Assessing the environmental effects of nanomaterials – dose metrics considerations

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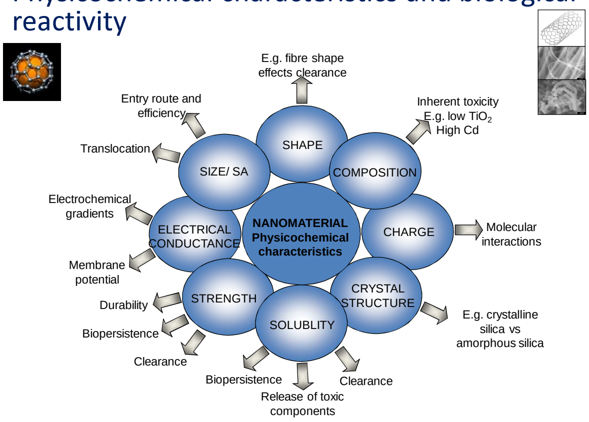
"Bridging nanoEHS Research Efforts"
US-EU Joint Workshop, Washington, 10-11 March 2011

Questions to be addressed in Session 3

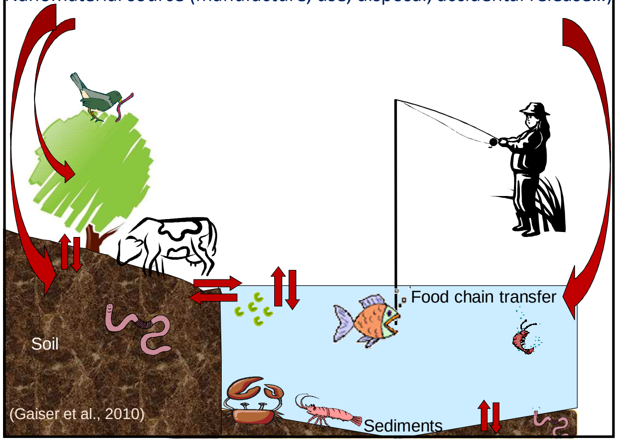
- What metrics are most scientifically accurate when relating dose to response in toxicity assessments?
- How are dose-response data best extended to determining environmental concern concentrations?

- Dose metrics are commonly reported as mass dose
- However, other dose metrics such as surface area dose or particle number dose have also been mentioned...

Physicochemical characteristics and biological reactivity

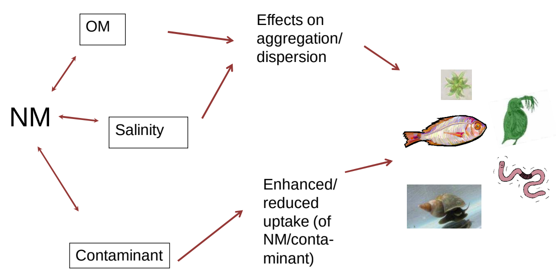


Nanomaterial source (manufacture, use, disposal, accidental release...)

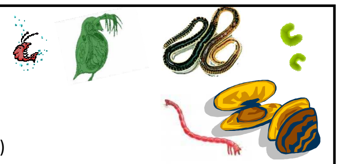


Fate of NM in the environment

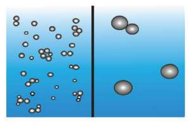
Interactions with other constituents and effects on bioavailability



Approaches



- What species?
- Endpoint – (mode of action?)
- Reported units – mass, surface area, particle number ....
- Acute/chronic
- Media composition – fresh/salt water; pH, OM, etc...
- Exposure conditions – temperature, light, shaking....
- Feeding/not feeding
- Bioaccumulation
- Population studies
- Food chain studies

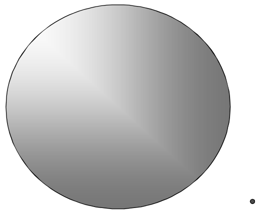


same mass concentration
(1 ppm)

Types of approaches

Surface area and surface atoms

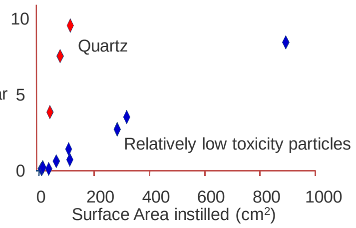
Smaller particles have larger surface area per equivalent mass



Diameter	100 µm	10 nm
Surface area	0.03 m^2/g	286 m^2/g
% atoms at surface	0.001 %	10.5 %

Stone and Kinloch Nanotoxicology (book), 2007, Ed Monteiro and Tran.

Surface area and inflammation



For relatively low toxicity particles (TiO_2 etc) there is a straight line relationship between surface area and lung inflammation.

Highly pathogenic particles with a highly reactive surface (eg quartz), are more inflammogenic in this model.

Duffin et al. 2002 Ann Occup Hyg 46 [suppl 1]; 242-245
Duffin et al. 2007 Inhal Toxicol 19 (10)

Surface area and inflammation

Six different carbonaceous particle types studied: PrintexG, Printex90, flame soot particles with different organic content (SootL, SootH), spark-generated ultrafine carbon particles (ufCP), and the reference diesel exhaust particles (DEP) SRM1650a. Mice were instilled with 5, 20, and 50 µg of each particle type, and bronchoalveolar lavage was analyzed 24 hr after instillation for inflammatory cells and the level of proinflammatory cytokines.

