Bundesinstitut für Risikobewertung

New Approach Methodologies (NAMs) for risk assessment of nanomaterials - insights from selected-research projects -

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Fibre and Nanotoxicology
Department of Chemical- and Consumer Safety

14.09.2023, NanoTrust

Overview

- Risk Assessment and New Approach Methodologies (NAMs)
  - Fibres
  - Nanomaterials
  - (Other) Advanced Materials

Risk Assessment: The current status

Substance Identity

Chemical Composition (Impurities?), Stability …

Exposure Assessment

Which dose? How often/ long? etc.

Hazard Identification

Target organs? Which adverse effects?

Dose Response Assessment

Threshold?

Risk characterization

Is there a risk? For whom? How big is the risk?

In total, 1.4 million animal uses for regulatory safety testing in EU (2020)

- 54 % human medical products
- 23 % veterinary medical products
- 9 % industrial chemicals
- 5 % plant protection products
- 4 % medical devices
- 3 % food
- 2 % feed (3.8%)
- 0.3% biocides

Regulatory Toxicology: OECD Test Guidelines

OECD Test Guidelines (TGs)

- considered adequate **for testing/ evaluation of chemicals**
- published by **O**rganization for **E**conomic **C**ooperation and **D**evelopment (OECD)
- accepted by regulatory agencies in many (including all OECD) countries
- guidelines are developed for international use

OECD Council Decision (1981): Mutual Acceptance of Data:

Test Data generated in any member country in accordance with **OECD TGs** and **GLP Principles** shall be accepted in other member countries

Mutual Acceptance of Data (MAD)

OECD Test Guidelines + **GLP Principles**

Acute toxicity (oral TG 420, TG 423)
Skin irritation/corrosion (TG 431, TG 439)
Eye irritation/corrosion (TG 437, 438, 460, 491, 492)
Skin sensitization (TG 442C, 442D, 442E)
Mutagenicity (TG 471)
Skin irritation /corrosion (TG 404)
Eye Irritation / corrosion (TG 405)
In-vitro cytogeneticity (TG 473, TG 487)
In-vitro mutation tests (TG 476, TG 490)
Acute toxicity (dermal, inhalation, TG 402, TG 436)
Subacute toxicity (TG 407)
Reproduction / Developmental Toxicity (TG 421, TG 422)
Repeated dose toxicity (TG 408)
Developmental toxicity (TG 414)
Extended One Generation Test (TG 443)
Chronic Toxicity (oral, TG 452)
Carcinogenicity (TG 451, TG 453)

***In vivo* test methods**

OECD member countries: 38

Australia, Austria, Belgium, Canada, Chile, Colombia, Costa Rica, Czech Republic, Denmark, Estonia, Finland, France, **Germany**, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Latvia, Lithuania, Luxemburg, Mexico, Netherlands, New Zealand, Norway, Poland, Portugal, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, UK, USA

New Approach Methodologies (NAMs)

NAMs

- can be broadly understood as *in silico*, *in chemico* and *in vitro* methods including new testing tools such as “high-throughput screening” or “high-content methods” like the various omics approaches
- Importantly, NAMs are more than just “alternatives” or “methods” (they are critically connected to “data”, “data models”/ “data structures” requiring harmonization to allow for data integration and data re-use), paving the way to modern data-driven approaches

NAMs have huge potential to substantially advance hazard and risk assessment in future.

Advantages

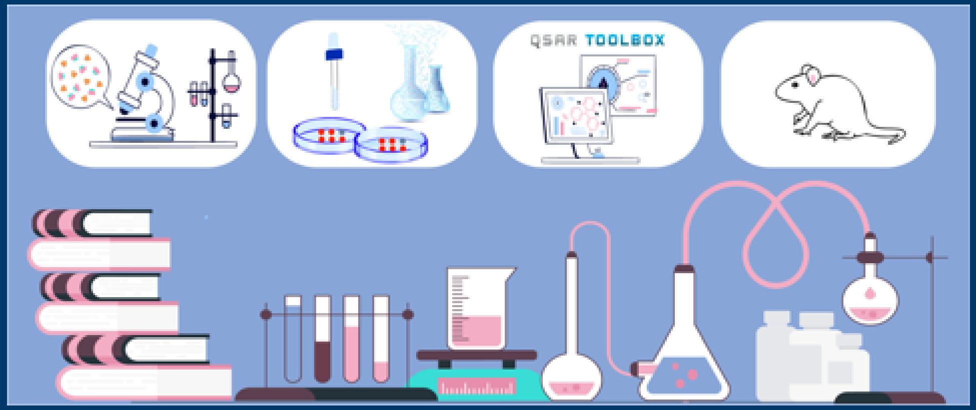
- higher efficiency
- less expensive (?)
- human- focused
- …
- **mechanistic insights**

Current regulatory applications

- Screening/ prioritization
- Replace animal tests (e.g. skin/ eye corrosion, sensitization)
- Higher tier endpoints remain challenging but NAMs can be applied in **Data Integration Approaches** such as
  - **Grouping and read-across**
  - **Integrated Approaches to Testing and Assessment (IATAs)**

Utilize NAMs in Data Integration Approaches (NAM – Frameworks)

Integrated Approaches to Testing and Assessment (IATAs)



OECD IATA Activities: https://www.oecd.org/chemicalsafety/risk-assessment/iata/

- **IATAs are flexible approaches for chemical safety assessment**
- IATAs integrate data from multiple methods/ sources, including NAMs
- provide a **logical combinations of tests and assessments** (outcome of one step determines the next); hence, the **overall assessment is conducted with a minimum of new experiments**
- Frequently IATAs are based on **Adverse Outcome Pathway (AOP) concepts**

