Scaffold Public Documents – SPD2

Innovative strategies, methods and tools for occupational risks management of manufactured nanomaterials (MNMs) in the construction industry

# BACKGROUND INFORMATION ON EXPOSURE, USE, AND HAZARD OF MANUFACTURED NANOMATERIALS IN THE CONSTRUCTION SECTOR

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Antti Karjalainen, Eija-Riitta Hyytinen, Hanna Lindberg, Tiina Santonen, Arto Säämänen, Helene Stockmann-Juvala, Finnish Institute of Occupational Health (FIOH)

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# 1. EXECUTIVE SUMMARY

Nanotechnology is one of the most active research areas with both novel science and useful applications that has gradually been established in the past two decades. Nanotechnology creates possibilities to produce construction materials with novel functionalities and improved characteristics. Applications of nanotechnology have been described for cement, wet mortar and concrete, paints, coatings, insulation materials, glass and infra-structural materials. The areas of applying nanotechnology in construction is mainly focused on lighter and stronger structural composites, low maintenance coating, better properties of cementitious materials, reducing the thermal transfer rate of fire retardant and insulations, and construction related nano-sensors.

The number of consumer products containing engineered nanoparticles is increasing rapidly and NPs are expected to play an important role in material design, development and production for the construction industry. Consequently, the potential for human and environmental exposure is also likely to increase rapidly. Generally, very limited information is supplied on the nano-additives in the Material Safety Data Sheets (MSDS) of the products, and many employers and workers are not aware of the usage of nanomaterials at their workplace.

At the moment, some of the engineered nanomaterials are extensively being studied for their potential health hazards, whereas for other engineered nanomaterials there is almost no toxicity data available in the open literature.

Of the materials being in the focus of the Scaffold project, extensive data is available on the toxic potential of amorphous silica, most likely due to the fact that it has already been used for decades. Amorphous silica may induce pulmonary inflammation. However, the effect seems to be reversible. Nano-sized $TiO_2$ is being intensively studied. There are some indications that it may cause pulmonary toxicity after repeated dose inhalation. *In vivo* studies performed with high doses also indicate a carcinogenic potential of $TiO_2$. Carbon nanotubes are also being intensively studied for their toxicity, due to the theory that they may behave in an asbestos-like manner. There are, however, a lot of different types of carbon nanotubes at the market, and it appears that the toxic effects may vary a lot depending on which type of material has been tested. Anyhow, it seems to be proven that some carbon nanotubes may induce lung inflammation, genotoxicity and carcinogenicity. In some studies, carbon nanotubes have even been shown as more potent than asbestos.

The toxic potential of carbon nanofibres, nanocellulose and nanoclays has only been investigated in a few studies. Due to their fibrous structures, it would be important to study the toxicity of nanocellulose and carbon nanofibres. As for carbon nanotubes, the tests of all these three types of engineered nanomaterials are complicated by the fact that they are containing not only one single substance, but groups of similar substances, which may still have marked differences in their physico-chemical properties, most likely also affecting their toxicological profiles.

Workers' exposure to nanoparticles may occur during production, handling and refinement, bagging and shipping, and processing. For the construction industry, the largest numbers of workers having a risk of being exposed are those who are involved in events associated with use and processing of the nanoparticles-containing materials. An exposure to engineered nanomaterials can in theory occur via inhalation, dermal, oral and ocular routes. Exposure

through inhalation of nanomaterial generating dust (e.g., from cutting, sanding, drilling or machining) or aerosols from paint-spraying are those most likely to pose health risks. Factors affecting exposure to engineered nanomaterials include, e.g., the amount of material being used, the degree of containment, and duration of use. So far, no occupational exposure limits have been given for any nanomaterial.

At present, there is only very limited information on the actual use of nanoparticulate products and about possible exposures to nanoparticles released from these products at the workplace in general, and in the construction field specifically.

A standard or an agreeable methodology for workplace measurements has not been established yet. The main reason is the difficulty in precise analysis of airborne particles in workplace, as nanomaterials have unique physico-chemical properties different from those of bulk materials. There is an on-going debate in the scientific literature about what are the relevant parameters to evaluate an exposure to nanoparticles. Particle number concentrations and particle number size distributions are the most commonly used metrics within the reviewed workplace and laboratory studies. A major drawback of current state of the art measurement devices is their lack of differentiation of background from nanomaterial related particles.

In order to limit the occupational exposure of the respiratory tract and the skin, several protective measures like substitution, technical, organizational and personal protection measures have to be applied, according to the precautionary principle and good practices for limiting dust exposure within the field of occupational hygiene. Elements of the risk management program should include guidelines for installing and evaluating engineering controls (e.g., exhaust ventilation, dust collection systems), the education and training of workers in the proper handling of nanomaterials (e.g., good work practices), and the selection and use of personal protective equipment (e.g., clothing, gloves, respirators).

There is limited information available to build well-informed exposure scenarios covering the life cycle of manufactured nanomaterials for uses which are known to exist. Most of the existing quantitative exposure data are associated with small-scale production of manufactured nanomaterials. There is particularly little information available on exposures to downstream users, i.e., consumer and occupational uses of preparations and articles containing manufactured nanomaterials. It is important to look at particles of all sizes, particularly since nanoparticles tend to agglomerate into larger particles.

According to an enquiry among the Scaffold partners and FIEC, the producers and other companies in the beginning of the life cycle of the manufactured nanomaterials seem to be most aware about the potential risks related to exposure to manufactured nanomaterials, whereas those using the products containing manufactured nanomaterials, e.g., at construction sites have not paid that much attention to the potential risks or to nano-specific risk management measures.

## 2. OBJECTIVES

The objective of this work within the Scaffold project was to collect and analyse the available data on exposure to manufactured nanomaterials (MNMs) the potential toxicity of those materials and risk management methods. The data was collected by systematic literature searches. The focus was on finding data specifically related to the use of MNMs in the construction field. However, very limit amounts of data were found, and thus also more general data was included. In the health hazard assessment part, toxicological information was assembled only on the nanomaterials selected for the Scaffold project.

In addition to the literature review, a questionnaire was distributed to the Scaffold industry partners, as well as to the European Construction Industry Federation (FIEC) in order to get an overview of the current status of awareness, exposure and risk management with respect to nanomaterials at workplaces within the construction sector.

The data presented in this report will be used as a background material for the further work being carried out within the Scaffold project, especially on risk assessment and on risk management and in the real case studies.

## 3. SCOPE

This work within the Scaffold project focused on the following issues, which are presented in this report:

1) Background information on the exposure levels of manufactured nanomaterials (MNMs) in general, and in the construction industry.
2) Brief screening of available toxicological data on the MNMs selected for the project.
3) Risk management measures and tools used in the construction field in general, and in relation to exposure to MNMs in particular.

# 4. INTRODUCTION

Nanotechnology is one of the most active research areas with both novel science and useful applications that has gradually been established in the past two decades (Zhi and Zhili 2008). The construction industry has started seeking out a way to advance conventional construction materials using a variety of MNMs (Lee et al. 2010). Nanotechnology seems to hold the key that allows construction and building materials to replicate the features of natural systems improved until perfection during millions of years (Pacheco-Torgal and Jalali 2011). Nanotechnology creates possibilities to produce construction materials with novel functionalities and improved characteristics. Applications of nanotechnology within the construction sector have been described for cement, wet mortar and concrete, paints, coatings, insulation materials, glass and infra-structural materials. (van Broekhuizen et al. 2011)

The areas of applying nanotechnology in construction is mainly focused on creating lighter and stronger structural composites, low maintenance coating, better properties of cementitious materials, reducing the thermal transfer rate of fire retardant and insulations, and construction related nano-sensors (Zhu et al. 2004; Zhi and Zhili 2008). Nanoparticles (NPs) exhibit different optical, electrical, magnetic, chemical and mechanical properties (Green and Ndegwa 2011) and they are being used in order to reduce the weight of concrete by using silica fume, to increase strength and elasticity of concrete, to save energy consumption of houses by improved performance of isolation materials, to improve weathering properties for exterior surfaces, as self-cleaning coatings for interior and exterior surfaces and window class, as traffic exhaust purification coatings for infrastructural works, to provide better crack resistance of polymer materials, as biocidal surfaces for walls of surgery rooms, to improve fire resistance of materials, etc. (van Broekhuizen et al. 2011).

The European Commission defines nanomaterials as particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1–100 nm (EC 2011).

Workplace exposure to NPs may have three main sources: (1) engineered NP (ENP), (2) process-generated NP (PGNP) or incidental NP, which include engine-generated NP (EGNP) and combustion-derived NP (CDNP), and (3) the environmental background NP. The environmental background concentration originates from natural sources (volcanism, weathering, etc.) and anthropogenic activities like combustion. (van Broekhuizen et al. 2012) In the Scaffold project, the focus is on exposure to ENPs.

## 5. USE OF MNMs IN THE CONSTRUCTION INDUSTRY

Compared to the nanoproduct development in total, the market share of nano-products in the construction industry is small and considered to be applied in niche markets (van Broekhuizen and van Broekhuizen 2009). Costs and the present uncertainty regarding long-term technical performance of nanoproducts are factors that have limited the use of nanoproducts in the European construction industry (van Broekhuizen et al. 2011). However, the number of consumer products containing ENPs is increasing rapidly (Green and Ndegwa 2011) and NPs are expected to play an important role in material design, development and production for the construction industry (van Broekhuizen and van Broekhuizen 2009). Consequently, the potential for exposure to humans and the environment is also likely to increase rapidly (NANEX 2010).

The most common MNMs in construction industry are carbon-based nanomaterials (e.g., carbon nanotubes/fibers and $C_{60}$ fullerene), metal oxide NPs (e.g., $SiO_2$, $Fe_2O_3$ and $TiO_2$) and metal NPs (e.g., Cu and Ag NPs) (Zhu et al. 2004; van Broekhuizen and van Broekhuizen 2009; Lee et al. 2010). Nanoproducts in the construction industry are currently mainly concentrated into four sectors: (1) cement-bound construction materials, (2) noise reduction and thermal insulation or temperature regulation, (3) surface-coatings to improve the functionality of various materials, and (4) fire protection (Greßler and Gazsó 2012). In 2009, coating products were identified to dominate the market, covering 68% of the total number of the identified nanoproducts. Concrete and cement products and insulation products made up for 12% and 7% of all the identified products, respectively. The 2009-survey indicated that 80% of the workers' representatives and 71% of the employers' representatives were not aware of the availability of nanomaterials and were ignorant as to whether they actually use nanomaterials at their workplace. (van Broekhuizen and van Broekhuizen 2009)

Generally, very limited information is supplied on the nano-additives in the Material Safety Data Sheets (MSDS) of the products. Current legislation does not oblige manufactures and suppliers to report the level of NPs contained in the product to the downstream user. Therefore, the market may face a growing number of downstream users who are not informed about the type and content of NPs in the products they use. There is also confusion about the definition of NPs, nanomaterials and nanoproducts, resulting in conflicting opinions about characterizing the supplied material as "nano" or not. It is concluded that communication about product performance and health risks of nanomaterials has to be improved in the supply chain. (van Broekhuizen et al. 2011)

Selected current and potential uses of MNMs in construction are presented in Table 1.

**Table 1**. Examples of MNMs used in construction (Lee et al. 2010)

| MNMs | Architectural/construction materials | Expected benefits |
|---|---|---|
| Carbon nanotubes | Concrete | Mechanical durability, crack prevention |
| | Ceramics | Enhanced mechanical and thermal properties |
| | NEMS/MEMS | Real-time structural health monitoring |
| | Solar cell | Effective electron mediation |
| $SiO_2$ NPs | Concrete | Reinforcement in mechanical strength |
| | Ceramics | Coolant, light transmission, fire resistant |
| | Window | Flame-proofing, anti-reflection |
| $TiO_2$ NPs | Cement | Rapid hydration, increased degree of hydration, self-cleaning |
| | Window | Superhydrophilicity, anti-fogging, fouling-resistance |
| | Solar cell | Non-utility electricity generation |
| $Fe_2O_3$ NPs | Concrete | Increased compressive strength, abrasion-resistant |
| Cu NPs | Steel | Weldability, corrosion resistance, formability |
| Ag NPs | Coating/painting | Biocidal activity |

## 6. EXPOSURE ROUTES AND FACTORS AFFECTING EXPOSURE

MNMs may be accidentally or incidentally released to the environment at different stages of their life cycle. Once in the environment, MNMs may undergo diverse physical, chemical, and biological transformations that change their properties, impact, and fate. (Lee et al. 2010)

Workers' exposure to NPs may occur during production, handling and refinement, bagging and shipping, production and processing of materials containing NPs (Hristozov and Malsch 2009; Kuhlbusch 2011). When a worker inhales dust containing NPs, the actual exposure depends on the structure and solubility of the dust. If the dust is insoluble, part of the NPs will remain embedded in the matrix and exposure will only be to the NPs of the surface of the dust grain. If the dust itself is soluble, there will be systematic exposure to the whole number of NPs contained by the dust grain. (van Broekhuizen and van Broekhuizen 2009)

In general, it is likely that processes generating nanomaterials in the gas phase (after removal of the nanomaterial from an enclosed generation system), or using or producing nanomaterials as powders or slurries/suspensions/solutions (i.e., in liquid media), pose the greatest risk for releasing nanoparticles. In addition, maintenance of production systems (including cleaning and disposal of materials from dust collection systems) is likely to result in exposure to NPs. Exposures associated with waste streams containing nanomaterials may also occur. (NIOSH 2009)

An exposure to ENMs predominantly can occur via inhalation, dermal, oral and ocular routes. The major possible portals of ENM entry are lung, skin, gastrointestinal tract, nasal cavity and eyes. (Yokel and MacPhail 2011) Exposure through inhalation of dust generated when processing materials (e.g., from cutting, sanding, drilling or machining) or aerosols from paint-spraying are the scenarios most likely to pose health risks (van Broekhuizen and van Broekhuizen 2009). Skin penetration may in theory play a role as well, but most studies have shown little to no transdermal absorption through healthy skin. However, the uptake via damaged skin cannot be ruled out. Oral (gastrointestinal) exposure can occur from intentional ingestion, unintentional hand-to-mouth transfer, from inhaled particles (>5 µm) that are cleared via the mucociliary escalator, and of drainage from the eye socket via the nasal cavity following ocular exposure. (Yokel and MacPhail 2011)

Critical factors affecting exposure to ENMs include the amount of material being used, the ability of the material to be dispersed (in the case of a powder) or form airborne sprays or droplets (in the case of suspensions), the degree of containment, and duration of use. In the case of airborne material, the particle or droplet size will determine the deposition of material. Respirable particles may deposit in the alveolar (gas exchange) region of the lungs, which includes particles smaller than ca. 10 µm in diameter. Approximately 30%–90% of inhaled nanoparticles are likely to deposit in any region of the human respiratory tract depending on, e.g., breathing rate and particle size. Even 50% of nanoparticles in the 10–100 nm size range may deposit in the alveolar region, while nanoparticles smaller than 10 nm are more likely to deposit in the head and thoracic regions. (NIOSH 2009)

Jobs and operations that may increase the likelihood of exposure to nanoparticles include for example (Schulte et al. 2008):

- Generating nanoparticles in the gas phase in non-enclosed systems increases the chance of aerosol release to the workplace.
- Handling nanostructured powders can result in aerosolization.
- Working with nanomaterials in liquid media without adequate protection (e.g., gloves) increases the probability of skin exposure.
- Working with nanomaterials in liquid during pouring or mixing operations or where a high degree of agitation is involved can cause the formation of airborne, inhalable, and respirable droplets.
- Conducting maintenance on equipment and processes used to produce or fabricate nanomaterials, or the cleanup of spills or waste material, pose a potential for exposure to workers performing these tasks.
- Cleaning of dust collection systems used to capture nanoparticles increases the potential for both skin and inhalation exposure.
- Machining, sanding, drilling, or other mechanical disruptions of materials containing nanoparticles can lead to aerosolization of nanomaterials.

