Environmental Behaviour and Effects of Nanoparticles in Organisms: Research and Data Needs for Regulatory Decision Making

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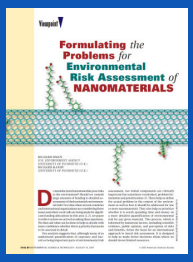
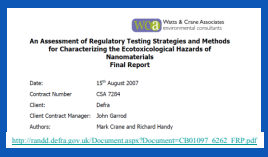
Problem Formulation in Hazard/Risk Assessment Reports



Owen and Handy (2007) Environmental Science & Technology, 41 (16): 5582-5588



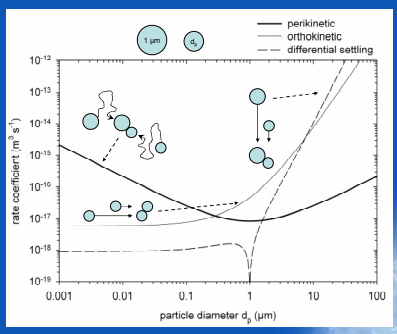
http://randd.defra.gov.uk/Document.aspx?Document=CB01097_6262_FRP.pdf

Owen et al., (2009). Strategic approaches for the management of environmental risk uncertainties posed by nanomaterials. In Nanomaterials: Risks and Benefits NATO Publications Series, pp. 369-384.

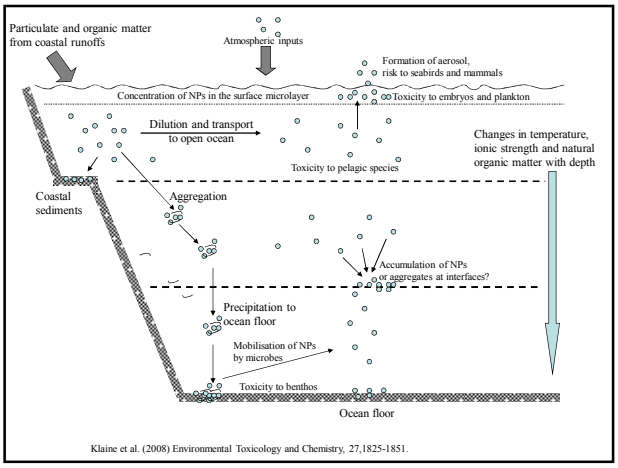
Environmental Chemistry of Nanoparticles

Fate and Behaviour
Environmental concentrations
Colloid particle chemistry
Uptake and Bioavailability

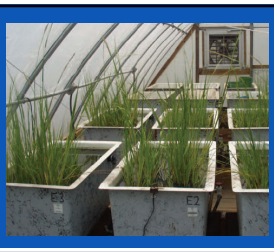
Nanoparticles Tend to Aggregate or Agglomerate in Natural Systems



Handy et al. (2008) Ecotoxicology, 17, 287-314



Klaine et al. (2008) Environmental Toxicology and Chemistry, 27,1825-1851.



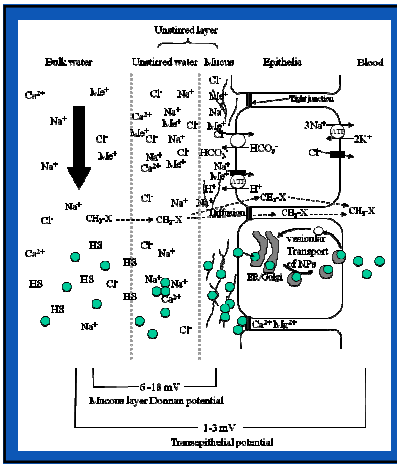
Gold NPs in Estuarine Mesocosms

Ferry et al., 2009. Nature Nanotechnology.

Table 1 | Distribution of gold in estuarine mesocosms after aqueous introduction.

Phase (g)	Gold (μg kg^{-1})[‡]		C_f^*	Per cent recovered gold in a given phase[‖]
	0 days	12 days		
Sea water (3.66 × 10^5)*	<LOD[‖]	0.42 ± 0.22	1.00	8.61 ± 4.51
Sediment (4.91 × 10^6)[†]	<LOD	13.9 ± 0.7	33.1	24.5 ± 1.23
Biofilm (1.01 × 10^3)*	12.2 ± 0.8	6.41 ± 0.28 × 10^3	1.53 × 10^4	61.0 ± 2.65
Spartina alterniflora (grass, 1.50 × 10^3)*	2.68 ± 2.01	3.45 ± 1.91	8.21	0.10 ± 0.06
Palaemonetes pugio (grass shrimp, 15.6)[†]	0.388 ± 0.30	48.1 ± 23.0	1.15 × 10^2	0.03 ± 0.01
Cyprinodon variegatus (GI tract and organs, sheepshead minnow, 22.5)[†]	0.964 ± 0.685	1.99 ± 2.34 × 10^2	4.74 × 10^2	0.31 ± 0.37
Ilyanassa obsoleta (snail, 5.5)*	<LOD	70.1 ± 33.2	1.67 × 10^2	0.05 ± 0.02
Mercenaria mercenaria (juvenile clams, 10.0)*	<LOD	9.57 ± 2.44 × 10^3	2.28 × 10^4	5.79 ± 1.48

