Karlsruhe Institute of Technology

DaNa 2.0
www.nanoobjects.info

# Supporting Risk Assessment of Nanomaterials with quality-Approved Information DaNa Literature Criteria Checklist

Dr. Clarissa Marquardt - Institute of Applied Computer Sciences (IAI)

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# Nanotechnology……

*Applications?*

Image source: fotolia.com

# Nanotechnology……

Worker

**SAFETY ?**

Environment

*Effects on……*

Image source: fotolia.com

# Nanotechnology……

Worker

**SAFETY ?**

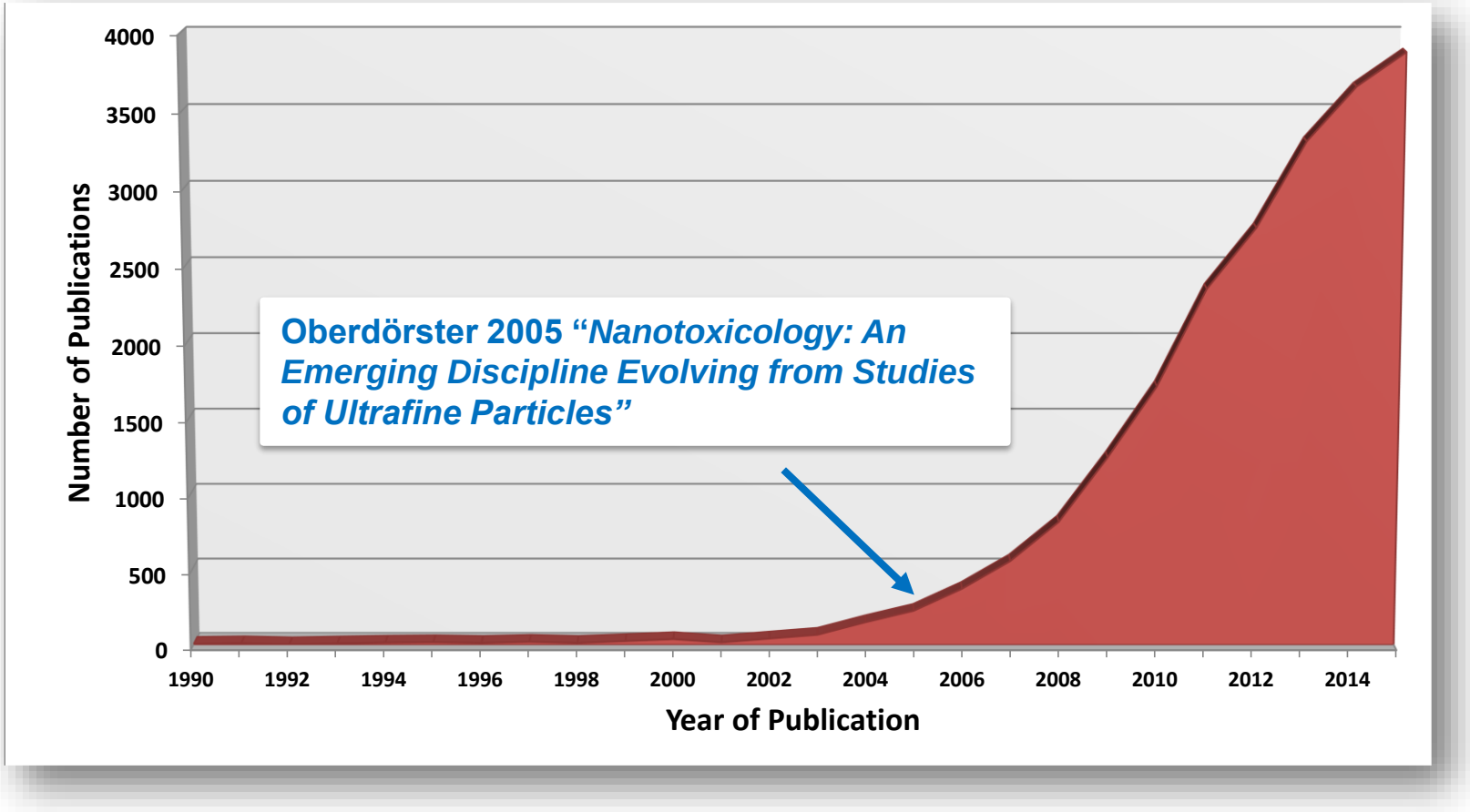
**Reliable Source of information?**

*Effects on……*

Image source: fotolia.com

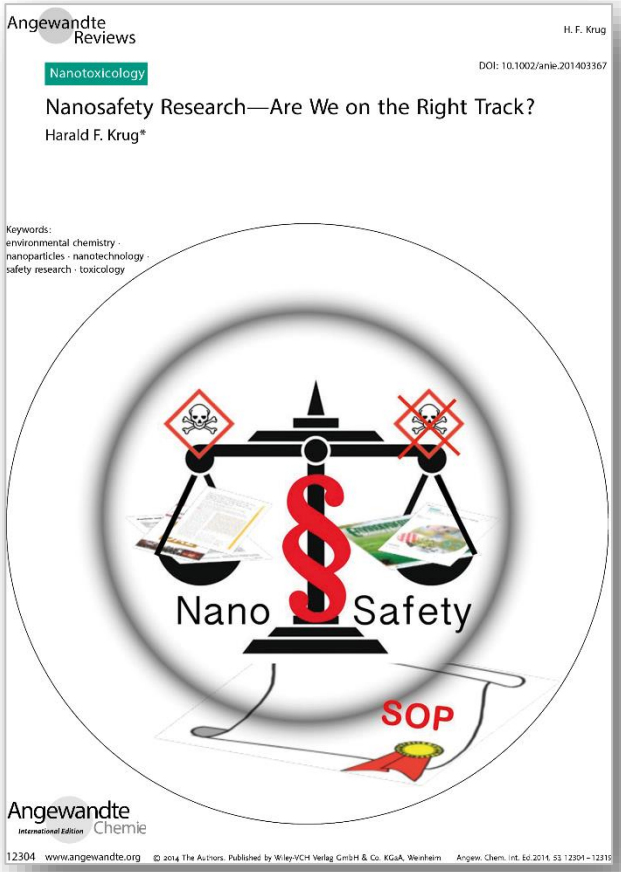
# Literature on Nanotoxicology

- Drastic increase in Number of Publications increases drastically




Source: Nau K. et al (2016), The DaNa2.0 Knowledge Base on Nanomaterials – Communicating current nanosafety research based on evaluated literature data. Journal of Materials Education Vol. 38 (3-4): 93-108 (2016)

# Quality & Usability of Publications




Krug H.F. (2014). **Nanosafety research-are we on the right track?** *Angew Chem Int Ed Engl,* 53(46): 12304-12319.

**Literature Review on 3 major uptake pathways & comparison Instillation vs Inhalation**

- Majority of studies was poorly conducted
- Characterisation of NM was insufficient
- Applied dosage in most cases under overload conditions

- **Results NOT applicable for risk assessment!!!**

# Characterisation…..

„*When things are large, they are what the are. When they are small, it‘s a different game:* ***they are what our measurements make them****.”*

(George M. Whitesides, No Small Matter. Science on the Nanoscale)

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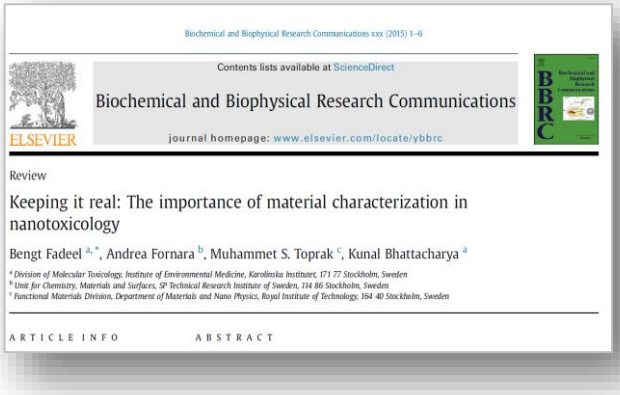

