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Current State of NanoEHS – an overview

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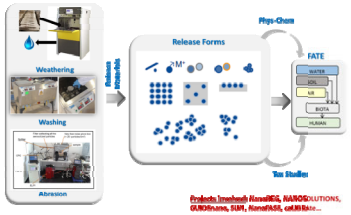
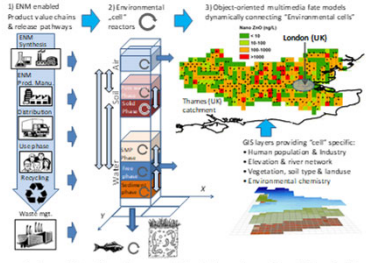
LEADERS IN IDEAS AND SOLUTIONS

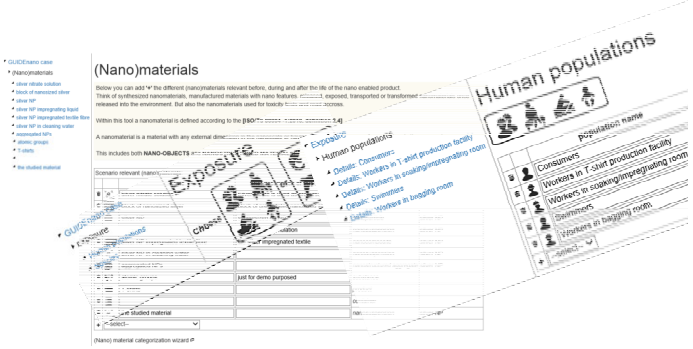
WG C: Exposure & Hazard assessment
Progress made 2014-2017

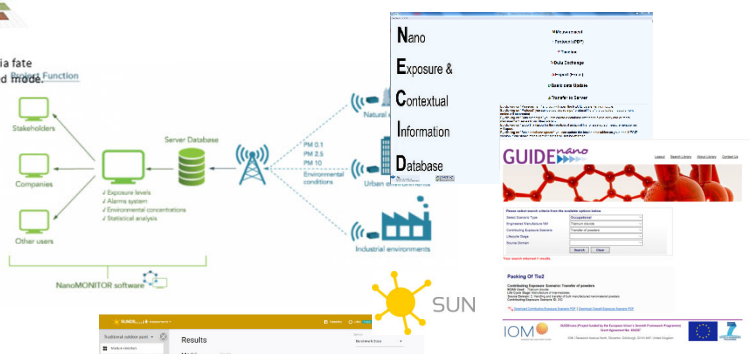
- Release
- Environmental fate
- Human Exposure
- Human Hazard

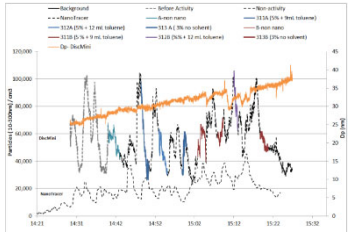


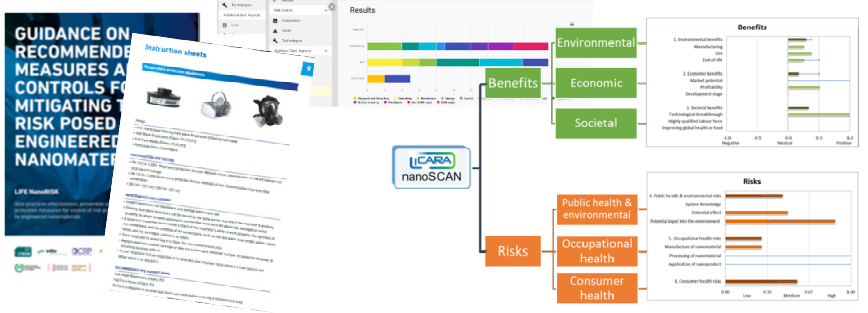


Conceptual workflow for a framework to deliver dynamic multimedia fate prediction both in a generalised model environment and GIS enabled mode.



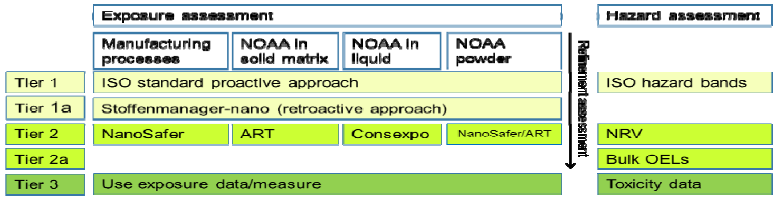




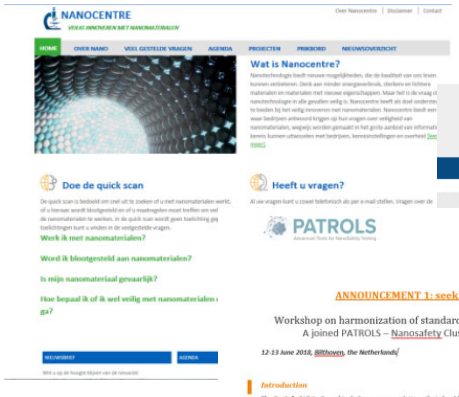


WG C: Exposure & Hazard assessment Future perspectives

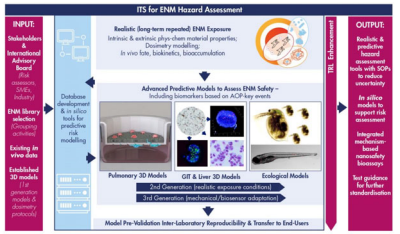
- Match with stakeholder needs
  - Workshops/webinars with / for industry



- Networking & Harmonisation
  - Joint project workshops



- Databases and data management
  - Harmonised collection and storage
  - Open access