# 7. POTENTIAL ADVERSE EFFECTS AND TOXICITY MECHANISMS

The occupational experiences with respirable quartz dust, asbestos and man-made mineral fibres dictate that the lung is the primary target organ for airborne fine particles in the workplace. Consequently, potential pulmonary effects of ENPs have been the main focus of the nanotoxicology studies over the last few years (Drew et al. 2009). Physico-chemical properties of ENMs can significantly influence their uptake. ENMs show greater uptake and are more biologically active than larger-sized particles of the same chemistry, due to their greater surface area per mass. These differences include a high rate of pulmonary deposition, the ability to travel from the lung to systemic sites, and a high inflammatory potential. Additional ENM characteristics that may influence their toxicity, besides size, include shape, surface functionalization or coating, solubility, surface reactivity (ability to generate reactive oxidant species), association with biological proteins, binding to receptors, and their strong tendency to agglomerate. (Yokel and MacPhail 2011)

For small airborne particles (fine particles: PM10 and PM2.5) the general rule has been as follows: the smaller the particles, the more deeply they can penetrate into the lungs before they deposit, the more severe their effect on health might be (van Broekhuizen and van Broekhuizen 2009). Of particular concern are findings from epidemiologic studies that provide strong evidence that inhaling ultrafine dust particles leads to oxidative stress in the lungs and, through ambient exposure, can lead to pulmonary diseases in occupational settings and excess mortality and morbidity in susceptible populations (Green and Ndegwa 2011).

For the exposure scenarios considered within the Scaffold project, the focus is on five different types of nanomaterials: silicon dioxide (amorphous silica), titanium dioxide, nanocellulose, carbon nanofibres, and nanoclays. The available toxicological data for each of the materials is reviewed in the following sections.

## 7.1 Silicon dioxide (amorphous silica)

### *7.1.1 Identity of the substance*

There are several different forms of silica (Figure 1). A common CAS number for all silicas is 7631-86-9. However, each different polymorph of silica has its own polymorph specific CAS number.

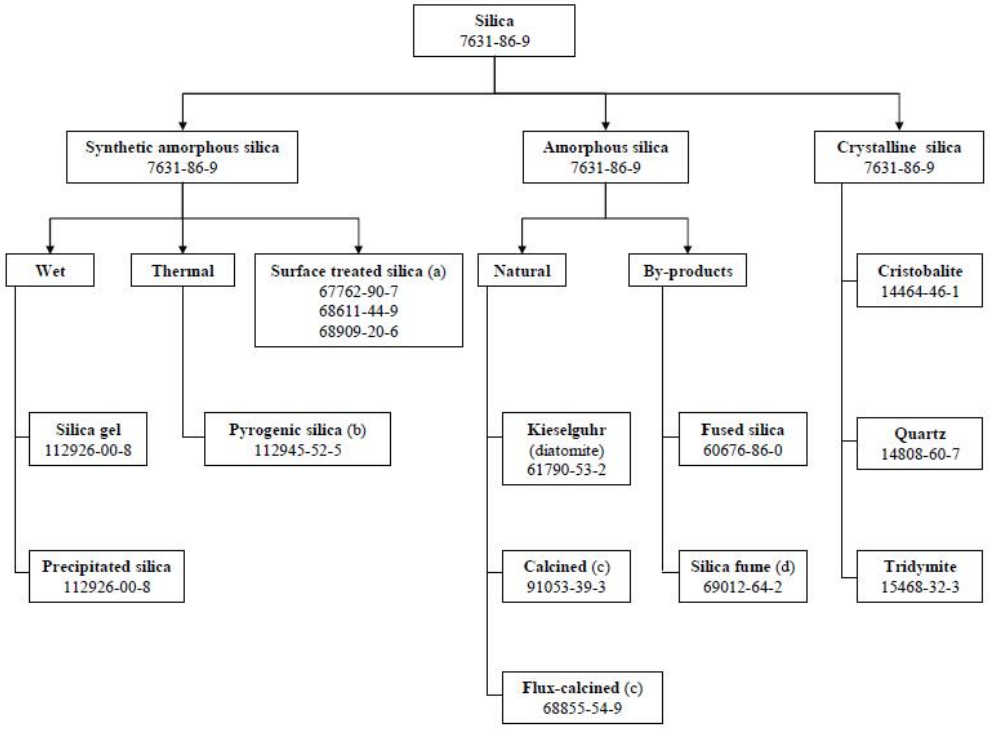
Crystalline silica is known from its carcinogenic properties and its ability to cause silicosis. Crystalline silica does not have a nanospecific structure and is out of scope of this review.

Amorphous silica can be divided to synthetic amorphous silicas, natural amorphous silicas (like diatomaceous earth) and by-products metal industry (silica fume). Natural forms of amorphous silica may contain impurities, particularly crystalline silica (OECD 2004; ECETOC 2006; Napierska et al. 2010). The physico-chemical properties and particle characteristics differ between different amorphous silica polymorps. Properties of synthetic amorphous silica, including particle characteristics, are presented in Table 2.

Silica fume has been often discussed in the context of nanotoxicology but it does not fall under the recent EU definition of nanomaterials (EC 2011).

Of different polymorphs of amorphous silica, synthetic amorphous silicas have been most widely studied on their toxic properties. Different production processes result in three principal forms of synthetic amorphous silicas: silica gel, precipitated silica and pyrogenic (fumed) silica, which have slightly differing physico-chemical properties from each other. These have been summarized in Table 2. The main properties of synthetic amorphous silicas likely to affect their toxicity include particle size, porosity and hydrophilic-hydrophopic properties and solubility. The number of silanol groups in the surface of amorphous silica particles affects the hydrophilicity of silica: increasing silanol group number resulting in higher hydrophilicity. (Napierska et al. 2010) Synthetic amorphous silicas can be also surface treated rendering them more hydrophobic (ECETOC 2006).

***Figure 1: Different polymorphs of silica with CAS numbers***




(a) All forms of SAS can be surface-treated either physically or chemically; most common treating agents are organosilicon compounds (Appendix B: Table B.2)
(b) Pyrogenic silica is also known as fumed silica in the English speaking countries
(c) Partial transformation into cristobalite
(d) By-product from electrical furnace

**Figure 1.** Different forms of silicas. Retrieved from ECETOC (2006).

Health and environmental effects of synthetic amorphous silica have recently been reviewed by OECD (2004) and ECETOC (2006). In general, since different forms of amorphous silicas have been at the market already for several decades, and they are used for a large number of different applications, including consumer uses, there are several *in vivo* and *in vitro* studies

available on their health effects (although not as much as on the health effects of crystalline silica).

**Table 2.** Properties of different types of synthetic amorphous silica (ECETOC 2006)

| | **Pyrogenic silica** | **Precipitated silica** | **Silica gel** |
|---|---|---|---|
| CAS No. | 112945-52-5 | 112926-00-8 | 112926-00-8 |
| EINECS No. | – | – | – |
| Description | Amorphous silicon dioxide particles from the flame hydrolysis of silicon tetrachloride. Also surface modified products available (hydrophobization by reaction with organosilanes or silicone fluids). | Amorphous silicon dioxide particles from the reaction of alkali metal silicate solution with mineral acid, followed by precipitation, filtration, drying and grinding. Also surface modified products available. | Interconnected random array of amorphous silicon dioxide particles from the reaction of alkali metal silicate solution with mineral acid, followed by drying. |
| Use | Fillers in the rubber industry. Thickening and free-flow agents. | Fillers in the rubber industry. Thickening and free-flow agents. | Desiccants, adsorbers, coatings, dentifrices. |
| Primary particle size | 0.005–0.05 µm | 0.005–0.1 µm | 0.001–0.02 µm |
| Aggregate size | 0.1–1 µm | 0.1–1 µm | 1–20 µm |
| Agglomerate size | 1–250 µm | 1–250 µm | NA |
| Pore size | Nonporous | > 0.03 µm | 0.0001-1 µm |
| Specific surface area | 50–400 $m^2/g$ | 30–500 $m^2/g$ | 250–1000 $m^2/g$ |
| Silanol group density | 1.3–2.5 $OH/nm^2$ | 5.0–5.7 $OH/nm^2$ | 4.6–7.9 $OH/nm^2$ |
| pH | 3.6–4.5 | 5–9 | 3–8 |

## *7.1.2 Toxicokinetics*

The data on the kinetics of synthetic amorphous silica in the lungs show rather consistently that, in contrast to crystalline silica, which exhibits a marked tendency to accumulate, amorphous silica reaches a plateau level at which elimination equates with deposition. After the cessation of exposure, synthetic amorphous silica is rapidly eliminated from the lung tissue. For example, Reuzel et al. (1991) exposed rats to two pyrogenic synthetic amorphous silica and one precipitated, hydrophilic silica for 6 hours/day, 5 days/week for 13 weeks at a concentration of 31 mg $SiO_2/m^3$ with recovery period up to 52 weeks. The particle size distribution was 1–120 µm. At the end of the exposure period, small amounts of silica could be detected in the lungs in all animals of the high dose group. Some trace amounts of silica were detected also 13 and 26 weeks after the exposure, but at the 39 week time point the levels had decreased to below the detection limit. The levels of silicon in the lungs and lymph nodes of the animals exposed to quartz (60 mg quartz/$m^3$) remained high during the whole post-exposure period (52 weeks). This difference between crystalline and amorphous silica can be at least partly explained by the better solubility of amorphous silica in lung tissue.

Silicon excretion after oral exposure has been studied in humans. In the studies by Lang (1966) and Langendorf and Lang (1967) (reviewed in ECETOC 2006) in humans, two types of synthetic

amorphous silica were administered to six volunteers each. The total urine was collected daily and analysed. During the four days post-treatment, significant changes of the renal $SiO_2$ excretion were not seen.
There is significant variability in the urinary excretion of silicon ion between individuals. This is probably due to variability in dietary intake. Since silicon in different forms is ubiquitous in the environment, various foods and drinking water contain various amounts of $SiO_2$ (see for example reviews by Jugdaohsingh 2007 and Martin 2007). Our normal dietary intake of silicon is between 20 and 50 mg Si/day (EFSA 2004). The available information suggests that silicon from the diet is fairly well absorbed, which is seen as a high proportion of dietary silicon excreted in the urine in one study the average of urinary excretion was 41% (Jugdaohsingh 2002). In blood, silicon exists as a monosilicic acid (Martin 2007). EFSA (2009) reviewed the use of silica in food additives and concluded that adding up to 1,500 mg $SiO_2$/day (equal to 700 mg/day) of silicon dioxide to food supplements is not a safety concern.

### *7.1.3 Acute toxicity*

Amorphous silica does not raise concerns for its acute toxicity. The OECD SIDS Initial Assessment Report (OECD 2004) has compiled the acute oral toxicity for rat studies conducted with synthetic amorphous silicas. In general, after a single oral administration of different types of synthetic amorphous silica, the $LD_{50}$ values were higher than the top doses applied and no deaths occurred and no signs of toxicity were seen during the observation periods. There were no lethal effects following inhalation exposure of rats to the highest technically feasible concentrations of 140 to 2,000 mg/$m^3$ of hydrophilic precipitated or pyrogenic silica. (OECD 2004)

### *7.1.4 Irritation and sensitization*

According to data presented in OECD (2004) and ECETOC (2006), synthetic amorphous silica does not raise concern for irritation to the skin and eyes under experimental conditions.
Amorphous silica has not been tested for its sensitizing properties but when taking into account the widespread environmental exposure to amorphous silica this endpoint is not a concern.

### *7.1.5 Repeated dose toxicity*

There is limited data available on the repeated dose toxicity of amorphous silica after oral exposure. Takizawa et al. (1988) performed a chronic oral feeding study in rats and mice with food-grade micronized silica gel. The length of the study was 93 weeks and 103 weeks, respectively (Takizawa et al. 1988). There were no biological or any other meaningful alterations in the body weight, food consumption or physical features of the exposed animals. No significant dose-related effects were seen at any dose level upon clinical laboratory examinations. The pathological examinations revealed no gross or microscopic changes in the tissues examined. The occasional presence of some neoplasms did not reveal any consistent, dose-related trends in any group.

More recently, So et al. (2008) fed nanosized (30 nm), microsized (30 μm) and "normal" silica to Balb/c and C57BL/6J mice for 10 weeks. After feeding, the blood was tested biochemically and hematologically. The nanosized silica fed group showed higher value of ALT than normal and microsized silica dieted groups. Otherwise, no difference between the groups in the tested endpoints was seen. HandE staining of the liver of the nanosized particle dieted group

indicated some fatty liver pattern while the contents of Si in the livers of the groups were almost the same. Total fed amount of silica was 140 g silica/kg mouse.

Lung effects after inhalation exposure have been the main concern related to the exposure to amorphous silica.

Groth et al. (1981) reported early nodular fibrosis in the lungs and effects on the lung function of monkeys with an Lowest observed adverse effect concentration (LOAEC) of 15 mg/m$^3$, with pyrogenic silica, corresponding to approximately 7–9 mg/m$^3$ of respirable particles <4.7 µm (mass-median aerodynamic diameter; MMAD).

Reuzel et al. (1991) reported inflammation, granulomatous lesions and interstitial fibrosis after exposure to synthetic amorphous silicas, with hydrophilic pyrogenic silica being the most potent. However, as opposed to quartz, these changes were (mostly) reversible after the cessation of exposures. The No observed adverse effect concentration (NOAEC) was 1.3 mg/m$^3$, LOAEC 5.9 mg/m$^3$. The primary particle size range was <6–45 nm, with a maximum aggregate/agglomerate size distribution of 10 µm.

