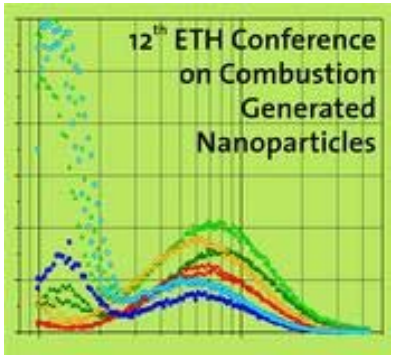
HelmholtzZentrum münchen
German Research Center for Environmental Health

iLBD
Institute of Lung
Biology and Disease




# *Deducing the inflammatory in vivo toxicity of combustion derived nanoparticles from in vitro data*

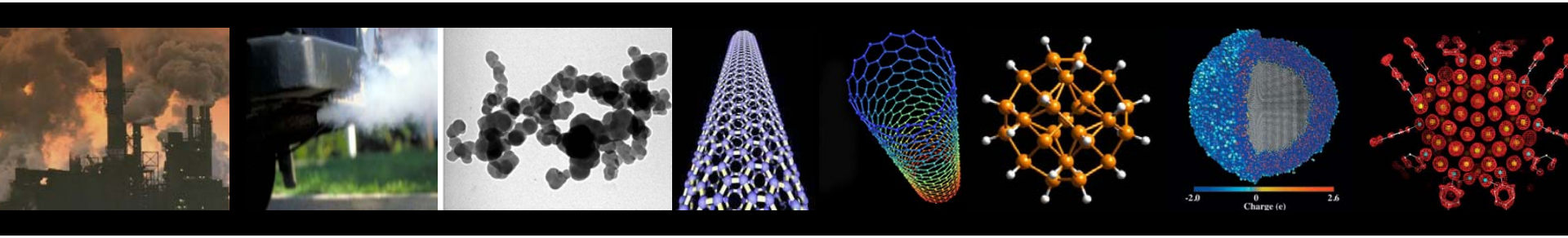
**13th ETH Conference on Combustion Generated Nanoparticles - Session 5B: Health Effects**
**Zürich, 23/06/2009**

T. Stoeger, O. Schmid, D. Dittberner, S. Takenaka, H. Schulz

CPC Comprehensive Pneumology Center

HELMHOLTZ ASSOCIATION

# Particle Toxicity: Where does it come from?




## Nanoparticle Parameters Relevant to Health:

**Solubility**

**Particle Size / Shape / Rigidity**

**Particle Concentration *(Dose)***

- Mass
- Number
- Surface Area

**Chemical Composition**

- Organic Compounds (PAH, Quinones)
- Metals (Iron, Zinc, Copper, ...)

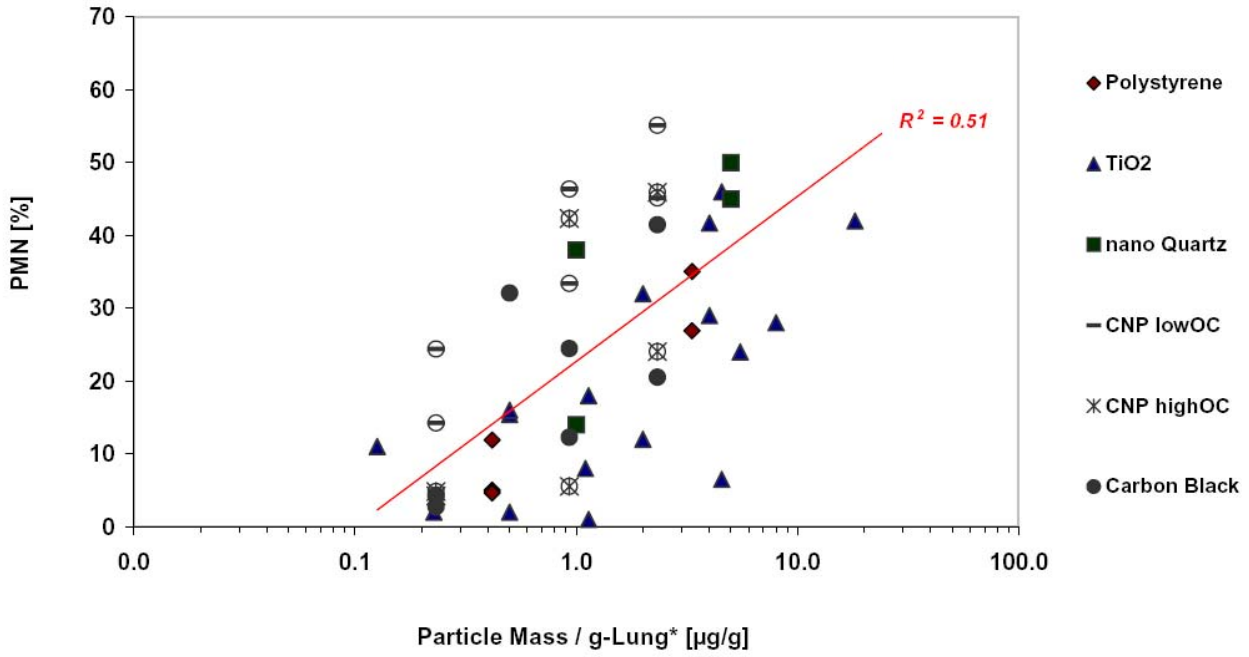
**Particle Reactivity**

- Bioavailability of NPs & Substances
- Surface Structure / Morphology
- Generation / Release of Radicals

# Dose Response Relations *(Animal Studies)*

Summary of 7 studies that analyzed the acute pulmonary response [%PMN] 24h after particle instillation in mice and rats

## Dose Metric: Mass




| Reference | Particle | Size Range [nm] | |
|---|---|---|---|
| Oberdörster-2005 | $TiO_2$ | 20-250 | mouse |
| Stoeger-2006 | CNP-lowOC | 9-50 | |
| Stoeger-2006 | CNP-highOC | 12-25 | |
| Brown-2001 | Polystyrene | 65-535 | rat |
| Dick-2003 | Carbon Black | 14 | |
| Dick-2003 | $TiO_2$ | 20 | |
| Höhr-2002 | $TiO_2$ | 25-180 | |
| Oberdörster-2005 | $TiO_2$ | 20-250 | |
| Warheit-2006 | $TiO_2$ | 300 | |
| Warheit-2007 | Quarz (Nano) | 12-50 | |