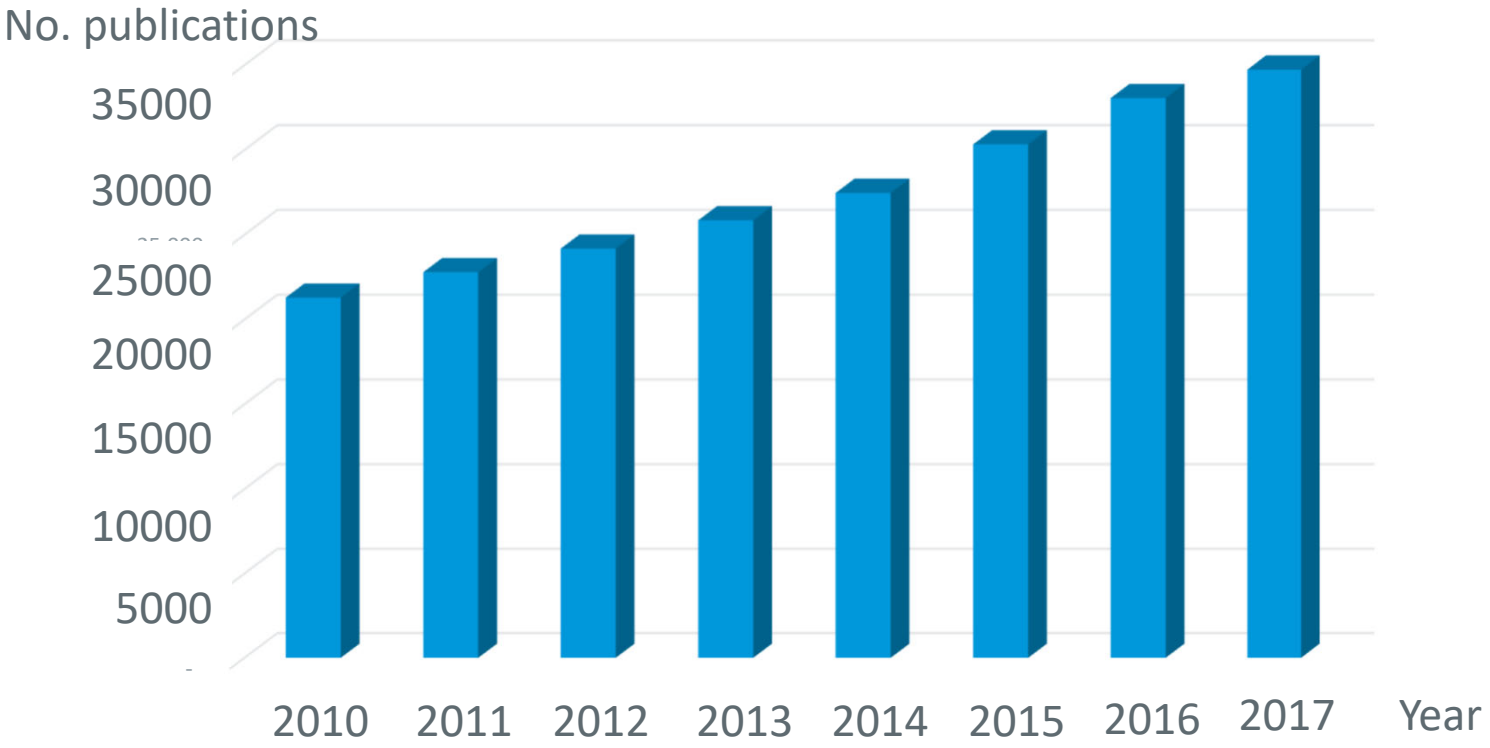
- Innovative methods for exposure & hazard testing

NanoEHS Publications

(Nanomaterial or Nanoparticle) + (Hazard or Toxicity)

All years 501 190 papers

Approx. 1600 per year increase



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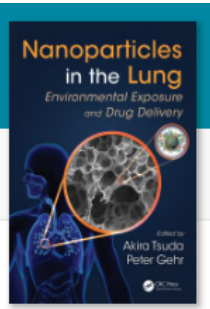
Publication patterns – gap analysis - 2013

ITS-NANO

	Biological impact						
Target	Biokinetics	Cytotoxicity	Inflammation	Ox. stress	Fibrosis	Genotox	Carcinogenicity
Lung	381	250	543	207	44	45	246
Liver	76	28	39	19	3	6	23
Spleen/immune	52	19	79	21	4	2	16
CNS	54	20	47	32	2	3	18
GI Tract	29	21	29	12	2	5	19
kidney	30	9	17	6	1	1	4
CV	138	84	219	83	18	10	60
Repro/dev	5	1	6	1	1	2	1
pleura (retention)	23	23	47	20	4	3	11



Nanoparticles in the Lung

Environmental Exposure and Drug Delivery

Edited By Akira Tsuda, Peter Gehr

< Back to book

Chapter 20

Nanotoxicology

By Dominique Balharry, Eva Gubbins, Helinor Johnston, Ali Kermanizadeh, Vicki Stone

Long-term studies missing

Why are long term studies missing?

- Usually conducted *in vivo*, using lots of animals and cost lots of money.
- Need to identify longer-term models and assays for *in vitro* study
- Jacobson et al. (NRCWE) – Repeated exposure to cells in culture in order to measure genotoxicity
- Kermanizadeh et al. (HWU) – Used protocol of Nicklas to treat 3D liver microtissues in vitro with nanomaterials up to 7 days
- Patrols will take this to 21 days for 3D lung and 3D Liver, and 5 days for 3D GIT