Arts et al. (2007) reported transient histopathological and BAL changes in rats exposed to ≥5 mg/m$^3$ of pyrogenic silica for 5 consecutive days. While quartz caused progressive changes in the lungs, synthetic amorphous silica caused only transient changes, which were resolved during the three-month follow-up period. The MMAD varied between 1.2 and 3.5 µm.

Based on these studies, the OECD SIDS (2004) on synthetic amorphous silicas showed in the following conclusions: The inhalation of respirable particles of synthetic amorphous silica produces a time- and dose-related inflammation response of the lung tissue in animal studies. A thirteen-week exposure to an average concentration of 1.3 mg/m$^3$ of a pyrogenic amorphous silica resulted in mild reversible pro-inflammatory cell proliferation rather than a pathologically relevant tissue change. Given the low-grade severity of this common lung-tissue response, 1 mg/m$^3$ can be established as the NOAEC and LOEC (sub-chronic, 13 weeks). The LOAEC was 5.9 mg/m$^3$ and the mid concentration produced clear signs of histopathological adverse effects. All of these effects were reversible following discontinuation of exposure.

No lung-tissue effects were observed following an exposure of five days to 1 mg/m$^3$ of the same silica. The LOAEL (5 d) was 5 mg/m$^3$.

These studies can be used to identify NOAEL and to set OEL for amorphous silica. In addition, there are several other studies employing either inhalation route or intratracheal administration and evaluating for example different inflammatory responses in the lungs. These studies can be regarded supporting the abovementioned conclusions.

Also *in vitro* studies on the cytotoxicity of different amorphous silica particles in different cell culture systems are available. These studies have been reviewed by Napierska et al (2010). In general, results vary with the particle types and cell type tested but it seems that both the particle surface area and particle surface properties (especially the number of silanol groups in the surface) play a role in *in vitro* cytotoxicity of amorphous silica particles (Napierska et al. 2010).

Epidemiological evidence from amorphous silicon manufacturing does not show any lung effects in humans. Some old studies from silicon metal/ferrosilicon industry with exposure to silica fume suggest adverse respiratory effects, that is, "ferroalloy disease" associated with amorphous silica fumes formed during the process. This disease was characterized by fibrotic

changes, which, however, seemed to regress when the exposure ceased. An association with metal fume fever was also suggested. The pathology of reported cases of "ferroalloy disease" within the metallurgical industry is unclear – whether it is related to these freshly generated fumes and repeated attacks of metal fume fever or to amorphous silicon dioxide particles is unclear. The confounding factors also include the co-exposing agents, like crystalline silica, which is used as a raw material and found in workplace air. In addition, no cases of "ferroalloy" disease have been reported anymore since the 1980s.

### *7.1.6 Reproductive toxicity*

There are limited data available on the fertility effects of amorphous silica. However, based on the acute and repeated dose toxicity data on synthetic amorphous silica, silicon ion is virtually non-toxic, showing no systemic toxic effects even at very high oral doses. No harmful effects on reproductive organs have been described in repeated dose toxicity tests. In addition, there is a dominant lethal study available on calcium silicate, which did not show any effects on male fertility (see OECD 2004; ECETOC, 2006). OECD (2004) concluded on the basis of weight of evidence that prolonged exposure to synthetic amorphous silica, applied before and during pregnancy at high doses, is not expected to produce harmful effects on the reproductive performance in experimental animals.

Developmental toxicity has been studied with hydrophilic silica gel (SYLOID® 244) (FDA 1973; see OECD 2004; ECETOC 2006). No embryotoxicity was observed and the number of external, visceral or skeletal abnormalities in the test groups did not differ from controls. Also, no maternal toxicity was seen even at the highest dose of 1600 mg/kg bw/day.

As a conclusion, amorphous silica does not raise concern for reproductive effects.

### *7.1.7 Genotoxicity*

There are several *in vivo* and *in vitro* studies available on the mutagenicity of amorphous silicas. These have been reviewed by OECD 2004 and ECETOC 2006. Most of these studies have not shown relevant positive responses. In one micronucleus study, a positive response was seen (Liu et al. 1996), but this was quite likely caused by the high concomitant cytotoxicity. There are contradictory results from Comet assays (Zhong et al. 1997; Barnes et al. 2008). One recent study (Gonzalez et al. 2010) with amorphous silica with particle size of 16, 60 and 104 nm showed a slight induction of micronuclei at non-cytotoxic dosed after amorphous silica exposure. Particle number and total surface area correlated with the amplitude of the effect. Likewise, alkaline comet assay and FISH-centromeric probing of micronuclei indicated a weak and not statistically significant induction of oxidative DNA damage, chromosome breakage and chromosome loss. Overall, the data on the genotoxicity of amorphous silica nanoparticles is inconclusive.

### *7.1.8 Carcinogenicity*

Tagizawa et al. (1988) fed mice and rats with micronized silica gel (Syloid) at dose levels of 0, 1.25, 2.5, and 5% for 93 and 103 weeks, respectively. No increases in neoplasms in any group were seen. However, as the group size was relatively small (20 animals/sex/dose in the recovery group), the study has limited sensitivity.

One old inhalation carcinogenicity test with amorphous silica in mice (Campell et al. 1940) showed an increase in lung tumours but the study cannot to be regarded reliable due to

technical deficiencies and especially due to the failure of substance identification. Intrapleural implantation of two different preparations of pyrogenic silica did not increase the incidence of tumours in one study (Stanton and Wrench, 1972). The International Agency for Research of Cancer concluded in their evaluation (IARC 1997) that amorphous silicas have been studied less than crystalline silica and they are generally less toxic than crystalline silica and are cleared more rapidly from the lungs. The conclusion of the IARC on amorphous silica was that there is inadequate evidence in humans and animals for the carcinogenicity of amorphous silica and amorphous silica is not classifiable as to its carcinogenicity in humans (Group 3).

Recently, Kolling et al. (2011) published a study in rats, in which they evaluated the induction of lung cancer after repeated intra-tracheal instillation of crystalline silica, amorphous silica (pyrogenic Aerosil 150, particle size 14 nm), carbon black and coal dust at the dose levels which were known to induce pulmonary inflammation. The dose for amorphous silica was 30 x 0.5 mg at the intervals of 14 days. The positive control, crystalline silica, elicited the highest inflammatory reactions in lungs, fibrosis, and the highest incidence of primary lung tumours (39.6%). After repeated instillation of soluble, ultrafine amorphous silica (15 mg) a statistically significant tumour response (9.4%) was observed. Tumour responses correlated with inflammatory responses in lungs. Overall, the results showed the relationship between tumour responses and non-cancerous effects (like inflammation and fibrosis) in rats and mechanisms related to lung overload, and persistent inflammation resulting in oxidative DNA damage were discussed.

Human epidemiological data is available only from ferrosilicon/silicon metal industry in which there is exposure to freshly generated ultrafine amorphous silica fumes (Kjuus et al. 1986; Langård et al. 1990; Hobbesland et al. 1999). These studies do not show increased incidence of cancer attributed to ultrafine silica fumes present in furnace work.

### *7.1.9 Conclusions*

The main concerns related to amorphous silica nanoparticles are their possible lung effects. In repeated dose inhalation toxicity studies, chronic inflammation and fibrotic lesions have been seen. These have, however, been reversible after the cessation of exposure, which differs from the lesions caused by crystalline silica. Limited data is available on the carcinogenicity of nanosized amorphous silica particles. In a recent study, a statistically significant tumour response was observed after repeated intratracheal administration of amorphous silica particles in rats. Tumour responses correlated with inflammatory responses in lungs and mechanisms like lung overloading may have played a role in tumour response. The data on the genotoxicity of amorphous silica particles is inconclusive.

## 7.2 Titanium dioxide

### *7.2.1 Introduction*

Titanium dioxide is the naturally occurring oxide of titanium. Often distinction is made by $TiO_2$ manufacturers between pigmentary and ultrafine grade. The primary crystal size typically ranges from 150 to 300 nm for $TiO_2$ of pigmentary grade and the surface area from 6 to 60 $m^2/g$. The ultrafine grade typically has a primary crystal size from 10 to 150 nm, and surface area between 50 and 200 $m^2/g$. The pigmentary $TiO_2$ has a white colour and is therefore widely used in paints etc. The ultrafine, including nano-sized, $TiO_2$ is transparent. In contrast to the

bulk $TiO_2$ (>100 nm) that is considered chemically inert, nano-scale $TiO_2$ can act as a photo-catalyst, and can generate reactive oxygen species upon illumination. A wide range of applications exist, exploiting the various properties of $TiO_2$ nanomaterials. In paints and for water treatment nano-sized $TiO_2$ is used as a photocatalysist producing reactive oxygen that may degrade other organics. Adding nano-$TiO_2$ into concrete aims to enhance its' durability and to maintain whiteness throughout the lifetime of the construct. In glass nano-$TiO_2$ is used for heat and fire protection and for its' self-clean properties. A number of other very diverse areas of application exist such as catalysts, toothpaste, sunscreens and other cosmetics, air filtration devices, semiconductors, etc. (van Brokhuizen et al. 2011)

## *7.2.2 Toxicokinetics*

Following an inhalation experiment in rats with $TiO_2$ particles of varying sizes, nano-$TiO_2$ accumulated in the lung accessing the interstitium to a greater extent compared to larger particles (Ferin et al. 1992).

In orally exposed (5 g/kg) mice, two nano-sized (20 nm and 80 nm) particles of $TiO_2$ were found to be distributed to the liver, spleen, lungs and kidneys two weeks post-exposure (Wang et al. 2007).

Following nasal instillation Wang et al. (2008a, b) reported accumulation of nano-$TiO_2$ in the brain of mice with the hippocampus and olfactory region being the major sites of accumulation. Takeda et al. (2009) administered titanium oxide (100 µg/day for 3–15 days, anatase, 25–70 nm) subcutaneously to pregnant mice. In 6-week-old mice NPs were identified in testis and in the olfactory bulb and cerebral cortex of brain.

Several studies have been conducted to evaluate the dermal absorption of $TiO_2$ *in vivo* (humans, porcine and rat) and *in vitro* (porcine and human skin) (e.g., Gamer et al. 2006; Mavon et al. 2007, Gontier et al. 2008; Adachi et al. 2010). The studies report none or negligible absorption through stratum corneum. The NANODERM European project (2002–2007) focused on skin penetration on nanosized $TiO_2$ from sunscreen formulations. It was concluded that the penetration was limited to the upper part of stratum corneum. The particle shape, formulation, and exposure time appeared not to have any significant effect.

## *7.2.3 Acute toxicity*

***Oral exposure***

Warheit et al. (2007) conducted an acute oral toxicity study of P25 $TiO_2$ (21 nm) according to OECD guidelines. A single dose (concentrations up to 5000 mg/kg) of the $TiO_2$ particles was administered by gavage to rats. No signs of acute toxicity were observed.

***Inhalation***

Grassian et al. (2007) exposed mice by whole-body inhalation to $TiO_2$ NPs (5 and 21 nm) for either four hours (acute) or four hours per day for 10 days (subacute). $TiO_2$ concentrations were 0.8 or 7.mg/$m^3$ for acute exposure, and 9 mg/$m^3$ for the subacute exposures. No adverse effects were observed after the acute exposure. A significant but modest inflammatory response (macrophage infiltration) was observed in the mice at 1 or 2 weeks after subacute exposures, with recovery at the 3rd week post-exposure.

***Intratracheal instillation***

In intratracheal instillation, test materials are dispersed in liquids and then directly instilled into the trachea of the test animal by using syringe or other similar instrument. Subsequently, the biological effects on the lungs and other organs at different time points are investigated. This method has been widely used as an alternative to the inhalation test.

In the study conducted by Ferin et al. (1992), rats were administered $TiO_2$ of various sizes via intratracheal instillation up to 1000 μg/rat. Examinations after 24 post-exposure indicated that nano-$TiO_2$ induced greater pulmonary inflammation response (characterized by neutrophil infiltration) than its fine counterpart. The clearance of smaller particles was slower.

Also in the studies of Renwick et al. (2004) nano-$TiO_2$ was more damaging in the lungs of rats than larger particles. Twenty-four hours after instillation, rats treated with nano-$TiO_2$ had induced neutrophil infiltration, elevated protein concentration (measure of epithelial permeability), and lactate dehydrogenase (an indicator of cytotoxicity).

Chen et al. (2006) exposed mice with 0.1 and 0.5 mg to ultrafine (19–21 nm) and determined their pulmonary toxicity. Morphology of emphysema-like alterations in the lungs was evident. An inflammatory response, as indicated by the infiltration of macrophages (that were particle laden), and upregulation of cytokines was also observed. Pott and Roller (2005) and Mohr (2006) included two $TiO_2$ NPs (21 nm and 25 nm) in the intratracheal instillation study. The former (dosage 15 x 0.5 mg, 30 x 0.5 mg) and latter (5 x 3 mg, 5 x 6 mg, 10 x 6 mg) induced lung tumours with incidences 0% and 7%, 52%, 67%, 70%, respectively.

In summary, in several studies there is clear evidence that nanosized $TiO_2$ is considerably more toxic than microsized $TiO_2$ (for example Ferin et al. 1992). In addition, it was found that the crystallinity (or the specific crystal form) of $TiO_2$ nanoparticles is thought to influence the toxicity, with the anatase form expected to be more toxic than the rutile form (Warheit et al. 2007). The pulmonary response to $TiO_2$ includes inflammation (Ferin et al. 1992; Renwick et al. 2004; Chen et al. 2006; Warheit et al. 2007; Grassian et al. 2007), epithelial damage, increased permeability of the lung epithelium, oxidative stress and cytotoxicity (Renwick et al. 2004), and morphological alteration within the lung (Chen et al. 2006).

## *7.2.4 Irritation, corrosivity and sensitization*

OECD guideline tests for dermal and eye irritation and dermal sensitization have been performed. No dermal irritation or sensitization was observed. Eye irritation test was negative (Warheit et al. 2007).

## *7.2.5 Repeated dose toxicity*

***Oral exposure***

Repeated dose toxicity (intragastric administration) studies with 5–7 nm of nano-titanium dioxide have been performed in mice (Cui et al. 2010; Duan et al. 2010). After administration of 5, 10 and 50 mg/kg bw for every other day for 60 days, hepatocyte apoptosis was observed in 10 and 50 mg/kg bw groups. Moreover, levels of oxidative stress markers were elevated (Cui et al. 2010).

