PUBLISHABLE EXECUTIVE SUMMARY

Development of an Integrated Platform for Nanoparticle Analysis to verify their possible toxicity and the eco-toxicity

Nanotechnologies are the emergent technologies of the 21st century. Europe enjoys a strong position in nanosciences that is being translated into a growing competitiveness of the European industry. The fast growth of nanotechnologies is not paralleled by an equally fast evaluation of their impact on the environment and on animal and human health. Nanotechnological industries and activities generate nanoparticles (NP) that can be employed in an extensive variety of objects of everyday life (cloths, glasses, tiles, paper, etc.), thus they can represent an unprecedented type of pollution, a stimulus of unknown consequences for the living organisms. No information is available about the consequences of exposure to NPs on the functions of cells, tissues and organs, and their possible impact on health.

The aims of the DIPNA project are to provide knowledge about the impact of metallic and ceramic NPs on different types of cells, and to design assays and instruments for field analysis of nanotoxicology.

The DIPNA project addresses the NMP Research Area NMP-2004-3.4.1.5-2 “Interaction of engineered nanoparticles with the environment and the living world.” The project aims at creating and validating instruments and assays, to assess the nanotoxicity of nanoparticles proposing new tests to verify their possible cytotoxicity and ways for detection of nanopollution in occupational sites, in order to promote prevention and nanosafety in NP manufacturing and handling. In this novel, multidisciplinary, multinational research project the Consortium aims at developing a precise knowledge on the impact of NP on human defence cells (nano-immunotoxicity). This knowledge will be validated in comparison with nano-genotoxicity and nano-carcinogenicity, *i.e.*, studying in parallel the activity of NP in a standardised transformation assay *in vitro*, to predict the potential risks of exposure to NPs for human health.

The scientific knowledge gained during this project will provide the ground for a development platform, aiming at standardising and field-validating prototypic assays and related instruments for biodetection of NP-associated health risks.

The coordinator is: **Dr. Antonietta M. Gatti**
Consorzio Nazionale Interuniversitario Sviluppo Materiali
University of Modena and Reggio Emilia – Italy

The **Consortium** is composed of 10 partners:

University of Salzburg - Austria
Fraunhofer Institute of Biomedical Engineering - Germany
Consiglio Nazionale delle Ricerche - Italy
Università della Magna Grascia - Italy
Grimm Aerosol - Germany
VITO n.v. - Belgium
CSEM SA - Switzerland
Institut Català de Nanotecnologia - Spain
Commission of the European Communities - Directorate General - Joint Research Centre, Ispra - Italy

The scientific objectives of the project are:

1. Definition of *in-vitro* systems of interaction of engineered NP with living cells, relevant to the assessment of immunotoxicity from single, chronic and repeated exposure. Use of human professional (T cells, dendritic cells, macrophages) and non-professional defence cells (epithelial cells from lung, gut, liver) as models of innate and adaptive immunity, inflammation and angiogenesis; use of mouse fibroblasts as model of cytotoxicity, genotoxicity and carcinogenicity.
2. Identifying the modes of NP-cell interaction: set up of one-to-one NP-cell systems (singlet interaction) to verify a dose-response *vs.* threshold effect
3. Applying the laboratory-developed cellular models to the field investigation.

The simulation tests of cell-NP interaction proposed here are based on a previous 3-year-long experience of "nanopathologies" (European Project Nanopathology FP5-QOL-2002-147), where non-engineered NP were found entrapped in human and animal pathological tissues (www.nanopathology.it).

The main goal will be the set-up of a standardisable nano-cytotoxicity tests with meaningful parameters of the nanointeraction, able to describe possible nano-effects.

