Ecotoxicology of Nanomaterials - Finding the best way forward

Teresa F Fernandes

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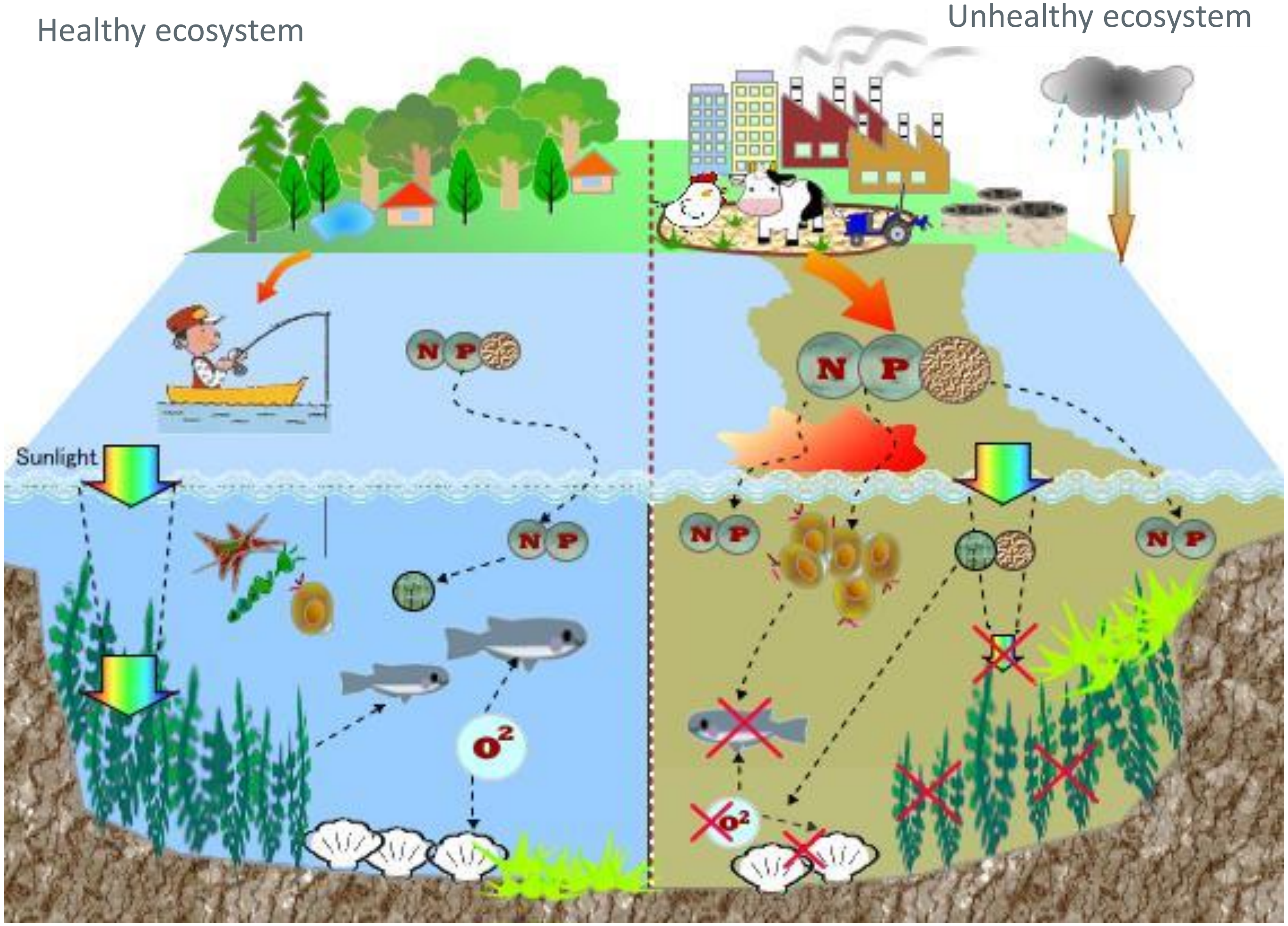
V. Stone Toxicology Award
19th April 2016

Overview

- Why consider the environment
- Nanomaterials through their life cycle
- Early investigations of effects of nanomaterials in the environment
- Increasing realism – link to industry and providing the environmental context
- Cross species studies
- Safety by design
- Using toxicity information in decision making
- Acknowledgements

Why should we care about the Environment?



http://ocean.nowpap3.go.jp/?page_id=926

Nanomaterials – A risk in the environment?

Risk = Hazard x Exposure

(Toxicity)

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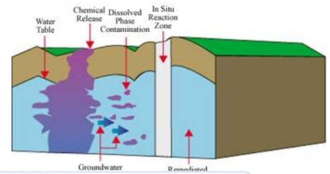
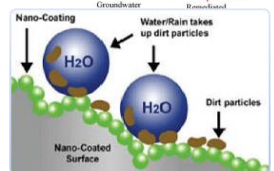
Nanotechnology Applications

Energy

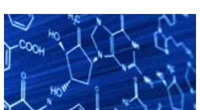
- More efficient and cost effective technologies for energy production
  - Solar cells
  - Fuel cells
  - Batteries
  - Bio fuels
- More energy efficient computers





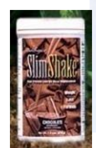
Environmental applications

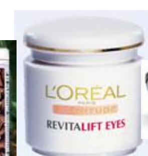
- Energy efficiencies
- Waste remediation
- Removal of contaminants from water
- Desalination
- Reducing car emissions, NO_x conversion

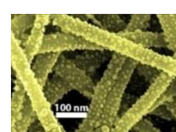


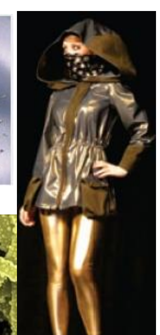
Medicine

- Cancer treatment
- Bone treatment
- Drug delivery
- Appetite control
- Drug development
- Medical tools
- Diagnostic tests
- Imaging







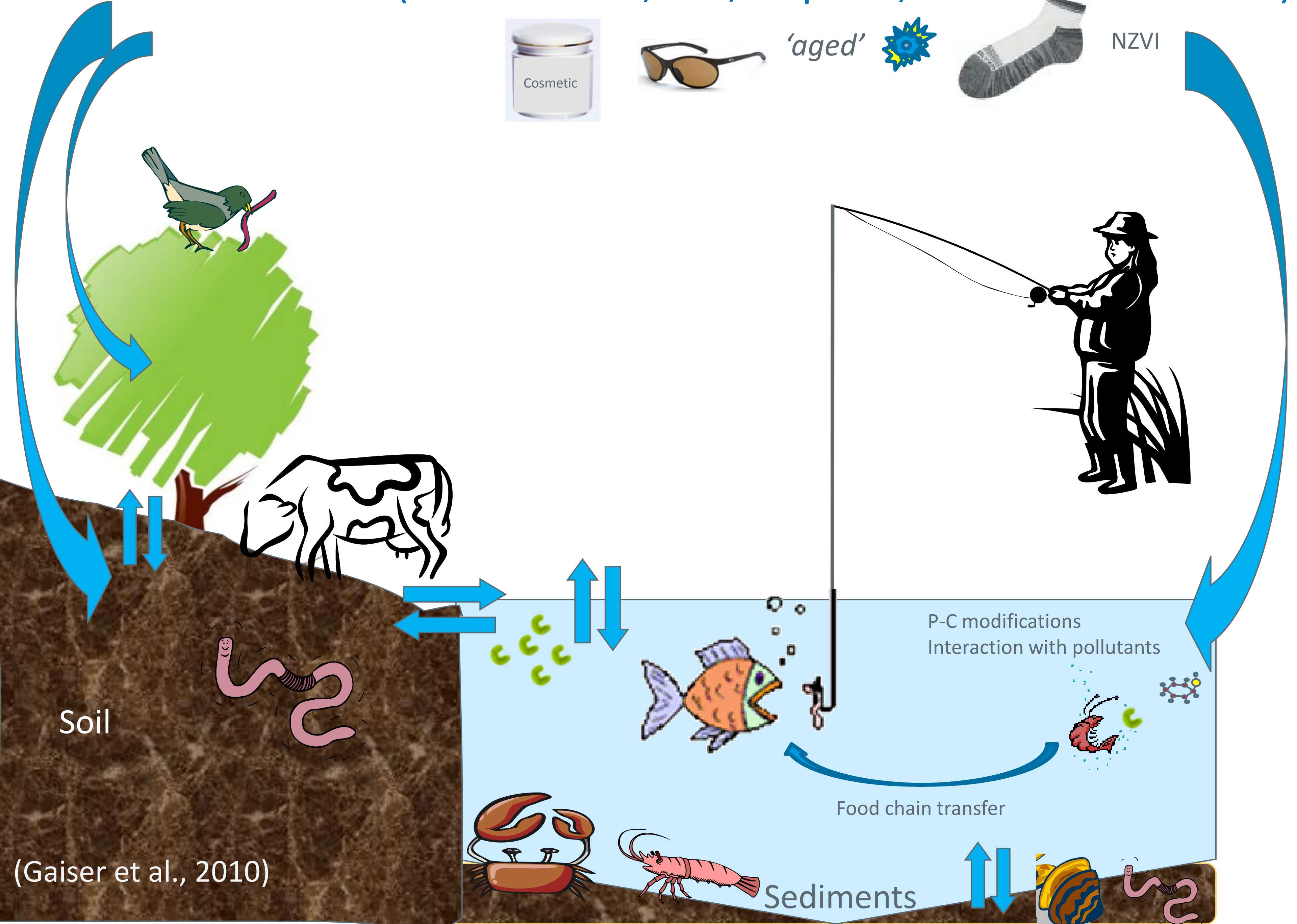


Consumer Goods

- Foods and beverages
  - Advanced packaging materials, sensors, and lab-on-chips for food quality testing
- Appliances and textiles
  - Stain proof, water proof and wrinkle free textiles
- Household and cosmetics
  - Self-cleaning and scratch free products, paints and cosmetics

Exposure / fate

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



Ecotoxicology- toxicology integration

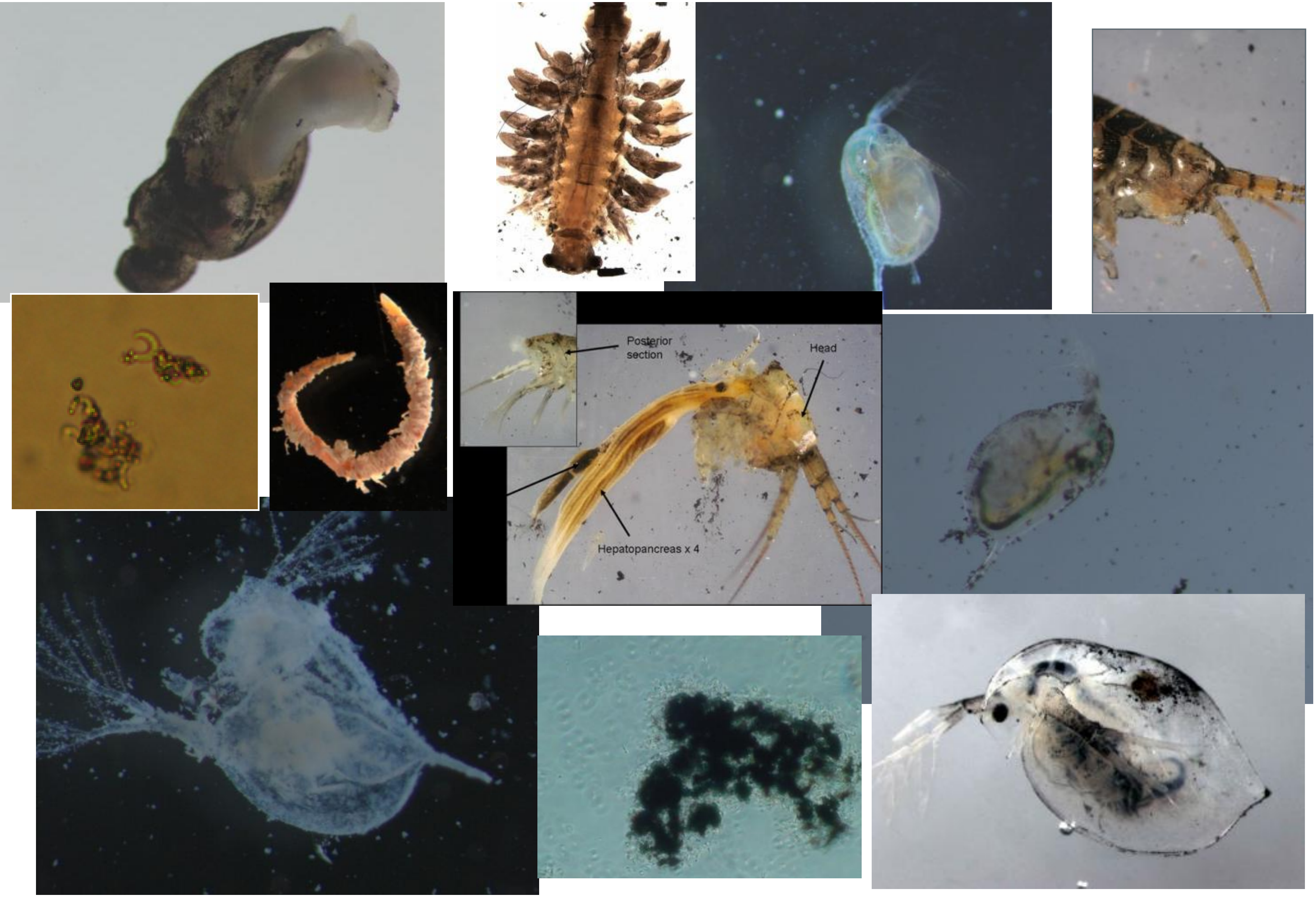
- Since more toxicological studies have been completed, the information gained from the toxicology can be used to inform ecotoxicology.

