Differentiation between sources of particle-induced oxidative stress: surface area versus organic compounds

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At present it is commonly hypothesised that the surface toxicity of soot particles originates from adsorbed redox-active components, which cause oxidative stress responses by reactive oxygen species (ROS) that in turn may lead to pulmonary or even systemic inflammation.
In this study we address the question whether the inflammatory response of mice to particle exposure can be predicted by the *in vitro* assessed oxidative potential of these particles. To this end we assessed the oxidative potency of six types of carbonaceous NPs (10 to 50nm in diameter; combustion and spark-discharge generated particles; 1 to 20% OC content) by measuring the consumption of an indicator antioxidant, *ascorbic acid*, in a cell free, physiologically buffered system. There was a good linear correlation between the *in vitro* oxidative potency of the different particles and their specific surface area. Furthermore, comparison of the oxidative *in vitro* effect and the *in vivo* inflammatory response (PMN influx into the lung 24h after intratracheal particle instillation) revealed a good linear correlation for five out of the six NPs investigated here, i.e., particle surface area can be directly related to the *in vitro* and *in vivo* response. The only exception was the SootH sample (high-OC flame soot; OC = 19%), for which the *in vitro* test underestimated its *in vivo* toxicity by a factor of 3. Since this was not observed for the other high OC sample investigated here (diesel exhaust particles (DEP); OC = 20%), the OC content alone could not account for this discrepancy. Hypothesizing that bioavailability of OC plays an important role, we searched for specific genetic expression markers by qPCR and immunoblotting of mouse lung samples to identify those particle types with *bioavailable* toxic organics. Among all candidates of inducible phase I and II detoxication enzymes our expression analysis detected only the cytochrome P450 oxidase Cyp1A1 to be significantly induced by the OC rich particles, namely SootH and weaker by DEP. Since metabolic activation of aromatic hydrocarbons by Cyp1A1 is known to generate intracellular oxidative stress, this suggests that bioavailibilty of OC may contribute to the *in vivo* inflammatory response of NPs.
In summary, adequate prediction of *in vivo* particle toxicity based on *in vitro* tests requires an *in vitro* test for the oxidative potential related to particle surface area combined with a test for the bioavailibilty of particle adsorbed bioactive compounds, such as Cyp1A1 expression.

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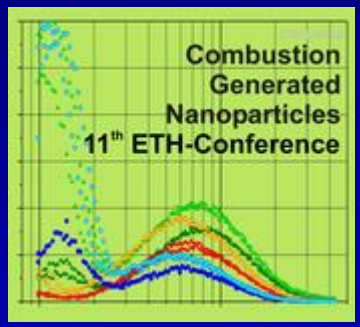
11th ETH Conference on Combustion Generated Nanoparticles

Zurich, 13th - 15th August 2007



Differentiation between Sources of Particle-Induced Oxidative Stress:

Surface Area versus Organic Compounds

Tobias Stoeger

National Research Center for Environment and Health

Institute for Inhalation Biology

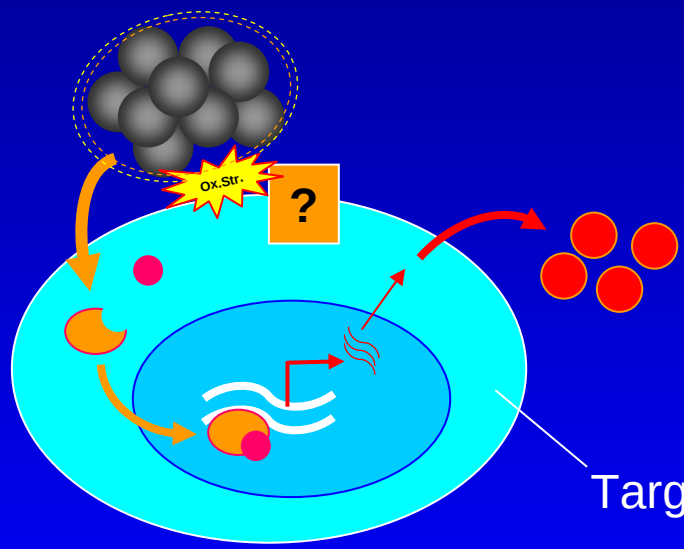
Particle Toxicity:
Hypothetical Mode of Action

Particles
(low solubility / low toxicity)

oxidative stress

inflammatory response

Lung Disease
(fibrosis, lung cancer…)



Proinflammatory Mediators:
Chemokines/Cytokines,
Prostaglandins …

Target Cell

particle deposition on cell surface → induction of oxidative stress ⇨ Redox-sensitive signalling cascade ⇨ proinflammatory gene expression → release of proinflammatory mediators

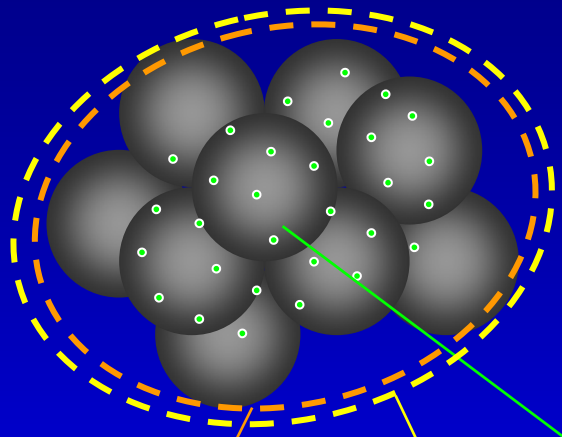
Sources of Particle Induced Oxidative Stress

Particles
(low solubility / low toxicity)

oxidative stress / ROS

inflammatory response

Lung Disease
(fibrosis, lung cancer…)



Sources for Oxidative Stress

structural surface properties

organic compounds (PAH)

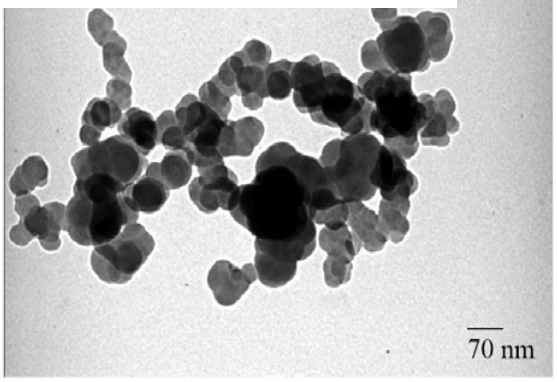
transition metals

Formation of Reactive Oxygen Species

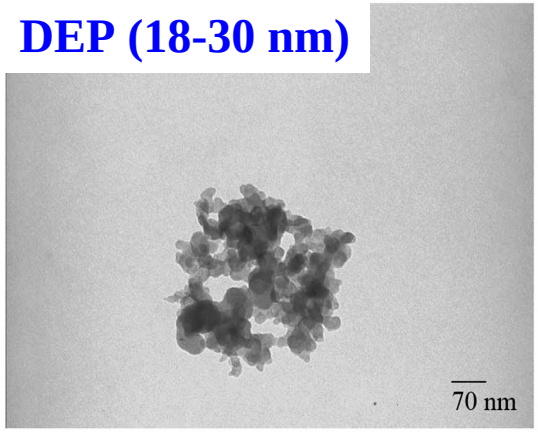
? Can we differentiate sources of oxidative stress / inflammation?

