Investigating the effects of nanomaterials on the environment

– current knowledge and future research needs

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Ecotoxicology- toxicology integration

- Since more toxicological studies have been completed, the information gained from the toxicology can be used to inform ecotoxicology.

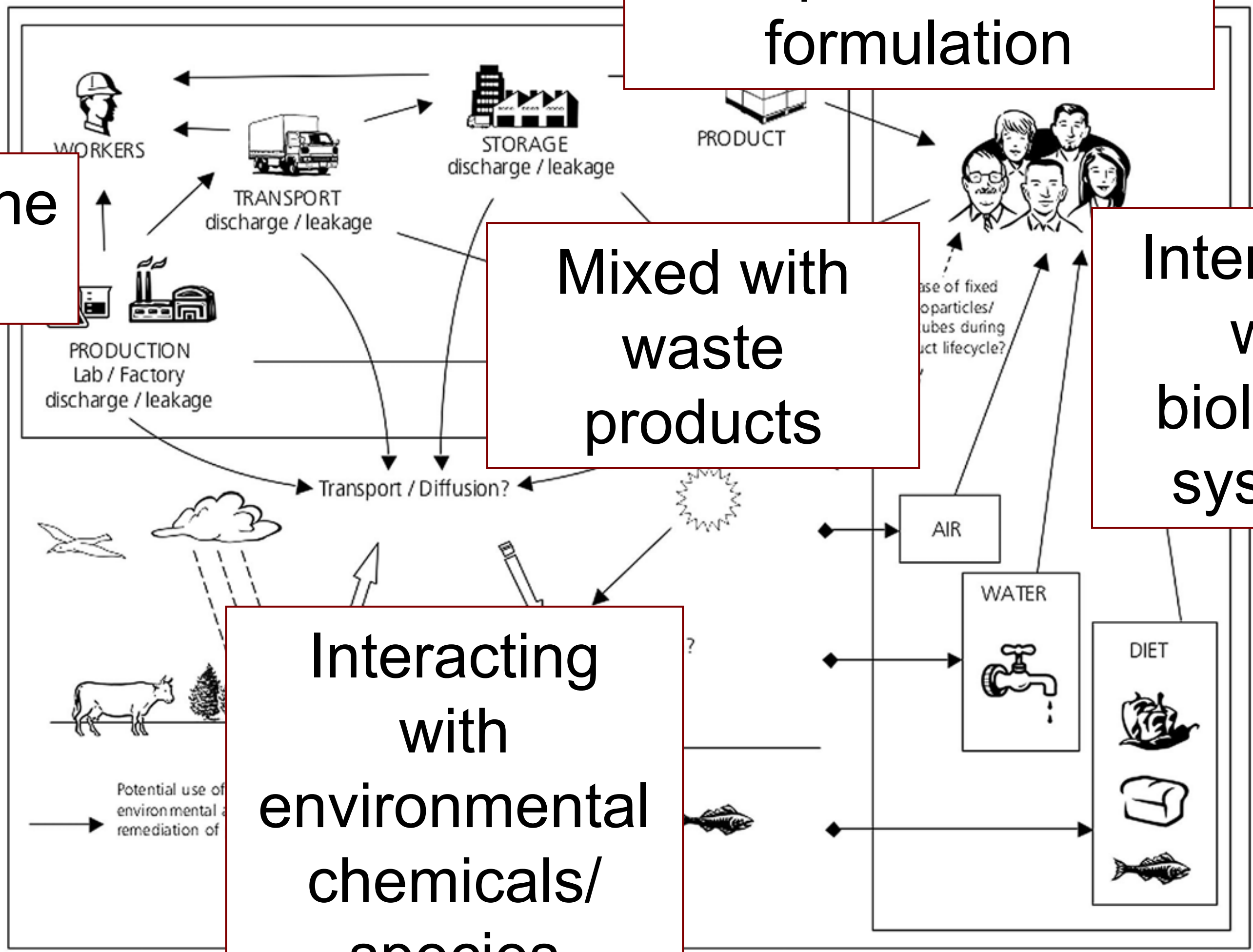
- The main findings of the toxicology can be broken down into two general areas:
  - (i) Physical and chemical characteristics
    - Size, surface area, dimensions,
    - solubility (biopersistence, durability),
    - aggregation/clumping, contaminants, composition.
  - (ii) Toxicological mechanisms
    - Free radical and reactive oxygen species production,
    - oxidative stress, inflammation, toxicokinetics (absorption, distribution, metabolism and excretion).

Nanomaterials – A risk in the environment?

Risk = Hazard x Exposure

(Toxicity)

Routes of release and hence exposure



Pristine NP

Incorporated into a formulation

Mixed with waste products

Interacting with biological systems

Interacting with environmental chemicals/ species

The Royal Society and Royal Academy of Engineers (2004)