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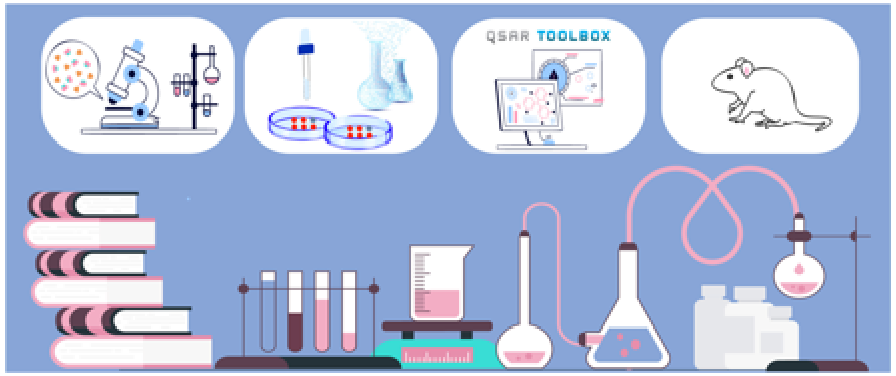
Risk Assessment: Integration of New Approach Methodologies (NAMs)

Developing/ integrating NAMs in chemical risk assessment is an important overarching goal in plenty of projects/ activities worldwide involving different stakeholders, agencies and organizations.

OECD IATA activities

OECD



Selected key projects/ initiatives

Tox21
PRECISION TOX
ONTOX
RISK HUNT3R
PARC

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EU PARC: EU Partnership for the Assessment of Risks from Chemicals

- A public-public **partnership** under Horizon Europe
- An initiative where the **EU**, prepared with early involvement of **Member States and Associated Countries**, together with public partners (EU and National Risk Agencies, Universities, Public Research Organisations), commit to **jointly support the development and implementation of a programme** of research and innovation activities in relation with **the assessment of risk of chemicals.**

PARC

www.eu-parc.eu

HORIZON EUROPE

➢ several BfR departments involved

Public- Public
Co-Fund Budget
EU 50/50 MS,AC
400 M€

Started : 01/05/2022
Duration : 7 years

~200 Partners

29 countries

24 Member States: Austria (AT), Belgium (BE), Croatia (HR), Cyprus (CY), Czech Republic (CZ), Denmark (DK), Estonia (EE), Finland (FI), France (FR), Germany (DE), Greece (EL), Hungary (HU), Ireland (IE), Italy (IT), Latvia (LV), Lithuania (LT), Luxembourg (LU), Netherlands (NL), Poland (PL), Portugal (PT), Slovakia (SK), Slovenia (SI), Spain (ES), Sweden (SE)
3 Associated countries: Iceland (IS), Israel (IL), Norway (NO)
2 Non-associated Third countries: Switzerland (CH), United Kingdom (UK)

3 European Agencies : European Environment Agency, efsa, ECHA European Chemicals Agency

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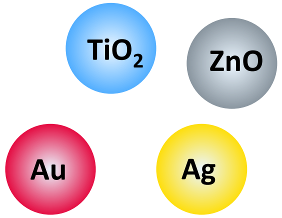
Opportunities & Challenges for Nanomaterials (NMs)

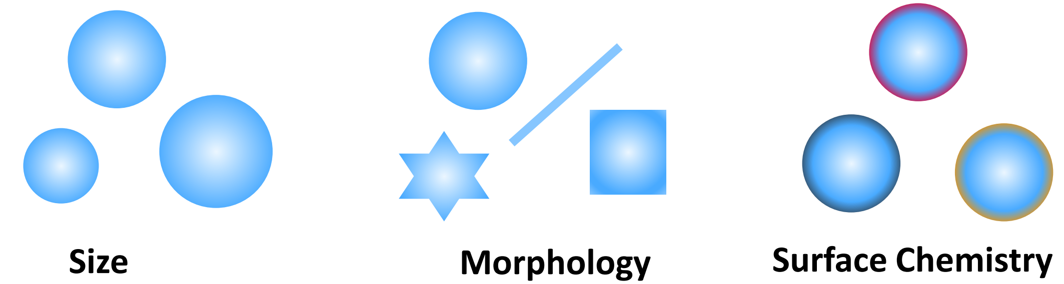
NMs are easily manufactured in plenty of variants and risk assessment requires data for each

- **Urgent demand for novel & more efficient approaches- but they have to be valid**
- **Critical bottleneck: Method validation for regulatory application (e.g. OECD TGs/ GDs) - lagging behind**

e.g. pigments



metals/ metal oxide



Particulate nature requires sophisticated physico-chemical characterization, not only of the pristine materials

Complex transformations need to be considered (e.g. agglomeration, dissolution, biomolecule corona)

Particulate nature renders in vitro assays more challenging (e.g. dispersion stability, interferences, dosimetry)

Establishing NAMs for Fibre Risk Assessment

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Fibres: Current Status and Challenges

Classical Fibre Toxicity Paradigm: **Respirable, long and biopersistent fibres are carcinogenic**