Investigated Carbonaceous Nanoparticles

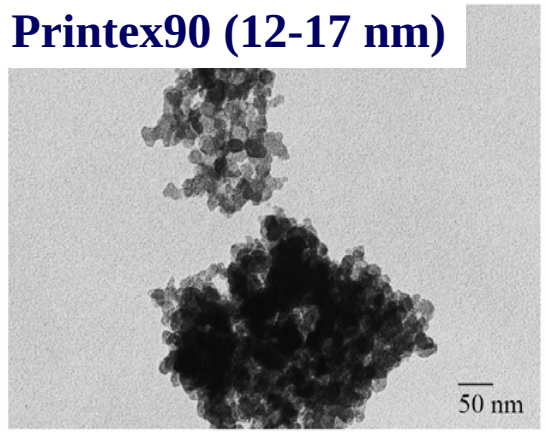
PrintexG (30-60 nm)



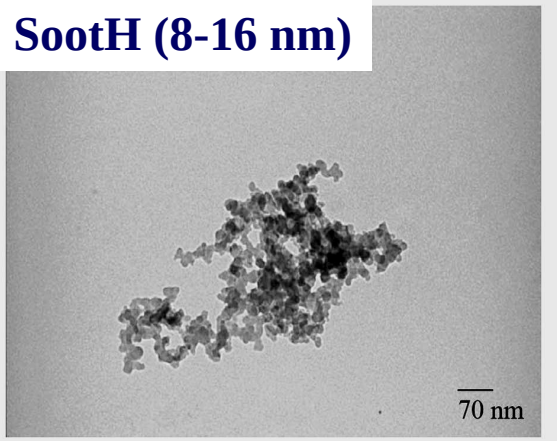
DEP (18-30 nm)



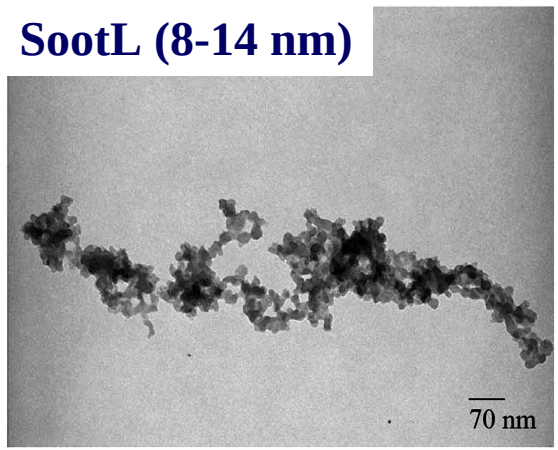
Printex90 (12-17 nm)



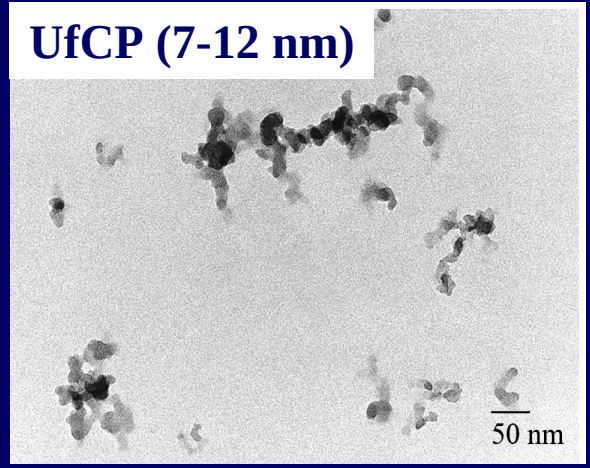
SootH (8-16 nm)



SootL (8-14 nm)



UfCP (7-12 nm)



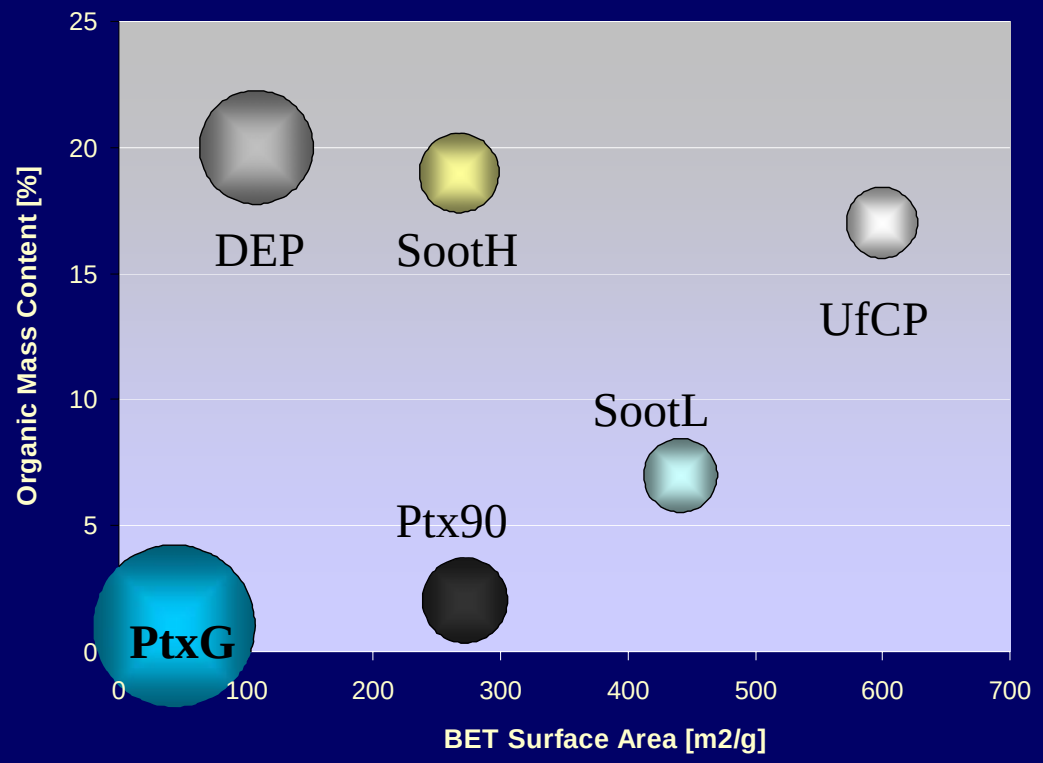
Investigated Carbonaceous Nanoparticles

Pigment Black	Spark Discharge	Flame Soot	Diesel Exhaust Particles
•Printex90 •PrintexG	•UfCP	•SootH •SootL	•DEP **SRM1650a**

Particle Characteristics

NPs	Diameter [nm]	Org. Content [%]	BET surface [m2/g]
DEP	25	20	108
PtxG	51	1	43
Ptx90	14	2	272
SootH	12	19	268
SootL	11	7	441
UfCP	10	17*	600

