Grant agreement number 2009 21 01

Physicochemical characterisation of MNs and exposure media

WP4 Leader
Keld Alstrup Jensen
The National Research Centre for the Working Environment

Co-funded by the Health Programme of the European Union

Main tasks

- To test and develop suitable methods and standard operation procedures (SOPs) for analysis and characterisation MN's and dispersions dispersions thereof
- To determine the intrinsic characteristics of nanomaterials selected for toxicological studies
- To test the homogeneity of the MN batches distributed
- Develop, test and verify highly suitable MN dispersion protocols to be used in toxicity testing

Co-funded by
the Health Programme
of the European Union

Examples of New SOPs

- **New procedures for establishment of nanoparticle dispersions was established using either a one-step direct stabilization by BSA or a three-step pH-BSA-pH stabilization (NRCWE and CEA + validation partners)**
- **Procedures were developed and tested for determination of primary and aggregate/agglomerate size-distribution using TEM (CODA-CERVA, IMC-BAS and INRS)**
- **Procedures for determination of average primary and aggregate size, number of primaries in aggregates and surface area in both powders and dispersions using SAXS were demonstrated (CEA)**
- **Procedure for identification and quantification of organic coatings or associated organic matter was established (NRCWE)**
- **Procedure for determination of dustiness using a Vortex Shaker was established (INRS)**
- **Two procedures were established to investigate the 24-hour hydrochemical reactivity and dissolution/biodurability of MN in various mediums. (NRCWE)**

Co-funded by
the Health Programme
of the European Union

Dispersion of the test materials for in vivo and in vitro toxicological tests

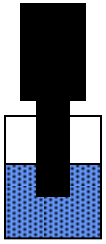
Co-funded by
the Health Programme
of the European Union

The NANOGENOTOX strategy for MN-dispersion

One dispersion protocol for all test systems!

Requirement

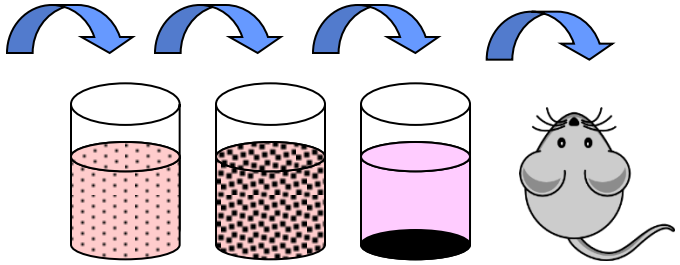
High concentration in a "physiologically" acceptable medium
Applicable for both hydrophilic and hydrophobic MN's



2.56 mg/ml MN Stock Suspension

(instilled, diluted or dosed into specific test mediums)

Different Exposure Systems



The NANOGENOTOX strategy for MN-dispersion

One dispersion protocol for

Requirement

High concent

Applic

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

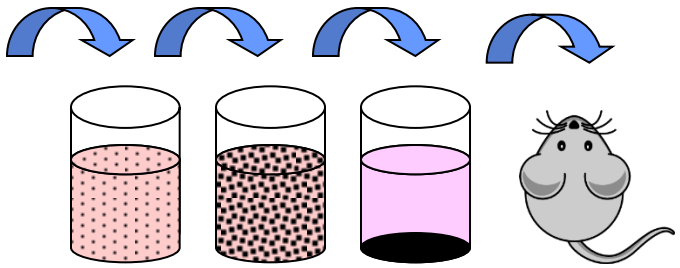
able medium

ophobic MN's

Different Exposure Systems

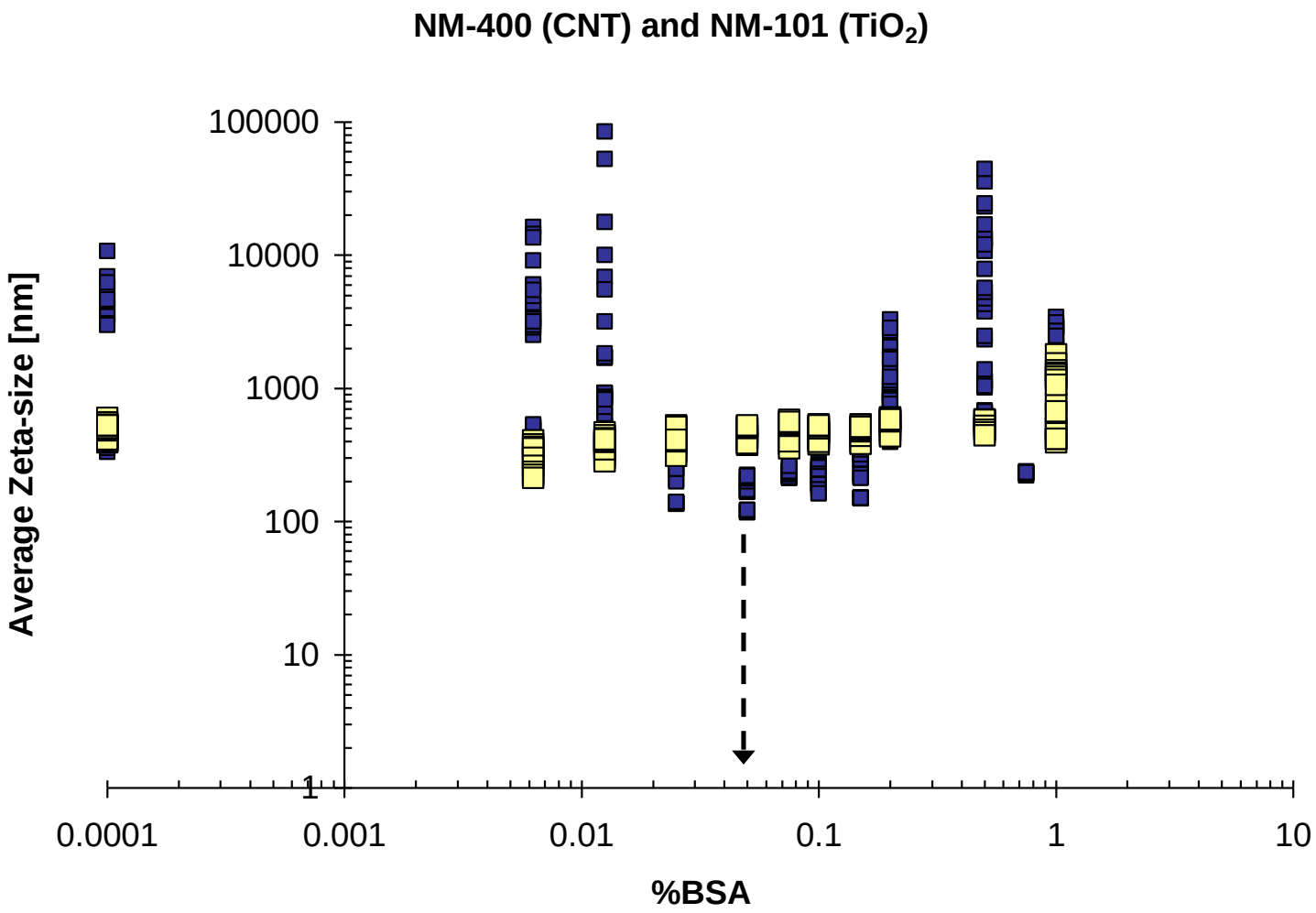
pension

diluted or dosed into specific test mediums)