Interactions of NM in the environment

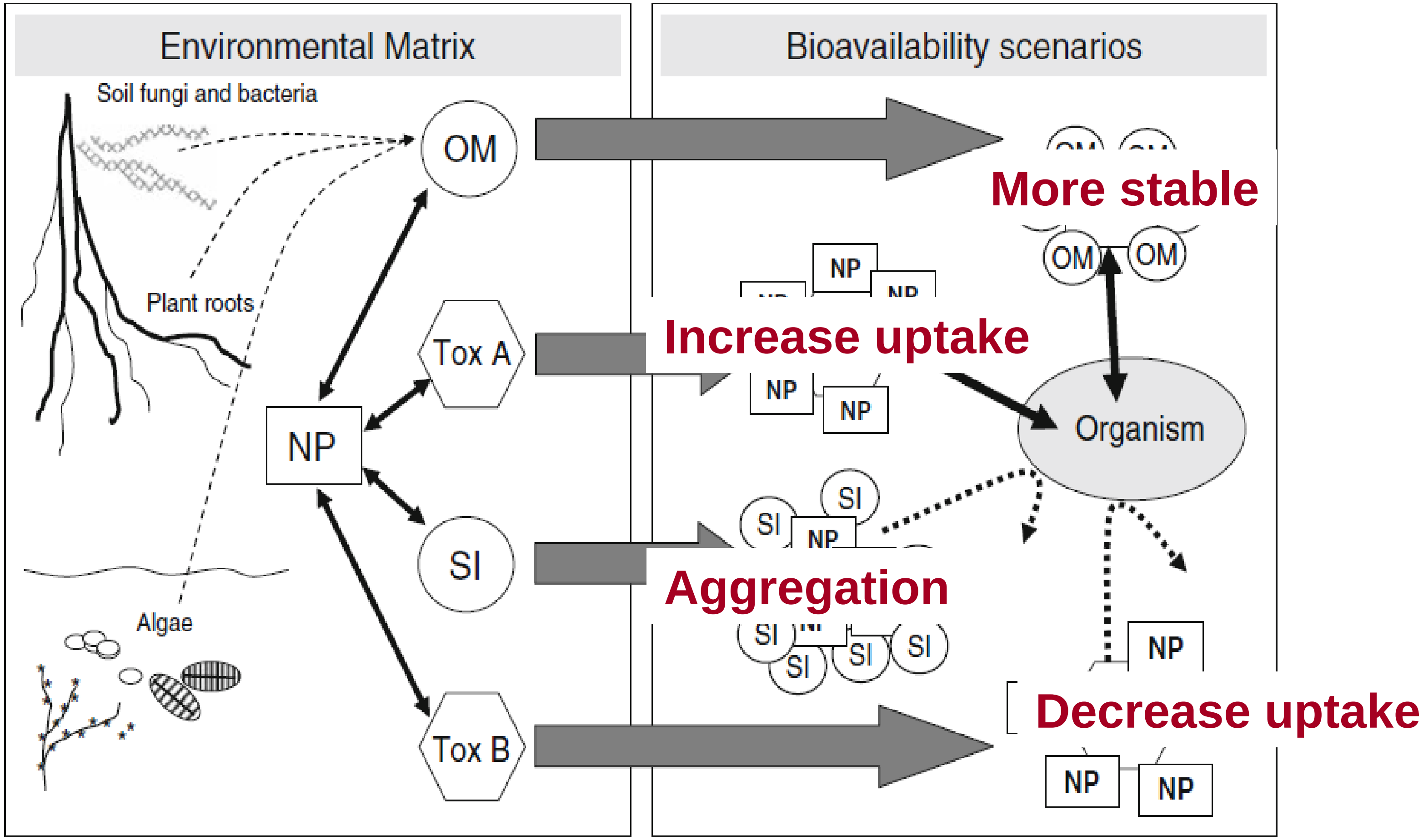
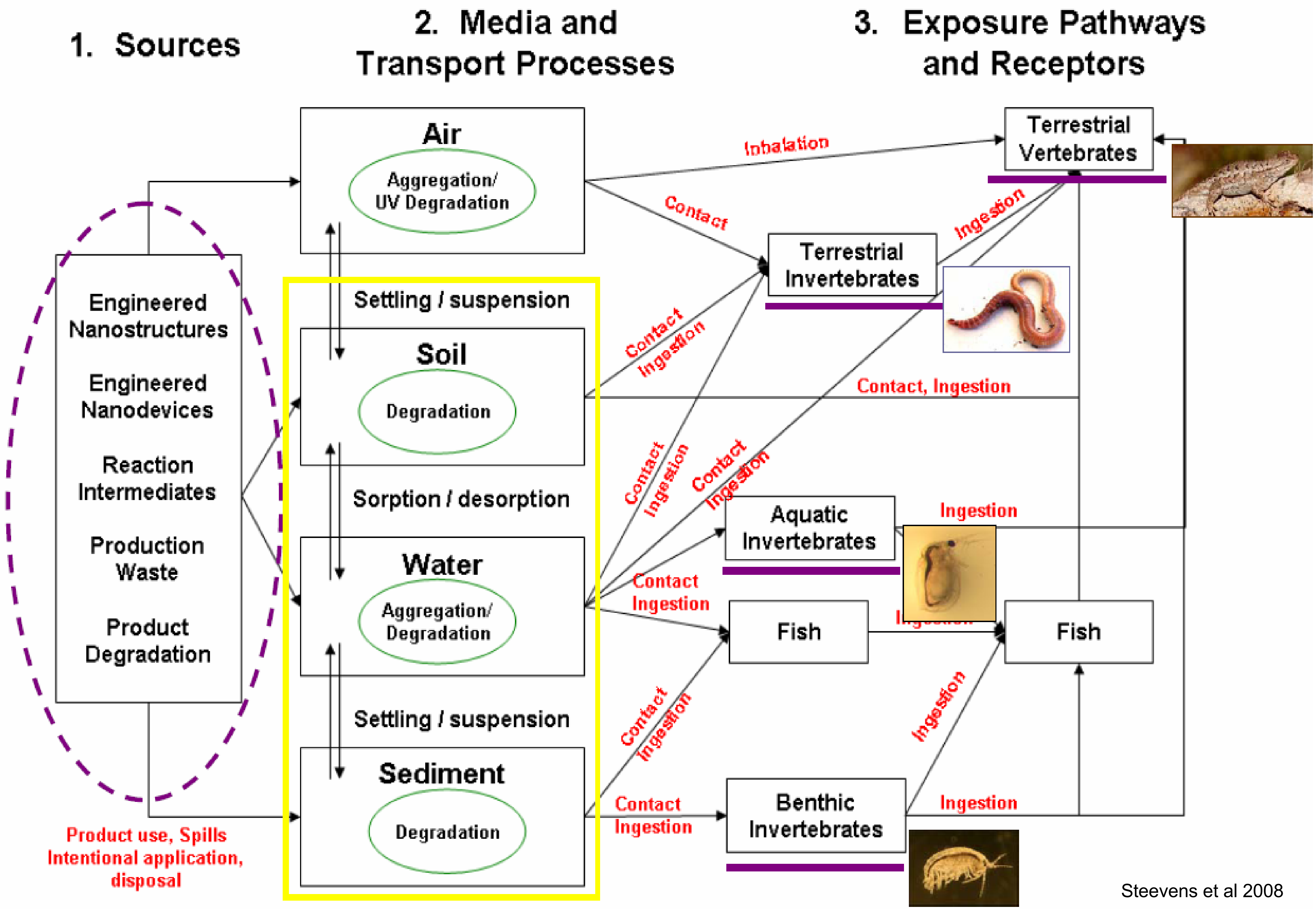


Fig. 3 Scenario of nanoparticles' (NP) interactions with toxicants (Tox A and B), salt ions (SI), and organic matter (OM) such as humic acids or compounds released by plants, fungi, bacteria, and algae. Some compounds present in environmental matrices might increase the NPs' stability (OM) and thus bioavailability (represented as solid arrows entering organisms), whereas others (salt ions) might foster the aggregation of NPs, thus reducing their bioavailability (represented as dotted arrows not entering organisms), or physically restraining NP-organism interactions. In other cases, NPs' bioavailability might be either increased or decreased

Navarro et al 2008

Conceptual Model



Steevens et al 2008


Posterior section
Physical impairment ?

