

Fate, behaviour, and toxicity of engineered nanomaterials in the environmental systems

Ndeke Musee

Nanotech Environ Impacts Res Group, NRE

nmusee@csir.co.za



Some questions: risk concerns



- At the time of introducing nanotechnology, were there indications of society's capability to address the technology's potential risks to human health and the environment?
- Has the society learned from previous malevolent technologies failures, and inability to reach their full potential despite good intentions? **IF YES**, can this catalyse motivate a different approach to nanotechnology development trajectory?
- How has risk knowledge (from previous technologies) has influenced approaches adopted to address the potential risks of nanotechnology??

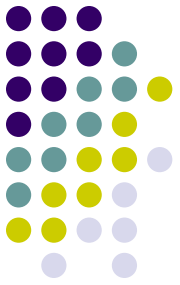
Lessons learned



- Asking more critical questions early on at R&D phase of a given technology related to risk management
- Development of research and governance collaborations across disciplines, departments, and international boundaries
- Development of process of establishing targeted research to generate relevant knowledge
- Engagement of diverse stakeholders (government, scientific, industry, civil society, etc)
- Interrogation of existing oversight mechanisms to establish their suitability within nanotechnology era

However, very slow progress....

One-way approach



R&D/M/PF



Patent



Industrial Production



Commercialization



Consumers health impacts

Environmental impacts



Risks reduction



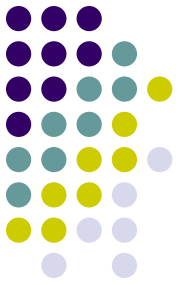
- Law suits
- Heavy penalties
- Jail terms.

Malevolent technologies are endless:

- Dichlorodiphenyltrichloroethane (DDT)
- Asbestos
- Chlorofluorocarbons (CFS)
- Genetically modified organisms (GMOs)
- Cell research
- Space programme**
- Mining (silicosis-related ailments)
- Nuclear waste

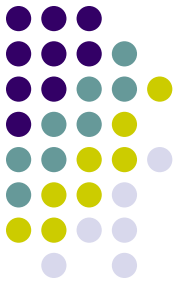
- **Lesson:** Missed early warnings became very costly to human health and the environment.
- Lessons learned too late!!

Environmental risks of ENMs



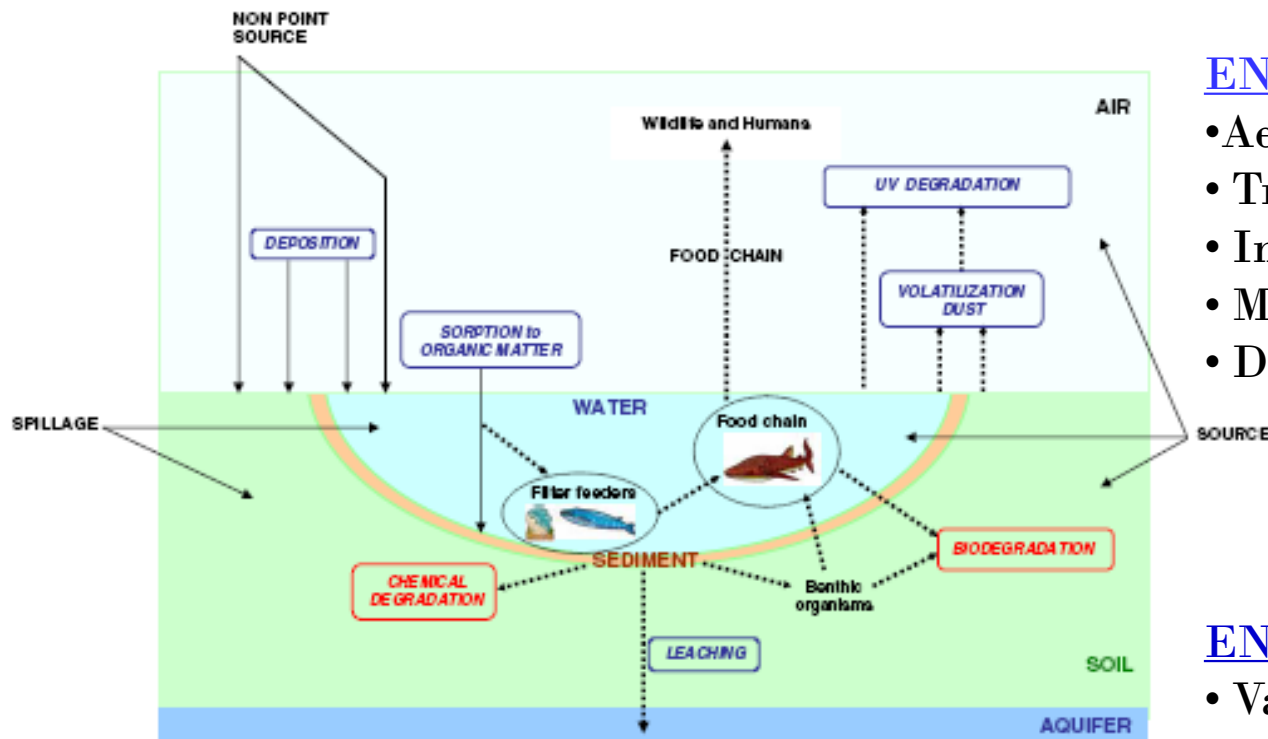
- Environmental risks of ENMs to biological organisms in the environment **MUST** satisfy two conditions: Hazard & Exposure
- Hazard (toxic effect): due to **nano/bio interface** –interactions of ENMs with: **Biomolecules, cell membranes, the cellular interior, organisms, tissues, organs, populations, and ecosystems.**
- Exposure: ability for ENMs (nanoscale toxicant) to be within the proximity of the organism or nano/bio interfaces. Depends on: **exposure media** (e.g. bacterial broth or yeast culture media), and **environmental factors** (e.g. type of water (sea, fresh or ground), pH, ionic strength, etc).

So, what do we know of ENMs risks



- We know we do not know enough on ENMs risks: many unknowns
- Window of opportunity to undertake risk assessment of ENMs rapidly closing (lost golden opportunity)
- Numerous ENMs tested have shown some form of toxic effects
- Fate and behavior of ENMs controls: hazard and exposure
- Collaborative approach under tier model, best suitable
- Increasing need & demand for legislative framework: not feasible presently

Environmental ENMs Pathways



ENMs in Air

- Aerosol
- Transportation: Long distances
- Indoor & ambient environment
- May aggregate & attach to dust
- Deposit in water &/or soil

ENMs in Water/Aqueous Environ

- Varied degrees of precipitation
- Varied Transport/bioavailability (stability dependent)
- Aggregation/agglomeration
- Interactions with aquatic colloids
- Possible deposition on sediments

ENMs in Soil

- Retention in soil matrix
- Break through on soil matrix
- Leaching into groundwater
- Possibility pathway to food chain

Farre et al., 2009, Anal Bioanal Chem

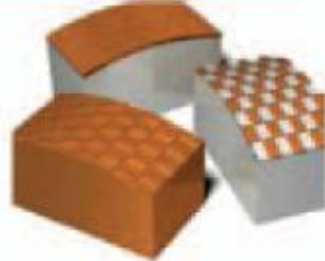


Exposure potential of ENMs estimation



Bulk-based NMs (one or multiphase)

EP: Very low to low



Structured surface, film or structured

EP: Very low to medium



Surface bound

EP: Low to high



NMs suspended in liquids

EP: Highly likely



NMs suspended in solids

EP: Medium to very high



Airborne/free ENPs

EP: Highly likely

Modified from Hansen et al. 2007 (Wischers and Musee, 2010)

EP: Exposure potential

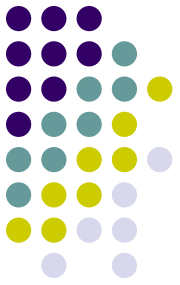
Fate of ENMs in the Environment



Key processes include:

- **Transformation**
 - Dissolution (pH, ionic strength, NOM, etc)
 - Surface coating (type of ligands, ENM type, etc)
 - Oxidation (NPs type, light, microorganisms, etc)
 - Photodegradation (light, etc)
- **Organism cleansing**
 - Uptake
 - Translocation
 - Transformation
 - Degradation

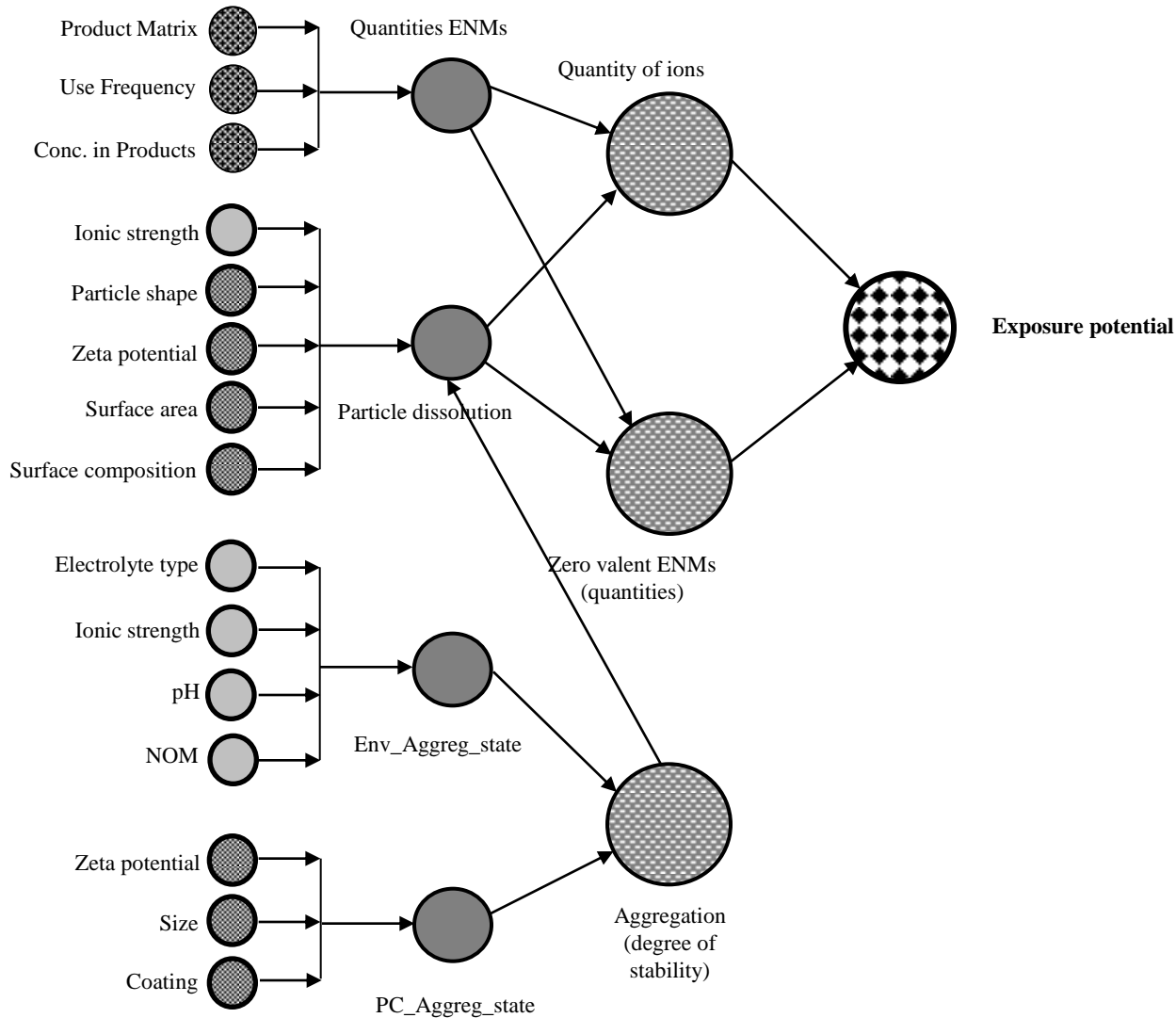
Influencing factors to ENMs fate in aquatic systems



- Agglomeration and /or aggregation
- Dissolution
- Chemical reactions (e.g. oxidation or reduction)
- Ligand exchange/coatings/functionalization
- Formation of new solid phases

Handy et al. 2008; Klaine et al. 2008; Navarro et al. 2008; Auffan et al., 2010; Bian et al., 2011; Liu et al., 2011.

Fate/exposure model



Proposed model for exposure estimation (Musee., 2012, unpublished)

Effect of pH, IS, NOM on Dissolution

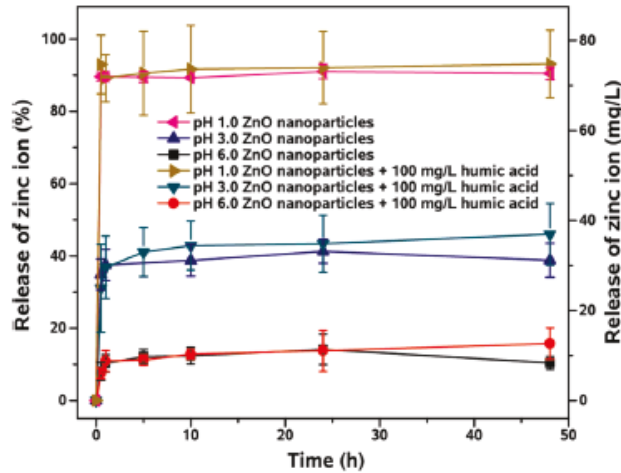
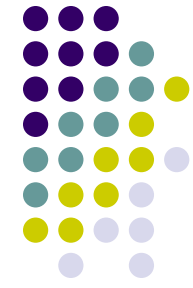


Figure 5. Time-dependent measurements of ZnO nanoparticle dissolution in the presence and absence of humic acid (100 mg/L initial mass concentration) at different pH (1.0, 3.0 and 6.0).