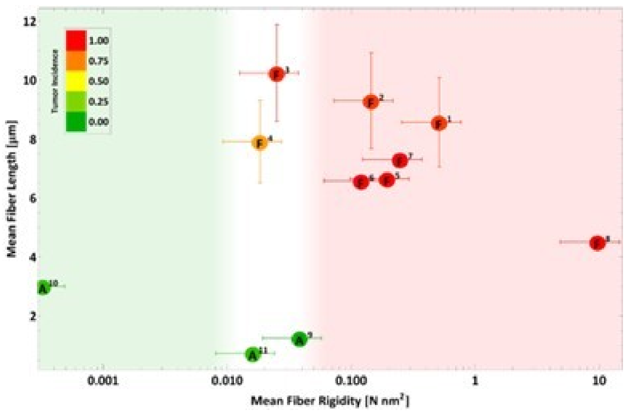
Asbestos associated pathologies:

- e.g. Fibrosis, Lung Cancer, Mesothelioma

Criteria (WHO):

- Respirability & Reaching distal lung (d < 3 μm)
- Failing alveolar clearance (L > 5 μm)
- High durability in water and biological environments

Nanofibres challenge the classical fibre toxicity paradigm:
Rigidity hypothesis: Long, rigid fibres withstand bending during phagocytosis; flexible, thin fibres curl up.



MWCNT i.p. testing data from literature

Threshold flexural rigidity of ca. 0.1 $\mathrm{N{\cdot}nm^2}$

- Rigidity threshold is material independent
- **Diameter threshold is material dependent**
- Additionally: highly ordered secondary structures

Available data from e.g. historic asbestos studies is limited due to lacking fibre characterization.
Crocodolite: $\mathcal{R} > 1\ \mathrm{N{\cdot}nm^2}$, Chrysotile: $\mathcal{R} \sim 0.2\ \mathrm{N{\cdot}nm^2}$

Research Focus 1: Establish IATAs for Fibres

Currently, risk assessment requires animal test, e.g. for carcinogenicity

Concerns: Ethical, Efficiency, Predictivity

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Contents lists available at ScienceDirect

NanoImpact

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

Research paper

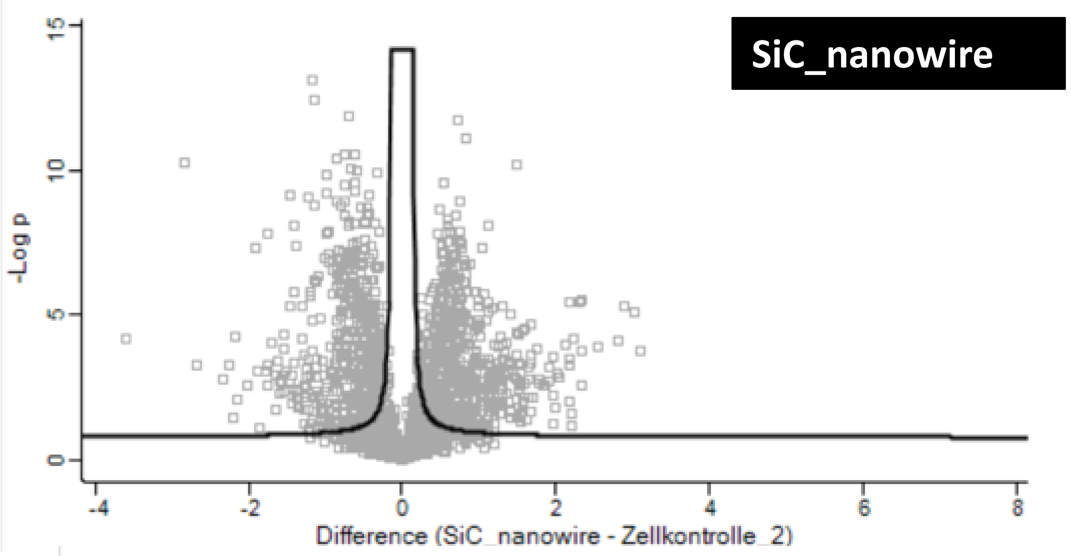
An integrated approach to testing and assessment of high aspect ratio nanomaterials and its application for grouping based on a common mesothelioma hazard

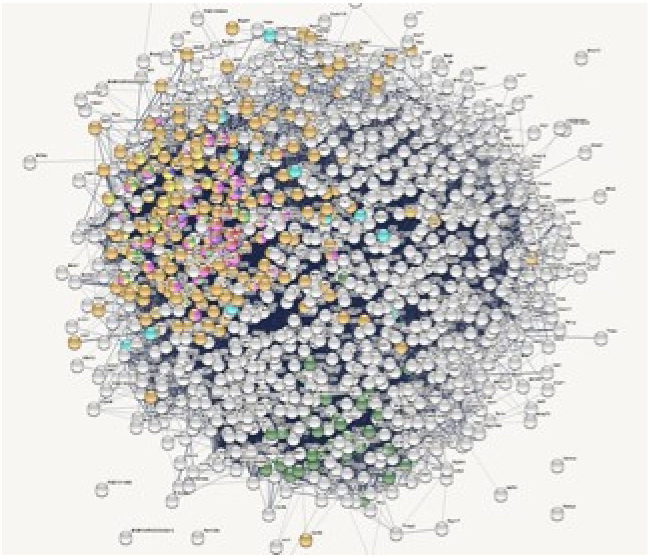
Fiona Murphy[a,*], Susan Dekkers[b], Hedwig Braakhuis[b], Lan Ma-Hock[c], Helinor Johnston[a], Gemma Janer[d], Luisana di Cristo[e], Stefania Sabella[e], Nicklas Raun Jacobsen[f], Agnes G. Oomen[b], Andrea Haase[g], Teresa Fernandes[a], Vicki Stone[a]

Integrated Approaches to Testing and Assessment (IATAs)

