

Technological Analysis

Industrial application
of nanomaterials –
chances and risks



With the support of the European Commission



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Technology analysis

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Front Page: left above: microelectronic clean room facility, left below: fluorescent cadmium telluride nanoparticles (source: University of Hamburg), right above: TEM image of agglomerated silicon carbide nanoparticles (source : CEA), right below: macrophage intaking ultrafine particles (source: GSF)

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Foreword

Nanotechnology is seen as one of the most relevant technologies for the 21st century. This opinion on nanotechnology is derived from its economic potential on new or optimised products as well as on the expected contributions for minimising ecological stress and consumption of resources. Nanotechnology does not only mean to make technology one step smaller, from micro- to nanotechnology. Nanotechnology rather prepares the way for handling and using quantum effects. Showing complete new characteristics and behaviours, nanomaterials are opening new product innovations e.g. for protection against sun, biochip makers or copy protection.

On the other hand there is an upcoming discussion about the potential risks of nanotechnology. Like in every early state of discussion on risks, fears, arguments and speculations are merging. Visionary aspects such as nanobots or grey-goo and questions about health and environmental implications of nanomaterials are named with the same breath like well known existing problems of nanoparticulate carbon emissions by car diesel engines. This discussion has given rise to a demand for a moratorium on nanotechnology by some NGOs.

Nevertheless we have to expect a large diffusion of nanotechnology based or nanotechnology related products and production processes in the coming years. The European Commission intend to support the gathering of scientific data in order to analyse chances and risks of nanotechnology as basis for matter-of-fact oriented public discussion. The intended result is seen in a reliable database and extensive safety for decision making in the sense of protection and regulation to a minimum extend.

The objectives of this report are to assemble available information from public and private sources on chances but also possible hazards involving industrial nanoparticle production, to evaluate the risks for workers, consumers and the environment, and to give recommendations for setting up regulatory measures and codes of good practice.



Dr. Dr. Axel Zweck

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1 INTRODUCTION

Atoms and molecules are the essential building blocks of all things. The manner in which things are “constructed” with these building blocks is vitally important to their properties and how they interact. Nanotechnology refers to the manipulation or selfassembly of individual atoms, molecules, or molecular clusters into structures to create materials and devices with new or vastly different properties. Nanotechnology is developing new ways to manufacture things. Since the late 90`s, nanotechnology has shot into the limelight as a new field with tremendous promise. The potential beneficial impact of nanotechnology on society has been compared with that of silicon and plastics. This new, “small” way of manipulating materials has already led to new research areas and the development of new products, which are available commercially.

Nanotechnology is a key technology for the 21st Century

Nanostructured materials play a key role in most of the nanotechnology based innovations. By tailoring the structure of materials at the nanoscale, it is possible to engineer novel materials that have entirely new properties. With only a reduction of size and no change in substance, fundamental characteristics such as electrical conductivity, colour, strength, and melting point – the properties we usually consider constant for a given material – can all change. Therefore nanomaterials show promising application potentials in a variety of industrial branches such as chemistry, electronics, medicine, automotive, cosmetics or the food sector. Nanotechnology here holds the promise for producing better goods with less input of energy and /or materials, developing specific drug delivery systems and lab-on-a-chip based diagnostics for a minimal invasive medicine, improving information and communication through smaller and more powerful electronic devices, etc. An optimistic view on these developments predicts that a new „industrial revolution“ will take place in the following decades.

Material properties can be tailored at the nanoscale

In recent times however, an increasing number of sceptical voices concerning nanotechnology can be heard in the public. Beside the discussion of risks of visionary developments like “nanobots” and “nanoassemblers” (Drexler 1986 and 1991, Joy 2000) most critics focus on potential health and environmental risks of nanomaterials. This can be illustrated with several articles in newspapers and top scientific journals (e.g. Service 2003, Malakoff 2003) discussing potential negative effects and risks of nanoparticle applications. Although, not very much has been published concerning specific nanomaterials, the potential health and environmental risks of nanoparticles respective ultrafine particles (UFP) with aerodynamic diameters < 100 nm, has gained public attention in the last years. In this discussion the terms ‘ultrafine particles’ - used in aerosol and epidemiology terminology - and ‘nanoparticles’ are often used interchangeably.

Public discussion on potential health and environmental risks of nanoparticles

Terminology of nanoparticles and nanomaterials is often ambiguous

Call for a moratorium on commercial production of nanomaterials

One of the sharpest critics of industrial nanoparticle applications is the Canadian-based non-governmental organisation ETC Group, which called for an immediate moratorium on commercial production of new nanomaterials and for a transparent global process for evaluating the socio-economic, health and environmental implications of nanotechnology (ETC 2002). The fear of risk associated with nanoparticle use is mainly caused by limited scientific knowledge about potential side effects of nanoparticle in the human body and the environment due to their special properties. Conventional compounds normally considered harmless might prove to be dangerous on a nanometer scale. For example nanoparticles can penetrate into body cells and even break through biological barriers (such as the blood-brain barrier).

Epidemiological studies show an association between number concentration of ultrafine particles in polluted air and health risks

Epidemiological studies have consistently shown an association between particulate air pollution and health, not only in exacerbations of illness in people with respiratory disease but also in rising numbers of deaths from cardiovascular and respiratory disease among older people. It has been proposed that the adverse health effect of particulate air pollution was mainly associated with the number concentrations of ultrafine particles (Oberdörster et al. 1994, Seaton et al. 1995) rather than the mass concentrations of coarser particle fractions. These epidemiological studies were conducted in the environmental context with traffic and industrial combustion processes being the main source of particulate matter in ambient air. So far, no epidemiological studies are available to describe the work place situation in regard to the production of nanoparticles. However, there have been some studies showing that nanoparticles, after deposition in the lungs, largely escape alveolar macrophage surveillance and gain access to the pulmonary interstitium with greater inflammatory effect than larger particles (Oberdörster 2001).

Few data available for physiological effects of nanoparticles

From occupational medicine it has been known for decades that particles deposited in the alveolar region of the lungs can lead to the development of chronic diffuse interstitial lung disease like silicosis and asbestosis. Recent findings from animal studies suggest a fast translocation of nanoparticles from pulmonary and gastrointestinal epithelium into the systemic circulation (Frampton 2001, Nemmar et al. 2002, Oberdörster et al. 2002) Also, there is some evidence that carbon nanoparticles can directly enter the brain via the respiratory nasal mucosa and the olfactory bulb (Calderon-Garciduenas et al. 2002, Oberdörster 2004). All these properties make the epidemiologically observed association of inhaled nanoparticles and adverse health effects biologically plausible. However, without hard data it is impossible to know what physiological effects will occur.

Although nanotechnology in most fields is still at an experimental stage, the next few years will probably see a dramatic increase in the industrial generation and use of nanoparticles (Mazzola 2003, Paull 2003).

Therefore impact of these materials on worker safety, consumer protection, public health and the environment will have to be considered carefully by legislation and regulation authorities.

1.1 Objectives of the report

The objectives of this report are to assemble available information from public and private sources on chances but also possible hazards involving industrial nanoparticle production, to evaluate the risks to workers, consumers and the environment, and to give recommendations for setting up regulatory measures and codes of good practice to obviate any danger.

The report gives information on characteristics of nanoparticles (size, shape, types, etc.), production methods, industrial application fields, characterisation and detection methods as well as a risk assessment including potential particle release and exposure, toxicological aspects and protective measures.

It has to be noted that this report focuses on the assessment of the production, handling and treatment, and use of nanoparticles in industrial processes and products, as well as in consumer products. Risks of other kind of ultrafine or nanoparticles e.g. from vehicle or power plant emissions will not be dealt with, although some of the knowledge and information acquired may be relevant.

1.2 Methods

The report summarises information from scientific literature, project studies within the partner organisations, environmental, health and worker protection associations as well as national and European legislation. Literature databases, proceedings of relevant workshops and conferences as well as internet searches and expert interviews were used as information sources.

The authors realise that in view of the broad scope and a very early stage of the discussion this report is rather a working-document that should be criticised and discussed to come to a better understanding of the topic. It has also to be mentioned, that many information concerning the development of nanomaterial based products are kept confidential by the involved companies, so this report may not represent the state-of-the-art in some areas.

2 CLASSIFICATION AND PROPERTIES

2.1 Classification of nanomaterials

All conventional materials like metals, semiconductors, glass, ceramic or polymers can in principle be obtained with a nanoscale dimension. The spectrum of nanomaterials ranges from inorganic or organic, crystalline or amorphous particles, which can be found as single particles, aggregates, powders or dispersed in a matrix, over colloids, suspensions and emulsions, nanolayers and –films, up to the class of fullerenes and their derivatives. Also supramolecular structures such as dendrimers, micelles or liposomes belong to the field of nanomaterials. Generally there are different approaches for a classification of nanomaterials, some of which are summarised in table 1.

Broad range of different nanomaterial classes

Classification	examples
Dimension <ul style="list-style-type: none"> • 3 dimensions < 100nm • 2 dimensions < 100nm • 1 dimension < 100nm 	particles, quantum dots, hollow spheres, etc. tubes, fibers, wires, platelets, etc. films, coatings, multilayer, etc.
Phase composition <ul style="list-style-type: none"> • single-phase solids • multi-phase solids • multi-phase systems 	crystalline, amorphous particles and layers, etc. matrix composites, coated particles, etc. colloids, aerogels, ferrofluids, etc.
Manufacturing process <ul style="list-style-type: none"> • gas phase reaction • liquid phase reaction • mechanical procedures 	flame synthesis, condensation, CVD, etc. sol-gel, precipitation, hydrothermal processing, etc. ball milling, plastic deformation, etc.

Classification approaches of nanomaterials

Table 1: Classification of nanomaterials with regard to different parameters

The main classes of nanoscale structures can be summarised as follows:

2.1.1 Nanoparticles

Nanoparticles are constituted of several tens or hundreds of atoms or molecules and can have a variety of sizes and morphologies (amorphous, crystalline, spherical, needles, etc.). Some kind of nanoparticles are already available commercially in the form of dry powders or liquid dispersions. The latter is obtained by combining nanoparticles with an aqueous or organic liquid to form a suspension or paste. It may be necessary to use chemical additives (surfactants, dispersants) to obtain a uniform and stable dispersion of particles. With further processing steps, nanostructured powders and dispersions can be used to fabricate coatings, components or devices that may or may not retain the nanostructure of the particulate raw materials. Industrial scale production of some types of nanoparticulate materials like carbon black, polymer dispersions or micronised drugs has been established for a long time.

Metal oxide nanopowders have already broad commercial applications

Another commercially important class of nanoparticulate materials are metal oxide nanopowders, such as silica (SiO_2), titania (TiO_2), alumina (Al_2O_3) or iron oxide (Fe_3O_4 , Fe_2O_3). But also other nanoparticulate substances like compound semiconductors (e.g. cadmium telluride, CdTe, or gallium arsenide, GaAs) metals (especially precious metals such as Ag, Au) and alloys are finding increasing product application.

Beside that, the range of macromolecular chemistry with molecule sizes in the range of up to a few tens of nanometers is often referred to as nanotechnology. Molecules of special interest that fall within the range of nanotechnology are fullerenes or dendrimers (tree-like molecules with defined cavities), which may find application for example as drug carriers in medicine.

2.1.2 Nanowires and -tubes

Carbon nanotubes are expected to have a big market potential in the future

Linear nanostructures such as nanowires, nanotubes or nanorods can be generated from different material classes e.g. metals, semiconductors or carbon by means of several production techniques. As one of the most promising linear nanostructures carbon nanotubes can be mentioned, which can occur in a variety of modifications (e.g. single- or multi-walled, filled or surface modified). Carbon nanotubes are expected to find a broad field of application in nanoelectronics (logics, data storage or wiring, as well as cold electron sources for flat panel displays and microwave amplifiers) and also as fillers for nanocomposites for materials with special properties. At present carbon nanotubes can be produced by CVD methods on a several tons per year scale and the gram quantities are already available commercially.

2.1.3 Nanolayers

Nanolayers are one of the most important topic within the range of nanotechnology. Through nanoscale engineering of surfaces and layers a vast range of functionalities and new physical effects (e.g. magnetoelectronic or optical) can be achieved. Furthermore a nanoscale design of surfaces and layers is often necessary to optimise the interfaces between different material classes (e.g. compound semiconductors on silicon wafers) and to obtain the desired special properties. Some application ranges of nanolayers and coatings are summarised in table 2.

Surface Properties	Application examples
<ul style="list-style-type: none"> Mechanical properties (e.g. tribology, hardness, scratch-resistance) 	Wear protection of machinery and equipment, mechanical protection of soft materials (polymers, wood, textiles, etc.)
<ul style="list-style-type: none"> Wetting properties (e.g. antiadhesive, hydrophobic, hydrophilic) 	Antigraffiti, antifouling, Lotus-effect, self-cleaning surface for textiles and ceramics, etc.
<ul style="list-style-type: none"> Thermal and chemical properties (e.g. heat resistance and insulation, corrosion resistance) 	Corrosion protection for machinery and equipment, heat resistance for turbines and engines, thermal insulation equipment and building materials, etc.
<ul style="list-style-type: none"> Biological properties (biocompatibility, anti-infective) 	Biocompatible implants, abacterial medical tools and wound dressings, etc.
<ul style="list-style-type: none"> Electronical and magnetic properties (e.g. magneto-resistance, dielectric) 	Ultrathin dielectrics for field-effect transistors, magnetoresistive sensors and data memory, etc.
<ul style="list-style-type: none"> Optical properties (e.g. anti-reflection, photo- and electrochromatic) 	Photo- and electrochromic windows, antireflective screens and solar cells, etc.

A vast range of functionalities and new physical effects can be achieved by nanoscale engineering of surfaces

Table 2: Tunable properties by nanoscale surface design and their application potentials

2.1.4 Nanopores

Materials with defined pore-sizes in the nanometer range are of special interest for a broad range of industrial applications because of their outstanding properties with regard to thermal insulation, controllable material separation and release and their applicability as templates or fillers for chemistry and catalysis. One example of nanoporous material is aerogel, which is produced by sol-gel chemistry. A broad range of potential applications of these materials include catalysis, thermal insulation, electrode materials, environmental filters and membranes as well as controlled release drug carriers.

2.2 Properties of nanomaterials

The physical and chemical properties of nanostructured materials (such as optical absorption and fluorescence, melting point, catalytic activity, magnetism, electric and thermal conductivity, etc.) typically differ significantly from the corresponding coarser bulk material. A broad range of material properties can be selectively adjusted by structuring at the nanoscale (see table 3).

Properties	Examples
Catalytic	Better catalytic efficiency through higher surface-to-volume ratio
Electrical	Increased electrical conductivity in ceramics and magnetic nanocomposites, increased electric resistance in metals
Magnetic	Increased magnetic coercivity up to a critical grain size, superparamagnetic behaviour
Mechanical	Improved hardness and toughness of metals and alloys, ductility and superplasticity of ceramic
Optical	Spectral shift of optical absorption and fluorescence properties, increased quantum efficiency of semiconductor crystals
Sterical	Increased selectivity, hollow spheres for specific drug transportation and controlled release
Biological	Increased permeability through biological barriers (membranes, blood-brain barrier, etc.), improved biocompatibility

Table 3: Adjustable properties of nanomaterials

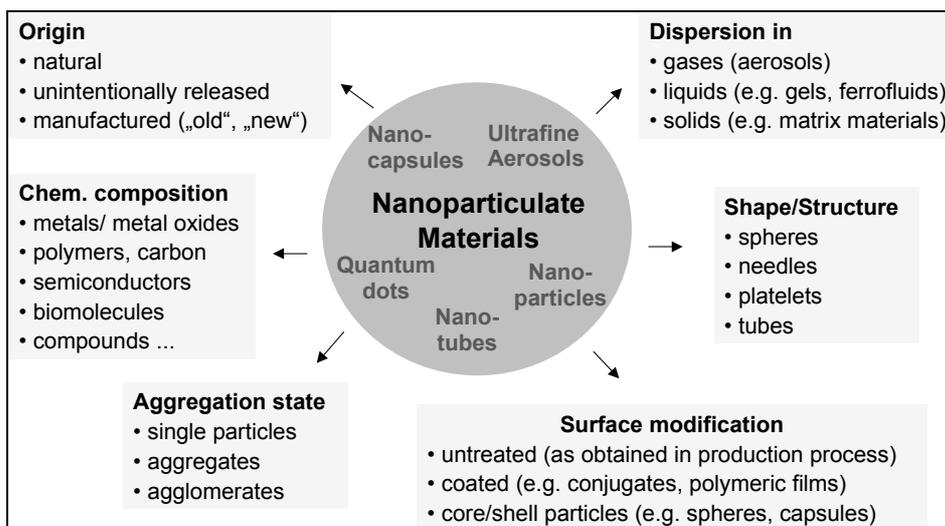
Special properties of nanomaterials are due to quantum effects and a large surface-to-volume ratio

These special properties of nanomaterials are mainly due to quantum size confinement in nanoclusters and an extremely large surface-to-volume ratio relative to bulk materials and therefore a high percentage of atoms/molecules lying at reactive boundary surfaces. For example in a particle with 10 nm diameter only approx. 20 per cent of all atoms are forming the surface, whereas in a particle of 1 nm diameter this figure can reach more than 90 per cent. The increase in the surface to volume ratio results in the increase of the particle surface energy, which leads to e.g. a decreasing melting point or an increased sintering activity. It has been stated that large specific surface area of particles may significantly raise the level of otherwise kinetically or thermodynamically unfavourable reactions (Jefferson 2000). Even gold (Au), which is a very stable material, becomes reactive when the particle size is small enough (Haruta 2003).

With precise control of the size of the particles their characteristics can be adjusted in certain borders. Though it is usually difficult to maintain these desired characteristics beyond the different manufacturing processes to the final product, because loose nano-powders tend to grow to larger particles and/or firmly connected agglomerates already at room temperature and thus losing their nano-specific characteristics. Therefore it is necessary to select or develop suitable production processes and further refining/treatment processes (e.g. coating of nanoparticles) to prevent or attenuate agglomeration and grain growth during generation, processing and use of nanomaterials (see also chapter 4.3).

2.3 Characteristics of nanoparticulate materials

In this report we focus on nanoparticulate materials which have structure sizes smaller than 100 nm in at least two dimensions. These nanoparticulate materials can have various shapes and structures such as spherical, needle-like, tubes, platelets, etc. Chemical composition is another important parameter for the characterisation of nanoparticulate materials, which comprise nearly all substance classes e.g. metals/ metal oxides, polymers, compounds as well as biomolecules. Under ambient conditions nanoparticles tend to stick together and form aggregates and agglomerates. These aggregates/ agglomerates have various forms, from dendritic structure to chain or spherical structures with sizes normally in the micrometer range. The properties of nanoparticles can be significantly altered by surface modification. For example, nanoparticles are often stabilised with coatings or molecule adducts to prevent agglomeration. For the characterisation of nanoparticulate materials it is further important in which medium the nanoparticles are dispersed e.g. in gaseous, liquid or solid phase. The following figure summarises relevant parameters for the characterisation of nanoparticulate materials.



Relevant parameters for the characterisation of nanoparticulate materials

Figure 1: Characterisation parameters of nanoparticulate materials (source: VDI-TZ)

Some examples of different types of nanoparticulate materials are presented in the following figure.

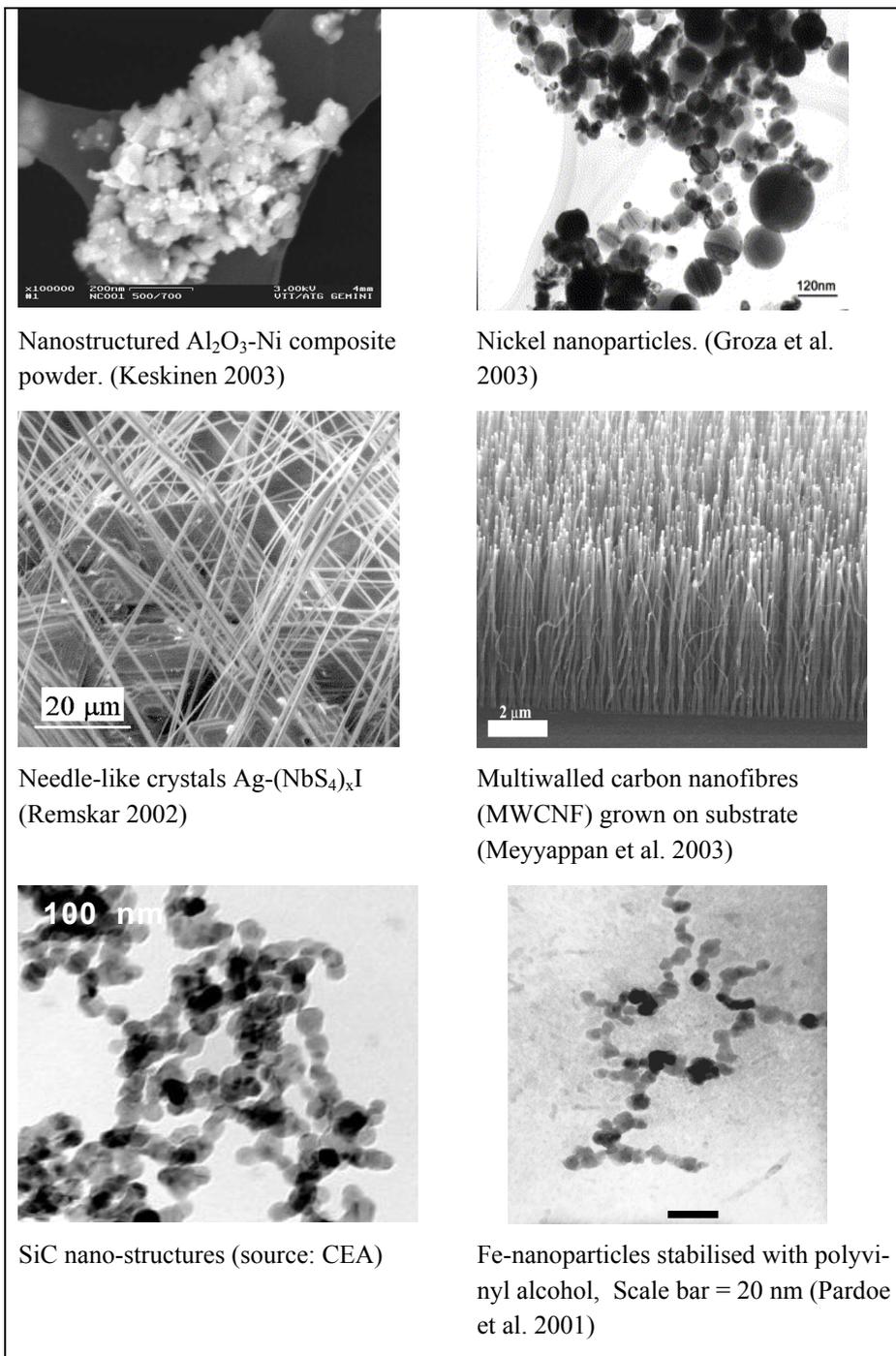


Figure 2: Electron microscopy images showing structure and shape of different nanoparticulate materials

3 INDUSTRIAL APPLICATIONS AND MARKET POTENTIALS

The production of nanomaterial based products involves several manufacturing steps. It usually starts with the production of nanoscaled particles from precursors or bulk materials, goes to master batches or dispersions which can be intergrated into commercial products to make semi-manufactured products and ends in products over a wide range of applications. The processing of nanoparticles depends on the basic formulation, solid as nanopowders or liquid as dispersions. Nanopowders can be used as fillers for different materials such as varnish, paint, plastics, etc. or they can be used as educts e.g. for the production of ceramics. Liquid nanodispersions can be integrated into other liquid systems such as paints or can be used to create new composites with new properties. The following figure shows a typical value chain of nanoparticulate material based products.

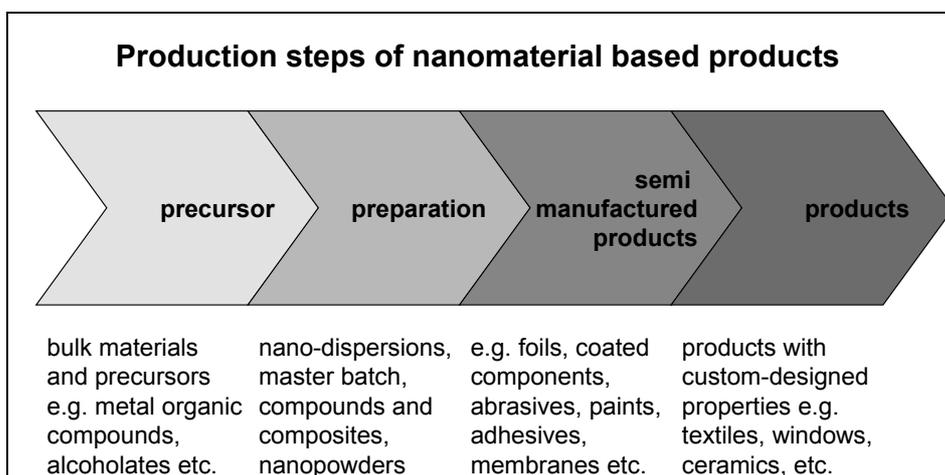


Figure 3: Production steps and value chain of nanomaterial based products

The following chapters summarise existing as well as potential applications of different types of nanoparticulate materials.

3.1 Nanoparticles

3.1.1 Metal oxides/metals

Metal oxides, in particular silica (SiO_2), titania (TiO_2), alumina (Al_2O_3), iron oxide (Fe_3O_4 , Fe_2O_3) at present occupy the first position in terms of economic importance within the range of inorganic nanoparticles. Also of increasing importance are mixed oxides, such indium-tin oxide (ITO) and antimony-tin oxide (ATO), silicates (aluminum and zirconium silicates) and titanates (e.g. barium titanate). While silica and iron oxide nanoparticles have a commercial history spanning half a century or more, other nanocrystalline metal oxides have entered the marketplace more

recently. Main applications fields of metal oxide nanoparticles are electronics, pharmacy/medicine, cosmetics as well as chemistry and catalysis.

In the range of cosmetics the most economic relevant application are nanoparticle-based sunscreens. Here nanoparticulate titania and zinc oxide are used as UV light absorbing components, which are transparent due to their small size and provide an effective protection. One marketing advantage of inorganic particles is the ability to provide broad-spectrum protection in a non-irritating sunscreen product. Certain organic active agents, including avobenzene, which provides full UVA shielding, can cause skin irritation. As a result, TiO₂ and ZnO are finding increasing application in sensitive skin and baby products and daily-wear skin lotions. One concern regarding the use of metal oxide nanoparticles, is that upon absorption of UV radiation, they release free radicals, which can damage DNA, and thus maybe prove to be carcinogenic. Therefore, suppliers of nanoparticles generally offer the particles with coatings, which cause the free radicals to recombine before entering the skin. However, recent concern about the fate of the particles when applied to the skin, as they possibly can penetrate much deeper than microparticles (see chapter 6.3), may complicate the use of organic and inorganic nanoparticles in cosmetics. Applications of nanoparticles in medicine are e.g. markers for biological screening tests (e.g. gold or semiconductor particles), contrast agents for magnetic resonance imaging (MRI) as well as antimicrobial coatings and composite materials for abacterial surfaces and medical devices (Salata 2004).