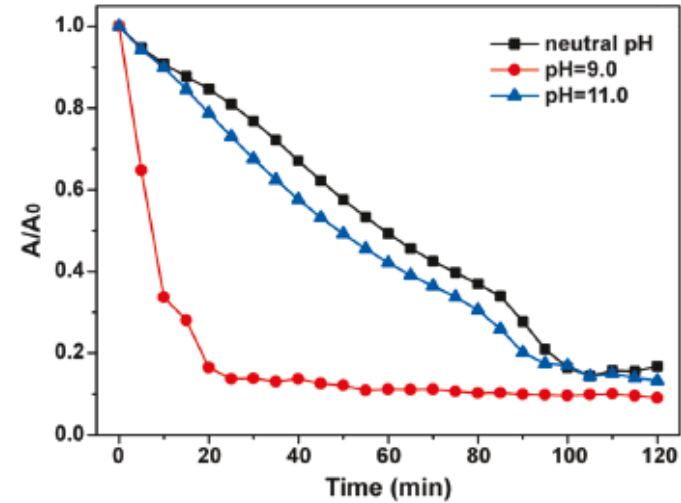
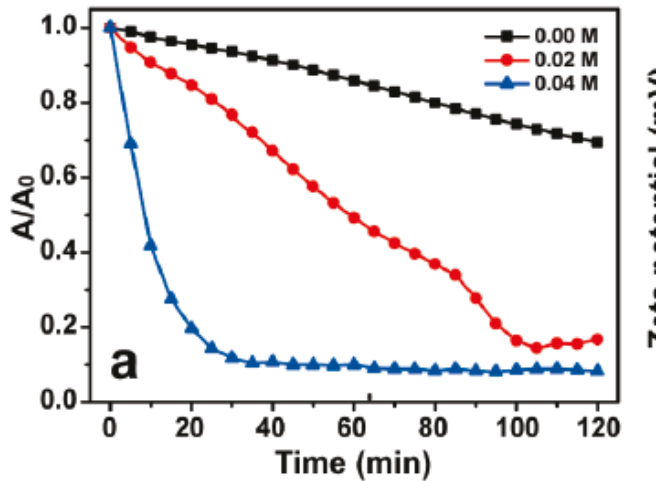


Figure 6. Sedimentation plots for ZnO nanoparticles at different pH (neutral pH, pH 9.0, and pH 11.0).



Bian et al., Langmuir, 2011,

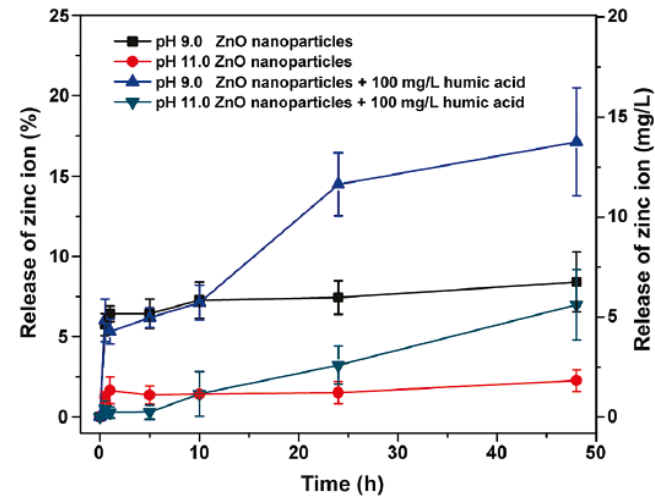
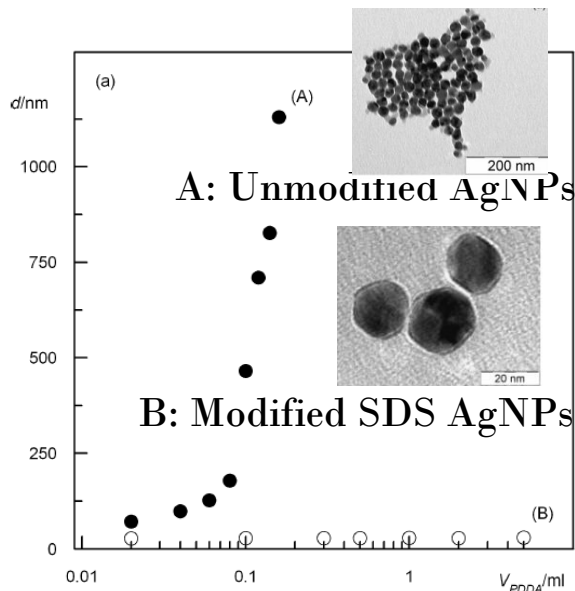


Figure 8. Time-dependent measurements of ZnO nanoparticle dissolution in the presence and absence of humic acid (100 mg/L initial mass concentration) at pH 9.0 and 11.0.

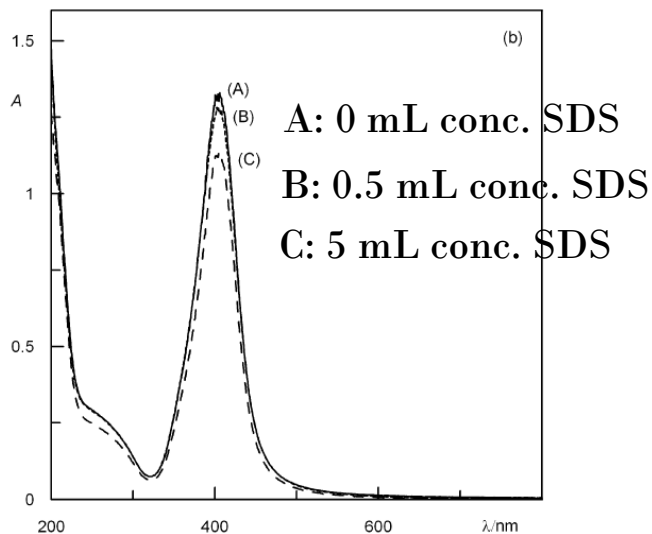
Effect of surface coating



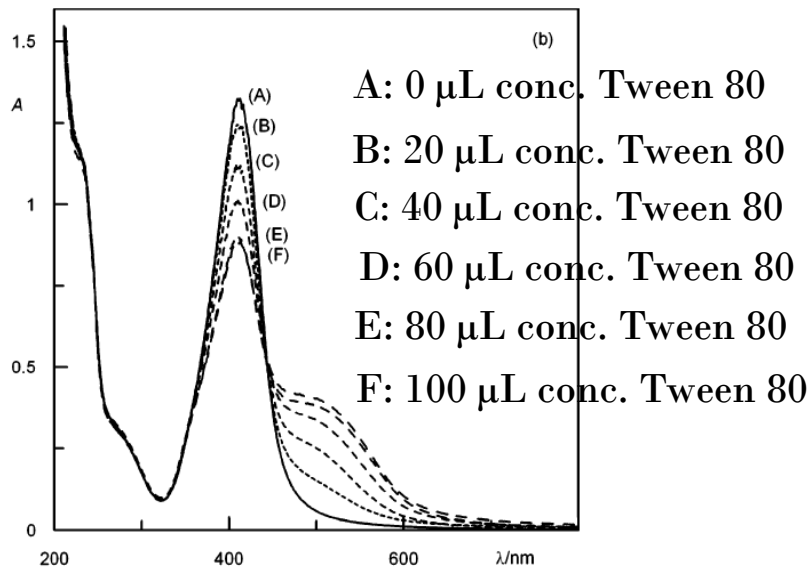
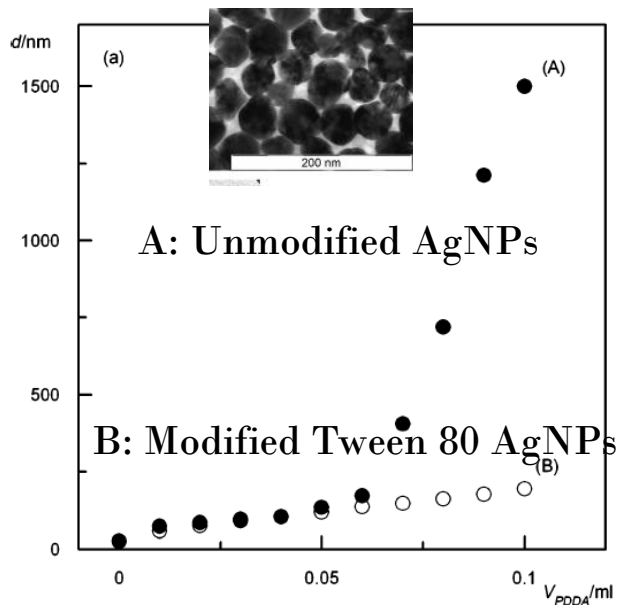
DLS size measurements



UV/Vis spectra



Steric effects due to EDL, increased zeta potential and enhanced AgNPs stability



Effect of Sulfidation to AgNPs Dissolution

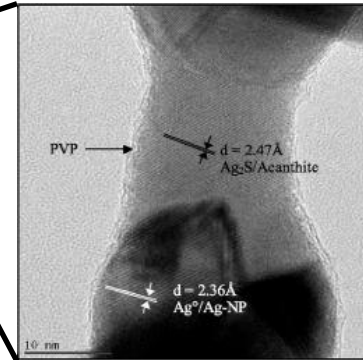
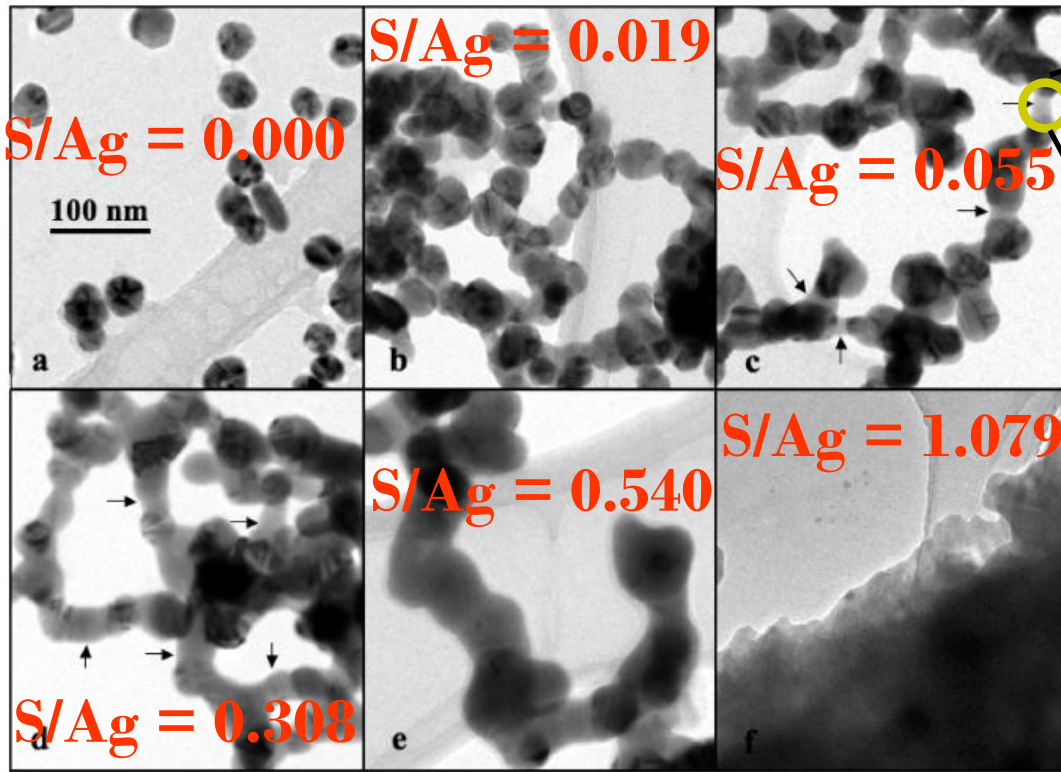
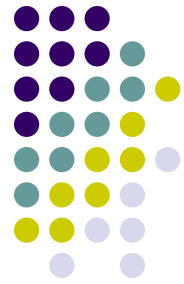
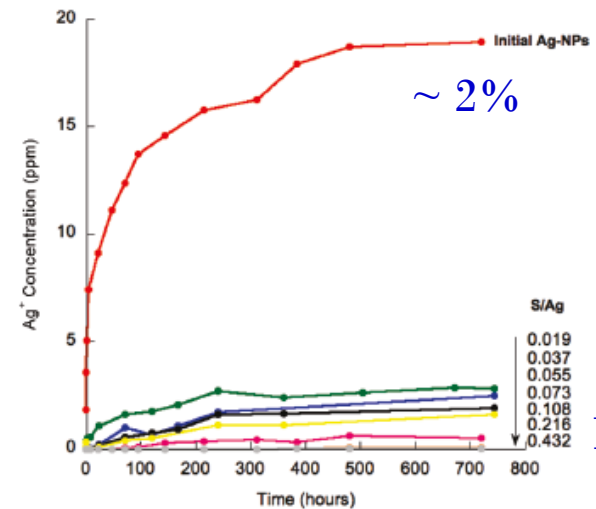


