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# Nanotoxicity of Graphene Oxide – Gold Nanohybrid to *Daphnia magna*

Taiwo Hassan Akere [a,b,*], Aline Maria Zigiotto de Medeiros [c,d], Diego Stéfani T. Martinez [c,d], Bashiru Ibrahim [a,b], Hanene Ali-Boucetta [b], Eugenia Valsami-Jones [a,*]

[a] School of Geography, Earth and Environmental Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom
[b] Nanomedicine, Drug Delivery & Nanotoxicology (NDDN) Lab, School of Pharmacy, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom
[c] Brazilian Nanotechnology National Laboratory (LNNano), Brazilian Centre for Research in Energy and Materials (CNPEM), Campinas, 13083-100, SP, Brazil
[d] Centre of Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), Piracicaba 13416-000, SP, Brazil

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ABSTRACT

The expanding use of hybrid nanomaterials in many applications necessitates evaluation of their environmental risks. This study investigates the acute toxicity and bioaccumulation of graphene oxide – gold (GO-Au) nanohybrid in neonates (<24 hrs old) of *Daphnia magna* after exposure to a wide range of concentrations (1–100 mg/L). No significant mortality or immobilisation was observed after the exposure period. Microscopic observation showed an uptake of the nanohybrid and internal damage in the gut of the exposed organisms. Bioaccumulation of the GO-Au nanohybrid also occurred in a concentration-dependant manner. Continuous evaluation of the environmental risks from exposure to this nanohybrid and other advanced materials is imperative to avert disruption to the ecosystem.

Graphene oxide – gold nanohybrids (GO-Au) are advanced materials that have enhanced functionalities and remarkable properties that make them useful for diverse applications (Amir et al., 2020; Santhosh et al., 2014). The nanohybrid, produced via the chemical reduction of chloroauric acid by sodium citrate, have been used in biosensing, cancer therapy and bioimaging (Neri et al., 2019; Al-Ani et al., 2017; Turcheniuk et al., 2015). However, their widespread usage also makes them a subject of concern for toxicologists due to their potential environmental release. As it is with the fate of many nanomaterials, it is unavoidable that these GO-Au nanohybirds, once commercialised, will be released to the aquatic ecosystem (Pretti et al., 2014). Potentially, this could setoff deleterious effects to biota at each level of the food chain (Guo et al., 2013). With the uncertainty around the toxicity of GO-Au nanohybrids, it is vital to understand whether or not they might induce any acute or chronic harmful effect on sensitive organisms such as *Daphnia magna*. In addition, since daphnia are known to bioaccumulate chemicals (Du et al., 2016; Zhu et al., 2010; Xiaohui et al., 2018), an evaluation of the bioaccumulation of GO-Au nanohybrid becomes necessary to gain insight into how the nanohybrid may spread through the food web. There is also a potential risk to human health through the consumption of contaminated seafoods such as clams and fish (Klaper and Lyman, 2011).

The nanotoxicity of GO-Au nanohybrid has not been investigated before. Hence, this research aims to evaluate the toxicity of GO-Au nanohybrid to neonates (< 24 hrs old) of *Daphnia magna* in borehole water. Specifically, this work focused on assessing the acute immobilization/mortality and bioaccumulation of the nanohybrid by daphnia neonates.

Acute toxicity testing was carried out according to the Organisation for Economic Cooperation and Development (OECD) acute immobilization test protocol (OECD, 2004). Daphnia neonates (<24 hr old) were exposed to a range of concentrations (1, 5, 10, 20, 50 and 100 mg/L) of GO-Au nanohybrid for 48 hrs in borehole water. Natural organic matter (NOM, final concentration: 20 mg/L) was used to stabilise the nanohybrid in the media. Record of mortality and immobilization was taken at 24 and 48 hrs. Protocol for the preparation of the test solutions is presented in supplementary information.

Bioaccumulation was evaluated in daphnia neonates exposed to different concentrations of GO-Au nanohybrid (1, 50 and 100 mg/L) for 7 days. The culture media was changed on day 3 and 5. On day 7, daphnia samples were collected and rinsed with ultrapure water. The organisms were split into 3 groups: the first group was processed for quantification of gold nanoparticles (AuNPs) using inductively coupled plasma mass spectrometry (ICP-MS), the second group was processed for

* Corresponding author.
*E-mail addresses:* txa880@bham.ac.uk (T.H. Akere), e.valsamijones@bham.ac.uk (E. Valsami-Jones).

