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Organisation for Economic Co-operation and Development

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**ENVIRONMENT DIRECTORATE  
JOINT MEETING OF THE CHEMICALS COMMITTEE AND  
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**ADDENDUM TO EXOTOXICOLOGY AND ENVIRONMENTAL FATE OF MANUFACTURED  
NANOMATERIALS: TEST GUIDELINES**

**Expert Meeting Report**

**Series on the Safety of Manufactured Nanomaterials  
No. 40**

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**OECD Environment, Health and Safety Publications**

**Series on the Safety of Manufactured Nanomaterials**

**No. 40**

**Ecotoxicology and Environmental Fate of Manufactured Nanomaterials:  
Test Guidelines**

**IOMC**

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

**Environment Directorate  
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT  
Paris, 2014**

***Also published in the Series of Safety of Manufactured Nanomaterials:***

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- No. 2, *Current Developments/ Activities on the Safety of Manufactured Nanomaterials: Tour de table at the 1st Meeting of the Working Party on Manufactured Nanomaterials (2006)*
- No. 3, *Current Developments/ Activities on the Safety of Manufactured Nanomaterials: Tour de table at the 2nd Meeting of the Working Party on Manufactured Nanomaterials (2007)*
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- No. 26, *Current Development/ Activities on the Safety of Manufactured Nanomaterials: Tour de table at the 7th Meeting of the Working Party on Manufactured Nanomaterials (2010)*
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- No. 34, *Current Development/ Activities on the Safety of Manufactured Nanomaterials: Tour de table at the 9th Meeting of the Working Party on Manufactured Nanomaterials (2012)*
- No. 35, *Inhalation Toxicity Testing: Expert Meeting on Potential Revisions to OECD Test Guidelines and Guidance Document (2012)*
- No. 36, *Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials (2012)*
- No. 37, *Current Developments in Delegations on the Safety of Manufactured Nanomaterials - Tour de Table at the 10<sup>th</sup> Meeting of the WPMN (2012)*
- No. 38, *Co-Operation on Risk Assessment: Prioritisation of Important Issues on Risk Assessment of Manufactured Nanomaterials - Final Report (2013)*
- No. 39, *Environmentally Sustainable Use of Manufactured Nanomaterials - Workshop held on 14 September 2011 in Rome, Italy (2013)*

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## FOREWORD

As part of its Programme on the Safety of Manufactured Nanomaterials, and in particular work on the testing and assessment of manufactured nanomaterials, OECD initiated a series of expert meetings to improve the applicability of the OECD Test Guidelines to nanomaterials. With this in mind, the Working Party on Manufactured Nanomaterials agreed to organise an expert meeting to address the Ecotoxicology and Environmental Fate of manufactured nanomaterials.

The OECD Expert Meeting on Ecotoxicology and Environmental Fate took place on 29th-31st January 2013 in Berlin, Federal Press Office. The event was hosted by the German delegation and funded by the German Federal Ministry of the Environment, Nature Conservation and Nuclear Safety (BMU) as well as the United States Environment Protection Agency (US EPA).

This document is an accompanying document to *Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines* [ENV/JM/MONO(2014)1], and includes the presentations given at the workshop. It is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, pesticides and Biotechnology of the OECD.

ENV/JM/MONO(2014)1/ADD

**PRESENTATIONS GIVEN AT THE OECD WORKSHOP ON ECOTOXICOLOGY AND ENVIRONMENTAL FATE**

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**Session 1: General Aspects of Environmental Toxicity and Fate of Manufactured Nanomaterials**



# Guidance on Sample Preparation and Dosimetry (GSPD)

## Overview, and Areas for Environmental Protocol Development

Phil Sayre, Ph.D. ([sayre.phil@epa.gov](mailto:sayre.phil@epa.gov))

Second OECD Parallel Workshop: Ecotoxicity and Environmental Fate  
29 January 2013

## Overview of the GSPD [ENV/JM/MONO(2012)40]

- A companion Guidance document to the OECD Test Guidelines, developed with consideration of the chemical and physical characteristics of nanomaterials
- Evolution from Sponsorship Program to the Present
- A living document
- Addresses all physical-chemical property, effects, and fate endpoints
- Utility of GSPD vs. amending individual OECD Protocols



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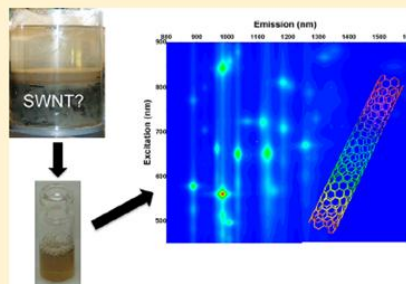
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## GSPD Section V.A: Physical-Chemical Properties

**ABSTRACT:** Near infrared fluorescence (NIRF) spectroscopy is capable of sensitive and selective detection of semiconductive, single-walled carbon nanotubes (SWNT) using the unique electronic bandgap properties of these carbon allotropes. We reported here the first detection and quantitation of SWNT in sediment and biota at environmentally relevant concentrations using NIRF spectroscopy. In addition, we utilized this technique to qualitatively characterize SWNT samples before and after ecotoxicity, bioavailability and fate studies in the aquatic environment. Sample preparation prior to NIRF analysis consisted of surfactant-assisted high power ultrasonication. The bile salt sodium deoxycholate (SDC) enabled efficient extraction and disaggregation of SWNT prior to NIRF analysis. The method was validated using standard-addition experiments in two types of estuarine sediments, yielding recoveries between  $66 \pm 7\%$  and  $103 \pm 10\%$  depending on SWNT type and coating used, demonstrating the ability to isolate SWNT from complex sediment matrices. Instrument detection limits were determined to be  $15 \text{ ng mL}^{-1}$  SWNT in 2% SDC solution and method detection limits (including a concentration step) were  $62 \text{ ng g}^{-1}$  for estuarine sediment, and  $1.0 \text{ } \mu\text{g L}^{-1}$  for water. Our work has shown that NIRF spectroscopy is highly sensitive and selective for SWNT and that this technique can be applied to track the environmental and biological fate of this important class of carbon nanomaterial in the aquatic environment.

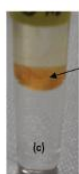


## GSPD Section V.A: Physical-Chemical Properties

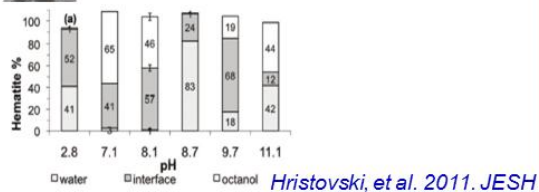
### Physical-Chemical Property Estimation: ISO and OECD Collaboration

#### • Octanol-Water Partition Coefficient

- Key to environmental partitioning, bioconcentration, and ecotoxicity
- Accumulation of insoluble nanomaterials at phase interfaces



- Different solutions:
1. Modification of traditional  $K_{ow}$  test with added distribution coefficients
  2. Nontraditional organic dye absorption test



#### • Radical Formation Potential

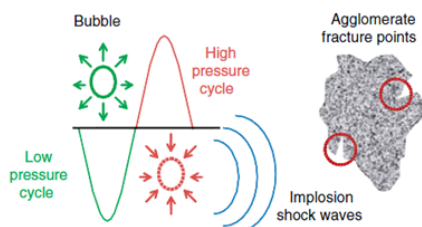
- Correlated with oxidative stress
- Oxygen singlets, superoxide, hydroxyl radicals, hydrogen peroxide; reactive nitrogen species
- Acellular assays:
  - Vitamin C yellowing
  - DCFDA dyes (dichlorodihydrofluorescein-diacetate) – several variants
  - FRAS (ferric-reducing ability of serum)
  - TBARS (thiobarbituric acid reactive substance assay; in presence of U.V.)

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## GSPD Section V.B.1: Preparation of Aquatic Media for Ecotoxicity Studies

### Preparation of TiO<sub>2</sub> Aqueous Stock Dispersions for Toxicological & Environmental Testing

- Alteration of stock solutions by sonication:
  - Sound waves → shock waves /  $T \leq 10,000^\circ \text{K}$
  - Undesirable alterations to nanomaterials
    - Reagglomeration, sintering of nanomaterials, scission of nanomaterials such as CNTs
    - changes in surface chemistry due to water-generated reactive oxygen species, degradation of other media components
- Protocol for dispersion of P-25 dry powder in deionized water
  - Uniform dispersion / Mean particle diameter ~70 nm, / Little undesirable changes / Reproducible due to standardized reagents, materials, equipment, and dispersion preparation steps / Specific for sonication conditions
  - Proposed calorimetric method to report sonication power, independent of instrument
- Extension to other nanomaterials for stock dispersion preparation
- Extension to dispersion in biological media



*NIST Special Publication 1200-3, in conjunction with CEINT*  
*Taurozzi, Hackley & Wiesner. 2011. Nanotoxicology*  
*Taurozzi, Hackley & Wiesner. 2012. Nanotoxicology*  
<http://www.nist.gov/mml/np-measurement-protocols.cfm>

## GNSPD Section V:B: Ecotoxicity & Sample Preparation

- Dispersion methods, suitable for chronic exposures:
  - Agitation (stirring, sonication)
  - Acid treatment
  - Dispersant use (NOM, Gum arabic, SDS, SDC, THF) often involves agitation

Table 1. Multiwalled nanotube (MWNT) particle characterization and *Daphnia magna* 96 h acute toxicity results<sup>a</sup>

Nanoparticle suspension	pH	Zeta potential	Hydrodynamic diameter	LC50 (mg/L)
MWNT in 18.8 mg/L DOC (SR-NOM)	8.08	NA	706.4 ± 35.9*	2.48
MWNT in 15.2 mg/L DOC (SR-NOM)	8.23	-28.9 ± 3.7	629.7 ± 26.8	1.90
MWNT in 10.4 mg/L DOC (SR-NOM)	8.29	NA	655.7 ± 27.6	2.25
MWNT in 5.1 mg/L DOC (SR-NOM)	8.25	-21.1 ± 3.8	655.5 ± 27.6	2.06
MWNT in 2.0 mg/L DOC (SR-NOM)	7.86	-26.5 ± 4.6	NA	2.78
MWNT in 15.1 mg/L DOC (ER-NOM)	8.61	-32.8 ± 4.14	703.3 ± 19.1*	4.09*
MWNT in 15.7 mg/L DOC (BR-NOM)	8.14	-30.6 ± 5.04	528.0 ± 24.2*	1.91

*Edgington, et al. 2010. Env. Tox & Chem.*



- Average NOM concentrations in U.S.: 1-10 mg/L
- Little effect on toxicity of **same NOM** @ different concentrations
- There is an effect of **different types of NOM** on toxicity
- ?Do CNTs represent a Category of Nanomaterials for Ecotoxicity?

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## Ecotoxicity – Sample Preparation & Category Approaches

**SAMPLE Preparation:** *Ma, et al. 2012a. ET&C*, with Robust Summary in TiO<sub>2</sub> Dossier

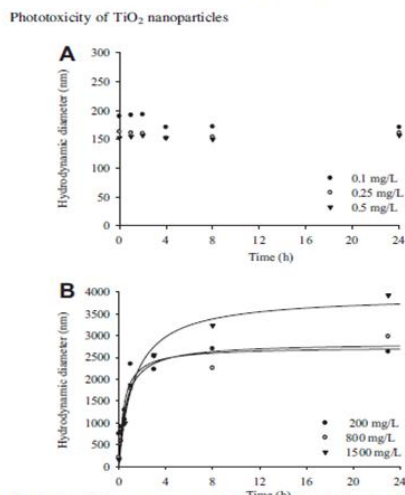


Fig. 3. Nano-TiO<sub>2</sub> exposure system characterization during the 48-h toxicity assay for *Daphnia magna*: (A) change of aggregate size during exposure under simulated solar radiation (SSR), (B) change of aggregate size during exposure under laboratory light, and (C) change of TiO<sub>2</sub> concentration during exposure under laboratory light.

- Aggregate size stable @ low concentrations/ionic strengths
- DLS, TEM, and Flame AAS analysis **during** tests (exudate effect)
- Exposure media replaced every 24h.

**?Category Approach?:** *Ma, et al. 2012b. ET&C*

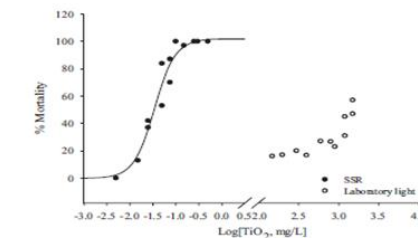
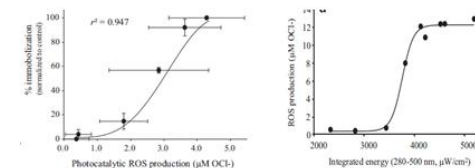


Fig. 4. Phototoxicity of nano-TiO<sub>2</sub> to *Daphnia magna* under simulated solar radiation (SSR) and laboratory light: median lethal concentration (LC50) (SSR) = 29.8 (95% CI, 24.8–35.1) µg/L; LC50 (Lab light) > 500 mg/L.



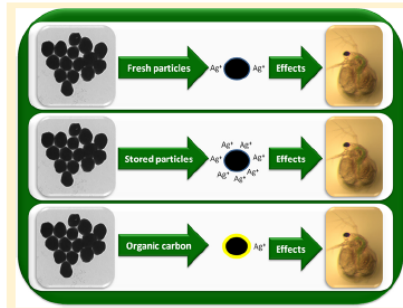
- ROS estimation method specific to TiO<sub>2</sub>: 3'[p-aminophenyl] fluorescein
  - Targets hydroxyl radicals and hypochlorite
  - more resistant to light-induced oxidation, rel. to other DCFD dyes
- MOA: ROS-induced damage to respiratory & ion exch. surfaces with SSR
- Photocatalytic ROS production: solar radiation & [TiO<sub>2</sub>]
- Estimation of Ecotoxicity based on ROS production
  - different crystallinities (surface properties, uptake/adsorption)
- Categories:
  - Titanium dioxide
  - Other insoluble metal oxides?

## GSPD Section III. Ecotoxicity Dosimetrics

- **Metrics:** Particle number, mass, (active) surface area, ion concentration
- **Mitigating Factors:** Photoactivation, presence of DOM and sulfides particle size, particle age...
- **Mechanisms of Action:** Direct (ROS, membrane deformation, protein denaturation, DNA damage by free radicals) and Indirect (particle oxidation and ion release)
- **Categories?** certain insoluble particles (CNTs, metal oxides), soluble metals
- Lessons from Human Health Inhalation studies?

*Kennedy, et al. 2012. ES&T*

with existing predictive toxicity models. This study investigated the implications of freshly prepared versus stored 20 and 100 nm nanosilver stocks to freshwater zooplankton (*Ceriodaphnia dubia*) in presence and absence of dissolved organic carbon (DOC). Results indicated that while the acute toxicity of nanosilver decreased significantly with larger size and higher DOC, storage resulted in significant increases in toxicity and ion release. The most dramatic decrease in



particle (2.0–2.4 fold) and dissolved silver (2.7–3.1 fold). While a surface area dosimetry presented an improvement over mass when DOC was absent, the presence of DOC confounded its efficacy. The fraction of dissolved silver in the nanosilver suspensions was most predictive of acute toxicity regardless of system complexity.

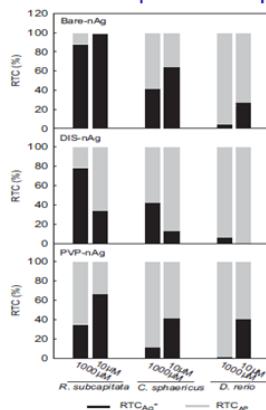
## GSPD Section III on Ecotoxicity Dosimetrics: Silver Ions or Particles? Mechanism of Action?

1. A series of studies compared the potency of uncapped nano-Ag to Ag<sup>+</sup> at acute and sublethal levels using *D. magna* & Fathead minnows (*Hoheisel, et al. ET&C, 2012*):

Overall, these studies did not provide strong evidence that nanosilver either acts by a different mechanism of toxicity than ionic silver, or is likely to cause acute or lethal toxicity beyond that which would be predicted by mass concentration of total silver. This in turn suggests that regulatory approaches based on the toxicity of ionic silver to aquatic life would not be underprotective for environmental releases of nanosilver.

Surface area normalizes for different sized particles: ion release ->gills->circulatory failure, or surface-mediated direct particle toxicity

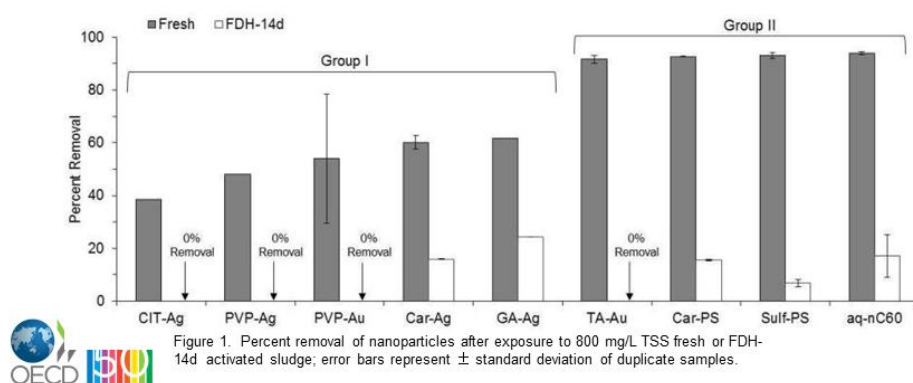
2. A second series of short-term/early life stage studies with uncoated, coated, & dispersed nano-Ag suggests ion vs. particle impacts may be associated with the trophic level of the aquatic test species (*Wang, et al. ET&C, 2012*):



- Efficient accumulation of Ag<sup>+</sup> by some algae
- Cladocera ingest particles which dictate toxicity
- Particles can be accumulated in chorion of zebrafish embryo to block pore canals and generate higher levels of ROS than Ag<sup>+</sup>

## GSPD Section C2.2 - Degradation & Transformation: Wastewater Treatment Removal of NMs

- OPPTS 835.1110, Activated sludge sorption isotherm
  - Test guideline traditionally used to estimate removal of soluble chemicals during waste water treatment due to sorption to sludge
  - Guideline underestimates NM sorption to sludge (*Kiser, et al. 2012. ES&T*)
    - FDH Sludge → Bacterial Biosurfactants → Decreased Sorption of NM to Sludge
  - Use of fresh sludge shown to be predictive of nanomaterial removal
- Unclear which NM properties best predict removal
  - Functionalization; other material properties including size, density, zeta potential



## GSPD Section C1.1: Aggregation/agglomeration & Sorption in Ground & Surface Waters → Environmental Fate

- High Throughput Screening alternative to Packed Columns (OECD Protocol Lacking):
    - Faster
    - Easily standardized: available 96-well plates; NIST polystyrene microspheres
  - Assesses retention/mobility of nanoparticles in porous media
  - Highest potential throughput for NPs that absorb light or fluoresce (plate reader)
  - HTS system tested with SWCNTs & fullerenes to represent a range of properties
  - River sediments and high purity quartz tested → Data useful for Model Building
- Robust Summary in SWCNT Dossier: [Bouchard, et al. 2012. Water Res.](#)

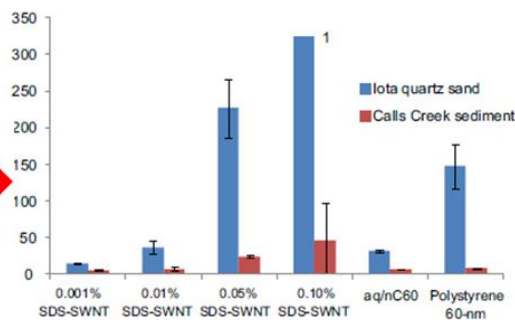
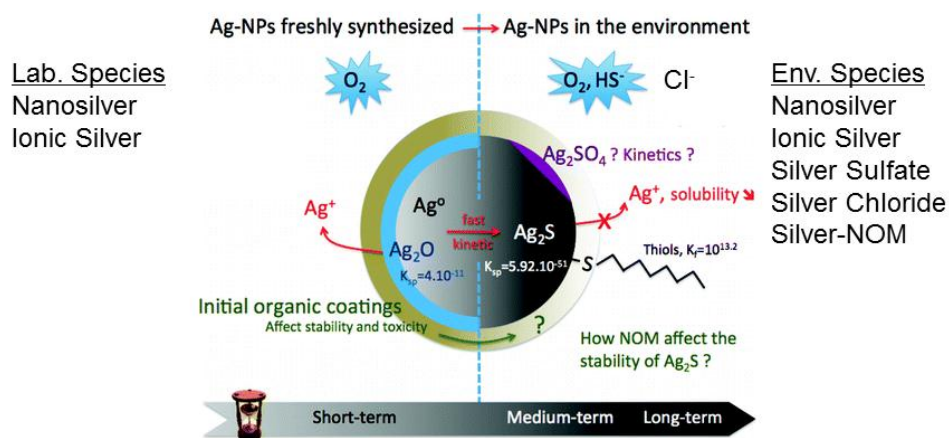


Fig. 5 – The mean and 95% confidence limits of the distance required to achieve a 3–log reduction



## GNSPD Section C2.1: Abiotic Transformation

### Dissolution Kinetics of Nanosilver



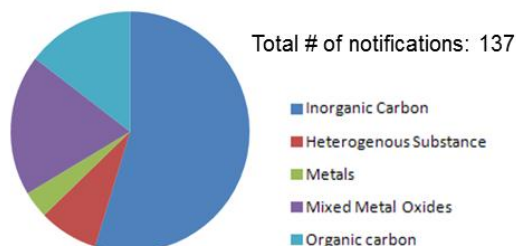
*Levard, et al. 2012. ES&T*



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## Areas of Interest

- Sample Preparation and Dosimetry for Aquatic Toxicity Tests
- Dissolution Kinetics of Nanosilver
- 
- Inherent Biodegradation (OECD 302B)
- Removal of Nanomaterials during Wastewater Treatment
- Nanomaterial Categories
- 
- Nanomaterials for Pesticidal and Industrial Uses:
  - Silver and copper (FIFRA)
  - Industrial uses (TSCA) →



15

Ahtiainen Jukka | 29.1.2013

## ***The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN***

The logo for TUKES (Finnish Environment Institute) is displayed in a white, rounded, lowercase font against a teal-to-white gradient background.