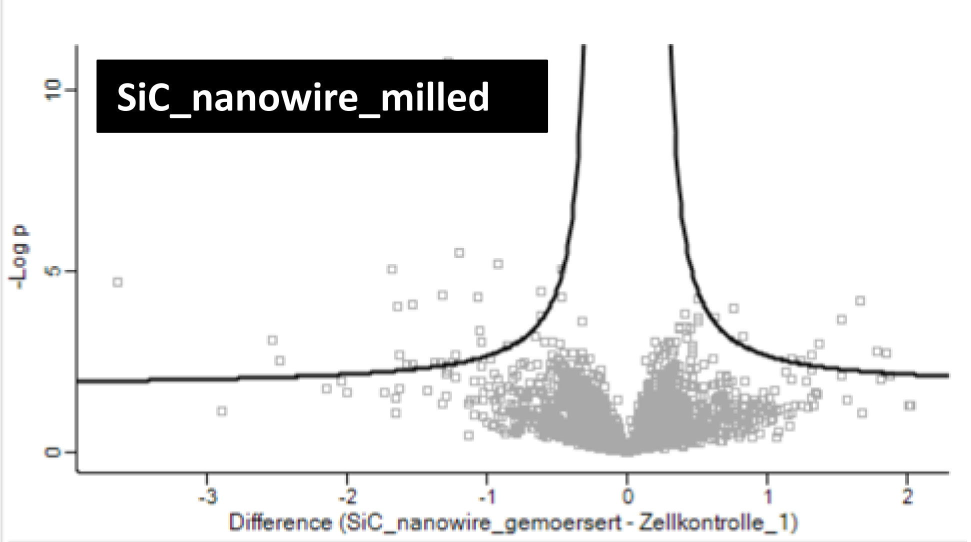
Morphology	Electron Microscopic Techniques	✓
Durability (Persistence)	Dissolution in biological media	(✓)
Rigidity (Nanofibres)	No validated test method Diameter Threshold (only for MWCNT)	– (✓)
Toxicity	Biomarker from Omics Integration (Macrophage-Assays, more complex cell models)	?

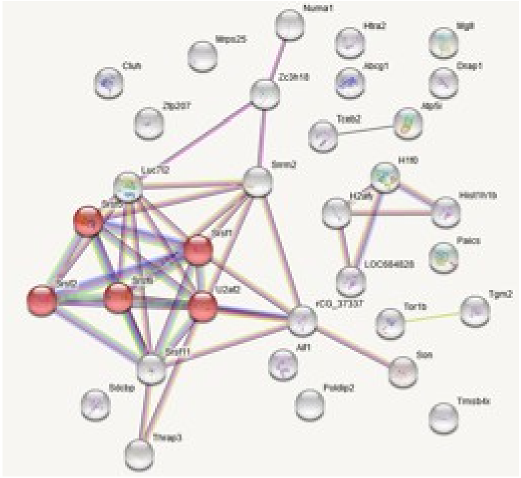
Research Focus 1: Establish IATAs for Fibres





NR8383 cells
(rat alveolar macrophages)
45 µg/ml, 18h



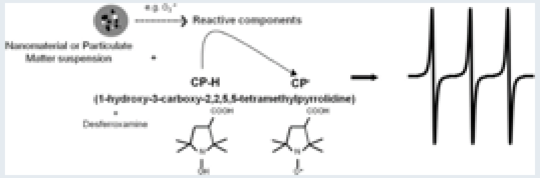


Establishing NAMs for Nanomaterial Risk Assessment

Research Focus 2: Establising NAMs – Example Reactivity/ Ox. Stress

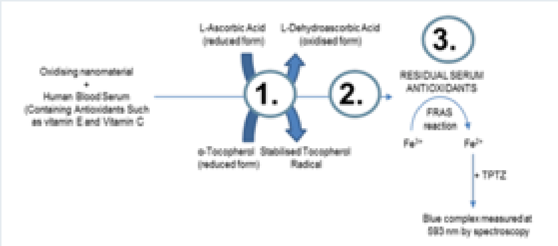
- Reactivity/ oxidative stress is a very important parameter as NMs are often more reactive – yet validated methods are missing
- In several projects different methods were developed- partially SOPs are available now, some of them verified in ILC

Electron Spin Resonance (ESR)



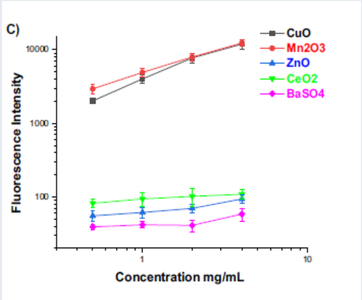
Hellack, Nickel, Schins, J Nanopart Res (2017)

Ferric Reduction Ability of Serum (FRAS) assay

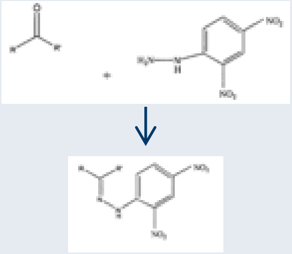


Gandon et al., J. Phys. (2017)

DCFH-DA assay



Protein carbonylation assay



Castegna, et al. (2003)

Interlaboratory Comparisons (DCFH-DA assay)

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Toxicology Mechanisms and Methods

Taylor & Francis

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/itxm20

Development of a standard operating procedure for the DCFH$_2$-DA acellular assessment of reactive oxygen species produced by nanomaterials