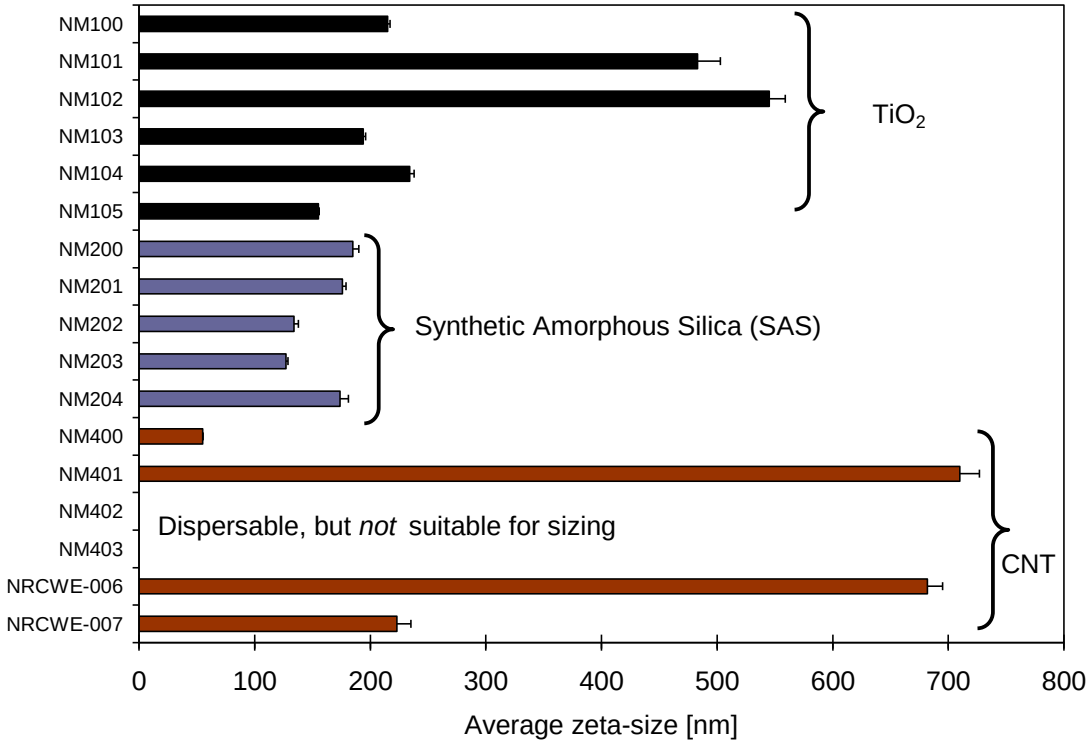
Selection of BSA concentration

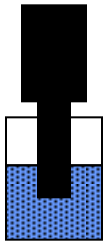
Grant agreement number 2009 21 01



d by
the Health Programme
of the European Union

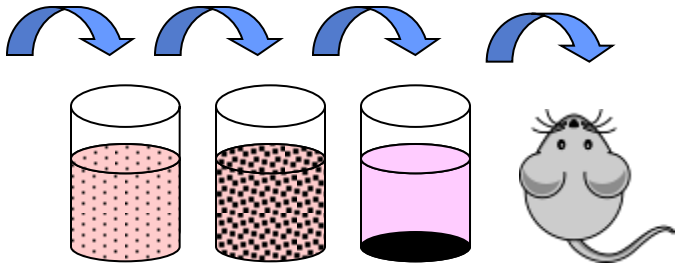
2.56 mg/ml in 0.05% w/v (Bovine) Serum Albumin (0.5 % EtOH pre-wetting for all)





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

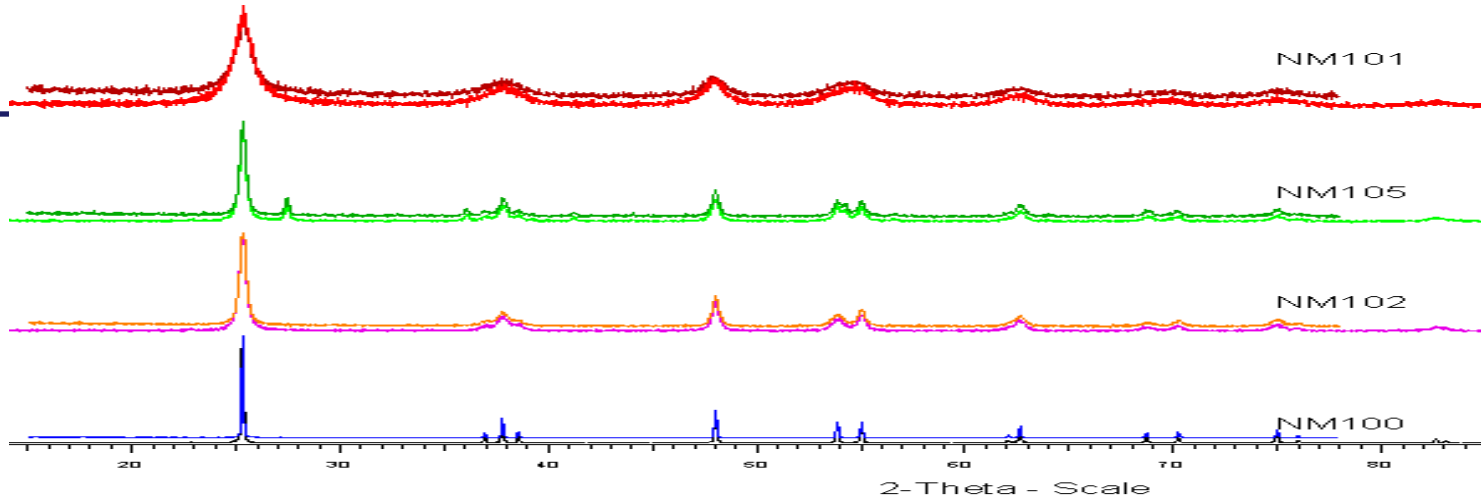
Different Exposure Systems



Primary physico-chemical characterization of NANOGENOTOX MN samples – Selected Major Conclusions

Co-funded by
the Health Programme
of the European Union

XRD

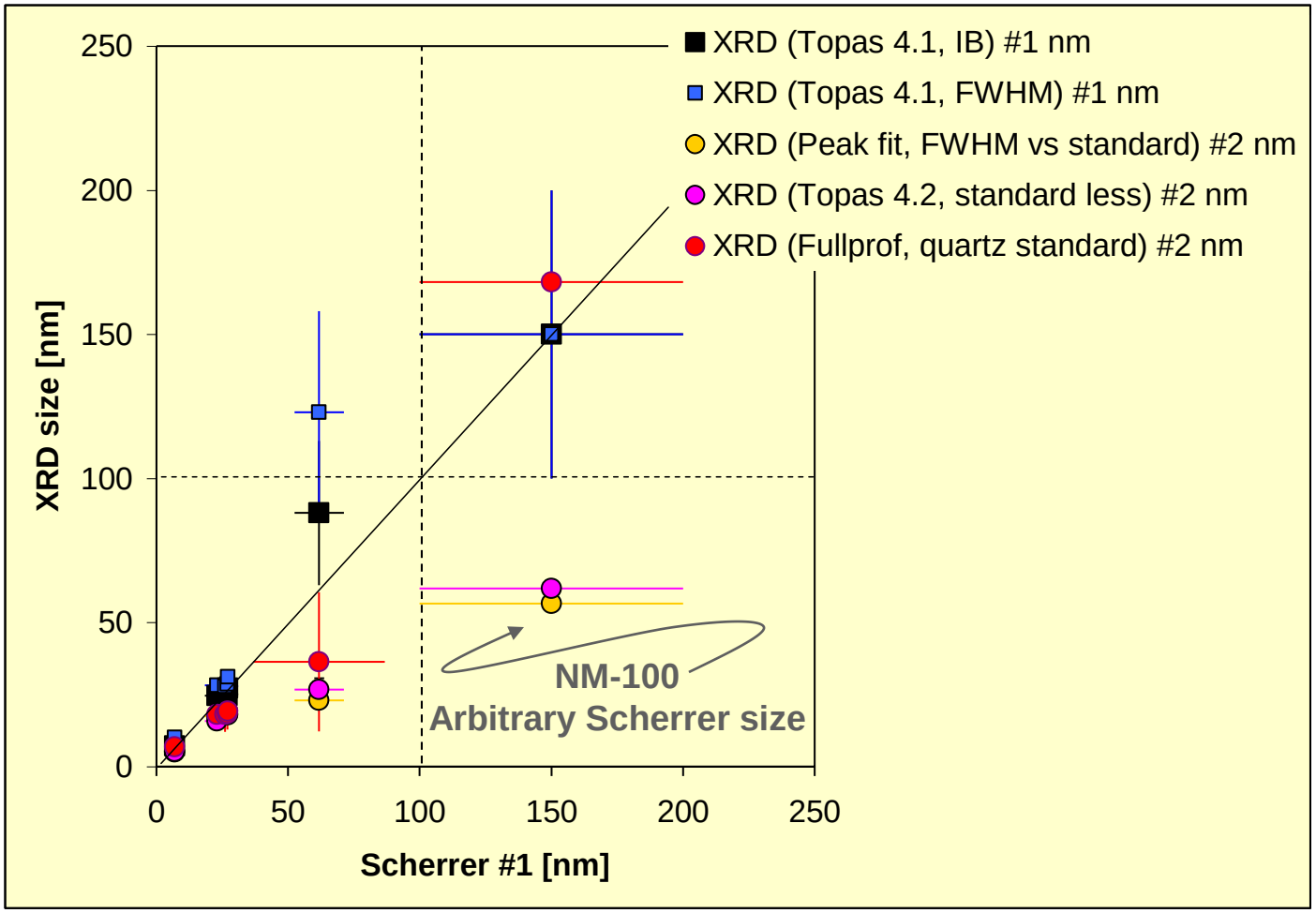


- TiO_2 (size only valid until 100 nm; size may vary with method of analysis; IMC-BAS XRD-size data are systematically smaller than LNE and NRCWE XRD-size data)
- SAS (generally amorphous, but Na_2SO_4 and AlO(OH) were observed in several samples by NRCWE. The type of sample mount and sample size may determine Limit Of Detection: Large Al-holder vs. Quartz-plate)
- CNT (A primary XRD peak can be observed, but it can probably not be used for reliable sizing of CNT diameter/wall thickness)