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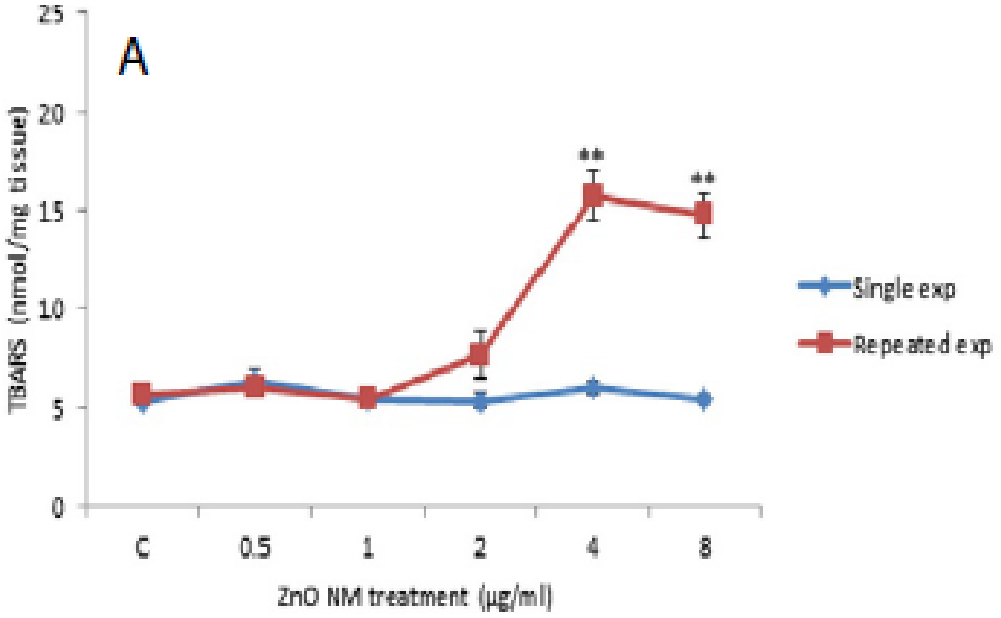
Kermanizadeh *et al. Particle and Fibre Toxicology* 2014, **11**:56
http://www.particleandfibretoxicology.com/content/11/1/56

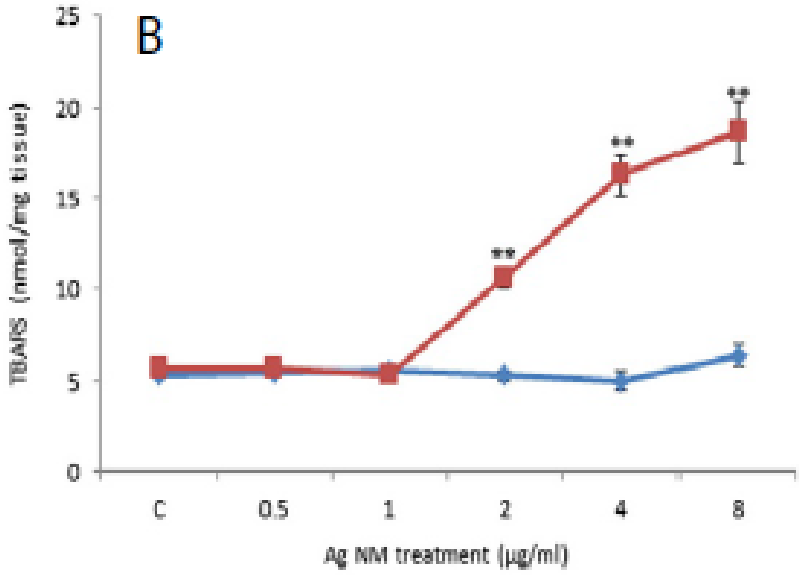
PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Hepatic toxicology following single and multiple exposure of engineered nanomaterials utilising a novel primary human 3D liver microtissue model

Ali Kermanizadeh[1,2*], Mille Løhr[1], Martin Roursgaard[1], Simon Messner[3], Patrina Gunness[3], Jens M Kelm[3], Peter Møller[1], Vicki Stone[2] and Steffen Loft[1]





Repeated exposure = 7 days

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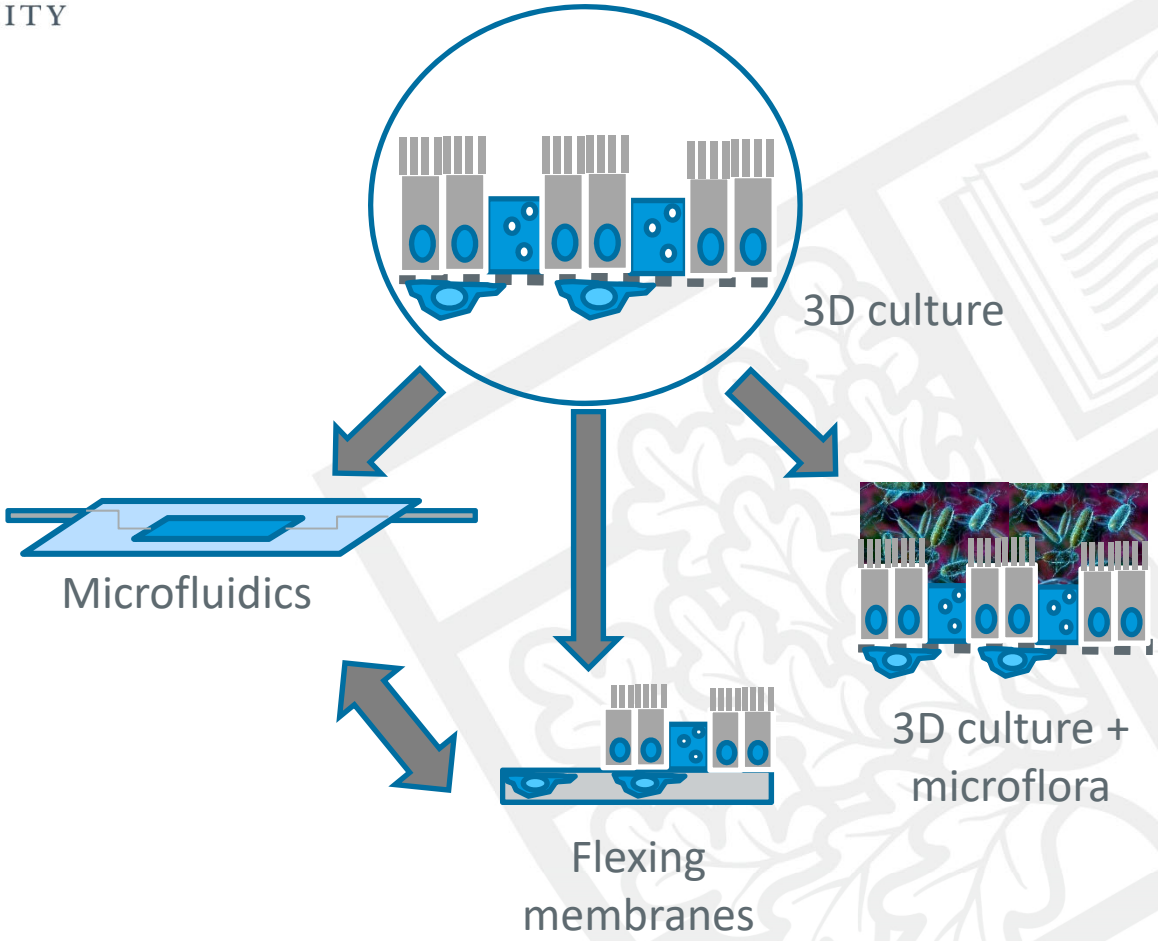
PATROLS
Advanced Tools for NanoSafety Testing



