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LEADERS IN IDEAS AND SOLUTIONS

Developing a Framework to support a streamlined Risk Assessment Framework to Nanomaterials via facilitation of Grouping and Read-Across

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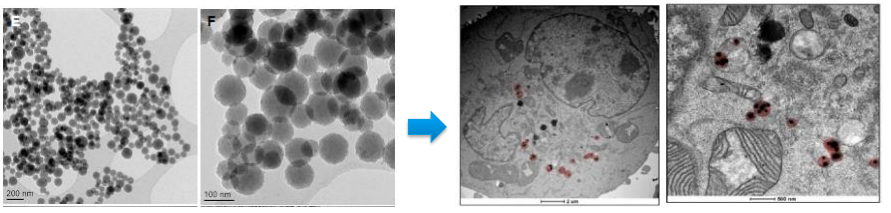
The Nanotechnology Industry

- Nanotechnology is a Key Enabling Technology (KET) - considered the next global frontier of science
- The global nanotechnology market is projected to grow to $173.95 billion by 2025
- Expected to provide major economic & social benefits.
- Hurdle: uncertainty regarding the potential risks posed by NM exposure and the lack of appropriate tools for NM safety assessment

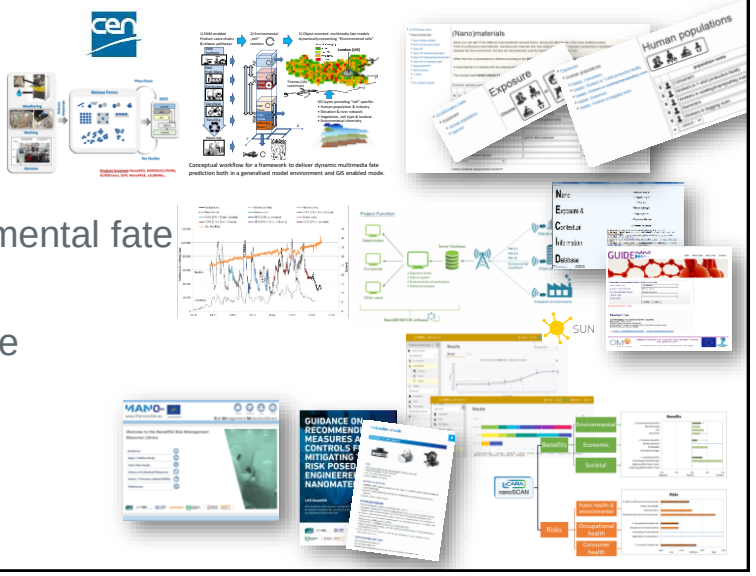


Content

- How much information is available?
- A gap analysis of current knowledge
- Structuring the knowledge gaps into a strategy
- Gracious – Grouping based upon hypotheses
- Well justified NanoEHS hypotheses
- How can we use this information?

Exposure & Hazard assessment Recent progress

- Release
- Environmental fate
- Exposure
- Hazard

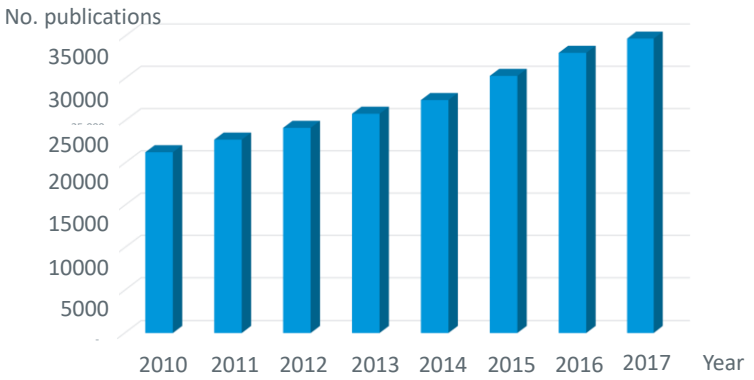


NanoEHS Publications

(Nanomaterial or Nanoparticle) + (Hazard or Toxicity)

All years 501 190 papers

Approx. 1600 per year increase



NanoEHS Publications


BEILSTEIN JOURNAL OF NANOTECHNOLOGY

NanoE-Tox: New and in-depth database concerning ecotoxicity of nanomaterials

Katre Juganson[1,2], Angela Ivask[1,3], Irina Blinova[1], Monika Mortimer[1,4] and Anne Kahru[1]

Full Research Paper

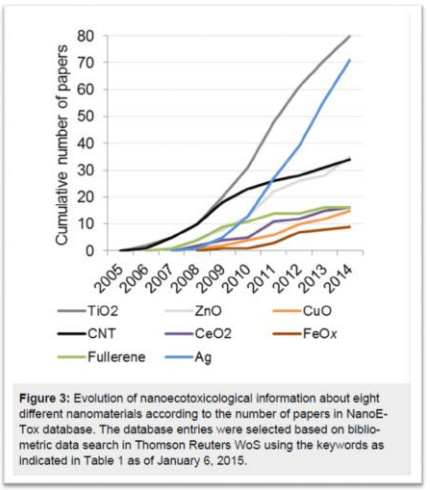


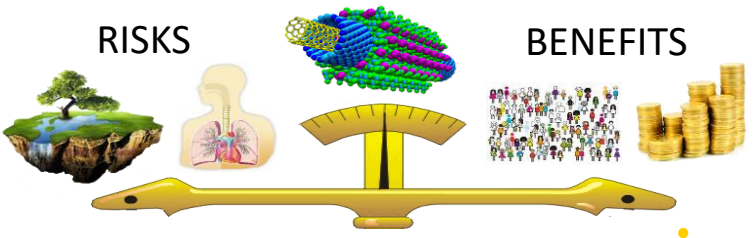
Figure 3: Evolution of nanoecotoxicological information about eight different nanomaterials according to the number of papers in NanoE-Tox database. The database entries were selected based on bibliometric data search in Thomson Reuters WoS using the keywords as indicated in Table 1 as of January 6, 2015.