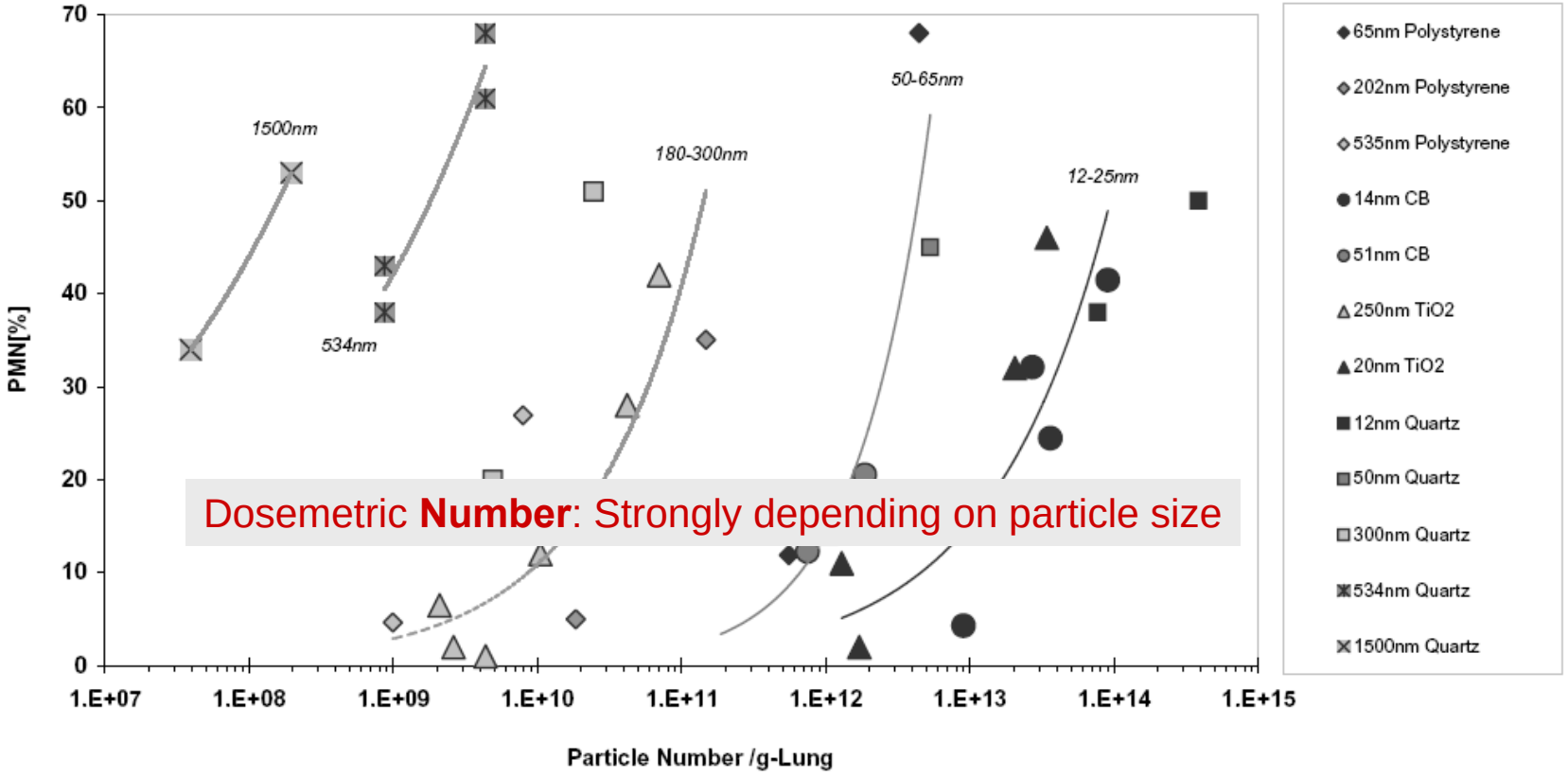
Dosmetric **mass** explains *"only"* about 50% of response variability

*Stoeger in preparation*

# Dose Response Relations *(Animal Studies)*

Summary of 7 studies that analyzed the acute pulmonary response [%PMN] 24h after particle instillation in mice and rats

## Dose Metric: Particle Number



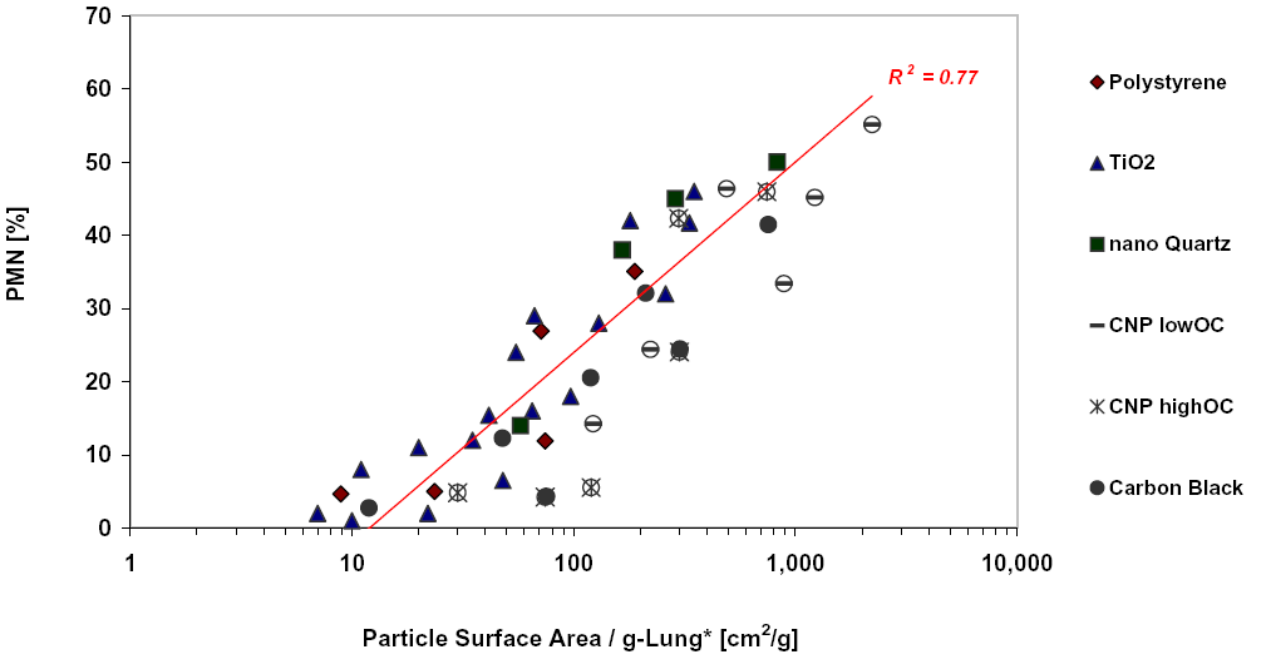

Dosemetric **Number**: Strongly depending on particle size

*Stoeger in preparation*

# Dose Response Relations *(Animal Studies)*

Summary of 7 studies that analyzed the acute pulmonary response [%PMN] 24h after particle instillation in mice and rats

## Dose Metric: Particle Surface Area

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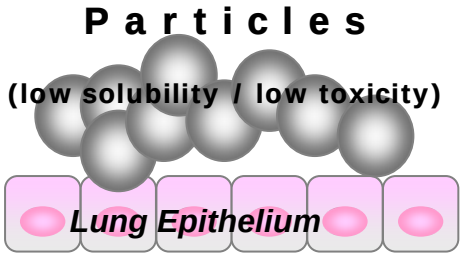
| Warheit-2007 | Quarz (Nano) | 12-50 | rat |

**⇒ Particle Surface Drives Particle Toxicity!**

*Stoeger in preparation*

# Partice Toxicity According to the Oxidative Stress Paradigm



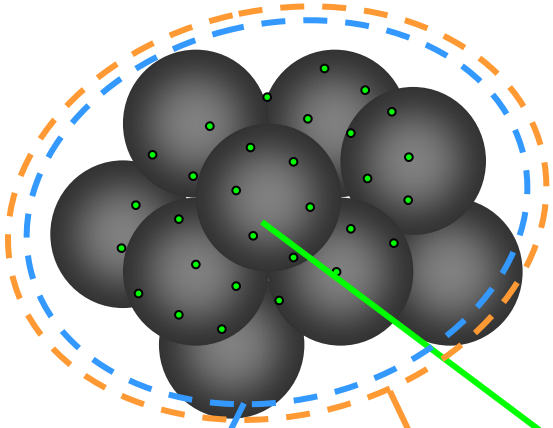

PM-Cell Interactions

Oxidative Stress / ROS

Inflammatory Response

**Lung Disease**
**(fibrosis, lung cancer…)**

**…Cardiovascular Disease**



Sources for Oxidative Stress

structural surface properties

organic compounds (PAH)

