
Risk Assessment of Nanomaterials: Summary and Conclusions of a MAK Working Group

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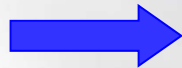
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Requirements for risk assessment of nanomaterials

- ▣ Identification of nanomaterials with relevant human exposure, e.g.
 - Titanium dioxide, silizium dioxide, zink oxide, aluminum oxide, cerioxide, silver oxide, carbon black, carbon nanotubes...



Partly chemicals with known toxicological properties, used also in nanoscale dimensions

Requirements for risk assessment of nanomaterials

Particle characterization with respect to:

- ▣ Solubility, also in biological fluids
- ▣ Size, shape
- ▣ Degree of agglomeration/aggregation, also in biological fluids
- ▣ Surface and surface reactivity
- ▣ Surface modification
- ▣ „Coating“
- ▣ ...

Requirements for risk assessment of nanomaterials

- **Establishment of methods for reliable measurements to quantify**
 - External exposure (also in relation to „background“ exposure)
 - Internal exposure
- **Biopersistence and/or systemic bioavailability after**
 - Inhalative exposure
 - Oral exposure
 - Dermal exposure

Requirements for risk assessment of nanomaterials

- ▣ Identification of relevant endpoints for risk assessment and threshold value setting **under relevant exposure conditions:**
 - Lung toxicity (chronic inflammation, carcinogenicity)
 - Kardiovascular diseases?
 - Neurotoxicity?

Distinction of nanomaterials for toxicological risk assessment

Grouping of nanomaterials to establish general approaches for risk assessment:

- ❖ Granular biopersistent particles with no or little additional chemical toxicity („inert“ particles)
- ❖ Metal-based nanoparticles
- ❖ Coated nanoparticles, e.g. „quantum dots“
- ❖ Fibre-like nanomaterials, e.g. nanotubes
- ❖ Nanoparticles as delivery systems, e.g. drug delivery
- ❖ ...

Nanoparticles as delivery systems

- ▣ Drug delivery, delivery of bioactive food components
 - ❖ Affects mainly the bioavailability and/or target organ of delivered compounds
 - ❖ Risk/benefit dependent on delivered compound
- ➔ ❖ No nanomaterial-specific risk assessment required (case-by-case evaluation)

Fibre-like nanomaterials, e.g. nanotubes

- ▣ May exert fibre-like characteristics, depending on actual material
- ▣ Risk assessment based on dimensions and biopersistence



Case-by-case evaluation required

Coated nanoparticles, e.g. „quantum dots“

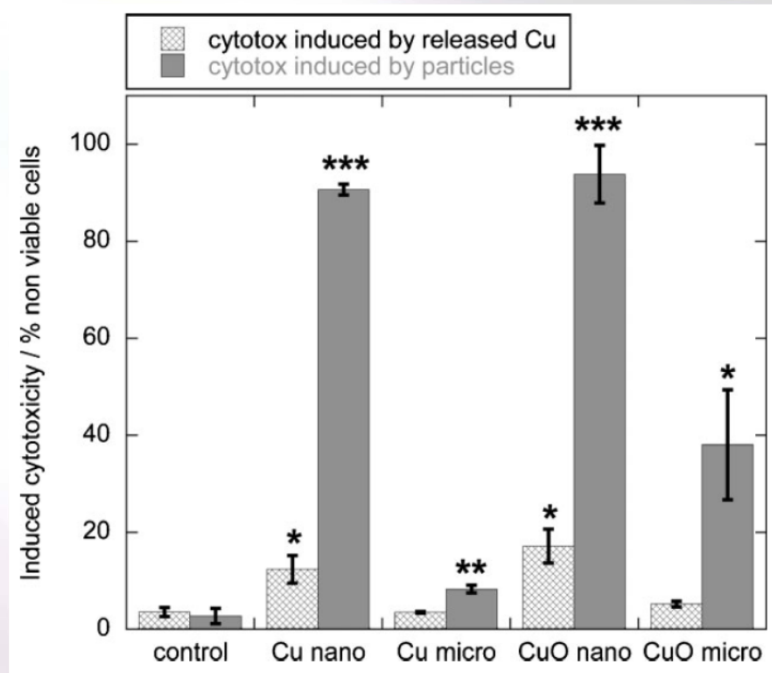
- ▣ Contain toxic metals and/or essential trace elements
- ▣ Manifold applications, e.g., semiconductors, medical diagnosis
- ❖ Affects the bioavailability and/or target organ of delivered compounds