In the field of catalysis the biggest market volume can be assigned to porous catalysts support for car exhaust catalysts. Nanoporous alumina here serves as supporting material for noble metal catalysts, which were finely dispersed on to the substrate. Nanoparticles will also find increasing applications as catalysts in PEM fuel cells and hydrogen reformers. The Business Communication Company (BCC) estimates the world market volume of metal oxide and metal nanoparticles at 750 mill. EURO in 2005. Table 4 gives an overview on applications in different industrial branches.

Electronic, optoelectronic magnetic applications	Biomedical, pharmaceutical cosmetic applications	Energy, catalytic structural applications
<ul style="list-style-type: none"> • Chemical–mechanical polishing • Electroconductive coatings • Magnetic fluid seals and recording media • Multilayer capacitors • Optical fibers • Phosphors • Quantum optical devices 	<ul style="list-style-type: none"> • Antimicrobials • Biodetection and labeling • Biomagnetic separations • Drug delivery • MRI contrast agents • Orthopedics/implants • Sunscreens • Thermal spray coatings 	<ul style="list-style-type: none"> • Automotive catalyst • Membranes • Fuel cells • Photocatalysts • Propellants • Scratch-resistant coatings • Structural ceramics • Solar cells

Table 4: Current and emerging applications of nanoparticles (source: Rittner 2002)

3.1.1.1 Carbon

Nanostructured carbon comprise long established mass produced materials like carbon black as well as relatively new compounds like fullerenes and carbon nanotubes (CNT). At present, conventional materials like carbon black are clearly dominating the world market with a sales volume of about 5 billion EURO (SRI 2002). Carbon black consists of chainlike aggregates of carbon nanoparticles, which have an average primary particles size of a few nanometers and are mainly used as fillers for rubber in car tyres or pigments in toners for photocopiers.

For CNT, which can occur single- or multiwalled, a big market potential is forecasted due to their outstanding properties, e.g. extremely high tensile strength (theoretically approx. 100 times stronger than steel) and excellent thermal and electric conductance (CMP 2003). The main barrier to a broad economic use of carbon nanotubes, e.g. in sensor technology, electronics (CNT based connects and transistors), composite materials (e.g. electrically conductive polymers) or flat screens (electron emitters in field emission displays) is due to the high price of approx. 150 EURO per gram for single wall CNT (Loefken and Mayr 2003). The high price reflects the early undeveloped stages of industrial production and purification. While the present market potential of CNT lies within the range of some million EURO, very optimistic prognoses forecast a world market size of 1 billion EURO already for the year 2006 (Fecht et al. 2003). However, these predictions will strongly depend on whether a cheap production of carbon nanotubes on an industrial scale can be implemented and significant performance gains in comparison with conventional products can be achieved.

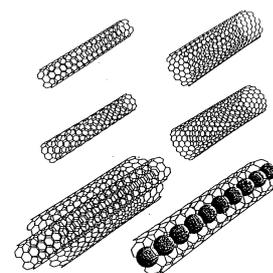


Fig. 4: Different modifications of carbon nanotubes, (single-walled, multi-walled, filled with metal atoms, etc.)

3.1.2 Nanoclays

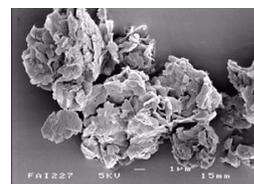
Nanostructured organically modified layer silicates (nanoclays) have been used for some time as fillers in polymers for improving barrier characteristics (e.g. gas tightness), as flame-retardant and also as mechanical reinforcement. Although some products are already on the market, problems during the manufacturing process as well as the relatively high price and only moderate performance gains impair a broad economic application of these materials. Up to the year 2006 the world market size for nano-layer silicates is estimated at 21 million EURO (SRI 2002).

3.1.3 Organic nanoparticles

Organic nanoparticles with economic relevance can be classified as follows (Horn und Rieger 2002):

- Polymer nanoparticles/-dispersions

Nanoclays as fillers for polymers



showing the morphology of nanoclay particles (source: IRC London)

Nanostructured
vitamines, pigments
and drugs with an
improved
effectiveness

- Micronised drugs and chemicals (vitamines, pigments and pharmaceuticals)
- Macro molecules (e.g. dendrimers)
- Micells, liposomes

Currently only micronised drugs, vitamins and polymer dispersions have a significant economic contribution. Through micronisation of organic compounds such as vitamins, pigments and pharmaceuticals, which often have a low solubility in water and require special formulation procedures when applied in aqueous solution, the increased surface-to-volume ratio improves the water solubility significantly and thus optimises the physiological (in pharmacy, cosmetic, crop protection, nutrition) or technological effectiveness (e.g. in lacquers and printing inks). Such nanoparticles can be made by mechanical milling or precipitation and/or condensation of colloidal solutions. The world market potential for organic nanoparticles (in particular vitamins) has been estimated to approx. 1 billion EURO in the year 2002 (Ebenau 2002).

A still larger market with approx. 15 billion EURO in the year 2002 exhibit aqueous polymer dispersions (Distler 2002). These material class is long established in industry but can be optimised by application of modern nanotechnological procedures, e.g. increasing the solid content due to a controlled particle size distribution, selective surface modification of the polymers or the production of nanocomposites by mixing with organic or inorganic fillers. Such polymer dispersions offer broad application fields, e.g. as binders in colors and lacquers, adhesives for labels and tapes or as coating systems for textiles, wood or leather. Beside that, aqueous polymer dispersions are more environmentally benign than products, which are based on organic solvents.

Organic macromolecules such as dendrimers and hyperbranched polymers (e.g. on polyurethane basis) are used in the niche markets but might have a promising future (Bruchmann 2002). Application potentials of dendritic molecules can be seen for example as supports for catalysts or pharmaceutical active substances (Drug Delivery) or as cross-linking materials for scratch-proof autolacquers or printing inks. The world-wide market potential of dendrimers is estimated at 5-15 million EURO in the year 2006 (SRI 2002).

3.2 Nanocomposites

Nanoparticles and –fibers are often used as reinforcement for other material classes such as polymers, ceramic or metals to yield nanocomposites with special properties.

3.2.1 Polymer nanocomposites

Polymer nanocomposites comprises block copolymers as well as polymer materials, which are doped with ceramic, silicates, metal or also semiconductor nanoparticles. The incorporation of nanoparticles into the polymer matrix serves the improvement of material properties e.g. (thermo-)mechanical and electronic characteristics. The following examples can be mentioned:

- Nanoclay doped polymers for improvement of barrier properties (e.g. gas tightness), as flame retardant or mechanical reinforcement
- Nanoparticle doped epoxies as insulation for electric car cables or for improved resins in coils
- Electric conductive polymers, e.g. doped with carbon black or henceforth with carbon nanotubes, for applications as electrostatic shielding of electronic devices, etc.
- Nanoparticle doped (e.g. silver) polymers with antimicrobial properties for applications in medicine and hygiene

In the medium-term a strong market growth is expected for the world market of polymer nanocomposites from 13 mill. EURO in 2001 up to 250 mill. EURO in 2006 (SRI 2002).

3.2.2 Metal matrix composites

By reinforcement of metals with ceramic fibers, in particular silicon carbide, but also alumina oxide or aluminum nitride, their thermo-mechanical properties can be improved significantly. Such metal matrix composites (MMC), e.g. SiC in aluminum alloys or TiN in Ti/Al alloys, possess due to their high heat resistance, hardness, thermal conductivity, controllable thermal expansion and low density, a high potential for structural applications in aerospace or the automotive sector.

3.2.3 Ceramic nanocomposites

Within ceramic nanomaterials a special focus lies on the production of controlled micro/nano-structured grain sizes, the production of gradient materials as well as application of nanostructured coatings and surface functionalisation. One objective is the improvement of thermomechanical properties, fracture toughness and formability ("super-plasticity") of this brittle material class. In addition, the sintering temperatures and the consolidation time of ceramic materials can be reduced by applying nanopowders, which saves not only money but also allows new manufacturing techniques like coprocessing of ceramics and metals. Ceramic nanopowders meanwhile can be manufactured with high chemical purity and adjustable powder grain size. Both gas or liquid phase processes are used for the production of ceramic nanopowders, for non-oxidic powders (e.g. Si_3N_4 , SiC, TiCN) preferentially gas phase processes and for oxidic powders (e.g. Al_2O_3 , SiO_2) also sol gel

Nanoparticle filled polymers with improved mechanical and electric properties

procedures. A further relevant topic are nanostructured gradient materials, in which the gradient can be adjusted both regarding thermomechanical or chemical properties. These materials could be used for example in the production of photonic structures in optical data communication or in the production of micromechanical and microelectronic components with a high degree of miniaturisation.

3.2.4 Application fields

The following table gives an overview on potential markets, market segments and products based on nanoparticulate materials.

Automotive industry <ul style="list-style-type: none"> • lightweight construction • painting (fillers, base coat, clear coat) • catalysts • tires (fillers) • sensors • Coatings for wind-screen and car bodies 	Chemical industry <ul style="list-style-type: none"> • fillers for paint systems • coating systems based on nanocomposites • impregnation of papers • switchable adhesives • magnetic fluids 	Engineering <ul style="list-style-type: none"> • wear protection for tools and machines (anti blocking coatings, scratch resistant coatings on plastic parts, etc.) • lubricant-free bearings
Electronic industry <ul style="list-style-type: none"> • data memory (MRAM, GMR-HD) • displays (OLED, FED) • laser diodes • glass fibres • optical switches • filters (IR-blocking) • conductive, antistatic coatings 	Construction <ul style="list-style-type: none"> • construction materials • thermal insulation • flame retardants • surface-functionalised building materials for wood, floors, stone, facades, tiles, roof tiles, etc. • facade coatings • groove mortar 	Medicine <ul style="list-style-type: none"> • drug delivery systems • active agents • contrast medium • medical rapid tests • prostheses and implants • antimicrobial agents and coatings • agents in cancer therapy
Textile/fabrics/non-wovens <ul style="list-style-type: none"> • surface-processed textiles • smart clothes 	Energy <ul style="list-style-type: none"> • fuel cells • solar cells • batteries • capacitors 	Cosmetics <ul style="list-style-type: none"> • sun protection • lipsticks • skin creams • tooth paste
Food and drinks <ul style="list-style-type: none"> • package materials • storage life sensors • additives • clarification of fruit juices 	Household <ul style="list-style-type: none"> • ceramic coatings for irons • odors catalyst • cleaner for glass, ceramic, floor, windows 	Sports /outdoor <ul style="list-style-type: none"> • ski wax • antifogging of glasses/goggles • antifouling coatings for ships/boats • reinforced tennis rackets and balls

Table 5: Overview on applications of nanomaterial based products in different areas

4 PRODUCTION METHODS

There are two general ways available to produce nanomaterials (Moriarty 2001, Schmid et al. 1999) as shown in the following figure. The first way is to start with a bulk material and then break it into smaller pieces using mechanical, chemical or other form of energy (top-down). An opposite approach is to synthesise the material from atomic or molecular species via chemical reactions, allowing for the precursor particles to grow in size (bottom-up). Both approaches can be done in either gas, liquid, supercritical fluids, solid states, or in vacuum (Mayo 1993, 1996). Most of the manufacturers are interested in the ability to control: a) particle size b) particle shape c) size distribution d) particle composition e) degree of particle agglomeration.

Two basic approaches to produce nanomaterials: „top-down“ and „bottom-up“

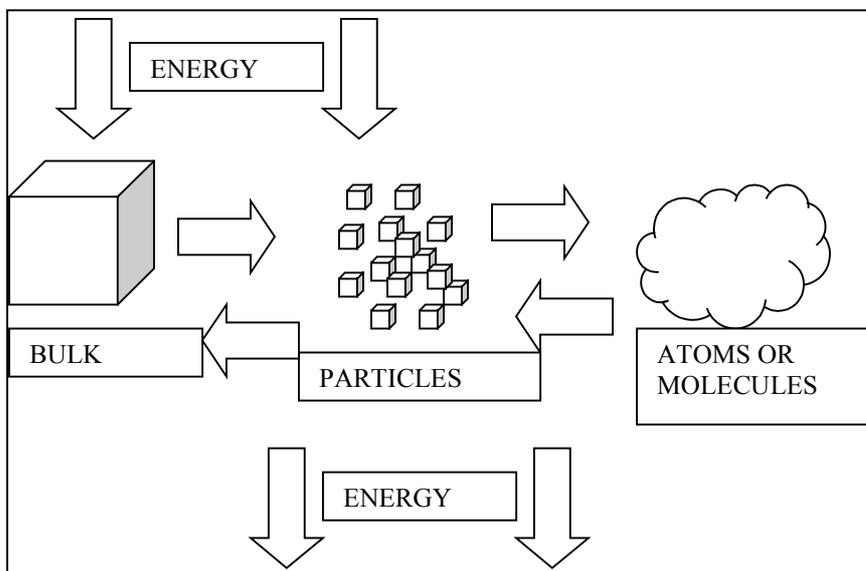


Figure 6: Two basic approaches to nanomaterials fabrication: top-down (shown here from left to the right) and bottom-up (from right to the left)

4.1 Top-down approaches

Methods to produce nanoparticles from bulk materials include high-energy ball milling, mechano-chemical processing, etching, electro-explosion, sonication, sputtering and laser-ablation. These processes are done in an inert atmosphere or in vacuum. Immediately after processing nanoparticles are very reactive and can easily form agglomerates. If a reactive gas is present some additional reactions may occur. This can be used to coat nanoparticles with a material that would prevent further interaction with other particles or the environment. In the following a more detailed description of the basic nanomaterials manufacturing techniques from bulk to nano are presented below.

4.1.1 Mechanical milling

Mechanical milling is a process which is routinely used in powder metallurgy and mineral processing industries. In this process, mixtures of elemental or prealloyed powders are subjected to grinding under protective atmosphere in equipment capable of high-energy compressive impact forces such as attrition or shaker mills.

A variety of ball mills have been developed for different purposes including tumber mills, attrition mills, shaker mills, vibratory mills, planetary mills, etc. Powders with typical particle diameters of about 50 μm are placed together with a number of hardened steel or tungsten carbide (WC) coated balls in a sealed container which is shaken or violently agitated. Since the kinetic energy of the balls is a function of their mass and velocity, dense materials are preferable to ceramic balls. During the continuous severe plastic deformation associated with high-energy mechanical attrition, a continuous refinement of the internal structure of the powder particles to nanometer scales occurs.

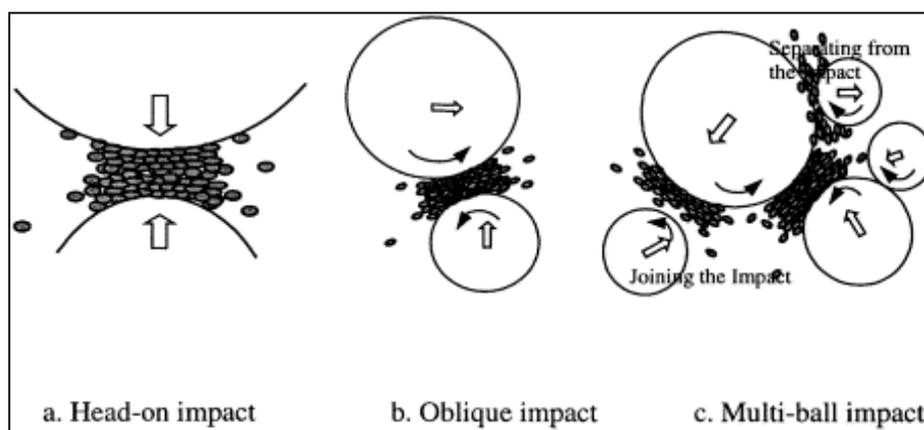


Figure 7: Schematic diagram showing the different forms of impact which might occur during high-energy ball milling (Zhang 2003)



placing a milling vial into planetary type ball mill. Starting powder and milling balls are inside tightly closed milling vial filled with inert gas (source: VTT)

When a single phase elemental powder or intermetallic compound powder is milled, the grain size of the powder particles continues to decrease until it reaches a minimum level – in the range of 3–25 nm. For some intermetallic compounds, the powder becomes amorphous beyond this point. For intrinsic brittle powders, such as silicon powder or carbide and oxide powders, the reduction of the grain size is a natural outcome of the transgranular fracturing and cold welding, and the minimum grain size is determined by the minimum grain size which does not allow nucleation and propagation of cracks within grains. No study has been seen which attempts to theoretically determine this minimum grain size. Very important advantage of the mechanical milling process is that the processing temperature is low, so the newly formed grains grow very slowly.

Mechanical attrition methods allow the preparation of alloys and composites which can not be synthesised via conventional casting routes, e.g. uniform dispersions of ceramic particles in a metallic matrix and alloys of metals with quite different melting points with the goal of improved strength and corrosion resistance. Mechanical attrition has also gained a lot of attention as a nonequilibrium process resulting in solid-state alloying beyond the equilibrium solubility limit and the formation of amorphous or nanostructured materials for a broad range of alloys, intermetallics, ceramics and composites (Edelstein and Cammarata 1996).

High-energy mechanical milling is a very effective process for synthesizing metal–ceramic composite powders as it allows incorporation of the metal and the ceramic phases into each powder particle, as shown schematically in the figure below.

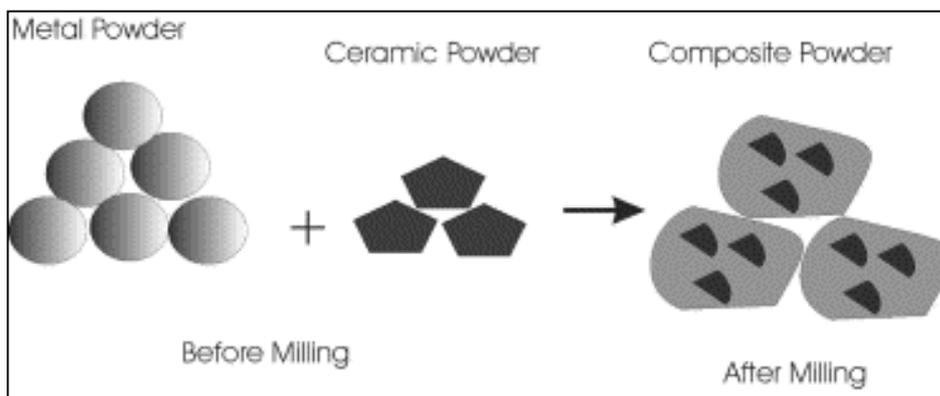


Figure 9: Schematic diagram showing the formation of composite powder after high-energy mechanical milling (Zhang 2003)

As previously mentioned high-energy mechanical milling can be used to produce nanopowders. There are two routes for producing nanopowders using mechanical milling: (a) milling a single phase powder and controlling the balance point between fracturing and cold welding, so that particles larger than 100 nm will not be excessively cold welded; and (b) producing nanopowders using mechanochemical processes.

Mechanochemical Processing (MCP) is a novel, cost effective method of manufacturing a wide range of nanopowders. MCP can most simply be described as the use of a conventional ball mill as a low temperature chemical reactor. It is important to realise that the ball mill is not being used as a simple grinding tool. Instead, the ball mill increases the reaction kinetics in the reacting powder mixture as a result of the intimate mixing and refinement of the grain structure to the nanometer scale, allowing the reaction to occur during the actual milling. Chemical reactions, which normally require high temperatures, are thus activated during milling. This is the key element of the MCP technology.

To produce nanoparticles of a specific material, a suitable precursor is chosen. Often a particular product can be produced from a range of precursors allowing the process to be optimised to use industry standard precursors to reduce cost. Oxides, carbonates, sulphates, chlorides, fluorides, hydroxides or other compounds are all candidates for use as the precursor material. The chosen precursor is then milled with an appropriate reactant. The resulting product phase is formed as individual single nanometer sized grains in a by-product matrix. After milling a low temperature heat treatment is often used to ensure the reaction is complete before the by-product is removed, leaving the pure, non-agglomerated nanopowder, which consists of dispersed nano-sized particles of 1-1000 nm in diameter (Froes et al. 2001).

One simple example is described as follows: The process starts by high-energy milling a mixture of FeCl_3 powder and Na pieces. The milling induces a reaction between FeCl_3 and Na, forming Fe nanoparticles mixed with NaCl. The NaCl can be easily leached out from the powder by using water, and Fe nanopowder is produced (Ding et al. 1995).

4.1.2 Etching (chemical)

A combination of lithographically defined patterning with etching is a basis of microelectronics. Regular arrays of the nanometer-sized structures can be produced on a planar substrate. Unmasked electrochemical or photo-electrochemical etching can be used to produce regular arrays of shapes within nanometer range. For example, layers of porous silicon are formed by electrochemically etching the crystalline silicon wafers, employing a mixture of hydrofluoric acid and ethanol as an electrolyte. Another example is porous alumina.

4.1.3 Electro-explosion (thermal/chemical)

Electro-explosion involves providing a very high current over a very short time through thin metallic wires, in either an inert or reactive gas, such that extraordinary temperatures are achieved. The wire is converted into a plasma state, but the plasma is contained and is in fact compressed by the very high fields produced during the pulse. The very high currents heat the wire to 20.000 – 30.000 degrees, and at these temperatures the resistivity of the metal becomes virtually infinite, terminating the flow of current. At that point the electromagnetic field disappears and superheated metal plasma expands with supersonic velocity creating a shock wave in the ionised gas surrounding the wire. The extremely fast cooling (10^6 to 10^8 deg/sec) rate provides ideal conditions for stabilisation of different metastable structures.

The process of electro-explosion of wire has prepared metallic powders of approximately 100 nanometers, where an electric power impulse is applied to the wire under argon pressure. The resulting powders have

greater chemical and metallurgical reactivity as compared to other powders. They also have internal strain and surface energies that are released as the powders go through a transformation from their active as-produced state to form sub-micron spheres. When turned into pellets and heated to their transition temperatures, which is ordinarily well below their melting points, they will release heat to cause the compacts to "self-sinter". Their reactivity allows alloying to occur at substantially reduced temperatures. Examples include a mixture of electro-exploded aluminium and amorphous boron, which react to form aluminium diboride by igniting the pellet with an electric wire, and where a pellet of electro-exploded copper and zinc will react at 200° C to form brass directly (Argonide 2004).

4.1.4 Sputtering (kinetic)

The impact of an atom or ion on a surface produces sputtering from the surface as a result of the momentum transfer from the incoming particle. Unlike many other vapour phase techniques there is no melting of the material. Sputtering is done at low pressure on a cold substrate.

4.1.5 Laser ablation (thermal)

In laser ablation, pulsed light from an excimer laser is focused onto a solid target inside a vacuum chamber to "boil off" a plume of energetic atoms of the target material (Ullmann et al. 2002). A substrate positioned to intercept the plume will receive a thin film deposit of the target material. This phenomenon was first observed with a ruby laser in the mid-1960s. Because this process then contaminated the films made with particles, little use was found for such "dirty" samples.

A broad range of nanoparticulate materials can be obtained by laser ablation

Laser ablation method has the following advantages for the fabrication of nanomaterials:

a) the fabrication parameters can be easily changed in a wide range b) nanoparticles are naturally produced in a laser ablation plume so that the production rate is relatively high c) virtually all materials can be evaporated by laser ablation. A modification of this technique includes laser ablation of microparticles (LAM), which helps to reduce size dispersion¹.

4.2 Bottom-up approaches

Methods to produce nanoparticles from atoms are chemical processes based on transformations in solution e.g. sol-gel processing, chemical vapour deposition (CVD), plasma or flame spraying synthesis, laser pyrolysis, atomic or molecular condensation. These chemical processes

¹ http://www.ph.utexas.edu/~laser/laser_stuff/nano1/Nano-Web.html

rely on the availability of appropriate “metal-organic” molecules as precursors. Sol-gel processing differs from other chemical processes due to its relatively low processing temperature. This makes the sol-gel process cost-effective and versatile. In spraying processes the flow of reactants (gas, liquid in form of aerosols or mixtures of both) is introduced to high-energy flame produced for example by plasma spraying equipment or carbon dioxide laser. The reactants decompose and particles are formed in a flame by homogeneous nucleation and growth. Rapid cooling results in formation of nanoscale particles.

These are chemical processes to materials based on transformations in solution such as sol-gel processing, hydro or solvo thermal syntheses, Metal Organic Decomposition (MOD), or in the vapour phase chemical vapour deposition (CVD). Most chemical routes rely on the availability of appropriate “metal-organic” molecules as precursors. Among the various precursors of metal oxides namely metal b-diketonates and metal carboxylates, metal alkoxides are the most versatile. They are available for nearly all elements and cost-effective synthesis from cheap feedstock have been developed for some.

Two general ways are available to control the formation and growth of the nanoparticles. One is called arrested precipitation and depends either on exhaustion of one of the reactants or on the introduction of the chemical that would block the reaction. Another method relies on a physical restriction of the volume available for the growth of the individual nanoparticles by using templates.

4.2.1 Sol-gel

The sol gel technique is a long established industrial process for the generation of colloidal nanoparticles from liquid phase, that has been further developed in last years for the production of advanced nanomaterials and coatings (e.g. Yu. 2001, Fendler 2001, Meisel 1997). Sol-gel-processes are well adapted for oxide nanoparticles and composites nanopowders synthesis. The main advantages of sol-gel techniques for the preparation of materials are low temperature of processing, versatility, flexible rheology allowing easy shaping and embedding. They offer unique opportunities for access to organic-inorganic materials. The most commonly used precursors of oxides are alkoxides due to their commercial availability and to the high liability of the M-OR bond allowing *facile tailoring in situ* during processing.²

Sol-gel process is a long established method for producing nanopowders

² <http://www.solgel.com/articles/jun02/preintro.asp>

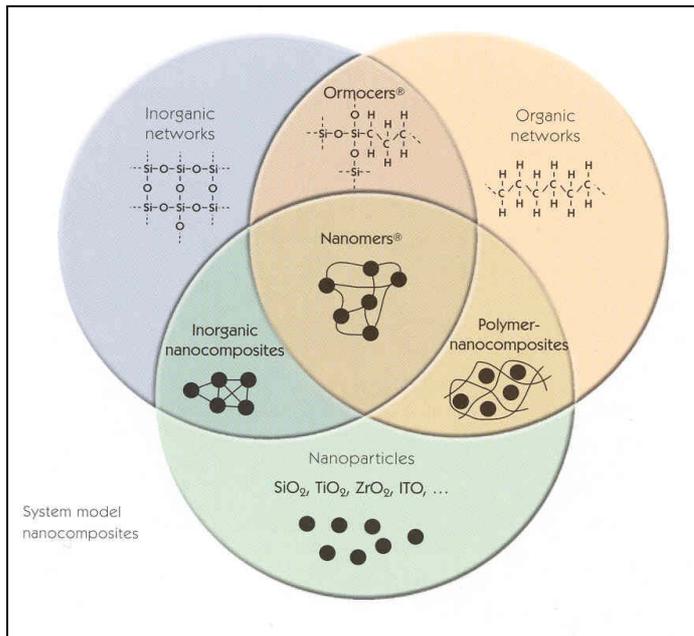


Figure 10: System model for nanocomposites produced by sol-gel (source: Fraunhofer IST).

