



Recent developments in nanotechnology

– links between human and ecosystem health

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Named: 25 environmental threats of the future

20 March 2008

NewScientist.com news service

Catherine Brahic



Forget genetically modified crops - the great environmental concerns of the future should be

nanomaterials, manmade viruses and biomimetic robots.

So say researchers, policymakers and environmental campaigners, who have identified 25 potential future threats to the environment in the UK, which they say researchers should focus on. In addition to well-publicised risks such as toxic nanomaterials, the acidification of the ocean and increasingly frequent extreme weather events are cited.

Sutherland et al (2008) Future novel threats and opportunities facing UK biodiversity identified by horizon scanning. *J. Applied Ecology*

Named: 25 environmental threats of the future

Nanotechnologies:



Opportunity	Threat	Research needs
Binding of other substances reduces their impact on biodiversity. Opportunities for bioremediation	Toxicity, physical impacts and increased bioavailability	Characterize particles, identify sources and transport pathways, determine and quantify effects and their mechanisms, assess biodiversity impacts. Bioremediation techniques. Understand drivers for development, use and regulation

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Sutherland et al (2008) *J. Appl. Ecol.*

What is 'nano'?

•The prefix 'nano-' specifically means a measure of 10^{-9} units, the nature of this unit being determined by the word that follows. Thus a nanosecond is 10^{-9} seconds and a nanometre is 10^{-9} metres.

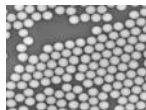
•**Nanomaterial:** Any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less.

European Commission Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), November 2007
http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_012.pdf

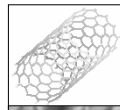
Nanomaterials diversity



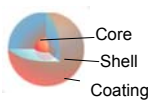
C₆₀ Buckyball
Fullerenes



Polystyrene
Beads



Carbon
nanotubes



Quantum Dots



Nanoparticle
gold

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Why 'nano'?

Manipulating materials at this level results in materials that exhibit desirable properties such as:

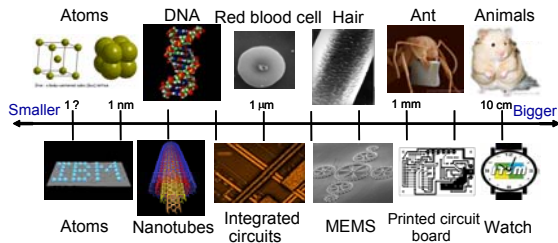
- Improved catalysis;
- Improved mechanical properties;
- Improved optical properties;
- Improved electrical conductivity;

Etc.

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Nanotechnology - How small is nano?

Natural structures



Artificial structures

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Manufactured Nanomaterials

- A new form of materials:
e.g. carbon, silica, metals and metal oxides, polymers, composite materials
- Fast growing sector of industry:
already a multibillion US\$ market expected to grow to 1 trillion US\$ by 2015
- Already used in many consumer products and numerous new applications about to appear in the market
- Nanotechnology has a huge range of opportunities in many areas, including information technology, medical advances and environmental benefits

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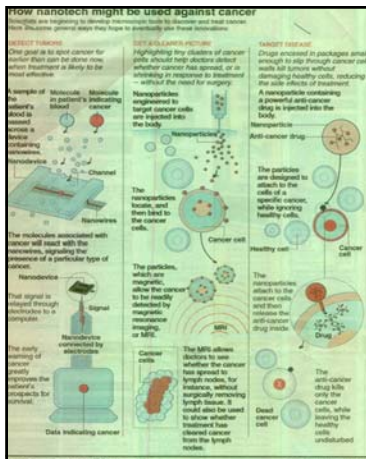
The Benefits of Nanotechnology



- Better medicines with fewer side effects
- Clean up of contaminated land
- Financial gains
- Ropes to tether satellites to the Earth
- Clean water for everyone
- Clothes that don't smell
- Smaller electronic gadgets
- Suntan lotion that's not white and sticky
- Prevention of MRSA infections
- Stain resistant clothing
- Increased shelf-life of food