Important points and gaps

- How do assay preparation/conditions affect fate/exposure/hazard
- What P-C properties of NMs/media are key on fate/exposure/hazard
- Long term
  - Costly, complex, artefacts, ethics
  - Need to identify suitable longer-term models and assays (e.g. for *in vitro* studies)

SUN (Sustainable Nanotechnologies)

Industry can avoid environmental, health and safety liabilities from nanomaterials, and nanotechnology innovation can be promoted effectively if an integrated approach is adopted, addressing the entire product lifecycle, balancing risks from nanomaterials with the social and economic benefits from their applications.



Ca' Foscari University of Venice

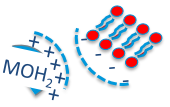
Professor Antonio Marcomini
Dr. Danail Hristozov

SUN
Sustainable Nanotechnologies Project

Safety by Molecular Design

DESIGN

***GRANULATION** from NANO to nanostructured MICRO powder to **CONTROL** powder aereosolisation (EXPOSURE POTENTIAL)*



***SURFACE CHARGE MODIFICATION** to **CONTROL** interaction with CELLS membranes (EXPOSURE/HAZARD POTENTIAL)*

***ANTIOXIDANT MOLECULES COATING** to NEUTRALISE **ROS** free radicals and **CONTROL** OXIDATIVE STRESS (HAZARD POTENTIAL)*

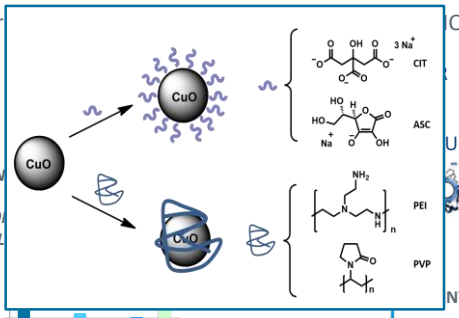
TESTING strategy for ...IONS

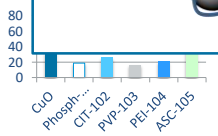
INTRINSIC PROPERTIES

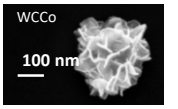
EVOLUTION IN

EVALUATIO

ION DISSOL







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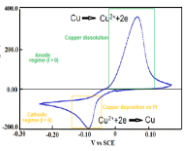


...NTS

PERFORMANCES

National Research Council of Italy
Istec Institute of Science and Technology for Ceramics

Materials

$CuSO_4*5H_2O$
(BDH Prolabo®)

pristine CuO NMs
(PlasmaChem)

Stock suspensions were prepared in Milli-Q water.
Before use, NMs suspension was sonicated twice for 8 minutes in a bath sonicator (Jacobsen et al. 2010).

"Safe by design" CuO Nanomaterials prepared by ISTEC-CNR (Faenza, Italy)

Introduction of coatings by self-assembling

Suspension agents

- MilliQ Water
- **Phosphate Buffer**

PO_4^{3-}

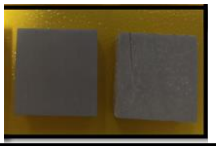
PVP (Polyvinylpyrrolidone)

ASC (-) (Na ascorbate)

$CuO\ (PO_4^{3-})$

- $CuO\text{-}PVP\ (PO_4^{3-})$
- $CuO\text{-}PVP\ (H_2O)$
- $CuO\text{-}ASC\ (PO_4^{3-})$
- $CuO\text{-}ASC\ (H_2O)$

Before use, the suspensions were vortexed thoroughly.



CuO_Acryl_FP

Embedded in a matrix and fragmented.
High unstable polydisperse suspension of fragmented product. Before use, NMs suspension was sonicated for a minute.

Lymnaea stagnalis

- Epibenthic species with a wide distribution;
- Good biomonitor for metal pollution;
- Has been widely used in ecotoxicological studies, but very little in the assessment of potential effects of nanomaterials;
- OECD model species for reproduction studies (OECD, 2016);
- Cultured in OECD 203 medium.

Credit: Danae Patsiou

Credit: Valentina Ricottone

Discussion and Conclusions

- Overall Cu salt is more toxic compared to CuO NMs
- Juvenile snails are more sensitive compared to adults exposed to either Cu ions or CuO NMs.
- In the chronic exposure, fecundity inhibition was a likely secondary effect due to inhibition of feeding rate.
- Toxicity of CuO NMs could not be attributed only to copper dissolution.
- The presence of the phosphate buffer seems to have a potentiation effect on the toxicity of the CuO NMs. It is possible that copper-phosphate complexes were formed, precipitated and ingested by snails.
- Overall, only CuO-PVP NMs in MilliQ water have met the criteria of “safe by design”.
- No toxicity due to exposure to CuO-FP highlights the need of more studies using “transformed” NMs rather than pristine NMs, as more representative of real exposure scenario.

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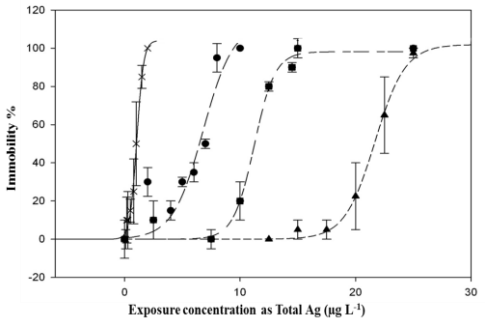
Effects of differently coated Ag NPs on *Daphnia magna*



$AgNO_3$ (**X**), PVP-Ag NPs (●), Cit-Ag NPs(■) and PEG-Ag NPs (▲); Error Bars: 95% CI.