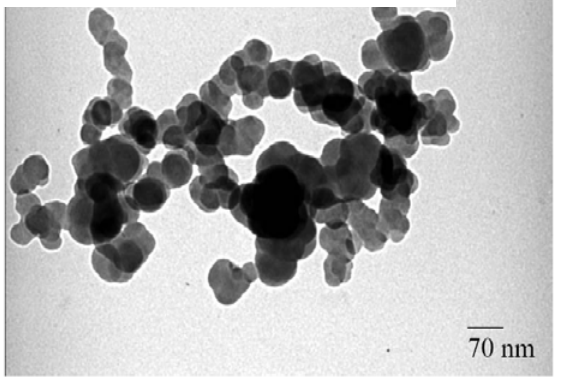
transition metals

Formation of Reactive Oxygen Species

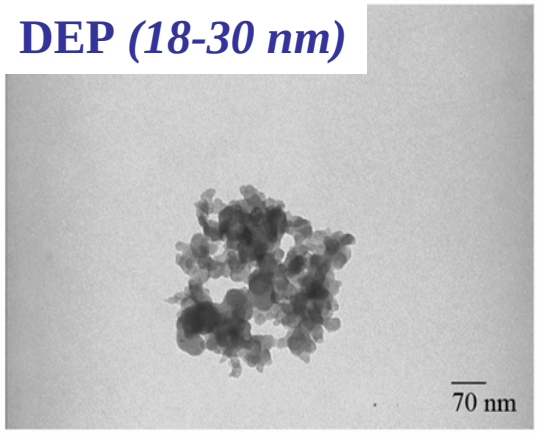
***? Can we differentiate sources of oxidative stress / inflammation?***

