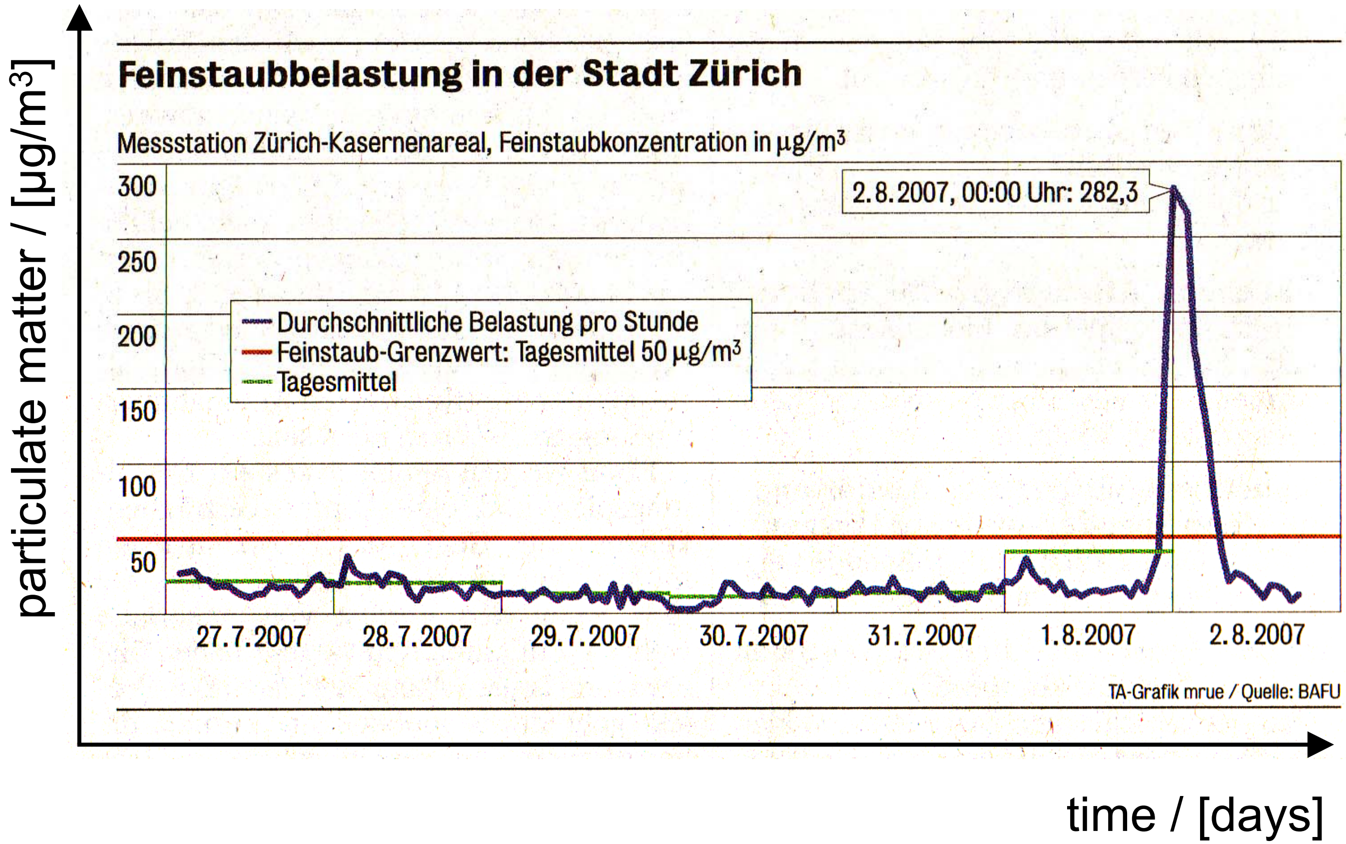
Exposure of engineered nanoparticles to human lung epithelial cells:

Influence of chemical composition and catalytic activity on oxidative stress

Ludwig Limbach, Robert Grass, Peter Wick,
Arie Bruinink, Wendelin Stark

ETH Zurich
Empa St. Gallen

1st august generated particles



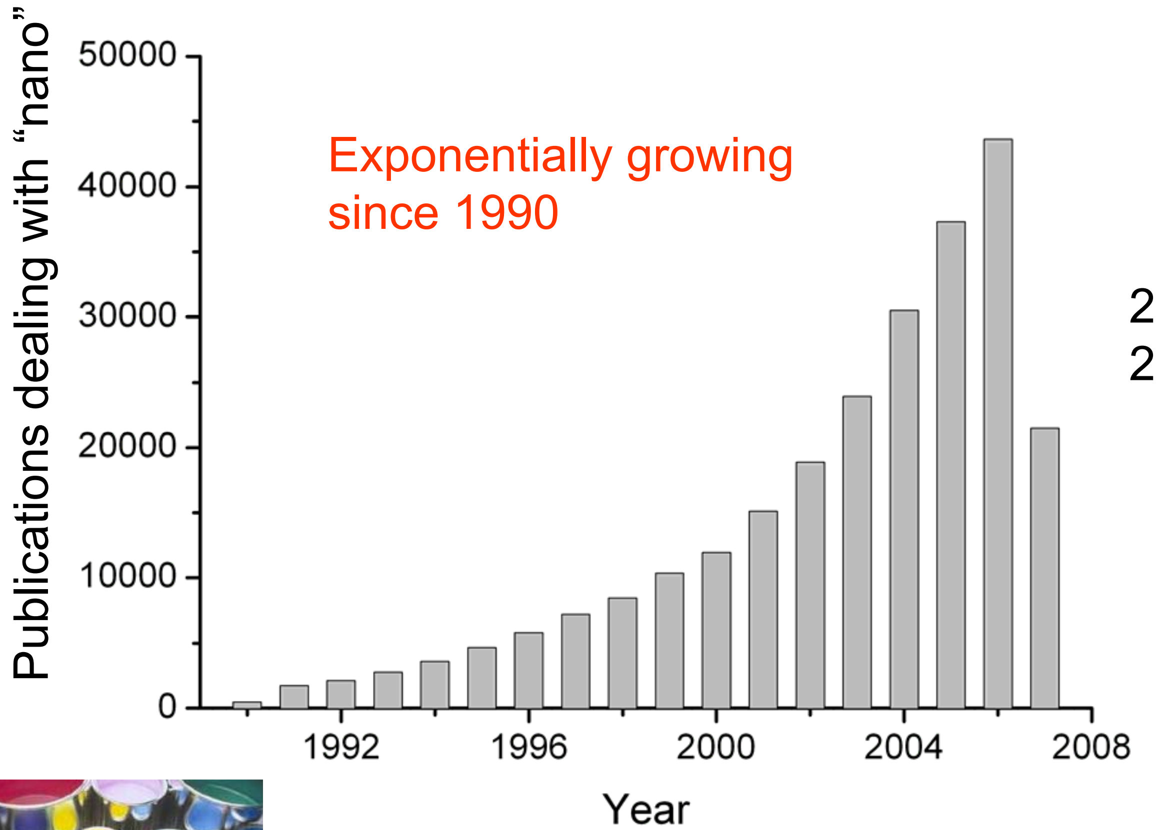
Particulate matter => engineered nanoparticles

Tagesanzeiger, 03. August 2007

Agenda

- increasing market of engineered nanoparticles
  - examples
- quantitative uptake of nanoparticles in cells
- Reactive Oxygen Species (ROS) generation
  - nanoparticles setup (controls and references)
  - results and discussion
- outlook for a safe development of nanomaterials

Nano is growing



2007 already
21'500 publications

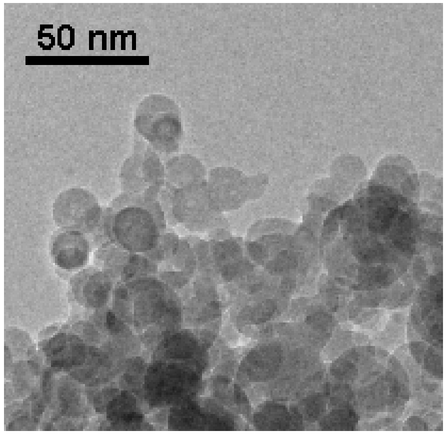
Few products on the market availble, more will come

Two expamles for possible "nano" products are…

synthetic nano-bone cement

Fully synthetic implant materials

- Amorphous TCP:
  - High surface area
  - Faster conversion to apatite
- Bioactive glasses:
  - High in vitro bioactivity
  - Applications in dentistry

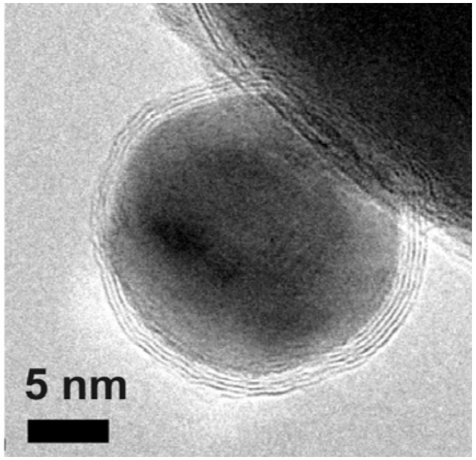


shorter recovery after operations

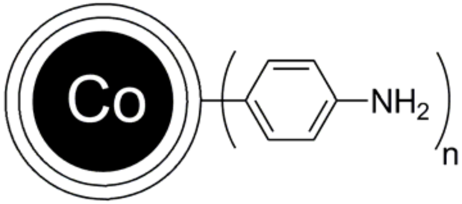
Stark, W.J., et al., ICP Patent, WO2005087660.
Loher, S., et al., *Chem. Mater.*,17(1): 36-42 (2005)
Brunner T.J. et al., *Chem Commun*, 13, 1384-6 (2006)

Coated cobalt nanoparticles

- **magnetic separation**
  - organic chemistry
- **magnetic purification**
  - water treatment
  - antibodies



functionalization of nano-magnets (linker)

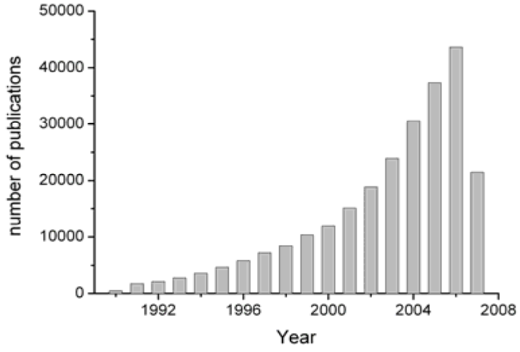


Rate determining step
Purification or separation

Grass R. N., Athanassiou E. K., Stark W. J., *Angew. Chem. Int. Ed.* **2007**, 4996-9 (2007)

Uncertainties of engineered nanoparticles

Seeing opportunities of these nanoparticles



is there an uncertainty or a risk for human or the environment

regarding engineered nanoparticles knowing results of studies and parallels with particulate matter?

What happens when nanoparticles come in contact cells?

How could we look at?

What kind of effect could we see?

How fast do nanoparticles enter living cells?

