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Toxicological Assessment of (silver) Nanomaterials: Challenges and Pitfalls

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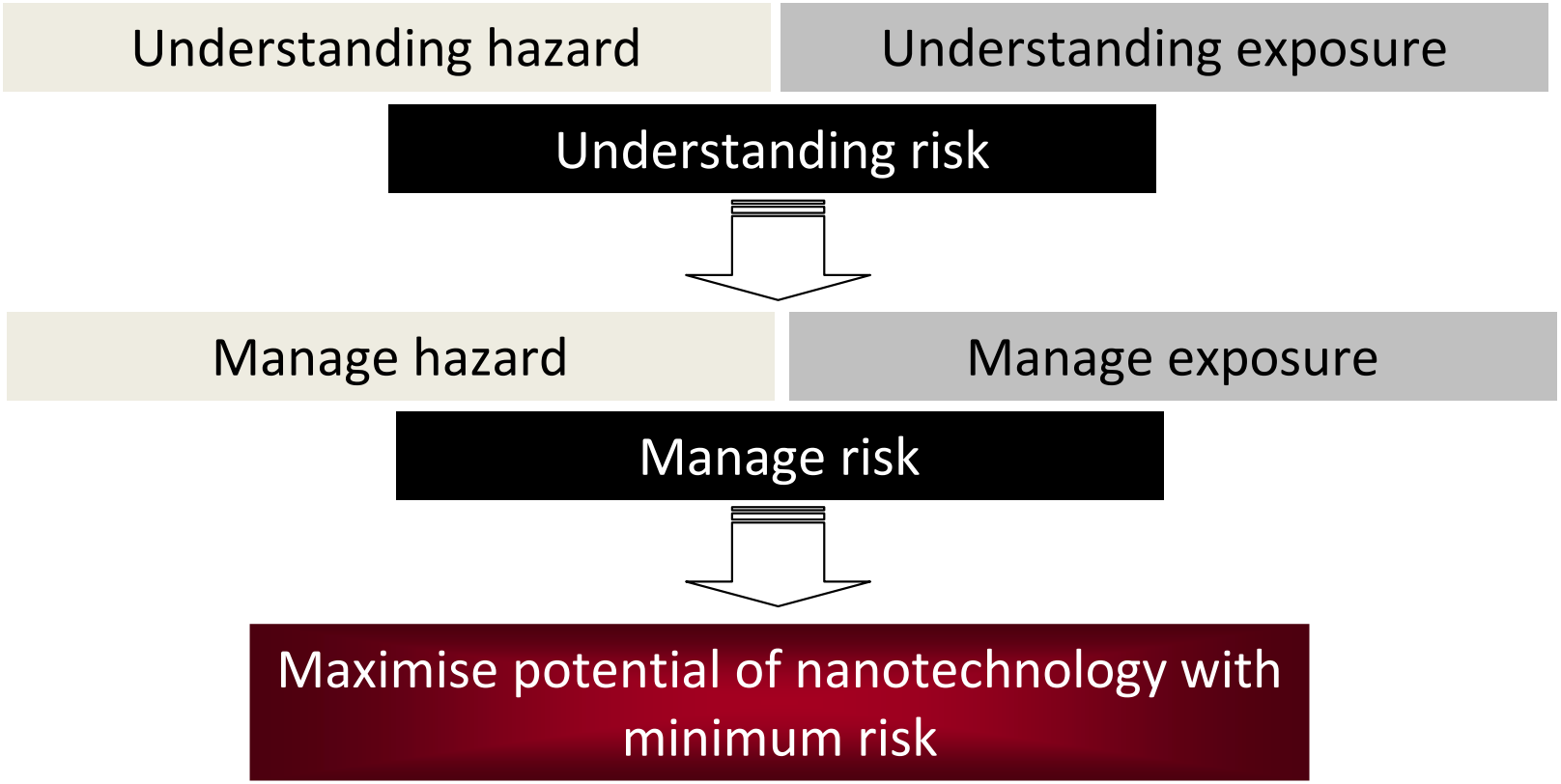
Nanotoxicology

- The behaviour of NMs has been demonstrated to be **strikingly different** to that of their larger counterparts
  - This **drives their exploitation**: many industries (textiles, cosmetics, food, electronics, pharmaceutical) want to harness these properties so NMs will be exploited within many different products
  - This **promotes concern** surrounding the exposure to NMs
  - **Nanotoxicology** is concerned with investigating the toxicity of **engineered** NMs to human health and the environment

NanoSilver

- Silver NMs are incorporated into **diverse products**
  - Use and production expanding so potential for human and environmental exposure is increasing
- Exploitation mainly derives from its **antimicrobial properties**
  - Toxic to bacteria – what about other targets?
  - Bacterial resistance to silver NMs?
  - Silver is a known to exhibit adverse effects
  - argyria (skin discolouration), aquatic toxicity
- Investigating the toxicity of silver NMs is essential
  - Size dependent effects?