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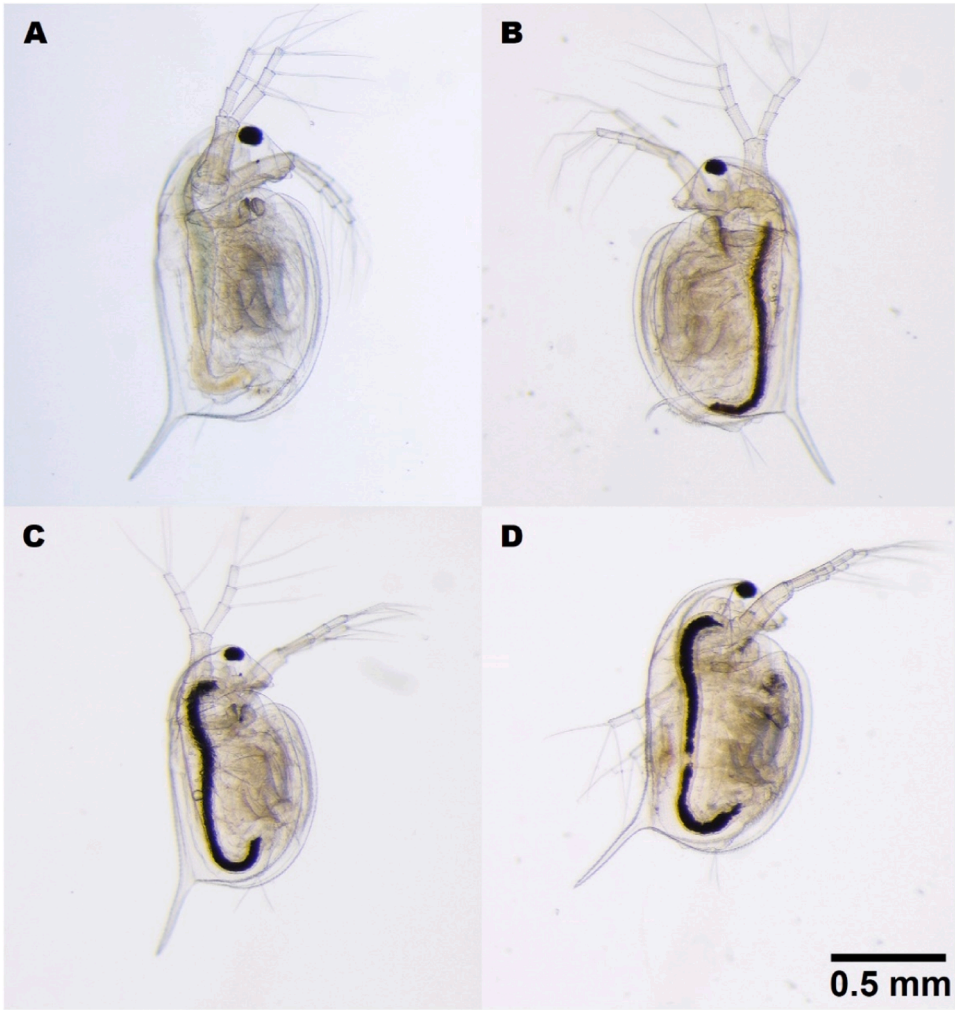


**Fig. 1.** Light microscope images of daphnids after exposure to different concentrations of GO-Au nanohybrid. (A) control, (B) 1 mg/L treatment, (C) 50 mg/L treatment (D)100 mg/L treatment.

imaging using transmission electron microscopy (TEM), and the last group was imaged (whole organism) using a light microscope.

The daphnia neonates in all the exposure concentrations had 100% survival except 100 mg/L where 93% survival was recorded. The result indicates that the GO-Au nanohybrid did not induce any lethal ecotoxicological effect (mortality and immobilization) to daphnia neonates at the tested concentrations (1–100 mg/L) in borehole media. According to the United Nations' global harmonised system of classification and labelling of chemicals (United Nations 2011), the GO-Au nanohybrid is not considered hazardous to the aquatic environment. A combination of factors could possibly explain the low toxic effect of GO-Au nanohybrid on the neonates. The organisms may have depurated the nanohybrid, as is common with daphnia (Guo et al., 2013; Lv et al., 2018). Also, biodegradation of the GO-Au nanohybrid may have occurred in the digestive tract of the exposed daphnids. Some organisms such as insects and microorganisms have been reported to biodegrade graphene oxide nanocomposites (Fan et al., 2017; Liu et al., 2022). In addition, the presence of NOM in the media could mitigate the toxicity of the GO-Au nanohybrid (Zhang et al., 2019).