- 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).

Early nano ecotoxicology studies

- Back in the early 2000s it was becoming apparent that the nanotechnology sector was increasing at a very fast pace, with wide ranging applications

- The cross link between human nano toxicology and environmental nanotoxicology was obvious


Posterior section
Head
Hepatopancreas x 4

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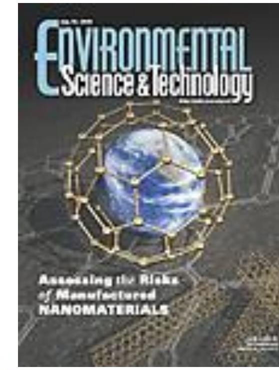
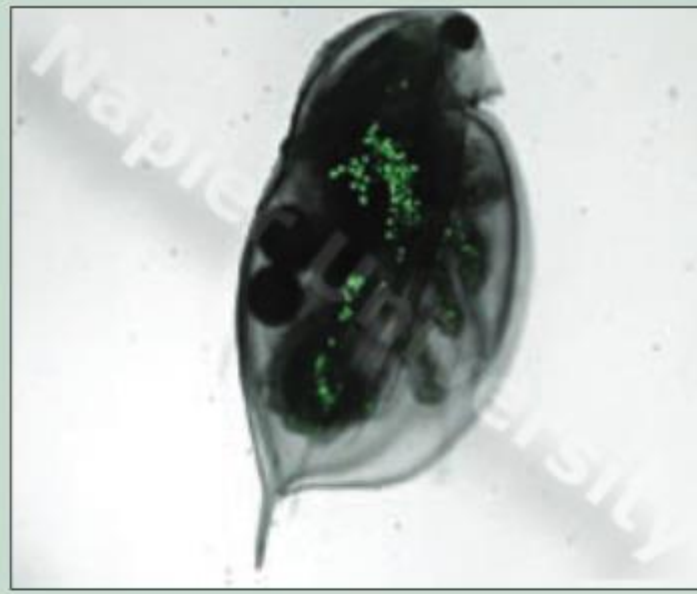
Environmental Science & Technology



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*).

Fernandes and her team started with immature (neonates) and adult daphnids swimming in an environment containing nanoparticles of titanium dioxide and carbon black. Both the immature and adult water fleas ingested the particles immediately. Graduate student Philipp Rosenkranz captured images of the carboxylated, fluorescently tagged particles in an adult's gut as well as in its fatty-lipid storage droplets. A neonate, <1 d old, also ingested the particles, as documented in images that the team plans to publish later this year.


TERESA FERNANDES, COPYRIGHT NAPIER UNIVERSITY (U.K.)

The researchers expected the particles to be readily seen in the adult animal's gut, but they wanted to determine whether the daphnid would excrete them immediately or store them elsewhere internally. "Within an hour of ingestion, one of the photographs indicated that the nanoparticles had been translocated to other parts of the body," Fernandes says. "They're going to use those storage deposits eventually," she says, "however, this requires further work to determine the relevance to nanoparticle safety." —NAOMI LUBICK

CSL defra

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CORRIERE DELLA SERA / SALUTE

AMBIENTE ANCORA POCHE LE RICERCHE SULLA TOSSICITÀ DEI NANOMATERIALI PER GLI ORGANISMI VIVENTI

Dove vanno a finire le particelle?

Sono già usate nell'industria. Ma non si sa con quali conseguenze

Nanoparticelle ben visibili in un organismo vivo. Le ha fotografate per la prima volta Teresa Fernandes della Napier University di Edimburgo all'interno di un crostaceo di acqua dolce molto impiegato negli studi sull'inquinamento delle acque, la Daphnia magna. La foto, presentata dalla ricercatrice all'incontro sui rischi delle nanotecnologie organizzato di recente a Bruxelles dalla Commissione europea, sembra testimoniare come la ricerca tossicologica oggi non riesca ad andare al di là di una «fotografia» del problema. Le nanoparticelle entrano all'interno delle cellule, questo è ormai dimostrato, ma che cosa significa in termini di rischio? «Non lo sappiamo — risponde Gunter Oberdorster, dell'università di Rochester, Stati Uniti, che al convegno belga ha tenuto una relazione in proposito —. Siamo a conoscenza del fatto che le nanoparticelle disperse nell'aria penetrano in profondità nell'albero respiratorio, possono venire assorbite, entrare in circolo e diffondersi in vari organi. Ma che cosa identifica la tossicità di una nanoparticella rispetto alla massa di quelle che vengono inalate?». Ma se la mancanza di test di laboratorio affidabili è ancora un problema per capire i

F.P.
26 ottobre 2008

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 28, No. 10, pp. 2142–2149, 2009

Printed in the USA
0730-7268/09 $12.00 + .00

A COMPARISON OF NANOPARTICLE AND FINE PARTICLE UPTAKE BY *DAPHNIA MAGNA*

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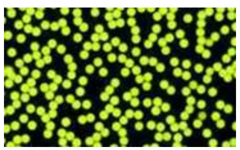


Abstract—The use of nanoparticles in various applications is steadily on the rise, with use in a range of applications, including printer toner, sunscreen, medical imaging, and enhanced drug delivery. While research on human effects via, for example, inhalation is relatively well developed, the environmental assessment of nanoparticles is in its infancy. In the present study, we assessed the uptake and quantitative accumulation, as well as the depuration, of a model nanoparticle, a 20-nm fluorescent carboxylated polystyrene bead, in the aquatic invertebrate *Daphnia magna* and compared it to a larger, 1,000-nm particle. Using confocal microscopy, rapid accumulation in the gastrointestinal tract was observed within an hour of exposure to both particle sizes in both adults and neonates. Fluorescence could also be observed in the oil storage droplets, suggesting that both particle sizes have crossed the gut's epithelial barrier. Quantification of fluorescence of both sizes of particles showed that although uptake of the 20-nm particles was lower in terms of mass it was equal to or greater than 1,000-nm particle uptake when expressed as surface area or particle number. Depuration was relatively rapid for the 1,000-nm beads, decreasing by more than 90% over 4 h. In contrast, depuration of the 20-nm beads was less extensive, reaching 40% over 4 h. Transmission electron microscopy confirmed uptake of 1,000-nm beads, but uptake of 20-nm beads was inconclusive since similar-sized inclusions could be observed in control treatments.

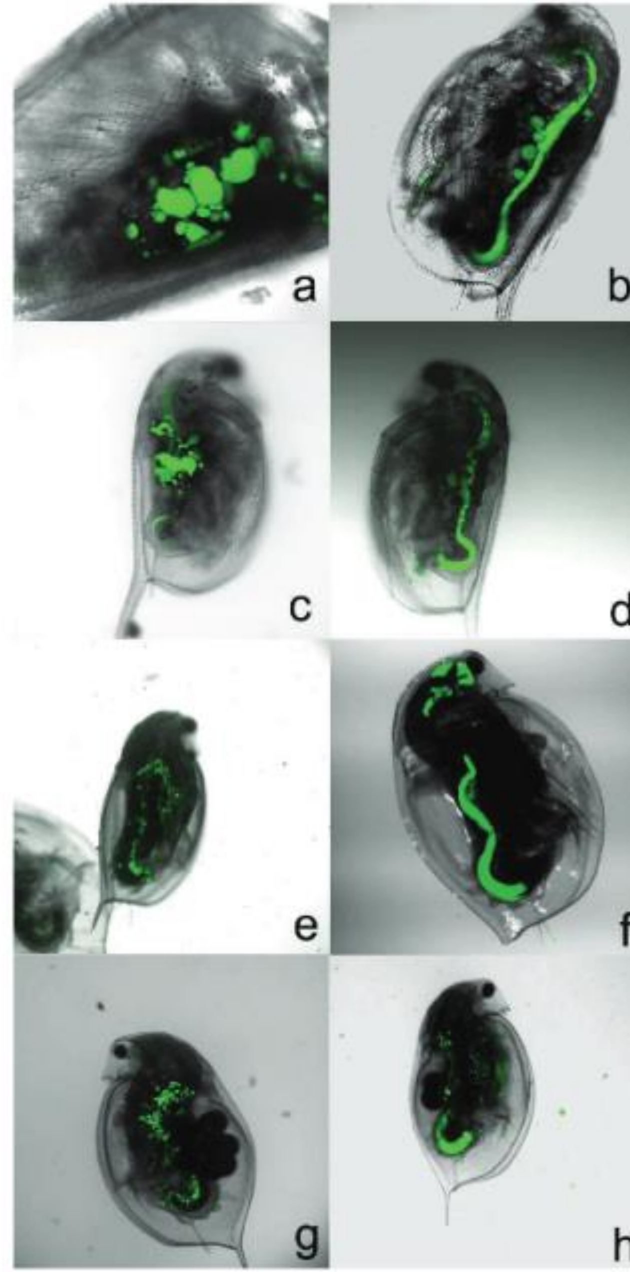
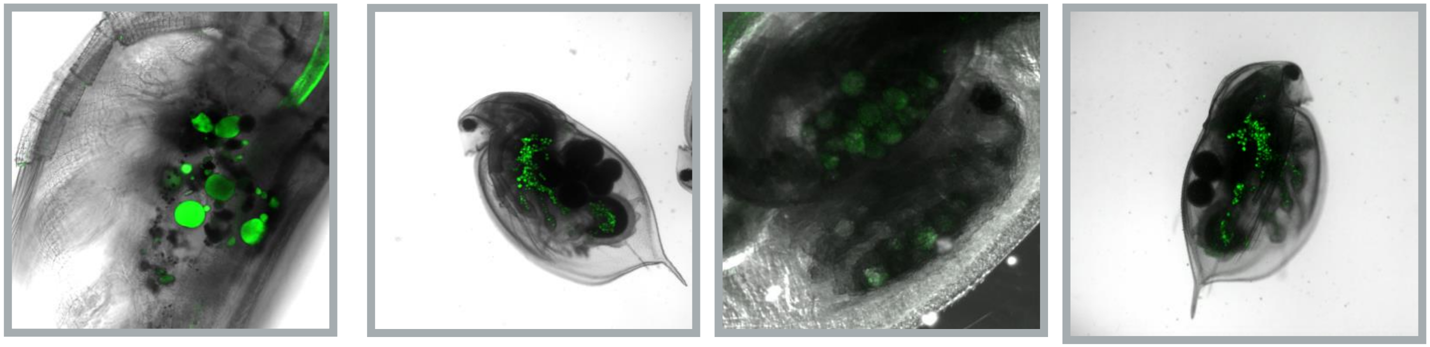
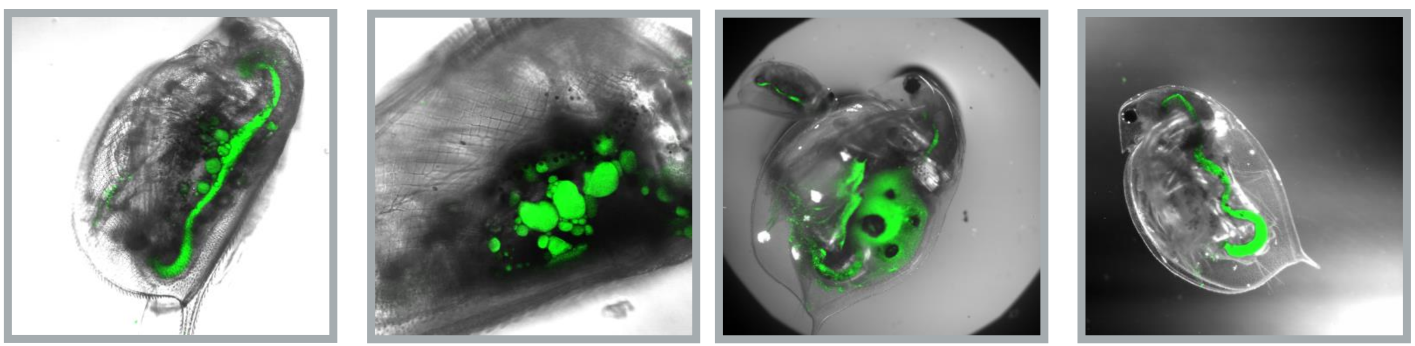


Fig. 1. Uptake of 20- (**a**, **c**, **e**, **g**) and 1,000-nm (**b**, **d**, **f**, **h**) fluorescein isothiocyanate–labeled polystyrene beads in neonate (<24 h old at start of experiment, a–d) and adult (e–h) *Daphnia magna* over 24 h as observed by confocal microscopy. Shown are pictures after 30-min (a, b, e, f) and 12-h exposure (c, d, g, h). Magnification was ×200 in panel a, ×100 in panels b to d, and ×25 in panels e to h.