*The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN*

### **Content**

- **Nanomaterials in the environment–**
  - *what do we know about fate and effects?*
  - *What we would need to test*
- **Applicability of OECD test guidelines and EU test methods for the assessment of nanomaterials -**
  - *Ecotox test applicability*
  - *Environmental fate test applicability*
- **OECD Test Guideline Programme - how to handle the guidance**
  - *TG development and guidance documents and MAD*
  - *Guidance for similar matrices and test or for nanomaterial groups?*

The logo for TUKES (Finnish Environment Institute) is displayed in a teal, rounded, lowercase font.

MAD = Mutual Acceptance of Data

■ *Nanomaterials in the environment – fate and effects?*

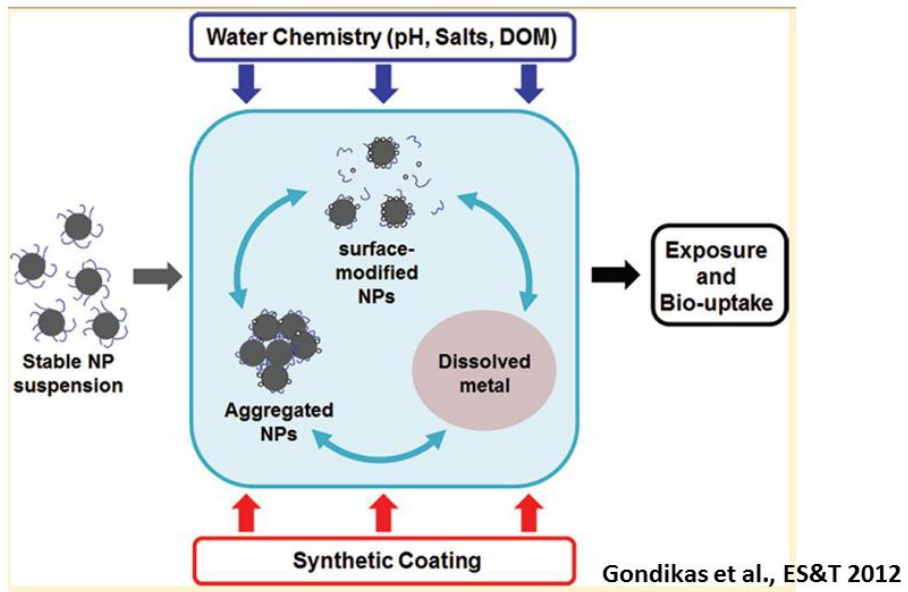


***Nanomaterials in the environment – fate and effects?***

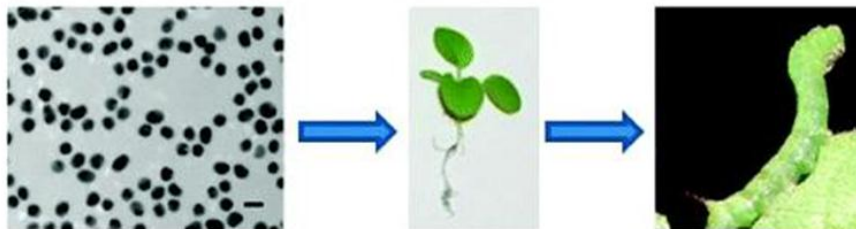
- ***Theoretically most nanomaterials would be probably bound to each others, other particles and colloids (humic acids)***
  - *Sediment as a sink- re-suspension?*
  - *Wastewater treatment: bound to sludge - soil exposure - reactions (Ag-S)*
  - *Surface layer of marine waters (NOM, surface charge)*
  - *Plankton and plankton (particulate) eaters*
  - *Physical interactions e.g. with fish gills*

NOM = natural organic material

*Nanomaterials in the environment*



*Biomagnification of nano-Au*



Judy et al., ES&T 2011

**BMF**  
5 nm: 6,2  
10 nm: 11,6  
15 nm: 9,6

## ■ *Applicability of testing protocols and methods to NM testing*



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*The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN*

### ***General applicability of test methods for NM testing***

- Biological “endpoints” or measurement variables are relevant and applicable for NM testing e.g.
  - *Number of offspring in reproduction tests*
  - *Bioaccumulation into tissues*
  - *CO<sub>2</sub> production in biodegradation test*
  - *Many apical endpoints in mammal tests*
- Some new “nanorelevant” endpoints might be needed
- Dosing and dosimetry of the test material and NM detection and characterization very important – Guidance to be developed
- With guidance and OECD test guideline- can MAD be still applied?

**MAD = Mutual Acceptance of Data**

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*The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN*

## ***Do we need new information and endpoints for NMs and in which area?***

- **Degradation and bioaccumulation**
  - Existing test could be OK (detection issue)
  - Enhanced screening tests maybe needed
- **Physical-chemical properties**
  - - yes we do, urgently!- but who will harmonize these?
- **Toxicology and Ecotoxicology**
  - Many of the existing endpoints are OK, but some additional could be considered

---

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*The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN*

## ***Methods for environmental fate - degradation***

### **Biotic degradation**

- Many methods exist and CO<sub>2</sub> production can be measured e.g. from labelled material but...
- Most current carbon-based NMs tend to be of an inorganic nature- but more biobased are coming, and there are coatings
- Assessment of transformation and metabolites instead of the rate of mineralization can be more relevant

### **Abiotic degradation and transformation**

- New test protocols and novel detection and characterization methods are needed

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*The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN*

## **Methods for environmental fate - bioaccumulation**

### **Bioaccumulation test methods:**

- From water to fish (OECD TG 305 + dietary exposure)
- Accumulation into sediment worms (OECD TG 315)
- Accumulation into earthworms (OECD TG 317)

### **Detection methods**

- Radio-labelling e.g. C<sup>14</sup> for carbon containing NMs
- Neutron activation of metal NMs
- ICP –MS, HPLC, FFF-ICP-MS
- Electron microscopy



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## **Methods for biotic effects**

### **Sample preparation of the NM**

- Information and characterization of the original material
- Dispersion techniques (sonication, stirring, direct addition into soil...)
- Characterization of stock dispersions and dosed samples

### **Test media and conditions during the test**

- Ionic strength can affect NM agglomeration
- Calcium concentration and hardness
- pH and alkalinity affects especially charged NMs
- Dissolved organic matter can act as dispersant or cause agglomeration



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*The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN*

## **Current OECD guidance on NM testing**

- **Preliminary review of OECD test guidelines for their applicability to manufactured nanomaterials (2009)**
- **Guidance manual for the testing of manufactured nanomaterials: OECD sponsorship programme – rev1(2009)**
- **Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials (GNSPD-2010)- now revision published 2012**
- **Dispersion protocols for in vitro testing (on-going work)**
- **Development of integrated testing strategy for NM testing (started)**

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*The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN*

## **Current needs for OECD guidance on NM testing**

- Some test guidelines may need small revisions e.g. in scope and applicability
- Dispersion protocols needs to be harmonized (on-going work with some knowledge to be published)
- Guidance documents for various areas (e.g. inhalation toxicity, soil and aquatic ecotoxicology...)
- Guidance for various groups of materials (+)
- Development of integrated testing strategy for NM testing

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■ **OECD Test Guideline Programme**  
**- how to handle the guidance**

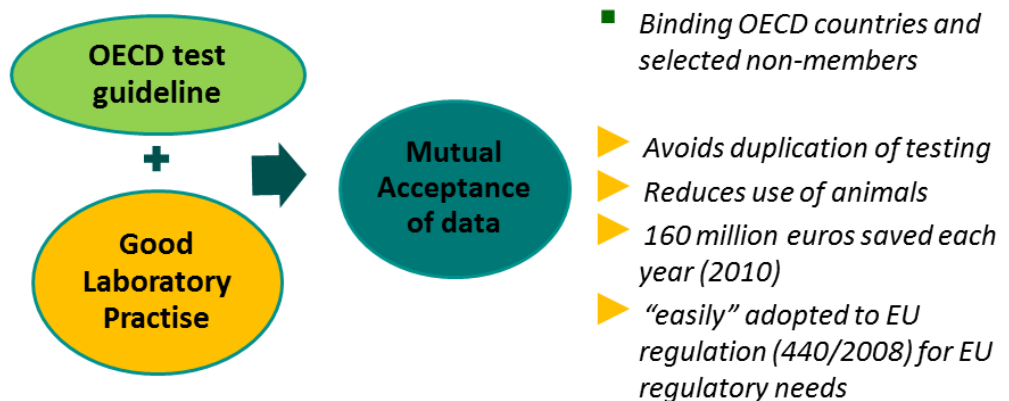
- *Tools to address regulatory data requirements*



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*The OECD Test Guideline Program (ecotox and fate)-appropriateness of TGs/ Needs for testing MN*

**Why to have OECD Test Guidelines or EU methods (also) for NM testing?**



The OECD Test Guideline Program (ecotox and fate)- appropriateness of TGs/ Needs for testing MN

## OECD Test Guidelines and Guidance Documents

### TEST GUIDELINE

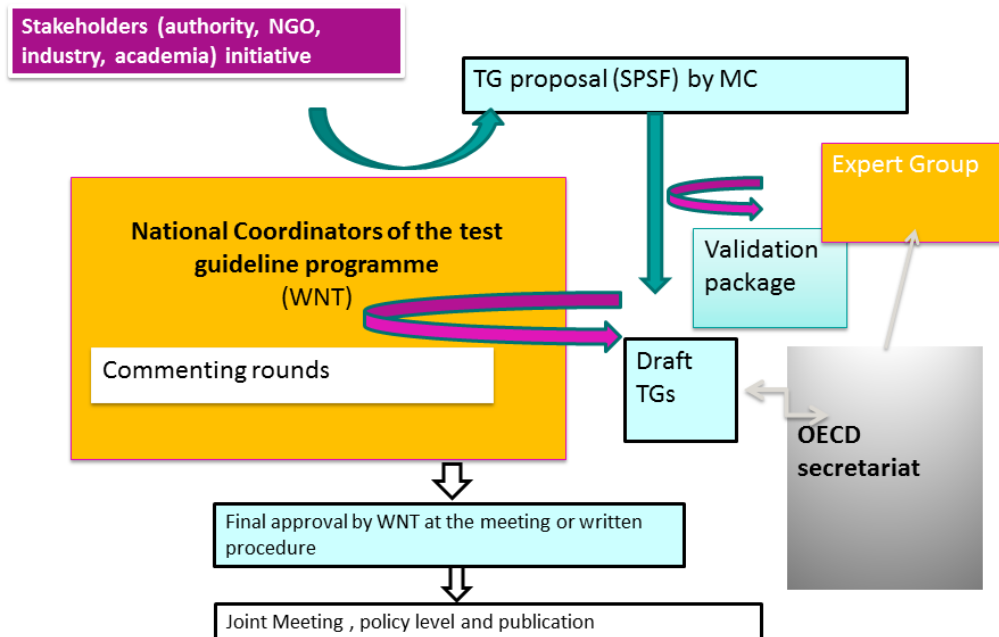
- Regulatory need
- **Covered by MAD**
- Fixed test protocol with validity criteria
- Thorough validation needed with fixed protocol
- Takes time and resources and cumbersome to update

### GUIDANCE DOCUMENT

- Regulatory need
- Not covered by MAD
- Can be a test method, or it provides technical guidance for the use of test guideline
- Validation could be limited
- Faster to develop and revise



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## OECD Test Guideline Programme - how to handle the guidance or guidance documents

- TG development and guidance documents (GDs) and MAD
- If the guidance given in the GDs can be seen **as refinement** of the test guideline, should the result be still under MAD?
- Or should the NM specific guidance be inserted as annex in the TG?
- Guidance for similar matrices and test or for nanomaterial groups?

	Metals	Metal oxides	CNTs	Fullerenes			
Soil tests							
Sediment tests							
Aquatic tests							
Bioaccumulation							
Degradation							



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*Thank you for listening!*



# Ecotoxicology of NM – Knowledge, Challenges, and Perspectives

Richard. D. Handy

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**RESEARCH  
WITH  
PLYMOUTH  
UNIVERSITY**

OECD meeting Berlin, 29-30<sup>th</sup> January 2013

## Aims

- Knowledge – summarise what we know, but more importantly, what we don't know.
- Challenges for ecotoxicity testing.
  - Detection in tissues.
  - Getting the exposure methods to work.
- Perspectives
  - Progress with modifying regulatory tests.
  - Making use of the fundamental science to underpin regulatory test assumptions.
  - Protecting most of the organisms most of the time?

## What do We Know so Far?

- Toxicity/Survival:
  - Acute lethal concentrations at mg/l levels, data sets weak on many organisms and chemistries.
  - Chronic toxicity data sets lacking.
- Growth and Reproduction:
  - Species may grow normally, but reproduction can be affected.
- Target Organs and sublethal effects:
  - Evidence of effects on all the major body systems, mechanisms not understood.
  - Uptake mechanisms partially documented, ADME poorly understood.

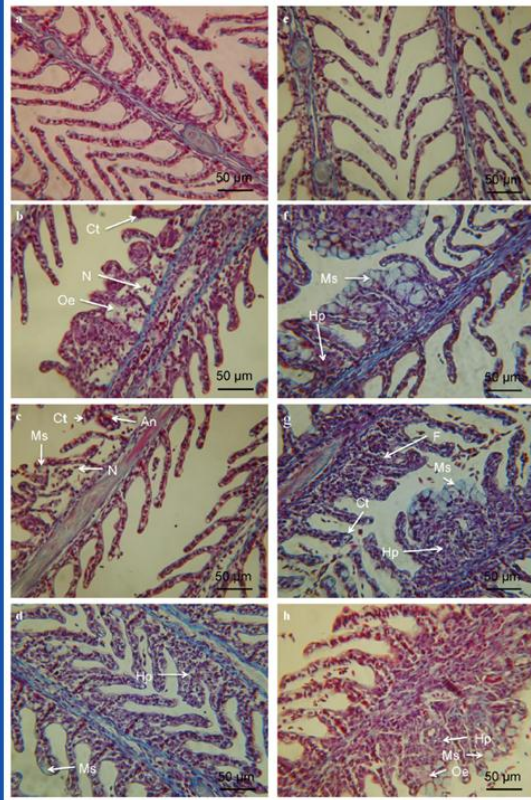
## Acute Toxicity Data For Fish

Nanoparticles	LC <sub>50</sub>	Species	Toxic effects	Authors
Nanoscale zinc oxide (nZnO) in aqueous suspensions ZnO/ bulk (Particle sizes)	1.793 mg/L 1.550 mg/L for 96 h	Zebrafish	Both ZnO and ZnO/ bulk aqueous suspensions delayed embryo and larva development, decreased their survival and hatching rates, and caused tissue damage.	Zhu <i>et al.</i> (2008)
Nanocopper suspension in water, resulting in 50-60% of added mass leaving the water column.	1.5 mg/L for 48 h	Zebrafish	Lethality associated with histological and biochemical changes in gills	Griffitt <i>et al.</i> (2007)
Nanosilver suspension, and sonication.	40 µg/L For 48 h	Zebrafish	Both silver and copper caused toxicity in all organisms.	Griffitt <i>et al.</i> (2008)
Nanocopper	60 µg/L	Zebrafish		Griffitt <i>et al.</i> (2008)
nC <sub>60</sub> aggregate in suspension	1.5 mg/L For 96 h	Zebrafish	nC <sub>60</sub> delayed embryo and larval development, decreased survival and hatching rates, and induced pericardial edema.	Zhu <i>et al.</i> (2007)
C <sub>60</sub> fullerene solubilized by tetrahydrofuran (THF), or solubilized by stirring in water	0.5 mg/L For 48 h.	Fathead minnow	100% mortality within 18 hours. 1 mg/L C <sub>60</sub> stirred in water did not lead to any mortality within 48 hours.	Zhu <i>et al.</i> (2006)

Review, Handy et al. 2011. J Fish Biology

# Gill Injury in Trout from Nano Copper

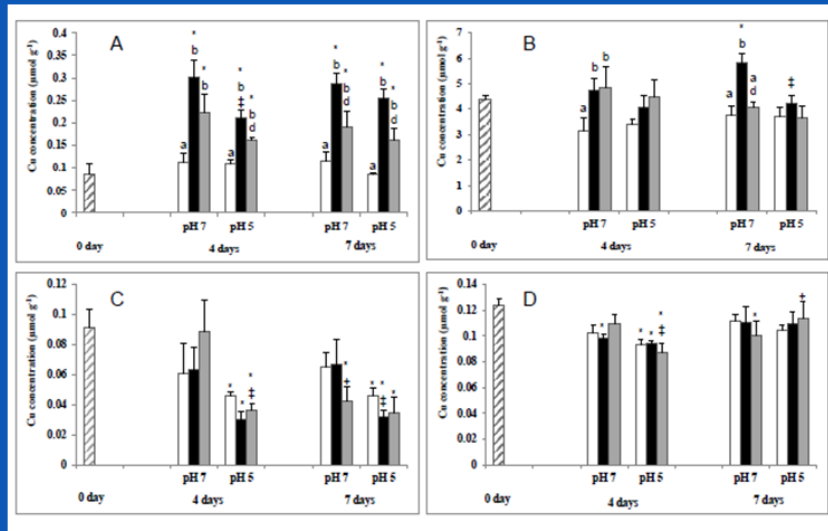
Al-Bairuty et al. *Aquatic Toxicology* 126 (2013) 104–115



Four days: (a) control, (b) 100 µg/l Cu as CuSO<sub>4</sub>, (c and d) 100 µg/l Cu as Cu-NPs. For day 10: (e) control, (f) 20 µg/l Cu as CuSO<sub>4</sub> (g) 20 µg/l Cu as Cu-NPs and (h) 100 µg/l Cu as Cu-NPs.

