

Assessment of a new tool for the eco-toxicological evaluation of engineered nanomaterials based on *in vitro* approaches

Azucena Bermejo-Nogales, M.L. Fernández-Cruz, J.M. Navas

Departamento de Medio Ambiente, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Carretera de la Coruña, Km 7.5, 28040 Madrid, Spain.

In recent years the increase in the industrial production of engineered nanomaterials (ENM) has raised serious concerns about the possibility of release into the environment. As a consequence, there is a need of appropriate approaches for the environmental and health assessment of ENMs. The use of *in vitro* studies utilizing cultured cells to predict fish responses appear as a suitable tool for determining the cytotoxic effects induced by a broad range of compounds with the reduction in the use of research animals. The aim of this study is to determine the cytotoxic effects of a broad array of ENM, cerium dioxide, multiwall carbon nanotubes (MWCNT), silicon dioxide, silver, titanium dioxide and zinc oxide (obtained from the JRC Repository) in the topminnow fish (*Poeciliopsis lucida*) hepatoma cell line (PLHC-1) as an *in vitro* toxicology model. Cytotoxicity was evaluated with classical toxicity assays performed on the same set of cells with fluorescent dyes (alamarBlue, CFDA-AM and neutral red). In parallel, interference with the assays and physico-chemical characterization of ENM has been performed. The results show that ENM have in general a low degree of fluorescence interference and different levels of aggregation in the culture medium. Moreover, PLHC-1 cells cytotoxic effects were mainly produced by silver and zinc oxide whereas the rest of ENM show negligible effects on cell viability. This study shows the appropriateness of this tool as an *in vitro* model to shed light on the toxicity of ENM to be used in the framework of intelligent testing strategies.