$AgNO_3$ > PVP-Ag NP > Cit-Ag NP > PEG-Ag NP

Khan et al (2015)

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Effects of differently coated Ag NPs on *Chlorella vulgaris*

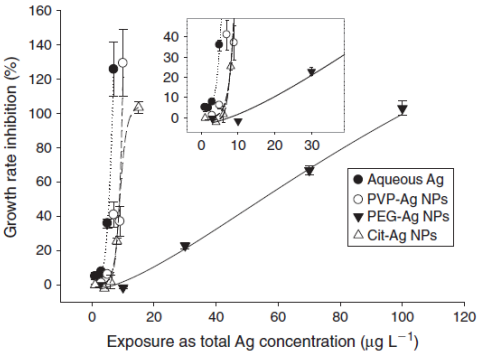

Fig. 1. Growth inhibition of *Chlorella vulgaris* after 72 h of exposure to aqueous Ag and Ag nanoparticles (NPs) (mean ± s.d., $n = 3$).

$AgNO_3$ ~ PVP-Ag NP ~ Cit-Ag NP > PEG-Ag NP

Kalman et al (2015)

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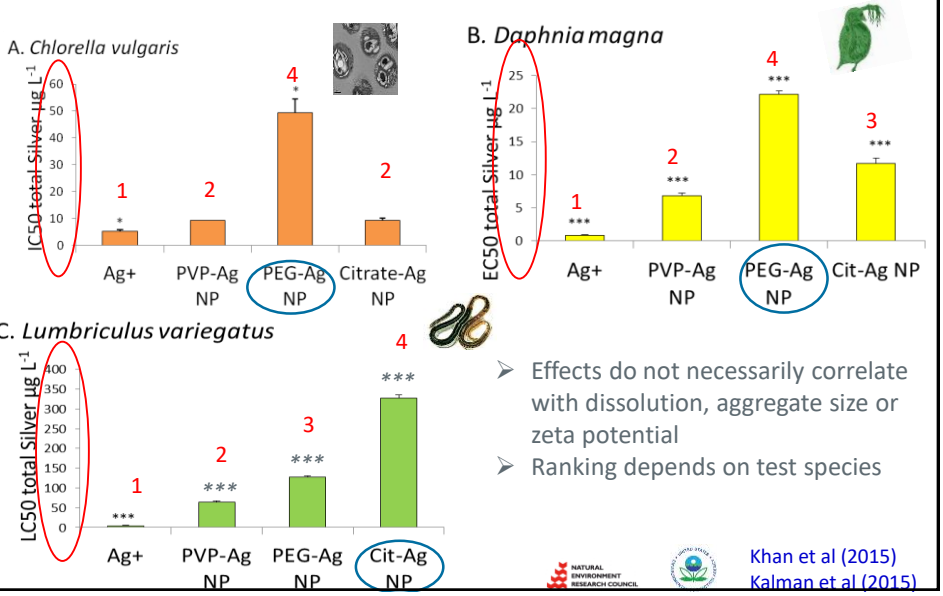
Acute Toxicities of different forms of Ag NPs (~ 10 nm) to 3 standard species



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

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

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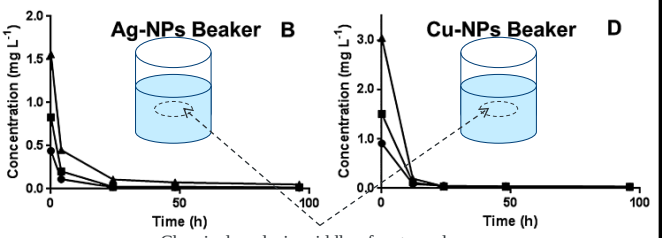
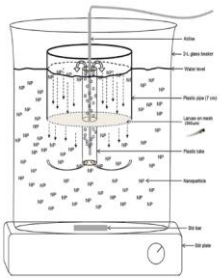
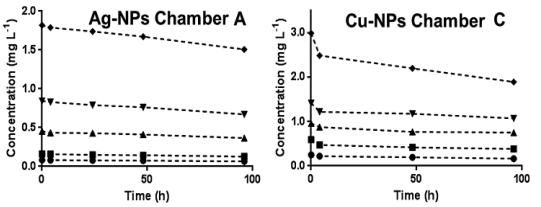
Aqueous Phase Exposure of Zebrafish - Dispersion

Particles visible on bottom of beaker



Chemical analysis middle of water column





Boyle et al., 2015

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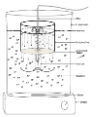
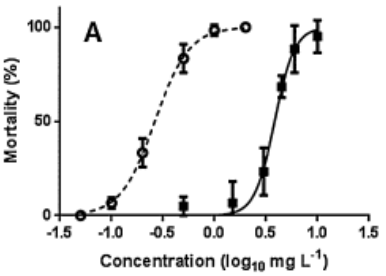
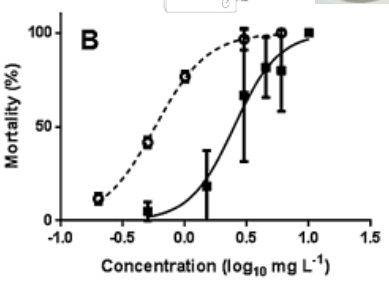
Aqueous Phase Exposure of Zebrafish - Dispersion



