A common European approach to the regulatory testing of nanomaterials

Standard Operating Procedure

Title	***SOP for probe sonicator calibration of delivered acoustic power and de-agglomeration efficiency for ecotoxicological testing***
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A common European approach to the regulatory testing of nanomaterials

SOP for probe sonicator calibration of delivered acoustic power and de-agglomeration efficiency for ecotoxicological testing

Work in progress (revision is being tested)

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Version 3

Date: 15 Jan, 2015

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Tested and commented by NANoREG participants:

SINTEF, NMBU, HW, Tekniker and Leitat

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1 Introduction

1.1 Background

One of the key requirements in comparative testing is that all preparation and subsequent characterization procedures are as harmonized and inter-calibrated as possible. This is of outmost importance for preparing aqueous batch dispersions for ecotoxicological testing where differences in the initial hydrodynamic particle size-distributions are important determinants for the rate of agglomeration and sedimentation, and ultimately also likely for the biological effects observed. It has been recognized that the acoustic energies and effective de-agglomeration effects delivered by different brands of sonifiers, and even the same brands and models, are rarely fully comparable. Moreover, small differences in the procedures which can arise due to different operators, water qualities, operation temperatures etc. may also play a role on the results.

1.2 Purpose and background for the SOP

The purpose of this SOP is to enable an easy-to-use and still reliable procedure for calibration of probe-sonicator amplitudes and duration of sonication for selected NANoREG batch dispersion protocols using probe sonicators.

The SOP has been developed for NANoREG purposes and consists of two parts:

1) a calorimetric method for initial calibration of the delivered acoustic energy by adjustment of the probe sonicator amplitude

and

2) a subsequent procedure for calibrating the effective level and quality of the dispersions.

The calorimetric procedure is based on a initial protocol prepared by Katrin Löschner and Manuel Correia, (DTU Food, Mørkhøj Denmark), who use a calorimetric approach for benchmarking probe sonicators in Nanolyse based on the work by Taurozzi et al. (2012). This protocol was mutually improved in collaboration between NRCWE (Keld Alstrup Jensen and Yahia Kembouche) and DTU (Katrin Löschner and Manuel Correia) as part of the NANoREG and NANODEFINE SOP harmonization attempts.

The second part of the protocols is derived from the work in NANOGENOTOX (Jensen et al., 2011) where the duration of sonication using different probe sonicators were identified by determination of the required sonication time resulting in similar hydrodynamic size-distribution of a nanomaterial. It was found that the latter approach is a necessary add-on owing to operator factors as well as deviations in media and instrumental deviations in configuration which may occur. If this does occur, the calorimetric procedure may not be a sufficient quality control.

2 Materials and Chemicals

- Water; thermally equilibrated to fume-hood air temperature (Nanopure-filtered water or MilliQ-filtered water or similar; resistivity 18.2 MΩ cm).
- Laboratory balance with maximum weight boundary greater than 700 g and a weighing accuracy of ±0.1 g or better.
- Pipettes with the ability to draw various suitable volumes of water.
- 600 mL Borosilicate glass beaker, tall form (height 150 mm and 80 mm in diameter), with spout. (similar to VWR catalogue number 213-1174).*

- Probe sonicator with the 13 mm probe diameter specified in the NANOREG-ECOTOX dispersion protocol. If the probe diameter deviates from 13 mm, different de-agglomeration efficiency is expected and may result in difficulties in matching the de-agglomeration performance.
    - The probe-sonicator must be placed in a fume hood or under strict local exhaust ventilation to avoid spread and inhalation exposure to aerosolized droplets (nanomaterial dispersions).
- Digital thermometer with measurement accuracy better than ±0.1°C (e.g. Testo 735-2, Testo AG or a thermometer associated with the probe sonicator).
- Powder sample NM200 (Synthetic amorphous silica) for benchmarking de-agglomeration efficiency.
- All chemicals and equipment requested by the NANOREG-ECOTOX dispersion protocol.

**Optimally, the beaker should be thermally insulated, but the reference values for such a beaker have not been established yet and the added benefit is considered minor for short durations of sonication.*

3 Procedure

As mentioned above the calibration procedure is divided into two steps described below.

3.1 Calibration of delivered acoustic dose from calorimetry

1. Draw Nanopure or MilliQ-filtered water (or similar quality with resistivity of 18.2 MΩ cm) into a large glass flask (it is recommended to draw at least 3 L so you have enough for at least 6 runs).