Image of nanobridge (Ag_2S)

TEM images of initial and sulfidized Ag-NPs

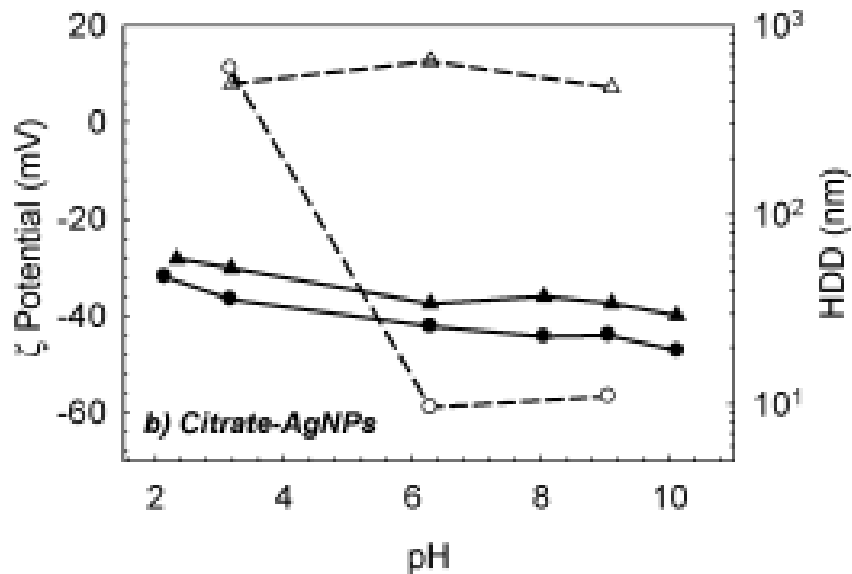
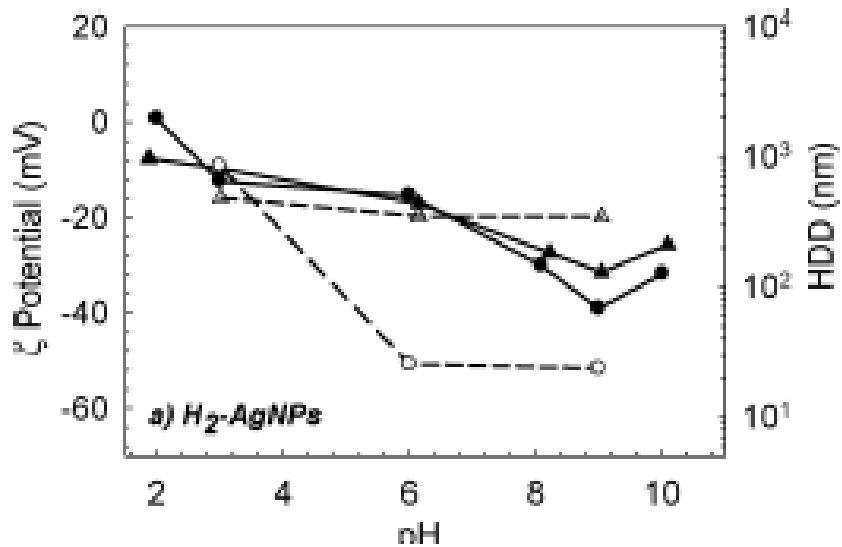


Negligible

Levard et al., 2011, Environ. Sci. Technol

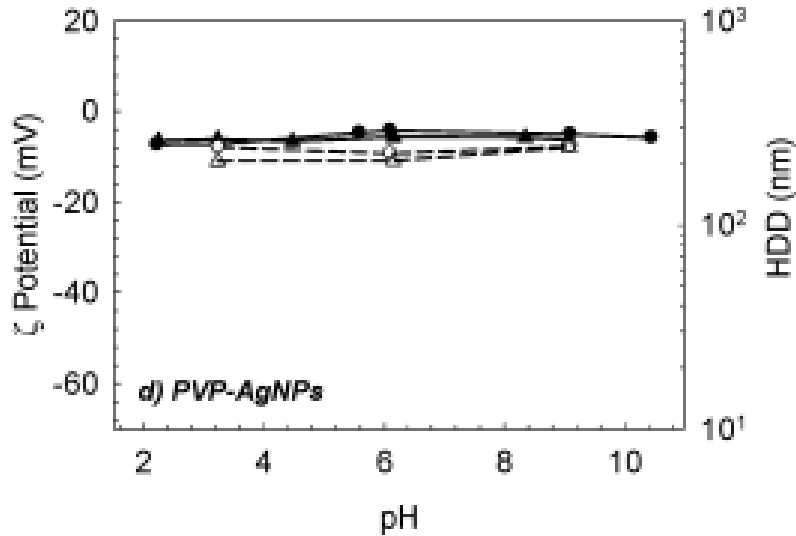
Figure 6. Dissolution rate measurements of Ag-NPs before and after reaction with increasing concentrations of aqueous Na_2S . (Initial Ag-NPs concentration for dissolution rate measurements was 1000 ppm in 0.01 M NaNO_3 , pH = 7).

Effect of pH, IS, electrolyte to zeta potential & aggregation

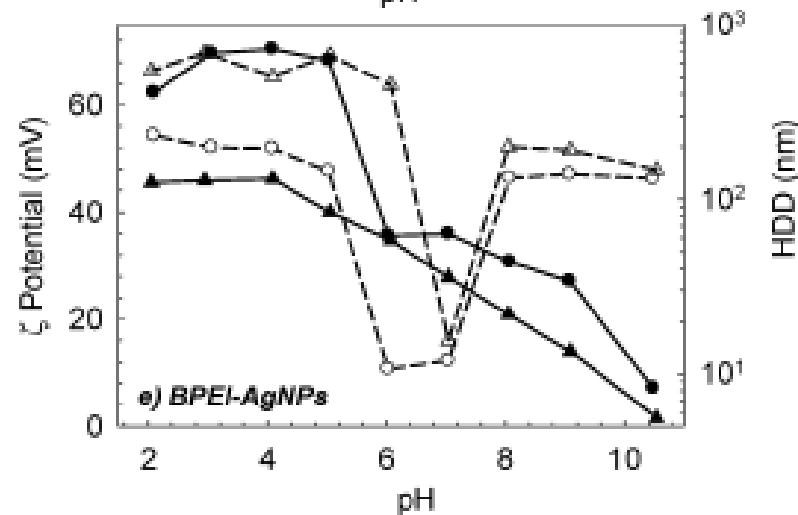


Uncoated AgNPs (H_2 -AgNPs) and electrostatically stabilized (citrate and $NaBH_4$ -AgNPs) aggregate at higher ionic strengths (monovalent) and/or acidic pH (3.0) conditions, or at any conc. of divalent electrolyte (e.g. Ca^{2+})

Effect of pH, IS, electrolyte to zeta potential & aggregation



PVP coated AgNPs (sterically stabilized AgNPs) WERE NOT AFFECTED BY ionic strength, pH and electrolyte type, hence no aggregation observed.



Surface charge and aggregation of the BPEI coated AgNPs varied according to the solution pH

Key findings: summary



- Capping agents dramatically influences surface behavior and aggregation of AgNPs.
- Type of stabilizing mechanism profoundly effects aggregation potential of AgNPs.
- Thus, fate and transport of AgNPs are closely associated with the chemistry of the capping agent (similar effect on AuNPs – Stankus et al., 2010) both in natural and engineered environments
- Acidic and/or with high ionic strength (e.g. with divalent cations) environments promotes NPs aggregation and settling may occur.
- Elevated levels of Cl^- may increase the stability of uncoated AgNPs in the presence of silver ions and at low ionic strength conditions.

Toxicity of ENMs

Ecotoxicology and Environmental Safety 74 (2011) 416–423



ELSEVIER

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Assessment of the effect of nanomaterials on sediment-dwelling invertebrate *Chironomus tentans* larvae

P.J. Oberholster^{a,b,1}, N. Musee^{a,*}, A.-M. Botha^c, P.K. Chelule^d, W.W. Focke^e, P.J. Ashton^a

^a CSIR Natural Resources and the Environment, P.O. Box 395, Pretoria 0001, South Africa

^b Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, P/Bag X04, Onderstepoort 0110, South Africa

^c Department of Genetics, University of Stellenbosch, Private Bag X1, Matieland 7601, South Africa

^d Department of Public Health, University of Limpopo, MEDUNSA Campus, MEDUNSA 0204, South Africa

^e Built Environment and Information Technology, University of Pretoria, Hillcrest, Pretoria 0002, South Africa

ARTICLE INFO

Article history:

Received 8 January 2010

Received in revised form

9 November 2010

Accepted 21 December 2010

Available online 7 January 2011

Keywords:

Nanomaterials

Chironomus tentans

Bioassays

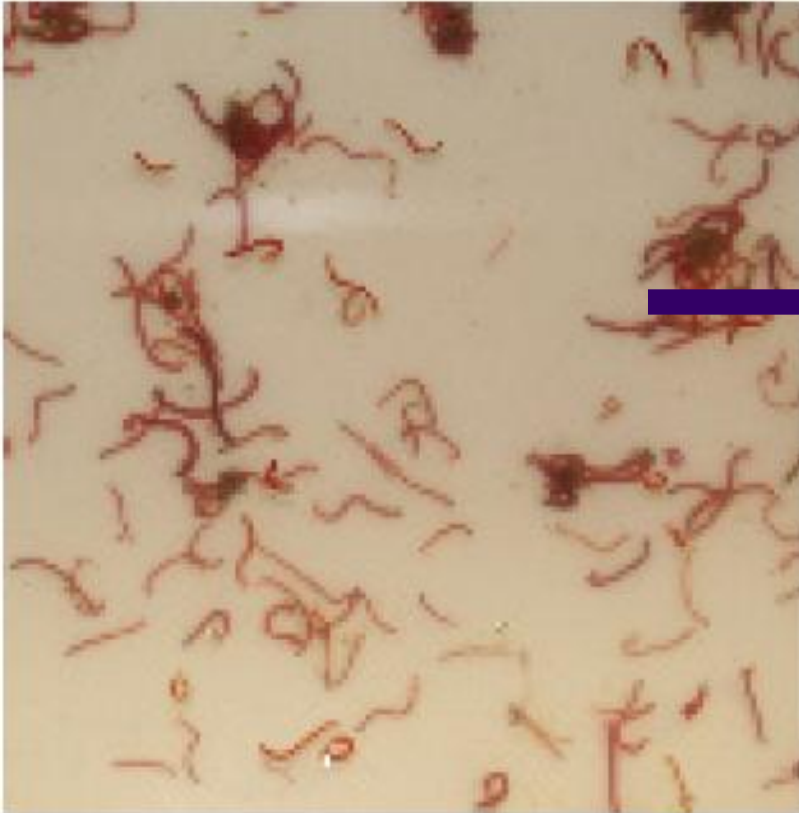
Ecotoxicology

DNA damage

ABSTRACT

Studies were conducted to determine the effects of a panel of seven nanomaterials (NMs), namely: α -alumina, γ -alumina, precipitated silica; silica fume, calcined silica fume, colloidal antimony pentoxide (Sb_2O_5), and superfine amorphous ferric oxide (Fe_2O_3), on sediment dwelling invertebrates *Chironomus tentans* under controlled laboratory conditions. Percentage survival, enzyme activities, growth development, and DNA fragmentation parameters were studied as acute, biochemical, and physiological toxicities of NMs, respectively. Quantitation of catalase and peroxidase enzyme activity demonstrated that toxicant stress of the NMs increased enzyme activity in a concentration dependent fashion across all treatments. The percentage growth length of the test specimens exposed to different NMs was significantly reduced compared to the negative control while only five concentrations were not in the toxic range, namely; Fe_2O_3 (5 $\mu\text{g}/\text{kg}$); silica fume (5 $\mu\text{g}/\text{kg}$, 50 $\mu\text{g}/\text{kg}$); Sb_2O_5 (5 $\mu\text{g}/\text{kg}$) and calcined silica fume (5 $\mu\text{g}/\text{kg}$). Genotoxic stress assessed by use of DNA laddering showed complementary findings to the other ecotoxicological endpoints tested in this study—the percentage survival and growth length inhibition.