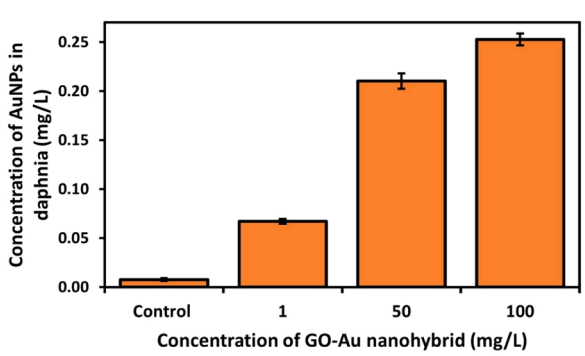


**Fig. 2.** Quantification of AuNPs in digested Daphnia magna samples after exposure to different concentrations of GO-Au nanohybrid for 7 days. Results represent the mean ± standard deviation of three individual replicates.

Morphological observation of the daphnia after exposure to GO-Au nanohybrid was carried out using a light microscope to enhance our understanding of the uptake and transport of the nanohybrid within the organism. As can be observed in Fig. 1, the GO-Au nanohybrid is present in the gut of the exposed daphnids except in the control. Also, the nanohybrids in the gut of the daphnids in the 50 and 100 mg/L treatments are noticeably more concentrated than those of the 1 mg/L exposure. These visual observations conform with the result of the AuNPs quantification in exposed daphnids. In addition, exposure of daphnia to potentially toxic elements and nanomaterials have been reported to have effects on embryonic development and induce tail loss and carapace shedding (Djekoun et al., 2015; Giardini et al., 2015; Ellis et al., 2020). However, the images show that the daphnids in all the treatments in this study have not lost body parts, agreeing with the