Matthew Boyles, Fiona Murphy, William Mueller, Wendel Wohlleben, Nicklas Raun Jacobsen, Hedwig Braakhuis, Anna Giusti & Vicki Stone

- https://doi.org/10.1080/15376516.2022.2029656

Application for NM Grouping

nanoGRAVUR

ELSEVIER

NanoImpact

Volume 19, July 2020, 100234

Research paper

Nanomaterial categorization by surface reactivity: A case study comparing 35 materials with four different test methods

Aileen Bahl [a], Bryan Hellack [b c], Martin Wiemann [d], Anna Giusti [a], Kai Werle [e], Andrea Haase [a 1], Wendel Wohlleben [e 1]

https://doi.org/10.1016/j.impact.2020.100234

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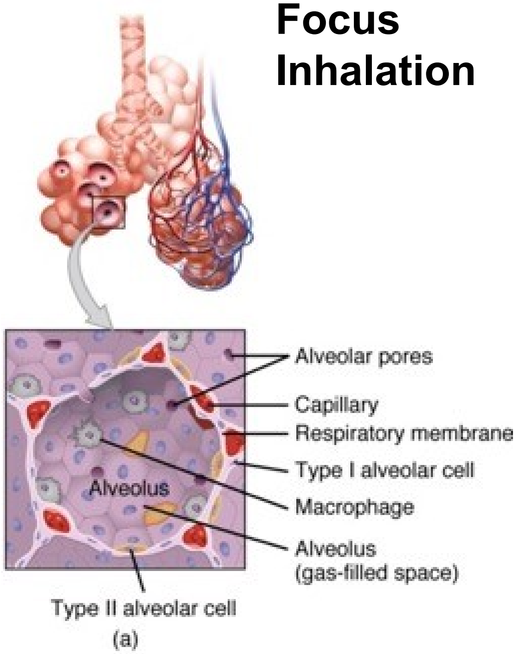
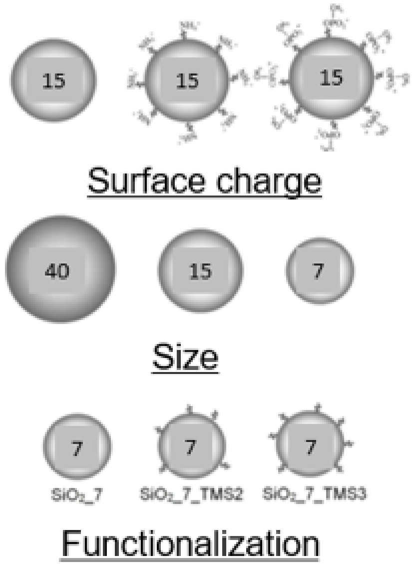
Research Focus 3: Characterising NM MoA by omics approaches

