In vivo and *in vitro* genotoxicity of new ultra-small non-magnetic iron oxide nanoparticles with potential use in Biomedicine.

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

Ultra-small (<10 nm) non-magnetic iron oxide nanoparticles, with a core similar to ferrihydrite and coated by tartaric and adipic acids (TA-Fe NPs), were designed a few years ago with a high potential in Biomedicine, as an anemia treatment and/or a drug nanocarrier. They showed a high cellular uptake, low or null toxicity and low solubilization rate. Moreover, in rat models, they reach the small intestine and iron was absorbed at high levels (79%), without effects on cell viability, DNA damage (*in vitro* on cultured cells) or lipid peroxidation, and with rather low levels of reactive oxygen species (ROS) production. Despite these promising data, to assure their biosafety for clinical uses, more complete genotoxicity assays must be performed. Because of that, in this work we have analyzed the possible genotoxicity of these TA-Fe NPs both *in vivo*, using *Drosophila melanogaster* as model organism, and the eye SMART assay to detect induction of mutation and/or recombination in somatic cells, and *in vitro*, using several human cell lines, and the Comet assay to detect induction of DNA strand breaks.

The SMART assay was performed in efficient and deficient nucleotide excision repair conditions (NER^+ and NER^-, respectively), and with chronic and surface treatments, with NP concentrations between 0.1 and 5 mM. Methyl methanesulfonate (MMS; 2.5 mM) was used as positive control. The alkaline Comet assay was performed in A2780, Caco-2 and Hep92 cell lines with concentrations of NPs between 0.25 and 2 mM, in 3 h treatments; 0.25 mM MMS was used as positive control.

Results showed that TA-Fe NPs are genotoxic *in vivo*, with surface treatments and doses over 2 mM, without detected toxicity, in NER^+ conditions. However, no genotoxicity was detected in chronic treatments in the same repair conditions, nor in NER^- conditions, independently of the treatment. *In vitro*, these NPs showed genotoxicity in all the analyzed cells, but only with the highest (no toxic) concentrations. These results demonstrated that these NPs might not be safe enough to be used for anemia treatment. However, they might still be employed as nanocarriers for antitumour drugs, such as cisplatin, because in this case their genotoxicity could represent an added value.