Potential risk depends on extent of release of toxic metals ions (e.g., cadmium) or essential metal ions with potential for metal overload (e.g., iron, manganese)

Metal-based nanoparticles

Example: Nanoscale vs. microscale copper oxide particles



Karlsson et al., 2008; Midander et al., 2009

Comparison of cytotoxicity of micro- and nanosized Cu and CuO particles and by the released copper fraction after 4 h incubation of A549 cells:

- Higher toxicity of nanosized material
- Not due to dissolution of particles outside the cell
- Probably uptake of both microscale and nanoscale particles by endocytosis, but increased intracellular dissolution and liberation of Cu ions

Nanoscale vs. microscale copper oxide particles

Research needs:

- Further mechanistic studies with respect to bioavailability in cell culture experiments
- Studies in experimental animals to elucidate the relevance of the effect for in vivo-conditions
- Dose-response studies in experimental animals to identify NOEL/NOAEL

➔ „Threshold“ effect plausible since Cu is an essential trace element

Microscale vs. nanoscale granular biopersistent particles

GBS microscale

- Lung as critical target organ upon inhalation
 - Carcinogenic in experimental animals on high-exposure conditions (particle overload)
 - Proposed mechanism: chronic inflammation, macrophage activation, secondary genotoxicity
-
- ➔ Candidate for MAK carcinogenicity category 4
 - ➔ NOAEL and thus MAK/OEL should protect from chronic inflammation

Microscale vs. nanoscale granular biopersistent particles

GBS microscale



GBS nanoscale

- **Quantitative or qualitative differences in toxicity and mode of action?**
 - Differences in uptake/passage through biological membranes?
 - Differences in toxicokinetics?
 - Differences in receptor interactions?
 - Additional target organs other than lung (cardiovascular system, brain)?
 - Chronic inflammation most sensitive parameter? Critical effect also for carcinogenicity?
 - Differences in genotoxicity (direct vs. indirect genotoxicity)?
- **In case of primarily quantitative differences, which parameters are decisive for setting OELs (e.g., mass, surface area, single particles, aggregates)?**

Microscale vs. nanoscale GBS: Genotoxicity

GBS microscale

- **secondary**, as a consequence of chronic inflammation



GBS nanoscale

- **secondary**, as a consequence of chronic inflammation
- **some evidence for additional primary genotoxicity** from cell culture studies in the absence of inflammatory cells, depending on particle size:
- **direct interaction with genomic DNA?**
- **interactions with mitotic spindle?**

Relevance in vivo unclear (no data available)

Genotoxicity would need to be determined in different cell types, e.g. of lung tissue after inhalation

Uptake of nanoparticles via dermal exposure

- ▣ Uptake of NP through damaged skin much higher as compared to intact skin
- ▣ Quantitative data concerning uptake through intact skin missing or not suitable due to missing controls



- So far no evidence for considerable uptake of NP through intact skin
- Research need taking into account background NP exposure

Additional target organs in case of nanoparticles?

Discussed target organs in addition to lung upon inhalation:

- Cardiovascular system
- Brain

Epidemiology:

- Some indications from environmental exposure towards ultrafine particles with respect to an increase in cardiovascular diseases;
- However, due to mixed exposure, data not suitable for risk assessment towards specific nanoparticles at the workplace

Microscale vs. nanoscale granular biopersistent particles: experimental systems

Hazard:

- ▣ Type of effects similar:
 - Inflammation
 - Oxidative stress
- ▣ Toxicokinetic differences:
 - Deposition behaviour in the respiratory tract and mechanisms related to clearance, cell entry and translocation to secondary organs (in case of microscale particles restricted to overload conditions)
 - Binding of selected proteins to NP (protein corona) may determine the biokinetic fate