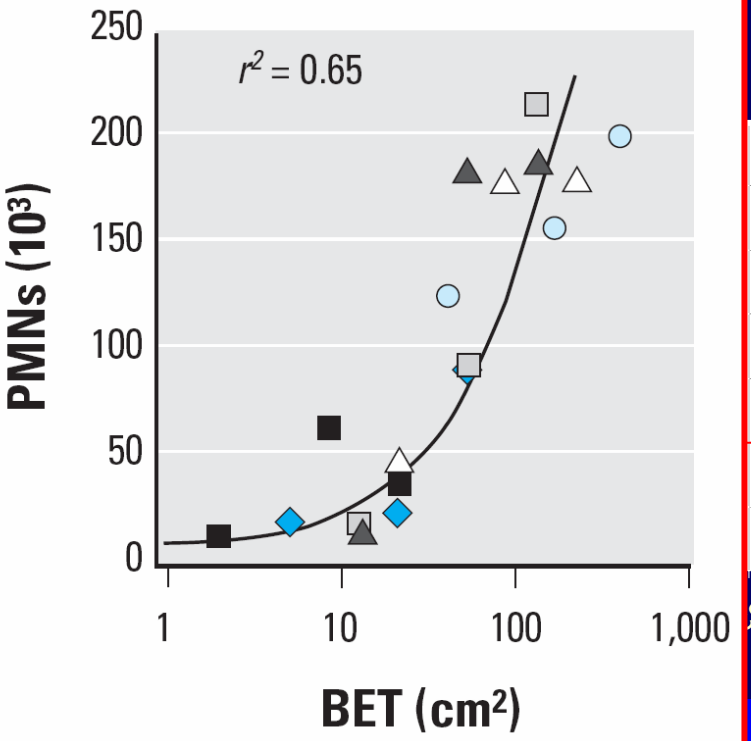
* rather <4%, *Frampton 2004*



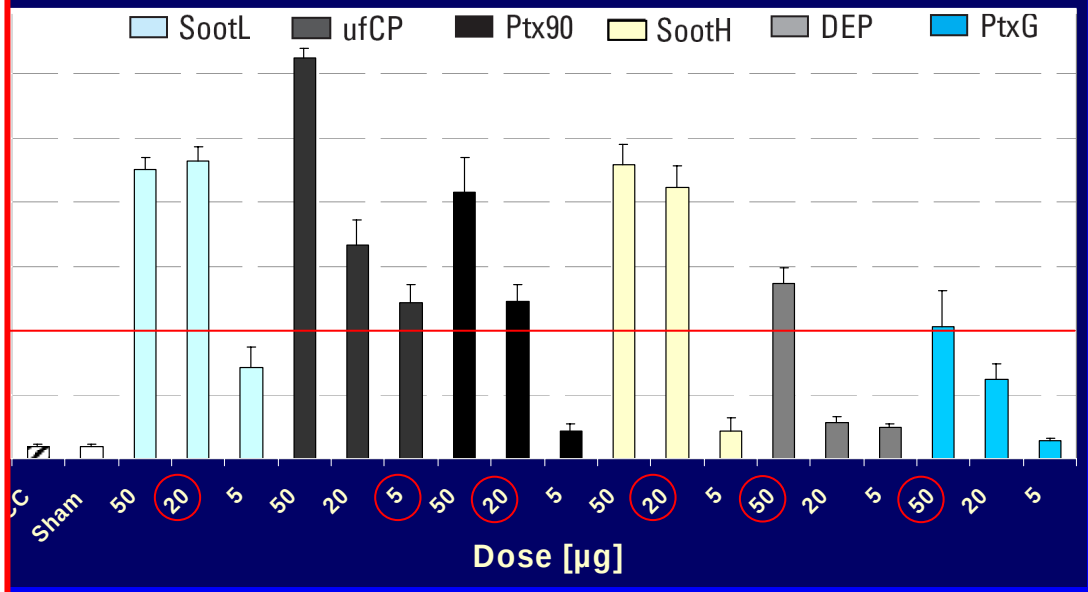
In Vivo Toxicity in Mice

Proinflammatory Effects of Intratracheal Instilled NPs

Surface Area Correlation



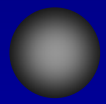
PMN Influx in BAL 24h after Instillation



Stoeger et al. EHP 2006

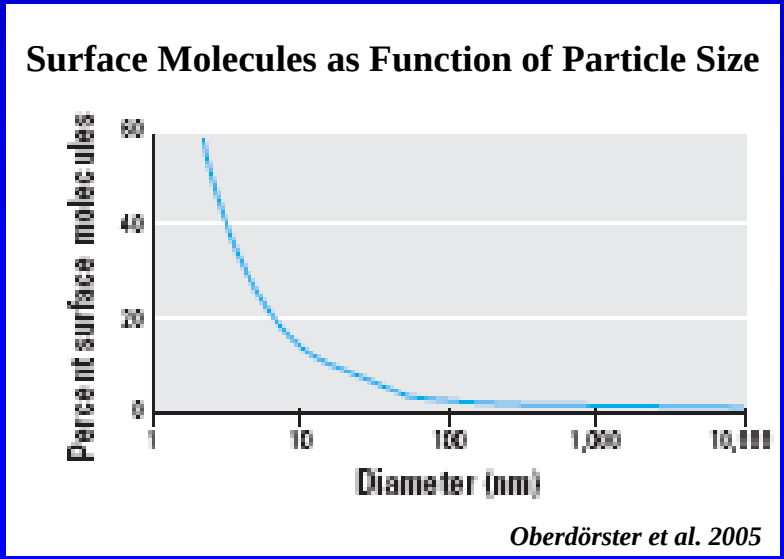
Surface Area Drives Pulmonary Inflammation

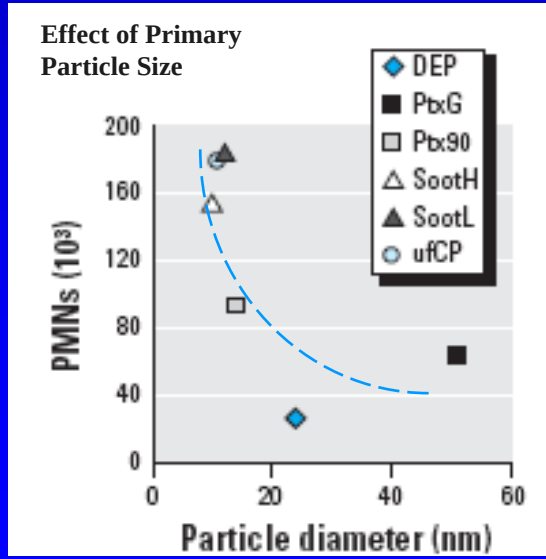
Surface Toxicity



= Mass =
<< Surface <<

	Dose:	Related to Number of:
• Soluble Matter:	Mass	Reactive Molecules
• Insoluble PM:	Surface Area	Reactive Surface Molecules