Maximising the potential for nanotechnology by minimising the risk

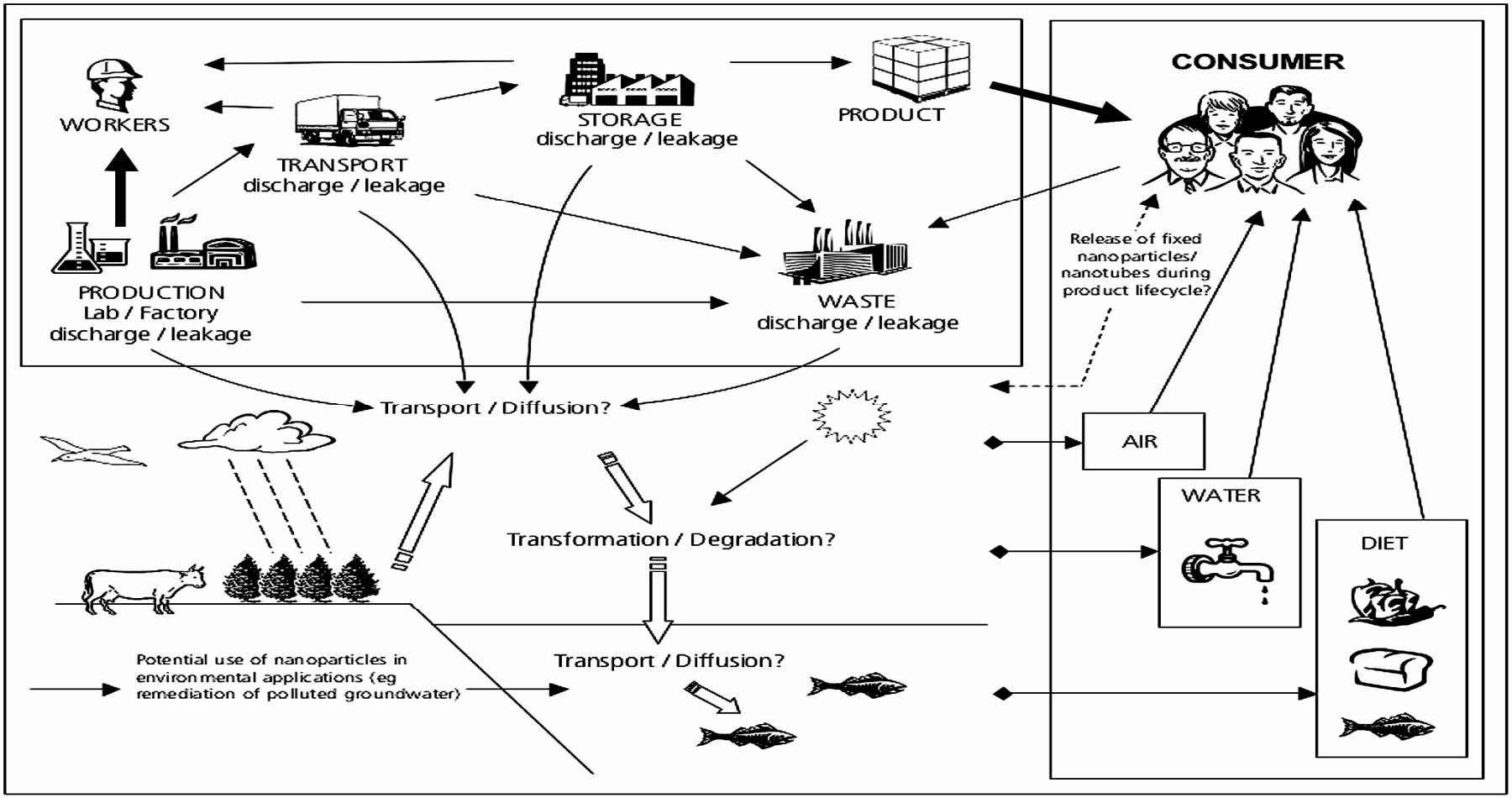


Identification of the Risks posed by NMs

Risk = hazard x exposure

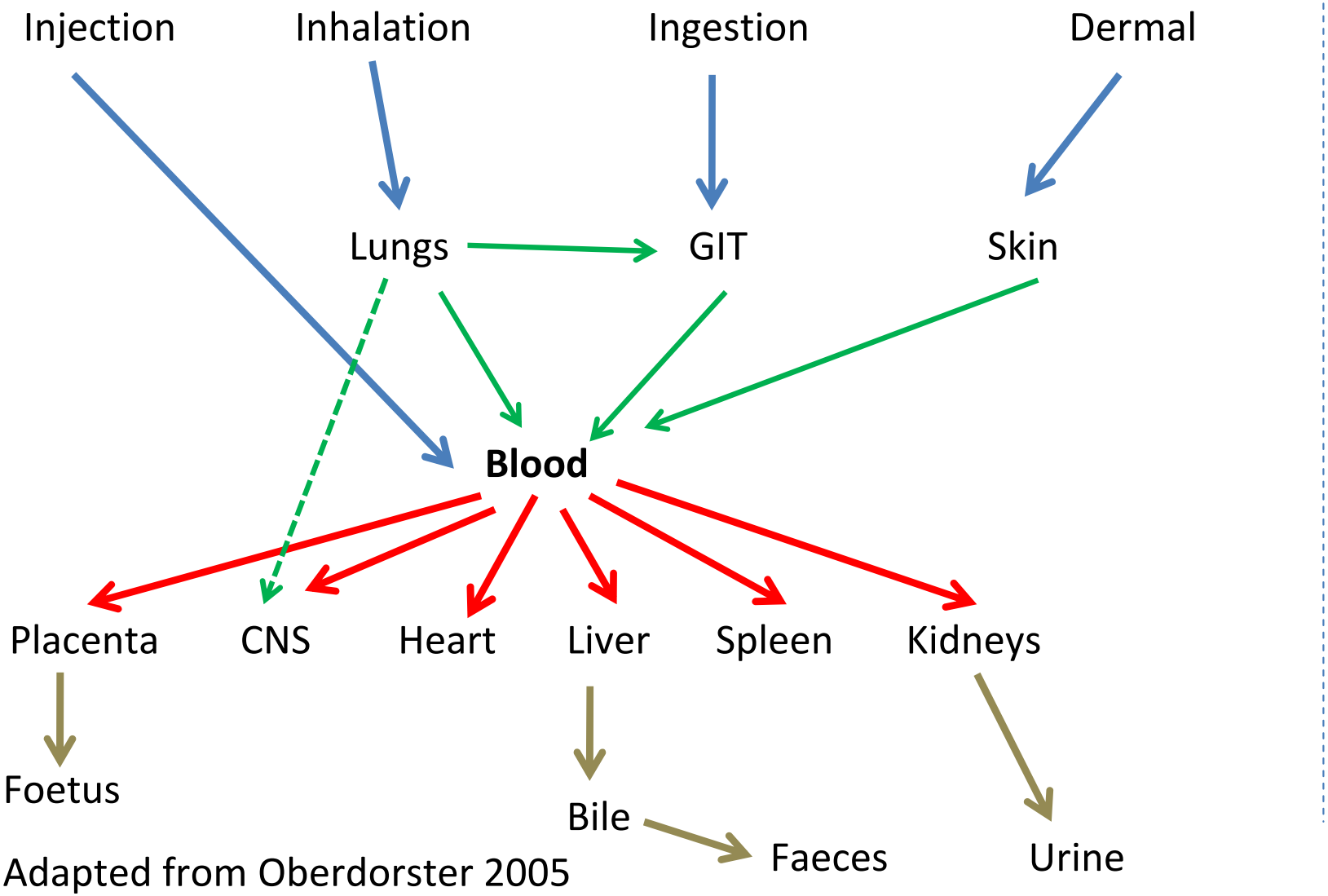
- **Exposure** must occur for a substance to present a risk to human health or the environment
- Human and Environmental risk assessments cannot be conducted for nanomaterials:
  - Lack of **exposure** information
    - Require a greater understanding of the fate and behaviour of NMs in the environment: predictive models?
    - Need robust methodologies to assess exposure in different environmental matrices
    - Require information on production and use of silver NMs
  - Lack of **hazard** information
    - Limited studies have looked at the adverse impact of NMs
    - No standardised tests to assess the safety of NMs

Exposure to NMs: Life cycle perspective



Royal Society *et al*., Report 2004

Distribution of NMs following exposure



Adapted from Oberdorster 2005

NM Fate

Exposure

Exposure Site

Translocation and Distribution

Elimination

Hazard Investigations

- The is a drive to **harmonize the safety testing** of NMs
  - OECD sponsorship programme
- NMs may pose unique hazards not currently considered within 'traditional' safety tests, and existing guidelines may need to be adapted to test NM safety
  - Need to determine the applicability of existing safety tests to NMs
- There are a number of **obstacles** that need to be overcome to achieve this:
  - How should NMs be characterised?
  - How should NMs be dispersed? How does the NM form change following exposure?
  - What concentrations of NMs should be used in toxicity tests?
    - What dose metric is best to use for NM exposures?
  - What experimental models should be used?
  - What endpoints should be assessed?

Physico-chemical characterisation

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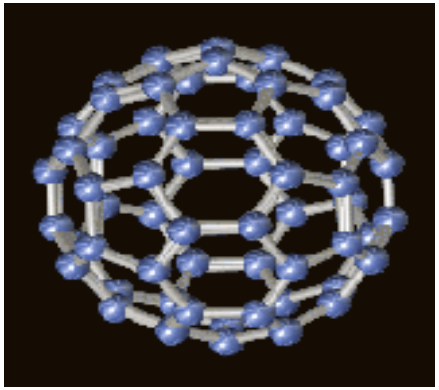
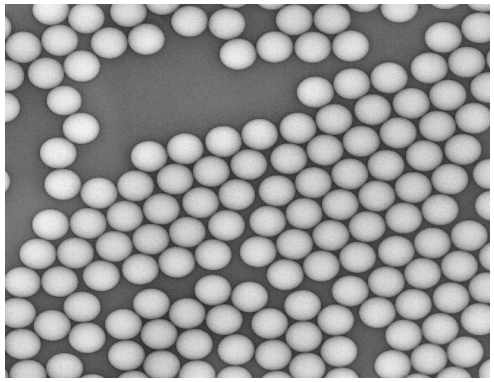
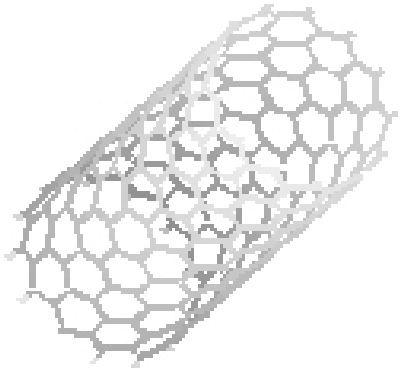
Nanomaterials



C_{60}
Fullerenes

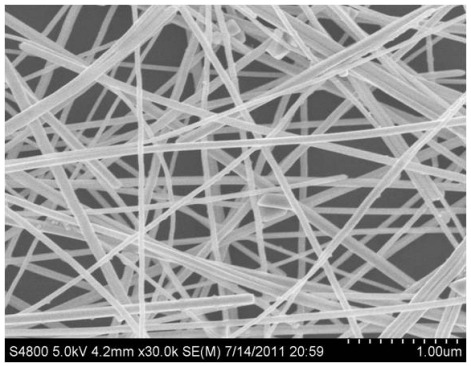


Polystyrene
Beads

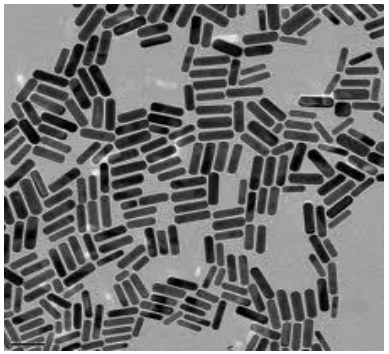


Carbon Nanotubes

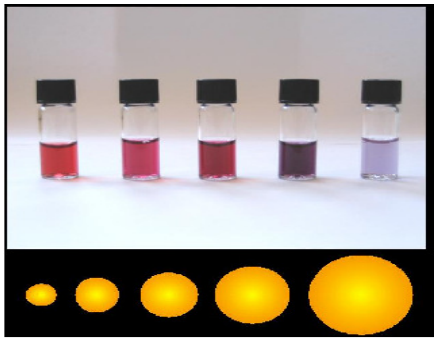
Quantum Dots



Silver Nanowires



Gold nanorods



Nano Gold

Characterisation

- There is no comprehensive list of the properties of NMs which require characterisation within toxicology investigations
  - Lack of standardised techniques to characterise NMs
  - No related reference materials
- Attributes of materials that should be routinely assessed include:
  - Size, surface area
  - composition (purity)
  - morphology
  - crystal structure
  - aggregation/agglomeration
  - surface chemistry
  - solubility
  - charge

Many approaches can be used to investigate these (which one(s) is most suitable?)