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Repeated exposures
Longer term effects…

GRACIOUS Grouping Framework

- **Aim GRACIOUS Framework:** support practical application of grouping of nanomaterials/nanoforms (NFs)

- Potential applications:
  - To facilitate targeted testing or targeted risk assessment
  - To fill a data gap in a regulatory dossier
  - To develop precautionary measures
  - To steer safe innovation/safe-by-design
  - To advance understanding of scientific mechanisms

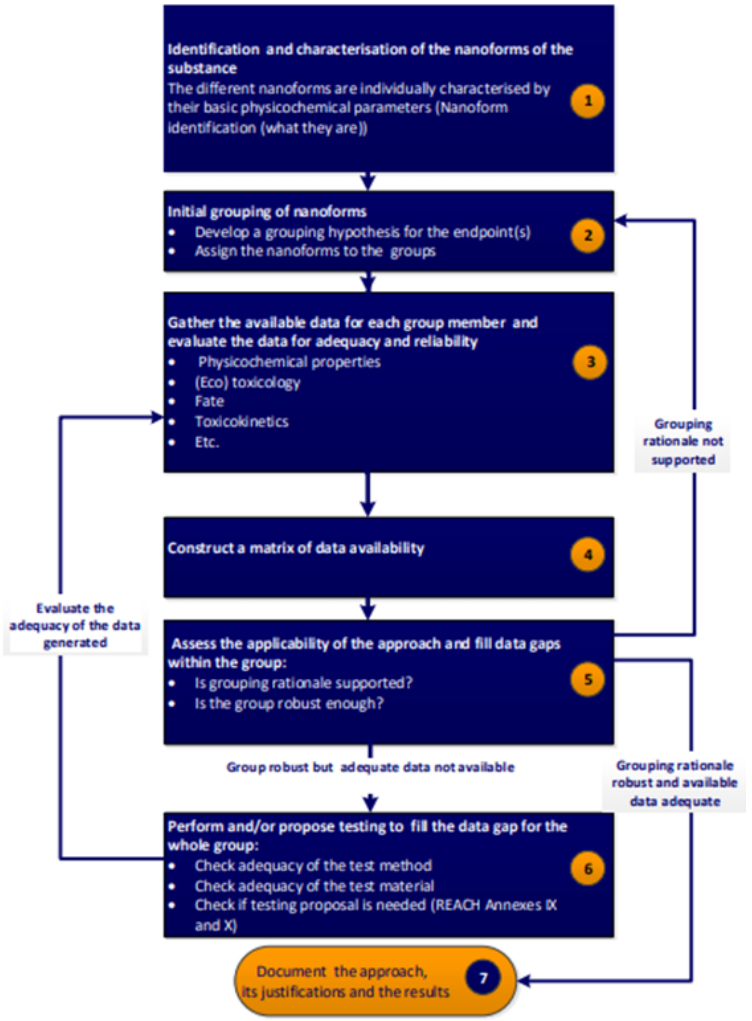
Project Overview

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



Project Overview

About Gracious

Development of a h
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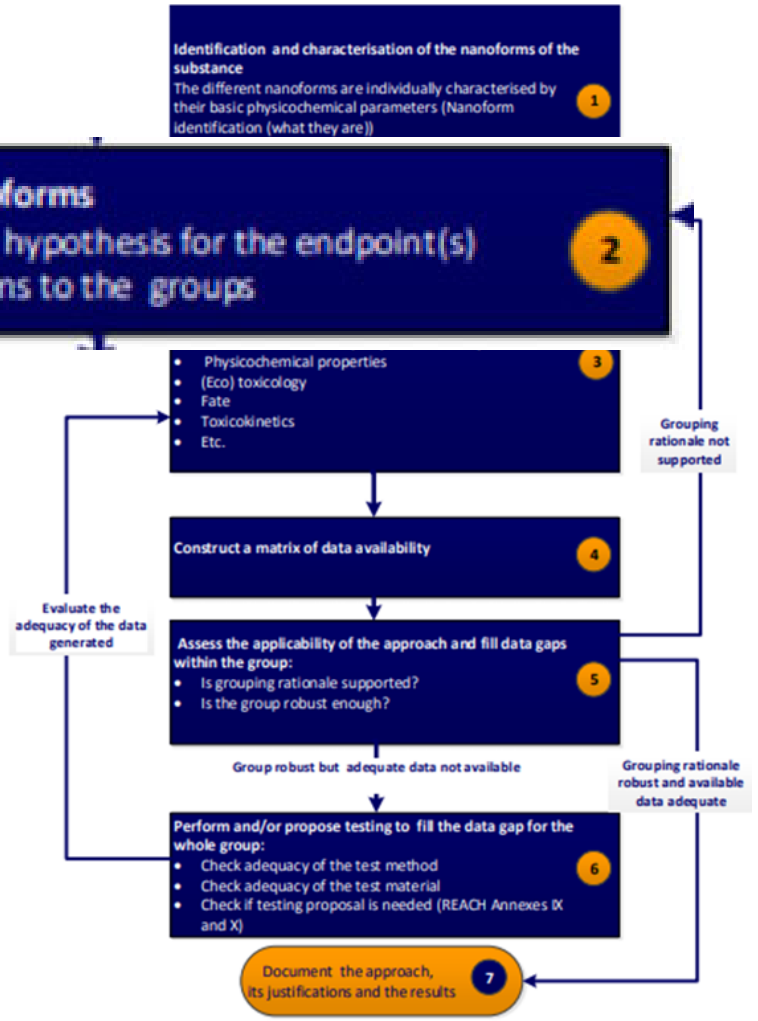
ECHA Guidance on Grouping suggests that Grouping should be Hypothesis driven.