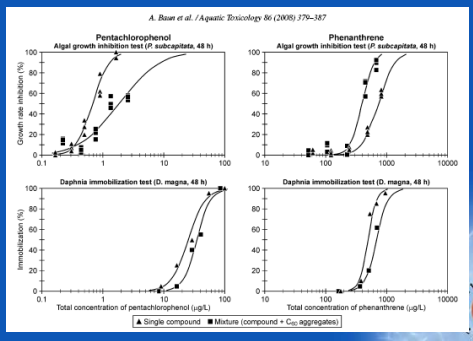
*Estimated mass of a phase in grams. †Measured mass of a phase in grams. ‡Gold atom content in ppb at $t = 0$ and $t = 12$ days based on dry weight for non-aqueous samples. §Concentration factor: $C_f = C_{gold}/C_{water}$ at $t = 12$ days. ‖Mass balance and relative error estimated from measured mass of water and sediment, with an assumption of 2 mm photosynthetic biofilm thickness throughout, and water contents of 36% (sediment), 67% (biofilm), 64% (*Spartina*), 80% (*Palaemonetes*), 72% (*Cyprinodon*), 36% (*Ilyanassa*) and 46% (*Mercenaria*)[28–29]. Limit of detection (LOD) for this method is 18.0 ± 0.5 pg kg^{-1}. All concentration measurements report the grouped mean of three separate samples per tank ($n = 9$) averaged across the replicate tanks accompanied by the pooled standard deviation.



External Chemistry and the Uptake Mechanisms

Handy et al. (2008) Ecotoxicology, 17, 396–409.

NPs as a Delivery Vehicle for Other Toxic Chemicals.



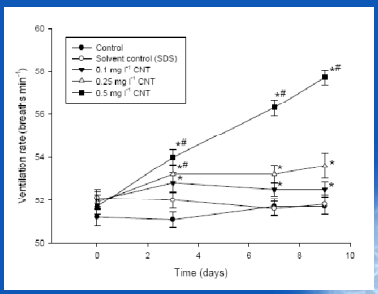
Knowledge Gaps & Research Needs: Fate and Behaviour

- Fate and settling behaviour modelling beyond single parameters in DLVO theory.
- User friendly computer software for predicting particle behaviour in experimental media (FW, SW, salines, mineral media, agar, etc)
- Particle size distribution in complex matrices of natural nanoscale materials (soil, food items, organisms).
- Detection limits are not sensitive enough: increase x100 fold to reach environmentally relevant concentrations.
- Measured rates of delivery for co-contaminants

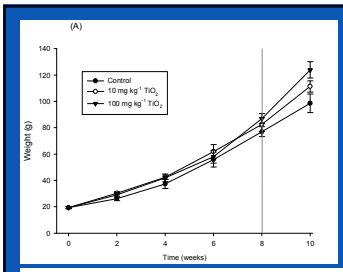
Biological Effects of Nanoparticles

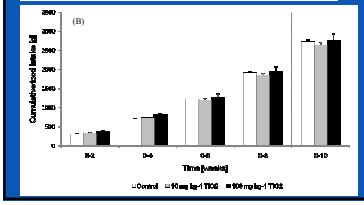
- Acute toxicity in high mg/l range.
- Fish toxicology: pathologies in all the major body systems.
- Toxic processes known: respiratory & ionoregulatory toxicity, oxidative stress, genotoxicity, etc
- Rare to find unique "nano-specific" toxic effects (vascular brain injury in fish, mechanical suffocation in invertebrates).

Carbon Nanotubes Are A Respiratory Toxicant To Rainbow Trout



Smith et al. (2007) Aquatic Toxicology, 82, 94-109.





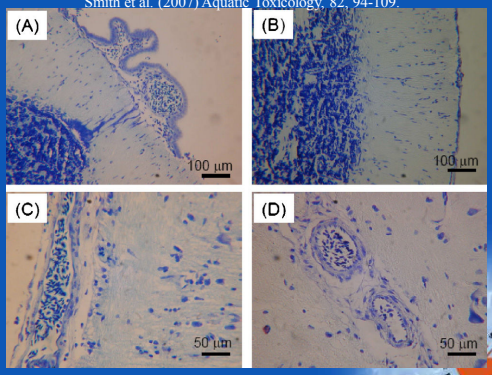
Growth & Food Intake

Ramsden et al. (2009) Ecotoxicology18:939-951.

