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SCIENCE FOR A SUSTAINABLE DEVELOPMENT

Physico-Chemical Determinants of Toxicity : A Rational Approach Towards Safer Nanostructured Materials

S²NANO

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FINAL REPORT PHASE 1
SUMMARY

PHYSICO-CHEMICAL DETERMINANTS OF TOXICITY : A RATIONAL APPROACH TOWARDS SAFER NANOSTRUCTURED MATERIALS

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A. Context

At present, nanomaterials have invaded nearly all industrial domains and are already in numerous consumer articles. Their production and marketing will further increase over the next few years. During these activities, employees, consumers and the general public in the broad sense, will potentially be exposed to these nanomaterials. The possible impact on health is therefore clearly an issue. In fact, the same physico-chemical properties that make nanomaterials so attractive from a technological point of view, could be dangerous to human health. In particular, the high level of physico-chemical reactivity of nanomaterials is also likely to occur in the biological tissues with which the nanoparticles come into contact. An assessment of the toxicological properties of nanomaterials is therefore urgently required. The available fragmentary data confirms these fears, and we currently know that nanoparticles have a toxic effect on the respiratory system, but probably also on the cardio-vascular system and on the central nervous system.
Considering the great diversity of existing and future nanoparticles, the task of determining the dangers of each of these substances on health will be huge, and probably disproportionate to the means available. It is therefore necessary to develop more generic approaches based, in particular, on knowledge of the properties of nanoparticles, which determine their toxic activity.

B. Aims

The aim of the S²NANO programme is to understand how nanoparticles exercise their toxic effects, and to identify the physico-chemical determinants.
This approach will contribute to:

- a better knowledge of the mechanisms that influence the interactions of nanomaterials with cells and biological tissues,
- improving the metrological approach towards nanoparticles, based on more relevant parameters than the mass dose, such as the surface or the number of particles or their morphology.

This programme will provide solid scientific bases to allow the sustainable development of nanomaterials and to provide statutory stakeholders as well as industrialists with recommendations based on evidence for the production and control of safer materials. It deals exclusively with aspects of human health, and does not address ecotoxicological aspects.

The experimental model selected to carry out the research is based on silica nanoparticles (SNP). There are many reasons for this choice, in particular:

- the possibility of synthesising a large range of SNP relatively easily by varying one property at a time,
- the great purity with which such SNP can be generated,
- the insolubility of SNP which allows the effect of the nanoparticles to be studied *per se* by excluding the impact of elements or molecules that could be made soluble in biological environments,
- the possibility of easily dosing the silicon in biological environments exposed to these SNP, as a marker of the dose in the biological target,
- the partner responsible for the preparation of the SNP has a great deal of experience in these nanomaterials.

C. Conclusions

During the first two years of the project (phase I), the research teams set up a real multidisciplinary approach, which provided for dialogue and reciprocal enrichment between the biologists and chemists taking part in the network.
The tools allowing the research to be carried out were set up first; SNP were synthesised and carefully prepared and cellular models were developed to study the effect of these SNP.
A unique series of 17 monodispersed SNP was synthesised by varying the size, surface and/or porosity of the nanoparticles (Table 1).
In order to be able to study the biological effects of these individual SNP and to avoid the formation of aggregates, a special effort was made to obtain a formulation that would allow a suspension of individual SNP to be maintained in an aqueous environment, including in the cellular culture medium. Some SNP were also prepared in conditions guaranteeing the near absence of endotoxins in order to be able to study the impact of these SNP on the delicate and highly sensitive immunological processes of this type of contamination. These preparations were very carefully characterised and this work was the subject of an initial scientific publication (Thomassen *et al.* 2009).
Cellular models were developed for the *in vitro* study of:

- the genotoxic effects on a human pulmonary epithelial cell line,
- the effects on a human endothelial cell line and the impact on coagulation,
- the activation of a macrophage line and the production of inflammatory mediators.

Before implementing these cellular models, it was necessary to adjust the SNP dosimetry problems and to check which fraction of the dose implemented was biologically active. We were able to show that the nominal dose (all the SNP present in the culture medium) accounted for their cytotoxic activity in the three cellular lines used as a model. This data was the subject of a second publication (Lison *et al.* 2008).

Before dealing with more complex cellular phenomena, we studied the cytotoxic activity of the SNP regarding the three target-cellular lines. The smallest SNP turned out to be the most cytotoxic, and the particle surface (rather than the mass) turned out to be the parameter that best accounted for the cytotoxic activity of the SNP. This data was also the subject of a publication (Napierska *et al.* 2009). Additional work showed that the physico-chemical determinants of the cytotoxicity varied according to the cellular line studied and it therefore appeared to be highly improbably that a single characteristic of the SNP would be able account for their cytotoxicity. A particularly interesting observation concerns the protective impact that the microporosity of the SNP seems to have on the macrophage line, but not on the other lines.
To study the genotoxic activity of the SNP, it was necessary to respect a certain number of quality criteria guaranteeing the scientific validity of the data obtained. For this purpose, a critical review of the literature was carried out in order to discover the most important aspects to be taken into account in nanogenotoxicology. This work was the subject of a scientific publication (Gonzalez et al. 2008). Among other things, the possible interaction of nanoparticles with the genotoxicity tests must be envisaged. Within this context, the interaction of the SNP with glycosylase in the comet test and the cytochalasin-B with the internalisation of the SNP in the micronucleus test was studied. The genotoxicity study results indicate that the SNP cause clastogenic and aneugenic effects; these effects are all the more significant in the smallest SNP. Data obtained during this first phase also suggests that the SNP act via an interference with the cellular microtubules.

D. Contribution of the project within the context of scientific support for a sustainable development policy

It is essential to take into account the possible impacts of nanotechnologies on the environment and human health early on, in order to guarantee the sustainable development of this new field of economic activity, and to avoid catastrophes such as those we have recently experienced, for instance, in the case of genetically modified organisms.

Within the spirit of sustainable development, the acceptance of new nanomaterials by the general public will indeed largely depend on the ability to integrate the (eco)toxicological aspects of the first phases of industrial development. The S²NANO project allows toxicological problems to be anticipated, and therefore has a major socio-economic value.

E. Keywords

Nanomaterials – Nanoparticles – Nanotoxicology – Nanogenotoxicology