4.2.2 Aerosol based processes

Aerosol based processes are a common method for the industrial production of nanoparticles (e.g. Gurav 1993, Kammler 2001, Pratsinis 1998). Aerosols can be defined as solid or liquid particles in a gas phase, where the particles can range from molecules up to 100 μm in size. Aerosols were used in industrial manufacturing long before the basic science and engineering of the aerosols were understood. For example, carbon black particles used in pigments and reinforced car tires are produced by hydrocarbon combustion; titania pigment for use in paints and plastics is made by oxidation of titanium tetrachloride; fumed silica and titania formed from respective tetrachlorides by flame pyrolysis; optical fibres are manufactured by similar process (Kodas and Hampden-Smith 1999).

Traditionally spraying is used either to dry wet materials or to deposit coatings. Spaying of the precursor chemicals onto a heated surface or into the hot atmosphere results in precursor pyrolysis and formation of the particles. For example, a room temperature electro-spraying process was developed at Oxford University to produce nanoparticles of compound semiconductors and some metals. In particular, CdS nanoparticles were produced by generating aerosol micro-droplets containing Cd salt in the atmosphere containing hydrogen sulphide.

4.2.3 Chemical vapour deposition

CVD consists in activating a chemical reaction between the substrate surface and a gaseous precursor. Activation can be achieved either with

temperature (Thermal CVD) or with a plasma (PECVD : Plasma Enhanced Chemical Vapour Deposition). The main advantage is the non-directive aspect of this technology. Plasma allows to decrease significantly the process temperature compared to the thermal CVD process. CVD is widely used to produce carbon nanotubes (Meyyappan et al. 2003).

4.2.4 Atomic or molecular condensation

This method is used mainly for metal containing nanoparticles. A bulk material is heated in vacuum to produce a stream of vaporised and atomised matter, which is directed to a chamber containing either inert or reactive gas atmosphere. Rapid cooling of the metal atoms due to their collision with the gas molecules results in the condensation and formation of nanoparticles. If a reactive gas like oxygen is used then metal oxide nanoparticles are produced.

The theory of gas-phase condensation for the production of metal nanopowders is well known, having been first reported in 1930³. Gas-phase condensation uses a vacuum chamber that consists of a heating element, the metal to be made into nano-powder, powder collection equipment and vacuum hardware.

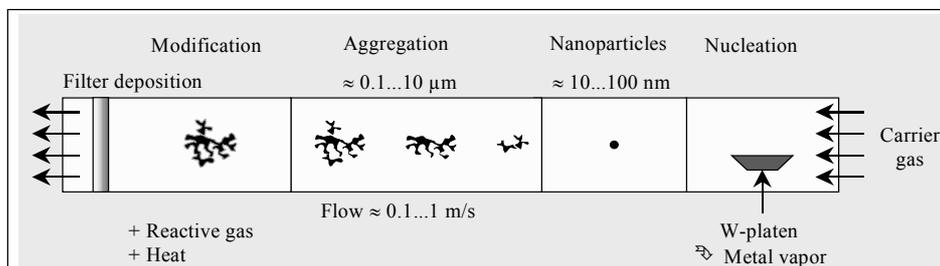


Figure 11: Principle of Inert Gas Condensation method for producing nanoparticulate material (source: FHG-IFAM, Bremen)

The process utilises a gas, which is typically inert, at pressures high enough to promote particle formation, but low enough to allow the production of spherical particles. Metal is introduced onto a heated element and is rapidly melted. The metal is quickly taken to temperatures far above the melting point, but less than the boiling point, so that an adequate vapour pressure is achieved. Gas is continuously introduced into the chamber and removed by the pumps, so the gas flow moves the evaporated metal away from the hot element. As the gas cools the metal vapour, nanometer-sized particles form. These particles are liquid since they are still too hot to be solid. The liquid particles collide and coalesce in a controlled environment so that the particles grow to specification, remaining spherical and with smooth surfaces. As the liquid particles are

³ A.H. Pfund, Phys. Rev. (1930)1434

further cooled under control, they become solid and grow no longer. At this point the nanoparticles are very reactive, so they are coated with a material that prevents further interaction with other particles (agglomeration) or with other materials.

4.2.5 Supercritical fluid synthesis

Methods using supercritical fluids are also powerful for the synthesis of nanoparticles. For these methods, the properties of a supercritical fluid (fluid forced into supercritical state by regulating its temperature and its pressure) are used to form nanoparticles by a rapid expansion of a supercritical solution. Supercritical fluid method is currently developed at the pilot scale in a continuous process.

4.2.6 Spinning

An emerging technology for the manufacture of thin polymer fibers is based on the principle of spinning dilute polymer solutions in a high-voltage electric field. Electro spinning is a process by which a suspended drop of polymer is charged with thousands of volts. At a characteristic voltage the droplet forms a Taylor cone, and a fine jet of polymer releases from the surface in response to the tensile forces generated by interaction of an applied electric field with the electrical charge carried by the jet. This produces a bundle of polymer fibers. The jet can be directed to a grounded surface and collected as a continuous web of fibers ranging in size from a few μm 's to less than 100 nm.⁴

4.2.7 Use of templates

Any material containing regular nano-sized pores or voids can be used as a template to form nanoparticles. Examples of such templates include porous alumina, zeolites, di-block co-polymers, dendrimers, proteins and other molecules. The template does not have to be a 3D object. Artificial templates can be created on a plane surface or a gas-liquid interface by forming self-assembled monolayers (Huczko 2000).

⁴ <http://www.polymers.dk/research/posters/ElectrospinningSUH.pdf>

Self Assembly of
nanomaterials using
biomolecular
recognition and
selfordering
principles

4.2.8 Self-assembly

Nanoparticles of a wide range of materials- including a variety of organic and biological compounds, but also inorganic oxides, metals, and semiconductors- can be processed using chemical self-assembly techniques (Meier 2000, Zhang 2002, Shimizu 2003, Shimomura 2000, Tomalia 1999, Fendler 2001). These techniques exploit selective attachment of molecules to specific surfaces, biomolecular recognition and selfordering principles (e.g. the preferential docking of DNA strands with complementary base pairs) as well as well-developed chemistry for attaching molecules onto clusters and substrates (e.g. thiol (-SH) end groups) and other techniques like reverse micelle, sonochemical, and photochemical synthesis to realise 1-D, 2-D and 3-D self-assembled nanostructures. The molecular building blocks act as parts of a jigsaw puzzle that join together in a perfect order without an obvious driving force present. Long-term and visionary nanotechnological conceptions however go far beyond these first approaches. This applies in particular to the development of biomimetic materials with the ability of self organisation, self healing and self replication by means of molecular nanotechnology. One objective here is the combination of synthetic and biological materials, architectures and systems, respectively, the imitation of biological processes for technological applications. This field of nanobiotechnology is at present still in the state of basic research, but is regarded as one of the most promising research fields for the future (European Commission 2001).

4.3 Stabilisation and functionalisation of nanoparticles

Due to their high reactivity nanoparticles have a high tendency to build aggregates resp. agglomerates, which could lead to a loss of the desired properties. Therefore it is often necessary to stabilise the nanoparticles with additional treatments. The commercial success or failure of nanoparticles in a particular application usually depends upon the ability to prepare stable dispersions in water or organic fluids with controlled rheology. In turn, the ability to prepare stable nanoparticle dispersions with controlled rheology is enabled by tailoring nanoparticle coatings. On the other hand coating nanoparticles with another material of nanoscale thickness is a simple way to alter the surface properties of nanoparticles. Core-shell structured nanoparticles have been shown to display advanced optical, mechanical and magnetic properties.

One common method to stabilise or modify the reactivity of the nanoparticle is the encapsulation with a molecular or polymeric layer. A thin polymeric shell enables compatibility of the particles with a wide variety of fluids, resins and polymers (Bourgeat-Lami 2002). In this way, the nanoparticles retain their original chemical and physical properties, but the coating can be tailored for wide variety of applications and

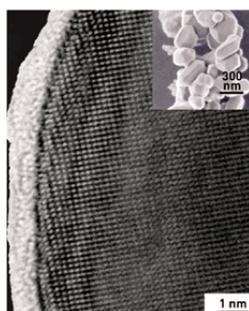


Fig. 12: Titania particle coated with a nanometer thick silica layer, the inset shows the particle morphology, (source: American Chemical Society)

environments, ranging from extremely non-polar (hydrophobic) to very polar systems (Gerfin et al. 1997). One example is the Discrete Particle Encapsulation (DPE) method patented by Nanophase Technologies Corporation⁵.

Another way to ensure the stability of the collected nanoparticle powders against agglomeration, sintering, and compositional changes is to collect the nanoparticles in a liquid suspension. For semiconducting particles, stabilisation of the liquid suspension has been demonstrated by the addition of polar solvent; surfactant molecules have been used to stabilise the liquid suspension of metallic nanoparticles (Sailor and Lee 1997). Alternatively, inert silica encapsulation of nanoparticles by a gas-phase reaction and by oxidation in colloidal solution has been shown to be effective for metallic nanoparticles (Mulvaney et al. 2000). For carbon nanotubes which are usually generated as mixtures of solid morphologies that are mechanically entangled or that self-associate into aggregates it is often necessary to disperse the CNT in fluid suspensions to obtain a regular orientation in the composite material resulting in unique mechanical or electrical characteristics. Milling, ultrasonication, high shear flow, elongational flow, functionalisation, and surfactant and dispersant systems are used to affect the morphologies of carbon nanotubes and their interactions in the fluid phase (Hilding et al. 2003).

Collecting nanoparticles in liquid suspension to prevent agglomeration

Nanoparticles dispersed in aqueous solutions also tend to build aggregates due to attractive van der Waals forces. By altering the dispersing conditions repulsive forces can be introduced between the particles to prevent the aggregation. There are two general ways of stabilising nanoparticles in aqueous solutions. Firstly by adjusting the pH of the system the nanoparticle surface charge can be manipulated in such way that an electrical double layer is generated around the particle. Overlap of two double layers on different nanoparticles causes repulsion and hence stabilisation. The magnitude of this repulsive force can be measured via the zeta potential. The second method involves the adsorption of polymers onto the nanoparticles in such way that the particles are physically prevented from coming close enough for the van der Waals attractive force to dominate. This is termed steric stabilisation. A combination of these two mechanisms is called electrosteric stabilisation and occurs when polyelectrolytes are adsorbed on the nanoparticle surface (Caruso 2001).

⁵ www.nanophase.com

5 CHARACTERISATION AND DETECTION TECHNIQUES

One essential prerequisite for the development, manufacturing and commercialisation of nanomaterials is the availability of techniques, which allow the characterisation of their physical, chemical and biological properties on a nanoscale level. Powerful analytical detection and characterisation methods are also the basis of a risk assessment of nanomaterials to investigate how nanomaterials behave under different chemical and physical conditions, how they move and distribute in different environmental compartments like water, soil and air and how they interact with the biosphere and the human organism.

Meanwhile there is a considerable arsenal of detection and characterisation methods for nanomaterials. These methods are normally used in research laboratories for the study of nanomaterial properties. However, most of them are not suitable for the realisation of systematic on-line measurements for safety analyses (i.e. detection in a continuous mode in industrial environment). For example, microscopy methods as well as X-rays spectroscopies are very powerful methods for the determination of nanoparticles characteristics but their use requires large instruments, UHV (Ultra-High Vacuum) and extensive sample preparation. Moreover, they are not adapted to continuous analysis for safety purposes. In the following sections the specification and limitations of the main methods used for nanomaterial characterisation will be briefly summarised.

Most detection methods are not suitable for a continuous online monitoring of nanoparticles

5.1 Atomic structure and chemical composition

The following paragraph presents some methods for the determination of atomic structure and chemical composition of solid or liquid nanomaterials. Though the techniques presented below are not specifically used for nanomaterials, they can provide valuable information on nanoscale material properties, which can differ significantly from the bulk properties.

5.1.1 Spectroscopic methods

Spectroscopic methods such as vibrational, nuclear magnetic resonance, X-ray and UV spectroscopies have been extensively used for the characterisation of nanomaterials. The following paragraphs summarise some examples.

5.1.1.1 Vibrational spectroscopies

Vibrational spectroscopies comprise Fourier Transform Infrared (FTIR) spectroscopy and Raman Scattering (RS). These two methods are used to investigate vibrational structure of molecules or solids. FTIR is well adapted for organic compounds and is extensively used for the

characterisation of carbon nanoparticles for the detection of fullerenes or Polycyclic Aromatic Hydrogenated species (PAH). Both methods can be performed on dry powders or on liquid suspensions. For FTIR, the absorption spectra can be deduced from transmission measurements through a KBr pellet with entrapped nanoparticles or directly on nanoparticles in a reflection mode measurement (DRIFT).

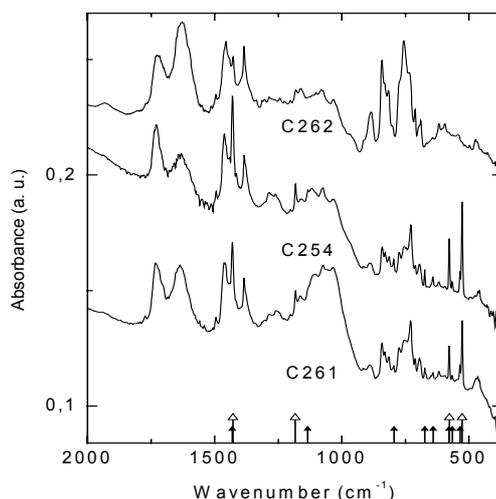


Figure 13: FTIR spectra of different laser-synthesised carbon black samples with varying fullerene content, fullerene signatures are indicated by the arrows (Tenegal et al. 2003)

FTIR can be also used to determine the the crystallisation and grain sizes in ceramic powders e.g. Si/C/N composites, where the spectra of the nanostructured powders differ significantly from the coarser bulk material (Dez et al. 2002).

5.1.1.2 Nuclear magnetic resonance

High resolution liquid and solid state NMR is another tool that has been widely adapted for the characterisation of nanomaterials. To be mentioned here are the characterisation of zeolites (Zhang et al. 1999), the investigation of nanoscale effects such as hydrogen-bonding and transfer (Limbach 2002) or the surface properties and chemistry of nanolayer systems (Liu et al 2003).

5.1.1.3 X-ray and UV spectroscopies

X-ray and UV spectroscopies are used to investigate the electronic structure of materials to deduce their atomic structure. Core levels, valence and conduction band are probed using an X-ray or a UV excitation. X-ray Photoemission Spectroscopy (XPS) or Electron Spectroscopy for Chemical Analysis (ESCA) refers to the photoemission of electrons produced by a monochromatic X-ray or UV beam (Briggs

1983). XPS spectrometers measure the kinetic energies of the electrons. Due to the limited mean free path of the electrons in matter, only few nanometric layers are investigated. Since binding energies are highly sensitive to chemical bonding, a map of the bonding configuration is obtained for surface layers. With the photoemission cross-sections, chemical compositions of the surface material can be calculated and compared to bulk chemical compositions. Surface values can differ significantly as shown in the following table for Si/C/N/O nanopowders (Gheorghiu et al. 1997).

	Si	C	N	O
Chem. anal. (bulk)	40 %	21 %	37 %	2 %
XPS (surface)	31.6 %	32.6 %	28.2 %	7.6 %

Table 6: XPS chemical composition. O and C atoms are concentrated at the surface of the nanopowders

Another X-ray method used to investigate the conduction band of materials is X-ray Absorption Spectroscopy (XAS), which involves Extended X-ray Absorption Fine Structure (EXAFS) and X-ray Absorption Near Edge Structure (XANES). The principle is based on the absorption of a monochromatic X-ray beam by a core shell electron of selected atomic species inside a sample. By changing the energy of the incident beam around the binding energy, modulation of the absorption cross section are observed and interpreted by an interference phenomenon between the wave associated with the emitted electron and the scattered waves emitted by the neighbouring atoms. XAS methods are selective and well adapted for samples with low crystallinity. They are local order techniques useful to follow the early stages of the crystallisation of amorphous nanoparticles. The absorption spectra can be measured by recording either the attenuation of an incident beam (transmission), the electron yield or the fluorescence yield. In the two last cases, modulations of the yield with the energy of the incident beam are that of the absorption cross section. For the electron yield, the information is more sensitive to surface for the same reasons as for XPS. Measurements using the fluorescence yield are efficient to probe the local environment of atomic species in diluted samples. They can be performed on dry powders or on liquid suspensions. However, an important drawback is that performing XAS measurements require Synchrotron Radiation Facilities (SRF).

5.1.2 X-ray and neutron diffraction

To characterise nanoparticles atomic structure at larger scales, diffraction techniques appear as the most powerful methods. They are based on the

diffraction of an incident beam (X-ray, neutrons) by reticular planes of the crystalline phases inside a sample. The beam (X-ray or neutrons) is diffracted at specific angular positions with respect to the incident beam depending on the phases of the sample. When crystal size is reduced toward nanometric scale, then a broadening of diffraction peaks is observed and the width of the peak is directly correlated to the size of the nanocrystalline domains (Debye-Scherrer relation).

XRD and neutron diffraction are complementary methods used to obtain a contrast effect on the diffracted beam. Indeed, diffracted intensities are modulated by weighting factors, which differ between X-ray and neutron due to the difference in the nature of interaction.

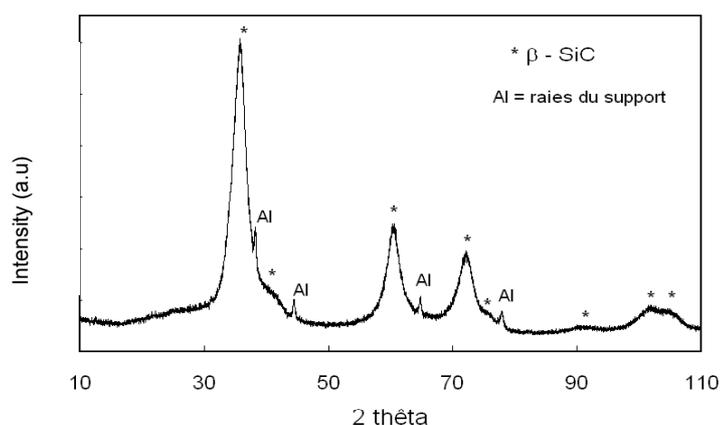


Figure 14: XRD pattern of a Si/C/N nanopowder. The broad peaks correspond to the crystalline β -SiC phase. The Debye Scherrer analysis gives a crystal size of 5 nm β -SiC crystals are mixed with an amorphous phase (source: CEA)

If the crystal size continues to decrease, peaks become broader until they transform into a smooth oscillation (for amorphous structures), which can be measured only with a sufficient bright beam of X-rays. Then, the modulations give rise to a total radial distribution function by a Fourier transformation. In this last case, the method is called Wide Angle X-ray Scattering (WAXS) and is performed only on a SRF as for XAS. Neutron diffraction requires also neutron facilities. At small angles (SAXS and neutron), particles size can be obtained. XRD, WAXS, SAXS and neutrons can be performed on dry nanopowders or on liquid suspensions.

5.2 Determination of size, shape and surface area

5.2.1 Electron microscopies

Electron microscopies are methods of choice to investigate particles size, shape and structure and also agglomerates. They regroup two techniques: Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM).

In SEM experiments, electrons emitted from a filament are reflected by the sample and images are formed using either secondary electrons or backscattered electrons. However, in the case of SEM, a field emission microscope (FE-SEM) is necessary to investigate the nanometric scale (electrons are emitted from a field-emission gun). FE microscopes could reach resolutions of the order of 1 nm using a cold cathode. If they are equipped with an Energy Dispersive Spectrometer (EDS), chemical composition can be obtained. Then, size distribution, shape and chemical composition of nanoparticles can be investigated by FE-SEM.

In TEM experiments, electrons pass through the sample and the transmitted beam is used to build the images. As for FE-SEM, shape and size distribution can be obtained by TEM. Size distribution (with low statistic) can be obtained by counting the number of particles as a function of the size on the micrograph. At lower magnifications, the way in which nanoparticles are connected can be observed. Then, qualitative information about the agglomerates structure is deduced from observations.

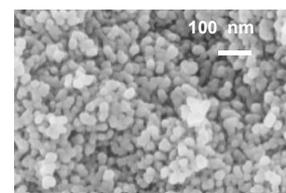


Fig. 15: FE-SEM image of cold-compacted Si/C/N nanopowders (source: CEA)

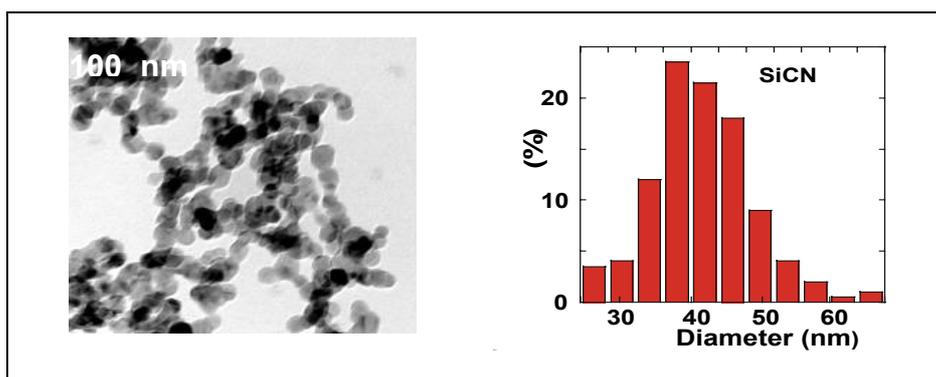


Figure 16: left: shape determination of SiC nanostructures, right: size distribution of Si/C/N nanoparticles derived from TEM Measurements (source: CEA)

The TEM resolution is below 1 nm for the High Resolution Microscopes (HRTEM). High resolution is performed to look at crystal quality and interfaces. EDS can be used for chemical composition determination.

STEM microscopes are field emission gun scanning/transmission electron microscopes. The STEM combines the features of both TEM and SEM. Analysis can be performed in transmission mode or in scanning mode. In scanning mode, a high brightness source produces a focused beam with high current density and small diameter for EDS microanalysis. Spatial resolution for microanalysis (about 2 nm for thin specimens) is much better than it is for microanalysis in SEM for bulk samples (about 0.5-3 microns). With STEM, EELS (Electron Energy Loss Spectroscopy) can be performed. This method allows measurements of the concentration profile of nanoparticles.

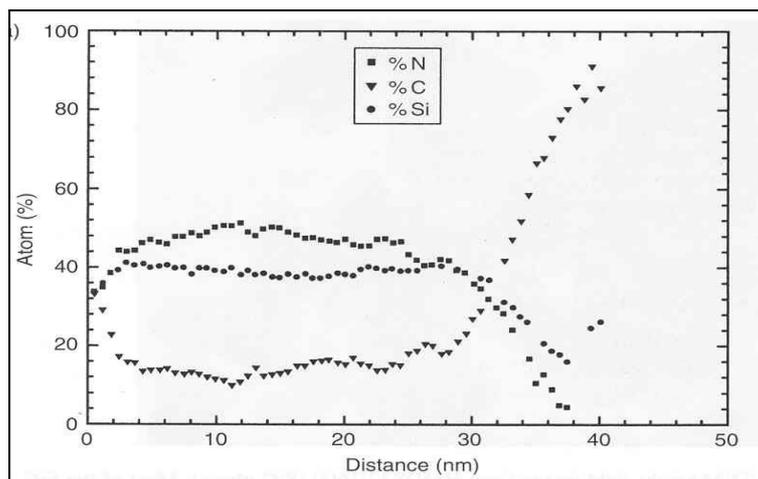


Figure 17: Concentration profile obtained by EELS for one nanometric grain of one Si/C/N nanopowder (Monthieux et al. 2000, data from N. Lebrun, LPS, Paris, France)

SEM and TEM are performed on dry powders. For SEM, environmental microscopes are available to perform analysis on wet samples e.g. in biology and medicine. Electron microscopies are powerful methods to investigate size distribution, shape, chemical composition but also phases analysis (nature and repartition) but require an extensive sample preparation.

5.2.2 BET and pycnometry

Specific surface area and density of nanoparticles are obtained using the Brunauer Emmet Teller (BET) method and helium pycnometry. BET is based on the measurement of the adsorption isotherm of an inert gas (N_2) at the surface of the particles. The surface determination is performed for an adsorbed volume corresponding to a monomolecular adsorption. Helium pycnometry is based on the variation of the helium pressure (in a calibrated cell) produced by a variation of volume. Nanoparticles are pre-compacted into small pellets and put into the cell. Helium pycnometry is a measurement of the true bulk density of the particles if they do not contain closed pores. BET and He pycnometry are performed on dry powders. By coupling the results obtained by these two methods, an average grain size can be calculated if the particles are isolated, spherical with a monodisperse size distribution. The value of d given by BET and helium pycnometry can be compared to the value obtained with electron microscopies. If the diameters are equal, then grains do not contain any open porosity. If the value of the model is quite smaller than that observed by microscopy, then particles contain open porosity. BET and helium pycnometry are performed on outgassed dry powders.

5.2.3 Epiphaniometer

The epiphaniometer (Gäggeler et. al. 1989) is an instrument developed at the Paul Scherrer Institute in Switzerland that measures the surface concentration of aerosol particles in both the nuclei and accumulation mode size ranges. The epiphaniometer is most sensitive to particles in the accumulation mode, but Gäggeler et al. (1989) reported successfully measuring silver particles between 20 and 90 nm that were agglomerates formed from smaller primary silver particles. The maximum concentrations the epiphaniometer can handle is not reported, but it is capable of measuring low atmospheric particle concentrations found in remote locations. In an epiphaniometer, aerosol is passed through a charging chamber where lead isotopes created from a decaying actinium source are attached to the particle surfaces. The particles are transported through a capillary to a collecting filter. The epiphaniometer uses a surface barrier detector to measure the level of radioactivity of the particles collected on the filter. The amount of radioactivity is proportional to the particle's Fuchs surface area and follows Fuchs theory of attachment of radioactive isotopes. Because of the short half-life of the lead isotopes, the filter does not become saturated and essentially real-time radioactivity measurements can be made. Although not clearly stated in the studies, the surface barrier detector measures radioactivity and must be related to the size of particles being sampled to get a measure of surface area of the particles. A pre-classifier, such as a DMA, may be used before the epiphaniometer to allow a determined range of particles to enter the instrument.

5.2.4 Laser granulometries and Zeta potential

Laser granulometries are statistical methods for the determination of quantitative particles size distributions. These methods are based on the diffraction/scattering of a laser beam by particles in stable suspensions. The first method is based on laser diffraction. The diffraction pattern (width of the ring and intensity) is directly connected to the particles size.

However, sizes lower than $\lambda/20$ are not observable by this method. Practically, only particles with sizes higher than 80 nm can be characterised using laser diffraction. Quantification of the size distribution using the Mie theory can be performed if refractive indexes (particles and solution) are known.

The second method more adapted to ultrafine particles is based on photon correlation (PCS). This method measures at selected angles the variation of the scattered intensities (due to the Brownian motion of particles) as a function of time⁶. An autocorrelation function giving changes of intensity as a function of time is measured and size distribution is extracted. As for

⁶ A. Rawle, "PCS in 30 minutes", info-brochure Malvern Instruments Ltd., U.K.

laser diffraction, PCS requires the prior knowledge of solution viscosity and refractive index. PCS is highly sensitive to the presence of agglomerates. If agglomerates are present, the autocorrelation function will be dominated by their signal. The sensitivity of the detection is highly dependent on the size of the particles/agglomerates. Sizes as low as 1 nm can be characterised by PCS. Both methods (laser diffraction and PCS) are well adapted for diluted or ultra-diluted stable suspensions. They work for dense, spherical low-absorbing particles.

