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**Nanotechnology in medical applications:  
possible risks for human health**

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## Abstract

### **Nanotechnology in medical applications: possible risks for human health**

While products based on nanotechnology are actually reaching the market, sufficient knowledge on the associated toxicological risks is still lacking. Reducing the size of structures to nanolevel results in distinctly different properties. As well as the chemical composition, which largely dictates the intrinsic toxic properties, very small size appears to be a predominant indicator for toxic effects of particles. Based on these conclusions, the development of specific guidance documents at a European level for the safety evaluation of nanotechnology products applied in medical technology is strongly recommended and the need for further research in the field of nanotoxicology is clearly identified.

For medical applications, immobilized nanostructures inside or on surfaces of medical devices such as surgical implants are expected to pose a minimal risk as long as they remain fixed. Release due to continuous chemical processes and/or mechanical stress at the interface of implants and surrounding tissues might yield potential risks, however. For medical applications utilising free nanoparticles or nanostructures, for example novel drug delivery systems, the specific toxicological properties have to be investigated. It is insufficient to rely on knowledge of the classical toxicity testing of chemical(s) and materials when the risks of nanoparticles and/or nanostructures have to be assessed.

From a regulatory point of view, a risk management strategy is already a requirement for all medical technology applications. With regard to applications utilizing nanotechnology this is considered sufficient, as long as manufacturers, notified bodies and competent authorities are made aware of the need to carry out a dedicated (nano)toxicological risk assessment.

Keywords: nanotechnology; nanotoxicology; nanoparticle; risk management; medical technology.



## Rapport in het kort

### Nanotechnologie in medische toepassingen: mogelijke gezondheidsrisico's

Er is te weinig kennis over de toxicologische risico's van nanotechnologische producten, terwijl deze al wel op de markt verschijnen. Het verkleinen van structuren tot nano-niveau resulteert in het ontstaan van andere eigenschappen dan men alleen op grond van de chemische samenstelling zou verwachten. Ook de afmeting blijkt namelijk een belangrijke parameter voor de toxiciteit van deeltjes te zijn. Op grond van deze conclusies wordt sterk aanbevolen om, bij voorkeur op Europees niveau, specifieke richtsnoeren te ontwikkelen voor de veiligheidsevaluatie van nanotechnologie toepassingen in het veld van de medische technologie. Daarnaast wordt de noodzaak voor verder onderzoek op het gebied van de nanotoxicologie duidelijk vastgesteld.

Voor toepassingen waarbij de nanostructuren vast zitten op het oppervlak of in het materiaal, zijn de risico's naar verwachting minimaal, zolang ze niet uit het materiaal kunnen vrijkomen. Voor toepassingen van vrije nanodeeltjes of nanostructuren, zoals bijvoorbeeld in bepaalde "drug delivery" systemen, moeten de specifieke toxicologische eigenschappen expliciet onderzocht worden. Het is onvoldoende om te vertrouwen op de basiskennis over toxiciteit van materialen en stoffen wanneer de risico's van nanodeeltjes en/of nanostructuren moeten worden beoordeeld.

Volgens de regelgeving voor medisch technologische producten is een fabrikant verplicht een risico management strategie te hanteren. Ook voor producten waarbij nanotechnologie wordt toegepast is dit voldoende, mits fabrikanten, aangewezen instanties en overheden zich bewust zijn van de noodzaak om een specifieke (nano)toxicologische risicobeoordeling uit te voeren.

Trefwoorden: nanotechnologie; nanotoxicologie; nanodeeltje; risicomangement; medische technologie.



## Preface

This report describes the possible risks for human health related to the application of nanotechnology in medical practice. This review was performed on the request of the Department of Pharmaceutical Affairs and Medical Technology of the Ministry of Health, Welfare and Sports in the Netherlands. The information gathered here is presented as basic information to staff of this department who are involved in determining Dutch policy on medical technology issues, but it may also be useful for other parties in the fields of nanotechnology and/or medical technology. Simultaneously, a second report was put together, which elaborates on the state of the art of materials and devices in the area of nanotechnology in medical applications.<sup>1</sup>

For the overview presented here, the scientific literature was evaluated and included when available either in journal publication or electronic prepublication on the internet before the 31<sup>st</sup> of March 2005.

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## 1. Introduction

Nanoscience can be defined as the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales where properties differ significantly from those at a larger particulate scale. Nanotechnology is then the design, characterization, production and application of structures, devices and systems by controlling the shape and size at the nanometre scale (The Royal Society and The Royal Academy of Engineering, 2004).

Nanotechnology is considered an emerging technology with enormous potential in a range of applications. In addition to various industrial uses, great innovations are foreseen in metrology, electronics, biotechnology, medicine and medical technology. It is anticipated that nanotechnology can have an enormous positive impact on human health. The potential medical applications are predominantly in diagnostics (disease diagnosis and imaging), monitoring, the availability of more durable and better prosthetics, and new drug delivery systems for potentially harmful drugs (Roszek, 2005; The Royal Society and The Royal Academy of Engineering, 2004). “Nano” means very small, and it comes from the Greek word “nanos”, meaning dwarf. It is also used as a prefix to indicate size in the series kilo-, milli-, micro-, nanometer. A nanometer is one thousand millionth of a metre,  $10^{-9}$  m. Nanoscale is generally considered to be at a size below  $0.1\mu\text{m}$  or 100 nm.

Nanostructures and particulates are well known in the biological field. Natural entities like viruses, and artificially constructed imitations of biomembranes such as liposomes are examples of products at nanolevel with a profound interaction with biological systems. However, recent progress in nanotechnology and nanosciences now indicates that we are entering a new area with large scale production and application of nanostructures and nanoparticles with a non-biological origin or history. Manufactured nanoparticles can be made from nearly any chemical, however, most nanoparticles currently in use have been made from transition metals, metal oxides, silicon, carbon, and various polymers (Duncan, 2003; Dreher, 2004; Ferrari, 2005). Manufactured nanoparticles can have physicochemical characteristics and coatings that give them unique electrical, thermal, mechanical and imaging properties which make them highly desirable for the aforementioned applications within medical technology.

However, the use of nanotechnology may be accompanied with yet unknown risks for health. Using very small particles, beyond a certain level (below a certain size) it seems that materials change, resulting in new characteristics, and old physical and chemical laws do no longer necessarily apply (Preining, 1998). The biological properties of such nanostructures/nanoparticles are largely unknown. Most research in the area of particles with regard to toxicity has been carried out in the field of ambient air pollution. Knowledge obtained in that area indicates that very small particles (ultrafine dust, nanoparticles) are not simply smaller particles of the same chemical entity with the same well known toxicological profile. There are indications that they behave differently compared to larger particles. The relatively enormous surface area compared to the actual volume seems to result in as yet largely unknown properties of nanoparticles.

The discussion on the safe use of nanotechnology has already started. The suggestion is that there is no such thing as “the nanotechnology”, but rather one should speak of various different forms of nanotechnology (nanotechnologies) each with its own separate area of applications and specific problems. (European Commission, 2004). The common feature of these nanotechnologies is the very small scale of the involved objects. Recently, various reports were published addressing the potential risks of nanotechnology, and the lack of

knowledge on possible adverse health effects (The Royal Society and The Royal Academy of Engineering, 2004; Hett, 2004; Rathenau Instituut, 2004; European Commission, 2004; Luther, 2004; Malsch, 2004; KNAW, 2004). Research on the adverse health effects of nanoparticles can in general be described as nanotoxicology (Donaldson, 2004; Oberdörster, 2005).

