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

Webinar 16 December 2020

Data requirements in Test Guideline and Guidance Document development for nanomaterials – next generation delivery of standards

Presenter:
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NanoHarmony Project
Task 1.9

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Task 1.9 Scientific basis for the development of technical recommendations for conducting assays with ENMs according to OECD TG 201, 202, 203.

***OECD TG 201*: Freshwater Alga and Cyanobacteria, Growth Inhibition Test**

***OECD TG 202:* Daphnia sp. Acute Immobilisation Test**

***OECD TG 203:* Fish, Acute Toxicity Test**

Task leader: INIA (National Institute of Agriculture and Food Research and Technology, Spain)
Partners: INERIS (French National Institute for Industrial Environment and Risks, France)
UAVR (University of Aveiro, Portugal)

Generate sufficient information and scientific evidences to support the development of guidance to be included as annexes to the OECD GD nº 317 July 2020 “Guidance Document on Aquatic and Sediment Toxicological Testing of Nanomaterials”

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Identification of difficulties and solutions Questionnaire to 31 experts

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ADAPTATIONS OF TG 201 ALGAL GROWTH INHIBITION TEST

a) Modification of the test medium described in OECD TG 201 or in the preparation of suspensions?
b) Adaptation of the test vessels?
c) Adaptation of the agitation system?
d) Modification of the lighting system?
e) Methods to determine algal biomass or concentration: microscope (manual cell counting using a hemocytometer), electronic particle counter, flow cytometer, determination of fluorescence *in vivo*, optical density, determination of Chlorophyll a *in vitro,* other?
f) Do you use extra controls besides the ones required in the TG (e.g. interaction of nanomaterials with algae, assessment of interferences in biomass measurements)?
g) expression of the exposure concentration: mass concentration, surface area, particle number concentration, particle size distribution, other?
h) Please add any other comment you think is relevant

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Identification of difficulties and solutions Questionnaire to 31 experts

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ADAPTATIONS OF TG 202 DAPHNIA SP. IMMOBILIZATION TEST

a) Modification of the test medium described in OECD TG 202, or in the preparation of suspensions?
b) Modification of the test vessels?
c) Modification of the overall exposure system (photoperiod, static, etc)?
d) Do you use extra controls besides the ones required in the TG?
e) Expression of the exposure concentration: mass concentration, surface area, particle number concentration, particle size distribution, other?
f) Please add any other comment you think is relevant

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Identification of difficulties and solutions Questionnaire to 31 experts

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ADAPTATIONS OF TG 203 FISH ACUTE TOXICITY TEST

a) Specific conditions of the aqueous media (hardness, pH, organic substances, others) to obtain stable suspensions?
b) Adaptation of the aquarium material?
c) Introduction of agitation systems?
d) Appropriateness of the limit test in non-stable suspensions?
e) Considerations on the stability/transformation of the nanomaterials within the 96 h exposure?
f) Criteria for the renewal of water in semi-static or flow-through conditions within the 96 h exposure?
g) Do you use extra controls besides the ones required in the TG?
h) Expression of the exposure concentration: mass concentration, surface area, particle number concentration, particle size distribution, other?
i) Please add any other comment you think is relevant

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Outcomes of the workshop

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Main points adressed

General for the three TGs

1) Procedures for preparing test dispersions/suspensions and series of concentrations

2) Stability of the dispersions/suspensions

3) Expression of results

Specific ones for TG 201

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1) Procedures for preparing test dispersions and series of concentrations

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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.

Main Conclusion for Regulatory Purposes:

The existing and recommended media in the test guidelines 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.

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1) Procedures for preparing test dispersions and series of concentrations