Laser granulometries are frequently coupled with Zeta potential analysers. Zeta potential is a measurement of charges carried by the particles in suspensions. The principle of the commonly used Zeta potential analyser is based on electrophoresis. Zeta potential measurements are used to characterise stability of the suspensions using electrostatic repulsion.

5.2.5 Elliptically polarised light scattering

A new method based on laser light scattering was recently developed to investigate size distribution, shape distribution but also agglomerates structure and size distribution (Pinar 2003). Unlike laser granulometries, the incident laser beam is elliptically polarised and modifications of the polarisation state due to sample are measured at specific angular positions. Polarisation analysis gives complementary information about shape and agglomerate structure.

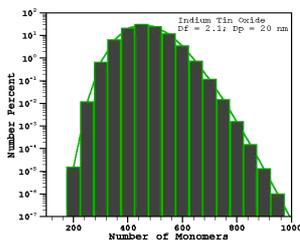


Fig. 19: Agglomerates size distribution for SnO₂ particles with a fractal dimension of 2.1

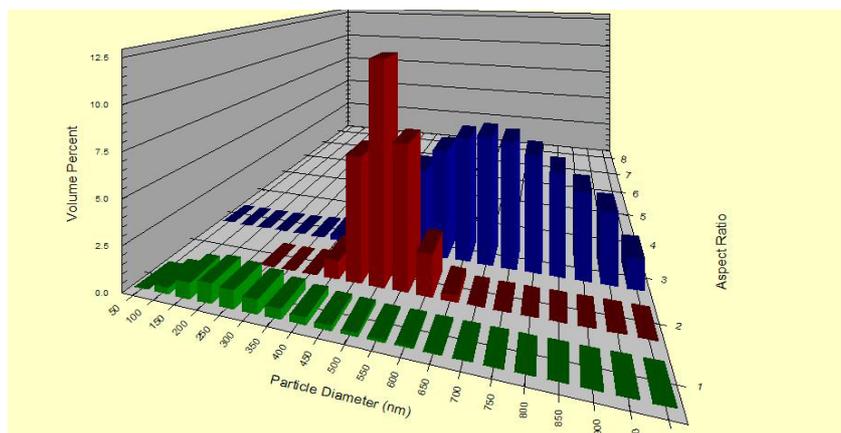


Figure 18: Size distribution is obtained for particles with different aspect ratio

The method determines the fractal dimension (D_f) of the agglomerates. This parameter is a function of the agglomerates structure (linear, porous, compact).

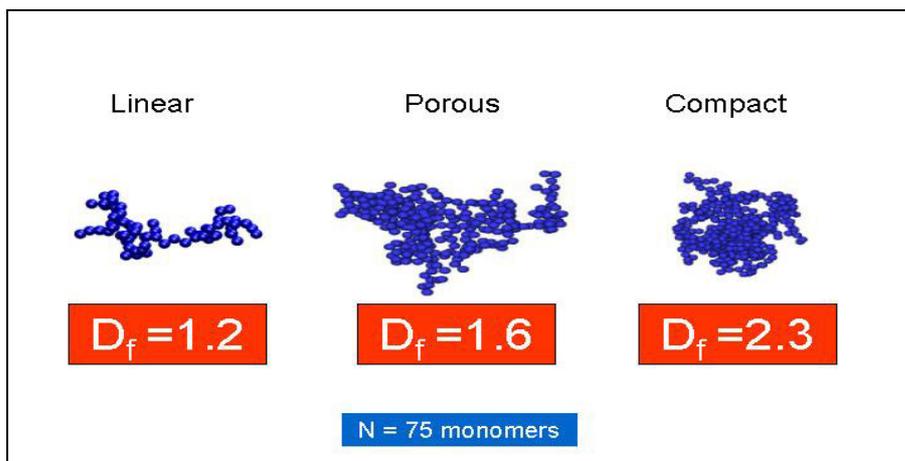


Figure 20: Different types of agglomerate structures

In the field of nanomaterial research, the way in which nanoparticles are agglomerated is important since many properties depend on this parameter. Agglomerates size can also be characterised by this method with an accessible range between 50 nm and 2 μm .

5.3 Determination of nanoparticles in aerosols

Most critical for assessing exposure of people and workers is the determination of nanoparticles in air. Whilst mass is the current metric for measuring exposure to the coarse aerosol fractions, there is evidence to suggest that it may not be so for the ultrafine particle fraction. Relevant metrics for nanoparticle exposures may be the number concentration, size distribution, surface area or morphology.

Technologies to measure these metrics for nanoparticles are not readily available, particularly in a form which may be used to measure personal exposure on a routine basis. Personal exposure measurements are preferred for workplace measurements as they give better exposure estimates and therefore lead to better risk assessment and risk management. Methods available for determining the number concentrations of airborne nanoparticles include the CPC (Condensation Particle Counter), which counts particle number, the SMPS (Scanning Mobility Particle Sizer and ELPI (Electrical Low Pressure Impactor) which count particle number and give number-weighted particle size distribution information. Both the SMPS and ELPI are relatively large and cumbersome. Although the CPC is much smaller, it is not a personal device and the lack of size discrimination is a major limitation. The situation with the measurement of particle concentration in terms of the surface area metric is even poorer. One instrument that has been specifically developed to measure the surface area of airborne nanoparticles is the epiphaniometer. This detects the radiation arising from radon isotopes attached to aerosol particles when exposed to a

Mass is not a suitable metric for measuring ultrafine particle exposure

Only few devices are capable of measuring number, size-distribution or surface area of ultrafine particles

radioactive lead source inside the instrument. It gives near real time information on the total Fuchs surface area of collected particles in the range 10 to 1000 nm. However, the instrument is large, with a registerable radioactive source and has never been fully developed from the experimental stage. Whilst rough estimates of surface area of airborne particles can also be obtained from number size distributions (as outlined above), there is currently no instrument (personal or static) suitable to measure the surface area-weighted particle exposures of workers.

At the present time, few techniques exist for the detection of ultrafine airborne particles in aerosols derived mainly from the automotive industry (OICA 2002) or from the biomass combustion (Johansson 2002). Methods for for real-time particle measurements to be mentioned here are:

- DLPI (Dekati Low Pressure Impactor)
- ELPI (Electrical Low Pressure Impactor)
- SMPS (Scanning Mobility Particle Sizer)

Specificities of the analyser give the following detection limits: DLPI and ELPI detect particles with size as low as 7 nm whereas SMPS can detect particles with diameter as low as 3 nm ^{7,8}. Methods by impaction (DLPI and ELPI) are based on inertial size classification (see figure below). The device have two co-linear plates of which one has a small nozzle in it. The sample aerosol passes through this nozzle at high speed and makes a sharp turn with the flow between the plates. Particles with sufficient inertia cannot follow the flow and impact on the second plate, particles with small enough inertia remain in the flow and are impacted at a subsequent stage.

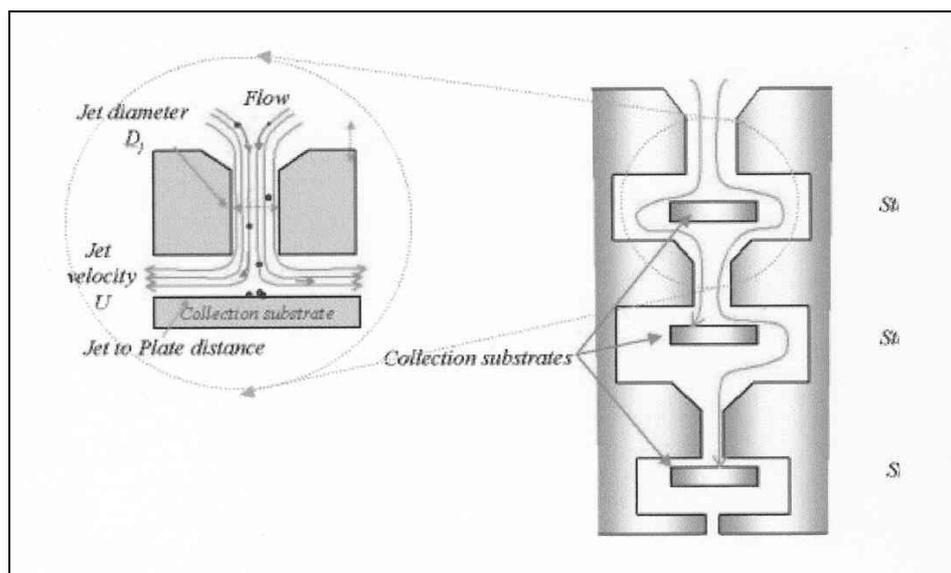


Figure 21: Principle of DLPI and ELPI analyser (source: www.dekati.com).

⁷ <http://www.dekati.com/dlpi.shtml>, <http://www.dekati.com/elpi.shtml>

⁸ <http://www.tsi.com/particle/downloads/brochures/3936.pdf>

The cut diameter for one impactor is defined as the size of particles collected with 50 % efficiency. Cascade impactor, as shown on the figure above, consist of several successive impactor stages with decreasing cut diameters. A DLPI impactor has 13 successive impactors. For DLPI, a gravimetric analysis is performed whereas for the ELPI, particles are preliminary charged before they enter the impactor and an electric analysis is performed resulting in a fast response time (Kerkinen et al. 1991, 1992). SMPS method allows number size distributions (from 3 to 1000 nm) and number concentrations measurements (Wang and Flagan 1990). The aerosol first passes through a single-stage, inertial impactor. This serves to remove large particles outside the measurement range that may contribute to data inversion errors caused by multiple charging. Then, particles of the aerosol are charged and enter the DMA (Differential Mobility Analyser). At this point, particles are separated according to their electrical mobility, by using their deviation in an electric field produced by a charged rod. Only particles within a narrow range of electrical mobility have the correct trajectory to pass through an open slit near the DMA exit. At the exit of the DMA, particles enter a Condensation Particle Counter (CPC). By changing the voltage of the rod inside the DMA, the entire size distribution can be measured.

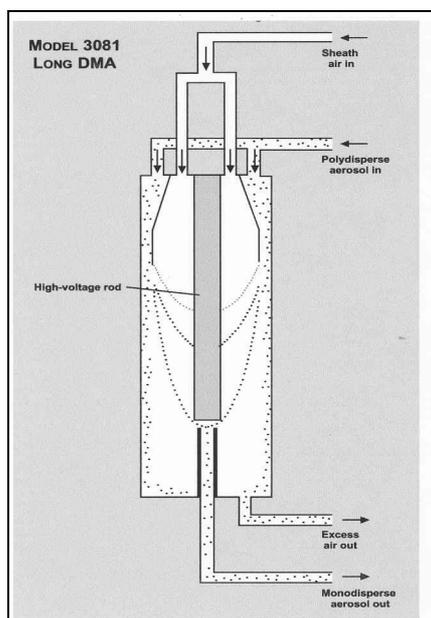


Figure 22: Principle of the DMA (Differential Mobility Analyser) of a SMPS analyser (source: www.tsl.com)

The CPC (or CNC) instrument is used to count the particles emitted from the DMA. The size-selected nanoparticles are flowing through a zone saturated by n-butanol vapours and subsequently cooled to cause the condensation of vapours at their surface (Harrison et al. 2000). Then particles are growing to the order of 10 μm in diameter at which they are efficient light scatterers. The particles can be counted as they pass through a light beam (low number densities – single count mode) or by

detecting the light scattered in a sensing zone (high number densities – photometric mode). Typically, size ranges between 3 and 20 nm can be characterised by CPC/CNC.

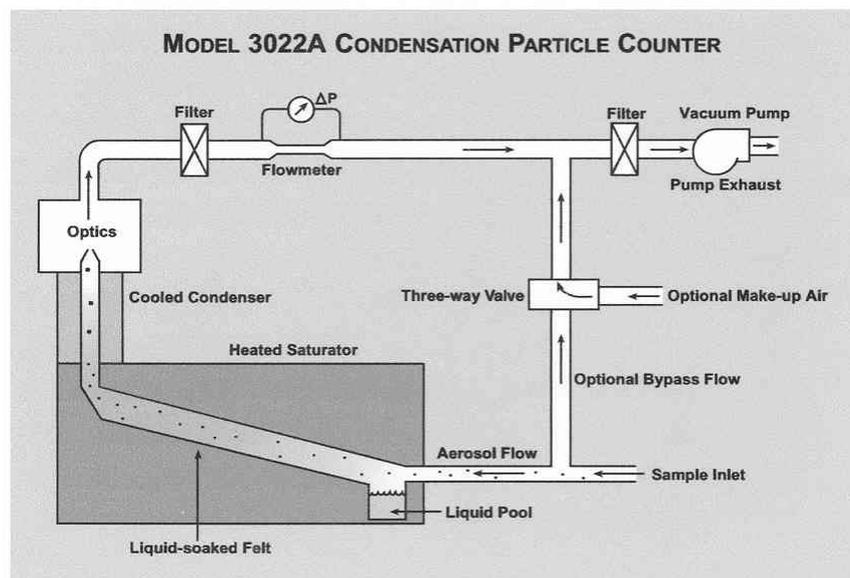


Figure 23: Principle of the Condensation Particle Counter (source: www.tsl.com)

Key benefits of the SMPS analyser are:

- fast results (about 60 s or less)
- high-resolution data, broad size range (3 to 1000 nm)
- wide concentration range (1 to 10^8 particles per cubic centimeter)
- simple control of operations.

As it has been shown by a round robin test, SMPS is a valid tool to measure size distribution and number concentrations of nanoparticles but only with uniform instrument parameters (Dahmann et al. 2001). Therefore there is an urgent need for the standardisation of measurement and sampling procedures and conditions. Furthermore the emergence of new sources of nanoparticle releases in industrial environments requires the setting up of well-adapted techniques for the detection of new types of nanoparticles. For analysers, the following specifications would be desirable:

- portable instruments
- adapted to aerosols and liquids
- continuous mode instruments
- real-time diagnostics
- personal exposure measurement systems

Among the existing characterisation methods, candidates for an online measurement system for nanoparticles in work place atmospheres could be based on light scattering methods (see chapter 5.2.4 to 5.2.5), which have specifications close to those given above and which can be also adapted to achieve detection in liquid effluents. For a detailed

Need for standardisation of measurement and sampling procedures

characterisation of size and morphology of nanoparticles only off-line measurement techniques such as Scanning Transmission Electron Microscopy (STEM), High Resolution Transmission Electron Microscopy (HRTEM) or Scanning Probe Microscopy techniques (SNOM, AFM) are available.

Table 7 gives an overview on characterisation parameters and respective measurement techniques for assessing nanoparticle exposure in aerosols.

Parameter	Measurement Techniques
Number Concentration	<ul style="list-style-type: none"> • Condensation Particle Counter (CPC)
Particle Number and number-weighted particles size distribution	<ul style="list-style-type: none"> • Scanning Mobility Particle Sizer (SMPS) • Electrical Low Pressure Impactor (ELPI)
Submicron particle surface area	<ul style="list-style-type: none"> • Epiphaniometer • Diffusion Charger
Size, morphology and surface properties (for collected particles)	<ul style="list-style-type: none"> • STEM • HRTEM • SNOM • AFM

Table 7: Parameters and measurement techniques for assessing nanoparticle exposures in the atmosphere (Harrison et al. 2000, Maynard 2000)

Beside the lack of personal exposure measurement systems also measurement standards for a reliable and comparable nanoparticle determination are presently not available. To perform an evaluation of the existing detection techniques, nanoparticles produced in industrial or pre-industrial environment must be completely characterised from their atomic structure to their agglomeration using a combination of the above mentioned complementary methods. The acquisition of detailed reference data as well as intercomparisons and round robin tests will be necessary to assess the reliability and the limitations of the applied detection techniques. Moreover, the understanding of the interaction mechanisms of nanoparticles with their environment requires a precise knowledge of the characteristics of the nanoparticles produced from pre-industrial and industrial processes.

5.4 Determination of nanoparticles in biological tissue

Most of the above mentioned detection techniques for nanoparticles are restricted to measurements in gaseous phase or solid phase. A critical point for assessing effects of nanoparticle exposure on living organisms and the environment are therefore measurement techniques which are capable of analysing liquid and/or biological samples. This is important because biological samples often require complex sample preparation steps before nanoanalytical methods such as electron microscopy can be

Cryo-TEM and STIM
for characteri-
sation of nanopar-
ticulate materials
in liquids and
biological tissue

applied. Methods for nanoparticle detection in biological samples to be mentioned here are Cryogenic Transmission Electron Microscopy (Cryo-TEM) and Scanning Transmission Ion Microscopy (STIM). These methods have been applied for example to assess, if nanoparticles used in cosmetics (sunscreens, etc.) can penetrate into the human skin and consequently may cause systemic effects (see chapter 6.3).

The Transmission Electron Microscope (TEM) that has been widely used in research in the fields of materials science and technology has now become capable of observing specimens at atomic resolution and is making valuable contributions to research and development of industrial products. In recent years, with the progress of molecular biology, the TEM has begun to be used in analyses of biological macromolecules such as proteins (enzymes) and viruses on the molecular level. Also, it is being applied to elucidating life phenomena, including those on the molecular level, as well as to development and improvement of industrial, pharmaceutical, and agricultural products. Also, recently, strong demands for TEM observation of three-dimensional structures of biological macromolecules in the hydrated state (the native state in living bodies) at atomic resolution have been made.

However, there are two difficult problems in using the TEM for these purposes. First, the path of the electron beam must be in vacuum and, therefore, the specimen to be observed must also be kept in vacuum. Second, damage of the specimen due to electron-beam irradiation is large and must be reduced considerably. A method for overcoming these difficulties and for observing the specimen at atomic resolution while keeping it in the hydrated state is the so-called ice embedding method for preparing a frozen specimen. Beiersdorf used the cryo-TEM method for demonstrating the non-penetration of titania nanoparticles into the human dermis (Pflücker et al. 2001).

Based on the idea similar to the Scanning Transmission Electron Microscopy the Scanning Transmission Ion Microscopy (STIM) was developed in the early 80's which uses an ion beam instead of an electron beam. The main advantage of STIM is the greater penetration depth that allows the analysis of much more thicker objects. This technique makes imaging as well as mass normalisation possible at resolution down to 100 nm (and less in future). STIM has been applied e.g. by the university of Leipzig for the investigation of percutaneous uptake of ultrafine TiO₂ particles (Menzel et al. 2004).

6 RISK ASSESSMENT

Risk assessment in general comprises several components including

- hazard identification
- hazard characterisation
- exposure assessment
- risk calculation

One the basis of a reliable risk assessment measures for risk management have to be undertaken comprising preventive measures, standardisation and regulation activities which are elaborated in chapter 7. The following figure gives an overview of different aspects and components, which have to be taken into account for the assessment and management of risks associated with industrial nanoparticle production and use.

<p>1 Hazard identification</p> <p>Particle Characteristics</p> <ul style="list-style-type: none"> • Aspect-ratio • Diameter (particle/aggregate) • Surface area/ properties • Water solubility • Chemical composition <p>Emission</p> <ul style="list-style-type: none"> • Production volume • Material flows • Potential particle release (production, use, disposal) <p>Health effects</p> <ul style="list-style-type: none"> • Humans • Experimental animals <p>Environmental effects</p> <ul style="list-style-type: none"> • Persistence • Biomagnification • Long range transport 	<p>2 Hazard characterization</p> <p>Epidemiological Studies</p> <ul style="list-style-type: none"> • Workers • Consumers • Exposed population <p>In vivo studies</p> <ul style="list-style-type: none"> • acute/chronic • different species <p>In vitro studies</p> <ul style="list-style-type: none"> • Human/ animal, different cell types • Models (lung, skin, systemic effects) <p>3 Exposure assessment</p> <p>Exposure routes</p> <ul style="list-style-type: none"> • Inhalation, dermal, ingestion <p>Environmental monitoring</p> <ul style="list-style-type: none"> • Biological uptake <p>Occupational monitoring</p> <ul style="list-style-type: none"> • Personal exposure 	<p>4 Risk calculation</p> <p>Susceptibility extrapolation models</p> <ul style="list-style-type: none"> • high dose → low dose • animal → human <p>Threshold value calculation</p> <ul style="list-style-type: none"> • Intake, immission concentration, maximum workplace concentration <p>5 Risk Management</p> <p>Preventive Measures</p> <ul style="list-style-type: none"> • Personal protection equipment • Modification of processes <p>Standardization</p> <ul style="list-style-type: none"> • Measurement techniques • Toxicological assessment <p>Regulation</p> <ul style="list-style-type: none"> • Exposure/ immission standards • Production standards/restrictions
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Figure 24: Components and aspects of risk assessment and management associated with industrial nanoparticle production and use.

It should be pointed out, that many of the above mentioned aspects concerning nanoparticles have not been investigated yet and are still unknown. The following chapter tries to give an overview on existing information with regard to the different steps of risk analyses. For the risk assesment it is useful to distinguish different types of nanoparticulate materials, whether they are dispersed in gaseous, liquid or solid phase, whether they occur as single-particles or as agglomerates or whether they are untreated or surface modified (see chapter 2).

Most critical with regard to potential health and environmental risk are nanoparticles dispersed in air (aerosols), because of their mobility and the possible intake into the human body via the lungs which represents the most critical exposure route for humans. On the other side nanoparticles dispersed in a solid matrix are much less likely to raise concerns because of their immobilisation.

Many aspects concerning risk assessment of nanoparticles are still unknown

As mentioned above aerosol nanoparticles tend to build larger agglomerates with sizes in the μm -range. So the question arises whether particle-size dependent phenomena like cell barrier crossing, etc. are still valid for engineered nanomaterials. To answer this question investigations have to be performed, e.g. if agglomerates can deagglomerate in the lung liquid or other biological liquids.

Natural aerosols also contain ultra-fine particles in high concentrations

Another point which has to be considered in the risk assessment of nanoparticles is the fact that natural aerosols also contain particles with sizes between $100\ \mu\text{m}$ to $10\ \text{nm}$ and even smaller (Primmermann 2000). The amount of nanoparticles is nearly the same in urban and rural areas with as much as 10^6 to 10^8 particles/liter air. Whereas, in rural areas the particles are mainly soil-derived and bioaerosols, in urban areas the particles are mainly composed of Man-made materials (combustion and mechanical abrasion, etc.) and bioagents. Hence one might expect that over millions of years e.g. the lungs had to adapt their natural exposed tissue to function and fulfil their work even in the presence of 10^6 - 10^8 nanoparticles/liter air and 10^2 particles/liter with a size between $100\ \text{nm}$ to $10\ \mu\text{m}$.

To sum up it has to be kept in mind when assessing risks of engineered nanomaterials that:

- At present combustion processes from traffic and energy generation as well as mechanical abrasion processes contribute much more to anthropogenic nanoparticle emissions than industrial nanoparticle production
- Industrial nanoparticulate materials usually build aggregates with sizes in the μm -range
- Also natural aerosols contain huge amounts of particles with sizes $< 100\ \text{nm}$

Nevertheless due to the fact that the next few years will probably see a dramatic increase in the industrial generation and use of nanoparticles and entirely new substance classes like carbon nanotubes are released into the environment, a careful risk assessment of engineered nanomaterials is obviously necessary.

6.1 Potential particle release

The objective of this part is to make an inventory of possible sources of potential particle release in nanoparticle production processes during the whole life cycle from nanoparticle generation to end products and finally disposal. Due to a variety of different production methods for nanoparticles, the process conditions vary widely and thus in principle the risk of a potential particle release has to be considered separately for each different process. However, most of the processes like plasma and laser deposition as well as aerosol process are usually performed in

evacuated or at least closed reaction chambers. Therefore, exposure to nanoparticles is more likely to happen after the manufacturing process itself except in the case of unexpected failure during the processing (e.g. chamber failures causing leakages).

6.1.1 Nanoparticle production

Processes working at high pressure (supercritical fluid for example) or with high energy mechanical forces (mechanical synthesis), particle release could occur in the case of failure of sealing of the reactor or the mills. Then large quantities of nanopowder could be released in a short time into the atmosphere. For laser processes (laser pyrolysis/ ablation), breaking of reactor laser windows (windows on the optical path of the laser beam) is a possible source of release.

Potential particle release in case of failure of reactor sealings or collecting apparatus

Moreover, when sealing is broken, reactive mixtures can be put in contact with air and in some case, violent chemical reactions can occur. For example when silane is used for the synthesis of silicon based nanopowders, the accidental contact with air provokes a spontaneous very exothermic reaction with oxygen and flame can appear inside the process unit or in the close environment.

Failure of collecting apparatus are also important sources of potential release during the processes. The collecting apparatus must be able to stop the nanoparticles and to evacuate effluents produced from the processes. If collecting apparatus is designed for the recovery of nanoparticles in a dry form (using filters for example), failure of filters efficiency could be a source of potential release towards the evacuation system (pumping unit for example). In order to avoid this kind of release, gas and aerosols treatments units must be connected at the exit of the plants to prevent release in atmosphere.

6.1.1.1 Mechanical processes

In mechanical milling processes raw material powders are usually crushed together with process control agents (PCA) under inert gas atmosphere. PCA can be in solid or liquid form. Risk arises when inert gas atmosphere is removed from the milling vial. Fine particles are very reactive at this stage. If the liquid PCA is used during the milling process, the nanoparticles stay stable in suspension until it is dried. After drying the suspension, release of nanoparticles to the surrounding atmosphere may occur.

6.1.1.2 Vacuum processes

In vacuum processes nanoparticles are formed inside the chamber and then collected on a substrate. These type of methods are e.g. CVD, PVD. The manufacturing process itself is safe, but opening the chamber may cause release of nanoparticles.

6.1.1.3 Spraying methods

Different kind of spray methods are used to produce nanoparticles. Typical examples of these methods are plasma spray synthesis, flame spray and laser pyrolysis. Liquid precursor/ fuel mixture is feeded into the flame. Nanoparticles synthesised in the flame are collected as powder e.g. in an electrostatic precipitator, baghouse filter or as a deposit on a substrate. Plasma spray synthesis has been used even in open atmosphere to produce nanoparticles. Flow velocity of nanoparticles in this process is high and collecting all of the produced nanoparticles is a highly demanding task. The manufacturing method is quite simple, unexpensive and also suitable for mass-production, but efficient and safe particle collecting system is required.

6.1.1.4 Sol-gel processes

Sol-gel processes are chemical methods based on hydrolysis or condensation reactions. They are well adapted for oxide nanoparticles synthesis. During the sol-gel processing nanoparticles are precipitated from solution. By controlling the amount of reactants or by using the chemical that blocks the reaction, precipitation can be arrested so that nanosize particles are formed. If the precipitated nanoparticles can stay in the solution, there will be no risk for release into the atmosphere. However, drying of this solution and collecting of dry nanoparticles will again arise risk for nanoparticle release.

