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Nanotechnology

– high risks with small particles?

A compilation of available knowledge concerning risks for health and environment from nanotechnology, and proposals on measures for how to fill the identified knowledge gaps

SWEDISH CHEMICALS AGENCY

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PREFACE

As a result of a government commission stated in the appropriation directions for 2007, the Swedish Chemicals Agency has made a compilation of available knowledge about the risks connected with nanotechnology. Measures to fill in knowledge gaps are proposed where such have been identified.

Jan Hammar was responsible for the commission at the Swedish Chemicals Agency, and. Agneta Falk Filipsson was project leader and responsible for carrying out the project and for writing and presenting the commission report to the Ministry of the Environment before 31 October 2007.

The SP Technical Research Institute of Sweden has contributed to the chapters on use areas and physical and chemical properties. The Swedish Defence Research Agency (FOI) has made amendments to the chapter on use areas and reviewed and commented on the report.

The report was also reviewed by external experts and representatives of concerned agencies.

This is an English translation of KemI Report 6/07.

CONTENT

SUMMARY

The rapid development of new fields of application, and a great lack of knowledge, calls for caution

The manufacture of nanomaterials is fast-growing industry, both in terms of total volume and the number of manufacturers. Strong growth may furthermore be expected over the next decade in the development of commercial products linked to nanotechnology, according to a number of studies from other countries. Knowledge about the possible health and environmental risks of nanotechnology is scarce. This applies equally to how humans and the environment might be exposed to nanomaterials and to what hazardous characteristics different nanomaterials have. Although there is insufficient evidence today to suggest that the development and use of nanomaterials brings particular health and environment risks, the rapid development in the area and the great lack of knowledge about risks calls for precautionary measures. This applies particularly to nanoparticles, as results from animal tests suggest that certain nanoparticles, when inhaled, could be harmful to human health.

More research about risks is needed

There is not enough knowledge about the environmental effects of nanomaterials, e.g. if they are absorbed by organisms in the environment and if they can bioaccumulate. It is furthermore likely to be a major technical challenge to develop methods for detecting nanomaterials in the environment. Humans may be exposed to nanomaterials in their work, as consumers, and via the environment. Since the respiratory system is likely to be the most important exposure route for nanomaterials, research has been mainly in this area. Once inside the respiratory tracts, nanoparticles appear to be capable of spreading to other parts of the body. There are also studies suggesting that nanoparticles can enter the brain via the olfactory nerve. However, the knowledge about harmful effects is very limited.

It is important that increased resources be allocated in Sweden to research about the health and environmental risks of nanotechnology. A Swedish strategy for nanotechnological development, which also covers research into health and environmental risks, should therefore be drawn up. It is furthermore important that methods for testing the hazardous properties with respect to human health and the environment are developed in international cooperation. Those risk assessments which can be made today are insufficient both due to the lack of data and to the difficulty in interpreting existing data. In risk assessments, it is important to consider the unique physical-chemical characteristics of nanomaterials, as well as new biological activity, new target organs, and unique routes by which nanoparticles can enter the human body and other organisms. A current example of this is that the EU's Scientific Committee on Consumer Products (SCCP) has said in a preliminary finding that it is regarded as necessary to reassess the risks of using the nanoform of titanium dioxide in cosmetic products, due to increased knowledge about nanoparticles, including the IARC's assessment that titanium dioxide could be carcinogenic to humans.

Companies are responsible for ensuring that human health and the environment are not damaged

Legislation places the same responsibility on companies with regard to nanomaterials as it does for chemical substances, chemical products and goods. However, the scientific limitations that exist today in assessing the risks imply that companies should apply special precautions in the development and use of nanomaterials in order to limit exposure to humans and the environment. This applies particularly to risks from the inhalation of nanoparticles.

Companies are obliged to classify substances themselves based on available information. In those cases where there is no information on a substance's health or environmental hazards, existing EU classification of health and environmental hazards for the substance at a larger scale shall apply for the nanoform as well.

Legislation needs to be extended

Although current legislation covers nanomaterials, regulations need to be made clearer as nanomaterials imply particular problems both in risk assessment and risk management. The rapid development of the area in combination with the great lack of knowledge about health and environmental risks call for precautionary measures. This is likely to involve complementing the EU regulatory framework with rules for nanomaterials, including rules about the way in and extent to which companies must test nanomaterials' health and environmental hazards. Affected government agencies will need to set up a working collaboration in order to provide the Government with a basis for upcoming reviews of EU rules.

In brief, the Swedish Chemicals Agency proposes that the Government:

- Instruct the Swedish Governmental Agency for Innovation Systems (VINNOVA) to draw up a Swedish strategy for nanotechnological research and development, which includes knowledge about risks to human health and the environment
- Allocate special research funds to the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) for research about the health and environmental risks of nanomaterials
- Instruct the Chemicals Agency to follow developments in the area and propose measures whenever it is justified, and to participate actively in the development of new or modified testing methods within the OECD cooperative framework
- Instruct the Chemicals Agency to produce a deeper analysis of the use of nanomaterials in chemical products and goods after consultation with the trade organisations concerned.
- Instruct the governmental agencies concerned to review the need for complementing existing legislation
- Arrange, in the context of Sweden's EU presidency in 2009, a workshop on how the health and environmental risks of nanotechnology should be dealt with by legislation

1. INTRODUCTION

The term nanotechnology was coined by Norio Taniguchi in 1974 to describe technology at the atomic level in semiconductor circuits (Taniguchi, 1974). However, the basis of the concept was described already 1959 by the physicist Richard Feynman (Feynman, 1959).

Nanotechnology is concerned with the creation and use of structures, devices and systems that have novel properties and functions because of their small size, and the ability to control and manipulate matter on an atomic and molecular scale (US EPA, 2007).

Nanomaterials have been used commercially since the 1930s. An example is silver in photographic film. Another important nanomaterial that has been produced in large volumes for a long time is carbon black. The annual production of carbon black today is over 10 000 000 tonnes (IARC, 2006), and the use is widespread. Almost all black paint comes from carbon black, and the substance is also used to improve mechanical strength and protect against breakdown by solar rays in plastics, as well as automotive tyres and other rubber products.