*Sediment toxicity of *Chironomus tentans**



0 days exposure



10 days exposure

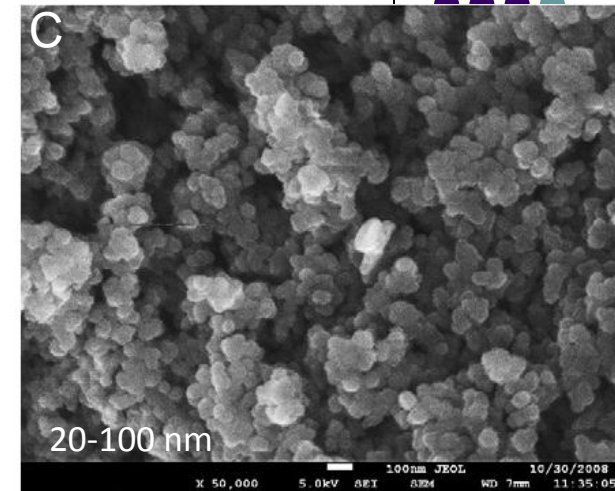
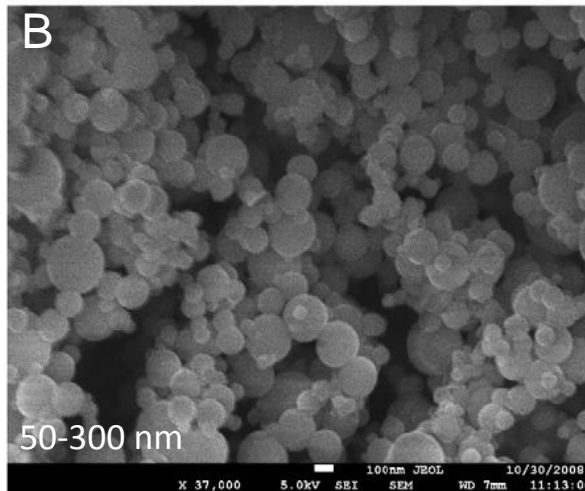
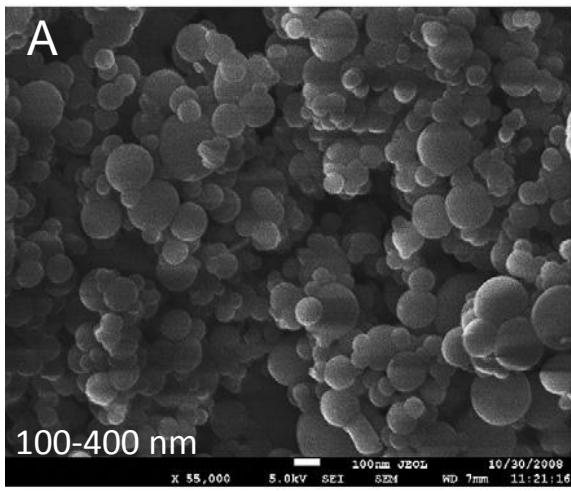


Table 2: Physicochemical properties and ranking of the essay findings [13].

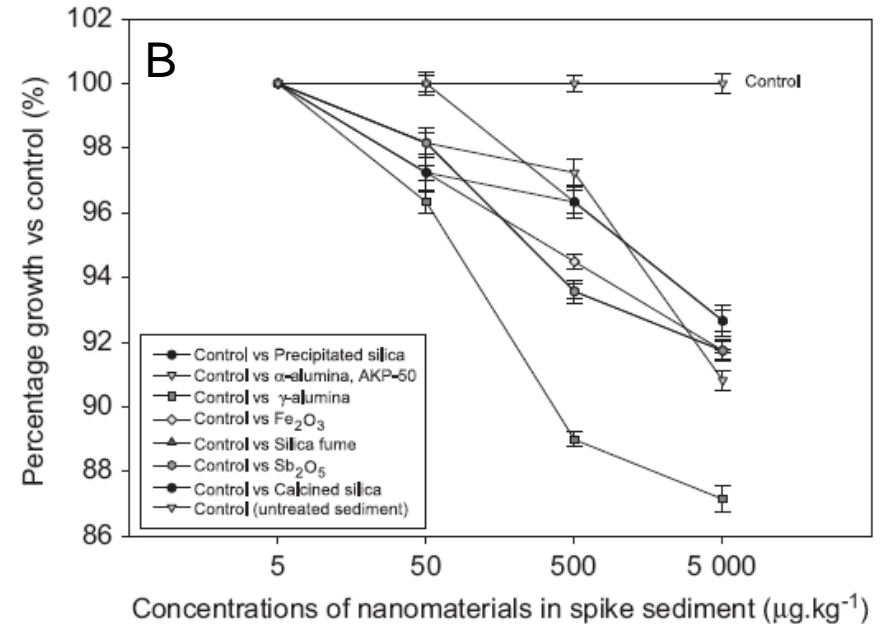
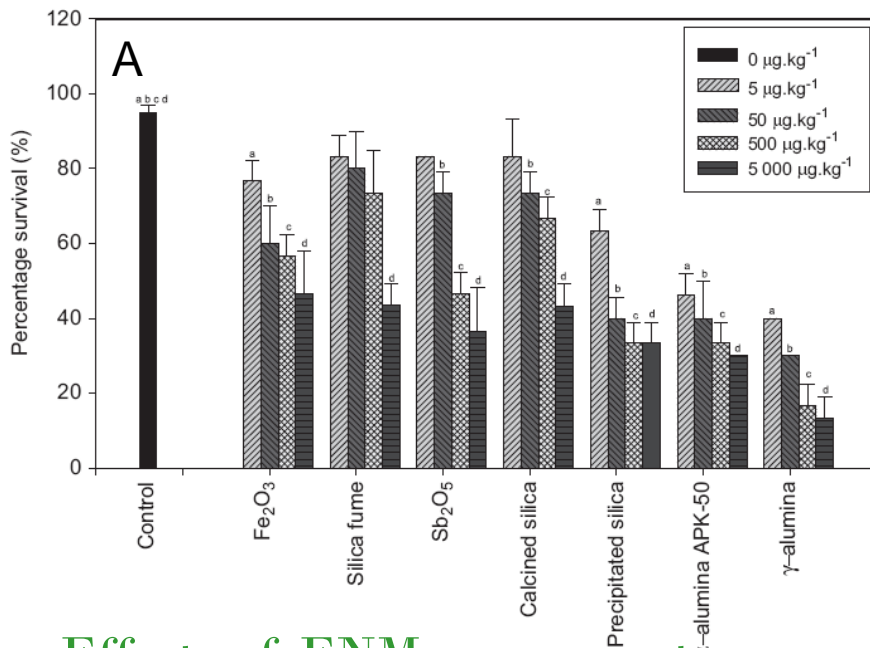
Test	Property/units	Fe ₂ O ₃	Silica fume (SiO ₂)	Calcined silica fume (SiO ₂)	Precipitated silica (SiO ₂)	Sb ₂ O ₅	α-alumina	γ-alumina
Zeta potential	mV	-18.3	-21.1	-14.6	-1.76	-23.4	19.7	-18.7
BET	Surface area (m ² /g)	235	24	17	157	3	13	72
	Density (g/cm ³)	5.2	2.10	2.1-2.4	2.0	4.07	3.6	3.97
Surface/volume ratio	m ² /mm ³	1274	50	41	314	12	47	286
XRD	Morphology	Crystalline	Amorphous	Amorphous	Amorphous	Crystalline	Amorphous	Crystalline
SEM	Particle shape	Spherical	Spherical	Spherical	Spherical	Mixture (spheres, irregular)	Spherical	Spherical
Zetasizer	Size (nm)	50-150	100-400	50-300	20-100	5000-15,000	20-50	80-400
Solubility	Degree	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble	Slightly soluble
Degree of dispersion	No units	Mono	Poly	Poly	Mono	Poly	Mono	Poly
Ranking of the toxic effects	Growth inhibition	4	7	6	3	5	2	1
	DNA damage	4	ND	ND	3	5	2	1
	Survival levels	5	7	6	3	4	2	1
	Enzymatic activities	4	7	6	3	5	1	2

ND: not detectable.

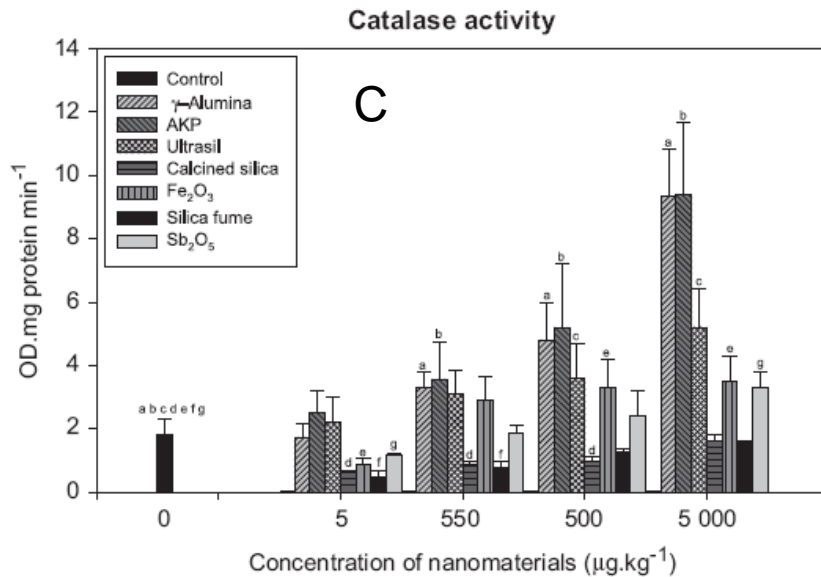
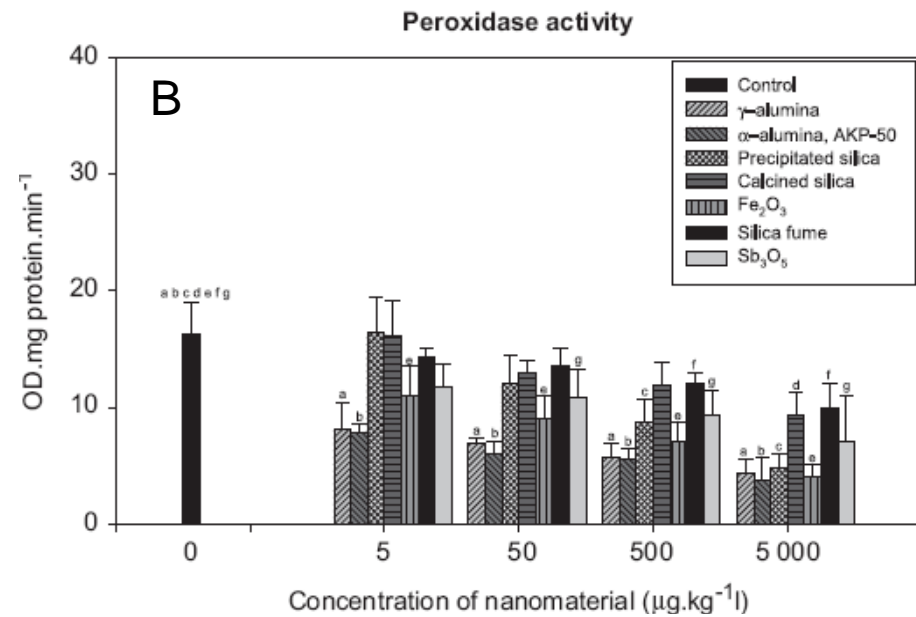
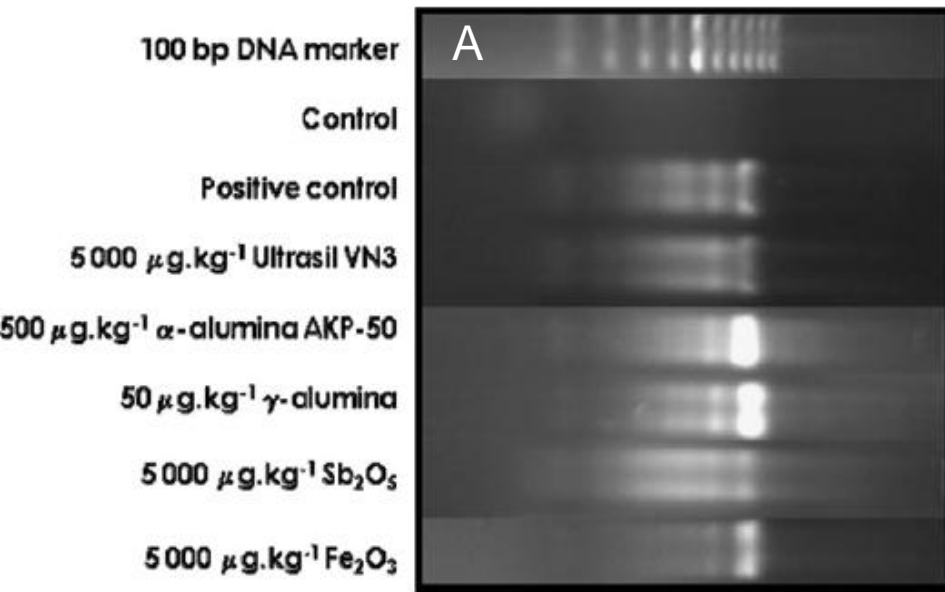
PC: Physicochemical properties



SEM images of: A: Silica fume, B: Calcined silica fume, and C: precipitated silica fume (Musee & Colleagues, Ecotocol Environ Saf, 2011)



Effects of ENMs on percentage survival (A) and growth inhibition percentage (B) of *C. tentans* (Musee & Colleagues, Ecotocol Environ Saf, 2011)



Results of DNA laddering (A) , peroxidate activity (B), and catalase activity (C) (Musee et al., Ecotox Environ Saf, 2011)



The effects of engineered nanoparticles on survival, reproduction, and behaviour of freshwater snail, *Physa acuta* (Draparnaud, 1805)