Size Dependent Particle Uptake by *D. magna* (observed via confocal microscopy)



20nm



1000nm

Rapid uptake of fluorescence in the gut and adjacent oil storage droplets can be observed.

(fluorescent green carboxylated nanospheres at 2.6 µg/L for 24 hrs)

Rosenkranz et al (2009)

Comparing the effects of 14 nm and 260 nm carbon black particles

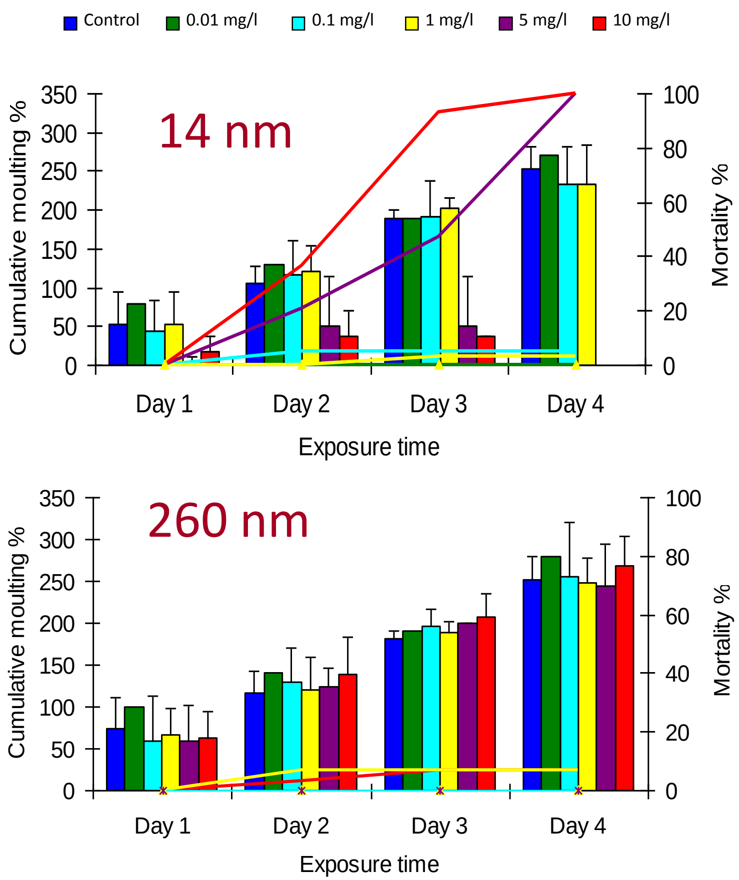


Fig.2: **Mortality (line)** and **cumulative moult** (column) after treatment with 14nm (top) and 260nm carbon black (bottom) in an acute, 96h exposure

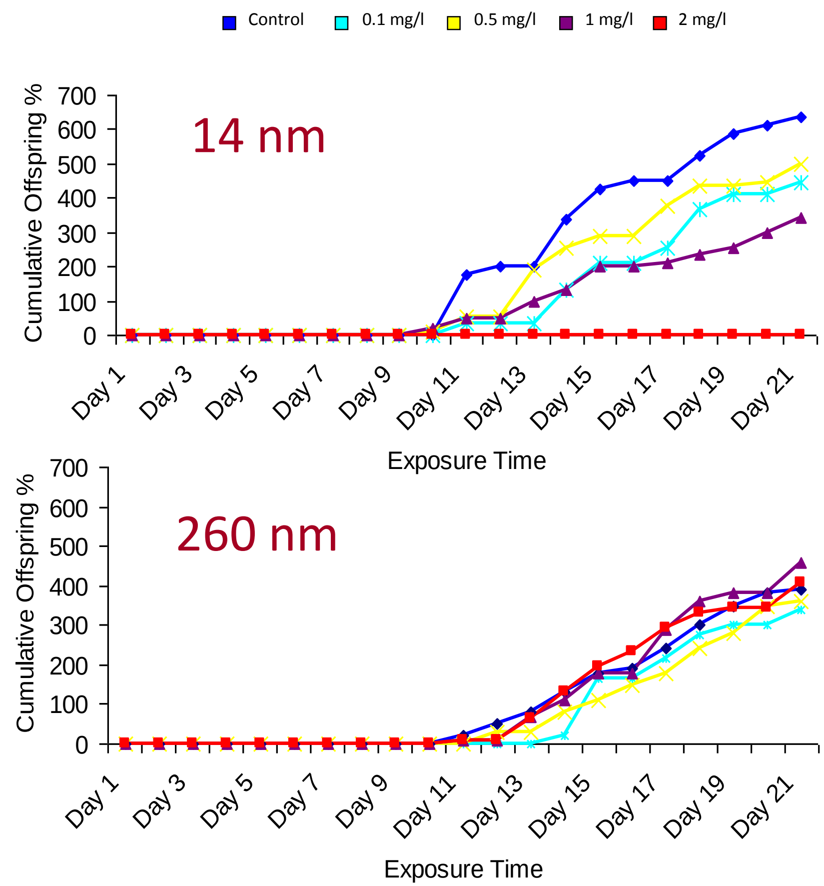
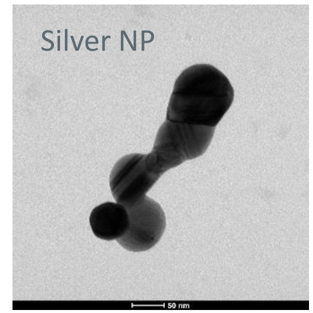
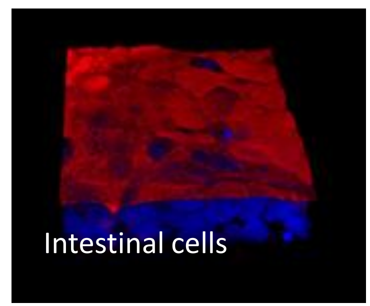


Fig.3: **Cumulative offspring** after treatment with 14nm (top) and 260nm carbon black in a chronic, 21 day exposure

Rosenkranz et al (2009)

Assessing human exposure, uptake and toxicity of NPs from contaminated environments





Project Aims:

To compare the uptake and relative sensitivity to nanoparticles of invertebrates, fish and human models

University of Birmingham

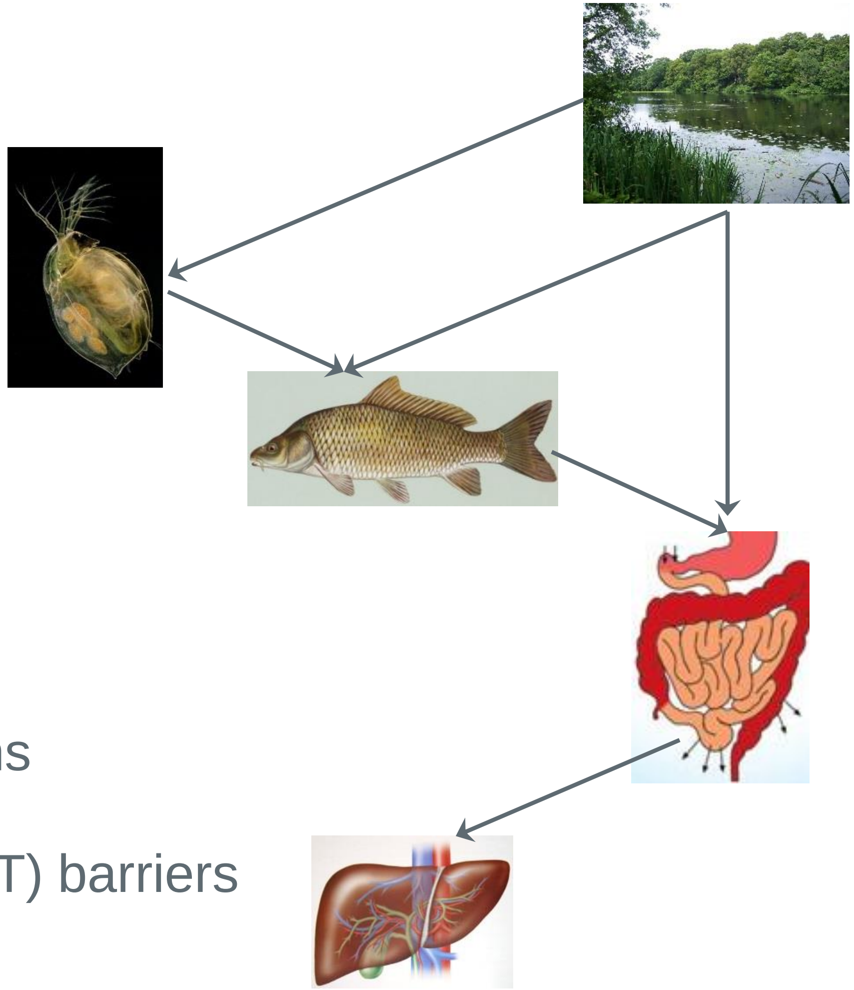
University of Bristol

Joint Environment and Human Health Programme (UK)

Assessing human exposure, uptake and toxicity of NPs from contaminated environments

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 (GIT) barriers
- Effects of NP in hepatocytes



Study Approach

System

1. Hepatocytes:
   Human (C3A), trout (*Oncorhynchus mykiss*) (primary)

2. Invertebrate - *D. magna*

3. Fish – carp (*Cyprinus carpio*)

Endpoints

1. Cytotoxicity (membrane integrity assessment)

2. Mortality, growth, moulting

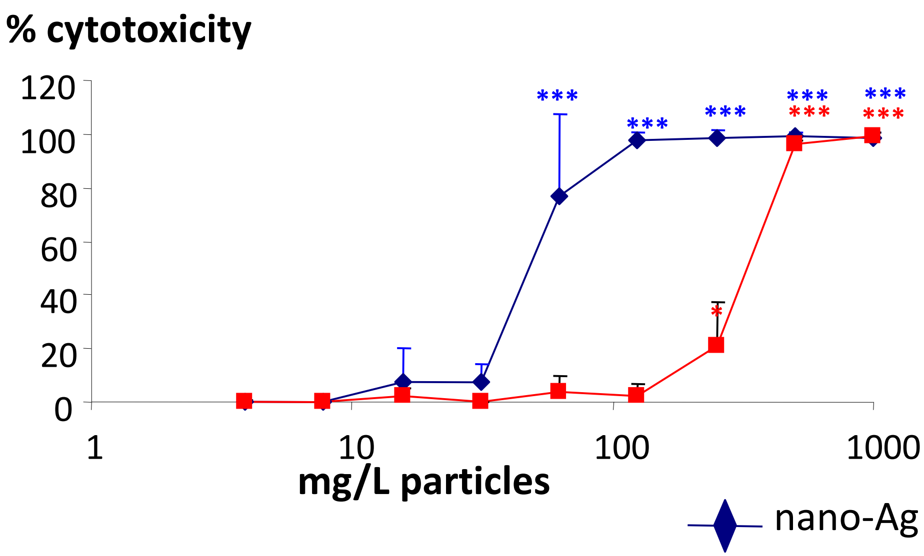
3. Bioavailability

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

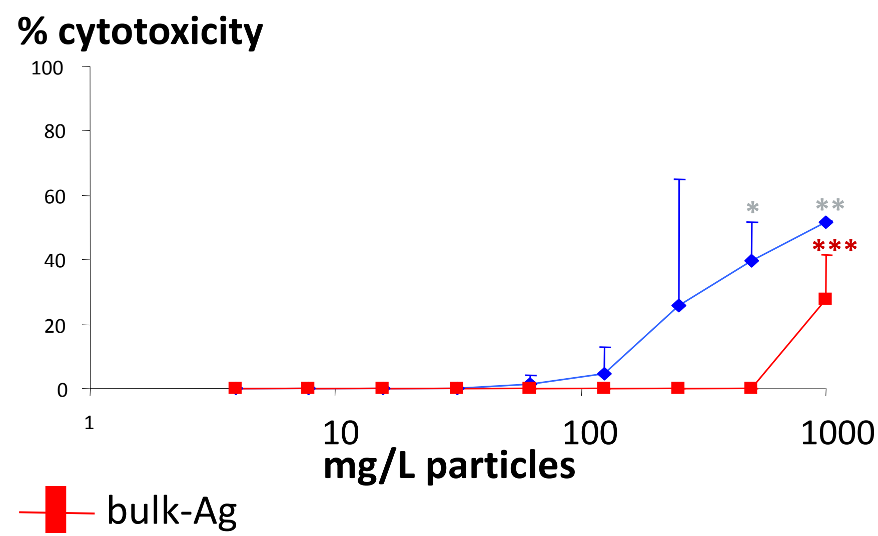
Assess transport through gastro-intestinal barriers