Biochemical and Biophysical Research Communications xxx (2015) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Review

Keeping it real: The importance of material characterization in nanotoxicology

Bengt Fadeel [a,*], Andrea Fornara [b], Muhammet S. Toprak [c], Kunal Bhattacharya [a]

[a] Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden
[b] Unit for Chemistry, Materials and Surfaces, SP Technical Research Institute of Sweden, 114 86 Stockholm, Sweden
[c] Functional Materials Division, Department of Materials and Nano Physics, Royal Institute of Technology, 164 40 Stockholm, Sweden

ARTICLE INFO ABSTRACT

Fadeel B., Fornara A., Toprak M.S., Bhattacharya K. (2015). Keeping it real: The importance of material characterization in nanotoxicology. *Biochem Biophys Res Commun,*. Doi: 10.1016/j.bbrc.2015.06.178

**Key challenge for nanosafety assessment & safe-by-design approaches is to**

- Understand which phys.-chem. properties are driving the nanomaterials’ toxicity
- Standardised testing methods & materials
- Dose & dosimetry in Nanotoxicology
- Naming & categorizing nanomaterials

# Requirements…

- Quality standards for (nano)toxicological studies
- Physico-chemical characterisation **fit-for-purpose** & **relevant** for addressed purpose
- Validated standard methods & reference materials
- Relevant models & concentrations

# Short Overview

Key Tasks of the DaNa - Knowledge Base Nanomaterials
(DaNa = Database Nanomaterials)

- Communication of current nanotechnology safety research
  - Scientific Literature Review & Quality Management
    - Collecting – Evaluating & Processing of Nanosafety Information for Website
    - for Interested Laymen, Stakeholders, Scientists
  - Website www.nanoobjects.info
- Support for German Nanosafety Research projects
  - NanoCare, NanoNature, ERA-Net SIINN

