Environmental risk assessment of nanomaterials and nanotech-based products

International Conference on Nanotechnology for Safe and Sustainable Development & Consultative Meeting on proposed Nanosafety Networking Platform
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3 May 2017

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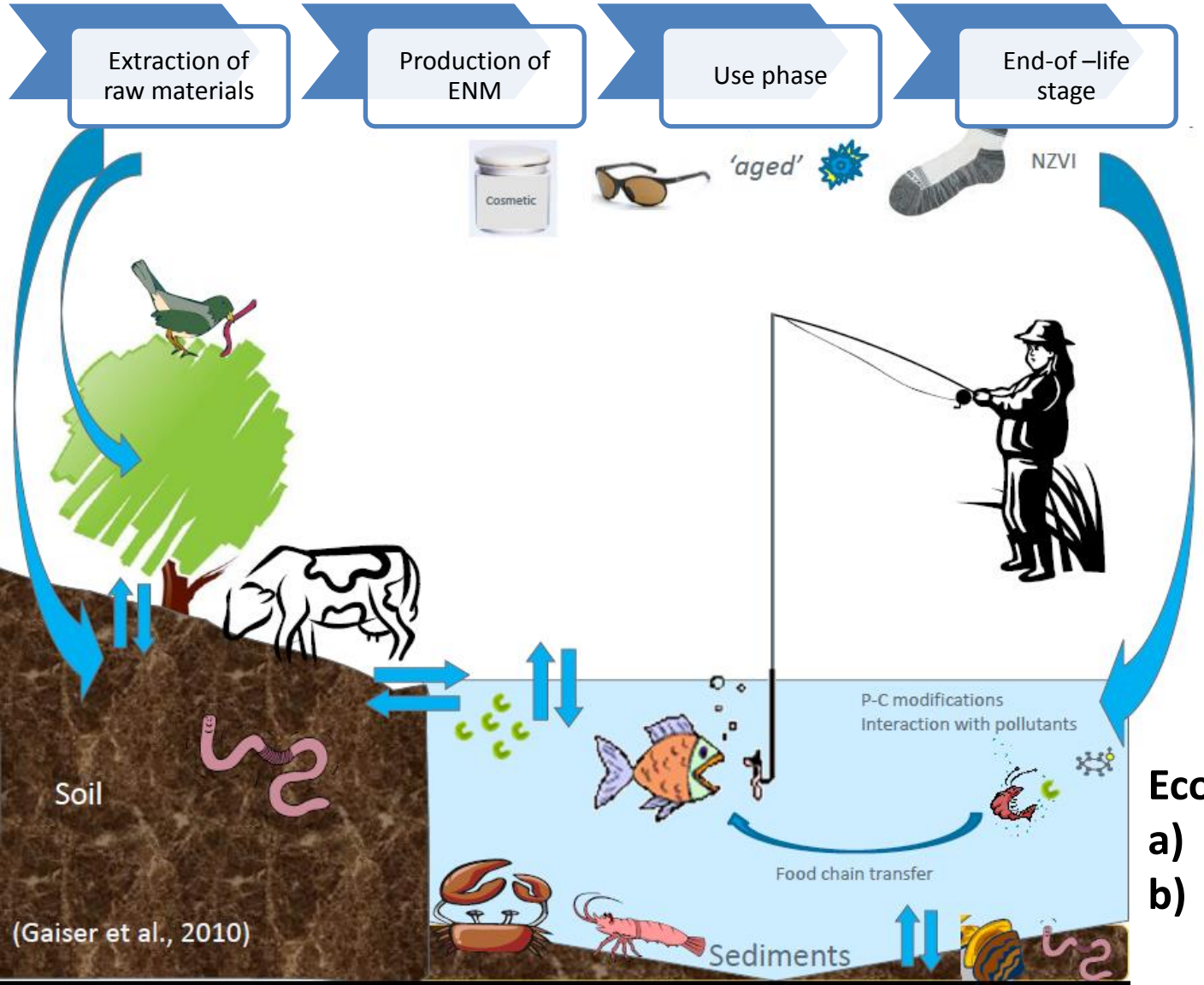
The release of nanomaterial into the environment