***Inhalation***

In the study by Ma-Hock et al. (2009), rats were exposed to 25 nm $TiO_2$ (2, 10, and 50 mg/$m^3$) by inhalation for five days. Morphological changes in the lungs were observed in the 50 mg/$m^3$ group. Dose-dependent increases in total cell counts and neutrophils in BALF, total protein content, enzyme activities and levels of a number of cell mediator were found. A LOAEC of 2 mg/$m^3$ was identified in this study.

In the study of Bermudez et al. (2004), rats, mice, and R hamsters were exposed to P25 particles for 13 weeks at concentrations 0.5, 2, or 10 mg/$m^3$). The P25 particles are $TiO_2$ particles that are manufactured by Degussa and consist of 80% anatase and 20% rutile. The have an average particle diameter of 21 nm. Pulmonary response was assessed up to 52 weeks post-exposure. It was demonstrated that the pulmonary response was stimulated by $TiO_2$ within mice and rats, but was absent in hamsters. At 52 weeks post-exposure, minimal to mild metaplastic changes and minimal to mild particle-induced alveolar septal fibroplasia were seen in rats. The effects observed were dose dependent. High concentrations (10 mg/$m^3$) of particles impaired their clearance from lungs in rats and mice. Pulmonary inflammation was evidenced by increased numbers of macrophages and neutrophils and increased concentrations of soluble markers (total protein and LDH) in BALF in rats and mice exposed to 10 mg/$m^3$. In rats responses were also observed in animals exposed to 2 mg/$m^3$. A NOAEC of 0.5 mg/$m^3$ was identified.

Systemic microvascular function was characterized after inhalation of fine and ultrafine $TiO_2$ aerosols. After 24 hours of inhalation (concentrations 1.5–12 mg/$m^3$) *in vivo* microscopy was performed. Significant microvascular dysfunction was observed in rats exposed to ultrafine particles as compared to microvascular function in control rats and those exposed to similar pulmonary load of fine particles (Nurkiewicz et al. 2008).

Rats were exposed via inhalation to 23 mg/$m^3$ (6 hours per day for 5 days per week for up to 12 weeks) of $TiO_2$ (21 nm, manufactured by Degussa) and 250 nm and examination of the consequences were evaluated over a 64-week post-exposure period (Ferin et al. 1992). Ultrafine $TiO_2$ induced a greater pulmonary inflammatory response (characterised by neutrophil infiltration), than its fine counterpart, which did not elicit any changes in the inflammatory status. Ultrafine $TiO_2$ particles were also find to remain within the lungs for longer (501 days) periods, following inhalation, than the fine particles (174 days), thus highlighting that the clearance of smaller particles form the lung was slower.

In summary, the pulmonary response of nano-$TiO_2$ after repeated exposure has been demonstrated to be inflammagenic. Moreover, some studies have demonstrated epithelial damage and cytotoxicity.

## *7.2.6 Genotoxicity*

***In vitro studies***

Evidence of genotoxicity has been found within *in vitro* studies. Anatase and uncoated forms seem to be more toxic as compared with rutile and coated NPs. One study reports hprt-mutations after intratracheal instillation of microsized $TiO_2$ (180 nm, anatase) (Driscoll et al. 1997).

Rahman et al. (2002) studied the potential of nano-$TiO_2$ to elicit chromosomal damage in SHE fibroblasts. The number of micronucleated cells was increased with the doses ≥1μg/$cm^2$. Moreover, elevated apoptosis within cells was detected. In peripheral lymphocytes and in lymphoblastoid cells micronuclei formation has shown dose-dependency (Wang et al. 2007; Sanderson et al. 2007; Kang et al. 2008). Exposing cells to 10 μg/ml $TiO_2$ was found to induce micronuclei in human bronchial cells (Gurr et al. 2005).

***In vivo studies***

Two *in vivo* genotoxicity studies of nano- $TiO_2$ have detected DNA damage after 5 and 7 days oral exposure (Trouiller et al. 2009; Sycheva et al. 2011). The lowest concentration to cause the effect was 40mg/kg bw.

Two short-term inhalation studies did not observe genotoxicity using concentrations up to 29 mg/$m^3$ (Landsiedel et al. 2010; Lindberg et al. 2012). Genotoxic effect in the offspring has been reported (Trouiller et al. 2009).

Both *in vivo* and *in vitro* studies have reported ROS formation and inflammation in association with genotoxicity suggesting that the effect may be driven via an indirect thresholded mechanism.

## *7.2.7 Carcinogenicity*

In a chronic inhalation study of Heinrich et al. (1995), rats were exposed to P25 -particles (manufactured by Degussa) for 2 years. The average exposure concentration was approximately 10 mg/$m^3$, and the cumulative particle exposure (g/$m^3$ x h) calculated by multiplying the particle concentration with the exposure time was 88.1. In comparison with the control groups, there was a significant increase in mortality, decrease in body weight, increase in lung weight, and decrease in clearance after three months of exposure. Incidence of lung tumours was significantly increased after exposure for 18 months or more. The relevance of these data for risk assessment is dubious due to the very high dose used. However, the International Agency for Research on Cancer has assessed $TiO_2$ (even the micro-sized form, if exposure is high enough) to be a Class 2B carcinogen (possibly carcinogenic to humans) (IARC 2010). The US National Institute of Safety and Health (NIOSH) has determined that inhaled $TiO_2$ is a potential occupational carcinogen and recommended an exposure limit of 0.3 mg/$m^3$ (NIOSH 2011).

## *7.2.8 Reproductive toxicity*

After subcutaneous injection of nano-sized titanium oxide to pregnant mice, several adverse effects on offspring have been reported in three publications. Takeda et al. (2009) demonstrated reduced sperm production and abnormal testicular morphology. In 6-week-old mice exposed prenatally to titanium dioxide, NPs were identified in testis and in the olfactory bulb and cerebral cortex of brain. Functional and pathological effects reported were: reduced sperm production, abnormal testicular morphology, aggregated in Leydig cells, Sertoli cells and spermatids. Changes in gene expression related to development and function of central nervous system has been reported by Shimizu et al. (2009). Yamashita et al. (2011) investigated biodistribution and toxicity of titanium dioxide (rutile, 35 nm). Mice treated with NPs had smaller uteri and smaller foetuses. Particles were found in the placenta, foetal liver and foetal brain. LOEC was 0.8 mg/ mouse.

Lung toxicity and developmental effects in offspring after exposing rats, for 11 days, 42 mg/m$^3$ with surface-coated $TiO_2$ were studied by Hougaard et al. (2010) and Halappanavar et al. (2011). Exposure resulted in changes in neutrophil, macrophage and lymphocyte amounts and increased levels of genes associated with acute-phase, inflammation and immune-response five days post-exposure. Prenatally exposed offspring displayed moderate neurobehavioral alterations.

## *7.2.9 Neurotoxicity*

As already described in the toxicokinetics section, Wang et al. (2008a, 2008b) showed that in, rats $TiO_2$ particles (80 nm/rutile and 155 nm/anatase) can be translocated from the nasal area to the central nervous system via the olfactory nerve and bulb. Accumulation of $TiO_2$ resulted in morphological alterations and loss of neurons in the hippocampus. In addition it was suggested that $TiO_2$ elicited oxidative stress within the brain due to the elevation of superoxide dismutase (SOD), and catalase activity, and evidence of increased lipid peroxidation and protein oxidation was found. Furthermore, an inflammatory response (indicated by IL-1ß, and TNFα) within the brain was stimulated by $TiO_2$ exposure. The adverse effects were more severe with the anatase $TiO_2$ particles.

## *7.2.10 Cardiovascular effects*

***In vitro studies***

Hefenstein et al. (2008) demonstrated that $TiO_2$ (up to 2.5 µg/ml) was able to affect cardiomyocyte electrophysiology and enhanced ROS production.

In the study of Jawad et al. (2012) contraction amplitude was reduced in conc. >100 µg/ml in rat myocytes. In hESC-cells 10 µg/ml reduced the beating rate significantly.

***In vivo studies***

Nurkiewicz et al. (2008) characterized systemic microvascular functionafter pulmonary exposure to fine and ultrafine $TiO_2$ aerosols. After 24 hours of inhalation *in vivo* microscopy was performed. Significant microvascular dysfunction was observed in rats exposed to ultrafine particles as compared to microvascular function in control rats and those exposed to similar pulmonary load of fine particles. Cardiovascular effects occurred at particle exposure concentrations below those causing adverse pulmonary effects. In a similar experimental settings NP exposure reduced microvascular nitric oxide bioavailability and altered COX-mediated vasoreactivity (Knuckles et al. 2012) and increased phosphorylation of p38, mitogen-activated protein kinase and cardiac troponin in the heart and substance P in nodose ganglia (Kan et al. 2012).

## *7.2.11 Toxicity in different cell types* in vitro

Several studies have shown various degrees of toxicity of nano-$TiO_2$ to epithelial lung cells (for example Gurr et al. 2005; Simon-Deckers et al. 2008). Signs of oxidative stress are often observed. Long et al. (2006) and Lai et al. (2008) have indicated that nano-$TiO_2$ can cause ROS driven toxicity *to* neural cells *in vitro*. Helfenstein et al. (2008) and Jawad et al. (2011) have observed adverse effects of nano-$TiO_2$ to heart cell function and increase in ROS production. Several studies (for example Renwick et al. 2001; Palomäki et al. 2010) suggest that nano-$TiO_2$ may affect different cell types of immune system.

### *7.2.12 Conclusions*

Several studies have shown clear evidence that nano-sized $TiO_2$ is considerably more toxic than micro-sized $TiO_2$. Among the $TiO_2$-induced adverse effects, respiratory tract is considered as the most critical site. The pulmonary response to $TiO_2$ is inflammation, epithelial damage, increased permeability of the lung epithelium, oxidative stress and cytotoxicity, and morphological alteration within the lung.

The genotoxicity of $TiO_2$ nanoparticles is thought to be driven by particle mediated reactive oxygen species production. The particles themselves are not thought to be inherently genotoxic, but may trigger genotoxicity via an indirect threshold driven inflammatory mechanism involving oxidative stress.

In rodents, nano-$TiO_2$ has been shown to be able to translocate into the central nervous system via axons of sensory neurons in the upper respiratory tract. In the human body, the relevance of transfer via this route is however questionable. Some evidence exists of a neurotoxic potential of nano-$TiO_2$.

A limited data suggest that $TiO_2$ nanoparticles may affect the cardiovascular system.

Dermal studies have shown little evidence for skin penetration after dermal applications of nano-$TiO_2$. However, there may be a risk associated with nano-$TiO_2$ applied to damaged skin.

Nano-$TiO_2$ has been classified as possibly carcinogenic to humans (Group 2B) (IARC 2010). The US National Institute of Safety and Health has determined that inhaled nano-$TiO_2$ is a potential occupational carcinogen and recommended an exposure limit of 0.3 mg/$m^3$.

## 7.3 Nanocellulose

### *7.3.1 Introduction*

Cellulose fibres are extensively used in paper production, cotton textiles, and as insulation and structural strengtheners in construction products. Despite the large scale use of cellulose fibres their possible toxic properties have not been as rigorously tested as for asbestos and other man-made fibres. Inhalation or instillation studies with cellulose fibres in rats and hamsters have shown that the fibres can cause different pathological changes like inflammation, granulomas, alveolitis, fibrosis and epithelial hyperplasia. (Milton et al. 1990; Hadley et al. 1992; Tatrai et al. 1995; Tatrai et al. 1996; Adamis et al. 1997; Muhle et al. 1997; Cullen et al. 2000) It has also been shown that intraperitoneal injections (3 injections spaced at weekly intervals) of high doses of cellulose fibres can cause tumours (sarcomas) in the abdominal cavity of male Wistar rats (Cullen et al. 2002b). In another study, a similar induction of tumours was seen in rats after intraperitoneal injection of cellulose fibres for 7 h a day, 5 days/week for periods of 1 day - 3 weeks (Cullen et al. 2002a).

The toxicological data on nanocellulose fibers is still very scarce because many of the new materials are still under development. There is no information about exposure to nanocellulose in industrial scale processes. In exposure scenarios, the most probable route for exposure of workers at workplaces would be via inhalation and skin. There is some concern that nanocellulose fibers could act similarly to other fibrous nanoscale structures such as

carbon nanotubes and asbestos causing fibrosis and cancer. When generalizing the available toxicity data on nanocellulose fibres, it is important to remember that the physico-chemical properties vary between different materials and also the test systems used are different.

### *7.3.2 Acute toxicity*

No data was available on acute toxicity after oral or inhalation exposure.

### *7.3.3 Irritation and sensitisation*

No data was found.

### *7.3.4 Repeated dose toxicity*

No data was found.

### *7.3.5 Genotoxicity*

Only a few studies on the possible genotoxicity of nanocellulose fibres exist, and the mechanism of material penetration into the cells is not known. de Lima et al. (2012) examined the genotoxicity of cellulosic nanofibres derived from white, brown, ruby, green cotton, and curaua in plants cells (*Allium cepa* roots) and lymphocyte- and fibroblast cell cultures *in vitro* by using chromosomal aberration and the single cell gel electrophoresis (comet) assays, as well as cytogenetic and molecular analyses. They concluded that in plant cells, the most genotoxic nanofibres were those derived from green, white, and brown cotton, and curaua, while in animal cells brown cotton and curaua fibers were the most harmful ones. Moreira et al. (2009) studied the genotoxicity of bacterial cellulose (*G. xylinus*) nanofibres *in vitro* by the Salmonella reversion assay and in chinese hamster ovary (CHO) cells by the comet assay. The conclusion was that the fibres tested did not induce mutations or DNA damage under the conditions tested. Kovacs et al. (2010) made an ecotoxicological characterization of nanocrystalline cellulose (NCC) using rainbow trout hepatocytes and nine aquatic species and founded that the NCC had low toxicicity. No genotoxicity (DNA strand breaks) was observed, as measured by the DNA precipitation assay.

### *7.3.6 Carcinogenicity*

No data was found.

### *7.3.7 Reproductive toxicity*

No data was found.

### *7.3.8 Cytotoxity data*

In one study, the inflammatory effects caused by microfibrillated cellulose (MFC; also referred to as nanocellulose) in cultured mouse macrophages and human monocyte derived macrophages were evaluated (Vartiainen et al. 2011). No significant exposure to MFC or inflammation in macrophages was detected, neither did the MFC cause acute environmental toxicity in the marine bacterium *V. fisheri*.

To obtain a biocompatible nanocellulose-polymer polypyrrole (PPy) composite, the effect of the composite aging on cell viability was studied *in vitro* using fibroblast and monocyte cell lines, and acutely *in vivo* in mice by intraperitoneal injection (Ferraz et al. 2012). The results indicated that the as-prepared composite did not induce any cytotoxic response *in vitro* or *in vivo*, but that the biocompatibility of the PPy composite depends largely on the rinsing and pretreatment as well as the aging of the material.

