Promoting the Implementation of Safe by Design

Prosafe's Review on Reliability of Methods and Data for Regulatory Assessment of Nanomaterial Risks – Part Environment

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Scientific Stakeholder Meeting on Nanomaterials in the Environment,
Umweltbundesamt Dessau-Roßlau, 10-11 October 2017

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The Status quo

- Increasing number of publications on nanosafety,
- More than 100 research programmes in EU and US over past decade,
- Regulatory relevance of the results mostly not examined,
- Contradicting and misinterpreted results.

Old publications:

- Insufficient characterisation,
- Fluctuating concentrations.

However: Scientists had to learn the specific behaviour of NM in test systems; they were not ignorant!

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A critical comment on toxicological results

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Krug, H. *Angew. Chem*. Int. Ed. 2014, 53, 12304 – 12319.
"Nanosafety Research—Are We on the Right Track?"

- „…*Systemic effects have been observed in only a small number of studies, but these results have not been found to be related to a specific "nano effect" of the ENMs ...*
- *Comparison of instillation and inhalation experiments: …The findings suggest an unspecific particle effect… Overall, the evaluated studies showed no indication of a "nanospecific" effect in the lung.*
- *It is frequently disregarded that specific ENMs can dissolve … in body fluids. This implies a complete new situation with no "nanotoxicity", but a more general element-related toxicity, which is described in the textbooks.*
- *… the "Babylonian diversity" in the applied methods allows no comparability between the studies, but explains the often contradictory results of several publications.*
- *The majority of studies did not consider the necessity to characterize the material properties of the ENMs used for the experiments. This considerably reduces the significance of these studies, in some cases to a total meaninglessness of the presented results.*"

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Nanomaterials vs. Conventional Chemicals

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Is Risk Assessment with Nanomaterials different?

- Nanomaterials are chemical substances!
- Risk paradigm holds for nanomaterials,
- Methods and tools for risk assessment apply (mostly).

Unique properties of nanomaterials:

- Characteristics not only dependant on chemical composition,
- Hazards and fate influenced by functionalities / varying phys-chem properties,
- Slow processes (mostly no equilibrium!),
- Different cellular uptake (e.g. pinocytosis),
- Changes from nanoform to nanoform,
- Changes throughout the life cycle.

→ Great variety makes read-across, grouping, tiered schemes necessary with higher relevance of *in vitro* tests, acellular assays, HTS, modelling and *in silico* approaches.

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Task Force of Experts

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Coordinators: Klaus Günter Steinhäuser & Philip Sayre

Fields of experience represented:

- Physicochemical Characterisation / Identification: Gregory Lowry
- Human Exposures through the Life Cycle (workplace and consumer): Thomas Kuhlbusch
- Environmental Exposures – Fate: Anders Baun
- Exposure and Fate Modelling: Bernd Nowack
- Ecological Effects: Anders Baun
- Health Effects and Biokinetics (*in vivo*): Günter Oberdörster
- Health Effects and Biokinetics (*in vitro*): Barbara Rothen-Rutishauser
- Computational Methods: Enrico Burello
- Risk Assessment (grouping / categorization): Agnes Oomen

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EU and US Programmes Sampled for Review

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- EU: Research programmes in the NanoSafety Cluster (NSC)

- NANoREG
- MARINA
- NanoValid
- SUN
- NanoDEFINE
- QualityNano
- EnvNano
- NanoMile
- NanoFATE
- NanoPUZZLES
- NanoSolutions
- Nanogenotox
- NanoHETER (SIINN)
- nanOxiMet (SIINN)
- NanoToxClass (SIINN)
- NanoTOES

BMBF programme:

- NanoGEM

US programmes:

- Duke University (CEINT)
- NIEHS
- NIOSH
- Army Engineer R&D Center
- Arizona State Univ.

- OECD: WPMN reports, TG and GD drafts

Approx. 1,000 documents examined and reviewed

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Criteria for the Review: Reliability und Relevance

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Test method validation is a process based on scientifically sound principles by which the reliability and relevance of a particular test, approach, method, or process are established for a specific purpose. **Reliability** is defined as the extent of reproducibility of results from a test within and among laboratories over time, when performed using the same standardised protocol. The **relevance** of a test method describes the relationship between the test and the effect in the target species and whether the test method is meaningful and useful for a defined purpose, with the limitations identified. In brief, it is the extent to which the test method correctly measures or predicts the (biological) effect of interest, as appropriate. **Regulatory need**, usefulness and limitations of the test method are aspects of its relevance. New and updated test methods need to be both reliable and relevant, *i.e.*, validated (OECD, 2005).