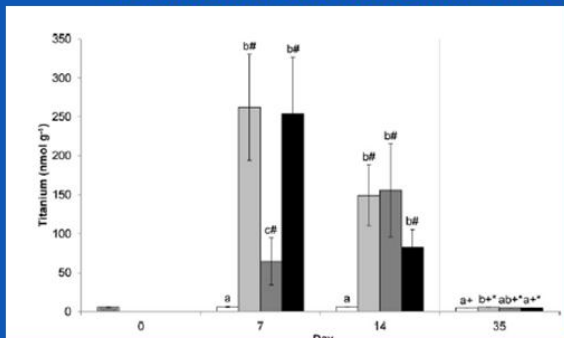
## pH Effect on Particle Toxicity? Metal accumulation in trout: CuSO<sub>4</sub> v CuNPs

Al-Bairuty and Handy Unpublished Data



Cu concentrations in (A) gills, (B) liver, (C) spleen and (D) kidney of trout after exposure to control (white bars) 20 µg/l of either CuSO<sub>4</sub> (black bars) or Cu-NPs (gray bars) for 4 and 7 days at pH7 and 5.

## Adult Zebrafish Exposed to 1 mg/l TiO<sub>2</sub> Retain Sufficient Health to Reproduce



Total titanium concentration in whole adult zebrafish digests.

Hatched bar (initial fish), clear bars (controls), light grey bars (1.0 mg/l bulk TiO<sub>2</sub>), dark grey bars (0.1 mg/l TiO<sub>2</sub> NP), black bars (1.0 mg/l TiO<sub>2</sub> NP). Data are mean ± SEM nmol Ti g<sup>-1</sup> dry weight, n = 6 fish per treatment.

**Table 1**  
Na<sup>+</sup>K<sup>+</sup>-ATPase activities at the end of the exposure period (day 14) in brain, gill and liver tissues of zebrafish.

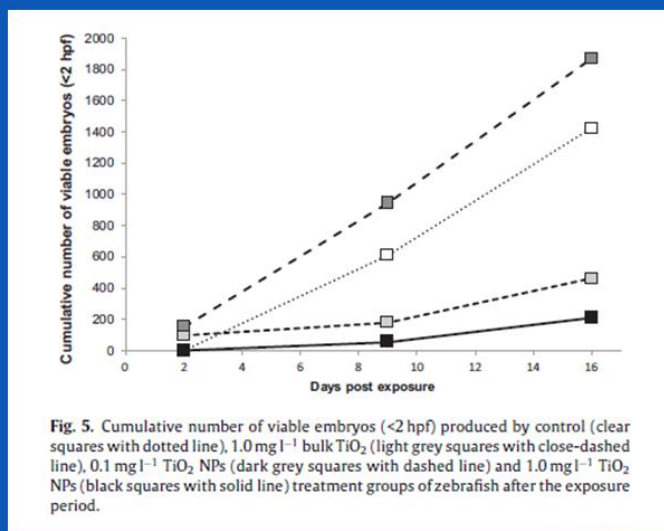
Treatment	Na <sup>+</sup> K <sup>+</sup> -ATPase activity at day 14 (μmol Pi mg protein <sup>-1</sup> h <sup>-1</sup> ) Tissue		
	Brain	Gill	Liver
Control	3.73 ± 0.15	1.91 ± 0.53	1.09 ± 0.13
1.0 mg l <sup>-1</sup> bulk TiO <sub>2</sub>	3.05 ± 0.03	1.84 ± 0.60	1.00 ± 0.00
0.1 mg l <sup>-1</sup> TiO <sub>2</sub> NP	4.23 ± 0.93	0.51 ± 0.41	0.66 ± 0.32
1.0 mg l <sup>-1</sup> TiO <sub>2</sub> NP	4.73 ± 0.93	1.36 ± 0.70	0.66 ± 0.29

Ramsden et al. 2013.  
Aquatic Toxicology  
126, 404–413



## Embryo Quality is Effected By Exposure. Latent Effect on Unexposed Embryos: TiO<sub>2</sub>

Ramsden et al. 2013. Aquatic Toxicology 126, 404–413

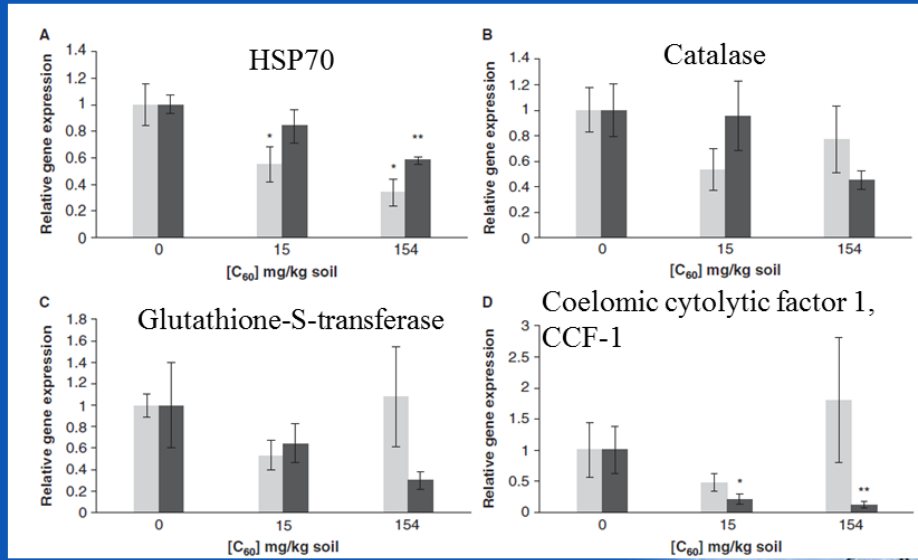


**Fig. 5.** Cumulative number of viable embryos (<2 hpf) produced by control (clear squares with dotted line), 1.0 mg l<sup>-1</sup> bulk TiO<sub>2</sub> (light grey squares with close-dashed line), 0.1 mg l<sup>-1</sup> TiO<sub>2</sub> NPs (dark grey squares with dashed line) and 1.0 mg l<sup>-1</sup> TiO<sub>2</sub> NPs (black squares with solid line) treatment groups of zebrafish after the exposure period.

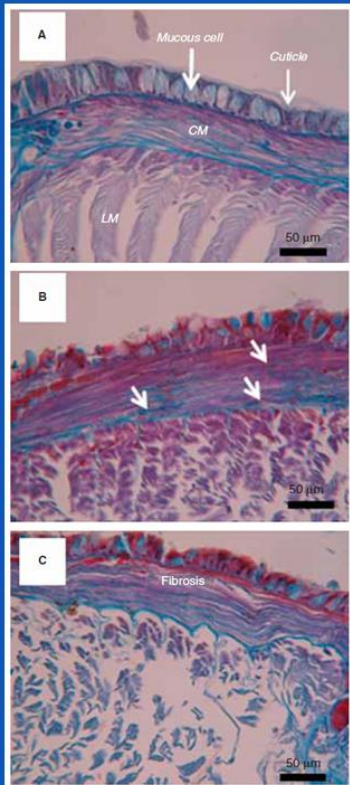


## Chronic Sublethal Effects: Gene Expression in Earthworms Exposed to C<sub>60</sub>

Van der Ploeg et al. 2012 Nanotoxicology



Four week exposure (light grey) Lifelong exposure (dark grey)



## Chronic Sublethal Effects: Integrity of the Epidermis in Earthworms Exposed to C<sub>60</sub>

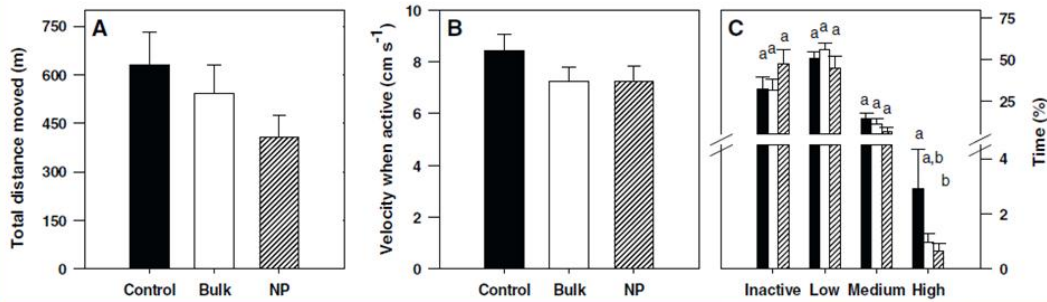
Van der Ploeg et al. 2012 Nanotoxicology

Transverse sections of segments from the anterior region of earthworms exposed to (A) control, (B) 15 and (C) 154 mg/kg of C<sub>60</sub> nanoparticles.



# Effect of TiO<sub>2</sub> on Swimming Behaviour of Trout

Boyle et al. (2013) Aquatic Toxicology 126,116–127

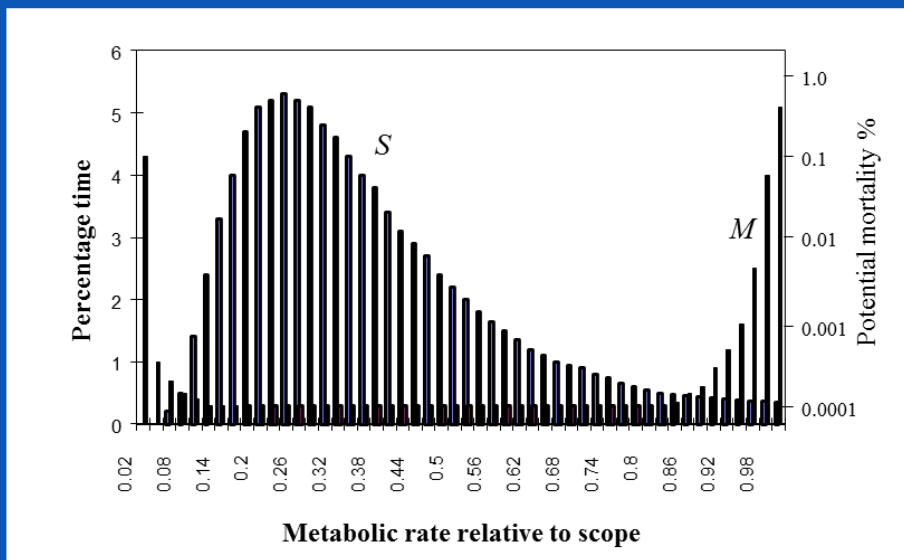


Swimming behaviour in rainbow trout exposed to control (closed bars), 1 mg/l bulk TiO<sub>2</sub> (open bars) and 1 mg/l TiO<sub>2</sub> NPs (hatched bars) for 14 days.

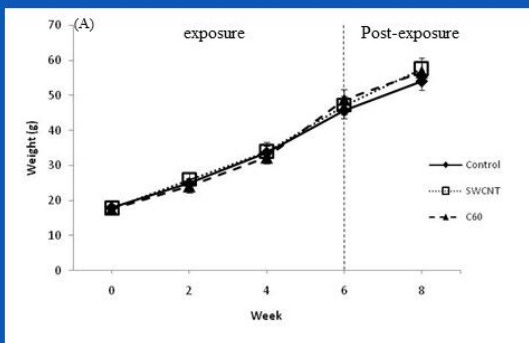


# Relationship Between Activity Level and Potential for Mortality

(after Priede 1977, Nature)

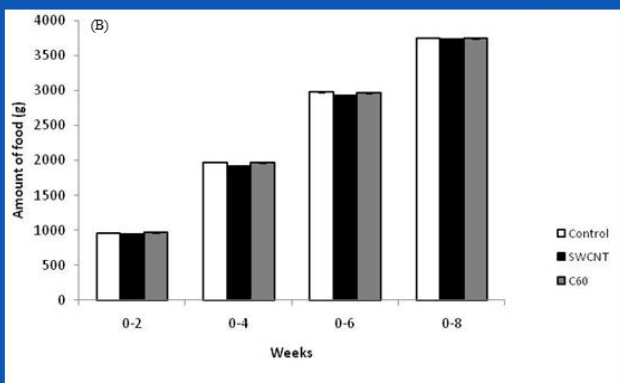


# Dietary Uptake and Food Chain Effects



## Dietary Carbon-based NPs Growth & Food Intake

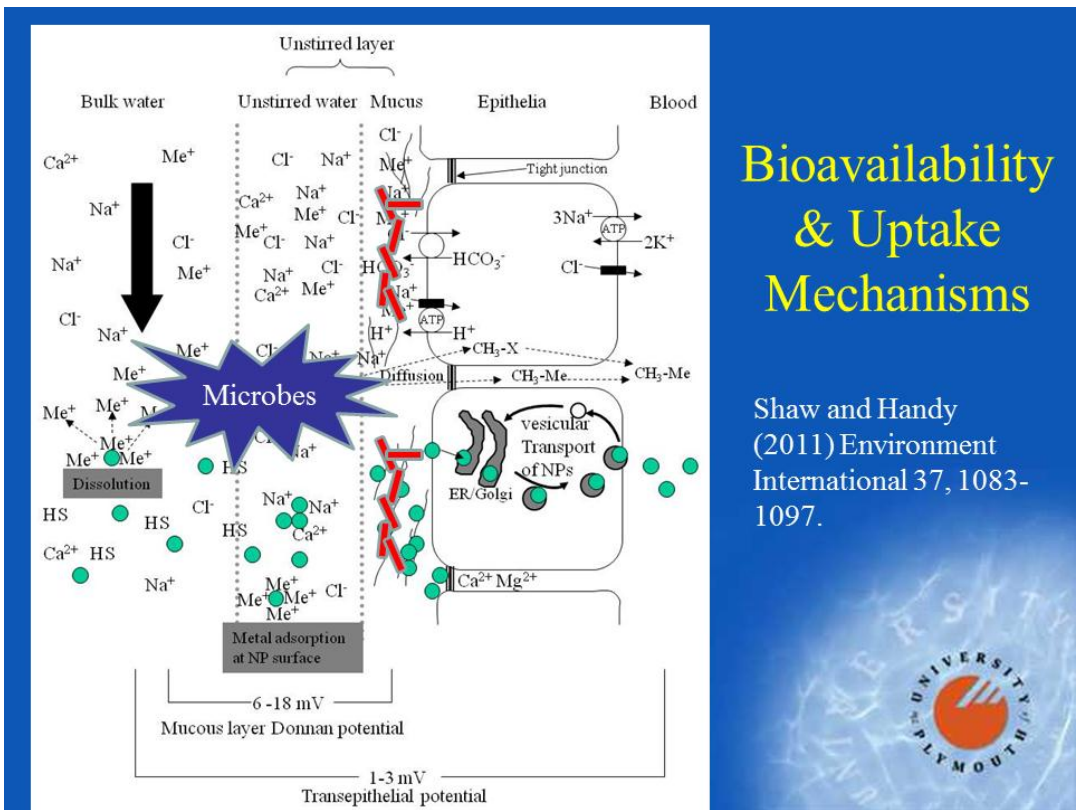
Fraser et al. (2010)  
Nanotoxicology



No statistical differences between treatments (ANOVA,  $P > 0.05$ )

No shape effect?





## Bioavailability & Uptake Mechanisms

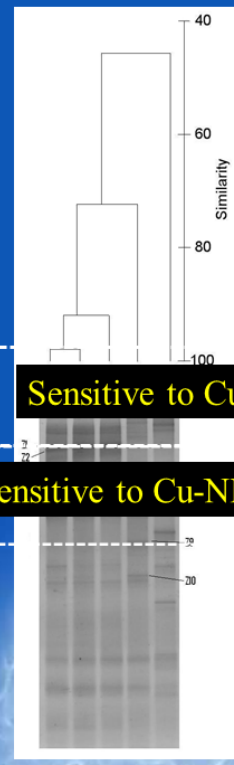
Shaw and Handy (2011) *Environment International* 37, 1083-1097.

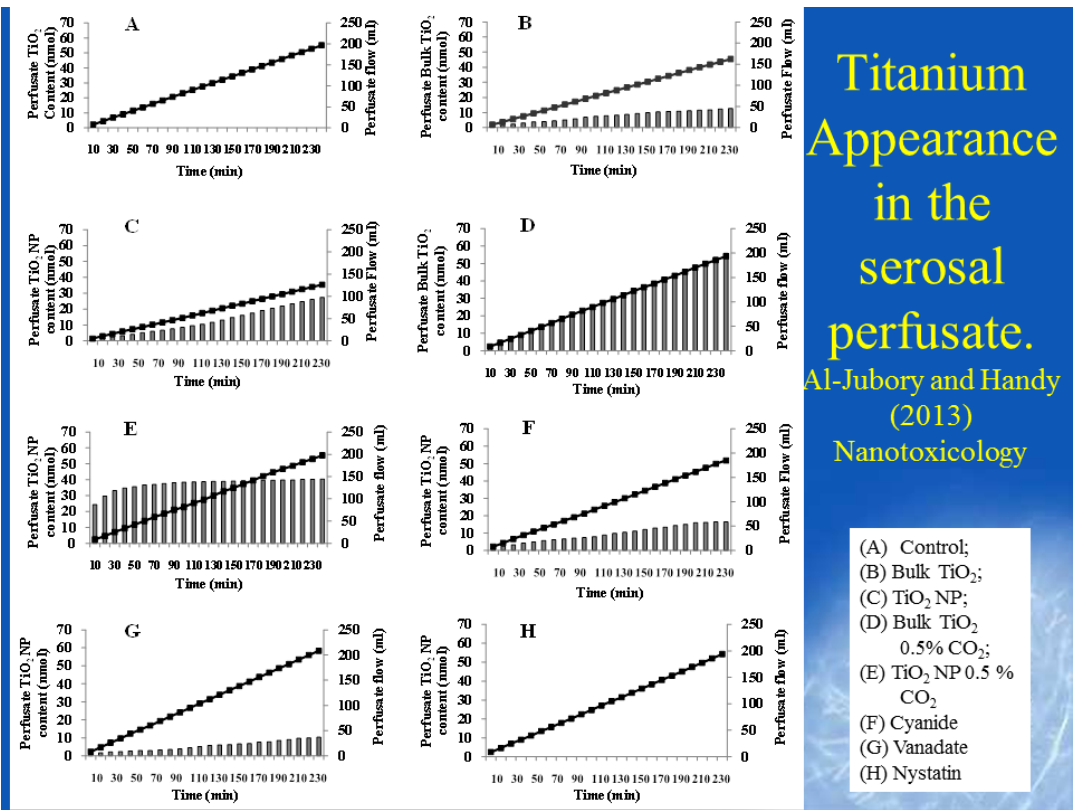


## Metal NPs Alter the Gut Microbes in Zebrafish

Merrifield et al. (2013) *Environmental Pollution* 174, 157-163  
 Representative Dendrogram and PCR-DGGE fingerprint

Code	Closest ID	Closest ID source	%
Z1	Uncultured bacterium clone	GIT of zebrafish	97
Z2	Uncultured bacterium clone	GIT of zebrafish	96
Z4	<i>Cetobacterium somerae</i>	GIT of fish	98
Z6	<i>Cetobacterium somerae</i>	GIT of fish	97
Z7	<i>Citrobacter freundii</i>	Estuarine sediments	95
Z9	Uncultured bacterium clone	GIT of zebrafish	97
Z10	Uncultured bacterium clone Sphingobacteriales	GIT of zebrafish	94
Z13	bacterium	Soil	100





## Flux Rates of Water and TiO<sub>2</sub> NPs Across Trout Intestine

Al-Jubory & Handy (2013) Nanotoxicology

Mucosal [TiO <sub>2</sub> ] (1mg l <sup>-1</sup> )	Net TiO <sub>2</sub> flux, $J_{net,TiO_2}$ (nmol g <sup>-1</sup> h <sup>-1</sup> )		Net water flux, $J_{net,H_2O}$ (ml g <sup>-1</sup> h <sup>-1</sup> )	
	Initial rate	Overall rate	Initial rate	Overall rate
Normal CO <sub>2</sub> Control	0.38 ± 0.26	0.43 ± 0.07	-6.22 ± 0.85	-7.50 ± 1.18
Normal CO <sub>2</sub> TiO <sub>2</sub> NP	2.58 ± 0.55*	3.97 ± 1.13*	-17.56 ± 4.00*	-18.01 ± 4.16*
Bulk TiO <sub>2</sub>	1.64 ± 0.78 #	1.42 ± 0.54 #	-26.28 ± 2.52*#	-28.54 ± 3.19*#
Low CO <sub>2</sub> Control	0.64 ± 0.41	0.20 ± 0.04	-5.04 ± 1.63	-4.15 ± 0.37
Low CO <sub>2</sub> TiO <sub>2</sub> NP	69.49 ± 61.57	9.48 ± 4.37	-19.14 ± 3.90*	-29.06 ± 12.34