The cytotoxic and (pro-)inflammatory responses induced by cotton cellulose nanowhiskers (CCN) were examined by using a 3D *in vitro* cell co-culture model of the human epithelial airway barrier (Clift et al. 2011). The results suggested that the CNN could elicit dose-dependent toxic responses, but that the effects were less pronounced than those caused by multiwalled carbon nanotubes (MWCNTs) and asbestos fibres.

### *7.3.9 Conclusions*

In summary, the few studies on nanocellulose toxicity conducted so far suggest that the different types of nanocellulose materials can be slightly toxic *in vitro* and *in vivo*, but the effect is milder than the one caused by MWCNTs and asbestos fibres. Further studies, especially *in vivo* and in mammalian cells, are necessary in order to make it possible to draw more firm conclusions about the toxic potential of nanocellulose. The upcoming results from the EU-funded SUNPAP project (Scale-Up Nanoparticles in Modern Papermaking, FP7), where one of the aims was to study the genotoxic and immunotoxic effects of selected nanocellulose materials both *in vitro* and *in vivo*, will give further insight into the toxicity of nanocellulose.

## 7.4 Nanoclay

### *7.4.1 Introduction*

Nanoclays are nanoparticles formed of layered mineral silicated and they are commonly blended with polymers to form nanocomposites. Nanoclays and polymer-layered nanocomposites are used in a wide range of applications, e.g., in the production of inks, paints, cosmetics, in water treatment applications, and food packaging products. As with other nanosized materials also nanoclays may have toxic effects which are not apparent in the bulk material. (Lordan et al. 2011)

The health effects of bentonite and kaolin clays, which are widely used in different industrial fields, have been fairly extensively examined. Bentonite is formed of highly colloidal and plastic clays composed mainly of montmorillonite. Kaoline is a mixture of different minerals with the main component being kaolinite. The large variability in composition of clay materials has, however been a challenge for the hazard characterization. The amount of crystalline silica, which is always present in clays, has often been the decisive factor in clay induced toxicity. As a summary, there is still very little information about the possible carcinogenicity or genotoxicity of bentonite and kaolin clays. Based on the available studies it has been shown that long term exposure to kaolin may lead to pneumoconiosis but that the potency is at least one order of magnitude less than quartz. Bentonite is likely to be less dangerous to humans than kaolin. (WHO 2005)

### *7.4.2 Acute toxicity*

An acute oral toxicity test in Sprague-Dawley rats showed a low toxic potential (LD50 >5700 mg/kg bw) of nanosilicate platelets (NSP) derived from natural montmorillonite clay (Li et al. 2010).

### *7.4.3 Irritation and sensitisation*

No data was found.

### *7.4.4 Repeated dose toxicity*

No repeated dose inhalation studies were found. Warheit et al. (2010) examined the toxicity of one type of nanoclay (sepiolite) by intratracheal instillation in male Crl:CD(SD) rats and it was found that the material could produce transient neutrophilic responses 24 hours post-exposure and occasional multinucleate giant cell formation at 1 week, 5 weeks, and 3 months post-exposure. In addition, inflammation occurred in centriacinar regions 24 hours post-exposure but the effects decreased in severity over time.

### *7.4.5 Genotoxicity*

Unmodified nanoclay (Cloisite Na+®) and organo-modified nanoclay (Cloisite® 30B) exposure did not induce mutations as measured by the Ames test or ROS production in a cell-free test system (Sharma et al. 2010). However, the unfiltered and filtered (particles above nanometre range removed) Cloisite® 30B particles induced DNA strand breaks in a dose-dependent manner in Caco-2 (human colon cancer) cells after 24 hours of exposure. The conclusion of the study was that the DNA damage observed was caused by the organo-modifier and not by the particles themself.

Lordan and Higginbotham (2012) reported that addition of 2.5% or 5% foetal calf serum (FCS) in the dispersion medium of unmodified nanoclay (Cloisite Na+) can influence the outcome of toxicity studies, showing that medium supplemented with 2.5% FCS or without FCS significantly inhibited cell growth in human monocytic U937 cells, while medium with 5% FCS had little effect on cell growth.

The genotoxic potential of nanosilicate platelets (NSP) derived from natural montmorillonite clay was addressed *in vitro* and *in vivo* (Li et al. 2010). The comet assay showed no DNA damage in Chinese hamster ovary (CHO) cells and no significant micronucleus induction in peripheral blood polychromatic erythrocytes of ICR mice or mutations by the *Salmonella* gene mutation assay were detected.

### *7.4.6 Carcinogenicity*

No data was found.

### *7.4.7 Reproductive toxicity*

No data was found.

### *7.4.8 Cytotoxicity*

Nanoclays have also been used in the production of bio-nanocomposites where chitin based polyurethane (PU) bio-nanocomposites are prepared using chitin, Delite HPS bentonite nanoclay enriched in montmorillonite, 4,4'-diphenylmethane diisocyanate and polycaprolactone polyol CAPA 231. The effect of nanoclay contents on mechanical properties and *in vitro* biocompatibility was investigated. Cytotoxicity evaluation using L-929 fibroblasts revealed that the final PU bio-nanocomposite, having 2% Delite HPS bentonite nanoclay contents, was ideal; increasing the amount of nanoclay also increased the cytotoxicity in fibroblasts. (Zia et al. 2011)

The toxic effects of unmodified nanoclay (Cloisite Na+®; mainly tactoid structures with lengths between 30–100 µm) and organically modified nanoclay (Cloisite 93A®; length 3–35 µm) were studied in human hepatoma HepG2 cells *in vitro* and the authors concluded that both materials were highly cytotoxic and that Cloisite Na+® was able to induce reactive oxygen species (ROS) generation (Lordan et al. 2011).

### *7.4.9 Conclusions*

So far, there is only very limited data available on the potential toxicity of nanoclays. Concerning the available toxicological data on nanoclays it is of importance to take into account that the physico-chemical properties usually vary between different materials and also the test systems used (e.g., dosing, *in vitro* cell culture/*in vivo* mice/rats, treatment time) are usually different, which makes the comparison of results challenging. As many nanoclays are modified to form nanocomposites, it seems important to elucidate if the modifiers are causing the toxic events or if the nanoclay itself can induce harmful effects. Repeated dose inhalation studies are needed to confirm the possible pulmonary toxicity of nanoclays.

## 7.5 Carbon nanotubes

### *7.5.1 Introduction*

The widespread use of synthetic carbon nanofibres (CNFs), including single- (SWCNT) and multi-wall (MWCNT) carbon nanotubes (CNTs), has raised safety concerns about their possible health effects. Especially CNTs with a high aspect ratio (length:thickness >3:1), consisting of long fibres (>20 µm) are of particular concern, since they might be able to induce lung cancer and mesothelioma in a manner similar to asbestos fibres (Linton et al. 2012). The mechanism, by which carbon nanofibres, including CNTs, could cause inflammation and ultimately cancer is, however, largely unknown. The reason for this is partially the large variability in the physic-chemical characteristics of the materials, including their length, diameter, surface area, density, shape, contaminant metals, and crystallinity. (Nagai and Toyokuni, 2010)

The toxicity of different types of CNTs has been studied fairly extensively during the past few years by using both *in vitro* cell line and *in vivo* animal test systems. Although the toxic potential of CNTs has been demonstrated, the results are sometimes conflicting and inconclusive and therefore more relevant data, especially on long-term effects, are still needed for a better hazard and exposure assessment. The main mechanism of CNT toxicity appears to be oxidative stress and inflammation, which can ultimately lead to genotoxic and carcinogenic responses. (Aschberger et al. 2010)

In the Scaffold project, CNTs are not specifically addressed as one of the focus areas. Instead, CNFs were selected as vital for the project. As the border between CNTs and CNFs is not always very clear, and as most toxicological tests have been carried out with CNTs, and not CNFs, this chapter summarizes very briefly the main observations and trends observed in the large amount of scientific data published on the toxicity of CNTs. The data on CNFs specifically is presented in section 4.6.

## *7.5.2 Acute toxicity*

There are no indications that CNTs would be acutely toxic or cause lethality.

## *7.5.3 Irritation and sensitisation*

There are no indications of CNTs being skin irritants or sensitizers.

## *7.5.4 Repeated dose toxicity*

Several *in vivo* studies with rats or mice exposed to MWCNTs and SWCNTs by short- and long term inhalation, or intratracheal instillation, have shown a significant ability to cause inflammation, fibrosis, granuloma formation, and immunosuppression after acute, sub-acute and sub-chronic exposure (Lam et al. 2004; Muller et al. 2005; Shvedova et al. 2005; Li et al. 2007; Mitchell et al. 2007; Nygaard et al. 2009).

However, *in vivo* studies indicating a low toxicity of CNTs have also been published (Fiorito et al. 2006; Davoren et al. 2007; Pulskamp et al. 2007; Muller et al. 2009). The obtained differences are most likely dependent on differences in the structural properties of the tested CNTs.

## *7.5.5 Genotoxicity*

In assessing the carcinogenic hazard of CNTs, the evaluation of genotoxic potential is of crucial importance and two main modes of action of CNTs have been proposed: generation of reactive oxygen species (ROS) generated by the particles themselves, upon particle-cell contact or due to particle-elicited inflammation or mechanical interference with cellular components (Gonzalez et al. 2008; Donaldson et al. 2010).

***In vivo studies***

Several *in vivo* genotoxicity studies with CNTs performed in rats and mice have been published. Intrapharyngeal instillation of SWCNTs to mice lead to aortic mitochondrial DNA damage (Li et al. 2007) and in C57BL/6 mice inhalation of SWCNTs was more effective than aspiration in causing K-*ras* mutations, an inflammatory response, oxidative stress, collagen deposition, and fibrosis (Shvedova et al. 2008).

Intratracheal instillation of SWCNTs caused inflammation and increased DNA damage in bronhoalveolar lavage cells of ApoE$^{-/-}$ mice (Jacobsen et al. 2009).

A single intra-tracheal administration of MWCNTs in rats induced a dose-dependent increase of micronuclei (MN) in lung alveolar type II cells (Muller et al. 2008). This type of MWCNTs also increased centromere-positive and -negative MN in rat epithelial RLE cells and human

epithelial MCF-7 cells *in vitro*, which indicated that MWCNTs can induce both clastogenic (chromosome disrupting) and aneugenic (aneuploidy inducing) events.

A single oral administration of SWCNTs increased the level of 8-oxo-2'-deoxyguanosine adducts in the liver and lung but not in colon mucosa of Fisher-344 rats (Folkmann et al. 2009).

A 5-day intraperitoneal administration of functionalized and non-functionalized MWCNTs induced chromosomal aberrations, MN and DNA damage in Swiss-Webster mice and DNA damage was also increased in isolated human lymphocytes *in vitro* (Patlolla et al. 2010).

Intratracheal instillation of MWCNTs in ICR mice induced DNA damage, oxidative DNA damage and *gpt* mutations in the lung cells (Kato et al. 2012). However, in Fisher-344 rats, oral exposure to SWCNTs or MWCNTs did not increase urinary mutagenicity assessed by the Ames test (Szendi and Varga 2008). There was neither any increase in MN or sister chromatid exchanges in isolated human lymphocytes from healthy donors exposed to the same materials; however, mitotic inhibition was observed in their lymphocyte cultures. MWCNTs neither induced MN in polychromatic erythrocytes of ICR mice (Kim et al. 2011; Ema et al. 2012).

***In vitro studies***

Several *in vitro* studies have also demonstrated the genotoxic potential of CNTs. SWCNTs induced DNA damage, as measured by the comet assay, in Chinese hamster V79 lung fibroblasts, but no mutations in *Salmonella typhimurium* strains YG1024 and YG1029 (Kisin et al. 2007). Long SWCNTs and graphite nanofibres induced DNA damage (comet assay) and MN (cytokinesis-block assay) in human bronchial epithelial BEAS 2B cells (Lindberg et al. 2009). In the FE1-Muta$^{TM}$ mouse lung epithelial cells line, SWCNTs increased the level of FPG sensitive sites/oxidized purines as determined by the comet assay (Jacobsen et al. 2008).

Both SWCNTs and MWCNTs induced DNA damage and activation of the transcription factors H2AX (phosphorylated histone H2AX) and PARP (poly ADP ribose polymerase) in normal and malignant mesothelial cells (Pacurari et al. 2008a; Pacurari et al. 2008b). MWCNTs were also observed to accumulate in cultured mouse embryonic stem (ES) cells and induce apoptosis, p53 activation, increased expression of DNA repair proteins, and a twofold increase in adenine phosphoribosyltransferase mutations (Zhu et al. 2007). MWCNTs induced DNA damage (Karlsson et al. 2008; Cavallo et al. 2011; Ursini et al. 2012) and micronuclei (Kato et al. 2012) in A549 cells, and in primary mouse embryo fibroblast cells moderately cytotoxic CNTs induced DNA damage (Yang et al. 2008a; Yang et al. 2008b). Purified SWCNTs and MWCNTs and functionalized SWCNTs induced MN in human lymphocytes and γH2AX foci in human fibroblast cells (Cveticanin et al. 2010). In mouse RAW 264.7 cells, SWCNTs and MWCNTs induced both MN and oxidative DNA damage (Migliore et al. 2010) and ROS release, necrosis, chromosomal aberrations and apoptosis (Di Giorgio et al. 2011). SWCNTs were found to cause DNA damage, MN and oxidative stress in human gingival fibroblasts (Cicchetti et al. 2011).

A few studies have also shown that CNTs might not all be genotoxic. MWCNTs (Di Sotto et al. 2009; Ema et al. 2012) or SWCNTs (Naya et al. 2011) were not mutagenic as measured by the Ames test. Neither *Xenopus laevis* larvae grown in the presence of double-walled CNTs showed any induction of MN in blood erythrocytes (Mouchet et al. 2008). MWCNTs (high aspect ratio 10–15 nm x 10 µm and low aspect ratio 10–15 nm x 150 nm) were neither genotoxic in Chinese hamster ovary CHO cells (Kim et al. 2011) or V79 cells (Wirnitzer et al. 2009) as measured by the chromosomal aberration assay or in cultured Chinese hamster cells as measured by the chromosomal aberration, MN and *hpgrt* mutagenicity assays (Asakura et al. 2010). SWCNTs

showed no induction of chromosomal aberrations in Chinese hamster lung fibroblasts or MN in polychromatic erythrocytes of ICR mice (Naya et al. 2011).

