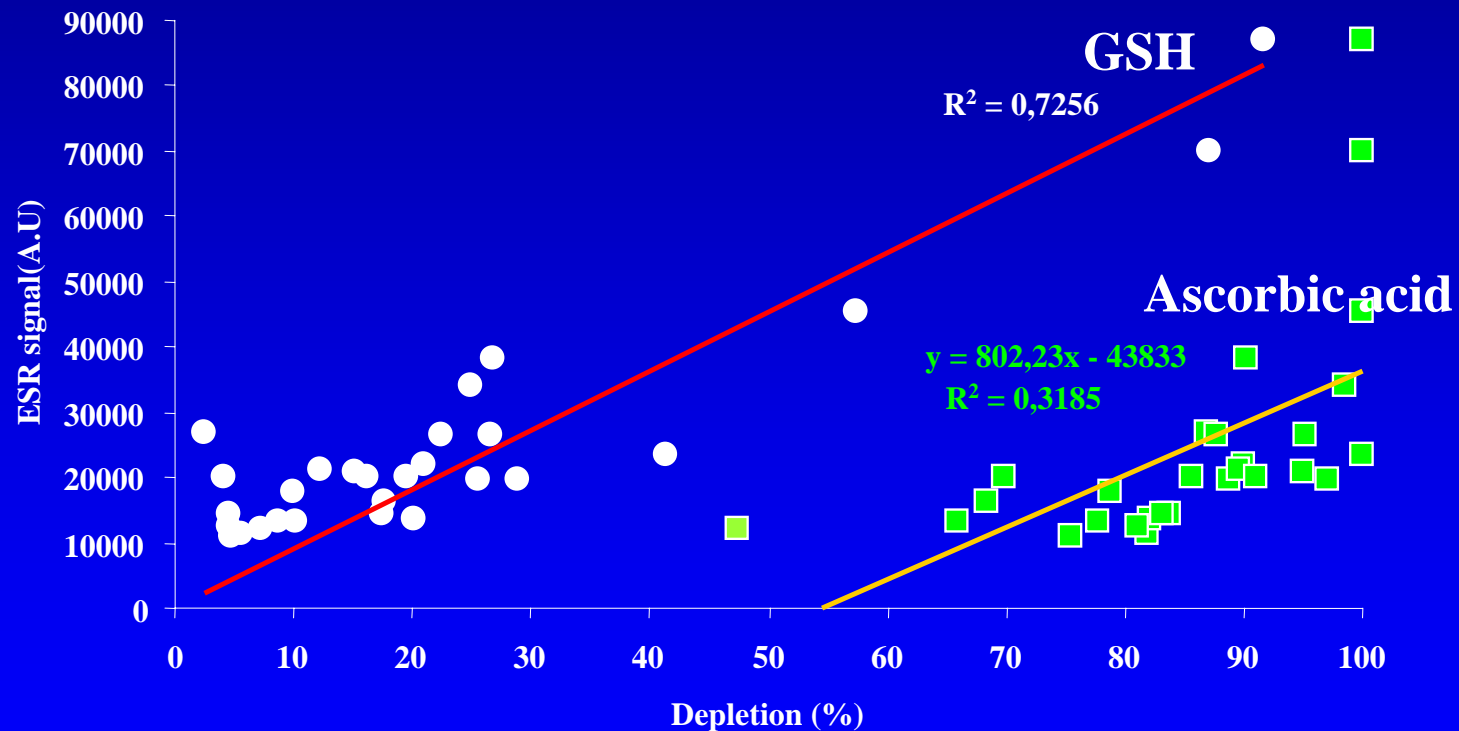


## Measure oxidant generating Capacity. EPR

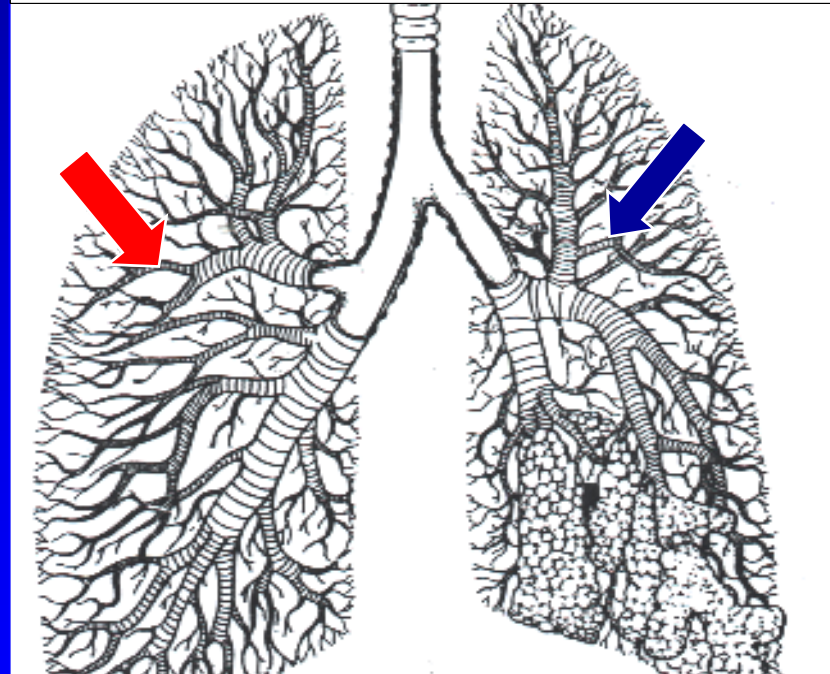