## Standardised Ecotoxicity Tests

Maintaining the dispersion  
Measuring concentrations in organisms  
(cause and effect)  
Metal salt controls and particle dissolution

### OECD210 Fish Test: $\text{TiO}_2$ NM101 Settles Rapidly in Freshwater.

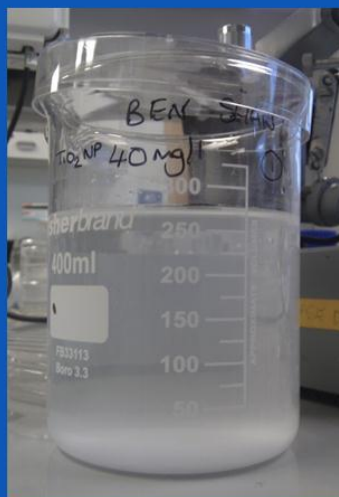
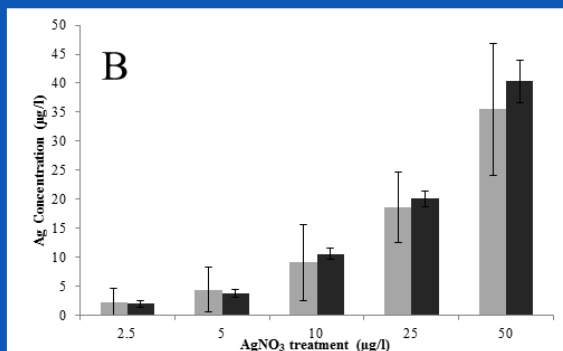
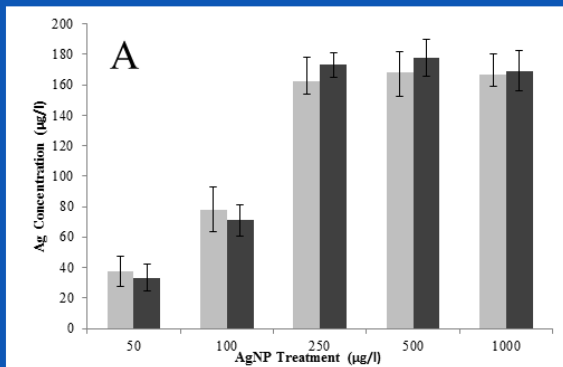


Figure. 1. Image showing the settling out of NPs in a beaker containing  $40 \text{ mg l}^{-1}$   $\text{TiO}_2$  NPs. Although not visible in this image, larvae generally swam at the top of the water column as soon as they were able.



## OECD 210 fish test: Total Silver Concentrations Immediately after Dosing & 24 h later.

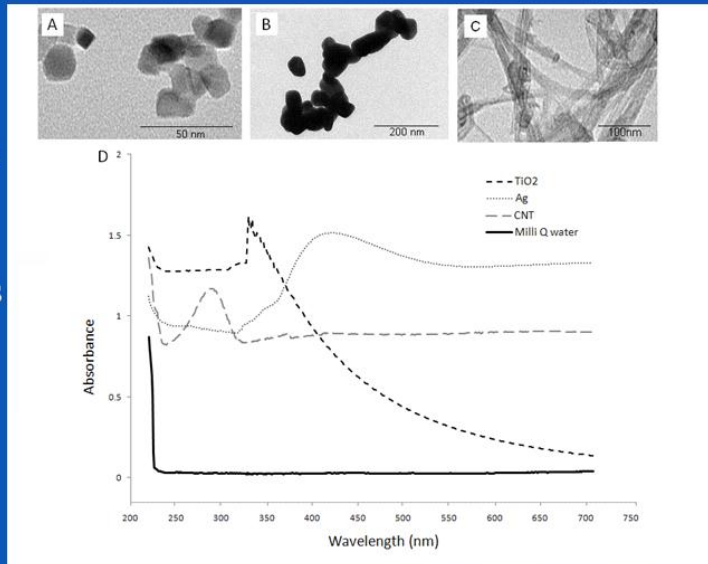
Figure 1. Total silver concentrations from exposure to (A) Ag NPs and (B) AgNO<sub>3</sub> reference, immediately after dosing (grey bars) and after 24 h just before the water change (black bars). The data are means and SD of n = 3 – 15 replicate measurements.

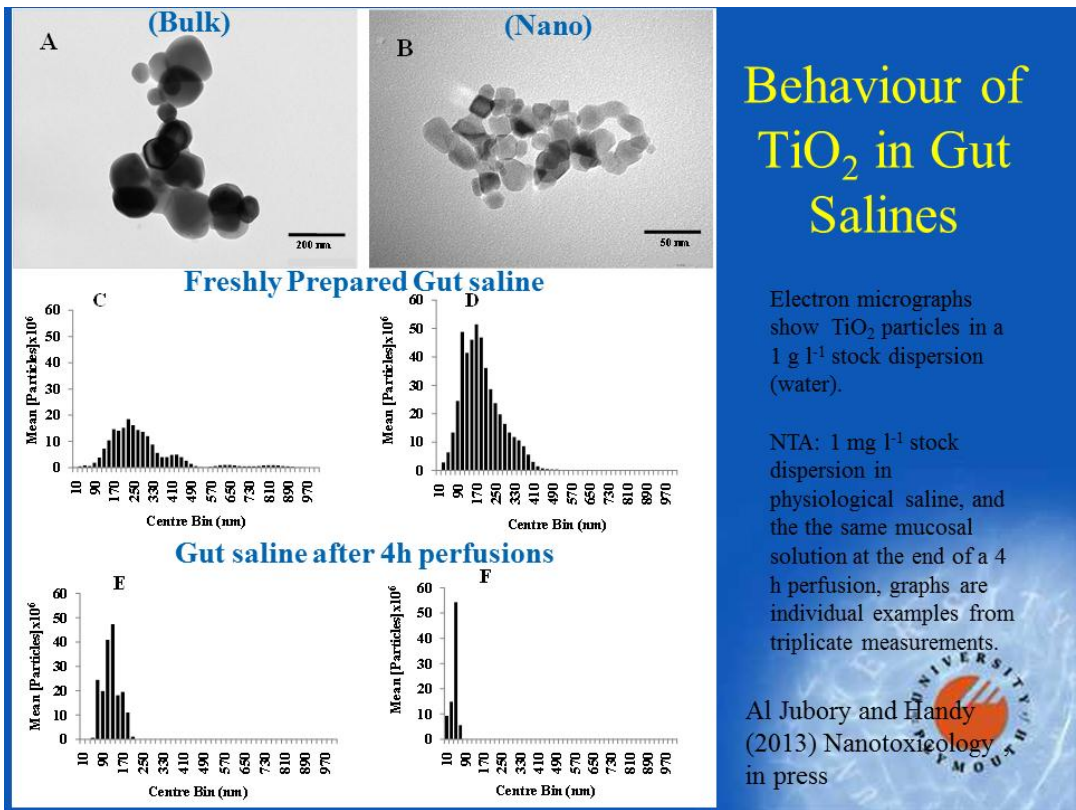


## Simple Optical Methods

Windeatt and Handy (2012) Nanotoxicology

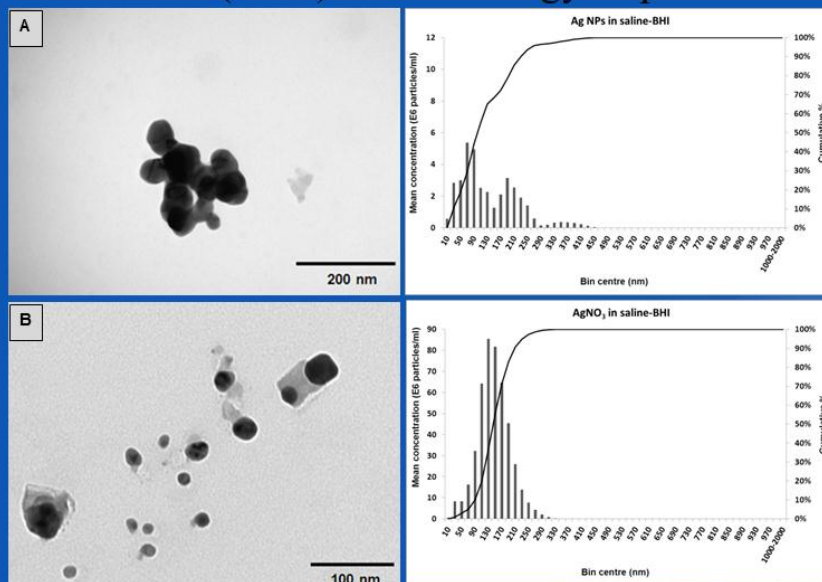
- Some materials have an absorbance peak that can be used to track concentration.
- Mostly only works down to about 1 mg l<sup>-1</sup> concs.
- Easy to do.

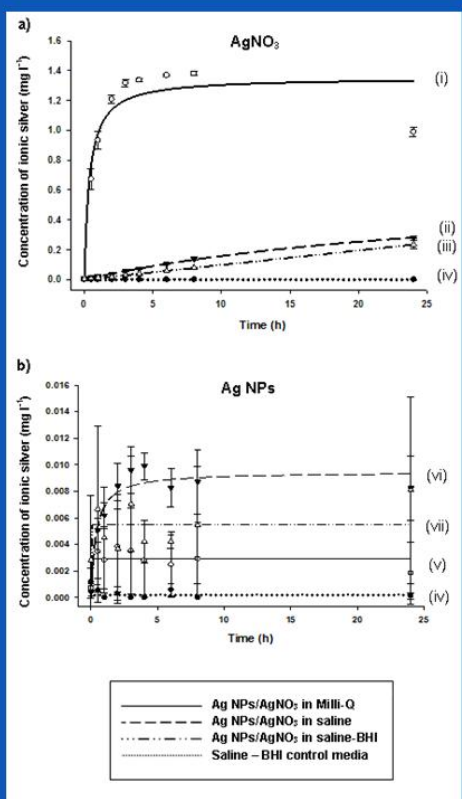




## Silver Nitrate Also Forms Nano scale Particles in Saline Conditions!

Besinis et al. (2013) Nanotoxicology in press





## Behaviour of Silver Nanoparticles Compared to Silver Nitrate

Besinis et al. (2013)  
Nanotoxicology, in press



## Rapid ICP-OES Method for Detecting Ti Metal from TiO<sub>2</sub> NPs in Fish Tissue

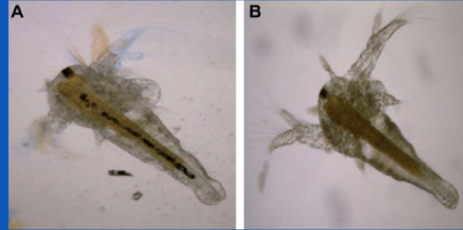
Shaw et al. (2013) Chemosphere, in press.

Table 1. Instrument and procedure precision in rainbow trout muscle and gill tissue spiked with 200 µg L<sup>-1</sup> TiO<sub>2</sub> NPs

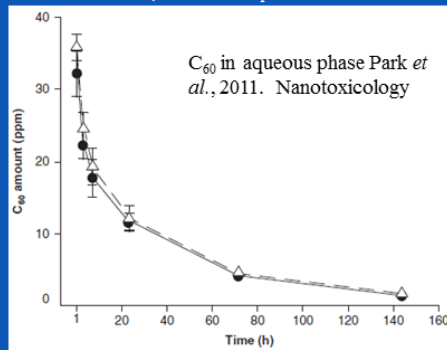
TiO <sub>2</sub> NP spike (µg L <sup>-1</sup> )	Tissue	Fish and sample Number	Ti metal (µg g <sup>-1</sup> )				SEM	Within-sample CV (%)
			Triplicate reading			Mean ± SD		
			1	2	3			
200	Muscle	Fish 1 sample 1	10.17	9.52	9.18	9.62 ± 0.51	0.29	5.23
200	Muscle	Fish 1 sample 2	10.34	10.41	9.74	10.16 ± 0.37	0.21	3.62
200	Muscle	Fish 1 sample 3	9.80	9.46	9.42	9.56 ± 0.21	0.12	2.18
200	Muscle	Fish 1 sample 4	10.64	10.45	10.89	10.66 ± 0.22	0.13	2.07
200	Muscle	Fish 1 sample 5	10.15	10.24	10.49	10.29 ± 0.18	0.10	1.71
200	Muscle	Fish 1 sample 6	10.67	10.47	10.44	10.52 ± 0.12	0.07	1.19
		Within-fish Ti metal Mean ± SD (SEM)	10.14 ± 0.50 (0.12)					
		Within-fish CV (%)	4.90*					
200	Gill	Fish 1 sample 1	14.18	15.23	14.92	14.78 ± 0.54	0.31	3.65
200	Gill	Fish 1 sample 2	14.89	15.04	15.00	14.98 ± 0.08	0.05	0.52
200	Gill	Fish 1 sample 3	13.37	14.44	12.84	13.55 ± 0.82	0.47	6.02
200	Gill	Fish 1 sample 4	12.62	12.73	12.60	12.65 ± 0.07	0.04	0.55
200	Gill	Fish 1 sample 5	14.32	13.65	14.15	14.04 ± 0.35	0.20	2.48
200	Gill	Fish 1 sample 6	13.02	13.26	13.19	13.16 ± 0.12	0.07	0.94
		Within-fish Ti metal Mean ± SD (SEM)	13.86 ± 0.93 (0.22)					
		Within-fish CV (%)	6.72*					

## Extraction of Carbon Fullerene (C<sub>60</sub>) into a Liquid Sample

- Sonication of tissue
- Repeated HNO<sub>3</sub> digestion
- Liquid sample dried
- Toluene to dissolve C<sub>60</sub>
- Toluene extracts analysed HPLC
  
- Water samples with nC<sub>60</sub>
  - Same as above, but no digestion



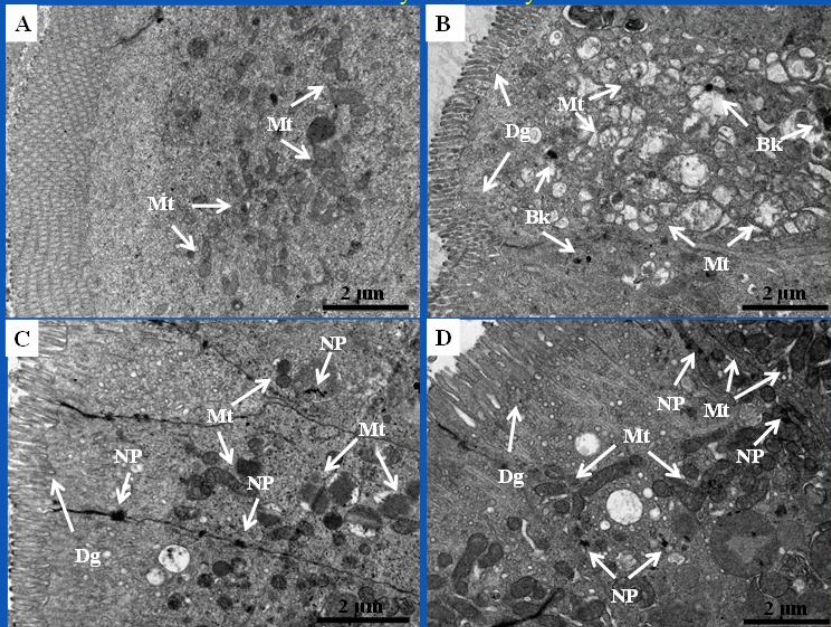
Park *et al.*, 2010. Chemosphere 81:1227-1232



Slide from Dr Ted Henry

## TiO<sub>2</sub> Particles in the Gut Epithelial Cells?

Al-Jubory and Handy

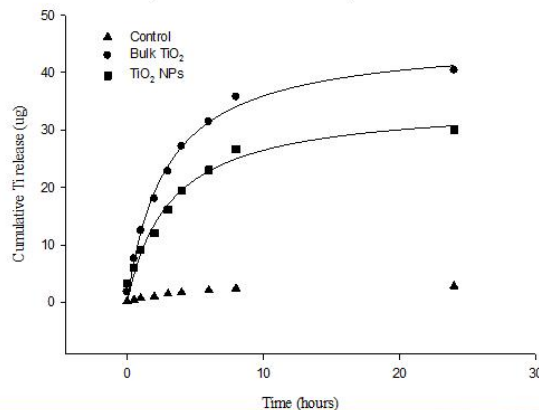


(A) control, (B) 1 mg l<sup>-1</sup> bulk TiO<sub>2</sub>, (C) 1 mg l<sup>-1</sup> TiO<sub>2</sub> NP, (D) 1 mg l<sup>-1</sup> TiO<sub>2</sub> NP gassed with 0.5 % CO<sub>2</sub>. TiO<sub>2</sub> nanoparticles (NP) and bulk (Bk), mitochondria (Mt), degeneration of the villi (Dg). Scale bar = 2 μm.

## Intracellular Saline Environment Causes the Dissolution of TiO<sub>2</sub>

Al-Jubory and Handy, 2013 Nanotoxicology, in press

Dialysis Expt: 8 ml of 100 mg l<sup>-1</sup> of TiO<sub>2</sub> NPs, bulk TiO<sub>2</sub> stock dispersions in saline. Beakers containing 492 ml of saline (total volume 500 ml).

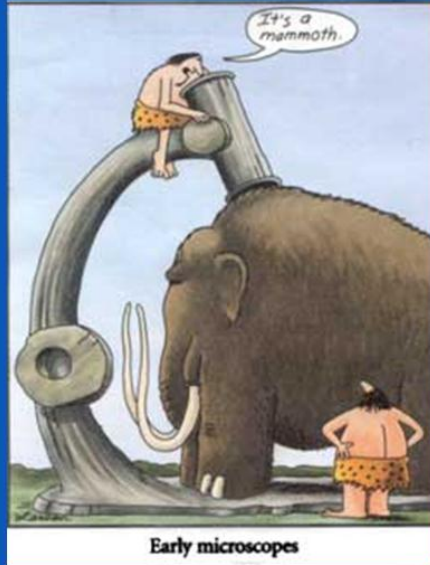


KCl-rich saline mimicking the intracellular environment at pH 7.2. For bulk:  $y = 46.04 \cdot \text{abs}(x) / (2.815 + \text{abs}(x))$ ,  $r^2 = 0.99$ . For TiO<sub>2</sub> NPs:  $y = 34.74 \cdot \text{abs}(x) / (3.126 + \text{abs}(x))$ ,  $r^2 = 0.99$ . The controls were at or below detection limits, but data points are shown for convenience.

## Perspective on Ecotoxicity Testing

- Progress is being made on exposure methodology and with the detection of NPs in the test systems as well as the organisms.
- Understanding of particle behaviour in ecotoxicity tests is quite good – we just need to control it!
- Some materials show greater toxicity than substances in the OECD sponsorship programme.
- Linking exposure and uptake with biological effect is still hampered by tissue detection issues, but there is progress.
- Keep an open mind for the “unexpected” in biological responses.