Ag-NPs

Cu-NPs

Boyle et al., 2015, Env. Toxicol. Chem. 34: 583-588

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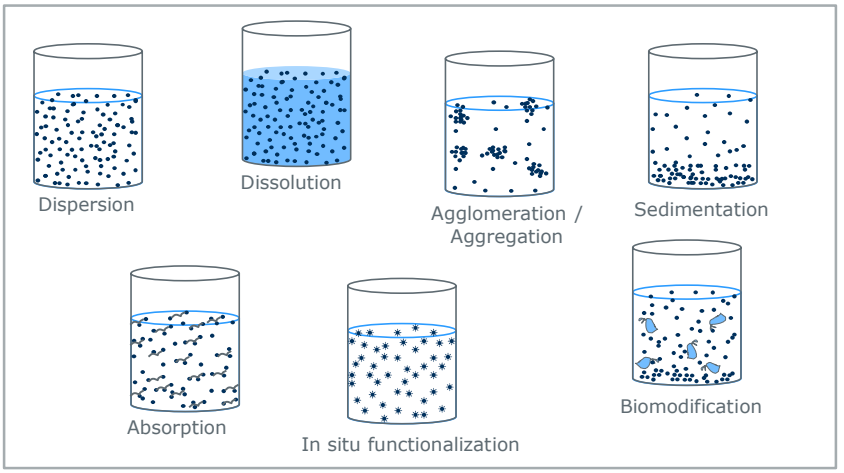
Transformation processes occur during the test…



Sørensen, Hjorth, Delgado, Hartmann, Baun (2015) Nanoparticle ecotoxicity – physical and/or chemical effects? Integrated Environmental Assessment and Management, 11, 722-724

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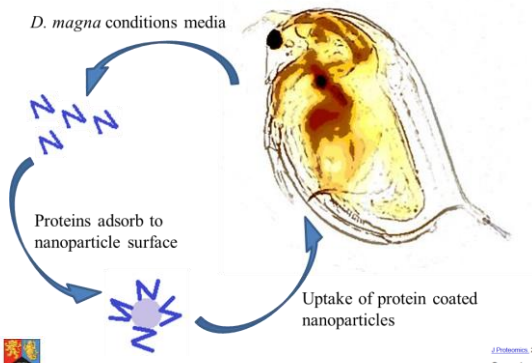
Role of secreted eco-corona on toxicity of NMs to *Daphnia magna*

FUTURE NANO NEEDS

- Organisms secrete biomolecules such as proteins, carbohydrates and lipids
- Create **eco-corona**- a layer of the biomolecules around the nanoparticle
- Change identity of NPs and interaction / toxicity / stability / uptake with organisms



- Characterised secreted corona (proteins, polysaccharides)
- Method development for small molecule (metabolite corona)
- Evaluated route of exposure to NPs (with/without food before, during and after exposure)

UNIVERSITY OF BIRMINGHAM

J Proteomics. 2016 Mar 30;137:45-51. doi: 10.1016/j.jprot.2015.09.005. Epub 2015 Sep 12.

Secreted protein eco-corona mediates uptake and impacts of polystyrene nanoparticles on Daphnia magna.

Nasser F[1], Lynch I[2].

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Nanotoxicology, May 2014; 8(3):334–348
© 2014 Informa UK, Ltd.
ISSN: 1743-5390 print / 1743-5404 online
DOI: 10.3109/17435390.2013.802387

informa healthcare

Concern-driven integrated approaches to nanomaterial testing and assessment – report of the NanoSafety Cluster Working Group 10

Agnes G. Oomen[1], Peter M. J. Bos[1], Teresa F. Fernandes[2], Kerstin Hund-Rinke[3], Diana Boraschi[4], Hugh J. Byrne[5], Karin Aschberger[6], Stefania Gottardo[6], Frank von der Kammer[7], Dana Kühnel[8], Danail Hristozov[9], Antonio Marcomini[9], Lucia Migliore[10], Janeck Scott-Fordsmand[11], Peter Wick[12], & Robert Landsiedel[13]

Integrated approach to NM testing and assessment – NanoSafety Cluster Working Group 10

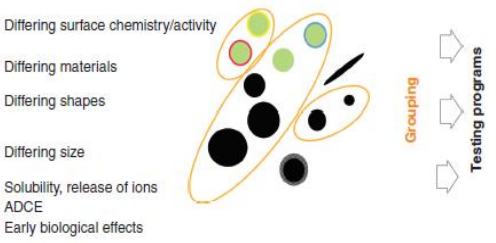

Figure 6. Illustration of grouping of NM based on material properties and/or biological effects. This schematic example shows three groups of NM and also NM not assignable to any group.

Advocated a tiered approach

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Recommendations for the human health and environmental hazard assessment of NM

Testing strategies

(1) Find an approach to deal with changing characteristics, and thus hazard, of NM during their life cycle.
(2) Integrate exposure, material properties, biopersistence, biokinetics (ADME/ADCE) as well as primary effect and apical effect testing into a concern-driven testing strategy that can be applied to an individual NM but also includes guidance for grouping of NM. Use all available information to identify relevant concerns and to choose the right studies to be performed. Fill in the criteria for the decision-making process of the concern-driven tiered testing strategy.
(3) Use grouping as an integral part of the testing strategy.
(4) Define adverse outcome pathways for different NM.
(5) Use data obtained in A.2, A.3 and A.4 to fill in criteria for grouping of NM.
(6) Define triggers for tier 2 and 3 for human health risk assessment.
(7) Define trigger values for environmental risk assessment

- Provide guidance on the extent of ecotoxicity testing (terrestrial vs. aquatic tests)
- Provide guidance on the consideration of accumulation (which criteria trigger accumulation testing in individual organisms or in the food web)
- Provide guidance on the selection of ecotoxicological tests for compliance testing.