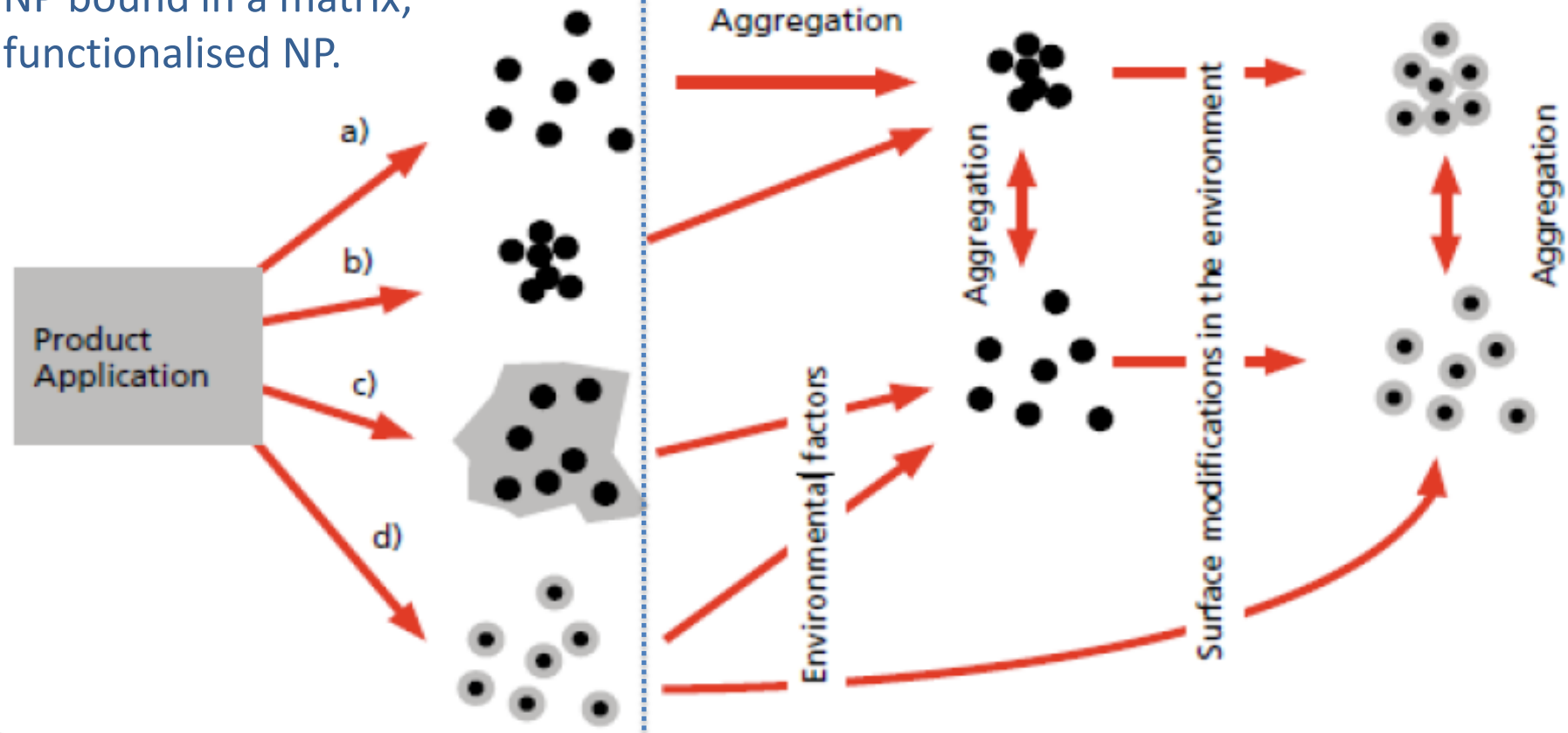
RISK= HAZARD x EXPOSURE

Ecotoxicology studies

a) **Aquatic system**
b) **Soil and sediments**

Release of nanoparticles (NP) are in different forms such as;

a) free NP;
b) aggregated NP;
c) NP bound in a matrix;
d) functionalised NP.



Environmental factors influence the agglomeration /deagglomeration of NP

Variability of L(E)C50 values within the same organism group

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Bulk metal oxides

No.	Group of organisms	Reference compounds		
		Bulk metal oxides		
		mg TiO_2/l Bulk TiO_2	mg ZnO/l Bulk ZnO	mg CuO/l Bulk CuO
1	Crustaceans	20000 (3)	0.48 (3)	127.8 (2)
2	Bacteria	20000 (1)	20.0 (3)	3758 (1)
3	Algae	**60** (1)	**0.052** (2)	**14.2** (1)
4	Fish	500 (2)	1.8 (2)	NF
5	Ciliates	NF	4.9 (1)	1947 (1)
6	Nematodes	137 (1)	2.2 (1)	NF
7	Yeasts	20000 (1)	134.4 (1)	1277 (1)
1-7	No. of data	9	13	6
1-7	Lowest L(E)C50	**60**	**0.052**	**14.2**
1-7	Most sensitive organisms	Algae	Algae	Algae
1-7	Classification (1-7)[b]	Harmful	Extremely toxic	Harmful
1-3	Classification (1-3)[c]	Harmful	Extremely toxic	Harmful
	Risk phrases (R)[d]	NF	R50/R53	NF
	Hazard class and category code (hazard statement code)[e]	NF	Aquatic Acute 1 (H400); Aquatic Chronic 1 (H410)	NF

