Session 3
Addressing gaps for conducting OECD TG 201, 202 and 203 assays with ENMs

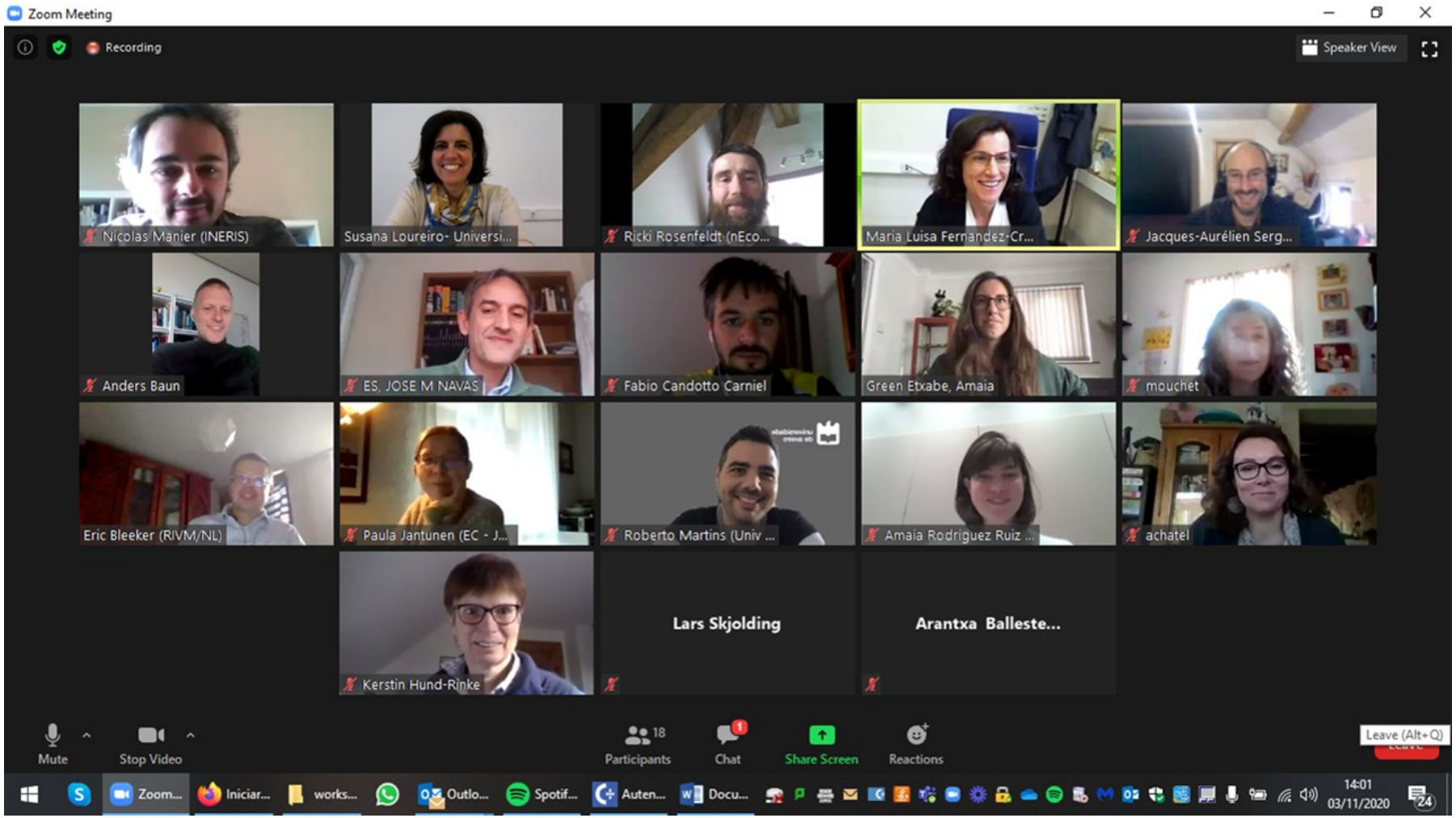
INIA, INERIS, UAVR

NanoHarmony

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

23 experts – Thank you!