N. Musee^{a,*}, P.J. Oberholster^b, L. Sikhwivhilu^c, A.-M. Botha^d

^a CSIR Natural Resources and the Environment, P.O. Box 395, Pretoria 0001, South Africa

^b CSIR Natural Resources and the Environment, P.O. Box 320, Stellenbosch 7599, South Africa

^c DST/CSIR Nanotechnology Innovation Centre, NCNSM, P.O. Box 395, Pretoria 0001, South Africa

^d Department of Genetics, University of Stellenbosch, Private Bag X1, Matieland, Stellenbosch 7601, South Africa

ARTICLE INFO

Article history:

Received 9 April 2010

Received in revised form 26 August 2010

Accepted 14 September 2010

Available online 12 October 2010

Keywords:

Sediments

Physa acuta

Antioxidant enzymes

Embryonic growth

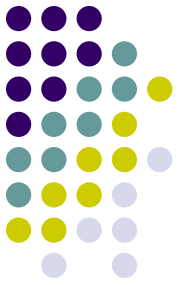
Hatchability

Snails

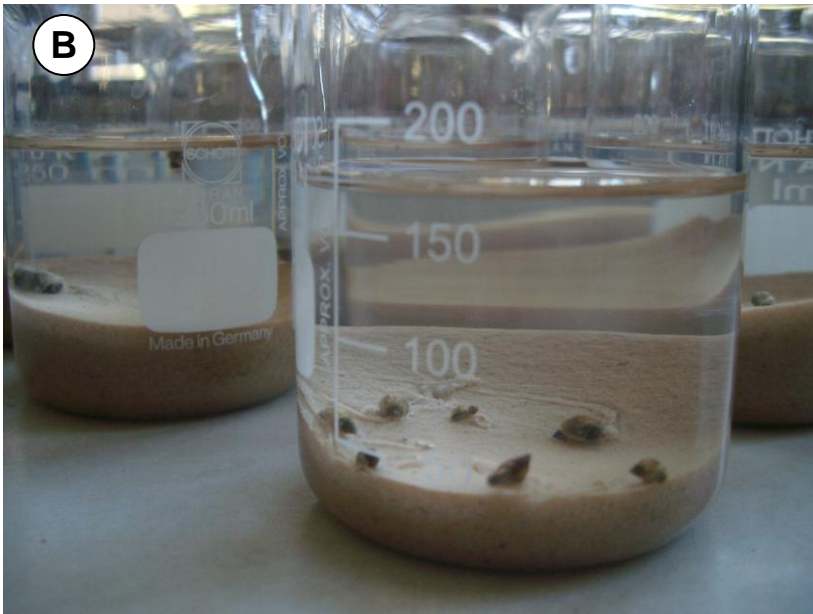
ABSTRACT

Increasing uses of engineered nanoparticles (ENPs) in commercial products and industrial applications has eventually resulted to their releases into atmospheric, terrestrial, and aquatic environments. However, knowledge gaps in ENPs toxicity, fate, and behaviour currently limit our ability to quantify risk assessment of materials with nanoscale dimensions, and therefore, the extent of the resultant environmental impacts remains unknown. In the present study, we evaluated the effects of γ -alumina, α -alumina, modified TiO_2 (M- TiO_2), and commercial TiO_2 (C- TiO_2) ENPs on the survival, behaviour, and early life stages of the freshwater snail *Physa acuta* (Draparnaud). The toxicity evaluation was carried out after spiking commercial sand with ENPs concentrations of 0.005, 0.05, or 0.5 g kg^{-1} . Our findings suggest that increases of γ -alumina and α -alumina concentrations at sub-lethal level concentrations caused significant reduction in the embryo growth rate and embryo hatchability. In addition, these ENPs induced observable developmental deformities of the embryos. In addition, toxicity evaluations using acute 96-h and chronic 28-d tests showed exposure duration may be a significant factor in ENPs-induced toxicity. Therefore, long-term exposure of aquatic organisms to ENPs – potentially can alter certain ecological populations at different trophic levels – and may compromise the entire aquatic ecological functionality. The percentage hatchlings in test chambers containing 0.5 g kg^{-1} γ -alumina and α -alumina concentration was 50% less to those observed in the controls. Our results suggest the embryonic growth and hatchability tests are useful endpoints in chronic sediment toxicity tests for determining the toxic thresholds of ENPs in sediment environment. Although no snail mortalities were observed during the static 96-h test containing sediment spiked with different concentrations of M- TiO_2 , C- TiO_2 , γ -alumina and α -alumina – the antioxidant enzymatic assay results indicated a significant change in antioxidant levels which altered peroxidation at 0.05 or 0.5 g kg^{-1} concentrations for both γ -alumina and α -alumina.

Exposure of ENMs to Physa acuta



Water Column Compartment



Water/sediment column compartment

Results



Table 1
Measured physicochemical properties of the ENPs.

Test	Property/units	C-TiO ₂	M-TiO ₂	α-Alumina	γ-Alumina
Zeta potential	mV	–	–	19.7	–18.7
BET	Surface area (m ² g ⁻¹)	50.3	301.7	13	72
XRD	Morphology	Crystalline	Crystalline	Amorphous	Crystalline
SEM	Particle shape	Spherical	Tubular	Spherical	Spherical
Particle size	Size (nm)	40–60 nm	7–11 nm	20–50	80–400
Solubility	Degree	Insoluble	Insoluble	Insoluble	Slightly soluble
Degree of dispersion	No units	Mono	Mono	Mono	Poly

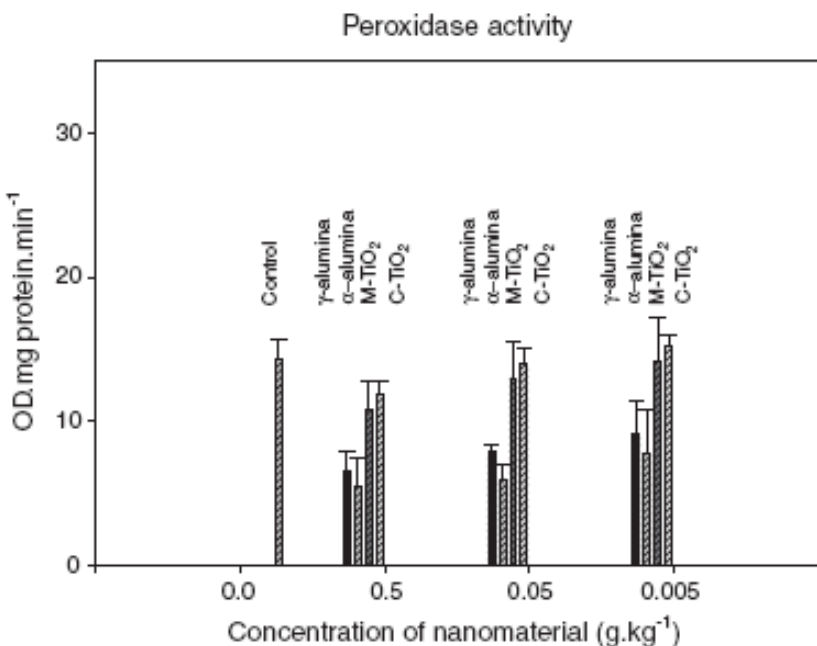
Notes: BET: Brauner, Emmett, and Teller; XRD: X-ray diffraction; SEM; scanning electron microscope.

Table 2

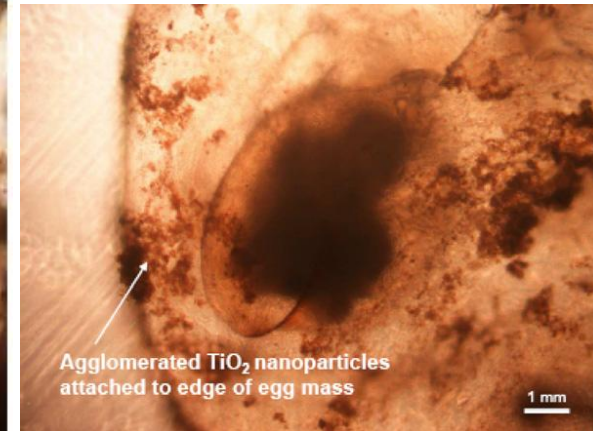
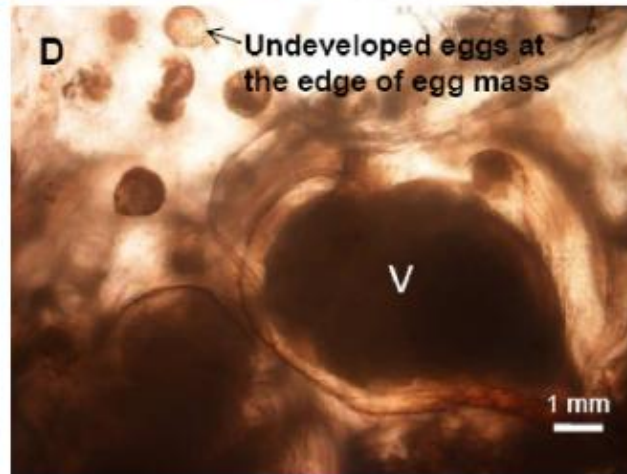
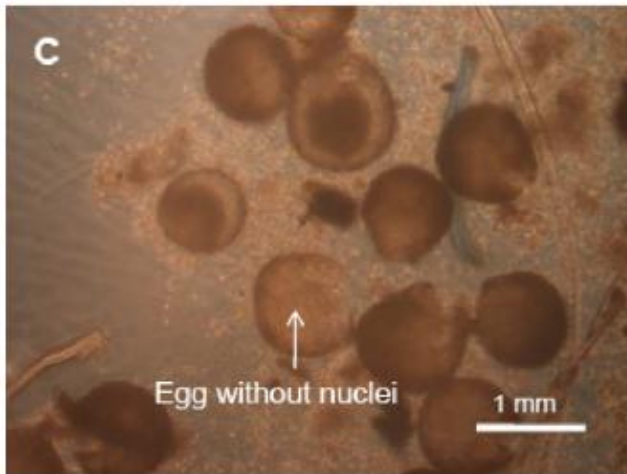
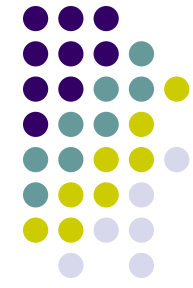
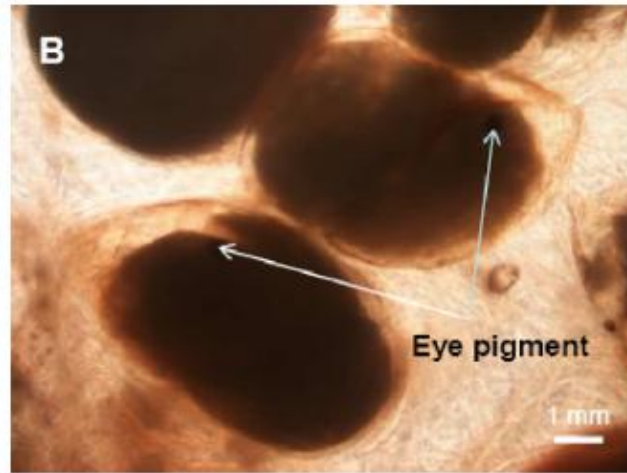
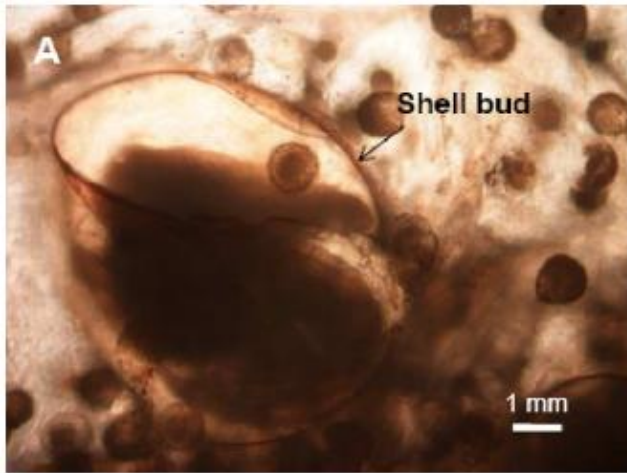
Egg production and hatching success for *Physa acuta* during 28-d period.

Concentration (g kg ⁻¹)	Number of egg masses from week 1 to 4	Number of eggs week 1 to 4	Mean (±SD) eggs per egg mass	Total (±SD) hatchlings in 4 weeks	Mean% per week
<i>M-TiO₂</i>					
0 (control)	11	201	18 (±2)	156 (±4)	19
0.005	10	200	20 (±3)	161 (±3)	20
0.05	11	198	18 (±1)	163 (±6)	20
0.5	11	194	17 (±2)	167 (±4)	21
<i>C-TiO₂</i>					
0 (control)	10	200	20 (±2)	147 (±4)	18
0.005	11	205	18 (±1)	162 (±4)	19
0.05	11	202	18 (±3)	157 (±5)	19
0.5	10	199	19 (±2)	164 (±6)	20
<i>α-Alumina</i>					
0 (control)	10	197	19 (±3)	161 (±4)	20
0.005	10	140	14 (±3)	112 (±5)	20
0.05	6	71	11 (±2)	40 (±3)	14
0.5	3	25*	8 (±1)*	8 (±1)*	8*
<i>γ-Alumina</i>					
0 (control)	11	198	18 (±2)	161 (±5)	20
0.005	9	131	14 (±3)	92 (±6)	17
0.05	5	49	9 (±2)	18 (±1)*	9*
0.5	3	22*	7 (±1)*	7 (±1)*	7*

* Significant difference compared with control ($P < 0.05$).