This overview will be limited to a survey of health based risks occurring after exposure to nanoscale materials and/or substances, especially in medical applications. Environmental aspects are not part of this study. Besides therapeutic applications, nanotechnology may also facilitate an improvement of medical care when applied in areas like diagnostics and monitoring devices. Indirect risks may be related to the uncertainties (false positive/negative responses) of such newly developed medical devices. Such indirect risks will not be included in this report.

## 2. Risk management

In order to guarantee the safe application of any technology, the associated risks need to be managed. In EN-ISO 14971 “Medical-Devices- Risk Management - Application of risk management to medical devices” the process of risk management is defined as the systematic application of management policies, procedures and practices to the tasks of analysing, evaluating and controlling risk (ISO, 2000, 2003). Risk management is a continuous process, described as a set of repeatable steps throughout the entire life cycle of medical devices, re-evaluation of all steps in this iterative process being essential. With regard to the safe application of nanotechnology in medical technology, various stakeholders are involved, including manufacturers, competent authorities and/or designated bodies, health care providers, and health care users (patients). During the risk management process, stakeholders identify each hazard, evaluate the risks, and implement and verify risk control measures one at a time. On top of this there is a need to step back, add up all the individual residual risks, and decide on the acceptability of risks and the need and possibility for further risk reduction: the overall risk evaluation. Risk evaluation is a judgement of whether a risk is acceptable, based on risk analysis and the current values of society. It is possible that the overall residual risk can exceed the stakeholders’ criteria for acceptable risk, even though individual risks do not.

The primary user of the risk evaluation and risk management is the manufacturer, because it is this party that is placing the product on the market and therefore has the obligation to ensure quality, including safety. However, a systematic approach to analysis, evaluation and control of safety problems may also be of use to other parties such as regulatory authorities. Risk assessment is at the basis of the risk management in answering the question whether there are potential risks involved with the use of a medical technology. As we are dealing with medical applications estimation of benefit is an important part of the risk assessment. If a manufacturer judges the overall residual risk to be acceptable in relation to the expected benefit of their product, taking also into account a comparison to available alternatives, the marketing phase can be entered. According to current regulations for most medical products, regulatory authorities and/or designated independent bodies (e.g. Notified Bodies) decide on the acceptability of the overall residual risk of the product by assessing the scientific evidence on safety and performance/efficacy as provided by the manufacturer. If the safety and performance/efficacy is deemed acceptable, market authorisation can be granted. It is important to realize that deciding on risk acceptability by manufacturers and authorities is an ongoing, iterative process. Once new information becomes available, for example in the post-production phase, the acceptability of risk should be re-evaluated .

The existing regulatory frameworks for marketing authorisation of medicinal products, medical devices, in-vitro diagnostics and combination products show differences, in particular with regard to the amount of direct control that is exerted by the authorities. For emerging technologies like nanotechnology, with their specific, new and partly unknown risks, the question has been asked whether the existing regulations for the different types of medical products are sufficient to guarantee the safe use of these technologies in practice. In order to answer this question, an assessment of the specific risks is needed. For the application of nanotechnology in medical technology the risks which are judged to need special attention are related to the toxicology of nanoparticles and nanostructures. Nanotoxicology has been proposed as an appropriate designation for this particular subcategory of toxicology (Donaldson, 2004; Oberdörster, 2005).

For toxicological risk assessment of the use of nanoparticles the classical pathway can be used with 1. hazard identification, 2. hazard characterization, 3. exposure assessment and 4. risk calculation as described by Luther (2004). The items 1-2 are related to the research involved to characterise the toxicological properties of a nanomaterials/structure, while the exposure data are needed to calculate the real risk for humans, as discussed in this report (Scheme 1).

<i>1. Hazard identification</i>	<i>2. Hazard characterisation</i>
Particle characterisation	Epidemiological studies
Aspect ratio	Workers
Diameter	Consumers
Surface area/properties	Exposed population
Water solubility	In vivo studies
Chemical composition	Acute/chronic
Emission	Different species
Production volume	Route of exposure
Material flows	In vitro studies
Potential particle release	Human/animal, different cell types
Health effects	Models (lung, skin, systemic)
Humans	
Experimental animals	<i>3. Exposure assessment</i>
Environmental effects	Exposure routes
Persistence	Inhalation, dermal, ingestion
Biomagnification	Implantation, parenteral
Long range transport	Environmental monitoring
	Biological uptake
	Occupational monitoring
	Personal exposure

*Scheme 1: Assessment of risk by identification and determination of the extent of possible hazards. Modified from Luther (2004).*

### 3. Nanoparticles and nanostructures

Nanoparticles and nanostructures can be prepared either by the “top down” technique starting with large particles and making things smaller by grinding or pulverizing, or the “bottom up” technique making things larger by building atom by atom or molecule by molecule. The limit to making things smaller seems to be reached, while making nanostructures by synthesis has just started. In the latter case avoiding random reactions and thus the control of the process is critical for the production of nanostructures. The development of enhanced microscopy techniques like Scanning Tunneling Microscopy and Atomic Force Microscopy, has facilitated the use of the bottom up process. Some uses of nanotechnology include:

Nanomaterials, used in nanostructured materials, coatings, electronics and active surfaces. Most of the times such nanoparticles/nanostructures will be fixed within or on the surface of materials.

Metrology, the science of measurement is extremely important for nanotechnology and nanosciences. It is especially the progress in this area that has made recent developments of nanoscience and nanotechnology possible, as we now have the tools to examine structures at nanoscale, but greater precision in metrology is still needed.

Electronics, optoelectronics and ICT will most likely greatly benefit from nanosciences in terms of further miniaturization of computer chips for data storage. Application of nanosciences is also expected in various areas of sensor technology.

Bionanotechnology and nanomedicine are rather promising areas for nanotechnology. Applications will probably include diagnostics, imaging techniques, materials for prosthetics, and drug delivery. Other applications may be as supporting structures in biomaterials and medical devices including the use in scaffolds for tissue engineering. Micro- and nanogrooves present on material surfaces may direct cellular growth, however, cell behaviour is also influenced by chemical coating (Barbucci, 2003).

A more comprehensive overview of the basics of nanostructures, the technologies they enable, and more particularly the applications in medical technology can be found in Roszek (2005).





## 4. Exposure

Nanoparticles are already part of the environment. They are present as part of the dust occurring or produced in mining industry or industrial processes, and released as particles in combustion processes from industry and traffic. So, inhalation is an important route of exposure for the general population. Another source of exposure for the population may be the (future) disposal and waste of nanotechnology products leading to more airborne particles, as well as particles in soil, and possibly drinking water. In addition, application of nanoparticles in various products such as cosmetics and food will result in exposure via skin and ingestion. For various medical technology applications such as medicines exposure via different routes including inhalation, ingestion, dermal, implantation and the parenteral route will occur.

Nanocrystalline silver is currently in use as antimicrobial agent. It is used in dressings for wound care in which there should be a delicate balance between the needed bacterial and unwanted cellular toxicity (Dowsett, 2003; Dunn, 2004; Lansdown, 2005). Besides the antimicrobial activity, it was suggested that the nanocrystalline silver may influence the inflammatory response in wounds and facilitates the early phases of wound healing (Wright, 2002). Also when added to bone cement antibacterial activity of nanocrystalline silver was retained without in vitro cytotoxicity (Alt, 2004).