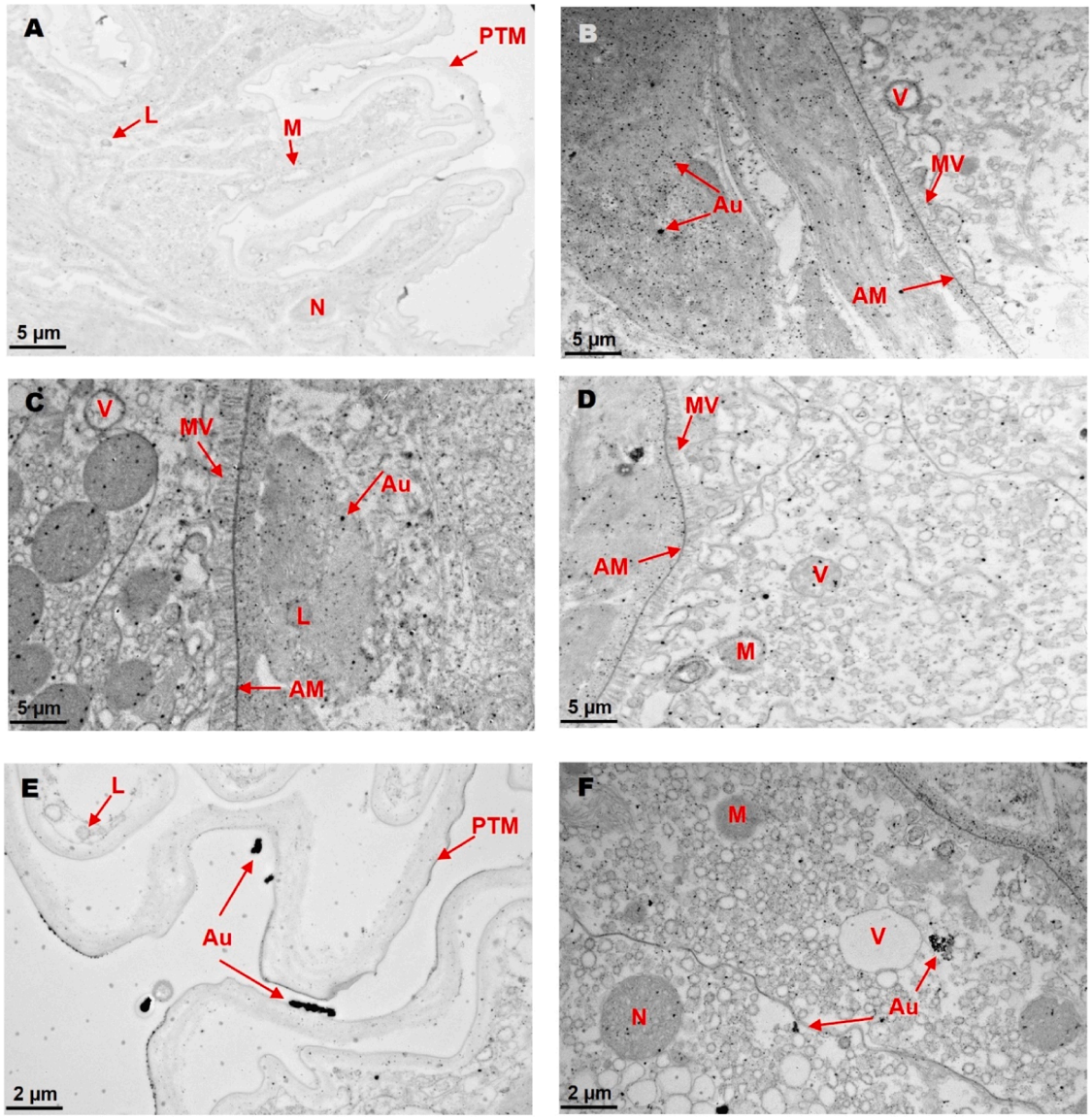


**Fig. 3.** Representative images of cross sections of daphnia neonates by transmission electron microscope (TEM) after exposure to GO-Au nanohybrid (*A* = control; *B* = 1 mg/L; C&*D* = 50 mg/L; E&*F* = 100 mg/L) KEY: microvilli (MV), lysosome (L), mitochondria (M), nucleus (N), peritrophic membrane (PTM), vacuole (V), apical membrane (AM), AuNPs (Au).

outcome of the acute toxicity test that the GO-Au nanohybrid did not cause any significant harm to daphnia at the tested concentrations.

Due to the difficulty in directly quantifying carbon nanomaterials in biological systems (abundant background carbon, absence of reliable quantification method etc.) (Yang et al., 2013; Xin and Wan, 2019; Wang et al., 2013), the concentration of AuNPs in the nanohybrid was used in this study as a chemical label for GO-Au nanohybrid. It has been previously shown that AuNPs were homogenously distributed on the GO-Au nanohybrid (Akere et al., 2023). Therefore, quantifying the amount of AuNPs in the exposed daphnids can be used to estimate the concentration of the GO-Au nanohybrid. The mean concentrations of AuNPs measured in 100µL of the digested daphnids ($n = 10$) exposed to 1, 50 and 100 mg/L of the nanohybrid were 0.0671 ± 0.002, 0.2103 ± 0.008 and 0.2526 ± 0.006 mg/L respectively (Fig. 2). This result confirms the bioaccumulation of the GO-Au nanohybrid by daphnia neonates, posing potential threat to the health and safety of the organisms above daphnia in the food chain. Besides induction of oxidative stress and immobilisation, bioaccumulation of toxic substances can cause adverse effects on reproduction and development in aquatic organisms (Fan et al., 2012; Cano et al., 2017).

To further evaluate the toxicity of GO-Au nanohybrid and its impacts on the organism's gut and internal organelles, TEM cross sectional images were taken and observed (Fig. 3). Common gut structures such as mitochondria (M) and lysosome (L) can be found in the control treatment (Fig. 3A). As expected, there were no traces of the GO-Au nanohybrid (as AuNPs) in the gut in the control treatment. However, AuNPs can be seen in the cross sections of all the remaining treatment samples (Fig. 3, B – F), confirming the uptake of the GO-Au nanohybrid by daphnia. This uptake is crucial and significant for bio-nano interaction which precedes the production of biological responses. In addition, internalization of the GO-Au nanohybrid may have led to over-generation of reactive oxygen species (ROS) which causes oxidative damage (Kwon et al., 2014). This sublethal effect could be responsible for the irregular shape and arrangement of organelles within the cells. An interaction of the GO-Au nanohybrid with the cells and formation of sizeable vacuole structures and lipid like cytoplasmic inclusions can be observed (Fig. 3C & F). GO-AuNPs can be seen within the cellular matrix (Fig. 3E), indicating that there may have been damages to the

peritrophic membrane. However, it is possible that a systemic effect may be produced on the whole organism following long duration exposures.

In conclusion, while neonates of *Daphnia magna* bioaccumulate GO-Au nanohybrid, significant acute toxic effects were not induced. This work represents a significant step in our understanding of the environmental risks associated with GO-Au nanohybrid. With the expanding use of GO-Au nanohybrid in various applications, there is a need for continuous evaluation of the nanosafety of the nanohybrid to the ecosystem and human health. Future research should focus on the chronic toxicity and sublethal effects (growth, reproduction, hormonal changes) of the GO-Au nanohybrid on daphnia.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2023.106552.

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