(Musee et al., Chemosphere, 2010)



A: Delayed hatching & anomalies of embryogenesis.

B: Normal eggs (control – hatches in 10 days)

C: No nuclei & no development after 4 weeks

D: Exacerbated delays in hatching process

Musee et al., Chemosphere, 2010

Fate of SiO₂ NPs in wastewater

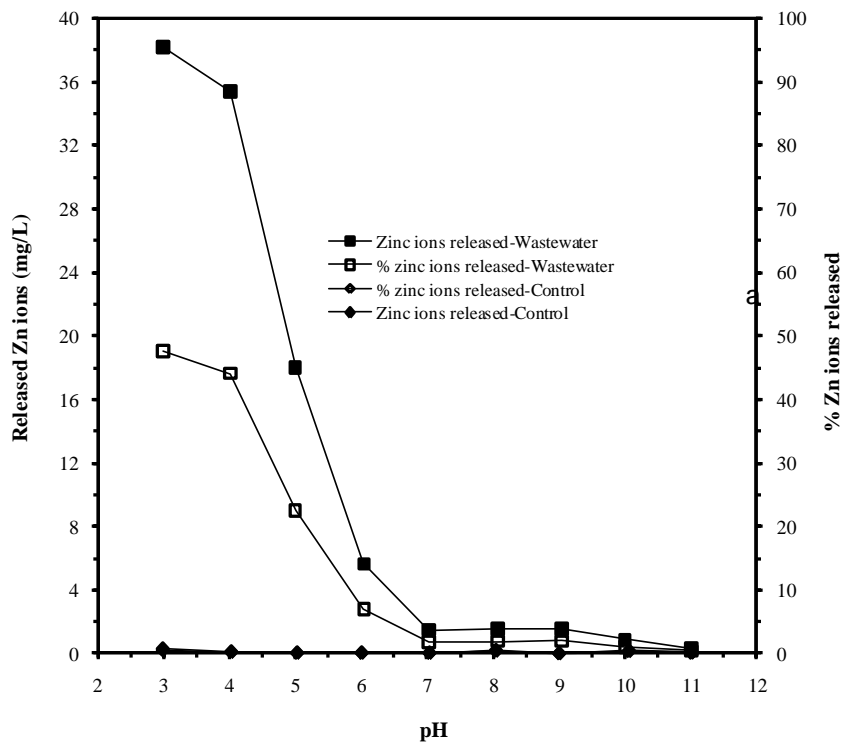


TABLE 2. Summary of Supplementary Tests of SiO₂NP Colloidal Stability in Electrolyte Solutions

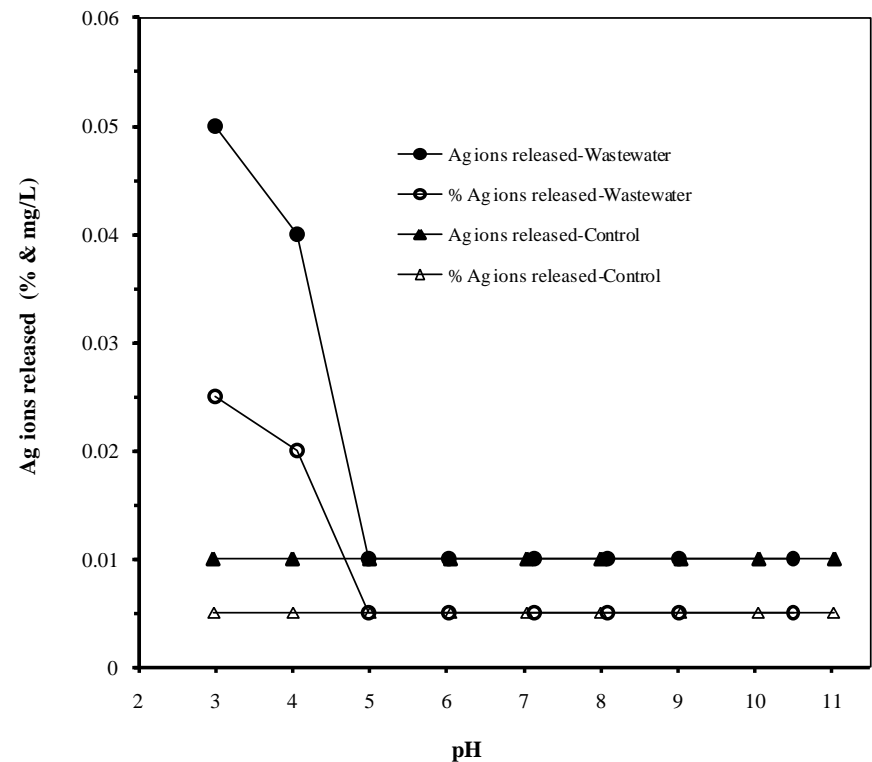
nanoparticle type	electrolyte solution	observations
unfunctionalized (uncoated) SiO ₂ NP	0.01 M La(NO ₃) ₃	rapid and significant flocculation within 8 min; complete flocculation after 30 min
	0.10 M La(NO ₃) ₃	slow flocculation over the first 30 min but incomplete
functionalized (Tween-coated) SiO ₂ NP	0.01 M NaCl	no effect after 72 h
	0.01 M La(NO ₃) ₃	no effect in first 5 min; slow flocculation over the next hour; complete flocculation after 2 h
	0.10 M La(NO ₃) ₃	no effect in first 5 min; slow flocculation over the next hour; complete flocculation after 2 h

Key findings:

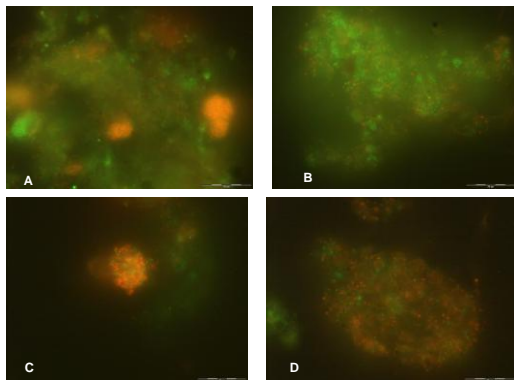
- Surface-functionalized SiO₂NPs likely to be removed via sedimentation to sewage sludge
- Uncoated SiO₂NPs will continue through the effluent stream (likely to go to secondary treatment stages)



Release kinetics of Zn²⁺ from ZnO NPs as a function of pH in wastewater



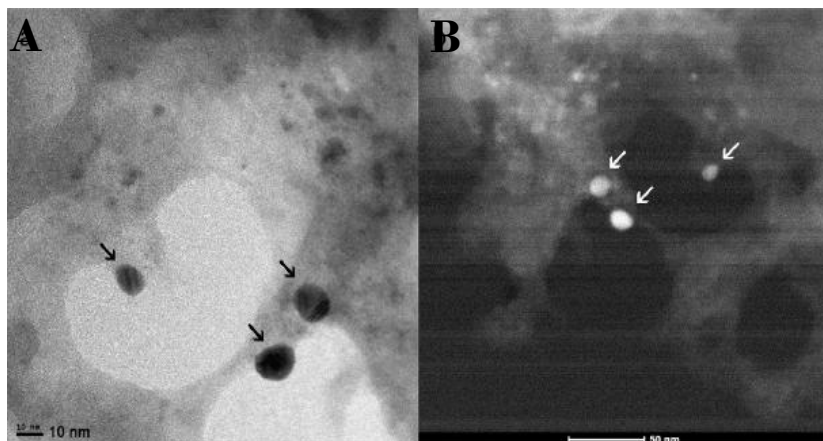
Release kinetics of Ag⁺ from AgNPs in wastewater as a function of pH



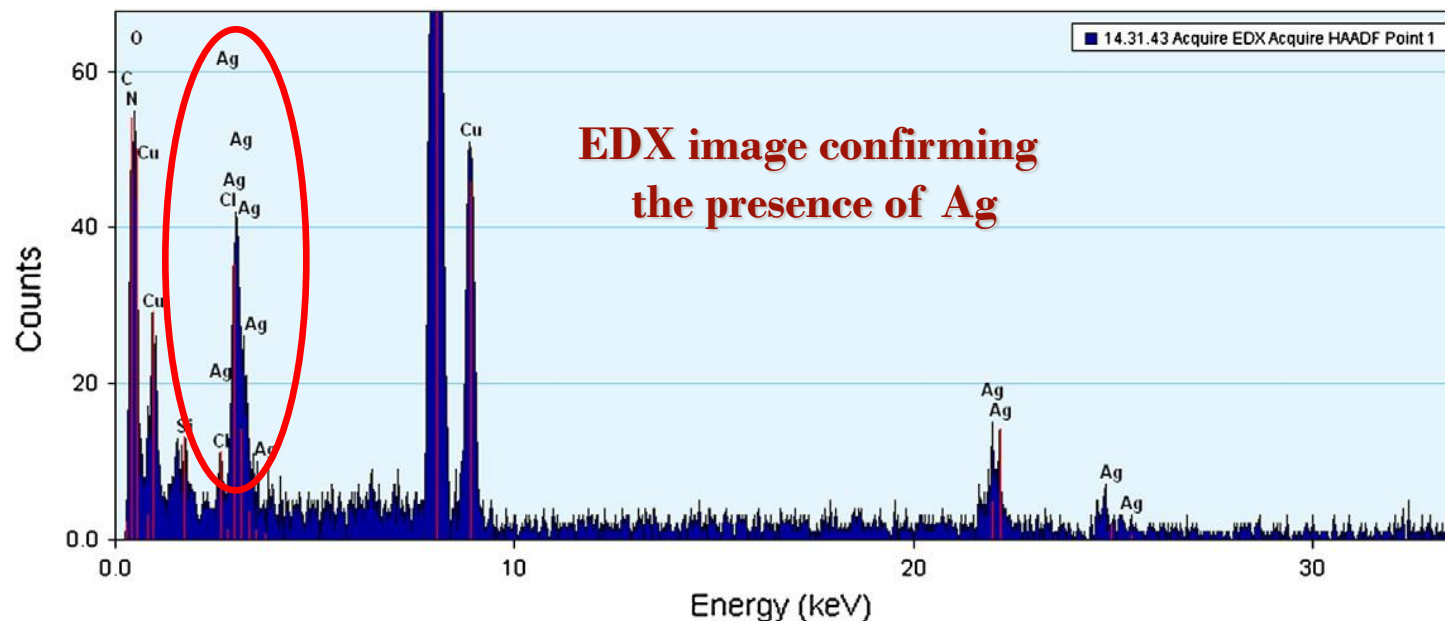
Dead/live baclight tests show enhanced dead Bacteria after exposure to NPs

Musee et al., 2012 (unpublished data)

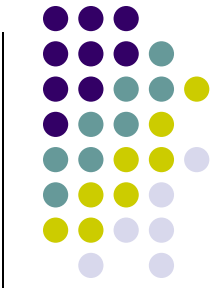
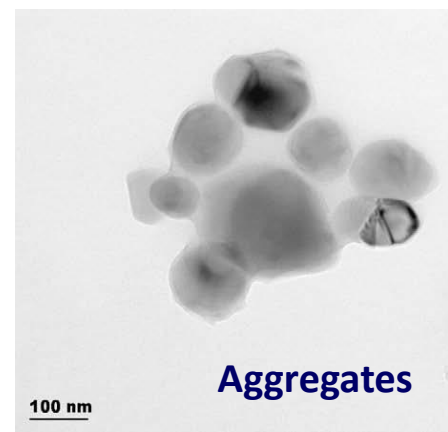
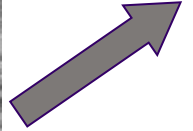
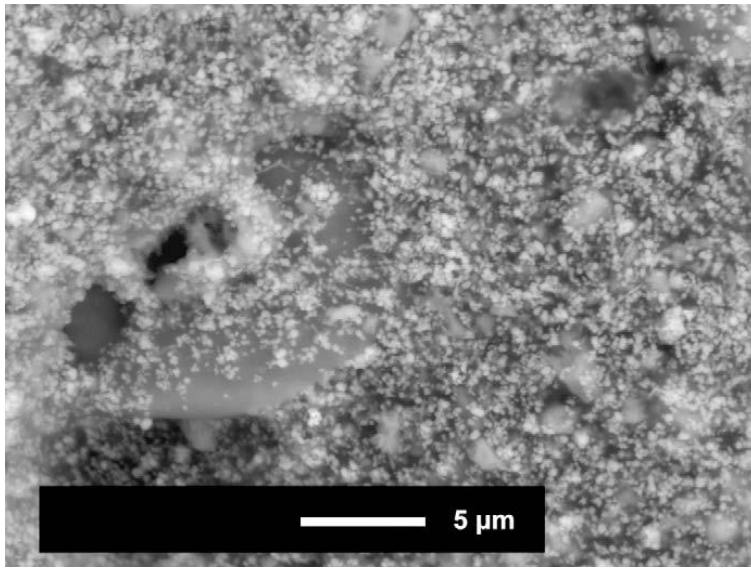
Fate of ENMs in actual environmental systems



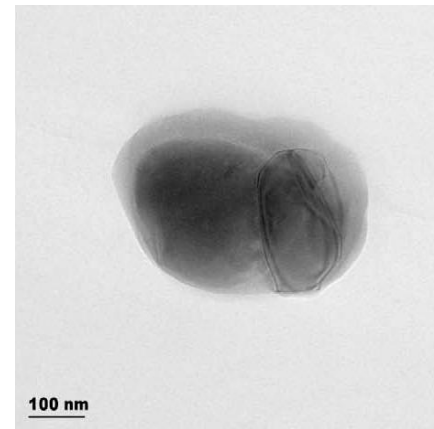
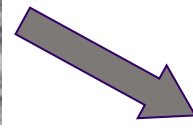
Average Ag-NP size in the effluent ~ 10 nm measured with TEM and STEM (St dev=3.2 nm, range 5–18 nm; n=26). The particles were spherical or irregular. TEM (a) and high angle annular dark field (HAADF) images; (b) of the nanoparticles (black and white arrows) in the washing machine effluent.