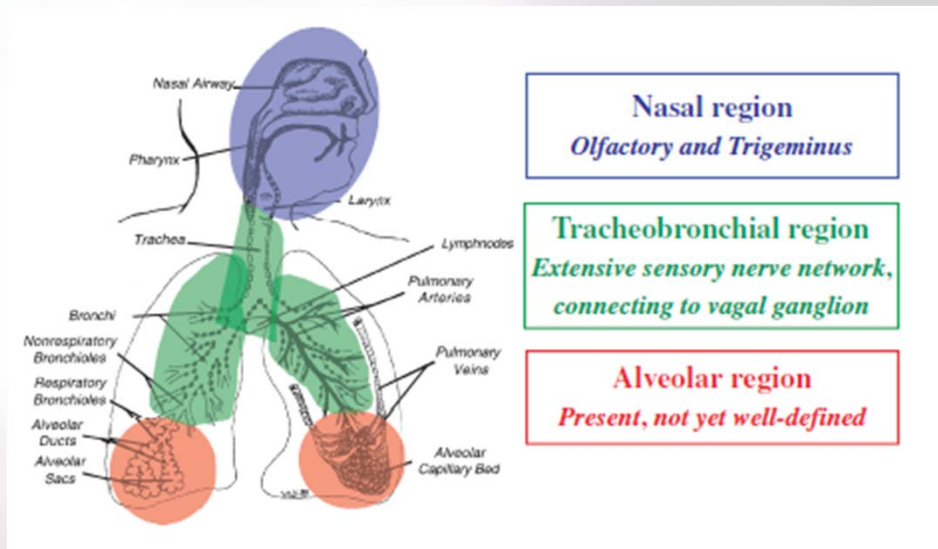
Risk:

- ▣ Consideration of biokinetics, exposure levels, delivered dose and dose rate
- ▣ Differences between inhalation studies and bolus application
- ▣ Adaptation at low dose after inhalation of NP (Oberdörster, 2009)
- ▣ Translocated fractions of inhaled nanoparticles to secondary organs < 1 %

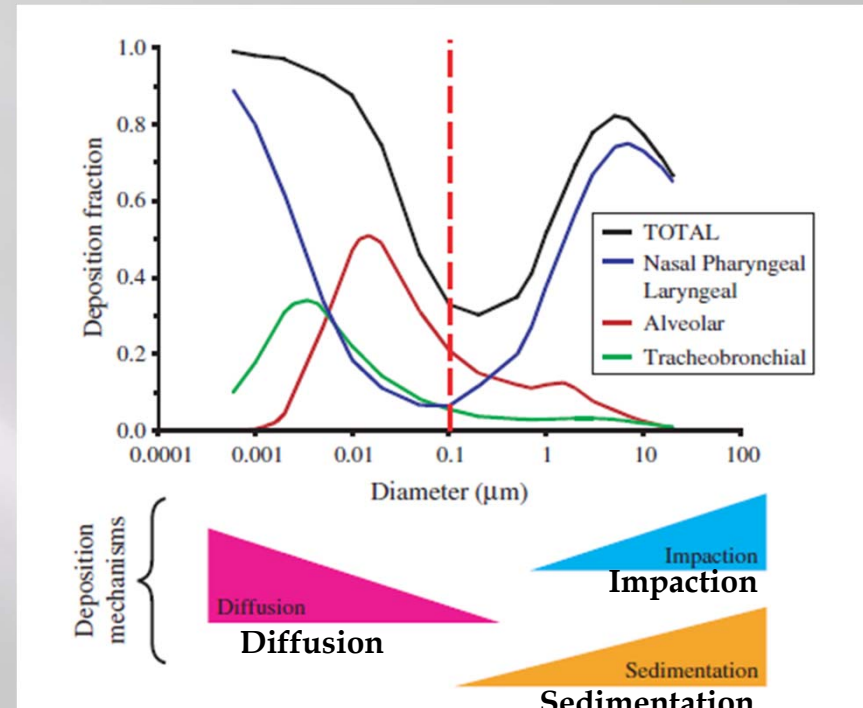
Neurotoxicity under relevant exposure conditions?

- ▣ Background:
 - Deposition of NP in the respiratory tract depends strongly on particle size
 - Small particles are largely diffusion-controlled and deposited in considerable amounts in the nasal cavity
 - ▣ May translocate with high velocity through neuronal axons and dendrites to the olfactory bulb

Neurotoxicity under relevant exposure conditions?