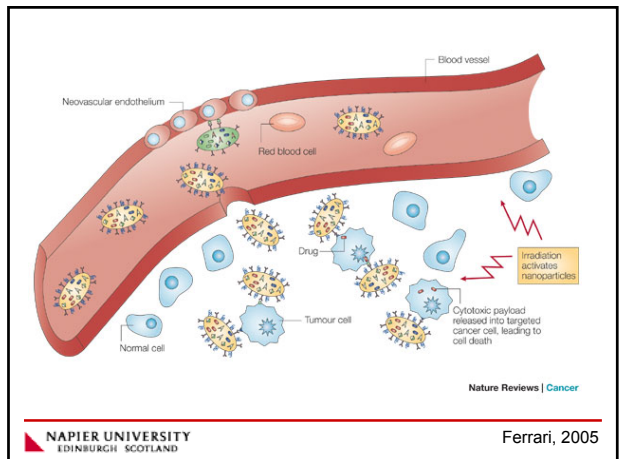
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<http://www.nanotechproject.org/>



How nanotech can be used in health

- Screening blood to detect molecules indicating cancer
- Tracking injected particles in blood which bind to cancer cells
- Drug delivery
- **Theranostics** (personalized diagnostic therapy)



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Nature Reviews | Cancer
Ferrari, 2005

Nanomaterials – A risk in the environment?

$$\text{Risk} = \text{Hazard} \times \text{Exposure} \quad (\text{Toxicity})$$

Raising the potential for Nano-Risk

There is now Global recognition that the safety of nanomaterials must be addressed due to their implementation in every day life.



Approaches to Risk Assessment



European Commission Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)

on the Assessment of the Suitability of the current Technical Guidance Documents approach for the assessment of risks posed by Nanomaterials (June 2007)

http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_010.pdf

SCENIHR opinion on risk assessment of chemicals

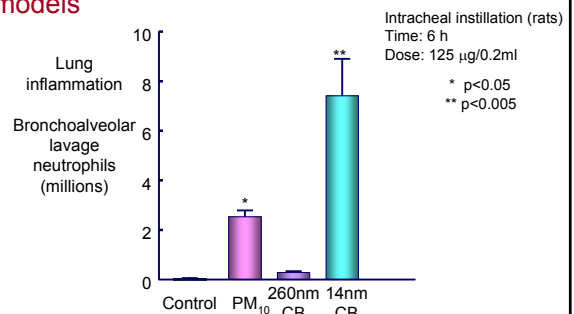
Based on the methods in the EU TGD on risk assessment

- **Hazards associated with nanomaterials can be identified**
- Nanomaterial formulations are not *per se* necessarily more toxic than formulations based on bulk substances > case-by-case
- **Appropriate metrics needed** (number concentration and surface area to complement mass concentration)
- Physico-chemical characteristics may change, e.g. agglomeration, dissociation, adsorption
- **There is a lack of standardised methods for the testing of properties of nanoparticles**
- Consideration should be given to **possible translocation** away from the portal of entry and passage across membranes may occur (e.g. across the blood-brain barrier)