Is

Particle Surface Toxicity

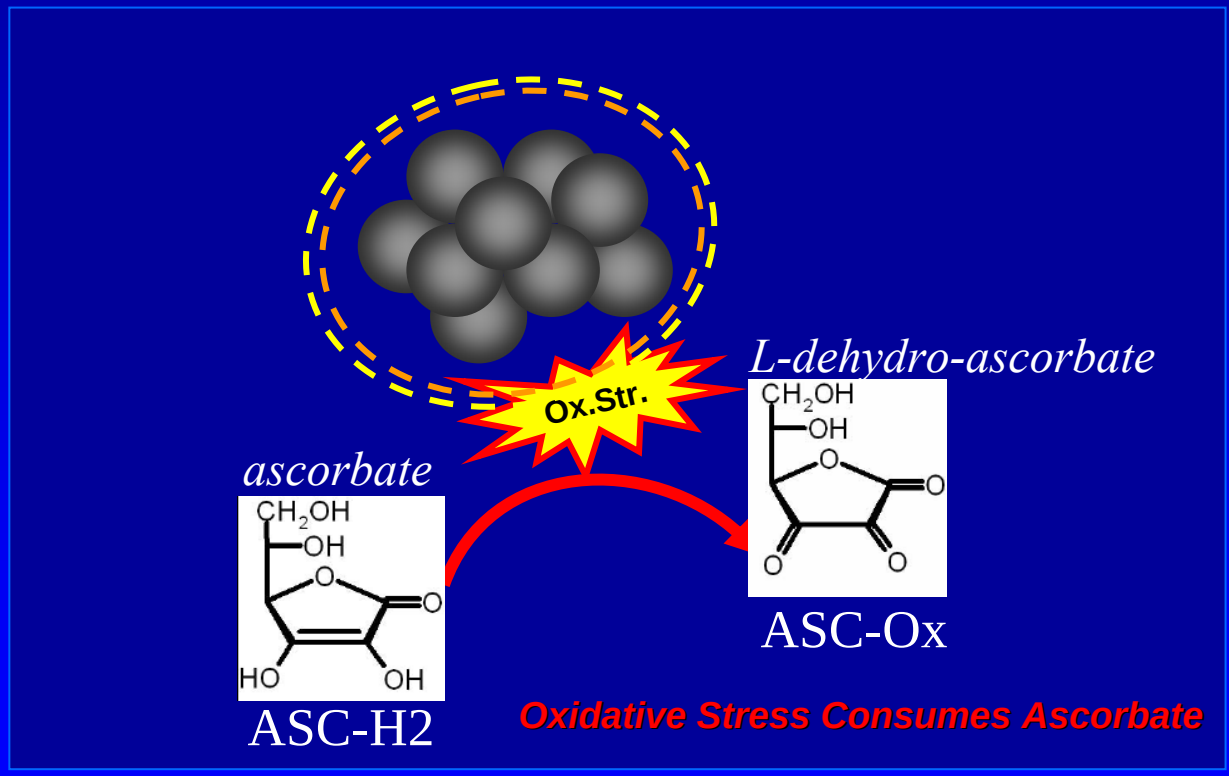
a consequence of

Particle's Own Oxidative Properties?

How to Assess 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 surface reactivity.

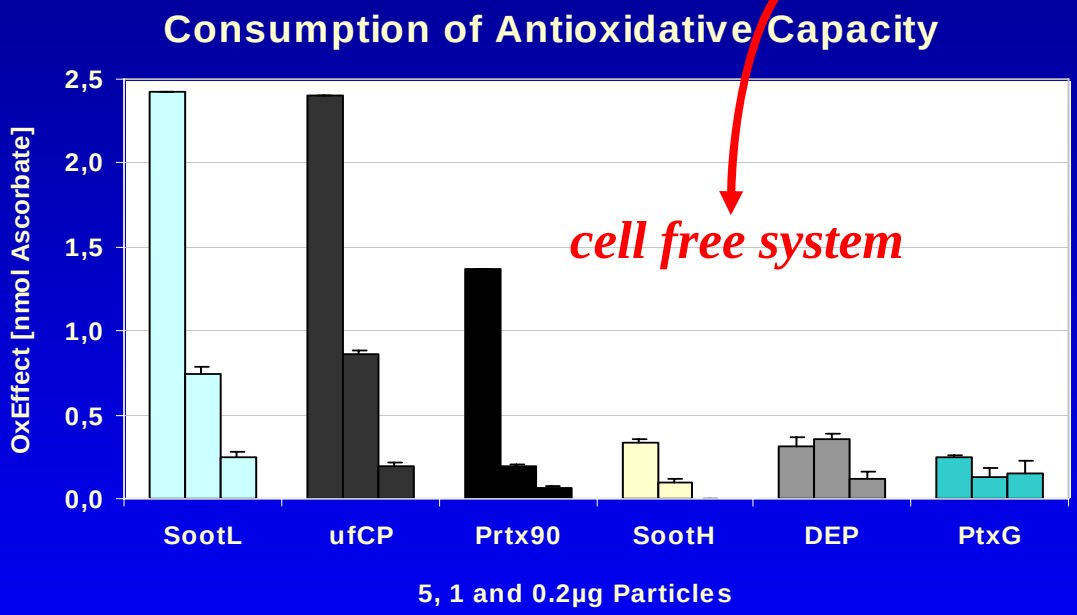


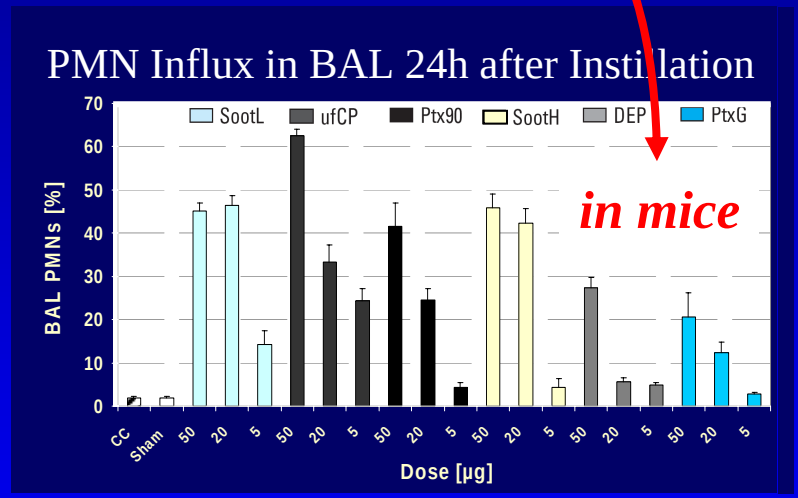
PHOTOCHEM quantiatation of antioxidative capacity

fast photochemical excitation of radical formation combined with sensitive luminometric detection

Oxidative Potency Assessed by the Consumption of Vitamin C *In Vitro*

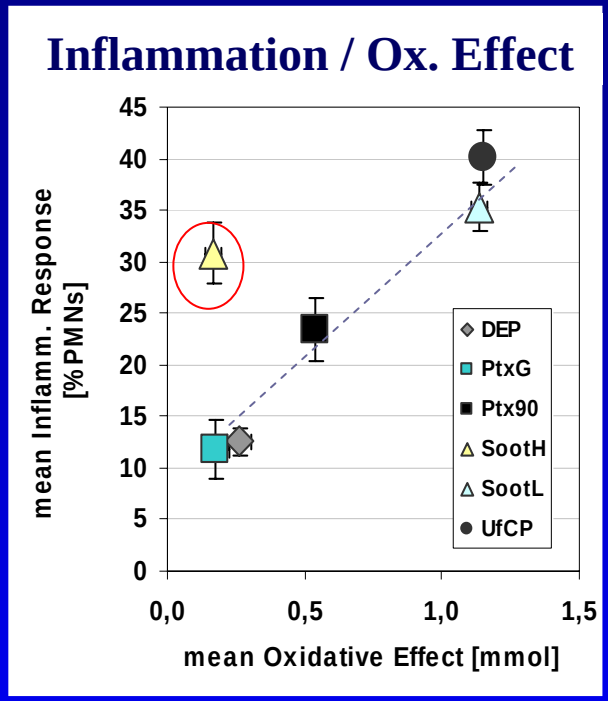
Association?