Co-funded by
the Health Programme
of the European Union

Relations between XRD-sizes

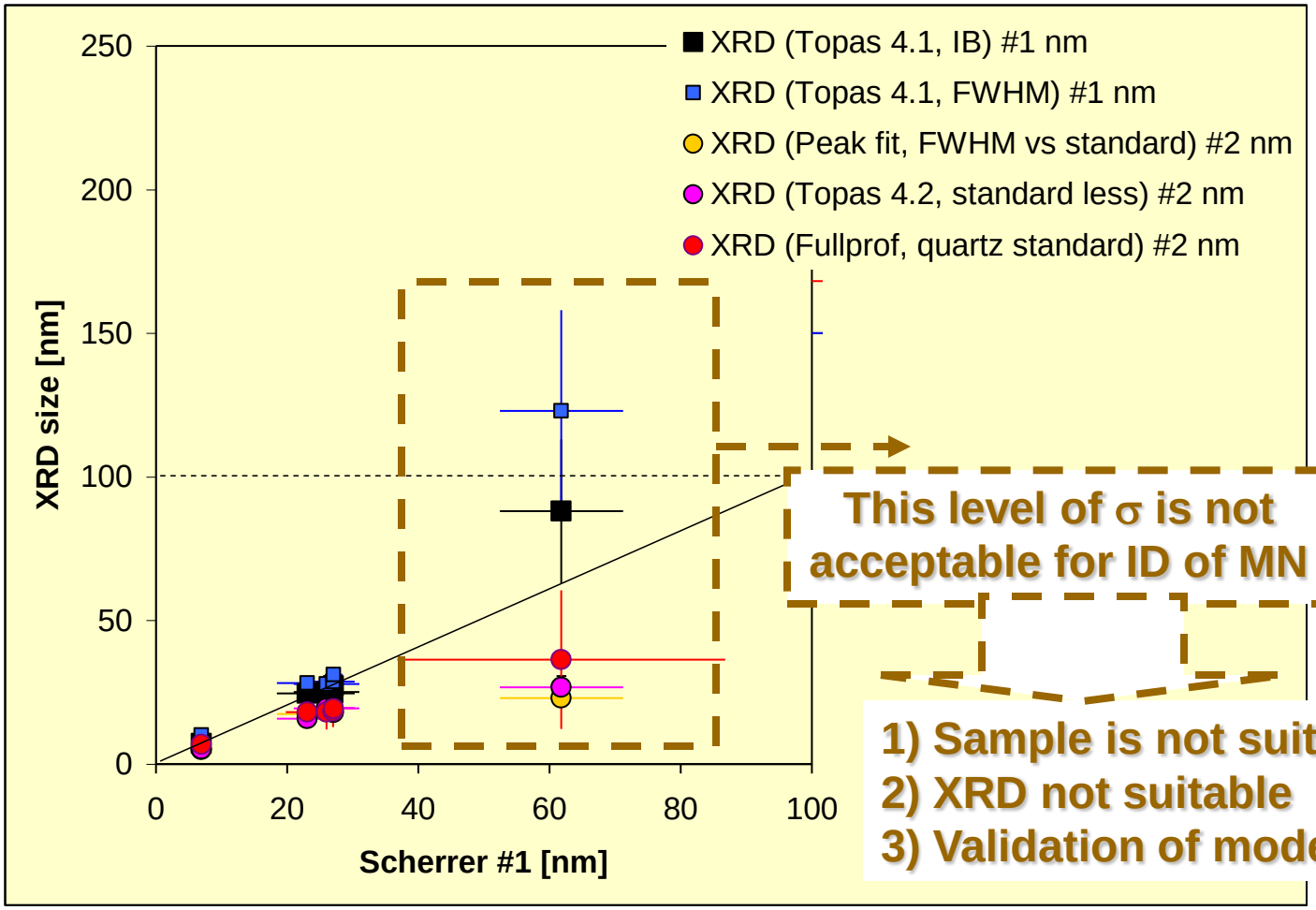
Grant agreement number 2009 21 01



Co-funded by
the Health Programme
of the European Union

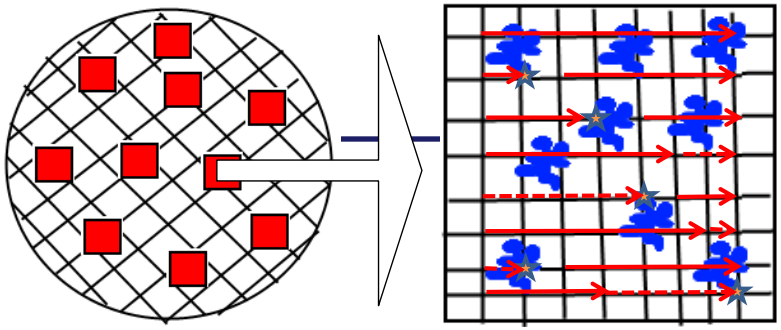
Relations between XRD-sizes

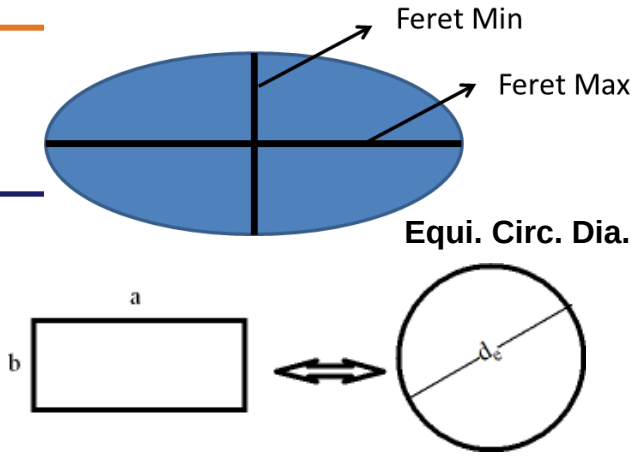
Grant agreement number 2009 21 01



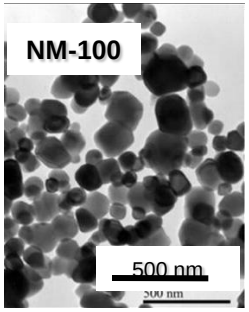
Co-funded by the Health Programme of the European Union

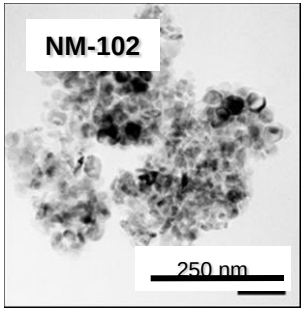
TEM

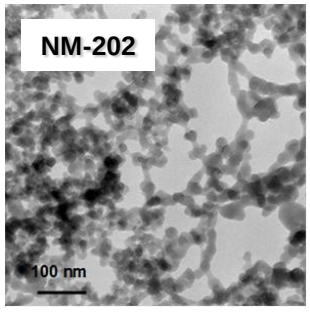


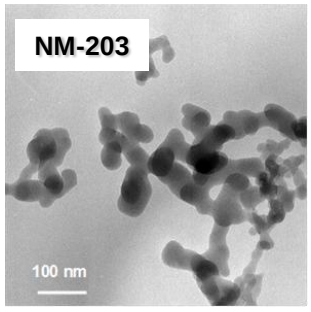


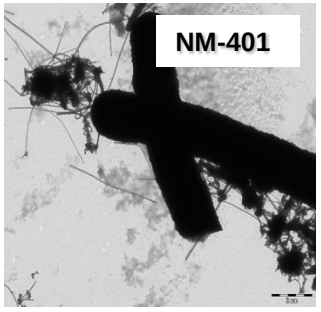
- Results from analysis of primary particle sizes showed general agreement between the different procedures.
  - Harmonization of reported dimensions is needed (e.g, Feret dim, PSD).
  - As for XRD, maybe greater variability with increasing particle size?
  - Primary sizes of our MN had too little variation for general comparison
- Challenges remain for complex morphologies (aggregates and high-aspect ratio nanomaterials!