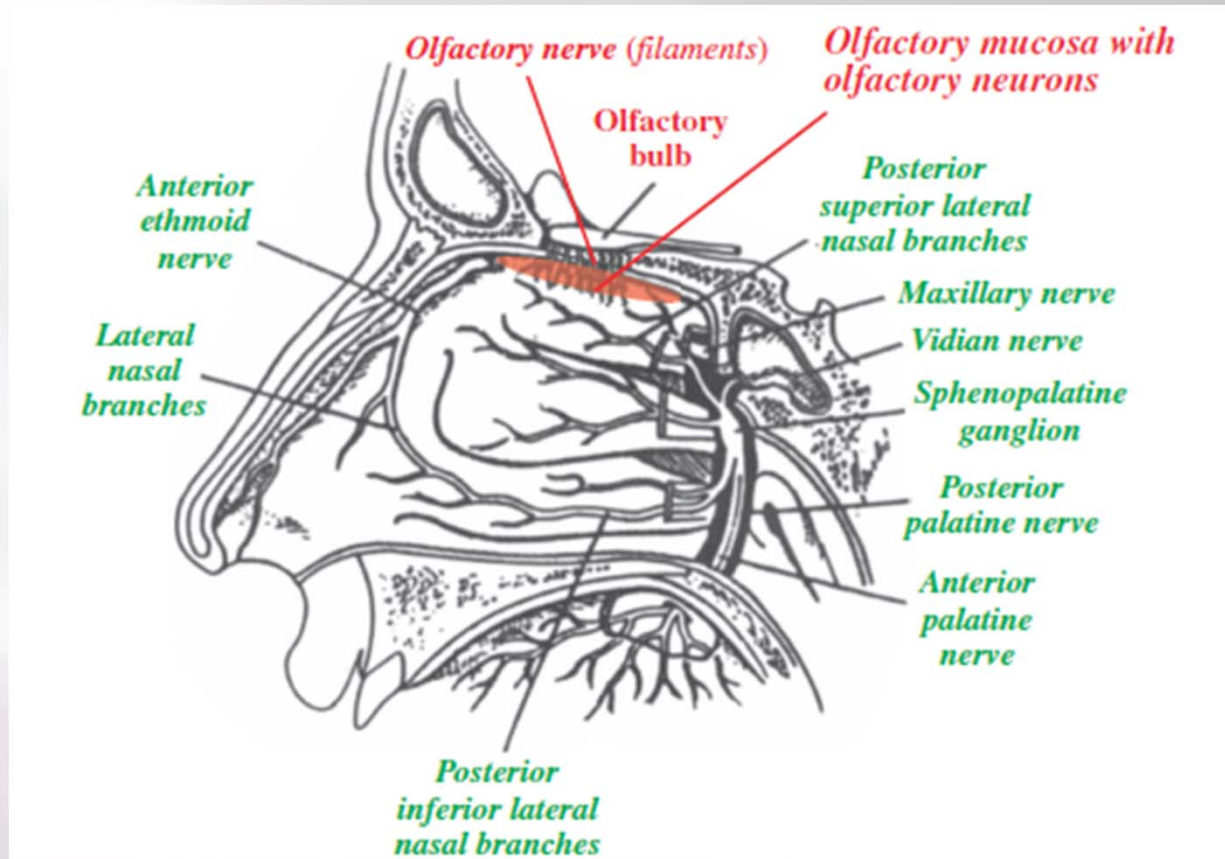


Sensory nerves in the respiratory tract, consisting of dense networks in the upper respiratory tract and tracheobronchial region and some in the alveolar region (from Oberdörster, 2009).



Predicted deposition fraction of inhaled particles in the human respiratory tract during nasal breathing; particles $< 0.1 \mu\text{m}$ deposited mainly by diffusion (from Oberdörster, 2009)