Nanostructures/particles are being developed for drug delivery purposes either as drug particle itself or as drug carrier (Cascone, 2002; Baran, 2002; Kipp, 2004). In addition, there are developments in the area of diagnostics and imaging, and bionanomaterials (Nanotech 2005 Meeting, May 8-12, Anaheim, USA). Quantum dots the size of about 15-20 nm were demonstrated experimentally to migrate to and accumulate in so called sentinel lymph nodes draining the site of injection (Kim, 2004; Soltesz, 2005). This would make the visualization of lymph nodes draining cancer sites possible.

An important application of nanoparticles/nanostructures in medical technology will be by creating or immobilizing coatings on implant material surfaces in order to improve cellular interaction and tissue integration. There is no reason to suppose that immobilized nanoparticles would pose a greater risk than larger scale biomaterials, however, since the properties of nanoparticles differ from their larger scale counterparts, biological responses to materials incorporating nanoparticles may also differ. An early risk might be related to the strength of fixation of the nanoparticles within or on the carrier material, and to the possibility of release or leakage from such structures. So, studies on fixation and stability should be performed to gain insight into possible release of nanoparticles. A late risk may be the production of wear debris during use. Wear debris in artificial hip joints is a common cause of local inflammation and osteolysis resulting in joint replacement failure (Ingham, 2005). The wear debris size may be in the submicron range as found in both ankle arthroplasties and hip replacements (Campbell, 1995; Tipper, 2000; Kobayashi, 2004; Visentin, 2004). In a simulation model for an artificial hip joint the surface damage of ultra high molecular weight polyethylene discs by scratching resulted in wear debris particles in the range of about 10 – 500 nm (McNie, 2000). Wear debris is known to accumulate around the implant site and is linked to tissue necrosis.

Exposure and thus risks arising from the production of free nanoparticles and nanotubes depend on the actual production techniques used. These risks are related to occupational exposure, and are therefore considered to be outside the scope of this report.

## 5. Toxicology of nanoparticles

Concern for the use of nanotechnologies is raised by some of the properties of nanomaterials such as surface reactivity and ability to cross cell membranes. Toxicity of nanoparticles and nanotubes cannot be predicted from the toxicity known for the chemical entity itself. However, the chemical composition certainly will have an impact on toxicity of nanoparticles (Donaldson, 2004). Reducing the size to nanolevel may change the physical state of particles resulting in activities distinctly different from larger particles (Preining, 1988). Size reduction to nanolevel is accompanied with a change in chemical reactivity and thus, presumably, toxicity. Some manufactured nanoparticles are more toxic per unit of mass than larger particles of the same material. This might be explained by the fact that toxic responses of nanoparticles seem to be related more to the surface area than to the mass of a particle. In addition to their inherent toxicity, dosage, uptake and metabolism, and distribution will determine whether there will be an adverse effect following exposure to nanoparticles.

Specific studies dealing with toxicity of manufactured nanoparticles are relatively scarce and are just emerging in the literature. Knowledge of the behaviour of nanoparticles mainly comes from studies on ambient air pollution where the toxicity of particles including ultrafine particles in the nanorange has been subject of study for some time (reviewed by Oberdörster, 1996, 2005; Borm, 2002, 2004; Donaldson, 2001a, 2003, 2004; Kreyling, 2004). Knowledge of the behaviour of particles is also available from pharmaceutical studies in which nanoformulation is used to overcome drug delivery problems with insoluble drugs. A recent paper extrapolated the knowledge in research in inhalation toxicology to the area of drug delivery (Borm, 2004). It was concluded that there is a wide gap between the two research areas, inhalation toxicology generally dealing with adverse effects of non defined particles in ambient air while research for drug delivery aims at well defined (in general) degradable nanoparticles with low or no toxicity. In addition, knowledge of the toxicity of fibres, such as asbestos dictates that careful consideration needs to be given to nanofibres. Since there are practically no toxicology studies available on the emerging applications of nanotechnology in medical technology, the above mentioned sources will be reviewed in the following paragraphs. In the absence of more relevant data, evaluation of toxicological risks related to applications in the medical field is only possible by extrapolation.

### 5.1 Toxicology of inhaled particles in ambient air

#### 5.1.1 Human health implications

Particle toxicity has been extensively studied in inhalation toxicology focusing on ambient air quality in relation to combustion processes. The concern for ambient air pollution has gradually moved from larger to smaller particulates, from  $PM_{10}$  (particulate matter of 10 micrometer or smaller) the fraction that penetrates beyond the larynx to the airways, to  $PM_{2.5}$  the respiratory fraction penetrating to the unciliated areas of the lung, and is now focusing on ultrafine particles with a size below 0.1 micrometer (100 nm). Generally, the  $PM_{10}$  particles are not very toxic in healthy people, but there can be dramatic effects in susceptible subgroups (Donaldson, 2001a). In environmental exposure, ultrafine particles are present at low concentrations in ambient air and their harmful effects are limited to

susceptible subgroups. Higher concentrations, however, such as those anticipated in production facilities for manufactured nanoparticles, would probably result in adverse effects even in healthy people. Part of the toxicity exerted by PM<sub>10</sub> particles was suggested to be mediated by the ultrafine fraction present, the mechanism being likely to be oxidative stress due to the large surface area of the particles (Donaldson, 2001a, 2003). Aerosols are known as a potential threat to health after occupational exposure (Vincent, 2000), while knowledge of the occupational exposure to ultrafine particles is just becoming of interest in terms of risk assessment.

Depending on its physicochemical composition particulate matter may have various health effects (Ghio, 2001; Medeiros, 2004). Ambient particulate air pollution was found to be statistically associated with cardiovascular morbidity and mortality (Pope, 2000; Samet, 2000; Peters, 2001; Brook, 2004). Populations exposed to air pollution episodes expressed abnormalities in several blood values such as in blood viscosity, fibrinogen and C-reactive protein which may be linked to vascular responses (reviewed by Donaldson, 2001b). So, the role of particulate matter (PM) as a type of air pollution with an influence on human health seems well established, although the mechanism of action is poorly understood (Englert, 2004; Brook, 2004). It is doubtful whether epidemiological studies can determine what kind of factors are responsible for the effects seen as the contribution from individual pollutants is difficult to discern. Both particle size and composition may be relevant but, for ultrafine particles, data on both exposure and human health effects are still limited.

Most airborne particulates were found to be aggregates ranging in aerodynamic diameters from a few nanometers to a few microns, containing as few as 2 nanocrystals up to several thousand nanocrystals or nanoparticulates. Elimination of these airborne nanoparticulates was suggested as an appropriate control mechanism to prevent respiratory illness (Murr, 2004). Although ultrafine (nano)particles in air can be used as an information source for particle toxicity, one has to be aware that particles in ambient air as part of pollution of combustion origin are coated with all kinds of reactive chemicals including biological compounds like endotoxin (Carty, 2003; Kreyling, 2004; Schins, 2004). So, the information obtained from ultrafine ambient air particles for nanoparticle toxicity should take into consideration the possible influence of particle composition.

### 5.1.2 Experimental evidence

*Deposition models:* Inhaled particulate matter can be widely deposited in the airways including nasal, tracheobronchial and alveolar regions depending on particle size as described in an ICRP model (ICRP, 1994). The deposition of particles with a size below 500 nm is likely to increase in all airway regions like bronchi and alveoli. With decreasing size there is a major increase in alveolar deposition (ICRP, 1994). An additional human and rat airway deposition model (MPPDep V1.1) was recently evaluated for deposition of soluble aerosol cadmium chloride (CdCl<sub>2</sub>) particles (Casseo, 2002). The model was found to predict accurately particle (CdCl<sub>2</sub>) deposition. Toxicity of the soluble model CdCl<sub>2</sub> particles in the range of 30 nm to 1500 nm was concluded to depend on the amount of material deposited. The about 33 nm size particles showed the highest Cd deposition and the highest level of lung toxicity as indicated by the increase in N-acetyl glucosaminidase and protein leakage in the bronchoalveolar lavage fluid.