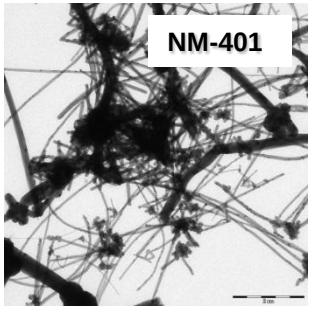






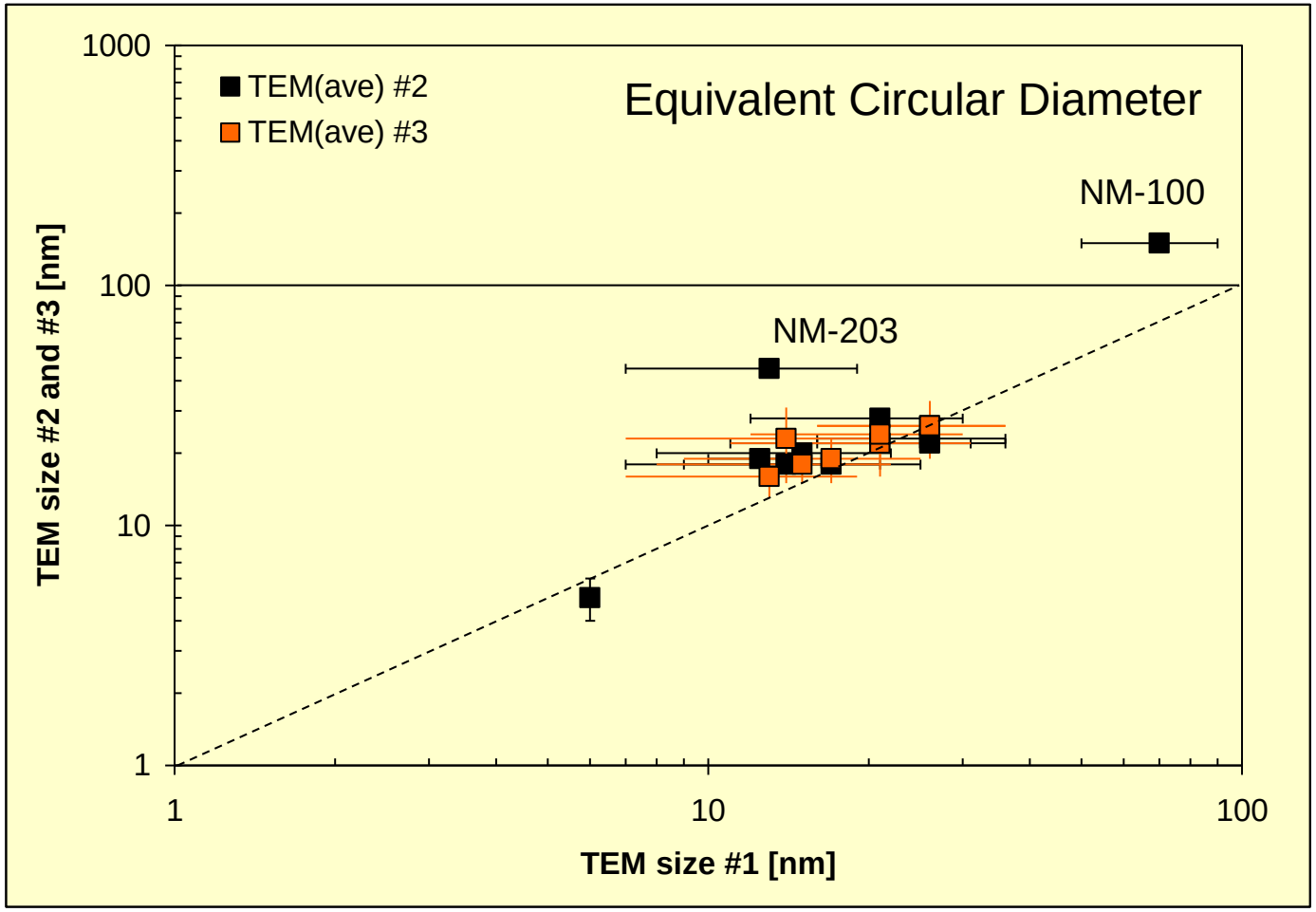






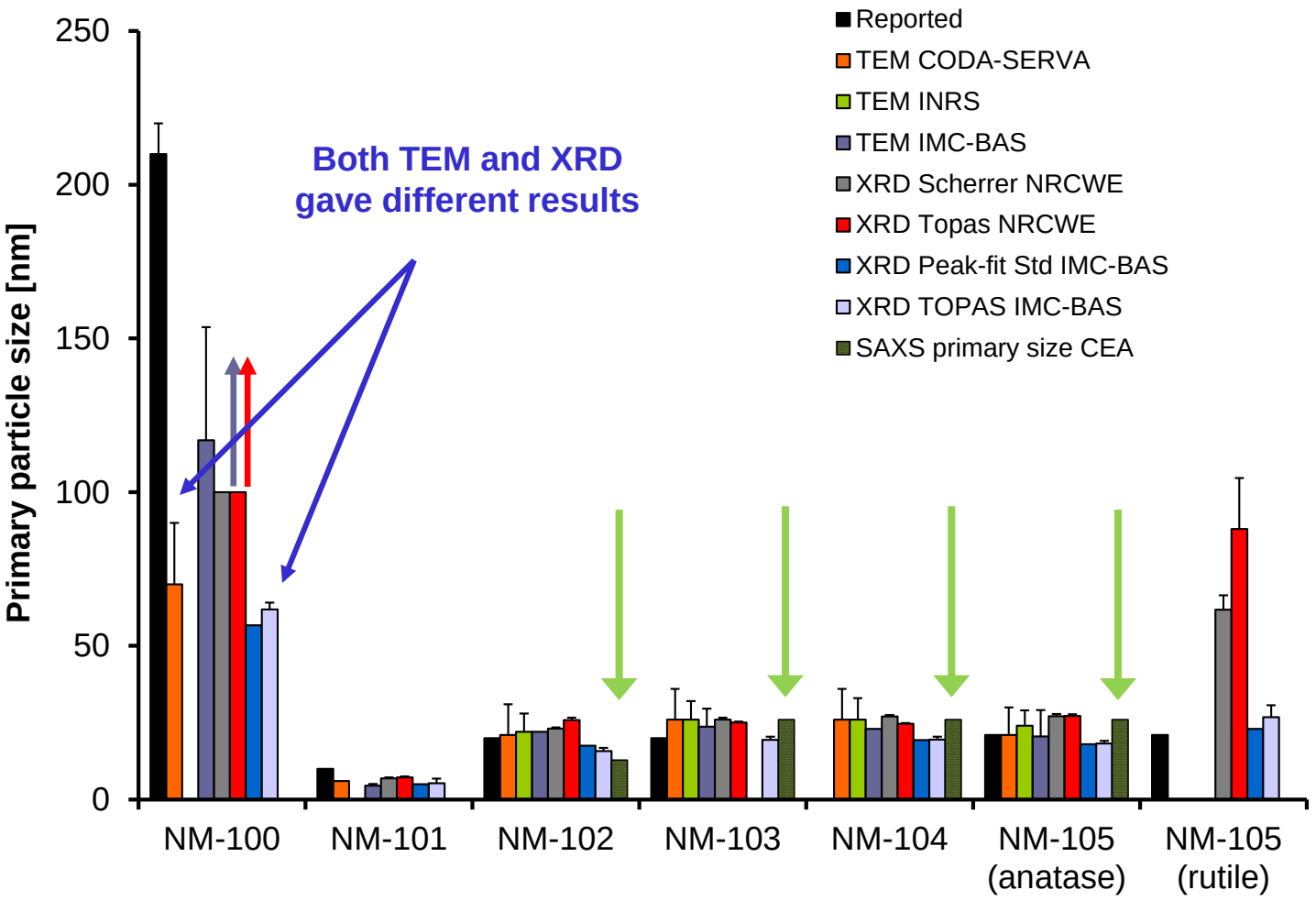
Co-funded by
the Health Programme
of the European Union

