The NanoCare project: a German initiative on health aspects of synthetic nanoparticles

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Abstract. Nanotechnology is increasingly considered to be the future technology. It will enable science and industry to provide new and better product solutions for the society. NanoCare is a German project, funded by the German Federal Ministry of Education and Research (BMBF), which aims to broaden knowledge about synthetic nanomaterials with regard to the potential impacts of nanomaterials on human health. 13 partners from industry, universities and research institutes are contributing their expertise to this partnership. The work plan of the NanoCare project is composed of three different parts: (1) the generation, (2) the management, and (3) the transfer of knowledge. The production of synthetic nanoparticles, the subsequent analysis of primary particles, aggregates and agglomerates, as well as the behaviour in biological media and effects on biological systems are focused in the generation of knowledge. In addition to the production and characterization of new synthetic nanoparticles (metal oxides like zirconium dioxide or zinc oxide), titanium dioxide and Carbon Black will be established as reference materials. This enables the comparison of the results of all partners of our project. Various analytical methods for characterization will be applied, for example: transmission and scanning electron microscopy, inductive coupled plasma mass spectroscopy (ICP-MS), atomic absorption spectroscopy (AAS) and the Brunner-Edward-Teller method (BET). In vitro studies will systematically investigate biological mechanisms of action of nanoparticles and the dependency on their size, shape, zeta potential and other important properties. In vitro data will be complemented by in vivo studies. Another work package deals with the measurement of working place exposure and agglomerate stabilities. Established measurement devices and methods will be developed further in order to determine aerosols and nanoparticles directly at the workplace during ongoing work processes. The stabilities of the agglomerated nanoparticle powders are additionally investigated with three different methods to assess deagglomeration probabilities which also influence the possible exposure. Data created within the NanoCare project consortium will be interpreted together with information from literature and then published for the public in a data base on the World Wide Web (www.nanopartikel.info). Furthermore, the results will be presented and discussed with the interested public, politicians and non-governmental organizations (NGOs) at dialogue events. Together with two other BMBF-funded projects (INOS, TRACER) NanoCare will help to standardize analytical procedures and will substantially increase knowledge about the biological activities of nanomaterials.
**Organization of the NanoCare project**

NanoCare is organized in different work packages (WP, Figure 1). An internal and an external council act as advisors. The objectives of the WP 1-5 intend to produce (primary) data for workplace-based health effects of nanoparticles. These data will be interpreted together with information from literature and then published for the public in a data base on the World Wide Web by WP 6. Furthermore, the results will be presented and discussed with the interested public, politicians and non-governmental organizations (NGOs) at dialogue events (WP 7).

![Figure 1. Organization chart of the NanoCare project.](image)

WPs 1 & 2 deal with the identification, preparation, standardization and characterization of nanoparticles. The production of synthetic nanoparticles is not new and numerous particles are already used in different applications. Nano-scaled Titanium dioxide and Carbon Black for example are well characterized. For that reason these particles serve as reference materials within the NanoCare project and will be compared with other novel and/or modified synthetic particles. The industrial partners of the project chose and produced inorganic and hardly soluble materials (Table 1). The control and adjustability of surface chemistry during the production process of these novel nanoparticles results in specific characteristics and opens new applications.

| Table 1: Particles used within the NanoCare project in alphabetical order. |
|---|
| **Material** |
| AlOOH (Boehmite) |
| BaSO₄ |
| CeO₂ |
| SrCO₃ |
| ZnO |
| ZrO₂ |
| mixed oxides (Ti-Al-Zr, Ti-Zr) |
| TiO₂ (reference material) |
| Carbon Black (reference material) |