Human nasal cavity

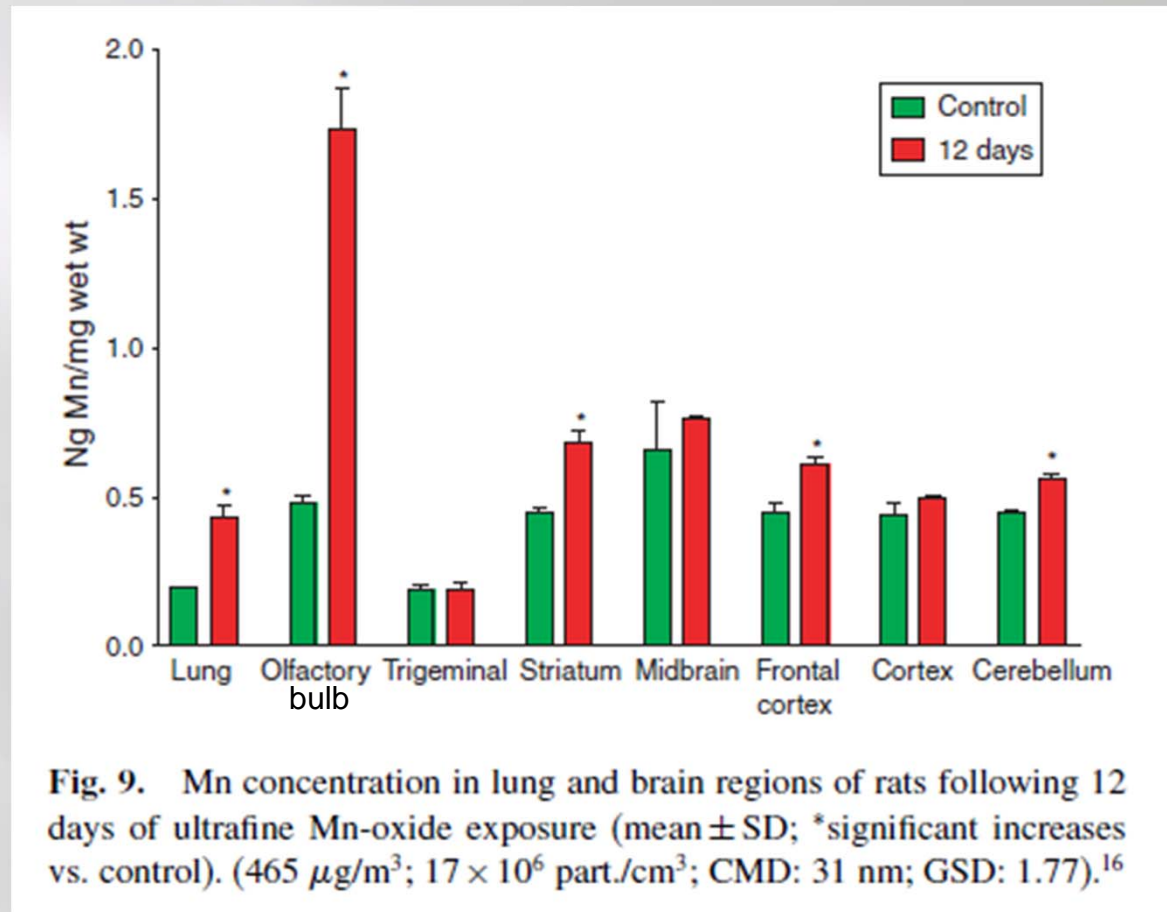


Outline of the human nasal cavity indicating olfactory and trigeminal nerve supply of nasal olfactory region and turbinates (from Oberdörster, 2009)

Neurotoxicity under relevant exposure conditions?

Example:
inhalative
exposure towards
MnO (31 nm)