Characterisation

- Ideally, an extensive characterisation of NMs would be performed within all hazard investigations
    - This is not realistic due to financial and time constraints and required access to advanced instrumentation and specialist technical expertise
- Use more than one technique to confirm findings due to the limitations associated with existing techniques
- Characterisation in ‘as produced’ and ‘as tested’ forms required
- Need this information for promoting the safe design of NMs

Dispersion of NMs for hazard investigations

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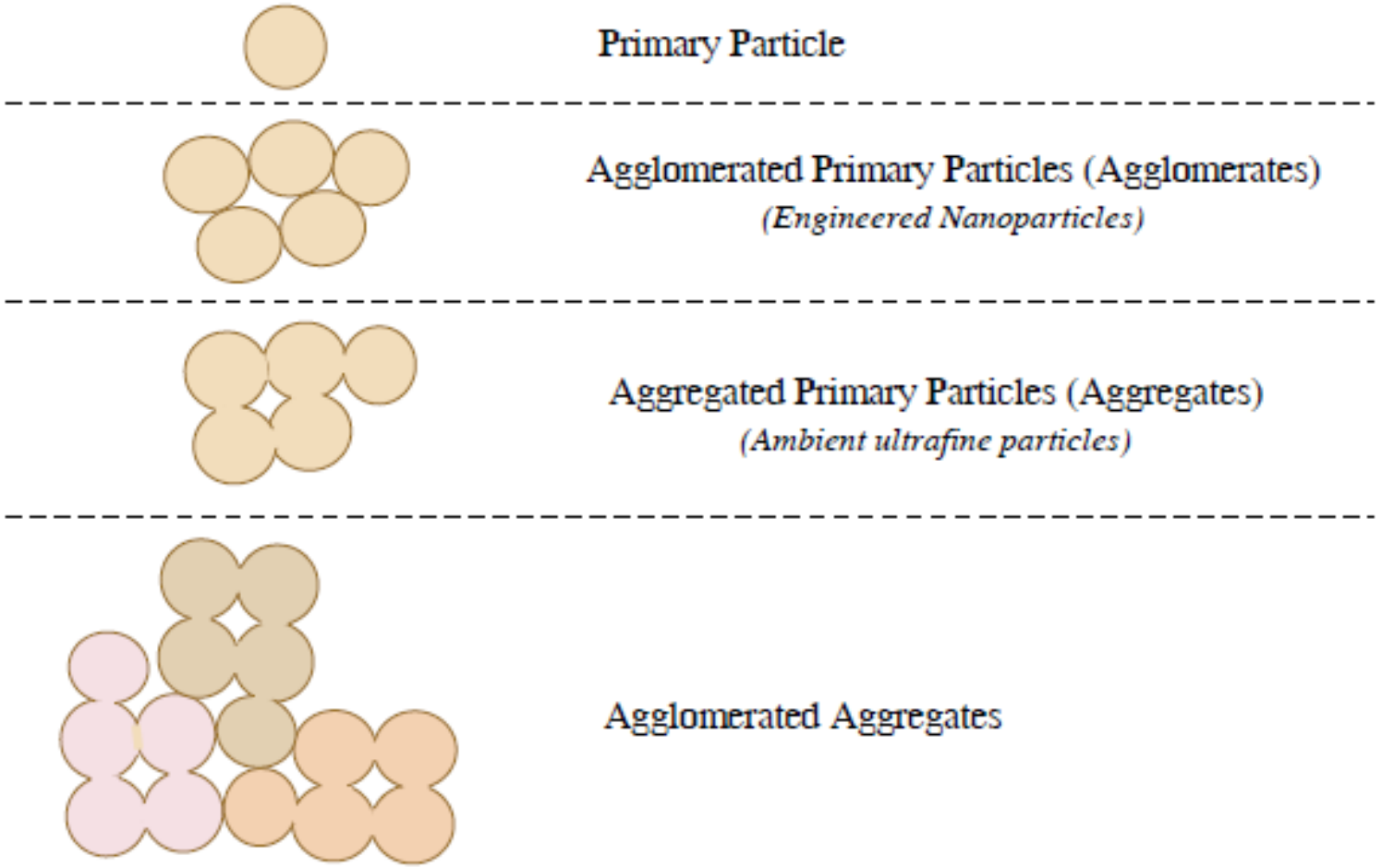
NM agglomeration / aggregation

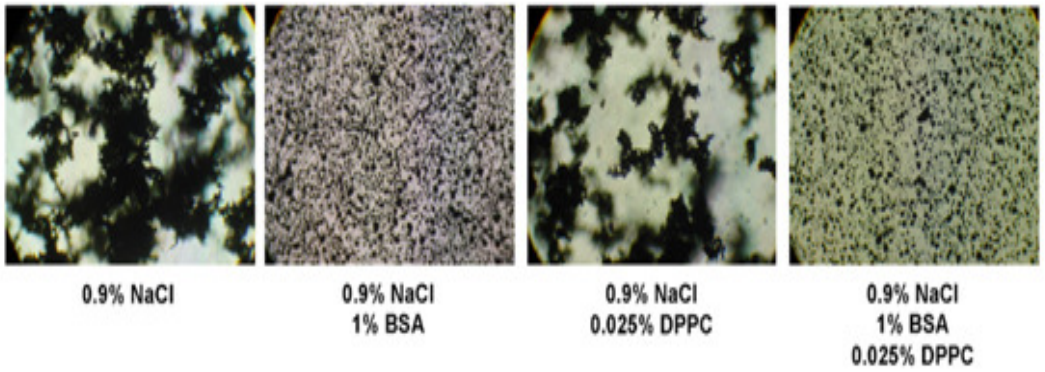
- NMs have a tendency to interact to form larger structures
- The terms agglomeration and aggregation are often used interchangeably to describe the attractions that hold together a collection of particles.
- However it is more appropriate to consider NM aggregation and agglomeration as distinct phenomena
- **Agglomerates** are formed by clusters of NMs that are held together by electrostatic interactions (Oberdorster, Stone and Donaldson, 2008).
- **Aggregates** are formed from fused NMs (by covalent bonds or sintering) that are not easily separated, and it is also possible for aggregates of NMs to agglomerate (Oberdorster, Stone and Donaldson, 2008).