2. Place the large glass flask in the fume hood where the probe sonicator is located until the water reaches the fume hood temperature. This will normally take a few hours.*

3. Fill a 600 mL glass beaker with 500 mL of the thermally equilibrated water
    a. Measure and document the temperature using a digital temperature sensor with an uncertainty of less than 0.1°C.
    b. Carefully, determine the mass of the 500 mL water with an uncertainty of less than ±0.1 g using a top loading balance (tare with empty beaker).

4. Place the 600 mL beaker in the sonicator chamber and immerse the tip of the sonicator probe to a position of 2.5 cm below the liquid surface.
    a. It is recommended to stabilize the beaker using a hook/clamp, so it cannot move during the sonication and measurement (especially at high power). See example in Figure 1.

5. Mount the temperature probe (connected to a temperature meter and data logger depending on the type) at a depth of 2.5 cm and 1 cm away from the sonicator probe.
    a. The temperature should be measured with an uncertainty smaller than 0.1°C.
    b. The temperature probe must be fixed using a clamp so it will stay at the same position and distance from the tip of the probe-sonicator probe during the entire experiment.

6. Select the lowest sonicator output setting with effect (e.g. “amplitude” or “% of amplitude”; usually set by a dial in the sonicator power module). Operate it in continuous mode and record the water temperature increase for the initial 6.5 minutes with a time-resolution of no more than 30 seconds. Always start with the lowest amplitude to avoid potential artefact from heating up of the probe and probe chamber.

7. Increase the amplitude to ca. 20% of the maximum and repeat the test described under point 6 (use a new 600 mL beaker!). Record the water temperature increase for the initial 6.5 minutes with a time-resolution of no more than 30 seconds

Perform the experiment for the start condition twice before continuing to point 7 (use a new 600 mL for each experiment!)

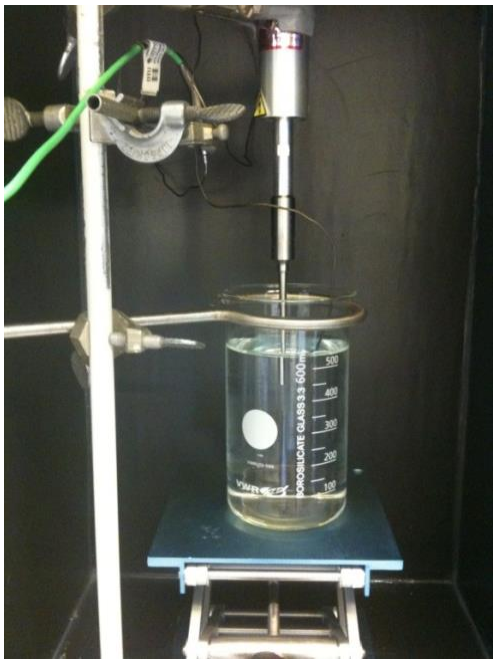

Figure 1. Setup for the measurement of probe sonication calorimetric curves (photo: Kindly provided by Katrin Löschner and Manuel Correia, DTU Food Denmark).

8. Open the Excel file “Template for Probe-Sonicator Calibration”. Enter the requested sonicator data, experimental data and conditions, the recorded temperature and time data for the lowest and ca. 30% maximum amplitude settings in the relevant sheets. This allows automatic plotting and calculation of the delivered acoustic power (Pac).Calculation of delivered acoustic energy using the calorimetric approach:
    a. Perform quality check of the measured temperature profiles from the automatically generated Temperature-time diagrams. Check that the time-temperature curve appears to have the same slope from the first measurement and onwards. The linear fit should have a R^2-value > 0.990 between the measured temperature in °C as function of time in seconds (both repeated data sets) using least squares regression. (Data are illustrated in Figure 2). If these criteria are not satisfied, your temperature reading or equilibration of the water temperature or the probe itself may affect the results and a new data-set may be required.
    b. Based on the slope of the linear regression curve, the delivered acoustic power is calculated according to the equation:

$$P_{ac}(Watt) = \frac{\Delta T}{\Delta t} MC_p ,$$

where $\frac{\Delta T}{\Delta t}$ is the slope of the regression curve, *T* is the temperature (K), *t* is the time (sec), C_P is the specific heat of the liquid (4.18 J/g*K for water), and *M* is the mass of liquid (g). The values for each run are given in cell C31, F31, and I31 in the Excel template and the average with standard deviation is given in cell B33 and B44, respectively.