Cytotoxicity (LDH*) of Ag

C3A human hepatocytes



Primary trout hepatocytes



LD$_{50}$ (Ag$_{nano}$) ≈ 50 mg/L

LD$_{50}$ (Ag$_{bulk}$) ≈ 300 mg/L

LD$_{50}$ (Ag$_{nano}$) ≈ 1000 mg/L

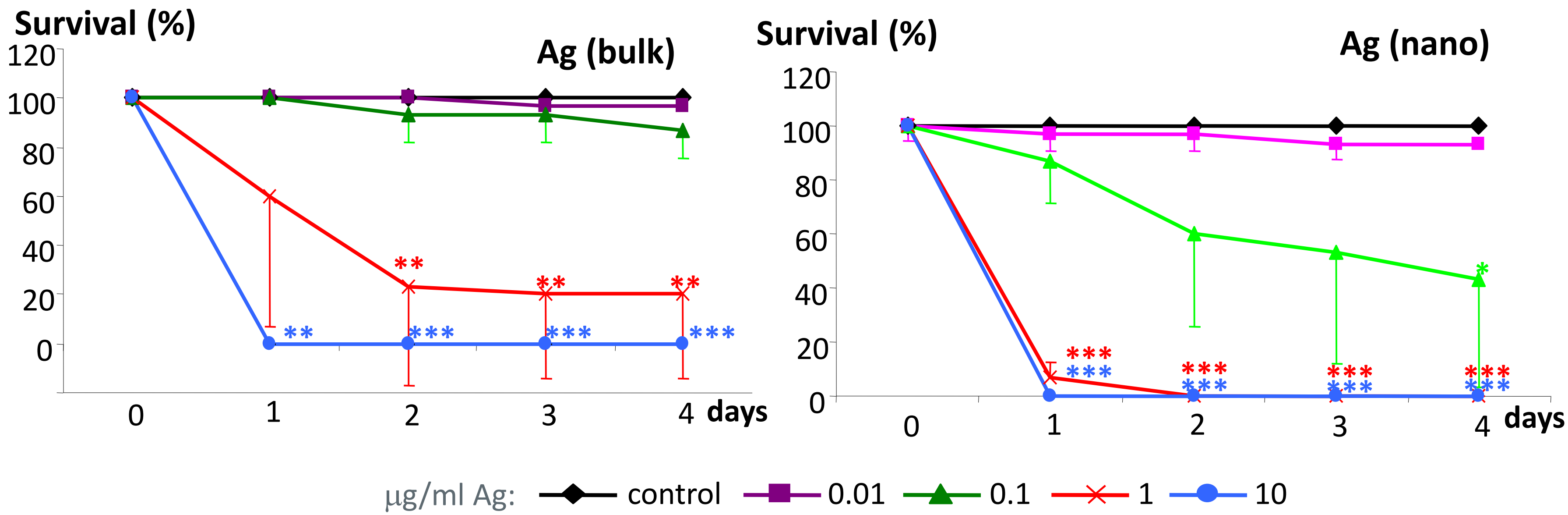
LD$_{50}$ (Ag$_{bulk}$): >1000 mg/L

→ Dose- and size-dependent toxicity

→ Ag less toxic in trout hepatocytes

* Release of enzyme **lactate dehydrogenase** into the surrounding medium upon rupture of the plasma membrane - marker for **cell toxicity**

Toxicity of Ag particles to *D. magna*



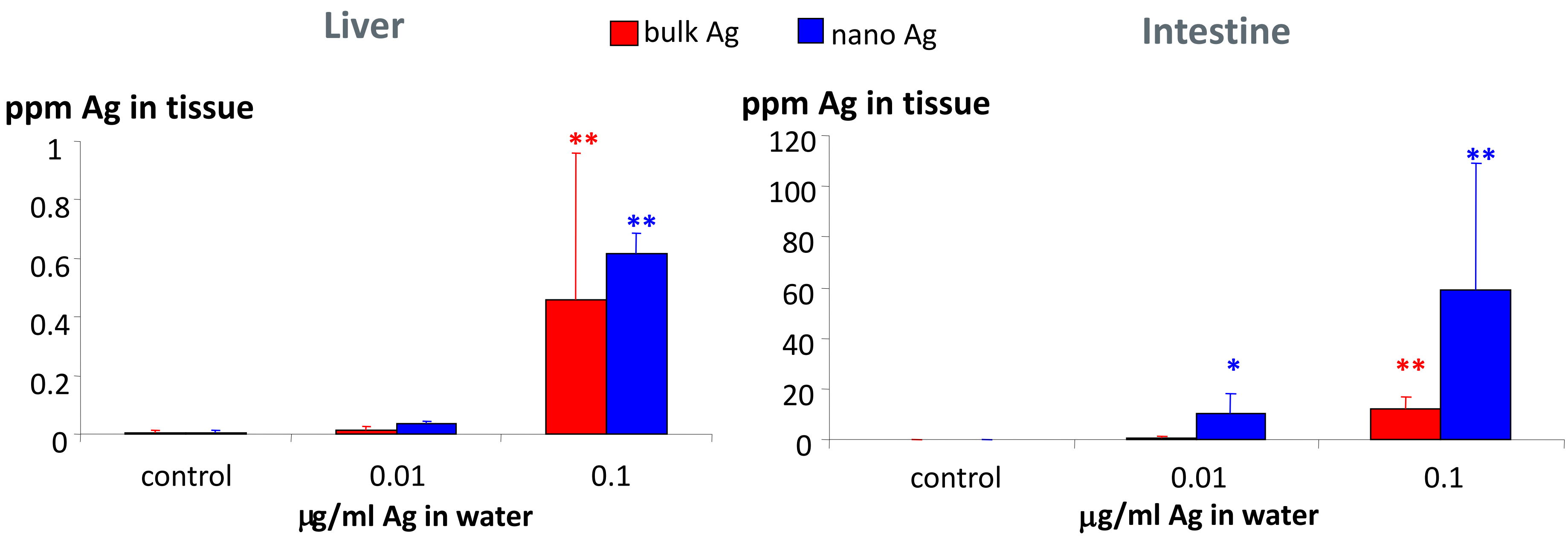
Bulk Ag:

- 100 % mortality at 10 mg/L
- 80 % mortality at 1 mg/L
- No significant mortality at 0.1 and 0.01 mg/L over 96 h
- LC_{50} (48hrs) ≈ 0.7mg/L

Nano-Ag:

- 100 % mortality at 10 & 1 mg/L
- 60 % mortality at 0.1 mg/L
- No significant mortality at 0.01 mg/L
- LC_{50} (48hrs) ≈ 0.3mg/L

Bioavailability of Ag in Carp (21 day exposures)



Ag increased in carp liver for both particle sizes (0.1 mg/L)

Ag uptake at 0.01 mg/L (nano) and 0.1 mg/L (bulk and nano)

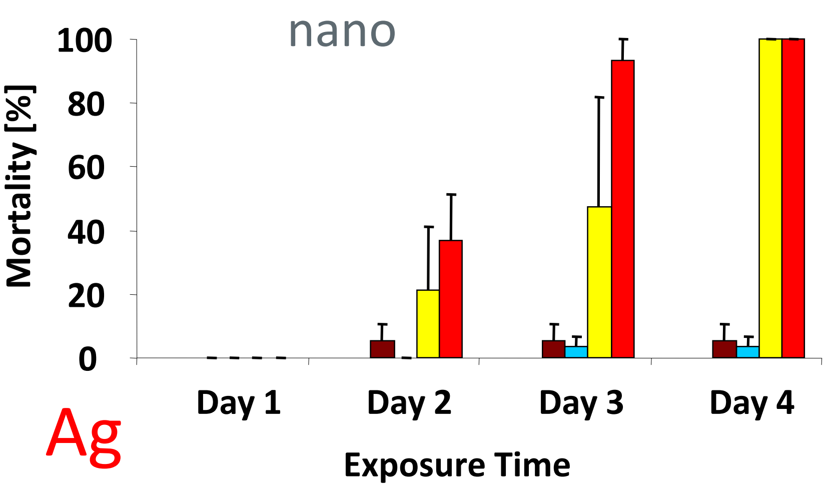
Highest concentration of all tissues

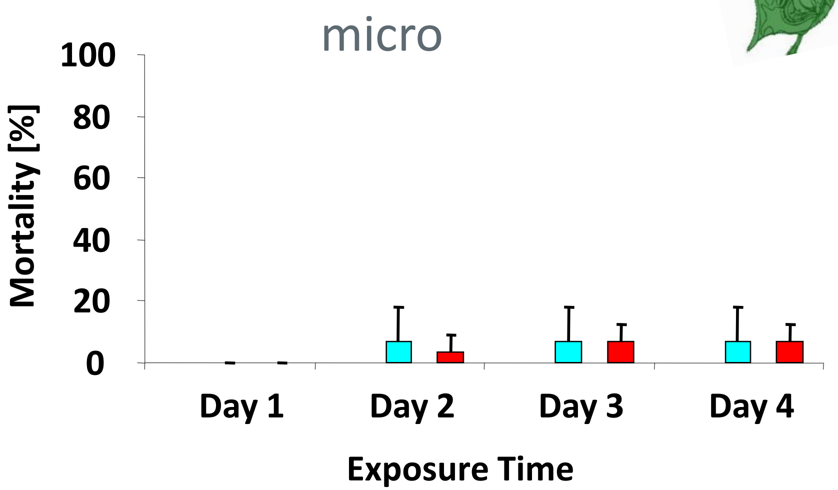
Results obtained for gall bladder and gill showed enhanced values but much variability

Mortality of mass dose in 96h acute tests with micro and nano particles of carbon black (14 and 260 nm) and silver (35 nm and ~1um)

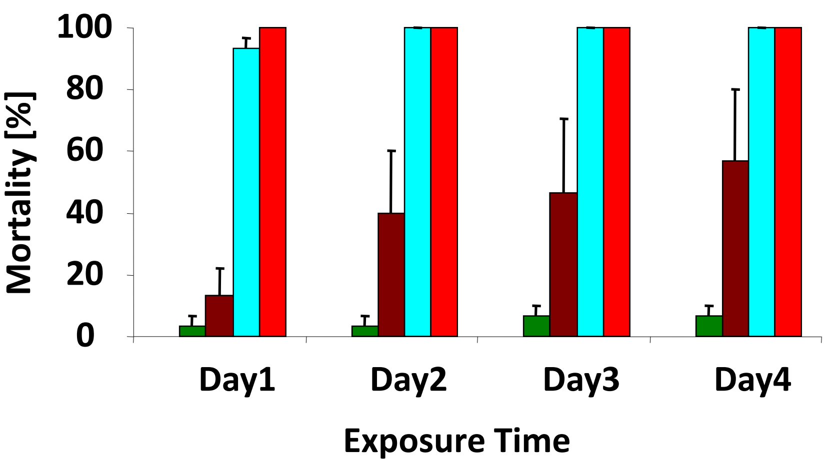
Control | 0.01 mg/l | 0.1 mg/l | 1 mg/l | 5 mg/l | 10 mg/l

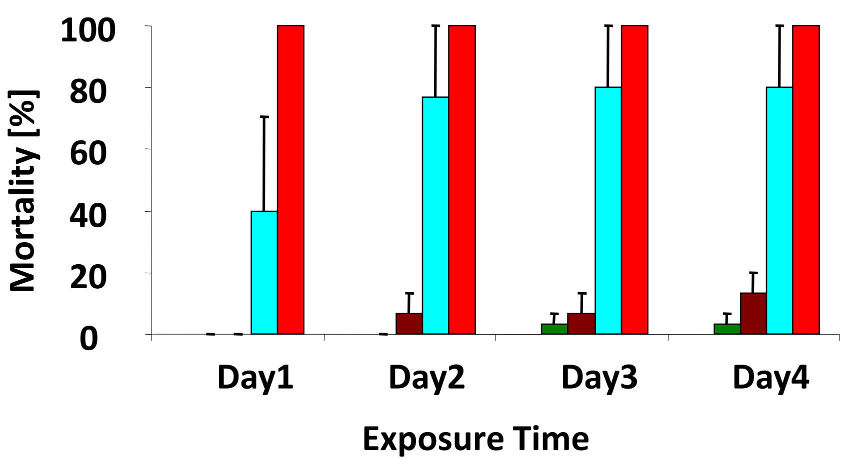
CB





Ag





Gaiser et al (2011a, b)