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FKZ 03X0131

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Confédération suisse
Confederazione Svizzera
Confederaziun svizra
Federal Office for the Environment FOEN
Federal Office of Public Health FOPH

# Project Team

## Core Team

DECHEMA

KIT Karlsruher Institut für Technologie

Empa Materials Science and Technology

HELMHOLTZ ZENTRUM FÜR UMWELTFORSCHUNG UFZ

## External Experts

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

baua: Bundesanstalt für Arbeitsschutz und Arbeitsmedizin

UNIVERSITÄT SALZBURG

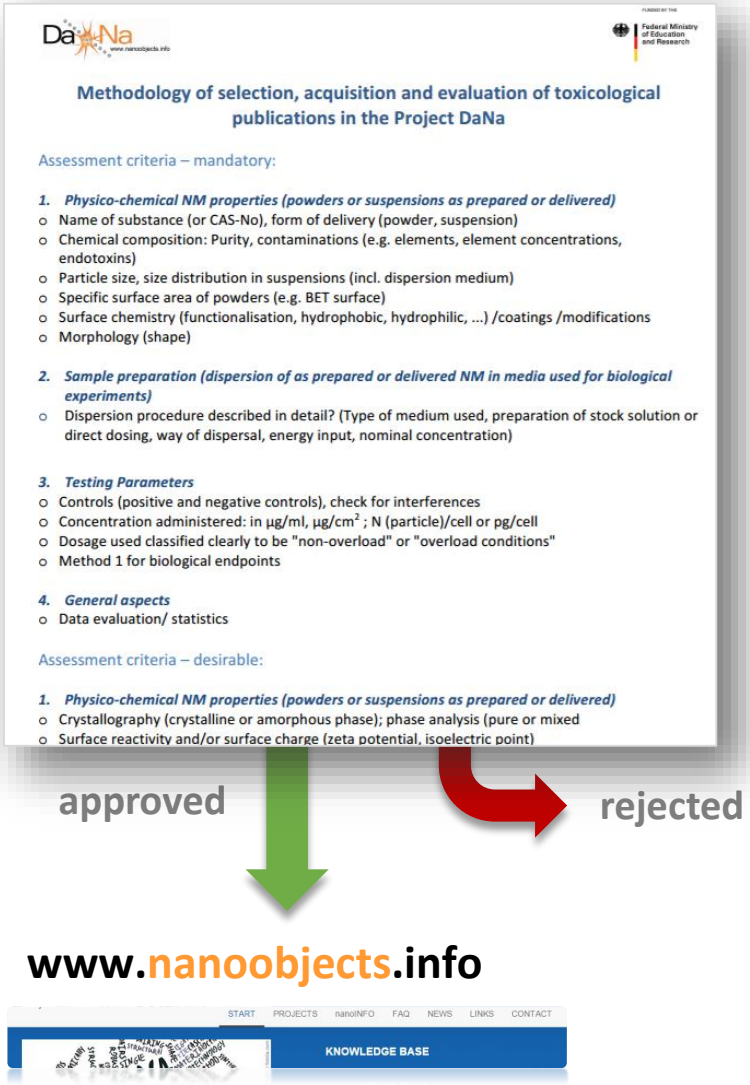
Technical University of Denmark DTU

UNIVERSITY OF EXETER

Empa Materials Science and Technology

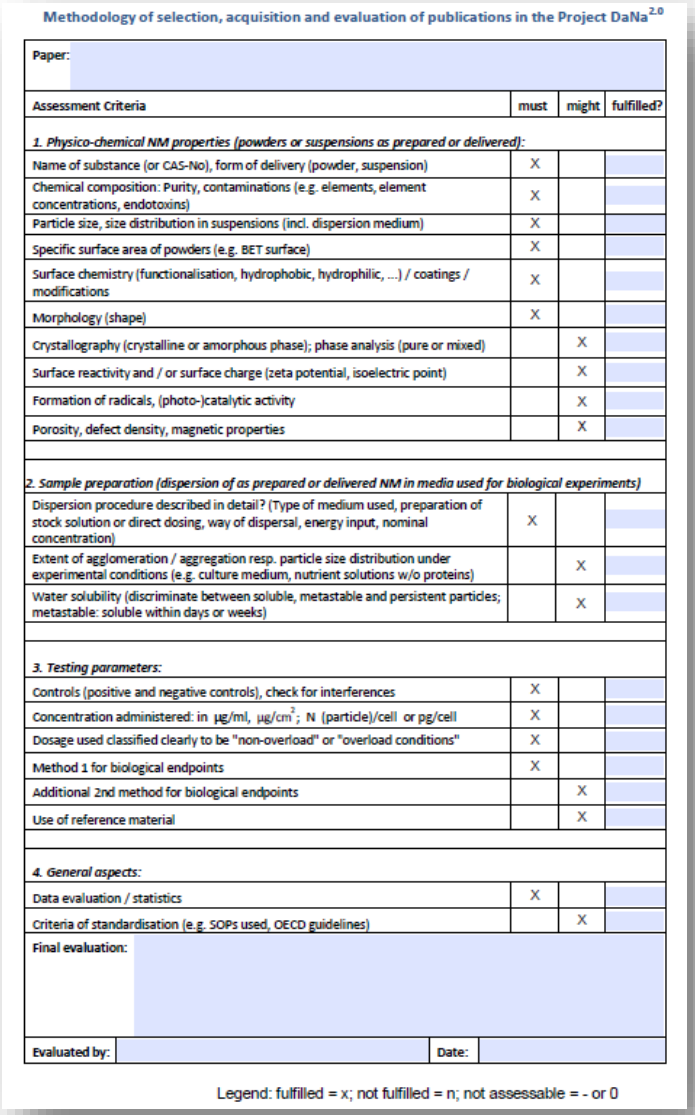
# Literature Quality Management

- Evaluation of peer-reviewed literature with publicly available quality criteria
  - Topics Human- & Eco-Toxicology
  - Sorting of approved & rejected literature using the DaNa Literature Criteria Checklist
  - > 800 quality-approved literature citations on the website



# DaNa Literature Criteria Checklist

→ Mandatory & Optional Criteria



**Methodology of selection, acquisition and evaluation of publications in the Project DaNa[2.0]**

| Paper: | | | |
|---|---|---|---|
| **Assessment Criteria** | **must** | **might** | **fulfilled?** |
| ***1. Physico-chemical NM properties (powders or suspensions as prepared or delivered):*** | | | |
| Name of substance (or CAS-No), form of delivery (powder, suspension) | X | | |
| Chemical composition: Purity, contaminations (e.g. elements, element concentrations, endotoxins) | X | | |
| Particle size, size distribution in suspensions (incl. dispersion medium) | X | | |
| Specific surface area of powders (e.g. BET surface) | X | | |
| Surface chemistry (functionalisation, hydrophobic, hydrophilic, ...) / coatings / modifications | X | | |
| Morphology (shape) | X | | |
| Crystallography (crystalline or amorphous phase); phase analysis (pure or mixed) | | X | |
| Surface reactivity and / or surface charge (zeta potential, isoelectric point) | | X | |
| Formation of radicals, (photo-)catalytic activity | | X | |
| Porosity, defect density, magnetic properties | | X | |
| ***2. Sample preparation (dispersion of as prepared or delivered NM in media used for biological experiments)*** | | | |