The technological objectives of the project are:

1. Development of *in-vitro* simulation tests where a clear **interaction of cells with nanosized particles** occurs (as opposed to aggregates or clusters). This objective is of key importance. The scientific results available usually do not consider the state of NP after interaction with cells. Some results showing lack of toxicity of NP may in fact be explained by the lack of interaction with cells, due to aggregation in the culture medium with formation of microparticles that cannot be internalised.
2. Development of an *in-vitro* **system of single cell-to-NP interaction**. This objective is highly innovative and advanced from the technological point of view. It can be realised only with the aid of nanotechnologies. This detection system will reproduce the *in-vivo* conditions, providing an accurate description of the real NP-cell interaction.
3. Development of **new sensors for detecting specific NP-induced biological reactivity**, based on defined and validated sensible parameters. The results of the tests will represent the basic knowledge necessary to the construction of new sensors. These will form the ideal platform for new advanced technologies.
4. Assembly of a **system for detecting and analysing NP in industrial settings**. This system will allow risk assessment within the working environment of nanotechnological laboratories or factories and could eventually contribute to set up prevention measures, so reducing risks to human health.

The Project is divided into 6 workpackages:

WP1: Fabrication and characterisation of selected NP
WP2: Nanotoxicology: NP-induced alterations of human defence cell physiology
WP3: Single particle impact on cells
WP4: Integrated platform for monitoring NP effects upon chronic and repeated exposure
WP5: Field validation and development platform
WP6: Coordination, management, training, and public awareness

The project presents several points of **originality**:

1. New methods for detecting NP-cell interaction (single *vs.* multiple)
2. Methodology for assessment of dynamic interaction (chronic and repeated)
3. Dose-effect and uptake-effect relationships related to immunotoxicology, carcinogenicity and genotoxicity (*in vitro* assay).
4. Identification of cellular markers of NP interaction, as a function of their size and chemistry, in relation to their immunotoxicity, carcinogenic potential and genotoxic effects.
5. The development of nano-biosensors for the evaluation of cell functions after exposure to NP.
6. Strategy to detect and estimate indoor and outdoor NP as initial approach to risk assessment in industrial sites.

7. Feasibility of an integrated platform for development of NP biodetection tools for evaluating the possible health risk in the environment by combining different methods from NP aerosol characterisation (physical parameters) with biological methods.
8. The anticipation of a real need of the society, asking for a healthier environment.

The achievement of all the tasks will promote:

1. A consensus paper as a basis for **novel criteria for prevention** and environment/health protection;
2. Providing **new technological instruments for implementing safety** and preventive procedures;
3. Proposing new ways for **detoxification and bioremediation**;
4. Suggesting **new rules for a safer production** of nanotechnological devices, food, cosmetics and drugs.

Summary of the 1-year achieved results

The first year of the project which terminated at the end of October 2007 was characterized by the implementation of all the objectives expected for the first year.
Nanoparticles of Cobalt, Nickel, Iron oxide, Cerium oxide were synthesized in dry and wet formulations and used for toxicity tests. A set up of common protocols for analysis of NP effects on human professional and non-professional defence cells was developed by 4 partners on Jurkat cells, T cells, epithelial cells, monocytes, macrophages and dendritic cells and on human immune cells. Genotoxicity and carcinogenicity tests were performed using Balb/3T3, to verify: the cell-growth inhibition through classical toxicological tests of ISO/EN 10993 (MTT, BrU, and alamarBlue™ and WST-1), the production of intracellular produced ROS using the radical-sensitive dye H_2DCF-DA , the analysis of the membrane-marker proteins HLA-DR, CD86, CD83 and CD54 and the uptake by CD34-DC.
It was also evaluated the carcinogenic potential and genotoxicity by Colony Forming efficiency and Morphological Transformation Assay (CTA), by Comet assay, and Micronucleus test. Also tested were the cell activation, the mRNA expression of a series of genes (the inflammatory cytokines IL-1ß, IL-18, and IL-1F7 (all five isoforms), the anti-inflammatory cytokines IL-33 and IL-1Ra, the receptors of such cytokines IL-1RI, IL-1RAcP, IL-18Ra, IL-18Rß, T1/ST2, the orphan inhibitory receptor TIR8, caspase-1, IL-18BP) involved in the regulation of inflammation and innate defence responses.
The proposed tests verified the non toxicity of the NPs , but morphological studies verified that the cell uptake was not homogenous and in some cases there was no interaction of NPs with the cells.

Coordination: **Antonietta M. Gatti**

Laboratorio dei Biomateriali
CNISM- Dipartimento di Discipline chirurgiche
Università di Modena e Reggio Emilia
Via Campi 213 A – 41100 Modena – Italy
antonietta.gatti@unimore.it
www.dipna.eu