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Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

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English - Or. English

**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**ADDENDUM TO THE REPORT OF THE OECD EXPERT MEETING ON THE PHYSICAL
CHEMICAL PROPERTIES OF MANUFACTURED NANOMATERIALS AND TEST GUIDELINES:
PRESENTATIONS GIVEN AT THE WORKSHOP**

**Series on the Safety of Manufactured Nanomaterials
No. 41**

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OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 41

**Report of the OECD Expert meeting on the Physical Chemical Properties of
Manufactured Nanomaterials and Test Guidelines**



INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2014

Also published in the Series of Safety of Manufactured Nanomaterials:

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- No. 37, *Current Developments in Delegations on the Safety of Manufactured Nanomaterials - Tour de Table at the 10th Meeting of the WPMN (2012)*
- No. 38, *Co-Operation on Risk Assessment: Prioritisation of Important Issues on Risk Assessment of Manufactured Nanomaterials - Final Report (2013)*
- No. 39, *Environmentally Sustainable Use of Manufactured Nanomaterials - Workshop held on 14 September 2011 in Rome, Italy (2013)*
- No. 40, *Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines (2014)*

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The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 34 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

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This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

As part of its Programme on the Safety of Manufactured Nanomaterials, and in particular work on the testing and assessment of manufactured nanomaterials, OECD initiated a series of expert meetings to improve the applicability of the OECD Test Guidelines to nanomaterials. With this in mind, the Working Party on Manufactured Nanomaterials agreed to organise an expert meeting to address the physico-chemical Properties of Manufactured Nanomaterials and Test Guidelines.

The meeting was organised in close collaboration with the International Organization for Standardization Technical Committee on Nanotechnologies (ISO/TC 229). ISO has a vast experience in assessing the physico-chemical characteristics of nanomaterials. By working together, the workshop benefited from a broad range of experts on physico-chemical properties of manufactured nanomaterials in this field combining regulators and other governmental representatives, and well as industry and experts from academia.

The workshop on physico-chemical Properties of Manufactured Nanomaterials and Test Guidelines took place on 28 February and 1 March 2013 in Queretaro, Mexico. It was hosted by the Mexican Ministry of Economy and the National Metrology Center (CENAM).

This document is an accompanying addendum to the *Report of the OECD Expert meeting on the Physical Chemical Properties of Manufactured Nanomaterials and Test Guidelines* [ENV/JM/MONO(2014)15], and includes the presentations given at the workshop. It is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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ANNEX IV. PRESENTATIONS

Session I. Welcoming Remarks

Hubert Rauscher: Characterizing Nanomaterials for Regulatory Safety Assessment



Characterizing Nanomaterials for Regulatory Safety Assessment

Hubert Rauscher
European Commission
Joint Research Centre

OECD Expert Meeting on Physical-chemical Properties of
Manufactured Nanomaterials and Test Guidelines

28 February 2013
Queretaro, Mexico

28/02/2013 H. Rauscher, JRC

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Outline

- Nanomaterial characterization:
 - regulatory purposes & relevant properties
- Consequences for measurements (Example: Size)
- Reference materials & sample preparation
- Dosimetry

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Nanomaterials need to be safe

- Uncertainty about the safety of NMs
- Nanomaterial characterization – different from classical chemicals
 - basic science
 - specific applications
 - **Safety**
 - product development,...

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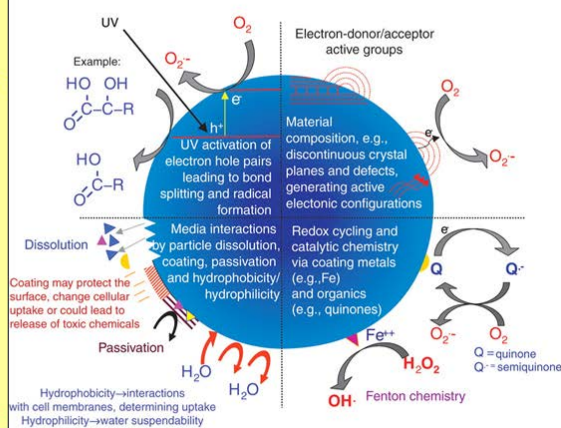
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NM Properties ↔ Possible Interactions NM-Tissue ↔ Hazard

- Size and Shape**
- Size distribution
 - Shape
- State of Dispersion**
- Agglomeration/Aggregation
 - (Water) solubility
 - Surface charge (Zeta potential)
- Surface Area and Porosity**
- Crystallinity**
- Crystalline phase and crystallite size
- Surface Chemistry**
- Surface composition
 - (Photo-) catalytic properties
 - Reactivity
 - Adsorption/desorption of molecules
- Other physico-chemical properties**
- Electro-optical properties



A. Nel et al., Science 311,622-627 (2006)

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Which physico-chemical properties are relevant for safety assessment (1) ?

Lists of physicochemical properties have been recommended:

- **OECD WPMN**: List of endpoints for the sponsorship program - ENV/MONO/(2010)46
- **ISO**: Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment - TR 13014 (2012)

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Which physico-chemical properties are relevant for safety assessment (2) ?

- **US EPA**: Nanotechnology White Paper - EPA 100/B-07/001 (2007)
- **US FDA**: Draft Guidance for Industry: Safety of Nanomaterials in Cosmetic Products (2012)
- **SCENIHR** (Scientific Committee on Emerging and Newly Identified Health Risks): Risk Assessment of Products of Nanotechnologies (2009)
- **SCCS** (Scientific Committee on Consumer Safety - EC): Guidance on the Safety Assessment of Nanomaterials in Cosmetics (2012)
- **EFSA** (European Food Safety Authority): Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain (EFSA Journal 2011;9(5):2140)
- **Other national authorities**: Germany, Netherlands, ...
- **Research consortia**: NanoImpactNet, NanoReg,...

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Physico-chemical properties

Property	Organization
Chemical composition, impurities	all
Size or particle size distribution	OECD, ISO, EPA, NanoImpactNet, FDA, SCENIHR, EFSA, SCCS
Aggregation, agglomeration, dispersibility, stability	OECD, ISO, EPA, FDA, NanoImpactNet, SCENIHR, EFSA, SCCS
Zeta potential (surface charge)	OECD, ISO, FDA, EPA, NanoImpactNet, SCENIHR, EFSA, SCCS
surface chemistry, functionalization, coating	OECD, ISO, EPA, NanoImpactNet, FDA, SCENIHR, EFSA, SCCS
(Water) Solubility or dispersibility but see ENV/JM/MONO(2012)40	OECD, ISO, EPA, NanoImpactNet, FDA, SCENIHR, EFSA, SCCS
Specific surface area	OECD, ISO, EPA, NanoImpactNet, FDA, SCENIHR
Shape or representative SEM img.	OECD, ISO, EPA, FDA, SCENIHR, EFSA, SCCS

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Physico-chemical properties

Property	Organization
Octanol-water partition coefficient	OECD, EPA, SCENIHR
crystallite size, dustiness, photocatalytic activity, redox potential, radical formation potential	OECD, SCENIHR, EFSA, SCCS
Porosity	OECD, FDA, SCENIHR
Crystallinity, crystal phase	OECD, FDA, SCENIHR
Catalytic activity	FDA, SCCS, EFSA
Density	FDA, EFSA, SCCS
Optical absorption, electrolytic conductivity, pH value of suspension	NIST, EFSA, SCCS
Specific electrical conductivity, structure, melting point, boiling point, vapour pressure, reactivity, stability	EPA

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Interesting observations

Observation	Organization
Information on manufacturing process required	US-FDA (Cosmetics) US-FDA (Food)
1. in the raw material form as manufactured 2. after addition to a final cosmetic formulation 3. during toxicological investigations	SCCS (for cosmetics)
1. Prior to use in applications (=raw material) 2. In food/feed applications 3. During toxicological testing 4. In biological tissues (ADME testing)	EFSA (for food)

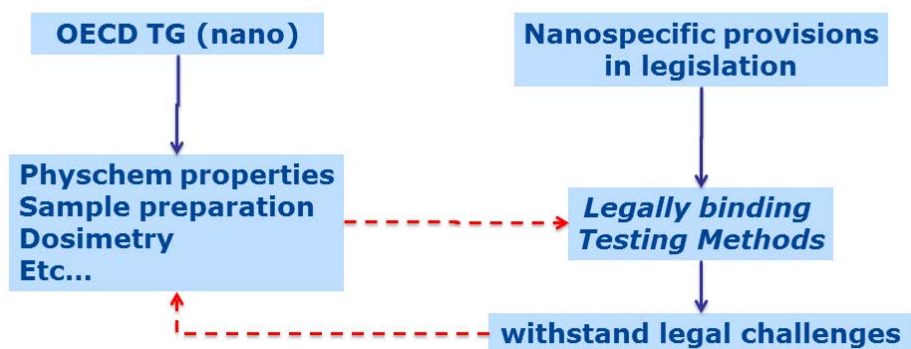
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OECD TGs – Regulatory Implications Consequences for physico-chemical characterization



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Legal obligations to report physico-chemical data (examples)

France: Environmental bill ("Grenelle 2")

- by order: name, composition, average size and size distribution, agglomeration/aggregation, shape, coating (if relevant)
- If possible: crystalline state, specific surface area, surface charge
- Detailed description of methods

EU – Cosmetics: Regulation (EC) No 1223/2009

- identification of the nanomaterial; specification; quantity; toxicological profile; safety data and exposure

Australia: NICAS (guidance)

- Size, size distribution, production method, shape, crystalline phase, agglomeration/aggregation, surface charge, surface chemistry,...

EU – Chemicals: REACH (European Chemicals Agency)

- sample preparation, nano-specific considerations for size, size distribution, shape, surface area

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Consequences for characterisation requirements

- Example: **Size**
- Considered as relevant for safety assessment by "all":
 - OECD, ISO, EPA, NIST, NanoImpactNet, FDA, SCENIHR, EFSA, SCCS
- Explicitly addressed in EU legislation
- Used as identifier for nanomaterials in (almost) all definitions
e.g., the EC Definition for regulatory purposes:

"Nanomaterial" means a natural, incidental or manufactured material containing particles, in an **unbound state** or as an **aggregate** or as an **agglomerate** and where, for 50 % or more of the particles in the **number size distribution**, one or more external dimensions is in the size range **1 nm - 100 nm**.

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Some resulting measurement requirements

- Determination of the **constituent (primary)** particles of the material
The constituent particles are often aggregated or agglomerated
- Determination of the **external dimensions** of the (constituent) particles
Particle size measurements assess the external dimensions of particles
- Address particle **size distribution** (by number)
e.g., "Determination whether 50% or more of the particles have an external dimension in the size range 1-100 nm"



EUR 25404 EN
July 2012

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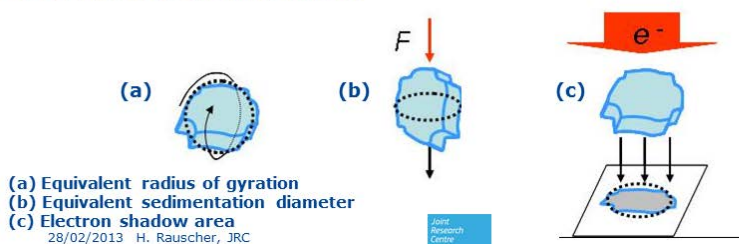
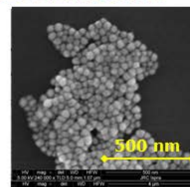
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Generic issues for reliable particle size determination

- Sampling
 - the sample(s) to be measured must be representative
- Sample preparation
 - Agglomerates and aggregates
 - Dispersion
- Conversion of mass based measurements to number-based size distribution
 - Based on assumptions; prone to errors
- Size values are method-defined



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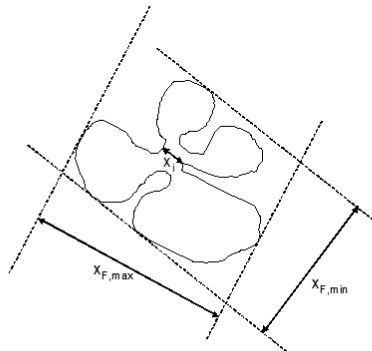
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Size – which external dimensions?

ISO/TC 24/SC4 (the ISO SC responsible for particle characterisation):
'linear dimension of a particle determined by a specified measurement method and under specified measurement conditions'



There are many possibilities

- Central part (x_i)?
- Minimum Feret diameter ($x_{F,min}$)?
- Maximum Feret diameter ($x_{F,max}$)?

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100 nm

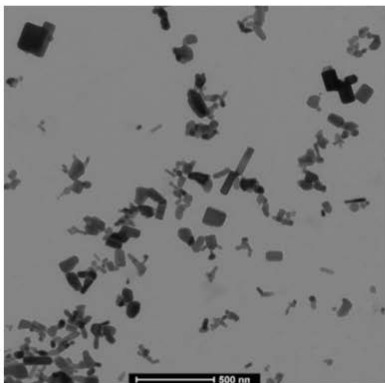
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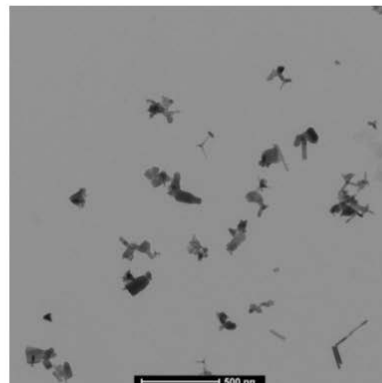


Example: ZnO from the NM series

NM 110



NM 111



Sample preparation: EUR 25066 EN - 2011

Non-spherical, polydisperse, aggregated particles, picture representative?

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Source: EUR 25066 EN

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ZnO NM 110 and NM 111 Size analysis

Method	NM-110 [nm]	NM-111 [nm]
<i>Size measurement on dry powders</i>		
TEM , Feret average	147 ± 149	141 ± 103
SEM , Feret	151 ± 56	141 ± 66
<i>Size measurement on suspensions</i>		
DLS , mean particle size	275 ± 4	253 ± 1
CLS (in distilled water)	193 ± 3	-
XRD (crystallite size)	41.5	33.8

The size values are method-defined

Source: EUR 25066 EN

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Method name	Measurement range and medium	size distribution of raw data	Can deal with particular types of nanomaterials?				Standards available?
			poly-dispersity	non-spherical particles	low-density materials	aggregates	
Electron microscopy	1 nm and higher; dry	number-based	😊	long 😊 flat 😞	😊	😊	yes
Dynamic light scattering	5 nm to 500 nm; suspension	(no distribution, or scattering intensity-based)	😞 😞	😊	😊	😞 😞	yes
Centrifugal liquid sedimentation	20 nm and higher; suspension	extinction intensity-based	😊	😊	😊	😞 😞	yes
Small-angle x-ray scattering	5 nm and higher; suspension	scattering intensity-based	😊	😊	😊	😞 😞	yes
Field-flow-fractionation	1 nm to 200 nm; suspension	(depends on detector)	😊	😊	😊	😞 😞	no
Particle tracking analysis	25 nm and higher; suspension	number-based	😊	😊	😊	😞 😞	no
Atomic force microscopy	1 nm and higher; dry	number-based	😊	😊	😊	😊	yes
X-ray diffraction	1 nm and higher; dry	(no distribution measured)	😊	😊	😊	😊	yes

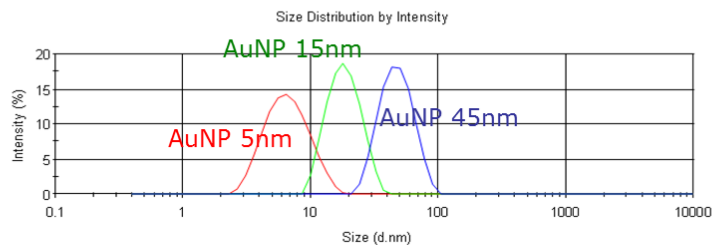
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From: Linsinger T. et al., EUR 25404, 2012
doi:10.2787/63490

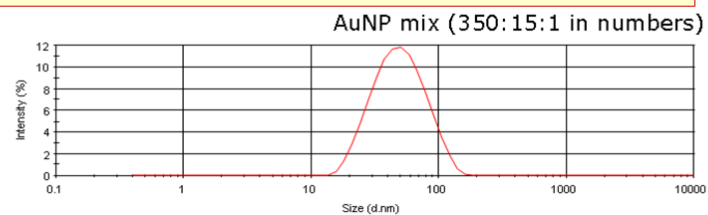
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NP Mixtures: Dynamic Light Scattering