# Investigated Carbonaceous Nanoparticles

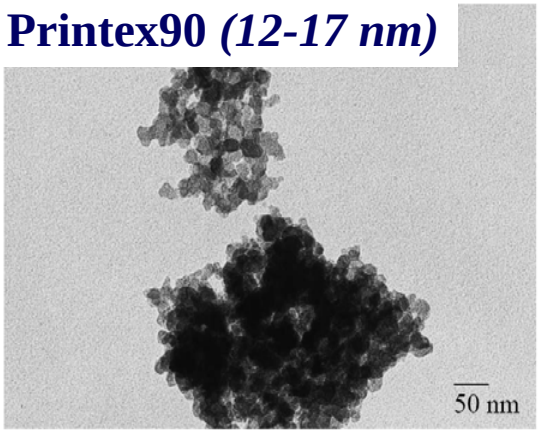
**PrintexG** ***(30-60 nm)***



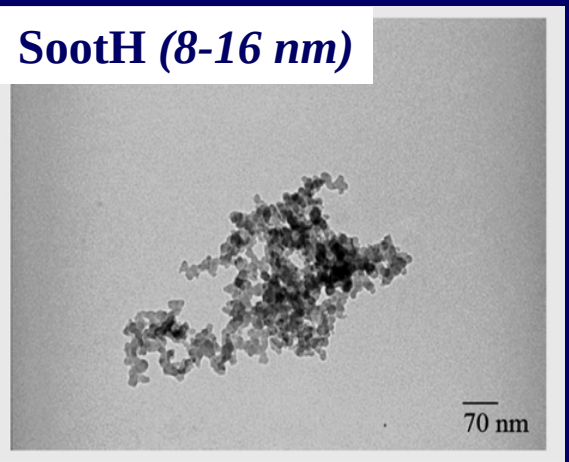

**DEP** ***(18-30 nm)***



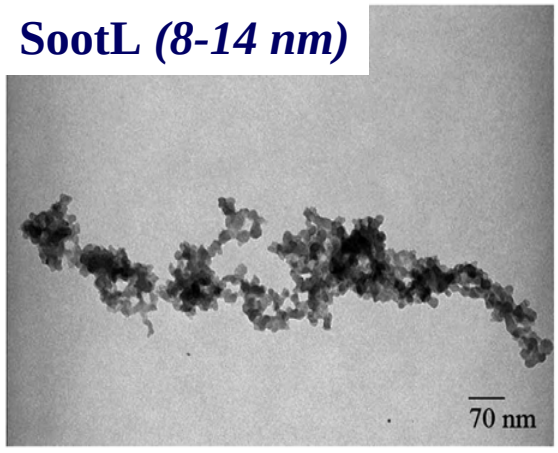

**Printex90** ***(12-17 nm)***



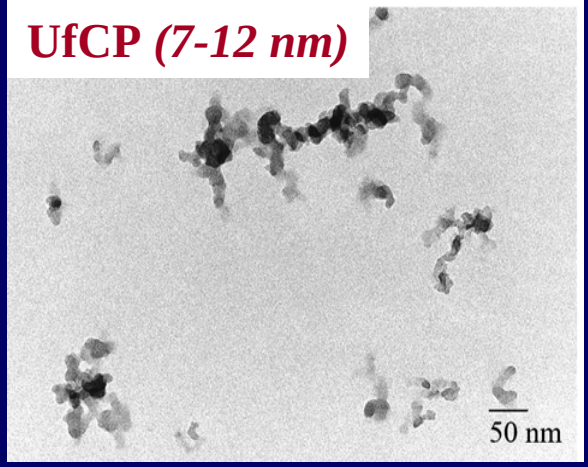

**SootH** ***(8-16 nm)***




**SootL** ***(8-14 nm)***




**UfCP** ***(7-12 nm)***

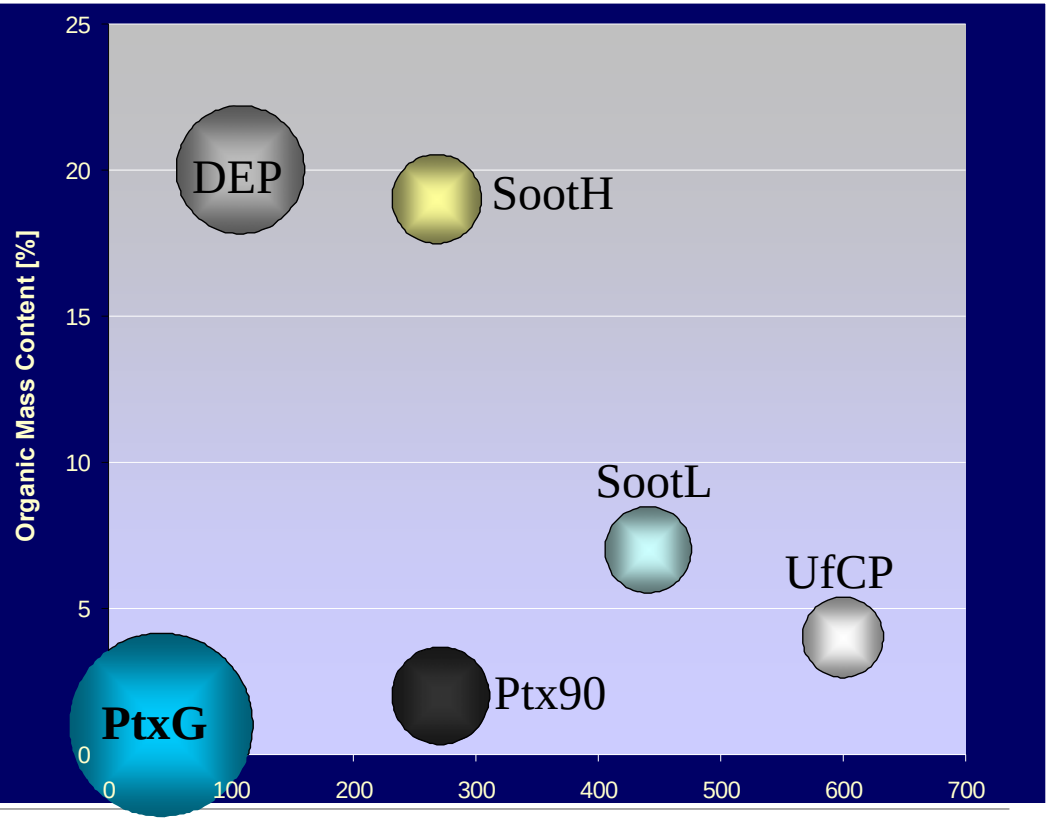


# Investigated Carbonaceous Nanoparticles

| Pigment Black | Spark Discharge | Flame Soot | Diesel Exhaust Particles |
|---|---|---|---|
| • Printex90<br>• PrintexG | • UfCP | • SootH<br>• SootL | • DEP<br>**SRM1650a** |

## Particle Characteristics

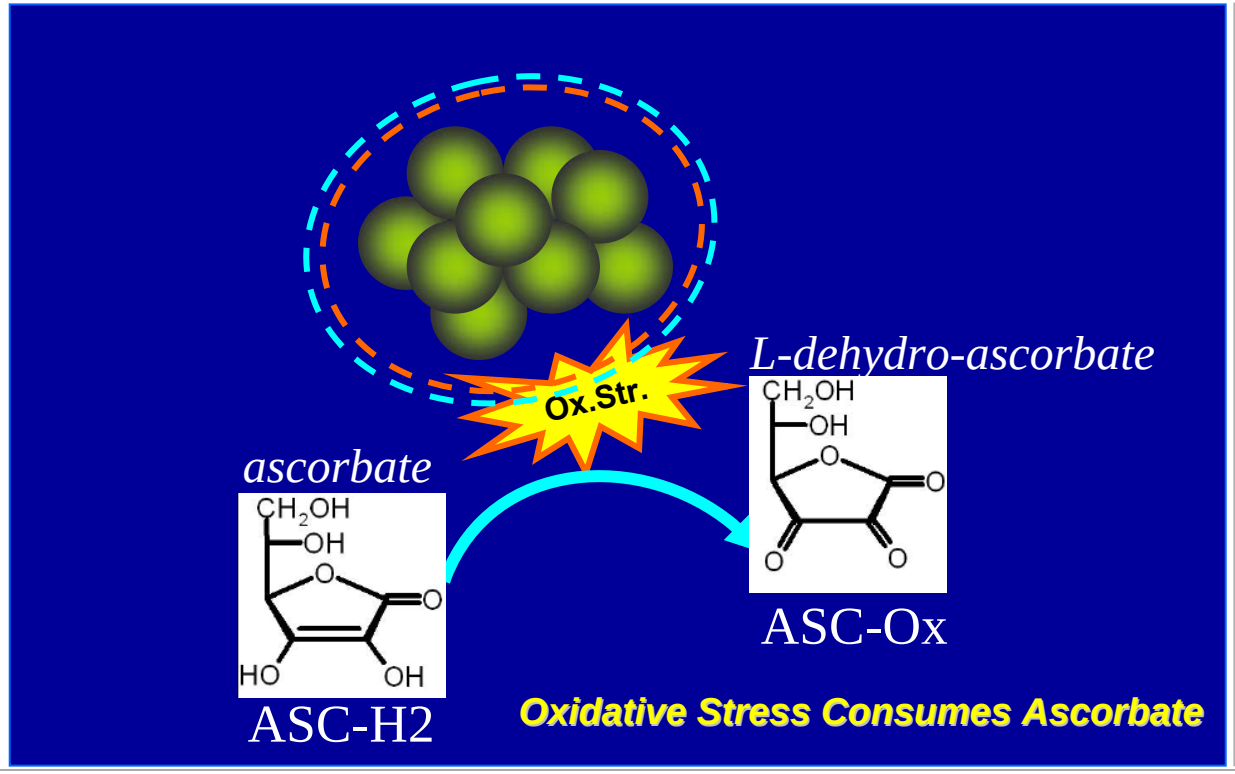
| NPs | Diameter [nm] | Org. Content [%] | BET surface [$m^2/g$] |
|---|---|---|---|
| DEP | 25 | 20 | 108 |
| PtxG | 51 | 1 | 43 |
| Ptx90 | 14 | 2 | 272 |
| SootH | 12 | 19 | 268 |
| SootL | 11 | 7 | 441 |
| UfCP | 10 | <4 | 600 |



# How to Assess the 'Oxidative Reactivity' of Nanoparticles?

**Oxidative potency of NPs assessed in a <u>cell free system</u>:**

Consumption of the anti-oxidative capacity of *ascorbate* as a measure for the oxidative reactivity.




**PHOTOCHEM quantification of antioxidative capacity**

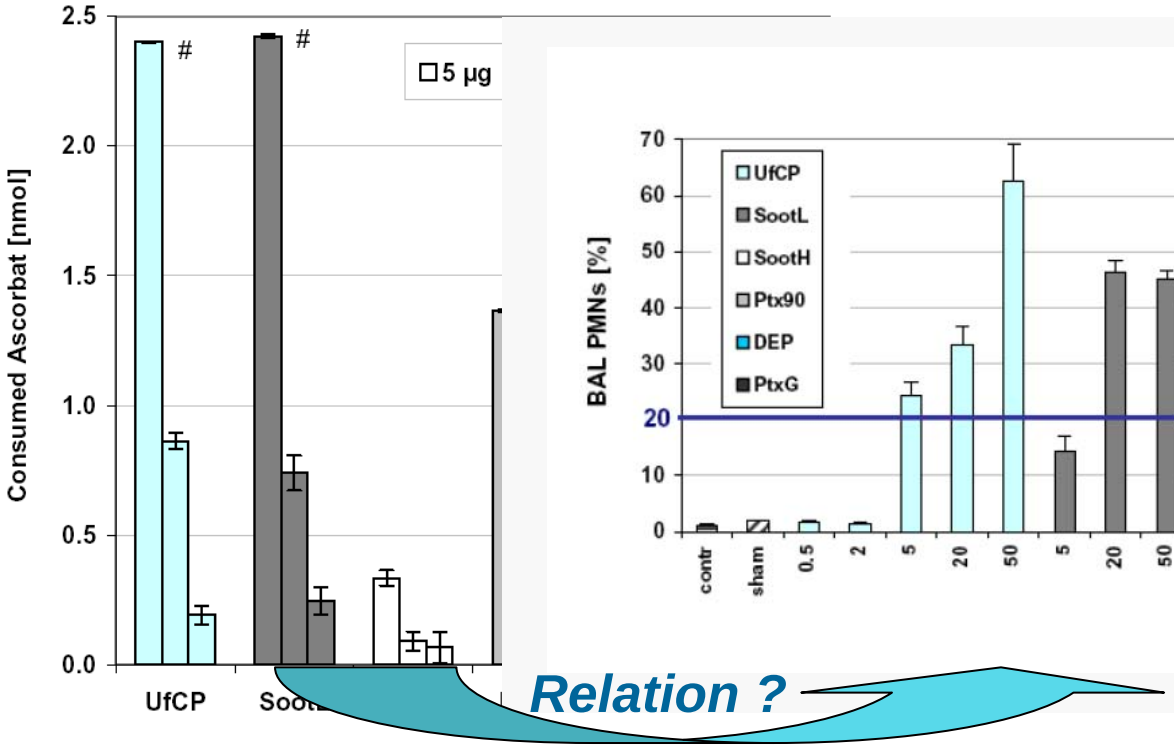
fast photochemical excitation of radical formation combined with sensitive luminometric detection