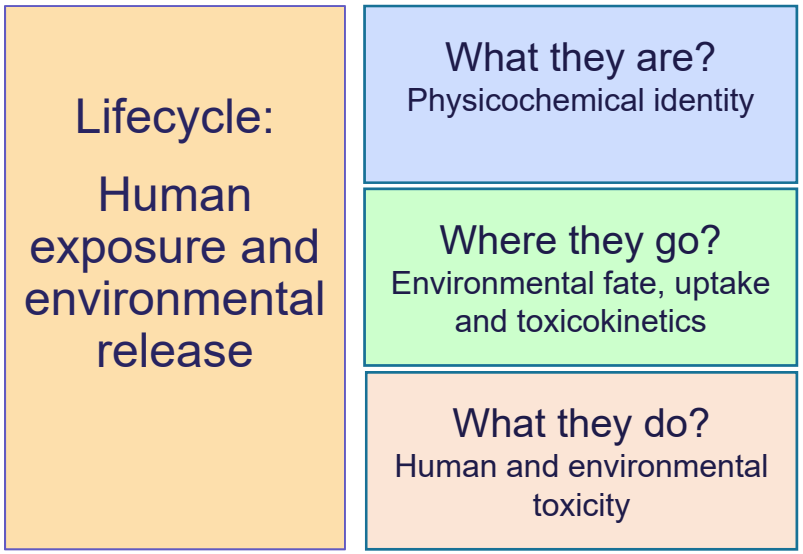


https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf

GRACIOUS framework –

Hypotheses Generated via knowledge and gap analysis

- 16 human health covering inhalation, ingestion & dermal routes of exposure
- 7 environmental covering water, sediments, soil and air
- Each hypothesis supported by tables summarising evidence from peer reviewed sources in order to judge the strength of each hypothesis
- 4 hypotheses with clear implications identified
  - DISS
  - HARN
  - D5NM
  - SNEP



Initial Overall Hypothesis example

Purpose: Targeted testing, regulatory, safe-by-design, precautionary

Context: Occupational, consumer, environmental

Input from life cycle

Exposure during production of NM or incorporation into other products or use of NM containing products

Type of Exposure

Workplace atmosphere, outdoors atmosphere, water, soil as waste

Level of exposure

Context dependent

What they are?

NM with a high dissolution rate

Where they go?

Context dependent: inhalation, ingestion, dermal deposition

Water, soil, aquatic and terrestrial organisms

NM will dissolve quickly after uptake and distribute similar to ions of the same chemical composition released from non-nanomaterials

What they do?

Toxicity of those NMs will be determined based on the ion toxicity, expect hazards similar to those of ions (as identified by GHS/CLP)

Potential implications:

If in group:

Regulatory: develop read-across argument on hazard based on ionic composition and know toxicity for regulatory use (Tier 2).

Targeted testing: focus on location of ion release as toxicity can probably be predicted based on dissolution rate, location of ion release and the toxicity of the released ions

Safe-by-design: consider the use of materials that release less toxic ions or on rapid dissolution to prevent

Initial Overall Hypothesis example

NM with a high dissolution rate.

Context dependent: inhalation, ingestion, dermal deposition

Water, soil, aquatic and terrestrial organisms

NM will dissolve quickly after uptake and distribute similar to ions of the same chemical composition.

Toxicity of those NMs will be determined based on the ion toxicity, expect hazards similar to those of ions.

Regulatory: develop read-across argument on hazard based on ionic composition and know toxicity for regulatory use (Tier 2).

Targeted testing: focus on location of ion release as toxicity can probably be predicted based on dissolution rate, location of ion release and the toxicity of the released ions

Human Inhalation Hypothesis example

Purpose: Precautionary, safe-by-design, regulatory, targeted testing

Context: Occupational, inhalation study

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)

What they are?

High aspect ratio, rigid NM with low dissolution rate and aerodynamic diameter to allow deposition in the distal lung

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.

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

Potential implications:

If in group:

Regulatory: develop read-across argument on hazard for regulatory use and compare to relevant

Human Inhalation Hypothesis example

Purpose: Precautionary, safe-by-design, regulatory, targeted testing

High aspect ratio, rigid NM with low dissolution rate and aerodynamic diameter to allow deposition in the distal lung.

A small proportion of HARN deposited (approx. 1%) will translocate to the pleural cavity.
Fibres > 5um in length will be retained in the pleural cavity….

Cause frustrated phagocytosis… result in chronic inflammation, mesothelial proliferation, fibrosis and mesothelioma

Regulatory: develop read-across argument on hazard for regulatory use and compare to relevant

Human Dermal Hypothesis example

Purpose: Regulatory

Context: Consumer, occupational

INMut from life cycle	**What they are?**
NMs incorporated into a consumer product applied to the skin (e.g. personal care products, cosmetics, sunscreens) **Type of exposure** Exposure to NMs in an occupational setting Dermal exposure	NMs larger than 5 nm that are not flexible
	Where they go? When reaching healthy skin, will not penetrate the stratum corneum, and will not permeate skin to access systemic circulation in a proportion larger than 0.1% of the external dose
	What they do? Will not cause particle/nano specific toxicity

Potential implications:

If in group:

Regulatory: conclude no systemic exposure to NM which may lead exposure-based waiving of testing for systemic toxicity (there could be exposure to ions if they dissolve).