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.)

http://pubs.acs.org/subscribe/journals/esthag/40/i14/html/071506news3.html

Effects of nanoparticles on different species

Terrestrial

Microorganisms

Plants

Invertebrates

Vertebrates

Aquatic

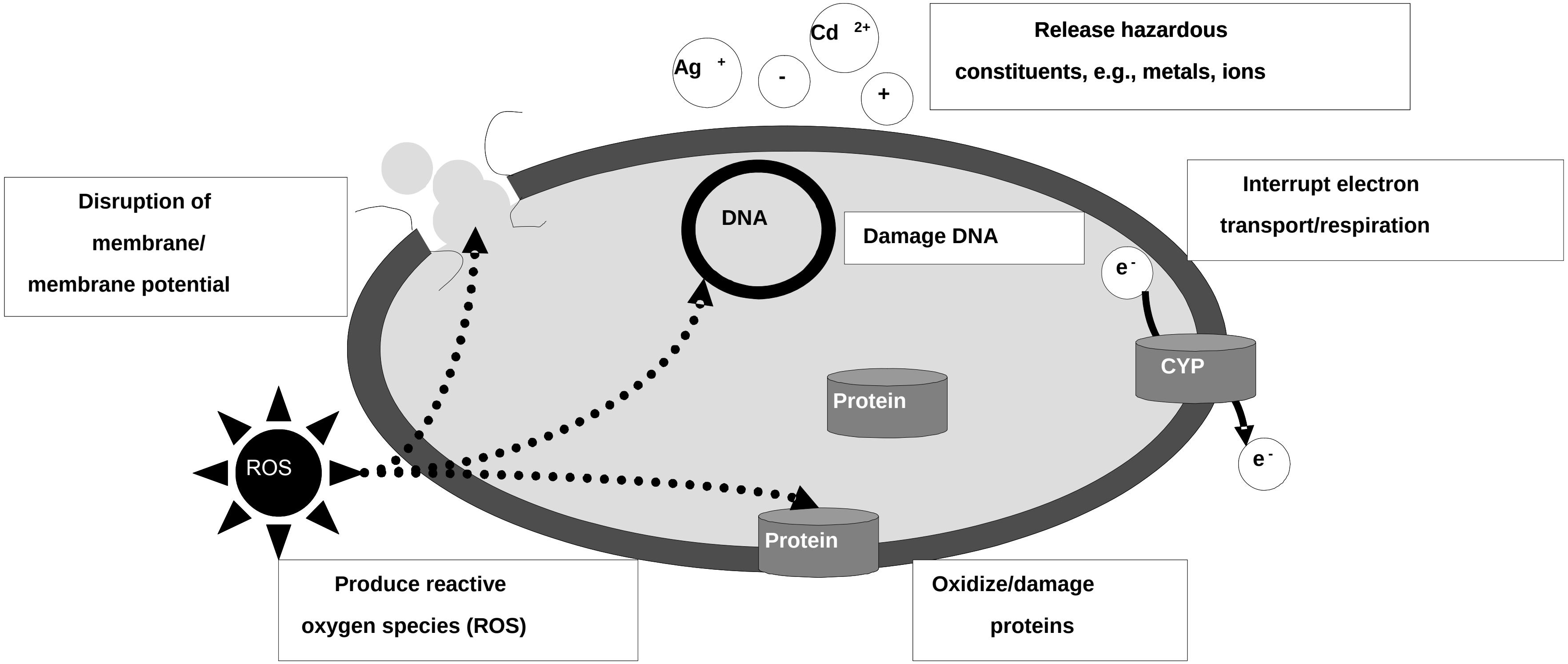
Which species should we study
– which are most likely to be exposed?

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

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.



Klaine et al (2008) Env. Toxicology and Chemistry

Vertebrates (1)

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

Particles	C_{60} suspended in tetrahydrofuran (THF; heterocyclic organic compound, $(CH_2)_4O$). 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 C_{60} into lipid-rich environments. •Poor antioxidant defence of neural tissue

➢ THF used – Therefore difficult to interpret

A note on particle preparation

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

Aim: To investigate the behaviour of colloidal C_{60} 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-C_{60} with negative charge, but more charged in THF.
- THF remains within n-C_{60} cluster.

Discussion:

- n-C60 acquires charge from organic solvents and by hydrolysis.
- Possible to disperse n-C_{60} without a solvent.
- Experiments using THF need to be re-interpreted.