### *7.5.6 Carcinogenicity*

One very important study demonstrating the possible carcinogenic capacity of long MWCNTs (MITSUI MWCNT-7) was done by Takagi et al. showing that a single intraperitoneal injection in *p53*+/- mice induced mesothelioma even more effectively than crocidolite asbestos (Takagi et al. 2008). Recently the same type of MWCNTs were shown to be able to activate the NLRP3 inflammasome in a similar manner as asbestos (Palomäki et al. 2011) and induce mesothelioma in *p53*+/- mice in a dose-dependent manner by so called frustrated phagocytosis (Takagi et al. 2012).

Another study examining the exposure of the mesothelial lining of the body cavity of C57BL/6 mice to long MWCNTs, which contained a substantial proportion of fibres longer than 20 µm, also resulted in an asbestos-like, length-dependent, pathogenic behaviour (Poland et al. 2008). However, no carcinogenic response was detected in rats after a single intraperitoneal injection of another type of MWCNTs (diameter 11.3 nm, length 0.7 µm) with and without defects and the authors suggested that the lack of response could be due to insufficient sustainability of the inflammatory reaction in the peritoneal cavity or that the MWCNTs used did not contain a sufficient number of long nanotubes (Muller et al. 2009).

### *7.5.7 Reproductive toxicity*

No relevant data was found.

### *7.5.8 Cytotoxicity*

Several *in vitro* studies have demonstrated that both MWCNTs and SWCNTs can induce oxidative stress, induction of inflammatory cytokines, cytotoxicity, apoptosis and altered protein expression in various cell types (Shvedova et al. 2003; Cui et al. 2005; Jia et al. 2005; Bottini et al. 2006; Witzmann and Monteiro-Riviere 2006; Sharma et al. 2007).

### *7.5.9 Conclusions*

Different types of CNTs have been tested for their toxicity in several studies, due to the fact that it has been assumed that they may behave similarly as asbestos fibres. The reported data shows large varieties in responses depending on which types of CNTs have been tested. There are indeed clear indications that some materials may induce pulmonary inflammation and may be genotoxic and carcinogenic.

## 7.6 Carbon nanofibres

### *7.6.1 Introduction*

Carbon nanofibres (CNFs) typically have a diameter of 50–200 nm and structurally they resemble MWCNTs. The primary characteristic that makes them different from CNTs is the

graphene alignment – if the graphene plane and fibre axis do not align, the structure is defined as a CNF. It is less expensive to produce CNFs as compared to CNTs and they are used, for example, in composite materials to improve strength, stiffness, electrical conductivity, or heat resistance. (Kisin et al. 2011) Despite the widespread use of CNFs, their toxicity has not been extensively studied.

### *7.6.2 Acute toxicity*

No data was found.

### *7.6.3 Irritation and sensitisation*

No data was found.

### *7.6.4 Repeated dose toxicity*

A subchronic inhalation toxicity study (DeLorme et al. 2012) of inhaled vapour grown carbon nanofibres (CNF) (VGCF-H) was conducted where male and female Sprague Dawley rats were exposed nose-only (6 h/day, for 5 days/week) to 0, 0.50, 2.5, or 25 mg/m$^3$ VGCF-H over a 90-day period and evaluated 1 day later. In addition, groups of 0 and 25 mg/m$^3$ exposed rats were evaluated at 3 months post exposure. The aerosol exposures induced concentration-related small, detectable accumulation of extra-pulmonary fibres with no adverse tissue effects. At the two highest concentrations, inflammation of the terminal bronchiole and alveolar duct regions was observed. At 3 months post exposure, the inflammation in the high dose was reduced. After 90 days after the ending of the exposure, bronchoalveolar lavage fluid biomarkers were still increased at 25 mg/m$^3$, indicating that the inflammatory response was not fully resolved. The NOAEC for VGCF-H nanofibres was considered to be 0.5 mg/m$^3$ (4.9 fibres/cc) for male and female rats.

In another study (Yokoyama et al. 2005), so called hat-stacked CNFs (length 100 nm-1 μm, diameter 30-100 nm) were implanted in the subcutaneous tissue of Wistar rats and the rats were sacrificed at 1 and 4 weeks after implantation (Yokoyama et al. 2005). The results indicated that some CNFs were incorporated in lysosomal vacuoles of phagocytes but no severe inflammatory response was detected.

C57BL/6 mice were exposed by pharyngeal aspiration to respirable CNFs (120 μg/mouse), SWNCTs (40 μg/mouse) or asbestos (120 μg/mouse) and groups of mice were sacrificed on days 1, 7, and 28 post-exposure (Murray et al. 2012). Pulmonary inflammatory and fibrogenic responses to CNF, SWCNT and asbestos varied depending upon the agglomeration state of the particles/fibres. Foci of granulomatous lesions and collagen deposition were associated with dense particle-like SWCNT agglomerates, while no granuloma formation was seen after exposure to CNFs or asbestos. Interstitial fibrosis was increased 28 days post SWCNT, CNF or asbestos exposure. Exposure to SWCNT, CNF or asbestos resulted in oxidative stress and local inflammatory and fibrogenic responses were accompanied by modified systemic immunity.

### *7.6.5 Genotoxicity*

Kisin et al. (2011) studied the genotoxic potential of carbon-based nanofibres (Pyrograf(R)-III) in lung fibroblast (V79) cells and a comparison was made with asbestos fibres (crocidolite) and SWCNTs. DNA damage and micronucleus induction were found after exposure to all tested materials with the strongest effect seen for CNF. CNF induced predominantly centromere-

positive MN in primary human small airway epithelial cells (SAEC) as tested by the fluorescence in situ hybridisation technique indicating that the fibres induced aneugenic (chromosome loss) events in the cells.

### *7.6.6 Carcinogenicity*

No data was found.

### *7.6.7 Reproductive toxicity*

No data was found.

### *4.6.8 Cytotoxicity*

The cytotoxic potential of carbon-based nanofibres (Pyrograf(R)-III) were examined in lung fibroblast (V79) cells and a comparison was made with asbestos fibres (crocidolite) and SWCNTs (Kisin et al. 2011). A concentration- and time-dependent loss of V79 cell viability after exposure to all tested materials in the following sequence was seen: asbestos>CNF>SWCNT. Also cellular uptake and generation of oxygen radicals was seen in the murine RAW264.7 macrophages following exposure to CNF or asbestos but not after administration of SWCNT.

CNF exposure did not significantly affect cell viability or increase ROS production in mouse keratinocytes (HEL-30) (Grabinski et al. 2007). CNFs (Pyrograf Products, Inc.) were seen to decrease the viability of H596 lung tumour cells in a dose-dependent manner and also after modifying the surface of CNFs, a marginal increase in their toxicity was observed compared to the situation before surface modification (Magrez et al. 2006).

Two types of CNFs (NF1, platelet and herringbone structure; NF2, platelet structure) did not increase toxicity in human peripheral mononuclear cells isolated from healthy donors as measured by the lactate dehydrogenase (LDH) assay nor did they induce apoptosis or necrosis in THP-1 (human acute monocytic leukemia cell line) cells (Brown et al. 2007). However, the NF1 appeared to increase $O^-_2$ production in the mononuclear cells and inhibited the phagocytosis capacity of THP-1 cells suggesting that the toxicity of CNFs may depend on their graphene structure (Brown et al. 2007).

CNFs have also been proposed as a possible new orthopaedic/dental implant material and therefore the influence of CNF wear debris on osteoblast (bone-forming cell) viability was evaluated (Price et al. 2004). The results showed concentration- and time-dependent decreases in cell viability after CNF treatment but most importantly the CNFs were less cytotoxic to osteoblasts compared to the larger diameter conventional carbon fibres suggesting the possible efficient use of CNFs in orthopaedic implants.

### *7.6.9 Conclusions*

CNFs have been studied for their potential health hazards only in a limited amount of studies. Based on the available data, there are indications that these materials may cause pulmonary inflammation. One study also indicates a possible genotoxic potential of CNFs. Therefore it would be critical to carry out further studies on the toxic effects of these materials.

# 8. OCCUPATIONAL EXPOSURE TO NANOPARTICLES

## 8.1 Limit values

So far, no regulatory occupational exposure limit values (OELs) have been given for any nanomaterial by the European Union or any national OEL-setting authority (van Broekhuizen et al. 2012). NIOSH (2011) has proposed a recommended exposure limit (REL) for $TiO_2$ nanoparticles in workplace air on the basis of available toxicity data: 0.3 mg/m$^3$, as time-weighted average (TWA) for up to 10 h per day during a 40 h working week. NIOSH (2010) has also published a draft standard for exposure to carbon nanotubes and carbon nanofibres, based on available toxicity data. The proposed value is 7 µg/m$^3$.

A critical question for nanoparticles is what to use as an OEL. OELs from for a bulk form of a material do in most cases not appear appropriate for the nanoparticle form. Although size-specific OELs may be needed for nanoparticles, size is not the only factor that needs to be considered. Chemical composition, surface reactivity, solubility, aggregation or agglomeration, and other physico-chemical factors also can influence the toxicity of nanoparticles. To establish OELs, nanoparticle exposure-disease relationships need to be defined, and the levels to which risks should be controlled need to be identified. Complexity is added when nanoparticles are incorporated into the complex matrix of a product. (Schulte et al. 2008)

Provisional nano reference values (NRVs) have been suggested in Germany and the Netherlands. NRVs are recognised by Dutch authorities as an acceptable tool for precautionary risk management. (van Broekhuizen et al. 2012). The NRVs are based on the precautionary principle and are not health-based. A NRV is defined as a warning level and refers to the ENP concentration in the workplace atmosphere, corrected for the background particle concentration. It is intended to be a warning level to trigger a thorough assessment of nanoparticles at the workplace. The NRVs are quantified as 8-h TWA. See table 3. (van Broekhuizen et al. 2011; SER 2012)

**Table 3.** Nano reference values (NRVs) (van Broekhuizen et al. 2012; SER 2012)

| Description | Density | Benchmark level (8-h TWA) | NP type |
|---|---|---|---|
| Rigid, biopersistent nanofibres for which effects similar to those of asbestos are not excluded | | 0.01 fibers/cm$^3$ | SWCNT or MWCNT or metal oxide fibres for which asbestos-like effects are not excluded |
| Biopersistent granular nanomaterial in the range of 1–100 nm | >6000 kg/m$^3$ | 20000 particles/cm$^3$ | Ag, Au, $CeO_2$, CoO, Fe, $Fe_xO_y$, La, Pb, $Sb_2O_5$, $SnO_2$ |
| Biopersistent granular nanomaterial in the range of 1–100 nm | <6000 kg/m$^3$ | 40000 particles/cm$^3$ | $Al_2O_3$, $SiO_2$, TiN, $TiO_2$, ZnO, nanoclay carbon black, $C_{60}$, dendrimers, polystyrene, nanofibres for which asbestos-like effects are excluded |
| Non-biopersistent nanomaterial in the range of 1–100 nm | | Applicable OEL | Fats, NaCl |

## 8.2 Measurement issues

At present, there is only very limited information about the actual use of nanoparticulate products and about possible exposures to NPs released from these products at the workplace. Exposure to engineered NPs in practice is limitedly reported in scientific literature for research activities and to an even lesser extent for workers in NPs manufacturing or nanoproducts' use. (van Broekhuizen et al. 2011) To use nanotechnology safely, it is necessary to precisely control exposure to engineered nanomaterials during production or application processes as workers can be directly exposed to nanomaterials for a long time (Park et al. 2009).

Although many organizations in the world have researched various methods for the workplace exposure assessment of nanomaterials, and different approaches for strategies to workplace measurements have been proposed (e.g., by NIOSH, ECHA, IUTA/BAuA/BG RCI/VCI/IFA/TUD and nanoGEM), a standard or an agreeable methodology has not yet been established. The main reason is the difficulty in precise analysis of airborne particles in workplace, due to the fact that nanomaterials have unique physico-chemical properties, different from those of bulk materials (Park et al. 2009). Since there is no generally established and validated method for nanoparticle occupational hygiene measurements, it is essential for scientists to produce data about the suitability of the aerosol measurement instruments for real-time nanoparticle exposure estimation (Leskinen et al. 2012).

The current measuring methodology recommended by research organizations mostly is a modified form of conventional measuring methods for micro-sized materials. Importantly, it is a key point whether the equipment precisely and accurately can measure nano-sized materials (Park et al. 2009). ENPs can be measured in the workplace using a variety of instrumentation, including: condensation particle counter (CPC); optical particle counter (OPC); scanning mobility particle sizer (SMPS); electric low pressure impactor (ELPI); aerosol diffusion charger; and tapered element oscillating microbalance (TOEM), which vary in complexity and field portability. Unfortunately, relatively few of the instruments are readily applicable to routine exposure monitoring due to non-specificity, lack of portability, difficulty of use, and high cost. (NIOSH 2009)

There is an on-going debate in the scientific literature about what are the relevant parameters to evaluate an exposure to nanoparticles. At the moment, there are no generally agreed parameters which should be measured to define the nanoparticle concentration in the workplace air. Nor is there an agreement on which instruments should be used to carry out these measurements. (Leskinen et al. 2012). Number, mass and surface area exposure concentrations have been suggested as metrics for exposures to ENPs (van Broekhuizen et al. 2012). Particle number concentrations and particle number size distributions are the most commonly used metrics within the reviewed workplace and laboratory studies. Personal exposure approaches are either based on personal devices and samples or real measurements combined with the recording of personal activity patterns, to allow the calculation of personal exposure. (Kuhlbusch et al. 2011). A large number of equipment able to measure nanoaerosols is available on the market. The majority is designed for laboratory use, but newly developed equipments are easily transportable and easy to use (Nanosafe-June 2008). A major drawback of current state of the art measurement devices is their lack of differentiation of background from nanomaterial related particles (Kuhlbusch et al. 2011). The on-going FP7 project Nanodevice is focusing on the development of easy-to-use devices for measurement of NP concentrations in the workplace air.

Different approaches can be pursued to derive exposure relevant information in workplaces: (a) Studies based on real workplaces and (b) process based studies in simulated workplaces and of simulated work processes. The major advantage of the prior approach is that data from real work conditions are obtained. (Kuhlbusch et al. 2011). However, most research groups have conducted exposure assessment under controlled conditions in the laboratory. These results may be helpful to understand mobility of airborne nanomaterials emitted in the process. (Park et al. 2009). The simulations in laboratories allow the clear differentiation of a release from the investigated process from background aerosols coming from other sources than the process. Moreover, process based studies in simulated workplaces and of simulated work processes also enable investigations on how variances in handling and process conditions influence release rates. (Kuhlbusch et al. 2011). However, there is a gap in exposure feature between produced nanomaterials in laboratory and generated nanomaterials under production processes in the fields (Park et al. 2009).