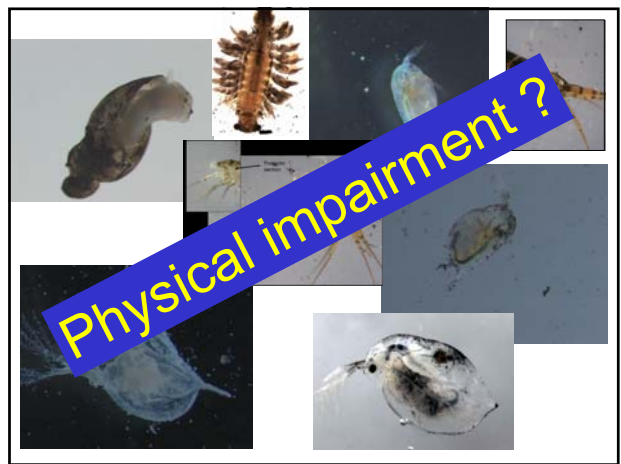
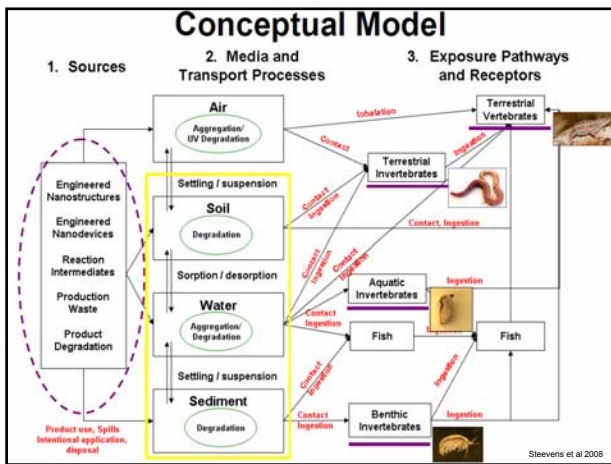
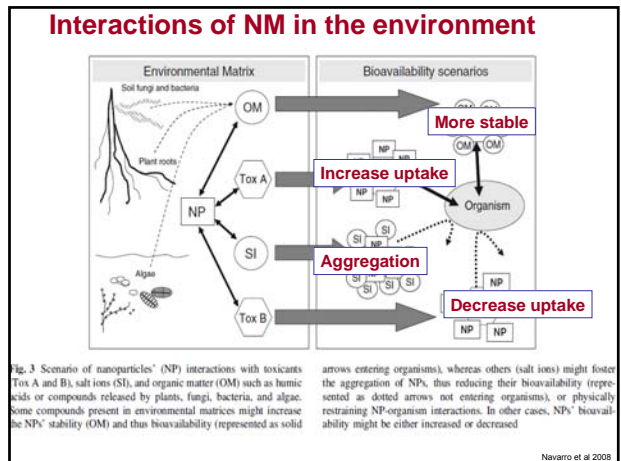
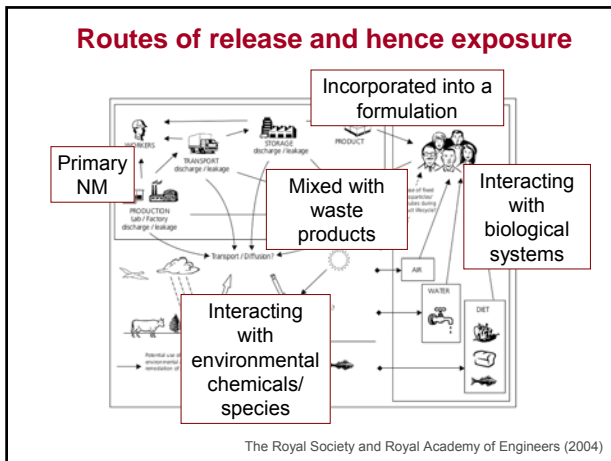
Ecotoxicology- toxicology integration

- Since more toxicological studies have been completed, the information gained from the toxicology can be used to inform ecotoxicology.
- The main findings of the toxicology can be broken down into two general areas:
 - (i) Physical and chemical characteristics
 - Size, surface area, dimensions,
 - solubility (biopersistence, durability),
 - aggregation/clumping, contaminants, composition.
 - (ii) Toxicological mechanisms
 - Free radical and reactive oxygen species production,
 - oxidative stress, inflammation, toxicokinetics (absorption, distribution, metabolism and excretion).

Particle induced inflammation in vertebrate models



Li et al. 1997 Env. Health Perspectives



Environmental Science and Technology, Vol 40, Issue 14 (2006)

Still life with nanoparticles

For the first time, researchers have captured an image of nanoparticles inside a whole, live organism. Nanoparticles have been photographed previously in cells in vitro, but this image, which was presented at the Society of Environmental Toxicology and Chemistry Europe meeting in May by Teresa Fernandes of Napier University (U.K.), captures the tiny particles inside a daphnid or water flea (*Daphnia magna*).

Teresa Fernandes, copyright Napier University (U.K.)

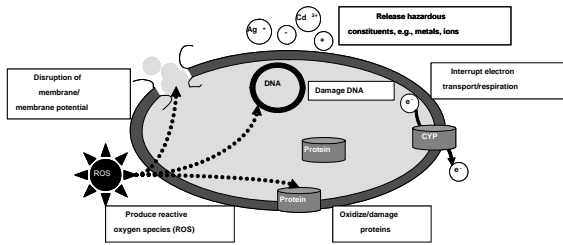
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<http://pubs.acs.org/subscribe/journals/esthag40i14/html/071506news3.html>

Effects on microbes

- A range of studies taken from the literature
- Over 30 papers or so published to date
- Materials studied include: fullerenes, CNT, metals, metal oxides, quantum dots
- **Bactericide, viricide, reactive oxygen species production, oxidative damage, cell membrane damage, inhibits grow (via interference with energy metabolism), cytotoxic**
- Range of target species, nanomaterials and endpoints still narrow

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 Klaine et al (2008) Env. Toxicology and Chemistry

Possible mechanisms of nanomaterial toxicity to bacteria. Different nanomaterials may cause toxicity via one or more of these mechanisms.