material

↓

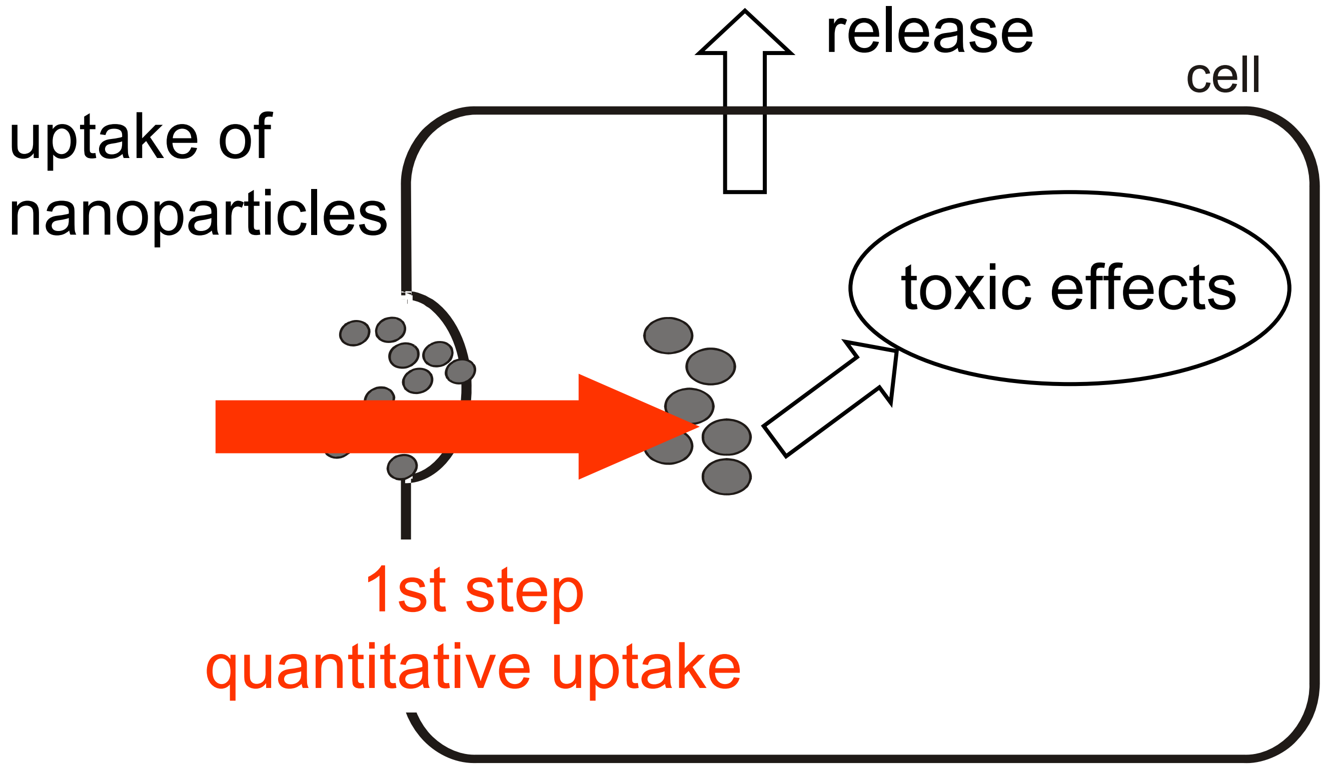
in vitro experiments

↓

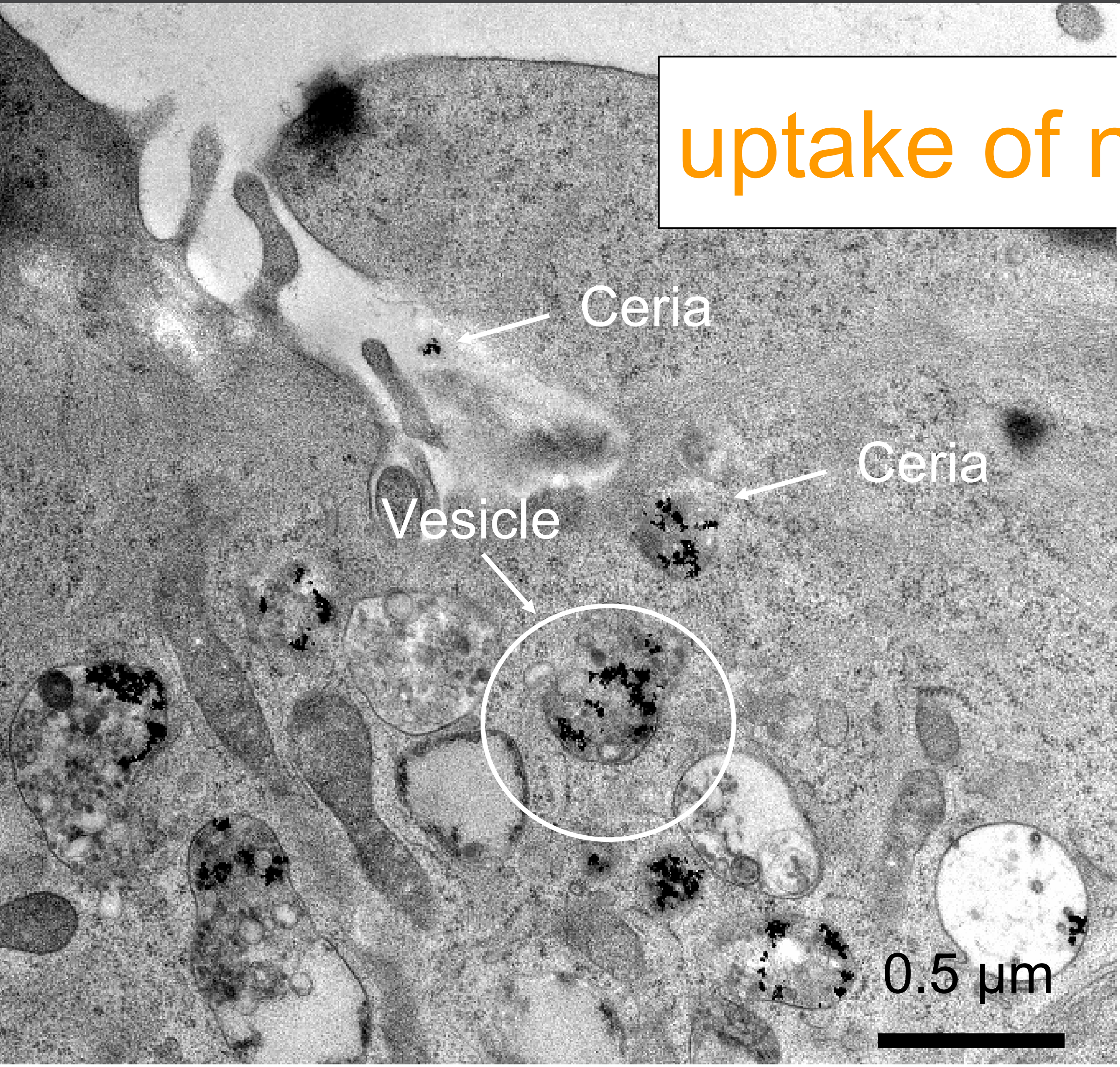
in vivo experiments

↓

predict effects on humans



uptake of nanoparticles



Kirchner et al. 2005 Nano Letters

Wilhelm et al. 2003 Biomaterials

Chithrani et al. 2006 Nano Letters

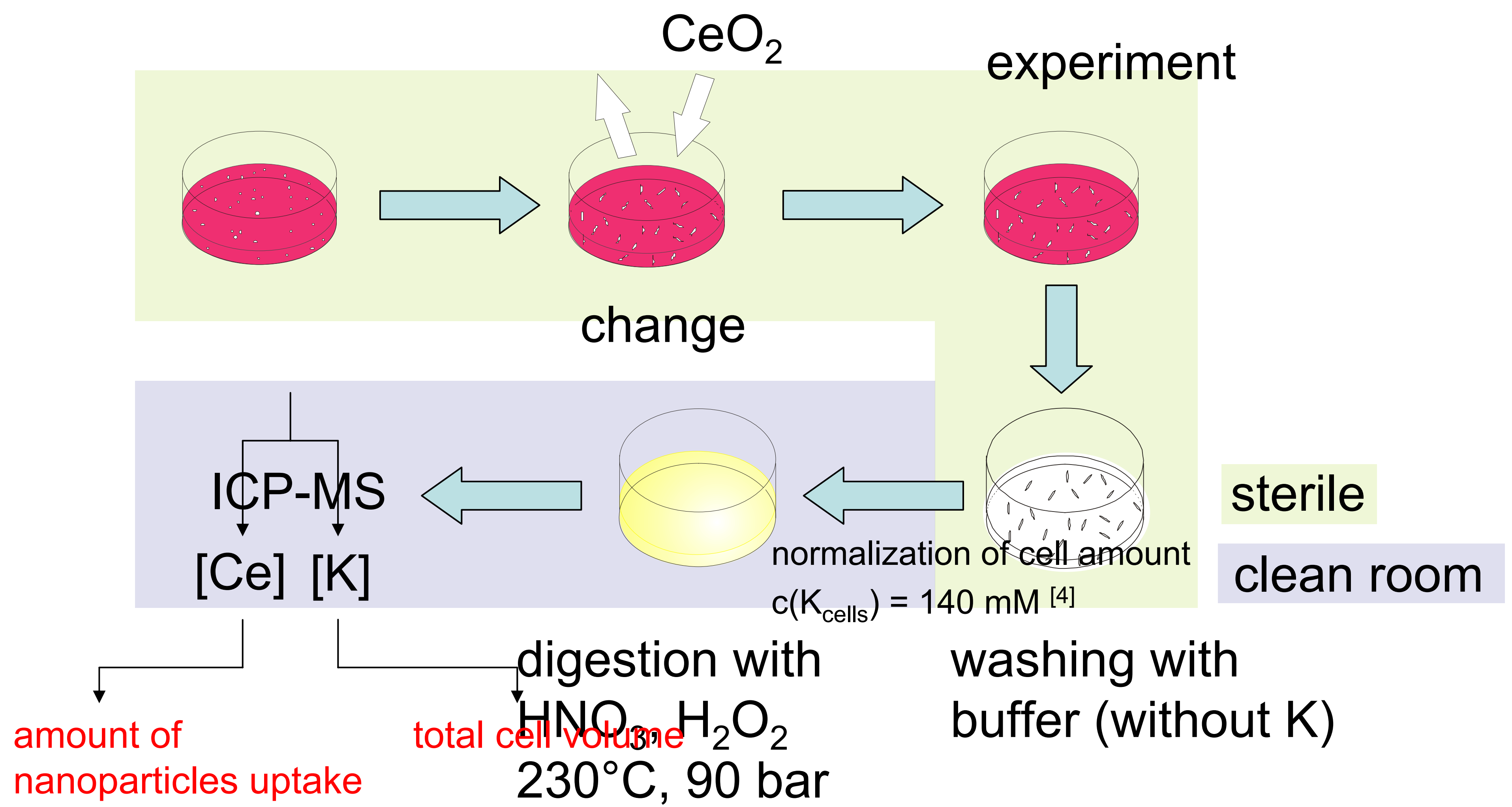
Gupta et al. 2005 Biomaterials

Rothen-Rutishauser 2007 Envi. Sci Tech.

well investigated
and fast uptake

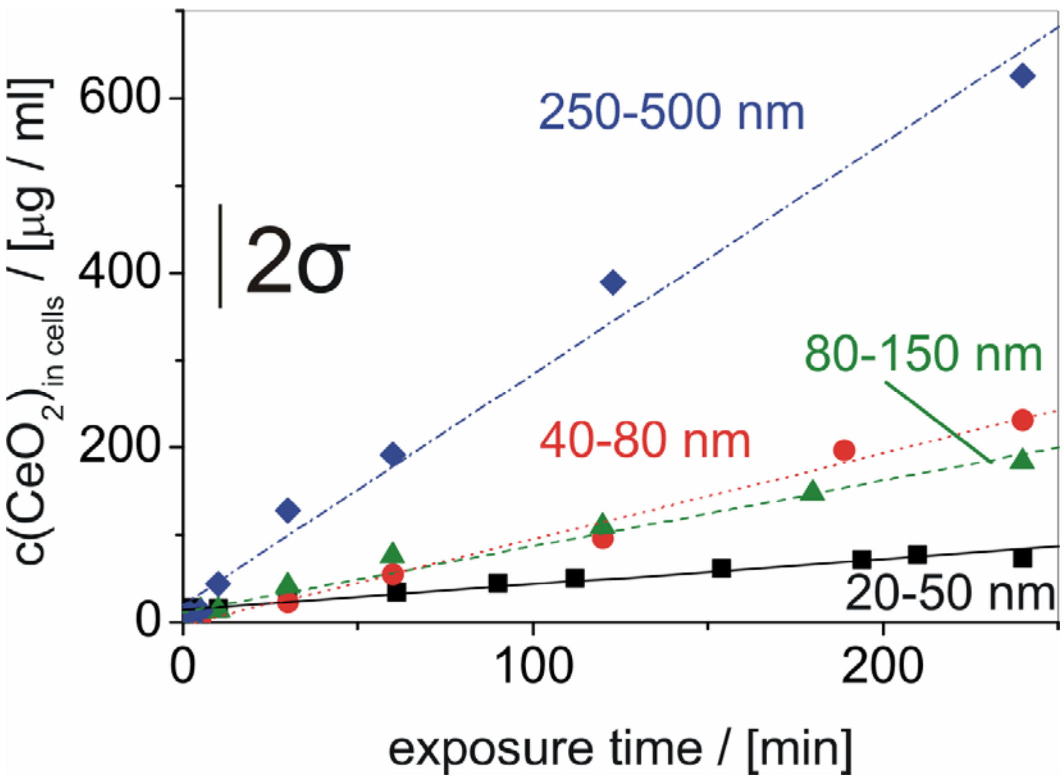
L.K. Limbach, Y. Li, R.G. Grass, T.J. Brunner, M. Hintermann, M. Muller, D. Gunther, W.J. Stark, 2005 Environ. Sci. Tech

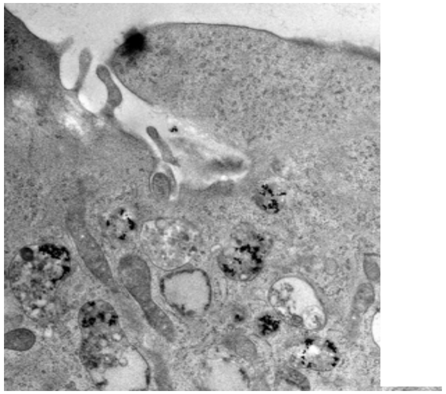
Experimental setup



B. Alberts et. al., *Molecular biology of the cell*

How fast is CeO_2 uptake size dependent?