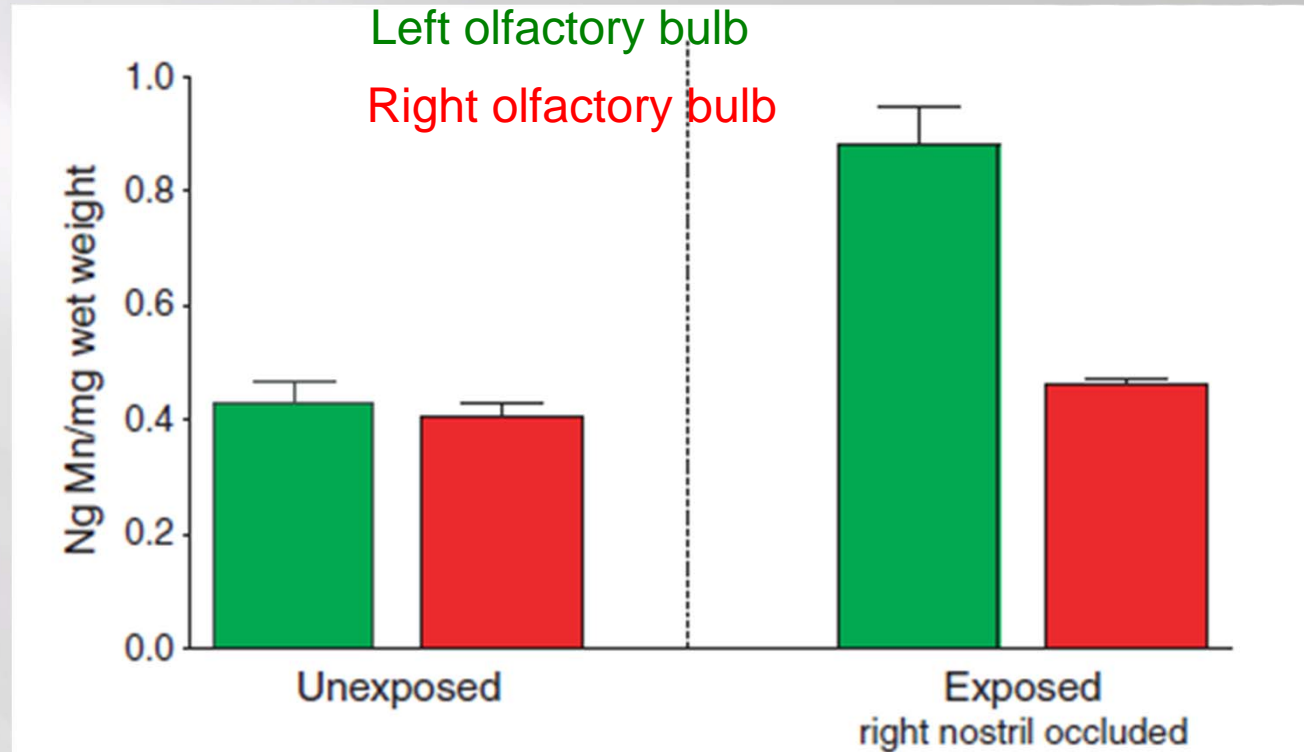
From Elder et al., 2006;
cited in Oberdörster 2009



Higher accumulation of Mn in the olfactory bulb as compared to the lung

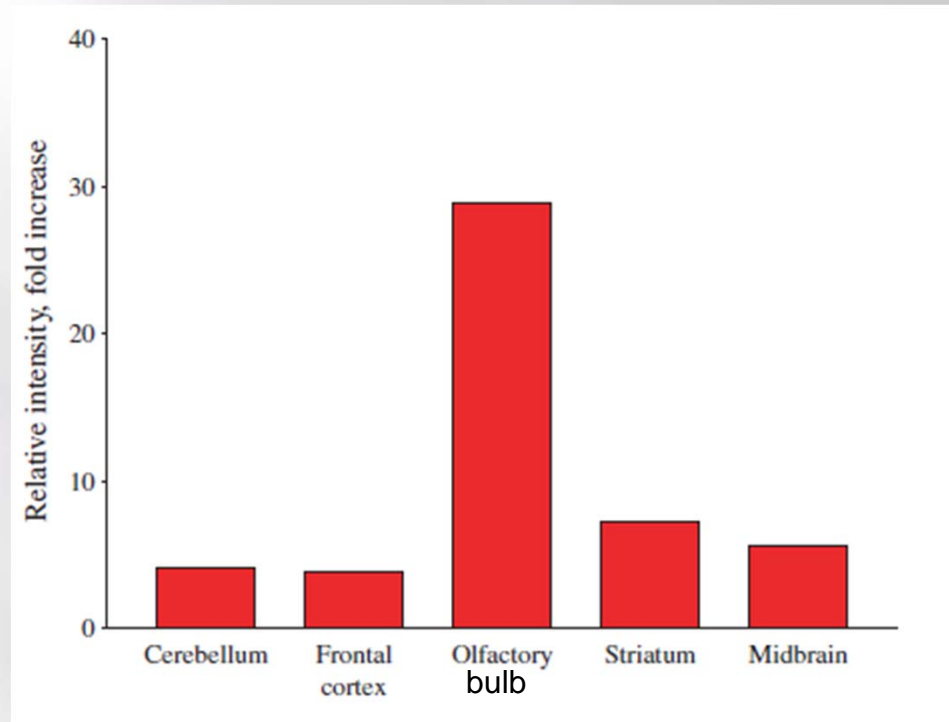
Neurotoxicity under relevant exposure conditions?

Example: inhalative exposure towards MnO (30 nm)



Deposited Mn from MnO translocating from olfactory mucosa to olfactory bulb (from Oberdörster, 2009)

Neurotoxicity under relevant exposure conditions?



Changes of TNF alpha expression in brain regions after 12 days exposure to ultrafine MnO in rats (from Oberdörster, 2009)

Neurotoxicity under relevant exposure conditions?