6.1.2 Collection of nanoparticles

Risks are increasing during the collect of nanoparticles particularly in a dry form. When opening collecting apparatus or reactors, nanoparticles can be released and travel in air due to their high volatility. In gaseous atmosphere the behaviour of dry nanoparticles is primarily determined by the balance between attractive and lift forces. Gravity force has no noticeable effect on nanoparticles. Therefore nanoparticles in gaseous atmosphere will not settle down easily and may stay in the air as impurities for a long time causing health risks via inhalation. Drastic effects can be observed for metallic or non-oxide nanoparticles due to the high pyrophoricity of dispersed nanopowders.

6.1.2.1 Dust explosion

When handling small particles there may arise risk for dust explosion, especially in the case of metal powders. During various manufacturing processes, dust or dust clouds may be generated. Once dust has formed into the proper mixture with air, it can be ignited by energy from various internal or external sources. Figure 25 summarises the general conditions necessary for a dust explosion and/or fire to occur.

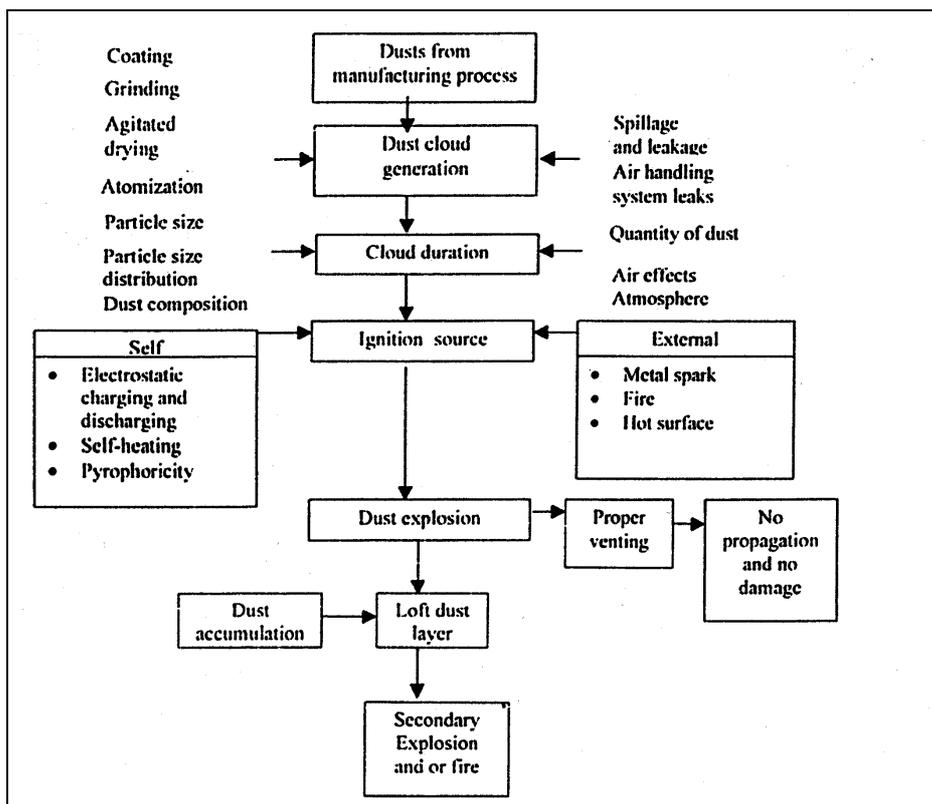


Figure 25: Summary of conditions required for dust explosion with metal powders (Dahn et al. 2000)

Many chemical and physical material properties, various atmosphere conditions, the type and magnitude of energy of the ignition source determine whether a dust cloud ignites and how intense the explosion output is.

6.1.2.2 Ventilation

During collection of solid nanopowders special care must be taken with regard to ventilation at the working place. Air streams could disperse nanopowders to form aerosols. This could occur if fume cupboards are in the proximity of pieces covered with nanopowders (parts of the reactors or of the collecting apparatus, filters).

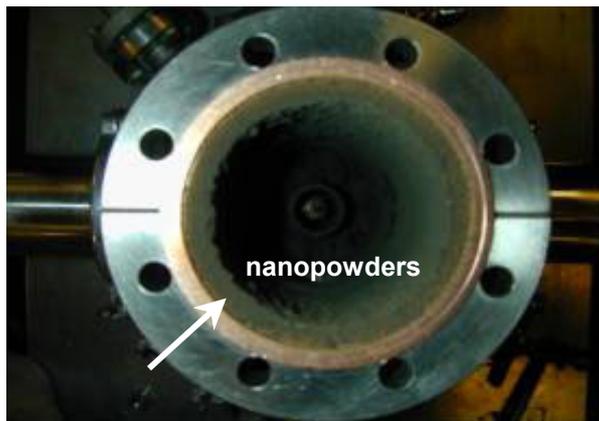


Figure 26: Nanopowders deposit in a small laser-pyrolysis reactor (source: CEA). The white arrow shows the deposit of nanopowders on the wall of a laser pyrolysis reactor

6.1.3 Cleaning operations

Nanoparticle release can also occur during cleaning operations of reactors, after the disassembling, when nanoparticles have to be removed from stainless steel pieces, windows or filters. Cleaning is usually performed using solvents or water, tissues, brushes or sponges, which are then discarded in garbage cans. Optimisation of reactor design to limit the diffusion of nanoparticles towards the walls during production would be a solution (confinement of the synthesis reaction and of the nanoparticles flow for flow methods) but cleaning of the collecting apparatus remains a critical point.

6.1.4 Handling and conditioning operations

Once nanoparticles have been collected, other risks appear during their handling and conditioning to form final products. For example release of nanoparticles while producing ceramic pieces (coating, composites) is possible, particularly when the compressed nanopowders (“green compacts”) or coatings are formed (spray-drying, screen-printing, spraying, tape and slip casting, cold pressing). For cold pressing, large amount of nanopowders can be released if dry powders are used (dye filling, grinding, sieving, pumping operations). The “green forms” as intermediate products are further processed by consolidation through heat treatment (sintering) performed in closed furnaces/presses. Releases are possible into the pumping systems. In this last case, particles can be released with the emanation of gaseous species. Gas can condense in cold parts of the sintering processing unit and possibly reacts with specific parts of the equipments. As for synthesis process, particles can be released during the cleaning of materials used to elaborate final products (grinder, sieve, glassware, furnaces).



Fig. 27: Silicon oxide condensation on the cold part of a thermogravimetric analyser from vapours produced in large quantities by the thermal treatment of silicon based nanopowders (Doucey 1999)

6.1.5 Waste disposal

Waste disposal concerns the whole production equipment that is in contact with nanopowders at the different steps from their production to their integration in consumer products. For example, during cleaning operation of the plants, brushes, sponges or tissues are used with solvents or water to clean the reactors or the collecting apparatus and filters. When nanopowders are collected, scrapers or spatulas are used and then cleaned using single-use tissues and solvents. Liquids (solvents or water) used for rinsing containers or glassware, which might contain nanoparticles, are usually evacuated into drain hoses. Disposal of the waste might be a potential source of nanoparticle release into the environment (air, soils and water) if no special care is taken with traceability and final storage or combustion of the wastes. However, recycling processes (such as distillation for solvents) exist and are used in chemical industry and in research laboratories. In order to prevent release of nanoparticles into the environment, effluents containing nanoparticles have to be treated and preferentially recycled. Nanoparticles in the effluents could be recovered or neutralised (regarding the possible health hazards) by provoking their growth for example (by a thermal or chemical treatment).

6.1.6 Final product utilisation

When final nanoparticle based products are obtained, risks depend on the way in which nanoparticles are integrated. For nanostructured materials (composites, coatings), nanoparticles are linked to a matrix (ceramic, polymer, metals) by a thermal treatment at high temperature and therefore risks of nanoparticle release are expected to be low. However, under wearing conditions (cutting tools/various mechanical solicitations and high temperature) particle release is likely to occur but dissociation of matter at the nanometric scale is unlikely.

6.1.7 Specific cases

6.1.7.1 ODS (Oxide Dispersion Strengthened alloys)

Oxide Dispersion Strengthened (ODS) alloys have been developed to improve the high temperature properties (mainly creep resistance) of corresponding conventional metallic materials in order to use them in higher temperature range applications as structural and corrosion resistance materials. The improved properties result from the dispersion of ultrafine oxide particles of few tens nanometers in the metallic matrix having grains size of either few tens microns or several millimetres. For instance, commercial Ni-based and Fe-Cr alloys ODS alloys (MA-956 from INCO, PM2000 from PLANSEE) are generally reinforced with Y_2O_3 particles and used in their recrystallised state with very large

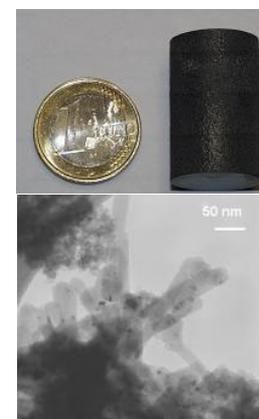


Fig. 28: above right: fibrous Si_3N_4/SiC nanocomposite elaborated from Si/C/N nanopowders (coin as a scale), below: TEM observation of a grinded fragment of the nanocomposite shown above (source: CEA)

grains. Another benefit of oxide reinforcement sometimes lies in the presence also of coarser particles of few hundreds nanometres which are effective in pinning the grain boundaries so that a fine microstructure can be obtained with grain size of 1 μm . This allows obtaining high mechanical properties at low temperatures (below 400° C) together with a significant ductility as it is the case for the FeAl intermetallic alloy developed by CEA (FeAl₄₀ Grade 3) for use in transportation (aeronautic and automotive) and power generation industries since it exhibits very interesting specific mechanical properties - thanks to its low density - and high corrosion resistance in hot aggressive environments.

The oxide content of the ODS alloys is generally in the order of 1 vol.%. The fine dispersion can be obtained by internal oxidation such as in Al₂O₃ reinforced Cu-based alloys, but in most cases it is obtained by powder metallurgy techniques consisting in milling together the oxide reinforcement powder (with particle size < 1 μm) with the alloy elemental powders (mechanical alloying) or pre-alloyed powder (mechanical milling) in a dry atmosphere (argon or hydrogen). The milled powder is nanostructured with grains of few tens of nanometres while the particle size distribution is of several tens of microns. Depending on the nature of the reinforcement oxide, the milled powder can be fully metallic (dissolution of Y₂O₃ by milling) or a nanocomposite (no dissolution of MgAl₂O₄). The powder is then degassed and consolidated by hot extrusion or hot isostatic pressing before subsequent cold or hot forming with conventional techniques (forging, rolling, etc.). In cases where oxide particles were dissolved during milling, they precipitate during the hot consolidation step. Once formed, oxide particles are stable and cannot be dissolved by heat treatments. They can only coarsen, but only by heat treatments at temperatures higher than the one used for the consolidation of the powder and generally they remain submicronic.

At the manufacturing step, release of particles may occur during the handling of the oxide powder before milling, but relevant powder inlet systems can prevent this from occurring. The particles contained in the machining chips can be released after dissolution of the metallic matrix by long-term exposure to corroding environments (Cl⁻ containing environment for non stainless Fe-based ODS alloys). On fine-grained materials machining chips are very small (powder-like) and are more prone to release particles by dissolution of the matrix. But due to the high cost of machining ODS alloys, net-shape and near-net-shape manufacturing techniques are preferred as much as possible thus resulting in a self-limitation of chip production. The main source of particles release may occur in working conditions through wear and corrosion (dissolution of the matrix).

6.1.7.2 Metal hydrides

Nanostructured metal hydrides have a potential as efficient hydrogen storage medium for supplying fuel cells (PEMFC) especially in mobile applications, which require increased safety and high energy storage density. Mg shows a high weight capacity, but too low rates of charge/discharge of hydrogen. In order to improve the reaction kinetics, nanostructured MgH_2 (or Mg alloy hydride) composite powders have been designed e.g. by Hydro-Quebec (and are now commercialised by HERA), where the powder particles should have an average size ranging from 0,1 to 100 μm , with a microstructure made of crystallographic grains having sizes ranging from 3 and 100 nm and with clusters having size of 2 to 200 nm of an activating phase (such as V and C) dispersed inside the powder particle. The Mg composite powder is best obtained by reactive milling of an Mg powder in H_2 under 4 bars at $300^\circ C$, together with graphite and vanadium powders. An alternative but longer and more expensive process, consists in first hydriding the Mg powder and then milling it under argon at room temperature with graphite and vanadium powders. The powder is then packed in tanks by cold pressing (and eventually partial sintering).

Generally there are limited risks of nanoparticle release during the composite powder synthesis, as both the raw and final powders are of micrometric size. But nanoparticles may be released due to cracking, which results from the repeated (through charging/discharging cycles) high volume change induced by the metal/hydride transformations.

The recycling or management of waste products would require specific attentions in order to prevent explosions, which could occur when a large amount of air or water comes suddenly (by rupture of the safety packaging) in contact with the active material (either in its metallic or hydride state) due to its high specific surface (combined with the high exothermal potential of the reactions in case of Mg storage material).

6.1.7.3 Nanotubes

Since the discovery of carbon nanotubes, which can occur multi-walled (MWCNT) or single-walled (SWCNT), a great deal of progress has been made in this area. Meanwhile a variety of synthesis methods for carbon nanotubes exists (see table 8) and the production level will soon reach an industrial scale (Mitsui 2002).

Process	Temp. (°C)	Pres. (mbar)	Yield (%)	Characteristics
Arc discharge ⁹	4000	700	15-25	MWCNT, Soot (amorphous carbon, graphite)
Laser ablation ¹⁰	1000-3000	200-400	60	SWCNT
Catalytic CVD (Thermal, PE-HF & DC-HF)-CVD ¹¹	500-1000	200-500	>60	MWCNT
CVD in gaseous phase ¹²	800-1000	-	30-60	MWCNT Soot
Fluidised-bed CVD ¹³	450-750	-	60	MWCNT (Soot)

Table 8: Synthesis methods for carbon nanotubes

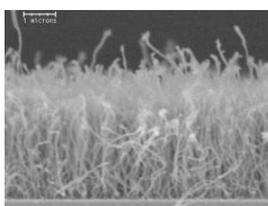


Fig. 29: SEM micrograph of the MWCNT grown on silicon substrate



Fig. 30: SEM micrograph of MWCNT produced by arc discharge (purified MWCNT)

When SWCNT are produced, most of them occur in bundles, each bundle consisting of tens of SWCNT. SWCNT have a diameter of about 1 nm to 5 nm with length of several micrometers. In addition, bundles are randomly oriented. MWCNT have a diameter from 10 nm to 60 nm with length of several microns. The figures presented below shows MWCNT grown by CCVD process on Si substrate and morphology of purified MWCNT.

Recently some studies have been performed on an evaluation of the aerosol release during the handling of carbon nanotube material (Baron et al. 2003, Maynard et al. 2004). Certain circumstances (e. g. vigorously agitating carbon nanotube material or removing spilled nanotube material with a vacuum cleaner) can lead to the generation of ultrafine particles and an increase in the aerosol number concentration. However, concentrations generated while handling material in the field were very low and it was unclear whether these particles represented nanotubes, catalyst particles (used in the manufacturing process) or other kind of carbonaceous particles, because with the applied measurement techniques it was not possible to distinguish particle shapes.

6.1.7.4 Carbon black

Carbon black is one of the most important nanoparticulate material with regard to production volume and is used for example in automobile tyres or copy toners. The carbon black manufacturing process consists of three process steps: reactor, pelletizing, bagging. The primary particles with sizes between 1 and 500 nm very rapidly form aggregates due to the high particle number concentration in the closed reaction area which are firmly held together by Van der Waals forces. Before bagging, these aggregates collected on filters and pelletised to very large agglomerates

⁹ Shi et al. 1999

¹⁰ Tess et al. 1996

¹¹ Ishima et al. 2000

¹² Andrew et al. 1999

¹³ Wang et al. 2002

for greater ease of handling. A particle exposure to workers is most likely to occur during the bagging of the materials because the first stages of the production line (reactor and pelletiser) are closed systems.

6.1.7.5 Surface treatment

In this paragraph, we will focus therefore on particles release problems during PVD surface treatments. Industrial Physical Vapour Deposition (PVD) equipment used for hard coatings deposition in mechanical (cutting and forming tools) as well as in decorative applications is shown in the following figure.



Figure 31: PVD equipment used for mechanical (left) and decorative applications (right)

Large size chambers are required in order to increase productivity in these batch systems. Two types of reactions can lead to powders or nanopowders synthesis in these large chambers during PVD processing, particularly when cathodic arc evaporation technique is used. On one hand, reactions in homogeneous phase occur between metallic vapours and reactive gas and, on the other hand, reactions in heterogeneous phase occur on the chamber walls and on the substrates between the specimen and the surfaces. These phenomena are very important in an industrial environment because of the large chamber sizes. Operators have therefore to clean the chambers and the substrates holders after each run. Table 9 summarises the maintenance operations of an industrial PVD reactor.

Operations	Frequency
Blowing of the substrate holder with compressed air	4 – 10 times per day
Chamber walls cleaning with a vacuum cleaner	4 – 10 times per day
Substrates holder cleaning with a vacuum cleaner	4 – 10 times per day
Stripping (chemical) of sheet metal (chamber walls)	2 times per month

Table 9: Example of all maintenance operations in an industrial PVD plant, operations frequency is also specified



Fig. 32: Industrial thermal chemical vapour deposition (CVD) furnace

Chambers cleaning techniques can vary from a plant to another but operators should always wear gloves and mask (nose and mouth). In some cases a load cooling (500 °C to room temperature) with pressure cycles (300 – 700 mbar) is performed after each batch, in order to speed up the cooling of the reactor. This operation favours unsticking coatings, which lead to particles release. Chamber walls cleaning is necessary two times per month in order to prevent a process drift. This operation can be achieved by a chemical stripping but it is sometimes impossible to remove coatings from the walls via a chemical way depending on the product composition. In that cases it is necessary to brush the chamber walls by means of a metallic brush leading to a high particle release.

Figure 32 shows an industrial thermal Chemical Vapour Deposition (CVD) furnace. High temperatures (950 – 1100°C), which are used to activate the deposition reaction between gaseous precursors and substrates surface, lead to nanoparticle generation.

Figure below shows a deposit inside the reactor that contains nanoparticles. Gloves and a gas mask are used by the operator during reactor cleaning (see figure 33).



Fig. 33: Gloves and gas mask are necessary for cleaning after a thermal CVD cycle



Figure 34: Particle formation after a thermal CVD cycle (980 °C)

Vacuum processes for microelectronic applications are performed in clean rooms; particles size and quantity are strictly controlled. Moreover the vacuum chambers are relatively small and adjusted to flat silicon substrates which are closed to the PVD source or the CVD precursors injection. In this case, operators clothes are worn to prevent products pollution.



Figure 35: Microelectronic clean room facility (left) and deposition chamber (right)

6.1.8 Conclusion and recommendations

Due to a variety of different production methods for nanoparticles no general statement with regard to risks of a potential particle release can be made. Nevertheless, it can be stated that most of the production processes are usually performed in closed systems resp. reaction chambers. Therefore the risk of particle release during production is relatively low with exception of unexpected failure (e.g. chamber failures causing leakages) or potential problems during the starting and stopping of continuous processes. Exposure to nanoparticles is more likely to happen after the manufacturing process during collecting, handling, conditioning and packing of the nanoparticles and cleaning operations of the process equipment. A preferable way to collect nanoparticles is in liquid suspensions by developing collecting apparatus able to put nanoparticles in the desired liquid either during the production (flow apparatus) or at the end of the process. Wherever feasible, the collection of nanoparticles in well-adapted liquids could help to minimise risks of particle release and worker exposure.

6.2 Exposure assessment

6.2.1 Work place

When evaluating the worksite, exposure assessment is an absolutely necessary part of understanding the health effects and designing an appropriate preventive response. The review of plant operations provides the opportunity to observe work activities by job category and department along with the potential for exposure. Observation of work activities may help identify workers at increased risk of exposure and, potentially, health problems. Plant operations during different shifts have to be reviewed to determine, if significant shift differences exist.

Nanoparticle exposure of workers in conventional industrial processes

Occupational health may be affected through the emission of nanoparticles or -fibres from the exhaust of particle and fibre production processes, from leaks in closed processes, during cleaning and maintenance of production equipment, with the handling of the product and by other processes/machines in the working areas. Exposure of workers to nanoparticles could occur not only during intended industrial production of nanoparticles but also in conventional industrial processes like welding, smelting, soldering, heating, laser ablation, combustion.

Workplace aerosols with a significant nanoparticle number concentration could be roughly categorised as follows (Vincent and Clement 2000):

- Fumes from hot processes (e.g. smelting, refining of metals, welding, etc.) with particle sizes usually not much greater than about 1 μm and going down to a few nanometers (i.e. the size of primary particles produced by nucleation).
- Fumes from combustion processes (e.g. transportation, carbon black manufacture, etc.) usually associated with incomplete combustion, again with particle sizes not much greater than about 1 μm but going down to a few nanometres
- Dust and sprays from mechanical processes (e.g. mining, textiles, chemical manufacture and transportation, construction industry, agriculture, etc.)
- Bioaerosols (e.g. agriculture, biotechnology, natural aerosols emitted by plants and trees, etc.) where some particles (e.g. viruses, endotoxins, etc.) may be as small as a few tens of nanometres

Only few working areas and industrial processes have so far been investigated in view of release of and exposure to nanoparticles or -fibres (nanotubes). One example is the determination of size characteristics of particles emitted during the bagging of various kinds of carbon blacks in three carbon black plants. The investigations conducted by the University of Duisburg showed that bagging of the carbon black material increases number concentration of particles mainly with sizes $> 0,4 \mu\text{m}$ but does not increase number concentrations of ultrafine particles (Kuhlbusch et al. 2002).

Recently some studies have been performed on an evaluation of the aerosol release during the handling of carbon nanotube material, a special class of nanomaterials (Baron et al. 2003). These studies have been complemented by a field study in which airborne and dermal exposure to SWCNT was investigated while handling unrefined material (Maynard et al. 2004). Certain circumstances (e.g. vigorously agitating carbon nanotube material or removing spilled nanotube material with a vacuum cleaner) can lead to the generation of ultrafine particles and an increase in the aerosol number concentration. However, concentrations generated while handling material in the field were very low and it was unclear whether

Workplace exposure during production and handling of carbon nanotube material

these particles represented nanotubes, catalyst particles (used in the manufacturing process) or other kind of carbonaceous particles, because with the applied measurement techniques it was not possible to distinguish particle shapes. The studies also stated a high potential for exposure to unprotected skin during material handling and recommends some protective measures for workers. Glove deposits of SWCNT during handling were estimated at between 0.2 mg and 6 mg per hand.

Other examples of nanoparticle measurements in workplace atmospheres have been published, where an unintended nanoparticle exposure occurs in conventional industrial processes like welding processes in a shipyard (Wehner et al. 2001), ski hot-waxing using fluor powder (Hämeri et al. 1996) or the use of diesel-powered vehicles in confined industrial spaces e.g. mines (Knight et al. 1983).

Because of the lack of information about nanoparticle exposures at workplaces some national programmes have been established to gather relevant data in this area. The BIA in Germany conducts a long-term programme aiming at the cataloguing of ultrafine particle exposures in selected workplace atmospheres. First measurements have been made by means of a SMPS (Scanning Mobility Particle Sizer) e.g. in the following areas:

- Welding processes
- Hard and soft soldering
- Laser ablation
- Plasma cutting
- Grinding
- Milling
- Powder coatings
- Printing and copying
- Bakery

6.2.2 Ambient environment

Inhaled particulate matter has been associated with both acute and chronic health effects. Concerns about these effects derive primarily from epidemiologic studies that associate short-term increases in particle concentration with increases in daily mortality from respiratory and cardiovascular diseases. The effects of variations in daily air pollutants on mortalities have been reported from Europe (Anderson et al. 1996, Spix et al. 1993, Touloumi et al. 1994) as well as from the United States (Dockery et al. 1993, Samet et al. 2000, Schwartz 1994) but also from Southeast Asia (Kwon et al. 2002, Vajanapoom et al. 2002, Wong et al. 2002, Xu et al. 1994) and New Zealand (Hales et al. 2000).

Because airborne particles do not constitute a uniform population, various types of particulate air pollution have been addressed in epidemiological studies, the main ones being TSP (total suspended

Long-term programme in Germany for cataloguing ultrafine particle exposure at selected workplaces

Ultrafine particles contribute much to the number concentration of particles in aerosols but their mass share is negligible

particulate) and PM₁₀ (particulate matter with an effective aerodynamic diameter less than 10 µm). In recent years, many studies have targeted fine particulate matter, i.e. particles with an aerodynamic diameter less than 2.5 µm (Murphy et al. 1998, Pope et al. 1995, Schwartz et al. 1996, Schwartz and Neas 2000). Based on epidemiologic evidence and results from animal studies on the potential toxicity of ultrafine particles, recent epidemiologic studies focus on the health effects of particles which are less than 100nm in diameter (De Hartogg et al. 2003, Klot et al. 2002, Pekkanen et al. 2002, Pekkanen et al. 1997, Penttinen et al. 2001, Peters et al. 1997, Tiitonen et al. 1999). Primary ultrafine particles are formed during gas-to-particle conversion or during incomplete fuel combustion. Car traffic is an important source of particulate emissions (Franck et al. 2003, Wehner et al. 2002). The number concentration of these small particles exceeds usually that of larger ones in the urban area, but their contribution to the total mass concentration is relatively low. Therefore, measurements of number size distributions down to a few nanometers are essential to describe traffic emissions. With the improvement of measurement techniques (Wichmann et al. 2000, Wiedensohler et al. 2002), clearer effects were observed with smaller particle sizes. However, most of the studies are ongoing and only few results are available so far (Ibald-Mulli et al. 2002, Pekkanen et al. 2002, Peters et al. 1997).

6.3 Toxicological assessment

In the following a toxicological risk assessment of nanomaterials is described based on peer reviewed publications and research papers. Since not much literature is available on the health risk of engineered nanomaterials, literature for nanoparticles and/or fibres from other sources such as diesel exhaust particles (DEP), textile flock, silica, asbestos, man-made fibres, etc. has been used (see review and original research papers e.g. Akerman et al. 2002).

An important point of discussion concerning nanomaterials is the penetration into the human body. Our knowledge in this field mainly comes from drug delivery (pharmaceutical research) and toxicology (xenobiotics) studying the pharmaco-(or toxico-) kinetics of these compounds. The effect of nanomaterials on organs “inside” the body (e.g. liver and brain) and the blood have been studied from the few publications on the penetration of nanomaterials through the lung, skin or intestinal barrier and on the existing literature on deliberately administered nanomaterials such as in drug delivery studies.

In this report, the most attention has been paid to the pulmonary exposure. This route of exposure is probably the most troublesome, because nanomaterials can, in comparison with other - larger - solid materials, enter easily deep into the lung tissue; the surface area of the alveolar epithelium is large and thin and consequently relatively defenceless compared to skin and intestines.

6.3.1 A brief biological background

Our three main contact sites with the environment are the skin, lungs and intestinal tract. These three organs have on the one hand, a barrier function in order to protect the body against hazardous compounds from the environment, and, on the other hand, a transport function to allow the organism to take up water, nutrients and oxygen.

The human skin functions as a strict barrier and no essential elements are taken up through the skin (except for UV-radiation necessary to build up vitamin D, and oxygen via the retina). The lungs exchange oxygen, carbon dioxide with the environment, during breathing some water escapes via the heated, and water saturated exhaled air. The intestinal tract is in close contact with all the materials taken up orally; here all nutrients (except gasses) are exchanged between the body and the environment.