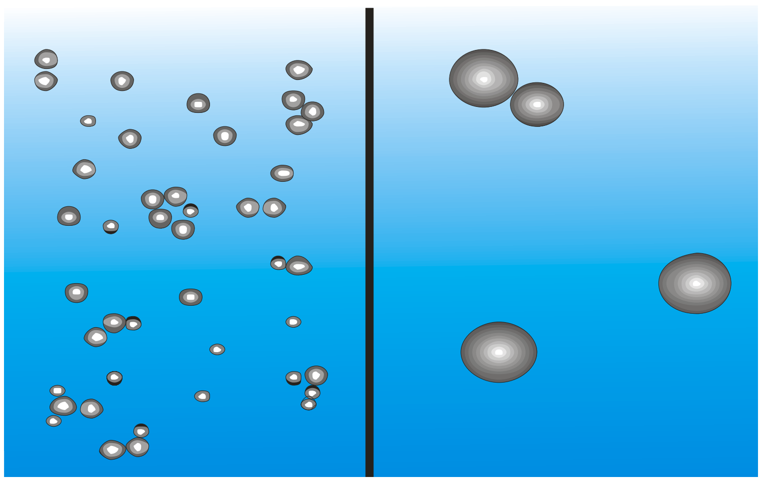
-no saturation within four hours

-linear uptake

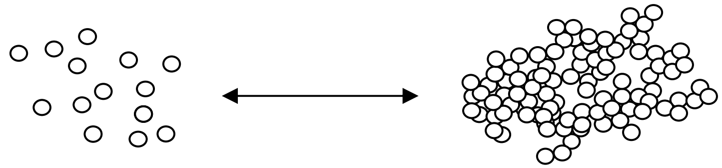
exposure concentration **1 ppm** (µg/ml)

L.K. Limbach, Y. Li, R.G. Grass, T.J. Brunner, M. Hintermann, M. Muller, D. Gunther, W.J. Stark, 2005 Environ. Sci. Tech

Quantitative description of particle agglomeration



same mass concentration
(1 ppm)

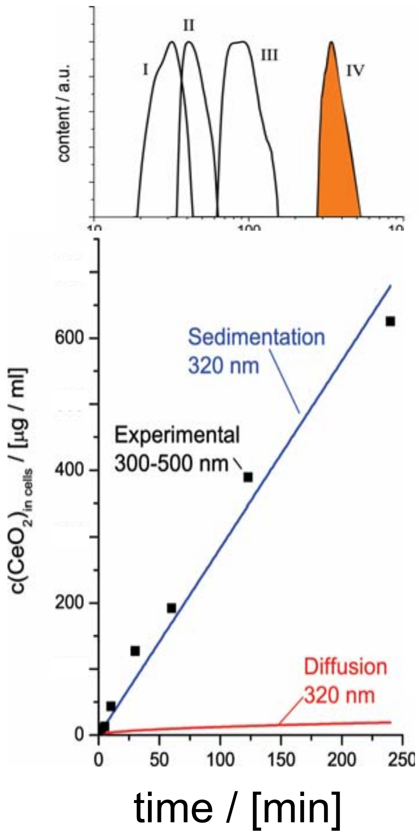


$$\frac{dn_t}{dt} = -\frac{1}{2}\frac{\beta}{W}n_t^2$$

n_t = number concentration
β = aggregation rate constant
W = stability ratio

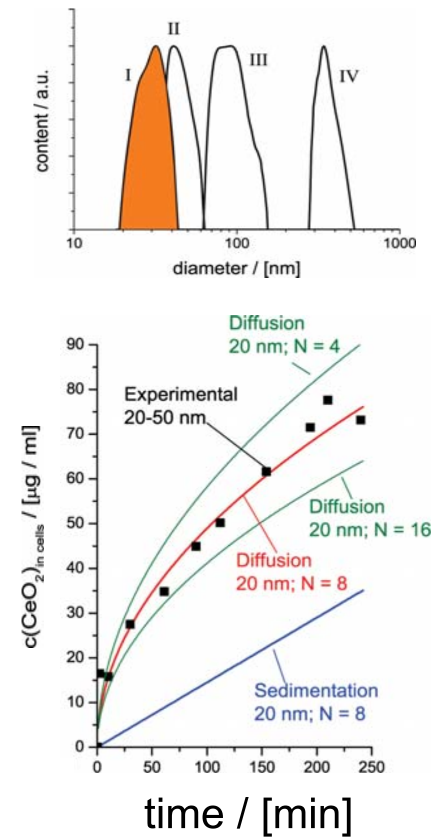
Limbach LK, Li Y, Grass RN, Brunner TJ, Hintermann MA, Muller M, Gunther D, Stark WJ, *Environ. Sci. Technol.* **2005**

Size specific particle transport mechanism



- ← large particles: sedimentation
- → small particles: diffusion and agglomeration

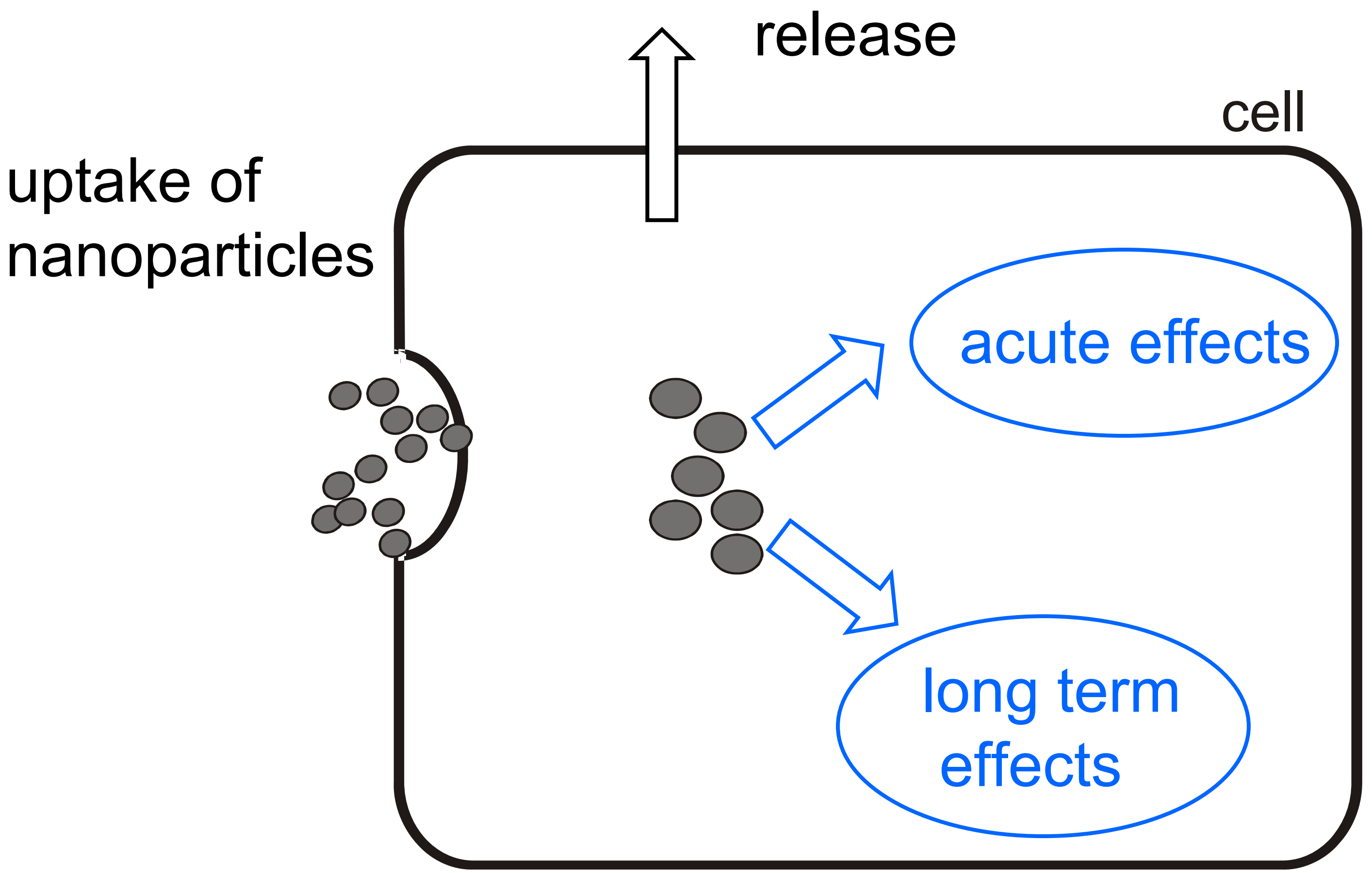
quantitative model for nanoparticles uptake in cells



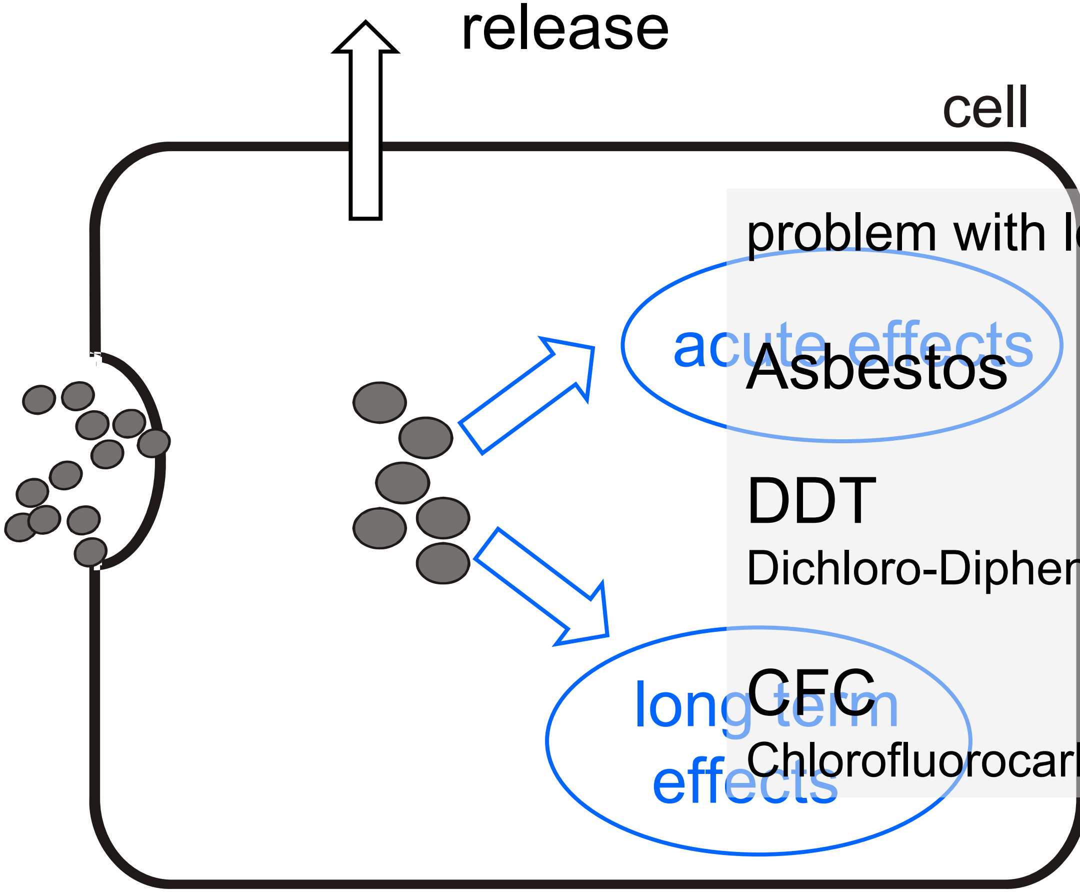
250-500 nm

20-50 nm

What happens after entrance?



Long term effects?



problem with long term effects

Asbestos

DDT

Dichloro-Diphenyl-Trichloroethane

CFC

Chlorofluorocarbons

Nanoparticles associated risk

acute effects

→ apoptosis
→ necrosis

→ solubility and intracellular ion release

Brunner et al. 2006 Environ. Sci. Tech
Braydich-Stolle et al. 2006 Toxicol. Sci.
Kirchner et al. 2005 Nano Letters