NanoHarmony



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Main points adressed

NanoHarmony

General for the three TGs

- ✓ Procedures for preparing test dispersions/suspensions and series of concentrations
- ✓ Stability of the dispersions/suspensions
- ✓ Expression of results

Specific ones (mainly for TG 201, 203)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Procedures for preparing test dispersions and series of concentrations

NanoHarmony

OECD
Organisation for Economic Co-operation and Development

ENV/JM/MONO(2020)8

Unclassified — English - Or. English
20 July 2020

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

GUIDANCE DOCUMENT ON AQUATIC AND SEDIMENT TOXICOLOGICAL TESTING OF NANOMATERIALS

Series on Testing and Assessment
No. 317

Figure 4. Examples of methods for spiking Manufactured Nanomaterials into test media in the exposure vessels.

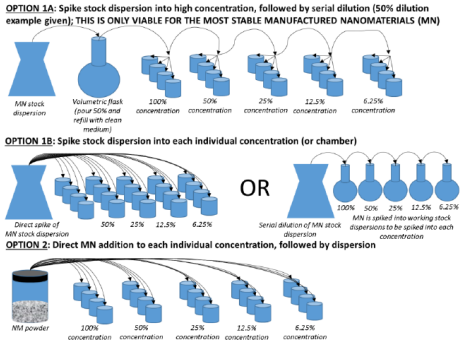
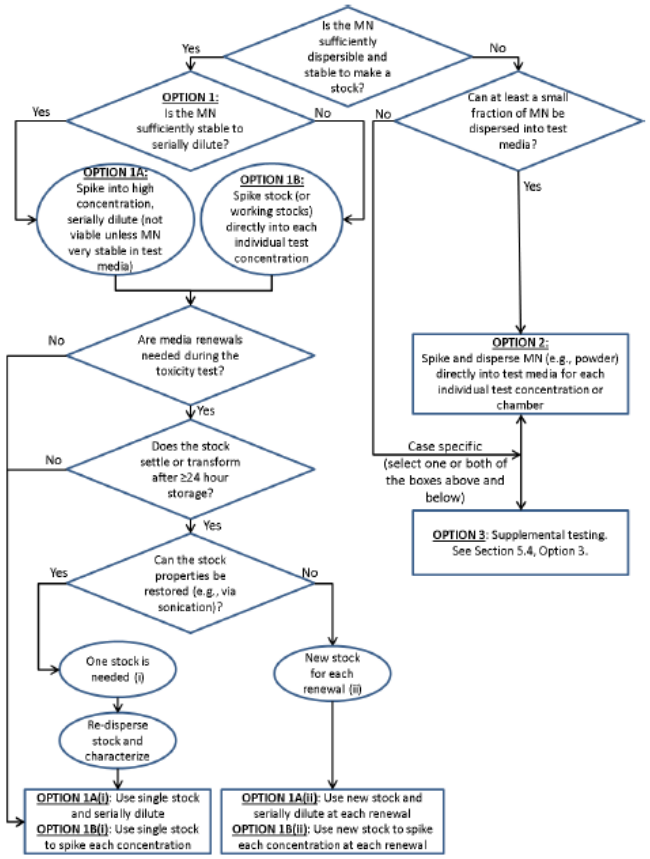


Figure 3. Flowchart to inform decisions on how to prepare MNs stock dispersions and spike MNs into test media.



OECD
Organisation for Economic Co-operation and Development

ENV/JM/MONO(2020)9

Unclassified — English - Or. English
20 July 2020

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

GUIDANCE DOCUMENT FOR THE TESTING OF DISSOLUTION AND DISPERSION STABILITY OF NANOMATERIALS AND THE USE OF THE DATA FOR FURTHER ENVIRONMENTAL TESTING AND ASSESSMENT STRATEGIES

Series on Testing and Assessment
No. 318

Other documents:

ECETOC Technical report from dec 2018 : TR 132 – An evaluation of the challenges and limitations associated with aquatic toxicity and bioaccumulation studies for sparingly soluble and manufactured particulate substances
https://www.ecetoc.org/publication/tr-132-an-evaluation-of-the-challenges-and-limitations-associated-with-aquatic-toxicity-and-bioaccumulation-studies-for-sparingly-soluble-and-manufactured-particulate-substances/

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Procedures for preparing test dispersions and series of concentrations

NanoHarmony

Needs for appropriate test media/dilution water

Main Aim: to enhance the stability of the test suspension, thus enabling a more reproducible result and exposure.