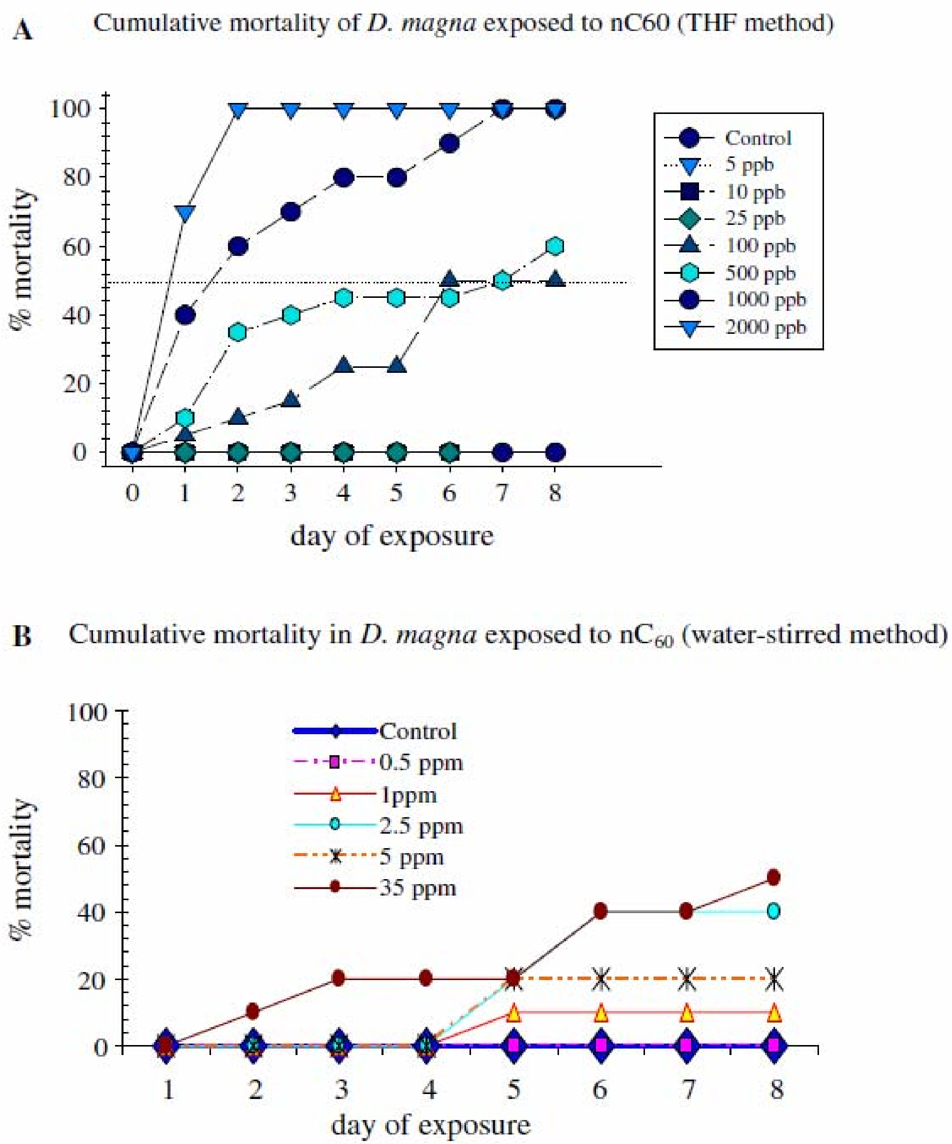


Fig. 1. Mortality curves for *Daphnia magna* exposed to either THF- (A) or water-stirred-nC_{60} (B). The THF-nC_{60} is at least one order of magnitude more toxic than the water-stirred-nC_{60}, highlighting the importance of relevant preparation techniques for aquatic toxicity testing. The 48 h LC_{50} for THF-nC_{60} was calculated to be 0.8 ppm (800 ppb), and the 48 h LC_{50} for water-stirred-nC_{60} was >35 ppm.

Does THF affect toxicity?

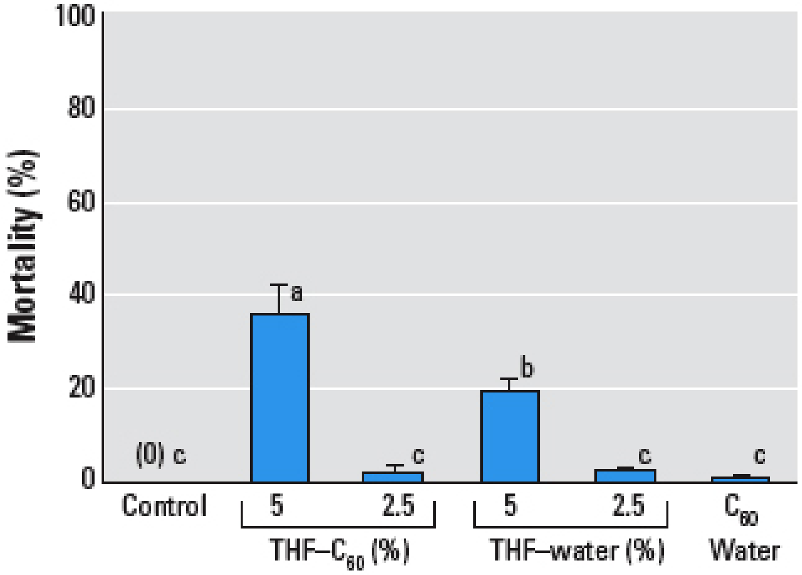


Figure 4. Mean mortality (± SD, n = 3) of larval zebrafish in control (fish water) and experimental treatments after 72-hr exposure. Larvae that survived exposure in the control, THF–C_{60} (2.5%), THF–water (2.5%) and C_{60}–water treatments were used for microarray analyses. Significant ($p < 0.05$) differences in mortality among groups are indicated by different letters.

Zebrafish exposed C_{60} with/without THF

Higher mortality on C_{60}-THF treatment. Gene expression studies indicated most differences found in THF-C_{60} and most of these were similarly expressed in fish exposed to THF-water. Toxic effects were linked to a THF-degradation product, (**γ-butyrolactone**) rather then to C_{60} (GS-MS, tox studies)

Henry et al (2007) Attributing Effects of Aqueous C60 Nano-Aggregates to Tetrahydrofuran Decomposition Products in Larval Zebrafish by Assessment of Gene Expression. Environmental Health Perspectives, 115(7)

Vertebrates (2)

Smith et al 2007
Aquatic Toxicology 82: 94-109

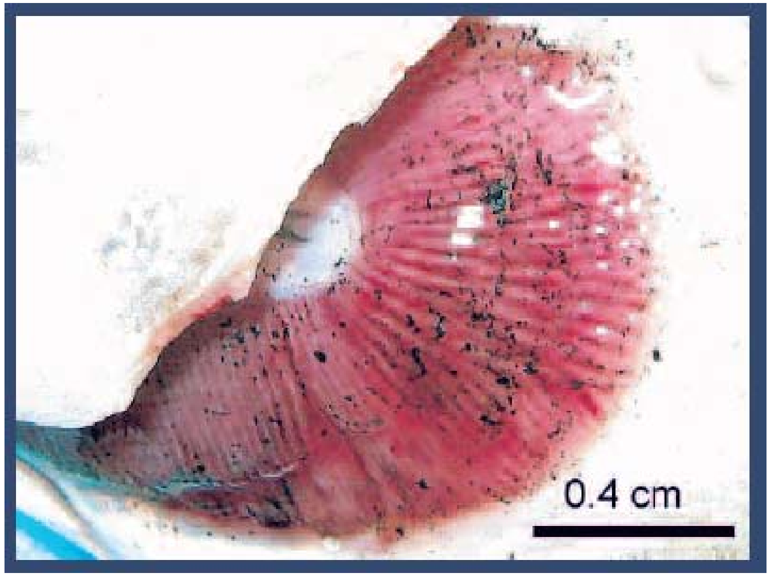
Particles
Single Walled Carbon Nanotubes (SWCNT)
1.1nm diameter x 5-30 μm length
[SDS (sodium dodecyl sulphate) and sonication]
0, 0.1, 0.25, 0.5mg/L up to 10 days