Farkas. et al. Environ. Int. (2011).

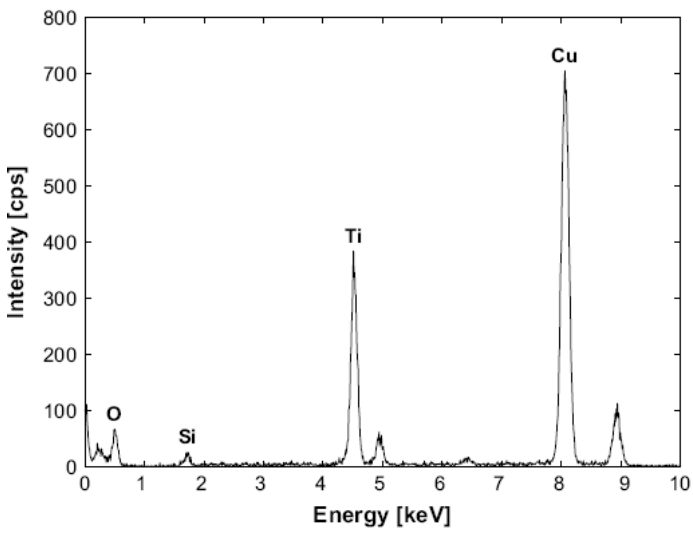


TEM bright field image of TiO₂ particles from the runoff of the new facade



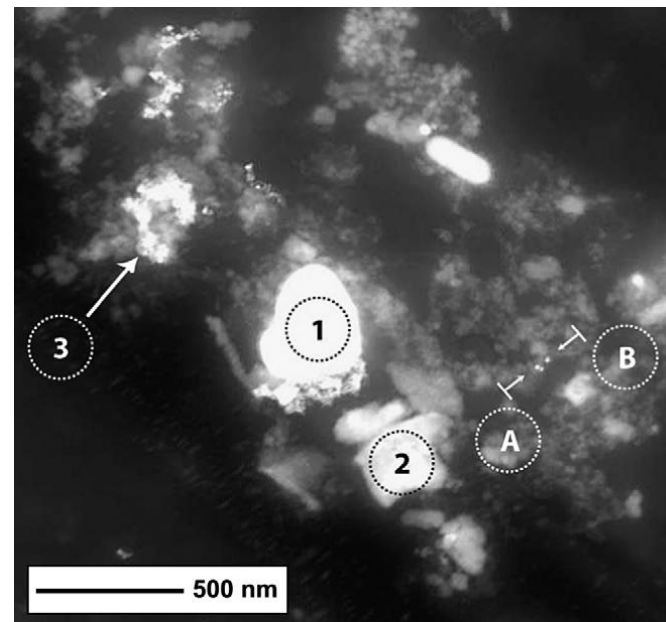
SEM-BSE image of the aged façade (white spots represent nTiO₂ particles).

Synthetic nTiO₂ within a size range of a few tens to a few hundreds of nm in diameter were successfully detected and identified in the environment using a combination of analytical electron microscopy (TEM-EDX) and bulk chemical (ICP-MS) methods.



Kaegi et al. Environ. Poll. 156 (2008) 233–239

TEM-EDX of TiO₂ particles detected in the runoff of the aged facade.



Model house with panels with the Ag-NP paint

TEM image (HAADF-STEM) of the Ag-NP

- Findings provide first direct evidence for the release of Ag-NP from a typical outdoor application to the aquatic environment.
- About 30% of the Ag-NP initially contained in the paint were lost within one year of exposure
- Ag-NP were attached to an organic binder from the paint and released mostly as composite colloids.
- Microscopic findings clearly showed the difficulties encountered in detecting, or monitoring ENMs in the environment

Kaegi et al. Environ. Poll. 158 (2010) 2900 – 2905

Nanowastes classification



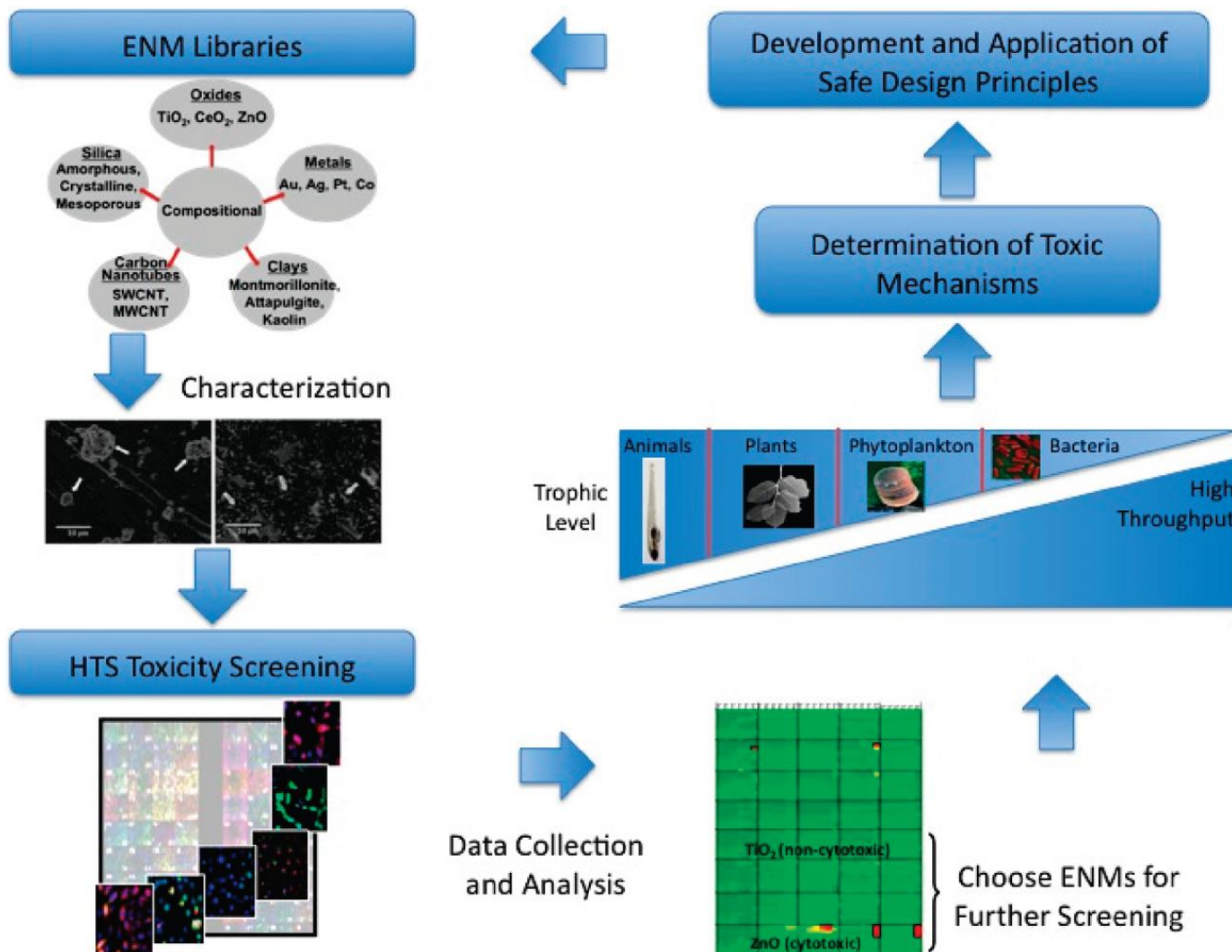
Table 4

Few examples of risk profiles of nanoproducts and/or applications containing different NMs at the disposal life cycle phase.

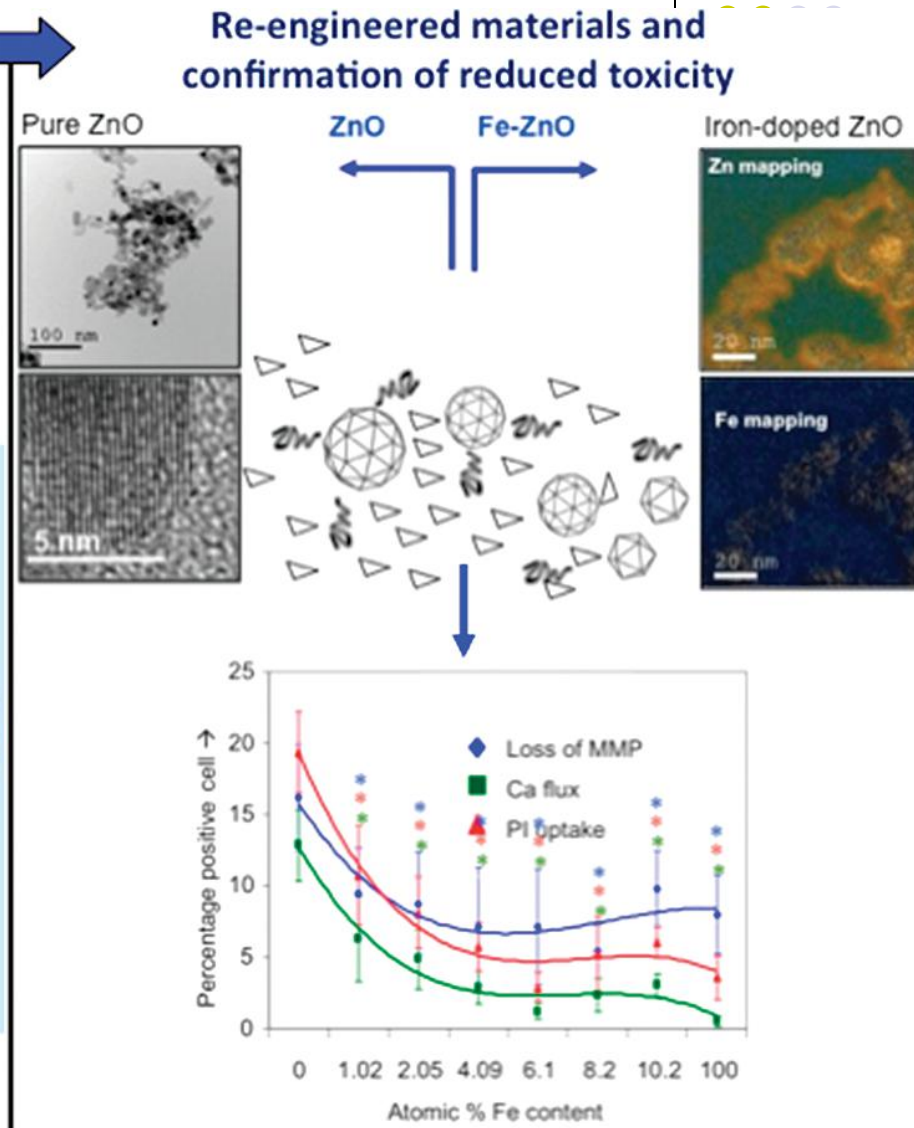
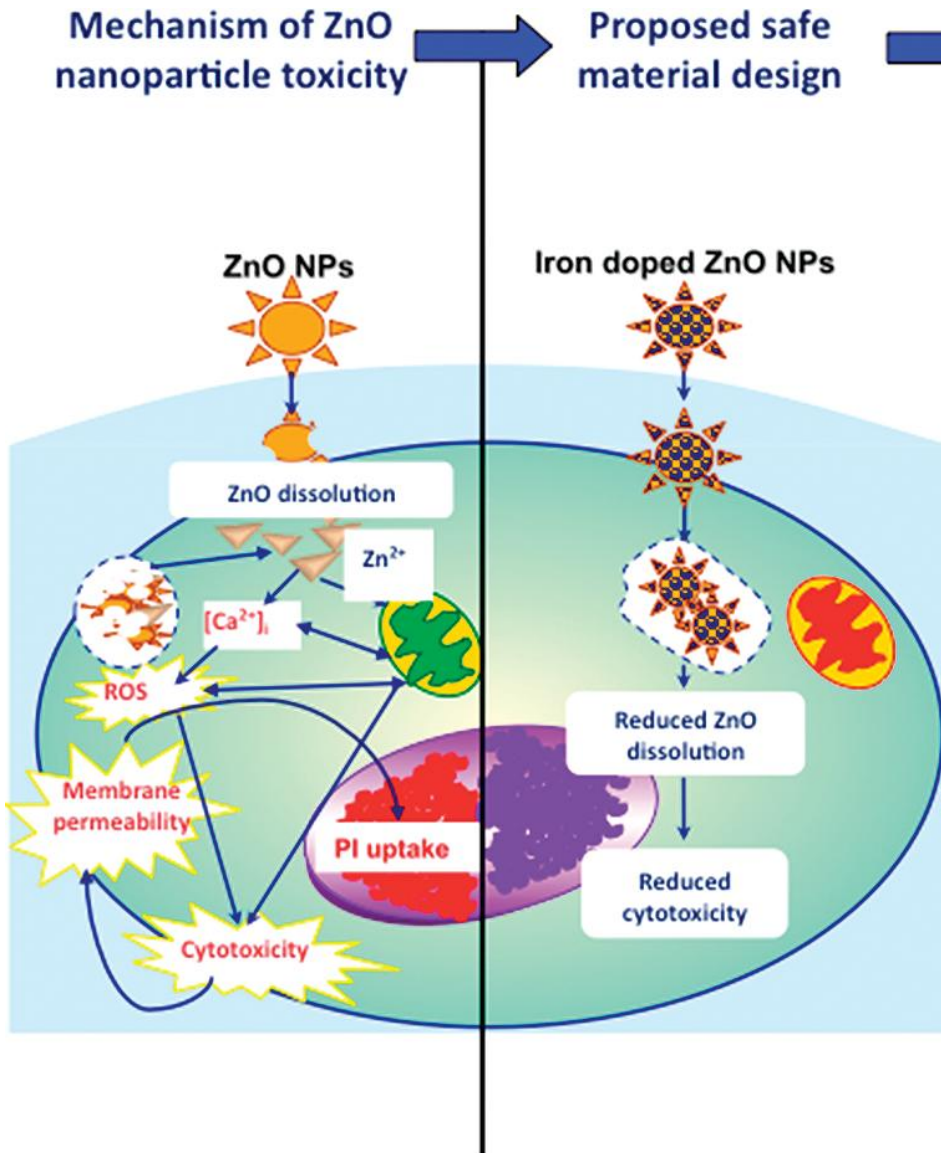
Application	NMs	Hazard	Exposure potency	Risk at disposal	Potential nanowaste class
Personal care pro.	Ag	Medium	High	Medium	Class II/Class III
	Fullerenes	High	High	High	Class IV/Class V
	Fe ₂ O ₃	Medium	High	Medium	Class II/Class III
	TiO ₂	Low	High	Low	Class I
Food/ beverages	TiO ₂	Low	Medium	Low	Class I
	ZnO	Medium	Medium	Medium	Class II/Class III
	Fullerenes	High	Medium	High	Class IV/Class V
Sunscreen lotions	Dendrimers	Medium	Medium	Medium	Class II/Class III
	ZnO	Medium	High	Medium	Class II/Class III
	TiO ₂	Low	High	Low	Class I
	Fullerenes	High	High	High	Class IV/Class V
Automobile parts	Dendrimers	Medium	High	Medium	Class II/Class III
	SWNCT	High	Medium	Medium	Class II/Class III
	MWNCT	High	Medium	Medium	Class II/Class III
	Nanoclays	Low	Medium	Low	Class I
Polishing agents	Fullerenes	High	Medium	Medium	Class II/Class III
	TiO ₂	Low	High	Low	Class I
	ZnO	Medium	High	Medium	Class II/Class III

