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Towards reliable risk assessment for nanomaterials: evaluating the ecotoxicology of graphene oxide

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Markovic, M., Andelkovic, I., Shuster, J., Janik, L., Kumar, A., Losic, D., and McLaughlin, M. J. (2020) Addressing challenges in providing a reliable ecotoxicology data for graphene-oxide (GO) using an algae (*Raphidocelis subcapitata*), and the trophic transfer consequence of GO-algae aggregates. *Chemosphere*, 245: 125640.

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Nanomaterials such as graphene oxide (GO) have potential for a myriad of industrial applications due to their physical, chemical, mechanical and structural properties. However, due to these unique attributes, assessing the toxicity of GO — necessary to meet relevant regulatory requirements — remains challenging. A study now seeks a robust, reliable methodology for evaluating the ecotoxicity of GO, using an alga (*Raphidocelis subcapitata*) and a shrimp species (*Paratya australiensis*) to evaluate exposure risk.

Graphene comprises a single-layer sheet of bonded carbon, arranged in a honeycomb-like lattice. This material is a promising candidate for use in applications such as electronics, composite materials, energy generation and storage, sensors and biomedicine; and has experienced a surge in popularity and manufactured volume in recent years. However, the increasing prevalence of graphene in commercial products means that the nanomaterial has an increasing number of pathways into the environment, from the production line (dust, wastewater) to transport, direct use and disposal.

Safety regulators are struggling to outpace such development, and standardised methods to quantify and monitor environmental exposure to nanomaterials are lacking. However, recent policy updates include protocols for nanoparticles and materials from the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation; these protocols have been adapted based, inter alia, on input from the EU FP7 NANoREG, Environmental Effects and Risk Evaluation of Engineered Nanoparticles (ENVNANO), and Horizon 2020 PROSAFE projects, which have collectively evaluated methods of risk assessment for nanomaterials to provide recommendations for policymakers and regulators.

The ecotoxicological risks posed by graphene nanomaterials (GNMs) — i.e. their potential to affect and stress ecosystems — have been assessed in the laboratory, but can vary widely depending on the type of GNM studied and the structure of the sample (e.g. number of layers, shape, surface chemistry, size). The most common form of GNM studied in aquatic toxicology is GO, as this material is more dispersible in water than other forms.

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To assess the risks associated with GO, this study sought to explore toxicologically significant GO levels, characterise how a sample's physical properties (e.g. its size or shape) affects such toxicity and qualify the stability and behaviour of GO suspensions in different media. The researchers investigated the differences in GO toxicity using two different media and the algae *Raphidocelis subcapitata* and studied the adverse effects of GO-algae aggregates and trophic transfer on the shrimp species *Paratya australiensis*. As algae are primary producers at the base of aquatic food webs, any adverse effects of GNMs at this level could influence entire ecosystems (including if passed up trophic levels in a way that causes bioaccumulation, or if they cause algae populations to shrink to become insufficient to support higher trophic levels of the food chain).

The researchers selected two culture media (modified and standard) in which to compare the effects of GO on the freshwater *Raphidocelis subcapitata* algae. GO-laced and GO-free media of each type were inoculated with the alga and the cultures were monitored to assess the effects of GO exposure on the algae, and the concentration of the GO.

The results showed that the composition of the test media, and the presence (or lack) of algae, affected the physical attributes of the GO sheets in the media in terms of size, surface area and shape. This highlights the need to consider the transformation of GO under experimental conditions in ecotoxicological studies, say the researchers.

Additionally, the presence of GO (with suspension concentrations of 2 and 8 milligrams per litre) appeared to adversely affect algal cell structure, and caused algae to combine with GO to form aggregates — effectively removing them from the water column and thus potentially impacting subsequent tiers of the food chain. To evaluate the toxicity of GO, the researchers found that the number of algal cells per millilitre proved to be a reliable marker for GO's effect on algal growth. However, they also used fluorescence as a screening tool, and evaluated how the GO inhibited algal growth relative to control conditions. Across the different media, toxicity inhibitory concentration (IC50)[1] values for the algae after 96 hours (with fluorescence as an endpoint) ranged from 1.76 (in the reference (standard) medium) to 4.96 milligrams per litre (after GO exposure in the modified algal culture medium — a more environmentally relevant scenario)[2]. The starting density of algae was always 10 000 cells per millilitre.

Secondly, shrimps of the species *Paratya australiensis* were fed GO-exposed algae in the form of GO-algae pellets for a period of 14 days, and the number of dead, alive, or physically affected individuals recorded daily. There was no statistically significant difference between the control

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and treated groups of shrimps in survival, moulting (shedding of exoskeleton), or amount of food intake. Shrimp are known to be sensitive to polluted freshwater environments, but exhibited no signs of stress, internal GO accumulation, or food avoidance as a result of the GO exposure.

Overall, the study provides guidance on a number of key points in assessing the properties, behaviour and potentially adverse effects of GO — including trophic transfer of such material (e.g. shrimps consuming GO-exposed algae) and changes in the material's physical properties (e.g. size) in certain conditions. The researchers emphasise the need for further research to standardise the characterisation of graphene nanomaterials, particularly regarding the use of environmentally relevant media, in order to establish environmentally acceptable levels of these materials.

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1. Toxicity inhibitory concentration 50, or IC_{50}, is the concentration at which a substance prevents 50% of cellular growth or yield (in this case) compared to the control.

2. Current modelling suggests that the concentration of a range of nanoparticles in the aquatic environment can range from ng/L to µg/L. The IC_{50} value in this study is an order of magnitude higher — measured in mg/L.