The histology of the environmental contact sites of these three organs is significantly different. The skin of an adult human is more or less 2 m² in area, and is at most places covered with a relatively thick first barrier (10 µm) which is built of strongly keratinised dead cells. This first barrier is difficult to pass for ionic compounds as well as highly water-soluble molecules. Varying impermeability is depending on anatomic site, age, etc.

The lung consists of two different parts, the airways (transporting the air in and out the lungs) and alveoli (gas exchange areas). The airways are a relatively robust barrier, with an active epithelium protected with a viscous layer of mucus. In the gas exchange area, the barrier between the alveolar wall and the capillaries is very thin. The air in the lumen of alveoli is on average only 0.5 µm away from the blood. The large surface area of the alveoli and the intense air-blood contact in this region is often illustrated by the metaphor, that one wineglass of blood is spread over the surface of a tennis court. This specialised air exchange barrier consists of three layers: epithelial cells, connective tissue (interstitium) and a layer of endothelium (blood vessel wall). Although some defence mechanisms are active in the alveolar tissue, it is less well protected against environmental damage compared to the airways.

The intestinal tract is a more complex barrier – exchange site, it is the most important portal for macromolecules to enter a biological system. From the stomach, only small molecules can diffuse through the epithelium. The epithelium of the small and large intestines is in close contact with ingested material in order to allow exchange from nutrients. Several uptake systems have been described in detail and numerous molecules have been designed in order to enter the body more efficiently, this knowledge can be used to predict the behaviour of new compounds in the intestinal tract. In regard to the behaviour and translocation of particulate matter, much less information is available.

Three main possible entry routes for nanoparticles into the human body: lungs, skin and intestinal tract

The skin is a tightly closed barrier in direct contact with the environment

The lungs, more specific the alveoli, are the most vulnerable in contact with nanomaterials

The intestinal tract allows transport of soluble molecules and dispersed materials; it is a portal for food and drugs

6.3.2 Lung

6.3.2.1 Inhalation of solid (poorly soluble) material and pulmonary clearing

The pathogenicity of inhaled solid material (particles or fibres) depends primarily on achieving a sufficient lung burden (Moolgavkar et al. 2001). The lung burden is determined by the rates of deposition and clearance. Logically, for any dust or fibre, a steady-state dose level will be achieved when the rates come into balance. This is only true when the solid material neither interferes with the clearance mechanisms nor possesses any toxicity. In respect to the burden, the chemical and physical properties of the material itself are important insofar as they influence deposition and clearance rates.

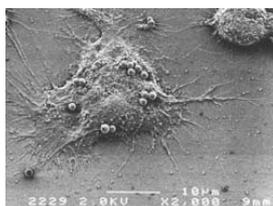
Spherical solid materials can be inhaled when their aerodynamic diameter is less than 10 μm . The smaller the particulates the deeper they can travel into the lung, particles smaller than 2.5 μm will even reach the alveoli. Ultrafine particles (nanoparticles with an aerodynamic diameter of less than 100 nm) deposit mainly in the alveolar region. Fibres are solid materials with a length to diameter ratio of at least 3:1 (occupational safety and health standard). The penetration into the lungs of each fibre can be described through its aerodynamic diameter. In general, fibres with a small diameter will penetrate deeper into the lungs, while very long fibres ($\gg 20 \mu\text{m}$) are easily stuck in the higher airways (see reviews: Lippmann et al. 1990).

The mucociliary escalator dominates the clearance from the (upper) airways; clearance from the deep lung (alveoli) is predominantly by macrophage phagocytosis. The mucociliary escalator is an efficient transport system pushing the mucus, which covers the airways, together with the trapped solid materials towards the mouth. The phagocytosis of particles and fibres results in activation of macrophages and induces the release of chemokines, cytokines, reactive oxygen species, and other mediators; this can result in sustained inflammation and eventually fibrotic changes.

The phagocytosis efficiency can be affected by the (physical-chemical) characteristics of the solid material (see below); moreover, fibres too long to be phagocytised (fibres longer than the diameter of the alveolar macrophage) will only be cleared (very) slowly.

From experimental laboratory exposure studies, it has been shown that if the inhaled concentrations are low, at which the deposition rate of the inhaled particles is not more than the mechanical alveolar macrophage-mediated clearance rate in the lung, retention half time of about 70 days (steady-state lung burden during continuous exposure) is found. If the deposition rate of the inhaled particles exceeds this clearance rate, the retention half time is significantly increased, reflecting an impaired or

Ultrafine particles deposit mainly in the alveolar region of the lungs



intaking ultrafine particles (source: GSF)

prolonged alveolar macrophage-mediated clearance function with continued accumulation of lung burden, which can lead to an overload.

Inhaled fibres

Fibres, that are persistent in the alveoli, can interact with the pulmonary epithelial cells or even penetrate the alveolar wall and enter the lung tissue. These fibres are often described as being in the “interstitial” where they may lie between or within the cells making up the alveolar walls. Materials that stay long time in the body are defined as “biopersistent”. The biopersistence is by itself a health hazard (via epigenetic pathways), which can be potentiated by the presence of mutagenic substances in the material (e.g. leaking metals), increasing the risk of developing cancer.

Recently, it has been reported that nanotubes show a sign of toxicity (Service 2003). This has been confirmed by two independent publications by Warheit et al. (2003) and Lam et al. (2003), respectively in rats and mice. Both reported the finding of granulomas, and some (interstitial) inflammation. The research group of Warheit concluded that these findings (multifocal granulomas) may not have physiological relevance, and may be related to the instillation of a bolus of agglomerated nanotubes. The other group concluded that the results show that if carbon nanotubes reach the lungs, they are much more toxic than carbon black and can be more toxic than quartz. These studies have to be read with some prudence because a study by NIOSH showed that no or only a small fraction of the nanotubes in the air can be inhaled (Baron et al. 2002).

Carbon nanotubes can cause severe lung damage, but exposure assessment indicates low risk for inhalation

Role of particle size

Clearance from the lung is not only dependent on the total mass of particles inhaled but depends strongly on the particle size – and, by implication, on particle surface, as shown in the following studies.

A subchronic 3 months inhalation exposure study to ultrafine (~20 nm) and fine (~200 nm) TiO₂ particles (Oberdörster et al. 1994) showed that the ultrafine particles cleared significantly more slowly, showed more translocation to interstitial sites and to regional lymph nodes compared to the 10-fold larger TiO₂ particles. Comparing similar - in size and composition - carbon black particles, but having a significant different specific surface area (300 versus 37 m²/g), it was found that the biological effects (inflammation, genotoxicity, and histology) were similar per specific surface area but not per particle mass.

Similar findings were reported in earlier studies on tumorigenic effects of inhaled particles. Some studies showed that tumour incidence correlates better with specific surface area than with particle mass (Driscoll et al. 1997, Oberdörster and Yu 1999). Comparing the health effects of chronically inhaled TiO₂ particles with a distinct different calibre's, it is remarkable that the low exposure (10 mg/m³) study

Some studies showed that biological effects of inhaled particles correspond with their specific surface area

(Heinrich et al. 1989) results in a greater lung tumour incidence than the high exposure (250 mg/m³) study (Lee et al. 1986). The inhaled particles in both studies consisted of aggregated primary particles, with an aerodynamic diameter that was probably not very different. The primary particle sizes of the low dose study was 20 nm, while it was appr. 300 nm in the latter study.

It must be noted that some types of particles will deposit in a different manner compared to most types because of some specific characteristics, such as very high or low density, specific dimensions, irregular shape, explicit hydrophobicity or hydrophilicity, etc.

6.3.2.2 Particles surface and biocompatibility

Technical reports on the surface properties of nanoparticles, both physical and chemical, stress that nanoparticles differ from bulk materials and that their properties heavily depend on the particle size. Therefore, it is not correct to assume that the nanoparticles are merely small crystals. The ratio of total molecules in a nanoparticles over the number of molecules at surface is low. Independently of the particle size, two parameters play a dominant role: The charges carried by the particle in contact with the cell membranes and the (chemical) reactivity of the particle (Lee et al. 1986).

Surface charges

Polycationic macromolecules show a strong interaction with cell membranes in vitro. A good example can be found in the Acramin F textile paint system. Three polycationic paint components exhibited considerable cytotoxicity (LC50 generally below 100 mg/ml for an incubation of 20-24 hours) in diverse cell cultures, including primary cultures of rat and human type II pneumocytes, and alveolar macrophages and human erythrocytes. The authors speculated that the multiple positive charges play an important role in the toxic mechanism (Hoet et al. 1999 and 2001).

Studying the biocompatibility (cytotoxicity) of polycationic materials (Fischer et al. 2003) as a function of the molecular weight, it was found that with increasing molecular weight some macro-molecules such as DEAE-dextran, poly-L-lysine (PLL) (Morgan et al. 1988 and 1989), dendrimers (Haensler and Szoka 1993) and PEI (Fischer et al. 1999) become more toxic; however, these findings apply only for polymers from same structure, but not for different types of polycations. Consequently, to explain the toxicity of polymers with different structures further parameters have to be taken into account. Dekie (Dekie et al. 2000) concluded that the presence of a primary amine on poly l-glutamic acid derivatives has a significant toxic effect on red blood cells causing them to agglutinate.

Not only the type of amino function but also the charge density resulting from the number and the three-dimensional arrangement of the cationic residues is an important factor for cytotoxicity. Ryser (Ryser 1967) suggested that a three-point attachment is necessary for eliciting a biological response on cell membranes, and speculated that the activity of a polymer will decrease when the space between reactive amine groups is increased. The arrangement of cationic charges depends on the three-dimensional structure and flexibility of the macromolecules (Ryser, 1967) and determines the accessibility of their charges to the cell surface. Branched molecules were found to be more efficient in neutralising the cell surface charge than polymers with linear or globular structure, rigid molecules have more difficulties to attach to the membranes than flexible molecules (Singh et al. 1992). Therefore, high cationic charge densities and highly flexible polymers should cause higher cytotoxic effects than those with low cationic charge densities, even more globular polycationic structures (chSA, PAMAM) were found to be polymers with a good biocompatibility (low cytotoxicity), whereas polymers with a more linear or branched and flexible structure (DADMAC, PLL, PEI) showed higher cell damaging effects.

The structure and the surface charge of some specific types of particles can be linked to their cytotoxicity

Surfactant interaction and surface chemistry

Geiser (Geiser et al. 2003) studied the influence of the particle surface chemistry on the immersion into the lung's surface-lining layer. They found that particles, regardless of the nature of their surfaces, will be submersed into the lining layer after their deposition in small airways and alveoli. This displacement is promoted by the surfactant film itself, whose surface tension falls temporary to relatively low values (Gehr et al. 2000, Geiser et al. 2003)

On the other hand reactive groups on a particle surface will certainly modify the biological effects. For silica, it has been shown that surface modification of quartz affects its cytotoxicity, inflammogenicity, and fibrogenicity. These differences are mainly due to particle surface characteristics (Schins et al. 2002). Specific cytotoxicity of silica is strongly correlated with the surface radicals and iron derived reactive oxygen species (ROS) and are considered to be the key factors in the development of fibrosis and lung cancer by this compound (Fubini 1997).

6.3.2.3 Systemic translocation of particles

The impact of inhaled particles on extrapulmonary organs has only recently been recognised. Most research has concentrated on the possible consequences of particle related malfunction of the cardio-vascular system, such as heart rate variability, coagulation, etc. (Yeates and Mauderly 2001). Recent data support the concept that the autonomic nervous system may be a target for the adverse effects of inhaled

Cardio-pulmonary hazard arises from lung inflammation and translocation of nanomaterial to the bloodstream.

particulates (Gold et al. 2000, Liao et al. 1999, Nemmar et al. 2001). The biological plausibility is not well documented. Two complementary hypotheses are proposed: the first hypothesis explains the observed effects by the strong (and persistent) pulmonary inflammatory reactions in the lungs, leading to the release of mediators (see above), which may influence the heart, coagulation, or other cardiovascular endpoints; the second hypothesis is that nanoparticles translocate from the lungs into the systemic circulation and thus, directly or indirectly, influence haemostasis or cardiovascular integrity.

In the evaluation of the health effects of inhaled nanoparticles the translocation to the systemic circulation is an important issue. Conhaim and co-workers (Conhaim et al. 1988) found that the lung epithelial barrier was best fitted by a three-pore-sized model, including a small number (2%) of large-sized pores (400-nm pore radius), an intermediate number (30%) of medium-sized pores (40-nm pore radius), and a very large number (68%) of small-sized pores (1.3-nm pore radius). The exact anatomical location of this passage, however, remains to be established (Hermans et al. 1999). Until recently, the possible passage of xenobiotic particles did not attract much attention, although, the concept is now gaining acceptance in pharmacology for the administration of macromolecular drugs by inhalation.

Inhaled nano-particles can migrate from the lungs to the circulation.

Nemmar et al studied the particle-translocation of inhaled ultrafine ^{99m}Tc-carbon particles to the blood. They observed that a small part of these particles, which are very similar to (the ultrafine fraction of) actual pollutant particles, diffuse rapidly – within 5 minutes - into the systemic circulation. Therefore, the authors concluded that it was unlikely that phagocytosis by macrophages and/or endocytosis by epithelial and endothelial cells are (solely) responsible for particle-translocation to the blood.

The literature on the translocation of very small particles from the lungs into the blood circulation is limited and still conflicting. A recent study has reported deposition and clearance for 2 h of an ultrafine (60 nm) technetium-^{99m}-labelled aerosol in human volunteers. No significant radioactivity was found over the liver (1-2 % of the inhaled radioactivity) but, unfortunately, no radioactivity measurements in blood were reported (Brown et al. 2002). In agreement with findings of Nemmar et al, Kawakami et al. (1990) have reported the presence of radioactivity in blood immediately after inhalation of ^{99m}Tc-technegas in human volunteers. It is also known that aerosolised insulin gives a rapid therapeutic effect (Steiner et al. 2002) although the pathways for this translocation are still unclear. In addition to human studies, in experimental animal studies (Kreyling et al. 2002, Lippmann 1990, Nemmar et al. 2001, Oberdörster et al. 2002, Takenaka et al. 2001) have reported extrapulmonary translocation of ultrafine particles after intratracheal instillation or inhalation. However, the amount of ultrafine

particles that translocate into blood and extrapulmonary organs differed among these studies. It has also been shown that, following intranasal delivery, polystyrene microparticles (1.1 μm) can translocate to tissues in the systemic compartment (Eyles et al. 2001). A recent study (Kato et al. 2003) has the merit of having provided, for the first time, morphological data showing that inhaled polystyrene particles are transported into the pulmonary capillary space, presumably by transcytosis.

Another alley of translocation from the lungs towards other organs has been undertaken by Oberdörster et al (2001). In inhalation experiments with rats, using ^{13}C -labelled particles, they found that nano-sized particles (25 nm) could be found in several organs 24 hours after exposure. Most extraordinary finding was that particles were found in the CNS. The authors examined this phenomenon more detail and found that particles, after being taken up by the nerve cells, can be transported via the nerves (in this experiment via the olfactory nerves) at a speed of 2.5 mm per hour (Oberdörster et al. 2002).

6.3.2.4 Thrombosis and lung inflammation

Epidemiological studies have reported a close association between particulate air pollution and cardiovascular adverse effects such as myocardial infarction (Peters et al. 2001). The latter results from rupture of an atherosclerotic plaque in the coronary artery followed by rapid thrombus growth because of exposure of highly reactive subendothelial structures to circulating blood, thus leading to additional or complete obstruction of the blood vessel. This will be discussed in chapter 6.3.6 “Body distribution and systemic effects”.

6.3.2.5 Fibre biopersistence

Long non-phagocytizable fibres (in humans longer than 20 μm) will not be effectively cleared from the respiratory tract. The main determinants of fibre biopersistence are species specific physiological clearance and fibre specific biodurability (physico-chemical processes). In the alveoli the rate at which fibres are physically cleared depends on the ability of alveolar macrophages to phagocytose them. Macrophages containing fibres longer than their own diameter may not be mobile, and be unable to clear these fibres from the lung. The biodurability of a fibre consists of: a) dissolution and leaching, b) as well as mechanical breaking and splitting.

Long fibres in the lung can disintegrate, leading to shorter fibres that can be removed by the macrophages. Biopersistent types of asbestos, where breakage occurs longitudinally, result in more fibres of the same length but smaller diameter. Amorphous fibres break perpendicular to their long axis (ILO 1990, Searl 1994), resulting in fibres that can be engulfed by the macrophages.

Cancer risk of inhaled fibres/ particles

The longer insoluble materials persist in body tissue the higher the risk of developing adverse effects

It is self-evident that the slower the fibres are cleared (high biopersistence), the higher is the tissue burden; and the longer the fibres reside in a tissue the higher is the probability of an adverse response. A milestone was set by Stanton et al. (1977, 1972) who undertook a series of experiments with 17 samples of carefully sized fibrous glass. They found that for mesothelioma induction in rats, the peak activity was in the fibres greater than 8 μm in length and less than 1.3 μm in diameter. These findings are known as the “Stanton hypothesis”. However these results do not strictly indicate that all fibres longer than the lower threshold are equally active or that shorter fibres are not, although fibres less than 5 μm in length did not appear to contribute to lung cancer risk in exposed rats (Berman et al. 1995). Risk appears to increase with length, with fibres more than 40 μm in length imposing the highest risk. This has been recently reviewed by Schins (2002).

6.3.3 Intestinal tract

6.3.3.1 Translocation

Particles are taken up by the intestinal tract

The gastrointestinal uptake of inert particulate matter (as opposed to viral / bacterial uptake) is a widely accepted phenomenon. Particulate uptake does take place, not only via the M-cells in the Peyer's patches (PP) and the isolated follicles of the gut-associated lymphoid tissue, but also via the normal (intestinal) enterocytes. There have been a number of excellent reviews on the subject of intestinal uptake of particles (Florence and Hussain 2001, Hussain et al. 2001)

Uptake of inert particles has been shown to occur transcellularly through normal enterocytes and PP via M-cells, and to a lesser extent across paracellular pathways (Aprahamian et al. 1987).

Delivery to specific tissues or cells depends on the physico-chemical characteristics of the material

Already in 1926 it was recognised by Kumagai that particles could translocate from the lumen of the intestinal tract via aggregations of intestinal lymphatic tissue (Peyer's patches or PP), containing M-cells, specialised phagocytic enterocytes. This observations have now been reported in different species from salmon to chicken (Hussain et al. 2001). Initially it was assumed that the PP did not discriminate strongly in the type and size of the absorbed particles. It has been shown that modified characteristics, such as particle size (Hillyer and Albrecht 2001), the surface charge of particles (Jani et al. 1989), attachment of ligands (Hussain and Florence 1998, Hussain et al. 1997) or coating with surfactants (Hillery et al. 1994), offers possibilities of site-specific targeting to different regions of the intestinal tract, including the PP (Woodley 2002).

The kinetics of particle translocation in the intestine depends on diffusion and accessibility through mucus, initial contact with enterocyte or M-cell, cellular trafficking, and post-translocation events.

Charged particles, such as carboxylated polystyrene nanoparticles (Jani et al. 1989) or those composed of positively charged polymers exhibit poor oral bioavailability through electrostatic repulsion and mucus entrapment. Szentkuti (1997) determined the rate of particle diffusion across the mucus layer to the enterocyte surface with respect to both size and surface charge of the particles. In brief, Szentkuti (1997) observed that cationic nanometer-sized latex particles became entrapped in the negatively charged mucus, whereas repulsive carboxylated fluorescent latex nanoparticles were able to diffuse across this layer. The smaller the particle diameter the faster they could permeate the mucus to reach the colonic enterocytes, 14-nm diameter permeated within 2 min, 415-nm particles took 30 min, while 1000-nm particles were unable to pass through this barrier. Within the time of the experiment (30 min) none of the particles was endocytosed by the enterocytes despite the fact that the latex nanoparticles preferentially bound the cell surface more strongly than the mucus. After a larger time window (oral gavage for several days) a sparse accumulation of charged particulates in the lamina propria was found compared to uncharged latex nanoparticles in the same size range (Jani et al. 1989).

Particulates, once in the sub-mucosal tissue, are able to enter both lymphatics and capillaries. Particles entering the lymphatics are probably important in the induction of a secretory immune responses while those which enter the capillaries can reach different organs. In a study, the body distribution after translocation of polystyrene particles was studied in some detail. Polystyrene spheres (ranging from 50 nm to 3 μ m) were fed by gavage to female Sprague Dawley rats daily for 10 days at a dose of 1.25 mg/kg. As much as 34 % and 26% of the 50 and 100 nm particles were absorbed respectively. Those larger than 300 nm were absent from blood. No particles were detected in heart or lung tissue (Jani et al. 1990).

6.3.3.2 Intestinal Translocation and Disease

Crohn's disease is characterised by transmural inflammation of the gastrointestinal tract. It is of unknown aetiology, but it is suggested that a combination of genetic predisposition and environmental factors play a role. Particles (0.1-1.0 μ m) are associated with the disease and indicated as potent adjuvants in model antigen-mediated immune responses. In a double-blind randomised study, it has been shown that a particle low diet (low in Ca and exogenous microparticles) alleviates the symptoms of Crohn's disease (Lomer et al. 2002).

Some human diseases can be linked with uptake of particulate matter

Although there is a clear association between particle exposure, uptake and Crohn's disease, little is known of the exact role of the phagocytosing cells in the intestinal epithelium. It has been suggested that the disruption of the epithelial barrier function by apoptosis of enterocytes is a possible trigger mechanism for mucosal inflammation. The patho-physiological role of M cells is unclear; e.g., it has been found that in Crohn's disease M cells are lost from the epithelium. Other studies found that the endocytosis capacity of M cells is induced under various immunological conditions, e.g. a greater uptake of particles (0.1 μm , 1 μm and 10 μm diameter) has been demonstrated in the inflamed colonic mucosa of rats compared to non-ulcerated tissue (Kucharzik et al. 2000, Powell et al. 2000) and inflamed oesophagus (Hopwood et al. 1995).

Diseases other than of gut origin also have marked effects on the ability of gastro-intestinal tract to translocate particles. The absorption of 2- μm polystyrene particles from the PP of rats with experimentally induced diabetes is increased up to 100-fold (10% of the administered dose) compared to normal rats (Mc Minn et al. 1996). However, the diabetic rat displayed a 30% decrease in the systemic distribution of the particles. One possible explanation for this discrepancy is the increased density of the basal lamina underlying the GI mucosa of diabetic rats that may impede particle translocation into deeper villous regions. This uncoupling between enhanced intestinal absorption and reduced systemic dissemination has also been observed in dexamethasone treated rats (Limpanussorn et al. 1998).

6.3.4 Skin

6.3.4.1 Translocation of particles from topical exposure to dermis or epidermis

The skin is an important barrier, protecting against insult from the environment. The skin is structured in three layers: the epidermis, the dermis and the subcutaneous layer. The outer layer of the epidermis, the stratum corneum (SC), covers the entire outside of the body. In the SC we find only dead cells, which are strongly keratinised (horny). For most chemicals the SC is the rate-limiting barrier to percutaneous absorption (penetration). The skin of most mammalian species is, on most parts of the body, covered with hair. At the sites, where hair follicles grow, the barrier capacity of the skin differs slightly from the "normal" stratified squamous epidermis.

Most studies concerning penetration of materials into the skin have focussed on the fact whether or not drugs penetrate through the skin using different formulations containing chemicals and/or particulate materials as vehicle. The main types of particulate materials commonly used are: liposomes; solid poorly soluble materials such as TiO_2 and

polymer particulates and submicron emulsion particle such as solid lipid nanoparticles. The penetration of these particulate carriers has not been studied in detail.

6.3.5 Solid materials

TiO₂ is a particle, which is often used in sunscreens, absorbing the light and therefore protecting the skin against sunburn or genetic damage. It has been reported by Lademann et al. (1999) that TiO₂ in sunscreens (micrometer-sized particles) penetrates the human stratum corneum and even into some hair follicles - including their deeper parts. However, the authors did not interpret this observation as penetration into living layers of the skin, since this part of the follicular channel (the acroinfundibulum) is covered with a horny layer barrier too (Lademann et al. 1999). Several studies have been conducted showing no or very little penetration of the skin by TiO₂ nanoparticles (Plücker et al. 2001, Menzel et al. 2004). However, in a recent review it is stated that: “very small titanium dioxide particles (e. g. 5–20 nm) penetrate into the skin and can interact with the immune system.” (Kreilgaard 2002).

Uptake of poorly soluble nanomaterials via the skin is questionable

Tinkle et al. (2003) demonstrated that 0.5- and 1.0- μm particles, in conjunction with motion, penetrate the stratum corneum of human skin and reach the epidermis and, occasionally, the dermis. The authors hypothesised that the lipid layers within the cells of the stratum corneum form a pathway by which the particles can move (Menon and Elias 1997) into the skin and be phagocytized by the Langerhans cells. In this study the penetration of particles is limited to particle diameter of 1 μm or less. Nevertheless, other studies reported penetration through the skin using particles with diameters of 3-8 μm (Andersson et al. 2002, Lademann et al. 2001) but only limited penetration was found often clustered at the hair follicle.

Penetration of non-metallic solid materials such as biodegradable poly(D,L-lactic-co-glycolic acid (PLGA) microparticles, 1 to 10 μm with a mean diameter of 4.61 ± 0.8 μm was studied after application on porcine skin. The number of microparticles in the skin decreased with the depth (measured from the airside towards the subcutaneous layer). At 120 μm depth (viable dermis) a relative high number of particles was found, at 400 μm (dermis), hardly none, but still some microparticles were seen. At a depth of 500 μm no microparticles were found (de Jalon et al. 2001).

In the skin of individuals, who had an impaired lymphatic drainage of the lower legs (e.g. endemic elephantiasis), soil microparticles, frequently 0.4-0.5 μm but as larger particles of 25 μm diameter, were found in the in the dermis of the foot. The particles are seen to be in the phagosomes of macrophages or in the cytoplasm of other cells. The failure to conduct lymph to the node produces a permanent deposit of silica in the dermal

tissues (a parallel is drawn with similar deposits in the lung in pneumoconiosis). This indicates that soil particles penetrate through (damaged) skin, most probably in every individual, and normally are removed via the lymphatic system (Blundell et al. 1989, Corachan 1988).

Liposomes

Liposomes penetrate the skin in a size-dependent manner. Micro-sized, and even submicron sized, liposomes do not easily penetrate into the viable epidermis, while liposomes with an average diameter of 272 nm can reach into the viable epidermis and some were found in the dermis. Smaller sized liposomes of 116 & 71 nm were found in higher concentration in the dermis (Verma et al. 2003).

Emzaloid particles, a type of submicron emulsion particle such as liposomes and niosomes, with a diameter of 50 nm to 1 μm , were detected in the epidermis in association with the cell membranes after application to human skin. The authors suggested that single molecules, which make up the particles, might penetrate the intercellular spaces and, at certain regions in the stratum corneum, are able to accumulate and reform into microspheres. In a subsequent experiment, it was shown that the used formulation allowed penetration of the spheres into melanoma cells, even to the nucleus (Saunders et al. 1999).