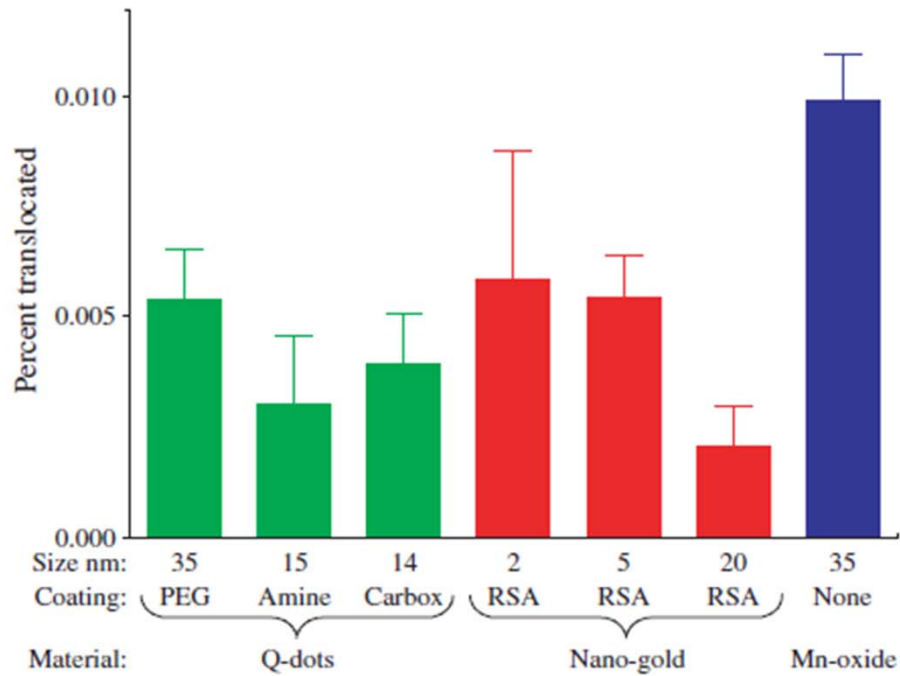


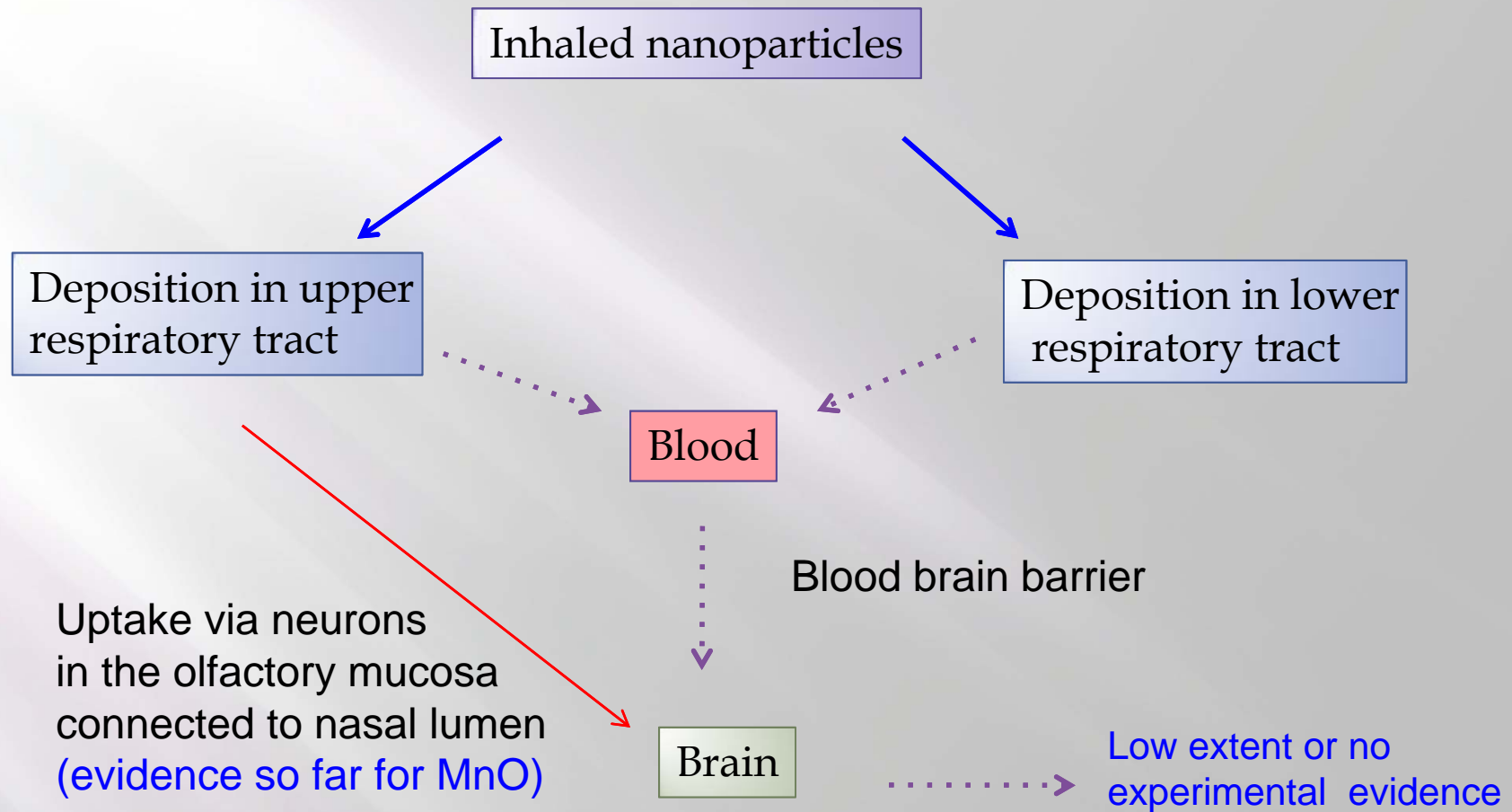
Fig. 12. Nanoparticle translocation to olfactory bulb 24 hrs after left intranasal instillation in rats. Percent of total instilled dose of nanogold particles (10 μg) of different sizes coated with rat serum albumin (RSA), and Cd-Se/Zn-S quantum dots (5 μg Cd) with different surface modification measured in the olfactory bulb. Intranasally instilled nano-Mn-oxide (10 μg) (see Fig. 9) is shown for comparison.