*Model particulates:* For evaluation of air particulate toxicity ultrafine (nanoscale) model particulates were used including polytetrafluoroethylene (PTFE), polystyrene (PS), titanium dioxide (TiO<sub>2</sub>), carbon black, cobalt, nickel and latex (Oberdörster, 2000; Donaldson, 2000;

Dick, 2003; Nygaard, 2005). Ultrafine titanium dioxide ( $\text{TiO}_2$ ) at high doses induced more bronchoalveolar inflammation than fine  $\text{TiO}_2$  when rats were exposed to an equal concentration (Ferin, 1992; Oberdörster, 1994, 2000). Similar results were obtained for ultrafine PTFE (Oberdörster, 2000) and polystyrene (Brown, 2001). These studies indicated that materials that are normally low in toxicity at larger sizes, could be toxic when administered in a nanoformulation as ultrafine particles. Also for low doses with ultrafine and fine carbon black and latex particles similar results were obtained (Li, 1999; Donaldson, 2000, 2001a; Wilson, 2002; Renwick, 2004). For ultrafine nickel (size about 20 nm) an enhanced lung inflammation and toxicity was observed compared to standard sized (average diameter 5  $\mu\text{m}$ ) nickel as indicated by an increase of total protein, lactate dehydrogenase, and neutrophils in the bronchoalveolar lavage fluid (Zhang, 2003). Thus, for inhalation exposure it can be concluded that ultrafine or nanoparticles show an increased toxicity compared to larger particles of the same chemical composition. The increase in lung inflammation for ultrafine particles compared to fine particles was noted when doses were expressed as mass. In contrast, when doses were expressed as surface area similar responses were observed for both fine and ultrafine particles (Oberdörster, 2000). Also when comparing toxicity differences between  $\text{TiO}_2$  and  $\text{BaSO}_4$  dose response relationships were similar when compared at a dose expressed as surface area burden (Tran, 2000). Besides size also the chemical nature has an impact on the induced lung inflammation after intratracheal instillation. Instillation as ultrafine Ni was more toxic than ultrafine Co, ultrafine  $\text{TiO}_2$  being the least toxic (Zhang, 1998). The ranking of toxicity was reflected in the capability of the materials to induce free radical damage to plasmid DNA, indicating that free radical generation may underlie these observed differences in toxicity.

*Particulate migration:* After deposition, migration of ultrafine particles may occur to the lung interstitium and into the blood and liver (MacNee, 2000; Oberdörster, 2000, 2002). In the liver a substantial amount of particles could be detected (Oberdörster, 2002). Ultrafine particles of carbon and platinum (as part of the urban atmospheres), PTFE particles and titanium dioxide particles were investigated (Oberdörster, 2000). However, for ultrafine insoluble irridium particle sizes of about 15 and about 80 nm clearance was found to be primarily via the airways into the gastrointestinal tract. Only a minimal fraction of <1% of the particles was translocated into secondary organs like liver, spleen, heart and brain. Of these, the about 80 nm sized particles were an order of magnitude less common than the about 15 nm sized particles indicating the importance of size even within the nanometer area (Kreyling, 2002). The presence of low numbers of particles in organs like the liver and spleen could be attributed to translocation from the lung. Particles were not dissolved nor absorbed from the gut (Kreyling, 2002). Circulating particles (after iv administration) accumulated in liver and spleen and were retained there. Also in man passage of ultrafine inhaled particles into the bloodstream was demonstrated although most of the inhaled particles stayed in the lung (Nemmar, 2002).

Another route of translocation from the airways may be by neuronal uptake. Ultrafine  $^{13}\text{C}$  particles with a size about 35 nm were detected in the brain olfactory bulb after inhalation exposure. The route of brain entry was suggested to be by migration along the olfactory nerve into the olfactory bulb of the brain after deposition on the olfactory mucosa in the nasal region (Oberdörster, 2004). Inflammatory biomarkers like Interleukin 1 $\alpha$  (IL1 $\alpha$ ) and Tumor Necrosis Factor  $\alpha$  (TNF $\alpha$ ) were increased in the brain of mice exposed to ambient air particulate (fine and ultrafine, size below 2500nm, or ultrafine size below 180 nm) matter compared to controls (Campbell, 2005). The ambient air concentrates used, contained a high proportion of metals, which were suggested to be responsible for the inflammatory responses. It is unknown whether this leads to potentially adverse consequences, but it certainly warrants

further studies. In view of the induction of inflammatory cytokines a relation with neurodegenerative disorders might be considered as was suggested by Campbell (2005).

*Systemic effects:* In animal studies vascular effects such as thrombosis were observed for intratracheally administered ultrafine (about 60 nm) positively charged amine modified polystyrene particles but not for about 400 nm sized particles (Nemmar, 2003). Both about 60 nm and about 400 nm sized positively charged polystyrene particles induced pulmonary inflammation, so inflammation and thrombogenesis do not seem to be coupled. In a recent study in rats ultrafine carbon particles (about 38 nm) were found to induce a mild but consistent increase in heart rate (Harder, 2005). The ultrafine carbon induced only a low grade pulmonary inflammation. The effect on the heart rate could not be related to blood hypercoagulability. So, it is not clear whether hypercoagulability is linked to cardiovascular responses after exposure to ultrafine particles (reviewed by Donaldson, 2001b).

*Mechanism of toxicity:* Several possibilities have been postulated as mechanisms of action for particulate toxicity, including injury of epithelial tissue (Pagan, 2003), inflammation, oxidative stress (Nel, 2001; Donaldson, 2001a, 2003), and allergy (Dybing, 2004). At the cellular level oxidative stress is considered to be of importance (Donaldson, 2001a,b; Oberdörster 2005). Ultrafine- and/or nano-particles induced oxidative stress responses in keratinocytes, macrophages and blood monocytes after in vitro exposure (Shvedova, 2003; Brown, 2004). In a recent study gene expression profiles indicated that pulmonary injury and inflammation are likely to be due to increased expression of an oxidative stress response and subsequent contributions from cytokines and chemokines after exposure to urban particulate matter (Kooter, 2005). Interestingly expression of antioxidant genes was also found to respond to the inhalation injury. An increase in gene expression of antioxidant enzymes was demonstrated in alveolar macrophages after in vivo exposure to ultrafine TiO<sub>2</sub>, but this was not sufficient to counteract the occurring lipid peroxidation and hydrogen peroxide generation (Afaq, 1998). Thus, the overall resultant effect was an apparent induction of oxidative stress in the cells.

In conclusion, toxicology has identified adverse health effects of ultrafine (nanosized) ambient air particles including possible targets like the cardiovascular, central nervous and immune system. However, clear epidemiological evidence for adverse health effects (toxicity) of ultrafine ambient air particles at relevant doses is lacking. Whether a similar situation exists of toxic effects after exposure to engineered nanoparticles used in medical technology for production of medicinal products and/or medical devices is not yet known.