long term effects

→ mutagenicity
→ teratogenicity

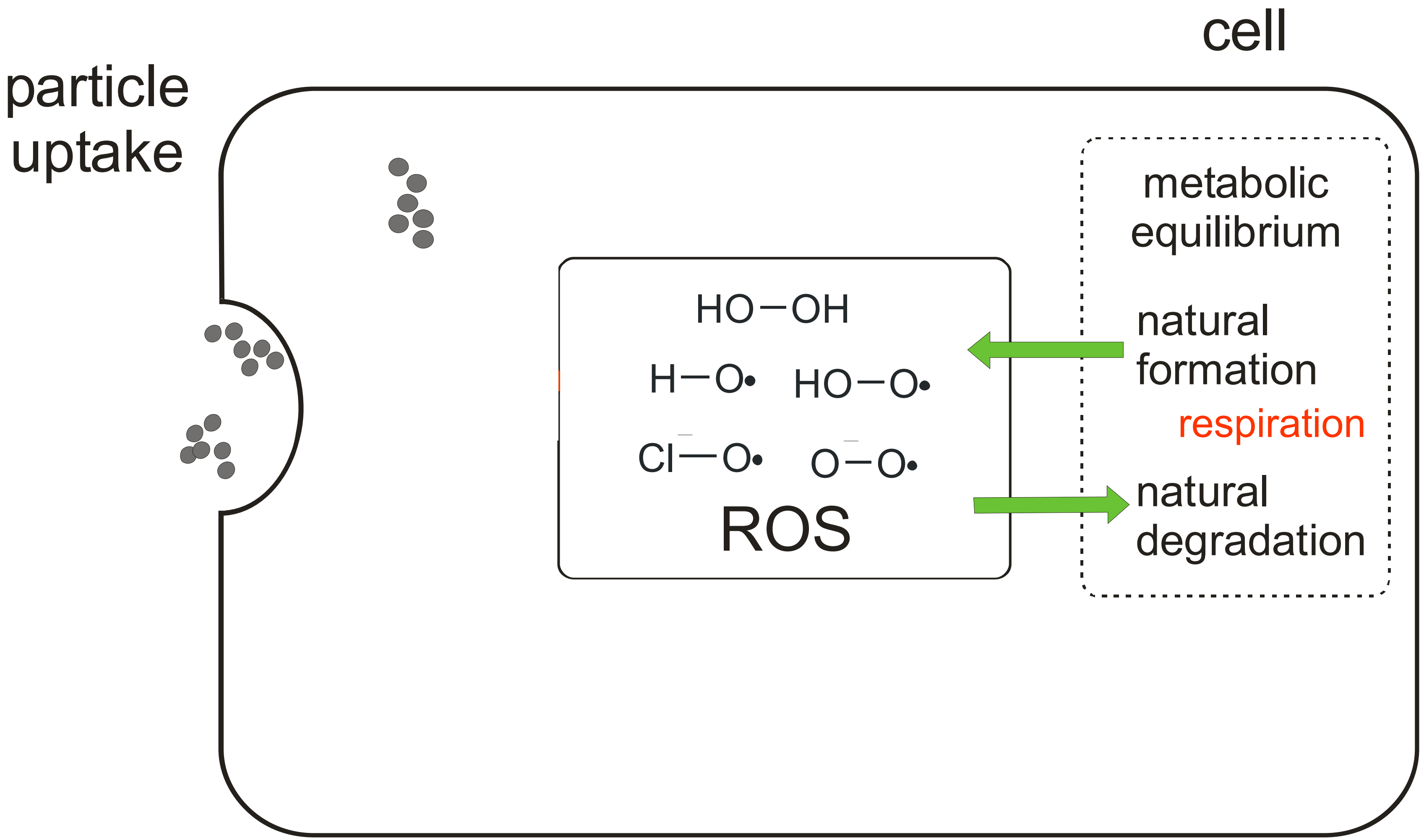
→ redox potential

→ reactive oxygen stress

→ ion release

LK Limbach, P Wick, P Manser, RN Grass, A Bruinink, WJ Stark, Environ. Sci. Tech. **41** (11): 4158-4163 2007

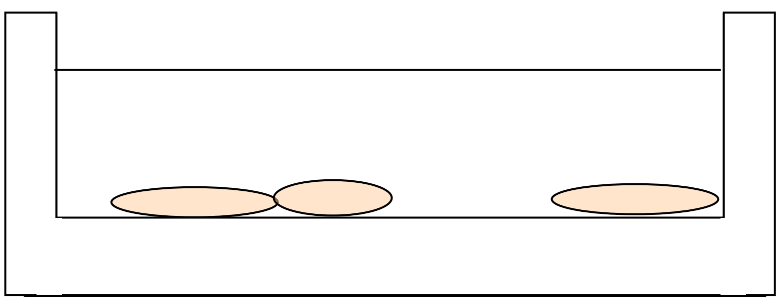
Reactive Oxygen Species formation



Two different test systems

in vitro

A 549

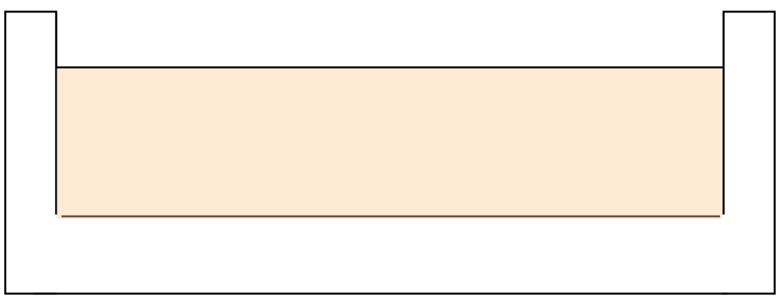


reaction space: cells

incl. natural formation / natural defense

material properties

cell free system



reaction space: whole well

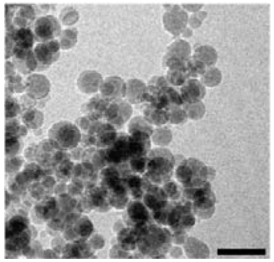
simulation of the ROS direct formation

Set of investigated nanoparticles

titania	iron oxide	cobalt oxide	manganese oxide
TiO_2	Fe_2O_3	Co_3O_4	Mn_2O_3

measured as:
- pure oxide
- Ti, Fe, Co and Mn in Silica (0.5%, 1,6%)
- Fe, Co, Mn Ions ($FeCl_3$, $MnCl_2$, $CoCl_2$)

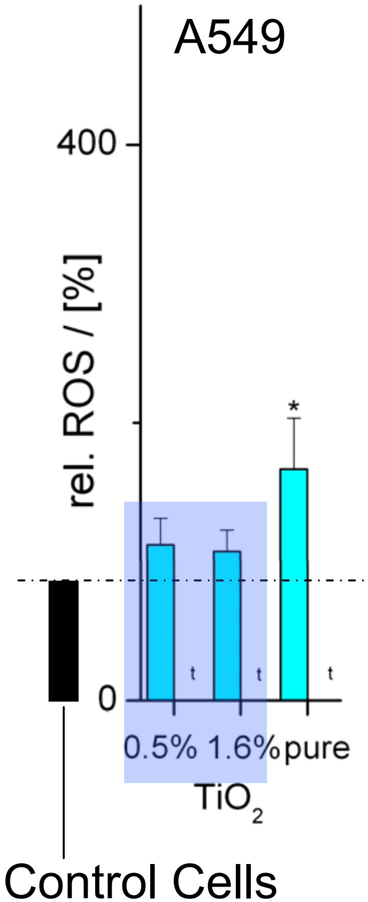
reference
- untreated cells (in vitro)
- pure silica (cell free)

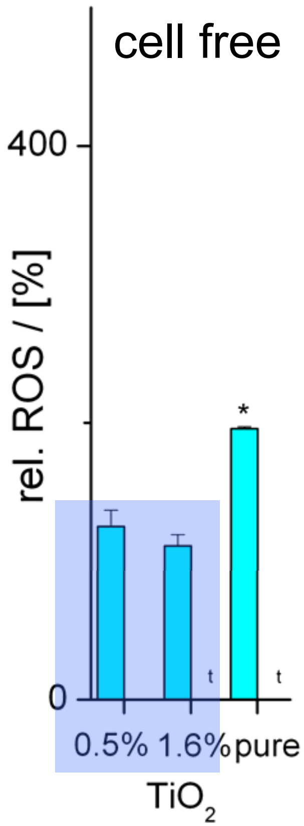


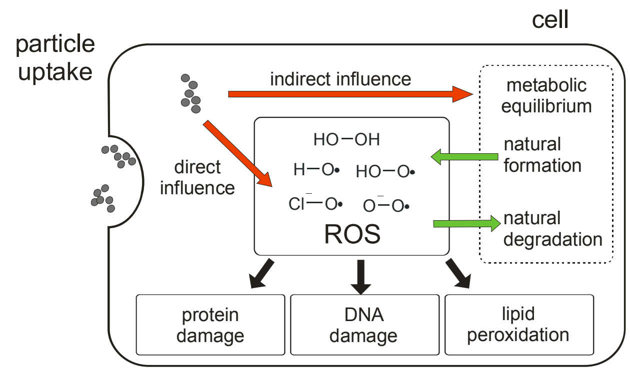
⇒ Similar size, shape, morphology and state of aggregation due to flame spray process
⇒ 20-80 nm nano-particles

