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INTERLAB STUDY ON NANOTOXICOLOGY OF REPRESENTATIVE GRAPHENE OXIDE

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Graphene oxide (GO) is a material with potential in many different applications and with thermal stability (Zhou et al. Sci. Rep. 3:2484 (2013)). GO is not considered till now as representative compound in the actual studies in the EU (e.g. REACH, OECD). Recently, European Commission's JRC Institutes IRMM (Belgium) and IHCP (Ispra, Italy) introduced the term 'representative test material' (RTM), and provided a frame for its use (Roebben et al. J. Nanopart. Res. 15, 1455 (2013)). The definition of RTM is a material from a single batch, homogeneous and stable with respect to one or more specified properties (ISO/AWI TS 16195). The ISO Tech. Comm. Nanotechnol. (TC 229) has recently published a new standard: ISO/TR 16197:2014 – Nanotechnologies – Compilation and description of toxicological screening methods for manufactured nanomaterials. This standard is a Technical Report related to *in vitro* and *in vivo* methods that can be useful for the toxicological and ecotoxicological measurement, complementing the ISO/TS 27687:2008 and ISO/TS 80004-1. In view of these documents, our Brazilian Network on Nanotoxicology-GIGENANOTOX (MCTI/CNPq) decided to follow and evaluate these new rules for a selected representative compound, GO, by different nanotoxicological assays. The graphene sample GO:Single-layer graphene oxide, purity 99%, thickness 0.7-1.2 nm (AFM); ~300-800nm X&Y dimensions is the standard size <450 nm & 1-20 μm lateral dimensions. Cheap Tubes Inc., Bratleboro, USA was selected for our study. Exhaustive characterization of GO was afforded. It exhibited thermal stability over 60^{o}C and it was suspended in deionized water after ultrasonication (1 mg/mL) (stable 10 days). All the biological fluids used in the different assays were used as control of the colloidal suspension stability. Then, all the studies were carried out within that stability period. The cytotoxicity assays were carried out by the Rezasurin reduction, MTT and flow cytometry assays in mouse embryonic fibroblast cells (3T3), human keratinocytes (HaCaT), colorectal cancer cells (Caco-2/HCT 116), Lewis lung cancer cells (3LL), acute myeloid leukemia cells (KG-1, Jurkat, Kasumi-1) and chronic myeloid leukemia cells (K562, Lucena) and no significant toxicity was found after exposition to 0.1-100 μg/mL for 24 and 48 h. Breast cancer cells, MCF-7, showed a 20% reduction on cell viability at 24 and 48 h. No cytotoxicity were found in lymphocytes, Chinese hamster ovary cells (CHO) and human macrophage cell line (U937) at 0.1-50 μg/mL, but 30-50% survival inhibition was observed at 100 μg/mL. A dose-dependent increase in apoptosis was observed in some cells (Kasumi-1, Jurkat and K562 cells). In the case of CHO and 3T3 cells, greater levels of necrosis with increasing concentrations of GO (>50 μg/mL) were observed. Genotoxic study using the Comet assay showed slight DNA damage in lymphocytes at all concentrations tested, while more significant effects were observed in 3T3 cells, being worst in CHO cells. Econanotoxicity was carried out by lethality assays in the nematode *Caenorhabditis elegans* and in the freshwater coelenterate *Hydra* with no signs of toxicity at concentrations varying from 0.1-50 μg/mL of GO. However, death and disintegration of *Hydra* was observed after exposition to 100 μg/mL for 72 h. In *in vivo* studies, no changes in biochemical parameters of Fischer 344 rats were observed after the i.p. administration of GO, suggesting that it is less efficiently removed by the reticuloendothelial system (RES), probably due to agglomeration after the i.p. injection, thus leading to low absorption. Black agglomerates were indeed found in the intraperitoneal cavity of rats injected with GO. This was also observed previously in Balb/c mice receiving GO i.p. (Yang et al., Biomaterials 34, 2787 (2013)). However, in Fisher 344 rats-bearing prostate tumors, treatment with GO negatively affected the hepatic parameters, whilst in the renal ones, an improvement was observed. Studies are in progress to understand the mechanisms involved in the uptake of GO by RES. The validation of all of these assays involving GO will be discussed in details in the presentation.

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