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

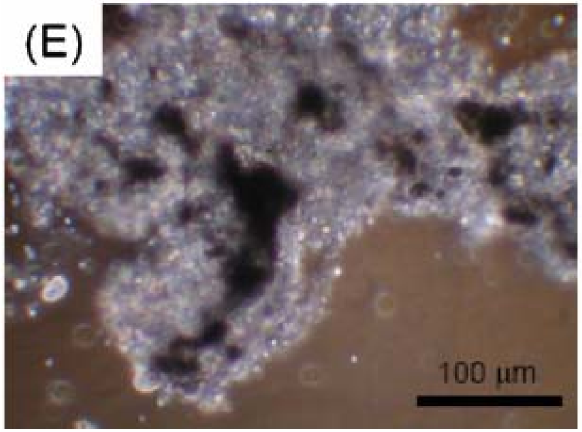
Effect of Single-Walled Carbon Nanotubes (SWCNT) to rainbow trout



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.

(E)



Smith et al (2007)

Vertebrates (3) – polystyrene beads

Kashiwada 2006
Environ. Health Persp. 114: 1697

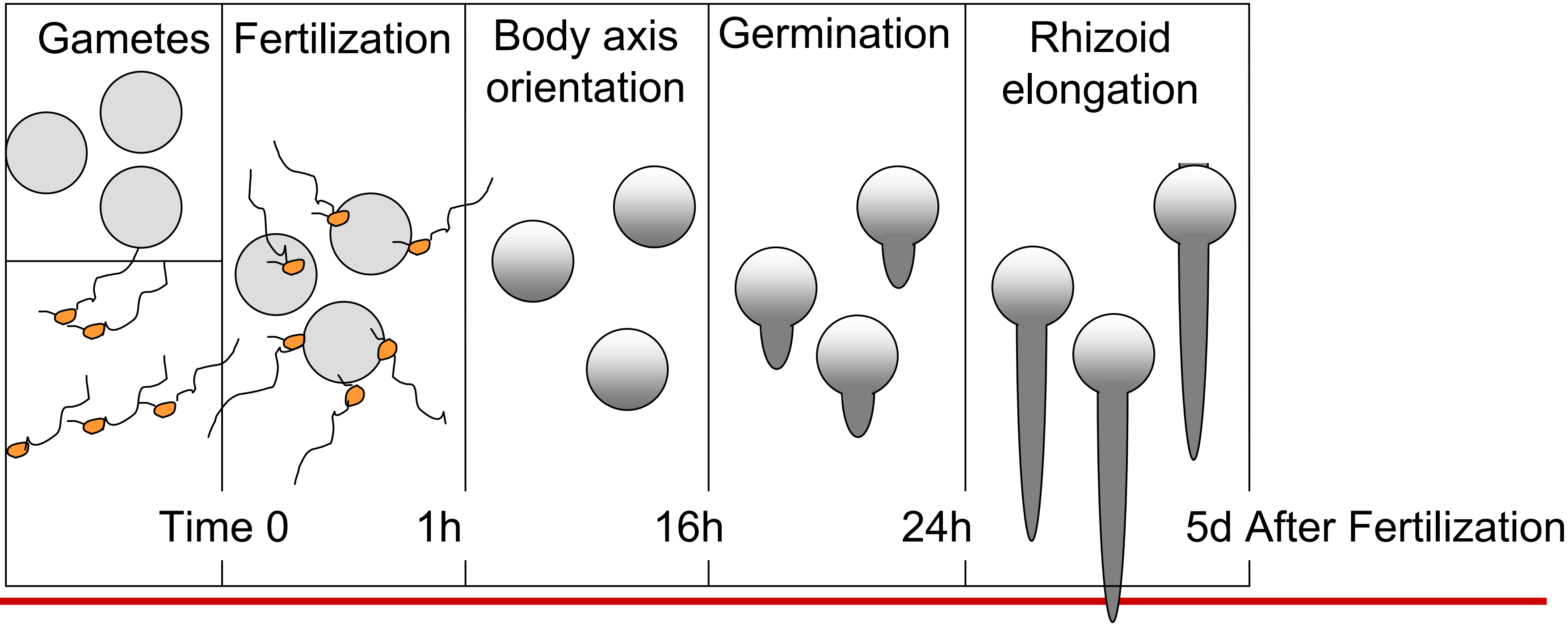
Particles	Polystyrene (latex beads) 39.4 – 42000nm
Species	Fish – Japanese Medaka
Results	Egg – all absorbed into chorion, 474nm highest bioavailability 39.4nm shifted into yolk and gall bladder during embryonic development Adult - 39.4nm accumulated in gills and intestine Also detected in brain, testis, liver and blood

Marine macroalgae

Nielsen et al *Nanotoxicology* (2008)

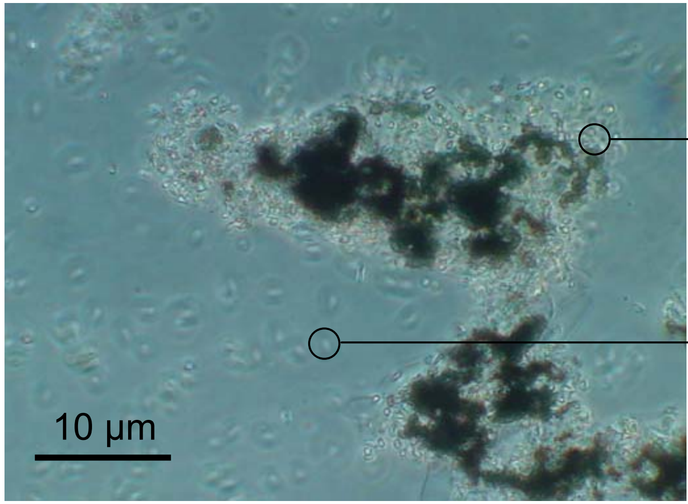
Particles: CB 14nm diameter (Degussa Printex 90)
0.1, 1, 10 and 100 μg/ml
Dynamic Light Scattering charaterisation

