A common European approach to the regulatory testing of nanomaterials

Validated protocols for test item preparation for key in vitro and ecotoxicity studies
Deliverable 2.6

Introduction

Most assays for eco- and in vitro toxicological testing of nanomaterials require dispersion of solid particles into liquid test mediums. The way this is done strongly affects the outcome of (eco-)toxicity tests. Standardisation of the dispersion procedures is therefore the first indispensable step towards establishing reliable and comparable toxicological testing. It is a prerequisite for comparative analyses of results from different laboratories and different toxicological assays and for the regulatory testing of nanomaterials.

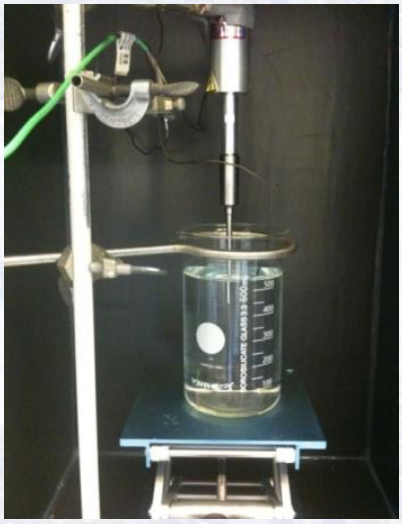
To ensure that for the NANoREG project such comparative analysis of results would be possible, a Guidance Document (July 2014) has been established during the preparatory phase of the project describing -among others- dispersion procedures and characterisation requirements to be applied by all partners. This deliverable describes the results of further elaboration and validation of the dispersion procedures. The final result is a set of validated protocols that can be used as a solid basis for the regulatory (eco-)toxicological testing.

Description of Work

For NANoREG, the management committee decided that for selection of candidate dispersion protocols they should have:

- Documented applicability to several different types of NM
- Demonstrated use in at least two to three laboratories with comparable results
- No known serious interference with the NM and bioassays to be conducted

For in vitro studies, only the ENPRA and NANOGENOTOX dispersion protocols had been documented to work for both hydrophobic and hydrophilic NM as well as metallic and carbonaceous materials. For ecotoxicological studies it was initially decided to use a NOM water dispersion protocol for carbon nanotubes and pure water for all other NM. This decision was later refined to add an ethanol pre-wetting step in the water-based protocol to allow dispersion of hydrophobic NM.



Setup for the measurement of calorimetric curves of probe-sonication (photo kindly provided by DTU Food Denmark)

Since all the selected dispersion protocols apply probe-sonication for dispersing the NM, it was also found necessary to establish and validate a probe-sonicator calibration procedure. This procedure was developed in collaboration with the EU FP7 NANODEFINE project.

All selected protocols were analysed, discussed and optimised based on protocol testing within a core test team of NANoREG partners before they were distributed to the NANoREG consortium as part of the NANoREG Guidance Document July 13, 2014. Validation of the performance of the dispersion protocols were subsequently made on all core MNM by the core test team. All WP5 partners performing toxicological studies were also asked to submit their probe-sonicator calibration date resulting in a total data set from 26 partners for the analysis.