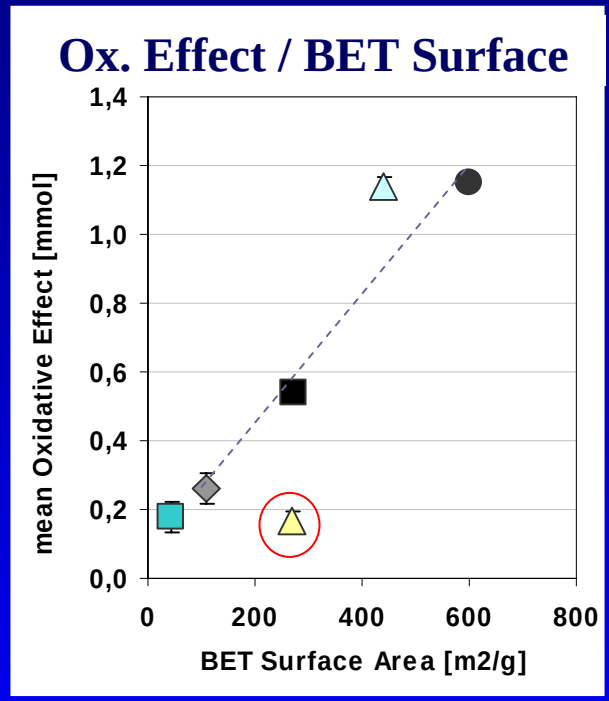


In Vitro Oxidative Potency as Predictor for Inflammatory *In Vivo* Response!?

Correlations: *In Vivo* / *In Vitro* Effects and Surface Area / *In Vitro* Effects



SootH:
Oxidative effect underestimates inflammatory response ~2fold



SootH:
Surface area overestimates oxidative effect ~3fold

Only SootH

⇒ Inflammatory response: not explained by oxidative potency

⇒ Oxidative potency: not explained by particle surface area

Because of Organic Mass Content?

Pigment Black	Spark Discharge	Flame Soot	Diesel Exhaust Particles
•Printex90 •PrintexG	•UfCP	•SootH •SootL	•DEP **SRM1650a**

Particle Characteristics

NPs	Diameter [nm]	Org. Content [%]	BET surface [m2/g]
DEP	25	20	108
PtxG	51	1	43
Ptx90	14	2	272
SootH	12	19	268
SootL	11	7	441
UfCP	10	*17 (4)*	600

TG-EGA-MS

Conflict: OC-DEP = OC-SootH

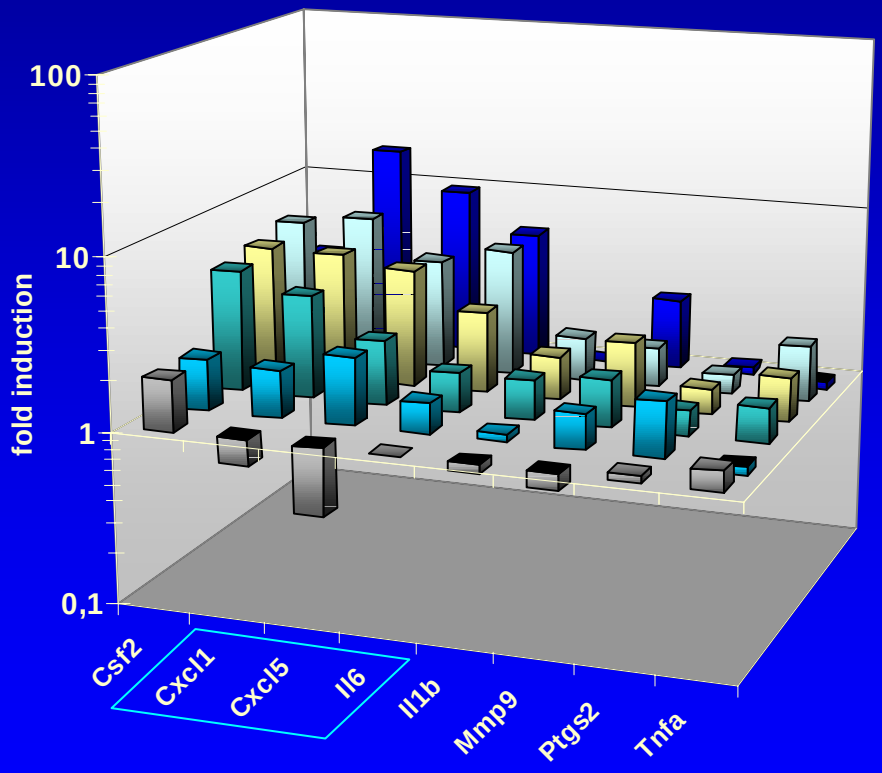
! OC does not give information about bioavailability or “toxicity” of particle adsorbed compounds!

⇒ need for a biologic marker for bioavailability

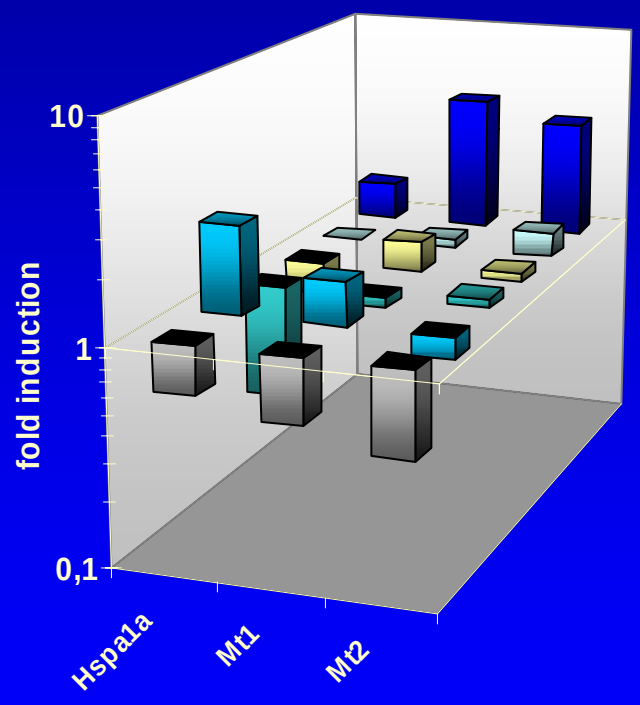
Expression Profiling to Identify Genes Suitable as Marker for Bioavailable Organics

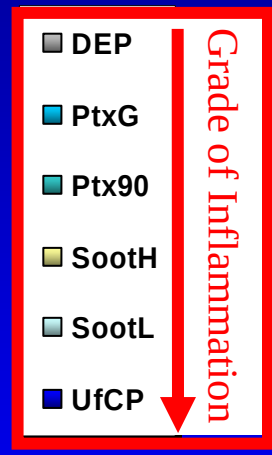
Lung RNA extraction 24h after Particle Instillation