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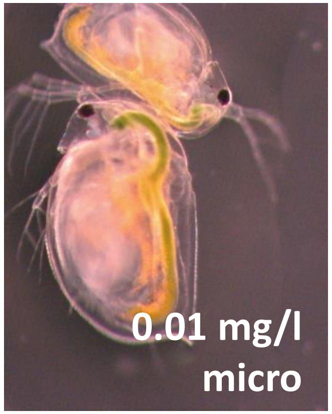
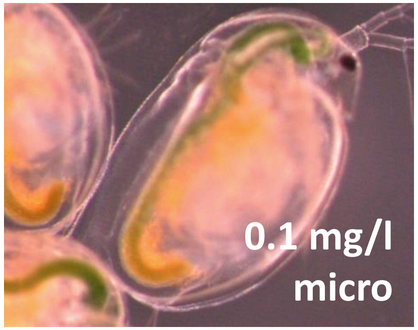
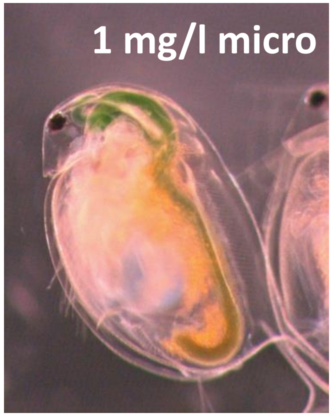
Toxicity: CeO_2, *Daphnia magna*

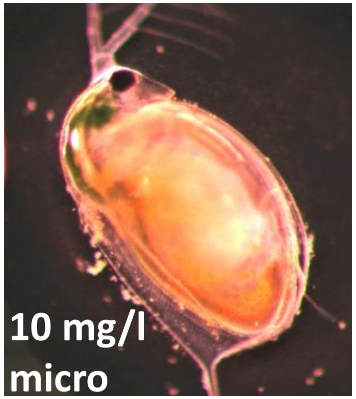
control

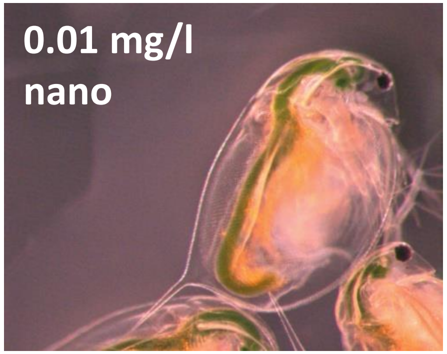
Gaiser et al 2012a
Gaiser et al 2012b

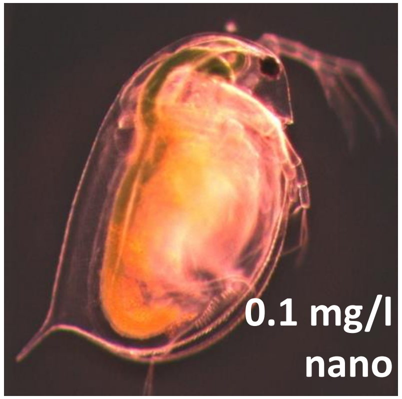


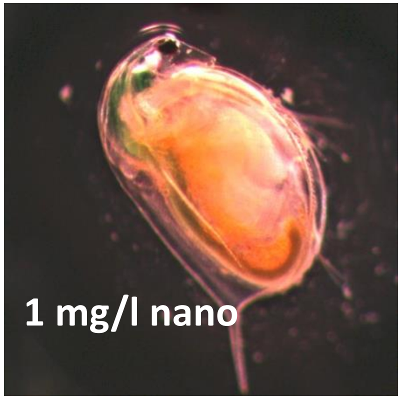


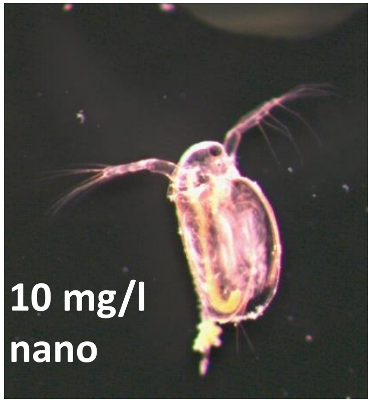










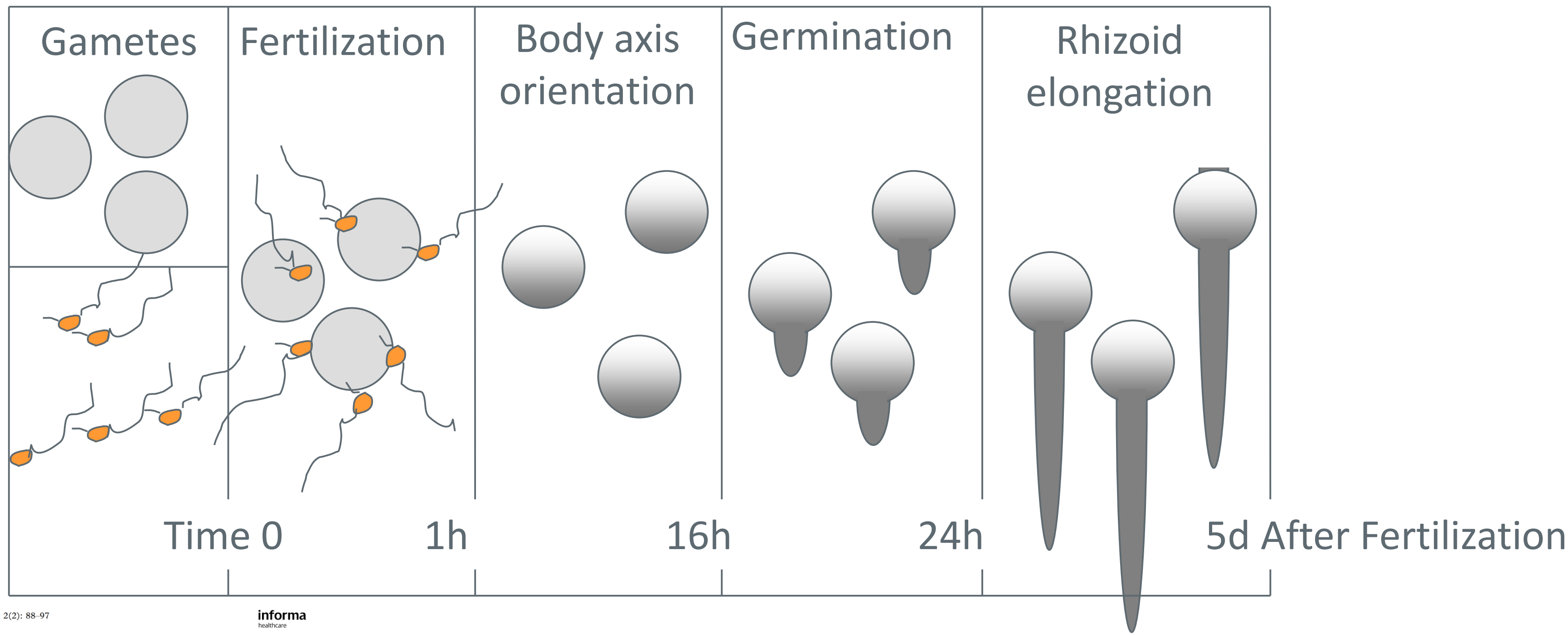


NATURAL ENVIRONMENT RESEARCH COUNCIL

Marine macroalga

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

Organism: Macroalga *Fucus serratus*

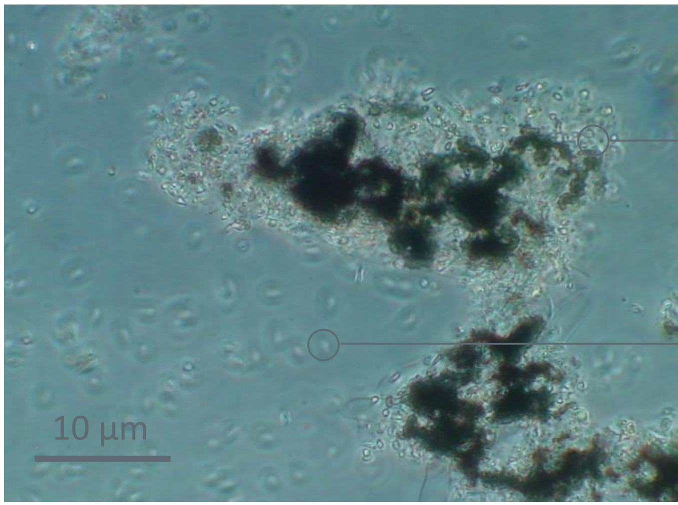


Nanotoxicology, June 2008; 2(2): 88–97
informa healthcare

Interactions between carbon black nanoparticles and the brown algae *Fucus serratus*: Inhibition of fertilization and zygotic development

HANNE D. NIELSEN[1], LORRAINE S. BERRY[2], VICKI STONE[1], TREVOR R. BURRIDGE[3], & TERESA F. FERNANDES[1]

Effects of CB NPs on marine macroalga



Spermatozoid *adsorbed* to CB aggregate

Free swimming spermatozoid

Physical restriction, shading?

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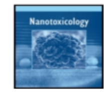
HANNE D. NIELSEN[1], LORRAINE S. BERRY[2], VICKI STONE[1], TREVOR R. BURRIDGE[3], & TERESA F. FERNANDES[1]

Macroalga treated with carbon NPs

Nanotoxicology
Volume 2, Issue 2, 2008



Original
Interactions between carbon black nanoparticles and the brown algae *Fucus serratus*: Inhibition of fertilization and zygotic development

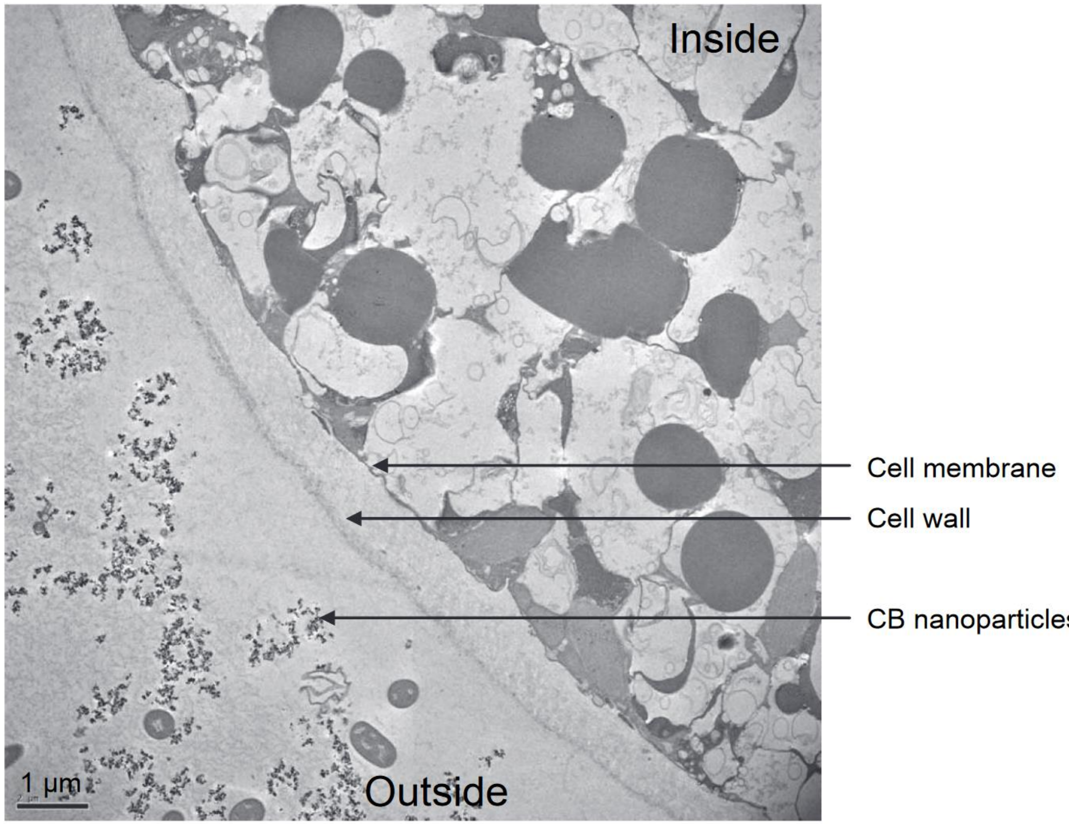


Figure 8. Example of cross section of *F. serratus* zygote after 20 h exposure to 50 µg ml^{-1} CB. There is no sign of uptake of CB particles (lower left corner) in the zygote