## **5.2 Toxicity of nanoparticles used as drug delivery system**

*Carriers for drug delivery:* Nanostructures/particles can be used for drug delivery purposes either as drug itself or as drug carrier (Cascone, 2002; Baran, 2002; Duncan, 2003; Kipp, 2004). Especially for cancer therapy and diagnostics, nanotechnology may be of importance, although many challenges still need to be solved (Ferrari, 2005). In addition, nanostructures are investigated for gene delivery purposes as well (Kneuer, 2000; Salem, 2003; Ravi Kumar, 2004; Gemeinhart, 2005; Yoo, 2005; Roy, 2005), applications being plasmid DNA administration for vaccination (Salem, 2003; Cui, 2002, 2003; Zhang, 2005) and cancer therapy (Ramesh, 2004; Gordon, 2005). Gene transfer was accomplished both in in vitro and in vivo models using various types of nanoparticles. A clinical trial with gene therapy aimed

at determining safety and tolerability was performed in cystic fibrosis patients (Konstan, 2004). Generally the therapy was well tolerated and gene transfer was established.

Research for nanoparticles for drug delivery purposes includes various formulations: albumin (Damascelli, 2003), chitosan (Dyer, 2002; Huang, 2004), poly(D,L-lactic-co-glycolide)acid (PLGA) (Panyam, 2002; Weissenbock, 2004), solid lipid formulations (Muller, 1997, 2000; Wissing, 2004), cetyl alcohol/polysorbate nanoparticles (Koziara, 2004), hydrogels (Gupta, 2004b), gold (Hainfeld, 2004; Paciotti, 2004), polyalkylcyanoacrylate composites (Cruz, 1997; Olivier, 1999; Kreuter, 2003), magnetic iron oxide (Gupta, 2005), methoxy poly(ethylene glycol)/poly( $\epsilon$ -caprolactone) (Kim, 2003), and gelatin (Cascone, 2002). Most of these compounds are (bio)degradable polymers resulting in drug release after degradation. For doxorubicin clinical studies with nanoparticles (polyisohexylcyanoacrylate nanoparticles) were performed (Kattan, 1992). Also albumin nanoparticles are subject of clinical studies for anticancer drug delivery purposes (Damascelli, 2003). Not all of the nanoparticle formulations mentioned are nanoparticles in the sense that their size is below 100 nm. Studies for drug delivery include particles up to several hundreds of nanometers. The route of administration may be oral, parenteral (subcutaneous, intramuscular, intra-arterial, intravenous) and via the skin.

*Distribution:* The aims for nanoparticle entrapment of drugs are either enhanced delivery to, or uptake by, target cells and/or a reduction in the toxicity of the free drug to non-target organs. For these aims, creation of long-lived and target-specific nanoparticles is needed. One of the problems is the entrapment of nanoparticles in the mononuclear phagocytic system as present in the liver and spleen (Lenaerts, 1984; Gibaud, 1996; Demoy, 1997; Moghimi, 2001). However, liver targeting of nanoparticles may be favourable when treating liver diseases like tumor metastasis or hepatitis. Also oligonucleotides for modification of gene expression were demonstrated to migrate into the liver when bound to biodegradable polyalkylcyanoacrylate nanoparticles (Fattal, 1998). Surface modification with PEG resulted in prolonged presence in the circulation by inhibiting recognition and phagocytosis by the mononuclear phagocytic system (Bazile, 1995; Peracchia, 1999). Besides reduction of therapeutic efficacy, liver entrapment may also have an adverse effect on liver function. For cyanoacrylate and polystyrene nanoparticles (about 214 nm and about 128 nm, respectively) transient liver alterations were observed after acute and chronic intravenous administration (Fernandez-Urrusuno, 1995, 1997). Inflammatory responses were characterized by secretion of acute phase protein  $\alpha$ 1-acid glycoprotein by hepatocytes (Fernandez-Urrusuno, 1995). In addition, antioxidant defenses of hepatocytes were depleted probably as a result of local release of oxidative species (Fernandez-Urrusuno, 1997).

Although nanoformulation is aimed at enhancing drug delivery without loss of drug activity, in a study comparing insulin-chitosan nanoparticles to chitosan solution and chitosan powder formulations the insulin-chitosan nanoparticles were less effective in terms of bioavailability and lowering blood glucose level in both a rat and sheep model (Dyer, 2002).

*Intracellular uptake:* For drug delivery not only organ or cellular targeting is of importance but also the fate of the nanoparticles within the cells. Particles generally end intracellularly in endosomes or lysosomes followed by degradation. For activity of the encapsulated drugs release into the cytosol is needed. However, for nanoparticles of about 20 nm also cellular uptake without contribution by endocytic mechanism was demonstrated (Edetsberger, 2005). Chemical characteristics such as surface charge may determine the fate of nanoparticles in cells. Poly(DL-lactide-co-glycolide) nanoparticles were found to be ingested by cells by endocytosis (Panyam, 2002; Konan, 2003). The escape from these endosomes into the

cellular cytoplasm was suggested to be caused by a change in surface charge from negative to positive of the PLGA nanoparticles resulting in cytoplasmic delivery of the incorporated drugs. The hypothesis that the positive surface charge influenced the escape of the endosomes was supported by data obtained with negatively charged polystyrene nanoparticles which did not reach the cytosol but remained in the endosomal compartment of the smooth muscle cells used in this study (Panyam, 2002).

One of the effects of nanoparticle formulation may be an increased cellular uptake. Encapsulation in sub 130 nm size PLGA particles increased cellular uptake of a photosensitizer, resulting in enhanced cytotoxicity in vitro (Konan, 2003). Toxicity of free nanoparticles was not determined in this study.

*Cellular targeting:* Specific targeting to retinal pigment epithelium cells in the eye is possible (Bourges, 2003). For very small quantum dots (<10 nm) specific targeting of peptide coated dots to the vasculature of lungs and tumors was observed (Åkerman, 2002). PEG coating abrogated uptake by the reticuloendothelial system of liver and spleen. In contrast about 40-50 nm magnetic nanoparticles coated with PEG were quite well taken up by endocytosis (Gupta, 2004a).

Surface modifications of nanoparticles offer possibilities for medical applications like drug targeting in terms of cellular binding, uptake and intracellular transport. Carbohydrate binding ligands on the surface of biodegradable and biocompatible poly(D,L-lactic-co-glycolide)acid (PLGA) nanospheres were found to increase cellular binding (Weissenböck, 2004). Such increased adherence may lead to an enhanced activity of the drug presented as or incorporated in nanoparticles. Coupling specific proteins such as antibodies to the nanoparticle surface may enable a more specific immunologically directed targeting of the particles (Nobs, 2004).