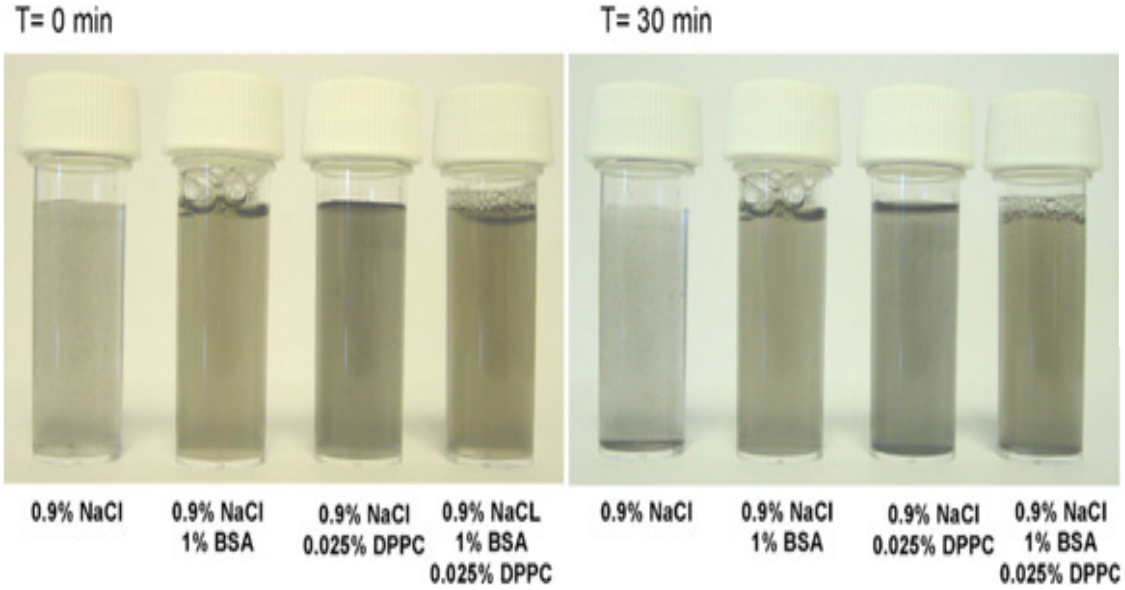
NM agglomeration / aggregation

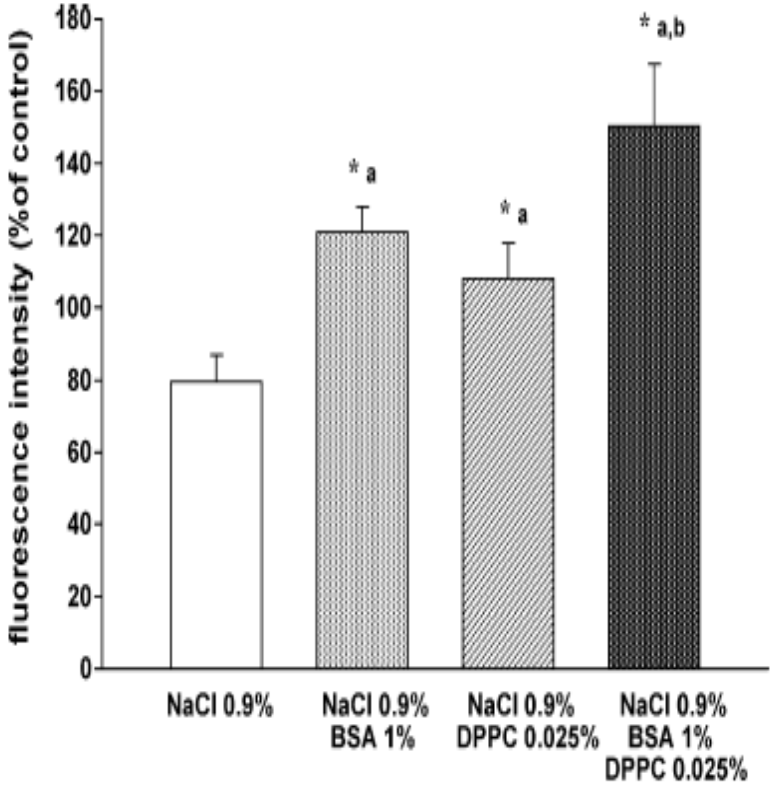


Oberdorster , Stone and Donaldson 2008

Dispersion of NMs – how the agglomeration/ aggregation of NMs can modify their toxicity



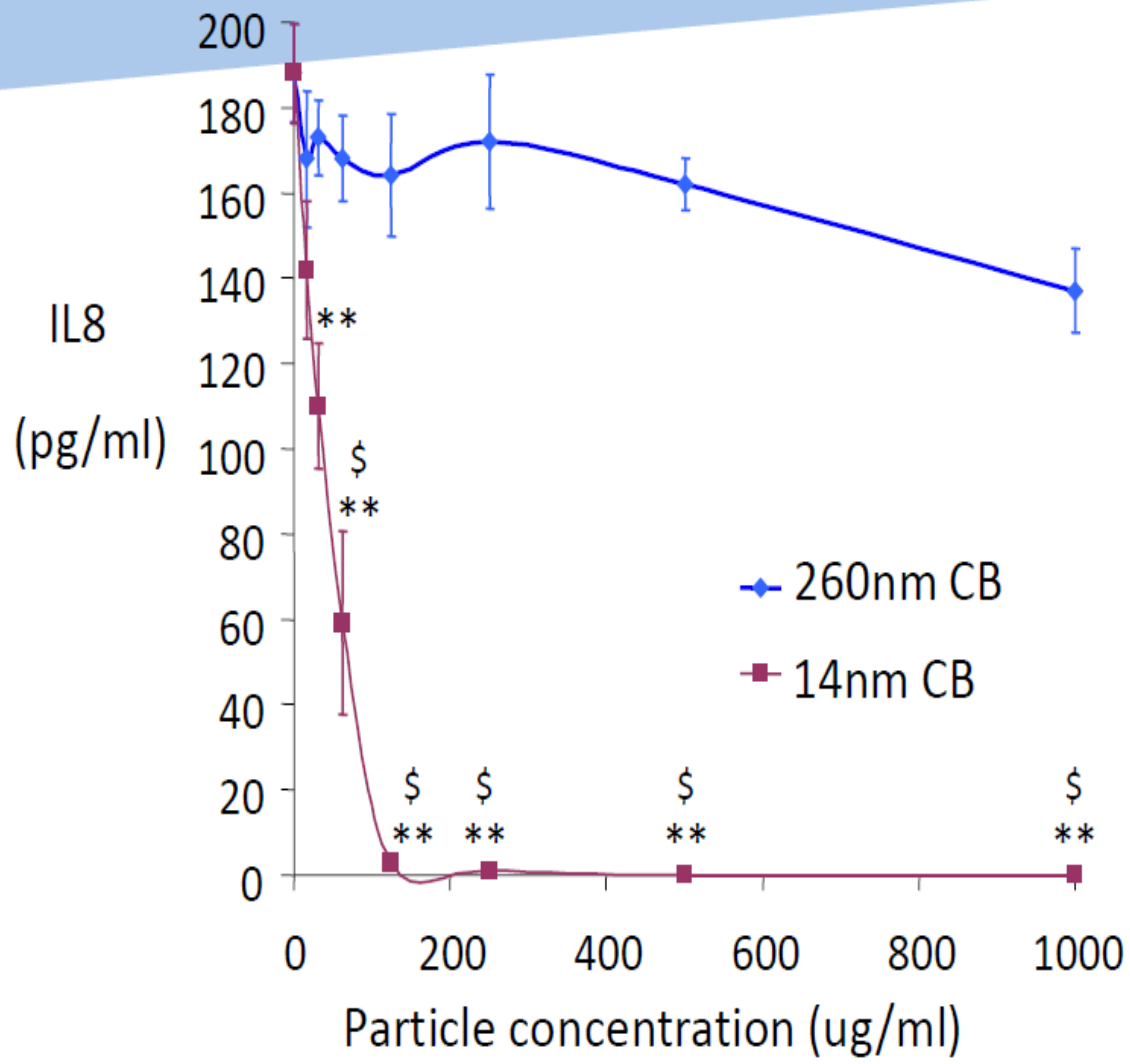




Foucaud *et al.*, (2007) *Toxicology Letters* 174; 1–9.

Interaction with biological molecules

IL-8 adsorption to the NM surface



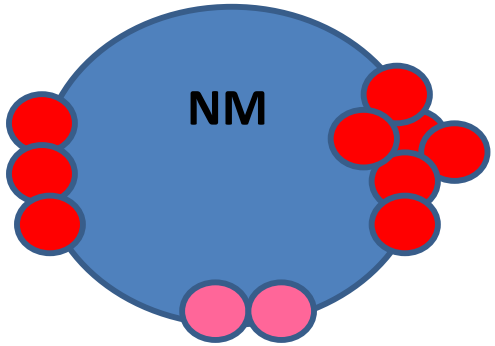
* p<0.05 vs control
** p<0.01 vs control
$ p<0.001 vs 260nm at the same concentration

Brown *et al.* 2010 Nanotechnol. 21 (21: 215104.

Consequences of NM-Protein interactions

Particle interference with toxicology assays

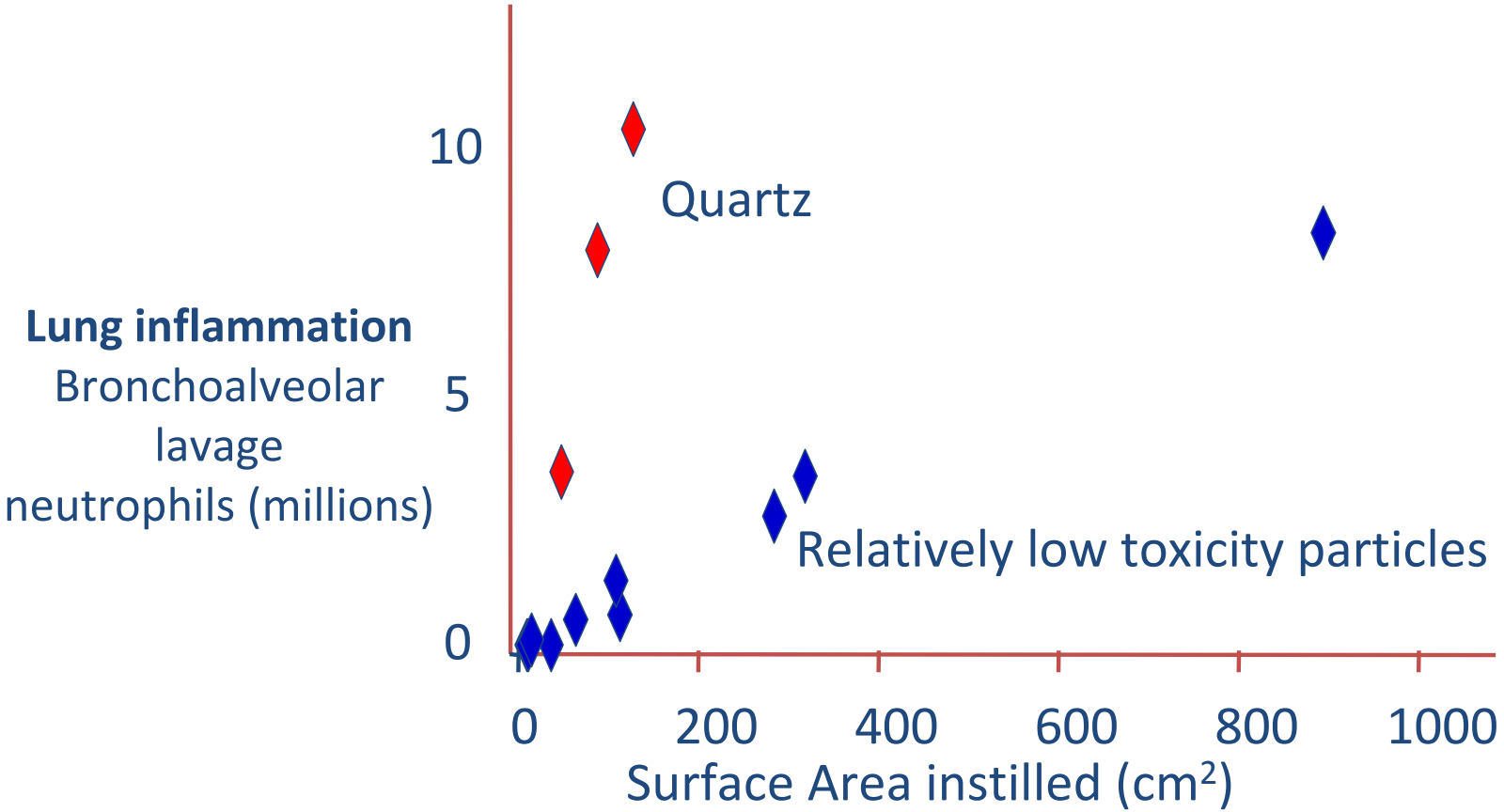
Proteins block the particle surface prevent surface related toxicity