Nano metal oxides

No.	Group of organisms	Inorganic nanoparticles			
		mg TiO_2/l Nano TiO_2	mg ZnO/l Nano ZnO	mg CuO/l Nano CuO	mg Ag/l Nano Ag
1	Crustaceans	67.7 (10)	0.62 (3)	2.65 (2)	**0.040** (1)
2	Bacteria	603 (4)	20 (3)	71 (2)	7.60 (5)
3	Algae	**65.5** (4)	**0.068** (2)	**0.87** (1)	0.23 (2)
4	Fish	300 (4)	1.9 (2)	NF	7.1 (1)
5	Ciliates	NF	5.4 (1)	156.5 (1)	39.0 (1)
6	Nematodes	80.1 (1)	2.24 (1)	NF	NF
7	Yeasts	20000 (1)	121.2 (1)	20.5 (1)	NF
1-7	No. of data	24	13	7	10
1-7	Lowest L(E)C50	**65.5**	**0.068**	**0.87**	**0.040**
1-7	Most sensitive organisms	Algae	Algae	gae	Crustaceans
1-7	Classification (1-7)[b]	Harmful	Extremely toxic	ery toxic	Extremely toxic
1-3	Classification (1-3)[c]	rmful	Ext ely to	ery toxic	Extremely toxic

Classification is based on <u>median L(E)C50 value of the mo</u>

<0.1 mg/l = extremely toxic to aquatic organisms;

0.1–1mg/l = very toxic to aquatic organisms;

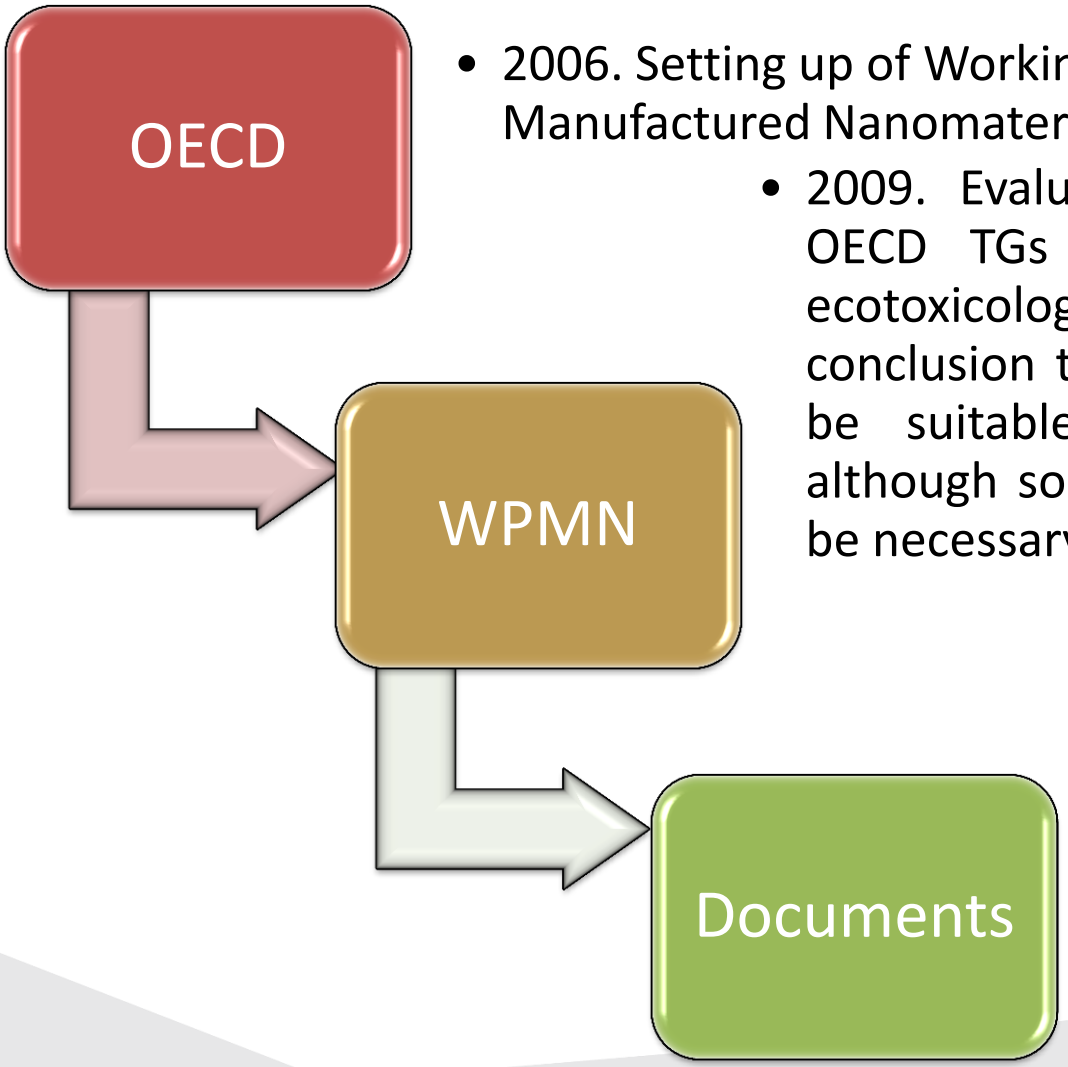
1–10 mg/l = toxic to aquatic organisms;

10–100 mg/l = harmful to aquatic organisms;

>100 mg/l = non-toxic to aquatic organisms.