Nanomaterials can be manufactured either by grinding down material at macro-scale to material at nanoscale (top-down) or by creating material at nanoscale from atoms and molecules (bottom-up). The challenge with regard to top-down is in being able to manufacture smaller and smaller structures with sufficient precision. The challenge with regard to bottom-up is in being able to make sufficiently large structures for them to be usable materials (Royal Society, 2004).

Nanomaterials can also be produced unintentionally, for example in the formation of ultrafine particles in combustion, or in natural processes. On a sunny day new formation of organic nanoparticles takes place through photo-oxidation of organic substances that are emitted from forests. Other natural processes that lead to the new formation of nanoparticles are volcanic eruptions. Minerals also occur naturally at nanoscale, formed both through breakdown processes (weathering) and through building-up (e.g. redox chemical) processes. Macromolecular breakdown products such as humus substances are present in very large quantities on earth and also occur at nanoscale. They also interact with nanominerals. In addition, all living organisms are made up of biological macromolecules which, like many viruses, can be regarded as a natural form of advanced nanoparticles.

Technical development in recent times has made it possible to study and manipulate matter at nanoscale, and research on and development of nanomaterials have increased greatly in recent years. The National Nanotechnology Initiative (NNI) in the United States, which was launched by the Clinton Administration in 2001, can be mentioned as an example. The task of the NNI is to coordinate nanotechnological research and development between government agencies (NNI, 2007). Twenty-five government agencies are currently taking part in the initiative, thirteen of which have separate budgets for nanotechnology. The NNI supports research and development in a broad area, from the research and development of technology, instrumentation, measurement and standards to research on risks to health and the environment. The 2008 budget for the federal research programme is almost USD 1.5 billion, which is more than three times as much as in 2001.

The reason why such great interest has been aroused in this particular field is that a substance can have completely different properties and combinations of properties than the same substance at larger scale. The principal reasons for the changed properties are an increased surface area per unit of mass when size decreases, and the fact that the quantum effects play a

greater role the smaller a particle becomes. Quantum mechanics states that the energy levels of an atom can assume certain discrete values and when the atoms change energy state energy in a particular quantity is released or absorbed (known as quanta). Quantum mechanics also states that matter at the very smallest dimensions can behave as waves (particle-wave dualism). The effect of quantum mechanics is that when relatively few atoms are combined into a particle that is smaller than approximately 10 nm, the electrical, optical, chemical or magnetic properties may differ markedly from those of equivalent larger particles.

The size range as such is thus not new or unique. What has driven development forward in recent years is that the methods for manufacturing and measuring materials at nanoscale have been refined. The use of intentionally produced nanomaterials is expected to increase sharply. It is also reasonable to assume that new manufacturing processes and areas of use will have to be taken into account in assessing possible risks to human health and the environment resulting from exposure to certain nanomaterials.

This report is largely based on existing reviews of risks associated with nanotechnology, for example Royal Society (2004), US EPA (2007), SCENIHR (2007) and Oberdörster *et al* (2005; 2007).

2. WHAT IS A NANOMATERIAL?

Nanomaterials comprise a heterogeneous group consisting of different substances that can vary in size (from 1 to 100 nm) and structure (e.g. particles and tubes). New nanomaterials with entirely new properties are being discovered and produced at an increasing pace, and the group is consequently expected to become even more heterogeneous in the near future. It is therefore difficult to make far-reaching generalisations about the physical and chemical properties of nanomaterials.

Several physico-chemical properties in addition to those usually used to characterise a substance will probably be needed to characterise nanomaterials. There is still insufficient knowledge to identify the properties that will need to be evaluated in risk assessment and classification of the hazardousness to health and the environment of nanomaterials. A property that has been mentioned in this connection is surface area per unit of weight or volume.

2.1 Size and definitions

The most common definition of nanotechnology is based on size. According to this definition, nanotechnology is research and development at atomic, nanometric or macromolecular scale if the size of the nanomaterial is less than 100 nanometres in at least one dimension. A nanometre (nm) is a billionth of a metre or 10^{-9} metre. Nanotechnology is concerned with the creation and use of structures, devices and systems that have novel properties and functions because of their small size, and in addition the ability to control and manipulate matter on an atomic scale (US EPA, 2007).

There is reason to distinguish different types of nanomaterials from the point of view of risk assessment and risk management. Several attempts have been made to create harmonised terms in this area. The American Society for Testing and Materials has drawn up standard terminology for nanotechnology in international cooperation (ASTM 2456-06, 2006). A more extensive collection of terms has been compiled by British Standards (BSI PAS 71:2005). It should be possible for several of the terms to be used in risk assessment.

Two terms that relate to the properties often associated with nanotechnology are transitive and non-transitive nanoparticles (ASTM 2456-06, 2006).

– *Non-transitive* nanoparticles are particles which, by definition, are nanoparticles but which do not exhibit size-related intensive properties. An example of particles that express non-transitive properties from a toxicological point of view is titanium dioxide. Although the toxicity (oxidative stress) of airborne nanoparticles of titanium dioxide is significantly higher than for larger particles, it has been found that toxicity in this case is linearly dependent on the aggregate surface area of the particles and that the properties of larger forms of the substance could be extrapolated down to the nano forms.

– *Transitive* nanoparticles are particles which, by definition, are nanoparticles that express a size-related intensive property. Examples of this are what are known as quantum dots (see section 2.2.3, below).

In nanotechnology these two definitions are based on the technical properties, i.e. the properties that are to be achieved by applying the material. In some cases toxicological or ecotoxicological properties may be linked to such technical properties, but it is possible that a nanoparticle that is transitive from a technical point of view is non-transitive from a toxicological/ecotoxicological point of view, or vice-versa.

Other terms that are of significance to risk assessment are:

– *Intentionally* produced and *unintentionally* formed (e.g. air pollutants) nanomaterials;

– *Free* nanomaterials that may enter the body, move and bioaccumulate in target organs or be dispersed in the environment and *fixed* nanomaterials that are embedded in a matrix and cannot move;

– *Nanostructured materials* which are constructed atom by atom or molecule by molecule and *nanostructured surfaces* which start with a substrate where different techniques are used to create a surface with nanoproperties;

– *Surface-treated* and *non-surface-treated* nanomaterials: a surface treatment (coating) can make a particle inert as long as the coating persists, but this can be changed for instance by the surrounding environment;

– *Short-lived* and *long-lived* nanomaterials: of particular significance in the transporting of pharmaceuticals into the body and for degradability in the environment.