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Development of a Roadmap

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10 July 2016

ProSafe Roadmap for Members of Task Force when Reviewing Data, Protocols, Reports and Guidance notes for Regulatory Relevance

Phil Sayre and Klaus Steinhäuser

- Question sets over the nine areas of concern oriented on regulatory relevance,
- Included as Annex 1 with the Joint Document.

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The Joint Document

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Reliability of Methods and Data for Regulatory Assessment of Nanomaterial Risks

Final version
14 March 2017

Editors in Chief and Contributing Authors:
Phil Sayre and Klaus Günter Steinhäuser

Task Force Experts:
Anders Baun, Enrico Burello, Thomas Kuhlbusch, Gregory Lowry, Bernd Nowack, Günter Oberdörster, Agnes Oomen, Barbara Rothen-Rutishauser

Task Force Contributing Authors:
Barbara Drasler, Andrea Haase, Jerome Rose, Susan Wijnhoven, Teresa Fernandes, Lars Skjolding

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Scientific Conference with OECD

ProSafe

Promoting the Implementation of Safe by Design

29 November – 1 December 2016
OECD Conference Centre
Paris - France

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Relevant Physico-chemical Properties

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Physico-chemical properties on which the review is focused:

Intrinsic Particle properties (most relevant to regulatory definitions of a nanomaterial)	**Extrinsic** Particle Properties (medium-, and time-dependent)	
"What they are"	**"Where they go, and their persistence"**	**"What they do"**
	Properties and Processes	"Reactivity"
Particle size distribution (number average)	Biodurability *in vivo* or *in vitro*	ROS production and photoreactivity
Particle shape (e.g. aspect ratio)	Zeta potential	
Crystalline phase(s)	Density (including effects of milieu)	
Hydrophobicity	Surface affinity	
Chemical composition (impurities, surface chemistry)	Dustiness (depends on moisture)	
Rigidity	Dissolution rate in environment and in physiological fluids (acellular)	
Redox potential / band gap	Agglomeration /Hydrodynamic diameter (dispersion stability)	
Specific surface area		

Functional Assays

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- A functional assay provides a measure of a nanomaterials behaviour or rate in a specific system, e.g.
  - Surface affinity as a measure for environmental mobility,
  - Dissolution rate in relevant media as a measure for the fate and the bioavailability of MNs,
  - Cell-free assays to determine the ROS generation potential of NM surfaces,
  - Dustiness to determine the exposure at workplaces.
- Relevant (a) for characterization, (b) for estimating exposure and/or fate, (c) for prediction of inflammatory response, (d) for developing (Q)SARs, (e) as first tiers in risk assessment frameworks,
- Functional assays have added value, though will also need further evaluation of their reliability and predictivity.