Testing methods

(1) Designate potential testing methods applied to NM to be of use for each concern
(2) Define a list of NM as performance standards for testing methods
(3) (ideally covering different toxic effects and including positive and negative controls)
(4) Update and amend existing testing methods for specific needs of NM testing

- Provide guidance on NM dispersion and *in situ* characterisation in the test system
- Provide guidance on methods to study biokinetics (ADME/ADCE) and biopersistence, and on application of these data
- Provide guidance on development and application of testing methods as part of a testing strategy rather than as stand-alone tests
- Provide guidance for the simulation of increased environmentally relevant exposure; for example, application of natural stabilisers

(5) Update and amend existing testing methods in general (for NM and chemicals alike)

- Bronchoalveolar lavage in inhalation studies
- Extended histopathology (e.g., lung)
- Aquatic and terrestrial ecotoxicity tests

(6) Establish general concepts for NM (and other particles) effects

- Carcinogenicity
- Cardiovascular effects
- Epigenetic effects
- Immunological effects
- Reproductive toxicity, developmental toxicity

(7) Establish concepts for environmental monitoring to verify the effects detected in laboratory tests or simulation tests

Grouping of NM

(1) For grouping to be used as an integral part of the testing strategy, define and validate scientifically sound grouping criteria based on available data and material properties (metrology), biopersistence, fate, ADME/ADCE as well as primary and apical effects.
(2) Use quantitative structure–activity relationship (QSAR), if applicable.

Oomen et al (2014)

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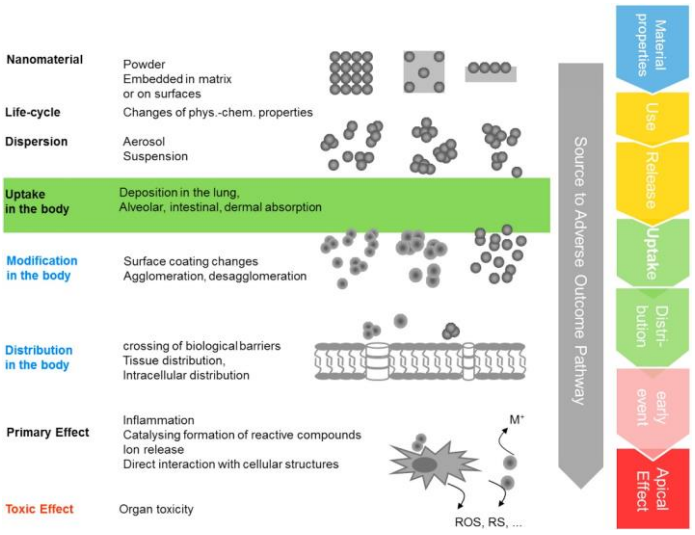
ELSEVIER
Environmental Pollution
Volume 218, November 2016, Pages 1376-1380

Concern-driven integrated approaches for the grouping, testing and assessment of nanomaterials

Robert Landsiedel



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Environmental Pollution
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Concern-driven integrated approaches for the grouping, testing and assessment of nanomaterials

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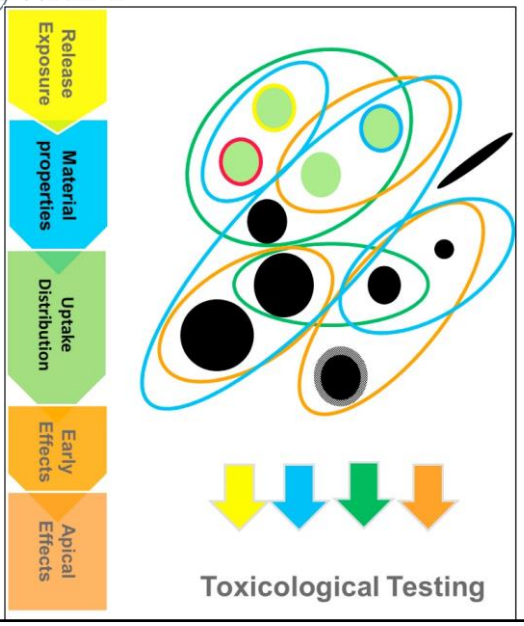

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Environmental Pollution
Volume 218, November 2016, Pages 1376-1380

Concern-driven integrated approaches for the grouping, testing and assessment of nanomaterials

Robert Landsiedel

4. Conclusion

Grouping of NMs by exposure, use and release should be applied to determine NMs of concern and can be complemented by data on NM's biopersistence and biokinetics. This information should be used to streamline testing to investigations that are relevant for NM risk assessment. Grouping of NMs by their biophysical interactions and early biological effects should form an intrinsic part of all subsequent tiers of the IATA* with the aim of refining concerns until risk assessment can be performed; *in vivo* studies for apical toxic effects could thus be minimized and restricted to nanomaterials serving as reference for a group or those nanomaterials which could not be assigned to a group. This approach to NM risk assessment serves to ensure product safety of a large variety of NM in different modifications, uses and life cycle stages, while at the same time reducing the need for animal testing and the costs and expenditure for testing.

*Integrated Approaches to Testing and Assessment

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Fast forward…

- A variety of publications exploring concepts, projects developing, practical work taking place…

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Perspectives of grouping

Hazard

Comparable PC-properties result in groups of ENM with similar hazard

READ-ACROSS -> Detailed

SEG4nano (sophisticated environmental grouping for nanomaterials)

Umwelt Bundesamt

"Grouping concept for metal and metal oxide nanomaterials with regard to their ecotoxicological effects on algae, daphnids and fish embryos" K. Hund-Rinke, K. Schlich, D. Kühnel, B. Hellack, H. Kaminski, C. Nickel. (2018) NanoImpact, 9,52-60.