This review relates to intentionally produced nanomaterials. In cases where naturally and unintentionally produced materials can contribute to increasing knowledge of intentionally produced materials, this knowledge has been included.

From the point of view of risk it is principally free nanomaterials that are of interest, but fixed nanomaterials that are released from their matrix might also be of concern.

2.2 Different types of nanomaterials

Nanomaterials include many different structures and sizes and they share the feature of being less than 100 nm in at least one dimension. The properties of the materials are affected among other things by how small they are and by the form they are in. A review of a broad division of different nanomaterials and a brief description of their possible areas of use follow below. Nanomaterials cannot be categorised according to chemical composition or structure or according to area of use. Several examples of how different areas can overlap are given instead.

2.2.1 Nanoscale in one dimension

Thin films, coatings and surfaces

One-dimensional nanomaterials such as thin films and coatings have been used for decades in the electronic, chemical and engineering industries. It is relatively well known how these are formed and function (Royal Society, 2004). Progress is continuously being made with regard to controlling composition and the properties of the surfaces.

Surface structures with strengthened properties such as large surface area and specific reactivity are routinely used in many areas, for example as fuel cells and catalysts. These properties make them also usable in the chemical and energy sectors, as higher activity and greater selectivity in reactors and separation processes lead to obvious economic benefits.

Nanocomposites of clay

Composites that contain nanoclays as reinforcing materials are now used in some commercial applications, for instance in car interiors. Techniques are based on the fact that it is possible, using solvents, to break clay particles up into their smallest constituents, which consist of sheets that may be as thin as one nanometre (Jakubowicz *et al*, 2004). A property the nanoclay can give a composite is dramatically increased gas-tightness, which can reduce material consumption in certain applications, e.g. food packaging.

2.2.2 Nanoscale in two dimensions

Carbon nanotubes

Carbon nanotubes, or cylindrical fullerenes, were first observed in 1991 (Iijima, 1991). A carbon nanotube is comparable to a disc of carbon atoms with a graphite structure, which is rolled up into a tube. Some tubes are open at the ends while others are closed. Nanotubes with just one wall (SWCNT = Single Wall Carbon NanoTube) are the simplest form. There are also nanotubes with several walls (MWCNT = Multi Wall Carbon NanoTube), i.e. several SWCNTs within one another. MWCNTs generally have several defects in the layers and walls, at molecular level, which means that they become weaker than SWCNTs. Both are a few nanometres in diameter and several micrometres to centimetres in length.

Carbon nanotubes have very high tensile strength, are very flexible and can also conduct electrical current extremely well. All these properties mean that they have many possible applications, for example in reinforced materials, sensors and electronics. They can be manufactured in several different ways, but carbon nanotubes have not yet been successfully manufactured in a particular size (diameter) with specific physical characteristics. The nanotubes have to be sorted, usually by wet-chemical methods. It is also reported that SWCNTs can interact with long-wave light, which is the reason why they have also been proposed for cancer treatment in a number of different ways (Kam *et al.*, 2005).

The similarity in shape of carbon nanotubes and asbestos fibres means there is some concern that nanotubes may cause damage to human health in an equivalent way.

Of the various nanomaterials it is carbon nanotubes that are present in the greatest number of products today, and the number of suppliers is increasing rapidly. World-wide production of carbon nanotubes is probably in the order of 100 tonnes per year.

Inorganic nanotubes

Inorganic nanotubes and inorganic C_{60}-fullerene-like materials (see section 2.2.3 below on C_{60} fullerenes) based on layers, for example of molybdenum disulphide were discovered shortly after the carbon nanotubes. They have excellent lubricant properties, high capacity to store hydrogen and lithium, are resistant to pressure waves and are catalytically reactive. These properties make them usable in several different areas.

Nanofilaments
Nanofilaments are ultrafine threads that combine into long threads. They can consist of a number of different materials. Semiconductor nanofilaments of various substances such as silica, gallium nitride and indium phosphide have suggested remarkable optical, electronic and magnetic properties.

Biopolymers
The possibility of combining nanotechnology and biotechnology in the future is expected to open up a number of scientific and technological opportunities. The variation and the possibility of identifying specific points on biopolymers as DNA makes it possible to organise nanomaterials in more advanced patterns where the DNA strand is, for example, covered by metals.

2.2.3 Nanoscale in three dimensions - nanoparticles

Nanoparticles are often defined as particles smaller than 100 nm in diameter. Intentionally produced nanoparticles are in a minority in comparison with naturally occurring and unintentionally formed nanoparticles.

Metals and metal oxides
For metal oxides it is principally the use in new cosmetic products that are mentioned, for example titanium dioxide and zinc dioxide in sunscreens. Other applications for metal oxides are as pigments in paints, or catalytic breakdown of dirt by giving the surface certain properties in concrete and window glass (NyTeknik, 2005).

Nanoparticles of pure metals can be used as such or in combination with other materials. Particles of pure iron have been shown to be effective in breaking down organic pollutants in soil and nanoparticles of silver and gold can be used in medical applications. An example is to bind antibodies to gold particles, enabling them to concentrate on malignant tumours. The method has to date only been tested in the laboratory, but the idea is that the particles could be used both for diagnosis and for various forms of treatment due to the fact that they can effectively be heated using infrared radiation.

Polymers
Nanoparticles of polymers have medical applications, among others. Polymeric nanoparticles have proved capable of transporting pharmaceuticals across the blood-brain barrier (Calvo *et al.,* 2001), and in the future it is thought that nanoparticles can be used to deliver pharmaceuticals directly to target organs. An example of such a use is specific administration of substances to remove cancer cells (McCarthy *et al.*, 2005).

C_{60} fullerenes
In the mid-1980s a new form of carbon known as fullerenes was discovered (Kroto *et al.*, 1985). It is the spherical form of fullerenes that was discovered first. The C_{60} form is approximately 1 nm in diameter and consists of 60 carbon atoms arranged as hexagons and pentagons which together look like a football. Several areas of use can be predicted for fullerenes: as carriers for other molecules, to lubricate surfaces, for drug delivery in the body and in electronic and optical equipment.

Dendrimes
Dendrimes are spherical polymer macromolecules which are used, for instance, in coatings and inks but have several interesting properties that may lead to use in other areas. They can be carrier molecules and could be used to deliver pharmaceuticals to the target organ or against environmental pollutants, as they are good at taking up metals.