Nano Ag> ZnO>CuO>TiO2

FROM ECOTOXICOLOGY TO NANOECOTOXICOLOGY



- 2006. Setting up of Working Party on Manufactured Nanomaterials (WPMN)
  - 2009. Evaluated the applicability of the OECD TGs and with a focus on the ecotoxicological test guidelines. The conclusion that the TGs are considered to be suitable for the testing of NMs; although some adaptations were found to be necessary.
    - 2012. No. 36, Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials.
    - 2014. No . 40, Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines

“Not all NP formulations have been found to induce a more pronounced toxicity than the bulk formulations of the same substance. This suggests that the evaluation of nanoparticle formulations should be carried out on a case by case basis.”

Source: SCENIHR Report (2007)

Analytical challenges associated with nanoecotoxicological studies

a) Dispersion protocols for aquatic ecotoxicology
b) Measurements techniques and metrics
c) Application of the ENM to the test system
d) Endpoints for environmental behaviour

The choice of appropriate **dispersion method** depends both on the test to be performed and the type of ENM to be dispersed.

Key parameters for the dispersion of ENMs:

(i) ultrasonication procedures (e.g. sonicator type, energy output, amplitude, and delivered energy),
(ii) water quality/composition (eg. influence bioavailability of metal ions)
(iii) pre-wetting steps (eg. use of ethanol for hydrophobic NMs)
(iv) stabilising / dispersing agents (eg. inclusion of NOM into test dilutions)
(v) stock concentrations (to ensure high monodisperity and whether the 80% target concentration in the test system; as suggested in TGs for chemicals is also an appropriate level for ENMs.

Measurements techniques and metrics

For example;

1. In situ measurement of anodic stripping voltammetry (ASV) with a hanging mercury drop electrode to determine the concentration of dissolved metal in metal oxide NP suspensions. (Jiang, C., 2016)
2. Use of recombinant microbial sensors (eg E. coli or yeast) to determine the concentration of dissolved metal in metal oxide NP suspensions. (Aruoja *et.al*, 2009)

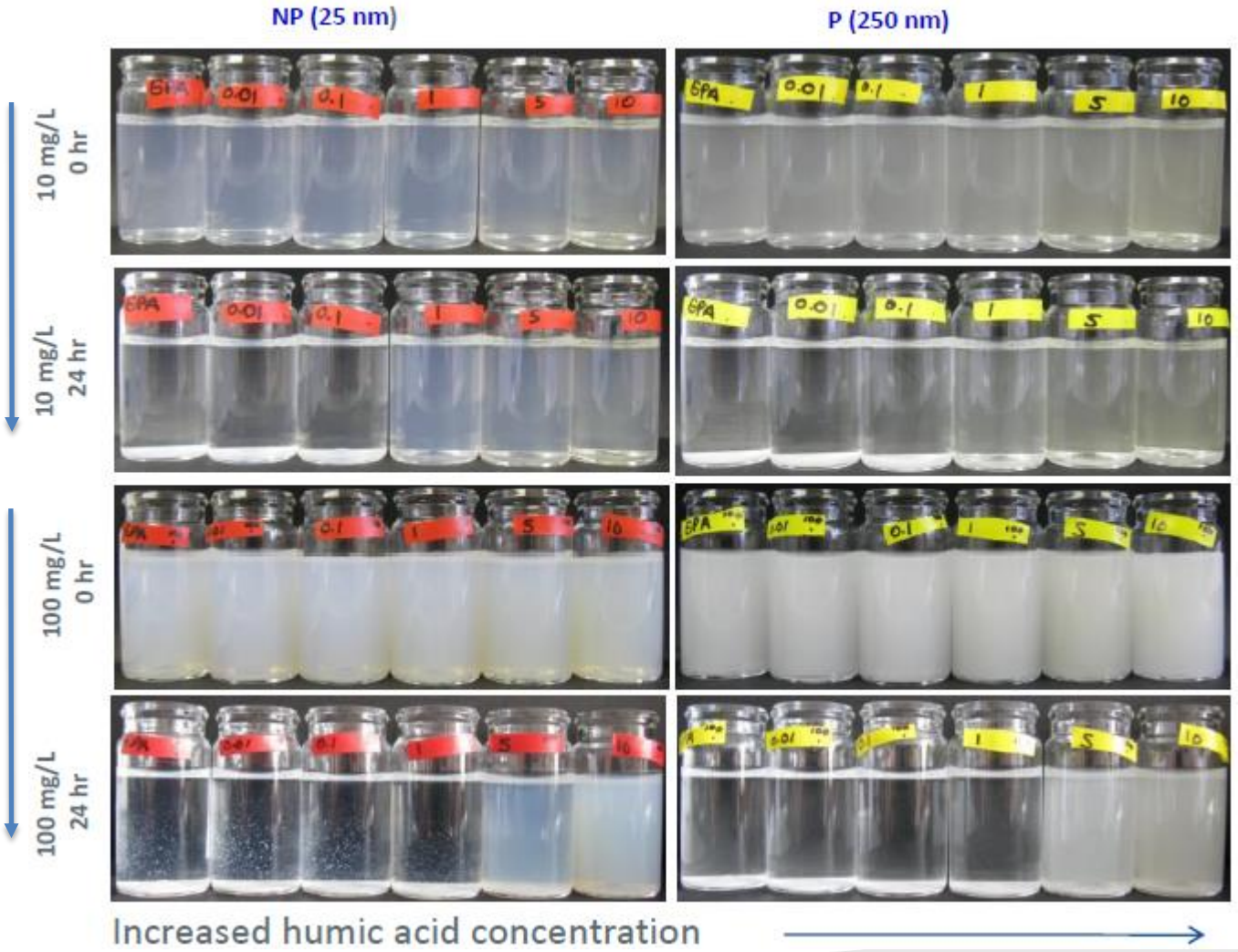
1. The mass metric appears not always to be the most appropriate or relevant one. Conversion between the metrics of mass, number and surface area remains challenging.