# Any Questions?



# Environmental fate of nanomaterials – knowledge, challenges, perspectives

Graeme Batley

CSIRO LAND AND WATER, Sydney, Australia  
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## Environmental fate of nanomaterials – knowledge, challenges, perspectives

### *Overview*

- Solubility
- Aggregation
- Behaviour in natural waters
- Degradation/transformation
- Sedimentation
- Bioaccumulation
- Fate in wastewater treatment plants
- Risk assessment
- Research needs



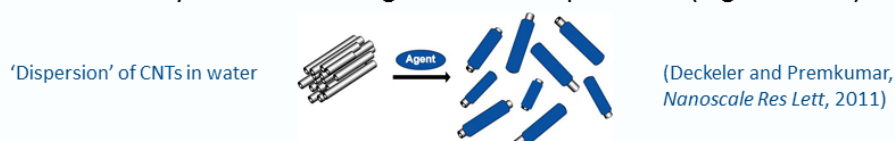
## Background to nanomaterial fate research

- Studies of nanomaterial fate in aquatic systems began around 2004
- Understanding of the behaviour of natural nanomaterials (aka colloids) was refined in mid 1990s (e.g. Buffle)
- Extension of colloid theory to nanomaterials in waters in mid 2000s
- Terrestrial systems have similar pattern: natural colloids (1996-2000), and nanomaterials (2008-)
- Studies in environmentally relevant natural systems (2010-)



## Nanomaterial solubility

- Carbon-based nanomaterials are virtually insoluble in waters
  - Fullerene solubility  $10^{-18}$  mol/L
  - Often 'solubility' means 'forming stabilised suspensions' (e.g. for CNTs)

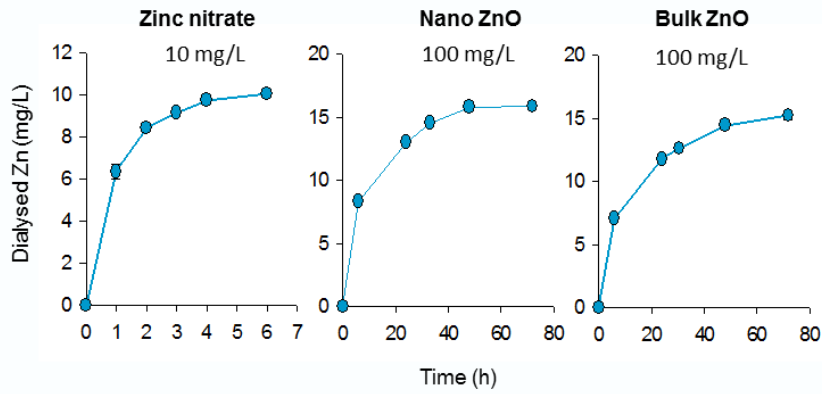


- Surface functionalisation enhances solubility
- Metal-based nanomaterials have finite, but often low, soft water solubility
  - $\text{CeO}_2$ :  $<1 \mu\text{g/L}$
  - Citrate and PVP-coated Ag:  $\approx 100\text{-}500 \mu\text{g/L}$
  - Exception is ZnO:  $7\text{-}12 \text{ mg/L}$



## Nano ZnO solubility

Synthetic soft water pH 7.5



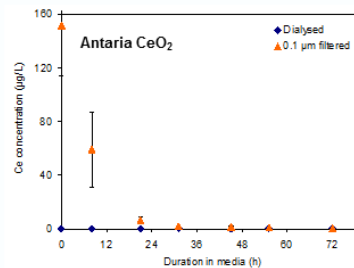
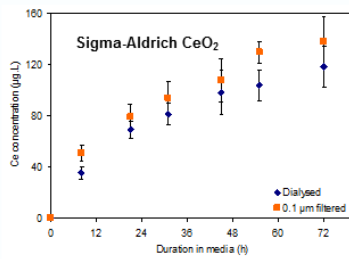
Zn is toxic to most freshwater aquatic species at < 5mg/L

(Franklin et al. *ES&T*, 2007)



## Nano CeO<sub>2</sub> solubility

Low chloride soft water pH 6.5      40 mg/L suspensions



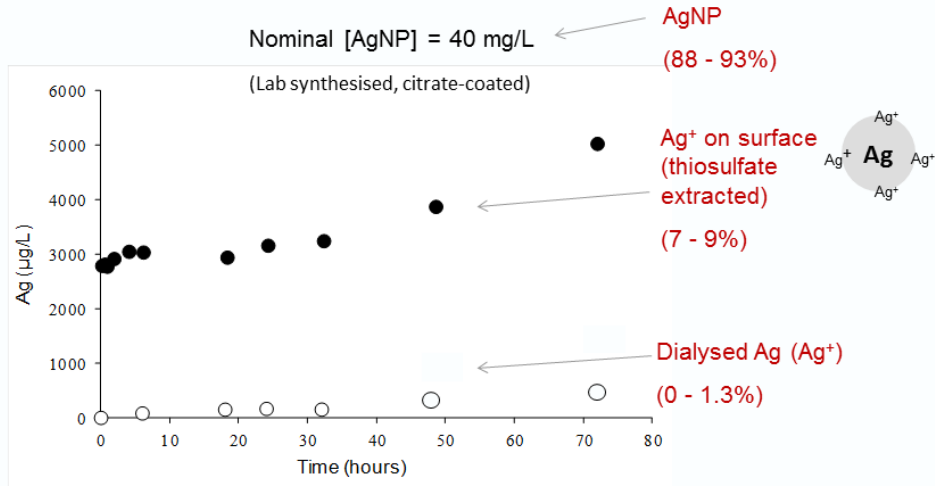
- Micron-sized CeO<sub>2</sub>  
72-h solubility ~130 µg Ce/L

- Nano CeO<sub>2</sub>  
72-h solubility <1 µg Ce/L

(Angel et al. 2011)



## Silver nanoparticle solubility

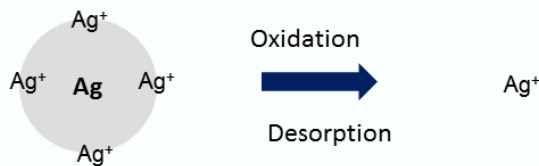


Low chloride soft water pH 7.5



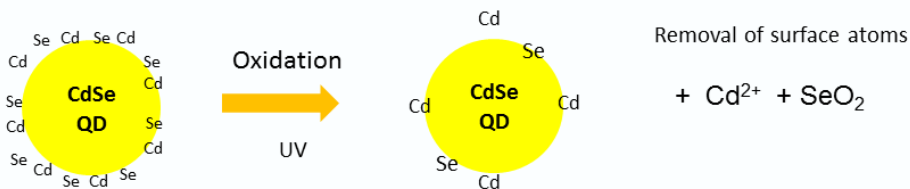
## Solubility via surface oxidation

- Nano Ag releases Ag<sup>+</sup>



- CdSe quantum dots release Cd<sup>2+</sup>

250 mg/L → 80 mg Cd/L

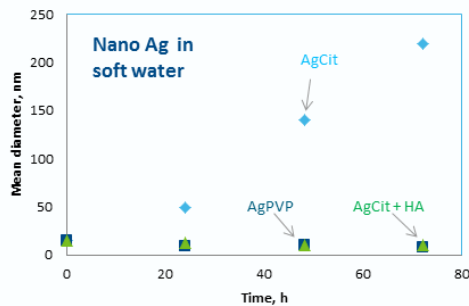


(Derfus et al., *Nano Lett.*, 2004)



## Nanomaterial aggregation

- Aggregation is dependent on:
  - nanomaterial formulation, surface coating and surface charge, and steric effects
  - ionic strength and pH of water
- Many assessments of nanomaterial toxicity are using nano-aggregates >100 nm



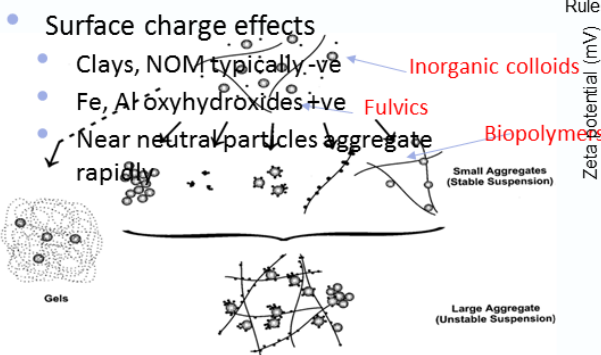
NM	Nominal size, nm	Initial size in soft water, nm
CeO <sub>2</sub> Antaria	34	164
CeO <sub>2</sub> Umicore	12	570
Ag Cit	14	17*
Ag PVP	15	16

\* Rapid aggregation to mm size in seawater

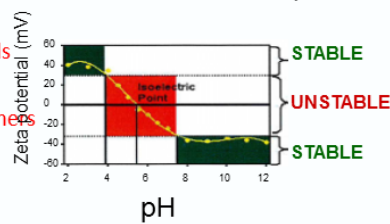


## Natural colloid aggregation

- Natural colloids comprise clays, hydrous Fe, Mn oxides, NOM, exopolymers
  - Smallest colloids (<100 nm) disappear first (by aggregation); the largest particles sediment
  - Left with particles 10-1000 nm

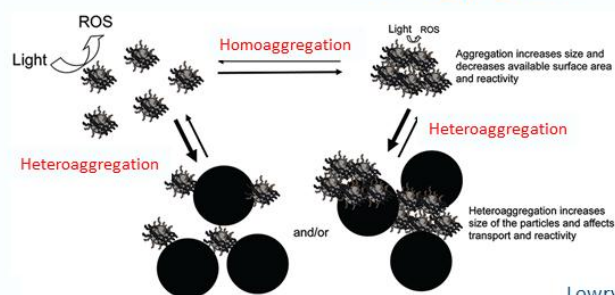


Rule of thumb for electrostatic stability is +/- 30 mV



## Homoaggregation and heteroaggregation

- Studies in synthetic solutions deal largely with **homoaggregation**
- In natural waters and soil solutions, **heteroaggregation** dominates



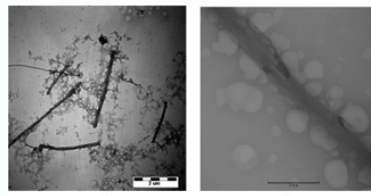
Lowry et al., *ES&T*, 2012

- Theoretical approaches (DLVO) not successful predictors of aggregation due to polydispersity, non-spherical shapes, and varying properties of NMs
- Further complicated by surface coatings



## Stabilising nanomaterial suspensions

- By modification of surface properties
  - Addition of surfactants to fullerenes, CNTs
  - Surface oxidation or functionalisation of CNTs
  - Surface coatings on metal and metal oxide nanos (citrate; PVP etc)
- Stabilisation by natural organics (humic, fulvic and tannic acids)
  - MWCNTs (enhanced dispersion, both steric and electrostatic contributions). Increased adsorption of HA with ionic strength
  - With nano  $\text{TiO}_2$ , Au, Ag,  $\text{Fe}_2\text{O}_3$ , HA interactions both hydrophobic (high pH) and surface charge related (low pH)
  - Disaggregation of  $\text{Fe}_2\text{O}_3$  at neutral pH (Baalousha et al. *ET&C*, 2008)



TEM of MWCNT and HA (Hyung and Kim, *ES&T*, 2008)



## Nanomaterials in natural water systems

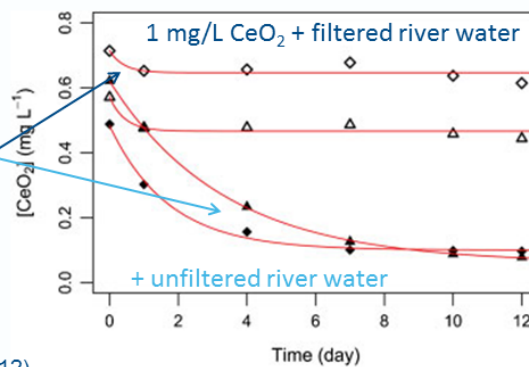
- Natural colloids in freshwaters: 1-20 mg/L (higher in soil waters)
  - Fulvic and humic acids (NOM) (<50 nm)
  - Exopolymers (fibrillar colloids) (<50 nm)
  - Hydrrous Fe and Mn oxides (nm- $\mu$ m)
- Expected environmental concentrations of nanomaterials in freshwaters: <20  $\mu$ g/L (50-1000 fold less than colloids)
  - Sizes 20-500 nm
- Adsorption of NM to NOM/Fe oxides is likely rather than adsorption of NOM to NM seen in lab studies



## Nanomaterial aggregation in natural waters

- **Heteroaggregation** with natural colloids in unfiltered river water leads to sedimentation of nanomaterials
- In filtered river water, CeO<sub>2</sub> is stabilised against sedimentation by NOM – **homoaggregation** in first day

(Quik et al., *ET&C*, 2012)



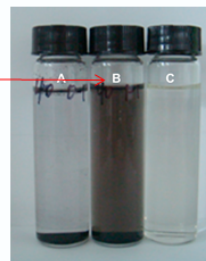
- Later studies with nano Ag, CeO<sub>2</sub> and C60 showed 8-100% removal depending on water characteristics
  - High DOC stabilised against sedimentation

(Quik et al., *SETAC Berlin*, 2012)



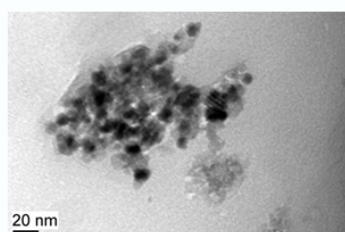
## Carbon nanotubes in natural waters

- CNTs unable to be stabilised in natural waters
  - Partially stabilised at high TOC (>16 mg/L) after vigorous agitation
  - Some anionic and neutral surfactant-facilitated CNT suspensions are stable (Lin et al. *Env Pollut*, 2010)
- 
- Heteroaggregation with natural colloids is major pathway for CNTs in aquatic systems
  - Need for the development of novel and more accessible experimental techniques for the study of heteroaggregation between NPs and other colloidal particles (Chen et al. *Env Chem*, 2010)



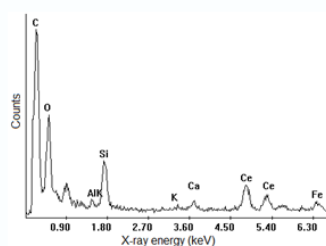
## Nanomaterials in soils

### Aggregation of CeO<sub>2</sub> and soil colloids



TEM image of a CeO<sub>2</sub>NP aggregate in 0.45 μm filtrates of soil spiked with 12.6 mmol/kg CeO<sub>2</sub>NP

Cornelis et al., *ES&T*, 2011



EDS spectrum of the aggregates shows peaks of Al, Ca, Fe, K, and Si that suggest that the aggregates also contain natural colloids



## Degradation/transformation of nanomaterials

### Abiotic degradation

- Hydrolysis – few data
  - Metal oxides not affected but maybe coatings are
  - C60, CNTs might react slowly, adding -OH or -COOH
- Photodegradation – data needed
  - Metal oxides not affected but maybe coatings are
  - C60 rapidly degrades in sunlight,  $t_{1/2} = 20$  h unknown products (Hou and Jaever *ES&T* 2009)
  - CNT possibly similar

### Oxidation/reduction

- Nano CeO<sub>2</sub> - CeIV/CeIII mixture in nanos – increased CeIV with added humics

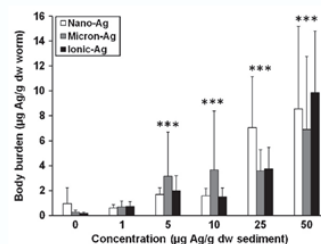
### Biodegradation

- Effect on coatings
  - Polymer coatings (PEO) on NMs can be degraded by microorganisms (Kirschling et al. *ES&T*, 2011)
- Carbon-based materials might biodegrade - testing needed



## Sedimentation of nanomaterials

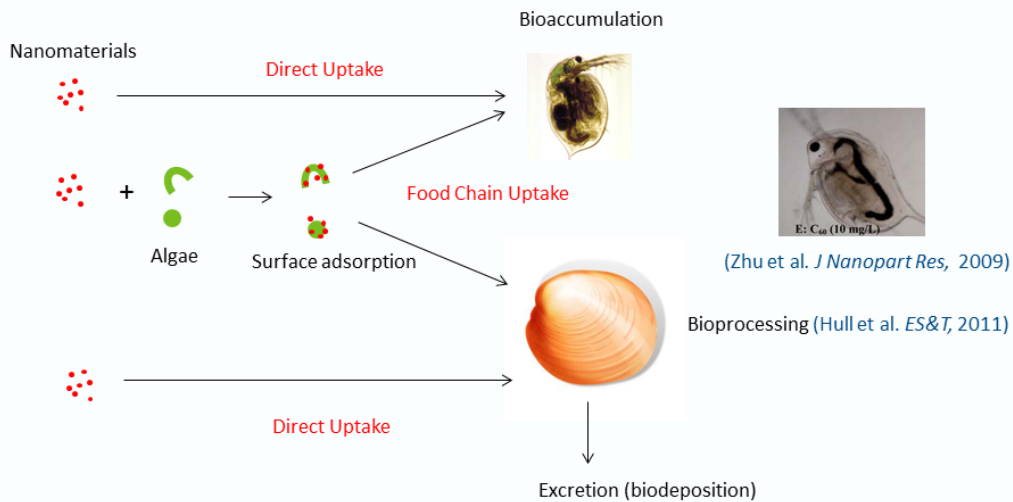
- Sediments are ultimate repository of nanomaterials in aquatic systems
  - But they are most likely not present as nanoparticles
- Binding to sediments (sorption isotherms)
  - C60 and CNTs expected to bind to NOM in sediment
- Bioaccumulation from sediments
  - In polychaetes, CNTs accumulate around gut lumen but no uptake into gut tissues - simply voided (Galloway et al. *Env Pollut* 2011)
  - Silver bioaccumulation in polychaetes
  - No other nano studies reported



(Cong et al. *Aquat Tox*, 2011)



## Bioaccumulation of nanomaterials

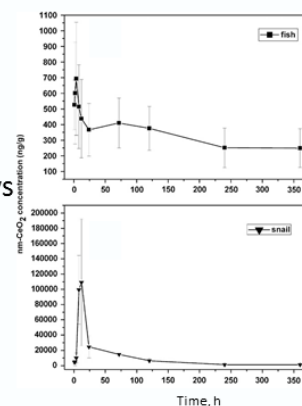


Is bioaccumulation of nanoparticles different to that of dissolved forms?



## Some bioaccumulation findings

- Gold NP uptake in clam (*Corbicula fluminea*) limited to digestive tract with excretion (changes in shape and size) (Hull et al. *ES&T*, 2011)
- CuO uptake in mussels (*Mytilus galloprovincialis*) in digestive gland but 'Trojan horse' delivery of Cu<sup>2+</sup> (Gomes et al. *ES&T*, 2011)
- CeO<sub>2</sub> uptake in fish, snails, but fast elimination
- High snail uptake from surface sediments (Zhang et al. *Chemosphere*, 2011)
- More studies needed to determine uptake pathways and mechanisms and sites of accumulation



## Nanomaterial fate in wastewater treatment

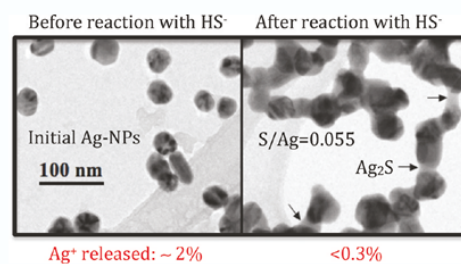
### Removal efficiency

- 90-97% efficiency for <100 nm particle removal (Mueller and Novak, *ES&T*, 2008)
- Measured  $\text{TiO}_2$  removal  $82 \pm 21\%$  for 10-50  $\mu\text{g/L}$  in effluent (Kiser et al., *ES&T*, 2009)
- Surfactants prevent aggregation
- Activated sludge isotherms?