Quantum dots
Nanoparticles of semiconductors, known as quantum dots, were created in the early 1980s. They are densely packed semiconductor crystals that consist of several thousand atoms. If they are made sufficiently small, the quantum effects limit the energies at which electrons can exist. As energies are related to wavelength, this means that optical properties that vary with size can be obtained. There is great potential for new uses for example in lasers for optical communication, or as low-energy light sources.

2.3 Physical and chemical properties

Existing nanomaterials make up a heterogeneous group, from organic molecules and molecular aggregates to metal oxides and quantum dots which are metals in various compounds. In addition, new nanomaterials with entirely new properties are being discovered and produced at an increasing pace, and the group is consequently expected to become even more heterogeneous in the near future. It is therefore difficult to make far-reaching generalisations about the physical and chemical properties of nanomaterials. As well as size and shape, the type of binding and oxidation number of the substances that build up nanomaterials will be of significance for their chemical properties.

The area is principally defined by the size of nanomaterials having one or more dimensions in the area smaller than 100 nm. The discussion below relates to how size can affect physical and chemical properties so that they differ from a material at larger size.

The physical and chemical parameters which it is important to know about in the risk assessment of substances at micro-scale include melting point, boiling point, vapour pressure, octanol-water partition coefficient (log Kow) and solubility in water. There are established and harmonised test methods for these parameters in the EU ("OECD Guidelines for the Testing of Chemicals" and Directive 67/548/EEC).

The parameters above may be important for certain nanomaterials, but other properties such as particle size, size distribution, shape and other morphological properties, proportion of particles in relation to agglomerates (state of dispersion), ratio of surface area to volume, magnetic properties, surface charge, surface coating and conductivity are expected to be more important for the majority of nanomaterials (US EPA, 2007; Powers, *et al.*, 2006).

A given nanomaterial may in many cases be manufactured by a large number of different processes that produce derivatives of the same material. Single-walled carbon nanotubes (SWCNT) can be manufactured, for example, by four different processes that can generate products with different physical and chemical properties (e.g. size, shape, composition) and potentially different ecotoxicological and toxicological properties (US EPA, 2007).

It is thus possible that new harmonised test methods and modification of test methods that already exist will be required in order to be able to fully characterise nanomaterials.

Quantum effects
With regard to materials at nanoscale the situation moves between traditional physical laws and quantum mechanics. The 5-20 nm size range in particular represents a limit in relation to when a material can be regarded as particles in the traditional sense and when the properties of the individual atoms contained start to play a greater role (Preining, 1998). For larger particles, the properties of the particles can be related to diameter, volume or area. In the 5-20 nm range each individual building block becomes so important that the properties of the particle cannot be described with continuous variables. At this size the physical, chemical and biological properties of the materials may differ substantially from the properties of individual atoms and the bulk material, i.e. the material in the form of larger particles (NNI, 2004). This

makes it difficult to predict what causes a certain effect and means that materials at nanoscale may display unexpected properties that do not occur at larger scale.

Examples are particles of gold that may assume different colours, such as orange, purple or greenish depending on size. Other examples are particles of titanium oxide and zinc oxide, which are white powders at larger particle sizes but become colourless in emulsions when they are produced at nanoscale.

Further examples are the quantum effects achieved in the 'quantum dots'. The individual particles may be put into different electrostatic states and act as transistors. Their high quantum yield (colour strength when exposed to light) additionally makes them suitable in different analyses, e.g. imaging of disease markers by fluorescence microscopy. It should be pointed out that quantum dots of for example CdSe are too toxic for them to be usable in humans for this purpose.

Effects of specific surface area

Particles at nanoscale generally have a large specific surface area, i.e. surface area per unit weight or volume. For some applications of nanotechnology, quantum effects are not relevant. Instead the aim is to produce as large a surface as possible with for example catalytic or adsorptive activity. This may apply to nanoparticles of platinum in vehicle exhaust catalytic converters, or of metallic iron, which could be used for the decontamination of polluted soil.

The large specific area and therefore increased reactivity may also mean that combustible nanoparticles have an increased tendency to explode by self-ignition in comparison with the equivalent substance at microscale. The UK Health and Safety Laboratory of the UK Health and Safety Executive has also recommended that research on the explosiveness of nanoparticles should be given priority as very little work has been published in this area (Pitchard, 2004). It may be mentioned that there is great interest from various organisations in energetic materials (rocket fuel and explosives) containing additives of various nanoparticles in order to increase the performance or energy content of the material.

The large specific surface area is also significant for the aggregation properties of the nanomaterials, i.e. their tendency to clump together. Nanomaterials are so small that forces between molecules such as ionic bonds, hydrogen bonds, dipole-dipole and van der Waals forces become large or dominant in comparison with other physical forces such as gravitation or kinetic energy. Nanomaterials therefore often have a strong tendency to aggregate. An example of the strength of the intermolecular forces in nanomaterials is gecko feet, which are covered with nanofibres. They attach to the surface so effectively that the gecko is able to climb walls and ceilings of almost any material. Increased reactivity as an effect of the increased specific area can be regarded as an example of a non-transitive nanoparticle. Although the effect is significantly greater at nanoscale, it also exists for the larger forms and to some extent can be extrapolated.

The ability of nanomaterials to clump together may also affect their solubility in water. An example of this is C_{60}-fullerene which in particulate form has very low solubility in water ($<10^{-9}$ mg/l). In contact with water, however, it forms water-stable colloidal aggregates consisting of C_{60} with reported diameters around ~5-500 nm. These aggregates permit concentrations of up to 100 mg/l, which is ~10^{11} times more than the molecular solubility indicates (Fortener, *et al.*, 2005).

3. AREAS OF USE

There are existing and future uses for materials based on nanotechnology in most areas of application, and their use is expected to increase substantially in the next few years.

In many applications nanoparticles are used in a way that entails them coming into direct contact with humans (cosmetics, pharmaceuticals). In other applications free nanoparticles or nanofibres are only used during the manufacture of a product (automotive paints, sports items, batteries etc.) to be sold to and used by the consumer as fixed particles or fibres in materials. It should be mentioned that in most cases in which nanotechnology is used in articles it is not evident that this is the case from the product description given to consumers and other users.

