## Methods Harmonization for Nanomaterial Testing

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## FIGURE 1

Daphnia magna exposed to TiO2 (NM 101,104) (TG 202), with details on the full gut and NMs' attachment to antenna.

## FIGURE 2

Effective dispersion and contamination procedure of fish feed for the TG 203 toxicity test with fish. Procedure involves accurate sonication, dispersion and stabilization of NMs in fish feed.

The nanotechnology's success in applying nanomaterials (NMs) in several products may lead to their release into the environment. Consequently, there is an increasing need to evaluate their potential environmental and human risks. However, the wide variety and distinct physicochemical properties of NMs make their environmental risk assessment very challenging. The existing guidelines do not account for all the nano-specificities, leading to insecurities and uncertainties regarding NM's hazard. Several problems have been reported when using aquatic ecotoxicity Test Guidelines (TG) to nanomaterials (NMs) required in REACH, CLP and other regulations leading to the need to adapt the protocols of the TG 201 Freshwater Alga, Growth Inhibition Test; TG 202 Daphnia sp. Acute Immobilization test; TG 203 Fish, Acute Toxicity Test.



Therefore an overarching aim has been set to harmonize experimental methods to enlarge the scientific basis for the eco-toxicological testing of NMs and generate technical recommendations. This will also support the development of guidance included as annexes to the "Guidance Document on aguatic and sediment toxicological testing of nanomaterials" (OECD Guidance Document N.º 317) and in the work programme of the OECD. For that, the identification and collation of data, protocols, and SOPs, and improvements to the TGs for NMs testing have been carried out in different laboratories to check appropriateness, in close collaboration with a working group at OECD, using several JRC NM references: TiO<sub>2</sub> (NM 101, 104), ZnO (NM 110, 111), SiO<sub>2</sub> (NM200), MWC nanotubes (NM400, 401), BaSO<sub>4</sub> (NM220) and Bentonite (NM600). This approach includes the exposure characterization and the close relation with the induced effects. For the Daphnia TG 202, NMs stability and behaviour during exposure are tested and advised to adapt accordingly. The feed contamination characterization of the fish TG 203 is being assessed to ensure stability during exposure.

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