9. Determine the third setting for estimation of the correct amplitude settings like this:
    a. If the P_{ac} for the lowest and 20% amplitude setting are both lower than the required 7.35±0.05 Watt, project the required amplitude setting (assume linear relationship) to reach the requested 7.35±0.05 Watt. Repeat the test described under point 6 with the amplitude setting obtained by extrapolation twice (use a new 600 mL for each experiment!).
    b. If the P_{ac} for the minimum and 20% amplitude setting is lower and higher than 7.35±0.05 Watt, respectively, interpolate (assume linear relationship) the amplitude setting to achieve the requested acoustic power of the probe. Repeat the test described under point 6 (use a new 600 mL beaker!) with the amplitude setting obtained by interpolation extrapolation twice (use a new 600 mL for each experiment!).
    c. If the P_{ac} for the lowest amplitude setting is higher than 7.35±0.05 Watt, then calculate the sonication time (t in s) to deliver a total energy of 7056±103 J using the equation: t(s) = 7056 J/P_{ac}(W). Use the P_{ac} value which was determined for your lowest amplitude setting for the calculation (You may use the sheet “Amplitude and time estimation” in the Excel template for your calculations). Then proceed to step 2; performance testing in Section 3.2 using the calculated sonication time and the lowest amplitude setting (Section 3.2). Note: The 7056±103 J corresponds to the total delivered acoustic energy in the ENPRA and NANOGENOTOX dispersion protocols.

10. Enter the requested sonicator data, experimental data and conditions, the recorded temperature and time data for the third amplitude setting tested in point 9 in the empty sheet of the “Template for Probe-Sonicator Calibration” to automatically plot and calculate the delivered acoustic power (P_{ac}) for this last setting. Again P_{ac} for each run are given in cell C31, F31 (and I31) in the Sonicator Calibration Excel template and the average with standard deviation is given in cell B33 and B44, respectively.

11. Go to the sheet “Amplitude and Time Estimation” in the “Template for Probe-Sonicator Calibration” Excel file to identify the setting required to deliver Pac = 7.35±0.05. Watt. The “Amplitude Estimation” sheet contains the calculated Pac and a column for your amplitude settings and a graph with a regression curve for the amplitude versus the Pac in your test (see example Figure 3).

    Check that the settings entered are correctly displayed (percent, absolute amplitude or normalized arbitrary scale 1, 2, 3, 4, ….. for numeric or letter scales) and continue when the

format is acceptable for plotting and calculations (Check that the values are correctly displayed in the template plot and for calculations of the settings.)

Decision tree for selection of amplitude settings

a. If the sonicator is able to produce a Pac = 7.35±0.05 Watt, use the calculated output setting and proceed to step 2; performance testing (Section 3.2).

b. If the maximum amplitude setting of the sonicator does not reach P_{ac} = 7.35±0.05 Watt, calculate the sonication time (t in s) to deliver a total 7056±103 J using the equation t(s) = 7056 J/P_{ac}(W). Use the P_{ac} value which was determined for your highest amplitude setting for the calculation (You may use the sheet "Amplitude and time estimation" in the Excel template for your calculations). Then proceed performance testing using the calculated sonication time and the highest amplitude setting (Section 3.2). 7056±103 J corresponds to the total delivered acoustic energy in the ENPRA and NANOGENOTOX dispersion protocols over a 16 min period.

