Inhaled nanomaterials

Valorisation paragraph

This thesis focuses on the effects of nanomaterials after inhalation. In this paragraph, the value of this thesis for the society and economy is discussed.

In the society, there are concerns that nanotechnology poses risks to human and environmental health. For many people nanotechnology and related terms are difficult to understand as nanomaterials and nanotechnology cannot be seen by the naked eye. In the meantime, nanomaterials are used in a growing number of consumer products because of their unique physicochemical properties that are related to their small size. These unique properties can be beneficial - for example nanomaterials are used to develop efficient solar cells, dirt-repellent textiles, anti-cancer drugs and fast computers - or they might introduce health risks. There is need for adequate and efficient risk assessment of nanomaterials to keep up with the growing number of products containing nanomaterials. Currently, there are 292 personal care products on the market that claim to contain nanomaterials (Nanotechnologies, 2014). People can be exposed to these products daily, while the safety of these nanomaterial containing products is not tested. There are many concerns in the society that a long-term exposure to nanomaterials will induce adverse effects in the future; we just do not know this yet. Especially inhalation of nanomaterials is of concern given the vulnerability of the lungs and the experiences in the past with asbestos. The challenge is to assure the safety of nanomaterials and nanotechnology without hampering the development of new products and services of which the society can benefit.

At the moment, each nanomaterial with slightly different characteristics, e.g. 20 nm vs. 50 nm, spherical vs ellipses, coated vs. non-coated, should in principle be assessed separately. Given the large number and variety of nanomaterials, it is not feasible to test each nanomaterial with slightly different characteristics to identify their hazard. In this thesis, we identified the particle surface area that reaches the alveoli as a suitable dose descriptor to describe the acute effects of silver nanoparticles both after *in vivo* inhalation and in lung epithelial cells *in vitro*. The identified dose metric can be used in the risk assessment of silver nanoparticles to predict the acute effects of uncoated silver nanoparticles of different sizes that have not been tested yet, thereby reducing the number of studies needed to assess the risks of nanomaterials. For example the release of a pro-inflammatory cytokine in the lung fluid was increased by 20% after exposure to 1.7×10^9 nm^2 silver nanoparticles per cm^2 of alveoli, irrespective of the particle size. For 74 nm silver nanoparticles that not have been tested in our *in vivo* study, the release of this marker will probably also increase by 20% after exposure to 1.7×10^9 nm^2 silver nanoparticles per cm^2 of alveoli. For the risk assessment of nanomaterials that differ in other characteristics like chemical composition, coating or surface charge, the

dose metric can be identified - in studies that test nanomaterials that vary in a single characteristic - to be able to predict the effects of other nanomaterials that not have been tested. In this way, information on the hazard of nanomaterials can be efficiently gathered without testing all nanomaterials separately.

To perform risk assessment of nanomaterials without stopping innovation in the field of nanotechnology, there is need for new strategies that efficiently gather hazard information on nanomaterials. Next to this, a clear communication between industry, safety assessors and legislators is needed. Risk assessment of nanomaterials should not be based on a checklist of experiments that are required for other chemicals. Efforts should be made to optimize experiments for nanomaterials by focusing on nanomaterial characterization, dosimetry and the development of alternative methods. In order to make this work, industries should be transparent about the use of nanomaterials in products and possible exposure to both workers and consumers. In addition, safety assessors should be trained on nanomaterial characteristics and behaviour to be able to use this knowledge in the risk assessment of nanomaterials. Finally, legislators should be more flexible in accepting risk assessment information and strategies that are not based on checklists of (animal) experiments that are required for conventional chemicals. In this thesis, we present a new testing strategy to assess the acute effects of nanomaterials after inhalation without using animals. This testing strategy can help to speed up the risk assessment process and thereby the development of new nanomaterials and nanotechnology-enabled products.