Protein binding to the NP results in the bolus delivery of protein to the cell surface

Proteins are denatured preventing protein activity

Surface Properties



Duffin et al. 2002 Ann Occup Hyg 46; 242-245

In Vivo vs In Vitro

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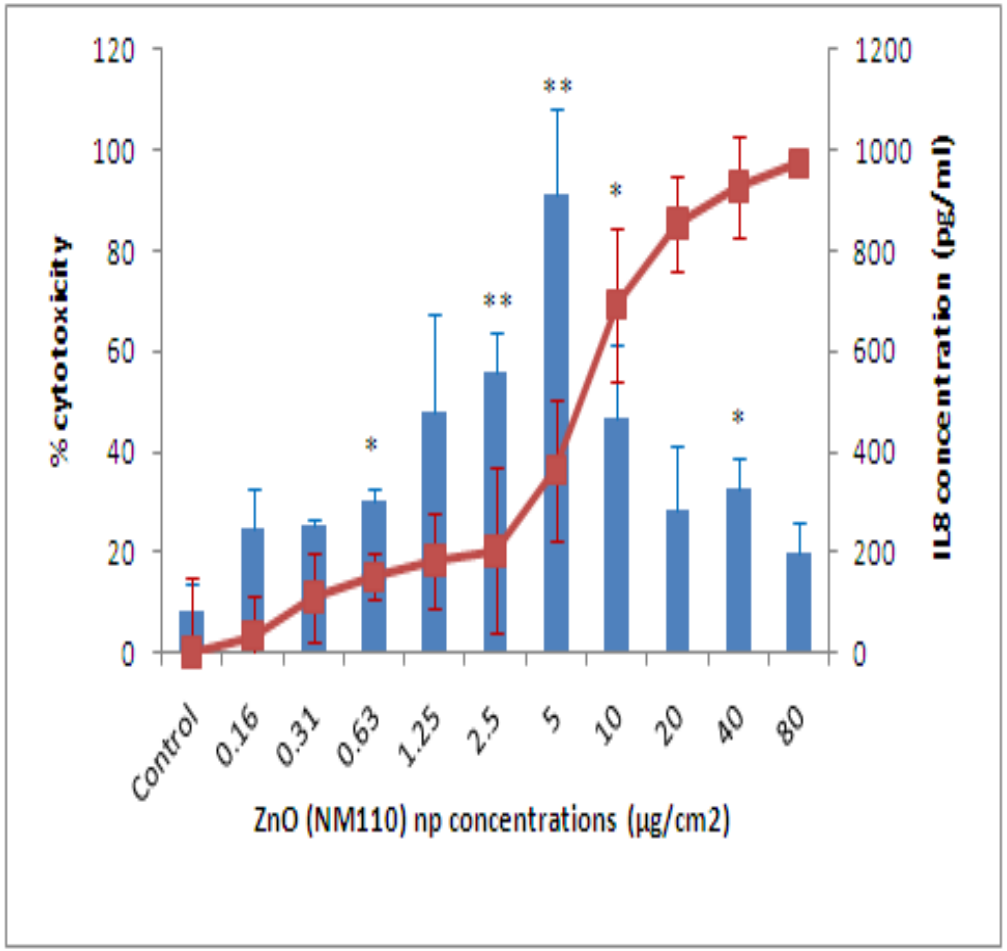
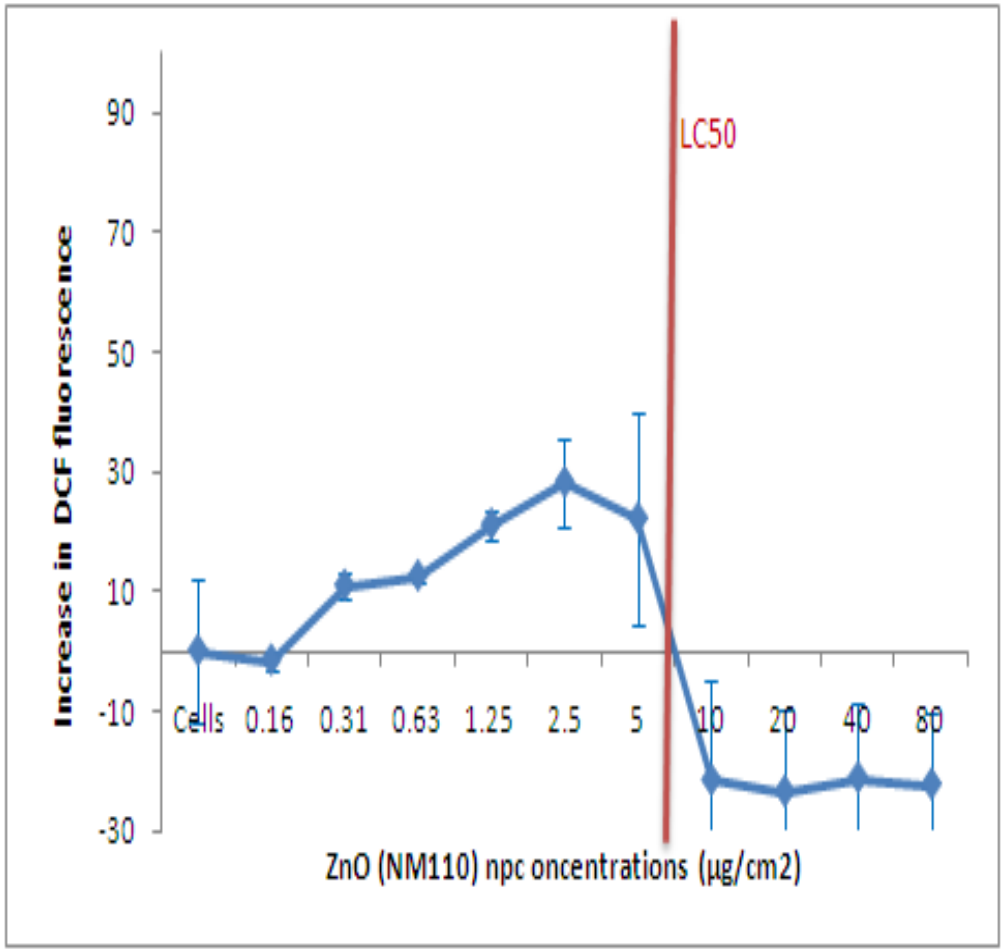
ENPRA: high toxicity group