3.1 Introduction

There are existing and future uses for materials based on nanotechnology in most fields of application. All industries and sectors may be affected, from foods, medicinal products, cosmetics etc. to construction and the transport sector. The most common nanomaterials that occur in various consumer products are described below, with the focus on nanomaterials that are used either as free particles, in suspensions, or additives to a liquid or other matrix (for example as fillers in polymer materials).

3.2 Nanoparticles

Nanoparticles based on carbon, such as carbon black, have long been in widespread industrial use, particularly in tyre manufacturing, but they have only recently been categorised as nanoparticles. Another example is silicon oxide particles with particle sizes down to 10 nm, which have long been in extensive use as consistency agents (thickeners) in paints.

Nanoparticles of silver commonly occur in various products, and their use is increasing. The purpose of using nanoparticles of silver is to obtain an antibacterial effect, although it should be pointed out that silver also has antibacterial properties at larger scale. Examples of uses in which it is claimed that nanoparticles of silver have antibacterial properties are in medical products (patches, dressings, catheters), clothing textiles (“anti-odour”) and in refrigerators, washing machines and air filters for domestic use.

Another nanoparticle used commercially in larger quantities, mostly in surface coatings, plastics and rubber, is calcium carbonate. Particular mention can be made of use in PVC products such as window profiles and tubes where nano-$CaCO_3$ has increased impact strength six-fold.

Titanium dioxide in the form of nanoparticles also got a number of uses, for example in self-cleaning windows. Use is made of the fact that nanoparticles of titanium dioxide catalyse chemical reactions when they are exposed to UV light. By coating a surface with a transparent film of such particles, dirt that has attached to the surface can be rinsed away by rain after it has undergone catalytic breakdown. Addition of titanium dioxide and zinc oxide in nanoform in a sunscreen means that ultraviolet light is absorbed effectively, while the sunscreen becomes translucent to visible light and therefore invisible. In frying pans a grainy surface of ceramic particles of titanium dioxide and aluminium dioxide prevents burning and aids washing. Other uses for ceramic nanoparticles are scratch-resistant spectacles, crack-resistant paints, anti-graffiti wall coatings, dirt-repellent fabrics, toothpastes, cleaning agents, ceramic coatings for solar cells etc.

Liposomes are spherical aggregates made up of organic molecules, often lipids. They can be made hollow and in sizes down to a few tenths of a nanometre. Liposomes and other molecular aggregates at nanoscale are increasingly used in cosmetic products, for the release of vitamins or other substances with a claimed health effect. Because of their small size they are claimed to be able to deliver the active substance more efficiently to where it is destined to go in the body. Cosmetic products containing C_{60} fullerenes ("carbon footballs") have also started to appear on the market.

Nanoparticles are increasingly being used in biomedicine. An example is fluorescent nanoparticles (quantum dots and dye-doped silicon dioxide particles), which are surface treated with molecules that makes the particle attach to specific target molecules in the tissue. This can consequently be imaged by using a fluorescence microscope. In the same way surface-treated gold particles a few nanometres in size are used to visualise specific molecules by electron microscopy. Another example is magnetic nanoparticles for the analysis of biological samples. The particles have surfaces that are tailored to bind specific molecules from a liquid sample. In the binding of molecules the size of the particles is changed sufficiently for it to be possible for their changed mobility in a magnetic field to be detected.

Nanoparticles of iron have proved effective for the breakdown (oxidation) of certain organic soil pollutants (e.g. solvents that have leaked from industrial plants). The nanoparticles are injected directly into the soil and the breakdown takes place on site, instead if it being necessary to excavate the soil for decontamination or destruction.

3.3 Nanotubes/nanofibres

A nanotube is a cylinder, usually of the element carbon, but it may also be of various inorganic substances such as boron nitride, silica, titanium dioxide, tungsten disulphide and molybdenum disulphide. Non-carbon-based nanotubes, particularly those consisting of semiconductor materials, are often called nanofilaments.

As nanotubes have small dimensions, the laws of quantum mechanics govern the electrons in these, and many new electronic effects have been observed. There is no good theory yet for conductivity in tubes or collections (bundles, tangles) of tubes. Composites based on carbon nanotubes accounted for 15% of all nanocomposites manufactured in 2005, but this proportion is expected to decrease due to a faster rate of increase for the other types of nanocomposites.

There are many potential uses, but high price is delaying the development of products based on carbon nanotubes. There is, however, a growing market for carbon nanofibres (which are not hollow) because they are far easier to manufacture and consequently much cheaper. The following uses are already on the market or may become relevant in the near future:

Waterproof and strong clothing, strong and light sports equipment such as tennis racquets, golf clubs, balls, cycle parts, high-strength fibres, compositions and various filters. Carbon nanotubes have attractive electrical properties and could be used in electrical circuits, but much work remains to be done, as it is still difficult to control what type of nanotube arises in manufacturing.

Carbon nanofibres are already used today by the car industry in applications that utilise their electrical conductivity and mechanical strength. They are used for example in fuel hoses and as an undercoat in the painting of plastic components (handles, rear-view mirrors, bumpers).

Another type of nanomaterials is polymer-based nanofibres. Their areas of use are medical applications, filtration, barrier, hygiene articles, compositions, clothing, insulation and energy

storage. Examples of possible uses for nanofibres are protective clothing, filters, cosmetic products, electronics, optics, sensors and patches.

3.4 Nanolayers/nanoclays

Certain types of clays, particularly montmorillonite (MMT) have been most used as additives in polymers thanks to their ability to improve many properties of materials at the same time, such as mechanical strength and stiffness, fire resistance and dimensional stability. In packaging in particular there are interesting applications where barrier properties are important. It is possible to prevent diffusion of water, gases and perfumes through the packaging material. This is due to the fact that the clays consist of thin sheets with very large expansion in the plane, which means that foreign substances have to take a very long detour to pass through the film. In addition the clay layer has a very large surface, which is bound to the molecules of the polymer matrix and means that the polymer is "stiffened". This implies that it becomes more difficult for foreign substances to move through the polymer.

Many studies have shown that a small proportion of clay particles is sufficient to bring about a very great improvement in barrier properties. Because the clay layers are very thin they do not disperse light, i.e. the transparency of a polymer that is filled with clay particles does not change significantly. The stiffness and strength of the material is improved without any lowering of the toughness of the material.