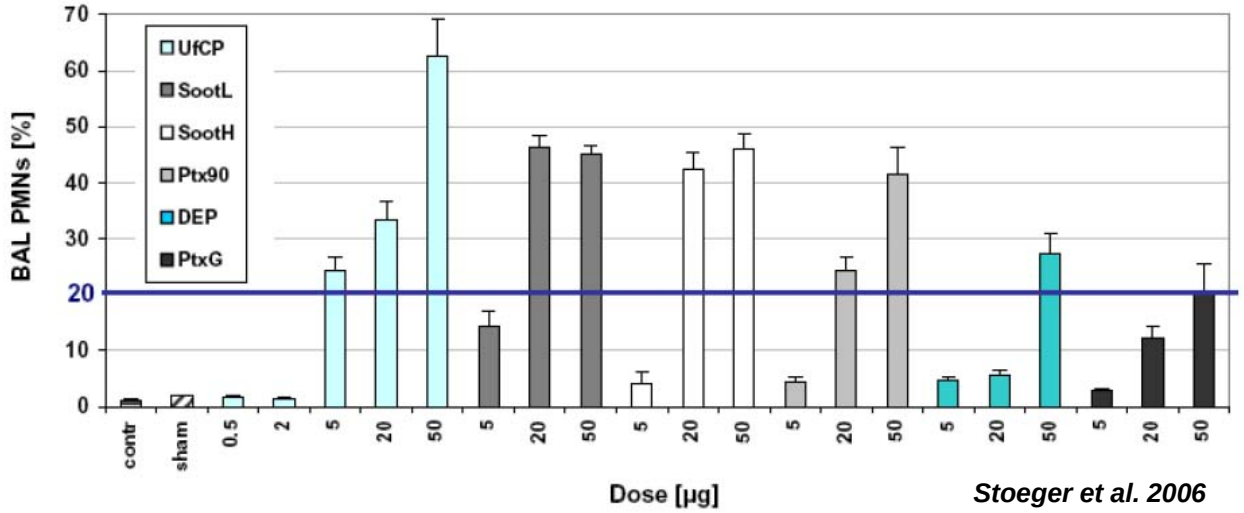
# Oxidative Potency of the Six Carbon Nanoparticles