Note : Dosimetry refers to estimating or measuring the amount (in terms of mass, number, surface area, volume, etc.) of a particle at a specific biological target site at a particular point in time.

Stability of TiO_2 in organic matrix

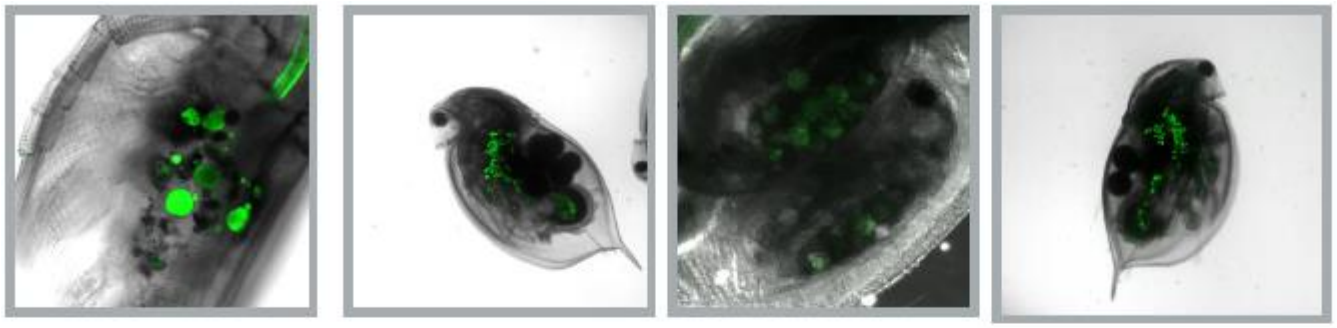
For the same type of NOM, TiO_2 NP disaggregation rates increased with NOM concentration.

The study suggests that typical environmental concentrations of (eg. humic acids) are sufficient to stabilize TiO_2 NPs, even if present at high concentration and that they can play important roles in the disaggregation and dispersion of already formed nanoparticle aggregates.

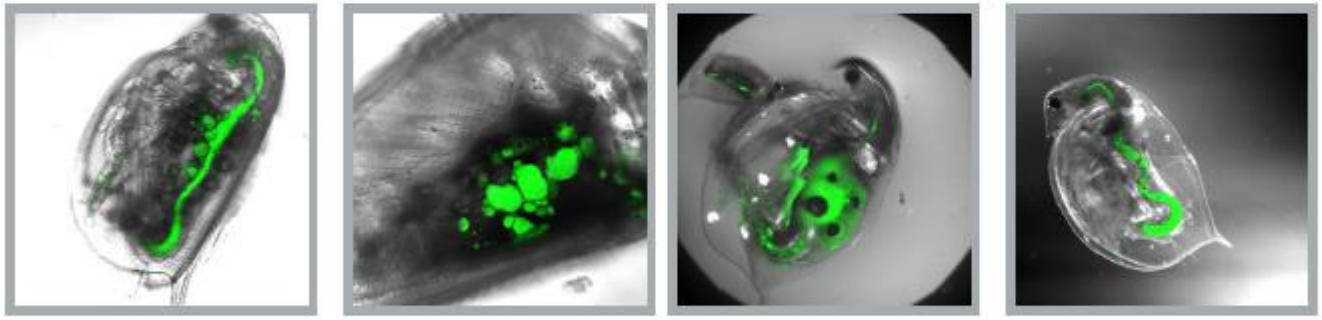


Source: Loosli, F *et.al* (2013)