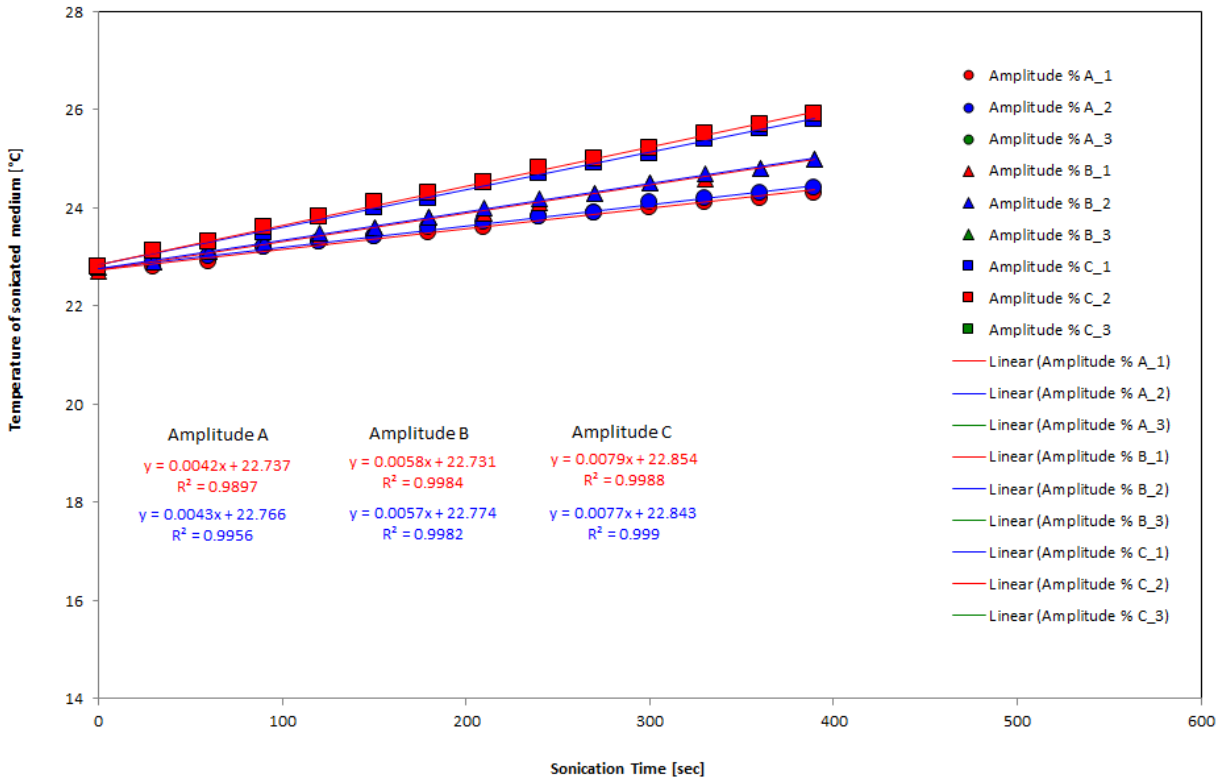

Figure 2. Temperature increase as function of sonication time using a 20kHz Branson S-450D (Branson) with a 13 mm disruptor horn at different sonicator probe amplitude (10, 15 and 20%) settings.

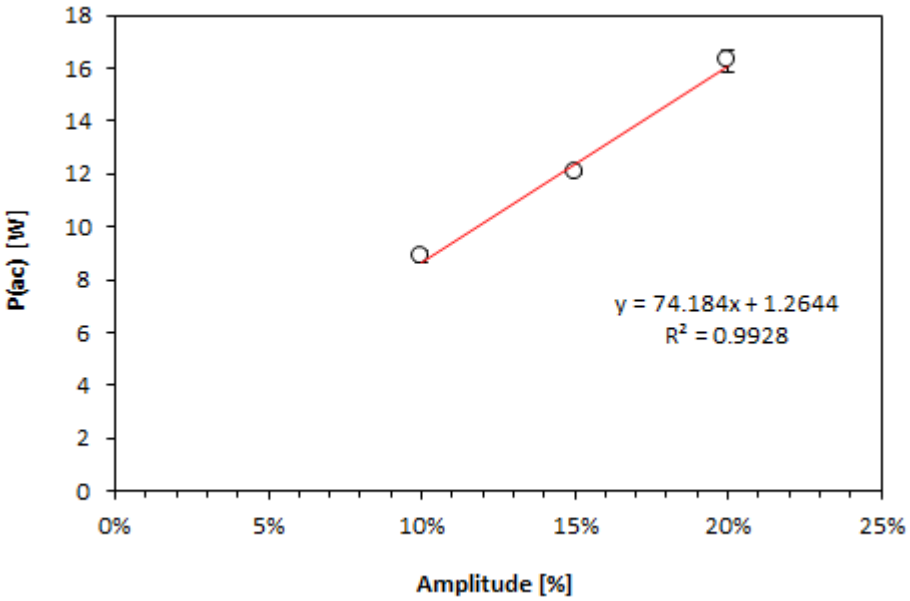

Figure 3. Probe amplitude setting plotted as function of calculated delivered acoustic sonication power P_{ac} (in this example: percent amplitude for the Branson 450S). Error bars indicate the standard deviations in the experiment. The linear regression function can be used to calculate the setting required to reach the required delivered acoustic sonication power (in this case the Branson must be set to 10% amplitude and operated for 13 min 16 sec to deliver 7.35±0.05 Watt and an accumulated dose of 7056±103 J to the batch dispersion).