## *Particles Differ in Their Oxidative Reactivity / Potency*

**Cell Free Assay:** *"Oxidative Effect"*

**Result from Animal Exposure:** *"Inflammatory Effect"*
*inflammatory efficacy (dose per 20%PMN)*



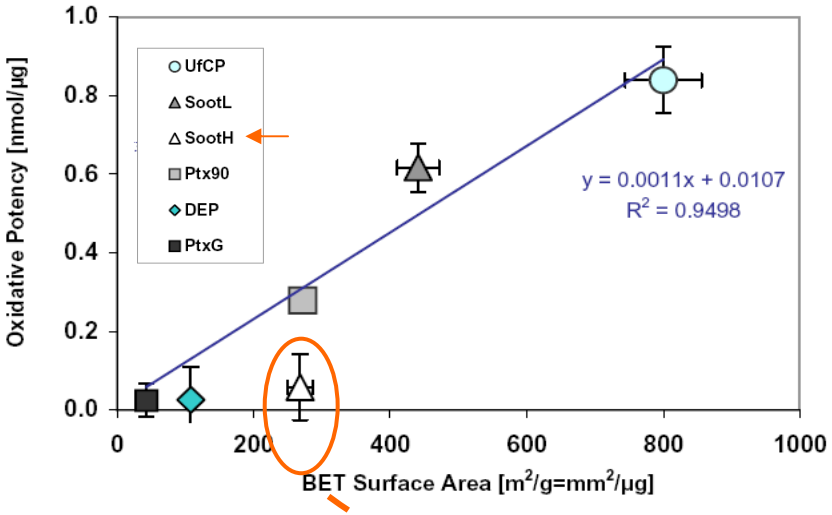




*Stoeger et al. 2006*

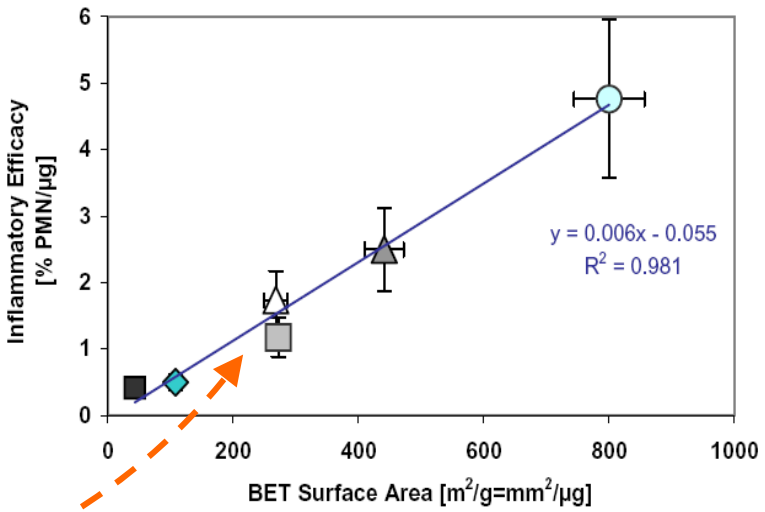
***Relation ?***

# ‘Oxidative Potency’ and ‘Inflammatory Efficacy’ of NPs as Function of BET Surface Area

***Cell Free***




***In Vivo***

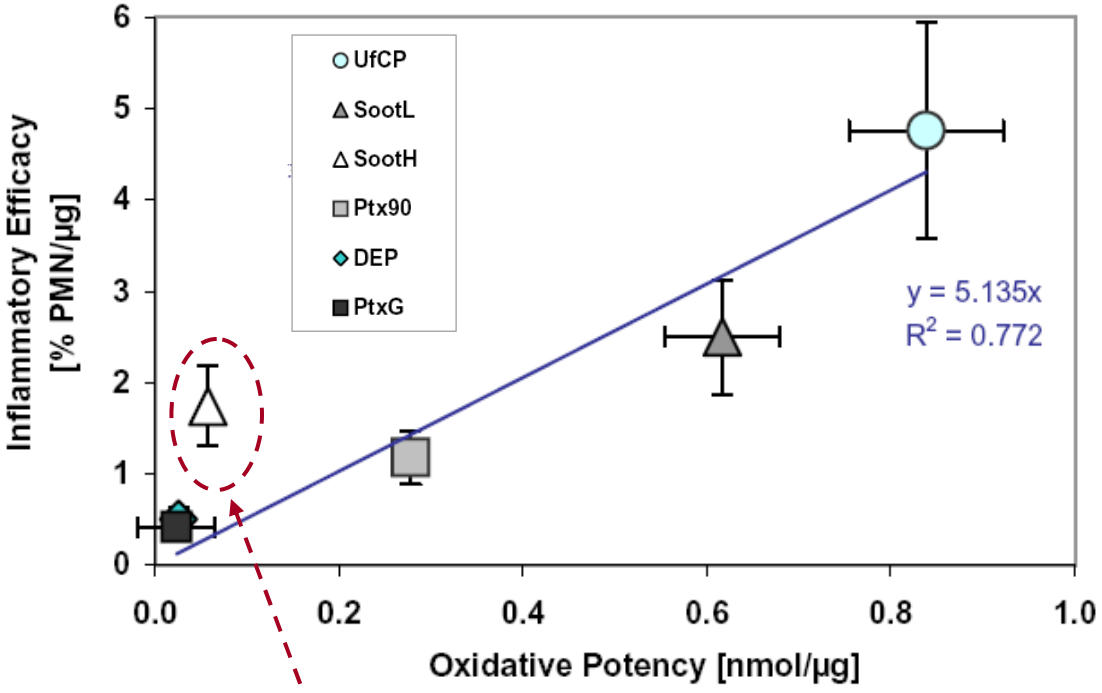



***Inflammatory Efficacy ($I_{Eff}$): Effective dose (µg/mouse) causing an inflammatory effect of 20% PMNs.***

**SootH Particle Surface has been *activated* ?**

*Stoeger et al. 2008*

# Can 'Oxidative Potency' Predict the 'Inflammatory Efficacy' of Nanoparticles?

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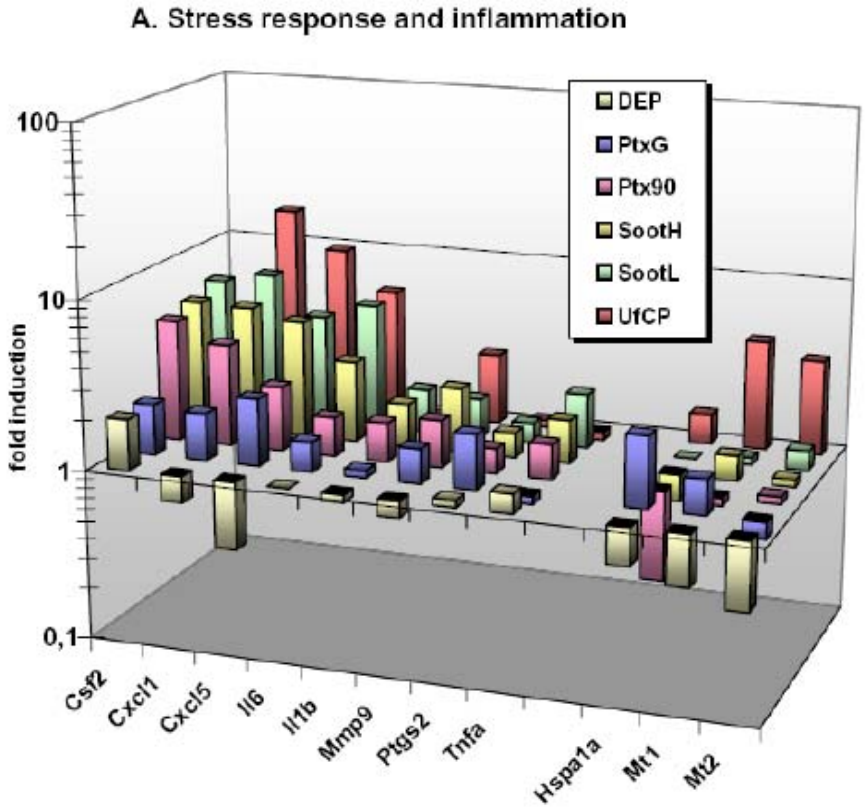
**All but not *SootH* particles follow the relation!**
***Organic Fraction?***

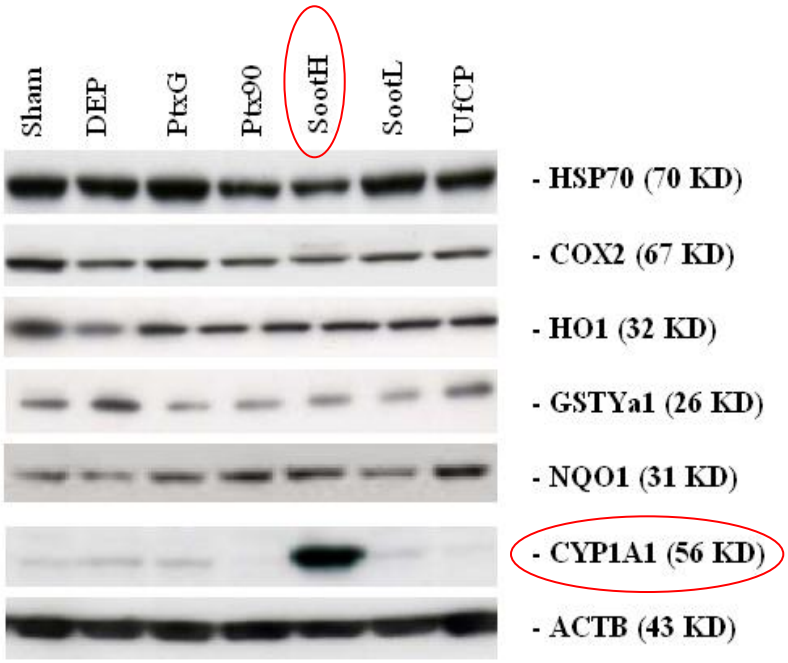
# Bioavaliability of Organic Compounds Investigated by Gene Expression Analysis



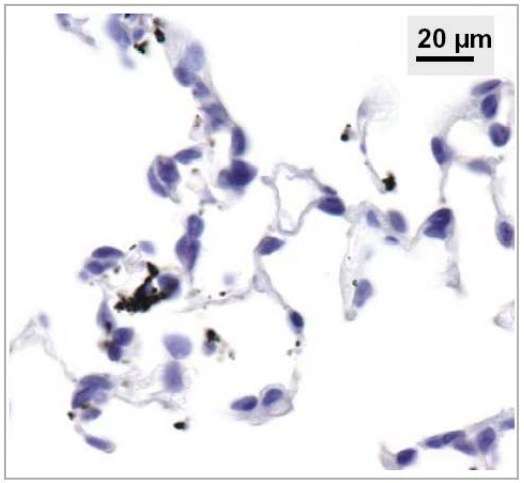

**Cyp1a1 expression matches well with the "Oxidative Potency vers. Inflammatory Efficacy" discrepancy**

# PAH-rich SootH but not SootL induced CYP1A1 Expression in Lungs of instilled mice => Biomarker

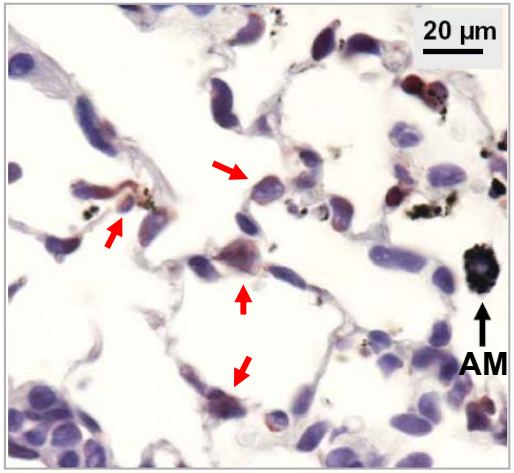
## Protein Expression (Western Blot)