Methods of synthesis, source materials and modifications are equally defined to ensure that all project partners work with the same material, hence the comparability of the results is guaranteed. Therefore, methods and measuring equipments will be standardized. Importantly, agglomeration- and aggregation-behaviour of the primary particles as well as the stability of the agglomerates and aggregates will be considered, both in aerosols and in solution. To assess the risk potential of nanoparticles the physico-chemical characteristics as well as the behaviour in physiological media and biological test systems will be analyzed.
WP 3 and WP 4 deal with the biological effects. In WP3 the toxicity of synthetic nanoparticles is investigated in various cell-based in vitro systems. In vitro experiments are carried out with cell cultures of different types and origins, with different methods of analysis and with various particles to enable a broad evaluation of the adequate test system. For this purpose, fifteen cell lines representing the organs lung, skin and gastrointestinal tract as well as cells of the immune system were selected. Cell biological and toxicological methods are applied and, if necessary, adapted to the needs of particle testing. The aim is the development of standard operation procedures (SOPs) for toxicological in vitro particle testing. An SOP dealing with the preparation of suspensions of nanoscaled metal oxides for biological testing has already been published [1]. A second SOP concerning phagocytes is published on our web page [www.nanopartikel.info/methoden.html].

Other main aspects of this work package are the investigations of the cellular uptake and the crossing of the pulmonary air-blood-barrier by nanoparticles. An additional aim is the evaluation and pre-validation of in vitro cell systems as an alternative for animal experiments. Thereby, the localization of particles at or in cells is investigated with different nano-biophysical methods like AFM/FS. Furthermore, the in vitro vector model, which is based on primary rat macrophages and mimics some of the elemental cell effects related to fine dust toxicity, is adapted to its use with nanomaterials [2]. With this model system effects on the cellular metabolism, stimulatory effects and particle-induced ROS secretion, can be analyzed simultaneously and a biological screening and monitoring of the potential hazard of nanomaterials is possible. This represents a helpful tool to correlate in vitro data with results from in vivo animal experiments.

Animal studies were carried out within the work package 4. In this WP in vivo inhalation studies (5 days of inhalation and 3 weeks observation) as well as 28-day subacute inhalation toxicity studies (inhalation: 6 h/d, 5 day/week on 4 consecutive weeks) with selected particles are performed in rats to analyze pulmonary toxicity. To date, titanium dioxide, zinc oxide, zirconium oxide, cerium oxide and carbon black were used in the short term animal studies, while AlOOH was investigated in the subacute inhalation study. Technical procedures of atmosphere generation and determination of particle size were established and standardized. There is evidence that nanomaterials show a tendency to form agglomerates or aggregates in the test atmosphere and that only a small part of the particles is nanoscaled [3]. An SOP concerning this short-term inhalation study for testing of nanomaterials has already been published [4].

WP5 "Reality – Exposure measurements at workplaces" is divided into three parts: (1) stabilities of agglomerates under mechanical stress and modelling of physical particle dynamics; (2) development and standardization of measurement devices and methods; (3) measurement and characterization of possible exposure to aerosols and nanoparticles directly at the workplace during ongoing work processes.

First results of the dusting experiments (with low mechanical stress) show that there is a release of nanoscaled particles subjected to the particle powder [unpublished data]. Several SOPs concerning the activities in this work packages are in preparation.

WP6 & 7: Knowledge transfer

The third pillar of the project encompasses the research, compilation and interpretation of published data from the literature and internal data of the toxicity of nanomaterials. This information will be addressed to the public and published on the web site www.nanopartikel.info (in German language). We established this “knowledge base” (Wissensbasis) and focus on the materials analyzed within the NanoCare project. We also aim to answer to frequently asked questions and give the opportunity to ask an expert.

The main concern of the NanoCare project focuses on the allocation of a competent and coherent knowledge base about chances and security aspects of nanomaterials for stakeholder, politicians, NGOs and interested layman. The organization and realization of two stakeholder dialogs (2007 and
2009) and three citizen dialogs (2008 in Hamburg, Munich and Dresden) is a further important part of this work package.

We prepare high quality results due to the manifold competences of the NanoCare partners. All together we work on the basis of an objective and targeting discussion about potential chances and risks of nanomaterials. More than 70 NanoCare members contribute to this aim coordinated by a project leader and his management assistant at the research center Karlsruhe.

Taken together, the efforts of the NanoCare project are to ensure that new scientific insights into the effects of synthetic nanoparticles on human health are efficiently and adequately communicated to the public.

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