# HISTOLOGICAL AND ULTRASTRUCTURAL OBSERVATIONS OF *DAPHNIA MAGNA* EXPOSED TO DIAMOND NANOPARTICLES

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Regarding Nanomaterials and Society we are in the midfield between risks and benefits. Nanomaterials (dimensions up to 100 nm) have unique properties / functionalities and have been introduced in the market in goods with applications in different areas from health to environment. Nanomaterials do not behave in a predictable way, and expected hazards at the nanoscale are different from the ones known at a macro/micro-scale and a knowledge gap on the potential health and environmental effects of Engineered Nanoparticles was identified. The way to solve the question is through interdisciplinary and multidisciplinary research work. Uptake of nanoparticles into the aquatic biota is of major concern and not clearly understood. New and adapted methods and tools are being considered in environmental toxicity studies with nanoparticles. The study of cellular biology through light and electron microscopy is one of the possible ways to evaluate distribution within the body and target organs.

Diamond nanoparticles have great interest in industrial (polishing, oil and electrolytes additives, dry lubricants) and medical applications (coatings, biosensors, etc.). This work aims to assess potential effects of diamond nanoparticles in *Daphnia magna* at histological and ultrastructural levels. Particles were obtained from Nanocarbon Research Institute (Japan), average size of 20 nm. A nanodiamond aqueous suspension was prepared by 24h-sonication and gravimetric and zeta potential determinations were done. Adult females were exposed for five days to a concentration of 105 mg/l nanodiamond. The assessment of effects on *Daphnia magna* included light microscopy and Transmission Electron Microscopy (TEM) observations.

After the experimental period, *D. magna* individuals were collected and fixed for microscopic analysis. For light microscopy individuals were fixed in Bouin-Hollande's solution (48 h). After that, samples were washed in formic acid solution and then dehydrated in a progressive series of ethanol. Then samples were xylene impregnated, embedded in paraffin and processed following the usual histological techniques. Sections of 5-7 thickness µm were stained routinely with haematoxylin and eosin, mounted with DPX resinous media and examined using a light microscope (DMLB model, Leica microsystems, Germany). For TEM, individuals were primary fixed in glutaraldehyde, postfixed in $OsO_4$ and uranyl acetate and dehydrated trough a graded ethanol series. Samples were infiltrated and embedded in an Epon-Araldite resin (Mollenhauer). Ultra-thin sections were collected, viewed and photographed in a JEOL 100SX electron microscope operated at 80 Kv.

Obtained results showed no difference between controls and exposed *Daphnia* on survival and reproduction. Light microscopy observations of exposed females showed that diamond nanoparticles adhere to the exoskeleton surface and that nanoparticles and

food accumulate, within the gastrointestinal tract when compared to control females. In some individuals histological observations showed degeneration of the gut cells. TEM observations of cross-sections through the gut of *Daphnia*, showed distension of the intestinal contents with thinning of the cell layers of the gut's wall of the treated animals when compared to the controls. The gut contents of treated animals showed the presence of numerous clumps of particles morphologically compatible with the diamond nanoparticles. Microorganism presence was increased in these samples. The epithelial lining of the gut appeared intact although distended and a few lisosome-like structures with heterogeneous contents were observed in the basal region of the cells.
*Daphnia magna*, an ecological significant organism, proved to intake particles/aggregates of nanodiamond. Effects in cell composition of the lumen can point out to digestion and absorption injuries. The possibility of translocation of the diamond nanoparticles from the digestive tract into other parts of the body, which would lead to accumulation, must be further investigated. Research on new criteria in assessing effects of nanoparticles, e.g. histological studies with light and electron microscopy, is needed.

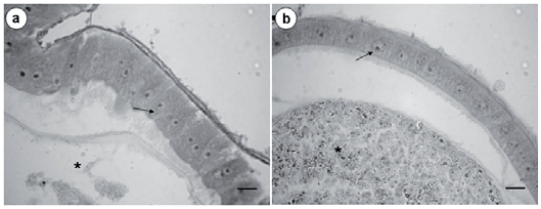


Fig 1. Light Microscopy - Overview of sections of *D. magna* intestinal tract: (a) control individual showing normal gut cells and an empty lumen containing food residues; (b) exposed individual showing clumps of nanoparticles mixed with food. Gut cells (arrow); Intestine lumen (*).

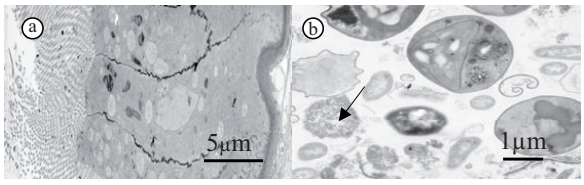


Fig 2. TEM – Sections of *D. magna* intestinal tract: (a) control individual showing normal gut epithelium (b) exposed individual showing gut contents with microorganisms and nanoparticle clumps (arrow).