Synthetic nanoparticles usually exist as agglomerates, which are made up of a varying number of small primary particles. The size, shape, and morphology can vary between different nanoparticles. This poses a significant challenge for the measurement methods as the particle properties affect the behaviour of the particles within the measurement instruments and human body. As the particle diameter decreases, the specific surface area increases exponentially. Therefore, the physico-chemical properties of these particles, or materials containing them, are substantially different from those of bulk materials. (Leskinen et al. 2012). For ENPs, more profound investigation is needed and different properties, such as particle size distribution, surface area/volume ratio, shape, electronic properties, surface characteristics, state of dispersion/agglomeration, and conductivity need to be studied. The high complexity and great diversity of ENPs, however, make their characterization very difficult. (Hristozov and Malsch 2009)

According to Savolainen et al. (2010), some of the real challenges ahead for ENM monitoring and health risk assessment are as follows: (a) to redesign “ENM-capable” instruments already in laboratory use into portable and affordable devices, (b) to expand the sensing technology available for ENM detection by adopting new options with realistic potential for real-time measurement and compact design; and (c) to extend the metrics into new areas such as CNT shape identification and catalytic properties. In the future, it will be increasingly important to have devices providing real-time, on-line data.

## 8.3 Exposure measurements

Aitken et al. (2004) identified potential exposure scenarios regarding the manufacture and use of ENPs, and studied the production processes of fullerenes, CNTs, metals and metal oxides. The four main groups of ENP production processes were identified: vapour deposition, gas-phase, colloidal and attrition processes. It was concluded that all production processes can potentially result in occupational exposure through inhalation, dermal or ingestion routes.

Maynard et al. (2004) assessed the propensity for aerosol particles to be released during agitation and measured the size of particles released into the air while SWCNT material was removed from production vessels and handled prior to processing. Airborne concentrations of SWCNT were estimated to be <53 $\mu g/m^3$, while hand glove deposits of SWCNT during handling were estimated to be 0.2–6 mg per glove. According to the study, occupational exposures of SWCNTs are most likely to occur during handling and bagging of the materials and there is high risk of dermal exposure.

Han et al. (2008) measured occupational exposures in the production cycle of MWCNTs. Air samples were taken and the MWCNTs in the samples were counted. Most of the MWCNT exposure levels (max. 0.43 mg/$m^3$) were lower than the current threshold limit value (TLV) for carbon black (3 mg/$m^3$).

Fujitani et al. (2008) compared the particle size distributions and morphology of aggregated and agglomerated fullerenes at the production facilities of Frontier Carbon Corporation in Japan, during work and non-work periods. The concentration of particles with diameters less than 50 nm was not higher during the removal of fullerenes from a storage tank for bagging and weighing than prior to the activity.

Demou et al. (2008; 2009) demonstrated that, during production, the number concentrations of NPs were higher than the concentrations in the background.

Plitzko (2009) measured $TiO_2$ in low-volume production. Measurements were conducted during actual production and also during cleaning of the system. No significant increase of NP number was measured during handling of NPs. However, it was possible to observe process-related increases during cleaning and production in mass concentration of NPs (10-fold and 4-fold increases, respectively). However, filter sampling showed that the NPs originated from combustion furnaces of the installation and from diesel engines.

Brouwer (2010) and Kuhlbusch et al. (2011) have written reviews about exposure monitoring of nanoparticles at the workplaces. There is still lack of quantitative exposure measurements from the real workplaces. The first review found a total of 15 exposure studies monitoring NPs at the workplaces, and the second one found a total of 25 workplace monitoring studies. The comparison of the different studies is also hard, because of the different measurement metrics and methodologies, and lack of comparability (e.g., calibration, detection limits, uncertainty and background noise).

Leppänen et al. (2011) measured exposure to nanosized $CeO_2$ during an enclosed flame spray process used for coating and surface modification of materials. The average particle number concentration varied from 4700 - 210 000 $cm^{-3}$ inside the enclosure, to 4 600 - 14 000 $cm^{-3}$ outside the enclosure. Average mass concentrations were 320 and 66 µg/$cm^3$, respectively.

Koivisto et al. (2012) studied four different sessions during a working day: $TiO_2$ NP production, $Cu_xO_y$ nanocoating and two different $Mn_xO_y$ nanocoatings. The background concentration was 17 800 particles/$cm^3$ in the process room. During $TiO_2$ production, the average number concentration was 101 000 $cm^{-3}$ and average mass concentration was 440 µg/$cm^3$.

Curwin and Bertke (2011) assessed exposure to various metal oxides in seven facilities that produce or use nanoscale metal oxides, including the oxides of titanium, magnesium, yttrium, aluminium, calcium, and iron. Workers had the greatest potential for exposure during handling processes. However, in area sampling the production processes generally had higher particle and mass concentration of NPs than handling processes.

A summary of mass concentrations and particle number concentrations of the abovementioned studies is presented in Table 4.

**Table 4.** Mass concentrations and particle number concentrations for nanomaterial oxides measured at workplaces

| Work process | Metal oxide | Mass concentration ($mg/m^3$) | | Particle number concentration ($particles/cm^3$) | |
|---|---|---|---|---|---|
| | | Background | Work process | Background | Work process |
| Cleaning of the pyrolysis system | $TiO_2$ | 0.1–0.2 | 1.7 | 7 000 - 20 000 | 22 000 |
| Production (flame pyrolysis) | $TiO_2$ | 0.1–0.2 | 0.4 | 7 000 - 20 000 | 21 000 |
| Flame spray process** | $CeO_2$ | – | 0.07 | – | 4 600 - 14 000 |
| Handling | $TiO_2$ (<0.3 µm) | 0.0004 | 0.001 (0.053)* | 8 000 | 13 000 |
| Production | $TiO_2$ | – | 0.44 | 17 800 | 101 000 |
| Production | $TiO_2$ (<0.3 µm) | 0.0004 | 0.006 (0.010)* | 8 000 | 38 000 |
| Production (gas-phase manufacturing) | metal based NPs | 0.052 | 0.188 | 8 500 | 59 100 |
| Production | metal oxides | 0.009 | 0.463 | 2 100 | 106 000 |

* the concentration is measured from workers' breathing zone
**work process indicates the situation outside the enclosure

The report by van Broekhuizen et al. (2012) presents data on NP levels. The main results are summarized in table 5.

**Table 5.** Background-corrected number of nanoparticles at some workplaces (van Broekhuizen et al. 2012)

| Event | Particles per $cm^3$ | | |
|---|---|---|---|
| | Min | Max | AM |
| 1. Electroplating plant | 0 | 121 021 | 47 592 |
| 2. Manufacturing nano-wall paint | 0 | 270 135 | 17 316 |
| 3. Manufacturing pigment concentrates for plastics | 0 | 6 226 237 | 189 134 |
| 4. Production of non-reflective glass | 0 | 35 126 | 6 997 |
| 5. Manufacturing fluorescent tubes | 0 | 11 044 905 | 565 075 |
| 6. Manufacturing non-nano alkyd paint | 5 205 | 3 106 170 | 203 976 |
| 7. Long-term wear lubrication | 0 | 63 380 | 15 240 |
| 8. Vehicle refinishing | 0 | 303 764 | 17 933 |

*AM*, arithmetic mean
The work situation in which the peak particle number concentration was reached at different events:
1. Blue passivating bath, nano
2. Full period batch manufacturing
3. Mixing nano-ZnO
4. Background
5. Adjusting device
6. Batch manufacturing/talc (solid)
7. Heavy machines
8. Spraying conventional coating

There are very limited amounts of data available on exposure to ENMs within the **construction sector**. Van Broekhuizen et al. (2011) measured exposure to dispersed NPs during use of nanoproducts on two different companies for the following working situations: spraying a liquid window coating, applying a cement repair mortar and nano-concrete filling. Personal exposure assessment and source identification measurements were carried out during all these activities. All the calculated 8-h TWA exposures remained well below the NRV level (see section 5.1). The background concentration, the use of electrical equipment, heaters, diesel aggregates and smoking were identified as potential confounding factors in ENP measurement. The main findings have been collected into table 6.

**Table 6.** Exposure measurements to NPs at some construction sites (van Broekhuizen et al. 2011)

| Working situation | ENP | Measurement location | Workers exposure to nanoparticles | | |
|---|---|---|---|---|---|
| | | | Min ($N_p$/cm$^3$) | Max ($N_p$/cm$^3$) | AM ($N_p$/cm$^3$) |
| 1. Company 1: Spraying Self-cleaning Coating | $TiO_2$ (anatase) | Personal exposure during spray activities | 9 512 | 16 337 | 12 219 |
| | | Background | 7 195 | 15 696 | 11 898 |
| 2. Company 2: Location 1 Mixing mortar | $SiO_2$ (amorphous) NanoCrete R4 | Personal exposure: (NanoCrete mixing) | 45 429 | 641 074 | 199 508 |
| | | Background | 6 177 | 73 928 | 20 763 |
| 3. Company 2: Location 2 Mixing and handling Repair mortar | $SiO_2$ (amorphous) NanoCrete R4 | Personal exposure: (NanoCrete mixing) | 6 107 | 71 519 | 13 983 |
| | | Background | 5 964 | 13 310 | 8 844 |
| | | Background in workers canteen | 59 957 | 115 011 | 79 619 |
| | | Direct emission mixer | 6 896 | 114 962 | 49 978 |
| 4. Company 2: Location 3 Drilling cured concrete mortar | Up-wind location | Drilling in NanoCrete concrete, near field | 7 416 | 52 732 | 29 545 |
| | | Drilling in normal concrete, near field | 7 886 | 20 068 | 15 960 |
| | | Drilling machine idle-running | 9 743 | 83 545 | 39 033 |
| | Down-wind location | Drilling in NanoCrete concrete, near field | 7 043 | 164 424 | 70 981 |
| | | Drilling in normal concrete, near field | 10 075 | 66 079 | 22 889 |
| | | Drilling machine idle-running | 10 656 | 572 410 | 195 616 |
| | | Background | 5 611 | 11 346 | 7605 |

$N_P$/cm$^3$, number of nanoparticles/cm$^3$
*AM*, arithmetic mean
NanoCrete R4, the mortal material

The *near field* is defined as a distance of 1–2 m from the activities with dispersive use of nanomaterials. The background for situations 1, 2 and 3 was measured preceding the activities using ENPs. The background for situation 4 was measured at larger distance in up-wind position. Direct emissions from idle-running electrical equipment were measured without the use of products containing ENPs. The nanomaterial used in situation 1 concerns a waterborne suspension of Nano-$TiO_2$, while situations 2 and 3 concern the mixing of dry nanomaterial. Situation 4 concerns release of NPs from drilling activities in cured concrete.

Savolainen et al. (2010) commented that there is a need to characterize the different ENM exposure scenarios. One should be able to assess the exposure to fresh ENM emitted from different production processes as well as from exposure settings in which exposure to "aged" ENM takes place, e.g., during handling and packing of produced, partly agglomerated and aggregated ENM takes place. Examples of exposure scenarios dealing with exposure to fresh

ENM include process disturbances and leaks, during which ENM being produced are released into the workplace air. Exposure to both types of ENM may take place during maintenance of production facilities and storage sites of freshly produced ENM. They noted that it is important to obtain data from these types of exposure settings to be able to assess true exposure levels of ENM in workplaces.

The NANEX project (2010) aimed to develop a catalogue of exposure scenarios for MNMs taking account of the entire lifecycle of these materials. NANEX focused on carbon nanotubes (CNTs), nano-sized titanium dioxide (nano-$TiO_2$) and nanosized silver (nano-Ag). In total, 62 exposure scenarios were developed using publicly available data and data collected in several large-scale sampling campaigns. 57 scenarios were related to occupational exposure and 5 to consumer exposure. As there was little or no empirical exposure information available for the development of consumer exposure scenarios, exposure estimate for these scenarios were based on exposure estimation models and default or worst case assumptions. In contrast, all of the occupational exposure scenarios were developed using measurement data from the literature or from sampling campaigns. Based on the information collected during the project, a comprehensive analysis of key data gaps and research needs was carried out.

Exposure scenarios generally include information on the substance, the process and activities, the presence of any risk management measures, and estimates of exposure and are therefore an important tool for managing exposure. Within the REACH regulation (EU 2006), exposure scenarios are used to describe the operational conditions and risk management measures that are required to ensure that exposure levels are safe. Although the exposure scenarios developed within the NANEX project do not describe safe use of the MNMs (as defined under REACH), they rather describe the existing exposure situation (i.e., no risk assessment was carried out). Although several exposure scenarios were developed in the project, they were consistently characterized by missing information or information of dubious accuracy and reliability. Most studies reported in the literature or as part of the measurement campaigns, had an explorative character and were focused on concentration/emission analysis. These studies did not include most of the information that is necessary to build exposure scenarios (e.g., amount used, frequency of activities). Therefore, it was not possible to infer meaningful or generalizable exposure characterizations for these exposure scenarios. (NANEX 2010)

In conclusion, the following observations were made within the NANEX (2010) project:

- There is limited information available to build well-informed exposure scenarios covering the life cycle of MNMs for uses which are known to exist.
- Most of the existing quantitative exposure data are associated with small-scale production of MNMs.
- There is particularly little information available on exposures to downstream users, i.e., consumer and occupational uses of preparations and articles containing MNMs.
- Literature-based studies often do not include descriptions of contextual details, such as room size, presence of ventilation, or typical frequency and duration of an activity.
- Studies assessing inhalation exposure to MNM rely on real-time particle counters to measure exposure, yet these instruments cannot distinguish MNMs from background particles. There is an urgent need to develop more selective instrumentation.
- It is important to look at particles of all sizes, particularly since nanoparticles tend to agglomerate into larger particles.
- The current state-of-the-science does not allow for a detailed comparison of data between studies due to differences in particle properties, measurement techniques, and reporting metrics.

# 9. RISK MANAGEMENT MEASURES

In order to limit the occupational exposure via the respiratory tract and the skin, several protective measures like substitution and technical, organizational and personal protection measures have to be applied. In addition, classification, labelling and occupational exposure limits are important instruments of risk management, but critically depending on the availability of studies on toxicity. (EU-OSHA 2009).

Risk management programs are an important part of an overall occupational safety and health program for any company or workplace that produce or use nanomaterials. These methods should form a continuous cyclic process, providing solutions to correct the problems of potential exposure sources. Operations and job tasks that have the potential to aerosolize nanomaterials (e.g., handling dry powders, spray applications) deserve more attention and more stringent controls than the nanomaterials embedded in solid or liquid matrixes. Risk management programs should include guidelines for installing and evaluating engineering controls (e.g., exhaust ventilation, dust collection systems), the education and training in the proper handling of nanomaterials (e.g., good work practices), and the selection and use of personal protective equipment (e.g., clothing, gloves, respirators). (Schulte et al. 2008, Cornelissen et al. 2011).

The order in which risk management actions are usually taken in occupational hygiene is shown in table 7.