### Transformations

- Fullerenes aggregated and removed (Hyung and Kim, *ES&T*, 2009)
- CNTs , quantum dots?
- Silver sulfidation to  $\text{Ag}_2\text{S}$  in S-rich environments – detected in sludge (Kim et al. *ES&T*, 2008)

Levard et al. *ES&T*, 2011



## Toxicity of nanomaterials to sewage bacteria

- Ag,  $\text{TiO}_2$ , ZnO, C60 and CNTs have all been shown to be toxic to activated sludge bacteria
- Possibility for compromised bacterial activity in WWTP, resulting in the discharge of partially treated chemicals (Musee et al. *J Environ Monit*, 2011)



## Nanomaterial exposure assessment

- **Models of exposure in waters** (Boxall 2007; O'Brien and Cummins 2009, 2010, 2011; Gottschalk et al. 2011)
  - Attempt to consider life cycle and environmental fate
  - Usually black-box semi-quantitative and subjective
  - Based on best available data
  - Kinetic model of agglomeration has difficulty with NOM-NM interactions (Arvidsson et al. *HERA*, 2012)
  - **Apart from accidental spillage, effluents from wastewater treatment plants were predicted to be the largest source of NMs**
  - TiO<sub>2</sub> 1-10 µg/L; Ag 10-100 ng/L; CeO<sub>2</sub> 0.1-1 µg/L
- Need to populate models with environmentally relevant data



## Fate-based ecological risk assessment

### In freshwaters

- Based on PEC/PNEC ratios

	PEC, µg/L	PNEC, µg/L	Reference
TiO <sub>2</sub>	1-10	40	Batley and McLaughlin, 2008
CNT	<0.1		
Ag	0.03-0.3	1	Mueller and Nowack, 2008
CeO <sub>2</sub>	0.1-1	3000	O'Brien and Cummins, 2009

- Too early to develop PNECs for soil exposures as most experimentation was conducted under non-relevant conditions
- Sludge to land may need assessment



## Research needs for nanomaterials in waters

W= well studied; M= moderately studied; L= limited data

Fate topic	Synthetic solutions	Natural waters	Research needs
Solubility	W	L	Product specific studies
Aggregation	W	L	Modelling behaviour in natural waters
Photodegradation	L	L	Data gap but minor issue
Biodegradation	L	L	For carbon-based NM and coatings
Bioaccumulation	L	L	Food chain studies (uptake pathways)
Sediment behaviour	L	L	Assumed low bioavailability?
WWTP fate	-	M	Need to better define discharge concentrations
Exposure data	-	L	Life cycle studies; realistic field data
Risk assessment	L	L	Needs effects data
New materials	L	L	Consumer products; semiconductors;



## Overall findings and research needs

- Need for improved fate studies in natural water, sediment and soil systems that are **realistic and environmentally relevant**
  - Avoiding possible artifacts of high concentrations
- Need for more toxicity testing to better define no effect concentrations
- Minimal environmental risk from engineered nanomaterials with current usage
  - May change if there is widespread usage



# Thank you

**CSIRO Land and Water**

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[www.csiro.au](http://www.csiro.au)



**Session 2: Environmental Toxicity and Fate of Manufactured Nanomaterials – Compartment Water**

## Stimulus Presentation Degradation/Transformation of NM

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Jed Costanza, Ph.D.

US EPA Office of Chemical Safety and Pollution Prevention  
OECD Expert Meeting on Eco-toxicity and Environmental Fate  
January 29, 2013  
costanza.jed@epa.gov

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### Office of Chemical Safety and Pollution Prevention (OSCPP)

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- Protect people and the environment from potential risks of chemicals through:
  - Toxics Substances Control Act (TSCA)
    - Evaluate safety of “new chemical substances” before they enter into US Commerce
    - Maintain TSCA inventory of chemicals in US Commerce
  - Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)
    - Pesticides to be “registered” before being sold in US
    - EPA must find that use “will not cause unreasonable adverse effects on the environment” – the safety determination



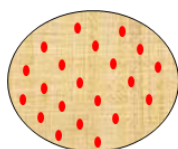
## Nanosilver Registration

- EPA conditionally registered nanosilver product “HeiQ AGS-20” on December 1, 2011
  - First and only pesticide registration known to contain nanosilver or nanomaterial
  - EPA used available data and conservative assumptions to evaluate the risk from exposure to textiles treated with nanosilver
  - Potential to reduce amount of silver in textiles
  - Granted a conditional registration for AGS-20 as a textile preservative while requiring generation of new data over a 4 year time period and occupation protection measures



2

## AGS-20 Exposures

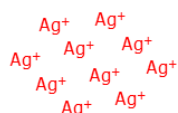


**Nanosilver-Silica Composite**

- Nanosilver sintered to SiO<sub>2</sub>
- 1,000 nm overall diameter



**Nanosilver – 1 to 10 nm (avg)  
and in the 50 nm range**



**Ionic silver**

Required New  
Risk Assessment

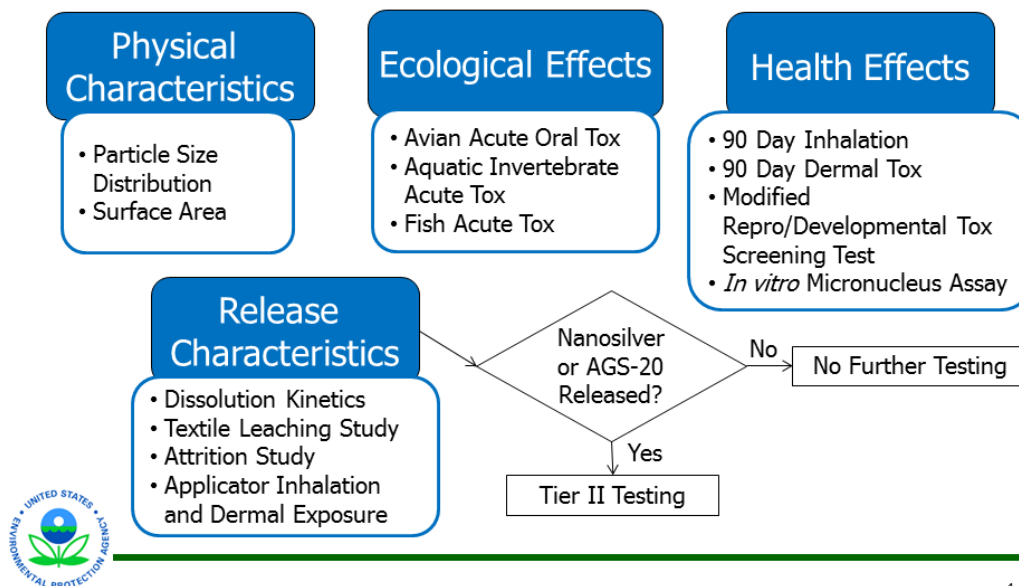
Existing Risk  
Assessment



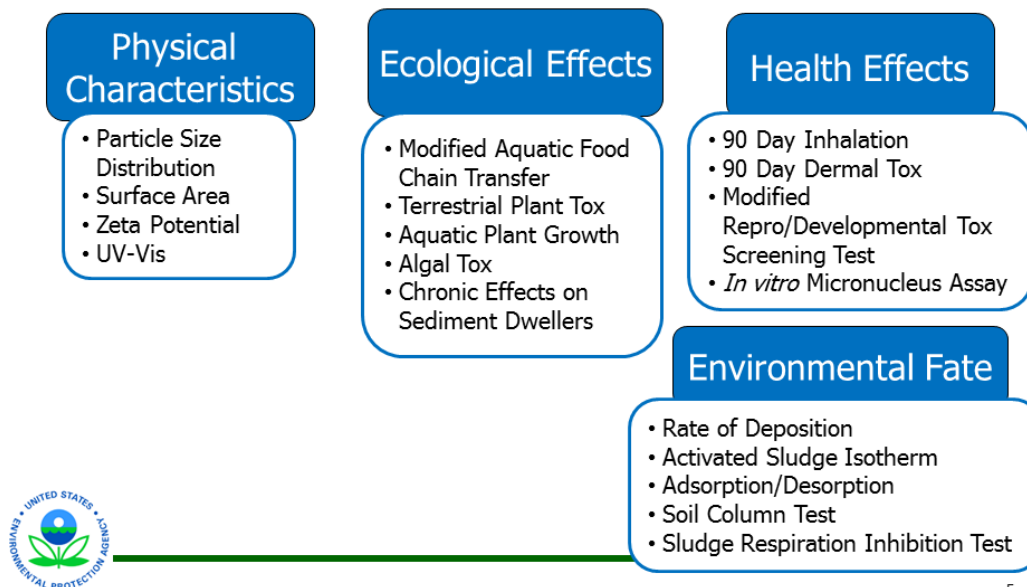
(See Egger et al. *Appl. Environ. Micro.* **2009**, 75:2973 for AGS-20 details)

3

## Required Tier I Data for AGS-20



## Tier II Data if Nanosilver Released from AGS-20



## Testing Progress

- Testing must be completed by dates given in an enforceable schedule



6

## Dissolution Kinetics

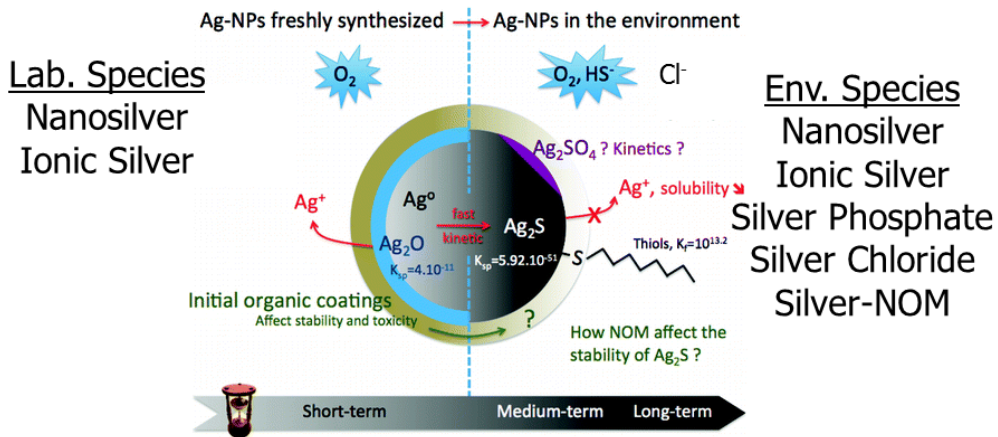


Image Source: Levard et al. *Environ. Sci. Technol.*, **2012**, 46:6900  
Focus Issue: Transformation of Nanomaterials in the Environment

7

## Dissolution in Aqueous Suspension

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- Rate at which nanosilver transforms into ionic silver in aqueous suspension
  - Increases with decreasing pH and increasing temperature (Liu and Hurt, *Environ. Sci. Technol.*, **2010**, *44*, 2169)
  - Decreases with increasing diameter (Zhang et al. *Environ. Sci. Technol.*, **2011**, *45*, 4422; Ma et al., *Environ. Sci. Technol.*, **2012**, *46*, 752)
  - Follows first-order kinetic model (Liu and Hurt, *Environ. Sci. Technol.*, **2010**, *44*, 2169; Lee et al., *Environ. Toxicol. Chem.* **2012**, *31*, 155)
- Rate at which nanosilver *composite* transforms to ionic silver in aqueous suspension
  - Immediate fast ionic silver release followed by prolonged slow release (Sotiriou and Pratsinis, *Environ. Sci. Technol.*, **2010**, *44*, 5649)



## Dissolution Kinetics Protocol

---

- Flask with 0.5 mg/L nanosilver (~ 60 bill particles)
- Rate and form of silver released in:
  - deionized water,
  - natural river water,
  - and moderately hard reconstructed water.
- Darkened temperature controlled chamber on a flatbed shaker at 150 rpm
  - Experiments conducted in dark because nanosilver not likely to be exposed to sunlight after reaching wastewater treatment facilities or after discharge to water bodies (sediment bound)



## Dissolution Kinetics Protocol (cont.)

- Sample collection frequency to determine kinetic model or time to 50% dissolution
- Sample Fractionation
  - Collect sample through 0.45 µm filter for 450 to 10 nm particulate fraction
  - Filter half of sample through 3 or 10 kDa ultrafiltration membrane for ionic silver fraction
- Analytical

Method	Required/Recommended
ICP-MS for Total Silver	Required
Ion Selective Electrode (ISE) for ionic silver fraction	Recommended
UV-vis spectroscopy	Recommended
Particle Size Distribution	Required if Detected



10

## Biodegradation of Fullerenes

- Fullerenes unlikely to pass ready biodegradability test
  - 0% degradation for SWCNT, DWCNT, MWCNT, MWCNT-COOH, C60, and C70 in OECD 301D – Closed Bottle Test (Kümmerer et al. *Chemosphere*, **2011**, 82:1387–1392)
- Fullerenes may degrade during inherent biodegradability test
  - SWCNT and MWCNT were degraded by horseradish peroxidase when hydrogen peroxide present (Allen et al. *Nano. Let.*, **2008**, 11:3899; Zhao et al. *J. Phys. Chem. A* **2011**, 115:9536)
  - C60 degraded by ligninase enzymes (Schreiner et al. *Environ. Sci. Technol.* **2009**, 43: 3162)



11

## Closing

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- Environmental Fate methods are highly dependent on Physical-Chemical characterization techniques, for example:
  - Particle Size Distribution
  - Surface Area
  - Zeta Potential
  - UV-vis
  - Ion Selective Electrode (ISE)
- EPA believes establishing robust physical-chemical characterization guidelines is key to robust fate and ecotox guidelines







Rijksinstituut voor Volksgezondheid  
en Milieu  
Ministerie van Volksgezondheid,  
Welzijn en Sport

## Fate and Behaviour

Bioaccumulation in aquatic  
organisms

fish.photoshelter.com  
Copyright Eric Engbretson

Fate and Behaviour | 30 January 2013



## Introduction

- Bioaccumulation, often in fish
- Bioconcentration factor (BCF), measure for bioaccumulation

Legislation	B	vB	species
REACH	>2000	>5000	aquatic organisms
GHS	>500	-	fish
UNEP (Stockholm Convention)	>5000	-	aquatic organisms

- Test guidelines:
  - TG 305: Bioaccumulation in fish: Aqueous and dietary exposure
  - TG 315: Bioaccumulation in sediment-dwelling benthic oligochaetes
  - TG 317: Bioaccumulation in Terrestrial Oligochaetes

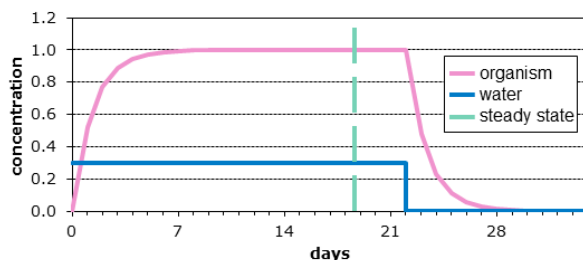


## Measuring bioaccumulation

- 2 methods:
  - 1) Rate constants ratio (kinetic)
  - 2) Concentration ratio (steady state)

$$BCF = \frac{k_1}{k_2} = \frac{[C_{organism}]}{[C_{water}]}$$

### Uptake & Depuration concentration



## Revision of OECD TG 305

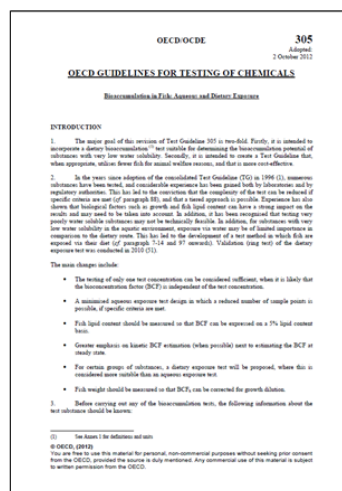
Published on October 2, 2012:  
[dx.doi.org/10.1787/9789264185296-en](http://dx.doi.org/10.1787/9789264185296-en)

### Major changes

- Inclusion of a "minimised" aqueous method
- Inclusion of a dietary exposure method
- Introduction

### Minor changes

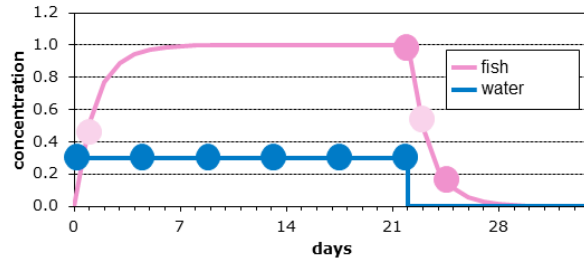
- Fish size and growth
- Solubility and use of solvents
- Fish lipids
- More focus on kinetic BCF





## “Minimised” aqueous method

### Uptake & Depuration concentration



- Minimised set of sampling points
- Kinetic calculation of BCF

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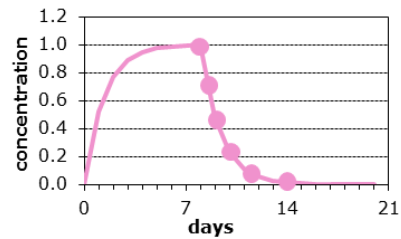
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## Dietary Exposure Method

- Kinetic approach
- Sampling in depuration phase only
- Assimilation efficiency ( $\alpha$ ) calculated from:
  - depuration rate constant ( $k_2$ )
  - fish concentration at end uptake ( $C_{0,d}$ )
  - food concentration ( $C_{\text{food}}$ )
  - feeding rate ( $I$ )
  - uptake duration ( $t$ )
- $I \times \alpha \approx k_1$
- BMF corrected for food/fish lipid and growth

### Uptake & Depuration concentration



$$\alpha = \frac{C_{0,d} \cdot k_2}{I \cdot C_{\text{food}}} \cdot \frac{1}{1 - e^{-k_2 t}}$$

$$\text{BMF} = \frac{I \times \alpha}{k_2}$$

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## Potential issues with nanomaterials in TG305 (1)

### Aqueous exposure

- Most nanomaterials are not/poorly soluble in water
- Can stable suspensions be used in these tests?
- Sorption (to fish, suspended matter, walls of test vessels, etc.)
- Dissolution rate, aggregation, sedimentation

### Dietary exposure

- Spiking of food (best method?)
- Stability of nanomaterials in/on food?



## Potential issues with nanomaterials in TG305 (2)

### General issues

- Duration of the test

### Sampling/dosimetry issues

- Analysis
  - Measurement of substance/particle in water/food
  - Measurement of substance/particle in test organism
  - Is substance/particle bioavailable
- Aggregation/disaggregation

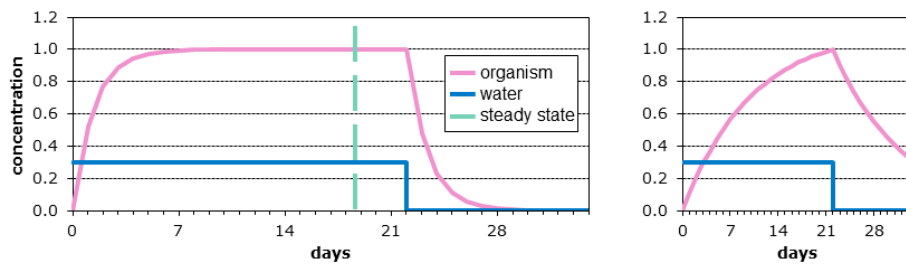


## Potential issues with nanomaterials in TG305 (3)

### Principle

- Passive diffusion of (organic) substances into (lipid) tissue
- (Ultimately) Thermodynamic equilibrium assumed

### Uptake & Depuration concentration



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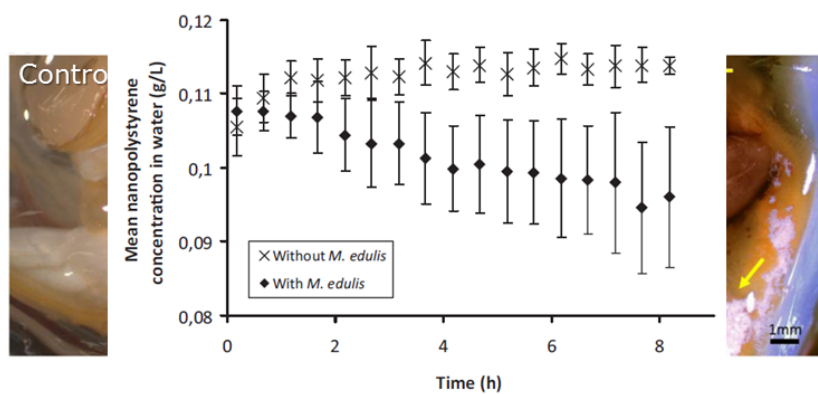
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## Other species more suitable for nanomaterials?