## Immunohistochemistry of Lungs *(S. Takenaka)*




SootL

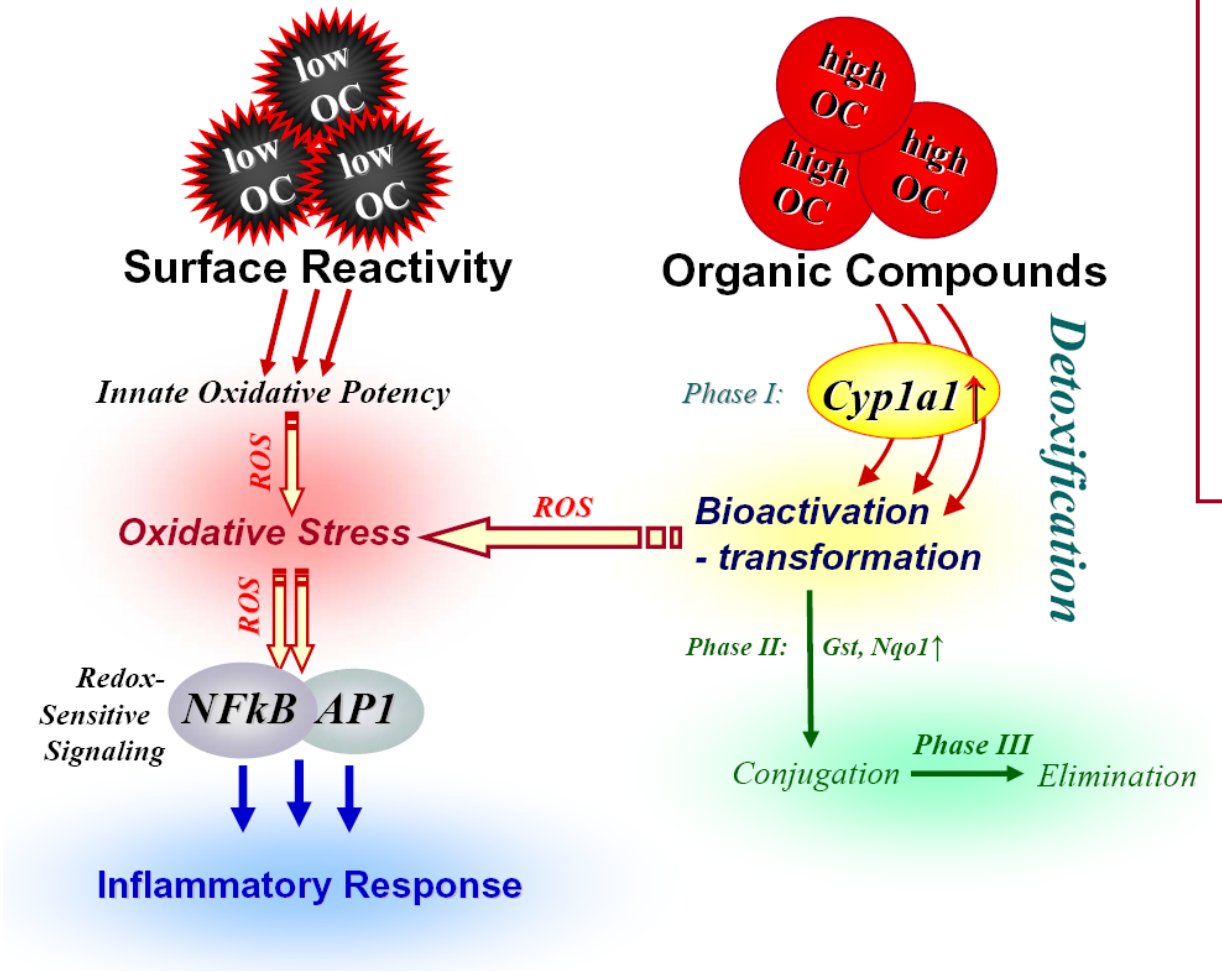


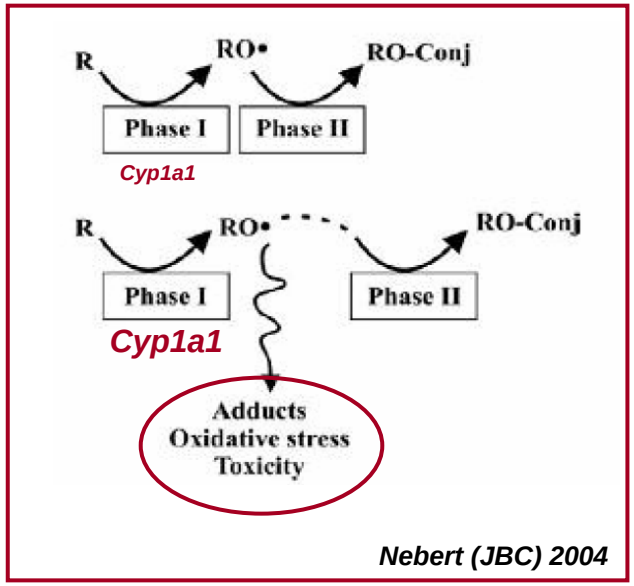

SootH

➔ CYP1A1 expressing alv. epithelial cells

*Stoeger et al. 2009*

# Pathways that Contribute to the Particle Induced Inflammatory Response

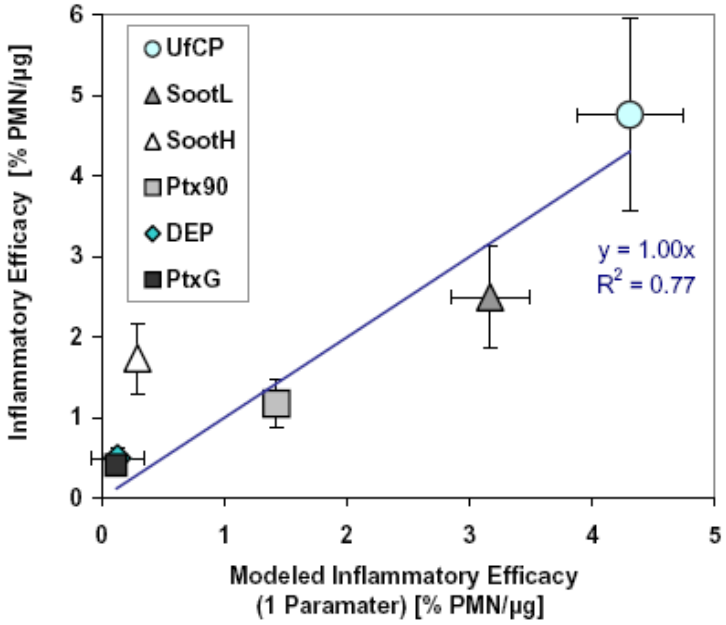



Nebert (JBC) 2004

# Quantitative Model for Inflammatory Efficacy: *A Two Pathway Concept*

**Oxidative Potency = Surface Reactivity Only:**

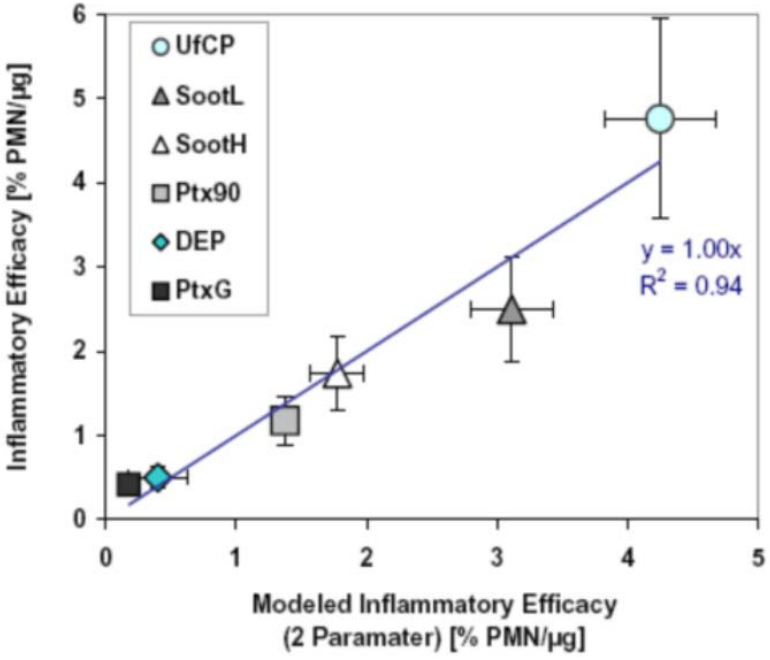



$$I_{Ef} = 5.14 Ox_{Pot} \left[ nmol/\mu g \right]$$

# Relative Contribution of 'Oxidative Potency' or '*Cyp1a1*-Pathway' to Particle Toxicity

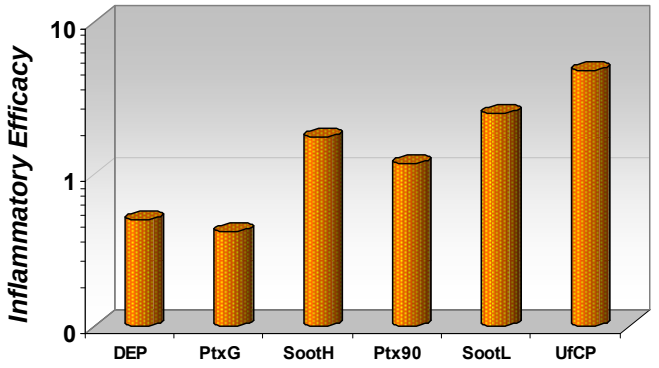
## Two Parameter Model

### Inflammatory Toxicity as a Product of: Surface Reactivity + Metabolic Activation

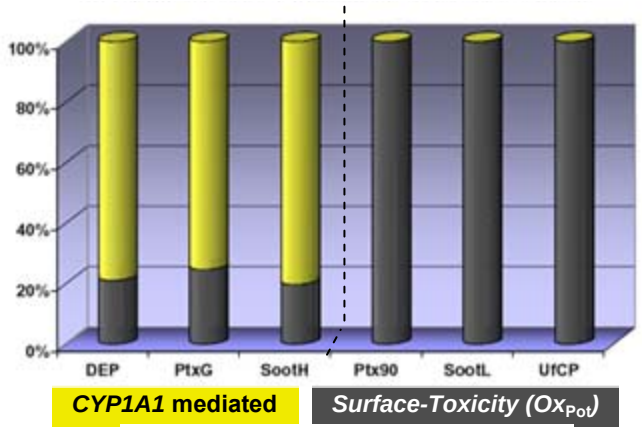



$$I_{Ef} = 5.05 Ox_{Pot}\,[nmol/\mu g] + 0.509\left(GE_{Cyp1a1} - 1\right)$$

Stoeger et al. 2009




### *Contribution to Inflammatory Efficacy*

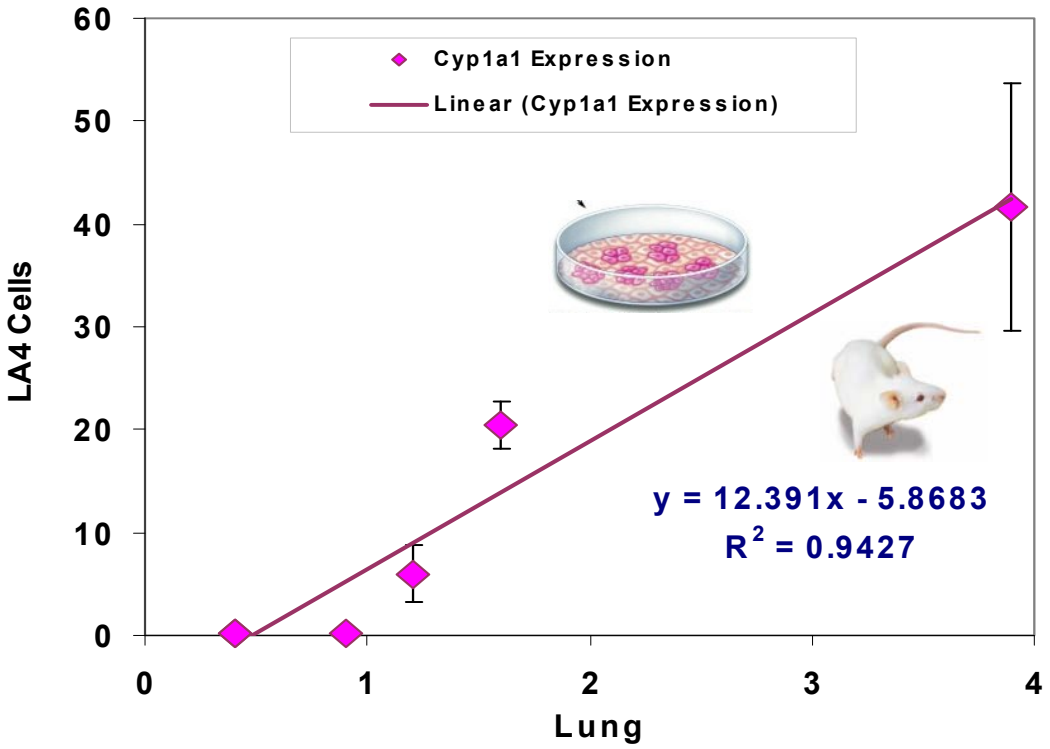



Stoeger unpublished

# Can we Predict the Toxicity from *In Vitro* Data Only?

=> *Find Cell Line with Lung-Like Cyp1a1 Inducibility*