Organism: Macroalgae *Fucus serratus*



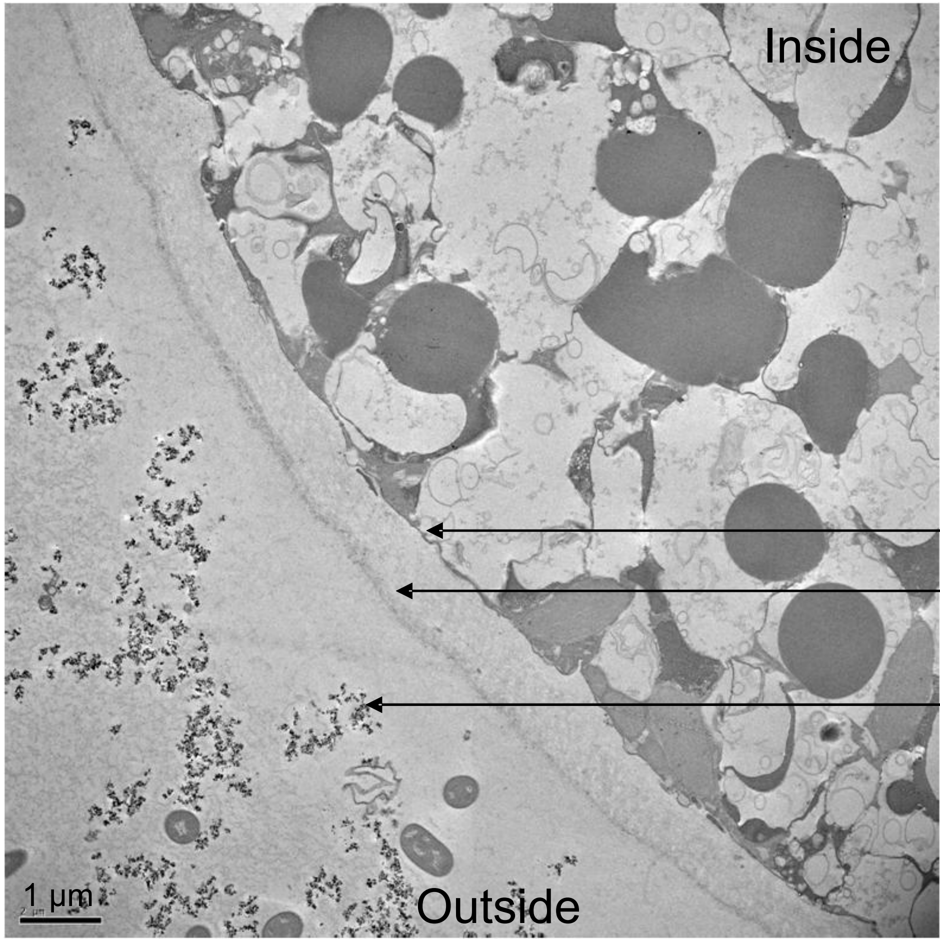
Macroalgae treated with carbon nanopartices

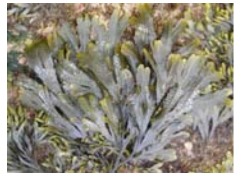
Nielsen et al 2008



Physical restriction?

Macroalgae treated with carbon nanopartices





Do NPs penetrate the cell wall?

Transmission electron microscopy

Macroalgae treated with carbon nanopartices

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 (?)

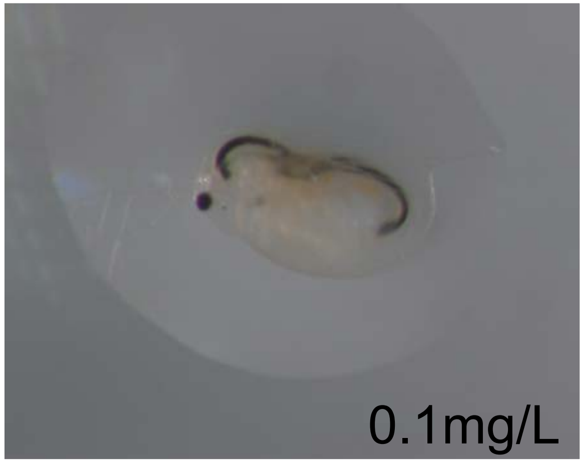
Aquatic Invertebrates

Fernandes et al (2007)