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

Brain Injury: Waterborne CNT

Smith et al. (2007) Aquatic Toxicology, 82, 94-109.



Blood vessel abnormality on the ventral surface of the cerebellum in a fish exposed to 0.5mg l⁻¹ SWCNT (panel A) compared to solvent control (panel B). Blood vessels in the ventral region of the brain were normal in samples from each treatment (panels C&D). 8µm thickness, toluidine blue.

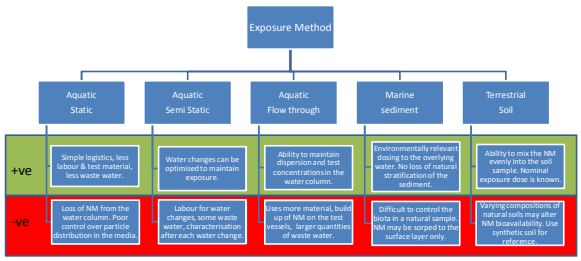
Knowledge Gaps on Biological Effects

- Knowledge on some body systems is "lacking": immune system, nervous system, muscle-skeletal (locomotion).
- Species sensitivity distributions-are we using the right species to test for toxic effects?
- Environmentally relevant exposures/chronic exposures.
- Dietary uptake/food chain effects.
- No data on reptiles, many birds, marine organisms from different Phyla

Ecotoxicology: Regulatory Needs

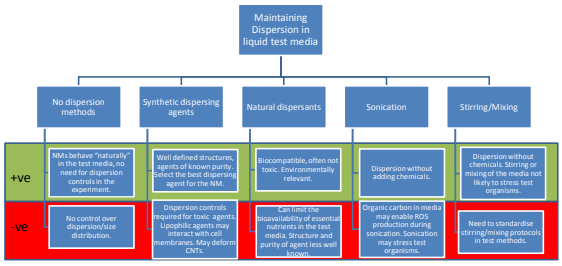
- Standardized ecotoxicity tests for NPs
  - SETAC Clemson workshop,
  - NanoImpactNet, Dublin Workshop
- Accept that some regulatory tests are fundamentally flawed/need major modifications for nano, make a new test (e.g., BCF tests).
- "Nano" tier in environmental monitoring schemes.

Figure 1



SETAC Clemson Workshop Handy et al. 2011 ET&C In review

Figure 2



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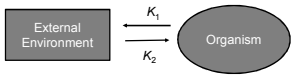
Bioconcentration (BCF) Fish Test is Very Problematic for Nano



Data Required for Modelling

External Environment

"Need to know" Information	Do we know this for ENMs?
Thermodynamic stability constants of the chemical species present	No, not measured. May need to do this for surface chemistry-but how?
Free ion or free solute concentration	Not appropriate, ENMs are not aqueous solutions but emulsions or dispersions.
Total concentration, preferably molar.	Inappropriate, only mass concentration (mg/l)
Effects of temperature, pH, DOC, Ca etc on free solute concentration.	No, not measured yet.
Effect of flow dynamics on removal of the substance from the external media (losses from bulk solution)	Yes, sometimes, but using a different set of rules to solutes.

Organism

"Need to know" Information	Do we know this for ENMs?
Surface adsorption of solute on to exterior of the organism (binding, but not true uptake)	No, not measured yet. Need a sensitive detection method for ENMs. Not clear that ENMs will fit the rules of adsorption chemistry for solutes.
Total concentration, preferably molar, in the organism.	Only mass concentration (mg/l).
Uptake rate measured and fitted, e.g., to Michaelis–Menten kinetic parameters (K_M and V_{max}).	No, not measured. Michaelis–Menten kinetics may be inappropriate.
Elimination rate measured as above, and net flux calculated.	No, not measured yet. No proof that unidirectional fluxes = net flux for ENMs.

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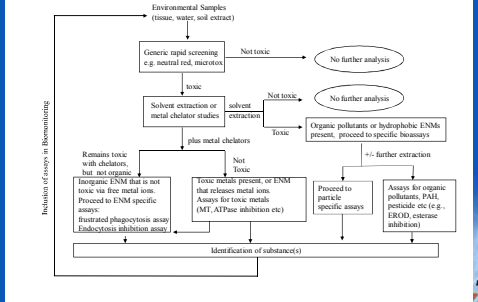
Ecotoxicological Screening And Diagnosis of Environmental Samples For Engineered Nanomaterials



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Bacterial Cell Wall is a Formidable Barrier to MNMs

Table 3. The bacterial envelope as a barrier to nanoparticles.