| Dispersion procedure described in detail? (Type of medium used, preparation of stock solution or direct dosing, way of dispersal, energy input, nominal concentration) | X | | |
| Extent of agglomeration / aggregation resp. particle size distribution under experimental conditions (e.g. culture medium, nutrient solutions w/o proteins) | | X | |
| Water solubility (discriminate between soluble, metastable and persistent particles; metastable: soluble within days or weeks) | | X | |
| ***3. Testing parameters:*** | | | |
| Controls (positive and negative controls), check for interferences | X | | |
| Concentration administered: in µg/ml, µg/cm²; N (particle)/cell or pg/cell | X | | |
| Dosage used classified clearly to be "non-overload" or "overload conditions" | X | | |
| Method 1 for biological endpoints | X | | |
| Additional 2nd method for biological endpoints | | X | |
| Use of reference material | | X | |
| ***4. General aspects:*** | | | |
| Data evaluation / statistics | X | | |
| Criteria of standardisation (e.g. SOPs used, OECD guidelines) | | X | |
| **Final evaluation:** | | | |
| **Evaluated by:** | | **Date:** | |

Legend: fulfilled = x; not fulfilled = n; not assessable = - or 0

http://www.nanopartikel.info/files/methodik/DaNa_criteria_checklist_2015_form.pdf

# DaNa Literature Criteria Checklist

Mandatory & Optional Criteria

- Physico-Chemical characterisation

Methodology of selection, acquisition and evaluation of publications in the Project DaNa 2.0

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| Particle size, size distribution in suspensions (incl. dispersion medium) | X | | |
| Specific surface area of powders (e.g. BET surface) | X | | |
| Surface chemistry (functionalisation, hydrophobic, hydrophilic, ...) / coatings / modifications | X | | |
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| Crystallography (crystalline or amorphous phase); phase analysis (pure or mixed) | | X | |
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| Formation of radicals, (photo-)catalytic activity | | X | |
| Porosity, defect density, magnetic properties | | X | |
| Use of reference material | | X | |
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Karlsruhe Institute of Technology