Effects of CB NPs on marine macroalga

Results: Carbon nanoparticles were found to influence *Fucus* embryos development, for example by:

- Reducing fertilisation success
- Changing orientation of the body axis

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informa healthcare

Interactions between carbon black nanoparticles and the brown algae *Fucus serratus*: Inhibition of fertilization and zygotic development

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Stability in media

- TiO_2 (25 nm) and (250 nm) 10 mg/L and 100 mg/L
- Suwannee River Humic Acid solutions of 0, 0.01, 0.1, 1, 5 and 10 mg/L

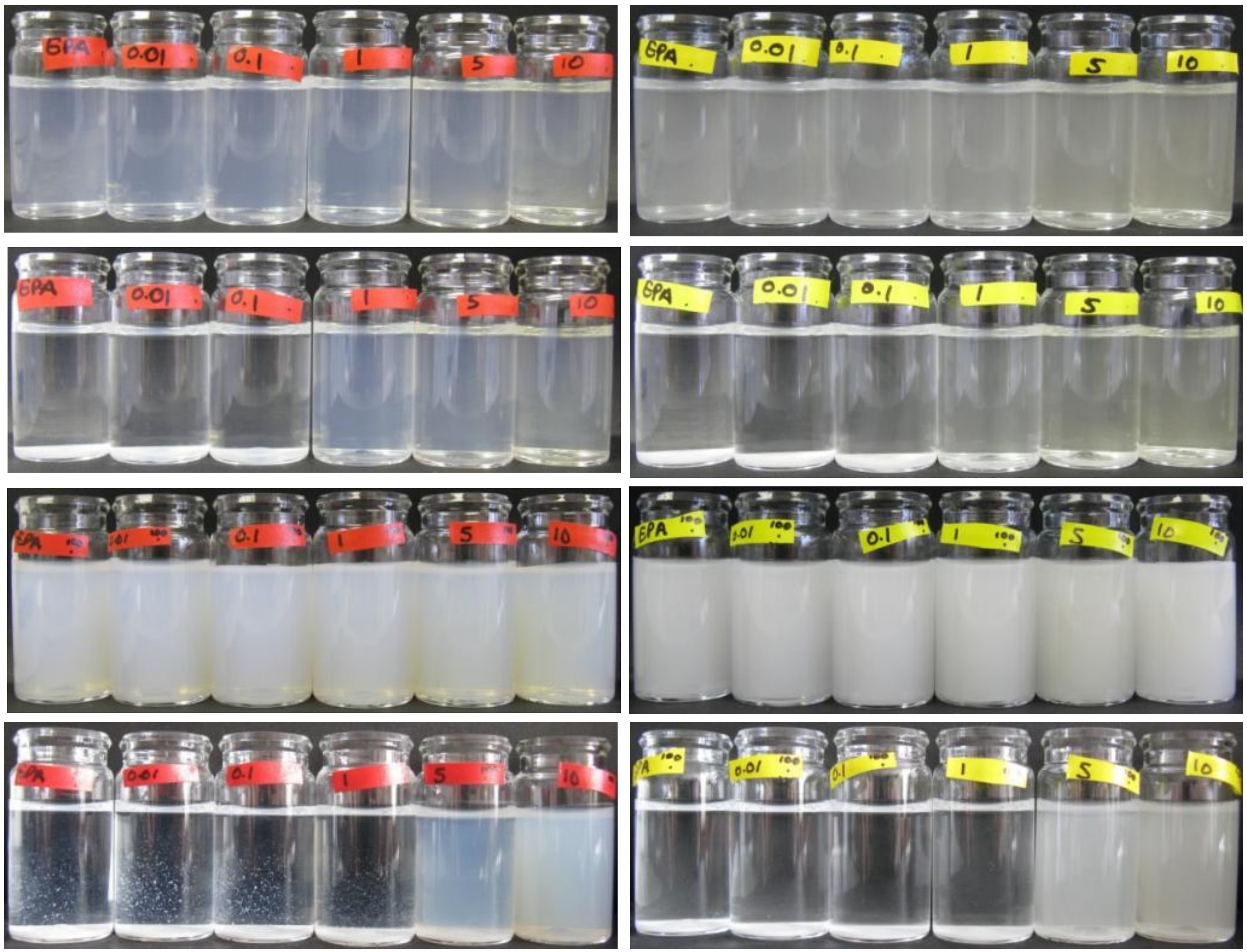
NP (25 nm) **P (250 nm)**

10 mg/L 0 hr

10 mg/L 24 hr

100 mg/L 0 hr

100 mg/L 24 hr

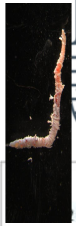


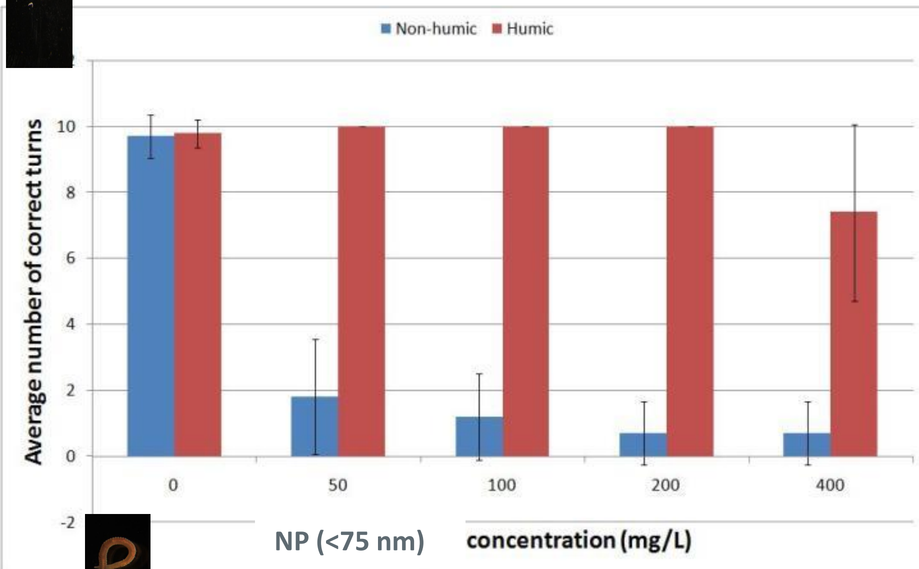
Increased humic acid concentration →

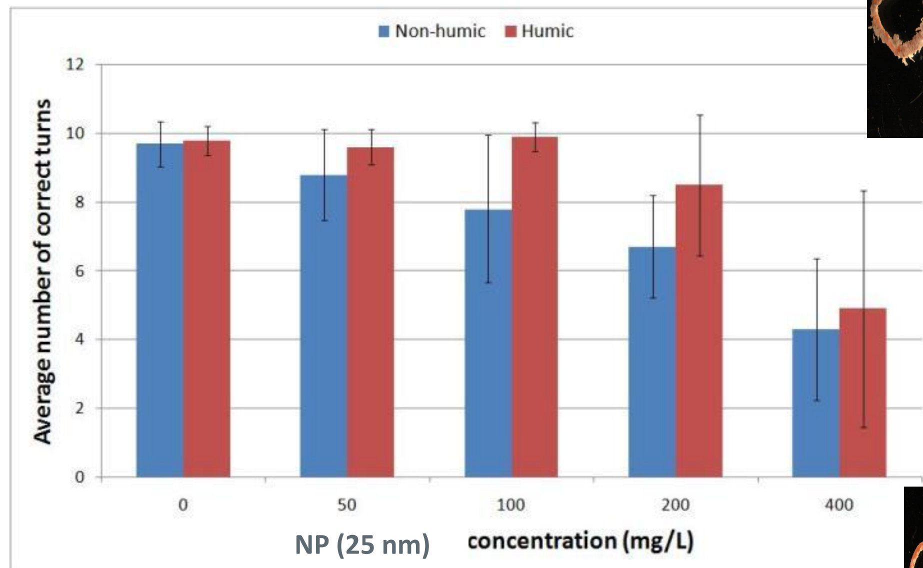
Jon Mullinger

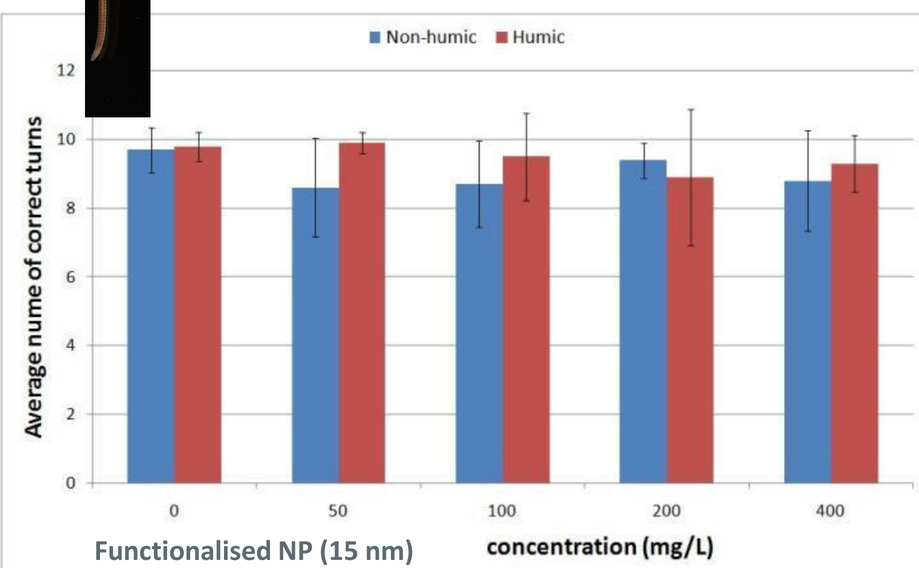
Lumbriculus variegatus sub-lethal results on exposures to TiO_2 particles

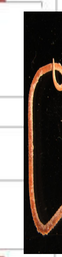
(without/with 5 mg/L humic acid; water only 4 day exposure)