Basically similar distribution for mixture and pure 45nm NP



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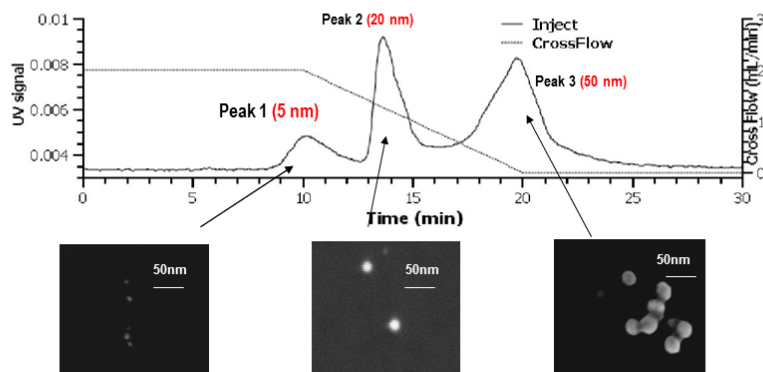
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Nanoparticles separation with Asymmetric Flow Field Flow Fractionation

Injection: 20 ml of nanoparticle mixture (5, 20, 50 nm)



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Recommendations for size measurements

- Provide dedicated guidance documents for *specific materials and sectors*
- Combine data from *several methods* with information on *manufacturing process*
- Carefully consider the *constituent particles/ aggregates* issue
- Perform *validation studies* and interlaboratory comparisons for a range of methods

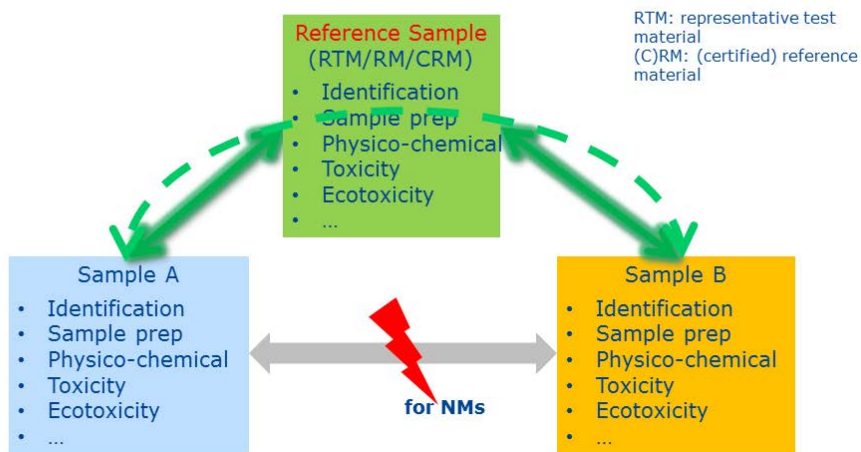
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Transferability of test results for safety assessment – the need for reference materials



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Sample preparation – Dispersion

- Recognized as critical by OECD WPMN
- Recognized as critical by most projects addressing NM characterization
- Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials - ENV/JM/MONO(2012)40

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Detailed and validated protocols for sample preparation are needed

Towards a method for detecting
the potential genotoxicity of nanomaterials

For example:



Final protocol for producing suitable
manufactured nanomaterial exposure media

—

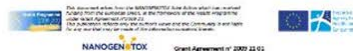
Web-report

The generic NANOGENOTOX dispersion protocol

—

Standard Operation Procedure (SOP)

October, 2011



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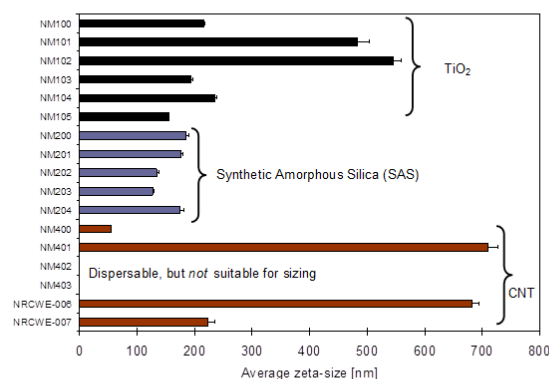
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Grant agreement number 2009 21 01

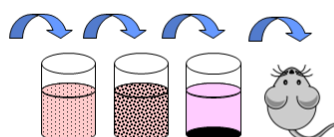
2.56 mg/ml in 0.05% w/v (Bovine) Serum Albumin
(0.5 % EtOH pre-wetting for all)

NM stability in specific test mediums may vary and should be verified case by case



2.56 mg/ml
NM Stock Suspension
400 Watt; 300 Hz (10% Ampl)
16 min cont. Sonication
Ice-water bath

Different Exposure Systems



NANOGENOTOX – External Evaluation – 29 November, 2012

by Keld Alstrup Jensen

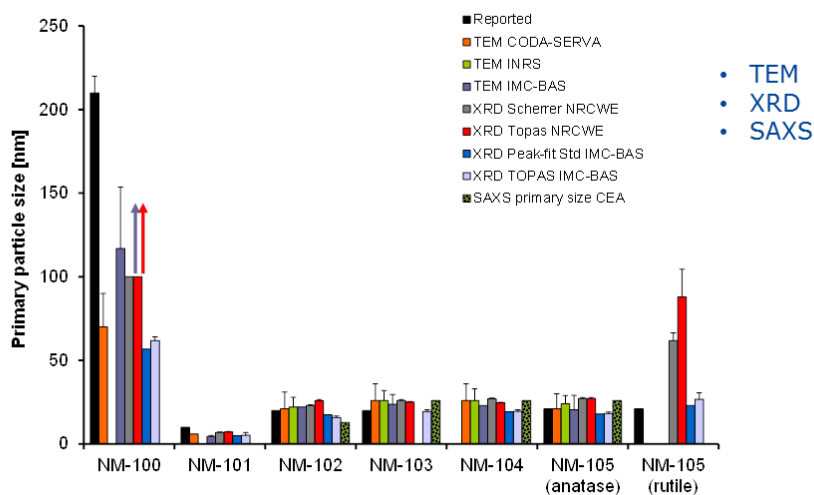


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Grant agreement number 2009 21 01

Primary sizes of the TiO₂ MN samples



- TEM
- XRD
- SAXS

NANOGENOTOX – External Evaluation – 29 November, 2012

by Keld Alstrup Jensen

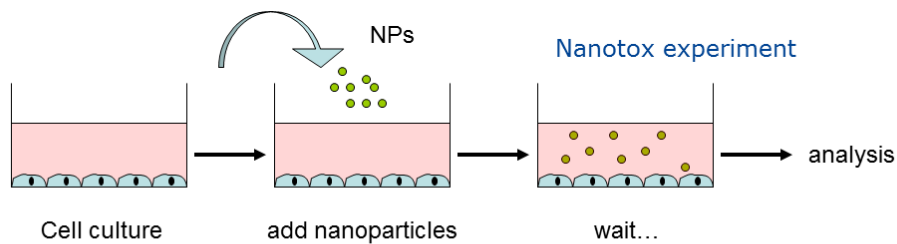
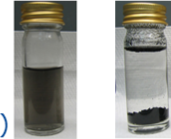


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Considerations on dosimetry

- Traditionally: **mass based** concentration (mg/ml)
- For NM also recommended in terms of **surface area** (cm²/ml)
or **particle number** (particles/ml): ENV/JM/MONO(2012)40
- Is that enough?



What is the NP dose?

Can we assume a homogeneous concentration of NPs throughout the experiment?
Do we have to take into account sedimentation effects?

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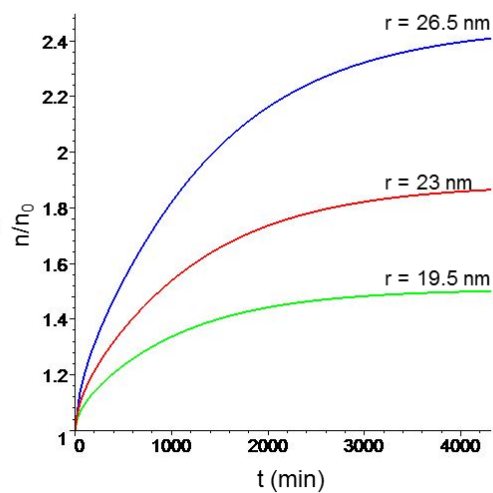
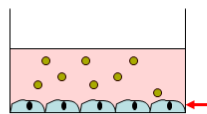
See also ACS NANO 5 (2011) 3766 27



Assay in multiwell plate:

96 well plate bottom
d = 6,35 mm,
100 µl
Co-Ferrite P601
72 hours

Gravitation
Buoyancy
Drag
Diffusion

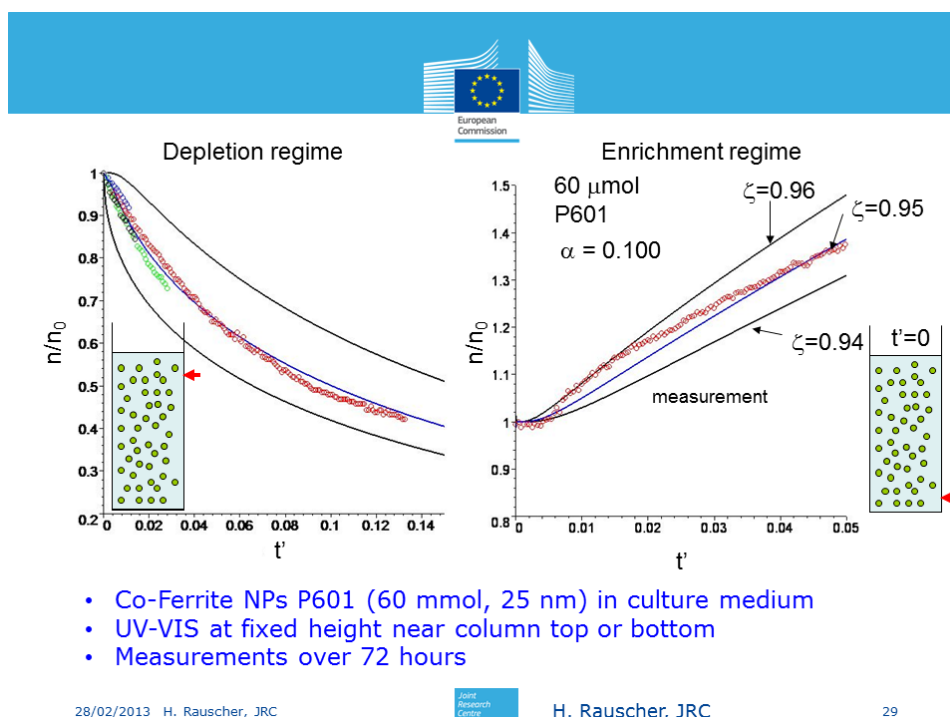


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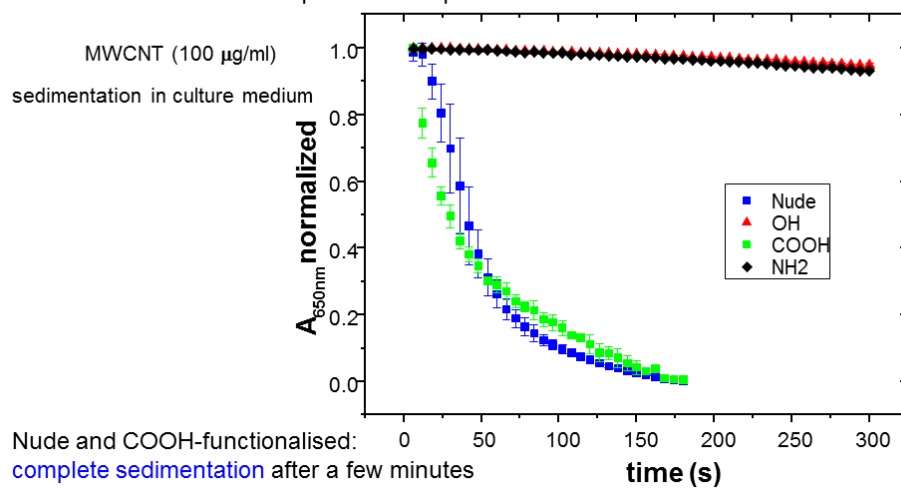
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Analogous analytical model for CNTs not possible – irregular shape
empirical extrapolation can be done



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- sedimentation effects can play a role
- this depends on the particle type, experiment duration and design
- equilibration time has to be considered
- *a priori*, concentration (molarity) is *not a good measure* for nanotox studies with NPs (and with other particles as well)
- better: area dose should be given, considering sedimentation effects

mass/area (e.g., mg/cm²) instead of **mass/volume** (mg/ml) is recommended if fast sedimentation is expected



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Conclusions

- NM characterization for regulatory safety assessment - challenging
- Physico-chemical properties have nanospecific aspects (size, settling,...)
- Suitable reference or at least benchmark materials must be used
- Nanospecific sample preparation protocols should be established

Nanospecific OECD TGs and guidance are urgently needed

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Session II. Test Guidelines on Physical-Chemical Properties

Yasir Sultan: Targeted Physical-Chemical Needs for Risk Assessment – An OECD WPMN Perspective



Working Party on Manufactured
Nanomaterials (WPMN)
Steering Group 6:
Risk Assessment of Nanomaterials

- Mandate
- Activities
- Moving Forward

Co-Chairs:
Yasir Sultan, PhD. (CAN)
yasir.sultan@ec.gc.ca

Carsten Kneuer, PhD.(GER)
carsten.kneuer@bfr.bund.de

1

SG6: Co-operation on Risk Assessment

The objective of this SG is to evaluate the risk assessment approaches for MNs and to identify opportunities to strengthen and enhance risk assessment capacity

Tasks:

- Consider *existing* strategies, methodologies and supporting tools
- Identify and consider any *unique issues* that Nanomaterials present for Risk Assessment
- Make recommendations to WPMN for addressing and filling identified gaps, and *consider*:
 - the need for *provision of guidance* on key issues
 - the development of *empirical evidence* to support this guidance



2

Typical challenges encountered in the risk assessment realm



3

Fate Determination

- Env. Fate of nanomaterials may be determined with the following considerations:
 - Surface species
 - Degradation of the material
 - Aggregation/agglomeration (and dis-aggregation)
 - Surface charge
 - Recognition that strictly inorganic nanomaterials will not benefit from standard biodegradation tests
 - Examining the potential for abiotic degradation

Are these considerations relevant/appropriate?

Are there additional considerations?



Read-across: Nano-to-Nano Extrapolation

- Read-across data play an important contribution to chemical risk assessments.
 - However, it is uncertain what role read-across data can play in the context of nanomaterials risk assessments:
 - Does “case-by-case” suggest generation of full datasets for any modification?
- Or, can data from
- a 20nm be used to assess the same material scaled up to 35 nm?
 - an uncapped material be used for the same material which is capped?

What information is needed to support read-across?



Metrics of Data Reporting

- It is recognised that there is uncertainty concerning the units of measurement used to communicate test results employed in risk assessments.
- It is expected that empirical results will continue to be reported in terms of mass based units.
- Risk assessments recommended to include discussion of any limitations this metric may present (e.g., considerations of surface area, number of particles, etc).

Should we continue using mass based units of measurement to report effects and exposure information?



6

SG6 activities



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SG6 Risk Assessment Workshop

- SG6 held a Workshop on *Risk Assessment of Manufactured Nanomaterials in a Regulatory Context* in 2009 in Washington DC
- Main workshop objective was to promote the further development of a draft Report on *Critical Issues in Risk Assessment of Nanomaterials*
- Case studies were reviewed for Carbon Nanotubes, Silver Nanoparticles and nanoscaled Titanium Dioxide
- Approaches to Risk Assessment of MNs were discussed (Risk Assessment Paradigm confirmed) and open questions identified
- Workshop report published 2010 as **ENV/JM/MONO(2010)10**



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Important Issues in RA of MN

Finalized 2011 and Published as **ENV/JM/NANO(2012)8**

Content:

1. General Introduction
 2. Background (Information on Current Risk Assessment Approaches and Nanomaterial Case Studies)
 - 3. Risk Assessment of Nanomaterials: Important Issues**
 - 4. Risk Assessment of Nanomaterials: Strategies and Approaches**
 5. Research Needed to Address Risk Assessment Issues
- Intention for this to be a living document with updates as needed/required



9

Risk Assessment Issues Prioritisation (2012/13)

Currently being finalized

Objective: to identify, from *Important Issues in Risk Assessment of MN*, key issues that require priority.

