

# Toxic effects caused by a long-term exposure of *Danio rerio* to TiO<sub>2</sub> nanoparticles.

Roberta Pecoraro<sup>1\*</sup>, Giuseppina Camiolo<sup>1</sup>, Angeliki Droutsa<sup>2</sup>, Daniele Tibullo<sup>1</sup>, Maria A. Buccheri<sup>3</sup>, Giuliana Impellizzeri<sup>3</sup>, Maria V. Brundo<sup>1</sup>, Fabio Marino<sup>4</sup> and Vittorio Privitera<sup>3</sup>

- <sup>1</sup> University of Catania (Italy), Department of Biological, Geological and Environmental Sciences, Italy
- <sup>2</sup> Agricultural University of Athens, Greece
- <sup>3</sup> CNR-IMM, Italy
- <sup>4</sup> University of Messina, Department of Veterinary Sciences, Italy

In the last years, metal oxide nanoparticles (NPs) are receiving increasing attention due to their different applications. NPs have unique physicochemical properties that differ substantially from their respective bulk materials of the same composition. According to several studies, titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) have short-term risks on *Danio rerio* (zebrafish). In the present study, we focus on the potential toxic effects that can be caused by a long-term exposure to titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) on zebrafish. The zebrafish, has long been recognized as a useful model organism in ecotoxicology. Zebrafish Wild-type AB strains, were obtained from the Sicilian Center of Experimental Ichthyopathology (CISS), University of Messina, Italy, where they were kept in a "Fish facilities" (Stand Alone Unit, Tecniplast), a closed-loop system for the continuous monitoring of vital parameters. Adult specimens of zebrafish were exposed to different concentrations of TiO<sub>2</sub> NPs (0,5-1 mg/L) (Degussa P25, Sigma Aldrich), for 90 days. Histological, immunohistochemical and molecular analysis were performed on their gills, gut, skin, muscles and liver. All samples analyzed by immunohistochemical investigation, showed a very clear positive response to Metallothioneins (Abcam, 1:500). Moreover, the positivity was confirmed by Western blot analysis (WB). Histological examination showed intestinal alterations and in particular, partial thinning of the folds and occasional flaking of epithelium and morpho-functional alterations in the gill epithelium. Histological analysis on muscles, liver and skin showed no alterations. In conclusion, the current study confirmed only a slight cytotoxicity of TiO<sub>2</sub> NPs on zebrafish adults after a long-term exposure, however, further and wider studies are required to establish their effects on other organs and to evaluate the severity of their toxicity.

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\* **Correspondence:** Dr. Roberta Pecoraro, University of Catania (Italy), Department of Biological, Geological and Environmental Sciences, Catania, I-95124, Italy, pecoraro\_roberta@libero.it