*Organ specific migration:* One of the advantages of the use of nanoparticles for pharmaceutical formulations might be the potential to cross the blood brain barrier (BBB) (Kreuter, 2001). However, this may also be the major drawback for systemic administration of nanoparticles in terms of potential brain toxicity. Such passage was suggested to be possible by the toxic effect of nanoparticles (about 200nm) on cerebral endothelial cells (Olivier, 1999), although for similar nanoparticles (about 300nm) this was contradicted (Kreuter, 2003), and not found for a different type of nanoparticles (Lockman, 2003). Physical association of the drug to the nanoparticles was necessary for drug delivery to occur into the brain (Kreuter, 2003). When nanoparticles with different surface characteristics were evaluated, neutral nanoparticles and low concentrations of anionic nanoparticles were found to have no effect on BBB integrity, whereas high concentrations of anionic nanoparticles and cationic nanoparticles were toxic for the BBB. The extent of brain uptake of anionic nanoparticles at lower concentrations was superior to neutral or cationic formulations at the same concentrations. So, nanoparticle surface charges must be considered for toxicity and brain distribution profiles (Lockman, 2004). Especially coating of the nanoparticles with the polysorbate (Tween) surfactants results in transport of drugs into the brain (Kreuter, 2004). The mechanism for transport was suggested to be endocytosis via the Low Density Lipoprotein (LDL) receptor of the endothelial cells after adsorption of lipoproteins from blood plasma to the nanoparticles (Kreuter, 2001, 2004)

Passage of the BBB may also be achieved by masking certain drug characteristics and thus circumventing certain functions in brain cells for removal of drug from the brain. The effect of entrapment of a cytotoxic drug paclitaxel (PX) in cetyl alcohol/polysorbate nanoparticles (PX Nanoparticles) was evaluated in an in situ rat brain perfusion model (Koziara, 2004). The results suggest that entrapment of paclitaxel in nanoparticles significantly increases the brain



drug uptake and its toxicity towards p-glycoprotein expressing tumor cells (p-glycoprotein is an efflux transporter associated with drug removal from the cells). It was hypothesized that PX nanoparticles limit paclitaxel binding to p-glycoprotein, which consequently would lead to higher brain and tumor cell levels of the otherwise effluxed drug.

After oral administration, only 10% of about 60 nm polystyrene particles were recovered from the tissue of the gastrointestinal tract. Most of these particles were present in lymphoid tissue such as Peyer's Patches and lymphoid aggregates in the large intestine (Hillery, 1994). After dermal administration skin permeation by nanoparticles was found for negatively charged particles of about 50 and 500 nm., while positively charged and neutral particles of all sizes did not. It was suggested that particle size was less important than the total charge explaining why both about 50 and 500 nm sized latex particles showed permeation and about 100 or 200 nm negatively charged particles did not (Kohli, 2004). A greater concentration of charge was suggested to be responsible for overcoming the skin barrier, explained for the about 50 nm particles as being the small size and large surface area, and for the about 500 nm explained by the high number of charged groups (Kohli, 2004). It can be expected that penetration of damaged skin will be easier. So, when nanoparticles are used in products contacting intact or damaged skin there should be particular consideration of risks related to skin permeation.

No data were identified to address the risk of adverse effects in the foetus due to transplacental passage.

Magnetic fields can be applied for selective localization of magnetic nanoparticles as was demonstrated in a rat tail model in which the drug concentration could be selectively increased in the specific tail segment with a reduction in other organs (Vyas, 1989).

Specific migration into draining lymph node is of importance for both treatment and diagnostic purposes. Nanoparticle formulations of polyisobutylcyanoacrylate (Nishioka, 2001) and fluorescent quantum dots (Kim, 2004; Soltesz, 2005) were shown to localize into such draining lymph nodes. Also uptake by endothelial cells can be used for diagnostic as well as therapeutic (prevention of restenosis) purposes (Davda, 2002; Uwatoka, 2003, Westedt, 2004).

*Toxicity:* The use of nanoparticles as drug carrier may reduce the toxicity of the incorporated drug. In general the toxicity of the whole formulation is investigated while results of the nanoparticles itself are not described. So, discrimination between drug and nanoparticle toxicity cannot be made. For indomethacin loaded nanospheres (size below 200 nm) composed of methoxy poly(ethylene glycol)/poly( $\epsilon$ -caprolactone) polymers the in vitro cytotoxicity was reduced when compared to free indomethacin, although some minor toxicity of 15-20% growth reduction was still present (Kim, 2003). In vivo acute toxicity studies found a LD<sub>50</sub> value of 1.47 g/kg, and 50% of this LD<sub>50</sub> value administered for 7 days did not induce acute toxicity in heart, lung, liver and kidney as determined 8 days after treatment (Kim, 2003).

Nanoparticle formulation does not always result in a change of toxic characteristics. For chitosan the same in vitro cytotoxicity for chitosan molecules and chitosan nanoparticles (about 100-350 nm) was found, while there was a clear difference in the uptake, the nanoparticle formulation showing an increased uptake (Huang, 2004).

The structure and properties of gold nanoparticles make them useful for a wide array of biological applications. Toxicity, however, has been observed at high concentrations using these systems. Goodman (2004) demonstrated by hemolysis and bacterial viability assays in vitro that for about 2 nm gold particles cationic particles were moderately toxic, whereas anionic particles were relatively nontoxic. Such very small about 2 nm gold nanoparticles were found to be non toxic when administered to mice for tumor therapy (Hainfeld, 2004).

Also for a thiol derivatized PEG colloidal gold nanoparticles with TNF an enhanced antitumor activity was reported when compared to free TNF (Paciotti, 2004). Topoisomerase inhibitors when formulated in lipid containing nanoparticles showed increased antitumor activity in an in vivo nude mouse xenografted human tumor model (Williams, 2003). Toxicity of the (empty) nanoparticles itself was not reported.

### 5.3 Additional factors for toxicity

*Size:* For particle toxicity two main factors are of importance one being size and the other the chemical composition, which is responsible for the intrinsic toxicity of the compound (Donaldson, 2004). Reduction in particle size to the nanolevel results in an enormous increase of surface area, so relatively more molecules of the chemical are present on the surface, thus the risk of enhancing the intrinsic toxicity. This may be one of the explanations why nanoparticles are more toxic per unit mass than larger particles of the same material when used on a mass base. Similar dose response relationship between particles of different sizes have been observed when the dose was expressed in surface area (Oberdörster, 2000). In another series of studies, doses of  $\text{TiO}_2$ , measured in mg induced more severe lung inflammation and particle lymph node burden than  $\text{BaSO}_4$  (Tran, 2000). These differences in severity of the response disappeared when the dose was expressed as surface area. These examples emphasize the fact that size is a relevant parameter for particle toxicity. In addition, the toxic effect of carbon black was more severe than titanium dioxide (Renwick, 2004). For both compounds ultrafine nanosized particles were found to induce inflammation and epithelial damage in lungs at greater extent than their fine counterparts. Thus, size and chemical composition, which dictates the intrinsic toxic properties of the chemical, are both of importance for the toxicity of particles.

*Chemical composition:* The chemical composition of the surface is important for the adverse effects of nanoparticles. Fractions isolated from particulate pollutants (diesel exhaust particles) were demonstrated to exert toxic effects on cells in vitro (Xia, 2004). So, besides the particulates also the chemical composition, or for air pollutants the chemical absorbants can be responsible for the toxic effects. For example for the coarse fraction of  $\text{PM}_{10}$  sampled ambient air particles the induction of inflammatory responses in the lung could be partially ascribed to the endotoxin contaminants present (Schins, 2004).

Ultrafine particles can interact with metals. Iron was able to potentiate the effect of carbon black nanoparticles, resulting in enhanced induction of reactive oxygen species (ROS) in a cell free system (Wilson, 2002). In addition, surface modification of nanoparticles can result in a diminishing of cytotoxicity. The in vitro cytotoxicity of superparamagnetic iron oxide nanoparticles could be abrogated by coating the nanoparticles with pullulan (Gupta, 2005). Also for dextran and albumin derivatised iron oxide nanoparticles a reduction in in vitro cytotoxicity was noted (Berry, 2003).

Nanoparticles can have additional, unwanted, effects beyond the intended use. Both  $\text{TiO}_2$  and  $\text{ZnO}$ , used in sunscreens as physical UV absorbers, were found to increase dermal uptake of several pesticides (Brand, 2003). Part of the activity could be ascribed to the 'inert' ingredients present, since the enhanced absorption could be eliminated by substituting the solvent. For polystyrene particles of about 100 nm an IgE adjuvant activity was observed in an animal model system of ovalbumin allergy (Nygaard, 2005).

