Exploring the potential of graphene nanomaterials as carriers for oil compounds: an *in vitro* toxicity approach

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Complex mixtures of pollutants are usually found in the aquatic environment. Polycyclic aromatic hydrocarbons (PAHs) are prioritary pollutants and main constituents of the water accommodated fraction (WAF) of oil. Graphene nanoplatelets can adsorb organic compounds thus being potentially useful in oil spill remediation. However, they could also act as vehicles of organic contaminants to aquatic organisms (the so-called "Trojan horse" effect) (1, 2). This study aimed to evaluate the potential "Trojan horse" effect of graphene nanoplatelets (graphene oxide (GO), GO-polyvinylpyrrolidone (GO-PVP) and reduced GO-PVP (rGO-PVP)) with adsorbed oil compounds from naphthenic North Sea crude oil WAF (3) using *in vitro* toxicity assays in hemocytes of marine mussels (4). Two approaches were tested to obtain graphene nanoplatelets with adsorbed oil compounds: filtration and centrifugation. Hemocytes were exposed to a wide range of concentrations of GO, GO-PVP and rGO-PVP with and without adsorbed oil compounds and to a series of WAF dilutions. After 24 h exposure, cell viability (MTT assay) and ROS production were assessed (4). Centrifugation (270*g* for 30 min) successfully separated WAF solution from graphene nanoplatelets with adsorbed oil compounds. This procedure was thus used for *in vitro* toxicity testing. Exposure to WAF decreased cell viability and increased ROS production in hemocytes starting at 25% WAF. In line with previous studies (4), GO, GO-PVP and rGO-PVP nanoplatelets were moderately toxic to mussel hemocytes and produced a significant increase in ROS production. In exposures to graphene with adsorbed oil compounds, hemocytes viability decreased at similar concentrations as in exposures to nanoplatelets alone. However, ROS production increased in hemocytes exposed to lower concentrations of graphene with adsorbed oil compounds (10 mg/L) compared to nanoplatelets alone (25 mg/L), indicating that adsorbed oil compounds increase nanoplatelets toxicity. In conclusion, a protocol to obtain graphene nanoplatelets with adsorbed oil compounds was established. Nanoplatelets with and without adsorbed oil compounds showed similar cytotoxicity to hemocytes but the ones with adsorbed oil compounds increased ROS production earlier, indicating that graphene nanoplatelets may act as "Trojan horse" carriers of oil compounds. This work was funded by the EU H2020 GRACE project (grant 679266), Spanish MINECO (project NACE, CTM2016-81130-R), Basque Government (consolidated research group IT810-13) and University of the Basque Country (UFI 11/37).

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