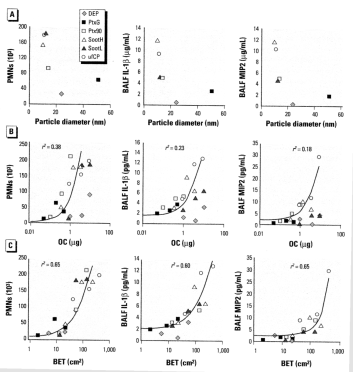
Table 1. Physical and chemical particle characteristics as determined by authors/suppliers.

Particle	Diameter (nm)	OC (%)	Surface area (m^2/g)
DEP	18–30/–	NA/20	NA/108
PrintexG	30–60/51	1/0.7	43/30
Printex90	12–17/14	2/1	272/300
SootH	8–16/–	19/–	268/–
SootL	8–14/–	7/–	441/–
ufCP	7–12/–	17/–	807/–

Abbreviations: –, not specified; NA, not analyzed. OC was measured according to the NIOSH 5040 method (Cassinelli and O'Connor 1998), and surface area was measured according to the BET method (Brunauer et al. 1938).

Stoeger et al. 2006. Instillation of Six Different Ultrafine Carbon Particles Indicates a Surface Area Threshold Dose for Acute Lung Inflammation in Mice. EHP 114; 328-333.

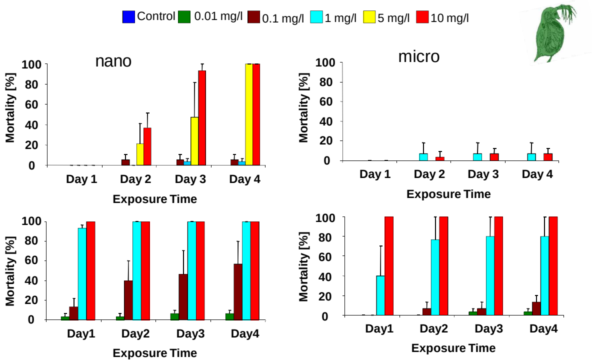
Surface area and inflammation



Relating the inflammatory effects to the particle characteristics: organic content, primary particle size, or specific surface area demonstrates the most obvious dose response for particle surface area. This study suggests that the surface area measurement developed by Brunauer, Emmett, and Teller (BET) is a valuable reference unit for the assessment of causative health effects for carbonaceous NPs.

Stoeger et al. 2006. Instillation of Six Different Ultrafine Carbon Particles Indicates a Surface Area Threshold Dose for Acute Lung Inflammation in Mice. EHP 114; 328-333.

Mortality of mass dose in 96h acute tests with micro and nano sized particles of carbon black (top) and silver (bottom)



Philipp Rosenkranz

Particles used in these experiments

CB: micro (260 nm) and nano-sized (14 nm)

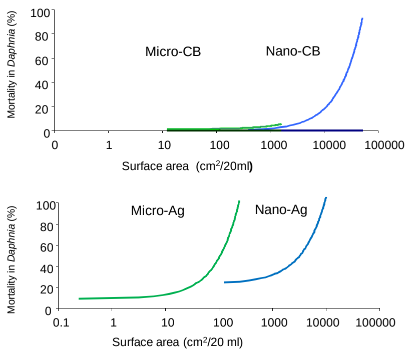
(8m²/g) (254m²/g)

Ag: micro (0.6-1.6 µm) and nano-sized (35 nm)

(1.2m²/g) (50m²/g)

Philipp Rosenkranz

Mortality as a function of surface area dose



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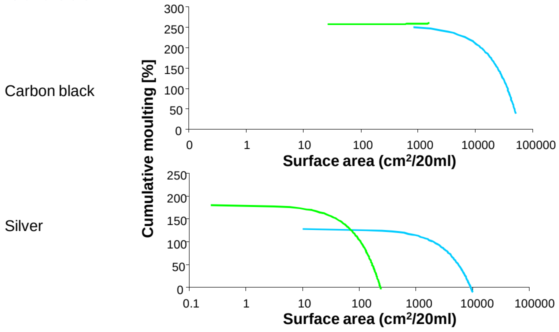
Cumulative moulting as a function of surface area dose



Philipp Rosenkranz

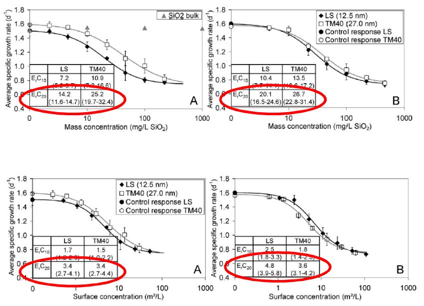
Conclusion from this study?

