# Further information about the assimilation and activity of ultra-small, non-magnetic iron oxide nanoparticles with potential use in biomedicine

A. Rodríguez-Pescador[1,2,3], L. Gutierrez-Romero[3,4], E. Blanco González[3,4], M. Montes-Bayón[3,4] and L. M. Sierra Zapico[1,2,3]

[1] *Department of Functional Biology (Genetic Area). University of Oviedo, C/ Julián Clavería s/n, 33006, Oviedo, Spain*
[2] *Oncology University Institute of Asturias (IUOPA)*
[3] *Institute of Sanitary Research of Principality of Asturias. Avda de Hospital Universitario s/n, 33011, Oviedo, Spain*
[4] *Department of Physical and Analytical Chemistry, Faculty of Chemistry. University of Oviedo. C/ Julian Clavería 8, 33006 Oviedo, Spain.*
*Email: UO278759@uniovi.es*

The use of iron oxide nanoparticles as therapeutic agents has been a field of interest in biomedicine for some time. Among the wide variety of these particles, the ultra-small (< 10 nm), non-magnetic ones, composed by a ferrihydrite core covered with tartaric and adipic acids (TA-Fe-NPs), are absorbed by the enterocytes as nanoparticles, because of their low solubilization rate in the rat intestinal lumen, and are solubilized inside the cells into free iron. However, since their solubilization might produce reactive oxygen species (ROS), that could be the origin of protein, lipid, and DNA oxidative damage, the assimilation of these TA-Fe-NPs by different cells, as well as their activity, in terms of cell survival and genotoxicity, both *in vitro* and *in vivo*, should be checked.

In this work, we determined both TA-Fe-NP cellular intake and their solubilization in different cultured cells, using HPLC with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) methodology. Cell viability and ROS production in these cells were analyzed after exposure with different concentrations, and the possible induced genotoxicity was evaluated with the Comet assay. *In vivo*, using *Drosophila melanogaster* as model organism, the TA-Fe-NP effects on viability and genotoxicity were studied, using the eye SMART assay, after treating larvae from efficient repair and nucleotide excision repair (NER) deficient strains, (*OK*-NER$^+$ and *mus201*-NER$^-$, respectively), in surface and chronic treatments.

Results show that the NPs solubilize at a slow rate, enter the cells, and increase their Fe content. In addition, TA-Fe-NPs do not impair cell viability and produce low levels of ROS in the studied cell lines. Moreover, in all these cells they induce significant but low increases of DNA damage, only with the highest tested concentration after 3h treatments. These increases, after 24h treatments, were only detected on NER deficient GM04312 cells. *In vivo*, these NPs increase the Fe level in treated larvae, do not show effects on viability and present genotoxic activity only in *OK*-NER$^+$ larvae, after surface treatments.

These data demonstrate that, despite preliminary indication against it, these TA-Fe-NPs might be a good option for the treatment of anemia.