The automotive industry throughout the world has started introducing nanomaterials to save weight, reduce air resistance, reduce fuel consumption and improve the cosmetic appearance of the vehicle. Wilson Double Core has provided tennis balls with a nanomaterial that causes the balls to retain gas pressure significantly longer than conventional ones. Similar materials are used in the running shoes for the same reason.

In a report from the University of Chicago from 2003 the consumption of all types of polymer/clay nanocomposites (PNC) in the United States was estimated, as well as the expected percentage increase by 2020. The greatest increase is expected in the automotive sector and in the area of consumer products (around 45% each). In addition, the proportion of polymers/clay nanocomposites in plastics is expected to increase from 0.3% in 2003 to around 14% in 2020.

Statistics on consumer products based on nanotechnology are given in an American inventory (www.nanotechproject.org/consumerproducts/). The inventory presents an approximate picture of the number of products, which are the largest areas of use and the increase in consumer products in recent years. The most commonly occurring substance in the products is silver (just under 100 products in 2007). The total number of products more than doubled between March 2006 and May 2007 and has now reached around 500 products. "Health and fitness" accounts for around half of these. In this area most products are in cosmetics and clothing (around 70 products each in May 2007).

There are no similar statistics for Sweden, but it is reasonable to assume that the use of products based on nanotechnology is increasing and will also continue to increase here.

4. EXPOSURE

Very little is known about the tendency of nanomaterials to be taken up by organisms in the environment. Studies on animals indicate that some nanomaterials can be taken up in biological tissues, but it is not possible to draw any general conclusions on the tendency of nanomaterials to bioaccumulate.

The studies that have been done indicate that dispersion, conversion and uptake in the environment are more complex for nanomaterials than for the substances that occur in molecular form. New tools will be needed to understand and estimate exposure. Knowledge and tools from adjacent areas of research can be used, but information on the extent to which these tools will suffice is lacking at present. New knowledge on mechanisms with regard to breakdown and dispersion in the environment will also be important in the work of devising relevant test methods for nanomaterials.

Available knowledge of human exposure to nanomaterials is limited. The knowledge cannot be extrapolated from substances at larger scale to the substances at nanoscale. Nor can general assumptions be made for all nanomatrials with regard to how and whether a substance is taken up.

Humans can be exposed to nanomaterials through their work, as consumers or via the environment. Unintentional uptake of nanomaterials can take place through inhalation, orally or, presumably, through the skin. Other routes can also be imagined, for example through the eye. In the future, humans may perhaps be exposed to nanomaterials in medicinal products.

Inhalation is regarded as being the most important exposure route for nanoparticles, and research has therefore primarily been carried out in this area. Nanoparticles may be deposited in the airways, and factors that influence where they end up include the properties of the particles (e.g. composition, size and solubility), the geometry of the airways and individual differences (e.g. breathing patterns).

Once they are in the airways nanoparticles can be distributed to other parts of the body. They can be transported across the epithelium out from the lung or be taken up by nerve endings in the olfactory mucosa and transferred to the brain via the olfactory nerve. The substances at nanoscale may cross the blood-brain barrier, and they might also contribute to other substances reaching the brain.

There are few studies concerned with the uptake of nanomaterials from the gastrointestinal tract, most showing that nanomaterials are taken up in the gastrointestinal tract and rapidly disappear from there. The skin may be an important uptake route but the extent of this and the underlying mechanisms are still unclear.

Nanomaterials may also cross various membranes in the cells, and have for instance been found in the mitochondria and cell nuclei, which means that the distribution may be very different for nanomaterials compared with materials at larger scale. This may be of significance to the toxicity of nanomaterials.

4.1 Environment

4.1.1 Sources

The area of use and the form in which the material occurs have a great impact on whether it is possible for the nanomaterials to reach the environment. There is a complete range here from fixed nanomaterials embedded in polymers, for example from carbon nanotubes in composite materials and carbon black in plastics to nanoparticles which are intentionally released into the environment for the decontamination of polluted soil.

Below are a number of examples of uses that already today lead to emissions and uses that can be expected to lead to emissions in the future. As both use and the number of areas of use are predicted to increase sharply, it can be expected that further uses might lead to emissions to the environment.

Discharges via sewage treatment plants
Spherical fullerenes and nanoparticles of titanium dioxide, zinc oxide and iron are used today in skin-care products, sunscreens and cosmetics (Teknologirådet, 2006; FOE, 2006; Royal Society, 2006). A large proportion of these probably reaches sewage-treatment plants and therefore they have the potential to reach the environment.

Releases in connection with use in sewage-treatment plants
A large number of different applications using nanotechnology in the treatment of wastewater have been proposed. Primarily nanoporous filter materials or fixed nanoparticles, but other conceivable applications that have been proposed are suspended free nanoparticles that are added to the water to be treated. Photolytic oxidation of organic substances using nanoparticles of titanium dioxide and ultraviolet light at sewage-treatment plants is under development.

Decontamination of polluted soil
An area of use that can be expected to increase is the decontamination of polluted soil with nanoparticles. Metallic iron in nanoparticle form has proved highly effective in catalysing the treatment of soil polluted by chlorinated organic substances (Zhang, 2003). The large ratio between surface area and mass in nanoparticles can also be used to immobilise heavy metals. In these cases it means that relatively large quantities of nanoparticles are released into the soil and will reach the groundwater.

Agriculture
It can be expected that nanotechnology will be applied to various pesticides in agriculture and forestry. The American pesticides industry has involved itself in the work launched by the Office of Pesticide Programs (OPP) under the EPA to judge whether new grounds for assessment are required for these types of materials. There are various conceivable ways of using nanotechnology in this area. The nanomaterial can itself function as a pesticide, as a carrier of pesticide to minimise unintentional releases or enhance the effectiveness of the active substance. This use may mean that nanoparticles are dispersed in the environment. Similarly there are a number of other possible applications in which nanoparticles can be used as carriers for the effective administration of different active substances as nutrients or growth agents (known as “probiotics”) (USDA. 2003).