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Klaine et al (2008) Env. Toxicology and Chemistry

Vertebrates (1)



Oberdorster E. 2004
Environ. Health Persp. 112; 1058-1062.

Particles C₆₀ suspended in tetrahydrofuran (THF; heterocyclic organic compound, (CH₂)₄O). Final suspension contained 30-100nm aggregates.

Species Juvenile largemouth bass

Protocol 48h 0.5 and 1.0 ppm

Results Increased lipid peroxidation in brain could be due to:
• Partitioning of C₆₀ into lipid-rich environments.
• Poor antioxidant defence of neural tissue

✗ THF used – Therefore difficult to interpret

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A note on particle preparation

Brant et al. 2005 Environ.Sci.Technol. 2005 6343-6351

Aim: To investigate the behaviour of colloidal C₆₀ prepared
➢ A. Using the organic solvent THF
➢ B. Stirred in water for several weeks (proposed to be more indicative of natural environment)

Results:
➢ Both procedures generate n-C₆₀ with negative charge, but more charged in THF.
➢ THF remains within n-C₆₀ cluster.

Discussion:
➢ n-C₆₀ acquires charge from organic solvents and by hydrolysis.
➢ Possible to disperse n-C₆₀ without a solvent
➢ Experiments using THF need to be re-interpreted.

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Vertebrates (2)



Smith et al 2007
Aquatic Toxicology 82: 94-109

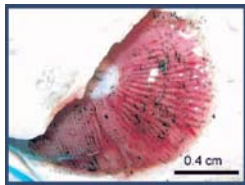
Particles Single Walled Carbon Nanotubes (SWCNT) 1.1nm diameter x 5-30 µm length [SDS (sodium dodecyl sulphate) and sonication] 0, 0.1, 0.25, 0.5mg/L up to 10 days

Species Fish - Rainbow Trout

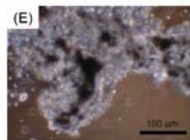
Results Dose dependent:
• Rise in ventilation rate, gill pathologies and mucus
• Lipid peroxidation in gill, brain and liver
• Increased gill and liver glutathione (due to low oxygen-induced stress in gills?)
• Brain pathology
• Aggressive behaviour

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Effect of Single-Walled Carbon Nanotubes (SWCNT) to rainbow trout



The surface of a rainbow trout gill showing how single-wall carbon nanotubes (in black) collect and stick to the mucus coat on the gill surface. Secreted fish mucus rapidly aggregated previously dispersed SWCNT on the surface of the gills (fish from 0.5 mg l⁻¹ SWCNT treatment);



Phase contrast photograph of a mucus smear (magnification ×40) showing aggregates of nanoparticles associated with the mucoproteins.

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Smith et al (2007)

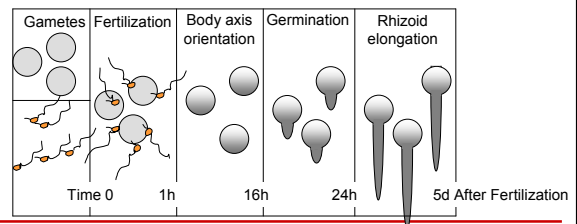
Marine macroalgae



Nielsen et al 2008, Nanotoxicology

Particles: CB 14nm diameter (Degussa Printex 90) 0.1, 1, 10 and 100 µg/ml

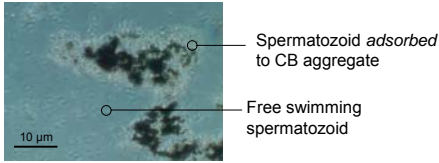
Organism: Macroalgae *Fucus serratus*
Dynamic Light Scattering characterisation



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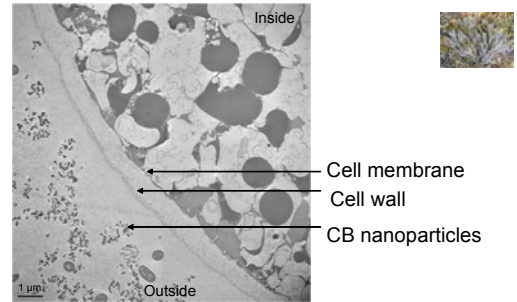
Macroalgae treated with carbon nanoparticles

Nielsen et al 2008, *Nanotoxicology*



Physical restriction?