To inform people about the results of this thesis, many chapters are also presented in peer reviewed journals, at conferences and in a short movie. Peer reviewed journals are mostly read by colleagues within the nanotoxicology research field. At conferences, oral presentations and poster presentations reach a broader audience within the nanomaterial and toxicology field. Especially within NanoNextNL - which is a consortium of more than one hundred companies, universities, knowledge institutes and university medical centres, aimed at research into micro and nanotechnology - there was great interest in risk assessment of nanomaterials. NanoNextNL funds many PhD projects that all are required to include risk assessment in their thesis. During a risk assessment and technology assessment (RATA) course organized by NanoNextNL, the research present in this thesis was used to explain the importance of risk assessment of nanomaterials to other PhD candidates that are involved in developing new nanomaterials and technologies. By including risk assessment in the development of nanomaterials and nanotechnology, products can be developed that are safe by design. This means that information on toxicity-determining characteristics of nanomaterials can be included in the development of new products. For example, the use of fibre shaped nanomaterials in sprays will be avoided as they are more hazardous compared to spherical particles. In addition, new nanomaterials can be tested in toxicity assays and subsequently modified until they induce minimal or no toxicity. NanoNextNL also encouraged collaboration between universities, institutes and industry resulting in sharing of knowledge between experts in the field of toxicology, risk assessment, physics, and chemistry. These collaborations will help future projects on

nanomaterials to include the expertise form different fields and will aid the development of nanomaterials that are safe by design. Next to this, the results of this thesis will be used as a case study in future workshops on dosimetry to inform other researchers about the importance to use the appropriate dose metric to describe the effects of nanomaterials.

This thesis also resulted in a close collaboration with the Karlsruhe Institute of Technology (KIT). The QualityNano (QNano) Transnational Access program of the QNano Research Infrastructure sponsored visits to KIT. This gave us the opportunity to work with a newly developed exposure system that is able to expose cells *in vitro* to nanoparticles at the air-liquid interface under temperature and humidity controlled circumstances. For studying the effects of nanomaterials after inhalation, such innovative systems are essential as they can mimic the *in vivo* inhalation more closely compared to conventional submerged exposures, especially regarding the deposited doses and dose rates.

To inform the general public about research that is performed on the toxicity of nanomaterials after inhalation, a short movie was released via the website of the National institute for Public Health and the Environment (RIVM):
http://www.rivm.nl/Documenten_en_publicaties/Algemeen_Actueel/Multimedia/Infectieziekten/Wetenschapscommunicatie/Inademing_van_nanomaterialen/Download/Inademing_van_nanomaterialen
This movie was specially made for the lay public as part of the project 'Jonge onderzoekers in beeld'. The purpose of this project was to provide opportunity for PhD students to present their research professionally and clearly to the general public, and to provide information to the public about the relevance to society of research conducted at the RIVM.

There are no specific regulatory frameworks or guidance documents for the risk assessment of nanomaterials (yet). In this thesis, a novel testing strategy is presented to assess the acute effects of nanomaterials after inhalation without using animals. If this testing strategy proves to be valid, a large number of nanomaterials can be tested. The testing strategy presented in this thesis focuses on the acute effects of nanomaterials after inhalation. A similar strategy can be designed for other exposure routes and effects, for example after oral exposure. By first determining the appropriate dose metric both *in vivo* and *in vitro*, preferably using available *in vivo* data, *in vitro* models can be validated. Next, these *in vitro* models can be used to determine the hazard of new nanomaterials after for example oral exposure. Information on the hazard of nanomaterials is essential for their risk assessment. In addition, the information can also be used to group nanomaterials by their hazard in order to find characteristics that are linked to hazard. This will help to develop and produce nanomaterials that are safe by design. This is beneficial to the economy as the development of nanomaterials and nanotechnology enabled products is a growing market. The society also benefits as safe nanomaterials can be used to develop beneficial products like sunscreens, medicines and computer technology while exposure to potentially harmful nanomaterials is minimized.