- Questionnaire circulated to all OECD WPMN delegation I/2012
- First Analysis of Responses and Preliminary Report II/2012
- Task Group for further analysis, especially on inter-linkage of issues was formed
- Final Analysis expected WPMN 11 (Feb 2013)



Risk Assessment Issues Prioritization

Section 1									
	ISSUE	II - Importance			III - Data availability			IV	V - Volunteer
		very high ¹	high ²	less	from SP	other source	not expected	related to issue no.	to lead / to contribute
PROBLEM FORMULATION									
1	Selection of assessment endpoints								
2	Testing plan								
HAZARD IDENTIFICATION									
3	Applicability of testing methods								
4	Endpoints assessed								
5	Target organs								
6	Effective dose								
7	External factors influencing toxicity								
8	Variability of external factors								
9	Definition of adversity								
10	Mechanistic considerations								
11	Non-nano to nano extrapolation								
12	Nano to nano extrapolation								
13	Nanomaterials acting as carriers								



Risk Assessment Issues Prioritization (2013)

- Purpose of this exercise is to foster projects to help guide risk assessments
 - 348 issues identified through the questionnaire indicating a very complex regulatory landscape for risk assessments
- As a first step, *issues* which were most interdependent were identified as priorities
- Three methodologies utilized based primarily on expert judgement
 - Data weighting
 - Cluster analysis
 - Mind maps



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Risk Assessment Issues Prioritization (2013)

- Data weighting

$$\text{Score} = \frac{10 \times \text{„very high“ scores} + 5 \times \text{„high“ scores}}{\text{total number of scores}}$$

Environment	Human Health
Physico-chemical properties	Physico-chemical properties
Quantifying risk	Applicability of testing methods
Applicability of testing methods	Effective dose
Non-nano to nano extrapolation	Non-nano to nano extrapolation
Hazard assessment for classification and labelling	Hazard assessment for classification and labelling
Applicability of tools for exposure modelling	Selection of assessment endpoint
Use of data on close analogues	Use of data on close analogues
Bioaccumulation	Dose metrics
Transformation, degradation and persistence	Linking material properties to ADME and toxic effects
Nanomaterial identification	Nanomaterial identification

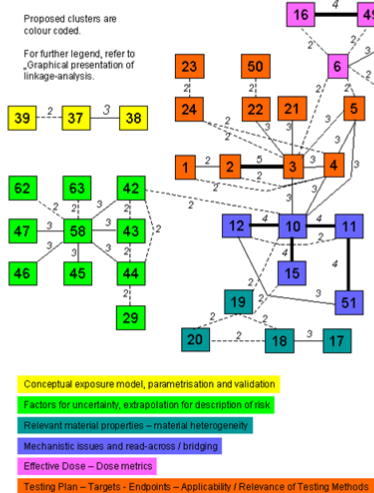


13

Risk Assessment Issues Prioritization (2013)

Cluster analysis

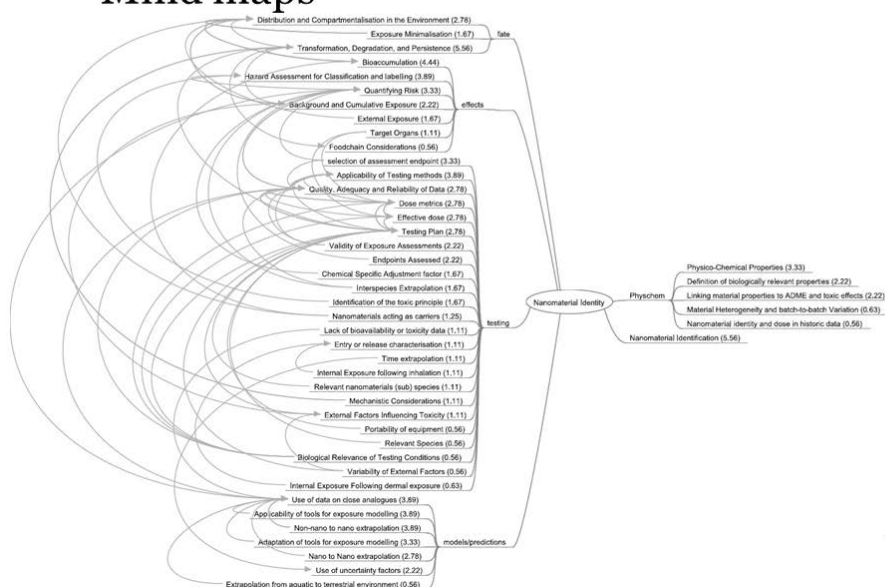
Graphical presentation of cluster-analysis



14

Risk Assessment Issues Prioritization (2013)

Mind maps



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Draft conclusions

- Issues which cross-cut the most categories were identified as priorities
 - However, this does not mean other issues are not priorities
- Physical-chemical properties (including nanomaterial identity) was identified as the most important priority to the RA community
 - i.e., how to characterize physical-chemical properties to inform on env. Fate and effects, e.g., how to use measure and use dissolution data to inform on aquatic, sediment, and/or soil partitioning
- Other high-level priorities identified through this exercise are Fate, Effects, and Testing



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Moving Forward

- SG6 would like to initiate projects which address how to use physical chemical data to inform risk assessment and risk management of nanomaterials
- Reasons to support this path forward include:
 - It is the category which may have the largest amount of available data
 - Physical-chemical characterization is a critical need to inform the other categories
 - There is still very little clarity on how physical-chemical endpoints can be used in risk assessments in a predictable manner
 - Working on this will increase collaboration and cooperation between SG6 and the other SGs (such as SG3) and will also link with the categories work being discussed



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Example of potential project being proposed

- How to address nAg dissolution in risk assessments
 - Primary literature reports varying aqueous dissolution data depending on the surface chemistry
 - Preliminary analysis indicates that the surface coating plays an important role in dissolution depending on type, concentration, and interaction with external parameters
 - Current risk assessment approaches derive no clear approaches to deal with varying dissolution rates and env. Fate and effects
 - SG3 data needed to increase data quality and to better recommend approaches



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Example of potential project being proposed

START	RISK ASSESSMENT Through examining NP Solubility		FINISH
Natural Waters Coated Ag-NPs enter environmental waters from various sources	FACTORS AFFECTING SOLUBILITY	TRENDS	Natural Waters Based on the coating agent, other NP characteristics and environmental conditions, certain percentage of the original Ag-NPs exists as Ag ions (amount differs for different coating agents, media, etc.)
	Coating Agent	Sterically Stabilizing (PVP, Tween) Electrostatically Stabilizing (SDS, BPEI) Electrostatically Stabilizing (Citrate, NaBH ₄)	
	% Coating	• Larger size/weight of coating inhibits dissolution	
	Particle Size	• Solubility increases as particle size decreases	
	Size distribution	• Higher Ag-NP concentrations → rapid aggregation → dissolution rates decline	
	Shape	• Spherical NPs dissolve to a greater extent than rod shaped NPs	
	Crystallinity & Crystal Structure	• Certain crystal faces more susceptible to dissolution • Example: {1 1 1} and {1 1 0} faces with lower coordination numbers dissolve faster than {1 0 0} faces of nanocrystals	
	Surface Morphology	• Particles' surface features with smaller radius of positive curvature (convex) are energetically unstable → Preferential dissolution and higher equilibrium solubility	
	Oxygen Levels	$2Ag_{(s)} + \frac{1}{2}O_{2(g)} + 2H^+_{(aq)} \rightleftharpoons 2Ag^+_{(aq)} + H_2O_{(l)}$ • Dissolution reduced under anaerobic conditions	
	pH Levels	• Increase in pH, reduces the amount of $H^+_{(aq)}$ available for the dissolution process → Lower dissolution	
	Sulfidation Reactions	• Higher extent of sulfidation (with HS/NaS) → Lower dissolution	
	Presence of Natural/Dissolved Organic Matter	• NOM adsorbs onto the particle surface providing a barrier to aggregation • Presence of cysteine can increase dissolution process	
	Presence of Electrolytes & their Catalytic role	• Higher degree of dissolution in electrolyte solutions than in DI water • Cl ions catalyze the oxidation of Ag-NPs	
	Contaminants	• Various reducing and/or stabilizing agents can have distinct effects on the toxicity of Ag-NPs depending on the properties on the contaminants (i.e. whether it contributes towards the oxidation of Ag-NPs or stabilizes them)	

Current Activities & Outlook

- SG6 to focus on specific guidance on important issues of nanomaterial risk assessment (*WPMN mid-term review*)
- To be achieved through:
 - Prioritizing issues / challenges to be addressed
 - Initiating projects on selected topics
 - Cooperating with WPMN research activities developing empirical science to support improved approaches to risk assessment methodology for nanomaterials
 - Interim project reviews / workshops
 - Project reports and OECD Risk Assessment Guidance



SG6 needs from the Sponsorship Program and Experts

- Involvement of experts on SG6 projects of mutual relevance
- Develop relevant test guidelines or guidance on how to conduct pchem tests
- Guidance on what physical-chemical parameters inform on environmental fate, including partitioning and bioavailability and how to conduct those tests





Thank you for your attention

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Angela Height Walker: ISO Guidelines

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FOR ECONOMIC
CO-OPERATION
AND DEVELOPMENT



ORGANISATION DE
COOPÉRATION ET
DE DÉVELOPPEMENT
ÉCONOMIQUES



OECD Expert Meeting on Physical-
chemical Properties of Manufactured
Nanomaterials and Test Guidelines
in collaboration with
ISO/TC 229: Nanotechnologies

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SESSION I. TEST GUIDELINES ON PHYSICOCHEMICAL PROPERTIES

ISO (TC229) Guidelines

*Outline existing and planned ISO documents for nanomaterials
relevant for physicochemical characterization*

Angela R. Hight Walker
representing

Toshiyuki FUJIMOTO

Convener, ISO TC229 – IEC TC113 /JWG2
National Metrology Institute of Japan, AIST

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ISO/TC 229 RESOLUTION FROM STRESA, ITALY

RESOLUTION 6/2012 (JWG2)

ISO/TC 229 commits to supporting the envisioned co-organization of a joint workshop on the physicochemical characterization of nanomaterials with OECD WPMN. ISO/TC 229 suggests scheduling the joint meeting just before the ISO/TC 229 meetings in Mexico in March 2013.

Approved unanimously



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ISO/TC 229 MEETING GOALS

- Respond to OECDs requests
- Prioritize measurement needs to feed our JWG2 Strategic Plan



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TERMINOLOGY

*Nanomaterials,
Nano-objects,
Nanoparticles,
Primary particle,
Nanostructured,
Nanotube*

*Guidance,
Test Guidelines,
Protocols,
Technical Reports,
Technical Specifications,
International Standard*

etc

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SCOPE OF ISO/TC 229 NANOTECHNOLOGIES

Standardization in the field of nanotechnologies that includes either or both of the following:

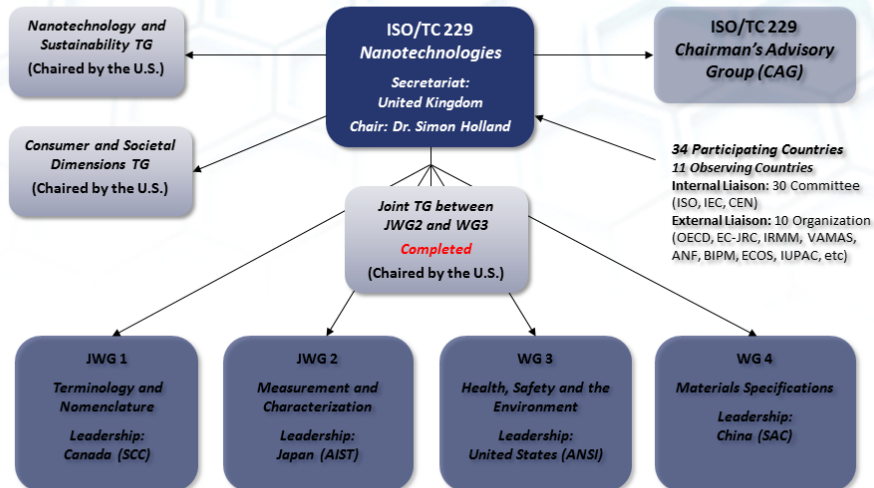
1. Understanding and control of matter and processes at the nanoscale, typically, but **not exclusively**, below 100 nanometers in one or more dimensions where the onset of size-dependent phenomena usually enables novel applications,
2. Utilizing the properties of nanoscale materials that differ from the properties of individual atoms, molecules, and bulk matter, to create improved materials, devices, and systems that exploit these new properties.

Specific tasks include developing standards for: **terminology and nomenclature; metrology and instrumentation**, including specifications for reference materials; test methodologies; modeling and simulations; and **science-based health, safety, and environmental practices and material specification.**

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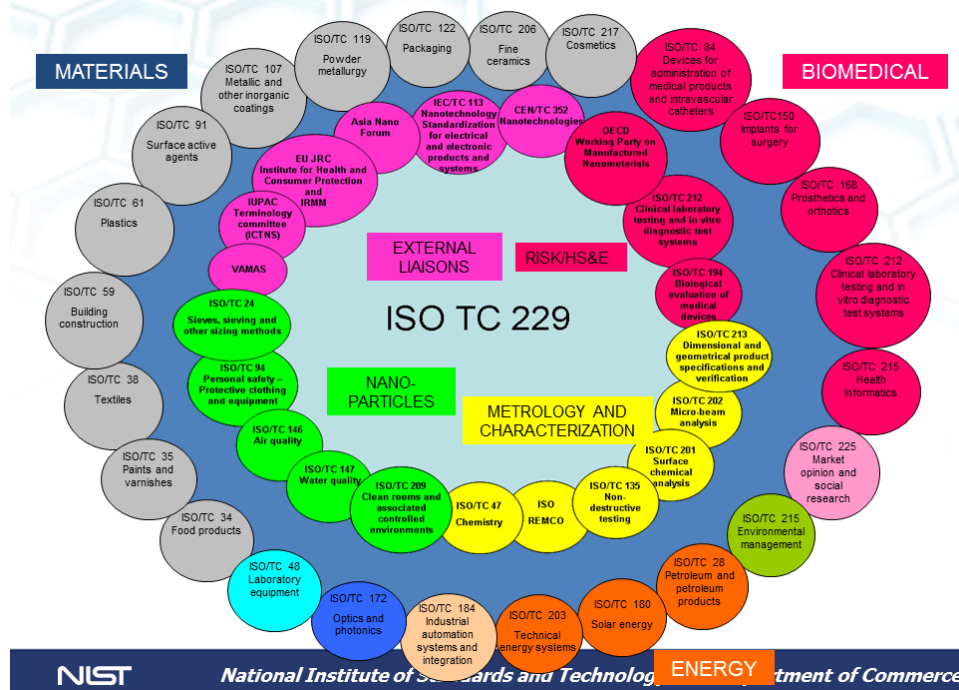
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ISO/TC 229 ORGANIZATION