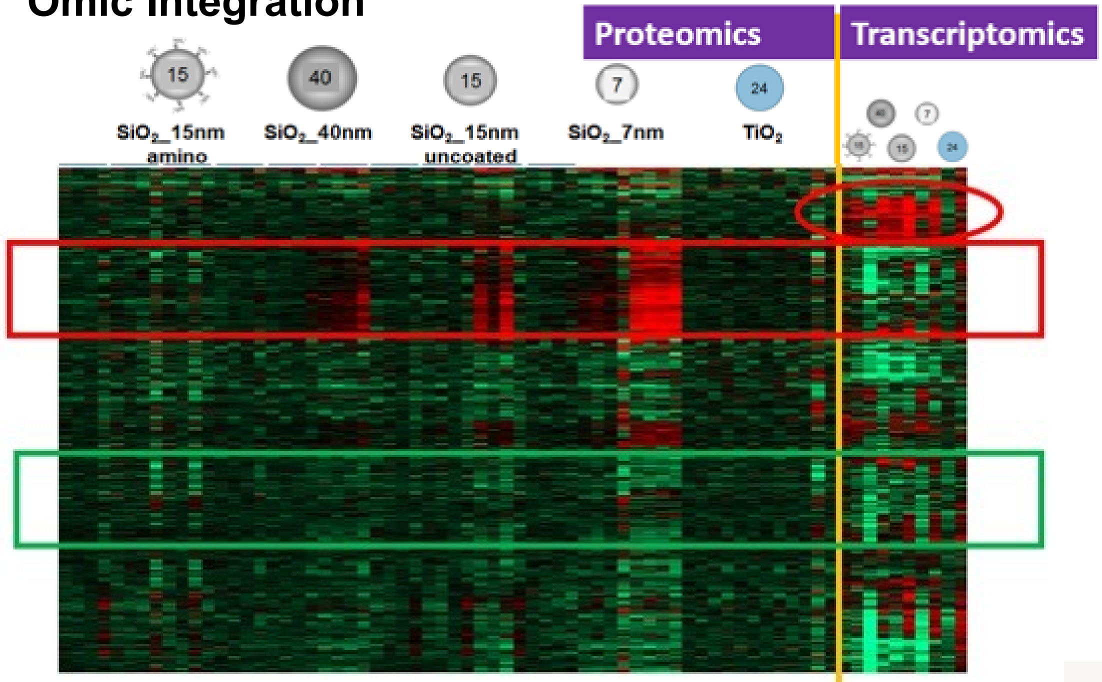
Focus Inhalation



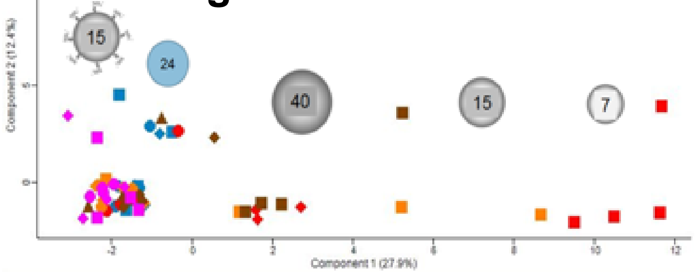
Material Selection

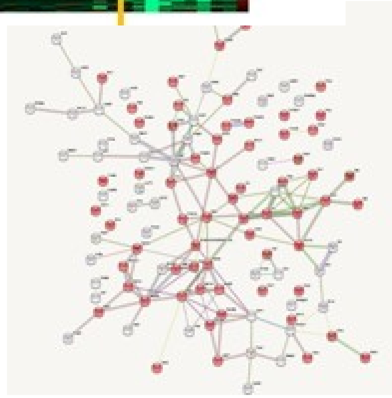


Omic Integration



Characterizing mechanisms/ Biological relevance





- Bannuscher et al. 2020 ***Nanotoxicology*** 14, 807-826. https://doi.org/10.1080/17435390.2020.1764123
- Bannuscher & Karkossa et al. 2020 ***Nanotoxicology*** 14, 181-195. https://doi.org/10.1080/17435390.2019.1684592
- Karkossa & Bannuscher et al. 2019 ***Particle and Fibre Toxicology*** 16, 38. https://doi.org/10.1186/s12989-019-0321-5

Research Focus 4: Data Infrastructure & FAIR Data

Vision: Enable the design and advanced safety assessment of innovative materials through data-rich concepts by establishing a reliable and sustainable research data infrastructure.

ANALYSIS
https://doi.org/10.1038/s41565-021-00911-6
nature nanotechnology

Towards FAIR nanosafety data

Nina Jeliazkova[1], Margarita D. Apostolova[2], Cristina Andreoli[3], Flavia Barone[3], Andrew Barrick[4], Chiara Battistelli[3], Cecilia Bossa[3], Alina Botea-Petcu[5], Amélie Châtel[4], Isabella De Angelis[3], Maria Dusinska[6], Naouale El Yamani[6], Daniela Gheorghe[5], Anna Giusti[7], Paloma Gómez-Fernández[8], Roland Grafström[9,10], Maciej Gromelski[11,12], Nicklas Raun Jacobsen[13], Vedrin Jeliazkov[1], Keld Alstrup Jensen[13], Nikolay Kochev[1,14], Pekka Kohonen[9,10], Nicolas Manier[15], Espen Mariussen[6], Agnieszka Mech[16], José María Navas[17], Vesselina Paskaleva[1,14], Aurica Precupas[5], Tomasz Puzyn[11,12], Kirsten Rasmussen[16], Peter Ritchie[18], Isabel Rodríguez Llopis[8], Elise Rundén-Pran[6], Romica Sandu[5], Neeraj Shandilya[19], Speranta Tanasescu[5], Andrea Haase[7] and Penny Nymark[9,10]

https://doi.org/10.1038/s41565-021-00911-6

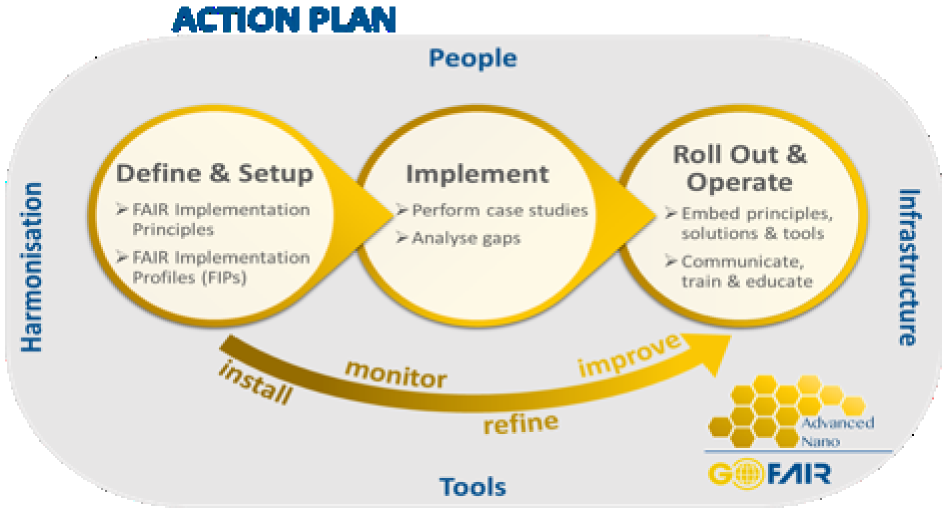
nanotoday
Volume 51, August 2023, 101923

From principles to reality. FAIR implementation in the nanosafety community

Verónica I. Dumit[a], Ammar Ammar[b], Martine I. Bakker[c], Miguel A. Bañares[d], Cecilia Bossa[e], Anna Costa[f], Hilary Cowie[g], Damjana Drobne[h], Thomas E. Exner[i], Lucian Farcal[j], Steffi Friedrichs[k], Irini Furxhi[l m], Roland Grafström[n v], Andrea Haase[a], Martin Himly[o], Nina Jeliazkova[p], Iseult Lynch[q], Dieter Maier[r], Cornelle W. Noorlander[c], Hyun Kil Shin[s]...Penny Nymark[v]

https://doi.org/10.1016/j.nantod.2023.101923

Advanced Nano FAIR Implentation Network



https://www.go-fair.org/implementation-networks/overview/advancednano/

EFSA NAMS4NANO - Integration of New Approach Methodologies results in chemical risk assessments (case studies addressing nanoscale considerations)