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Functional Assays

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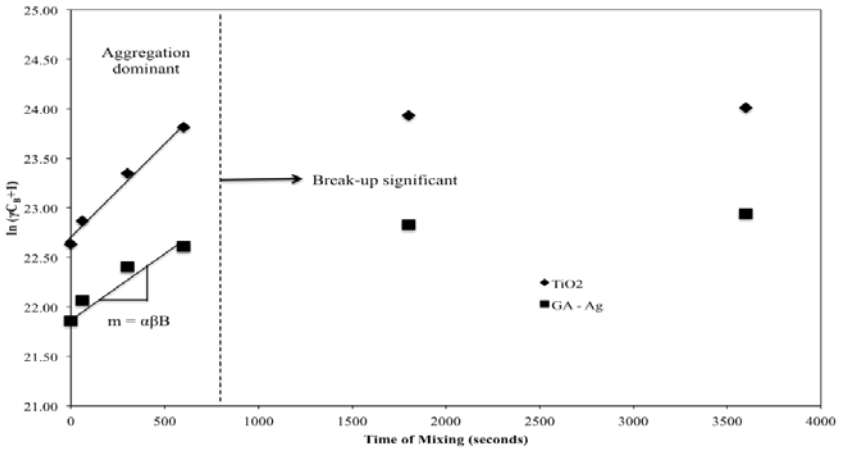
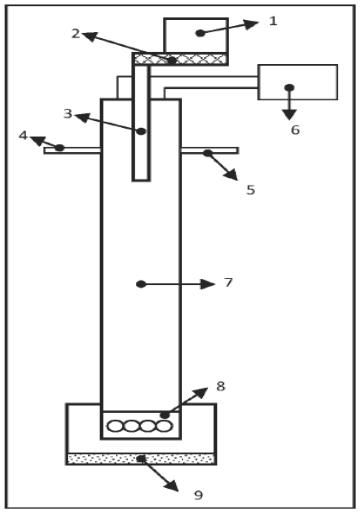
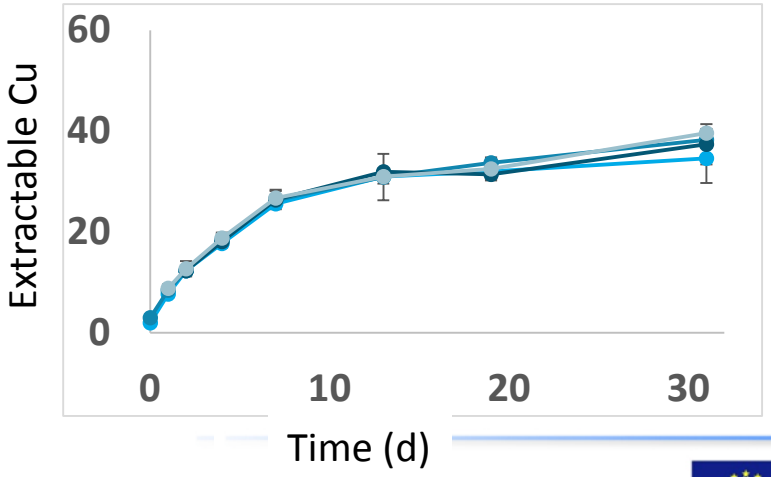
Surface affinity α (Barton et al, 2015)

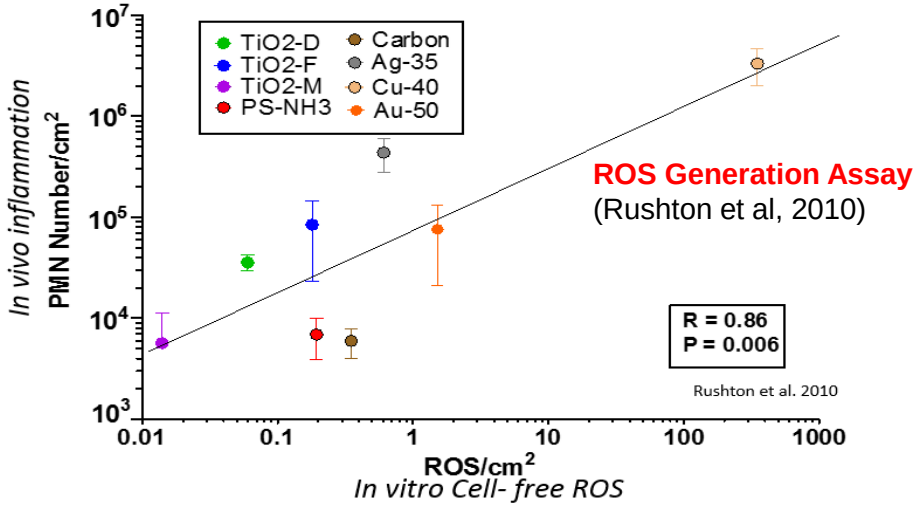




Continuous drop dustiness testing device (Dalman & Mons, 2011)

Dissolution rate (Hendren et al, 2015)





ROS Generation Assay (Rushton et al, 2010)

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Ecotoxicological Priorities

Method	Application	Recommendation
Draft OECD GD on aquatic toxicity testing	Performance of aquatic toxicity tests	Accomplish, make it as precise as possible
Preparation of stable dispersions		Use mechanical methods, sonication and pH changes; minimize addition of Natural Organic Matter (NOM)
Dissolution		Determine contribution of dissolved ions to toxicity but express result to the MN as a whole
Pelagic toxicity		Base set organisms are appropriate and sensitive, No extrapolation from acute to chronic possible, Sediment toxicity cannot be derived from pelagic toxicity
Sediment toxicity	Performance with sparingly soluble MN with high tendency to agglomerate	More tests necessary to choose most appropriate/sensitive organisms. Standardize/develop guidance for spiking procedure
Terrestrial toxicity		More tests necessary to choose most appropriate/sensitive organisms. Standardize/develop guidance for spiking procedure; Initiate an OECD GD analogous to the aquatic GD
Photoreactivity		Standardize illumination conditions for photoreactive materials
Avoidance of artefacts – Interaction of organisms with physical conditions	Various aquatic tests	Use devices which separate organisms from nanomaterials
Eco-corona, biokinetics, in vitro-tests		Basic research necessary

