National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Introduction

OECD Sponsorship Programme for the Testing of Manufactured Nanomaterials:

need for adequate and complete characterisation of NMs

to enable a further evaluation of their (toxicological) properties

Aim of the evaluation:

Evaluate **whether a specific method** applied to determine a certain physico-chemical property **is suitable for the specific property**

- for a specific nanomaterial
- for a (broad) range of different nanomaterials

Process of method evaluation

- Methods from dossiers of the Testing Programme (“May 2015”)
- Nominated experts from CA, EC, NL, US, JP, BIAC
- Evaluations of **test methods for physico-chemical properties**
  - Web-based questionnaire >> semi-structured answers
  - For each parameter each applied method evaluated at least once; extrapolation to other NM(s) where possible
  - Opinion of one or a few individual expert(s) >>
    Not necessarily represents consensus

- Final document scrutinised by experts from CA, DE, EC, NL, BIAC

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Thanks to all experts and reviewers!

Parameter	MWCNT	SWCNT	Ag	SiO_2	CeO_2	ZnO	TiO_2	Fullerenes	Dendrimers	Gold	Nanoclay
Chemical composition											
Aggregation/agglomeration											
Particle size distribution											
Crystalline phase											
Dustiness											
Specific surface area											
Water solubility/Dispersibility											
Zeta potential											
Photocatalytic activity											
Porosity											
Redox potential											
Radical formation potential											
Crystallite size											
Surface chemistry											
Pour density											
K_{ow}											

Summary of evaluation

Parameter	Broad range of NMs	Certain NMs only	Not suitable
Chemical composition	XPS	ICP/OES; EDX	
Aggregation / Agglomeration	AFM	TEM; SEM; DLS	Turbidity
Particle size distribution	CLS; TEM; SEM	DLS(+DOSY-NMR); DMA	Laser diffraction
Crystalline phase	XRD		Raman; TEM; SEM
Dustiness	(small) rotating drum	Continuous drop tester	Vortex shaker
Specific surface area		BET	SAXS
Water solubility / Dispersibility	Shake flask method	Spectrometry; filtr.+spectr.	
Zeta potential	ELS	Laser-Doppler electrophoresis	
Photocatalytic activity		Rhod.-B; DPPH; Hydroxyl generation; Orange II degr.	Degradation of aldehyde
Porosity	BET; BJH	Mercury porosimetry	
Redox potential			Potentiometry; Oxo-Dish-O_2
Radical formation potential	EPR / ESR	KI & optical absorbance	Benzoic acid PBS
Crystallite size	XRD		
Surface chemistry		XPS	EDX; Liquid chromatography

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Crystallite size	XRD		
Surface chemistry		XPS	EDX; Liquid chromatography

NO suitable method for Redox Potential

Conclusions

- Most parameters: **one or more suitable methods** available
  - some methods only applicable **subset of nanomaterials**
  - some methods only applicable **under certain conditions**

- Most methods **not standardised (yet)** (for nanomaterials)
  - sample preparation

- The **reporting** of methodology needs improvement
  - e.g. sample preparation, test conditions

Recommendations (1/2)

Chemical composition

- Energy dispersing X-ray analysis (EDX)
- Inductively coupled plasma/optical emission spectrometry (ICP/OES) (ICP/MS?)

Aggregation / agglomeration

- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)

Particle size distribution

- Dynamic Light Scattering (DLS)
- Centrifugal Liquid Sedimentation (CLS)
- Differential Mobility Analysis (DMA)
- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)

Recommendations (1/2)

Chemical composition

- Energy dispersing X-ray analysis (EDX)
- Inductively coupled plasma/optical emission spectrometry (ICP/OES) (ICP/MS?)

CEN develops

Aggregation / agglomeration

- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)

CEN & ISO develop

Particle size distribution

- Dynamic Light Scattering (DLS)
- Centrifugal Liquid Sedimentation (CLS)

ISO method available

- Differential Mobility Analysis (DMA)
- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)

Recommendations (2/2)

Crystalline phase

- X-ray diffraction (XRD)

Dustiness

- Small rotating drum
- (continuous drop tester)

Radical formation potential

- Electron paramagnetic resonance / electron spin resonance

Crystallite size

- X-ray diffraction (XRD)

Recommendations (2/2)

Crystalline phase

- X-ray diffraction (XRD)

Dustiness

- Small rotating drum
- (continuous drop tester)

Radical formation potential

- Electron paramagnetic resonance / electron spin resonance

ISO develops

Crystallite size

- X-ray diffraction (XRD)

Recommendations

- Sample preparation protocols
- Reference nanomaterials
- Standardised media
  - biologically relevant test media

Further details:

"EVALUATION OF METHODS APPLIED IN THE OECD-WPMN TESTING PROGRAMME – 1: METHODS FOR PHYSICO-CHEMICAL PROPERTIES"

Results: chemical composition

Broad range of NMs	Certain NMs only	Not suitable
▪ XPS	▪ ICP/OES ▪ EDX	

- Often very limited information on method used
- ICP/OES
  - generally accepted method
  - NOT all elements
- EDX
  - elements above carbon
  - NOT for complex composition / matrices, and large aggregates
- XPS
  - suitable method for determining chemical composition
  - NOT for coated materials