Daphnia magna

25nm TiO_2 for 48h

14nm CB for 48h



Comparing the effects of 14 nm and 260 nm CB

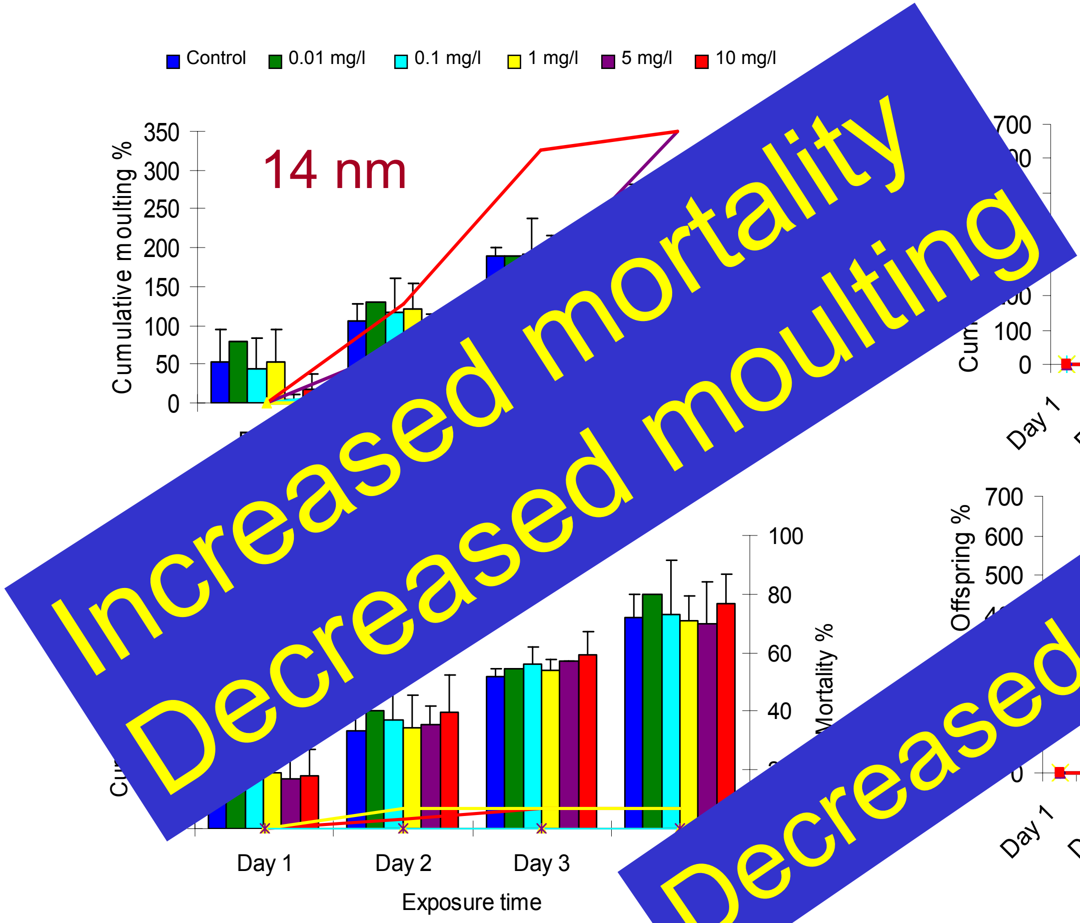


Fig.2: Mortality (line) and cumulative moult (column) after treatment with 14nm (top) and 260nm carbon black (bottom) in an acute, 96h exposure

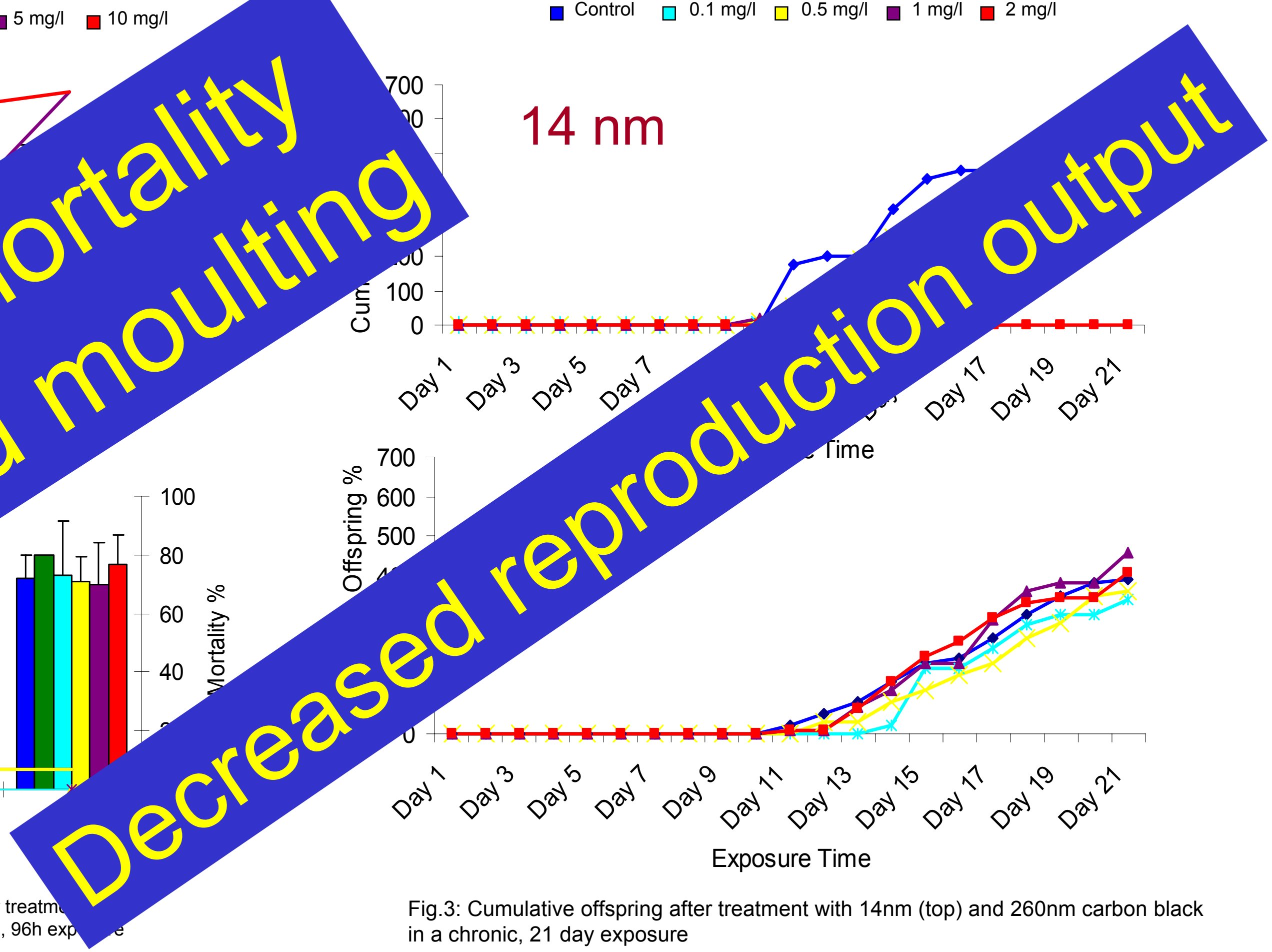
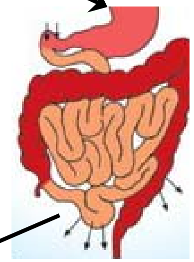
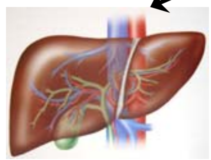


Fig.3: Cumulative offspring after treatment with 14nm (top) and 260nm carbon black in a chronic, 21 day exposure

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

Joint Environment and Human Health Programme (UK)

Collaboration: Univ. Exeter, Birmingham, Bristol

B. Geiser

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?
- Surface area may not be the key metric when expressing toxicity in nano-Ag

Effects on aquatic organisms

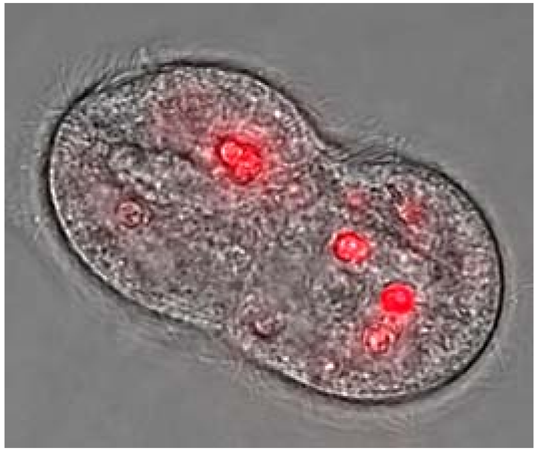
- A range of studies taken from the literature
- About 30 papers or so published to date – large output in 2007/08 (although some as reviews)
- Rapid uptake of NM; but also excretion?
- Wide range of results observed
- Indication of higher toxicity associated with exposures to nano, as opposed to micro sized materials
- Role of preparation method?
- Effects of metals – what is the role of dissolution ?