Macroalgae treated with carbon nanoparticles



Do NPs penetrate the cell wall?

Transmission electron microscopy

Macroalgae treated with carbon nanoparticles

Nielsen et al 2008, *Nanotoxicology*

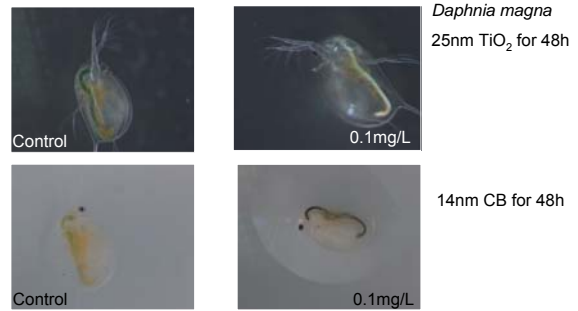
Result: Carbon nanoparticles may influence *Fucus* embryos development, for example by affecting:

- Sperm frequency
- Orientation of the body axis
- Germination and rhizoid elongation (?)



Aquatic Invertebrates

Fernandes et al 2007



P. Rosenkranz

Comparing the effects of 14 nm and 260 nm CB

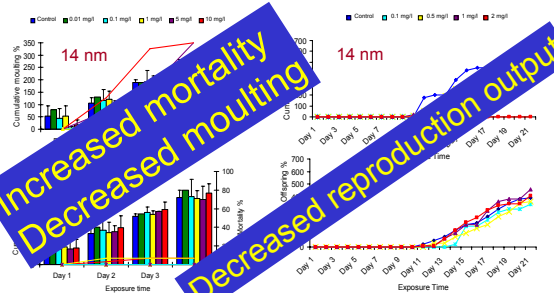


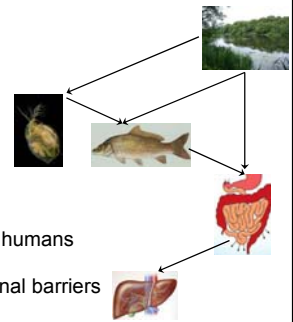
Fig. 2. Mortality (line) and cumulative moult (columns) after treatment with 14 nm (top) and 260 nm carbon black (bottom) in an acute, 96-h test.

P. Rosenkranz

Assessing effects of silver NP

Connecting ecotoxicology and toxicology of water-borne NP:

- Exposure of primary producers
- Exposure of invertebrates
- Exposure of fish
- Uptake into higher animals and humans
- Transport through gastro-intestinal barriers
- Effects of NP in hepatocytes



Study Approach

System

1. Primary producers (*Pseudokirchneriella subcapitata*)
2. Hepatocytes: Human (C3A), trout (*Oncorhynchus mykiss*) (primary)
3. Invertebrate - *D. magna*
4. Fish – carp (*Cyprinus carpio*)

Endpoints

1. Productivity, esterase activity
2. Cytotoxicity (membrane integrity assessment)
3. Mortality, growth, moulting
4. Bioavailability

Characterisation of particles in respective media or water (ongoing): concentration, aggregation, solubility

Assess transport through gastro-intestinal barriers

What have we learned?

- Nano-Ag (35 nm) **more toxic** than bulk-Ag (0.6µm-1.6 mm)
- Nano-Ag can be **accumulated** in organs (from fish studies)
- **Ingestion** is likely the main route of particle uptake in carp; transport through epithelium?