Comparison between laboratories



Co-funded by
the Health Programme
of the European Union

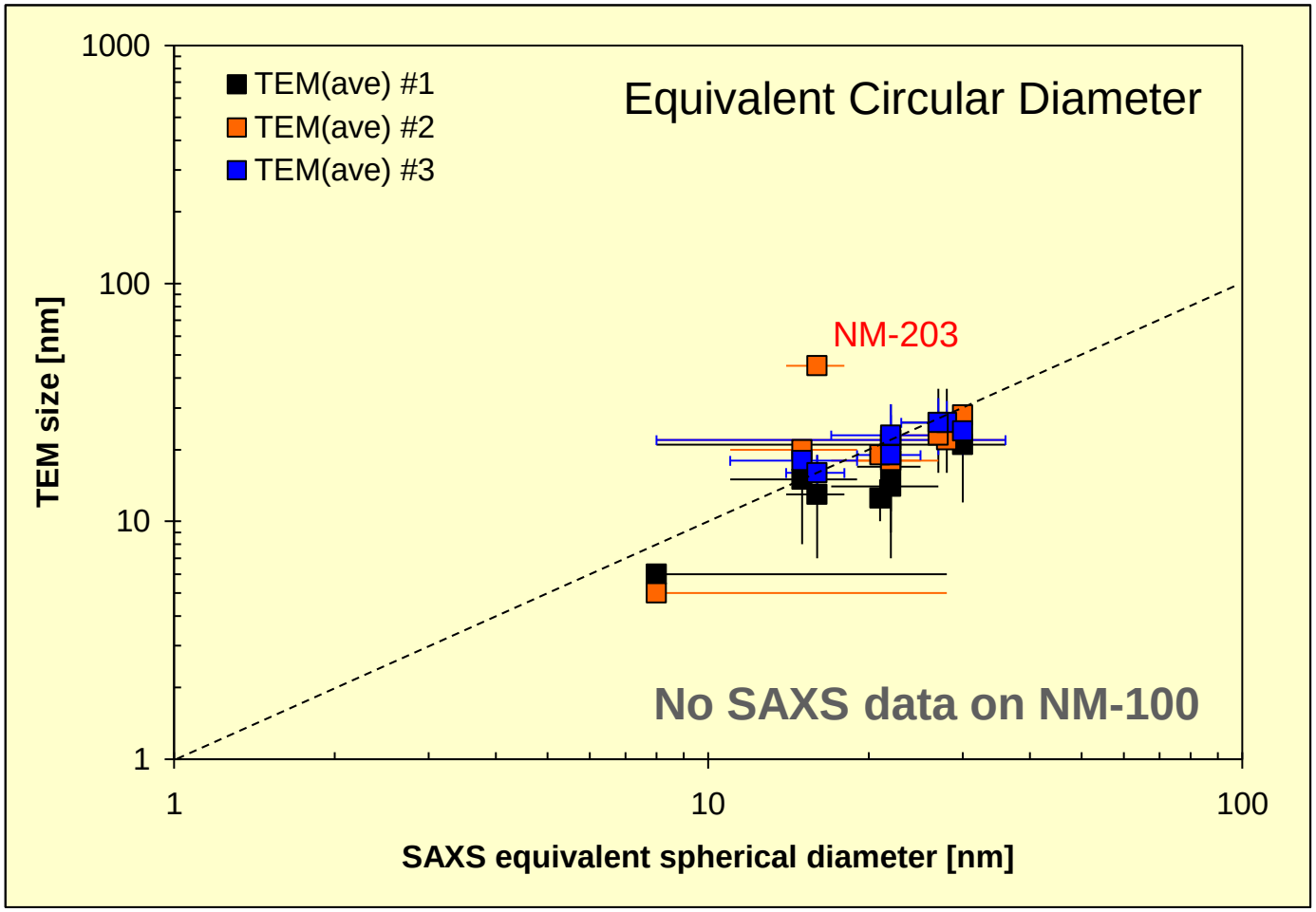
Primary sizes of the TiO_2 MN samples



Co-funded by the Health Programme of the European Union

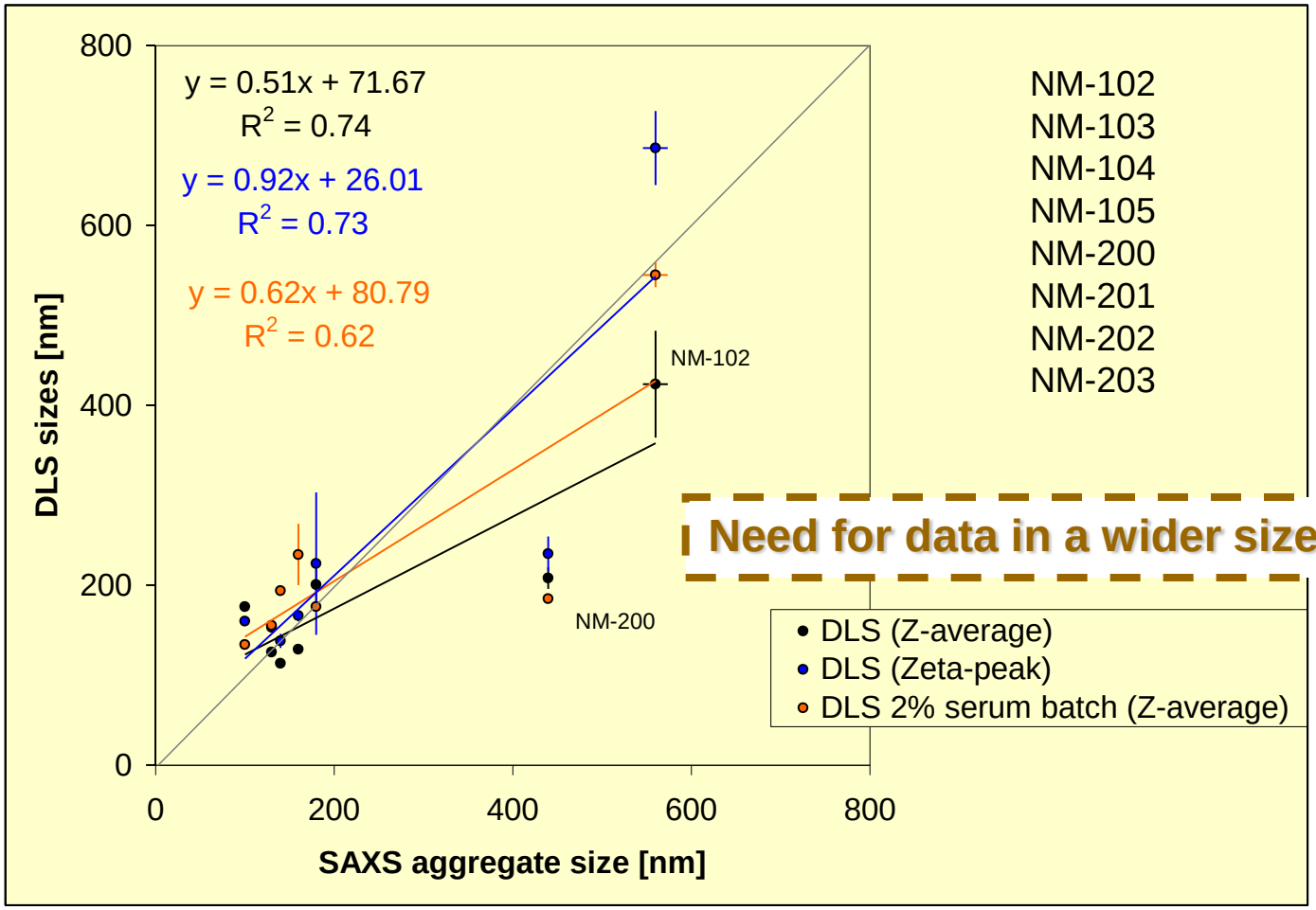
Comparison between SAXS and TEM

Grant agreement number 2009 21 01



Co-funded by
the Health Programme
of the European Union

"Aggregate" size by SAXS and DLS



Need for data in a wider size-range

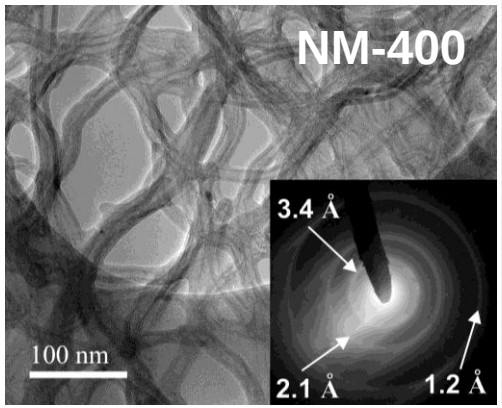
Co-funded by
the Health Programme
of the European Union