**Table 7.** Hierarchy of exposure controls* (NIOSH 2009. Cornelissen et al. 2011)

| Control method | Process, equipment, or job task |
|---|---|
| 1. Elimination | Change design to eliminate hazard |
| 2. Substitution | Replace a high hazard for a low hazard |
| 3. Engineering | Isolation/enclosure, ventilation (local, general) |
| 4. Administrative | Procedures, policies, shift design |
| 5. Personal protective equipment | Respirators, clothing, gloves, goggles, ear plugs |

*Control methods are typically implemented in this order to limit worker exposures to an acceptable concentration (e.g., occupational exposure limit or other pre-established limit).

## 9.1 Engineering controls

If the potential hazard cannot be eliminated or substituted by a less hazardous or non-hazardous substance, then engineering controls should be installed and tailored to the process or job task (NIOSH 2009). ENM exposure can be reduced through the use of engineering controls, such as process changes, material containment and enclosures, operating at negative pressure, compared to the worker's breathing zone; worker isolation; separated rooms; the use of robots; and local exhaust ventilation (LEV) (Yokel and MacPhail 2011). Engineering control techniques should be effective for capturing airborne nanomaterials, based on what is known of nanomaterial motion and behaviour in air. The quantity of the bulk nanomaterial, that is synthesized or handled in the manufacturing of a product, will significantly influence the selection of the exposure controls. Other factors that influence selection of engineering controls include the physical form of the nanomaterial, task duration, and frequency. (NIOSH 2009)

Process/source enclosure (i.e., isolating the ENM from the worker) can be aided by glove boxes, chemical fume hoods, biological safety cabinets (BSC), or an externally vented LEV system. However, one should also consider that these methods may release ENMs into the environment, potentially creating environmental pollution and loss of costly material. (Yokel and MacPhail 2011)

For removing nanoparticles from the exhausted air, an appropriate filtration system has to be used. High efficiency air filters can be classified into three groups: EPA (efficient particulate air), HEPA (high-efficiency particulate air) and ULPA (ultra-low penetration air), depending on their penetration obtained for the most penetrating particle size, from the range 120 to 250 nm. (EU-OSHA 2009)

NIOSH (2008) carried out a field study in a company that used gas-phase condensation reactors to produce manganese, silver, and cobalt nanoparticles in a diameter range from 15 to 50 nm. Investigations were done before and after the installation of portable fume extractor typically used in the welding industry. The fume extractor was attached to a HEPA filtered air-handler with a carbon pre-filter. Both direct sampling and filter-based sampling methods were used to determine the effectiveness of the implemented (LEV) control technology. The filter-based tests showed a reduction in ambient nanoparticles ranging from 74% to 96%, with a mean reduction of 88%. Direct-reading test reductions ranged from 78% to 100%, with a mean reduction of 96%. The transmission electron microscope analysis confirmed a notable increase in nanoparticle capture when the LEV was used.

Production of a nanocomposite material containing alumina in a polymer by a twin-screw extrusion process caused release of 5–20 nm and 50–200 nm alumina particles in the worker's breathing zone (Tsai et al. 2008a). Covering the top of the feeding throat and the open mouth of the particle feeder, thorough cleaning by washing the floor, and water-based removal of residual dust on all equipment significantly decreased airborne particles (Tsai et al. 2008a; Tsai et al. 2008b). The results suggested that some engineering controls may be appropriate to safely remove some airborne ENMs, including maintaining the room at negative pressure relative to the outside, avoiding the handling of dry ENMs, adequate ventilation, and containment of the ENM material during its use.

Tsai et al. (2009) measured the exposure to aluminium NPs during handling in fume hoods in two laboratory rooms. Concentrations of airborne particles with a diameter from five to 560 nm were measured using a fast mobility particle sizer (FMPS). The handling of the particles (transferring particles from beaker to beaker by spatula and by pouring action) took place 15 cm from the face of the hood. Variable factors studied were the hood design, transfer method, face velocity/sash location and vertical height. The authors concluded that more particles were extracted while the hood performed at highest face velocity of one m/s and as the sash was lowered to the operator's low chest height.

Bello et al. (2009) showed that wet cutting of a hybrid CNT in an epoxy resin or in a woven alumina fibre cloth using a cutting wheel with water to flush dust particles produced no significant increase of airborne 5 to 1000 nm particles in the operator breathing zone, whereas dry machining did.

Asikainen et al. (2009) studied dust exposure in four buildings during ten working phases. During many construction work tasks, dust concentrations at the breathing zone and in the working area could be decreased by using tools equipped with local suction. For example, during concrete grinding, the local exhaust decreased the dust concentration at the working area by more than 98%. The cleaning work with brush was very dusty, whereas the use of

central vacuum cleaner helped to maintain low dust concentrations, especially at the breathing zone. Natural ventilation (the balcony door was open) appeared to be the most effective in decreasing the dust concentrations in the working area during many dusty tasks. The evaluated portable filtration units were not effective enough to control dust when dust production was high. However, the use of portable filtration units is recommended if natural ventilation is not possible. Good maintenance of the working tools was found to be important. For example, 99.8% lower dust concentrations were measured during the cement glue grinding when the grinding tool with a HEPA-filter was just maintained. An insufficient mean to decrease exposure to dust and mineral fibres during insulating was achieved by choosing painted and denser insulation materials, but this did not reduce exposure sufficiently. On the other hand, the partitioning and pressurization of the cleaner areas were effective.

## 9.2 Administrative controls

Administrative controls are policies aimed at limiting worker exposure to a hazard, typically by altering the amount of time a worker is potentially exposed and by the implementation of good work practices (Schulte et al. 2008). When engineering controls are not feasible for reducing exposure, administrative controls should be implemented. These are policies and procedures aimed at limiting worker exposure to a hazard. These could include a nanoscale material hygiene plan; preparation, training in, and monitoring use of standard operating procedures; reduction of exposure time; modification of work practices; and good workplace and housekeeping practices. Biological monitoring and medical examination are other administrative controls. (Yokel and MacPhail 2011)

In the report by NIOSH (2009), good practices for management and workers, with respect to nanomaterials, are presented as follows:

“Management

- Educating workers on the safe handling of engineered nano-objects or nano-object-containing materials to minimize the likelihood of inhalation exposure and skin contact.
- Providing information, as needed, on the hazardous properties of the precursor materials and those of the resulting nanomaterials product with instruction on measures to prevent exposure.
- Encouraging workers to use handwashing facilities before eating, smoking, or leaving the worksite.
- Providing additional control measures (e.g., use of a buffer area, decontamination facilities for workers if warranted by the hazard) to ensure that engineered nanomaterials are not transported outside the work area.
- Providing facilities for showering and changing clothes to prevent the inadvertent contamination of other areas (including take-home) caused by the transfer of nanomaterials on clothing and skin.”

“Workers

- Avoiding handling nanomaterials in the open air in a ‘free particle’ state.
- Storing dispersible nanomaterials, whether suspended in liquids or in a dry particle form in closed (tightly sealed) containers whenever possible.

- Cleaning work areas at the end of each work shift, at a minimum, using either a HEPA-filtered vacuum cleaner or wet wiping methods. Dry sweeping or air hoses should not be used to clean work areas. Cleanup should be conducted in a manner that prevents worker contact with wastes. Disposal of all waste material should comply with all applicable Federal, State, and local regulations.
- Avoiding storing and consuming food or beverages in workplaces where nanomaterials are handled." (NIOSH 2009)

## 9.3 Personal protective equipment

Use of personal protective equipment (PPE) such as respirators, gloves, and protective clothing is the least preferred method for preventing worker exposure to a hazard, as it places the responsibility for preventing injury or illness on the worker. Given the uncertainties about the health risks for ENPs and limited data for determining the effectiveness of controlling workplace exposures, the supplemental use of PPE may be warranted for some job tasks where engineering controls cannot be used or where they are only partially effective in preventing airborne or dermal exposure. (Schulte et al. 2008)

### *9.3.1 Respirators*

The use of respirators is often required when engineering and administrative controls do not adequately keep worker exposures to an airborne contaminant below a regulatory limit or an internal control target. If worker exposure to airborne nanomaterials remains a concern after instituting control measures, the use of respirators can provide further worker protection. Several classes of respirators exist, that can provide different levels of protection when properly fit tested on the worker. (NIOSH 2009)

Major types of respiratory protection include dust masks, filtering facepiece respirators, chemical cartridge/gas mask respirators, and powered air-purifying respirators (Yokel and MacPhail 2011). The European Standards (EN 143 and EN 149) rank filtering facepiece (FFP) respirators as FFP1, FFP2, and FFP3, which are 80, 94, and 99% efficient, respectively, indicated by CE (for Conformité Européene) on complying products. Research of the effectiveness of respirators for exposure to NPs has focused on the collection efficiency of filters for NPs (Schneider et al. 2011). Particles >100 nm are collected on filter media by two mechanisms: inertial impaction and interception. Airborne nanoparticles behave much like gas particles. Particles <100 nm are collected by diffusion. Further work is warranted to understand the influence of the physico-chemical properties of ENMs, particularly size, charge, and shape, on their penetration through filtering facepiece respirators. (Yokel and MacPhail 2011)

The most penetrating particle size (MPPS) varies with type of filter media and the condition of respirator, especially for electrostatically charged filter media (observed range 30–100 nm). Other studies confirmed that these types of filters are less effective for nanoparticles compared with HEPA or ULPA-type of filters. (Schneider et al. 2011)

For ENMs, the MPPS has been noted to be ~40 to 50 nm. This is approximately the same size of spherical ENMs that appear to contribute to their greatest differences in biological systems, compared with solution and bulk forms of the same materials. This feature raises concern, because the sizes of ENMs that may have the greatest effects in people are those that are best able to penetrate filtering facepiece respirators. Yokel and MacPhail (2011) concluded that

until further results are obtained from clinical laboratory or workplace studies, traditional respirator selection guidelines should be used.

Evaluation of commercial filter media under harsh conditions, for example, high-face velocity, is needed. However, there is currently no evidence that the assigned protection factors for respirators deviate for nanoparticles compared with conventional particles. Coagulation or scavenging of nanoparticles can result in agglomerates with sizes around 200–400 nm, which is considered to be the MPPS for mechanical filters. (Schneider et al. 2011)

### *9.3.2 Protective clothing*

Currently, there are no generally accepted guidelines available based on scientific data for the selection of protective clothing or other apparel against exposure to nanomaterials. This is due in part to minimal data being available on the efficacy of existing protective clothing, including gloves. A challenge to making appropriate recommendations for dermal protection against nanoparticles is the need to strike a balance between comfort and protection. Garments that provide the highest level of protection are also the least comfortable to wear for longs periods of time, while garments that are probably the least protective are the most breathable and comfortable for employees to wear. The two primary routes of exposure to particulates for workers using protective clothing are direct penetration through the materials and leakage through gaps, seams, defects, and interface closure areas. (NIOSH 2009)

Penetration of 10 to 1000 nm NaCl through woven and fibrous fabrics showed a MPPS between 100 and 500 nm and maximum penetration of 50 to 80% (Huang et al. 2007). Comparison of graphite nanoparticle penetration through 650 µm thick cotton, 320 µm polypropylene, and 115 µm non-woven high-density polyethylene textile (Tyvek®) showed ~30, 12, and 4% penetration of the MPPS (~40 nm), respectively (Golanski et al. 2008). Tyvek® permitted ~3 orders of magnitude less penetration of ~10 nm titanium and platinum than cotton or 160 µm woven polyester (Golanski et al. 2010). A study of ten nonwoven fabrics under conditions simulating workplace ENM exposure, showed that penetration increased with increasing air velocity and particle size (to ~300–500 nm). Pore structure of the various fabrics greatly influenced penetration (Gao et al. 2011). Although nonwoven fabrics were much more effective in protecting workers from ENM exposure than woven fabrics, they are much less comfortable to wear, suggesting that improvements in fabric design or selection are needed to address this disincentive to use more effective PPE.

Based upon the uncertainty of the health effects of dermal exposure to nanoparticles, it is important to consider using protective equipment, clothing, and gloves in order to minimize dermal exposure. Special attention should be paid to preventing exposure of damaged skin. Until more scientific data is available, specific to the performance of protective clothing and gloves against nanomaterials, it is generally recommended that current industrial hygiene best practices should be followed. (NIOSH 2009)

# 10. SUMMARY OF SCAFFOLD ENQUIRY ANSWERS

Within the Scaffold project, an enquiry was sent out to the industrial project partners, as well as to the European Construction Industry Federation (FIEC), in order to get an indication on where industry stands with respect to exposure to ENMs at the workplace. This chapter summarizes the answers obtained from the partners and FIEC regarding the enquiry questions related to exposure of workers, risk management methods, and occupational safety.

Process stages at which workers are mainly exposed to nanoparticles:

- Post-treatment or adaptation of the MNMs to the products, the demolition and disposal.
- Application of mortar, prime coat, paints, roof membrane, concrete, and cutting bricks and tiles.
- Machining and cutting samples.
- Production of nanoparticles.
- Handling final products or parts, pieces, rests of final products and processing waste from constructions.

Main risk management methods currently in use:

- Preparing MNMs in stable aqueous solutions in order to prevent workers from inhalation of powder MNMs.
- Installing extraction direct to all machinery to take all dusts or debris out at source.
- Closing production system and producing NPs in one step without handling.
- Packaging of powder in a closed, well-equipped and ventilated fume cabinet.
- Personal protection equipment: respiratory protection (e.g., filters of protection levels P2, FFP2, P3 or FFP3), protecting gloves (at least two layers of gloves are recommended), protective equipment (non-woven fabrics).
- Measuring air concentrations of nanoparticles.
- Apparatus for monitoring hygiene, ventilation and air composition of space.
- Occupational health and safety system.

The main challenges/problems related to occupational safety and risk management regarding handling and use of nanomaterials:

- Preparing non-hazardous MNMs and preparing MNMs in form of safe products.
- Education/instruction.
- Preventing risks by using personal protection.
- Lack of knowledge about nanoparticles in building materials.
- Lack of knowledge about the risks of the different types of fibres.
- Lack of nano-specific standards.
- Difficulty of monitoring nanoparticles.
- Measuring of nanoparticles during different stages of process.
- Very high temperatures, fire or explosion danger, closed and not ventilated spaces in special conditions.
- Finding the right balance between an appropriate level of protection of workers without putting an unreasonable burden (administrative, economic and legal) on the shoulder of enterprises.

In general, the producers and other companies in the beginning of the life cycle of the MNMs seem to be most aware about the potential risks related to exposure to MNMs, whereas those using the MNM-containing products, e.g., at construction sites have not paid much attention the potential risks or to nano-specific risk management measures.

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