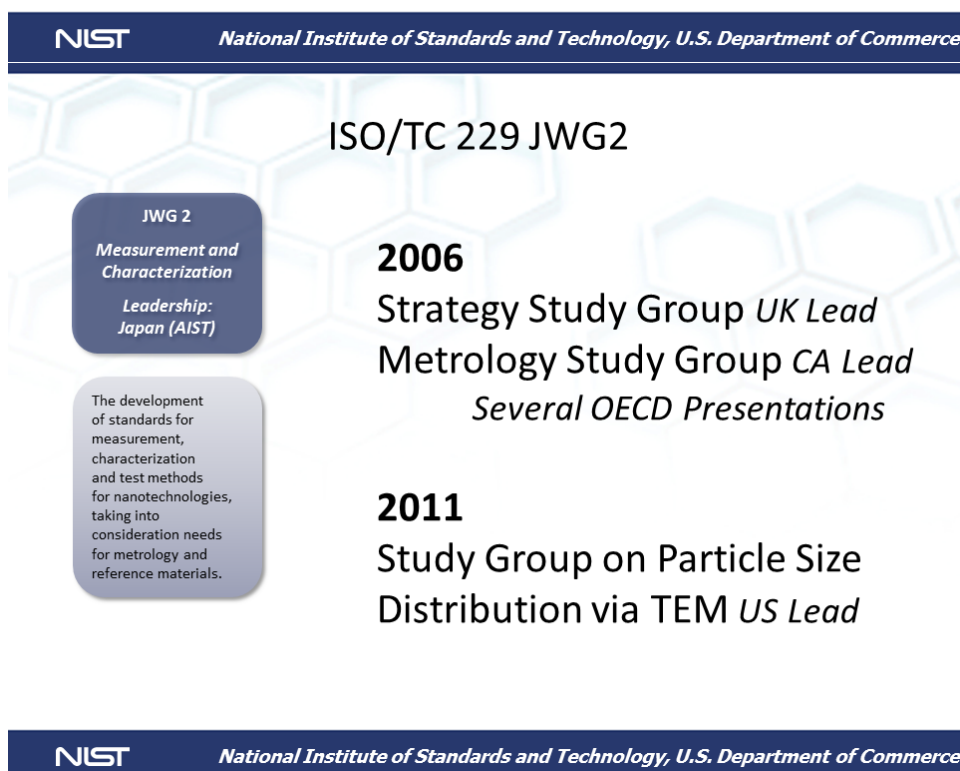
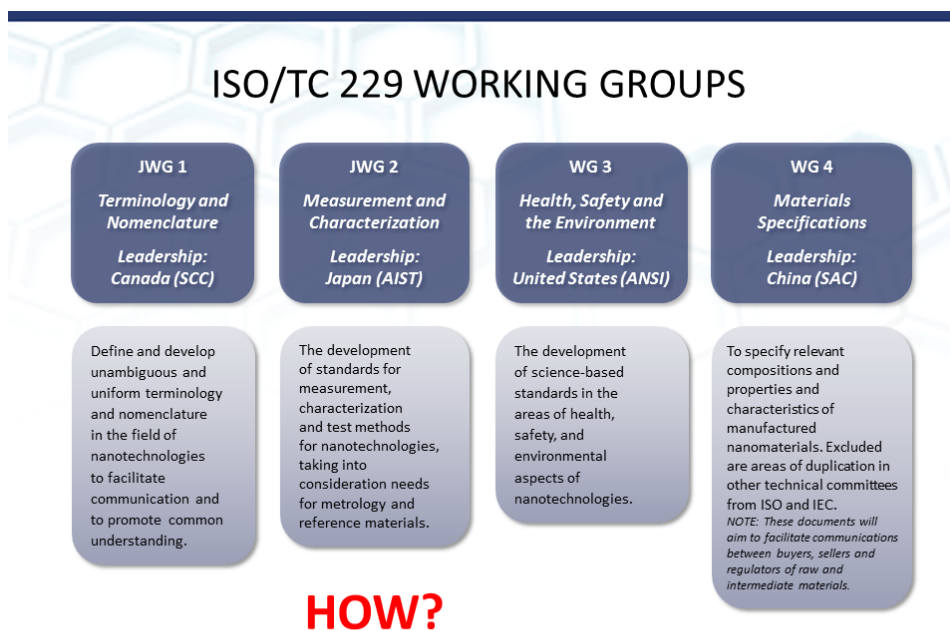
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METROLOGY STUDY GROUP

ISO/TC 229 N 673

ISO/TC 229 – IEC/TC113 / JWG2_N095rev1

Metrological check-list for use in the preparation and evaluation of ISO NWIPs and ISO WDs		
1	Has the system / body / substance that will be subjected to the measurement procedure, clearly been described, including its state? <u>Example:</u> 'suspension of nanoparticles'	Ⓢ D Ⓢ D
2	Is the definition of the system / body / substance not unnecessarily restrictive? <u>Example:</u> there is no need for a standard specific for 'metallic impurities in carbon nanotubes' if the proposed measurement method is equally applicable for any impurity in any matrix.	Ⓢ D Ⓢ D
3	Is the <u>measurand</u> clearly described? <u>Example 1:</u> 'average particle size in terms of particle volume' is different from 'average particle size in terms of particle number'	Ⓢ D Ⓢ D
4	Has it been clearly indicated whether the <u>measurand</u> is operationally or method-defined, or whether the <u>measurand</u> is an intrinsic, structurally defined property? <u>Example 1:</u> 'hydrodynamic diameter of particles in suspension' is method-defined: the motion of particles in the liquid depends also on the liquid in which the particles are suspended. <u>Example 2:</u> 'particle size as measured with a microscope in vacuum' is not method-defined: after degassing, the size of the particle measured is the same, independent on the type of microscope used (optical, electron, ...). <u>Note:</u> For a measurand that is method defined, an agreed documentary standard is needed to	Ⓢ D Ⓢ D

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STRATEGY AREAS OF JWG2 (2008)

The strategy and priorities draws heavily upon the results of the questionnaire survey (ISO/TC 229 N124)

- A – Standards for measurement and characterisation of **carbon nanotubes** and related structures
- B – Standards for measurement and characterisation of **engineered nanoparticles**
- C – Standards for measurement and characterisation of **coatings**
- D – Standards for measurement and characterisation of nanostructured materials (**composites and Porous structures**)
- E – Standards for **basic metrology** at the nanoscale
- F – Guidance for characterisation, specification and production of **reference materials**

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MATRIX APPROACH FOR SWCNTS

WG2_N007.12

Property Category	Method						
	SEM/EDX (Lead:USA)	TEM (Lead:USA, Co-lead:Japan)	Raman Spectroscopy (Lead:USA)	UV-Vis-NIR Absorption (Lead:Japan)	NIR-PL/ Fluorescence (Lead:Japan)	TGA (Lead:USA, Co-lead:Korea)	TG-MS (Lead:Japan)
Morphology	Tube structure, bundle thickness, orientation	Wall structure, amorphous carbon, metal catalyst coatings					
Purity	Non-carbon impurities	Tube surface cleanliness	Nanotube and non- nanotube carbon	Carbonaceous content (Quantitative) (Lead:USA)		Non-carbon content (Quantitative) Non-CNT content (Quantitative)	Non-carbon content (Quantitative)
Length and Diameter	Length and diameter	Tube diameter, metal cluster size	Diameter (Lead:Japan)	Diameter (Lead:Japan)	Diameter		
Tube Type			Metallic/ Semiconducting	Metallic/ Semiconducting (Lead: USA, Co-lead: Korea)	Chirality (Semi conducting tubes)		
Dispersability/ Solubility	Tube bundling			Tube bundling or separation (solution)	Tube bundling		
Additional						Oxidation/transition temperatures	Oxidation/transition temperatures

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MATRIX approach for SWCNTs

WG2_N007.12

Property Category	Method						
	SEM/EDX (Lead:USA)	TEM (Lead:USA, Co-lead:Japan)	Raman Spectroscopy (Lead:USA)	UV-Vis-NIR Absorption (Lead:Japan)	NIR-PL/ Fluorescence (Lead:Japan)	TGA (Lead:USA, Co-lead:Korea)	TG-MS (Lead:Japan)
Morphology							
Purity	PG2 2007.4 .13	PG1 2007.4 .11	PG8 2007.9 .3	PG3 2007.5 .1	PG4 2007.5 .1	PG7 2007.8 .23	PG6 2007.8 .20
Length and Diameter							
Tube Type	USA	USA Japan	USA	Japan	Japan	USA Korea	Japan
Dispersability/ Solubility							
Additional							

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MATRIX APPROACH FOR MWCNTS

Category	Property	Method
Purity	Moisture content	Weight loss measurement (*)
Purity	Ash content	Weight loss measurement (*)
Purity	Metallic residual content	XRF
Purity	Metallic residual content	ICP-AES
Purity	Volatile content	Weight loss measurement (*)
Purity	Polyaromatic hydrocarbons	GC-MS
Purity	Carbon materials excluding CNT	SEM and/or TEM
Physical properties	Burning properties	TG/DTA
Physical properties	Resiliency (Compression)	Density and resiliency
Physical properties	Bulk Density	Bulk density measurement
Physical properties	Specific gravity	Pycnometer
Physical properties	Specific surface area	BET
Physical properties	Stacking nature	XRD
Physical properties	Stacking nature	TEM
Physical properties	Electric resistivity	Volume resistivity
Geometrical property	Disorder	Raman Spectroscopy
Geometrical property	Morphology	SEM and/or TEM
Geometrical property	Morphology	Bending ratio (Hydrodynamic)
Geometrical property	Morphology	Bending ratio (SEM)
Geometrical property	Inner diameter	TEM
Geometrical property	Outer diameter	SEM and/or TEM
Geometrical property	Length	SEM and/or TEM

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MATRIX APPROACH for MWCNTs

Category	Property	Method
PG5 2007.5.31 registered as TS Japan PG9 (Shape Factor) 2007.12.11 Korea		

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MATRIX APPROACH for MWCNTs

Category	Property	Method
WG2_N007.12		
Nanotechnologies -- Characterization of multiwall carbon nanotube (MWCNT) samples (ISO/TR 10929) Nanotechnologies -- Characterization of multiwall carbon nanotubes -- Mesoscopic shape factors (ISO/TS 11888)		

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OTHER WORKING PROGRAMMES

PG No	Doc. No.	Title	Status
10	TS 12025	Nanomaterials — Quantification of nano-object release from powders by generation of aerosols	Published
11	IEC/TS 62622	Nanotechnologies – Description, measurement and dimensional quality parameters of artificial gratings	Published
12	TS13278	Nanotechnologies – Determination of elemental impurities in samples of carbon nanotubes using inductively coupled plasma mass spectrometry	Published
13	TS 16195	Nanotechnologies -- Guidance for developing nanoscale test materials in powder form for improving comparability	Printing Process
14	TS 17466	TS Use of UV-Vis Absorption Spectroscopy in the Characterization of Cadmium Chalcogenide Semiconductor Nanoparticles (Quantum Dots)	WD
15	TR 18196	Measurement Method Matrix for Nano-objects	WD

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Nanotechnologies — Measurement method matrix for manufactured nano-objects

Scope

This Technical Report provides a framework for identifying measurement methods for the physicochemical parameters of nano-objects that most commonly need to be measured.

Note 1: This technical report is limited to *commercially* available methods.

Note 2: Both standardized and unstandardized methods are listed in this document. Some methods are standardized for general nano-objects but some are written for specific nano-objects, e.g. for CNT.

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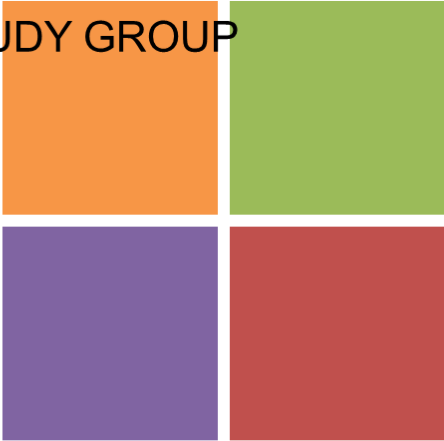
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Findings:

- Protocol conformed to ISO metrology checklist and VAMAS guidelines
- Round robin gave D_{fret} averages similar to reference material's nominal size
- Statistical analysis identified intra- and interlab variations in sample means, which appear to relate to image processing methods.

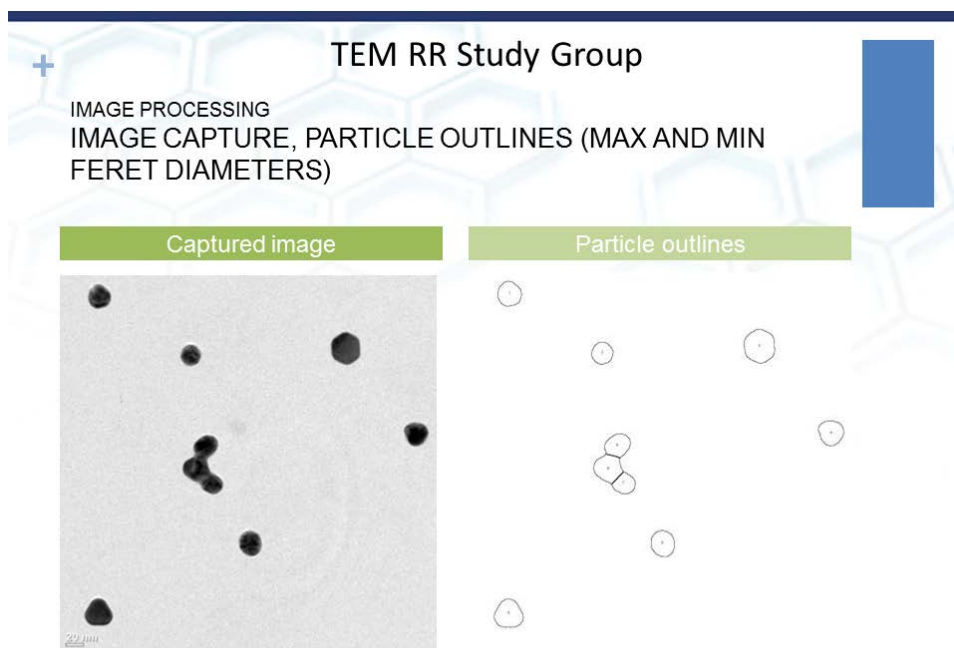
TEM RR STUDY GROUP



Next steps:

Study discrete nanoparticles (gold, and then colloidal silica), aggregated primary particles (titania and carbon black), and non-spheroidal discrete nanoparticles (nanorods and nanodisks). These will be used to develop generic guidelines, with specific case studies cited as appendices.

Also, we have more statistical work to do on the current data set; this will help establish statistical protocols for the next case studies.



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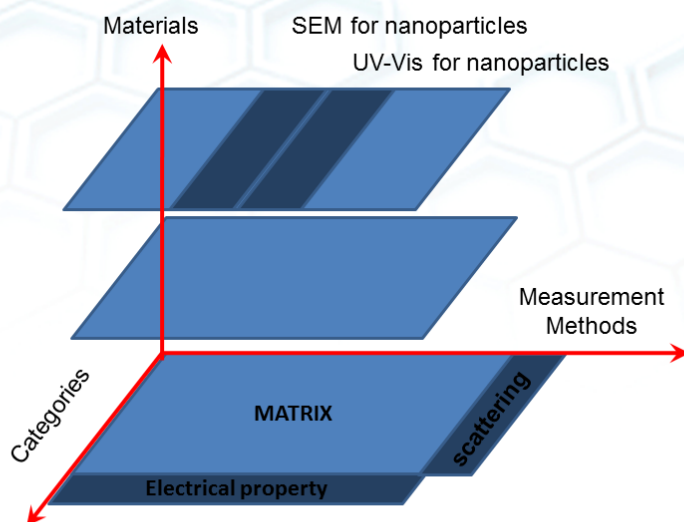
Possible Future JW2 Directions

Property Category	Method						WG2_N007.12
	SEMEDX (Lead:USA)	TEM (Lead:USA, Co-lead:Japan)	Raman Spectroscopy (Lead:USA)	UV-Vis-NIR Absorption (Lead:Japan)	NIR-PL/ Fluorescence (Lead:Japan)	TGA (Lead:USA, Co-lead:Korea)	
Morphology	Tube structure, bundle thickness, orientation	Wall structure, amorphous carbon, metal catalyst coatings					
Purity	Non-carbon impurities	Tube surface cleanliness	Nanotube and non-nanotube carbon	Carbonaceous content (Quantitative) (Lead:USA)		Non-carbon content (Quantitative) Non-CNT content (Quantitative)	Non-carbon content (Quantitative)
SIZE	Length and diameter	Tube diameter, metal cluster size	Diameter (Lead:Japan)	Diameter (Lead:Japan)	Diameter		
			Metallic/ Semiconducting	Metallic/ Semiconducting (Lead:USA, Co-lead: Korea)	Chirality (Semi conducting tubes)		
Dispersability/ Solubility	Tube bundling			Tube bundling or separation (solution)	Tube bundling		
Additional						Oxidation/transition temperatures	Oxidation/transition temperatures

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Possible Future JWG2 Directions



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POSSIBLE NEW WORK ITEMS TO BE DISCUSSED NEXT WEEK

- 9.1 Single Particle ICP-MS, NEN
- 9.2 Characterization of Zinc Oxide Nanoparticles/Quantum Dots using TEM, BIS
- 9.3 Nanocellulose characterization, CSA
- 9.4 Superhydrophobicity of Textiles - Characteristics and Measurement Methods, DIN
- 9.5 The Analytical methods and Tiered approach for Identifying Nanomaterials to the definition, JISC

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OTHER ITEMS THAT HAVE BEEN DISCUSSED AS IMPORTANT

Other target materials

particles, coating, composite and porous material
Silver, MWCNT, SWCNT, Liposomes, Quantum Dots
Nanocellulose, Aluminum pigments, Fuel cell catalytic-Pt, Graphene,
Nanoclays, Food-relevant

Other properties

Electrical, Magnetic, Mechanical, Optical
Sample Preparation/handling, General-Property Specific
also performance property Is there nano in there?
Concentration/Dose, Nanoparticle coatings, Surface topography,
Catalytic characterization

Other measurement methods

Partition function, Scattering(X-ray, neutron, light), SPM, XPS, XRD,
BET, AFM, Combination

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DISCUSSION POINTS

- How to deal with the lack of **scientific** readiness for guidelines/standards
- Sample heterogeneity limits applicability of assessing guideline
 - But can we use OECD data to assess a measurement?
- As it is VERY unlikely a single measurement will suffice for any endpoint
- “Dynamics”
- Can some “sample specific” bins be developed
- A publication is NOT validation
 - *But it is needed-how to achieve*
- Reference or representative materials
- Where to start-highest priority from OECD to 229

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THANK YOU!



