Effects of sublethal concentrations of fresh, aged TiO_2 and its bulk counterpart on marine mussels, *Mytilus* sp.

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After their release into the environment, engineered nanoparticles (ENPs) age and tend to form aggregates, which might increase their size and could make them less mobile. There is, however, limited data on how these physico-chemical changes alter the potential of ENPs to induce biological damage. In this experiment, we studied the biological responses of a marine bivalve, *Mytilus sp.* at sublethal concentrations of an environmentally relevant ENP, titanium dioxide (TiO_2). Mussels were collected from a UK reference site, acclimatised, and exposed to 10 mgL^{-1} of titanium dioxide (TiO_2), in either fresh ENP, aged ENP or bulk form, for 96h. Copper (40 μgL^{-1}) was used as a positive control.The ENPs and bulk TiO_2 were characterised using TEM. The biological responses included oxidative DNA damage, using the enzyme-modified comet assay, histopathological and histochemical analysis, metallothionein gene expression and fluorescent in situ hybridization, in gills and digestive glands. In addition, accumulation of metals in different tissues was determined using ICP-OES. Although the aged and fresh$nTiO_2$ showed significantly higher accumulation compared to bulk counterpart, the biological effects of several analyses suggest that the bulk form is more toxic than the two nano forms of TiO_2 (i.e. fresh or aged) used in the study. For oxidative DNA damage, however, all the treatment groups showed similar levels of damage compared to untreated mussels, suggesting a saturation level of induced DNA damage in these cell types for the concentration of TiO_2 used. Our integrated study suggests that for this ecologically relevant organism photo-catalytic aging of $nTiO_2$ does not significantly alter toxicity, and that bulk TiO_2 may be less ecotoxicologically inert than previously assumed.