Inflammation Pathway



Stress Response



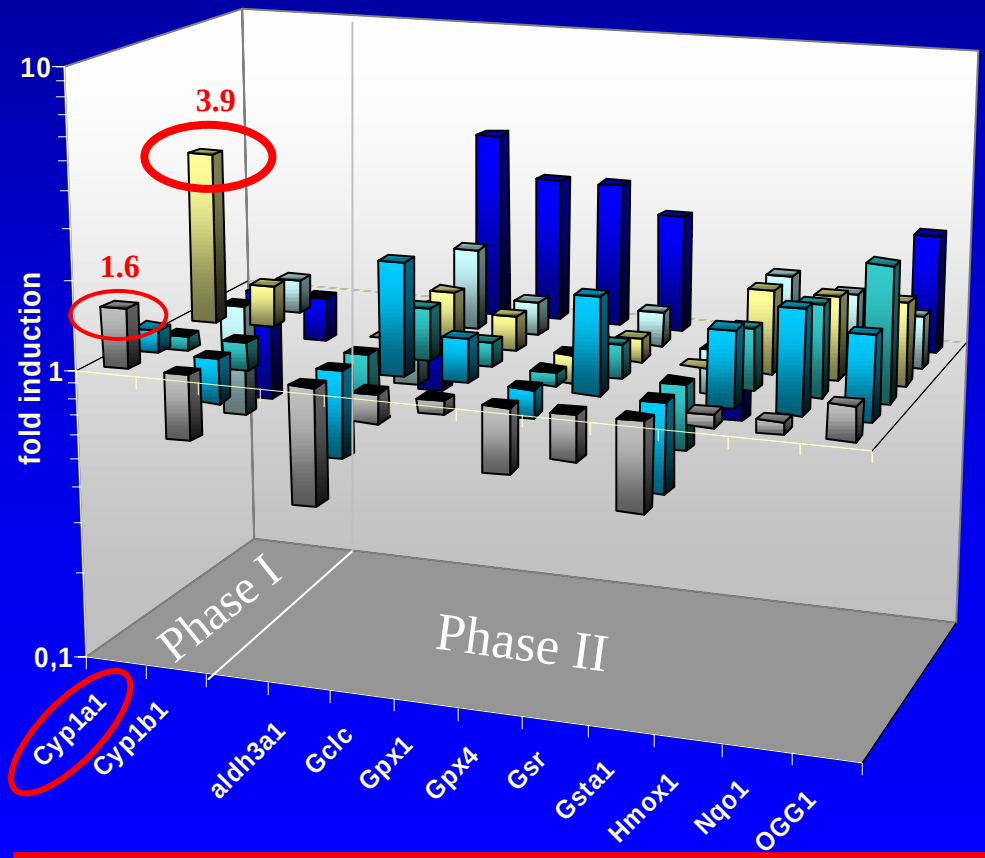


Non of the Selected Inflammation & Stress Response Marker Seems Specific for Bioavailable Organics

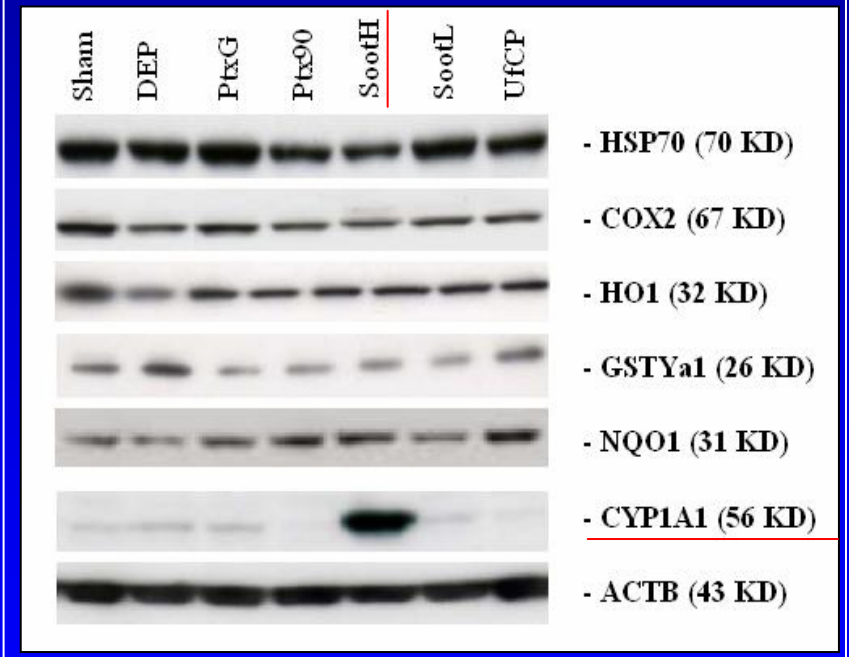
Expression Profiling to Identify Genes Suitable as Marker for Bioavailable Organics

Lung RNA extraction 24h after Particle Instillation

Phase I and II Detoxification



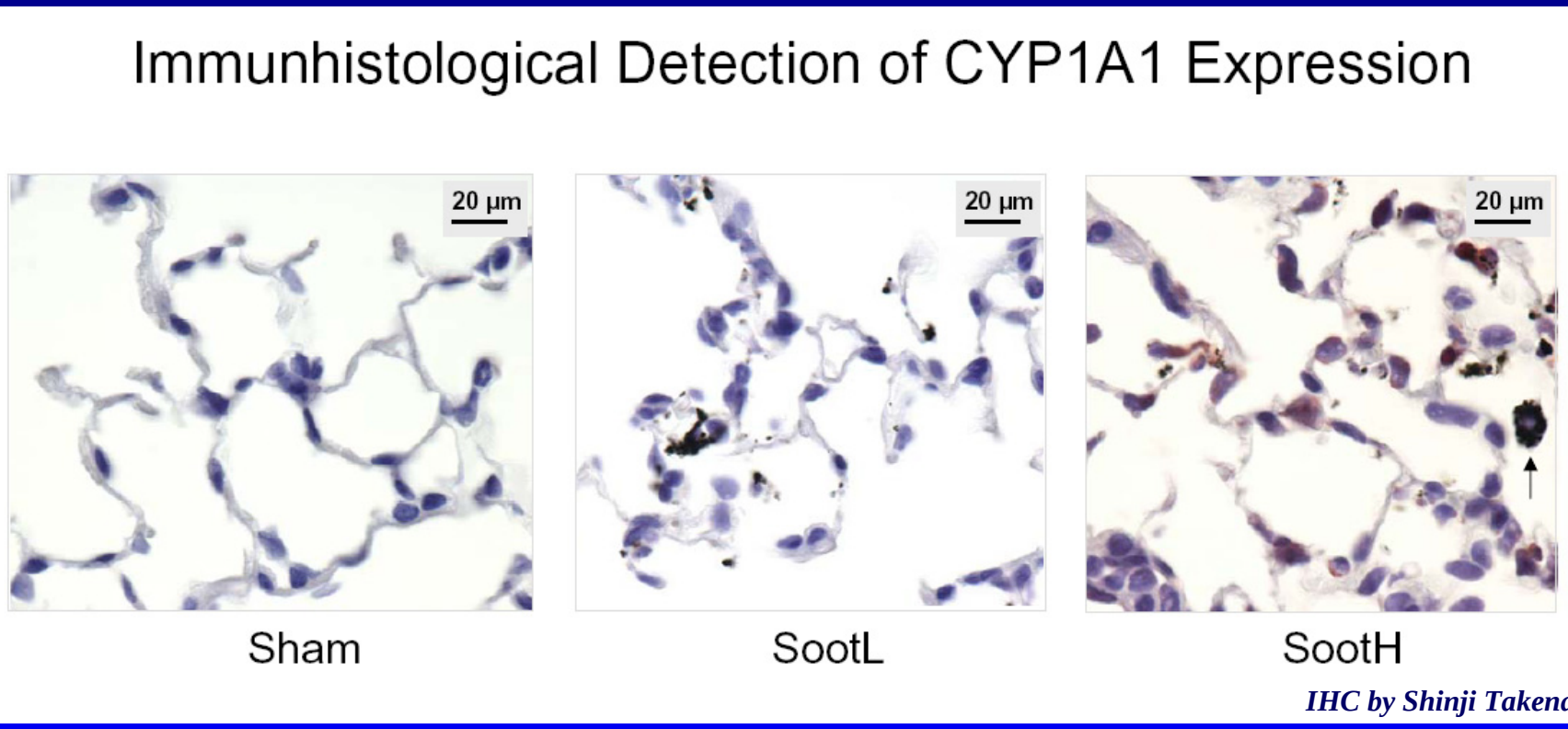
Protein Expression (Western Blot)