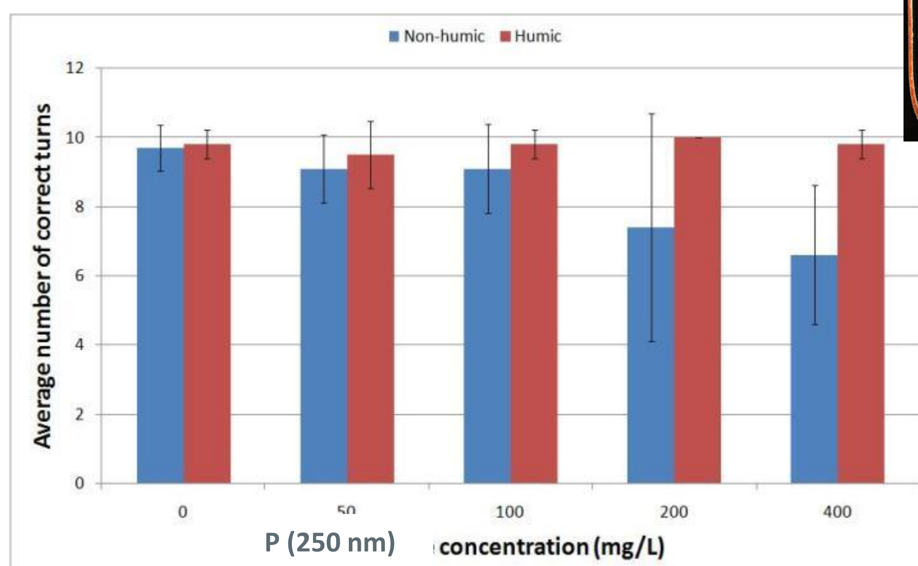












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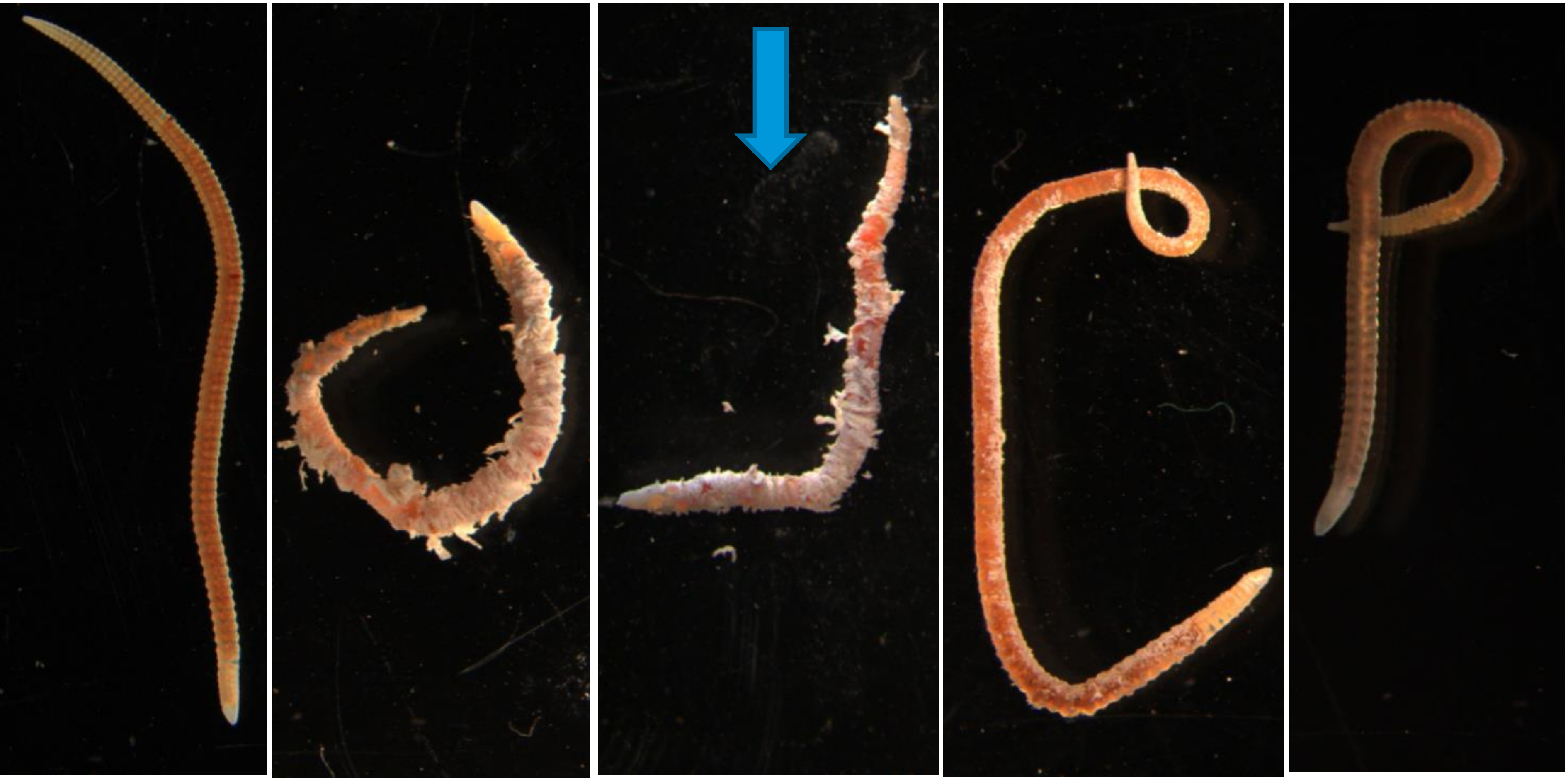
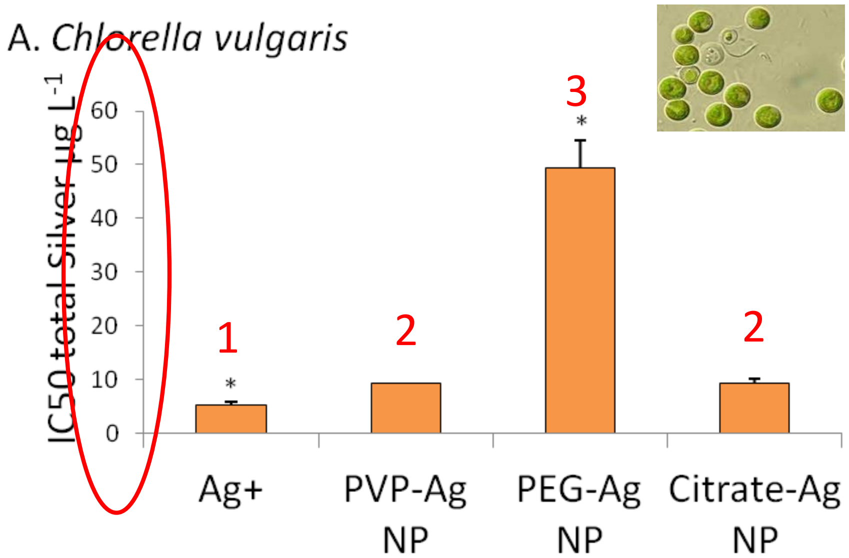
Lumbriculus variegatus (TiO_2)

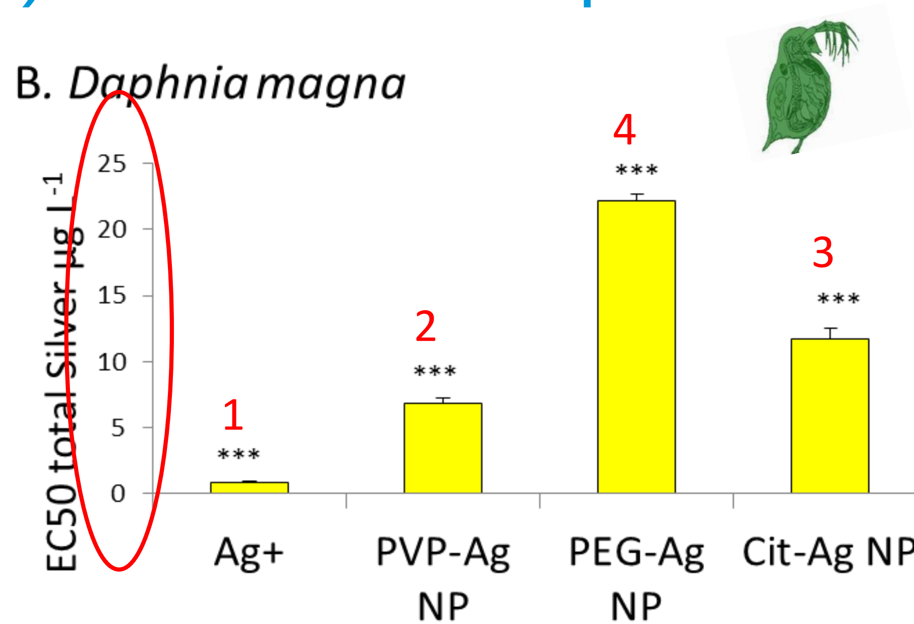


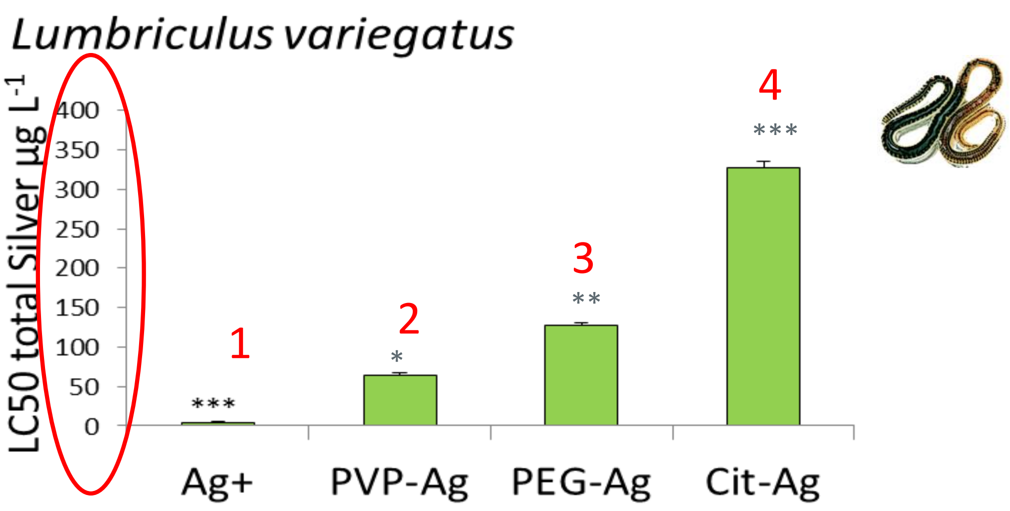
Control | NP (25 nm) | NP (<75 nm) | P (250 nm) | Modified NP (15 nm)*

*Consists of a TiO_2 core, an aluminium hydroxide and hydrated silica shell, and is functionalised with alginic acid.

Acute toxicities of different forms of Ag NPs (~ 10 nm) to 3 standard species







* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

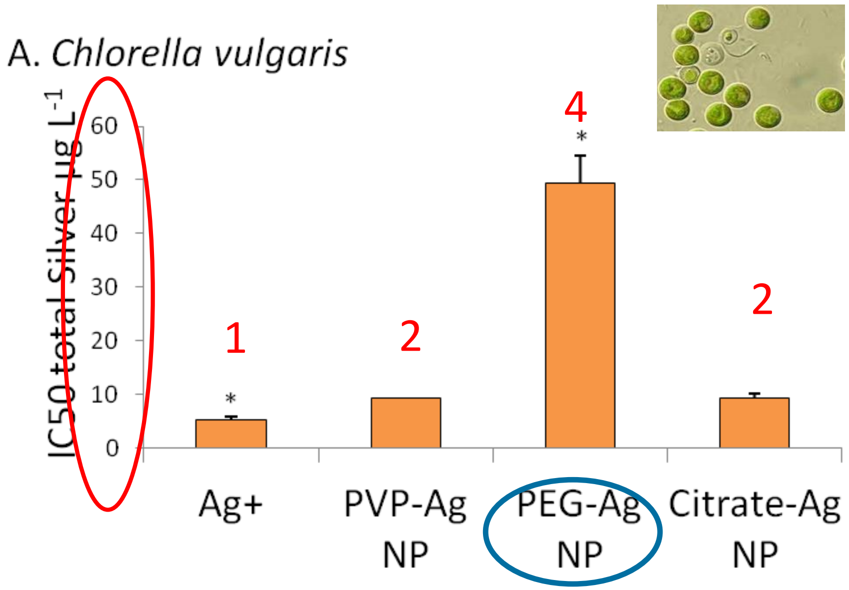
Ag^+ - exposure as silver nitrate
PVP – Polyvinylpyrrolidone
PEG – Polyethylene glycol
Cit - Citrate