For several different nanoscaled particles (PVC,  $\text{TiO}_2$ ,  $\text{SiO}_2$ , Co, Ni) only Co particles

induced toxicity in endothelial cells which was accompanied by induction of the proinflammatory cytokine IL8 (Peters, 2004). For other particles only TiO<sub>2</sub> and SiO<sub>2</sub> induced minor and profound IL8 releases, respectively. An explanation of the differences in cytotoxicity was not presented but might be due to both material differences and/or size difference at the nanolevel, as the particle size ranged from a mean diameter of 14 nm to 120 nm and even clusters of 420 nm (Peters, 2004). For microsized biomaterial particles, in vivo distribution was dependent on the composition of the material as of two polymers investigated, only PMMA (about 1.4 µm and about 6.4 µm) particles but not PS (about 1.2 µm, 5.2 µm and 12.5 µm) particles could be recovered from the spleen after intraperitoneal administration (Tomazic-Jezic, 2001). The PS particles regardless of size were accumulated primarily in the adipose tissue of the peritoneal cavity, with very few particles in the spleen.

Carbon fullerenes are lipophilic and localize into lipid rich regions like cell membranes and may be redox active (Colvin, 2003). In fish lipid peroxidation was induced in the brain after exposure to 0.5 ppm aqueous suspended colloids of uncoated nanosized (range 30 -100 nm) fullerenes (nC60) (Oberdörster, 2004).

*Geometric form:* A special category to consider for adverse effects are nanotubes or nanofibres of a few nanometers in diameter but a length which could be several micrometers. Risks should be considered bearing in mind the well known carcinogenic effects of certain asbestos fibre. In two recently published in vivo studies single-wall carbon nanotubes (SWCNTs) were demonstrated to induce lung granulomas after intratracheal administration (Lam, 2004; Warheit, 2004) indicating that these nanotubes can not be classified as a new form of graphite on material safety data sheets. On a dose per mass basis the nanotubes were more toxic than quartz particles well known for their lung toxicity. Carbon black, carbonyl iron and graphite produced no significant adverse effects (Lam, 2004; Warheit, 2004). Multifocal granulomatous lesions were observed without accompanying inflammation, cell proliferation or cytotoxicity which was suggested to indicate a potentially new mechanism of pulmonary toxicity and injury by the SWCNTs not following the normal paradigm of toxic dusts (Warheit, 2004). In vitro studies using a human keratinocyte cell line showed that carbon nanotube (SWCNT) exposure resulted in accelerated oxidative stress and cellular toxicity which may be interpreted as potential for dermal toxicity (Shvedova, 2003)

## 5.4 Additional human observations

Results obtained in various kinds of patients also can give information on the possible role of particles in diseases or disease processes. In patients with a variety of pathological conditions, nanoparticles could be detected in organs and in blood (Gatti, 2002, 2004a,b). Dental prostheses and previous medical investigations or treatments were suggested as the origin of some of the particles detected. Although no explanation can be presented for the relationship between the pathological lesion and the nanoparticle present, the phenomenon of particles being present warrants further studies to the role of these particles.



## 6. Toxicological testing

Assays described in various international guidelines can be used for the evaluation of adverse effects of medical technology products. For medicinal products safety evaluation is performed according to guidelines in the European Pharmacopoeia, while for medical devices, international standards published by the European Committee for Standardization (CEN, Brussels, Belgium) or the International Organization for Standardization (ISO, Geneva, Switzerland) can be used to demonstrate compliance with the European Medical Device Directive (European Commission, 1993). Although medical technology products may be produced or composed of nanostructures and/or particles the same basic principles apply. It is as yet not known whether the use of nanotechnology may pose specific problems in determining adverse effects of medicinal products and medical devices.

*Inhalation:* Endpoint testing for induction of oxidative stress, cell activation and signaling, and release of inflammatory mediators has replaced the classical tests for lung injury for evaluation of adverse effects after inhalation of particles. Ultrafine or nanoparticles pose a problem for determination of dose response relationships which are commonly sought in toxicology. Toxicology is normally based on the paradigm that health effects are correlated to the mass of the agent to which the individual is exposed resulting in an accumulated mass as internal or organ dose/exposure. For ultrafine/nanoparticles the concentration or number of particles, and the resulting total surface area, determines the interactions with biological systems. So, surface area and number of particles appear to be more reasonable parameters for expressing doses in terms of exposure than dosage in mass (Brown, 2001; Oberdörster, 2000; Höhr, 2002; Kreyling, 2003). For ultrafine TiO<sub>2</sub> significant species differences between rats, mice and hamsters were noted after inhalation exposure, the rat being the most sensitive (Bermudez, 2004). Pulmonary responses and dosimetry (particle retention and overload) were considered to be responsible for these differences.

*Mechanisms of toxicity:* Several hypotheses have been proposed for the adverse health effects of ultrafine particles as part of ambient air pollution (reviewed by Kreyling, 2004).

These hypotheses for adverse health effects of ultrafine particles include:

Hypotheses regarding exposure and distribution

- Deposition characteristics dependent on size
- Uptake by cells of respiratory epithelium
- Increased access to interstitial spaces
- Access to systemic circulation

Hypotheses regarding particle characteristics

- Large surface area for interactions with cells and tissues
- Complex formation with biomolecules
- Formation of increased level of radical species compared to larger particles
- Increased induction of oxidative stress
- Induction of oxidative stress by lipid peroxidation
- Induction of cellular DNA damage

Hypotheses regarding effects on organs systems

- Effects on the immune system
- Reduced function of macrophages, and reduced phagocytosis of particles themselves

- Reduced macrophage mobility and cytoskeletal dysfunction
- Increased pro-inflammatory activity
- Induction of proinflammatory cytokines and mediators
- Increased inflammatory effects
- Adverse effects on cardiac functions and vascular homeostasis

Some hypotheses suggested for ambient air ultrafine particles, such as increased reactivity resulting in adsorption of toxic substances, may be of limited or no relevance for engineered nanoparticles. Although such adsorption can not be ruled out, it is probably of less importance in the production and handling facilities of engineered nanoparticles compared to the ultrafine particles in ambient air. Limitations of the studies cited include the relatively high doses used, the short periods of time investigated, and/or artifacts occurring during sampling of the particles on filters (Wittmaack, 2002).

*Animal models:* The use of normal (healthy) animal models may hamper the interpretation of the results as some of the effects listed may only be a risk for susceptible organisms and predisposed individuals but not to healthy people (Kreyling, 2004). Inhalation toxicology for nanoparticles showed that nanoparticles are especially affecting people with existing disease. So, it was suggested that toxicological testing of nanoparticles should be performed in various models that reflect human disease (Borm, 2004). In an animal study it was found that age, co-pollutants and a compromised respiratory tract can modify the pulmonary inflammation and oxidative stress induced by ultrafine carbonaceous particles (Elder, 2000). For COPD (Chronic Obstructive Pulmonary Disease) the generation of free radicals on the surface due to the high reactivity of nanoparticles, and the induction of oxidative stress might contribute to the induction of inflammation (MacNee, 2003).