Western Blot affirms to RNA Data

Cyp1a1 as Marker for Bioavaliable Organic Compounds

Localization of Cyp1a1 by Immunohistochemistry



Detection of CYP1A1 positive cells only in SootH, and to a weaker extend in DEP, but **not** in SootL instilled lungs.

Role of CYP1A1 in PAH-Detoxification

Diagram of Oxidative Stress During Phase 1 + 2 Detoxification
(Nebert et al., JBC 2004)

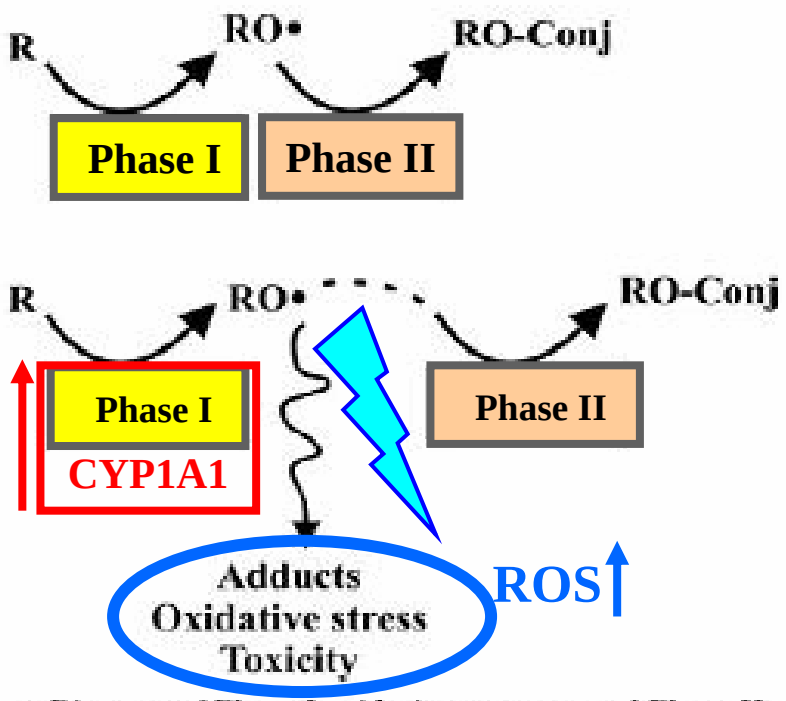
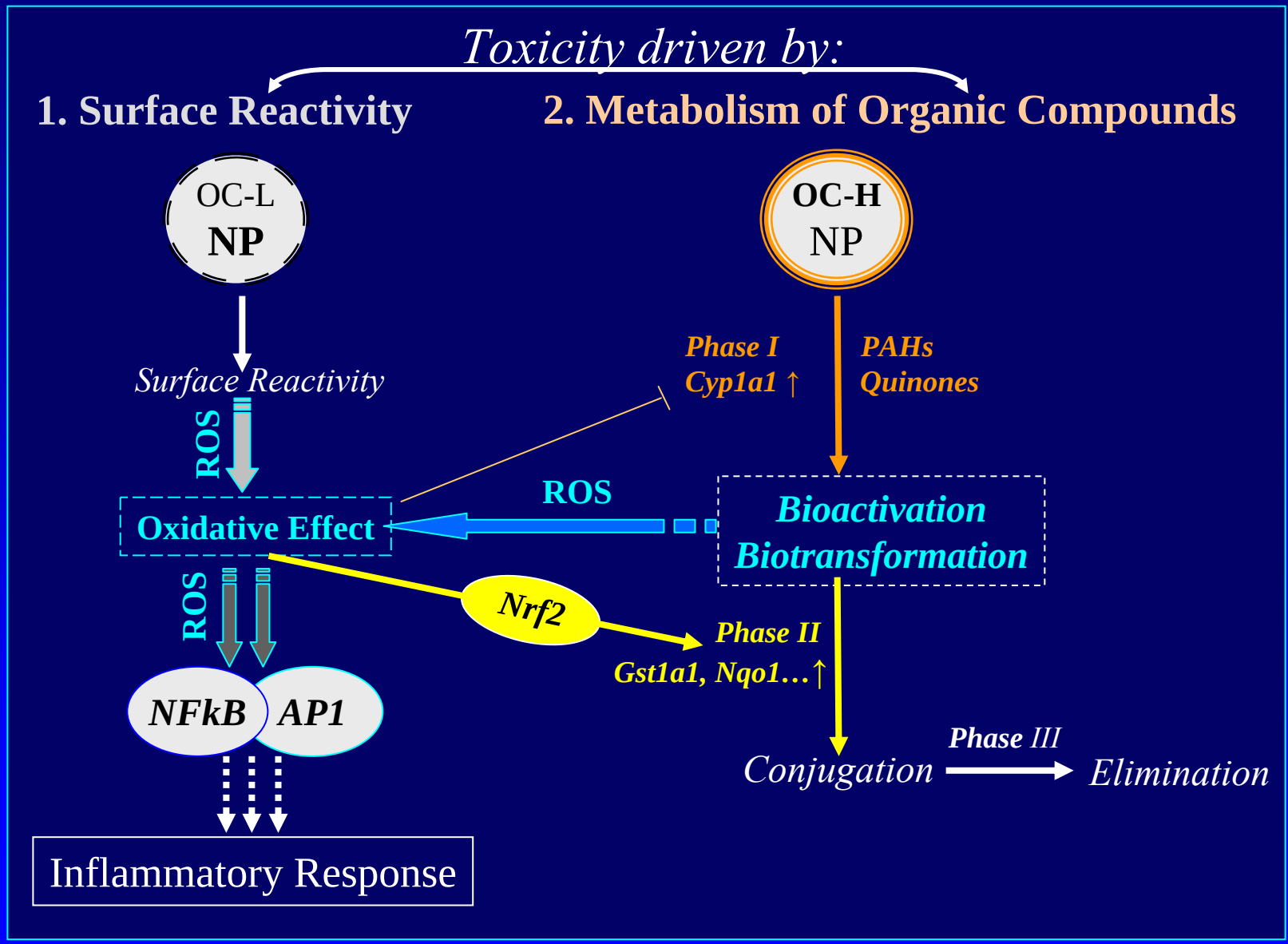