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ISO TC 229 JWG 2 MEASUREMENT AND CHARACTERIZATION

Published: 8 ISO characterization protocols

- Carbon Nanotubes; both single and multiwall
- Generic dimensional metrology

Strongly support other working groups, esp. WG3 and WG4

Under development:

- Quantum Dots metrology
- Generic Measurement Method Matrix
- Round robin study group to determine primary particle size distribution using transmission electron microscopy

New Areas of Future development:

- Graphene TR to be submitted this fall-*Joint with IEC TC113*
- *Concentration and NP Coating Metrology*
- *Nanocellulose*
- *Food specific nanometrology*



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Program of work in WQ2

PG			Status	Stage
1	TS 10797	TEM	Published	60.60
2	TS 10798	SEM	Published	60.60
3	TS 10868	UV-Vis-NIR	Published	60.60
4	TS 10867	NIR-PL	Published	60.60
5	TR 10929	MWCNT	Published	60.60
6	TS 11251	EGA-GCMS	Published	60.60
7	TS 11308	TGA	Published	60.60
8	TS 10812	Raman	CANCELLED	20.98
9	TS 11888	Shape Factor	Published	60.60
10	TS 12025	Nano object Release	Published	60.60
11	IEC 62622	Grating	Published	60.60
12	TS 13278	ICP-MS	Published	60.60
13	TS 16195	Nanoscale Test Materials	Printing Process	30.99
14	TS 17466	Q-Dots	WD	10.99
15	TR 18196	M3	WD	20.00

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nanoMark system

STRUCTURE OF NANOMARK

COMMITTEE

nanoMark Committee

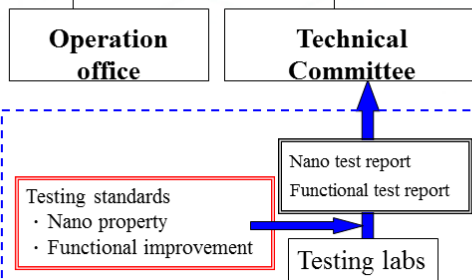
Started on Nov. 2004



**nanoMark
logo**

Meaning:

- 1.circular shape represents nanoparticles
2. ∞ implies unlimited possibilities of nanotech applications.



G.W. Peng (ITRI/Taiwan) 資料 SMAM-2(2006/05/25)

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Negative nano

Yellow Light for Nanotech Science 2004/07/29

LONDON, U.K.—Although “gray goo” made of self-replicating nanostuff is unlikely to doom the planet, some kinds of nanomaterials may indeed be hazardous and require a closer look, according to a 12-month study on nanotechnology published today by the U.K. Royal Society and the Royal Academy of Engineering. Overall, however, the report concludes that most nanotechnologies pose no new risk and no general moratorium is needed. Proceed with caution. Carbon nanotubes (left), quantum dots (inside cells, right), and nanoparticles show promise for many uses, but little is known about their impact on human health and the environment.

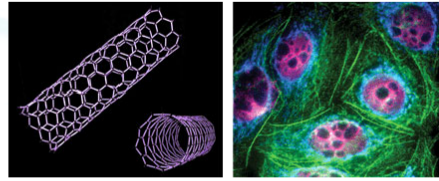
CREDIT: KELLY ET AL., *COMPUTING IN SCIENCE AND ENGINEERING* 3, 67 (2001)

Many products of nanotechnology, such as computer chips and self-cleaning windows, are no cause for new concern, says mechanical engineer

Ann Dowling, who led the study. **But two types of nanomaterial--loosely defined as stuff smaller than 100 nanometers--are worth worrying about, the panel found, because some evidence suggests that loose nanoparticles and nanotubes can readily penetrate cells and are toxic.**

For the environment, nanotechnology may be a double-edged sword. Nanoparticles hold promise in cleaning up oil spills and other environmental disasters. Yet some studies hint at possible danger to wildlife (*ScienceNOW*, 30 March). The U.K. study recommends that nanoparticles for remediation purposes “until it can be shown that the net benefits exceed the risks,” says panel chair. Carbon nanotubes and nanoparticles should treat them as if they were hazardous and minimize their release. The panel, which was commissioned by U.K. science minister David Sainsbury in July 2003, concluded that nanotechnology should be treated as new chemicals and their impacts on health and the environment be tested under existing U.K. regulations. The panel also concluded that nanotechnology should be tested under existing U.K. regulations, rather than the current philosophy in the United States, where, for instance, nanoscale particles of titanium dioxide (TiO₂) are not considered different from larger-scale particles of titanium dioxide.

A crucial first step in deciding how to regulate nanomaterials and products of nanotechnology is to define them. The U.K. study’s larger-scale counterparts, says physicist E. Clayton Teague, director of the U.S. government’s National Nanotechnology Initiative, is currently working on this, he adds.



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Nano toxicological problem

- **Unexpected adverse effects of nano-materials on human health are a serious issue that has attracted much attention.**

Cite this: *Metalomics* 2012, 4, 350-360

www.rsc.org/metalomics

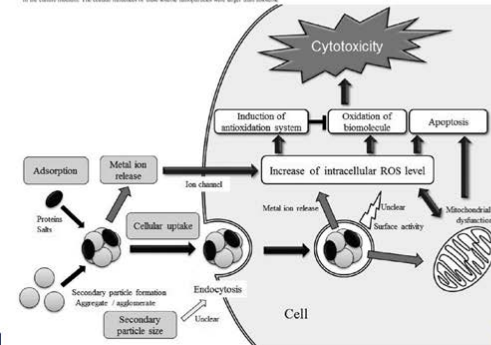
PAPER

Association of the physical and chemical properties and the cytotoxicity of metal oxide nanoparticles: metal ion release, adsorption ability and specific surface area†

Masaru Hori,^{1,2*} Kanako Fujita,¹ Haruhiko Kato,² Shigehisa Endo,¹ Keiko Nishio,¹ Lilian Kudo Kumaha,¹ Ayako Nakamura,¹ Arisa Miyazaki,¹ Shintaro Kuroguchi,¹ Yoshitaka Hayakawa,¹ Tetsu Naka,¹ Yumiko Yoshida¹ and Hiroshi Iwakura¹

Received 25th January 2012, Accepted 27th February 2012
DOI: 10.1039/c2mt00011a

Associations of cellular adhesion and physical and chemical properties were examined for 34 kinds of industrial metal oxide nanoparticles (ZnO, CuO, NiO, SnO₂, Fe₂O₃, CdO, MgO, V₂O₅, MnO, Ga₂O₃, Sb₂O₃, WO₃, Zn₂O, TiO₂, Co₂O₃, Ag₂O, Bi₂O₃, La₂O₃, Fe₃O₄ and amorphous SiO₂). We prepared a stable solution dispersion for each nanoparticle and examined the influence on cell viability and cytotoxicity were negative with physical and chemical characteristics. ZnO, CuO, NiO, MgO, and WO₃ showed a larger amount of metal ion release in the culture medium. The cellular influence of these oxide nanoparticles were larger than toxicity.



The long and short of carbon nanotube toxicity

Kostas Kostarelos

Toxicological and pharmacological studies suggest guidelines for the safe use of carbon nanotubes in medicine.

The unique physical, chemical and electronic properties of carbon nanotubes (CNTs) have generated much interest in their potential medical applications. Although most studies have assessed the pharmacological efficacy, stability and toxicity of CNTs in vitro, two recent reports, in the *Journal of Toxicological Sciences* and *Nature Nanotechnology*, explore

their carcinogenic risk in vivo. Notably, these studies reveal that CNTs delivered to the abdominal cavity of mice can induce a response resembling that associated with exposure to certain asbestos fibers. What is the significance of these findings for efforts to develop CNTs as delivery vehicles for therapeutic and diagnostic agents?

Carbon nanotubes are seamless cylindrical structures comprising single or multiple concentric graphene sheets. Applications of both single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs) have long been hampered by fears of toxicity because of their

CHAPTER 14

Toxicity Studies of Carbon Nanotubes

Jelena Kotonajic, Henri Szwarc and Fadi Masmaoui

Abstract
After decades, the potential and the growing use of CNT and their mass production have raised several questions about their safety and environmental impact. Research on the toxicity of carbon nanotubes has progressed and the data are still growing and subject to criticism. Preliminary results highlight the difficulties in evaluating the toxicity of this new and heterogeneous carbon nanomaterial family. A number of parameters including structure, size distribution and surface area, surface chemistry and surface charge, and agglomeration state as well as purity of the samples, have considerable impact on the toxicity of carbon nanotubes. However, available data clearly show that, under some conditions, nanotubes can cause the membrane lesions and suggest that even materials made the region they can induce harmful effects in inflammatory and fibrotic reactions. Therefore, more further studies on well-characterized materials are necessary to determine the safety of carbon nanotubes as well as their environmental impact.

Introduction
Along with fullerenes, carbon nanotubes (CNT) represent the third allotropic crystalline form of carbon. CNT as basically rolled sheets of graphene constituted by two and up to ten layers to half-fused hexagons (Fig. 1). Since their discovery in 1991, CNT with their unique structural, mechanical and electronic properties attracted considerable interest in many fields of materials science, including conductive and high-strength composites, energy storage and energy conversion devices, sensors, field emission displays, and radiation sources. Biological energy storage, and nanoscale-sized semiconductor devices, probes, as well as biomedical applications.¹⁻⁵

In the last decades, the potential and the growing use of CNT and their mass production have raised several questions about their safety and environmental impact. Since 2001, many researchers investigated the toxicity of CNT and derivatives. The results are highly contradictory, whereas some studies reported high toxicity, others affirm that the toxicity is low. This chapter presents a general review of toxicity studies on CNT and their derivatives. We will initially present the physical properties of these unique nanomaterials. Subsequently, this chapter will be divided in two subsections including in vitro and in vivo toxicity studies performed with pristine CNT and covalently modified or functionalized CNT (f-CNT).

Regulation of nanotechnology

2010 :

EC proposed RoHS directive of long MWCNT and silver nanoparticles

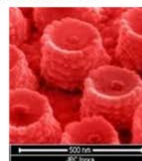
2011 :

The European Commission published the Recommendation on the Definition of Nanomaterial.

**What is a "nanomaterial"?
European Commission breaks new ground with a common definition**

— filed under: [nanotech](#), [nanotechnology](#), [nanomaterials](#), [nanosciences](#), [NANOHub](#)

Recommendation adopted by the European Commission today, based on scientific advice from the SCENIHR and the JRC



The proposed size limits for nanoparticles (1 to 100 nanometres); and the requirement that at least 50 % of the number of particles should be in this size range

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STRESA MEETING

2012.6.14-18 in Stresa, Italy (Host: JRC)
30 countries, 180 participants.

First meeting for Dr. Simon Holland as chair
JISC was assigned as CAG rotating member (2013-2014)

Draft team for labeling was organized.

Two items proposed by JISC were approved at the DTS ballot prior to the Stresa meeting. All comments were resolved and under publishing process.

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JWG1 (TERMINOLOGY)

<TOPICS>

- 4 New work item proposals.
- Systematic review for Terminology CNT will start.
- Cooperation with IUPAC for terminology PJ.
 - A Japanese expert will join the PJ.



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JWG2 (MEASUREMENT AND CHARACTERIZATION)

<TOPICS>

- Proposals from KATS, SAC are increasing
- Study Group was organized for "particle size distribution by TEM"
 - A Japanese expert joined the SG.

<ITEM from JISC>

- PG13(Test Material) was approved for the publication
 - All comments were resolved.



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WG3 (EHS)

<TOPICS>

Check LIST for Potential items were made.

"Nano-labeling" is discussed under ISO instead of CEN.

- A project meeting (WG3/PG14, PL: J-M Aubrant (AFNOR)) was held at Stresa meeting.
- A drafting team was organized. An expert from JISC joined the drafting team.

<Action from JISC>

NEDO-PJ was presented at Stresa meeting.



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WG4 (MATERIAL)

<TOPICS>

Two items proposed by SAC (CaCO_3 , TiO_2) were agreed to proceed publishing process.

- Published (14 Dec 2012)

<ITEM from JISC>

WG4/PG6 (Generic) was approved.

All comments were resolved at Stresa meeting. Revised one will be submitted to the Secretary of TC229 by early Feb.



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PUBLISHED ITEMS (2012.3-)

Reference	Document title	Stage date
ISO/TR 13014:2012	Nanotechnologies -- Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment	2012-05-08
ISO/TS 10797:2012	Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy	2012-05-29
ISO/TR 13014:2012	Nanotechnologies -- Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment -- Technical Corrigendum 1	2012-07-13
ISO/TR 11811:2012	Nanotechnologies -- Guidance on methods for nano- and microtribology measurements	2012-08-15
IEC/TS 62622:2012	Artificial gratings used in nanotechnology -- Description and measurement of dimensional quality parameters	2012-10-05
ISO/TS 12025:2012	Nanomaterials -- Quantification of nano-object release from powders by generation of aerosols	2012-10-29
ISO/TS 14101:2012	Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method	2012-11-05
ISO/TS 12901-1:2012	Nanotechnologies -- Occupational risk management applied to engineered nanomaterials - Part 1: Principles and approaches	2012-11-13
ISO/TR 13329:2012	Nanomaterials -- Preparation of material safety data sheet (MSDS)	2012-12-10
ISO/TS 11931:2012	Nanotechnologies -- Nanoscale calcium carbonate in powder form -- Characteristics and measurement	2012-12-14
ISO/TS 11937:2012	Nanotechnologies -- Nanoscale titanium dioxide in powder form -- Characteristics and measurement	2012-12-14

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SCHEDULE

- 2013, 4th March – 8th March
- Queretaro (Mexico)
- 2013, November
- XXX (Brazil) (P)
- 2014, November
- XXX meeting (India)



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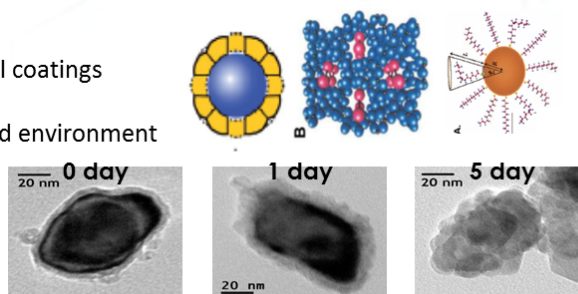
STUDY GROUP ON NANO-MATERIALS CHARACTERIZATION

OCTOBER 27, 2012 UPDATE
TC 201 Resolution No.9 (2011 Brescia)

ISO/TC 201 resolves to establish a study group on "nano-materials characterisation" with Dr. D. R. Baer as leader. Don.baer@pnnl.gov

What the big deal about nanomaterial characterization?