Similar results for coated ZnO and Ag NP

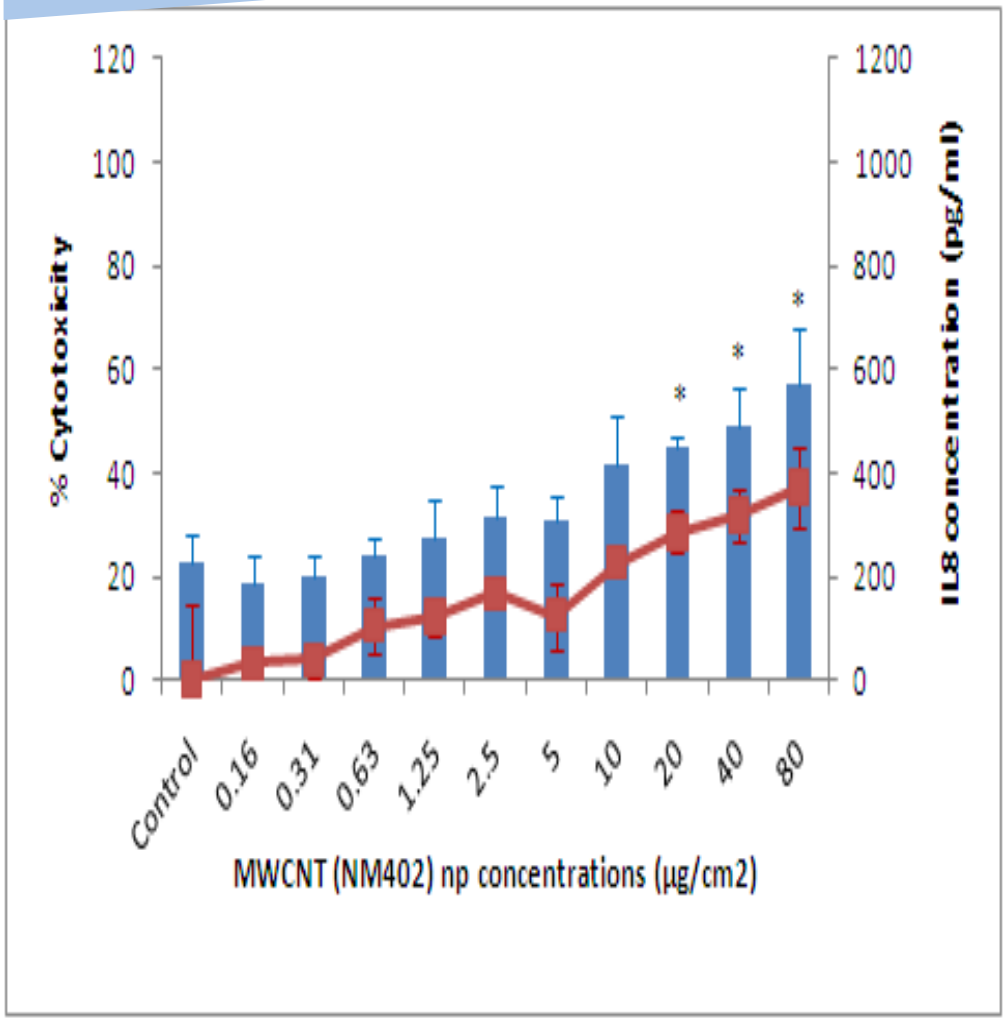


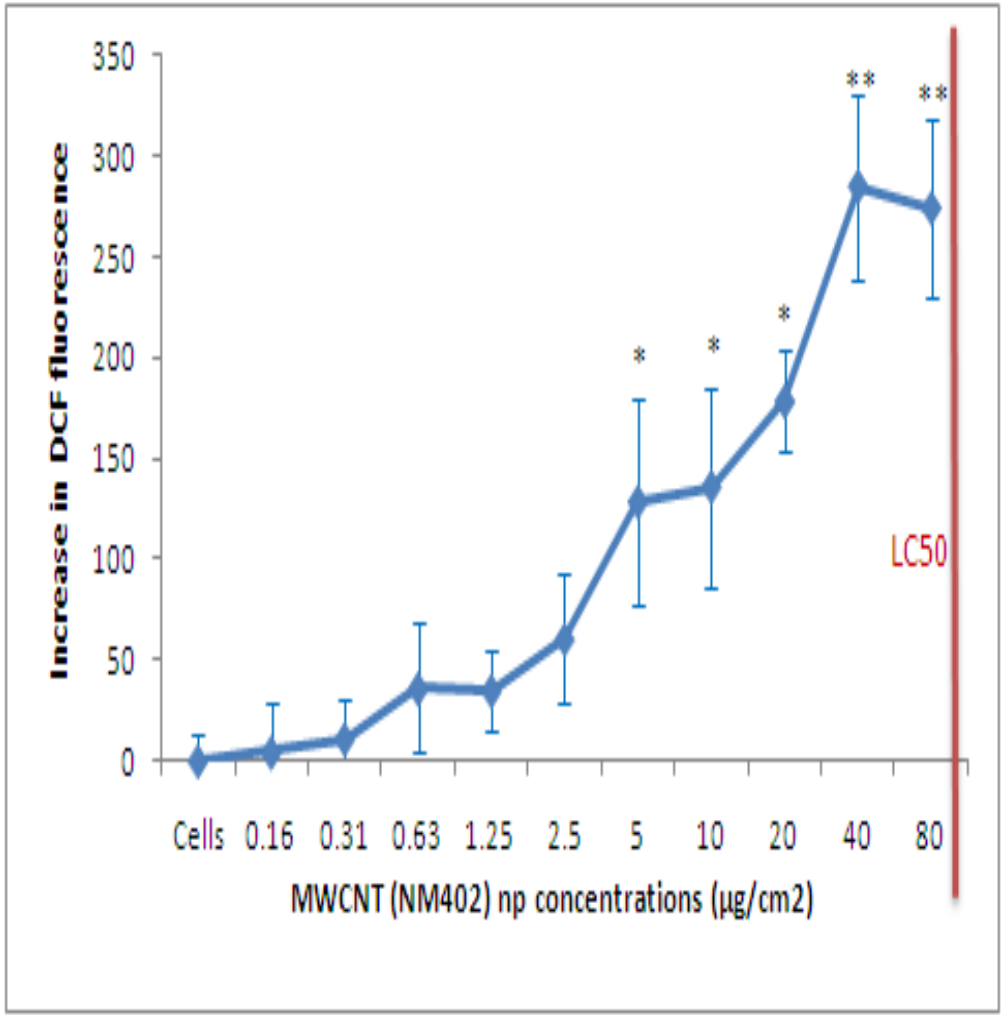


Kermanizadeh et al., (2012). Nanotoxicology. Online ahead of print

ENPRA – low toxicity NMs

Similar results for MWCNTs and TiO_2





Kermanizadeh et al., (2012). Nanotoxicology. Online ahead of print

ENPRA - Grouping of NMs based on their hazards

- The NM panel to be divided into **low toxicity** (TiO_2 and MWCNT) and a **high toxicity** (Ag and ZnO) groups.
  - The physico-chemical properties of NMs influence their toxicity
  - Different NMs may act via different mechanisms
- *In vitro* data corresponds well to the *in vivo* rodent studies
  - Suggests that the *in vitro* systems are effective, predictive models of NM toxicity
  - Supports the development of animal alternatives. This is essential from a financial and ethical perspective due to the vast number of diverse NMs in development and use.

Cross species comparison

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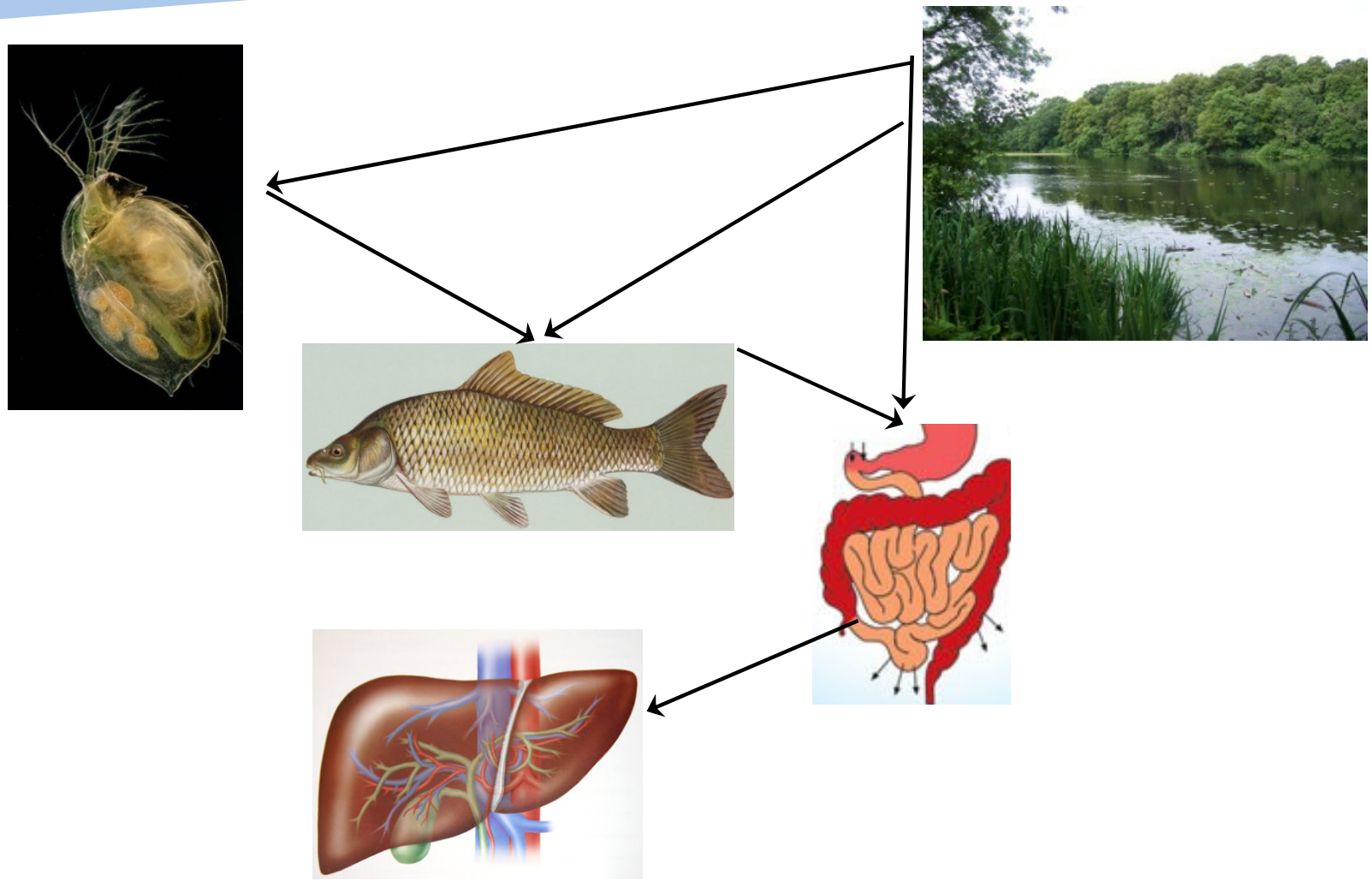
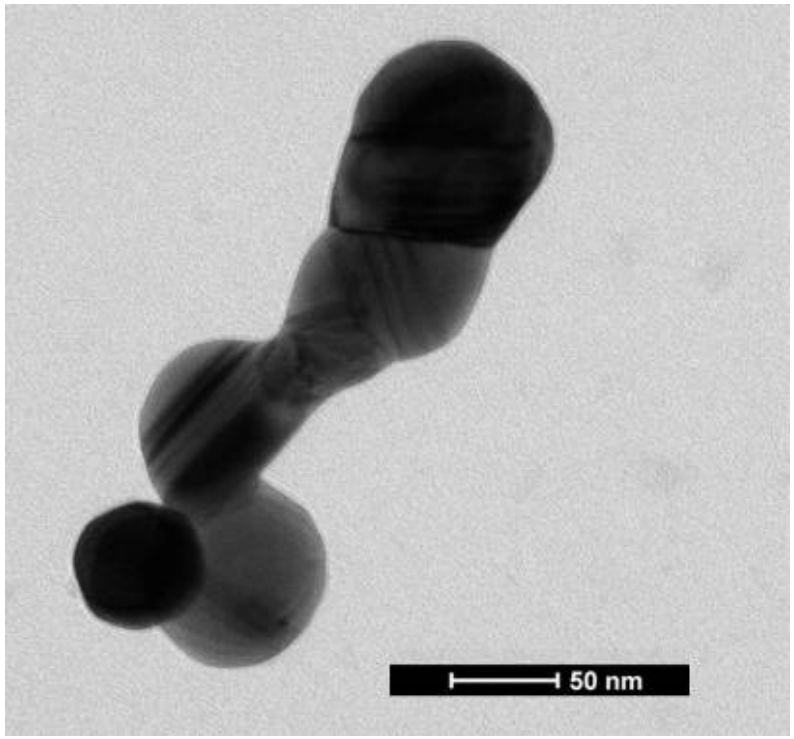
Exposure of different species: Interspecies comparison



