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Does ZnO nanoparticle size induce ultrastructural alterations in midgut of *Daphnia magna*?

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The growing use of nanoparticles (NPs) in a wide range of applications is inducing the scientific Community to investigate their possible effects on human health and ecosystems. During their life cycle, NPs will enter the environment, and experimental evidences have already shown that exposure to them is associated with an increased number of diseases, not only for aquatic organisms [1], but also for humans [2,3].

NPs have particular properties related to their nanosize. For instance, NPs of Zinc oxide (nZnOs) can protect human skin against UV radiation. For this reason they have a wide use in UV sunscreen products, but not only [4]. The application of this kind of cosmetics may lead to a release of these NPs into the aquatic compartments. Previous studies have reported the cytotoxic and teratogenic potential of nZnOs on aquatic organisms [4,5].

We used the water flea *Daphnia magna* to study ZnO effects *in vivo*, looking at the morphological alterations, the internalization patterns and the cellular localization of two differently sized ZnO particles.Five different nanoZnO suspensions (0.1, 0.33, 1, 3.3 and 10 mg/L) were tested. The results of the 48-h acute toxicity tests performed with ZnO < 100 nm (bZnO) and ZnO < 50 nm (sZnO), expressed as Immobilization Concentration (IC), showed slight effects with IC_{50} values of 3.1 and 1.9 mg/L for bZnO and sZnO, respectively. Specimens exposed to 1 and 3.3 mg/L have been analysed by transmission electron microscopy and nZnOs from both concentrations have been found into midgut cells: *i*) in the microvilli, bound to the cellular membrane; *ii*) in endocytic vesicles near the upper cell surface; *iii*) in some endosomes, as well as in mitochondria, in multivesicular and multilamellar bodies; *iv*) into the nucleus; *v*) free in the cytoplasm; *vi*) in the paracellular space between adjacent cells; *vii*) into the folded basal plasma membrane, and *viii*) in the gut muscolaris (Figure 1), suggesting that not only both nZnOs are able to interact with the plasmatic membranes of *D. magna* enterocytes, but also that they are capable to cross epithelial barriers. The ultrastructural changes increased with increasing concentrations and the worst morphological fields came from samples exposed to 3.3 mg/L of both nZnOs. No significant differences between the two nanomaterials have been detected. Particle size distribution inside cell compartments was very similar, only the intensity of the effect changed between the two nanoparticles (IC_{50} values were significantly different: χ^2=4.63, n=240, P<0.031). Data from ICP-OES analyses demonstrated that the maximum Zn^{++} concentration in our tested suspensions was 0.137 mg/L, which is below the reported NOEC for nZnO, suggesting that nZnO toxicity is not directly driven by the solubilized ions in the test solution. The large presence of NPs inside the midgut cells after only 48-h exposure to nZnOs and their effects on the intestinal barrier highlighted the toxic potential of these nanomaterials also suggesting that studies of chronic effects are needed.

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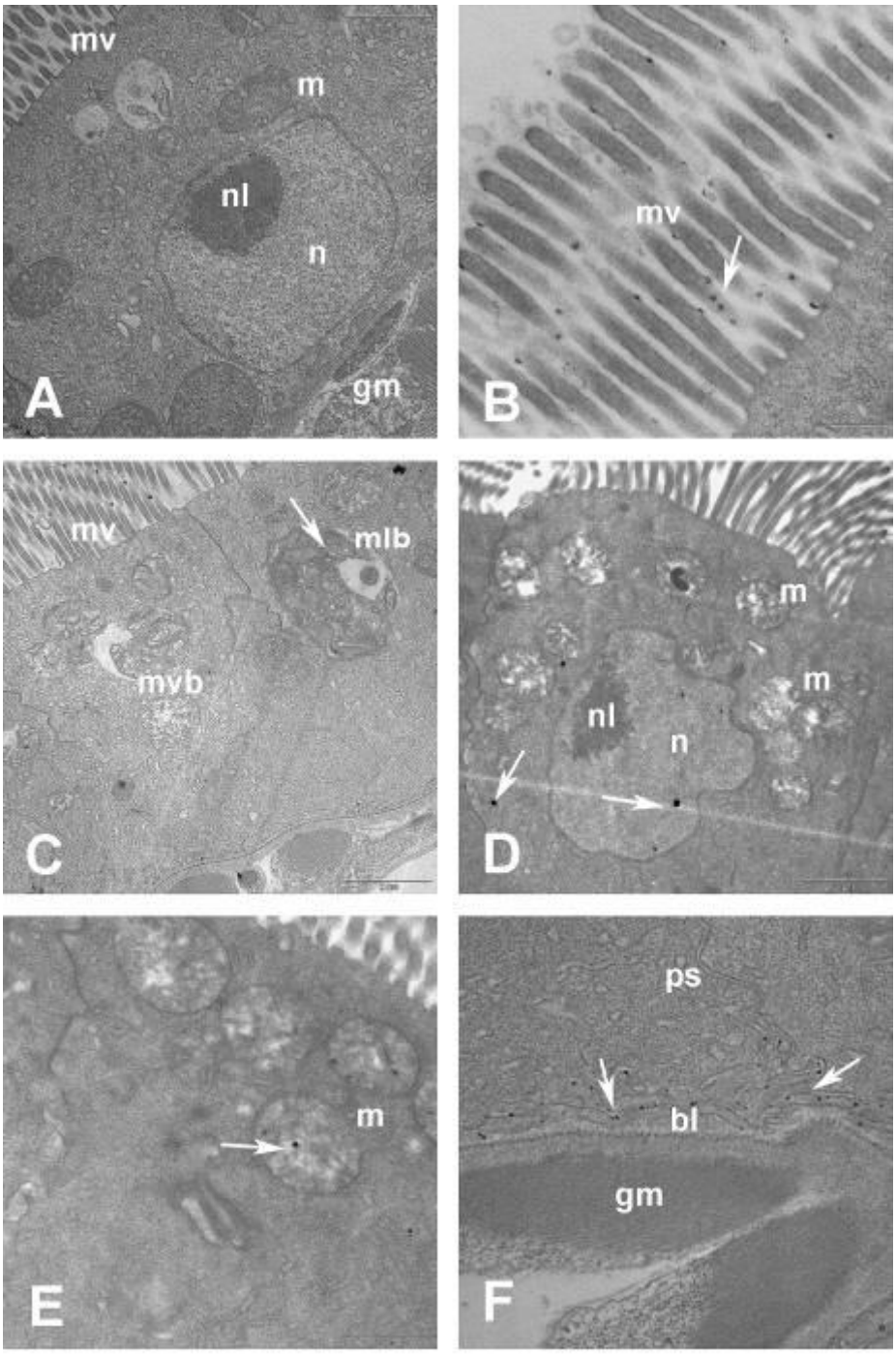

Figure 1. Ultrastructural analysis of enterocytes of 48-h D. magna. Control (A) and 1 and 3.3 mg/L exposed individuals (B-F) have been analysed by transmission electron microscopy and nZnOs from both concentrations have been found in different parts of the enterocyte: e.g into the microvilli (B), bound to the cellular membrane; in multivesicular and multilamellar bodies (C); into the nucleus and free in the cytoplasm (D); into the mitochondria (E); in the paracellular space between adjacent cells and into the folded basal plasma membrane (F). bl basal laminae, gm gut muscolaris m mitochondria, mlb multilamellar bodies, mv microvilli, mvb multivescicular bodies, n nucleus, nl nucleolus, ps paracellular space. The arrows indicate nanoparticles