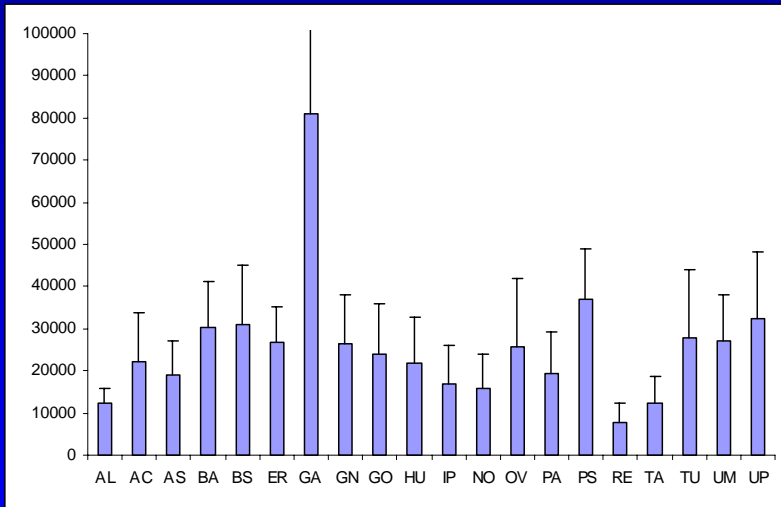
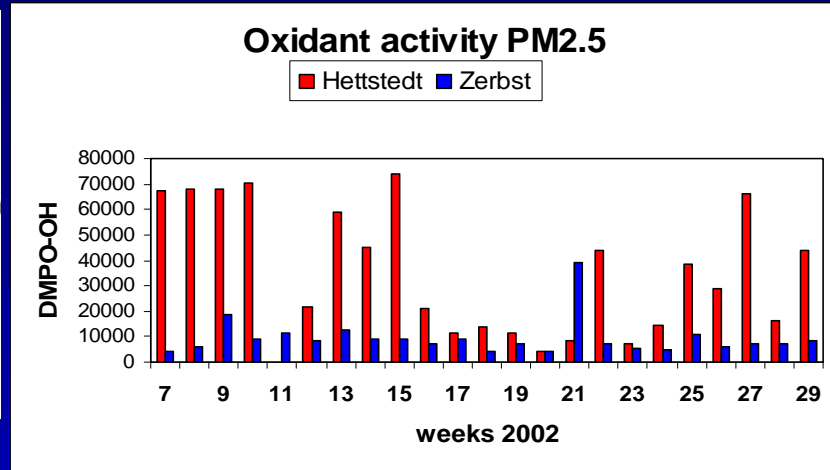
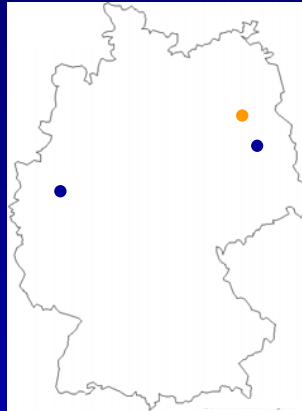