Budget:	5.3 Mill Euro (EFSA funding, GP/EFSA/MESE/2022/01)
Duration:	4 years (04.04.2023- 03.04.2027)
Consortium:	BfR (GER); ISS (IT); ANSES (FR); RIVM (NL); Sciensano (BE); WFSR (NL); LIST (LU)
Additional Partners:	JRC (EC); Singapore Food Agency (SING)
Subcontractors:	Fraunhofer ITEM (GER) – for Lot 1-3
	Uni Auckland (New Zealand); Uni Hasselt, Uni Politècnica de València – only for Lot 3

Lot 1: Review, Qualification System, Implementation Plan	**Lot 2: Risk Assessment Case Studies**	**Lot 3: Methodological Case Studies**
Coordination: A. Haase (BfR)	Coordination: A. Haase (BfR)	Coordination: F. Cubadda (ISS)

Overall aims:

- **Improve <u>general understanding on integrating NAMs in risk assessments</u>** (possibilities, challenges, uncertainties)
- **Explore <u>applicability of NAMs for risk assessment</u>** in specific case studies, mainly **for filling data gaps** in existing assessment by **integrating data** generated by NAMs with other types of data using **Integrated Approaches to Testing and Assessment (IATAs)**
- **<u>Further improve selected methodologies</u>**

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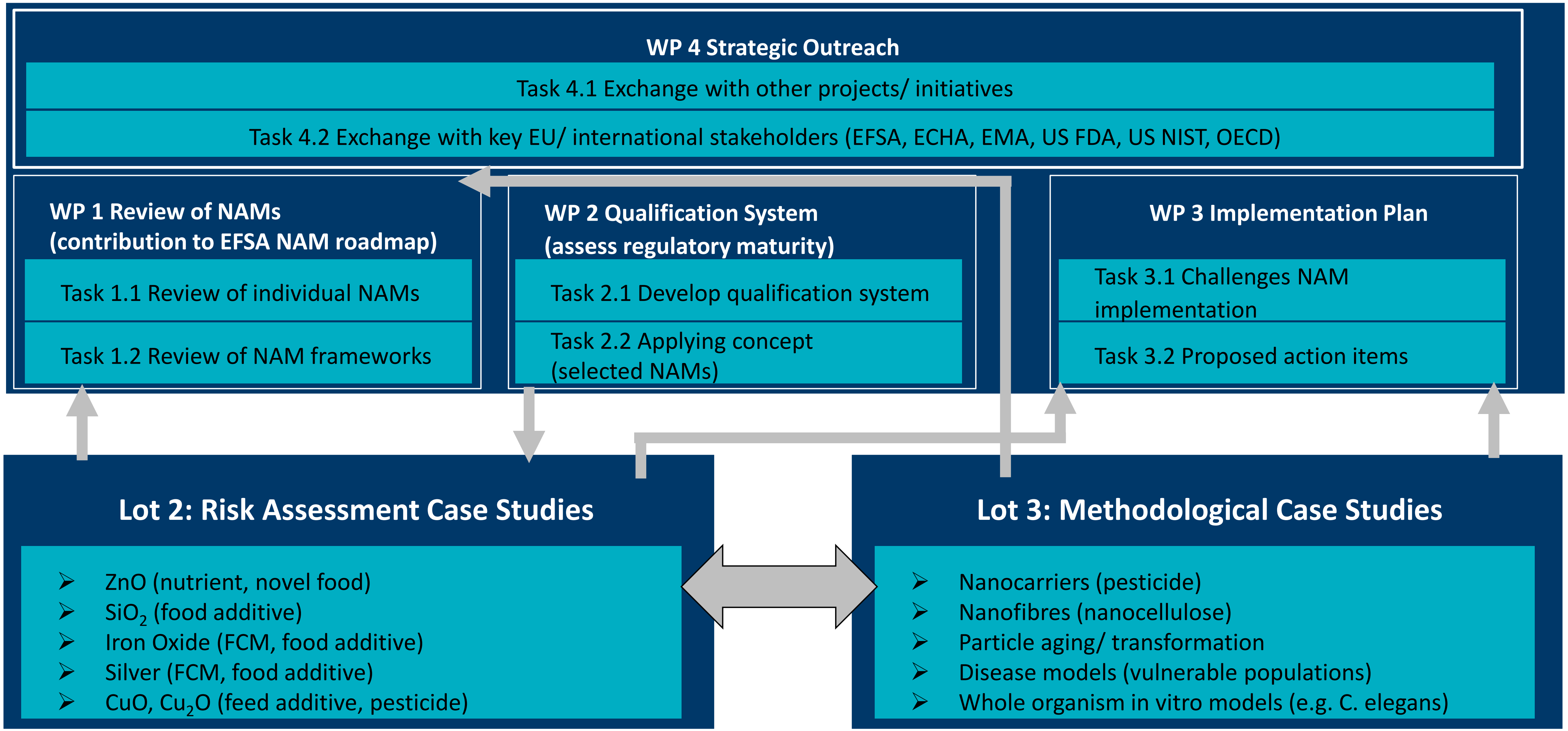
EFSA NAMS4NANO Project – Interaction between the Lots



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Establishing NAMs for Advanced Materials

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Advanced Materials (AdMa): Current Status and Challenges

➢ Material innovations are important drivers in many industrial sectors

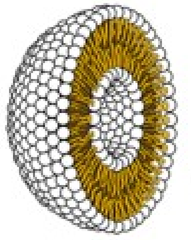
Working Definition
Advanced materials are materials that are rationally structured and designed through the precise control of their composition and internal or external structure in order to fulfil new functional requirements.