Complexity
Particles mostly surface
Deliberate and accidental coatings
Easily damaged
Can change with time and environment



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Increasingly recognized that NP
characterization is often inadequate

Tiny traits cause big headaches

Nanotech medicines held up by lack of particle characterization.
[Daniel Cressey](#) FROM NATURE NEWS SEPTEMBER 2010

"Characterization is the biggest challenge to this field," Simon Holland, GlaxoSmithKline

"Everybody accepts that as an academic community we haven't been characterizing enough," Kenneth Dawson, University College Dublin

<http://www.nature.com/news/2010/100914/full/467264b.html>

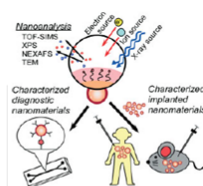
Nanoparticles designed for medical application

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DOI: 10.1002/adma.200701760

Nanobiomaterials and Nanoanalysis: Opportunities for Improving the Science to Benefit Biomedical Technologies**



By David W. Grainger* and David G. Castner

Nanomaterials advocated for biomedical applications **must exhibit well-controlled surface properties** to achieve optimum performance in complex biological or physiological fluids.

However, current literature is replete with many examples of nanophase materials, most notably nanoparticles, with **little emphasis placed on reporting rigorous surface analysis or characterization** needed to validate structure-property relationships for biomedical applications.

Advanced Materials, **2008**, **20**(5): p. 867-877

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MANY NANOPARTICLE ANALYSIS NEEDS AND CHALLENGES

What does the material look like?

- Particle/grain/film/structural unit size(s) & distribution
- Grain, particle, film morphology
- Agglomeration state/aggregation

What is the material made of?

- Bulk composition (chemical composition and structure)
- Bulk purity (including levels of impurities)
- Elemental, chemical and/or phase distribution (including surface composition and surface impurities)

How a material interacts with its surroundings?

- Surface area and surface charge
- Surface chemistry, including reactivity, hydrophobicity

Overarching considerations:

- Stability
- Environmental effects
- Functional behaviours.

Directly linked to TC201 techniques
Less direct link to TC201 techniques

<http://www.characterizationmatters.org> and

Boverhof, D.R. and R.M. David, Analytical and Bioanalytical Chemistry,

2010, **396**(3): p. 953-961, 144

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Activities initiated by email request for participation and identification of important topics

Dear Nanotechnology Researchers and ISO TC201 Participants,

ISO Committee TC201 on Surface Chemical Analysis has initiated a **study group** on nanomaterials characterization with a focus on methods within the purview of the committee. This activity follows a **technical report ISO 14187:2011** – surface chemical analysis – characterization of nanomaterials (see summary in Surface and Interface Analysis 44 (2012) 1305–1308). The technical report was prepared by TC201 with input and cooperation from ISO TC229 on Nanotechnology. The purpose of the study group is to assess the needs and opportunities for standards or guides related to nanomaterials from the **perspective of TC201 with the important input and coordination with ISO TC229**.

I am writing to request your input into this study group in two ways:

- 1) **Are you willing to participate in this group or do you have recommendations for other participants.**
- 2) **Suggest topics that you consider important and for which the time is appropriate for a standard or guide.**

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Email continued - Identifying topics

There are a number of groups interested in the characterization of nanoparticles and other nanomaterials. Based on some of these interactions a list of **possible topics for a standard or guides** is listed below.

Please indicate if you think any of these are particularly relevant or important or suggest other essential topics that we should consider:

- Guide for **preparing nanoparticles for surface chemical analysis**.
- Standard for **reporting the history and procedures** used to prepare nanoparticles for surface chemical analysis.
- Standard practice for determining **thickness of coating** on nanoparticles using XPS.
- **Other possibilities** for SIMS, SPM, or other methods?

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Interested Study Group Participants – 26 from 7 countries

Vikram Kestens	Belgium- JRC-GEEL
Gert Roebben	Belgium-JRC-GEEL
Wolfgang Unger	Germany-BAM
Thorsten Dziomba	Germany-Physikalisch-Technische Bundesanstalt
G. Sundararajan	India-ARCI
Ajay Karakoti	India-Battelle-India
Satya Kuchibhatla	India-Battelle-India
B. S. Murty	India-IITM
BLV Prasad	India-National Chemical Laboratory
Daisuke Fujita	Japan-NIMS
Toshiyuki Fujimoto	Japan-NMIJ/AIT
Alex Shard	UK-NPL
Charles Clifford	UK-NPL
Frederick Klaessig	USA- PA bio-nano systems
Sudipta Seal	USA- University of Central Florida
Heather Banko	USA-ANSI
Vincent Smetkowski	USA-GE
Marina Dobrovolskia	USA-NCI
Angela Hight Walker	USA-NIST
Cedric Powell	USA-NIST
Don Baer	USA-PNNL
Galya Orr	USA-PNNL
Marvin Warner	USA-PNNL
Amy Walker	USA-Univ Texas- Dallas
James P. Hamilton	USA-Univ Wisc Platteville

Expressed interest
from TC 201,
TC229 and other
sources

More participants
welcome:
Don.baer@pnnl.gov

Will be formally
added to study group
by request by national
bodies to ISO.

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General Comments

“This is a **critical issue** for many technologies, and it seems to me that we need best practice guides and other guidelines for their analysis. I have worked with several companies in the last year, who have been struggling with characterization issues for nanomaterials. **Your suggested topics are absolutely critical.**”

“There is a cleft in our thinking between particulate materials that may be nanoscale and macroscopic surfaces that may have a nanoscale layer (film, coating, membrane, layer). Lost in this discussion is the fact that **particulate matter has a surface** that must be different than the substrate material **and how best to describe that surface chemistry.**”

“I wish I had time to participate on this effort. I am buried with other substantial advisory and committee work to a level that I cannot responsibly participate here. Never the less I have some suggestions “

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SUGGESTED AREAS WHERE STANDARDS OR GUIDES ARE NEEDED:

GENERAL ISSUES

I hear many voices from industrial people about the necessity of the guidance for determination of the material representative values from the measurements. So the preparation should not be only for the single measurement but also include a statistical consideration.

I believe there are several other critical issues that also need to be addressed. Broadly these are: 1) quantification, 2) data handling and analysis including kinetics, 3) appropriate methods/combinations for multi-technique analysis.

In my opinion, standards or guidance for the preparation of nanoparticles for surface analysis is the most urgent item.

Develop Standards for all those handling nanoparticles for investigating their interactions and impact in/on the environment and living systems.

Guide for preparing nanoparticles for surface chemical analysis
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SUGGESTED AREAS WHERE STANDARDS OR GUIDES ARE NEEDED:

COATINGS AND INTERFACES

- Standard practice for determining thickness of coating on nanoparticles using XPS
- Particularly interested in thickness determination of coatings on NPs.
- We should be certain that it includes the characterization of organic coatings, inorganic coatings, as well as oxides.
- It should also include techniques such as AES and APT
- How to define an interface when nanoparticles are in a matrix or when nanoparticles are coated

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SUGGESTED AREAS WHERE STANDARDS OR GUIDES ARE NEEDED: SPM **SIZE AND SIZE DISTRIBUTIONS**

- My suggestion, standard practice for determining dimensions and morphology of nanoparticles using SPM.
- It may be possible to initiate standardisation of SPM for nanoparticle size characterisation, for Example with prenormative work in VAMAS.
- Development of dependable procedures that lead to a “known” size distribution.
- When talking about size of a nanoparticle - how appropriate is it to use just one dimension - as often they
- What is (are) the essential technique(s) (Or their combinations) that should be used to report the size of nanoparticles are not necessarily spherical
- Shape dependency is another gray area

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Suggested areas where Standards or Guides are needed: **Other Topics**

- **Standard materials approvals** and availabilities for comparative studies
- My suggestion is more on the **colloidal chemistry** side, which means wettability (contact angles) and functionality (ability to undergo further reactions). **Dispersal** in the environment and disposition within the body will be a balance of hydrophilicity and surface reactivity of particulate matter.
- **Develop endotoxin (LPS) testing** of nanomaterials - this is a problem since the current LPS assay methods are colorimetric and we see anomalous results with current materials - something we are trying to write up and that the National Nanocharacterization lab (NCL) should be concerned with as well.

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- Because of importance and wide area of interest explore routes for **additional input/more participants**
- Register participants to study group
- **Circulate current feedback** to participants and request additional input and establish highest priorities
- **Communicate** with various groups with common interests (Technical advisory groups, OECD related meeting,)
- Discuss/**coordinate** needs and plans with ISO **TC229** through TC201 Liaison Officer Dr. Charles Clifford and TC 229 JWG2 Chairman Dr. Toshiyuki Fujimoto. Dr. Clifford will give a presentation at TC 229 next March
- Explore options for development of a PNWI related to **reporting sample history and handling and/or preparation of samples for surface analysis** (SC2 resolution)

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◆ Nanoparticle nomenclature (WG1)

■ size (other TC?)

▲ chemical purity (WG2)

× size distribution (Other TC?)

✱ degree of agglomeration /aggregation (WG2)

● shape (other TC?)

+ specific area (WG2)

- chemical structure (WG2)

= chemical functionality (WG2)

◊ core composition (WG2)

■ sampling methods for characterization (WG2)

▲ porosity (WG2)

× physical properties - electrical (3-8 years) (WG2/IEC?)

✱ strength of agglomerate/aggregates (3-8 years) (WG2)

● Specifications - metallic nano-particles: Ag, Al, Au, Co, Cu, Ni, Pd, Pt, Ru, Zn + Shape Memory and Magnetostrictive

+ Specifications - metal oxides: Al₂O₃, CeO₂, CuO, Fe₃O₄, ITO, NiO, SiO₂, TiO₂, WO₃, ZnO

- dispersability (3-8 years) (WG2)

= Specifications - other compound particles: AgCu, BaTiO₃, CuNi,MoS₂, WS₂, WSe₂ + "Polymers" and "Organic"

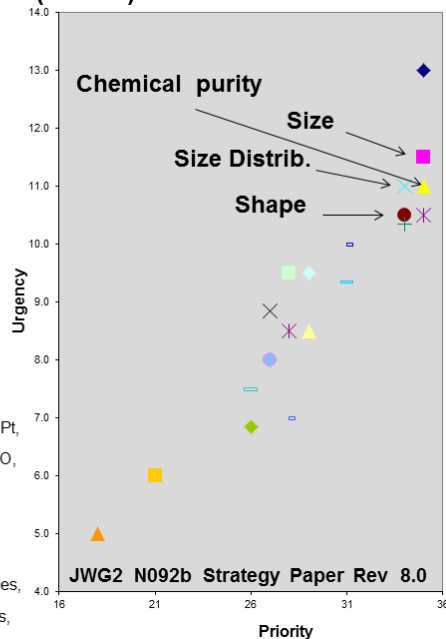
◊ degree of functionalization (3-8 years) (WG2)

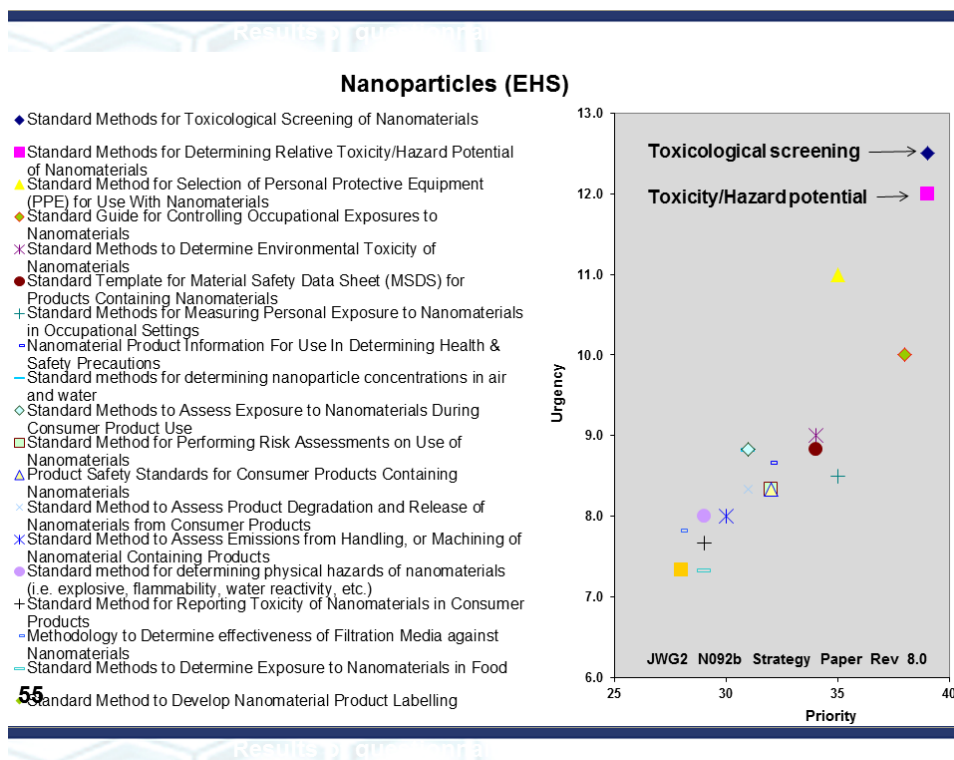
■ Specifications - functionalized particles: Au, Functionalized Fe oxides,

54 ■ Specifications - quantum dots (3-8 years): CdS, CdSe, CdTe, GaAs,

GaN, InAs, InP, Si based

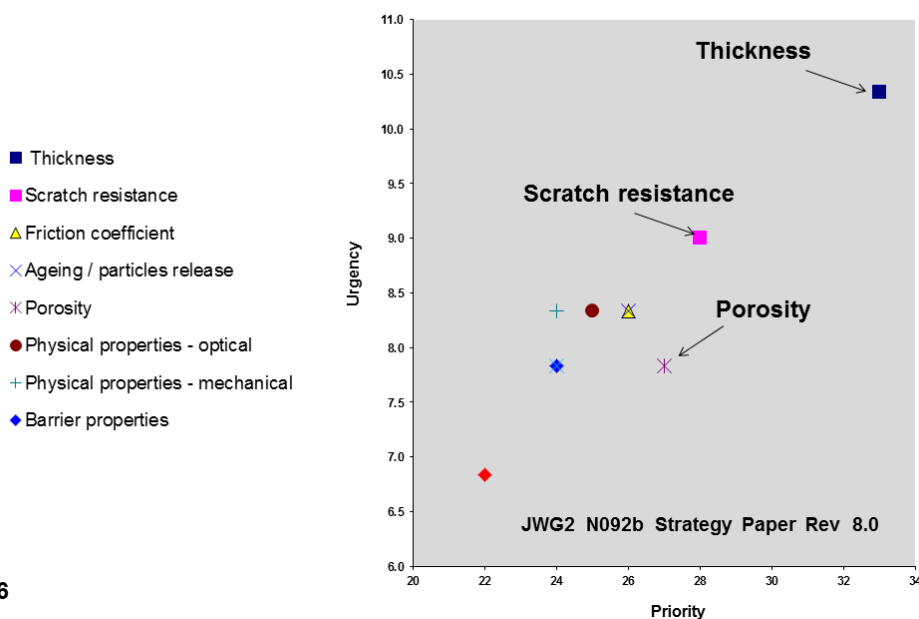
Nanoparticles (M & C)