### Mussels

- Nanoplastic in blue mussel



Wegner et al. 2012, Environ. Toxicol. Chem.; DOI: 10.1002/etc.1984

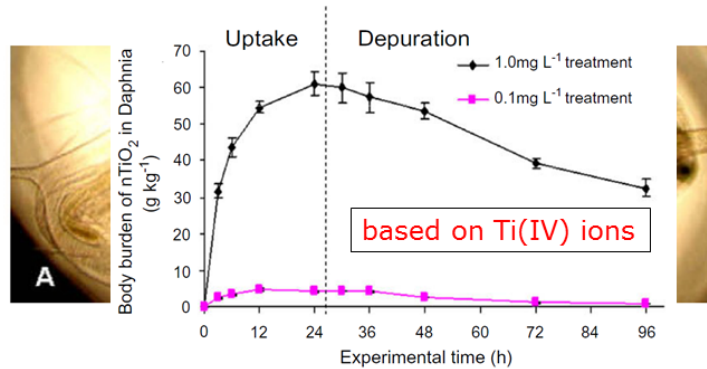
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## Other species more suitable for nanomaterials?

### Daphnids



Zhu et al. 2010, Chemosphere; DOI: 10.1016/j.chemosphere.2009.11.013



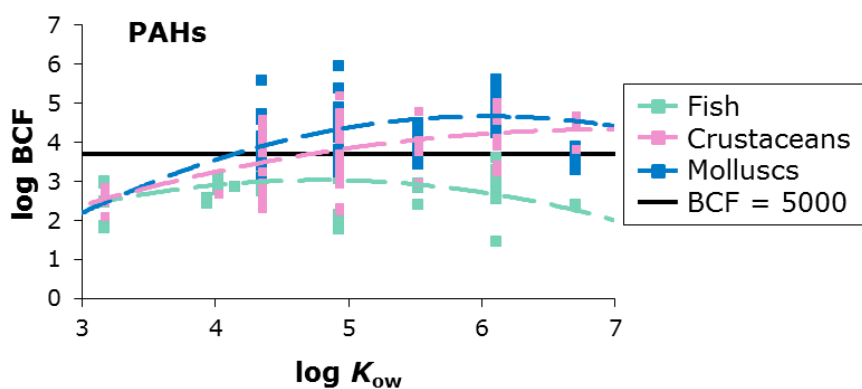
## Other species more suitable for nanomaterials?

- Mussels
- Daphnids
- Sediment species (benthic oligochaetes – TG315)  
*next session*



## Other issues with different test species

- Is it allowed by legislation?



Bleeker EAJ & Verbruggen EMJ, 2009. RIVM Report 601779002/2009: <http://www.rivm.nl/en/Search/Library>

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## Potential issues with nanomaterials in TG305

### Principle

- Passive diffusion of (organic) substances into (lipid) tissue
- (Ultimately) Thermodynamic equilibrium assumed

### Test setup

- Test duration
- Adding/maintaining compound in system (including water/food)
- Analysis of compound in water/food/organism (nano vs. non-nano)

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**Session 3: Environmental Toxicity and Fate of Manufactured Nanomaterials – Compartment Soil & Sediment**

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## **Break out group on ecotoxicity (compartment soil / sediment) – testing and characterisation**

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Kerstin Hund-Rinke

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### **Ecotoxicology**

#### **Soil – OECD TG ...**

- ... 207: Earthworm (mortality)
- ... 208 / 227: Plants (growth)
- ... 216: Microorganisms (N-transformation)
- ... 217: Microorganisms (C-transformation)
- ... 220: Enchytraeids (reproduction)
- ... **222: Earthworm (reproduction)**
- ... 232: Collembola (reproduction)

#### **Sediment – OECD TG ...**

- ... 218: Chironomids (reproduction)
- ... **225: Lumbriculus (reproduction)**

→ Challenge for ecotox-testing:  
Application of ENMs  
(soil / sediment composition)

→ Medium: Terrestrial testing - spiking of soil or feed  
Sediment testing - spiking of sediment or water

→ Procedure of spiking

→ Characterization of exposure (bulk, pore water, ion concentration) → discussion tomorrow

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## Aim of application of test guidelines

- Regulatory testing (→ decision)
  - Characterization of ENM → environmental impact, labelling
  - Pragmatic test design (suitable for routine application)
- Academic testing (→ understanding)
  - Environmental impact
  - Effect of different test conditions

→ Consequences, e.g.

- Procedure of application
- Test concentrations
- Which pragmatism is acceptable?

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## Ecotoxicology

### Soil – OECD TG ...

- ... 207: Earthworm (mortality)
- ... 208 / 227: Plants (growth)
- ... 216: Microorganisms (N-transformation)
- ... 217: Microorganisms (C-transformation)
- ... 220: Enchytraeids (reproduction)
- ... **222: Earthworm (reproduction)**
- ... 232: Collembola (reproduction)

### Sediment – OECD TG ...

- ... 218: Chironomids (reproduction)
- ... **225: Lumbriculus (reproduction)**

→ Challenge for ecotox-testing:  
Application of ENMs

- **Medium: Terrestrial testing - spiking of soil or feed**
- **Sediment testing - spiking of sediment or water**
- **Procedure of spiking**

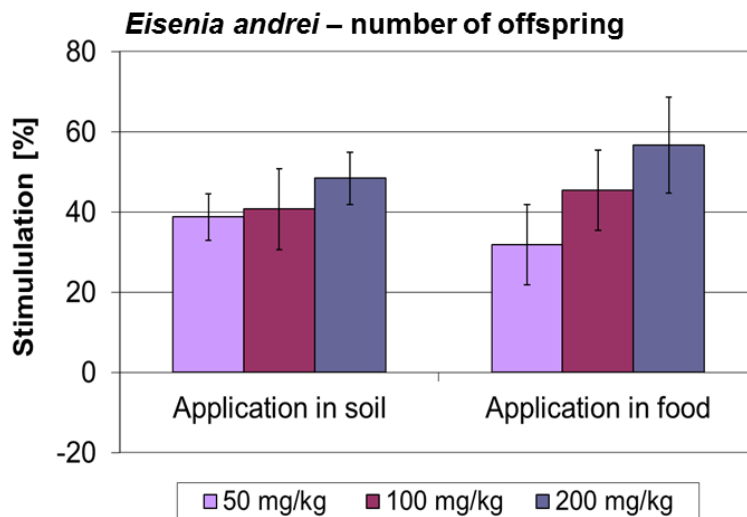
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## Spiking of soil vs. spiking of feed

	Soil / Sediment	Feed
Pros	<ul style="list-style-type: none"> <li>▪ According to guidelines / procedure established for conventional chemicals</li> <li>▪ Homogenous exposure; no / limited avoidance</li> <li>▪ Exposure can be quantified</li> <li>▪ Test design is comparable for all organisms</li> <li>▪ ...</li> </ul>	<ul style="list-style-type: none"> <li>▪ Simulation of exposure via feed</li> <li>▪ ...</li> </ul>

### Dry application “soil vs feed”: TiO<sub>2</sub> (P25)



- Spiked soil and spiked feed (cow dung) resulted in comparable effects  
→ Spiking should of soil be preferred. (?)

## Procedure of spiking: dry vs. wet

	Dry spiking	Wet spiking		
		Without dispers.	Water With dispersant	Soil extract
Pros	<ul style="list-style-type: none"> <li>High concentrations</li> <li>No chemical substance as carrier → no toxic interference</li> <li>Homogeneity using soil or silica sand as carrier</li> </ul>	<ul style="list-style-type: none"> <li>No interference</li> </ul>	<ul style="list-style-type: none"> <li>High test concentrations</li> <li>Stable dispersion</li> </ul>	<ul style="list-style-type: none"> <li>No chemical carrier (natural carrier: HA)</li> </ul>
Cons	<ul style="list-style-type: none"> <li>Low concentrations difficult to achieve</li> </ul>	<ul style="list-style-type: none"> <li>High test concentrations difficult to achieve (limitation: <math>WHC_{max}</math>, agglomeration)</li> </ul>	<ul style="list-style-type: none"> <li>Toxic interference with dispersant</li> <li>Functionalization of the surface</li> </ul>	

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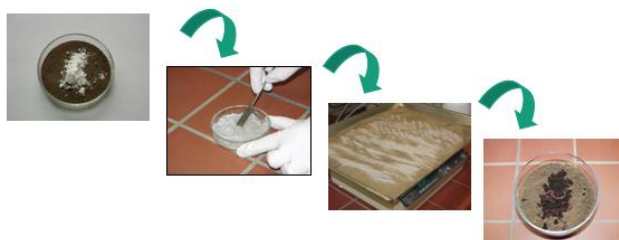


## Dry spiking: spiking procedure

Following the procedure for water insoluble chemicals:

→ mixture in a small amount of a solid carrier

→ addition to soil



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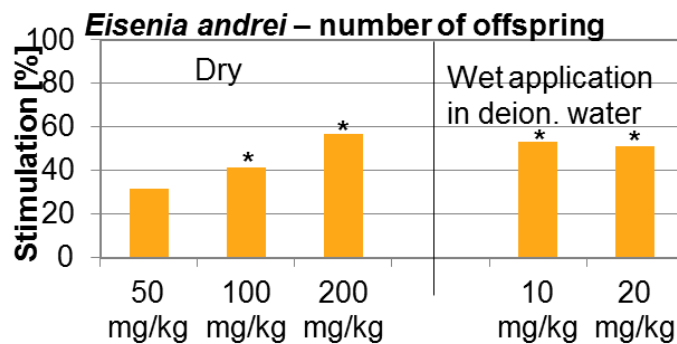
### Procedure of spiking: dry vs. wet

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### Application “dry vs. wet”: TiO<sub>2</sub> (P25)



- Dry application: concentration-effect relationship
- Wet application: increased effect → increased bioavailability but: no concentration-effect relationship
- Comparable results for plants, microorganisms (N- and C-transformation)
  - Dry spiking should be preferred. (?)
  - Exception: simulation of realistic pathways (?)

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## Application “dry vs. wet (deion. water) vs. wet (soil extract)”: Ag

Microbial N-transformation	Dry	Wet		
		Deion. water	Eluate of a sandy soil	Eluate of a loamy soil
EC50 – Day 3 [mg/kg]	<i>n.d.</i>	20.1 (16.3 – 25.8)	<i>n.d.</i>	17.3 (11.2 – 25.1)
EC50 – Day 8 [mg/kg]	10.8 (7.7 – 13.1)	14.1 (10.5 – 18.4)	11.5 ( <i>n.d.</i> – <i>n.d.</i> )	9.1 (3.5 – 12.1)
EC50 – Day 22 [mg/kg]	7.2 (7.1 – 7.3)	6.1 (6.0 – 6.2)	9.2 (8.5 – 9.4)	<i>n.d.</i>

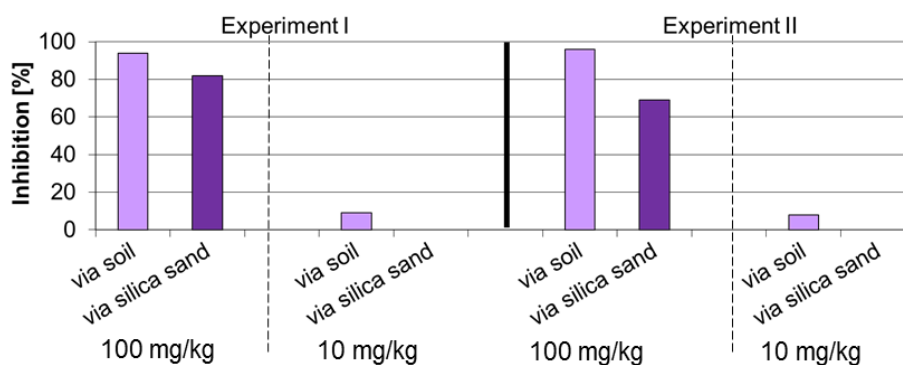
*n.d.*: not determined due to mathematical reasons or inappropriate data

- No obvious difference between dry and wet application
- No obvious difference between suspension in deionized water / soil eluate (sandy, loamy)
- Only due for ion releasing ENMs?  
→ Ion releasing ENMs and stable ENMs should be treated separately. (?)

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## Dry application with a solid carrier “soil vs. silica sand“: Ag soil microflora – nitrate formation rate



- Ecotoxicological results reproducible
- Homogeneity proved by chemical analysis
- Bioavailability of NM: soil > silica sand  
→ Soil as carrier should be preferred. (?)

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## Can we provide answers?

### Ecotoxicology

- Different procedures for different ranges of applications (regulatory t. / academic t.)
- Matrix of spiking: soil / sediment vs. feed
- Procedure of spiking: dry vs. wet
- Wet spiking
  - without vs. with dispersant
  - aqueous solution vs. soil extract
- Dry spiking: procedure of spiking
- One procedure for all ENMs?
  - Ion releasing ENM / stable ENMs
  - Coated ENMs / uncoated ENMs
  - Hydrophobic ENMs / hydrophilic ENMs

# Break Out Group Fate & Behaviour

Fate and behaviour  
OECD 106, 312, 315 and 317

Thomas Kuhlbusch IUTA (Ger)

Stimulus presentations:  
**Jason Kirby CSIRO (Australia)**  
**Anne J. Schneider RWTH (Ger)**

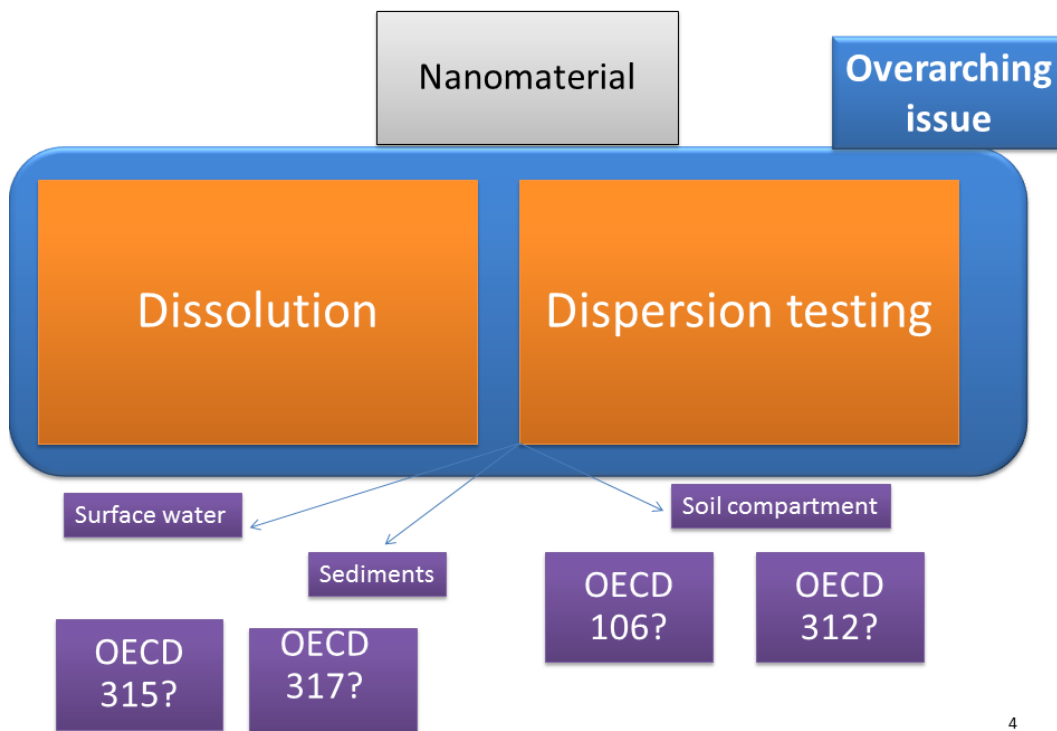
## Key topics

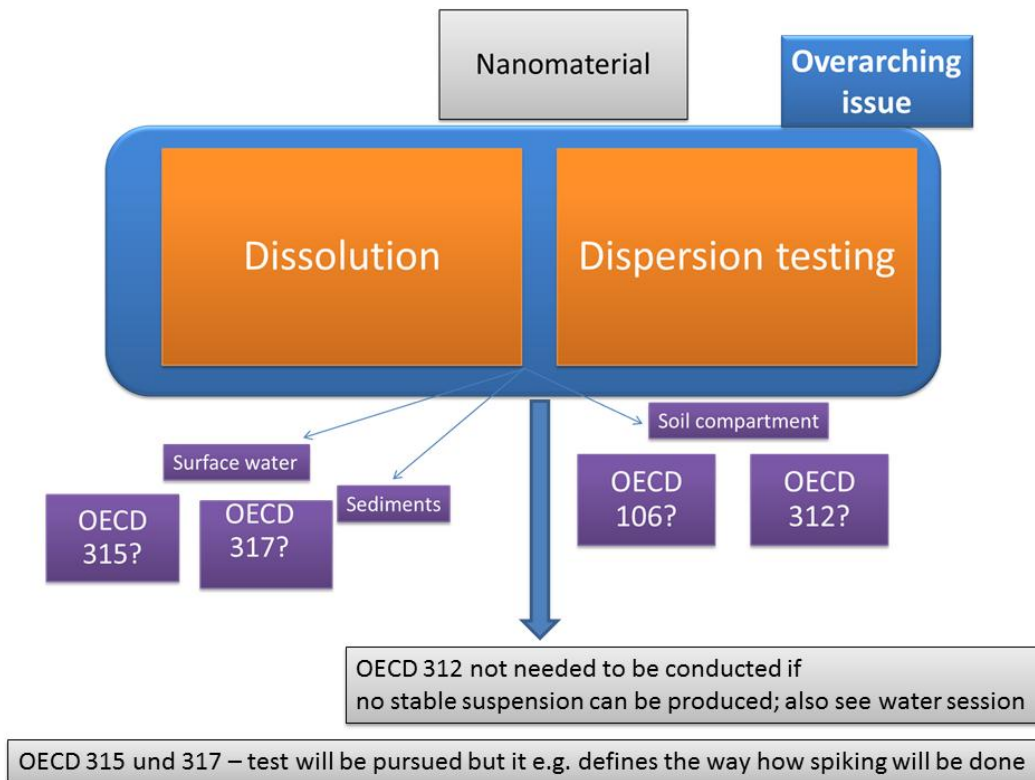
- Mobility of NM in soils
- Transformation of NM in soils
- OECD Tests
  - 106
  - 312
- Decision Tree: filling third tree suggested this morning by Break out group Eco-toxicology

## Summary of Fate & Behaviour for Soils

- Stimulus presentation Jason Kirby setting the basis for the discussion
- Background for all soil related studies: pathway / form of entry into the system

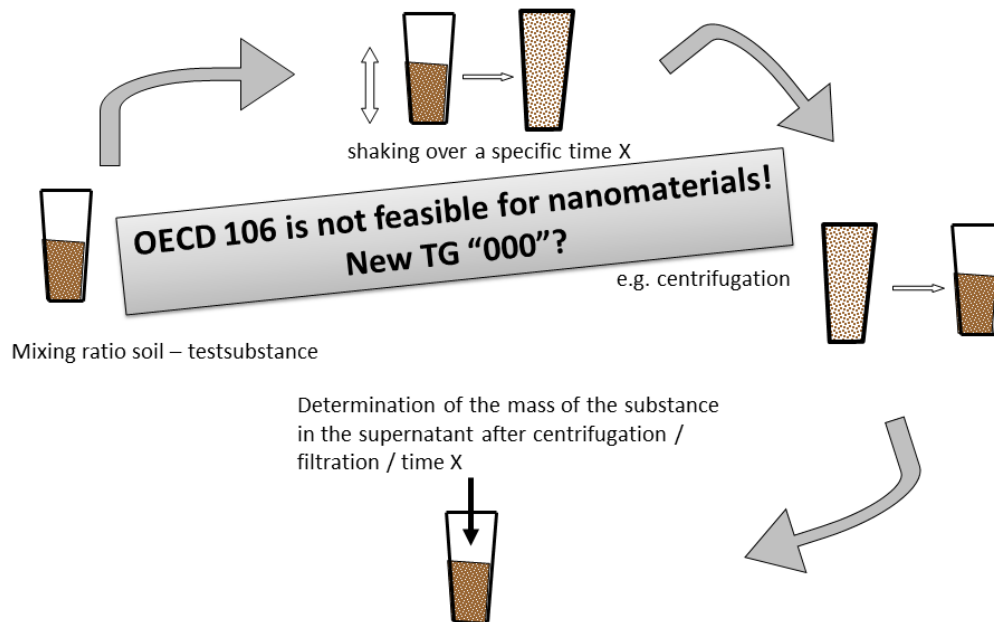
**Recommendation:** Review current guidance related to e.g. waste water treatment, nanofertilizers, nanopesticides.