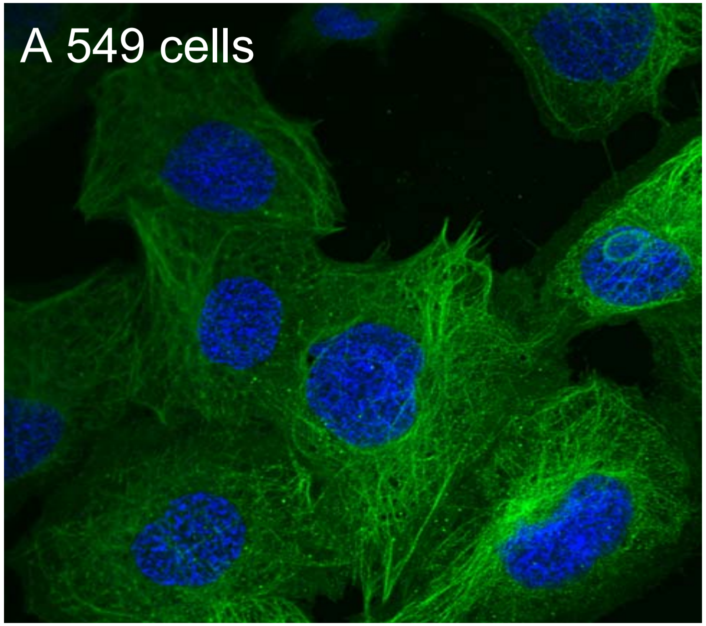
Titania - nanoparticles

in vitro | material properties

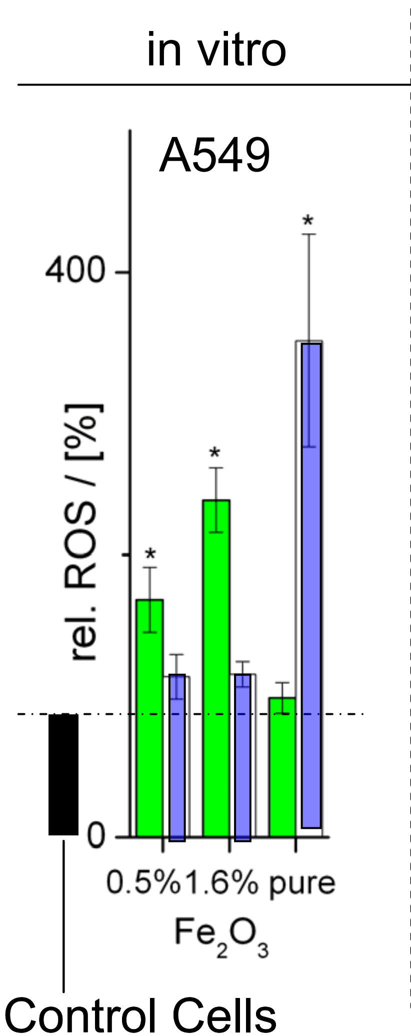


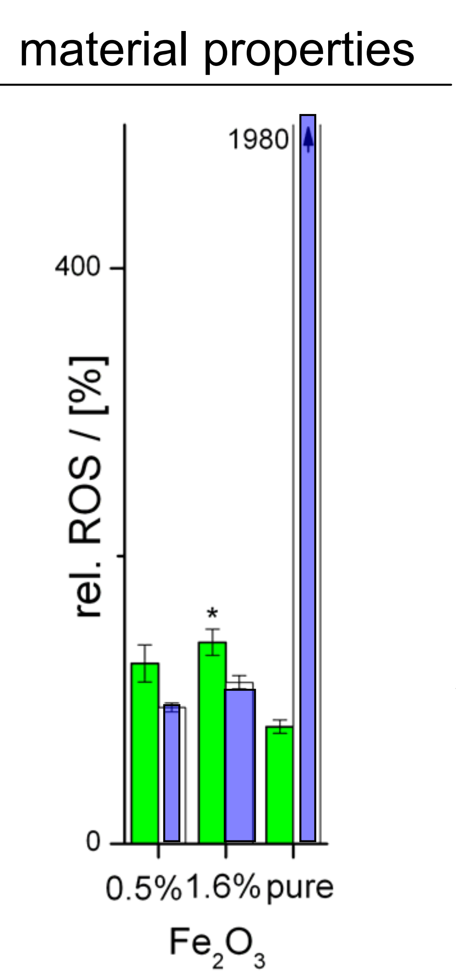


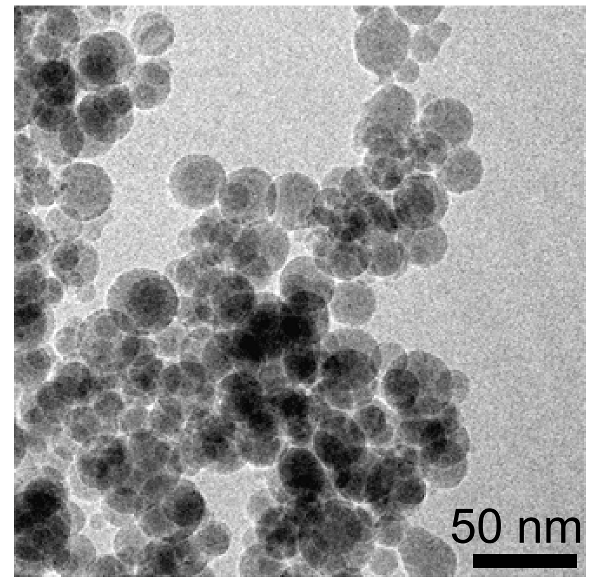




Iron oxide - nanoparticles





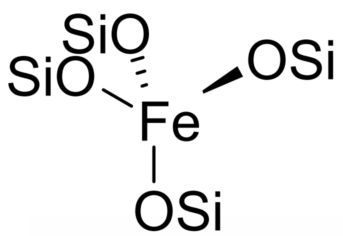


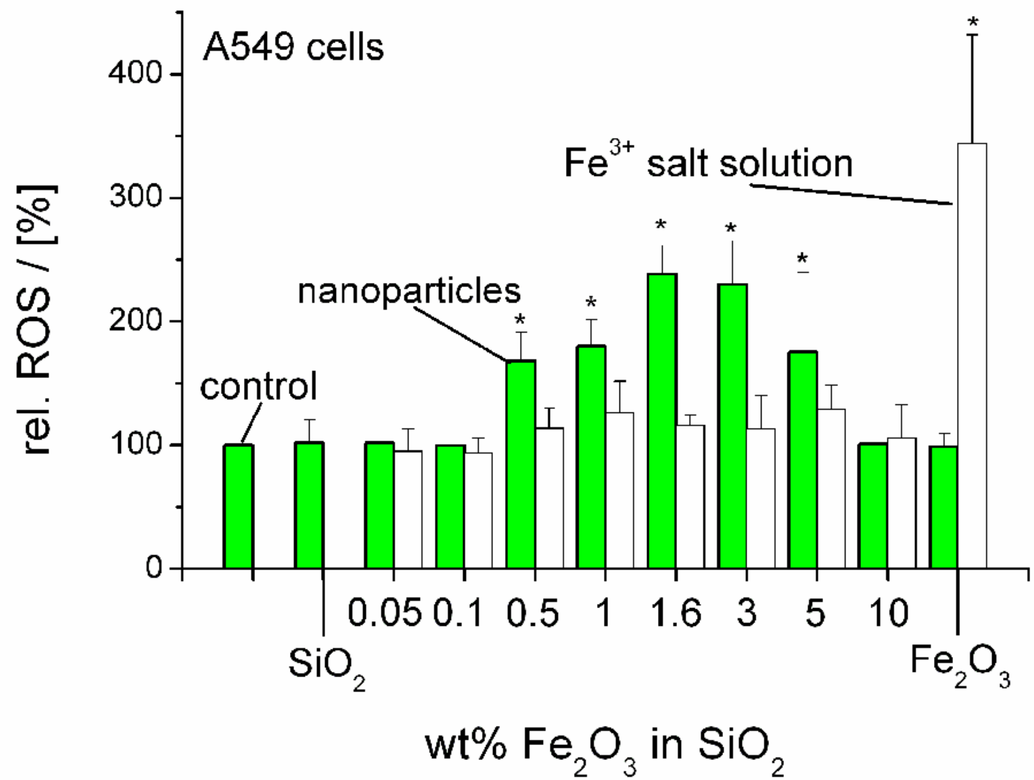
no ROS generated from pure iron oxide whereas iron ions induce ROS

surprisingly ROS generation of iron embedded in silica.

LK Limbach, P Wick, P Manser, RN Grass, A Bruinink, WJ Stark, Environ. Sci. Tech. **41** (11): 4158-4163 2007

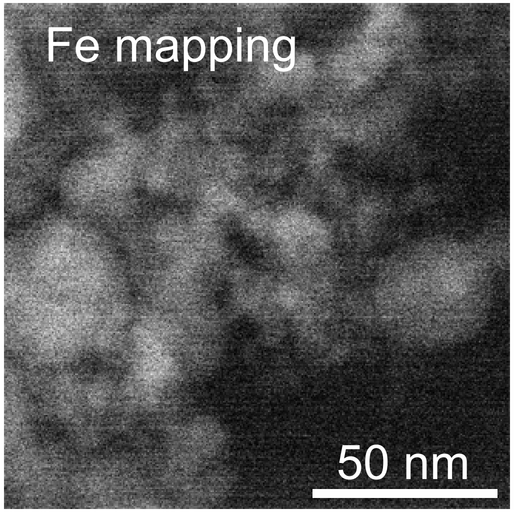
Iron oxide

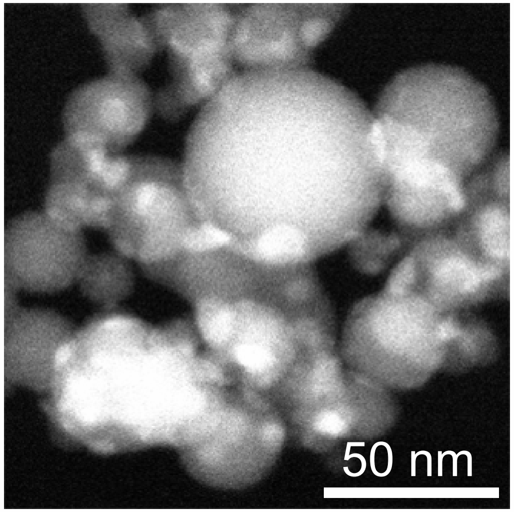




catalytic sites are "working" also in cells

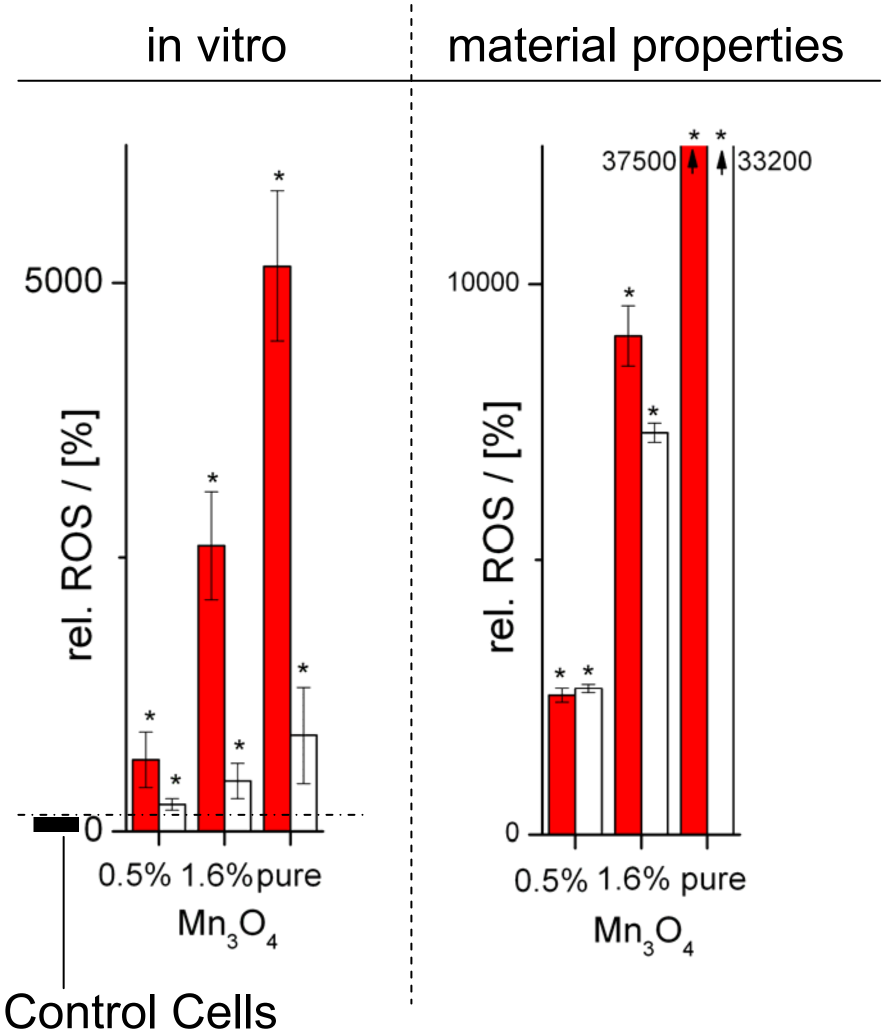
1,6% Fe_2O_3 in silica





10% Fe_2O_3 in silica

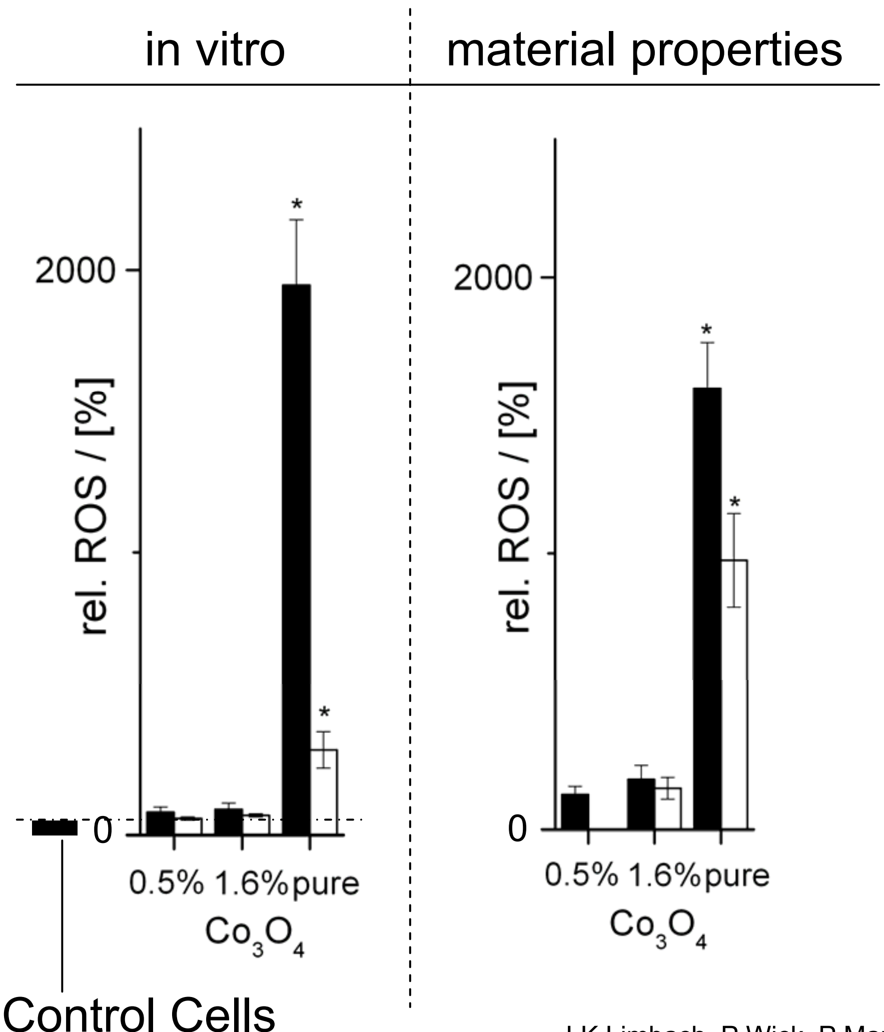
manganese oxide

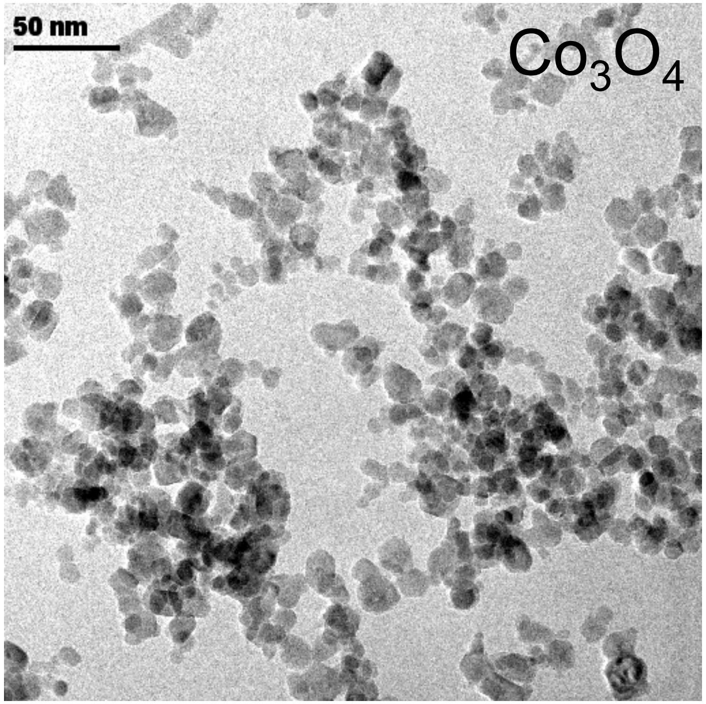


50 times increased ROS formation for manganese oxide nanoparticles, significantly more than the corresponding Mn-Ion concentration.

ROS generation of Mn even as low percentage bound in silica.

