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TiO_2 nanoparticles intake by fish gill cells following exposure

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Engineered nanomaterials such as nanoparticles (NPs) are increasingly being used for commercial purposes in products within medicine, electronics, sporting goods, tires, textiles and cosmetics. They comprise diverse types of materials from metals, polymers, ceramic to biomaterials and have been defined as particles with at least one dimension in the order up to 100 nm[1]. The higher toxicological potential of NPs is mostly due to their small size, wide surface, increase of their chemical reactivity and biological activity and the capacity to generate free radicals [1]. NPs also can have the ability to penetrate trough the biological barriers and to move easily through the biological systems. Therefore, is mandatory to assess the toxicity of these nanomaterials, since their industrial production and uses will also result in releases to the environment with unclear consequences [1,2,3].

The aim of the present work is to evaluate the toxicity of titanium dioxide (TiO2) NPs on gill gluthatione-S-transferase activity (GST), lipid peroxidation and on structure of the gills of two freshwater fish species (*Carassius auratus* and *Danio rerio*). Suspensions of TiO_2 NPs, with an average size of 21 nm, were prepared using distillate 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. The GST activity was determined by following the procedure described by Habig *et al*. [4] and lipid peroxidation was measured based on the *Thiobarbituric Acid Reactive Species* method [5]. The tissues were processed essentially according to Martoja and Martoja [6] 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 ultrastructural observations were performed using a Leica-ATC 2000 microscope and a JEOL 100-SX electron microscope respectively.

The results show a significant increase of GST in gills tissues for *C.auratus* exposed to 10 and 100 mg/L TiO_2 NPs and a decrease following the depuration period. With respect to *D. rerio* a significant increase was observed in fish exposed to 1, 10 and 100 mg/L TiO2 NPs. Lipid peroxidation are in agreement with GST results but showing a significant increase for fish (*C.auratus* and *D. rerio*) exposed to concentrations of 0.1 TiO_2 mg/L NPs and higher. Usually, the oxidative stress caused by exposure to TiO_2 NPs is attributed to hydroxyl radicals (OH) generated by photochemical (UV/vis) processes but it may be also related to specific properties of TiO_2 NPs such as size, surface area and solubility that can influence the degree of toxicity. The results from LM observations (Fig. 1) showed that exposure to TiO_2 NPs affected gill tissues, with changes being detected in both fish species exposed to 0.1 TiO_2 mg/L NPs and higher which is in accordance with biochemical results. Changes include different degrees of hyperplasia (from low to complete fusion of lamellae). The TEM analysis revealed that TiO_2 NPs were internalized by gills epithelial cells accumulating in vacuoles inside these cells (Fig. 2). After the depuration period it was observed that the capability for gills to recover was not complete. The results show a strong response to oxidative stress caused by exposure to TiO_2 NPs, possibly because they are in direct contact with the exposure medium and function as a first barrier against external aggression. However, the gills changes

observed following exposure and a partial recover after depuration suggest that TiO_2 NPs may cause deleterious effects in fish gills compromising fish homeostasis.

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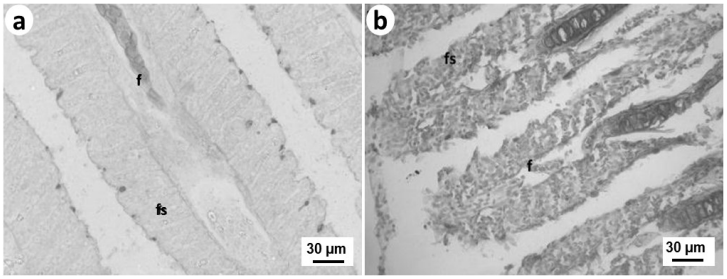

Fig 1- Light microscopy image from fish gills exposed to 100 mg/L TiO_2 nanoparticles for 21day. (a) *C. auratus* and (b) *D. rerio*. Legend: f, filament; fs, complete fusion of several lamellae.

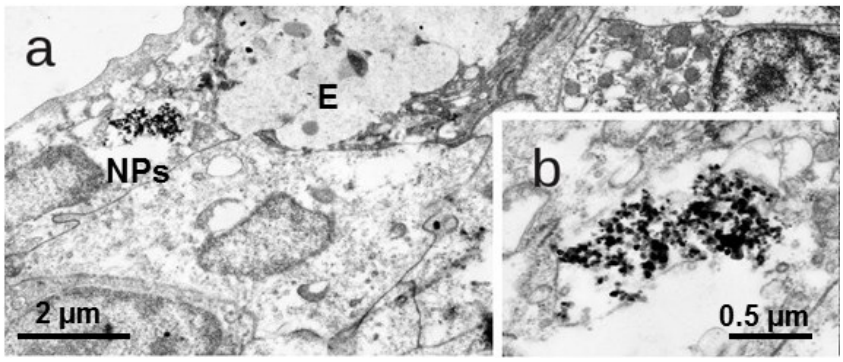

Figure 2- TEM image of fish gills exposed to 100 mg/L (21days); b) high magnification of the aggregate shown in (a). Legend: E (Gill epithelium); NPs (nanoparticles aggregates).

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