Identification of safer graphene related materials in the framework of the H2020 NanoReg2 project

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The main objective of this work is to identify those graphene related materials (GRM) with the lowest cytotoxicity among a group selected to in the framework of the H2020 NanoReg2 project. This project aims to develop and implement grouping and safe-by-design (SbD) approaches within regulatory frameworks and is sustained on three main pillars: safe product by design, safe use of products and safe industrial production. Taking all this into account, one case study in this project considers the production of a variety of GRMs in a real manufacturing process inside Grupo Antolin Ingeniería. In this case study it was essential to generate as fast as possible basic information about the toxicity of the different materials produced in order to identify those with the lowest toxicity so that safer by design approaches could be applied at the three mentioned pillars. For that, we considered that the use of in vitro approaches offered an invaluable tool allowing us to test rapidly a number of materials obtaining repetitive and reliable data. Since determining environmental hazards is an essential step in any nanomaterial risk assessment we considered that the use of fish cell lines could be more representative from an environmental perspective, than the use of cells of mammalian origin. Therefore, PLHC-1 (from liver *Poeciliopsis llucida*) and CLC (a carp macrophage cell line) were used. In addition, the PLHC-1 cell line was chosen because it has been used previously in the characterization of graphene oxide (GO) mechanisms of toxic action (Lammel and Navas, 2014). Suspensions of GRM were generated in cell culture medium using a protocol that guarantees the stability of the dispersion and minimize the sedimentation. The particle size distribution was established by means of dynamic light scattering at different times. Cells were exposed to a range of concentrations (being the highest, 200 µg/ml, diluted using a factor of two) of the selected GRMs for 24 or 72 h. Cytotoxicity was assessed by means of three different assays performed on the same set of cells and informing about disruption of the plasma membrane structure, alterations in the lysosomal functionality and modifications of the mitochondrial activity. Although, in general, time-dependent toxic responses were observed in both cell lines and some differences in the toxicity of the tested GRMs could be observed, however, toxicity was only pronounced at the highest concentrations. These results open the door fine tuning some manufacturing processes in order to generate safer products. This research is supported by the EU's Horizon 2020 research and innovation programme (NanoReg2, Grant Agreement n° 646221).

References

[1] Lammel T, Navas JM, Aquatic Toxicol. 150 (2014) 55