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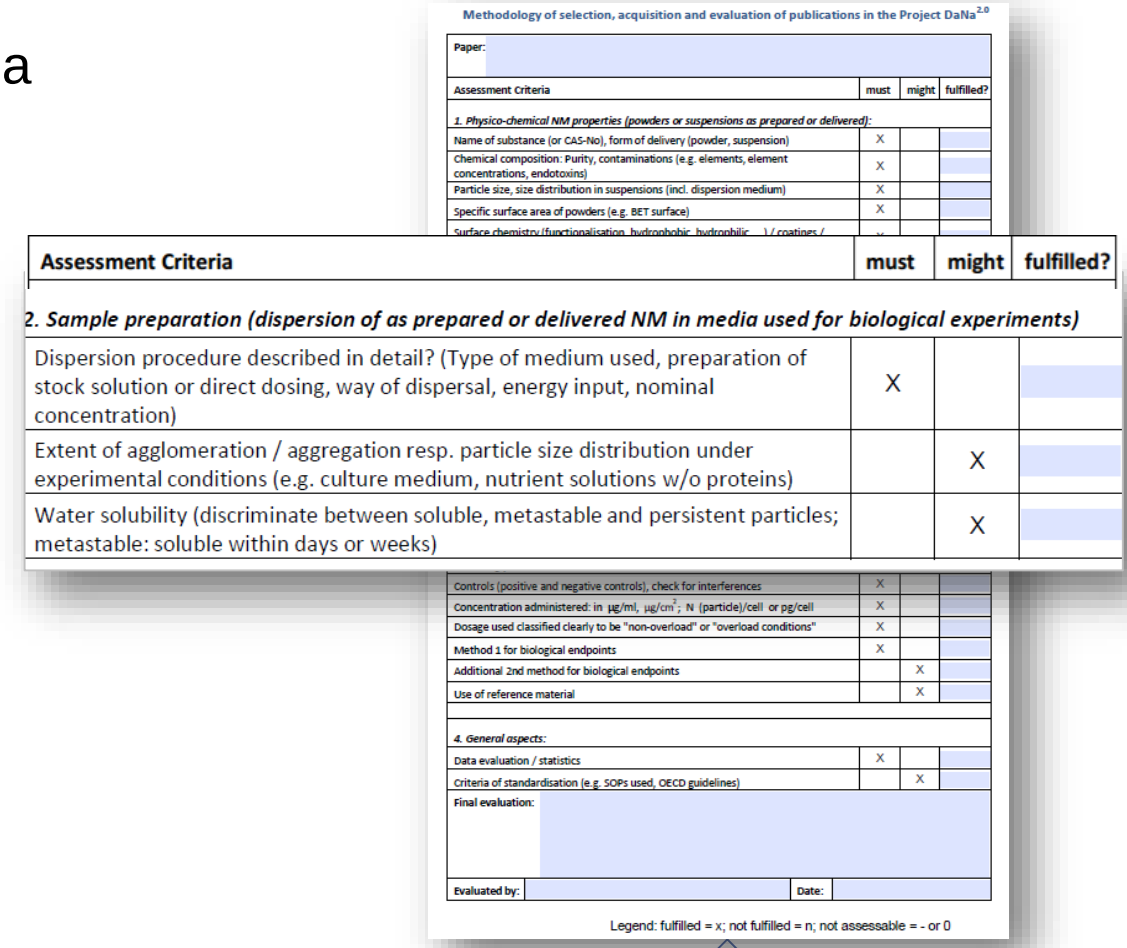
- Physico-Chemical characterisation
- Sample Preparation



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| Concentration administered: in µg/ml, µg/cm²; N (particle)/cell or pg/cell | X | | |
| Dosage used classified clearly to be "non-overload" or "overload conditions" | X | | |
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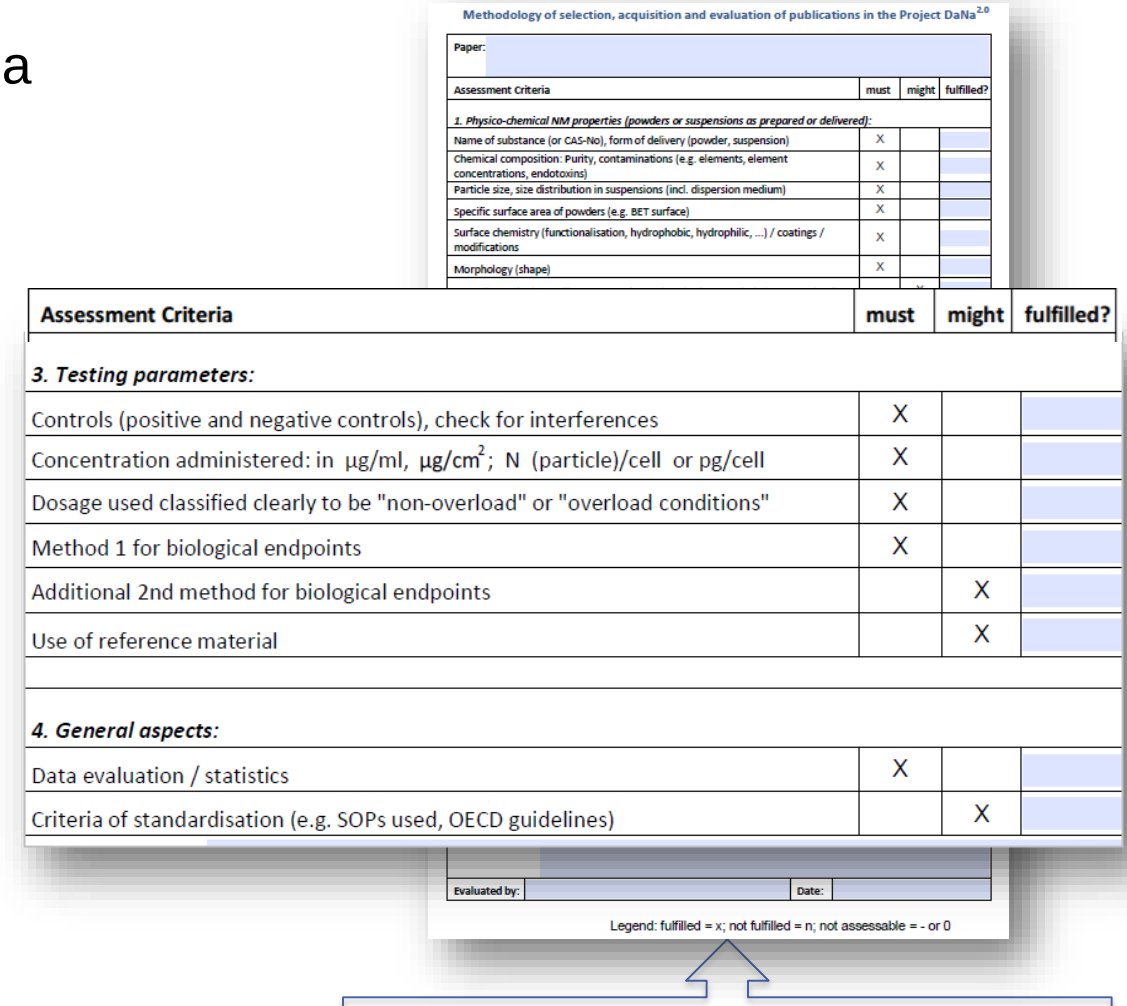
# DaNa Literature Criteria Checklist