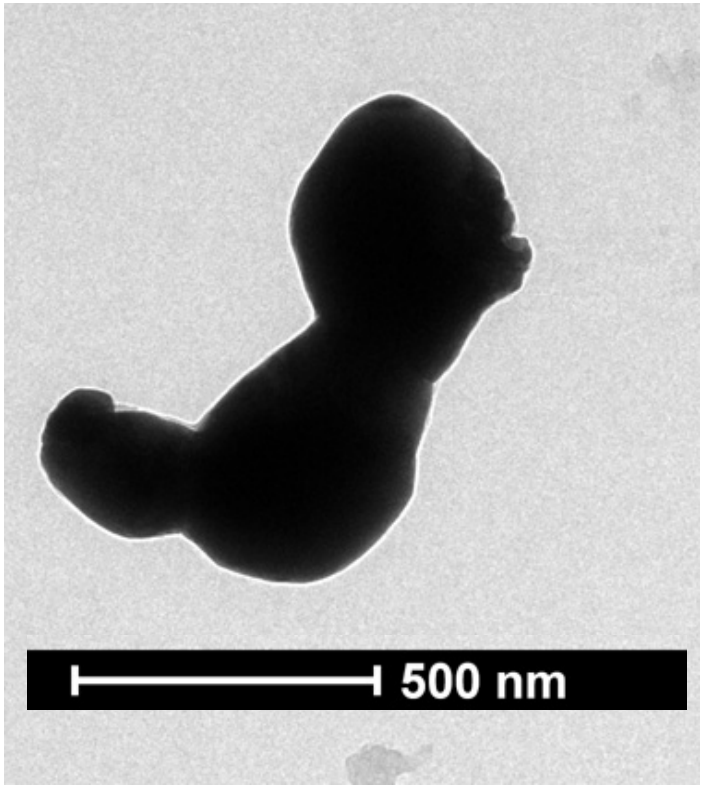
Uptake by multiple species from the environment and via ingestion: commonalities in the response?

Particle Panel

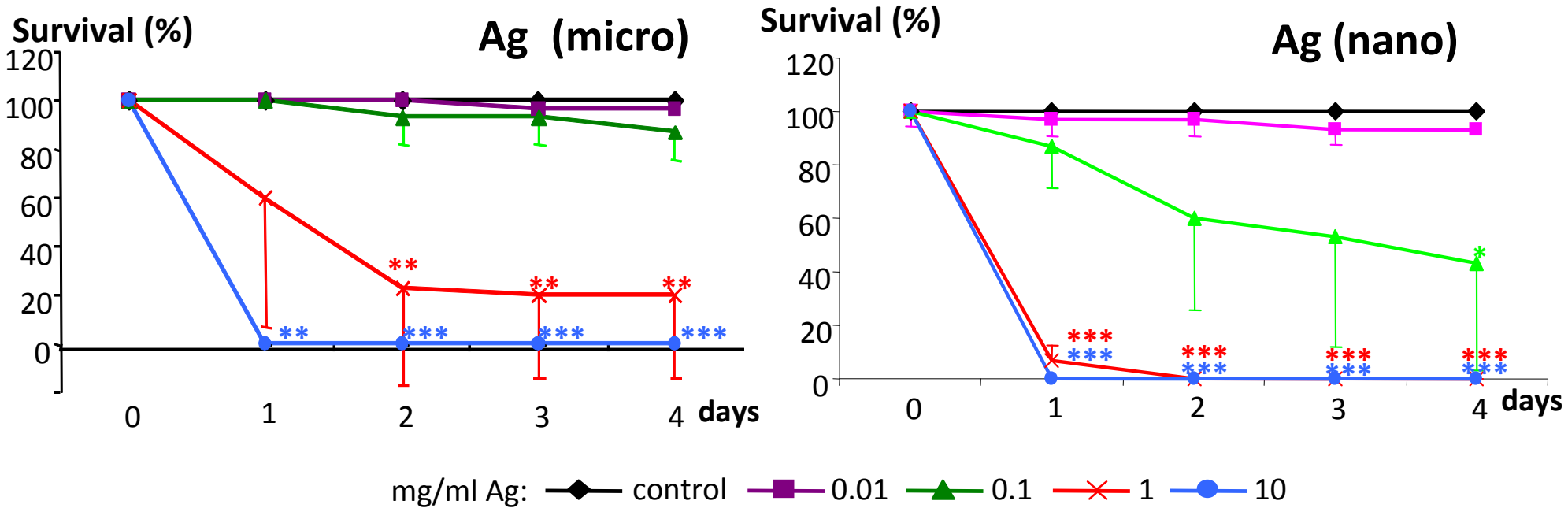
Nano Ag (TEM)



Micro Ag (TEM)