Risk

Combination of hazard grouping with grouping schemes for exposure (release + fate)

HIGHLY CONDENSED

AEG4nano
(aggregated environmental grouping for nanomaterials)

nanoGRAVUR

Publications under preparation

The road to NM grouping: NanoReg2

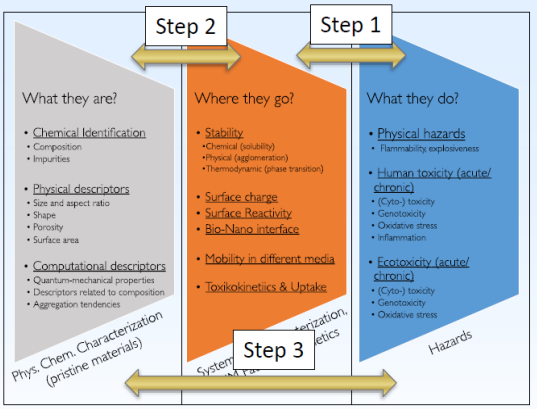
NanoReg2

I: Establish „similarity“ based on physico-chemical parameters considering

- chemical identity (composition, impurities...)
- physical identity: measured parameters, intrinsic & extrinsic parameters
- calculated (in silico) or semi-calculated (based on in silico analysis of measured parameters)

II: Establish correlations between physico-chemical information & toxicity in 3 steps

- use of in silico methods to establish these correlations



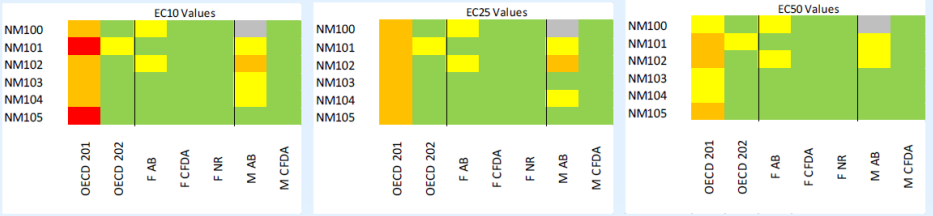
NanoReg2 WP1 OECD/NR2/GRACIOUS 2018

Haase et al (presentation OECD, NanoReg2, Gracious, Sept 2018)

NanoReg2

Example with TiO_2 NPs

Non toxic	> 100	Mod. toxic	1-10
Slightly toxic	10-100	Highly toxic	<1



- Alage growth inhibition test (TG201) EC_{10}, most sensitive endpoint. 2 groups: [101, 105] / [100, 102, 103, 104]
- Daphnia acute immobilization test (TG202). Low sensitivity. 2 groups: [101] / [100, 102, 103, 104, 105]
- *In vitro* FCL Alamar Blue test. Low senstiivty. 2 groups: [100, 102] / [101, 103, 104, 105]
- *In vitro* mussel cells Mussel Alamar Blue test, EC10, 3 groups: [102] / [101, 103, 104] / [105]
- *In vitro* CDFA-AM and NR in fish or mussel: no toxicity detected. No groups could be established.

Different grouping results depending on:
- Assay
- EC_x considered

Differences could be related to:
- Differences in test media
- Differences in mechanism of toxic action

Question:
- How to integrate in a grouping strategy, results obtained with different tests and EC_x?

Need to go further and analyse data considering phys-chem properties

Navas et al (presentation OECD, NanoReg2, Gracious, Sept 2018)

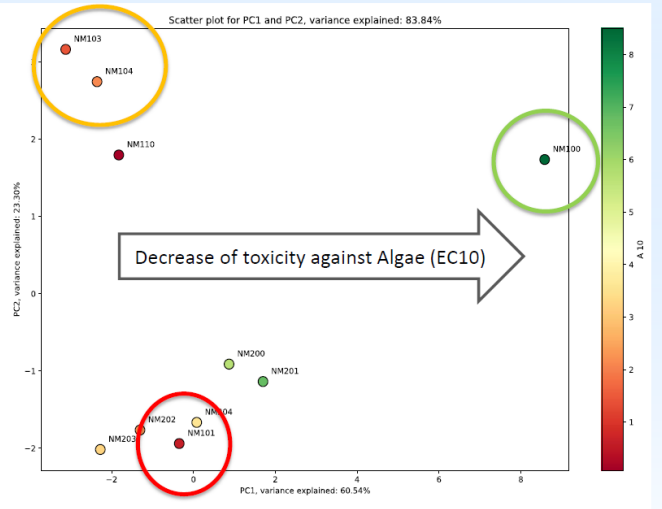
Similarity analysis of nanomaterials

NanoReg2

Example with algae test EC_{10} values

OECD 201	
	EC10
NM100	8,50
NM101	0,61
NM103	1,44
NM104	2,14

PC2: System dependent characteristics (*Z-average medium, DLS peak 1 in medium, Zeta pot medium*)



PC1: Pristine material characterization (*Elongation, Aspect ratio, Central Distance, Convexity, Shape*)

NanoReg2 WP1 OECD/NR2/GRACIOUS 2018

Navas et al (presentation OECD, NanoReg2, Gracious, Sept 2018)

gracious

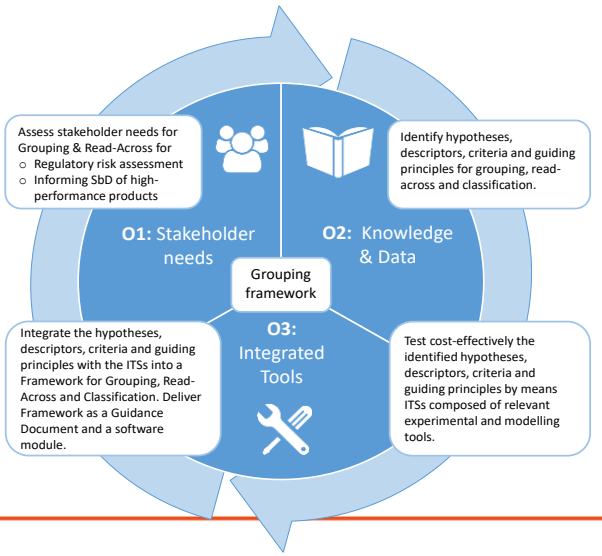
Draft GRACIOUS framework for grouping and read-across of nanomaterials for regulatory risk assessment and safe-by-design nano-enabled products

