

## **The challenge of ENMs in seawater: the case of nTiO<sub>2</sub> and the main knowledge gaps.**

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Engineered nanomaterials are at the forefront of ecotoxicologist agendas due to their increasing use in a broad range of industrial and domestic sectors. Actually, they are manufactured in increasing amounts year-by-year. Particularly, nanoscopic titanium dioxide (nTiO<sub>2</sub>) is adopted by a variety of industries mainly for catalysis and photocatalysis applications and as an additive in paints, papers, inks, plastics and various consumer products. So that it can be supposed that such widespread use of nTiO<sub>2</sub> may result to an increased environmental exposure that may reach unnegligible concentrations in surface waters posing a potential significant threat to aquatic ecosystems. Most part of literature on the ecotoxicity of nTiO<sub>2</sub> deals with aquatic organisms such as bacteria, algae, invertebrates, and fish, but there are also case studies considering cell lines and rodents. Mostly, testing species are from freshwaters. Little knowledge is available about saltwater species such as mollusc bivalves. For example, within our recent research activity on nTiO<sub>2</sub>, embryo toxicity effects were highlighted for both dark and light/dark scenarios (*in vivo* 48 h exposure of *Mytilus galloprovincialis* zygotes).

Nevertheless, data about potential seawater effects are still limited and hard to understand and compare due to a lot of gaps in the knowledge. Practically, the scientific community has just started tackling with various seawater exposure scenarios and does not yet agree upon neither the way the exposure is carried on nor how ENMs are characterised during testing. For example, taking into consideration the ENMs dispersing methods authors might suggest behaving naturally, using synthetic or natural dispersing agents, sonicating, stirring or mixing. Moreover, many analytical challenges have to be solved. For example, the complete characterization of ENMs in exposure media and organisms has to be achieved, because it is extremely relevant for comparative purposes, and the quantification methods currently applied for ionic chemical species might have to be revised and validated. Indeed, it seems that ENMs tend behaving in specific ways that could not be approached in the traditional way.