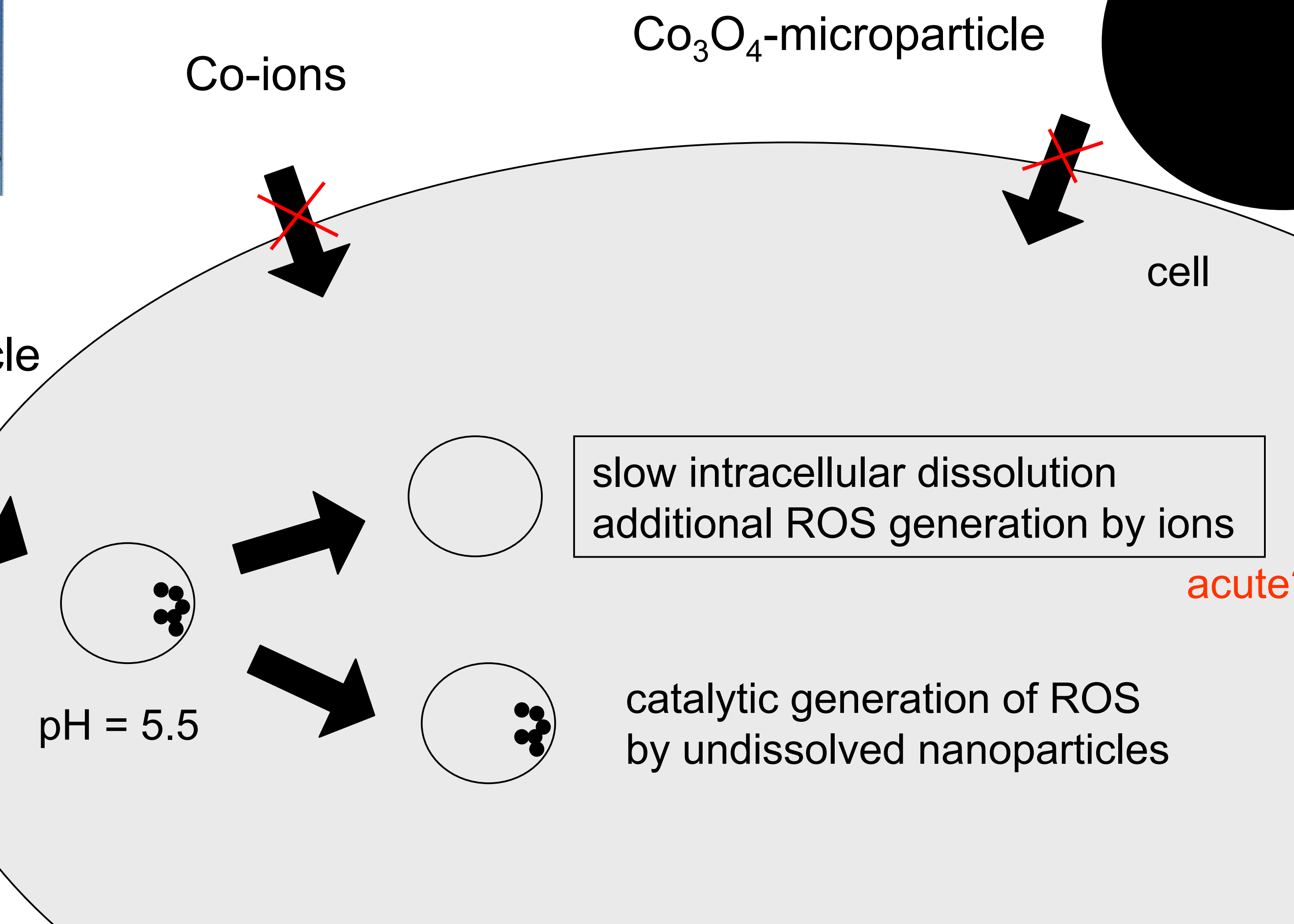
Cobalt oxide nanoparticles



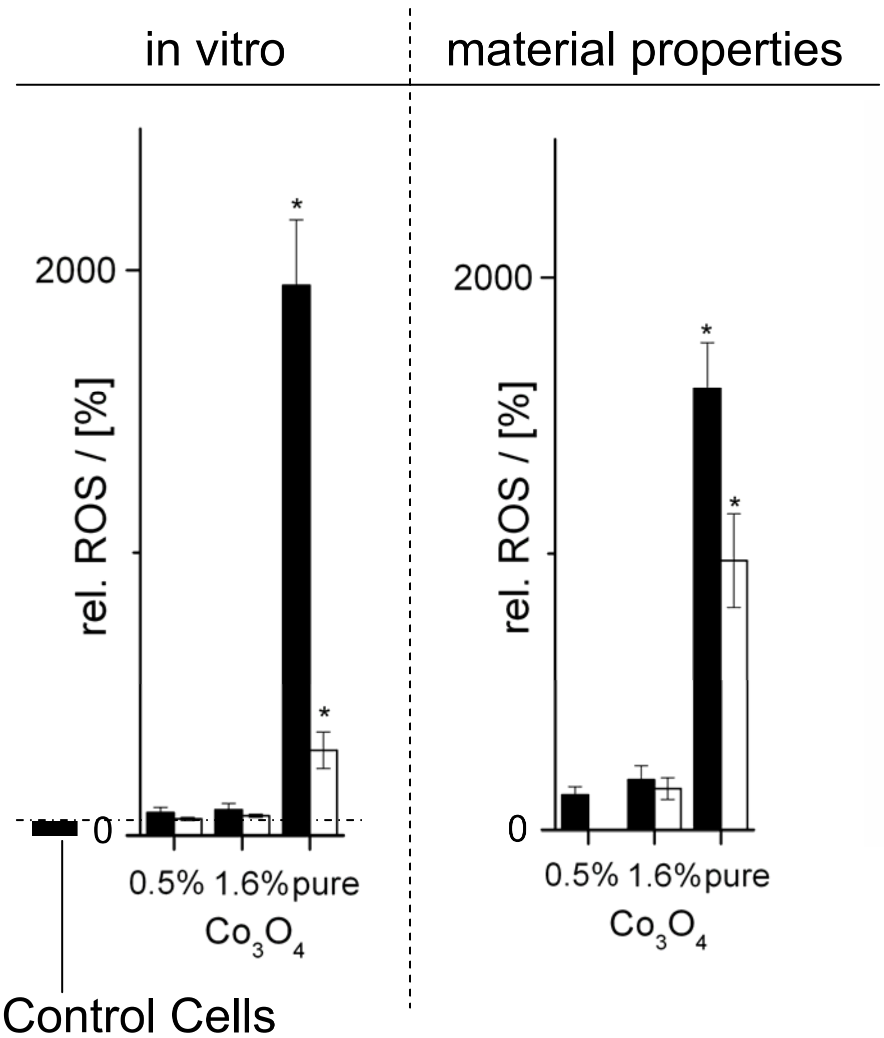


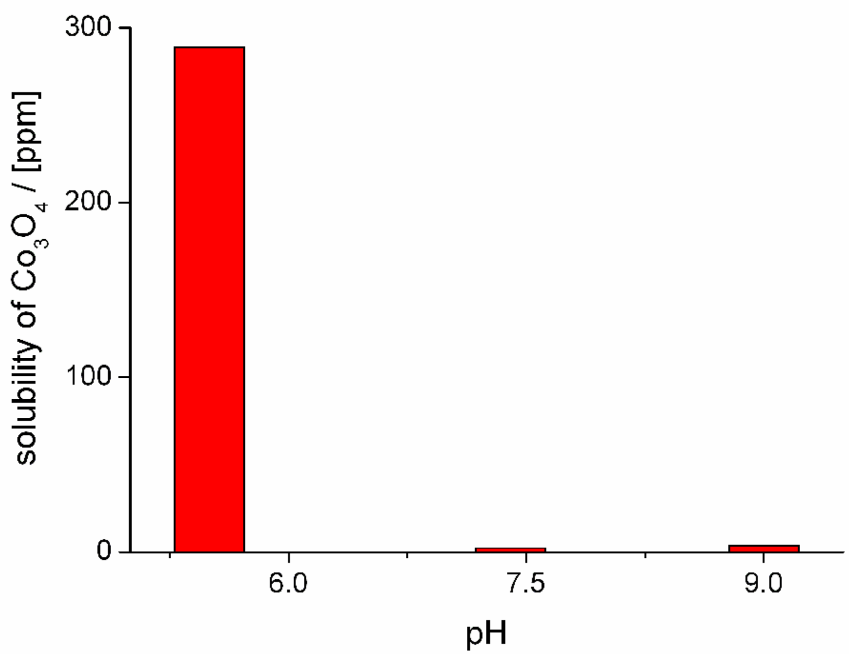
LK Limbach, P Wick, P Manser, RN Grass, A Bruinink, WJ Stark, Environ. Sci. Tech. **41** (11): 4158-4163 2007

Trojan horse mechanism



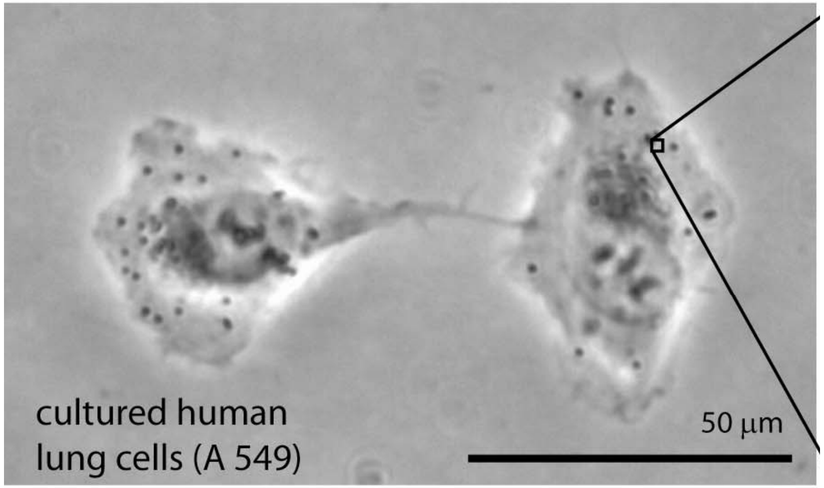
Cobalt oxide nanoparticles





Cobalt oxide slightly soluble at pH 5.5

Direct ROS generation

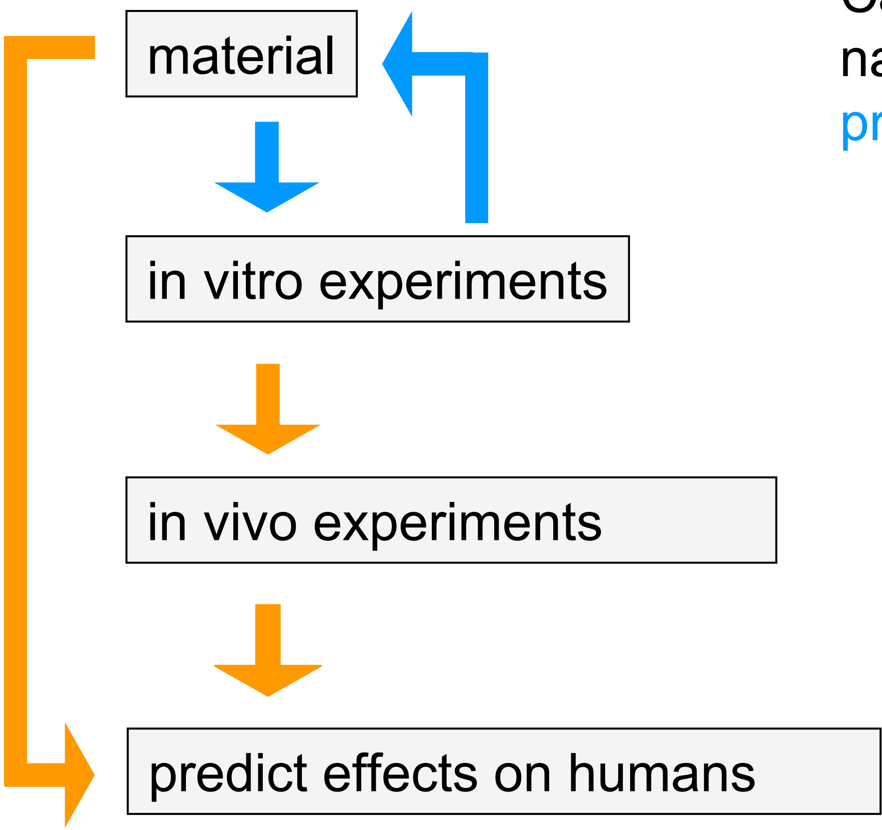


ROS generation is continuously until the particles are degraded or removed from the cells.

=> release of nanoparticles from cells is not well investigated

Residence time of particles in cells as a major parameter for early risk assessment

Further outlook



Can we predict possible damage of nanomaterials direct out of material properties?

- safe and sustainable development of nanoparticulate products
- No pricy down stream corrections (asbestos)
- gaining time and money
- classification of nanomaterials

Conclusion

- quantitatively ROS measurements of Mn, Co, Fe and Ti in human epithelia cells by an direct mechanism.
- catalytic sites are “working” in cells (Iron in silica)
- dissolving nanoparticles in cells can lead to additional ions effect (nano Trojan horse mechanism).

