

Evaluation of Toxicity of Cerium Oxide on Zebrafish Developmental Stages

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Abstract : Engineered Nanoparticles (ENPs) and Nanomaterials (ENMs) concern an active research area and a sector in full expansion. They have physical-chemical characteristics and small size that improve their performance compared to common materials. Due to the increase in their production and their subsequent release into the environment, new strategies are emerging to assess risk of nanomaterials. NPs can be released into the environment through aquatic systems by human activities and exert toxicity on living organisms. We evaluated the potential toxic effect of cerium oxide (CeO₂) nanoparticles because it's used in different fields due to its peculiar properties. In order to assess nanoparticles toxicity, Fish Embryo Toxicity (FET) test was performed. Powders of CeO₂ NPs supplied by the CNR-IMM of Catania are indicated as CeO₂ type 1 (as-prepared) and CeO₂ type 2 (modified), while CeO₂ type 3 (commercial) is supplied by Sigma-Aldrich. Starting from a stock solution (0.001g/10 ml dilution water) of each type of CeO₂ NPs, the other concentration solutions were obtained adding 1 ml of the stock solution to 9 ml of dilution water, leading to three different solutions of concentration (10⁻⁴, 10⁻⁵, 10⁻⁶ g/ml). All the solutions have been sonicated to avoid natural tendency of NPs to aggregate and sediment. FET test was performed according to the OECD guidelines for testing chemicals using our internal protocol procedure. A number of eight selected fertilized eggs were placed in each becher filled with 5 ml of each concentration of the three types of CeO₂ NPs; control samples were incubated only with dilution water. Replication was performed for each concentration. During the exposure period, we observed four endpoints (embryo coagulation, lack of formation of somites, failure to lift the yolk bag, no heartbeat) by a stereomicroscope every 24 hours. Immunohistochemical analysis on treated larvae was performed to evaluate the expression of metallothioneins (MTs), Heat Shock Proteins 70 (HSP70) and 7-ethoxyresorufin-O-diethylase (EROD). Our results have not shown evident alterations on embryonic development because all embryos completed the development and the hatching of the eggs, started around the 48th hour after exposure, took place within the last observation at 72 hours. A good reactivity, both in the embryos and in the newly hatched larvae, was found. The presence of heartbeat has also been observed in embryos with reduced mobility confirming their viability. A higher expression of EROD biomarker was observed in the larvae exposed to the three types of CeO₂, showing a clear difference with the control. A weak positivity was found for MTs biomarker in treated larvae as well as in the control. HSP70 are expressed homogeneously in all the type of nanoparticles tested but not too much greater than control. Our results are in agreement with other studies in the literature, in which the exposure of Danio rerio larvae to other metal oxide nanoparticles does not show adverse effects on survival and hatching time. Further studies are necessary to clarify the role of these NPs and also to solve conflicting opinions.

Keywords : Danio rerio, endpoints, fish embryo toxicity test, metallic nanoparticles

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