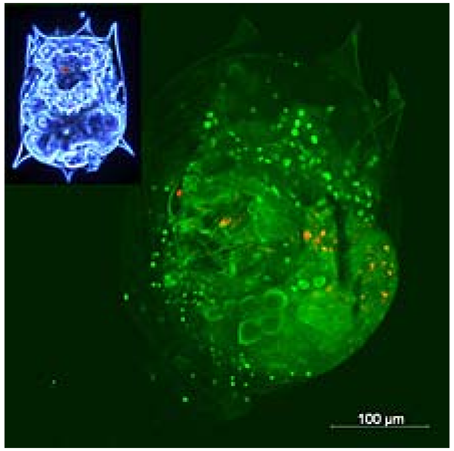
- Still unclear about mechanistic effects
- Very few studies on marine systems
- Range of target species, nanomaterials and endpoints still narrow

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?

Alga

Daphnia

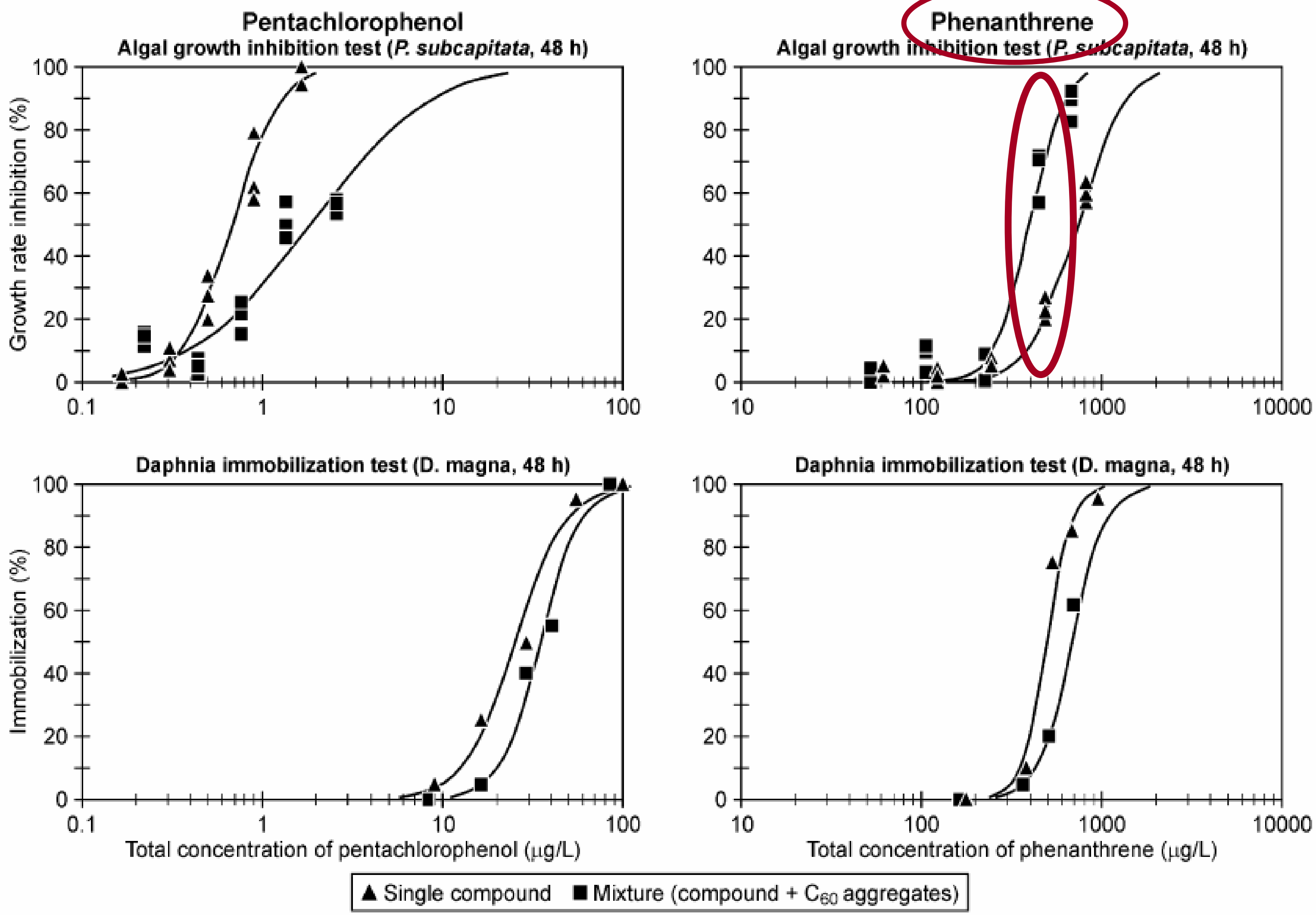


Fig. 4. Concentration–response curves for pentachlorophenol and phenanthrene with and without addition of 5–8 mg C_{60}/l (see Table 2), in toxicity tests with algae and daphnids. All concentrations are expressed as total concentrations in the test vessels.

What is NOT known?

- Uptake and translocation
- Depuration / excretion
- Generalised effects (regarding specific NMs & target species)
- Accumulation
- 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

Strategy

- Investigate range of particles (material, size, physical-chemical properties - REFERENCE)
- Characterise
- Agree standard methodologies for exposure
- Characterise (in exposure medium)
- Assess env. fate
- Determine environmental exposure
- Investigate routes of exposure in key species
- Investigate mechanisms of action of toxic effects
- Assess bioaccumulation potential
- Interaction with stressors

Effects of OM, Salinity, pH
Effects on soil/sediments

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

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CSL

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