Structure	*Archeae*	Gram positive bacteria	Gram negative bacteria	Nano Issue
Cytoplasmic membrane	Lipid bilayer of mainly glycerol-ether lipids. Contains membrane spanning proteins	Lipid bilayer of mainly glycerol-ester lipids. Contains membrane spanning proteins.	Lipid bilayer of mainly glycerol-ester lipids. Contains membrane spanning proteins.	Hydrophobic layers, pore sizes in proteins < 1 nm. Only lipid dispersible, or lipid coated ENMs may associate with later. Interactions of ENMs with peptidoglycans unknown. Hydrophobic ENMs less likely to penetrate this layer.
Murein layer	Absent	Relatively thick layer, 10-50 nm wide. Peptidoglycan, techioic acids, and polysaccharides. Polyanionic and hydrophilic.	Relatively thin layer, 2-3 nm wide. Mostly peptidoglycan. Polyanionic and hydrophilic.	
Outer membrane	Absent	Absent	A thin peptidoglycan layer, 7-8 nm thick. Contains lipopolysaccharides. Membrane spanning porins. Polyanionic and hydrophilic.	Hydrophilic ENMS likely to associate with the outer membrane. Porins too small (< 1nm pore) for NPs
S-layer	Glycoprotein coat sitting on the cytoplasmic membrane.	Glycoprotein layer covalently linked to the murein layer. Lattice structure with a pore size 2-8nm.	Glycoprotein layer covalently linked to the outer membrane. Lattice structure with a pore size 2-8nm.	S-layer interactions with ENMs not investigated. ENMs < 8 nm may theoretically penetrate the lattice.

Dublin Workshop: Handy et al. In review (NanoImpactNet)

Different Ways of Spiking Soils

Table 4. Advantages and disadvantages of different methods for spiking soils with MNMs, identified at the NanoImpactNet Workshop.

	Adding as powder	Adding in suspension without a dispersing agent	Add in suspension with a dispersing agent
Yield.	High concentrations possible (no limit)	Low concentrations (µg/l to mg/l range)	High concentrations possible (g/l range)
Ease of preparation.	Potential occupational hazards from dusts. Short preparation (hours).	Easy to apply, but potentially long preparation time for the stock dispersion (for stirring methods, up to months).	Easy to apply, and short preparation time (hours).
Control of the dosing.	If the soil is relatively dry and mixed with dry powder then a reasonable spread of the test material in the soil occurs.	Poor reproducibility of the stock dispersion could produce variable dosing. Depending on the hydroscopic nature and viscosity of the solution, and properties of the NMN, the material may not be evenly spread in the soil sample.	Improved reproducibility of the stock dispersion, and more chance that the test material will spread evenly in the soil sample. However, dispersing agents controls are needed in the test design.
Characterisation.	Possible in the stock dispersion, but not in the soil matrix.	Possible in the stock dispersion, but not in the soil matrix.	Possible in the stock dispersion, but not in the soil matrix.
Surface modification of the test material.	Weathering effects less likely with dry mixing.	Long preparation times of stock dispersions may lead to oxidation, hydroxylation or other chemical/physical modifications of the surface. Soil effect relative to the stock preparation effect on surface modifications are mostly unknown.	Short preparation times imply less likely to produce spontaneous changes in the particle surface, but dispersing agents will coat/modify the surface. Interaction of dispersing agent with the soil and particle surface will depend on soil type and the stability of any surface coating in the soil matrix.
Dosing for chronic tests.	Suitable dosing method, but MNM may age, particle ageing control should be included in the experimental design.	Suitable dosing method, but MNM may age, particle ageing control should be included in the experimental design.	Suitable dosing method, particle ageing may be different with dispersing agent present. Degradation of the dispersing agent is likely.

Dublin Workshop: Handy et al. In review (NanoImpactNet)

Clemson & Dublin Workshops: Some Key Findings

- Clarify or remove the "options" for altering lighting, shaking, mixing of test media in current standard protocols e.g., the algal growth test.
- Avoid using dispersing agents if possible.
- Technology gap in practical methods for confirming exposure and particle size distribution ***during*** experiments.
- Microbial assays that rely on the test substance penetrating the cell may not work! (false negatives in Ames test, Comet assay, BOD assay etc).
- The BCF and similar regulatory tests that rely on "steady state" concentrations are potentially seriously flawed for nano (not a "steady state" phenomena).
- Practical solutions
  - Shorter tests/different species or media.
  - Additional controls for shading, mixing etc.
  - DVLO software for predicting particle behaviour in media (Chappell & co workers).
  - New microbial assays based on the cell envelope
  - New "BCF"-like tests

Any Questions?