**Recommendation:**  
**Development of a consistent set of test guidelines for Dissolution and Dispersion testing → consequences for further testing in e.g. soil tests**

## OECD 106 – Adsorption / Desorption



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## Summary possible new TG

- Aim: Indication of mobility, benchmarking for expected environmental mobility in soils
- Results: Enables assessments through ranking, setting “reference points”
- Preamble on the use of this method essential

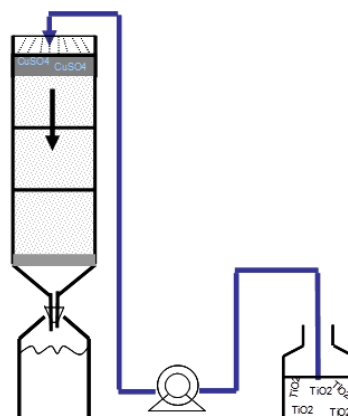
## Summary possible new TG (2)

- An approach was presented and discussed
- Several issues to be resolved e.g. “non-equilibrium method, shaking time, filtration vs. centrifugation, electrolyte, number concentration, how to add NM....

**Recommendation:** May be evaluated as a new test method. Needed evaluations have to be conducted including on the use of the results.

## OECD 312

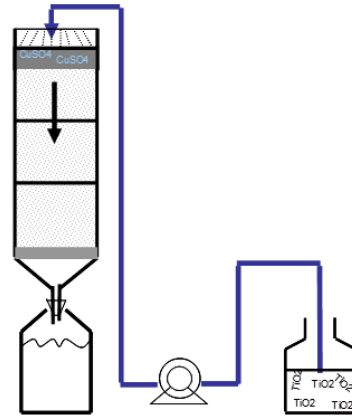
- Generally applicable
- Specific preamble and recommendation should be added
- E.g. definition on important parameters for soil selection, recommendation of a set of soil types, size distribution measurements before and after penetration of the column, NOM definition.....



- → for regulative purposes → average soils?

## OECD 312

**Recommendation:  
Amendment / Guidance on  
the use of OECD 312 for  
nanomaterials should be  
produced  
e.g. no dry spiking**



## Open questions, remarks

- No big mobility of NM seen and to be expected in soils
- Can / shall we define extreme cases? Or shall we define an average case?
- Test for remobilisation?
- Test for bioavailability? (e.g. extraction methods?)
- Discussion on soils / sediments!

## Key topics

- bioaccumulation of NM in soils
- transport and fate testing in sediments
- bioaccumulation in sediments
- OECD Tests
  - 317
  - 315
- Decision Tree: filling third tree?

## Overarching issues

- **Guidance** on measurement methods for different NM in different media → important for consistency
- **Guidance** on reporting of test materials and procedures in more detail
- **Guidance** on spiking - general advice but decision on which method to apply also dependent on the guideline

## Transport and fate testing in sediments

- Sediments are distinctively different from soils in view of e.g. bioturbation, turbulent transport (river), different NOM
- Still no specific missing guidelines were identified
- The important points specifically related to sediments are covered in e.g. OECD 315

### OECD 315

(bioaccumulation in sediments)

- Generally applicable
- What do we learn? How do we use the results in the regulative context?
- Body burden after a certain exposure period
  - partitioning into tissue
  - total body burden (skin, gut..)both should reported
- Spiking: preferably wet sediment spiking → reasons for dry sediment and water spiking
- BAF (see e.g. OECD 305 Annex 1) ongoing discussion

## OECD 315

(bioaccumulation in sediments)

**Recommendation:  
Amendment / Guidance on  
the use of OECD 315 for  
nanomaterials should be  
produced  
e.g. data interpretation, spiking  
..**

## OECD 317

(bioaccumulation in soils)

- Generally applicable
- Some points are not applicable for NM, other have to be more specified
- Body burden after a certain exposure period
  - partitioning into tissue
  - total body burden (skin, gut..)both should reported
- Spiking: Both, dry and wet spiking may be used in view of different bioavailability, wet possibly for better controlled size distributions

# OECD 317

(bioaccumulation in soils)

**Recommendation:  
Amendment / Guidance on  
the use of OECD 317 for  
nanomaterials should be  
produced  
e.g. body burden, spiking ..**

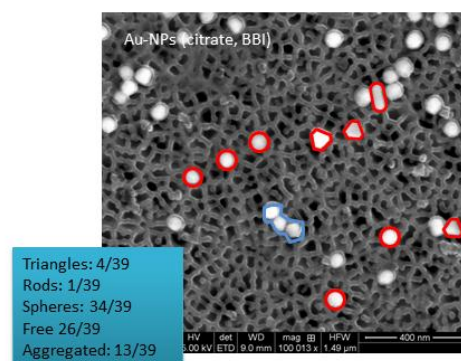
## Break out group – day one

- tired approach / decision tree
- First step: Tests for dissolution and dispersability
  - Second step: Tests agglomeration of NM in suspension
  - Third step: Tests biodegradation
    - Specific (bio)degradation tests for coated NM needed
  - Fourth step: Tests abiotic degradation

Applicability, available techniques and tools, available knowledge & test can be implemented

Still it is helpful to measure certain material properties, although we might incorrectly address some nanomaterials due to inappropriate methods

One nanomaterials alone might consist out of different fractions with Different properties (size, shape, compositions, coating) → internal heterogeneity





**Environmental behavior & fate testing**

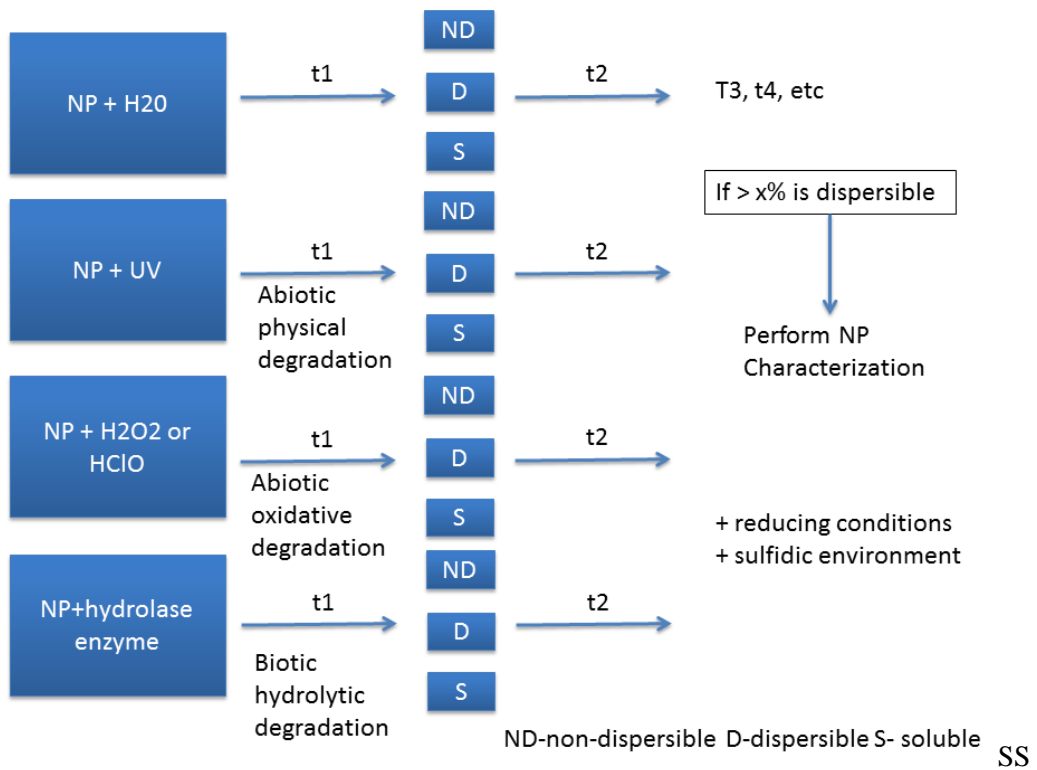
decision based, tired approach

**2a) Transformation/abiotic degradation**

**2b) biotic transformation**

Abiotic degradation test for NPs

- UV light (intensity, duration)
- anoxic / oxic conditions
- biodurability



## Break out group – day two – bioaccumulation TG 305

- BCF is not applicable!!!
- The aquatic exposure is not applicable, no homogeneous exposure can be guaranteed (heteroaggregation, attachment to the organisms)
- Dietary as worst case scenario
- Measurement is still a challenge – until more sensitive methods are available the background concentration compared to the concentration in the exposed organism should be measured
- Exposure time might be the same, but we will not reach a steady state
- Internalisation rate – useful ?
- In vitro tests for digestibility as pre-screening test

→ Annex to the TG for the testing of NM or

→ New TG for NM

## Session 4: Final Plenary



## Working Summary of Second OECD Parallel Workshop on Ecotoxicity and Environmental Fate

31 January 2013; Berlin

1

### 1<sup>st</sup> Plenary: Protocol Development - I

- ◆ Significant progress in NM detection methods for complex media at low concentrations:
  - ◆ FFF; ICPMS more approachable in near term
  - ◆ NIRF and solvent extract techniques developing
- ◆ Development of rate constants for aquatic fate modeling – in lieu of  $K_{ow}$  measurements:
  - ◆ Dissolution, transformation, heteroaggregation may be more important than homoaggregation and attachment efficiencies
- ◆ New Areas for Aquatic Toxicity:
  - ◆ chronic toxicity: growth, reproduction, energetics, new materials, diff. spp, environmentally realistic concentrats, ADME.....<sup>2</sup>



## 1<sup>st</sup> Plenary -- Protocol Development - II

- ◆ Modified 302B/ 310 biodeg tests
- ◆ Sludge sorption isotherm (non OECD guideline)
- ◆ Dissolution kinetics for soluble metals (non OECD guideline)
- ◆ Development of models for fate in receiving waters: need for WWT releases, photodegradation, biodegradation, new materials need to be tested – all @ environmentally-realistic concentrations
- ◆ Need for data on fate of sludge-applied NMs
- ◆ Significance of pristine vs aged NMs



3

## Aquatic Testing Protocol Development - I

- ◆ Focus initially on simpler/ fundamental protocols that (1) have data & (2) impact # of OECD TGs
  - ◆ **Decision tree approach: three tiers**
  - ◆ **Tier I: Preparation of stock/stem solutions** for aquatic toxicity and environmental fate (TiO<sub>2</sub>/NIST approach, MARINA). Anchors stock characteristics before dilution for comparability of results amongst tests; lessens unintended NP damage; repeatable (std. equipment, energy, monodispersion)
    - ◆ Stable suspension/size range for certain time
    - ◆ Protocols cover individuals nanomaterials, where NM data already exist (TiO<sub>2</sub>, CNTs, Silver)
    - ◆ May need to do pilot runs to determine applied E needed, stability with or without stabilizers (NOM, etc).

*Consideration should be given to a Categories' Approach*



4

## Aquatic Testing Protocols - II

### ◆ Tier II: Preparation of Exposure Solutions:

- ◆ Material properties will likely change on addition to more complex exposure media
- ◆ Accept more than 20% loss of NM during the test?
- ◆ May need test solution replacement (vs flow-through or agitation):
  - ◆ Run pilot to determine material loss (with or without organisms present?)
  - ◆ Consider alternative dosimetrics for varying exposure (nominal concentration as worst case; geometric mean; etc)



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## Aquatic Toxicity Protocols -- III

### • Tier III: Conducting the Test

- Media and particle specific decisions
  - Dosimetry: Measurement of particle #, mass, & surface area in test waters during the test (already in GSPD): measure relevant NM ions released; surface area metric may need further consideration
  - Results from high exposure concentrations should be considered in the context of more realistic (lower) environmental exposures
  - Consider testing with NMs which reflect most likely transformations after introduction into environment (species resulting from ligand binding).
- *Link Eco testing results with fate testing results via media preparation, etc.*



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## Aquatic Toxicity: Specifics – IV

- Algal tests
  - Assay should be tested in advance for lack of interference due to particle presence
    - Particles confound measurement of algal cell counts/biomass
    - Particles alter availability of photon energy (incr or decr. growth)
    - Photoreactivity should be considered (ex, ROS generation)
    - Material may affect nutrient availability (iron sequestration, etc.)
- Development of Categories should be considered
  - Based on material (metal, metal oxide, carbon, ...)
  - Based on MOA
  - Common approaches to test methods, and predictive toxicity



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## Aquatic Toxicity V: Protocol Updates' Process

- Decision Tree will be developed
- Decision Tree may become part of the GSPD
  - Prefer to update sections of GSPD over time
  - Greg Goss, Steve Diamond, Phil Sayre, Richard Handy?



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## Ecotoxicity in Soil and Sediment Species

- Test data limited; **OECD test protocols still applicable**
- Continue to move forward with both dry and wet spiking of soils and sediments; use stock solutions as in aquatic toxicity tests where feasible; advantages to both approaches enumerated
- Measurements of NM concentrations in soils/sediments needed (mass measurements feasible for some parent NM); soil and sediment pore water concentrations are also important but tech probs. exist. Few on-line measurement methods
  - Assessment of bioavailable fraction
  - Spiking of food, or spiking of sludge to add to soils
  - Consideration of different types of NMs
  - Consideration of different soil and sediment types/aging
  - Are we looking at the most appropriate endpoints?



Guidelines are applicable to other organisms

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## Ecotoxicology: Detection in Soils/Sediments

- Aggregation, soluble species, transformed species all affect bioavailability
- Total Metal determinations: acid and aqua regia extractions possible for Ag, but loss due to Cl- spp; backgr metals; radioisotopes effective
- EM+EDX (material specific) & EELS (redox specific) for bulk soil anal.
  - EM only useful to understand shape/agglomeration size; cautions on artifacts
  - Apply EM after initial screening to decrease labor? **Develop Tiered approach in genl**
  - EDX & EELS more academic application now (-> FFF-Single particle ICP-MS)
  - Alternative scrn approaches: X-ray powder diffraction (bulk) & XBS (speciation)
- Separation Techniques:
  - Pore water separation via filtration (consider attachm. to filters): interpretation of results difficult; regulatory utility
  - Centrifugation: better recovery, but filtration more convenient
- Soil pore water analysis: ICPMS + FFF vs. Single particle ICP-MS
  - Advantages & disadvantages outlined; may not be readily available methods
  - May be more relevant for some organisms depending on exposure mechanism
  - **NEED: No good correlation between pore water burdens and organism effects**
- Amount in organism likely key for regulat. purposes; but understanding state of NPs in soils/sediments is critical to inform overall results



**Guidance ( vs new protocol); consider regulatory needs**

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## Environmental Fate Protocol Development - I

### Fate and Degradation (Water)

- ◆ Develop a decision tree beginning with dispersion and dissolution (pH, NOM etc.) – tiered approach  
→ **also necessary as first steps before sediment / soil testing**
- ◆ Current OECD Dissolution TG not applicable
- ◆ Detect dispersion stability (aggregation rate constants...)
- ◆ Biodeg TGs basing on Oxidation of organic carbon only applicable in few cases (other methods needed to detect biodeg of organic coating)
- ◆ Abiotic degradation (hydrolysis, photodeg (TG 316, biodurability...))
- ◆ Pretreatment scenarios (H<sub>2</sub>O, UV, oxid., enzym., red.(?))



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## Environmental Fate Protocol Development -II

### Fate – Bioaccumulation (Water)

- ◆ BCF approach of TG 305 not applicable – no partitioning
- ◆ Dietary exposure (worst case?) in OECD 305 applicable but often no equilibrium reached → BMF cannot be determined
- ◆ Uptake rate? – NM may be only attached not incorporated → Internalization rate?
- ◆ Problems with analytical detection in particular in tissues
- ◆ In-vitro tests as pre-screening?
- ◆ Research necessary on uptake of NM by filtering organisms (mussels, crustaceans)



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## Environmental Fate Protocol Development -III

### Fate – Adsorption / Desorption (Soil/Sediment)

- ◆ Adsorption /desorption directed by porosity, charge, NOM (not organic carbon content)
- ◆ Mobility of NM in soil is expected to be very limited
- ◆ Necessary to determine size distribution of applied and eluted NM
- ◆ Application of dispersion more preferable than dry spiking
- ◆ OECD 106 should be replaced by another test for NM
- ◆ OECD 312 is generally applicable (preamble/ guidance necessary)
- ◆ If not dispersable → not necessary



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## Environmental Fate Protocol Development -IV

### Fate – Bioaccumulation (Soil/Sediment)

- ◆ OECD 315 and 317 generally applicable
- ◆ BAF is appropriate endpoint
- ◆ Specific guidance needed
- ◆ Wet spiking mostly preferable (may be reasons why not)
- ◆ Test purged and unpurged worms



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## Environmental Fate V: Protocol Updates' Process

- Develop guidance document for OECD 305 (dietary exposure of NM) – technical adjustments – endpoints to be determined
- Develop a tiered approach to determine fate of NM in Water (decision tree) as guidance document
- Develop a guidance document for pretreatment scenarios (Change of NM characteristics in environment)
- Develop new adsorption test replacing TG 106 as pre-test for TG 312 (?)



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## Environmental Fate VI: Protocol Updates' Process

- Develop guidance for nanorelevant aspects of TG 312
- Develop guidance for nanorelevant aspects of TG 315 and 317
- Develop.....



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## 2<sup>nd</sup> Plenary: Commonalities in Ecotox & Fate I (Water)

- Decision trees to move forward from regulatory perspective acceptable; should factor in knowns and unknowns, uncertainties
- Dosimetrics & regulatory needs: mass required, surface area, # concentrations, ion concentration also should be reported where available; indiv. regulatory needs should be considered (REACH, Other Country specifics)
- Media prep between Ecotox and Fate should be similar where feasible – at least same stem solutions
- Bioaccumulation via feeding studies assume that the particle has limited solubility.
  - Consider rate constant for accumulation on external prey organisms for food chain + digestibility test for predatory species → feeding study in tiered fashion?



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## 2<sup>nd</sup> Plenary: Commonalities in Ecotox & Fate (Water)-- II

- Use of NOM/other natural dispersants where dispersants are needed
- Stem solutions: do we prescribe a level of polydispersity?
  - Goals are consistency and repeatability: stable so that same amount goes to replicates per volume of stock
  - Polydispersity may uncouple the standardization across tests for ecotox and fate

*Protocols for Ecotoxicology and Fate of NMs may vary due to physical-chemical properties of individual materials*



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## 3<sup>rd</sup> Plenary session

- Analytics may further develop and become cheaper / better available
- Spiking should be similar in ecotox and fate tests – wet spiking of soil and sediment mostly favoured but there are cases where dry spiking is justified
- Selection of soils: artificial soil vs. sandy natural soil – 3-5 different natural soils should be selected and characterized.
- Bioavailability : no general approach? Needed?
- Aging in soil



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## Next Steps

- Workshop Summary and Process
  - Rapporteurs and Session Chairs provide summary information to Secretariat who will compile the Workshop Draft
  - Contributions as pdf files will be available
  - Report to WPMN 11
- Additions to the GSPD as draft annexes
  - Aquatic Toxicity Tiered Approach
  - Others??
- Commitments on Protocol Development
  - ???
- A Sincere Thanks to all Attendees and the Organizers ☺



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