If not in group: Consider alternative hypothesis (systemic exposure to NM cannot be excluded).

Human Dermal Hypothesis example

Purpose: Regulatory

Context: Consumer, occupational

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NMs > 5nm that are not flexible.

When reaching healthy skin, will not penetrate the stratum corneum and will not … access systemic circulation in a proportion larger than 0.1% of the external dose.

Will not cause particle/nano specific toxicity.

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Regulatory: conclude no systemic exposure to NM which may lead exposure-based waiving of testing for systemic toxicity (there could be exposure to ions if they dissolve).

If not in group: Consider alternative hypothesis (systemic exposure to NM cannot be excluded).

Solid matrix nano-enabled product

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.	*Precautionary approaches or safe-by-design:* Control-banding (Level 1), minimize exposure or adjustment of NEP.	• Incorporation of NF into the matrix of the NEP (g/g content, disperse state) • Resilience of matrix under relevant conditions

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NFs incorporated into a solid matrix.

The probability and form of release is mainly determined by the type of matrix, dispersion state of the NF in the matrix and use or aging process.

Hypotheses Evidence

HARN	Description of panel	PC	Model	Endpoints	Results	Ref
MWCNT	MWCNT Mitsui-7	Length Diameter Contamination	C57BL/6J mice were exposed by pharyngeal aspiration to 10, 20, 40 and 80 µg MWCNT	Morphometric methods were used to determine the distribution of MWCNT and the number of MWCNT fibre penetrations of three barriers: alveolar epithelium (alveolar penetrations), the alveolar epithelium immediately adjacent to the pleura (subpleural tissue), and visceral pleural surface (intrapleural space) at 1, 7, 28 and 56 d after exposure.	At 1 day 18%, 81.6% and 0.6% of the MWCNT lung burden was in the airway, the alveolar, and the subpleural regions, respectively. The density of penetrations increased to steady state levels in the subpleural tissue and intrapleural from day 28 - 56. At day 56 approximately 1 in every 400 fibre penetrations was in either the subpleural tissue or intrapleural space.	(Mercer, Hubbs et al. 2010)

>18 further references included in justification table

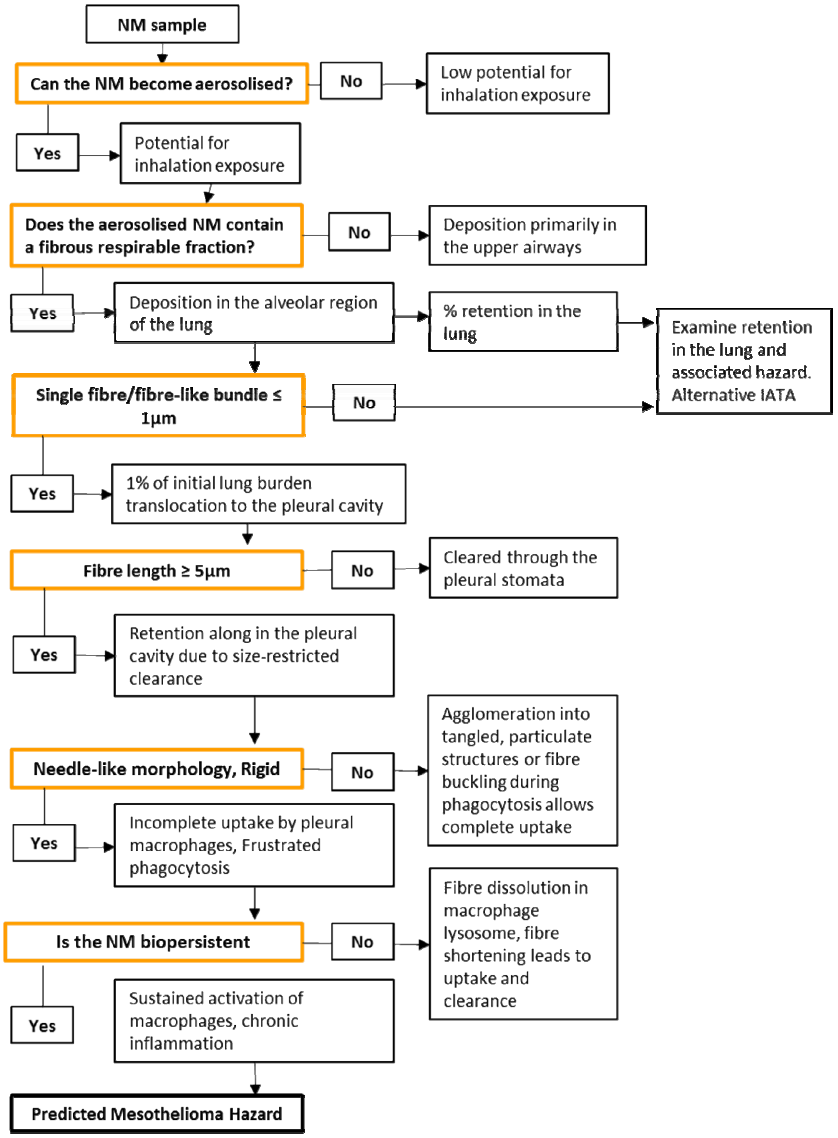
Integrated Approaches to Testing and Assessment

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

Grouping Framework Design

Integrated Approaches to Testing and Assessment

- The IATA format uses the format suggested by OECD
- Each IATA design is ‘science based’ and tailored to the specific hypothesis
- Allows identification of SOPs for each endpoint assessed
  - OECD, ISO, published



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Omics slide

- Omics approaches allow identification of mechanisms of action or adverse outcome pathways
- They therefore allow identification of biomarker 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

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Summary

- Long term studies are required in order to inform risk assessment of NMs
- In vitro models that allow longer term, repeated exposures are also needed, and are under development by PATROLS
- Up to 23 clear hypotheses identified by GRACIOUS that cover human and environmental hypotheses
- Only 4 of these can be considered, confident, well justified or with clear consequences.
- Hypotheses however are sufficient to allow generation of IATA's that allow the hypotheses to be tested.
- The IATA's currently consist of a mixture of OECD, ISO and published protocols – but gaps exist.
- Omics can inform endpoint identification.