Class I has lowest risk profile, Classes II and III exhibits moderate (medium) risk levels, and Classes IV and V have the highest degree of risk.

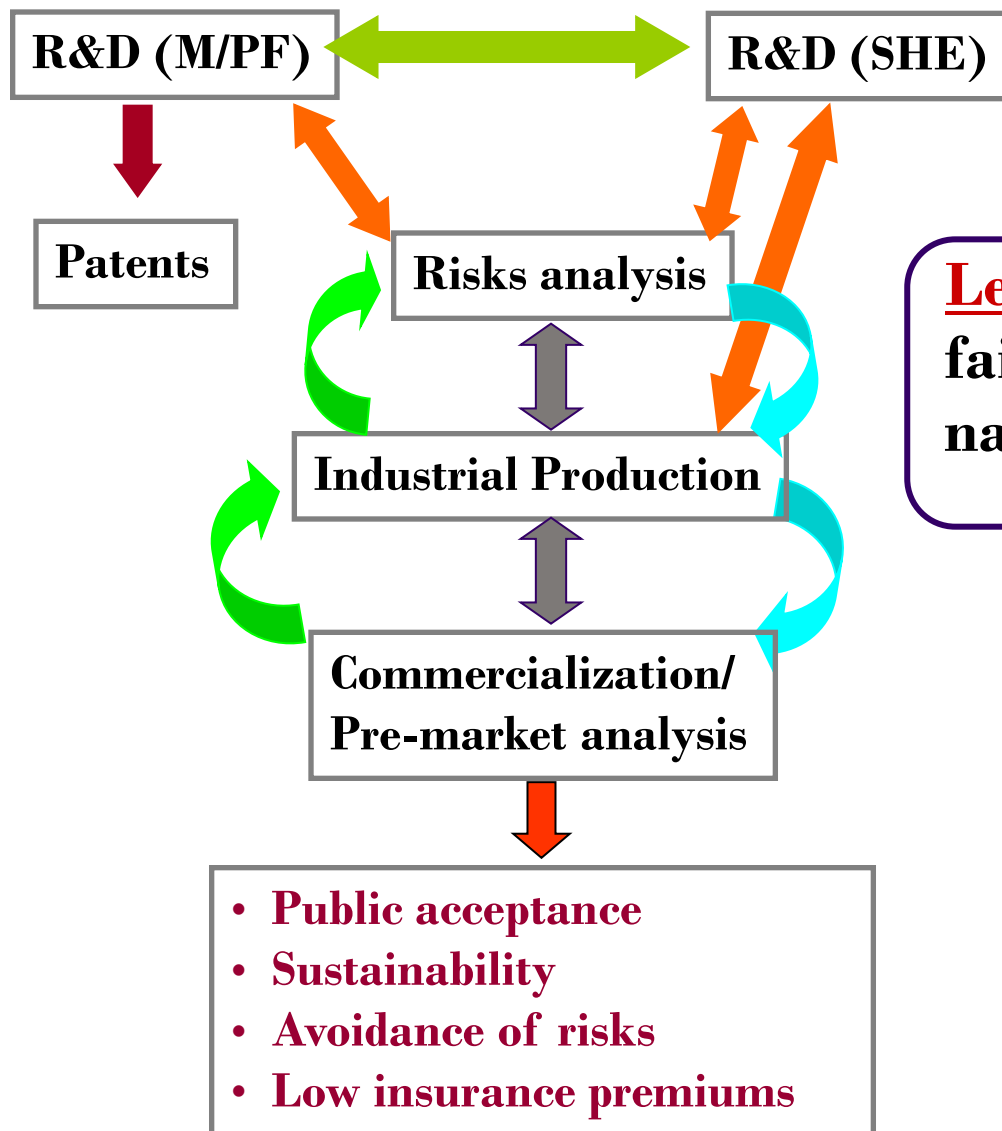
High throughput screening mechanisms



Safe design of ENMs



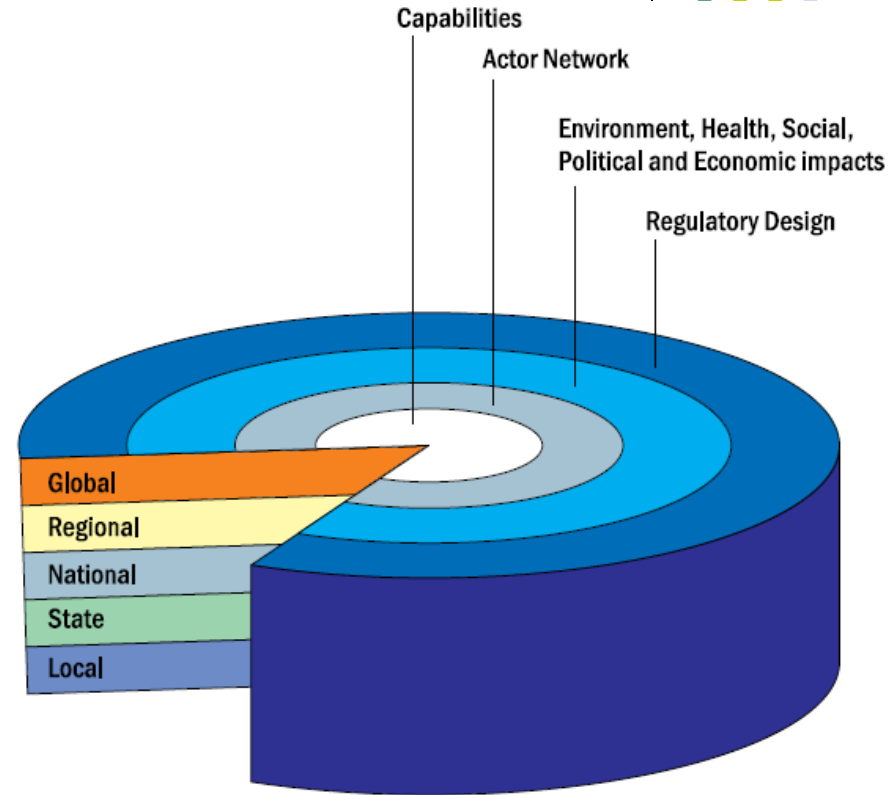
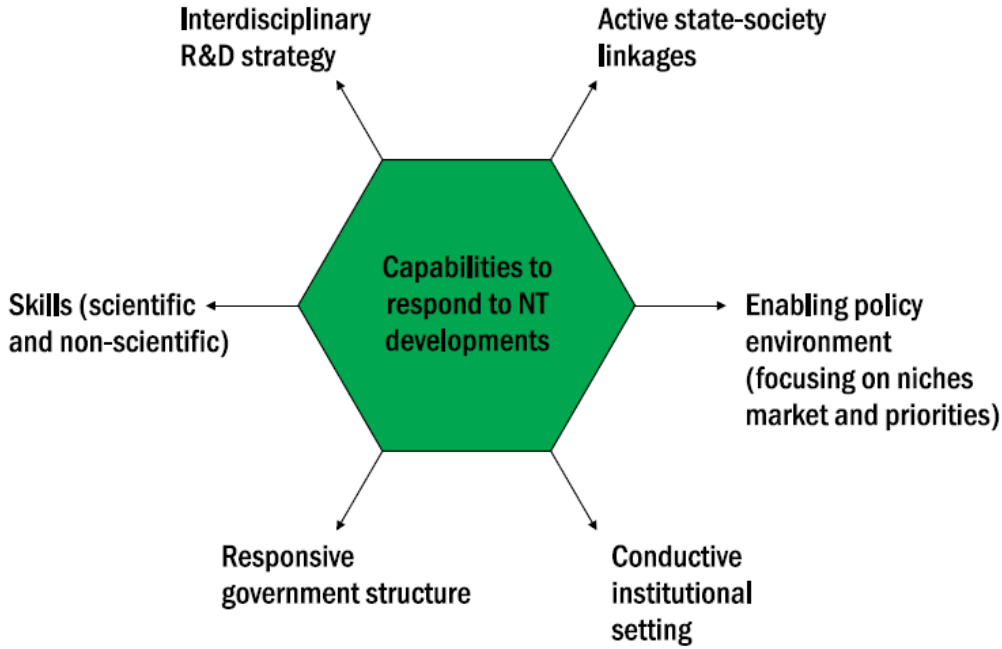
Proactive approach to Nanotech Sustainability



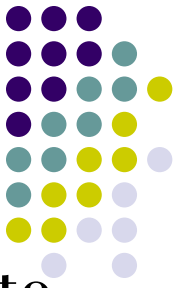
Lesson: Past technological failures can be avoided during nanotechnology era.

Musee et al., S. Afr. J. Sci. 2010

Future outlook



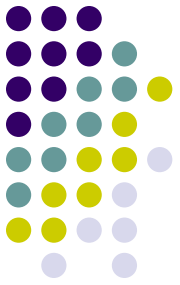
Paths Forward



- Focused integration of lab-based risk assessment research to nanoproducts impacts on actual environmental systems
- Formation of collaborations at national, regional, continental and global (including economic groupings) to address nanotech HSE aspects (e.g. policy/decision makers, scientists & engineers)
- Development of nanotech specific regulations to govern research and commercial applications of nanotechnology (e.g. products labeling). Challenges include:
 - Inadequate regulatory capacity
 - Information asymmetry
 - Financial constraints
 - Lack of expertise (technical know-how)
 - Absence of interagency coordination

The End....

But... Small Baby Steps moves on ...



Acknowledgements

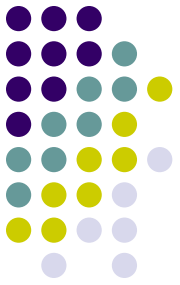


science
& technology

Department:
Science and Technology
REPUBLIC OF SOUTH AFRICA



And ... colleagues and students



Copyright © 2004 Creators Syndicate, Inc.

Thank you for your attention