Inter-comparison of the biological properties of nanomaterials just after dispersion and after its freezing (liquid N_2 / -80 ºC)

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To test the biological properties of nanomaterials they must be used just after its dispersion to avoid possible precipitations or agglomerations. This supposes to repeat this process any time a new experiment is running on. Thus, slight differences in the repeated procedure can suppose differences in the level of the achieved dispersion. This is particularly important when long-term or chronic exposures at sub-toxic doses are used; in these studies, lasting for months, the removal of the culture media (with the nanomaterial) every 3 days is required. This supposes the existence of a potential substantial variation between every process of dispersion. To avoid these problems we propose to freeze at -80 ºC different replicates of an initial dispersion that are thawed under request.

To demonstrate the efficiency of the proposed alternative we have compared the physico-chemical and biological properties of fresh and frozen nanomaterials. ZnO, TiO_2 and CeO_2 nanoparticles (NPs) were used. NPs characterization (size and morphology) was carried out by transmission electron microscopy (TEM). Furthermore, characterization of hydrodynamic size and zeta potential by dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) methodologies, respectively, was performed. Cell viability was determined by the Beckman counter method and cell uptake and ROS production was assessed by transmission electron microscopy (TEM) and flow cytometry (FC). Short-term exposures (24 h) to several doses and the BEAS-2B cell line (human bronchial epithelium cells) were used.

The obtained results show no significant differences between frozen and fresh NPs both in their physico-chemical characteristics and their biological effects. Furthermore, the results show that nano-ZnO and nano-TiO_2 are toxic for BEAS-2B cells, the first one being more toxic than the second one. Moreover, our results show the internalization of nano-TiO_2, nano-CeO_2 and nano-ZnO, although the last one (ZnO) in a minor extent, and that only nano-ZnO were capable to generate significant increase in the intracellular ROS production at the highest concentration.

This study would indicate that there are not differences between using fresh and frozen NPs and that using -80 ºC NPs in short and long-term exposure could minimize the variability between samples.