Karlsruhe Institute of Technology

## Mandatory & Optional Criteria

- Physico-Chemical characterisation
- Sample Preparation
- Testing Parameters
  - Biological assays
  - dose & dosage
  - controls,…
- General Aspects
  - Evaluation / Statistics
  - SOPs, Guidelines,…



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Evaluated by: Date:

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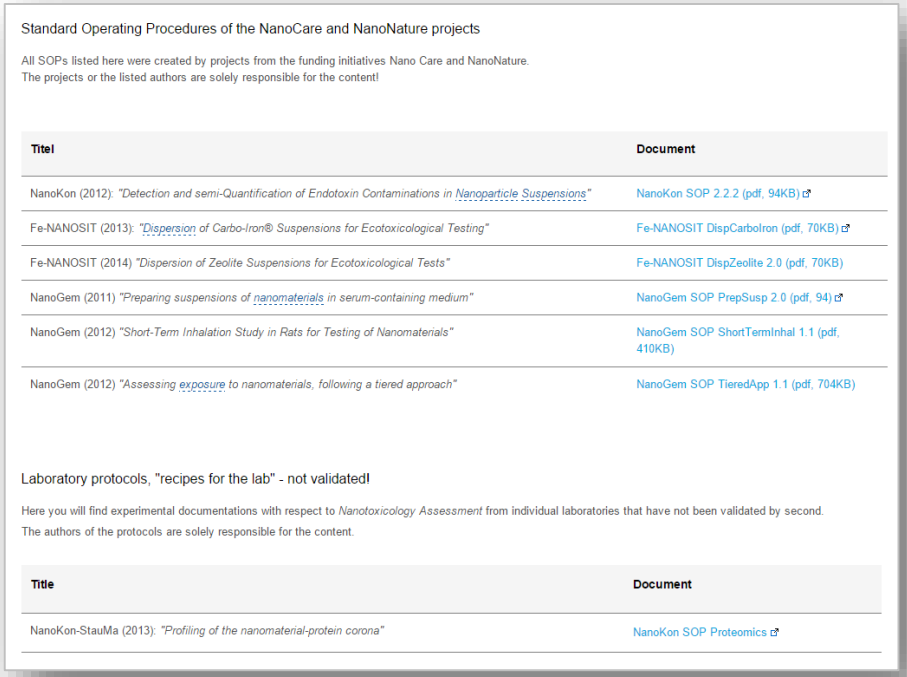
# Standard Operating Procedures (SOPs)

- DaNa SOP Template
- Validated SOPs & Lab-Protocols from German NanoSafety Research Projects
- Protocols from other projects (NANOMMUNE, V.I.G.O,…)