Mass versus oxidant generating capacity in one city, two locations over time

# PM induced OH generation (EPR) correlates with depletion of antioxidant substances



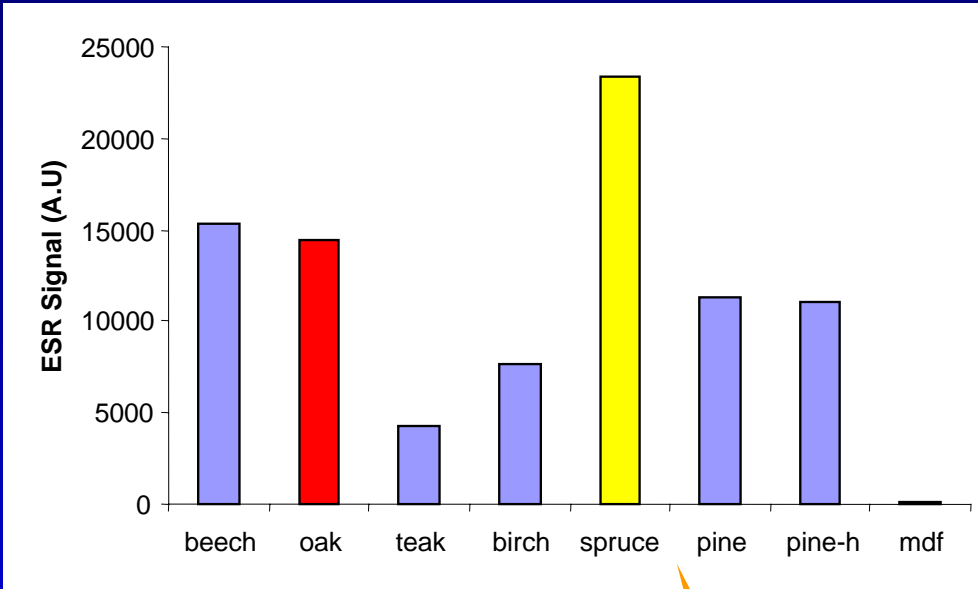
*Shi, Kelly, Mudway, Kuhlbusch and Borm (AIRNET, 2002)*



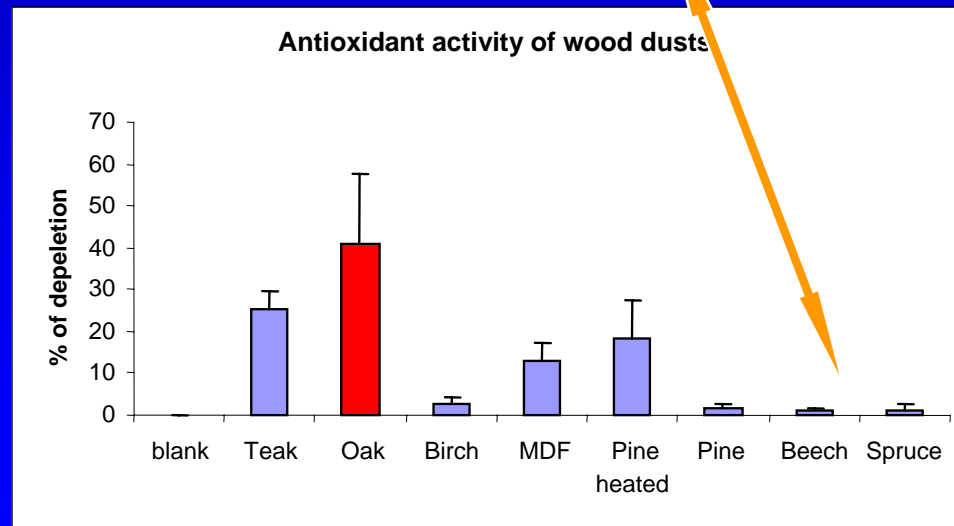
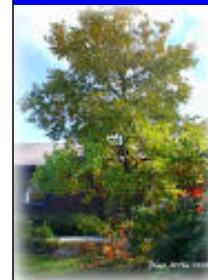
**ECRHS II: 2000- 2003**  
**20 cities, 70 samples PM2.5/city**  
**Link to composition as well as**  
**Health effects in population**