3.2 Calibration of de-agglomeration performance from benchmark data

After calibration of the probe sonicator amplitude (and potentially recalculating the duration of sonication) from calorimetric tests, a subsequent test is used to calibrate the effective de-agglomeration efficiency of NM200 (synthetic amorphous silica) following the NANoREG-ECOTOX dispersion protocol to be used. Based on the test results, the sonication time is adjusted until the accepted test results on three different sample vials show less than 12.5% deviation from the benchmark values in the hydrodynamic zeta-average diameter (Z_{ave}) and a polydispersivity index (PDI) not exceeding the ranges indicated below.

1. A batch dispersion of the NM200 is prepared following carefully the NANOREG-ECOTOX dispersion protocol. (Remember to be careful about the immersion depth of the sonication probe).

2. Program the manual measurement on the DLS before the samples are ready for analysis to reduce the waiting time from end of sonication to start of measurement.

    a. Enter the optical parameters for NM200 (refractive index = 1.544; absorption = 0.20)

    b. Select water as the medium

c. Set the temperature to 25°C and equilibrate the samples for 300 seconds before measurement

d. Select automatic for all measurement conditions; position, laser attenuation, number of runs and sub-runs, respectively. Do NOT select extended measurement time for large particles.

e. Enter that 10 repeated measurements should be made on the sample without pause

f. Use the general purpose algorithm for calculation of the DLS spectra

3. Immediately draw 1.0 mL of the NM200 batch dispersion by pipette and dose it into a polystyrene DLS cuvette.

4. After 5 minutes stabilization, insert the DLS cuvette in the Malvern DLS, name the sample: NM200NETX,##Watt;##Ampl,##min-vial###, where the ## are to filled out with your specific conditions.

    a. Example of the sample name given depending on protocol and information given on the probe amplitude.

        i. NM200NETX,BransonS-450D,300Watt;10%Ampl,13.25min-vial####

5. Press start to conduct the DLS analysis within 5 to 10 minutes after sonication so the total waiting time from end of sonication and end of thermal equilibration in the DLS is 10 to 15 minutes.

6. Repeat the experiment on two other NM200 vials.

7. Average the 10 repeated measurements for each of the three vials. You should obtain an average DLS intensity-weighted size-distributions comparable to the ranges one shown in Figure 4. If you do not get comparable spectra, be aware that the sonication may have accidentally failed, you may have outliers (due to variability in handling or the material), or your effective sonication efficiency is not yet within range. If a single outlier is observed, make a new dispersion to check if the problem is solved.