Wear from polymers and other matrices
An area of use for nanoparticles and nanofibres may be as reinforcement in polymers. Depending on use, wear can lead to nanoparticles being emitted and resulting in exposure close to the source. Global production capacity of carbon black today is 10 000 000 tonnes per year (IARC, 2006). 90% of the use is for reinforcement materials in rubber (ICBA, 2007), a very large proportion of which is accounted for by automotive tyres. Between 25 and 35% of the automotive tyre consists of carbon black (KemI, 1994). Nanoparticles are emitted when the tyres become worn during use, and it is possible that some of these come from the carbon black (Dahl *et al.*, 2006).

Emissions in the production of substances and manufacturing of articles
The level of production of carbon black is already high today, and it is estimated that the production of carbon nanotubes, nanofibres and similar materials for use in structural applications in the near future might be more than 10 000 tonnes per year (Royal Society, 2004). Emissions may also occur for other types of nanomaterials in connection with the production of the nanomaterial itself and in the further handling and production of particles.

4.1.2 Dispersion in the environment

It is not possible to say anything general about the dispersion of nanomaterials in the environment. As for all other substances, molecular (soluble or poorly soluble) and particulate (insoluble), the nanomaterials make up a highly heterogeneous group which will accommodate the whole range of properties that are important for their dispersion in the environment. Some will be readily degradable, while others cannot be broken down at all. It is possible that some will be resistant to aggregation, while others will rapidly attach to larger particles and precipitate from the atmosphere or sediment in lakes and watercourses.

Degradability

Nanoparticles have a large specific surface area ($m^2 \cdot kg^{-1}$) in comparison with microparticles, which can be imagined to result in a larger area of attack for degrading organisms and therefore more rapid degradation. There is a lack of data at present to make generalisations about a substance at nanoscale being more or less readily degradable than larger forms of the same substance.

Some nanomaterials consist of substances which in themselves are difficult or impossible to break down. Examples are inorganic substances such as ceramic substances and certain metal oxides. Some nanomaterials are unique molecular forms of substances, e.g. C_{60} fullerenes and carbon nanotubes, which are forms of carbon. This may also affect the degradability of the material.

It has been found that the carbon in C_{60} fullerenes is taken up by wood-degrading fungi, which is an indication that the fungi have the ability to break down these chemical structures (Filley *et al.* 2005). The chemical reactivity of these compounds is also relatively well known (see Weisner *et al.*, 2006 and references therein). It has additionally been shown that model enzyme systems can break them down. On the other hand, data are lacking on the degradability of C_{60} fullerenes, which would be directly applicable to risk assessment or other regulatory contexts.

The American EPA (US EPA, 2007) notes that there is insufficient information to make any predictions about the degradability of nanomaterials in general. This statement does not, however, apply just to nanomaterials but in principle to all chemicals. Degradability is one of the fundamental information requirements for general chemicals in the new EU legislation Reach (Regulation 1907/2006) and an important criterion for environmental classification in the Dangerous Preparations Directive 1999/45/EEC.

Via the atmosphere

The way in which aerosols behave is relatively well known on the basis of research on naturally and unintentionally produced particles, including nanoparticles. It should also be possible for a large amount of knowledge from this scientific area to be applied to unintentionally produced nanoparticles. Particles in the 1-100 nm size range, i.e. what is normally defined as nanoparticles, are generally short-lived in the atmosphere. These particles are so small that they are affected by the kinetic energy of the gas (“Brownian motion”) and they will therefore be combined relatively quickly and form larger, secondary particles. Particles in the 1-100 nm size range are known as Aitken particles or nucleation mode. Larger particles 100-1000 nm, are usually termed accumulation mode because they stay airborne longer than both larger and smaller particles, and these particles can consist of both primarily formed particles and loosely held-together secondary aggregates of smaller particles. Particles larger than 1000 nm are called coarse mode and are deposited more rapidly because of their size.

Some intentionally produced nanoparticles may have been surface-treated or chemically modified in order to counteract aggregation. These particles may have a substantially longer residence time in the atmosphere (and in water) than can be described by models developed for unintentionally produced and natural nanoparticles. These models may consequently result in underestimation of the risks associated with surface-treated or modified particles.

Via water

Some generalisations can be made with regard to dispersion in water, which are based on available knowledge. Nanoparticles sediment more slowly than larger particles of the same substance. Some of them can be assumed to have colloidal properties, that is to say they have hardly any sinking rate at all. On the other hand, their high surface area-mass ratio means that they have great potential to clump together to form large particles with a greater sinking rate and in this way are transported to the sediments. The effectiveness of this process depends on properties in the surface of the particle and environmental parameters, for example ionic strength and pH. There is some fundamental research in this area, studies having for instance been carried out on nanoparticles of C_{60} fullerenes and titanium dioxide (Fortner *et al.*, 2006, Dunphy Guzman *et al.*, 2006).

Some nanoparticles also have properties in themselves that mean their mobility increases. C_{60} fullerenes, which are around 1 nm, are insoluble in water but spontaneously form colloids with equilibrium concentrations in water of between 10 and 50 ppm. These concentrations are far above the solubility of polycyclic aromatic hydrocarbons, which are a group of closely related substances.

Finally, it has recently been shown that dissolved natural organic matter in lakes and watercourses can increase the mobility of carbon nanotubes (Hyung *et al.*, 2007). This is a process that has not yet been anticipated.

In soil

Very little is in general known about how nanomaterials move in soil. Their size means that adsorption and aggregation can be assumed to be greater than for larger particles. The American EPA also speculates that nanosize may mean that the particles are not captured by narrow passages between soil particles and therefore may be dispersed further (US EPA, 2007). Dispersion in soil may be of significance to how great a proportion of the material reaches the groundwater.

There are only a few reports concerned with the movement of nanoparticles in soil. It is reasonable to assume that the mechanisms and properties that govern binding between colloidal particles in general are also important to the aggregation and sorption of nanoparticles. Charge is one such example (Weisner *et al*., 2006).

Lecoanet *et al*. (2004) have shown that form and chemical modification play an important role in mobility. C_{60} fullerenes whose surface has been modified chemically to facilitate dispersion in water (known as fullerols) also had 100 times greater potential to spread in soil than the original form. Carbon nanotubes were also shown in the same study to have high potential to disperse in soil.

Dispersion models

Knowledge of dispersion in the environment is used to enable estimates to be made of the exposure situation for risk assessments of different substances and materials. Models based on theories on distribution equilibria for organic substances have been used to date in existing legislation for general chemicals and pesticides. These models have also been developed for application to metals. On the other hand, there are no models today that are used to estimate the exposure situation for particular insoluble substances.