Important Sectors for Advanced Materials (examples)

- Health Care & Medicine (e.g. new drug delivery systems)
- Building & Construction (e.g. new bio-inspired building materials)
- Energy (e.g. new battery materials)
- Transport (e.g. light-weight materials, new catalysts)
- Consumer Products (e.g. advanced surfaces, intelligent textiles)
- Packaging (e.g. renewable, recyclable materials, intelligent packaging)
- Agriculture (e.g. new formulation for biocides/ pesticides)
- Electronics

…

Source: Materials 2030 Manifesto, 07.02.2022



Liposome nanocarrier
Source: Wikicommons

Nanocellulose for portable paper solar cells
Source: Nogi et al. 2015 Sci Rep 5, 17254
https://doi.org/10.1038/srep17254

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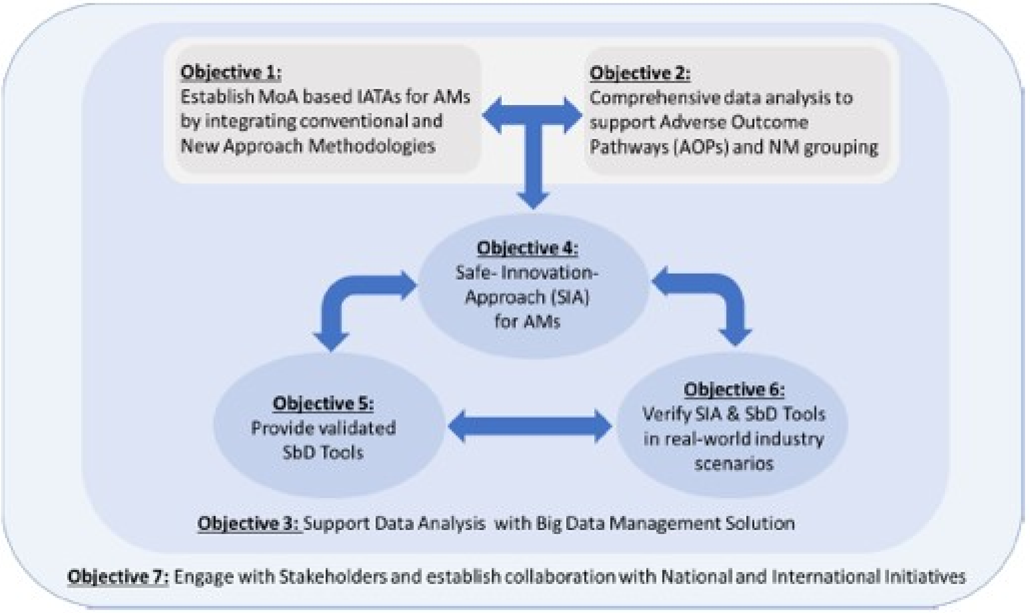
Research Focus 5: Establish IATAs for Advanced Materials

Advanced **H**igh **A**spect **R**atio and **M**ulticomponent Materials:
Towards comprehensive intel**L**igent t**E**sting and **S**afe by design **S**trategies

HARMLESS

InnoMatLife



Industrial relevant AM (broad material selection)

- **Pesticides** *(e.g. Metal-doped Imogolite)*
- **Paint formulations** *(e.g. Silica-polymer)*
- **Papermaking** *(e.g. Silica-rod-cellulose)*
- **Catalysts** *(e.g. perovskite)*
- **Solar cells** *(e.g. perovskite, Ag-nanowires)*
- **Facade insulation** *(e.g. fibre-supported aerogel)*

HELMHOLTZ MUNICH | BfR | TNO | NATIONAL RESEARCH CENTRE FOR THE WORKING ENVIRONMENT | BIO NANONET | DTU | INIA | IDEA Consult | BASF We create chemistry | Nouryon | LUNDS UNIVERSITET | baua: | cea | misvik biology | TEMAS Solutions

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Advanced Materials: German Interagency Working Group

Participants

Observer

- **Establish an Early Warning System**
  - Regulatory preparedness for materials innovations
  - Identify potentially critical materials/ applications early on
  - Identify data gaps, needs for research and method development
  - Check existing legislation and guidance documents for needs of adaptation

- **Ensure a regular and open communication**
  - Make use of complimentary expertise
  - Consider different perspectives from the beginning

- **Discuss Novel Concepts (e.g. SbD, SSbD)**

Chair: PD Dr. Andrea Haase, BfR

Meetings: since 2020, 2x per year

Thank you very much! Questions?

Unit Fibre and Nanotoxicology
Dr. Veronica Dumit
Dr. Mario Pink
Dr. Jutta Tentschert

Department 6 (Pesticides Safety)
Dr. Philip Marx- Stoelting
Dr. Vera Ritz

Group 4.I.5
Materials and Particulate Hazardous Substances
Dr. Dirk Brossel

IBE R&D Institute for Lung Health gGmbH
Prof. Dr. Martin Wiemann

Prof. Dr. Melanie Kah

Thanks to all partners in all projects!

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NanoCommons ID: 731032
NanoHarmony ID: 885931
Gov4Nano ID: 814401
gracious ID: 760840
HARMLESS ID: 953183

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nanoGRAVUR 03XP0002
InnoMatLife 03XP0216

Funding by EFSA GP/EFSA/MESE/2022/01

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