- future grouping of nanomaterials according to their chemical and physical material properties

Acknowledgments

Prof. W.J. Stark
R.N. Grass
N. Osterwalder
T.J. Brunner
L.K. Limbach
S.C. Halim
E.K. Athanassiou
O.D. Schneider
N. Lüchinger

Prof. D. Günther (ETHZ)

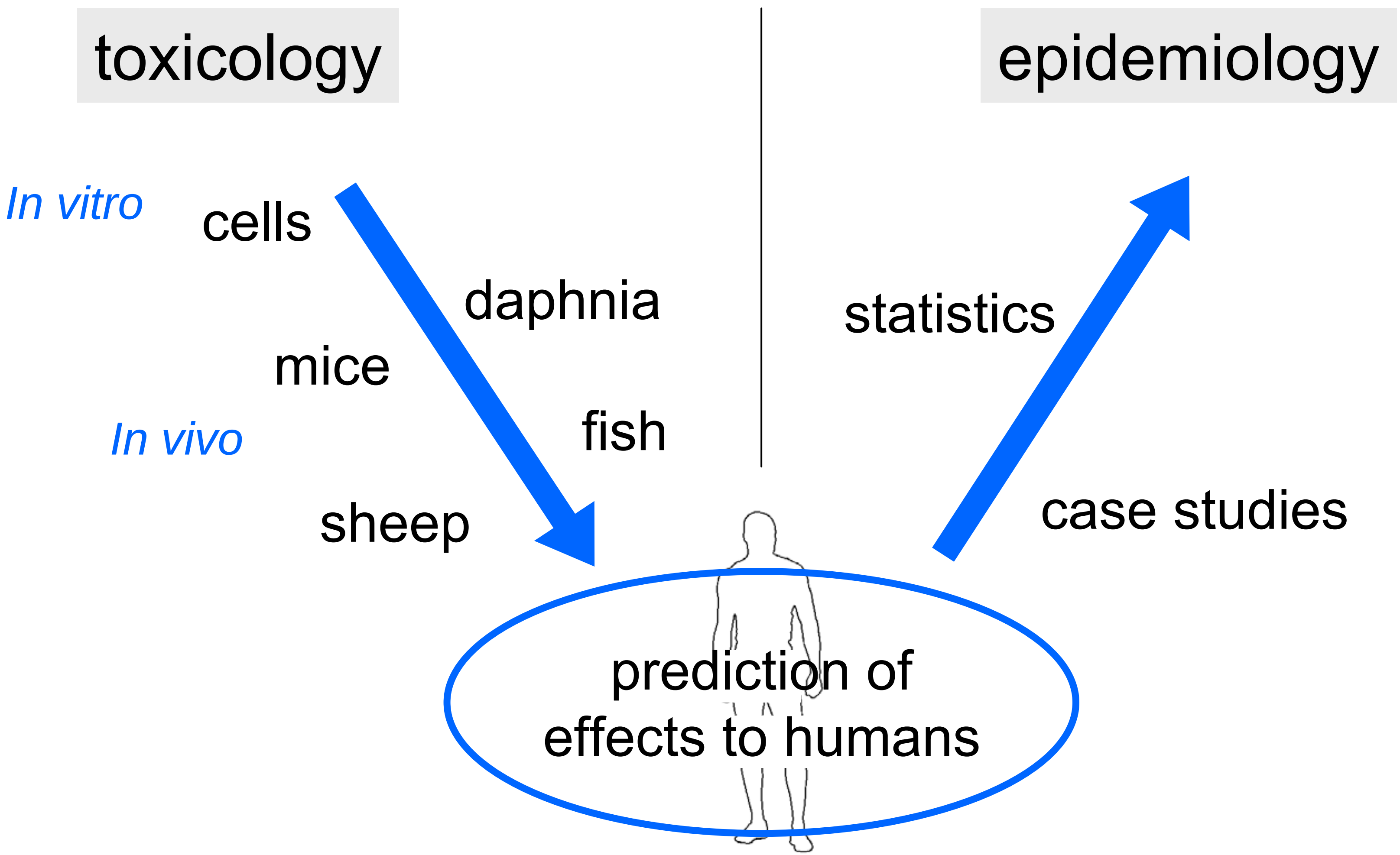
Empa St. Gallen
P. Wick
A. Bruinink
P. Manser

Financial support
BAG (Federal Office of Public Health, Switzerland)

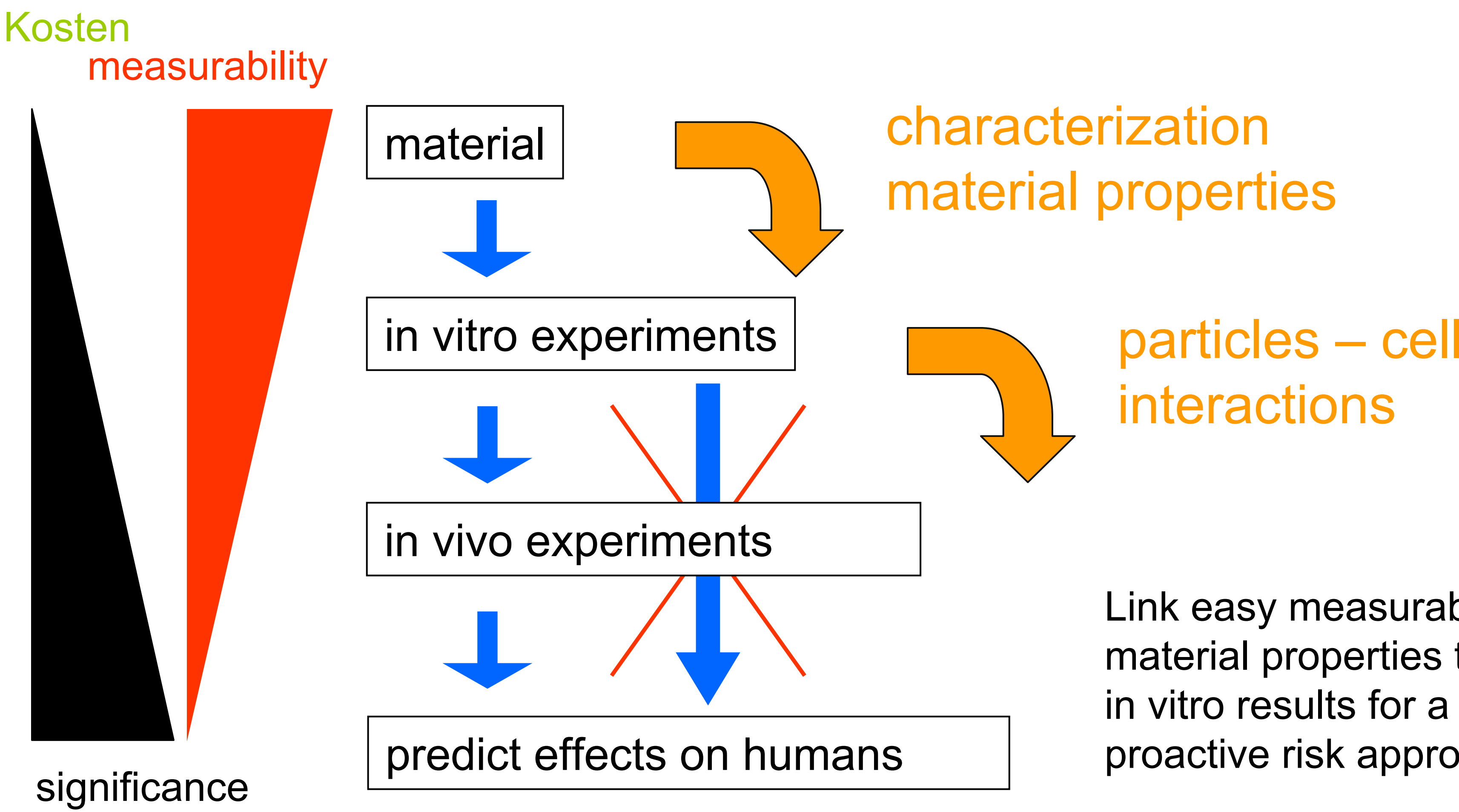
Thank you for your attention

Questions?

Are there indicators to problematic nanomaterials?

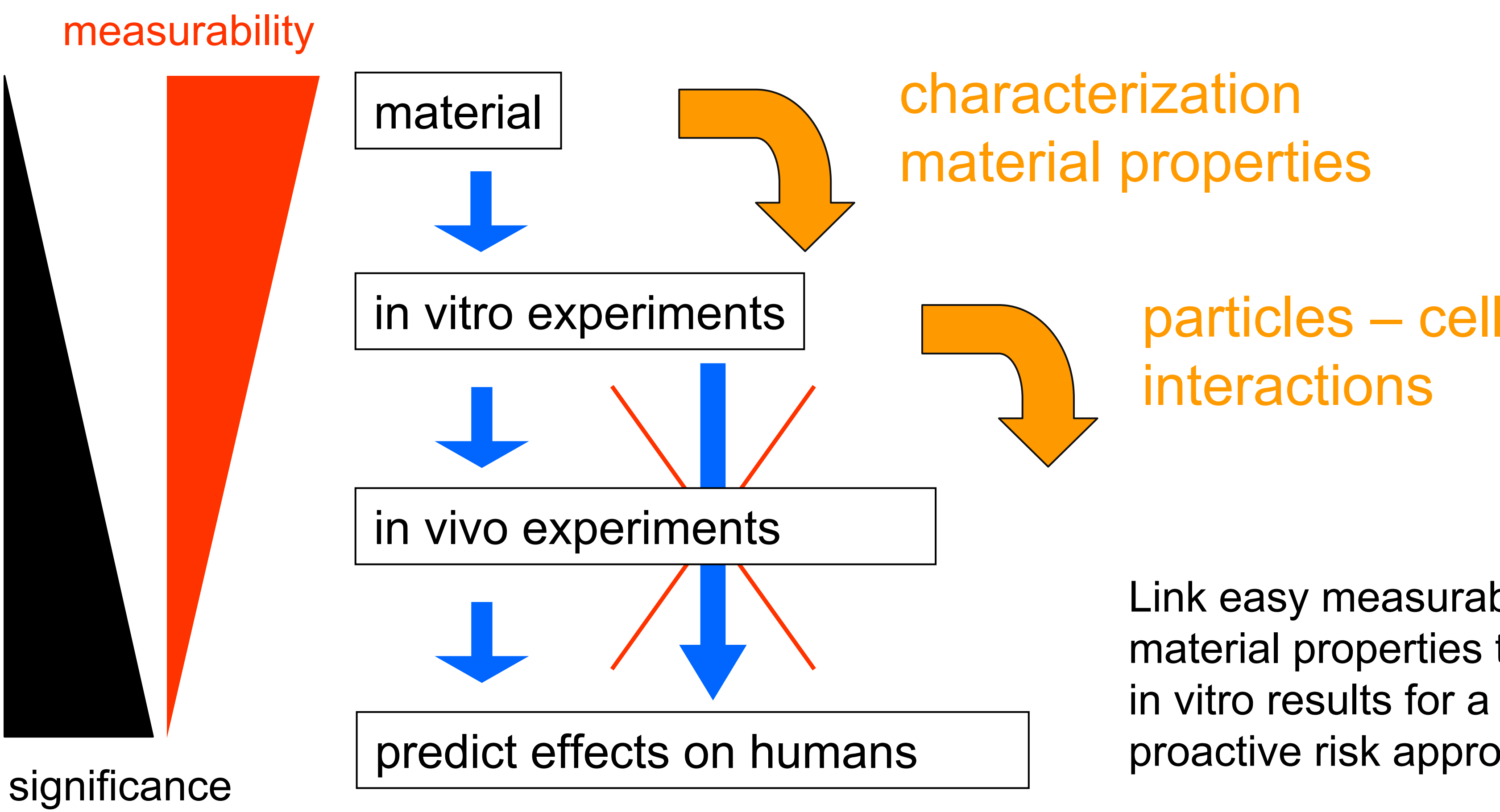


Hierarchy of toxicological tests



Link easy measurable material properties to in vitro results for a proactive risk approach

Toxicological pathway



Link easy measurable material properties to in vitro results for a proactive risk approach