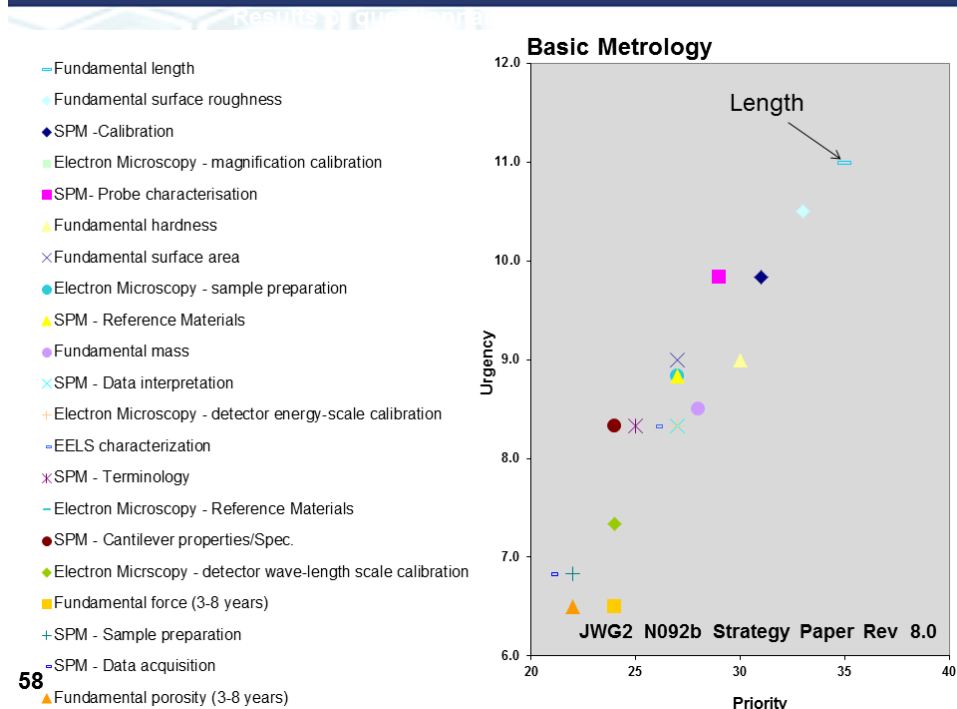
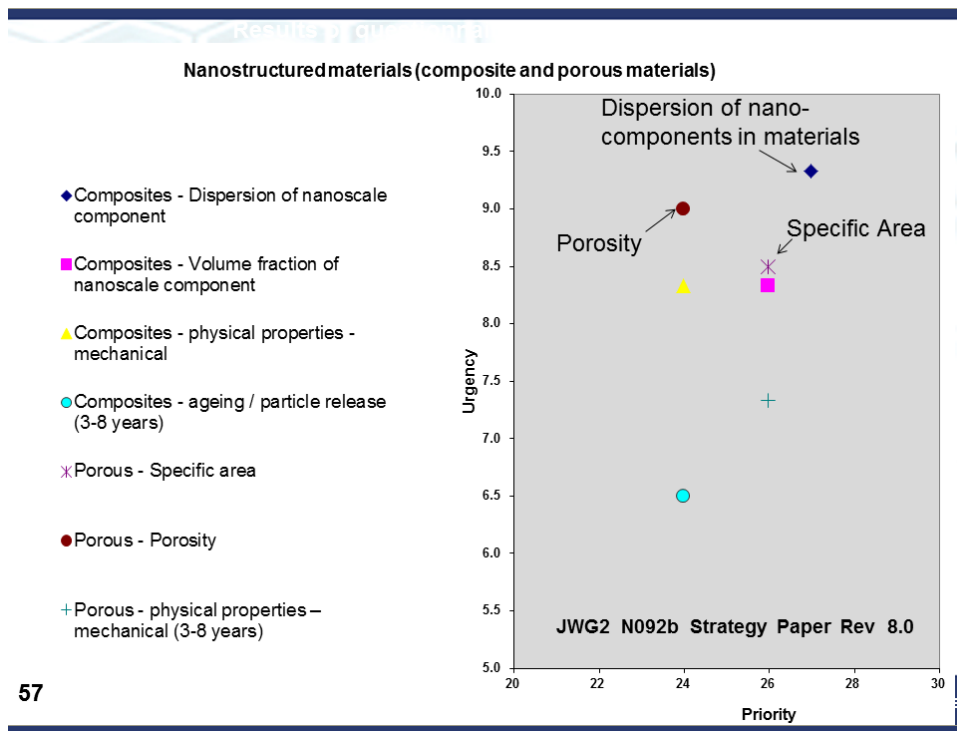


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Nanoscale Coatings



56

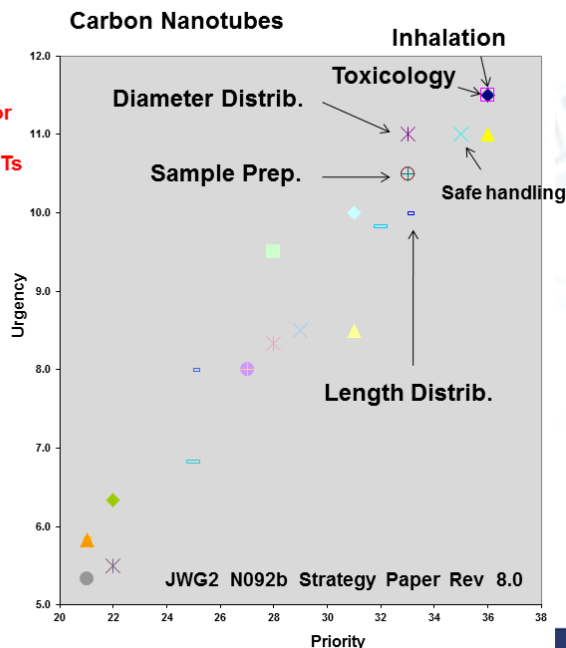


Most important nanotechnology breakthroughs since 2000

Highly efficient synthesis methods for carbon nanotubes initiated a brake through in the industrial usage of CNTs

- ◆ Inhalation testing (WG3)
- Toxicology testing (WG3)
- ▲ Exposure determination – ambient air (WG3)
- × Safe handling (WG3)
- ✱ Diameter distribution (WG2)
- Sampling methods for characterization (WG2)
- + Exposure determination – water (WG3)
- Length distribution (WG2)
- = Nomenclature (WG1)
- ◊ Safe disposal including destruction (WG3)
- Chemical structure (WG2)
- ▲ Eco-toxicology testing (3-8 years) (WG3)
- × Determination of nature and concentration of contaminants (WG2)
- ✱ Batch quality (WG2)

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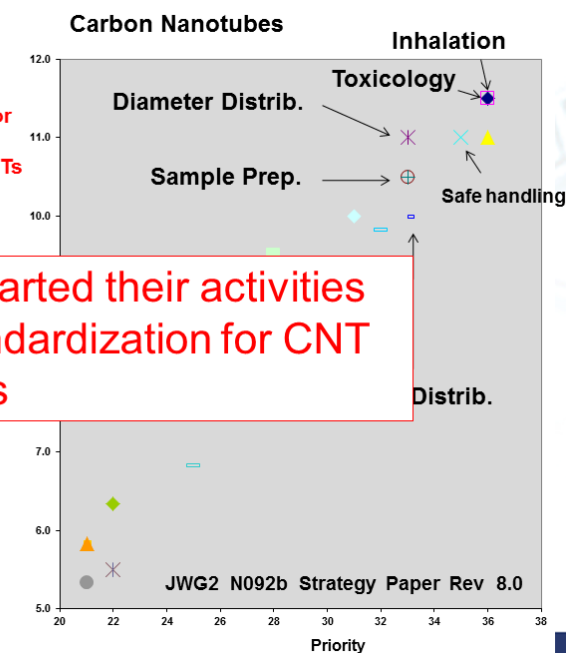


Most important nanotechnology breakthroughs since 2000

Highly efficient synthesis methods for carbon nanotubes initiated a brake through in the industrial usage of CNTs

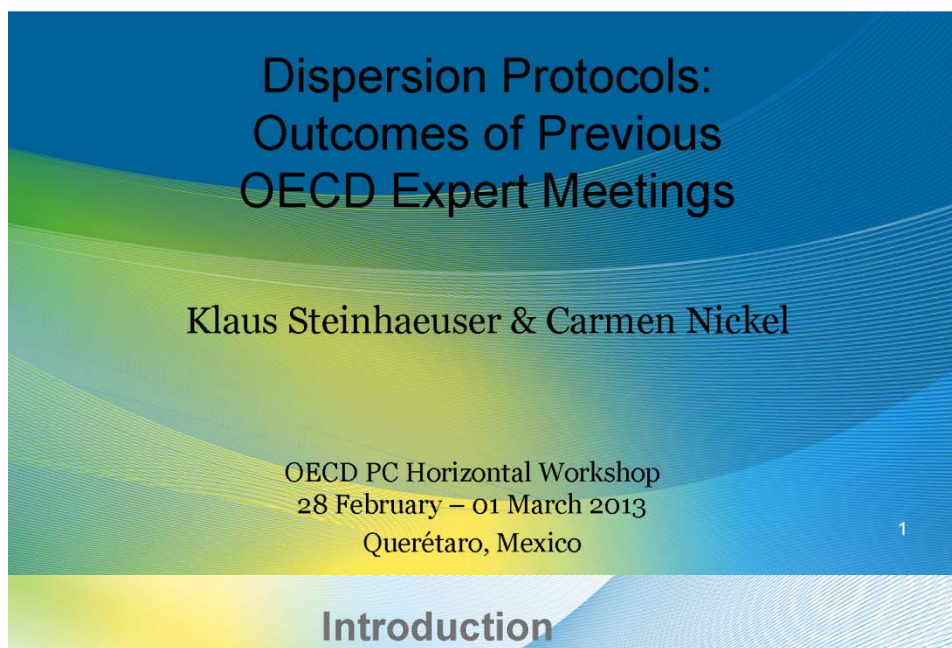
- ◆ Inhalation testing (WG3)
- Toxicology testing (WG3)
- ▲ Exposure determination – ambient air (WG3)
- × Safe handling (WG3)
- ✱ Diameter distribution (WG2)
- Sampling methods for characterization (WG2)
- + Exposure determination – water (WG3)
- Length distribution (WG2)
- = Nomenclature (WG1)
- ◊ Safe disposal including destruction (WG3)
- Chemical structure (WG2)
- ▲ Eco-toxicology testing (3-8 years) (WG3)
- × Determination of nature and concentration of contaminants (WG2)
- ✱ Batch quality (WG2)

60

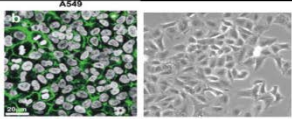
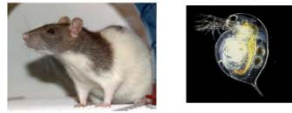


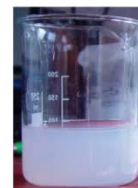
JWG2 started their activities with standardization for CNT materials

Klaus Steinhaeuser - Dispersion Protocols: Outcomes from Previous OECD Expert Meetings



Introduction

in vitro	
in vivo	
exposure (mobility availability, etc.)	



- Dispersion harmonisation necessary?
- Dispersion preparation → Properties → Effects
- Dispersion harmonisation possible?
- Comparable characterisation of used dispersions



OECD WPMN and its Sponsorship Program

OECD WPMN launched an experimental program with 13 representative manufactured nanomaterials (Sponsorship program) – Guidance: ENV/JM/MONO(2009)20/REV

16 PC parameters included:

- **Agglomeration/aggregation** - Water Solubility/
Dispersibility - Crystalline phase - Dustiness - Crystallite size - Representative Electron Microscopy (TEM) picture - Particle size distribution (dry and in relevant media) - Specific surface area - Zeta potential (surface charge) - Surface chemistry, where appropriate - Photocatalytic activity - Pour density – Porosity – Octanol-water partition coefficient - Redox potential - Radical formation potential

Program nearly completed: Evaluation and Integration Phase



OECD WPMN – Horizontal Workshops

– Horizontal workshops

- Inhalation Toxicity (19-20 October 2011 in The Hague, Netherlands).
- Environmental Fate and Eco-toxicity (29-31 January 2013 in Berlin, Germany).
- **Physical-chemical properties (28 Feb-1 March 2013 in Querétaro, Mexico).**
- Genotoxicity [September 2013, in Ottawa, Canada].
- Grouping of Nanomaterials [April 2014, in Washington D.C., USA].
- Toxicokinetics and Mechanistic Issues [early 2014 in Korea].



Overview of the GSPD [ENV/JM/MONO(2012)40]

Guidance for Sample Preparation and Dosimetry

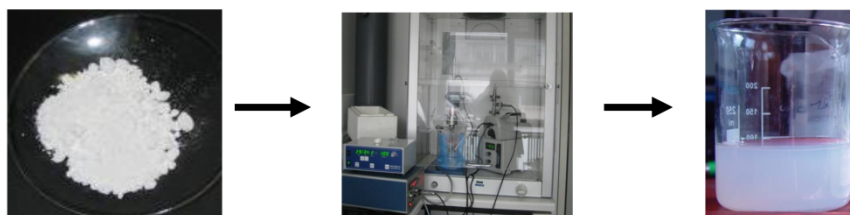
- A companion Guidance document to the OECD Test Guidelines, developed with consideration of the chemical and physical characteristics of nanomaterials
- Evolution from Sponsorship Program to the present
- Addresses all physical-chemical properties, effects, and fate endpoints
- A living document



GSPD: Stock dispersion preparation

ENV/JM/MONO(2012)40

- Supplementary guidance to OECD TGs on nano-specific test concerns
- Reproducible, representative and reliable preparation of dispersions
- Preparation of NM (stock) dispersion as fundamental step
 - Homogeneous distribution
 - Environmental like conditions
 - Characterisation of the starting conditions
- Standard operation procedure (NIST, PROSPECT, NANOGENOTOX, NANOMMUNE, ENPRA, MARINA, ...)



GSPD: Stock dispersion preparation

Stable dispersion (stability requirements based on e.g. agglomeration, zeta potential, time frame)

- Mixing
- Stirring
- Sonication
 - Waterbath
 - Probe
 - Cup horn
- Use of solvents or/and stabilising agents (e.g. Tween, NOM, PDMS,...)



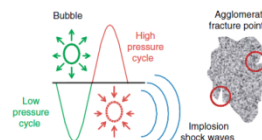
- Preparation steps depend on the specific material
- Preparation can affect the behaviour by e.g. structure defects, agglomeration, change of the functional groups



GSPD: Stock dispersion preparation

Preparation of a stable dispersion (stability requirements based on the following tests – e.g. agglomeration, zeta potential, time frame)

- If sonication has to be used for suspension preparation, the applied energy has to be determined
- Alteration of stock solutions by sonication:
Sound waves → shock waves / $T \geq 10.000^\circ \text{K}$
→ Undesirable alterations to nanomaterials:
Re-agglomeration, sintering of nanomaterials, scission of nanomaterials such as CNTs changes in surface chemistry due to water-generated reactive oxygen species, degradation of other media components.
- Test media quality (pH, ionic strength, NOM concentration) should be harmonised as much as possible between comparative studies



Dispersion characterisation

Parameters to characterise the stock dispersion based on the available instruments and chosen endpoints

- **Zeta potential**
- **Agglomeration**
- Particle size distribution
- Mass concentration
- Surface area
- Others e.g. length, morphology, surface functionality

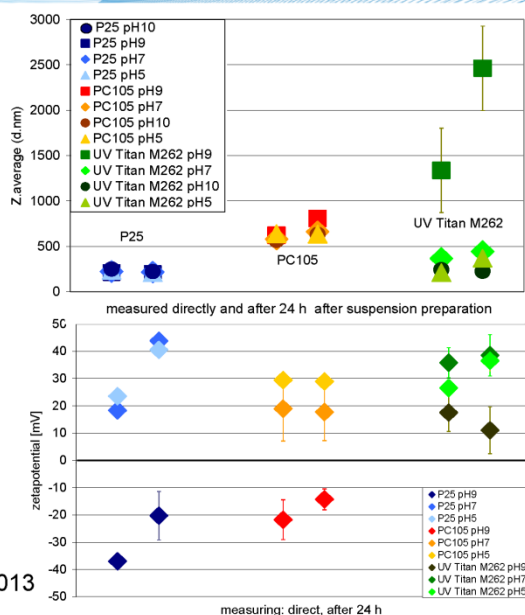
→ **Further Development of GSPD regarding measurement and data interpretation**



Dispersion characterisation - stability

Characterisation of the stability of the dispersion and its dependency on

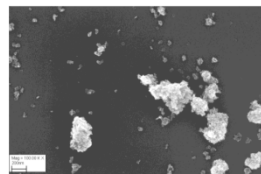
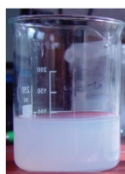
- Agglomeration
- Zeta potential