**_In Vivo_ (Mouse) – _In Vitro_ (LA4 Cells) Correlation of _Cyp1a1_ Gene Expression**




*Stoeger unpublished*

# *Conclusions for Combustion Derived Nanoparticles:*

- **Major contribution of 'Surface toxicity' to total particle toxicity**
- **Toxicity of combustion derived nanoparticles is not necessarily depending on organic contribution (bioavailability / bioactivity of OC?)**

  ***SootL (7% OC) even exceeds inflammatory efficacy of SootH (19% OC)***

⇒ ***Impact on toxicity of modern DEP! (low OC, high $Ox_{Pot}$?***
***Su et al. (Environ. Sci. Technol.) 2008: EuroIV-DEP more toxic than BS-DEP***

- **Toxicity or 'Inflammatory Efficacy' can be predicted by a two parameter, *in vitro* model that involves:**

  1. **Oxidative potency *(cell free assay)***
  2. ***Induction of Cyp1a1 gene expression (cell based in vitro assay)***

# Thank You For Your Attention

# &

# Thanks to:

## my colleagues at the Institute of Lung Biology and Disease (iBLD)

**… Shinji Takenaka, Otmar Schmid, Daniela Dittberner, Bärbel Ritter, Birgit Frankenberger, Ewin Karg, Holger Schulz …**