For regulatory purposes: the existing media should be used as much as possible to enable comparison between already existing data and new data, and also to enable the process for Read Across or QSAR modeling

Some recommendations:

- ✓ remove EDTA (already advised for metal ecotoxicity testing);
- ✓ use the usual media but in case you need to change, start by changing pH, then hardness and DOM content.
- ✓ NOM or DOM- avoid and only in specific cases (check Section 6: Conduct of the test, from Series Testing Assessment 317- Guidance Document)
- ✓ conditioning the medium with organisms for 24 hours before use (specific for daphnids, but may be interesting for other organisms).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Improvements for the test procedures to infer on comparable results (stable suspensions)

NanoHarmony

Characterize exposure: distinction between forms (e.g. ions, particles, solutes, complexes)

- ✓ DLS is not the best procedure - premise that all particles are spheres, measurement with light methods, influenced by particle size distribution.
- ✓ Media characterization with organisms: methods might not be able to discriminate between particles and those molecules/particles excreted.
- ✓ Add controls without organisms as a good principle to characterize exposure in time, and important for regulatory purposes. Check behavior, size distribution, in time.
- ✓ Different methodologies should be used to envisage an accurate exposure characterization.
- ✓ Analytical monitoring of exposure (initial, during, after test), at the end of the test, make a mass balance to see what was internalized/attached to the test organism or devise (aquarium, flask) and what was left in suspension.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Improvements for the test procedures to infer on comparable results (stable suspensions)

NanoHarmony

- ✓ **Surfactants:** avoid as much as possible. Even using surfactant controls, potential mixture toxicity effects may occur by interacting at the exposure (e.g. release of ions) or at the effect level.
- ✓ **Aquaria and flasks** should be chosen considering a stable exposure (low adsorption).
- ✓ **Limit test:** Limit 100 mg/L- we should be cautious, and let it depend on the range of concentrations where you can get stability and low aggregation. It can be that lower concentrations are toxic if the aggregates differ between concentrations.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Expression of results

NanoHarmony

- ✓ Mean concentration, particle number and surface area
- ✓ Mean concentration is essential for comparison with other data, but others like particle number and surface area may add value dependent of the NM specificity.
- ✓ Mechanical/physical effects should be reported.
- ✓ Distinguish between what is mandatory for regulatory purpose and what is good to have as an additional observation/endpoint

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Specific assays – OECD 201 main gaps

NanoHarmony

- Exposure system (Flask, volume, stirring procedure…)
  - ✓ Some concerns raised regarding the use of microplates (small volume, limited hydrodynamic and sedimentation);
  - ✓ The stirring parameters (rotary shaker or magnetic stirring) need to be more prescriptive
  - ✓ Smaller volume may be helpful to avoid the shading effect

- Algal biomass estimation → need to determine the limitation of the current methods
  - ✓ Direct biomass measurement is not appropriate and should be avoided;
  - ✓ Direct cell counts have some drawbacks depending on the NMs and their ability to interact and form hetero-agglomerates
  - ✓ Indirect estimation by autofluorescence measurement has limitations depending on the NMs tested and its concentration

  → Need to determine the limitations of the current methods

  → Measurement of the chlorophyll *a* concentration → good alternative to overcome the limitation due to hetero-agglomeration

  → Additional pre-studies may be needed for indirect counting methods based on autofluorescence *in vivo*

  → Decision tree to choose the most appropriate methods according to the NMs tested may be helpful

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Specific assays – OECD 203

NanoHarmony

Flow-through systems

- ✓ Flow through systems for exposure can bring several problems, like distributing unequally NM in aquaria or adsorption in tubes.
- ✓ To control exposure during testing, look at suspended, dissolved and settled particles, to infer on which will be the most responsible for the effects (this is also equally true for the other test systems in 201 and 202)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931