*In vitro research:* Ultrafine- and/or nano-particles induced oxidative stress responses in keratinocytes, macrophages and blood monocytes after in vitro exposure (Shvedova, 2003; Brown, 2004). A role for free radicals and reactive oxygen species was also suggested by in vitro studies in which antioxidants were able to block the particle induced release of TNF $\alpha$  from alveolar macrophages (Dick 2003). Also macrophage phagocytosis was impaired by ultrafine particles (Renwick 2001). For TiO<sub>2</sub> and ZnO nanoparticles, oxidative damage to DNA was demonstrated (Dunford, 1997; Rahman, 2002). Micronucleus formation, resulting from early genetic damage, and programmed cell death (apoptosis) resulting from DNA damage, was observed with the ultrafine but not fine TiO<sub>2</sub> (Rahman, 2002).

The type of cell under investigation may also be of importance for the ultimate effect of the particles investigated. For epithelial lung cells, either as cell line or primary rat type 2 cells, the coarse fraction of urban ambient air showed similar or higher potency to induce cytokine release and cytotoxicity compared to the fine and ultrafine fractions (Hetland, 2004). In contrast for TiO<sub>2</sub> nanotubes a more favourable cell growth and fibre formation was observed in bone regeneration when compared to the commonly used hydroxyapatite (Kubota, 2004). Human macrophages and osteoblasts showed a different behaviour towards nanotopography surfaces, macrophages showing preference for the nanosurface and being activated, while the osteoblasts moved away from the nanosurfaces (Rice, 2003).

Also in in vitro studies the importance of chemical composition was noted. Early (at 4h) neutrophil induction in lung lavages was observed for ultrafine carbon black and ultrafine cobalt particles, and at 18 h for ultrafine nickel particles (Dick, 2003). In contrast, for ultrafine TiO<sub>2</sub> no indications for induction of lung inflammation were observed at 18h.

In view of the specific characteristics demonstrated for nanoparticles and nanoparticle formulations the assays usually performed for medicinal products and medical devices may not be sufficient to detect all possible adverse effects of nanoparticles. Nanoparticles are very reactive and can interact with all kinds of endogeneous proteins and cells. So, based on experiences in inhalation toxicology, a series of tests was proposed for evaluation of the toxicity of nanoparticles used in drug delivery systems. These included tests for blood cell damage after intravenous administration, acute phase responses of hepatocytes or lung cells, permeability tests of endothelial cells, tests for destabilization of atheromatous plaques in animal models for atherosclerosis, tests for effects on the autonomic nervous system, tests for adjuvant activity in an immunization model, tests for immune activation by measuring T cell activity and cytokine induction in lymph nodes, tests for determination of surface activity and induction of oxidative stress in cell lines, tests for toxicity on various cell lines in vitro, and biopersistence (Borm, 2004).





## 7. Conclusions

The literature on toxicological risks of the application of nanotechnology in medical technology is scarce. Inhalation toxicology provides information on the biological behaviour of particles. Ambient air particles are not one single type of particle, and can contain all kinds of reactive substances including some of biological origin like endotoxin. So, results are not suitable for extrapolation to nanoparticles used in medical technology. Studies to the toxicity of model particles of pure chemicals in the lung are of relevance and show increased reactivity/toxicity of nanoparticles compared to larger particles. In the area of drug delivery using nanoparticles as drug carrier, toxicological studies with empty nanoparticles are limited. The emphasis is on the reduction of drug related toxicity masking possible nanoparticle related toxicity.

The results presented in this literature overview show that a knowledge of the toxicity of chemical(s) and materials is not sufficient for the assessment of the risk of that chemical when it is applied in the form of nanoparticles or nanostructures. It is generally accepted that nanoparticles pose a separate problem within the area of toxicology, designated as nanotoxicology. Reducing the size of particles or structures to nanolevel may result in specific properties and/or activities distinctly different from other formulations of the same chemical or material. Therefore, chemicals and materials in nanoformulation need to be evaluated for their activity and toxicity as nanoparticles. One cannot rely on safety data obtained with the chemical compound as such.

Chemical composition, which dictates the intrinsic toxic properties of the chemical is of significant importance in determining the toxicity of particles. However, as a general principle, reduction in size results in an increased reactivity, which may be expressed as increased toxicity after exposure. Observations with several chemicals show small (nano)particles to be more toxic than larger particles of the same chemical composition after inhalation exposure. Even materials of established low toxicity can become highly toxic when administered as nanoparticles. So, very small size seems to be a predominant parameter for toxic effects of particles. Whether this is true for all types of chemicals and/or for all kinds of applications is not known, and needs further investigation.

It is now being recognised that extremely small particles may pose a risk to health. However, the term ultrafine or nanoparticle suggests a kind of limit, currently being defined as smaller than 0.1  $\mu\text{m}$  (or 100 nm). This definition appears to be somewhat arbitrary in relation to the toxicological risk. It seems likely, however, that this limit is dependent on the type of nanoparticle/chemical. Further research is needed to establish whether such a limit exists.

Possible routes of exposure to medical technology products that incorporate nanoparticles or nanostructures are inhalation, ingestion, dermal contact, implantation and parenteral administration. After inhalation, translocation of nanoparticles can occur from the nasal compartment into the brain and from the lung into the body. There is a lack of knowledge on the fate of ingested nanoparticles and little work has been carried out to investigate other routes of exposure. The distribution of nanoparticles following uptake via various routes needs to be investigated. It has been demonstrated that nanoparticles can cross the blood brain barrier. Possibilities for transdermal and/or transplacental passage are largely unknown.

The parameters that determine the crossing of cellular barriers by nanoparticles need to be determined.

At this moment there are no general rules or scientific paradigms for estimating the toxicity of nanostructures/nanoparticles. There are several hypotheses on the mechanism of action of nanoparticles, but basic knowledge of their absorption, distribution, metabolism and excretion are lacking, and it remains to be determined whether these particles behave according to specific patterns. There are indications for toxic effects by means of induction of oxidative stress in lung cells. Whether other toxic mechanisms are of importance needs to be determined. In addition, as not all nanoparticles induce toxicity, the mechanism behind these differences needs elucidation. It is as yet unknown when is a compound in nanoparticle formulation is or is not toxic.

For medical applications immobilized nanostructures on surfaces may pose a minimal risk as long as they remain fixed on the surface. The potential risk would be related to the possibility of release from such structures depending on the strength of fixation of the nanoparticles within or on the carrier material. Such release may occur in the form of wear debris due to continuous chemical processes and/or mechanical stresses at the interface of implant and surrounding tissue. In addition, for implants wear particles may be generated with a size in the submicron range, including particles at nanolevel below 100 nm. Whether such wear particles have similar increased reactivity/toxicity as ultrafine ambient air particles or model polymer particles is unknown and needs further investigation.

From a regulatory point of view, the implementation of a risk management strategy that includes a risk assessment by the manufacturer, is already a requirement for all medical technology applications. Health care providers and European and National Authorities also have a significant role in the management of risks associated with healthcare technology. With regard to applications utilizing nanotechnology, this focus on risk management is considered sufficient, as long as all the stakeholders are made aware of the possible specific toxicological properties of nanostructures and nanoparticles. From our current knowledge of the effects of size reduction on material properties, we can conclude that an evaluation of the possible specific behaviour of nanostructures in the products needs to be incorporated into any risk assessment performed. Risk assessment needs to be carried out for each separate formulation of a nanosized product. One should not rely on existing knowledge of the toxicity of the constituent chemicals or materials but include particle size among the parameters to be considered during the risk assessment. This may result in the recognition of a new or additional risk to those who are exposed. It is strongly recommended that specific guidance at European level is developed, pointing out the above conclusions to the relevant stakeholders.

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