Environmental Fate Priorities

Method	Application	Recommendation
Draft OECD TG Sludge retention	Estimation of releases to water	Applicable, accomplish TG
Surface affinity / stickiness assay	Screening test of adsorption to sludge and of heteroaggregation	Applicable, validation and standardization recommended
Draft OECD TG Dissolution	Estimation of dissolution rate	Applicable, do not separate dissolved nanomaterials by filters with 0.45 µm pore size, accomplish TG
New OECD TG dispersion stability in simulated environmental media	Estimation of homoagglomeration	Applicable, TG accomplished
OECD GD for dispersion and dissolution of NM in aquatic media	Guidance for fate experiments with MNs	Should be developed, (currently on hold)
Test on heteroaggregation	Estimation of heteroaggregation	Further develop approaches aiming at standardization
Measurement strategy for agglomeration / aggregation / sedimentation	Higher tier assessment	Research necessary, can end in an OECD GD
OECD TG 312	Estimation of sorption on soil particles	OECD activity started, avoid too high $CaCl_2$ concentrations
Scheme to examine transformation of nanomaterials	Based on proposal of OECD expert meeting	OECD should develop a GD
OECD TG 307	Transformation in soil	Examine whether adaption of TG to nanomaterials is possible

Environmental Exposure Priorities (Modelling)

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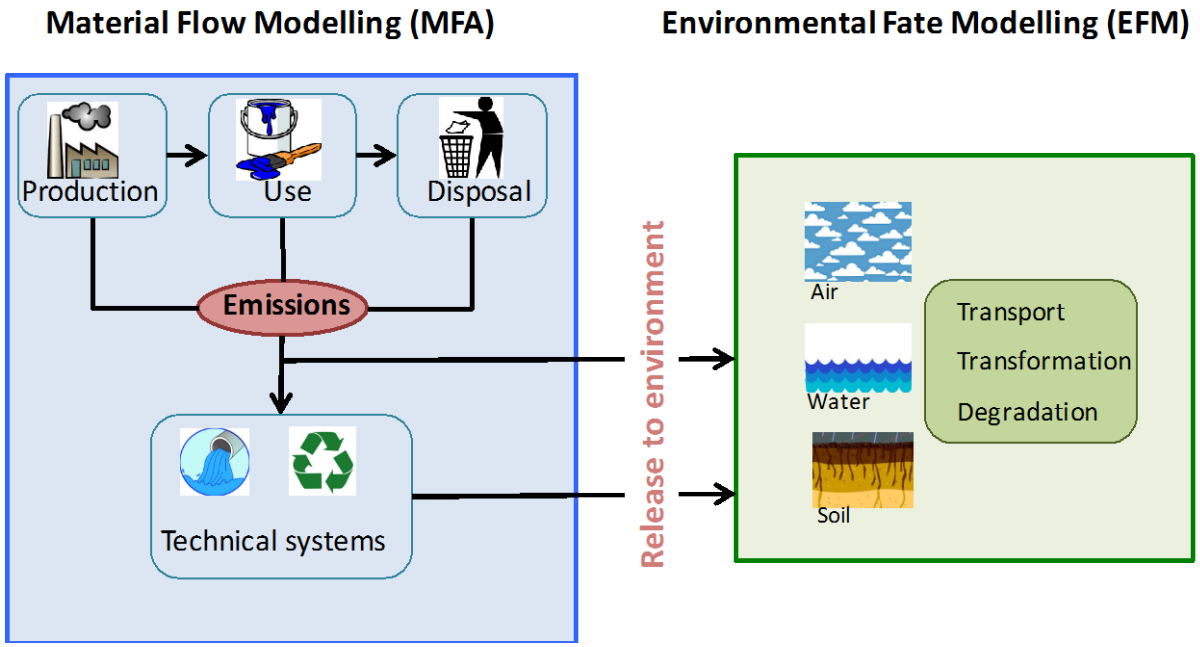
The relationship between MFA and EFM models
(from B. Nowack, 2017)



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Environmental Exposure Priorities (Modelling)