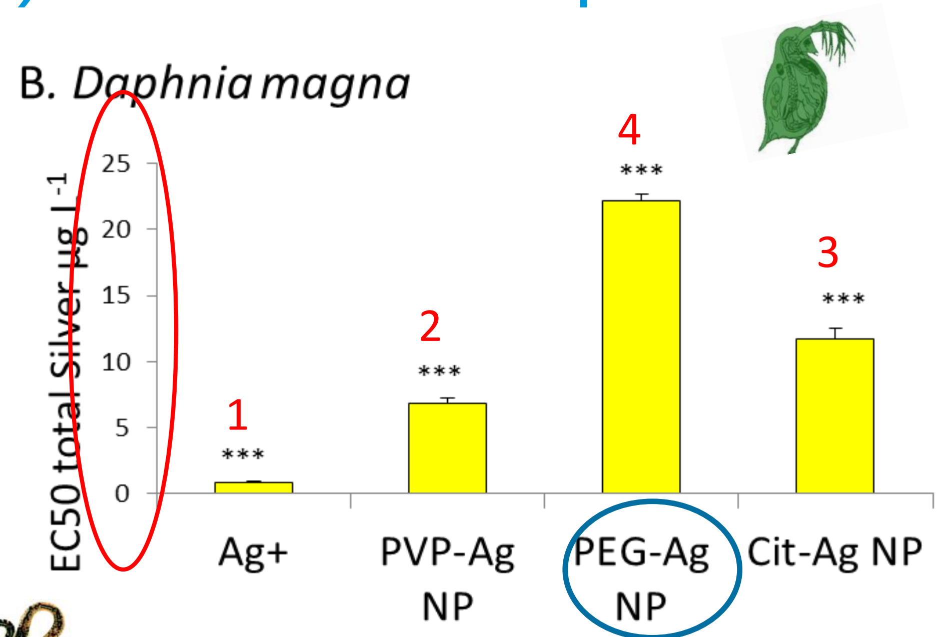
Kai Paul, Farhan Khan, Judit Kalman

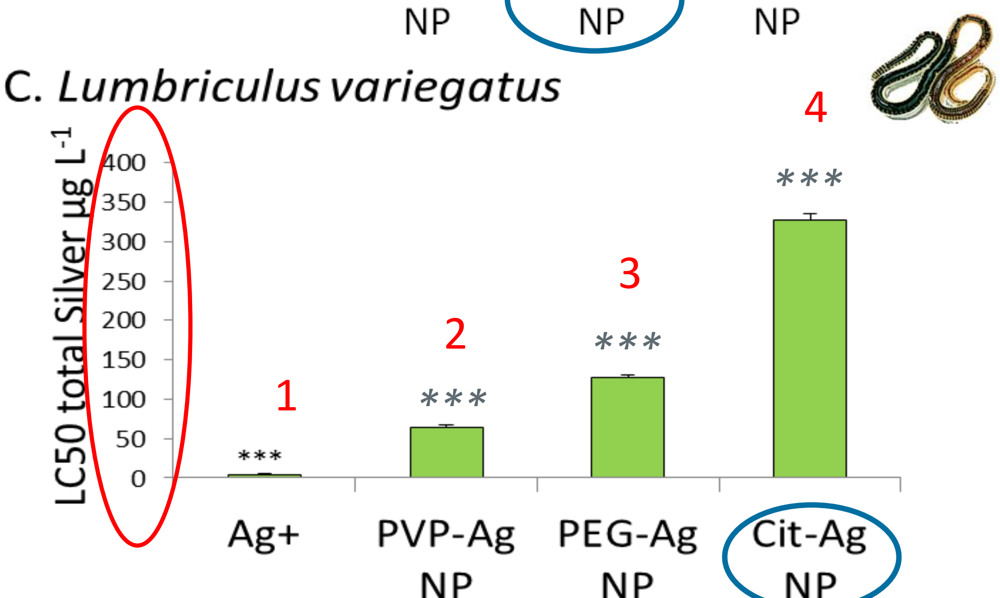
NATURAL ENVIRONMENT RESEARCH COUNCIL

Khan et al (2015)
Kalman et al (2015)

Acute toxicities of different forms of Ag NPs (~ 10 nm) to 3 standard species







- Effects do not necessarily correlate with dissolution, aggregate size or zeta potential
- Ranking depends on test species

Khan et al (2015)
Kalman et al (2015)

Effects of Ag NPs on *C. vulgaris*

- **Aqueous Ag** was **more toxic** than Ag NPs to *C. vulgaris*
- Transmission electron microscopy (TEM) images indicated uptake and **internalization of PVP-, and PEG-Ag NPs** into the cells of *C. vulgaris* **only at high exposure concentration**
- **Possible increase in membrane permeability** led to the internalization of NPs
- In general **uptake rate constant correlated well with toxicity** in *C. vulgaris*
- **Dissolution data do not relate to toxicity**, suggesting that dissolved Ag from Ag NPs not the only form that interact with the algae

Kalman et al (2015)

Effects of Ag NPs on *D. magna* (food vs aqueous Ag exposures)

- **Ag was assimilated from ingested algae by *D. magna***
- ***D. magna* assimilated Ag from ingested Ag NP-containing algae with a higher efficiency** than algae pre-exposed to $AgNO_3$
- Ag NPs were not eliminated completely from *D. magna*, suggesting **possible transport of Ag NPs** along the food chain
- In general, **food is the dominant pathway** of Ag uptake in the cases of Ag NPs.
- In the case of **aqueous Ag**, **water becomes the major source** to the overall accumulation

NanoBee project

Kalman et al (2015)

The project




- **NANOMICEX** - Mitigation of risk and control of exposure in nanotechnology based inks and pigments
- Partners from academia and industry
- Heriot-Watt University:
  - Hazard assessment of pristine and functionalised particles
  - Human and environmental exposure (and models)
  - Identification of high hazard particles
  - Impact of surface modifications:
    Decreasing particle toxicity
    Keeping pigment properties

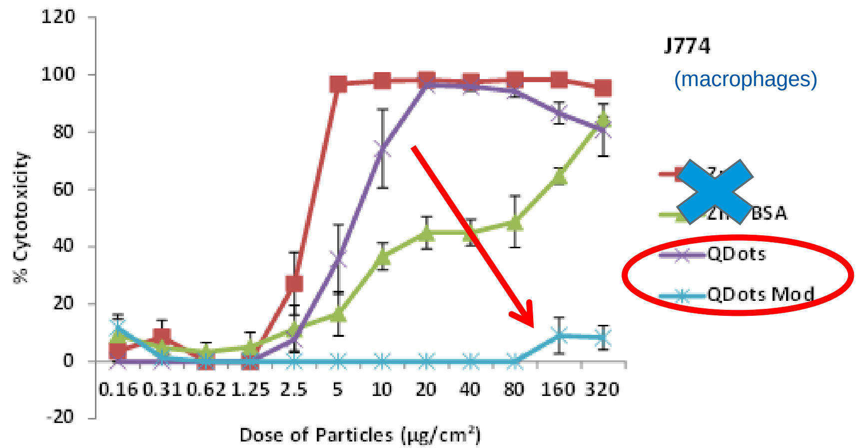
SEVENTH FRAMEWORK PROGRAMME

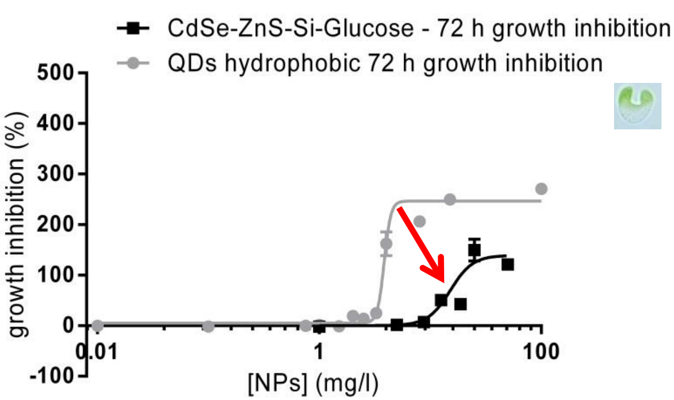
Ranking of particle acute toxicity

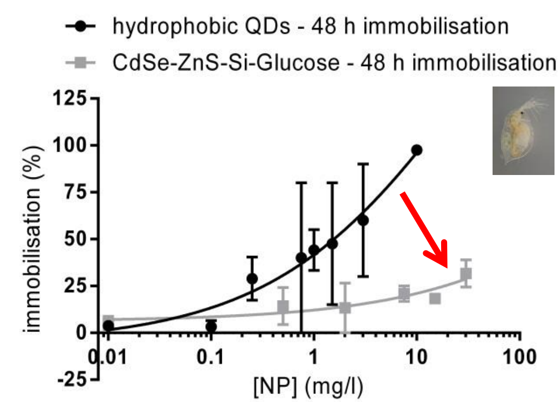
	Al_2O_3	Ag (hydrophilic)	CdSe (QDs)	$CoAl_2O_4$	Fe_2O_3	TiO_2	ZnO
P. sub-capitata							
L. varie-gatus							
D. magna							
J774 Macro-phages							
C3A Hepato-cytes							
A549 Alveolar epithelial type II cells							

Modified QDs – toxicity
(with ZnS shell and Silica-Glucose ligands)

- Modified QDs are less toxic than original QDs



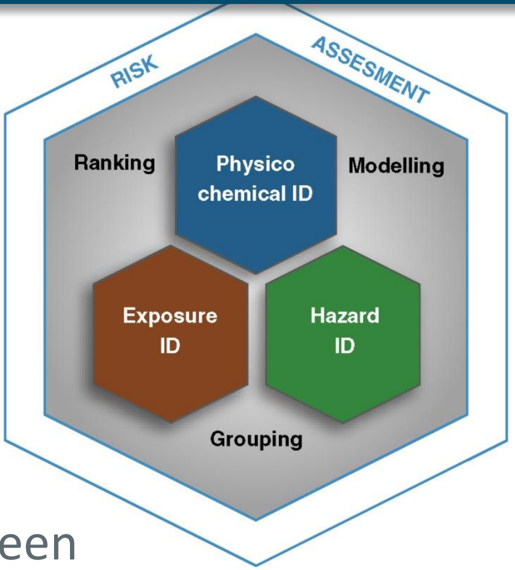




Research prioritization to develop an Intelligent Testing Strategy

A process that allows the risks of nanomaterials (NMs) to be assessed:

- Accurately
- Effectively
- Efficiently



- **Short term** - understanding of the connections between physicochemical, exposure and hazard IDs to enable **grouping/ ranking**.
- **Longer term** - development of **modelling** approaches for RA with a continual reduction in testing.
- In the **distant future** RA based on modelling and extrapolations and only if additional information is required with focused **physicochemical, exposure and hazard testing**.

Stone et al. 2014
Part Fibre Toxicol 11, 9.

ITS NANO

HERIOT WATT UNIVERSITY

OECD ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

OECD Work on the Safety of Manufactured Nanomaterials

Environment, Health and Safety Division
Environment Directorate
OECD

OECD Environmental Health and Safety Publications

Series on Testing and Assessment

No. XX

GUIDANCE DOCUMENT ON AQUATIC (AND SEDIMENT) TOXICOLOGICAL TESTING OF NANOMATERIALS

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris

September 2016

HERIOT WATT UNIVERSITY

European Commission

Health & Consumer Protection Directorate-General

Scientific Committee on Emerging and Newly-Identified Health Risks

SCENIHR

OPINION ON

THE APPROPRIATENESS OF THE RISK ASSESSMENT METHODOLOGY IN ACCORDANCE WITH THE TECHNICAL GUIDANCE DOCUMENTS FOR NEW AND EXISTING SUBSTANCES FOR ASSESSING THE RISKS OF NANOMATERIALS

Scientific Committees
- on consumer products
- on emerging and newly identified health risks
- on health and environmental risk

The SCENIHR adopted this opinion at the 19th plenary on 21-22 June 2007 after the public consultation

European Commission

Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Opinion on

Nanosilver: safety, health and environmental effects and role in antimicrobial resistance

Scientific Committees
- on consumer safety
- on emerging and newly identified health risks
- on health and environmental risks

SCENIHR approved this opinion at the 6th plenary of 10 -11 June 2014

OECD ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

OECD Work on the Safety of Manufactured Nanomaterials

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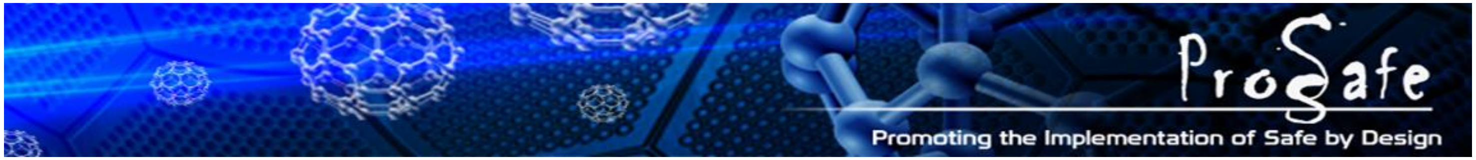
OECD

NANOREG



Summary

- Nanoecotoxicology has learnt a LOT from nanotoxicology
- Collaboration and interaction are essential
- The receiving environment affects fate, bioavailability and toxicity
- NMs can be taken up by the gut and sometimes translocated to other parts of the body (although not always)
- Respiratory surfaces (such as gills) are sometimes the most sensitive, but that is not always the case
- Some NMs can be passed on through the food chain
- Not all nanomaterials are equally toxic, but often toxicity in one model/species can be replicated in other models/species
- Physical impacts can be a problem but ‘toxic’ effects are also observed
- Population, food chain studies and interactions with other chemicals provide a means of assessing long-term and indirect effects
- Assay preparation and conditions, as well as reporting of any observed effects need to be considered carefully – certain methods are not appropriate to nanomaterial hazard studies!

Acknowledgements

Joint Environment and Human Health Programme, NERC, Unilever, CSL/FERA, EC FP7, Heriot-Watt University, Royal Embassy of Saudi Arabia, Danish Research Council, MASTS Prize Studentship, James Watt

Colleagues and research students:

Vicki Stone
Birgit Gaiser
Philipp Rosenkranz
Jon Mullinger
Shona O'Rourke
Helinor Johnston
Corin Liddle
Judit Kalman
Farhan Khan
Ted Henry
Hassien Alnashiri
Faisal Alqahtani
Tom Aspray

John Kinross
Florian Mallevre
Kai Paul
Mark Hartl
David Brown
Simon Little
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Well done and thanks, Vicki!

Toxicology Award 2015 Winner

Professor Vicki Stone
Heriot-Watt University

Awarded for pioneering transdisciplinary approaches to assessing the safety of nanomaterials