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Effects on aquatic organisms

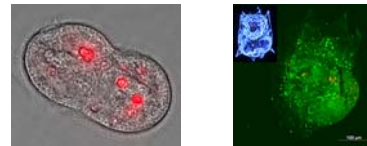
- A range of studies taken from the literature
- About 30 papers or so published to date – large output in 2007/08 (although some as reviews)
- Rapid uptake of NM; but also excretion?
- Wide range of results observed
- Indication of higher toxicity associated with exposures to nano, as opposed to micro sized materials
- Role of preparation method?
- Effects of metals – what is the role of dissolution ?
- Still unclear about mechanistic effects
- Very few studies on marine systems
- Range of target species, nanomaterials and endpoints still narrow

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Klaine et al (2008) Env. Tox and Chem.

Food chain effects?

R.D. Holbrook, K.E. Murphy, J.B. Morrow and K.D. Cole. Trophic transfer of nanoparticles in a simplified invertebrate food chain. *Nature Nanotechnology*, June 2008



Photomicrograph of ciliate *Tetrahymena pyriformis* (l.) during cell division with accumulated **quantum dots** (CdSe core and ZnS shell) appearing red and close up photomicrograph of rotifer *Brachionus calyciflorus* that preys on it (r., whole organism seen in upper left corner) with quantum dots assimilated from ingested ciliates appearing red. (Credit: NIST)

Escherichia coli → *Tetrahymena pyriformis* → *Brachionus calyciflorus*

Interaction with other chemicals?

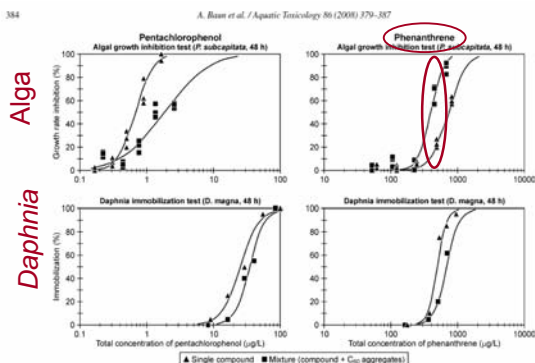


Fig. 4. Concentration–response curves for pentachlorophenol and phenanthrene with and without addition of 5–8 mg C_{50} /l (see Table 2), in toxicity tests with algae and daphnia. All concentrations are expressed as total concentrations in the test vessels.

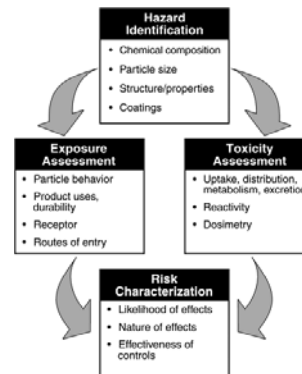
Still lacking information on...

- Uptake and translocation
- Depuration / excretion
- Generalised effects (regarding specific NMs & target species)
- Accumulation (excretion/depuration)
- Uptake via food (food chain effects)
- Mixtures (interactions with other chemicals)
- Procedural approaches, including protocols and characterisation (e.g. in soil systems)
- Much discussion regarding methodologies, protocols for exposure, rigorous characterisation and proper controls

The way forward

- Prioritise nanomaterial categories/groups
- Use of *reference* materials
- Agree standard methodologies for exposure
- Link cause-effect (link properties to effects – e.g. Kow?)
- Assess environmental fate – transfer across environmental compartments
- Life-cycle assessment

Risk Assessment Approach



(Tsuji et al. 2006)

EC asks for Scientific Opinion on Risk Assessment of Products of Nanotechnologies (26 Feb 2008)

<http://nanotech.lawbc.com/2008/02/articles/international/ec-asks-for-scientific-opinion-on-risk-assessment-of-products-of-nanotechnologies/>

The European Commission (EC) asked its Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) for a scientific opinion on the risk assessment of products of nanotechnologies. The request asks SCENIHR to **identify and assess new information and update its opinions on the potential risks of products of nanotechnologies, "in particular, with respect to characterisation, ecotoxicology and toxicology as well as exposure assessments"**. The EC's request for an opinion states that the update "should be done in a **step-wise manner taking into account the upcoming risk assessment demands related to specific nanomaterials and the evolving scientific information from various sources, including results from scientific research projects and activities of the European Technology Platforms related to the safety of nanomaterials**".

The deadline for the opinion is **November 2008**.

Full text at: http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_q_015.pdf

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 Iain Reid – *Lymnaea stagnalis* (Napier)
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SNIRC colleagues SCENIHR colleagues

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