Toxicity of Ag particles to Daphnia Magna

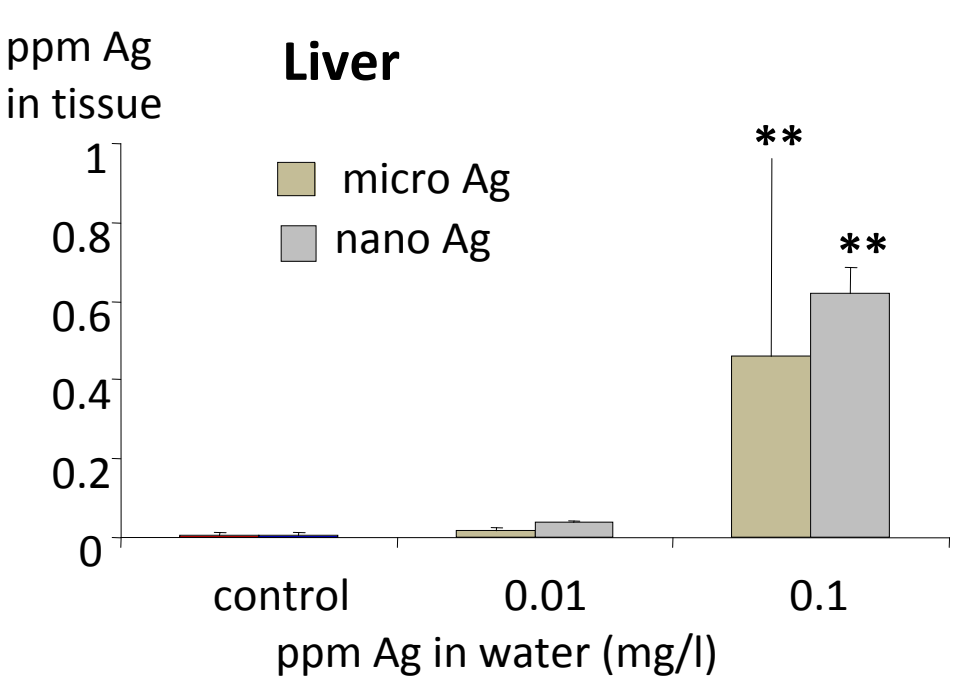


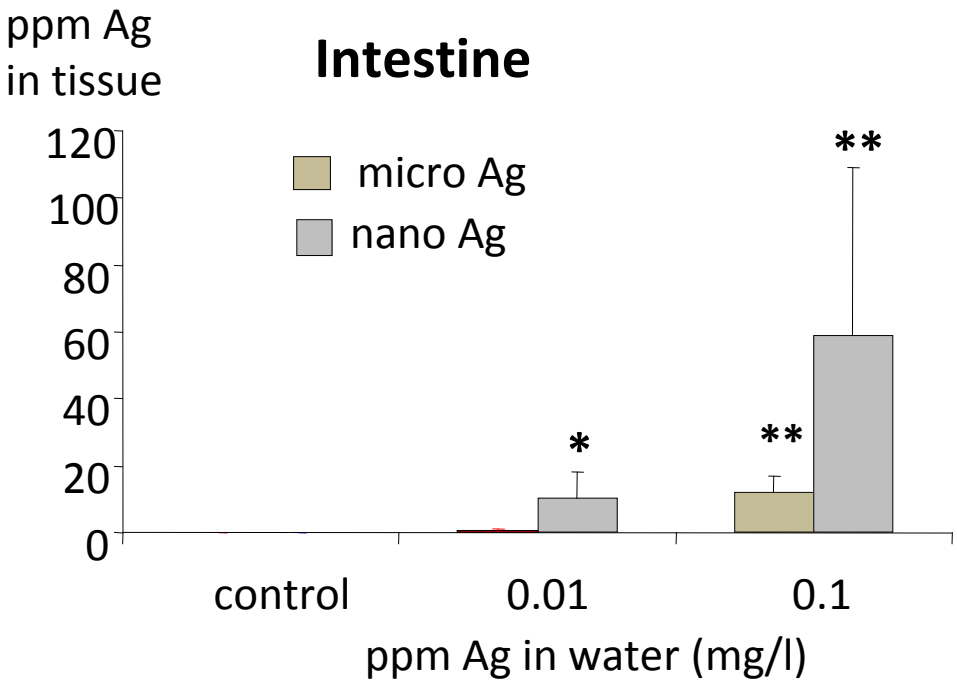
→ Both nano and micro Ag induce significant toxicity to *D. magna*

→ Nano Ag is more toxic than micro Ag

Gaiser et al, (2011). J Environ Monitor 13; 1227-35

Bioavailability of Ag in Carp (21 days)





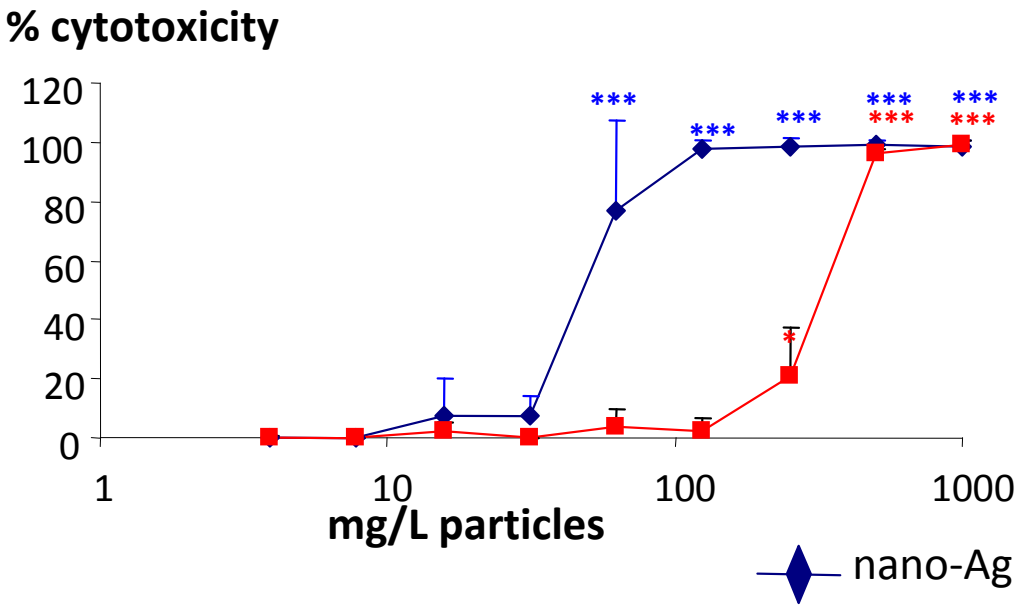
- Nano and micro, both lead to significant increase in Ag content of liver
- Also observe detectable uptake in gill and gall bladder tissue.

Gaiser et al, (2011). Env Tox Chem

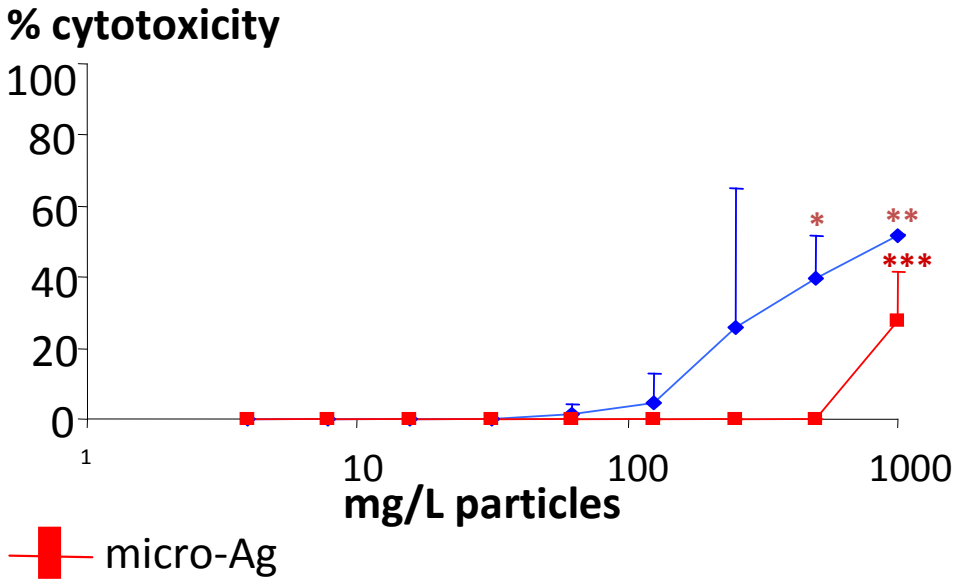
- Nano (0.01ppm) results in sig intestinal Ag content
- At 0.1 ppm micro and nano result in sig intestinal Ag content
- Highest concentration of all tissues
  - Suggestive of ingestion of particles

Cytotoxicity (LDH) of Ag

C3A human hepatocytes



Primary trout hepatocytes



→ Dose- and size-dependent toxicity

→ Trout hepatocytes less sensitive

Gaiser et al, (2011). J Environ Monitor 13; 1227-35

Cross species comparison

In a variety of models

- invertebrate, fish primary cells, human cell lines.

- Ag nanoparticles are more toxic than Ag microparticles
  - Therefore particle size influences toxicity.

- Ranking of toxicity is identical in all models tested

Summary

- The use of NMs in wide ranging applications is expanding
  - This is associated with the deliberate and accidental release of NMs into the environment
- **Physico-chemical characterisation** of NMs is required to identify what attributes of NMs drive their toxicity
- The **dispersion** of NMs in environmental media is a debatable issue
  - Physiologically relevant dispersants can improve NM suspension stability
- NMs **interact with biological molecules** following exposure
  - The exposure route can influence the subsequent toxicity of the NMs due to the acquisition of a different surface coating
- Silver NM **solubility** may influence their toxicity
- **Cross species comparisons** can investigate the toxicity of NMs
- Many obstacles exist to the testing of NM safety

Acknowledgements

- Prof Vicki Stone
- Dr David Brown
- Prof Teresa Fernandes
- Dr Birgit Gaiser
- Ali Kerminadezeh

Nanotoxicology Challenges

- **Harmonised approach** to NM safety testing achievable?
- **Experimental design** is key:
  - **Characterising NMs**
  - **Appropriate controls**
    - Investigate size dependency of effects
    - separate particle from ion effects
  - **Dispersing NMs**
    - NM agglomeration/aggregation, what is physiologically relevant?
  - **Dose Metric** and **Dose** of NMs administered
  - **Exposure Duration**
    - Acute vs chronic, repeat exposures?
  - **Interaction** of NMs with biological molecules
    - Physiological relevance and Interference of NMs with toxicology tests
  - ***In vivo* vs *in vitro* models**: correlation?
    - Correlation of findings?