Results: Aggregation / Agglomeration

Broad range of NMs	Certain NMs only	Not suitable
▪ AFM	▪ TEM ▪ SEM ▪ DLS	▪ Turbidity

- AFM
  - In solution
- TEM
  - Vacuum has influence; only 2D picture
- SEM
  - Similar as TEM; easier sample prep
  - NOT <10 nm
- DLS
  - Issue: particles vs. aggregates/agglomerates
  - Useful when maximum AND polydispersity index are given
- Turbidity
  - Only qualitative

Results: Particle size distribution

Broad range of NMs	Certain NMs only	Not suitable
• CLS • TEM • SEM	• DLS • DLS + DOSY NMR • DMA	• Laser Diffraction

- CLS
  - Mass based: errors in calibration and shape/size estimates
- TEM
  - Limited to primary particle size; only 2D picture
- SEM
  - Similar as TEM; easier sample prep; more accurate ~100 nm
- DLS
  - Issue: particles vs. aggregates/agglomerates; DOSY NMR only g/L
- DMA
  - Aerosols and suspensions; less issues with aggregates/agglomerates
- Laser diffraction
  - Only larger primary particles (> 50 nm)

Results: Crystalline phase

Broad range of NMs	Certain NMs only	Not suitable
• XRD		• Raman Spectral analysis • TEM • SEM

- XRD
  - A generally accepted and suitable method
- Raman Spectral analysis
  - May be suitable, but insufficient information for evaluation
- TEM
  - May be suitable, but insufficient information for evaluation
- SEM
  - May be suitable, but insufficient information for evaluation

Results: Dustiness

Broad range of NMs	Certain NMs only	Not suitable
• Rotating drum • Small rotating drum	• Continuous drop tester	• Vortex shaker

- rotating drum
  - Standardised method (EN-15051:2006)
- continuous drop tester
  - Standardized under EN-15051:2006
  - less suitable if caking or fluffy powders
- vortex shaker
  - Not representative for health-relevant dustiness

Results: Specific surface area

Broad range of NMs	Certain NMs only	Not suitable
	• BET	• SAXS

- BET
  - Standardised under ISO 9277:2010
  - Suitable unless gas is absorbed
  - For microporous solids specific adaptations are needed
- SAXS
  - Not recommended as a primary method

Results: Water solubility and Dispersibility*

Broad range of NMs	Certain NMs only	Not suitable
• Shake flask method	▪ Spectrometry ▪ Filtration and centrifugation	

- ▪ Shake flask
  - • Needs further evaluation
- ▪ Spectrometry
  - • Suitable for SiO_2, needs further evaluation
- ▪ Filtration & centrifugation
  - • Applicable to soluble nanomaterials
  - • Suitability for materials embedded in matrix?

* Strictly speaking water solubility and dispersibility are different parameters, but in practice difficult to distinguish.

Results: Zeta potential

Broad range of NMs	Certain NMs only	Not suitable
• ELS	▪ Laser Doppler Electrophoresis	

- ELS
  - Standardized under ISO 13099-2:2012
  - Suitable if dispersible in liquid
- Laser Doppler Electrophoresis
  - NOT for hydrophobic nanomaterials
  - NOT in high conductivity media

Results: Photocatalytic activity

Broad range of NMs	Certain NMs only	Not suitable
	▪ Rhodamine-B ▪ DPPH ▪ Hydroxyl generation under UV-light + EPR ▪ Orange II degradation + UV-Vis	▪ Degradation of acetaldehyde

- ▪ Rhodamine-B
  - NOT for coloured suspensions
- ▪ DPPH
  - NOT for coloured suspensions
- ▪ Hydroxyl generation
  - Suitable for TiO_2, insufficient information for others
- ▪ Orange II degradation
  - Suitable for TiO_2, insufficient information for others
- ▪ Degradation of acetaldehyde
  - Insufficiently quantitative

Results: Porosity

Broad range of NMs	Certain NMs only	Not suitable
• BET / BJH	▪ Mercury porosimetry	

- Interpretation depends on pressure/temperature and model used

- BET / BJH
  - Standardised under ISO 15901-2 (different calculations)
  - NOT for microporous nanomaterials
- Mercury porosimetry
  - Standardized under ISO 1590-1
  - NOT for metal-containing nanomaterials

Results: Redox potential

Broad range of NMs	Certain NMs only	Not suitable
		• Potentiometry • Oxo-Dish O_2-detection

- Potentiometry
  - Too sensitive to ions in test medium
- O_2-detection
  - O_2-levels may not correlate with redox potential

Results: Radical formation potential

Broad range of NMs	Certain NMs only	Not suitable
• EPR / ESR	• Potassium iodide and optical absorbance	• Benzoic acid PBS

- EPR / ESR
  - General suitable
- Potassium iodide and optical absorbance
  - Suitable for nanomaterials that generate hydroxyl radicals
- Benzoic acid PBS
  - Large influence of sample preparation and analysis conditions

- Which specific radicals should be measured?

Results: Crystallite size

Broad range of NMs	Certain NMs only	Not suitable
• XRD		

- XRD
  - General suitable
  - Crystallites sizes < 100 nm

Results: Surface chemistry

Broad range of NMs	Certain NMs only	Not suitable
	• XPS	• EDX • Liquid chromatography

- NOT distinction between core and specific surface

- XPS
  - Seems to be the only useable method