www.h2020gracious.eu

Coordinator: Vicki Stone, Heriot-Watt University

Project Overview

About Gracious



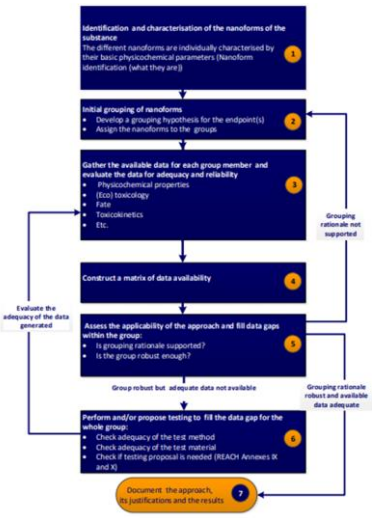
Project Overview

About Gracious

Development of a highly innovative science-based framework that supports the grouping and read-across of nanomaterials on the market and under development.

ECHA Guidance on Grouping suggests that Grouping should be Hypothesis driven.

https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf



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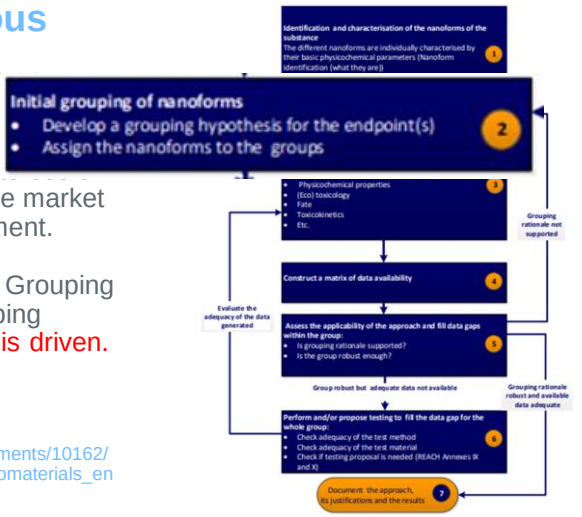
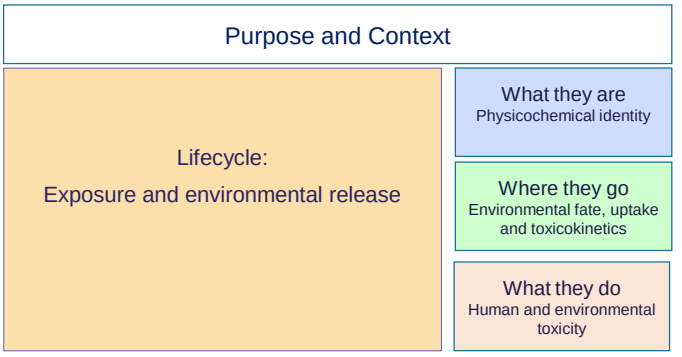


Grouping Framework Design

Hypothesis template



Hypothesis template

Purpose: Precautionary, Targeted testing, Regulatory, Safety by Design **Context**: Occupational, Consumer, Environmental	
Input from life cycle (WP2) • Physical form when being handled (powder, suspension/liquid/ embedded in solid matrix, …) • Stability (agglomeration, solubility…) • Exposure form (quasi-spherical, elongated, plate, pure, attached to a particle, embedded in a matrix, ionic form) • Intended use, specific process (occupational) • Environmental compartment where they are released (workplace atmosphere, outdoors atmosphere, water, soil as waste) • Population exposed • Exposure route • Exposure dose. This can be unfolded in several tiers: - Qualitative; unlikely, negligible, likely - Quantitative; short/peak exposure, long-term exposure	**What they are? (WP3)** Physicochemical identity **Where they go? (WP4)** Environmental fate, uptake and toxicokinetics **What they do? (WP5)** Human and environmental toxicity

Potential implications:
if in group:
if not in group:	

Grouping Framework

Hypothesis development

- General Hypotheses for grouping NM with high dissolution rates
- Human toxicokinetics
  - Inhalation route
  - Ingestion route
  - Dermal route
- Environmental Fate
  - Water
  - Sediment
  - Soil
  - Air

E.g. Long-term pulmonary retention of rigid, biopersistent HARN after occupational inhalation exposure will result in lung toxicity

E.g. Translocation to the pleural cavity of rigid, biopersistent HARN after occupational inhalation exposure will result in mesothelial toxicity

Detailed Hypothesis

<table>
<tr><td colspan="2">Purpose: Precautionary, safe-by-design, regulatory, targeted testing
Context: Occupational, inhalation study</td></tr>
<tr><td rowspan="3">Input from life cycle
Generated as a respirable aerosol during production or use
Type of exposure
Workplace atmosphere
Inhalation exposure
Level of exposure
Moderate, short peak exposure during handling dry powder
(e.g. bagging, pouring, weighing, spraying)</td><td>What they are?
High aspect ratio, rigid NM with low dissolution rate and aerodynamic diameter to allow deposition in the distal lung</td></tr>
<tr><td>Where they go?
A small proportion of HARN deposited in the distal lung (~ 1%) will translocate to the pleural cavity. Fibres ≥ 5 µm in length will be retained in the pleural cavity due to size-restricted clearance through stomata in the chest wall and diaphragm.</td></tr>
<tr><td>What they do?
Cause frustrated phagocytosis as pleural macrophages attempt to remove them and result in chronic inflammation, mesothelial cell proliferation, fibrosis and, overtime, mesothelioma</td></tr>
<tr><td colspan="2">Potential implications:
If in group:
Regulatory: develop read-across argument on hazard for regulatory use and compare to relevant source material e.g. asbestos.
Targeted tested: focus on location of HARN deposition, retention and endpoints related to persistent interaction between the HARN and cell/tissue/organism.
Safe-by-design: consider the use materials which are shorter, less rigid or biodegradable.
Precautionary: limit exposure/prevention of the generation of an aerosol.
If not in group: Consider alternative hypothesis/IATA</td></tr>
</table>