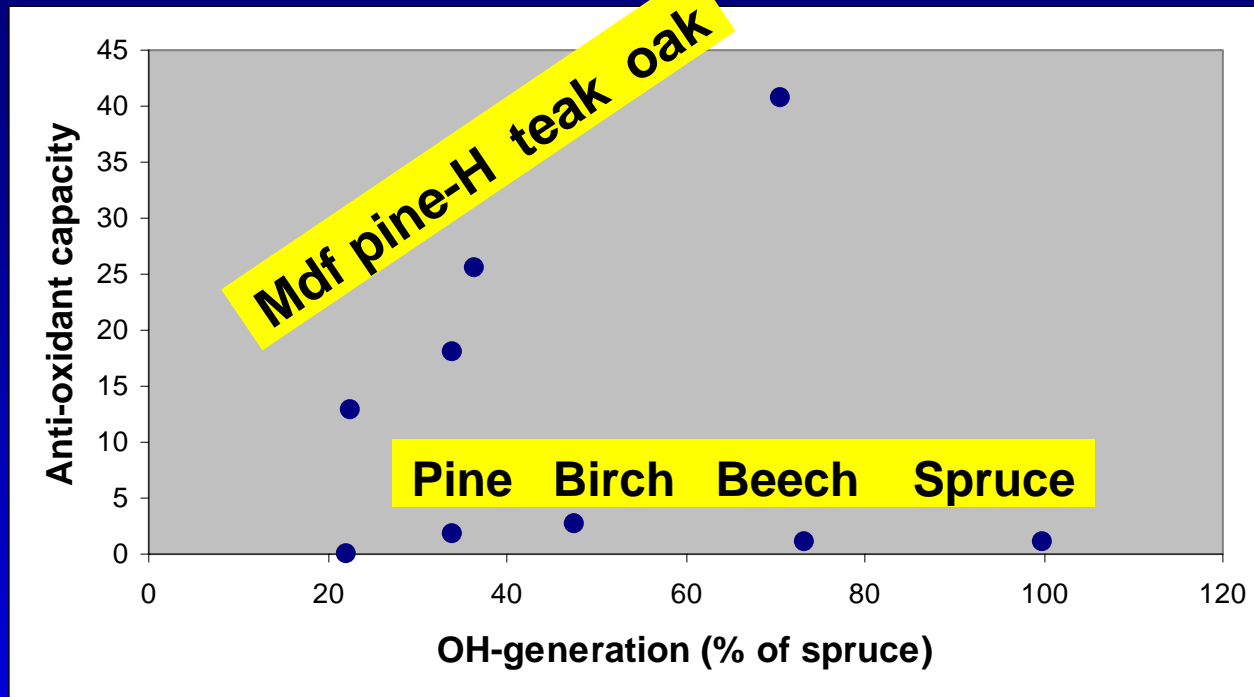
**Clinical study with PM2.5 in 16**  
**Volunteers, based on oxidant activity**