Soluble metal components

In a recent review by Hostynek (2003) it is concluded that the uptake of metals through the skin is complex, because of both exogenous factors, e.g. dose, vehicle, protein reactivity, valence and endogenous factors e.g. age of skin, anatomical site, homeostatic control, etc. Attempts to define rules governing skin penetration to give predictive quantitative structure–diffusion relationships for metallic elements for risk assessment purposes have been unsuccessful, and penetration of the skin still needs to be determined separately for each metal species, either by in vitro or in vivo assays.

6.3.5.1 Mechanical skin irritation

Glass fibres and rockwool fibres are widely distributed man-made mineral fibres because of their multiple applications, mainly as isolation materials, and have become important in replacing asbestos fibres. In contact with the skin, these fibres can induce dermatitis simply resulting from mechanical irritation. It has not been examined in detail what makes these fibres such a good irritants. In a occlusion irritant patch test in humans it was found that rockwool with a diameter of $4.20 \pm 1.96 \mu\text{m}$ was more irritating than those with a mean diameter of $3.20 \pm 1.50 \mu\text{m}$ (Jaakkola et al. 1994).

Although this is common knowledge, it is not clear what makes these fibres irritants. In search for reports on fibres with a diameter of < 100

nm no information has been found, indicating that some experimental work should be carried out on this matter.

6.3.6 Body distribution and systemic effects

6.3.6.1 Body distribution

The body distribution of particles is strongly dependent on the surface characteristics. In the following paragraph a few examples are shown:

1) Coating of poly(methyl methacrylate) nanoparticles with different types and concentrations of surfactants changes significantly their body distribution. Coating this type of nanoparticles with 0.1 % poloxamine 908 or more reduces their liver concentration significantly (from 75 to 13 % of total amount of particles administrated) 30 min after i.v. injection. Another surfactant, polysorbate 80, became only effective to change the characteristics of these nanoparticles above a concentration 0.5% (Araujo et al. 1999).

2) A different report shows that nanoparticles surface modified with a cationic compound, didodecyldimethylammonium bromide (DMAB), facilitates 7-10-fold the arterial uptake. The authors noted that the DMAB surface modified nanoparticles had a zeta potential of +22.1 +/- 3.2 mV (mean +/- sem, n = 5) which is significantly different from the original nanoparticles which had a zeta potential of -27.8 +/- 0.5 mV (mean +/- sem, n = 5). The mechanism for the altered biological behaviour is rather unclear, but surface modifications have potential applications for intra-arterial drug delivery (Labhasetwar 1998).

3) Oral uptake (gavage) of polystyrene spheres of different sizes (50 nm to 3 µm) in female Sprague Dawley rats (10 days at a dose of 1.25 mg/kg) resulted in systemic distribution of the nanoparticles. About 7% (50 nm) and 4% (100 nm), was found in the liver, spleen, blood and bone marrow. Particles larger than 100 nm did not reach the bone marrow, and those larger than 300 nm were absent from blood. No particles were detected in heart or lung tissue (Jani et al. 1990).

6.3.6.2 Inducing oxidative stress

It has been shown that nanoparticles that enter the liver, can locally induce oxidative stress. A single (day 1; 20 and 100 mg/kg) and repeated (14 days) i.v. administration of poly-isobutyl cyanoacrylate (PIBCA, a biodegradable particle) or polystyrene (PS, not biodegradable) nanoparticles induced in the liver a depletion of reduced glutathione (GSH) and oxidised glutathione (GSSG) levels, an inhibition of superoxide dismutase (SOD) activity and a slight increase in catalase activity. The nanoparticles did not distribute in the hepatocytes, implicating that the oxidative species most probably were produced by activated hepatic macrophages, after nanoparticle phagocytosis. Uptake

The body distribution of particles is strongly dependent on their surface characteristics

of polymeric nanoparticles by Kupffer cells in the liver induce modifications in hepatocyte antioxidant systems, probably due to the production of radical oxygen species (Fernandez-Urrusuno et al. 1997).

We have discussed above that nanosized particles in the lung can induce oxidative stress, via the pulmonary inflammatory response as well as via spontaneously surface related reactions.

6.3.6.3 Effects on thrombosis

Nemmar et al. studied the possible effects of particles on haemostasis, focusing on thrombus formation as a relevant endpoint. Polystyrene particles of 60 nm diameter (surface modifications: neutral, negative or positive charged) have a direct effect on haemostasis by the intravenous injection. Positively charged amine-particles led to a marked increase in prothrombotic tendency, resulting from platelet activation (Nemmar et al. 2002). A similar effect could be obtained after the intratracheal administration of these positively charged polystyrene particles, which also caused lung inflammation (Nemmar et al. 2003). It is important to indicate that the pulmonary instillation of larger (400 nm) positive particles caused a definite pulmonary inflammation (of similar intensity as 60 nm particles), but did not lead to a peripheral thrombosis within the first hour of exposure. This lack of effect of the larger particles on thrombosis, despite their marked effect on pulmonary inflammation, suggests that pulmonary inflammation by itself was insufficient to influence peripheral thrombosis. Consequently, the effect found with the smaller, ultrafine particles is most probably due, at least in part, to their systemic translocation from the lung into the blood. Using pollutant particles, namely diesel exhaust particle (DEP), it was shown that within an hour after their deposition in the lungs, DEP cause a marked pulmonary inflammation. Intratracheal instillation of DEP promotes femoral venous and arterial thrombosis in a dose-dependent manner, already starting at a dose of 5 µg per hamster (appr. 50 µg/kg). Subsequent experiments showed that prothrombotic effects persisted at 6 h and 24 h after instillation (50 µg/animal) and confirmed that peripheral thrombosis and pulmonary inflammation are not always associated (Moore et al. 2001).

Pro-thrombotic
effect of specific
nanoparticles is a
human health
hazard

6.3.6.4 Cell and tissue specific delivery

Cellular uptake

Reports on particle uptake by endothelial cells (Akerman et al. 2002, de Jalon et al. 2001), pulmonary epithelium (Boland et al. 1999, Hopwood et al. 1995, Juvin et al. 2002, Kato et al. 2003), intestinal epithelium (Florence and Hussain 2001, Hopwood et al. 1995), alveolar macrophages (Hoet and Nemery 2001, Lundborg et al. 2001, Mossman and Sesko 1990, Oberdörster 1995, Takenaka et al. 2001), other

macrophages (Blundell et al. 1989, Fernandez-Urrusuno et al. 1997, Lomer et al. 2002, Powell et al. 1996), nerve cells (Oberdörster et al. 2002) and other cells (Pratten and Lloyd 1997) can be found. In the context of this report we did not tackle this issue in detail.

Blood Brain Barrier passage of nanoparticles

Organ or cell specific nanoparticle-mediated drug delivery (Alyaudtin et al. 2001, Pulfer et al. 1999, Schroeder et al. 1998) is one of the promises of nanotechnology. It is hypothesised that transport of nanoparticles across the BBB is possible by either passive diffusion or by carrier-mediated endocytosis. Coating of particles with polysorbates, e.g. polysorbate 80, results in anchoring of apolipoprotein E (apo E) or other substances in the blood. These now “surface modified” particles seem to mimic LDL particles and can interact with the LDL receptor leading to uptake by endothelial cells. Hereafter the drug (which was loaded in the particle) may be released in these cells and diffuse into the brain interior or the particles may be transcytosed. Also other processes such as tight junction modulation or Pgp inhibition also may occur (Kreuter 2001). Oberdörster et al. (2002) reported the translocation of inhaled nanoparticles via the olfactory nerves.

Nanoparticles can penetrate into the brain via several routes

6.3.7 Conclusion

Although the contact with nanomaterials in the lungs and intestinal tract shows many similarities, from a toxicological point of view, a few remarks concerning the differences between inhalation and ingestion of nanomaterials can be made:

- In the intestinal tract a complex mix of compounds - such as secreted enzymes, ingested food, bacteria of the gut flora etc – is present, which can interact with the ingested (nano) material. Non-specific interaction reduce the toxicity of the ingested material. In vitro it has been described that particles are less cytotoxic when dosed in medium with high protein content. In the lungs, mucus or surfactant is present, in which antioxidants are present, but these can be easily neutralised when a high number of oxidative compounds is inhaled.
- The transit through the intestinal tract is a relatively fast process, the continuous decay and renewing of the epithelium reduces the half-life time of nanomaterials significantly. The presence of solid material in the lumen of the intestines will not automatically induce an inflammatory response. Inhaled materials < 10 µm and > 5 µm will not enter the alveolar spaces of the lungs, and therefore these will be cleared easily in healthy persons via the mucociliary escalator. Particles smaller than 5 µm, and certainly nanomaterials, will deposit in the alveolar space via Brownian movement. In the alveoli, materials that do not dissolve can only be removed via phagocytosis by macrophages or other cells, or via transportation through the

epithelium to the interstitium or systemic circulation. These processes are often accompanied by the onset of (persistent) inflammation. The particles itself can – depending on the physical-chemical characteristics of the material – remain for a long period in the alveoli.

- In the intestinal tract, the ingested materials are stressed from acidic (stomach) to basic conditions. The shift in pH changes the solubility and the ionic state of the material strongly (changing the surface characteristics). In the lungs, the milieu of the lumen is more constant.

From the literature analysis it can be concluded that:

- Particles in the nanosize range can certainly enter the human body via the lungs and the intestines; penetration via the skin is less evident although strong evidence exists that some known particles can penetrate deep into the dermis. The penetration is depending on the size and surface properties of the particles and also depends on the point of contact in the lung, intestines or skin
- The distribution in the body is strongly depending on the surface characteristics of the particle. It seems that size can restrict the free movement of particles.
- The target organ-tissue or cell of a nanoparticle needs to be investigated, certainly for potentially hazardous compounds. Before developing in vitro test it is essential to know the pharmaco-kinetic behaviour of different types of nanoparticles, therefore it would be wise to construct a good database on health risks of different nanoparticles.
- There is no universal “nanoparticle” to fit all the cases, each nanomaterial should be treated individually when health risks are expected.
- The health risks of inhaled fibrous material needs to be examined with care, because it is general accepted that fibres that are not cleared easily from the lungs can induce pulmonary disease.
- Until now not much data has been generated concerning the secretion of nanoparticles via the urine. It is probably of significant importance to know what is the half life time of a nanoparticle in the body and what is the effect of nanoparticles on the function of the kidney.

Uptake and distribution of nanoparticles in the body depends on characteristics of the particles and the portal of entry

6.4 Toxicological testing

The potential hazard of ultrafine particle and fibres is ‘a priori’ not predictable by the bulk physico-chemical properties. Both in-vivo and in-vitro methods can be used for the toxicity assessment of nanoparticles. Because in vivo experiments, using animal models, are expensive, slow and ethically questionable there is a strong demand for a low-cost high-throughput in vitro assay without reducing the efficiency and reliability of the risk assessment. The test method should be capable of studying the relationship between deposited particles and acute/chronic inflammation

Strong demand for low cost high-throughput toxicity assays

to determine which aspects of surface area (and other possible parameters) are best predictors of adverse health effects.

Although some test methods are already available (for example the „Vector-model“ which is an in-vitro system that can be used for qualitative and quantitative evaluation of the reactivity of alveolar macrophages e.g. metabolism, secretion of inflammatory mediator and secretion of DNA damaging reactive oxygen species) further work has to be done to establish a standardised test procedure to detect specific and non specific particle toxicity effects which could lead to a benchmarking of different particles. This could path the way to propose appropriate exposure limits and regulations according to nanoparticle emissions.

6.5 Preliminary scheme for risk assessment

In view of the fact that data on exposure assessment are lacking, a full risk assessment of nanoparticulate materials in most cases is not feasible at present. However a ranking of potential risks can be achieved by applying hazard trigger algorithms. Relevant factors which can give a first estimation of potential risks of nanomaterials/-particles are:

- Production volume
- Potential exposure to customers, workers, environment
- Potential aerosol release during production, handling, processing
- Solubility
- Aspect ratio (to distinguish between fibers and particles)
- Particle diameter (taking into account a potential deagglomeration in body liquids e.g. in the lungs)
- Toxicological and ecotoxicological parameters

A concept scheme for assessing the risks of nanomaterials is depicted in the following figure. This scheme is to be regarded separately from registration processes of new chemical substances in the frame of existing chemical regulations, because particle size does not play a role in current chemical legislation. Until now producers are not obliged to declare particle size of the substances in the frame of registration processes. Therefore, the proposed scheme should be applied also for already registered substances which are re-manufactured as nanoparticulate materials and therefore might differ significantly from bulk materials in their physical and toxicological properties.

Preliminary scheme
for risk assessment
of nanomaterials

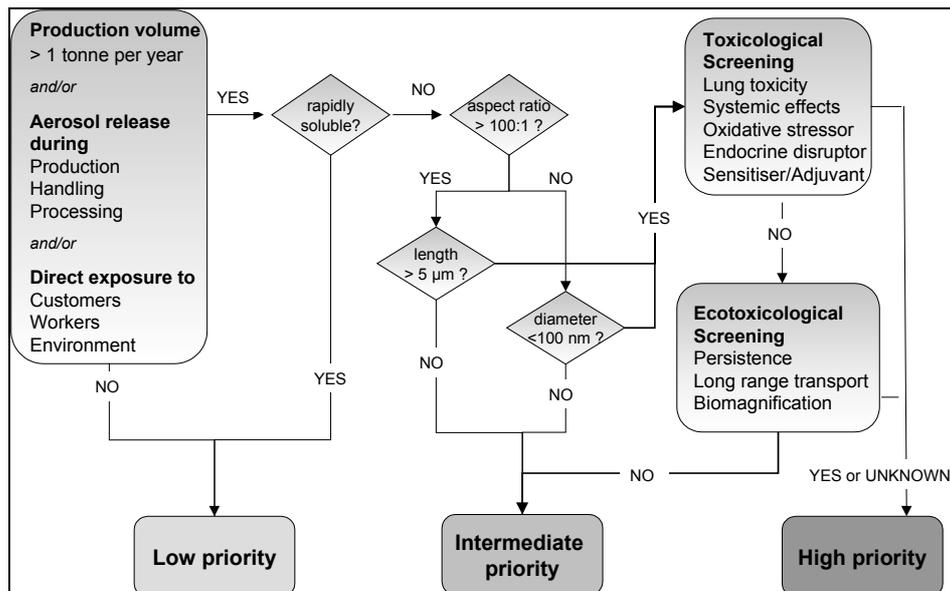


Figure 37: Scheme for a preliminary risk assessment of nanoparticulate materials (source: VDI-TZ, modified form Howard and de Jong 2004)

Further investigations could lead to more suitable parameters for risk assessing, e.g. surface properties (area, bioavailability). It can be assumed that many parameters of nanoparticulate materials with regard to toxicological and ecotoxicological properties will be unknown. Here standardised screening test would be of great use which can give a first assessment of potential risks. Nanoparticulate materials assessed with a high priority should be subject to further investigations and/or regulatory measures.

7 RISK MANAGEMENT

In the following chapter different measures for risk management of the production of engineered nanoparticles are pointed out. These comprise:

- Preventive measures at the work place
- Preventive measures for the environment
- Standardisation and regulation activities

Due to the lack of a reliable risk assessment for most kind of nanomaterials the implementation of risk management measures is presently at a very early stage.

7.1 Preventive measures at the work place

The production of nanoparticles normally requires complete physical enclosure; thereby reducing personal exposure of workers. However, handling and further processing of nanoparticles as well as cleaning operations of production equipment can lead to exposure of workers at the workplace. It is mainly for those situations the following paragraphs are intended for. Prevention of occupational exposure by control at the source of the hazard will generally be preferred to personal protective equipment. The assessment of airborne particulates, which can be inhaled or deposited on the skin, should be the main focus of the control system.

7.1.1 Process modification

Processes that are continuous, as opposed to intermittent or batch processes are likely to be less hazardous from an occupational exposure standpoint. Processes should be designed to contain nanoparticles within sealed or closed equipment to the greatest extent possible, to minimise the potential for emissions to the workplace.

7.1.2 Isolation of process

Computerised process control, automation of various production and maintenance procedures, and the general concept of remote processing help to isolate processes and equipment. Limiting employee access to certain areas during hazardous operations may also be an effective means of isolation.

7.1.3 Local exhaust ventilation

Local exhaust ventilation is probably the most commonly used and certainly one of the most versatile controls available. It involves a directed flow of air across an emission point and into a capture hood and ductwork system. Hood design depends on the physical configuration of the process equipment and emission characteristics. Designs may vary from freestanding hoods to complete process enclosure. Sufficient air

flow is necessary to capture and convey the nanoparticles into the hood and through the ventilation system to overcome the extraneous air patterns in the workplace. Booths in production areas that are equipped with a positively pressurised clean air supply might be helpful in limiting exposure to nanoparticles.

7.1.4 Work practices

Work practices are an essential adjunct to engineering control measures. Good work practices include structuring of standard operating and maintenance procedures that will minimise exposures. Ultimately, the individual worker shares a great deal of personal responsibility in preventing exposures. Lack of worker adherence to appropriate use of respirators, when indicated is likely to be a major factor in reducing the effectiveness of a respirator program. Workers must receive training regarding the need for respirators as well as their proper use. This training should include the nature of the respiratory hazard, the reasons why environmental controls are inadequate, the risks of health if respiratory protection is not used properly, and specific instructions regarding when respirators are to be worn. Not unexpectedly, the proportion of workers who use respirators is low when the health risk is associated with a cumulative effect of exposure and thereby perceived as remote. The worker's beliefs about the potential for discomfort or inconvenience and the attitude of co-workers are also important determinants of the intention to use a respirator.

7.1.5 Personal protective equipment

Personal protective equipment is another means of isolating the worker from the potential exposure. One of the major drawbacks to this type of control is that less emphasis is often placed on maintaining the general workplace free from contamination and placing the main responsibility for safety on the workers themselves.

7.1.5.1 Inhalation

Respiratory protection is generally used in situations where complete control is not achievable through feasible engineering measures. Respirators can remove nanoparticles from the surrounding air, supply breathable air from another source, or utilise a combination of both methods. Air-purifying respirators use facepieces of varying types: Quarter masks cover only the nose and mouth, half masks extend below the chin, and full masks also cover the eyes. Exhaled air leaves the mask through a one-way exhalation valve. Unless a powered blower is used to push air through the respirator's filter or cartridge, negative pressure is developed inside the mask when the wearer inhales.

There is a popular misconception that fibrous filters behave like a sieve where particles above a certain size are trapped and smaller particles pass through. While some filters such as membrane filters in liquids do function this way, fibrous air filters defy common sense by actually trapping smaller and larger particles more effectively than mid-sized particles. A fibrous filter is comprised of a large number of randomly oriented fibres. These fibres form a dense material or mat which captures and retains particles throughout its depth or thickness. The efficiency of entrapment is dependent upon the composition of the filter and the size distribution of the particles. Small densely packed fibers in the filter material increase the efficiency of the filter but also increase resistance to airflow. As the entrapped particle load on the filter increases, deposition efficiency and resistance increase. The higher the ambient concentration of particles and the greater the volume of air inhaled per minute through the respirator, the more quickly the filter becomes heavily loaded and requires replacement.

Three mechanisms are predominant in particle retention: interception, inertial impaction, and diffusion.

Interception occurs when a particle which is following a gas streamline comes within one particle radius of a filter fiber. The particle touches the fiber and is captured, thus being removed from the gas flow (see figure 38). For a given particle size, there are certain streamlines which will move close enough to a filter fiber so that the particle will be captured. Streamlines further than one particle radius away from a filter fiber will not contribute to the interception mechanism.

Inertial impaction occurs when a particle is so large that it is unable to quickly adjust to the abrupt changes in streamline direction near a filter fiber. The particle, due to its inertia, will continue along its original path and hit the filter fiber. This type of filtration mechanism is most predominant when high gas velocities and dense fiber packing of the filter media is present. Figure 39 illustrates this mechanism.

The **diffusion** mechanism of particle retention is the result of the Brownian motion of gas molecules. Small particles, with diameters in the range of $0.1 \mu\text{m}$ and below, tend to make random motions due to their interaction with the gas molecules. As these small particles are bumped by the gas molecules they too begin moving randomly about, bumping into other particles as well. Diffusion is predominant with low gas velocities and smaller particles. The smaller a particle is and the slower the flow, the more time it will have to zigzag around, thereby giving it much better chance of hitting and sticking to a filter fiber (see figure 40).

A graph showing how filter efficiency varies with particle size is shown in figure 41. As can be seen from the graph, a filter's ability to remove particles from a gas stream is directly related to the size of the particles in the stream. Large particles above $0.4 \mu\text{m}$ in diameter will be captured due

Particle retention mechanisms of fibrous air filters

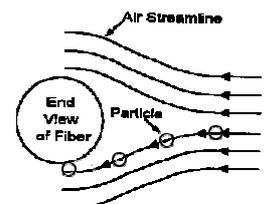


Fig. 38: Direct interception

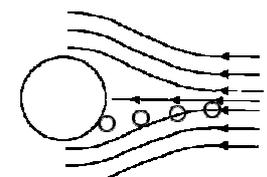


Fig. 39: Inertial Impaction

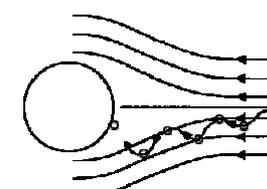


Fig. 40: Diffusion

to both the impaction and interception mechanisms. Medium particles, generally considered as the most penetrating, in the 0.1 to 0.4 μm diameter range, are captured by both the diffusion and interception filtration mechanisms. Small particles, below 0.1 μm in diameter, are captured by the diffusion mechanism.

The efficiency of a fibrous filter varies for different particles sizes and flow rates. It is meaningless to specify the efficiency of a fibrous filter without also stating the pertinent particle size and flow. For example, NIOSH defines a P100 (formerly HEPA) respirator filter to be at least 99.97 percent efficient for 0.3 μm particles at a flow rate of 85 liters per minute (lpm). Similarly, an N95 class filter must be at least 95 percent efficient against 0.3 μm particles at 85 lpm. The reason that a particle size of 0.3 μm is commonly referenced is because particles near 0.3 μm in diameter are more likely to get through the filter than any other size. In other words, it is the worst-case particle size. The filter's efficiency is higher at any other size. Comparing the lungs with a filter the same applies: the likelihood of ultrafine particles smaller than 100 nm to be deposited is greater than of particles around 300 nm in size.

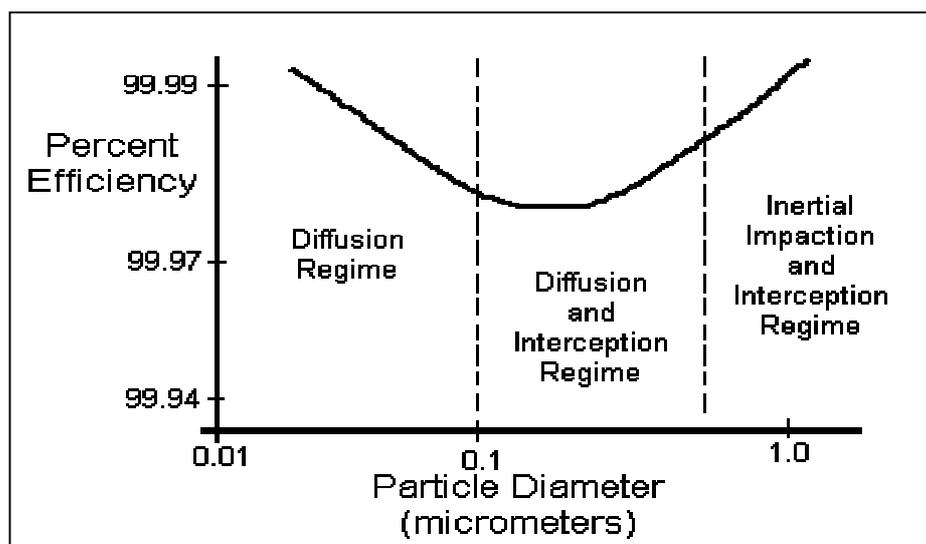


Figure 41: Filter efficiency versus particle size

7.1.5.2 Dermal contact and absorption

Although as route of exposure certainly less important than the lungs - the skin could still be a way of entry for nanoparticles into the human body. To prevent dermal absorption protective clothing, gloves, and head wear should be considered.

7.1.5.3 Eye contact

Eye contact can be prevented by wearing a full respiratory mask or glasses with side protection. People who wear contact lenses should

probably be advised to wear glasses instead, especially if they complain of sore and “dry” eyes.

7.1.5.4 Ingestion

Nanoparticles are added to processed food with no adverse health effects having been observed so far. On the other hand the chemical composition, metal content, and the biologic and immunologic properties of nanoparticles vary widely, and might well pose a risk. Also, nanoparticles will be ingested, once they have been cleared via the ‘mucociliary elevator’ from the tracheobronchial region of the lungs and were swallowed.

7.1.6 Control System

Appropriate surveillance of work area conditions and of worker exposure to ultrafine particles should be carried out. Regular measurements of the number concentration of nanoparticles are necessary not only where nanoparticles are produced, but also at exposed working places in the processing industries. The monitoring should cover conditions throughout a whole work shift as activities in the work area vary during the shift and change the hazard concentration.

Local exhaust ventilation should be preferred to personal protective equipment, wherever possible. If effective engineering controls are not feasible, appropriate respirators should be used. The importance of written standard operating procedures for the use of respirators is emphasised in OSHA 29 CFR part 1910.134 which also states that no one should be assigned a task requiring use of respirators unless found physically able to do the work while wearing a respirator. Furthermore, the wearer should receive fitting instructions including demonstrations and practice in wearing, adjusting and determining the fit of the respirator. Respirators in use should be inspected frequently to ensure that those selected for the job are being used and that they are in good condition.

7.2 Preventive measures for the environment

Ultrafine particles in the environment mainly stem from the traffic, especially diesel engine exhaust, and from other combustion processes like fossil-fuel power plants, and incinerators. It is yet unknown how much the production of nanoparticles and the use of products containing nanoparticles might contribute to environmental air pollution in the future.

Implementation of air quality standards is a high priority objective within the European Environment Action Programme

So far no plans exist to control ultrafine particles emissions

Environmental agencies around the world today regulate dusty pollutants on the basis of mass - not chemistry - and most governments focus on the particles easiest to catch and quantify: those that are 10 micrometers across (the PM-10 fraction), rather than 2.5-micrometer particles (PM-2.5). So far, no plans have been announced to control the fraction of ultrafine particles.

The Sixth Environment Action Programme (EAP), "Environment 2010: Our future, Our choice", includes Environment and Health as one of the four main target areas where new effort is needed. Air pollution is one of the issues included under Environment and Health. The objective considered in the Sixth Environment Action Programme is to achieve levels of air quality that do not give rise to unacceptable impacts on, and risks to, human health and the environment. The Community is acting at many levels to reduce exposure to air pollution: through EC legislation, through work at the wider international level in order to reduce cross-border pollution, through working with sectors responsible for air pollution and with national, regional authorities and NGOs, and through research. The focus for the next ten years will be the implementation of air quality standards and coherency of all air legislation and related policy initiatives.

Clean Air for Europe (CAFE)¹⁴ is a programme of technical analysis and policy development which will lead to the adoption of a thematic strategy on air pollution under the Sixth Environmental Action Programme in 2004. The programme was launched in March 2001. Its aim is to develop a long-term, strategic and integrated policy advice to protect against significant negative effects of air pollution on human health and the environment. The integrated policy advice from the CAFE programme is planned to be ready by the end of 2004 or beginning of 2005. The European Commission will present its thematic strategy on air pollution during the first half year of 2005, outlining the environmental objectives for air quality and measures to be taken to achieve these objectives.