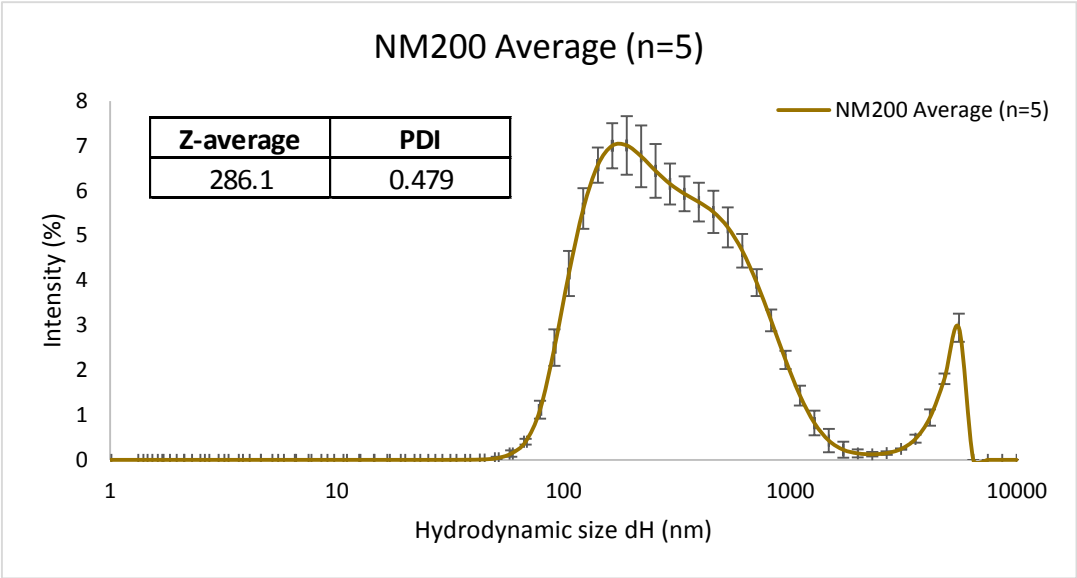

Figure 4. Hydrodynamic intensity-derived size-distribution of NM200 Milli-Q water by probe-sonication according to the NANoREG ECOTOX dispersion protocol (n=5). It is seen that a minor fraction of µm-size particles are present in the dispersion. This is unavoidable with the present powder material (and in fact most powder materials). The fraction of µm-size particles is, however, low and the size-spectrum is stable over time.

NB. Data in Figure 4. Were generated using a Branson S-450D (Branson) with a 13 mm disruptor horn operated at an amplitude of 15% for 13.15 min. These operating conditions were determined using the above procedure.

8. Evaluate the results for acceptance
    a. Calculate the mean average hydrodynamic zeta-size ($Z_{ave,mean}$) and the mean average PDI (PDI_{mean}) of the 10 repeated dispersion measurements in the three vials (i.e. the total data set is 30 measurements).
    b. Qualification of the sonication procedure
        i. If $Z_{ave,mean}$ lies between 260 and 310 nm and the PDI_{mean} is lower than 0.500, the calibration of the probe sonication is approved.
        ii. If $Z_{ave,mean}$ is smaller than 260 nm and the PDI_{mean} is greater than 0.500, the dispersion may be unstable and/or too polydisperse for comparability. Try to increase the duration of sonication and test if this change improves the comparability with the NM200 benchmark data. Continue until you meet the acceptable range. Aim for the mid-point values 285 nm and a PDI better than 0.500.
        iii. If $Z_{ave,mean}$ is smaller than 260 nm and the PDI_{mean} is smaller than 0.500, the de-agglomeration efficiency is too high. Try to reduce the duration of sonication and test if this change improves the comparability with the NM200 benchmark data. Continue until you meet the comparability requirements. Aim for the mid-point values 285 nm and a PDI better than 0.500. If the

target values are not reached, try to reduce the amplitude, if possible, and adjust the sonication time accordingly.

iv. If $Z_{ave,mean}$ is larger than 310 nm, the dispersion is too coarse. Try to increase the duration of sonication and test if this change improves the comparability with the NM200 benchmark data. Continue until you meet the comparability requirements. If the target range is not reached try to increase the amplitude, if possible.

v. If the target value cannot be reached, please consult.

9. End of probe calibration

4 Submission of calibration data

After completion of the calibration test, please send information on your specific probe sonicator, the probe applied, and your data for both the calorimetric calibration and the NM200 de-agglomeration test to andy.booth@sintef.no.

Templates will be provided for submitting the information from the calorimetric calibration tests.

DLS data will be submitted as the raw DTS files with a simple word or excel LOG describing the experiments, data-files and the data.

All contributors will co-author the intended scientific publication under lead by Keld Alstrup Jensen and Andy Booth. The tentative work title is: Testing a practical procedure for calibration the de-agglomeration performance of probe sonicators.

5 References

Jensen KA, Kembouche Y, Christiansen E, Jacobsen NR, Wallin H, Guiot C, Spalla O, Witschger O. Final protocol for producing suitable MN exposure media. Edited by Jensen KA and Thieret N, NANOGENOTOX deliverable report n°3: June 2011, 32 pp. http://www.nanogenotox.eu/index.php?option=com_content&view=article&id=136&Itemid=158

Taurozzi J, Hackley V, Wiesner M. Preparation of nanoparticle dispersions from powdered material using ultrasonic disruption. *National Institute of Standards and Technology*. 2012. Available at: http://nvlpubs.nist.gov/nistpubs/specialpublications/nist.sp.1200-2.pdf.