Link from cell data to materials properties

material

↓

in vitro experiments

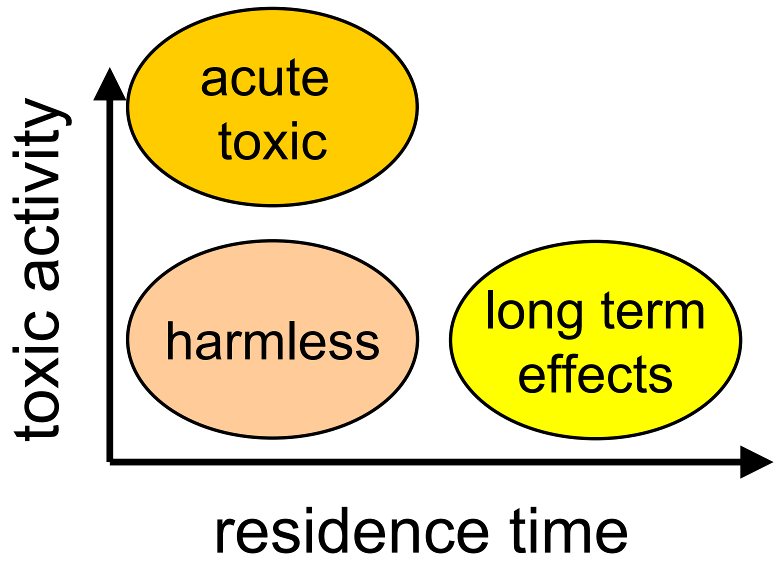
↓

in vivo experiments

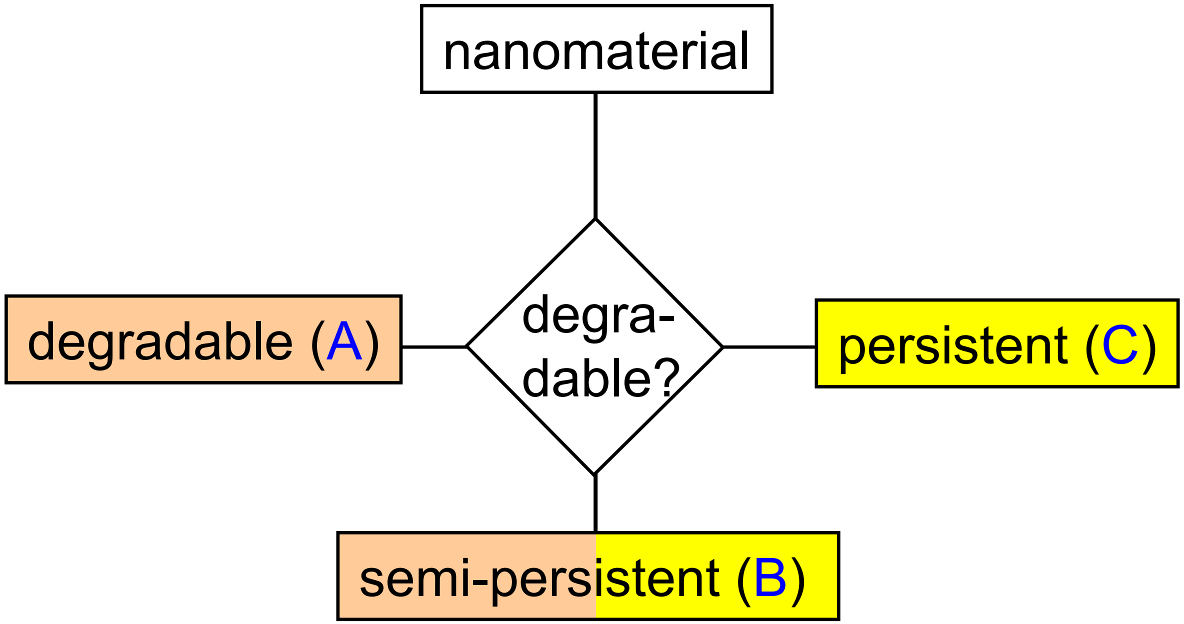
↓

predict effects on humans

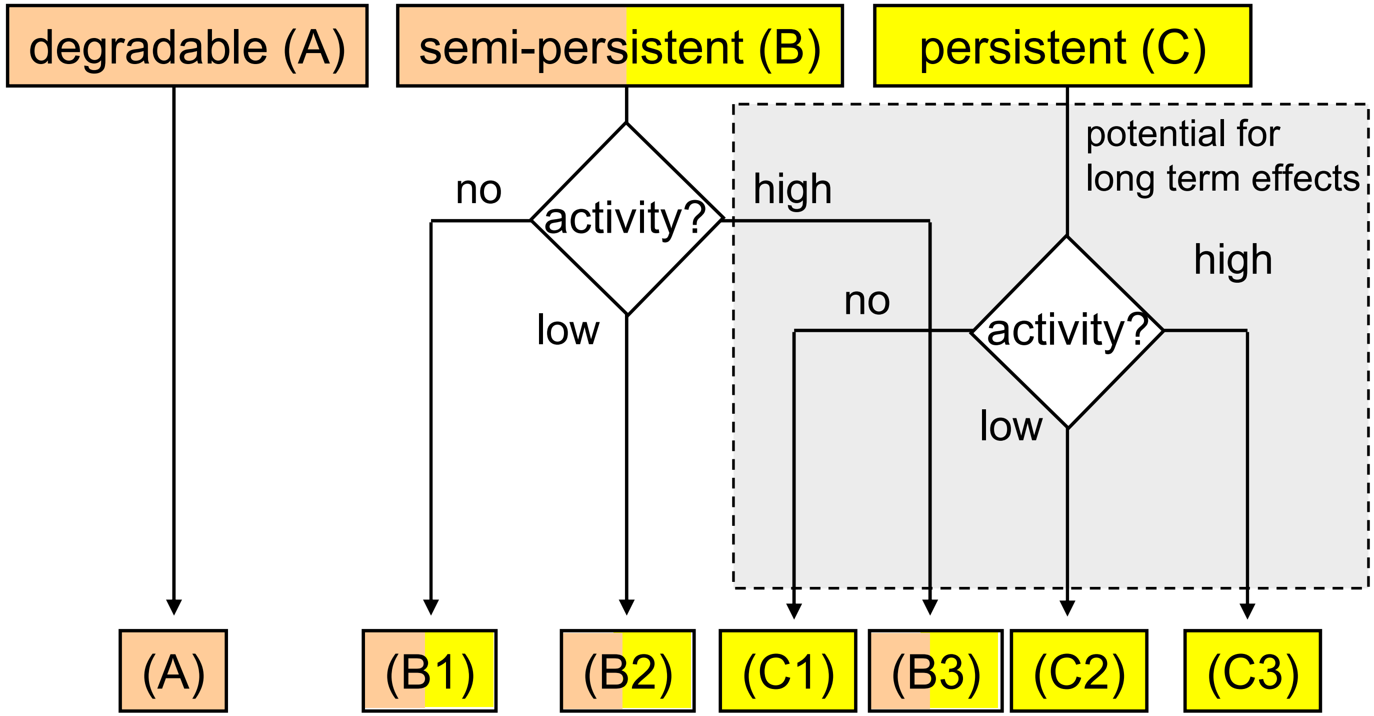
Can we predict possible damage of nanomaterials out of material properties?



is it degradable?



chemical activity



Possible pre-evaluation

risk := damage potential * occurrence probability

What are criteria for a pre-evaluation of the damage potential?

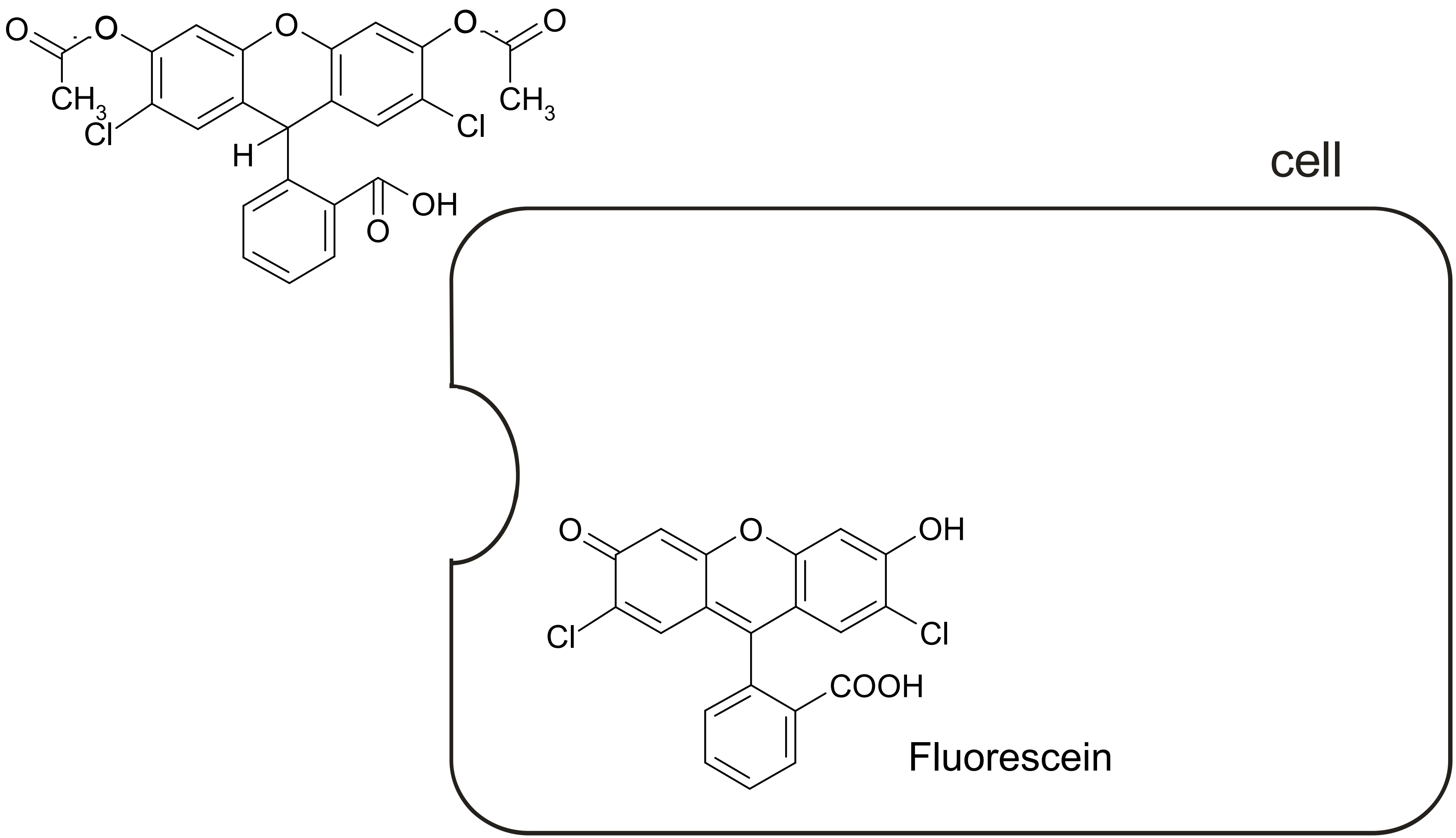
long term effects

=> long answer time

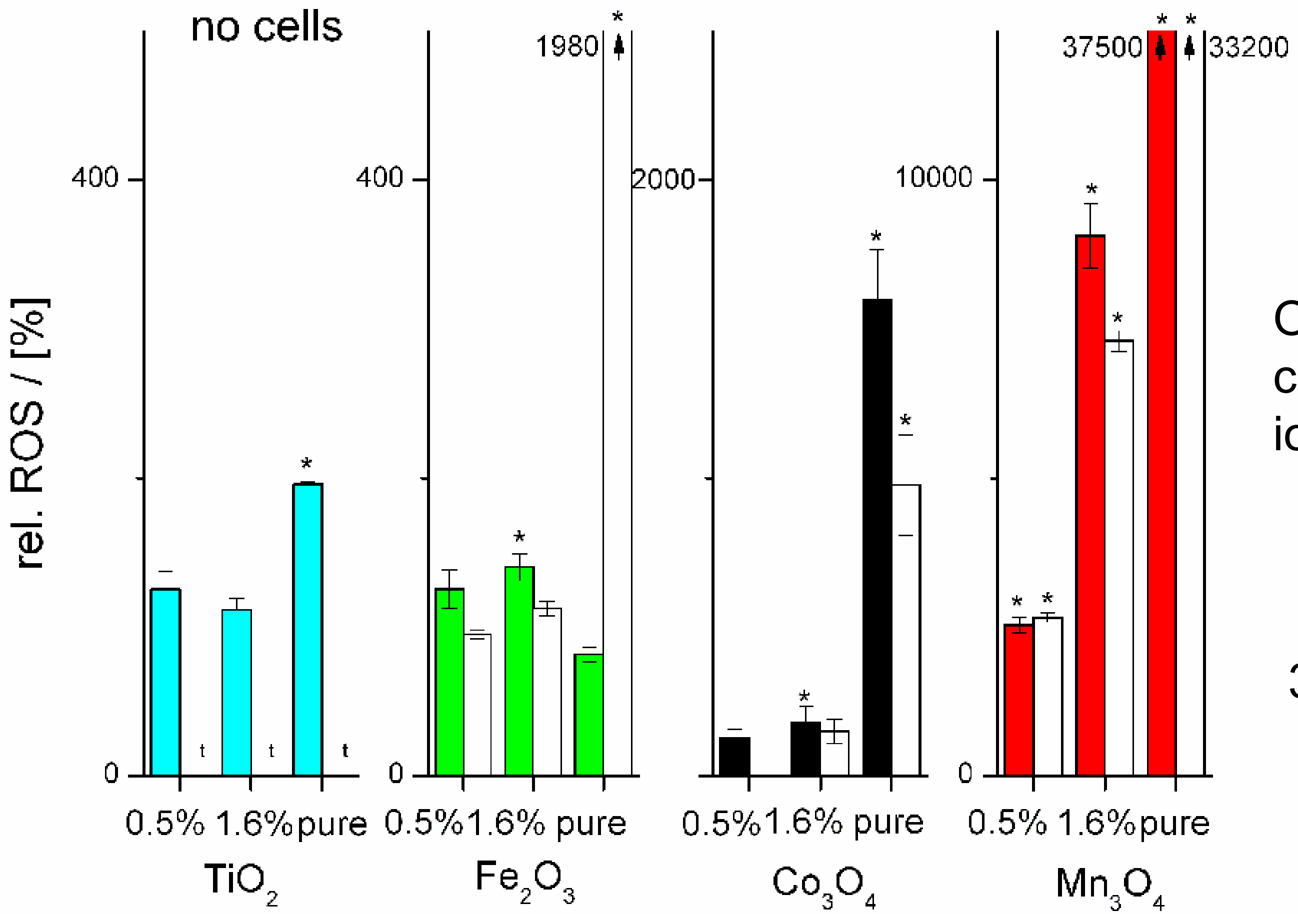
=> hardly measurable

- asbestos
- DDt
- CFC

Reactive Oxygen Species formation



Transition metals in lung cells



Oxide particles vs. corresponding ion concentration

30 ppm exposure

Other ROS precursor?