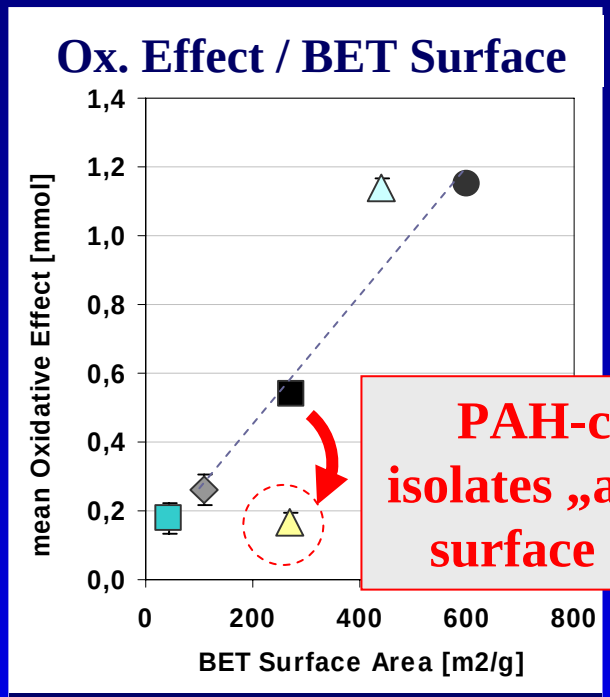
FIG. 4. Diagram of Phase I oxidative enzymes and Phase II conjugating enzymes that can be geographically subcellularly "tightly coupled" (*top*) or "loosely coupled" (*bottom*). *R*, any CYP1 substrate; *RO*•, reactive intermediate; *RO-Conj*, inactive product. Both Phase I enzymes and Phase II enzymes can be membrane-bound, both can be cytosolic, or one can be membrane-bound and the other cytosolic. Phase II metabolism includes glutathione *S*-transferases, UDP glucuronosyltranaferases, and various acetyl-, methyl- and sulfotranaferases (6, 10, 21, 59).

Gene	Pathway
Cyp1a1	detoxification phase I
Cyp1b1	detoxification phase I
Gclc	detoxification phase II
Gpx1	detoxification phase II
Gpx4	detoxification phase II
Gsr	detoxification phase II
Gsta1	detoxification phase II
Nqo1	detoxification phase II

Model for Particle Toxicity Related to Oxidative Stress

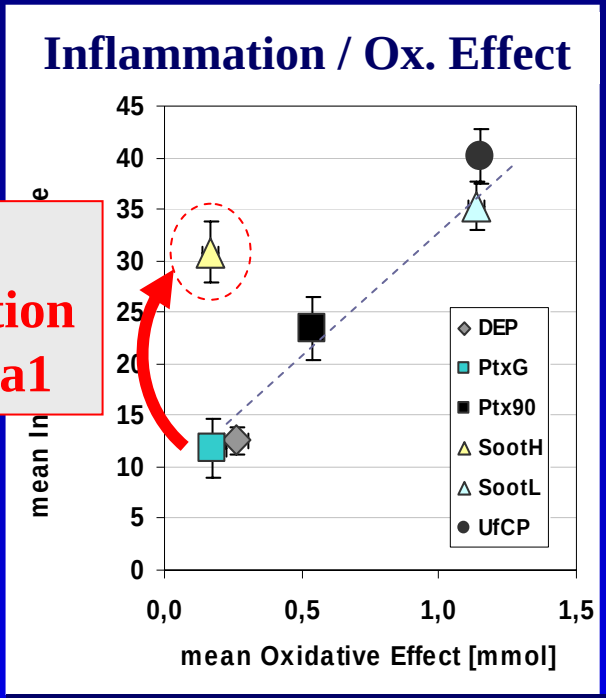


Possible Explanation of Discrepancy:



PAH-coat isolates „active“ surface area

PAH Bioactivation via Cyp1a1



- „Active“ carbon black surface coated with PAH.
- No ROS formation by PAH in cell free system (no Cyp1a1).

⇒ **Reduced oxidative power of SootH**

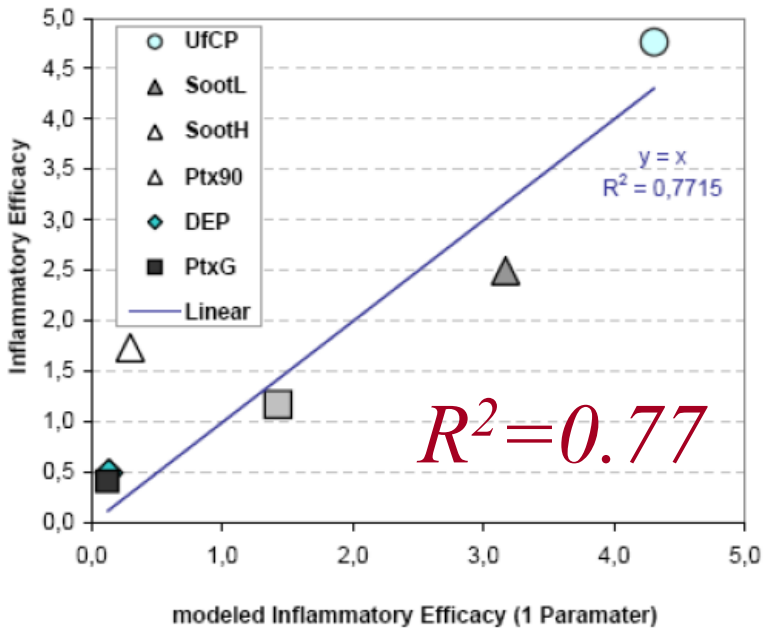
Biotransformation of surface bound PAH via Cyp1a1 generates ROS, which in turn induces inflammation.

⇒**Enhanced inflammatory response to SootH**

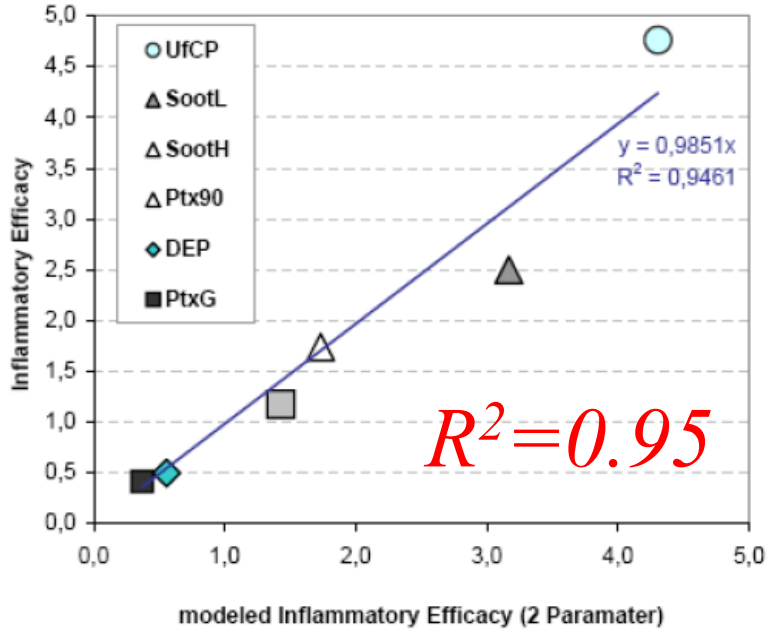
Conclusion:

Modelling Inflammatory Efficacy by One or Two Parameters

Oxidative Potential



Ox. Potential + Cyp1a1 Induction



Stoeger unpublished

Thank You !

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