
Does oxidation degree influence the toxicity of multi-layer graphene in *Xenopus laevis* larvae?

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Abstract

The attractive properties of carbon-based nanoparticles like multi-layer graphene (MLG) lead to their use in many application fields, whether they are raw or functionalized, such as oxidized. The production of these nanoparticles is expected to grow exponentially in a near future at the world scale, and may thus make MLG potentially bioavailable for the organisms. Very stable, these particles may finally contaminate the aquatic environment, which is a major receptacle of pollutants. The study of their impact on the ecosystems is thus essential.

This work focuses on the potential toxicity of MLG in *Xenopus laevis* larvae according to the standardized protocol (ISO 21427-1, 2006) to compare the impact of raw MLG with its oxidized counterpart: graphene oxide (GO). The exposure concentrations ranged from 0.1 to 50mg.L⁻¹. To check if the level of oxidation influences the observed toxicity, reduced graphene oxide containing respectively 4 and 17 % (atomic composition) of oxygen (rGO4%, rGO17%) were prepared by reduction of GO in hydrogen, and tested in the low concentrations range 0.05 to 1mg.L⁻¹.

Various endpoints have been investigated: mortality, growth inhibition and genotoxicity

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(induction of micronucleated erythrocytes). The results did not show any significant mortality. In contrast, a significant growth inhibition was observed from 10mg.L⁻¹ of raw MLG, and at 50mg.L⁻¹ of GO. On the contrary, growth significantly increased for larvae exposed to 1mg.L⁻¹ of rGO4% and rGO17% compared to the negative control. Significant genotoxicity was observed for GO at 0.1mg.L⁻¹ only.

The chronic toxicity observed in larvae exposed to high concentrations of MLG could be limited to physical effects such as intestinal obstruction, abrasive effects and nutrients deprivation. Respiratory deficiency may also occur in *Xenopus* because of its potential gill clogging. However, genotoxic effect could be attributed to intrinsic properties (surface chemistry) of GO, which loses its genotoxic potential when reduced.