Standard Operating Procedures of the NanoCare and NanoNature projects

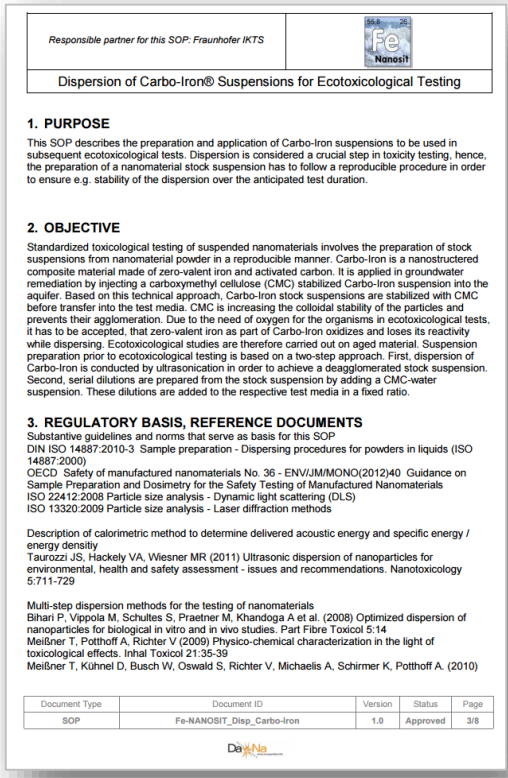
All SOPs listed here were created by projects from the funding initiatives Nano Care and NanoNature.
The projects or the listed authors are solely responsible for the content!

| Titel | Document |
|---|---|
| NanoKon (2012): *"Detection and semi-Quantification of Endotoxin Contaminations in Nanoparticle Suspensions"* | NanoKon SOP 2.2.2 (pdf, 94KB) |
| Fe-NANOSIT (2013): *"Dispersion of Carbo-Iron® Suspensions for Ecotoxicological Testing"* | Fe-NANOSIT DispCarboIron (pdf, 70KB) |
| Fe-NANOSIT (2014) *"Dispersion of Zeolite Suspensions for Ecotoxicological Tests"* | Fe-NANOSIT DispZeolite 2.0 (pdf, 70KB) |
| NanoGem (2011) *"Preparing suspensions of nanomaterials in serum-containing medium"* | NanoGem SOP PrepSusp 2.0 (pdf, 94) |
| NanoGem (2012) *"Short-Term Inhalation Study in Rats for Testing of Nanomaterials"* | NanoGem SOP ShortTermInhal 1.1 (pdf, 410KB) |
| NanoGem (2012) *"Assessing exposure to nanomaterials, following a tiered approach"* | NanoGem SOP TieredApp 1.1 (pdf, 704KB) |

Laboratory protocols, "recipes for the lab" - not validated!

Here you will find experimental documentations with respect to *Nanotoxicology Assessment* from individual laboratories that have not been validated by second.
The authors of the protocols are solely responsible for the content.

| Title | Document |
|---|---|
| NanoKon-StauMa (2013): *"Profiling of the nanomaterial-protein corona"* | NanoKon SOP Proteomics |



*Responsible partner for this SOP: Fraunhofer IKTS*

Dispersion of Carbo-Iron® Suspensions for Ecotoxicological Testing

**1. PURPOSE**

This SOP describes the preparation and application of Carbo-Iron suspensions to be used in subsequent ecotoxicological tests. Dispersion is considered a crucial step in toxicity testing, hence, the preparation of a nanomaterial stock suspension has to follow a reproducible procedure in order to ensure e.g. stability of the dispersion over the anticipated test duration.

**2. OBJECTIVE**

Standardized toxicological testing of suspended nanomaterials involves the preparation of stock suspensions from nanomaterial powder in a reproducible manner. Carbo-Iron is a nanostructered composite material made of zero-valent iron and activated carbon. It is applied in groundwater remediation by injecting a carboxymethyl cellulose (CMC) stabilized Carbo-Iron suspension into the aquifer. Based on this technical approach, Carbo-Iron stock suspensions are stabilized with CMC before transfer into the test media. CMC is increasing the colloidal stability of the particles and prevents their agglomeration. Due to the need of oxygen for the organisms in ecotoxicological tests, it has to be accepted, that zero-valent iron as part of Carbo-Iron oxidizes and loses its reactivity while dispersing. Ecotoxicological studies are therefore carried out on aged material. Suspension preparation prior to ecotoxicological testing is based on a two-step approach. First, dispersion of Carbo-Iron is conducted by ultrasonication in order to achieve a deagglomerated stock suspension. Second, serial dilutions are prepared from the stock suspension by adding a CMC-water suspension. These dilutions are added to the respective test media in a fixed ratio.