Nasal instillation:

Some translocation of further NP to the olfactory bulb, but very low percentage

(from Oberdörster, 2009)

Neurotoxicity under relevant exposure conditions?



Conclusions neurotoxicity

- ▣ In principle translocation through olfactory neuronal pathway for small particles (< 100 nm) possible
- ▣ Data showing significant accumulation in olfactory bulb after inhalation so far only available for MnO nanoparticles (33 nm; 0.01 - 5 mg/m²)
 - Higher accumulation of Mn in the olfactory bulb (4-fold) as compared to lung (2-fold)
 - Inflammatory response in the olfactory bulb with elevated Mn levels (eg., 30-fold increase in TNF α)

No comparable data suitable for risk assessment available for other nanoparticles

Microscale vs. nanoscale granular biopersistent particles

Research needs:

- Careful characterization of particles, also with respect to agglomeration/aggregation and also in relevant biological fluids
- Establishment of suitable cell culture systems to systematically elucidate the impact of composition, size, agglomeration/aggregation, shape, surface area etc. on cellular uptake, cytotoxicity, genotoxicity and other critical reactions

Microscale vs. nanoscale granular biopersistent particles

Research needs (continued):

- Comparison of microscale and nanoscale materials in **in vivo** systems **for relevant exposure conditions** with respect to
 - Target organ(s), deposition and clearance, identification of critical reactions
 - Discrimination between direct and indirect genotoxicity → cell-type specific determination
 - Dose-response-relationships, required to identify NOEL/NOAEL
 - Identification of parameters decisive for setting OELs (e.g., mass, surface area)?

Summary and conclusions

- Risk assessment of nanomaterials requires the same information as compared to microscale materials, but additionally comprehensive characterization of the particles under investigation
- Establishment of research strategies for groups of nanoparticles to systematically elucidate differences between nanoscale and microscale materials (cell culture, in vivo test systems; investigation of structure-activity-relationships)
- Identification of common criteria for risk assessment for groups of nanoparticles (e.g. nanoscale vs. microscale biopersistent particles; metal-based particles of different size; chemical or drug-containing nanomaterials)

Summary and conclusions

**Important are dose-response investigations
including low exposure conditions!**