BIO RIMA
BIOmaterial RIsk MAnagement

Integrated Risk Management Framework for nano(bio)materials used in medical devices and advanced therapy medicinal products

- Develop an **Integrated Risk Management (IRM) Framework.**
- Provide ready-to-use **Risk Management toolbox.**
- Provide **Decision Support System**, using validated tools and methods for materials, exposure, hazard and risk.
- **Scientific rationale** for selection.
- Enable **industries and regulators** to use tools for high-quality data and informed decision-making framework.

1st BIORIMA Stakeholder Workshop

6 November 2018 in Valencia, Spain

REGISTER NOW

www.biorima.eu
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Acknowledgements

Special thanks:
Araceli Sanchez
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Wendel Wohlleben
Helinor Johnston
Eric Bleeker
Dominique Balharry

ITS-NANO

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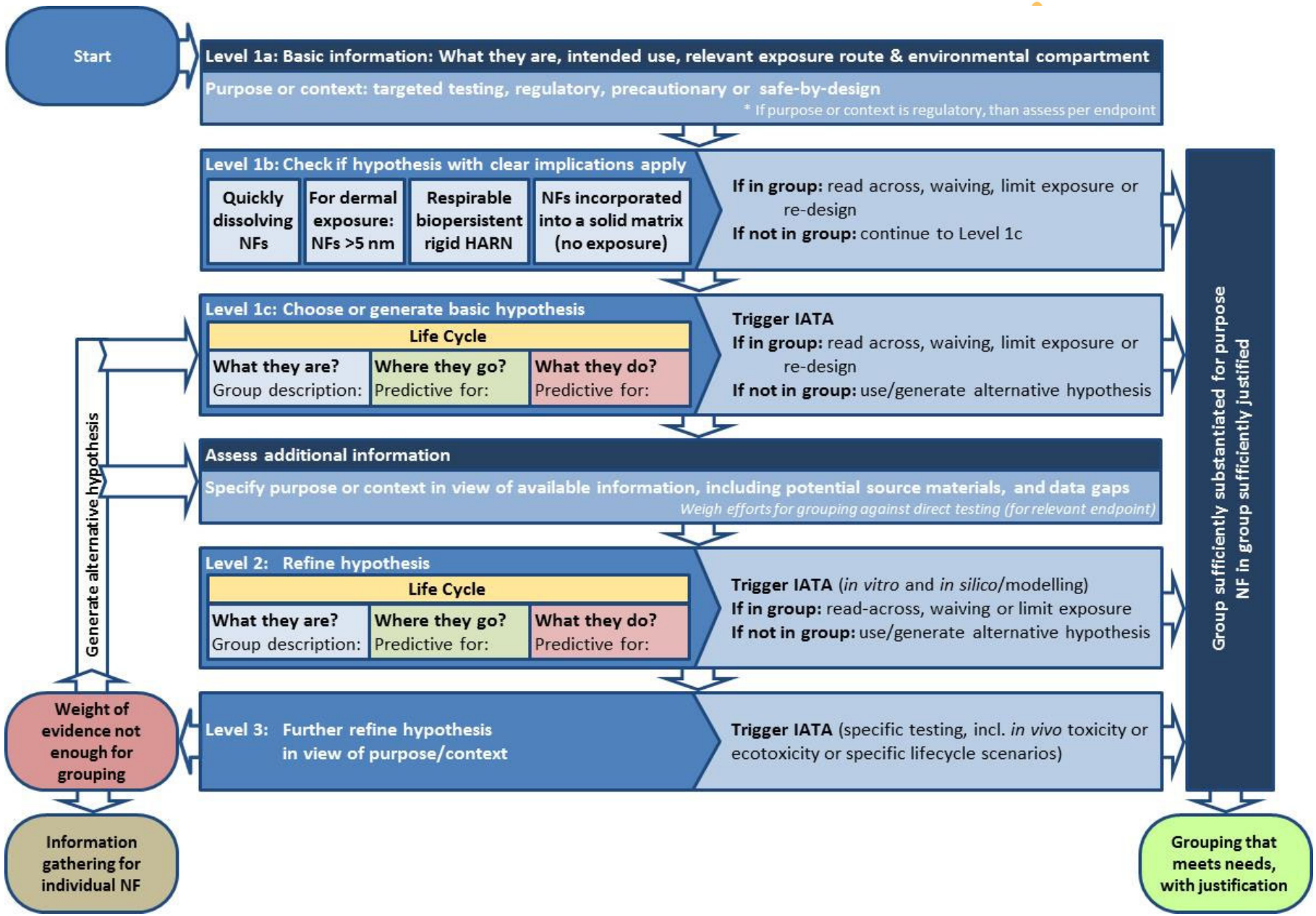
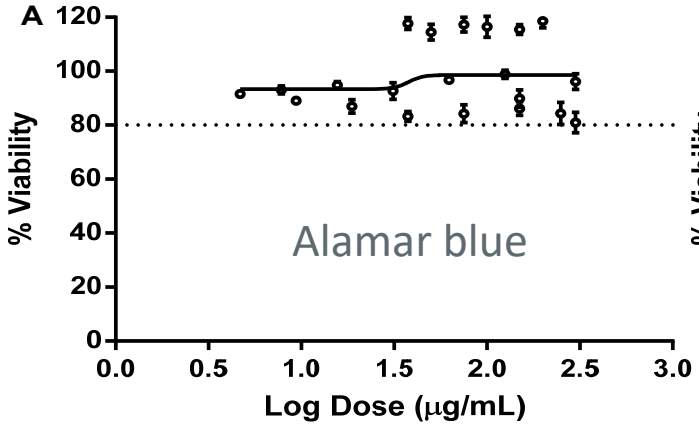
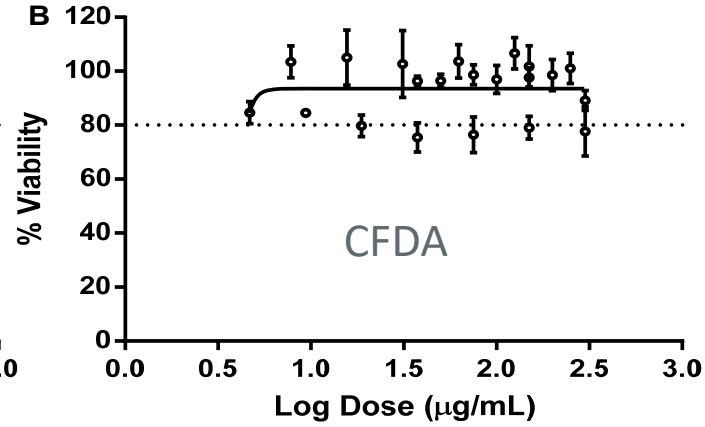
Thank You