Uptake of nanoparticles by *Daphnia magna* is size dependent



20nm



1000nm

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

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

Rosenkranz et al (2009)

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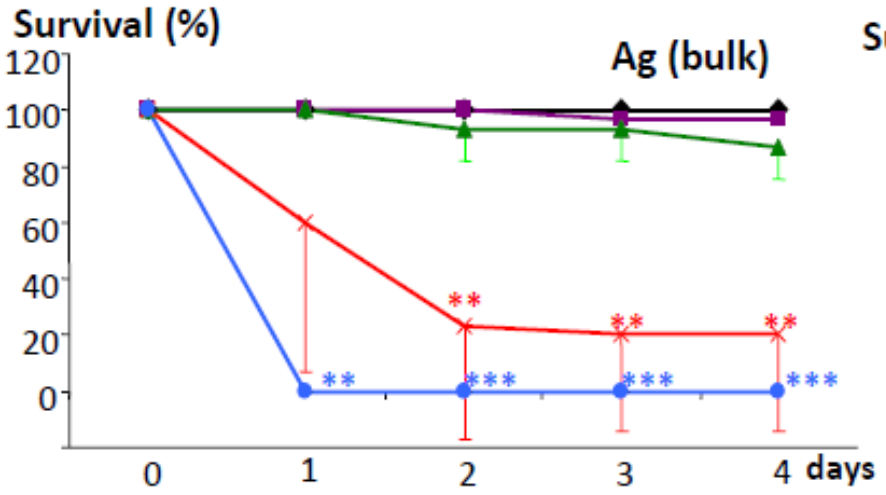
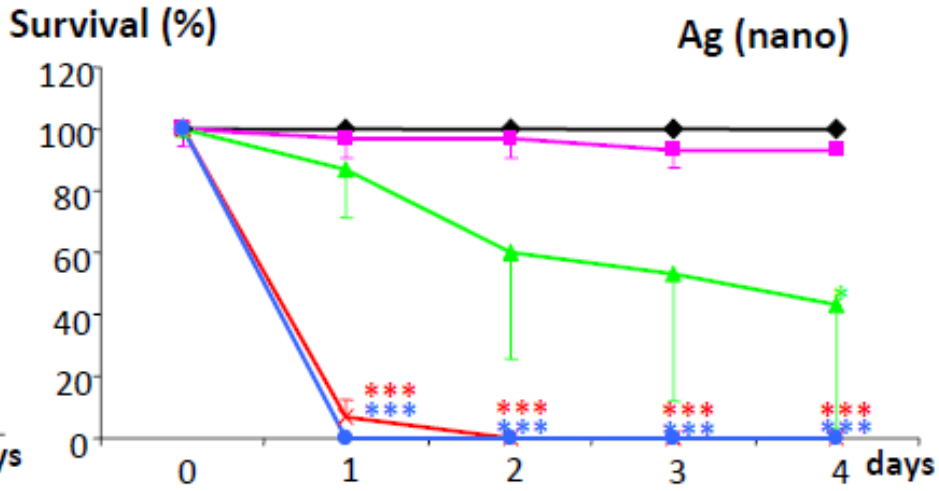
Toxicity of Ag particles to *Daphnia magna*





µg/ml Ag: control 0.01 0.1 1 10

Bulk Ag:

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

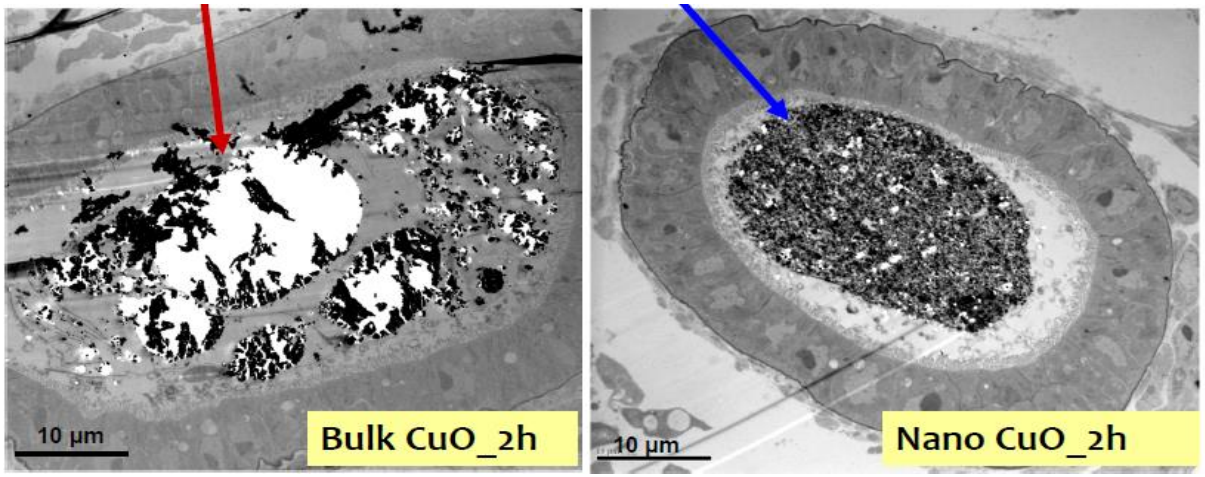
Nano-Ag:

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

Nanoecotoxicity and bioavailability of metal ions

Bulk CuO clump

Nano CuO dispersed



TEM images of the *Daphnia* midgut (Heinlaan *et.al*, 2010)

Chemical	*Daphnia magna* mortality LC_{50} (48 hrs) mg/L	Reference
ZnO	8.8 ± 1.4	Heinlaan *et.al* (2008)
Nano ZnO	3.2 ± 1.3	
CuO	164.8 ± 24.6	
Nano CuO	3.2 ± 1.6	

The Applicability of OECD Test Guidelines for ENM (OECD Doc. No 40)

		Applicability of OECD Test Guidelines	Recommendation
Aquatic system	Eco-toxicology	**TG 201** Freshwater Alga and Cynobacteria, Growth Inhibition **TG 202** *Daphnia sp.* Acute Immobilisation test **TG 211** *Daphna magna* Reproduction Test	Chronic toxicity testing should be conducted in environmentally realistic concentration. Effects of pristine vs aged ENM Detail study of ENM interaction with animal organs
	Environmental Fate & Behaviour	**TG 305** Bioconcentration: Flow through Fish test	Same stock suspension should be used for ecotox and fate analysis.
Soil and sediments	Eco-toxicology	TG 222 TG225	
	Environmental Fate & Behaviour	TG 312 TG 315 TG 317	

A summary of modification of ecotoxicity testing protocols with a focus on the information requested by regulatory bodies for safety assessment of NMs.

	OECD Test Guidelines	Issues	Proposed TG modification
1.	TG 201 Freshwater Alga and Cynobacteria, Growth Inhibition	NMs interfere with component of test medium Eg. EDTA – causing Fe precipitation	Use modified OECD M algal medium which; a) Exclude EDTA b) Increase algal growth by replacing $FeCl_3$ with $FeSO_4$ and 5 times phosphorus content as monobasic and dibasic salts
		NMs (eg. TiO_2) interfere with biomass cells counting via optical density (OD) reading and *in vivo* fluorescence	*In vitro* Chlorophyll (Chl a) determination via extraction followed by fluorometry measurement. * Can be inaccurate if toxicants affect the chlorophyll content of the algal cells.

	OECD Test Guidelines	Issues	Proposed TG modification
2.	TG 202 *Daphnia sp.* Acute Immobilisation test	Sedimentation of NMs & reduced exposure concentration for daphnids	Acquire basic information on the test material's point of zero change, pH range and suitable media composition that would lead to low aggregation in test solution. Increase NM dispersion via the use of very low ionic strength medium (eg. Very Soft EPA medium) Come up with evidence of internal uptake of NM by the test organism.

	OECD Test Guidelines	Issues	Proposed TG modification
3.	TG 210 Fish, early-life stage toxicity test	Sedimentation of NMs & reduced exposure concentration for fish larvae Fish larvae more sensitive than embryo to the tested NMs	Use of exposure chamber and renewal of water every 24 hours. Come up with evidence of internal uptake of NM by the test organism. Validate whether able to maintain the nominal concentration of NMs within ± 20% as prescribed in the TG.

Conclusion

1. For regulatory purposes, highly standardize procedures have to be applied to ensure comparability of results.
2. Technique and protocols for dispersing NMs are important before proceeding with the nanoecotoxicological studies.
3. Though the proposed OECD TG modification using various NMs produce reproducible results; however OECD level guidelines have yet to developed.
4. Environmental risk assessment also covers terrestrial ecotoxicology study and Environmental Fate & Behavior, both at aquatic and terrestrial ecosystems; hence other modified methodological approaches for testing NMs are also part initiative under the WPMN expert meeting.

Recommendation for collaborative work at national and international level

1. Sharing of expertise in analytical measurement verification.
2. Financial and capacity building funding to move from ecotoxicology to nanocotoxicology.
3. Engagement with other contract research organisations for studies related to Ecotoxicology and Environmental Fate & Behaviour

Reference

1. Hund-Rinke, K. *et.al* (2016). Regulatory ecotoxicity testing of nanomaterials – proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles. *Nanotoxicology*, 10:10, 1442-1447.
2. Cupi, D. *et.al* (2016). Influence of pH and media composition on suspension stability of silver, zinc oxide, and titanium dioxide nanoparticles and immobilization of *Daphnia magna* under guideline testing conditions. *Ecotoxicology and Environmental Safety* 127: 144–152.