## Wood dust OH- Generation



## Wood dust Tempol degradation

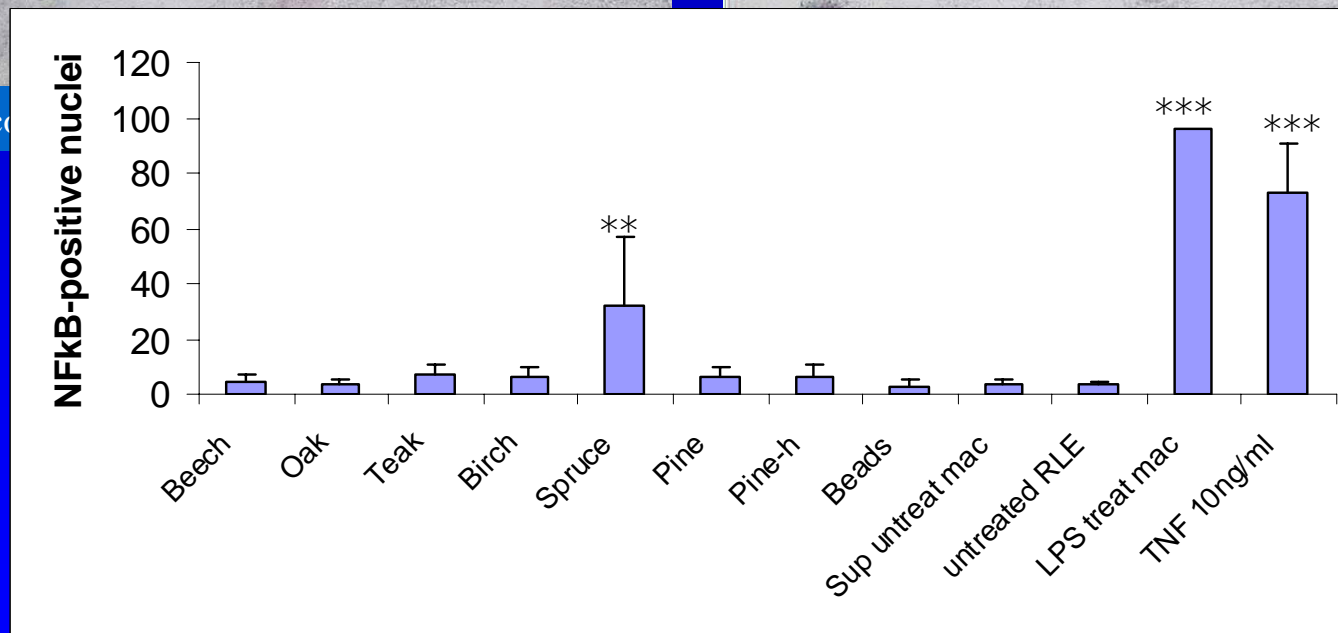
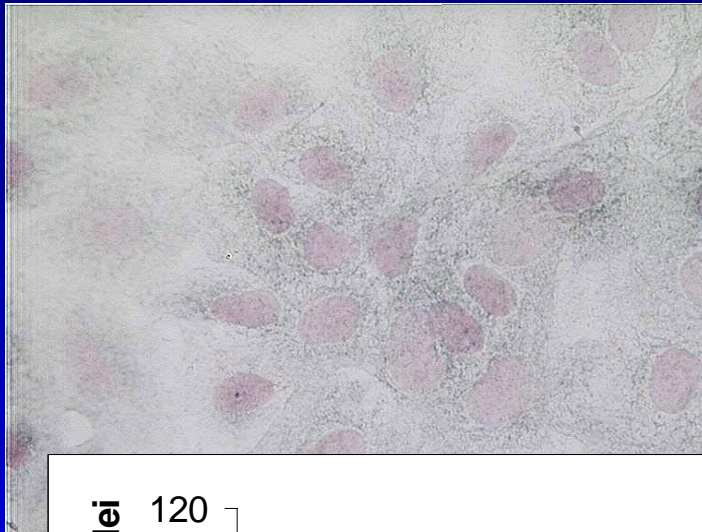




**Relation between anti-oxidant capacity and OH-generation  
Is complex:**

- some wood dusts with very low anti-oxidant capacity
- some wood dust positive relation between oxidant and anti-oxidant properties. Pro-oxidant mechanisms?

# Expression of NFkB (p65) in epithelial cells



**In conclusion:**

- **particles possess oxidant (and anti-oxidant) activity.**
- **mechanisms of acellular oxidant generation are dependent on particle surface chemistry and soluble components.**
- **Surface modification or impurities may dramatically affect oxidant generation.**

**Particle Oxidant generation is associated to biological effects:**

- **Activation of inflammatory response (quartz, PM) in vitro and in vivo (both rat and human, PM)**
- **Indirect and direct genotoxicity (quartz, PM)**
- **Activation of NFkB in vitro and in vivo (wood dust, quartz)**

**Current epidemiological studies investigate the relation between Particle oxidant generation and health effects such as lung function decline and asthmatic symptoms.**

## Contributors to this work:

- IUF: Roel Schins, Li Hui, Tingming Shi, Catrin Albrecht, Rodger Duffin
- ITEM (Hannover): Norbert Krug, Frank Kaufman
- UCLA: Nino Kuenzli, Thomas Gotschi
- Kings College: Frank Kelly, Ian Mudway
- LMU-Munchen: Fritz Kromcah, Huyan Long
- IUTA Duisburg: Thomas Kuhlbusch
- Un Maastricht: Ad Knaapen, FJ van Schooten