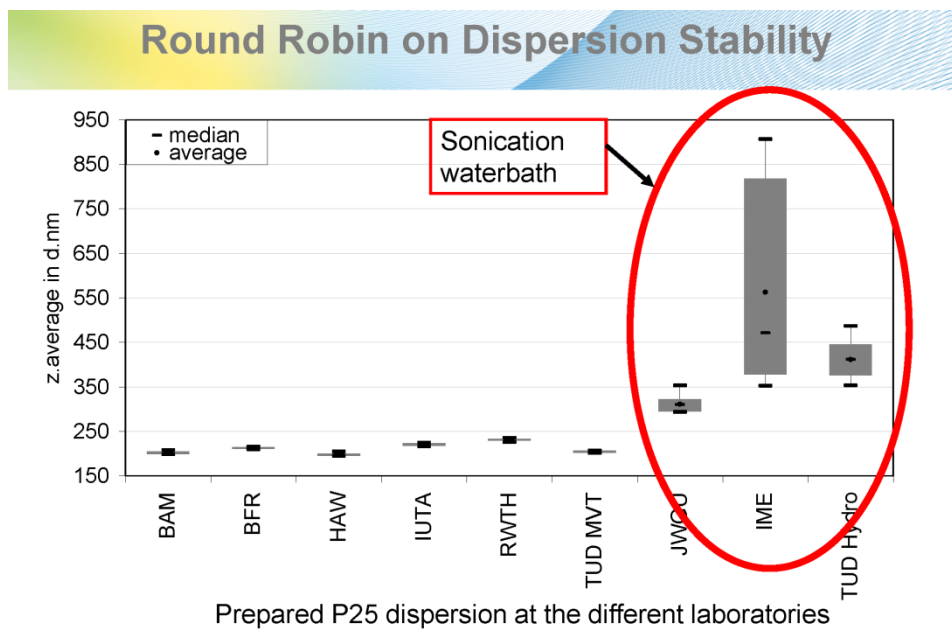


Round Robin on Dispersion Stability

Round Robin Test with 10 Different Laboratories

- Preparation according established SOP.
- Size distribution of the prepared dispersion using DLS
- Use of ultra pure water (HPLC-grade), same batch of the TiO_2 nanomaterials and other chemicals.
- Tests included:
 1. stable polystyrene dispersion
 2. stabilised TiO_2 – P25 dispersion with 100 mg/L were prepared at the IUTA and sent to all laboratories
 3. distributed TiO_2 – P25 powder was used for the preparation of a dispersion at each laboratory following the established SOP





Conclusions:

- No stable dispersion by sonication using a waterbath in this study with the used basic conditions
- If comparable equipment is used and the dispersion preparation follows a defined and appropriate SOP comparable and reproducible results can be achieved between different laboratories



Parameters for stock dispersions

Other parameters:

Pre-wetting with 0.5 vol% ethanol (or DI water)

- No pre-wetting or
- Pre-wetting of coated (hydrophobic) NMs or
- Pre-wetting of all NMs

Stock concentration

- Critical Coagulation Concentration (CCC)
- CCC dependent on the NM type and media conditions



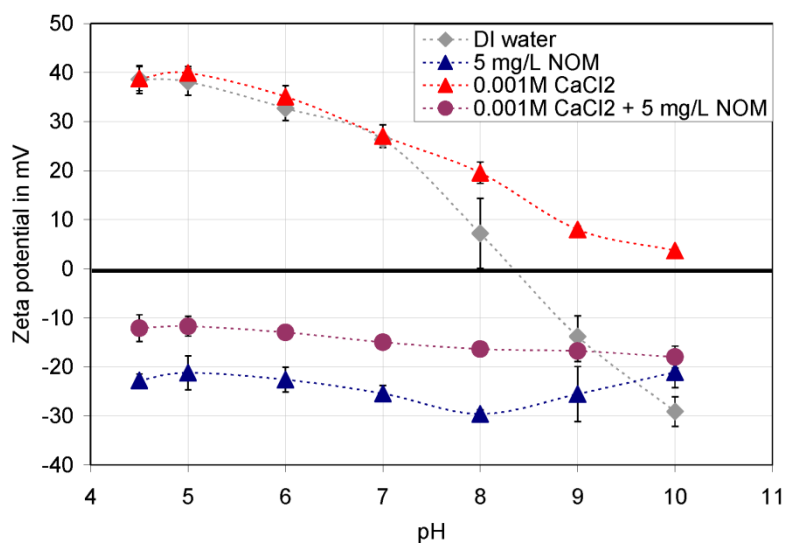
Preparation of Dispersions for Environmental Testing

Tiered approach

- Tier I: Preparation of stock dispersion
- Tier II: Preparation of Exposure dispersion
 - Material properties will likely change on addition to more complex exposure media (salts, buffer components, nutrients..)
 - Homogeneous exposure over longer time periods (Is more than 20% loss acceptable?)
 - Tests in more than one media, variation of different parameters (pH, IS, NOM content, NOM speciation [type of humic / fulvic acids, polysaccharides], hardness)
- Tier III: Conducting the test
 - Semistatic exposure preferred against flow-through

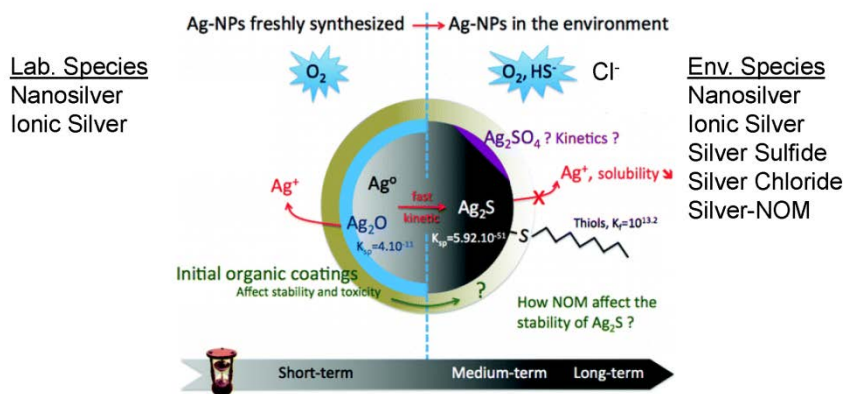


Preparation of Dispersions for Environmental Testing



Aging of Nanomaterials - Environmental Tests

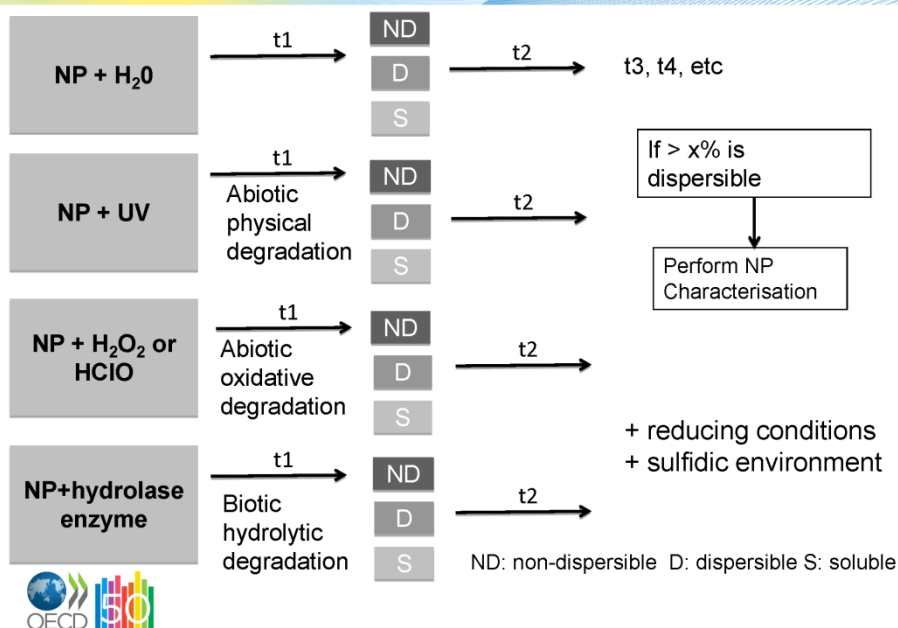
Dissolution Kinetics of Nanosilver



[Levard, et al. 2012. ES&T](#)



Aging of Nanomaterials - Environmental Tests



Application - Environmental Tests

Application of NM should be chosen in consideration of the relevant entry pathway

- Dry spiking
- Wet spiking
 - Spiking of water
 - Spiking of soil / sediment
 - Spiking of food

At Berlin Workshop:

- ➔ Application / Spiking: wet spiking should be favoured
- ➔ Dry spiking can be necessary for coated NM and should not be excluded completely
- ➔ Spiking of Food (TG 305)



Metrics - Environmental Tests

- Characterisation of the NM in the test system necessary
 - The "right" metric? Dependent on the question?
 - At this juncture mass should be used, but - if possible - other metrics have to be measured
 - Mass may be not sensitive enough to be linked with nano-related mode of actions
 - Other examples of relevant metrics (surface area, particle size distribution, surface reactivity, ion concentration)
 - Problem if soluble materials were tested
- At Berlin Workshop mass was preferred but other metrics should be additionally considered, where appropriate.
Determine concentration of dissolved metal ions (e.g. Ag, Zn)
- Clear prioritisation of important metrics should be made in OECD



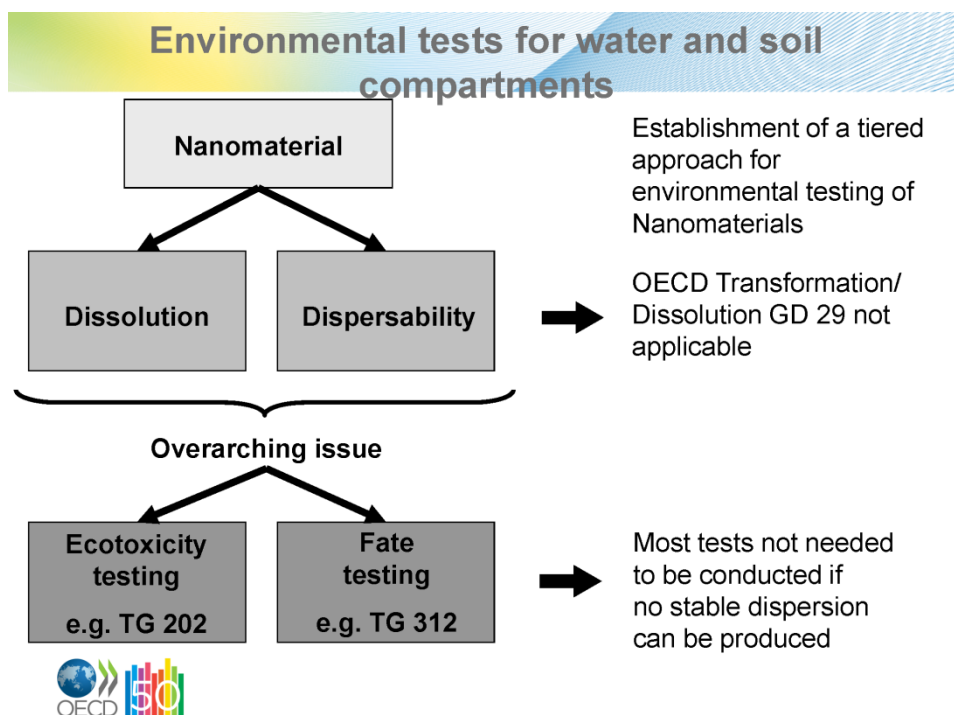
Environmental tests for water and soil compartments

→ Tiered approach/decision tree for fate (and ecotoxicity) tests

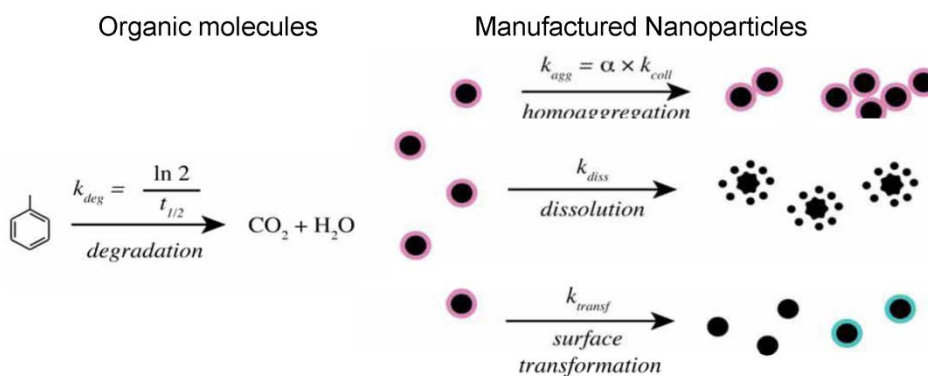
- | | |
|-----------------------|---|
| 1 st step: | Tests for dissolution and dispersability
(in different media - pH, NOM etc) |
| 2 nd step: | Detect dispersion stability (aggregation rate,...)
(in different media) |
| 3 rd step: | Tests biodegradation based on oxidation of organic carbon (only applicable in few cases - other methods needed to detect biodegradation of organic coating) |
| 4 th step: | Tests abiotic degradation by hydrolysis TG applicable;
uncertainty regarding photodegradation |

→ Guidance should be developed



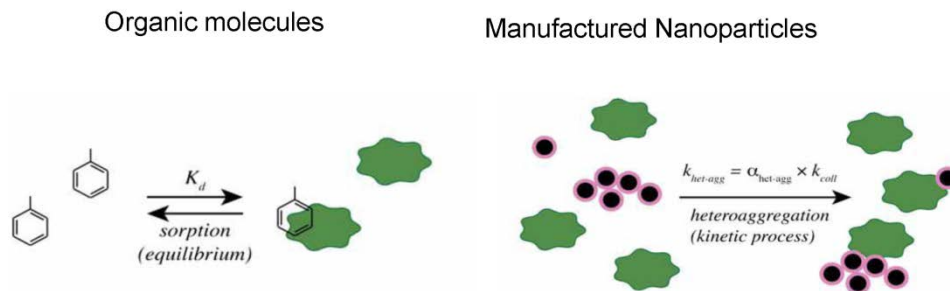


Processes: Transformation & Degradation



A. Praetorius et al., *Environmental Science & Technology* **46**, 2012, 6705–6713

Processes: Interaction with Suspended Particulate Matter (SPM)



A. Praetorius et al., *Environmental Science & Technology* **46**, 2012, 6705–6713



Modelling environmental fate of NM in aquatic compartment

Mass-Balance Model

Parameters required:

- rate constants for dissolution, transformation
- rate constants for homo- and heteroaggregation
- attachment efficiencies for homo- and heteroaggregation



Dispersions in Toxicology Testing

- In body fluids higher salinity. NM are often coated by proteins: protein corona → addition of proteins to test dispersions (BSA, FBS...) for *in vitro* tests
- Preparation of aerosols for inhalation toxicity tests:
 - OECD Workshop on Inhalation Toxicity 2011:
 - Aerolization by venturi and further deagglomeration by a jet mill
 - *ISO 10801: Generation of metal nanoparticles for inhalation testing using the evaporation condensation method*
 - Necessary desaggregation depends on exposure scenario (at production workplaces monodisperse NM may be required in contrast to uses of NM as powders where smaller aggregates are acceptable.
 - BALF (bronchoalveolar lavage fluid) analyses should be mandatory requirements in OECD inhalation TGs



Direct and indirect needs for Harmonization

Determination of dispersion stability and characteristics not only important for reproducible sample preparation for environmental tests! (Indirect needs)

- Dispersion stability TG necessary for determination of fate in risk assessment.
- Importance of dispersion characteristics for reliable grouping / read across

Identified „high level categories“ by SG 6 (February 2013)

- Physical chemistry - use of information about physical-chemical properties in risk assessment
- Environmental Fate and Effects – how to use physical-chemical properties to conduct and inform on environmental fate and effects endpoints



OECD Context

- The principle of **Mutual Acceptance of Data Agreement (MAD)** is the basis for internationally harmonised approaches for testing of chemicals
- The **OECD Test Guideline Program (TG)** is the **implementing tool of MAD** and should:
 - be scientifically sound and practical
 - contain appropriate balance between flexibility and harmonisation
- The applicability, reliability and relevance of TGs for testing and assessment of nanomaterials (as for any other chemicals) must be ensured.



Thank you for your attention

Thanks for support:

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