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Method	Application	Recommendation
DPMFA and LEARNano	MFA	Examine and validate these models for regulatory application
SimpleBox4Nano	EFM (1st tier)	Examine and validate the model for regulatory application within REACH
RedNano and NanoDuFlow	EFM (2nd tier)	Examine and validate these models for regulatory application where spatially or time-resolved information is needed; expand models aiming at inclusion of transformation processes in the environment
Registry on production and uses		Sample relevant information where legally possible aiming at validation of models by real world data
Cooperation of modellers with experimentalists		Design experiments which give modellers information to develop and validate their models
Measurement of ambient concentrations of nanomaterials, e.g. by FFF		Focus on measurements of release rates by weathering or leaching or measure near source

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Selected Areas for Future Research

- **Functional assays** on surface affinity and photoreactivity have to be further developed aiming at standardization.
- An assay to determine the likelihood of **heteroaggregation** as a major fate process in environment,
- Further develop and prioritize methods to determine **release rates** from products,
- Validated testing schemes to determine **aging,**
- **Adaption** of **soil and sediment tests** to nanomaterials (spiking!),
- Close **co-operation between modellers and experimentalists** to develop robust data sets with appropriate study design.

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Urgent need for test protocols and assessment tools

- Nanomaterials need specific (adapted/new) test protocols and assessment tools, particularly for the fate of NM,
- Scientific basis for suitable protocols and assessment instruments is now available,
- Tiered testing and assessment schemes that leverage *in vitro* tests, functional assays and other alternative approaches are needed for an affordable and scientifically sound risk assessment,
- Without proper assessment tools one cannot begin to assemble the appropriate hazard and exposure data to feed any risk assessment effort for regulatory review,
- No assessment scheme or framework can be perfect and cover all potential risks!
- Manufacturers need clear rules also for the development and application of Safe by Design (SbD) concepts.

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Conclusions

- Nanomaterials have lost **public attention** over the past years,
- The **scientific instruments** for regulatory risk assessment are **available** or will be available in near future. A solid basis for the regulation of manufactured nanomaterials is existing,
- **Validation of regulatory relevant methods** and their inclusion in regulations has just begun,
- Nanomaterials are reaching the market place in **increasing volumes** and **high product diversity**,
- Nanomaterials‘ **structure will become increasingly complex** in future; functionality will dominate properties of NM,
- **Keeping pace with scientific and technical progress** is a challenge for researchers and regulators and will make **further nanosafety research necessary.**

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Perspectives

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Future Trends

- Increasing **complexity** of nanomaterial structures,
- Increasing **functionalization** of surfaces,
- **Convergence of** ‚emerging technologies': Bio-, Nano- und Information technologies

Are we prepared? – May be NOT!

- What does this mean for the definition of a substance?
- How can you group nanomaterials by their functionalities?
- Relationship between intended functionality and unintended effects?
- Should we consider „new risks" which so far haven't played a role in chemical risk assessment? (e.g. with ‚Bionanos')?

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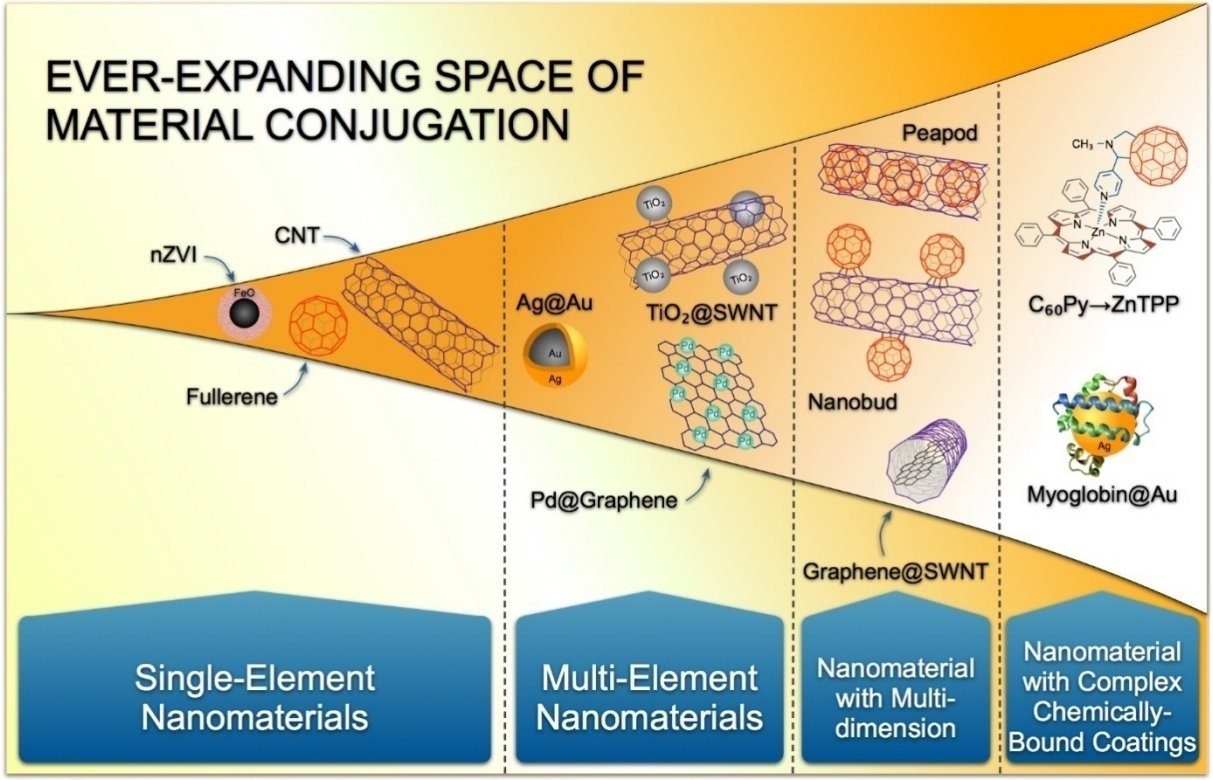
Perspectives