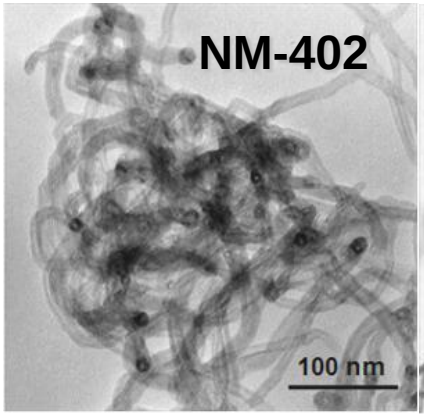
Primary Dimensions of CNT

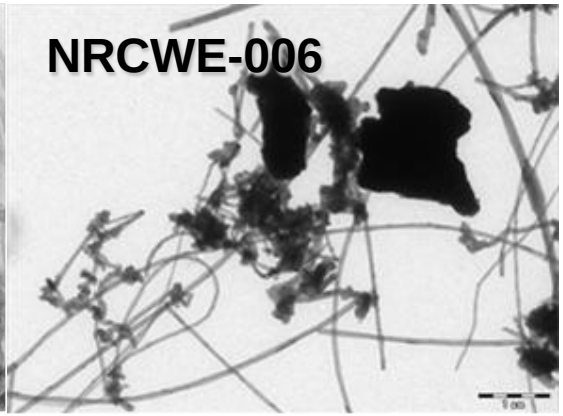
Lab	Thickness (nm) SD	Geodesic length (nm) SD	< 100 nm (%)	Aspect ratio*	n
NM-400 #1	11 ± 3	846 ± 446	100%	79 ± 50	20
NM-400 #2	16.2 ± 3.5				36
NM-401 #1	67 ± 24	4048 ± 2371	90%	66 ± 46	43
NM-401 #2	61.4 ± 24.4				358
NM-402 #1	11 ± 3	1372 ± 836	100%	125 ± 66	20
NM-402 #2	14.3±2.7				135
NM-403 #1	12 ± 7	443 ± 222	100%	42 ± 29	50
NRCWE-006 #1	74 ± 28	5730 ± 3674	87%	85 ± 63	56
NRCWE-007 #1	17 ± 7	465 ± 340	100%	30 ± 22	50

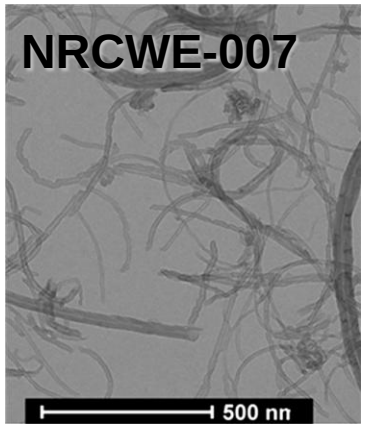
Co-funded by
the Health Programme
of the European Union

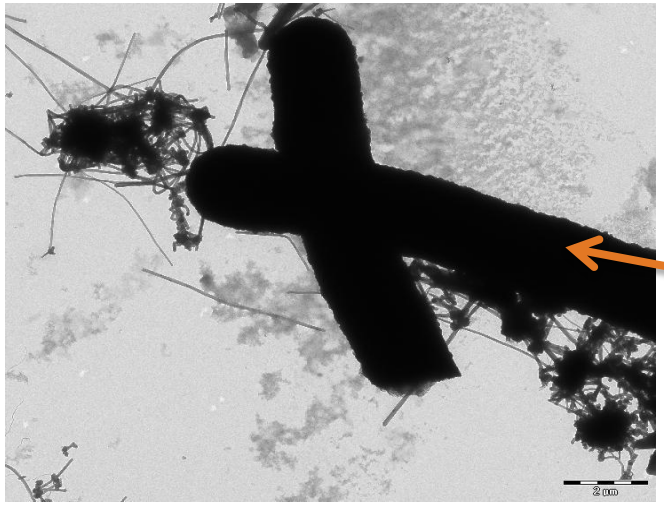
Primary Dimensions of CNT





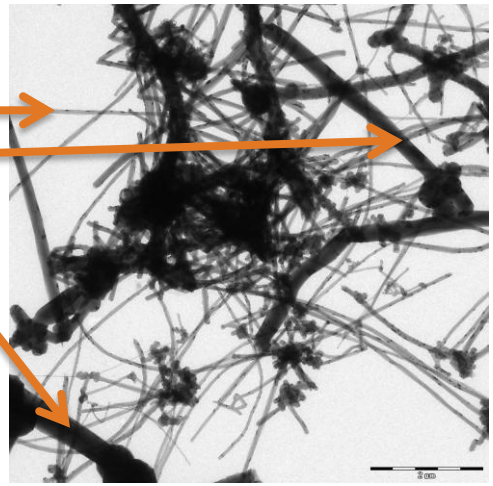






NM-401

Diameter
60 nm
260 nm
500 nm
2000 nm

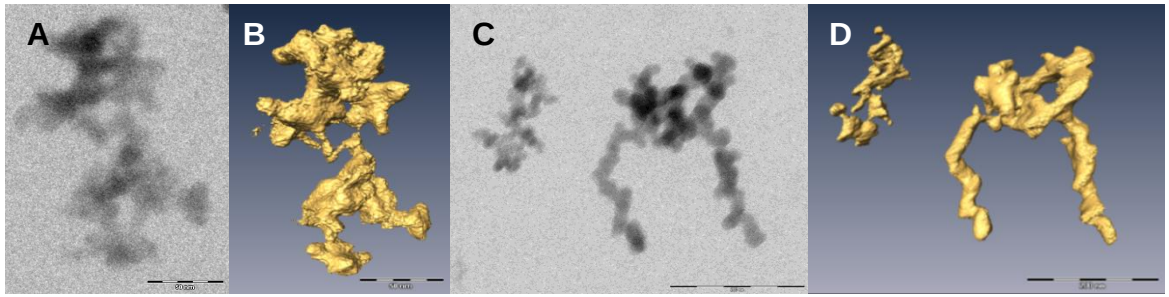


NM-401

Co-funded by
the Health Programme
of the European Union

Specific Surface Area (SSA)