Also other routes of potential environmental contamination has to be considered. It has been shown that natural enzymes can change the surface properties of nanoparticles such as fullerenes, which can form aqueous suspended colloids and become re-suspended after evaporation. In their native form, the small size, colloidal characteristics, and reactive surfaces of colloidal fullerenes make them ideally suited to carry toxic material over long distances. Thus, potentially, colloidal fullerenes could pollute aquifers. Manufactured nanomaterials therefore could have adverse impacts on aquatic organisms, and it is possible that effects in fish may also predict potential effects in humans (Oberdörster 2004). As countermeasures remediation treatments could oxidize the fullerene cage.

¹⁴ <http://europa.eu.int/comm/environment/air/cafe/index.htm>

These treatments should render the fullerene cage chemically inert as long as the treatment lasts, thereby reducing the overall potential biological activity of the treated nanoparticles. Of course environmental hazards and adequate countermeasures have to be investigated for different types of nanomaterials.

7.2.1 Conclusion and recommendations

Inhaled particulate matter has been associated with both acute and chronic health effects. Concerns about these effects derive primarily from epidemiologic studies that associate short-term increases in particulate matter concentration with increases in daily mortality from respiratory and cardiovascular diseases. But not much research has been done yet in regard to adverse health effects of nanoparticles with their unique characteristics, including increased adsorption of organic molecules and enhanced ability to penetrate cellular targets in the lung and systemic circulation. These properties and the well known fact that particles deposited in the gas-exchange region of the lungs can eventually lead to the development of chronic diffuse interstitial lung disease, make the epidemiologically observed association of inhaled nanoparticles and adverse health effects biologically plausible. Even in view of some remaining scientific uncertainty there is enough suspicion of harm to warrant preventive actions at the work place as well as in the ambient environment.

If nanoparticles are released during production, handling or further processing, exposure of workers can be prevented by the described measures. Generally the control at the source of the hazard should be preferred to personal protective equipment and respiratory protection should only be used in situations where complete control is not achievable through feasible engineering measures. If necessary, appropriate respirators can remove nanoparticles from the surrounding air and thereby protect the lungs (as well as the organism as a whole) from adverse health effects. To prevent dermal absorption protective clothing and gloves can be considered. Eye contact can be prevented by wearing a full respiratory mask or glasses with side protection.

7.3 Standardisation and regulation activities

7.3.1 Regulation framework and measures

At present no regulations exist which refer specifically to the production and application of nanomaterials or nanoparticles. A lot more knowledge has to be generated on how nanomaterial based processes and products may interfere with human health and the environment, before any regulation in this field can be established. In the future a major task will be to check if the existing legislation and regulation framework can cover

the range of nanotechnology or if and how it should be modified. With regard to legislation the following areas should be included into the discussion:

- Immission control
- Chemicals
- Labor protection
- Pharmaceutical and medicine
- Food, consumer goods and cosmetics
- Novel-Food

Within this legal framework there are manifold instruments to protect people and the environment against potential risks of nanomaterials and – particles- as for all hazardous substances in general (Paschen et al. 2003). Some of these optional measures, which differ significantly between different countries and economic areas, but are more or less harmonised on European Level, are summarised in table 10.

Legal framework
and optional
measures for
regulation of
nanomaterials

Area of interest	Safety Measure
Drugs	<ul style="list-style-type: none"> • Drug approval application process
Food	<ul style="list-style-type: none"> • Impose conditions for manufacturing processes • Obligations for indication and permission of producing and selling • Prohibitions • labelling and warning notices
Consumer products and cosmetics	<ul style="list-style-type: none"> • Product safety standards • Establish obligations for the indication and permission of producing and selling
Air pollution	<ul style="list-style-type: none"> • Limit values for emission and immission
Worker safety	<ul style="list-style-type: none"> • Exposure limits • Safety standards and guidelines
Hazardous substances and chemicals	<ul style="list-style-type: none"> • Registration of substances/chemicals • Guidelines for handling of hazardous substances

Table 10: Optional regulation measures for nanoparticle based products

The establishment of appropriate safety standards and regulations with regard to nanoparticles will strongly depend on reliable measurement techniques to assess air quality, workplace exposures, toxicological effects and the fate and transportation of nanoparticles in the environment and biosphere. Even though the mechanisms and particle characteristics causing negative health effects are heavily debated, it is necessary to determine all particle characteristics at various locations, e.g. in ambient air and working areas, to enable detailed toxicological studies and to produce a data base for particle exposure assessments. The problem is that so far all existing standards for the measurement of

Standardisation of
measurement
techniques and a
uniform nomencla-
ture as prerequi-
sites for regulation

particulate matter in the atmosphere like EN 13205:2002, ISO 14966:2002 or ISO 7708 do not include the range of ultrafine particles.

Another point is that at present no uniform nomenclature for nanomaterials exists. Only if various classes of substances are precisely defined, the results of risk assessment of different institutions or countries can be compared, thus facilitating progress in the clarification of potential risks. Without a consistent nomenclature regulative measures can not be implemented, and even the labelling of products becomes a difficult undertaking (Swiss Re 2004).

In the following the implications of nanomaterials and nanoparticles in the fields of worker safety, consumer protection, public health and the environment will be discussed in more detail.

7.3.2 Worker safety

The protection and safety of workers regarding exposures to hazardous chemicals at the workplace is regulated through national and international authorities which establish directives and guidelines on national, European or international level. On European level the directive 98/24/EC of 7 April 1998 regulates the protection of the health and safety of workers from the risks related to chemical agents at work (EC 1998). This framework directive obliges member states to establish limit values for hazardous chemicals taking into account the availability of measurement techniques and regulates the responsibilities of the employers to protect their workers. Such limit values are proposed by several national and international organisations like the World Health Organisations (WHO), the American Conference of Government Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA) or the MAK Commission/Germany.

As one class of nanostructured materials carbon black has been assessed by the International Agency for Research on Cancer (IARC) as well as by the MAK Commission in Germany as potentially cancer causing substance. However, a threshold value was not established due to the fact that the mechanisms of cancer generation and toxicity of nanostructured particles are not fully understood yet. It is still controversial if it is possible to establish health relevant safety threshold values at all for nanomaterials.

So far no limit values for nanomaterials have been proposed

But not only missing knowledge on toxicological mechanisms but also a lack of measurement standards hampers regulations for nanoparticulate materials. Existing standard with regard to aerosol exposure are based on mass concentrations of particulate matter with exception of fibrous aerosols which are assessed in terms of particle number. For example the CEN/TC137/WG3 (Workplace atmospheres – Airborne particles) has produced European Standard EN481 (Size fraction definitions for

measurement of airborne particles), which relates to the mass concentrations of the inhalable, thoracic and respirable fractions.

Ultrafine particles, however, represent only a small part of the mass fraction of aerosols but may significantly contribute to health effects due to their high number concentration and surface area. Because neither sufficient data about nanoparticle exposure of workers nor adequate measurement technology is available, there is no basis for the establishing of reasonable regulation measures at present. To tackle that problem some institutions like the BIA (Berufsgenossenschaftliches Institut für Arbeitsschutz, Germany) or the Health and Safety Laboratory (Sheffield, UK) started activities to collect data for a detailed assessment of nanoparticle exposures at work (Riediger and Möhlmann 2001, Wake et al. 2001). This topic will also be priority for the future work of the CEN/TC137/WG3. A similar programme is conducted in the USA by the National Institute for Occupational Safety and Health (NIOSH 2001).

Activities for
assessment of
nanoparticle
exposure at
workplaces in
Germany, UK and
USA

The ISO working group on particle size-selective sampling and analysis – workplace aerosols (ISO/TC146/SC2/ WG1) is currently working on a Technical Report entitled „Occupational ultrafine aerosol exposure characterisation and assessment“. This is currently under development, and is aimed at providing a basis for eventual ultrafine aerosol exposure standards. Also the European Committee for Standardisation has started activities relating to nanoparticle exposures within the frame of the working programme of the Technical Committee 137 (Workplace Exposures) and is in preparation of the following standards:¹⁵

ISO-Standard for
aerosol exposure
assessment in
preparation

- Workplace atmospheres - Measurement of the dustiness of bulk materials - Requirements and test methods (status: under development),
- Determination of diesel particulate matter - General requirements (status: under approval)

Beside the problem of determination of nanoparticle exposures there is another problem of adequate protection measures for workers which has to be taken into considerations by regulation authorities. The protection of workers against hazardous substances is regulated on European level by the Council Directive of the European Communities on the use by workers of personal protective equipment at the workplace (EC 1989). A possible intake of nanoparticles by workers could occur through inhalation, dermal absorption or oral ingestion. Some standards on European level for personal protection equipment already include the range of airborne nanoparticles such as EN 149:2001 and EN 143:2000 for particulate filters. As future actions, it should be proved for the different kind of nanoparticle exposures if the standard personal

¹⁵ http://www.cenorm.be/standardization/tech_bodies/cen_bp/workpro/tc137.htm

protection equipment such as respirators, masks, gloves, etc. provide sufficient protection.

The protection of workers during handling of nanoparticulate materials has to be ensured by Material Safety Data Sheets according to the relevant standards e. g. Commission Directive 2001/58/EC (EC 2001a). However, the knowledge on toxicological properties of nanomaterials is quite low, so these Material Safety Data Sheets are often based on predictions derived from the coarser bulk material. Here further investigations are required to take into account the special properties of nanoparticulate material.

Material Safety Data Sheets for nanomaterials often lack specific data concerning toxicological properties

7.3.3 Consumer protection

Standards for consumer products are an important mean to prove that products are safe and that a product fulfils the "state of the art" of technology. The General Product Safety Directive (EC 2001b) gives a framework for the establishment of product standards. Manufacturers and distributors of products in compliance with a standard limit their liability in case of accidents.

Nanoparticles find increasing applications in products in the range of electronics, pharmaceuticals, cosmetics, chemical-mechanical polishing or catalyses. In many applications nanoparticles are used as intermediate products or additives in industrial processes such as catalysts, polishing powders, etc., where no direct contact of nanoparticles with consumers can occur. In some consumer products nanoparticles are incorporated in a rigid matrix of other materials e.g. nanoparticle reinforced polymers used in foils and car parts or carbon black used in car tyres and copy toners. In most cases a contamination of consumers during product use is improbable but can occur through wear and abrasion of the product e. g. car tyre dust, which is assumed to be a source of potentially hazardous dust in cities.

A further class of consumer products uses nanoparticles as ingredients in food and cosmetic products, which get in direct contact with the human organism e. g. TiO₂ or ZnO nanoparticles as UV-absorbers in sunscreens. Sunscreens are among the most extensively used cosmetic preparations as well as to the concentrations of incorporated UV filter substances as to the surface area of application, i.e. they have the greatest potential for percutaneous absorption (SCCNFP 2002). In the last years a discussion of potential health risks of nanoparticles in sunscreens emerged (ETC 2003) because there were some indications that nanoparticulate TiO₂ has a much higher photo-reactivity than coarser powders and could cause DNA damages when directly introduced in human cells (Serpone et al. 2001). Therefore some nanoparticle producers have altered their particles to reduce or eliminate free radical production, either by coating the particles in organic or inorganic ingredients such as silica, by adding

antioxidants and vitamins to mop up free-radicals or by doping of the nanoparticles to shift the redox level.

At present no regulations concerning the use of nanoparticles in consumer products exist

Until now, no specific regulation has been established for the use of nanoparticles in consumer products. Within the range of chemical regulation producers are not required to declare the particles size of substances. Whether a substance is declared as a new substance (and therefore has to undergo a registration process which requires data on physical, chemical, toxicological and eco-toxicological properties) depends solely on its chemical formula. A new size or new physical property does not qualify a substance as a new one if the corresponding formula is already listed (Haum et al. 2004).

Within the range of cosmetics regulation the EU Scientific Committee for Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) issued an opinion after an off-record meeting with the cosmetics industry, that titanium dioxide particles are a safe component in sunscreen whether or not subjected to various treatments (coating, doping, etc.), irrespective of particle size (SCCNFP 2000). The US Food and Drug Administration (FDA) also does not distinguish between nanoparticles and their larger relations. In a final monograph on sunscreen ingredients, they determined that micronised titanium dioxide is not considered to be a new ingredient but a specific grade of the titanium dioxide originally reviewed by the panel. Pointing out that "fines" have been part of commercially used titanium dioxide powders for decades, they decided that nanoparticles were simply a refinement of particle size distribution (FDA 1999). Investigations of companies like Beiersdorf, a producer of nanoparticle based sunscreens, by means of electron microscopy give evidence that titanium nanoparticles can not penetrate the human skin (Pflücker et al. 2001). However, although a lot of scientific work has been done on whether nano-(or micronised) TiO₂ can penetrate human skin, there is still no consensus on this topic.

However, in the case of nanoparticulate ZnO also used in sunscreen formulations the situation is different. Here the SCCNFP concluded that for a proper safety evaluation an appropriate safety dossier on micronised ZnO itself, including possible pathways of cutaneous penetration and systemic exposure, is required (SCCNFP 2003). Although at present no regulations of nanoparticle use in cosmetic products exist, this gives an indication that for the future a stronger obligation for risk assessment of independent bodies and detailed toxicity studies for nanoparticles in cosmetic formulations can be expected.

7.3.4 Public health/ environment

Public health concerns related to nanoparticles could arise from emissions during production, use, recycling, disposal or combustion of nanoparticle based products. Most critical in view of negative impacts on

public health are nanoparticle emissions into ambient air and a possible intake by humans through their respiratory system. Epidemiological studies have shown an association between increased particulate air pollution and adverse health in susceptible members of the population, in particular the elderly with respiratory and cardiovascular diseases. This association has been found to be particularly relevant for the finer fractions of the airborne particles (PM_{2.5} and PM₁) (Peters et al. 1997, 2000, 2001, Dockery et al 1993, Ibald-Mulli 2002, Wichmann and Peters 2000, Maynard and Maynard 2002, Moshhammer and Nehberger 2003). This fraction is related more to human activity, industrialisation, etc. than natural sources and is therefore potentially more amenable to control measures (CONCAVE 1999).

A number of countries or international organisations have reviewed environmental particulate matter with the aim of establishing air quality standards. In the past these standards focused predominantly on PM₁₀ or Total Suspended Particulates (TSP).

In the United States, the EPA set National Ambient Air Quality Standards for particulate matter in 1971 and modified the standards in 1987. According to the 1987 standard of PM₁₀ the maximal allowable 24-hour concentration was set at 150 µg per cubic meter and the maximal annual mean was set at 50 µg per cubic meter. From 1988 to 1993, the averages of the annual mean PM₁₀ concentrations at 799 sites monitored by the EPA declined by 20 percent. Despite these improvements in air quality, Samet and coworkers reported associations between particle concentrations and the numbers of deaths per day in 20 of the largest cities and metropolitan areas in the United States from 1987 to 1994 with mean 24-hour PM₁₀ concentrations well below the standard. Responding to a substantial body of epidemiologic evidence, the EPA concluded in 1996 that PM_{2.5}, a correlated component of PM₁₀, was the size fraction better used as a surrogate for PM exposure linked to mortality and morbidity. In 1997, the EPA retained the PM₁₀ standards and promulgated new 24-hour and annual standards for PM_{2.5}, of 65 and 15 µg per cubic meter, respectively, based on consistency with the literature on health effects. Both the epidemiologic evidence and the new PM_{2.5} standard have been criticised. Arguing that the 1997 standards for ozone and particulate matter did not have an adequate scientific basis, industry groups sued the EPA in the Court of Appeals for the District of Columbia. In 1999, the court blocked implementation of the 1997 standards. Lacking knowledge of the harmful constituents of fine particles and the mechanisms by which they affect health, the EPA continues to propose standards based on particle mass. The final Directive of the European Union issued in 1999, no longer included PM_{2.5} values in the legislation (EC 1999). However, Member States are required to also sample and provide information on PM_{2.5} and the action

plans to reduce PM10 to the limit values given should include a concomitant reduction in the PM2.5 fraction.

At present no plans for implementation of ultrafine particle emission standards exist

With regard to ultrafine particles immission in ambient air neither standards exist nor will be established in the near future. Nonetheless the topic has gained attention and is under discussion in the respective working groups of the International Standardisation Organisation (ISO TC 146/ SC 3, ambient atmospheres) and the European Standardisation Committee (CEN TC 264, Indoor and Outdoor exposures). But before any regulations or standards can be established a lot more information has to be gathered on this topic. Estimation of the potential health risks associated with these new materials requires understanding of the mechanisms of ill health, the identification of some property or metric of the material which relates exposure to the material to health risk and some method for measuring exposure in relation to that metric. Once these are in place, it is potentially possible to define safe levels of exposure to these materials and to design control methodologies to enable exposures to be maintained at or below these safe levels. For nanoparticles, there is currently poor understanding of all of these issues.

More knowledge on toxicological mechanisms and a suitable metric for measuring exposure is necessary

Beside effects of airborne nanoparticles to public health also other negative impacts on the environment may arise from a release of nanoparticles into the environment in case of leakages or accidents during industrial nanoparticle production. For example potential environmental risks could be caused by deposition of nanoparticles in water and soil and subsequent bio-uptake and accumulation along the food chain. In considering the fate and transport of nanomaterials, there are some indications that nanomaterials can move easily through aquifers and soil. Due to their large and active surface for sorbing smaller contaminants nanomaterials could provide an avenue for rapid and long-range transport of waste in underground water like naturally occurring colloids (Colvin 2002). Another important factor for the assessment of possible environmental effects of nanomaterials is their biodegradation that controls their long-term persistence in the environment. Here also few data are available. To develop exposure guidelines as a first element of quantitative risk assessment of nanoparticles a lot more investigations have to be accomplished.

8 CONCLUSION AND RECOMMENDATIONS

In the following the key findings of the report will be summarised and some policy options for future actions and research programmes will be pointed out.

8.1 Key findings of the report

- Nanomaterials represent a large variety of different structure types, configurations and compound classes. From a commercial point of view long established nanostructured materials like carbon black, polymer dispersions or micronised drugs are most relevant, which have a world market volume of several billion EURO per year. In the range of nanoparticulate materials metal oxide nanopowders find increasing applications in commercial products, like sunscreens, cosmetics, catalysts, functional coatings, medical agents, etc. For the future a big market potential is predicted for other nanomaterials like carbon nanotubes or macromolecules like dendrimers. A large scale industrial production of different types of new nanoparticulate materials can be expected for the future.
- With regard to potential health and environmental risks dry powders of nanoparticulate materials are to be assessed as most critical, because they can easily form aerosols during production and handling processes, which might lead to human exposure or environmental contamination. Although recent studies showed that engineered nanomaterials usually form aerosols with particle aggregates in the μm -size range it is not clear whether these aggregates deagglomerate into nanoparticles or change their surface properties when they enter biological fluids (e.g. the lung liquid).
- At present unintentionally released nanoparticles generated by combustion processes from traffic or energy production, mechanical abrasion processes or conventional industrial processes (e.g. welding processes, laser ablation, plasma cutting, grinding and milling) contribute much more to anthropogenic nanoparticle emissions than industrial nanoparticle production. But due to the fact that the next few years will probably see a dramatic increase in the industrial generation and use of nanoparticles the impact of these materials on worker safety, consumer protection, public health and the environment will have to be considered carefully by legislation and regulation authorities.
- The risk of particle release during nanoparticle production seems to be low, because most processes take place in closed systems with appropriate filtering systems. Contamination and exposure to workers is more likely to happen during handling and bagging of the material and also during cleaning operations of the manufacturing equipment (e.g. reaction chambers). To avoid exposure workers should be equip-

ped with personal protective equipment such as respirators, protective clothing, masks and gloves. Generally the control at the source of the hazard should be preferred to personal protective equipment and respiratory protection should only be used in situations where complete control is not achievable through feasible engineering measures.

- Inhaled particulate matter has been associated with both acute and chronic health effects. Concerns about these effects derive primarily from epidemiologic studies that associate short-term increases in particulate matter concentration with increases in daily mortality from respiratory and cardiovascular diseases. It has been proposed that the adverse health effect of particulate air pollution was mainly associated with the number concentrations of ultrafine particles rather than the mass concentrations of coarser particle fractions.
- From the literature analysis it can be concluded that particles in the nanosize range can certainly enter the human body via the lungs and the intestines; penetration via the skin is less evident although strong evidence exists that some known particles can penetrate deep into the dermis. The penetration is depending on the size and surface properties of the particles and also depends on the point of contact in the lungs, intestines or skin. The distribution in the body is strongly depending on the surface characteristics of the particle. There is no universal “nanoparticle” to fit all the cases, each nanomaterial should be treated individually when health risks are expected. The health risks of inhaled fibrous material needs to be examined with care, because it is general accepted that fibres that are not cleared easily from the lungs can induce pulmonary disease.
- The potential hazard ultrafine particle and fibres is ‘a priori’ not predictable by the bulk physico-chemical properties. In view of the fact that data on exposure assessment are lacking, a full risk assessment in most cases is not feasible at present. To prioritise the work of decision makers a ranking of potential risks could be achieved by applying hazard trigger algorithms for assessing the risks of nanomaterials. Both in-vivo and in-vitro methods can be used for the toxicity assessment of nanoparticles. Because in-vivo experiments, using animal models, are expensive, slow and ethically questionable there is a strong demand for a low-cost high- throughput in-vitro assay without reducing the efficiency and reliability of the risk assessment. The test method should be capable of studying the relationship between deposited particles and acute/chronic inflammation to determine which aspects of surface area (and other possible parameters) are best predictors of adverse health effects.
- Beside the lack of personal exposure measurement systems also measurement standards for a reliable and comparable nanoparticle determination are presently not available. There is an urgent need for standardisation of measurement and sampling procedures and condi-

tions. To perform an evaluation of the existing detection techniques, nanoparticles produced in industrial or pre-industrial environment must be completely characterised from their atomic structure to their agglomeration using a combination of the above mentioned complementary methods. The acquisition of detailed reference data as well as intercomparisons and round robin tests will be necessary to assess the reliability and the limitations of the applied detection techniques.

- At present no regulations exist which refer specifically to the production and application of nanomaterials or nanoparticles neither for worker and consumer safety nor for environmental protection. Also in the frame of chemical legislation particle size does not play a role for the registration of new substances. A lot more knowledge has to be generated on how nanomaterial based processes and products may interfere with human health and the environment, before any regulation in this field can be established. In the future a major task will be if the existing legislation and regulation framework can cover the range of nanotechnology or if and how it should be modified.

8.2 Policy options

8.2.1 R&D-policy

The present, incomplete state-of-knowledge demands more toxicological and ecotoxicological data and the gathering of data on exposure. Existing programmes should be carried on and expanded. Some of the relevant topics include:

- Basic research on particle interactions at the nanoscale and development of modelling tools for production and handling of nanopowders and –particles
- Establishing relevant metrics of nanoparticle exposure (e.g. number concentration, deposited surface area) and development and/or standardisation of adequate detection techniques
- Gathering data on nanoparticle workplace exposures for selected industrial processes and developing appropriate personal exposure measurement systems
- Development and standardisation of a low-cost high-throughput in vitro assay for toxicological screening of nanoparticles to supplement or substitute animal testing.
- Further investigations of nanoparticle behaviour in the human body and in the environment, development of adequate measurement techniques (different types of particles, tissue, environmental compartments, etc.) and establishing of data bases with relevant information.

8.2.2 International standardisation

International standards (including a nomenclature for nanoparticles/nanomaterials), and guidelines would facilitate scientific exchanges, use of existing data, and intercomparisons of experimental results, strengthen consumer and environmental protection, and improve market transparency and facilitate trade. This concerns nanoparticle specific detection and measurement techniques, toxicological and ecotoxicological testing methods, good-working-practises as well as emission standards, etc.

8.2.3 International cooperation and initiatives

International pooling of health related information and monitoring the development of nanotechnologies should be encouraged. Joint projects of industry and scientific organisations on international and European level should be fostered as well as international networks of excellence.

8.2.4 Legislation

A focus should be laid on adapting existing legislation to match the requirements for a safe industrial use of nanoparticles and nanomaterials. Governments can draw here from the whole range of regulation tools already in place in the framework of chemical policy, worker safety regulation, consumer protection and handling of hazardous substances. A proactive approach should be taken to advance scientific knowledge, develop appropriate monitoring and warning systems and –if necessary– adjust existing legislation and regulation.

8.2.5 Risk communication

For non-scientists it is often unclear what nanotechnology actually is, what special qualities nanoproducts may have, and what possible risks are. The manufacturing processes and operating mechanisms of nanotechnological products remain largely inscrutable to observers, users and consumers. This may lead to uncertainty and scepticism in society, especially if the various risk aspects become the subject of public discussion. Therefore, an open public dialog with citizens and consumers is absolutely necessary as a basis for an objective judgement on nanotechnology and to avoid baseless fears. This dialog has to be a two-way process. Scientists, industrialists, and public servants need to understand the concerns of the general public. Conversely, the public should learn about the risks and benefits of nanotechnologies and participate fully in shaping nanotechnologies. The dialog with concerned industries should be strengthened. It should also allow other stakeholders to benefit from their experience and hopefully lead to the exchange of scientific information including toxicological and ecotoxicological data which were acquired or generated internally.

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LIST OF ABBREVIATIONS

AFM	Atomic Force Microscopy
BBB	Blood-Brain Barrier
CNT	Carbon Nanotube
CPC	Condensation Particle Counter
CCVD	Catalytic Chemical Vapor Deposition
CVD	Chemical Vapor Deposition
DEP	Diesel Exhaust Particle
DLPI	Dekati Low Pressure Impactor
DMA	Differential Mobility Analyser
DNA	Desoxyribo Nuclein Acid
ELPI	Electrical Low Pressure Impactor
EPA	Environmental Protection Agency
FED	Field Emission Displays
FE-SEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared
GMR	Giant Magneto Resistance
HRTEM	High Resolution Transmission Electron Microscopy
ISO	International Organization for Standardization
LDL	Low-Density Lipoprotein
MAK	Maximum Workplace Concentration
MBE	Molecular Beam Epitaxy
MCP	Mechanochemical Processing
MOCVD	Metal Organic Chemical Vapor Deposition
MRAM	Magnetic Random Access Memory
MWCNT	Multi Wall Carbon Nanotube
NGO	Non Governmental Organisation
NIOSH	National Institute for Occupational Safety and Health
ODS	Oxide Dispersion Strengthened
OLED	Organic Light Emitting Diodes
PCA	Process Control Agents
PCS	Photon Correlation Spectroscopy
PEM	Polymer Electrolyte Membrane
PP	Peyer's Patches
PVD	Physical Vapour Deposition
SCCNFP	Scientific Committee for Cosmetic Products and Non-Food Products intended for Consumers
SMPS	Scanning Mobility Particle Sizer
SNOM	Scanning Nearfield Optical Microscopy
STEM	Scanning Transmission Electron Microscopy
STIM	Scanning Transmission Ion Microscopy
SWCNT	Single Wall Carbon Nanotube
TEM	Transmission Electron Microscopy
TSP	Total Suspended Particulates

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Nanotechnology portal of VDI-TZ
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