**3. REGULATORY BASIS, REFERENCE DOCUMENTS**

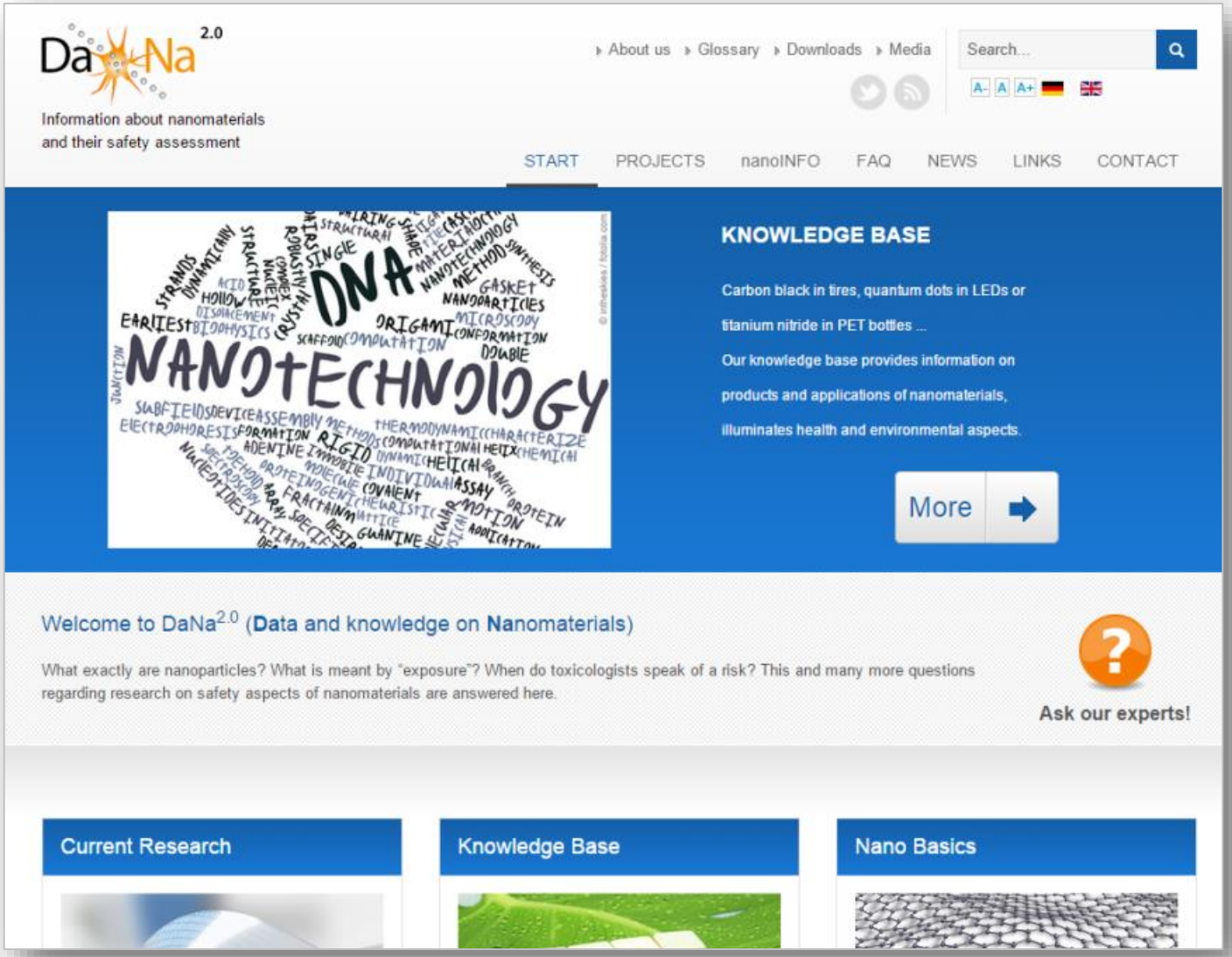
Substantive guidelines and norms that serve as basis for this SOP
DIN ISO 14887:2010-3 Sample preparation - Dispersing procedures for powders in liquids (ISO 14887:2000)
OECD Safety of manufactured nanomaterials No. 36 - ENV/JM/MONO(2012)40 Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials
ISO 22412:2008 Particle size analysis - Dynamic light scattering (DLS)
ISO 13320:2009 Particle size analysis - Laser diffraction methods

Description of calorimetric method to determine delivered acoustic energy and specific energy / energy densitiy
Taurozzi JS, Hackely VA, Wiesner MR (2011) Ultrasonic dispersion of nanoparticles for environmental, health and safety assessment - issues and recommendations. Nanotoxicology 5:711-729

Multi-step dispersion methods for the testing of nanomaterials
Bihari P, Vippola M, Schultes S, Praetner M, Khandoga A et al. (2008) Optimized dispersion of nanoparticles for biological in vitro and in vivo studies. Part Fibre Toxicol 5:14
Meißner T, Potthoff A, Richter V (2009) Physico-chemical characterization in the light of toxicological effects. Inhal Toxicol 21:35-39
Meißner T, Kühnel D, Busch W, Oswald S, Richter V, Michaelis A, Schirmer K, Potthoff A. (2010)

| Document Type | Document ID | Version | Status | Page |
|---|---|---|---|---|
| SOP | Fe-NANOSIT_Disp_Carbo-Iron | 1.0 | Approved | 3/8 |

# Project DaNa$^{2.0}$ www.nanoobjects.info



# Conclusions

- DaNa Literature Checklist – A tool for quality management of publications
- Increasing SOP collection
- DaNa Knowledge base – Reliable & objective information source
- Knowledge communication essential for any technology development

# Thank you for your Attention !

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clarissa.marquardt@kit.edu

www.nanoobjects.info

**@nano_info**