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Histological and biochemical effects of exposure to TiO_2 nanoparticles in livers of two freshwater fish species: *Carassius auratus* and *Danio rerio*

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Nanoparticles (NPs), particles with at least one dimension less than 100 nm, are used in many industrial applications and to produce new types of materials with unique physicochemical properties. The aquatic environment is commonly the ultimate recipient for NPs and there is uncertainty of exposure as understanding and data regarding the potential detrimental effects of NPs on aquatic biota are missing. In this study, titanium dioxide (TiO_2) was chosen for its potential use in technology and diverse industrial applications [1,2]. The objective of this work is to evaluate the toxicity of TiO_2 NPs on total liver glutathione-S-transferase (GST), lipid peroxidation and tissue structure of the livers of two freshwater fish species (*Carassius auratus* and *Danio rerio*).

Stock suspensions of TiO_2 NPs, with an average size of 21 nm, were prepared using distilled water and then ultrasonicated (10 min, 35 KHz). The suspensions were added to 10L of tap water in exposure tanks, to obtain nominal concentrations (0.01; 0.1; 1, 10; 100 TiO_2 mg/L). The test fish, *C. auratus* (N=144) and *D. rerio* (N=80), were randomly distributed by 6 exposure tanks and an additional tank with clean tap water was used as control. Fish were sampled after 7, 14, and 21 days. Six fish from both species were left for depuration in clean tap water during 14 days and then sacrificed. Immediately after sampling the fish were processed for enzymatic determination and histopathology. The GST activity was determined by following the procedure described by Habig et al. [3] and lipid peroxidation was measured based on the *Thiobarbituric Acid Reactive Species* method [4]. The tissues were processed essentially according to Martoja and Martoja [5] for light microscopy (LM). For transmission electron microscopy (TEM) the samples were fixed sequentially in glutaraldehyde, osmium tetroxide and uranyl acetate, dehydrated in ethanol and embedded in Epon-Araldite according to standard procedures. The histological and ultra-structural observations were carried out using a Leica microscope (Leica-ATC 2000) and a JEOL 100-SX electron microscope respectively.

The results showed increased activities of the GST in livers with increasing TiO_2 NP concentrations after 7 days of exposure, however after 14 days a trend to decrease was observed for both species. The GST results suggest that the increase of activity of these detoxification enzymes can be a response to oxidative stress caused by the generation of reactive oxygen species by the NP. On the other hand, size, chemical composition, surface area, shape, solubility and aggregation may also contribute for NPs toxicity. The results from lipid peroxidation showed an increase according to tested concentrations suggesting that TiO_2 NPs is able to cause cell damage and is in agreement with biochemical and histological findings. After 14 days of depuration, GST and lipid peroxidation levels were not significant different from controls suggesting that cells are able to recover in a certain degree. The results from LM (Fig. 1) showed that exposure to TiO_2 NPs affected liver structure, with more pronounced changes detected in livers from fish exposed to higher concentrations. Observed changes include tissue degeneration, inflammation and pyknosis among others. The TEM analysis revealed also severe changes in liver cells compatible with oxidative stress. Hepatocytes of treated fish showed glycogen depletion, swollen mitochondria and increased lysosomes, compared to controls. After depuration, some cells recovered nearly normal morphology, but retained the lysosomes, while others underwent necrotic changes (Fig. 2). Differences among the two species studied were of a quantitative nature, and more pronounced in *Danio rerio.*

The results suggest that potential risk to fish health exist related to the TiO2 NPs release to the aquatic environment and may cause deleterious effects in aquatic organisms. It is evident that the effects of TiO_2 NPs on environment is a matter of great concern and the precise mechanisms of toxicity of this and other types of NPs must be clarified.

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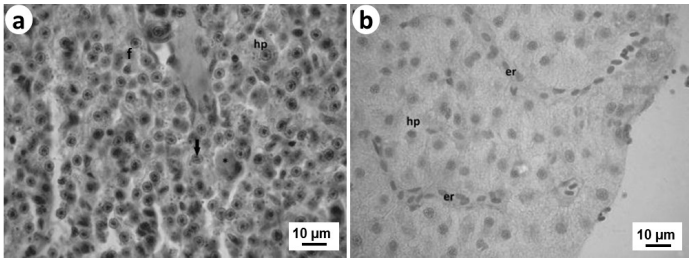

Fig. 1 – Representative LM image from fish liver (*C. auratus*) exposed to 100 mg/L TiO_2 nanoparticles for 21day. Legend: hp, hepatocytes; bi, bi-nucleated nuclei (arrowhead); er (erythrocytes); (*) macrophage. Staining: H&E.

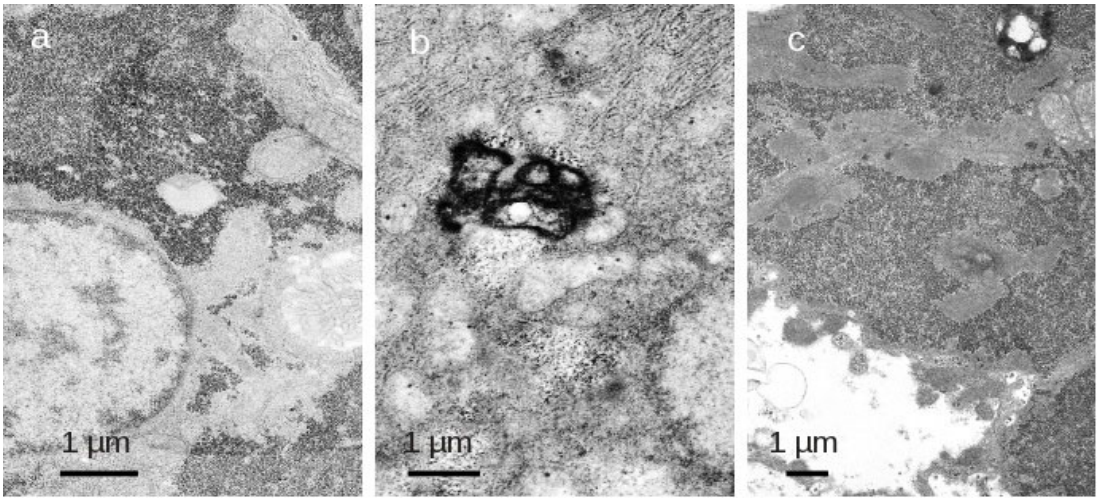

Fig. 2 – TEM of *Danio rerio* liver. a) control; b) exposed to 100mg/L (21 days); c) Depuration stage. G – Glycogen, M – mitochondria. L – lysosomes. N – necrotic cells.

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