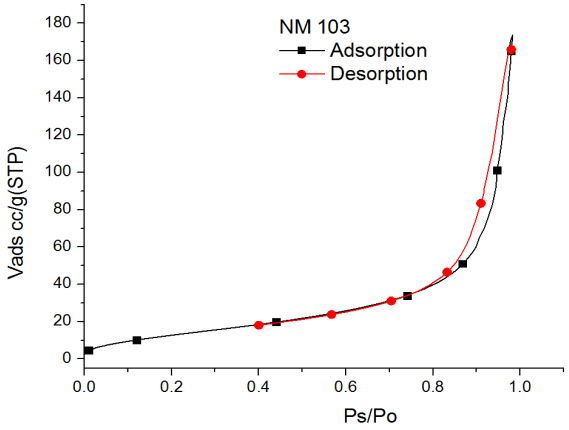
TEM dry powder and cryo

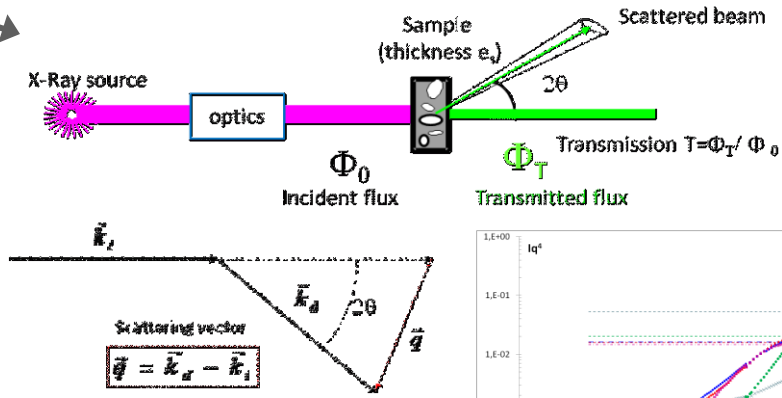


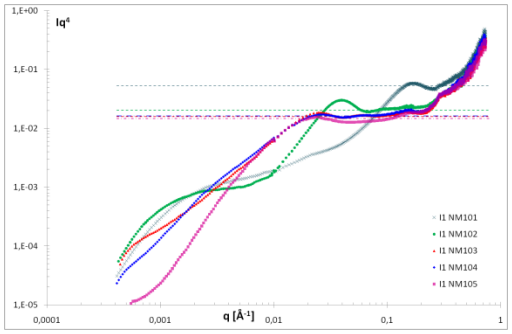
SAXS
BET

Dry powder and dispersions

Dry powder



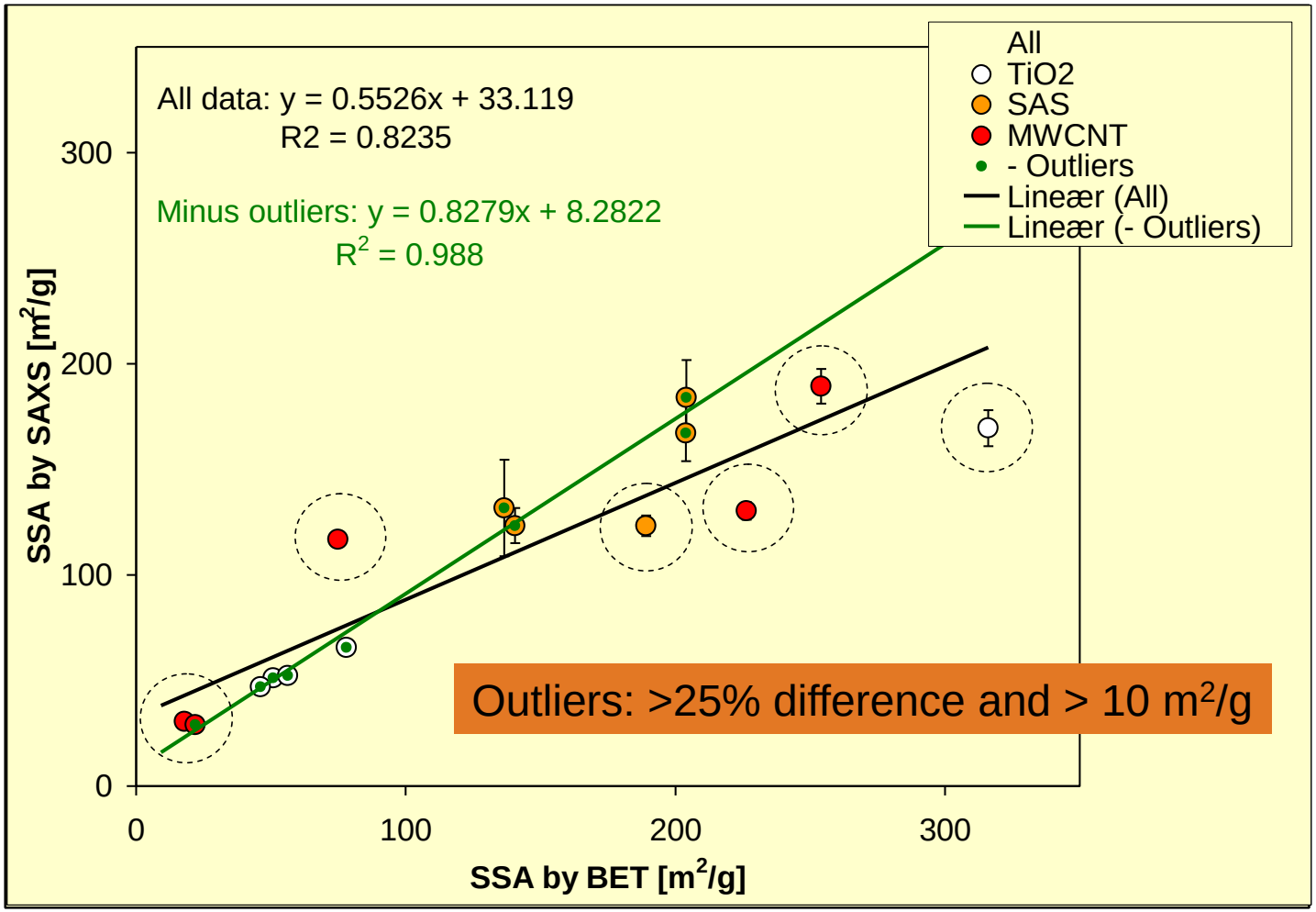




Co-funded by
the Health Programme
of the European Union

Specific surface areas all MNs

Grant agreement number 2009 21 01



Differences between BET and SAXS data may in part be due to challenges in mathematical procedures for data-treatment and material properties – e.g., inner and nano-porosity

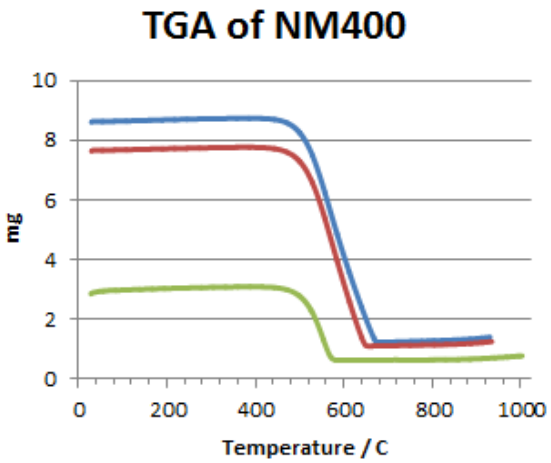
Chemical composition

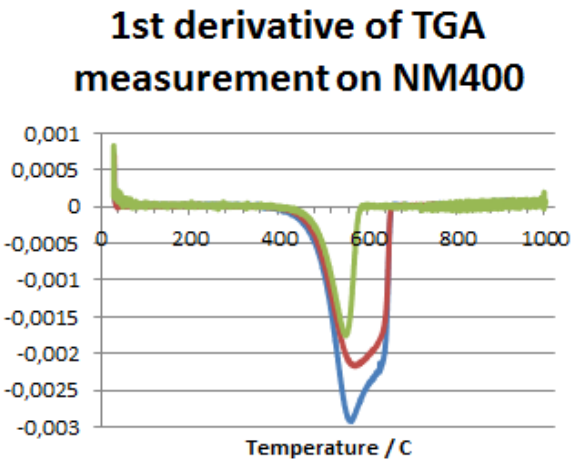
Strategy for the analysis

- Mass-loss in TGA
  - organic coating (or associated organics) in TiO_2 and SAS
  - incombustible residual in carbon-based MN
- Elemental analysis
  - general composition
  - catalysts
  - impurities
- Organic chemical analysis of MN with significant weight-loss
  - Organic coatings and functionalizations
  - Associated organic matter

Co-funded by
the Health Programme
of the European Union

TGA / DTA





- Very useful for identification of MN with potential presence of ”organic” coatings (or associated ”organics”)
  - NM101, NM103, NM104, NM204
- Very useful for analyzing the homogeneity (and apparent quality) of CNT
  - NM400, NM402 and NRCWE007 apeear to be inhomogeneous (> 10 – 15 mg)
- Very useful for determination of total mass of inorganic compounds in a combustable material such as CNT
  - CNT contained 3 – 18 wt% impurities (catalyst particles)

Co-funded by
the Health Programme
of the European Union

Elemental composition (EDS, ICP-MS, ICP-OES)

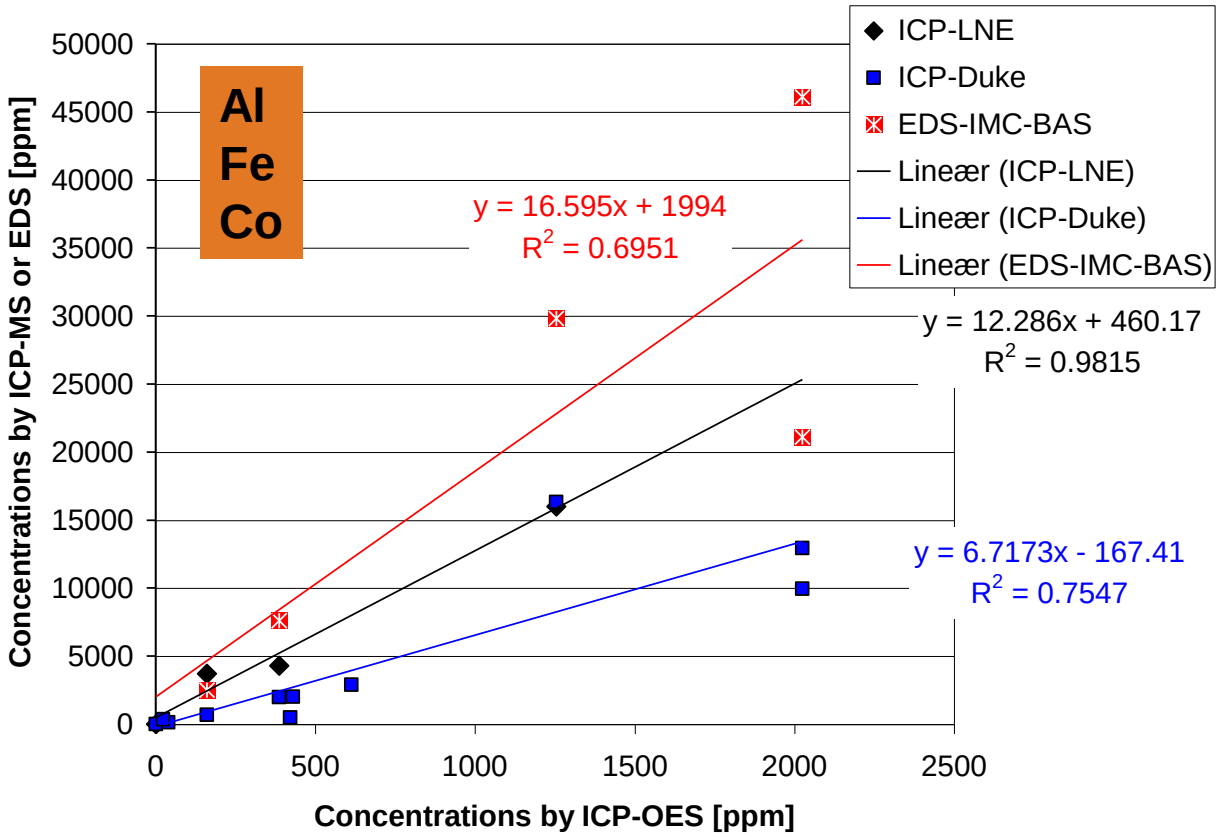
- **SEM EDS, ICP-MS and ICP-OES were conducted where SEM EDS is semiquantitative analysis of samples pressed into pellets**
  - TiO_2: general agreement in the major elemental impurities / coatings (Al and S), but Fe (EDS) were not detected in ICP-OES analyses.
  - SAS: was analysed in general agreement with major elemental impurities (Na, Ca, S, Al) between EDS and ICP-OES
  - CNT: The highest catalyst concentrations were detected by TGA and SEM EDS. Full recovery was not achieved in ICP-MS and ICP-OES analysis when using EDS and TGA analyses as benchmark data. However, there was general agreement in the detected main elements.