Grouping Framework

Grouping Hypotheses with clear implications

Group description and hypothesis	Potential implications/consequences	Relevant testing (in IATA where appropriate)
Quickly dissolving NFs (DISS): NF will quickly transform to the ionic or molecular form and have the same fate, kinetic and toxicity profile as the ionic or molecular form. *Scientific rationale:* Exposure to and uptake of the NF is negligible.	*Regulatory:* Read-across to the ionic or molecular form may be possible (in subsequent Level).	• Dissolution rate and transformation in water and relevant media.

Grouping Framework

Grouping Hypotheses with clear implications

Group description and hypothesis	Potential implications/consequences	Relevant testing (in IATA where appropriate)
NFs which are incorporated into a solid matrix (SNEP): NF will be released as free NF depending on the use/aging process & matrix. *Scientific rationale:* The probability and form of release is mainly determined by the type of matrix, dispersed state of the NF in the matrix and use or aging process.	*Precautionary approaches or safe-by-design:* Control-banding (Level 1), minimize exposure or adjustment of NEP. *Targeted testing:* Testing to assess concerns.	• Incorporation of NF into the matrix of the NEP (g/g content, disperse state) • Resilience of matrix under relevant conditions • Forms of release from NEP under relevant conditions

Integrated Approaches to Testing and Assessment

- Tiered streamlined approach to testing
- Spanning
  - physicochemical characteristics,
  - Data mining and *in silico* tools
  - Simple in vitro screening assays
  - Complex physiologically relevant in vitro models
  - In vivo (vertebrate and invertebrate) assessment
- Tailored – via Grouping approaches

Omics

- Omics approaches allow identification of mechanisms of action or adverse outcome pathways
- They therefore allow identification of targets for assessing hazard or efficacy
- The cost is coming down
- Large data sets generated
- Not yet a screening tool, more a guiding tool for identification of relevant endpoints to screen

OECD NanoReg2 GRACIOUS workshop Sept 2018

Stakeholder engagement

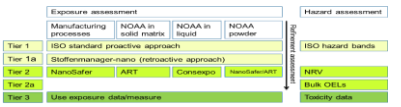
- EU regulatory bodies
  - E.g. ECHA,
- EU policy makers
  - E.g. EC
- European NS national regulatory bodies
  - E.g. RIVM, NRCWE
- International regulatory bodies
  - E.g. US EPA, Health Canada
- Industry bodies
  - E.g. NIA and BIAC
- Industry
  - E.g. BASF, Black Diamond
- Consultants
- E.g. Yordas, Blue Frog

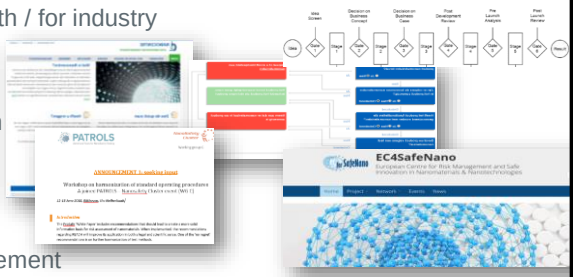
Summary

- We need to streamline hazard testing in order to improve efficiency and application of the 3Rs for risk assessment and risk benefit analysis
- The complexity and diversity of nanomaterials provides a challenge to streamlining
- GRACIOUS will generate a framework for grouping of nanomaterials and construction of Integrated Approaches to Testing and Assessment that will streamline risk assessment
- We have already lots of good knowledge regarding factors that influence toxic potential including size, shape, composition, charge, dissolution….

Exposure & Hazard assessment Future perspectives

- Match with stakeholder needs
  - Workshops/webinars with / for industry
- Networking & Harmonisation
  - Joint project workshops
- Databases and data management
  - Harmonised collection and storage
  - Open access
- Innovative methods for exposure & hazard testing





Acknowledgments

HERIOT WATT UNIVERSITY

QualityNano

nanoMICEX

FUTURE NANO NEEDS

HORIZON 2020

NANOSOLUTIONS

BIO BEAUTY
THE BIOPACKAGING SOLUTION FOR ORGANIC SKIN CREAM

NANOREG

MARINA
MANAGING RISKS OF NANOMATERIALS

SUN
Sustainable Nanotechnologies Project

nanoMICEX
European Project

Unilever

fera
The Food and Environment Research Agency

NanoImpactNet

NanoSafety Cluster

NATURAL ENVIRONMENT RESEARCH COUNCIL

SEVENTH FRAMEWORK PROGRAMME

Examples of relevant EU-funded projects

PATROLS
Advanced Tools for NanoSafety Testing

ProSafe

ENM
eNanoMapper

gracious

NanoFASE

ACEnano

BIORIMA

NANOINDEX

caLIBRAte
nano risk governance

NanoTEST

NanoReg2

NANORISK

nanoMONITOR

for SafeNano

CERASAFE

nanoMICEX

GUIDEnano

NANOSTREEM

NanoMILE

NanoCommons
Nano-Knowledge Community

HERIOT WATT UNIVERSITY

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