Need for guidelines specifically adapted for the toxicity testing of nanomaterials

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In the last two decades the scientific world has been involved in what we can call a Nanorevolution. Thanks to their surprising and promising physical and chemical properties, nanoparticles (NPs) have attracted the attention of scientists in different fields such as Electronic, Mechanic, Biomedicine and Diagnosis. In this way, the aim of the researchers is the development of appealing devices for a widespread range of applications.
However, this could mean that the use of NPs for commercial products will let these nanomaterials get in contact with Humans and Environment in a dose/time concentration ratio higher than the expected one. Moreover, the possible reactions and the effects that NPs can induce in human's health are still unknown.

Due to the starting use of NPs in commercial products, from electrical devices to food additive, and the increasing attention and warning of the consumers, the European Governments and the EE UU have focused their attention on the "NPs safety issue" in the last decade.

Nowadays, there are lots of validation tests to estimate and evaluate the impact of NPs in the humans and the environment.
These tests include *in vitro*, *in vivo* and *ecotoxicological* assays and, thanks to the results obtained, it is possible to have a general overview on the possible impact of nanocompounds.

The problem, anyway, is that the results obtained till now are chaotic and not well organized, due the lack of standard procedure protocols. That is the reason why the European Commission has located financial grants in the last Seventh Framework Program (*NMP.2012.1.3-3 Regulatory testing of nanomaterials*) to develop a way of standardized and put an order on the nanomaterial world. To help the understanding of the NPs interactions with cells and living systems, some groups of scientists are now using the QSAR ideas of the computational approach and have developed the so-called quantitative nanostructure-activity relationships (QNAR) modeling (Fourches *et al*., 2011).

In this work, we focus our attention on the difficulties in understanding the behavior that NPs can have into a living system. In our laboratory we have made *in vitro* and *in vivo* tests on different types of NPs: gold (with and without coating and with different sizes), cobalt ferrite, cerium oxide and graphene oxide NPs.

The first step was to perform *in vitro* tests of NPs taking into account size, chemical nature and eventual coating to evaluate the possible internalization, cytotoxicity, genotoxicity and embryotoxicity. The results show different effects depending on the type, size and coating (di Guglielmo *et al*., 2010) (Fig1). We performed a wide *in vivo* analysis (in *Rattus novergicus*) by exposing the animals to three types of NPs: gold NPs with and without coating and cobalt ferrite NPs. The obtained results showed differences in kinetic behavior and in deposition in different organs (Fig 2).
In particular, the inhalation exposure to gold NPs for 21 days demonstrates the presence of pneumoconiosis in all treated groups.

On the other side, cerium oxide NPs seems to alterate the DNA structure in the *in vitro* tests inducing genotoxic effects. Interestingly, these NPs in the presence of a well known oxidant compound are able to protect the cells from oxidative stress damages due to its chemical nature.

Seed germination test was performed to evaluate ecotoxicity of these NPs. Different effects were obtained according to different plant species tested. It was especially noticeable that smaller seeds showed a higher level of toxic effects than the bigger ones at the same concentration of nanoparticles.

Even graphene oxide NPs showed interesting results. This nanomaterial seems to be very well tolerated by cells as we have tested its cytotoxicity with MTT tests and it revealed no acute toxic effect. Anyway, it can produce a decrease in the % of viability when considering a chronic effect.

Concerning genotoxicity, a very high toxic response was obtained as it can be compared to the positive control used in the comet assay.

What we would underline is to focus the general attention on the characteristics, behavior and way of interactions of the NPs in the living systems and how these factors should be taken into consideration when we analyze the toxicological results.
Particular factors such as distribution, internalization and surface coating can modify the fate of NPs in the cells.

Differences in size, shape, coating and chemical nature increase the difficulty level for a unique, linear and coherent understanding of the results.
Finally, since the industrial and biomedical industries has a need for standards and well fixed parameters, it's necessary to start establishing the basis for a European guideline that should be followed Europewide.

References

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Figures

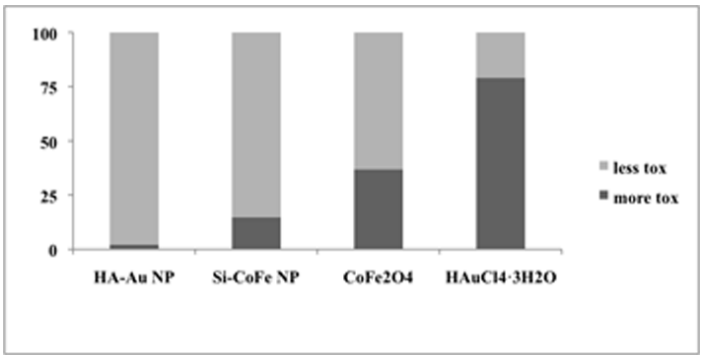

Fig 1.- Histogram of the potential degree of toxicity in embryos with the in vitro Embryonic Stem Cell Test.

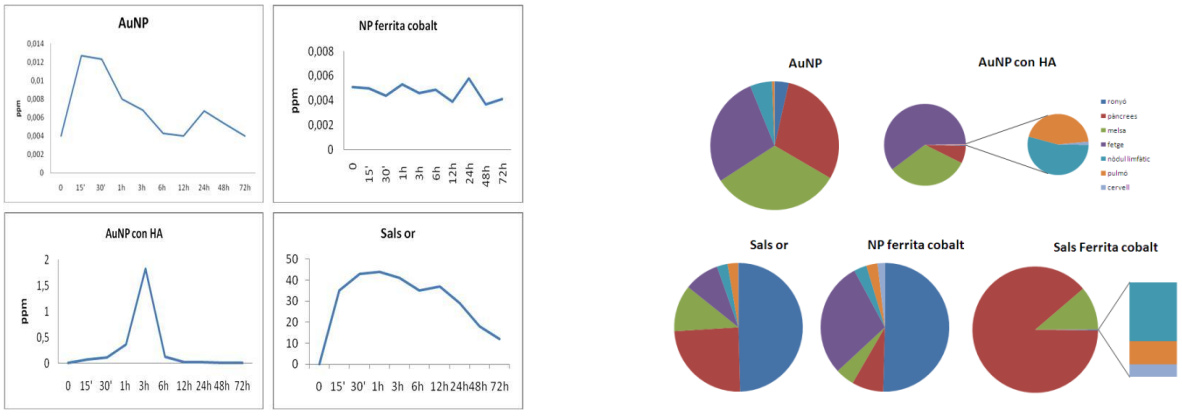

Fig.2.- Results of in vivo assays for blood kinetic (left) and body biodistribution of NPs (right).

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EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
A WAY TO MAKE EUROPE
This project has been co-funded by MICINN and ERDF
NanoSost, towards a sustainable, responsible and safe nanotechnology
PSE–42000–2008–003