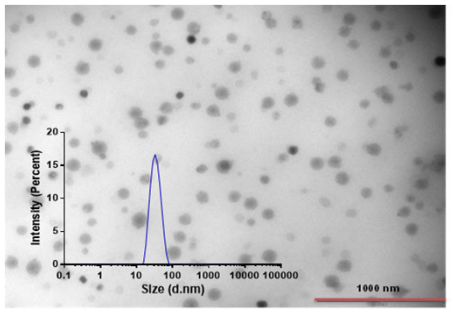

Start
Level 1a: Basic information: What they are, intended use, relevant exposure route & environmental compartment
Purpose or context: targeted testing, regulatory, precautionary or safe-by-design
* If purpose or context is regulatory, than assess per endpoint
Level 1b: Check if hypothesis with clear implications apply
Quickly dissolving NFs
For dermal exposure: NFs >5 nm
Respirable biopersistent rigid HARN
NFs incorporated into a solid matrix (no exposure)
If in group: read across, waiving, limit exposure or re-design
If not in group: continue to Level 1c
Level 1c: Choose or generate basic hypothesis
Life Cycle
What they are? Group description:
Where they go? Predictive for:
What they do? Predictive for:
Trigger IATA
If in group: read across, waiving, limit exposure or re-design
If not in group: use/generate alternative hypothesis
Generate alternative hypothesis
Assess additional information
Specify purpose or context in view of available information, including potential source materials, and data gaps
Weigh efforts for grouping against direct testing (for relevant endpoint)
Level 2: Refine hypothesis
Life Cycle
What they are? Group description:
Where they go? Predictive for:
What they do? Predictive for:
Trigger IATA (in vitro and in silico/modelling)
If in group: read-across, waiving or limit exposure
If not in group: use/generate alternative hypothesis
Weight of evidence not enough for grouping
Level 3: Further refine hypothesis in view of purpose/context
Trigger IATA (specific testing, incl. in vivo toxicity or ecotoxicity or specific lifecycle scenarios)
Group sufficiently substantiated for purpose
NF in group sufficiently justified
Information gathering for individual NF
Grouping that meets needs, with justification

Development of ε-decalactone based nanomaterials

C3A hepatocytes







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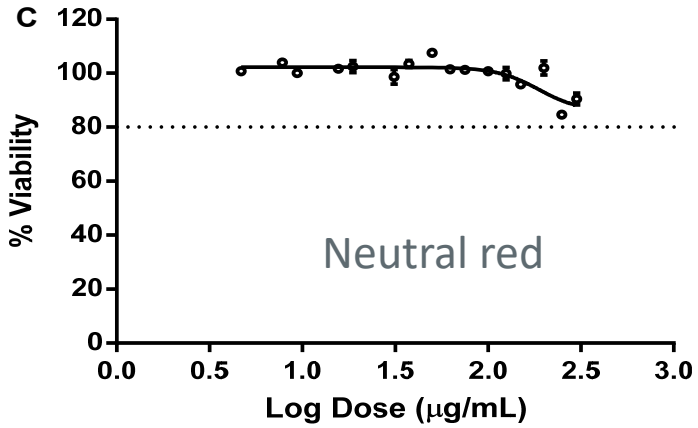

Hypothesis development

- General Hypotheses for grouping NM
- 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

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 Hypotheses with clear implications

Group description and hypothesis	Potential implications/consequences	Relevant testing (in IATA where appropriate)
Respirable biopersistent rigid High Aspect Ratio NFs (HARN): NF will translocate to the pleural membrane and lead to frustrated phagocytosis (uptake and clearance) by macrophages (immune cells) that subsequently can cause mesothelioma (cancer of pleural cavity around lungs). *Scientific/clinical rationale:* Mesothelioma.	*Precautionary approaches or safe-by-design:* Prevent/minimize exposure, or modify the NF/NEP to reduce hazard. *Targeted testing:* Testing to assess concerns. *Regulatory:* Read-across to asbestos (Level 1), or another rigid HARN (in subsequent Level) may be possible.	• Dissolution in fluids representative of lung lining and lysosomal fluid. • *In vitro* assessment of frustrated phagocytosis. • *In vitro* assessment of pro-inflammatory, pro-proliferative and genotoxic potential. • In later tiers (if applicable): *in vivo* translocation, *in vivo* inflammation and/or mesothelial cell proliferation.

Grouping Hypotheses with clear implications

Group description and hypothesis	Potential implications/consequences	Relevant testing (in IATA where appropriate)
NFs larger than 5 nm (D5NM): NF will not translocate across skin. *Scientific rationale:* If there is no translocation across intact skin in case of dermal exposure, systemic exposure via skin will not occur.	*Regulatory:* Waiving of endpoints related to systemic exposure.	• Size of the NF in relevant media • Translocation studies across skin (*in vitro*, *ex vivo*).