Co-funded by
the Health Programme
of the European Union

Method-depended variation for CNT

Grant agreement number 2009 21 01

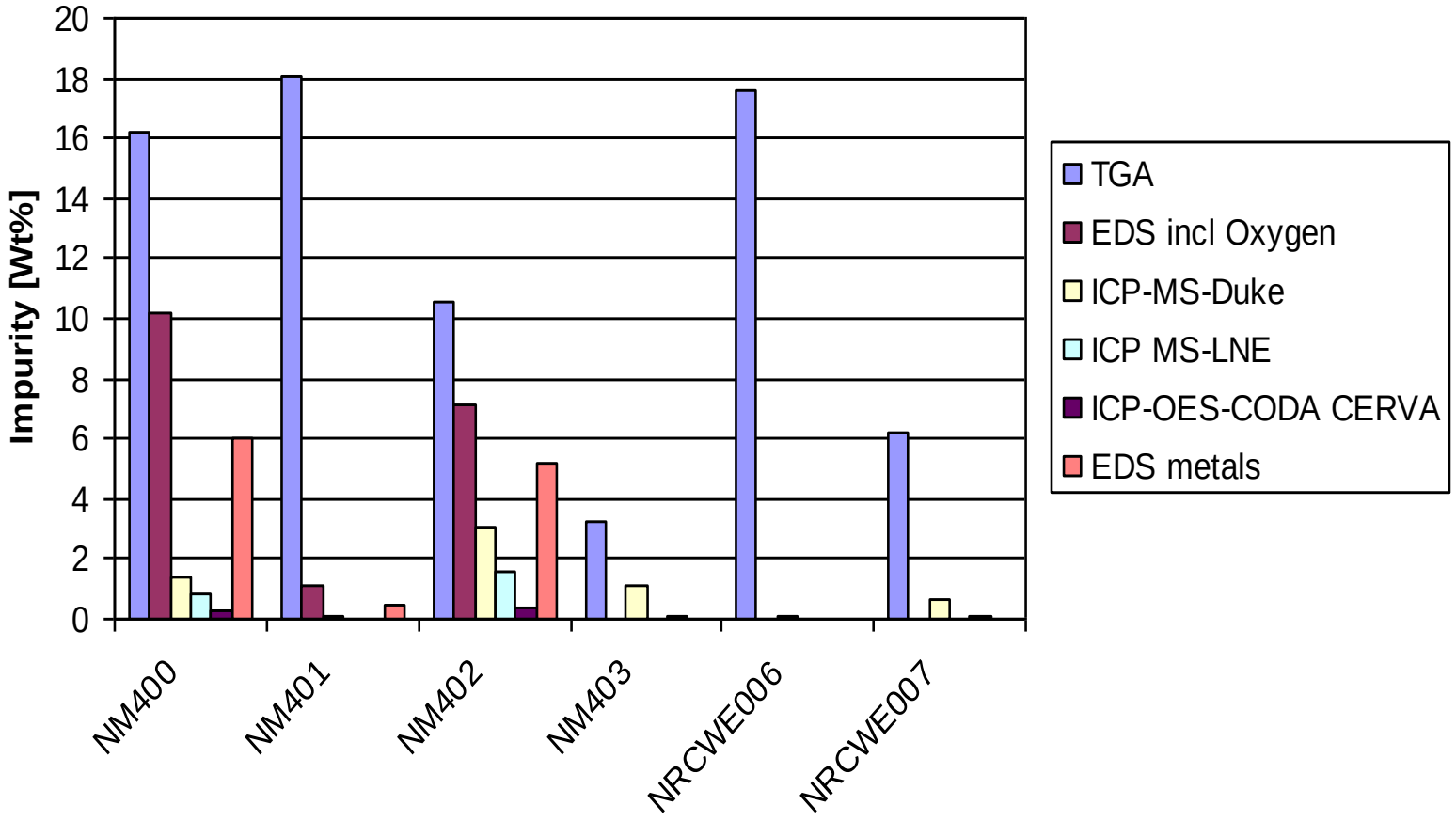
Apparent problems in getting agreement in quantitative elemental analysis of CNT is due to different extraction procedures



Co-funded by
the Health Programme
of the European Union

Chemistry of the CNT MN samples

Grant agreement number 2009 21 01



Co-funded by
the Health Programme
of the European Union

Main Conclusions

- **Dispersion protocol**
  - Generic protocol developed – d_H comparable to the primary aggregate sizes.
  - Stabilities of at least 1 hour for almost all dispersions allowing sufficient time for exposure.

- **Primary physicochemical characterization methods**
  - XRD sizes are method-dependent and uncertainty increases at the lower and upper end of the nano-range - harmonization and validation may be required.
  - TEM and DLS sizes generally comparable across laboratories. BUT size-range of tested MN was too narrow to investigate the upper and lower limit of the nano-range.
  - SAXS is a promising tool for SSA and size analysis of both primary particles and aggregates
  - TGA useful for ID of MN with associated ”organics” and residual catalysts in CNT.
  - Elemental analysis using digestion procedures should be improved.
  - Dustiness tests are useful for assessment of emission potentials and dust characteristics

- **The Phys-chem characteristics of the MNs**
  - TiO_2 and SAS MN are releatively homogenous MN, but some SAS contain minor Na_2SO_4 and AlO(OH) impurities (not homogeneously distributed).
  - The CNT were chemically and structurally inhomogeneous with 3-18 wt% catalyst (>10-15 mg needed for TGA).
  - Wide distributions were found in CNT tube diameters. Length measurements are uncertain.

Thanks for listening!

- National Research Centre for the Working Environment (NRCWE), Denmark
  - Keld Alstrup Jensen (WP-leader)
- Vetinary and Agrochemical Research Centre (CODA-CERVA), Belgium
  - Jan Mast
- Commissariat a l'Energie Atomique (CEA), France
  - Olivier Spalla
- Institut National de Rescherche et de Securite (INRS), France
  - Olivier Witschger
- Central Laboratory of Mineralogy and Crystallography (CLMC), Bulgaria
  - Boris Shivachev

- **Collaborating Partners**
  - Laboratoire National de Métrologie et d'Essais (LNE), France
  - Joint Research Centre (JRC, Ispra), Brussels
  - Duke University, USA

Co-funded by
the Health Programme
of the European Union

NANOGENOTOX

Grant agreement number 2009 21 01

NANOGENOTOX

WP 4: Physicochemical Characterisation of MNs and Exposure Media

Statement by M. A. Bader, BAM, Berlin

Co-funded by
the Health Programme
of the European Union

Statement on WP 4 Outcome

- **Detailed and unique SOP for nanomaterial dispersion to be used in toxicity testing has been provided.**
- **Different methods to characterise large volume batches of nanomaterial and dispersions thereof have been applied, useful and detailed SOPs have been provided recognising state-of-the-art.**
- **Nanomaterial characterisation, availability of stable and homogeneous dispersion and characterisation thereof is key issue:**
  The importance of phys-chem characterisation is recognised,
  WP objectives were achieved, outcome is relevant.
- **Restrictions: Choice of materials (TiO_2, SiO_2, CNT), dispersion (BSA), equipment (participating labs). The question of transferability of SOPs might arise.**

Co-funded by
the Health Programme
of the European Union

Recommendations / Input

- **Many different SOPs and guidelines are around: OECD, ISO, NIST, JRC, NANOMMUNE, …**
  Where and how do NANOGENOTOX results fit in?
  NANOGENOTOX guidance document suitable?
- **Industry and regulatory agencies rely on standards:**
  Your input in ISO, CEN and national standardisation committees is strongly recommended.
- **Development/application of certified nanoscale reference materials and validation of methods seem necessary to overcome discrepancies in results that are still observed.**
- **Some refinements in specific SOPs for material characterisation are suggested.**

Co-funded by
the Health Programme
of the European Union