A common European approach to the regulatory testing of nanomaterials

Development of an aquatic Mesocosms Platform Deliverable 3.5

Introduction

Current strategies for assessing the environmental safety of engineered nanomaterials (NMs) are largely based on ecotoxicology approaches focusing on the exposure of a single species or model organism to such materials. However such strategies separate the organism from the environment and community of organisms (*i.e*, the ecosystem) in which the environmental risks of NMs ultimately need to be understood. It therefore neglects the interplay of parameters such as aggregation state, sorption of (in)organic substances, oxidation-reduction potential, as well as ecological factors such as e.g. interacting organisms like microorganisms, and plants.

To achieve a more robust characterization of risk, the complex ecosystem-level interplay between the organisms, their environment, and the NMs needs to be taken into account. This can be done by the use of a mecocosm: *"an experimental system that simulates real-life conditions as closely as possible, while allowing the manipulation of environmental factors".*

This deliverable reports on the results of testing the applicability of laboratory-scale mesocosm facilities to serve as a platform for investigating the effects and impact of exposure of ecosystems to nanomaterials.

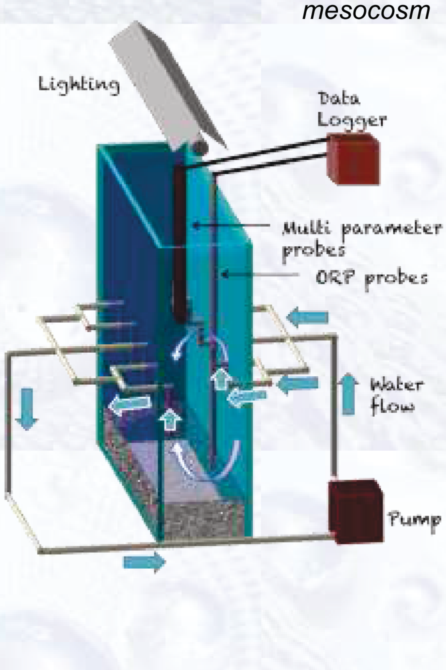
Description of Work

Three different types of mesocosms have been tested: a Freshwater mesocosms of 3 Liters and 60 Liters and a marine water mesocosm of 60 Liters. To take into account the various stages of the life cycle, the mesocosms were exposed to different pristine nanomaterials (CeO_2, TiO_2, Ag and ZnO) as well as to formulation products and released materials from nano-enabled products (diesel additive, Cement and PET and PP film.

The duration of the experiments varied from 20 days to 28 days; some with only a single pulse dosing, others with a multiple dosing.

Physical and chemical variables like temperature, pH, temperature and oxygen were measured frequently. Biological parameters like e.g. population changes and the distribution of nanomaterials (more specific the distribution of the added metals) over the different compartments also have been measured although less frequent.

Figure: 60 Liter Marine water mesocosm



Main results and evaluation

Feasibility of mesocosms for risk assessing

The developed experimental design, provides more realistic conditions of exposure by taking into account transformation of ENM; an aspect that has proven to be critical in assessing the potential and is missing many studies. It was successfully shown that mesocosms can be used to simulate a ENMs runoff rain or vent loading (e.g. single pulse) versus a continuous point source discharge such as wastewater treatment plant or industrial discharge (e.g. multiple dosing). A good reproducibility could be achieved across the 9-mesocosm setup on one site but also between two sites.

Such versatility and reproducibility allows for contrasting different environmentally relevant exposure scenarios. By creating representative conditions for environmental transformation and ecosystem exposure, such platform facilitates the integration of the ecotoxicological data into an environmental risk assessment model related to nanotechnologies based on reliable exposure and impact data.

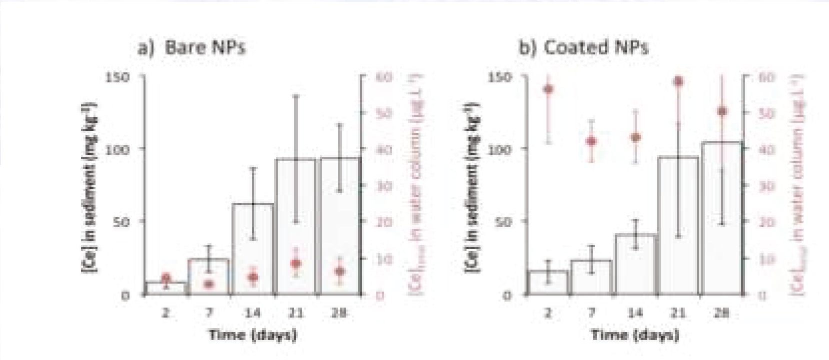
The complexity of food web and the species diversity in mesocosms is smaller than in real ecosystems. This exacerbates the exposure and impact in mesocosms compared to real natural environments. Consequently, mesocosms will provide the upper limit of the risks expected in the mimicked ecosystems.

Effects of investigated nanomaterials on the ecosystem

Biological assays performed with the experimental design have successfully shown that it was possible to assess the effects on benthic and planktonic invertebrates and microorganisms. Using a battery of biomarkers and (micro) biological assays at the individual, sub-individual, and community level, it was possible to observe effects in terms of oxidative stress and also changes in microbial diversity. For the first time these biological responses could be correlated to the transformation of the ENMs and the exposure pathways.

The work performed in the WP3 of NANoREG and presented in the deliverable 3.5 focused on the exposure assessment and not on the effect towards the organisms or on the ecosystem. Consequently, it was out of the scope of the work carried out to provide concrete results about the biological or ecological effects of the nanomaterials tested.

Differences in effects between pristine material, formulation products and released material from nano-enabled products



Distribution of Ce between water column and sediment

By thoroughly characterizing the exposure of the organisms and the transformation of ENMs in more realistic conditions, interesting different behaviours as a function of the ENMs life cycle stage could be observed. It was found that the kinetics and mechanisms of physico-chemical transformation were life cycle stage-dependent.

CeO_2 ENMS are a good example of ENMS for which the mechanisms and kinetics of transformation depends on the life cycle stages. Aggregation was found to be faster for the combusted diesel additives (because of size increase during combustion and the degradation of the organic matrix), compared to pristine nanoparticles, and citrate-formulated nanoparticles (which remained longer in the water column because of the negatively-charged surface). The dissolution also was influenced by size and surface properties. Because of their large specific surface area and the citrate-enhanced dissolution, the formulated nanoparticles were the more chemically unstable, followed by the pristine nanoparticles and the combusted diesel additives (which have the lowest specific surface area, and the slowest kinetics of dissolution).

Since these transformations govern the exposure of organisms (micro- versus macro-, benthic versus planktonic, filter feeders versus grazers…), it provides valuable information about the organisms that have to be tested in priority. However, as previously stated, it was out of the scope of the work carried out in the WP3 of NANoREG to provide concrete results about the biological or ecological effects.

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

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