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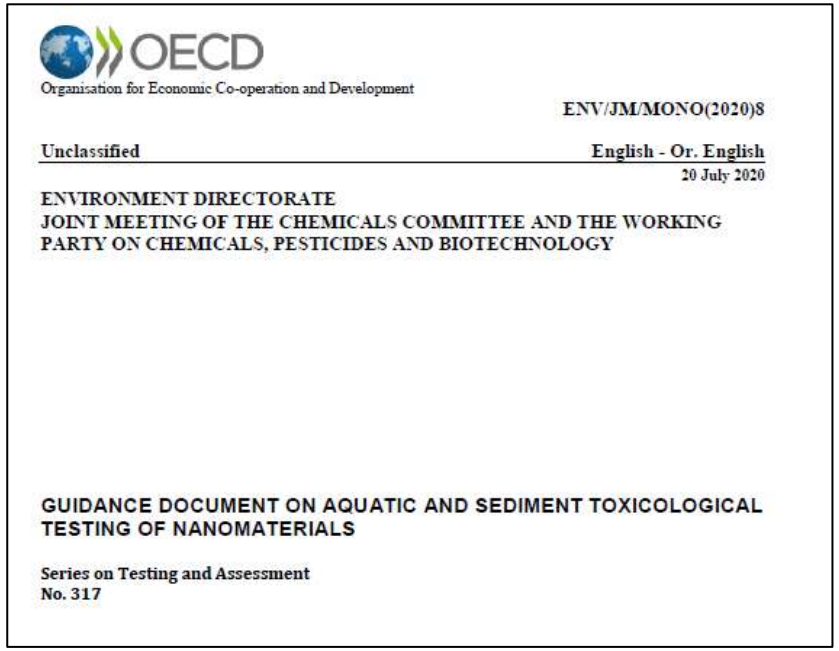
Some recommendations

Two Guidance Documents

as start points



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ENV/JM/MONO(2020)8

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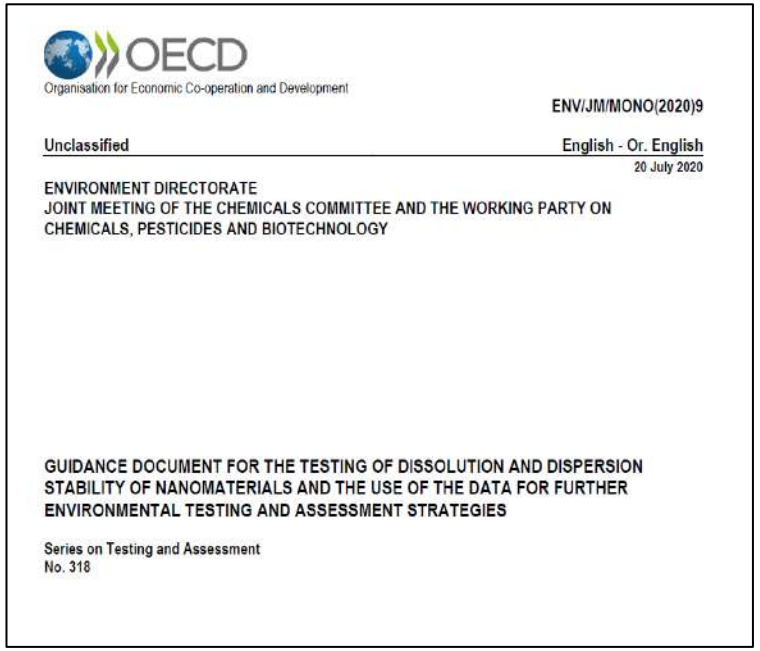
20 July 2020

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



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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

- ✓ 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)

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2) Stability of the dispersions/suspensions

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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 size distribution during the exposure period
- ✓ Different methodologies should be used to envisage an accurate exposure characterization
- ✓ Analytical monitoring of exposure (initial, during and at the end of the test): make a mass balance to see what was internalized/attached to test organism or devise (aquarium, flask) and what was left in suspension.

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2) Stability of the dispersions/suspensions

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Some recommendations

- ✓ 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.
- ✓ Vessels should be selected to limit adsorption
- ✓ Flow through systems for exposure can bring several problems, like distributing unequally NM in aquaria or adsorption in tubes.
- ✓ 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.

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3) Expression of results

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- ✓ 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.

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Specific assays – OECD 201 main gaps

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Exposure system (Flask, volume, stirring procedure…)

✓ Some concerns raised regarding the use of microplates (small volume, limited hydrodynamic, 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: some drawbacks depending on NMs and their ability to form hetero-agglomerates

✓ Indirect measurement by autofluorescence: limitations depending on the NMs tested and its concentration

→Measurement of the chlorophyll *a* concentration → may be a 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

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THANK YOU

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QUESTIONS, REMARKS, SUGGESTIONS WELCOMED

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