Product Safety and Hazard Assessment Section

INTEGRATED GLP STUDIES FOR PRODUCT REGISTRATION

VALUE PROPOSITION

- One-stop integrated service centre with strategic partners to meet regulatory needs of the local and export market
- **Compliance to OECD Principles of Good Laboratory Practice (Full Adherence to OECD Council Acts)**
  (Registration No. : National Pharmaceutical Control Bureau GLP 001 & Standards Malaysia GLP 004)
- MS ISO/IEC 17025 accredited laboratories (SAMM Certificate No. 195)
- Contract research service for product development

COMPLIANCE TO REGISTRATION REQUIREMENTS :

INDUSTRIAL CHEMICAL
REACH
Occupational Safety and Health
(Classification, Packaging and Labelling)
Regulations 1997

PESTICIDE
Pesticide Act 1974: Requirements
on the active ingredients and formulations

PHARMACEUTICAL
Control of Drugs and Cosmetics
(amendment) Regulations 2007

COSMETIC
Control of Drugs and Cosmetics
(amendment) Regulations 2007

ANY REGISTRATION REQUIREMENTS
that require pre-marketing safety data

PARTNERSHIP with OECD-COMPLIANT FACILITIES for Full Scope of Pre-Clinical Studies Required for a Product.

PRE-CLINICAL SERVICES include :

HEALTH HAZARD

- Acute Oral Toxicity
- Balb/c 3T3 NRU Cytotoxicity
- *In Vitro* Skin Corrosion Test
- *In Vitro* Skin Irritation Test
- Skin Sensitization: Local Lymph Node Assay
- *In Vitro* 3T3 NRU Phototoxicity Test
- Skin Absorption: *In Vitro* Method
- Bacterial Reverse Mutation Test
- *In Vitro* Mammalian Chromosome Aberration Test

EFFECTS ON BIOTIC SYSTEMS

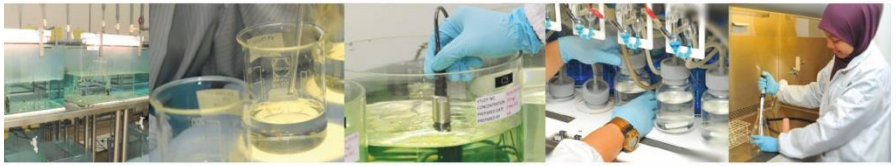
- Algae, Growth Inhibition Test
- Daphnia sp., Acute Immobilisation Test
- Fish, Acute Toxicity Test
- Terrestrial Plant Test Acute toxicology

DEGRADATION & ACCUMULATION

- Ready Biodegradability
- Ultimate Biodegradability
- Partition Coefficient P_{ow}

PHYSICAL CHEMICAL PROPERTIES

- pH
- Boiling Point
- Melting Point
- Specific Gravity
- Flash Point
- Relative Density
- Flammability
- Vapour Pressure
- Solubility
- Dissociation Constant
- Hydrolysis



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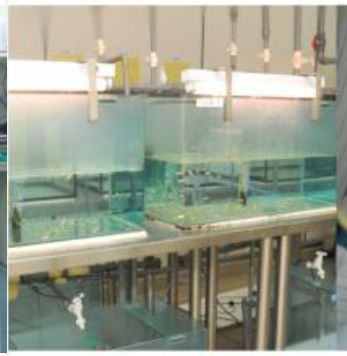
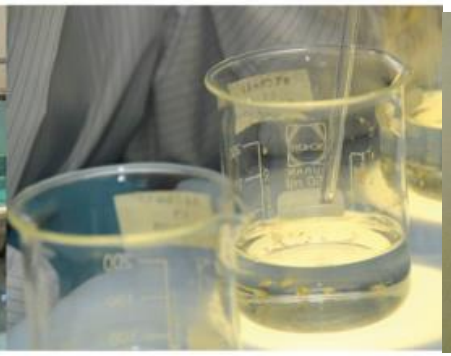
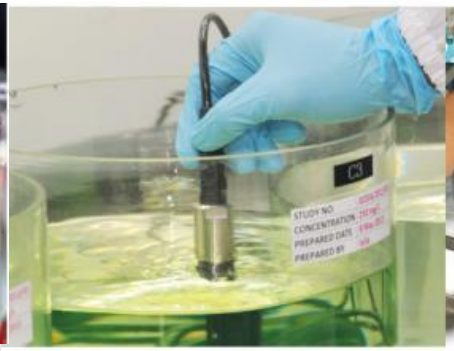
Product Safety and Hazard Assessment Section

ECOTOXICITY STUDIES	MUTAGENICITY STUDIES	ENVIRONMENTAL FATE
Algae Growth Inhibition	Bacteria Reverse Mutation	Biodegradation
Daphnia Immobilization		
Fish Toxicity		





Thank you

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