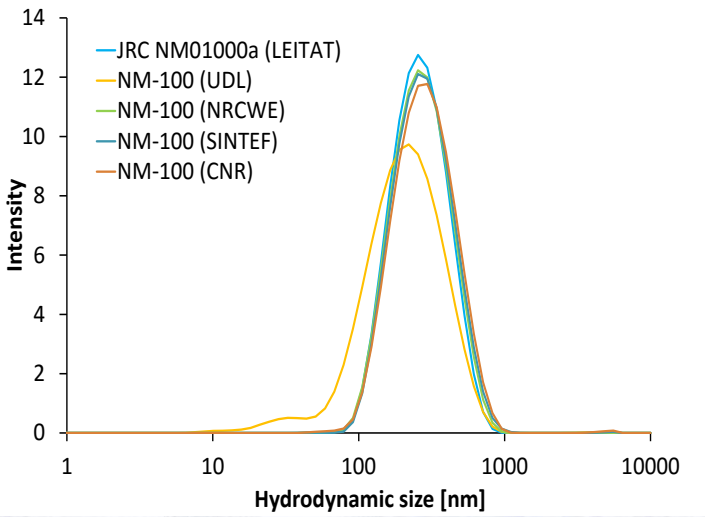
Main Results

- A protocol for calibration of probe sonicators using calorimetry and NM benchmark values following the NANoREG dispersion protocol, which was developed as part of the project.
- The refined generic NANOGENOTOX batch dispersion protocol for in vitro studies.
- A protocol for calibration of probe sonicators using calorimetry and NM benchmark values following the water-based ecotoxicological dispersion protocol, which was developed as part of the project.
- The water-based batch dispersion protocol for ecotoxicological studies, which was developed as part of the project.
- The enhanced NOM-water batch dispersion protocol for for ecotoxicological studies of hydrophobic NM, which was developed as part of the project.
- A harmonized protocol for Dynamic Light Scattering measurements to document the state of dispersions.
- All protocols are performance tested and validated using an interlaboratory comparison approach.

The results show that probe sonicators cannot be calibrated using a calorimetric approach alone. The introduction of dynamic light-scattering (DLS) performance benchmark values ensures comparability of batch dispersions across laboratories.

Based on interlaboratory comparisons, all dispersion protocols used in NANoREG enable establishment of comparable dispersions for granular NM. However, some variations were observed for some granular NM, which is generally ascribed to sample variability.

Due to the method, DLS results are generally poor for carbon nanotubes and nanocellulose samples and comparability between laboratories was generally poor. Optical microscopy screening of dispersions made with the NANO-GENOTOX dispersion protocol showed that only NM-411 (SWCNT) was poorly dispersed among all 19 NANoREG core test materials.

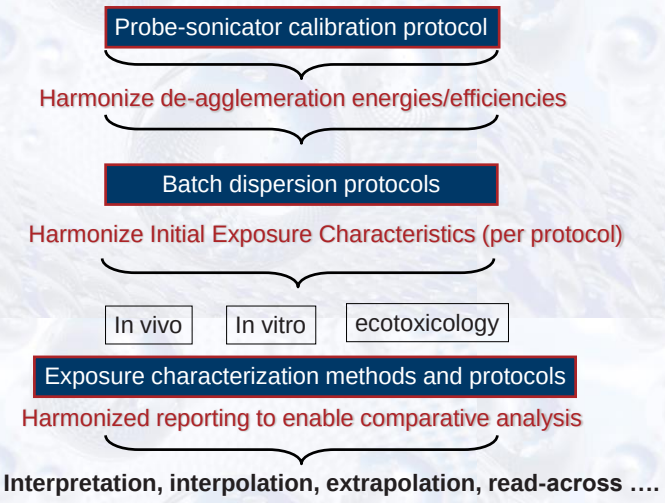


Intensity hydrodynamic size-distribution and tabulated data for NM-100 / JRCNM01000a. The size-spectra and PDI-values demonstrate that the sample disperses well following the NANO-GENOTOX dispersion

Evaluation of the results

The result of the deliverable documents that harmonisation of test item preparation can be reached. The methods or concepts established should be considered for adoption into guidance documents for toxicological testing following the approach outlined in the figure.

The interlaboratory comparison for documentation of the probe sonicator calibration procedure is extensive, with 26 partner contributions, and the procedure should be ready for general implementation.



The performance of the dispersion SOPs is generally convincing based on the 3 to 6 partner interlaboratory comparison. However, final recommendation on dispersion SOPs should await documentation of the implemented procedures after completion of the toxicological studies in WP3 and 5.

For more details about NANoREG please visit the official website www.nanoreg.eu.

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