Effects of carbon black are more closely linked to surface area dose then the ones obtained for silver

Mortality results obtained on exposures to CB suggest a positive relationship with surface area dose suggesting that the toxicity is surface area related

Silver shows a similar pattern for each particle size, but the model suggests that surface area dose is not the only factor responsible for the mode of Ag toxicity

Assessing toxicity – comparing mass dose with surface area dose.... *Pseudokirchneriella subcapitata* exposed to silica particles (12.5, 27 nm and < 62 um) for 72 hrs



EC_{20} sig. different when treatments are compared as mass concentration
➢PARTICLE TYPE DEPENDENT

EC_{20} NOT sig. different when treatments are compared as surface area concentration
➢SURFACE AREA DEPENDENT

Van Hoecke et al (2008)

Assessing toxicity – comparing mass dose with surface area dose.... *Daphnia magna* exposed to CeO_2 particles (14, 20 nm, 29 nm and bulk) for 21 days

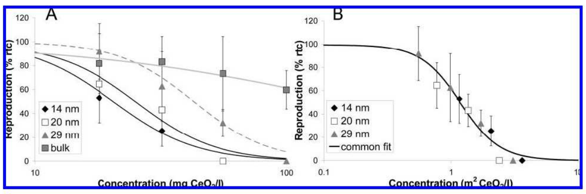


FIGURE 3. Concentration–response curves and calculated log–logistic fits of a 21 days reproduction test with *D. magna* obtained for 14, 20, and 29 nm CeO_2 NPs and CeO_2 bulk material. Concentration is expressed as mass (A) and as surface area (B).

- Pattern was different for the different particles when assessed as mass concentration.
- No differences between the different particles found when surface area concentration was used

Van Hoecke et al (2009)

Summary

- A range of physico-chemical characteristics influence nanomaterial toxicity
- The receiving environment affects fate, bioavailability and effects
- Assay preparation and conditions, as well as reporting of any observed effects need to be considered carefully
- Surface area and particle number dose metrics may be provide an interesting perspective when interpreting and reporting results from hazard studies
- A major issue to consider is how to measure accurately surface area and particle number in environmental matrices

Summary

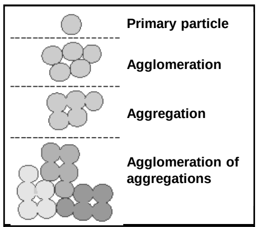
- BET is a method developed by Brunauer, Emmett and Teller for measurement of specific surface area and pore sizes of dry powders by gas sorption under high vacuum conditions
- BET measurements may not be accurate even in dry samples given that results depend on displacement of gas and their reproducibility will depend on assay conditions; BET is more appropriate for materials with homogeneous surfaces (?)
- ESA (Envelope Surface Area Analyzer)? The BET technique gives total surface area including that within the particles (if porous), while the ESA gives the surface area on the exterior of the particles, which is used to calculate the average particle size.
- Visual images can be used to estimate surface area

➢ *The specific surface area (SSA) measured by BET in a dry sample may not coincide with the apparent SSA in aqueous dispersion, especially for aggregating particles (Waychunas et al. 2005), although other methods for aqueous SSA may be used such as colourimetric titrations and nuclear magnetic resonance (NMR) measurements (Washton et al. 2008; Yukselen and Kaya 2006). The calculation of SSA is further complicated by the effect of shape and porosity on the SSA calculation.*

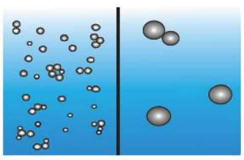
In: Ju-Nam et al (submitted to Nanotoxicology).

Important question

- What reliable measures exist for the accurate measurement of surface area and/or particle number in environmental matrices?

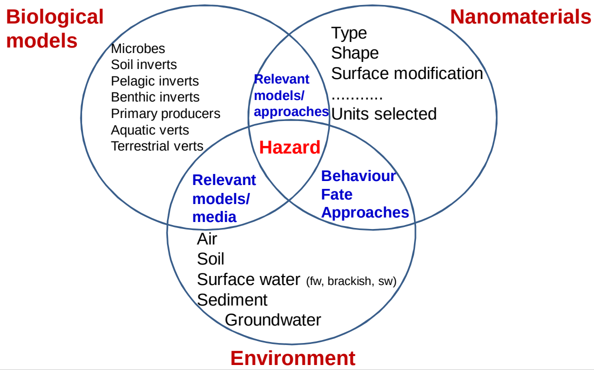


Oberdöster, G., Stone, V. & Donaldson, K., (2007) Toxicology of nanoparticles: A historical perspective. *Nanotoxicology*. 1(1) pp 2-25 .



same mass concentration
(1 ppm)

Challenges



Acknowledgements

Joint Environment and Human Health Programme (UK), Unilever, CSL/FERA

Colleagues and researchers at Heriot-Watt and Edinburgh Napier Universities:

Birgit Gaiser
Philipp Rosenkranz
Pooja Sainani
Jon Mullinger
Iain Reid
Shona O'Rourke
Hanne Nielsen
Helinor Johnston
John Kinross
Lesley Young
David Brown
Vicki Stone

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