Increasing complexity of nanomaterials requires an adaptable testing strategy for assessing nanomaterial fate and toxicity (from Saleh et al., 2015 *ES Nano* 2 11-18)

Interested?

The Results of the Review are Published in

- A Scientific Report of ProSafe (Joint Document, will be posted on: http://www.h2020-prosafe.eu/)
- As peer reviewed publications in a special issue of NanoImpact (9 out of 11 articles already accepted).

Please send an Email to:
klaus-g.steinhaeuser@posteo.de

Interested?

ProSafe Review ‚Reliability of Methods and Data for Regulatory Assessment of Nanomaterial Risks‘

List of Publications:

Research Report: ‚Reliability of Methods and Data for Regulatory Assessment of Nanomaterial Risks‘

Special Issue in NanoImpact (peer reviewed articles):

1. **Methods and data for regulatory risk assessment of nanomaterials: Questions for an expert consultation**
   Philip G. Sayre, Klaus Günter Steinhäuser, Tom van Teunenbroek (attached: Roadmap)

2. **Reliability of methods and data for regulatory assessment of nanomaterial risks**
   Klaus Günter Steinhäuser, Philip G. Sayre

3. **Progress towards standardized and validated characterizations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks**
   Xiaoyu Gao, Gregory V. Lowry

4. **Nanomaterial Exposures through the Life Cycle**
   Thomas A.J. Kuhlbusch, Susan W.P. Wijnhoven and Andrea Haase (under revision)

5. **Regulatory relevant and reliable methods and data for determining the environmental fate of manufactured nanomaterials**
   Anders Baun, Phil Sayre, Klaus Günter Steinhäuser, Jerome Rose

6. **Evaluation of environmental exposure models for engineered nanomaterials in a regulatory context**
   Bernd Nowack

7. **Regulatory adequacy of aquatic ecotoxicity testing of nanomaterials**
   Rune Hjorth, Lars M. Skjolding, Sara N. Sørensen, Anders Baun

8. ***In vivo* effects and biokinetics of inhaled nanomaterials**
   Günter Oberdörster (under revision)

9. ***In vitro* approaches to assess the hazard of nanomaterials**
   Barbara Drasler, Phil Sayre, Klaus Günter Steinhäuser, Alke Petri-Fink, Barbara Rothen-Rutishauser

10. **Review of (Q)SAR models for regulatory assessment of nanomaterial risks**
    Enrico Burello

11. **Risk assessment frameworks for nanomaterials: Scope, link to regulations, applicability, and outline for future directions in view of needed increase of efficiency**
    Agnes G. Oomen, Klaus Günter Steinhäuser, Eric A.J. Bleeker, Fleur van Broekhuizen, Adriënne Sips, Susan Dekkers, Susan P. Wijnhoven, Philip G. Sayre

Thank you

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- Tom van Teunenbroek and Aart Dijkzeul (ProSafe coordinators)
- James Baker, Joke Vroom and Yvonne Linnebank (ProSafe project office)
- All Task Force members and their supporters

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- The audience for its interest and patience!

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