

## Evaluation of the ecotoxicity and toxicity of graphene by a battery of multitrophic bioassays

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Currently, there are around nanomaterials a great interest due to their properties and the large number of applications they have. Nanomaterials are defined as natural or manmade materials, which have at least one of their dimensions between 1 and 100 nm, while nanoparticles (NPs) include those with at least two dimensions between 1 and 100 nm. While research in nanotechnology and nanomaterials has come a long way, research on its effects on life and health has not yet reached the same level. In this context, the concept of nanotechnology is defined as the study of the adverse effects of nanoparticles on organisms, populations, communities and ecosystems. The urgency of these studies is driven by the rapid exploitation of known applications for nanotechnology for commercial and industrial entities. One of the most nanomaterials currently used are metal oxides.

The aim of this paper is to evaluate the ecotoxicity in one multilayer graphene in different environmental compartments and in human health, with the purpose of detecting at what levels, these particles can be harmful when they are released to the environment.

For these bioassay of acute toxicity, were performed in the organisms *Daphnia magna*, *Selenastrum capricornutum*, *Vibrio fischeri* and *Eisenia fetida*. *Daphnia magna*, a freshwater filter-feeding crustacean, is one of the most sensitive organisms used in ecotoxicity experiments. In addition to their sensitivity, their representation in the trophic level and its importance in the marine ecosystem are the reasons why this species is selected as indicator. The trial follows the standard OECD 202 (OECD, 2004) Young daphnids, aged less than 24 hours at the start of the test, are exposed to the test substance at a range of concentrations for a period of 48 hours. Immobilisation is recorded at 24 hours and 48 hours and compared with control values. The evaluation of the acute toxicity in *Eisenia fetida* was performed following the instructions of the OECD 207 guide. *Pseudokirchneriella subcapitata* is a unicellular alga characteristic of lakes, rivers and freshwater ponds. To evaluate the toxicity of the materials in a kind of continental algae, was used the test Algaltoxkit FTM (MicroBioTests Inc., Gante, Bélgica). The test protocol provided by the manufacturer following the ISO 8692 standard and guide OECD 201. To evaluate the toxicity of the graphene in a detritivorous bacteria species was chosen *Vibrio fischeri* and was used the kit Toxi-Screening Kit<sup>TM</sup> (MicroBioTests Inc., Gante, Bélgica). The test protocol provided by the manufacturer followed the ISO 11348-1 standard. Finally, a quantification of cellular and genetic damage was performed by a cell viability assay and the comet assay in epithelial cell line A459.

## Figures

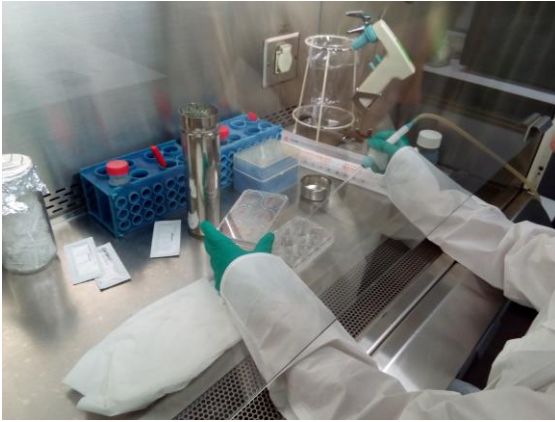


Figure 1: Cell Viability Assay

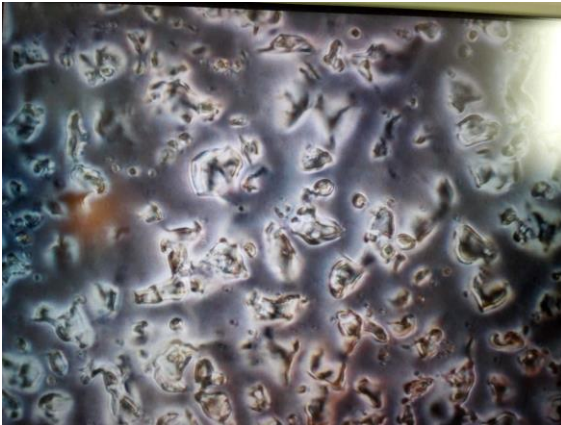


Figure 2: Cell Viability Assay