There is a large body of knowledge on which to build for atmospheric dispersion. In a similar way there is knowledge in areas such as aggregation of colloidal material in water and movement of colloidal material in groundwater. What is needed is to explore applicability and integrate knowledge from different disciplines to build exposure models for nanomaterials.

4.1.3 Bioaccumulation in organisms

Uptake in organisms is usually measured in the form of various uptake factors such as bioconcentration factor (BCF) or storage in food webs (biomagnification), which also indicates the potential of the substance to be stored in biological tissues. No study that quantifies uptake or bioaccumulation of nanomaterial in organisms was found in a review of published ecotoxicological studies. A toxicological effect, depending on where it occurs, may be an indication that uptake has occurred. The effects of C_{60} fullerenes that have been observed in carp (Oberdörster *et al.,* 2004) are a sign that these can be taken up by fish from surrounding water. Uptake of model nanoparticles at various sizes manufactured from polystyrene has also been observed in eggs of the Japanese rice-fish species (Kashiwada, 2006). The highest bioavailability among the sizes studied was shown by particles of 474 nm, while the smallest size (39.4 nm) was redistributed inside the embryo during early development. The results of the study by Kashiwada also suggest that the smallest nanoparticles in the study (39.4 nm) were taken up directly from the water via the gills of adult fish and reached the brain and gallbladder.

Another form of uptake that did not entail any storage in tissue has been observed by Roberts *et al.* (2007). Daphnia exposed to carbon nanotubes surface-treated with fats to improve distribution in water grazed on the carbon nanotubes and used the layer of fat as a source of food. When the layer of fat had been used up the carbon nanotubes clumped together in the gastrointestinal tract and the animals sank to the bottom, with resultant reduced survival and multiplication.

It can be concluded from studies of health effects of nanomaterials that many nanomaterials have the potential to cross biological membranes (see section 4.2.2 below). Nanoparticles such as C_{60} fullerenes also behave as fat-soluble substances in molecular form in the sense that they can dissolve in biological membranes.

To summarise, very little is known about the tendency of different nanomaterials to be taken up by organisms in the environment. There are indications that some nanomaterials might be taken up in biological tissues, but it is not possible to draw general conclusions about the tendency of nanomaterials to bioaccumulate.

4.2 Humans

4.2.1 Various exposure situations

Humans may be exposed to nanomaterials in various ways. There may be occupational exposure, indirect exposure via the environment (food, water, soil or air) or indirect exposure as a consumer. How use of nanomaterials proceeds and exposure takes place will be of crucial significance to where the substance ends up and what effects it has. Information about the deposition of the material and its subsequent fate is needed in order to be able to decide what dose an individual is exposed to. An important factor in assessing existing studies and producing new ones is that the surface area of particles appears to be a more relevant measure of dose than their mass with regard to calculating the dose-response relationship of nanoparticles.

Occupational exposure
Workers may be exposed to nanomaterials during the manufacture of nanomaterials and the formulation or final use of products containing nanomaterials. Exposure may also take place in the repair, destruction and recycling of products containing nanomaterials (US EPA, 2007; NIOSH, 2005). Occupational exposure to nanomaterials demands special attention as it takes place more often and at higher concentrations than other exposure. An occupational group that is often forgotten when discussing occupational exposure to nanomaterials is research scientists who study and develop nanomaterials.

There are several possible sources of exposure for each manufacturing process. Most processes are, however, stated as taking place in enclosed systems, and exposure of workers therefore principally takes place during handling (transfer and unloading), packing and cleaning (US EPA, 2007).

In the formulating of nanomaterials into products, exposure is most likely to happen during the handling of materials and cleaning of equipment, while exposure in industrial end-use greatly depends on the area in which the product is to be used. If the product for example is to be sprayed on manually workers often have a higher level of exposure.

In order to be able to handle nanomaterials safely it is important to conduct more research on how effective existing protective equipment is with regard to exposure to nanomaterials, and if necessary adapt or develop new equipment specifically for this area of use (NIOSH, 2007).

Exposure of the general public
The general public can be exposed through direct use of products containing nanomaterials. There are already consumer products today in many different areas of use, for example electronics, medicine, cosmetics, chemistry and catalysts, and the number of products and areas of use is expected to increase. Widespread environmental exposure can also be expected from varying consumer use.

The general public may also be exposed to nanomaterials in air, drinking water, food or soil if nanomaterials are emitted into the environment. Information is lacking with regard to the quantitative estimation of emissions to the environment.

Emissions from accidents during manufacturing or transport, or in natural disasters, may also lead to exposure of the general public.

There is, in some respects, great variation in sensitivity between different individuals in the general population, and individuals do not all respond in the same way to exposure to particles (US EPA, 2004). Examples of sensitivity groups are children, the elderly or persons with chronic diseases related to impaired lung function, the cardiovascular system and the immune system. This should be borne in mind when considering the risks of nanomaterials.

4.2.2 Uptake, distribution and excretion

Inhalation
The airways are important with regard to exposure to nanomaterials, and large differences have been reported between particles at nanoscale and larger particles with regard to how they are deposited in, taken up in and removed from the airways (Oberdörster *et al.*, 2005).

How far down in the airways the particles reach and how the body gets rid of them depends on how large they are and may be of crucial significance to how they are spread, as well as for what effects the particles will cause. It is usually said with regard to larger particles that the smaller the particles are the further down in the airways they can reach. Various model

calculations have shown that nanomaterials can end up anywhere in the airways: including nasopharynx, bronchi and alveoli regions (Yeh *et al.,* 1996).

The principal mechanism that decides where the nanoparticles end up is considered to be diffusion, as a result of them colliding with air molecules (Oberdörster *et al.,* 2005). Factors that are important for larger particles, for example gravitation (Kreyling and Scheuch, 2000; Schlesinger *et al.,* 1997; US EPA, 2004), on the other hand, are not considered to affect where the nanomaterials end up to the same extent (Oberdörster *et al.,* 2005). It has been proposed that knowledge from aerosol science can be applied to understand how airborne nanoparticles behave in the airways (Pritchard, 2004). Aerosols are principally affected by particle size and forces such as gravitation and diffusion. Other factors that may affect nanoparticles are aggregation, whether they can dissolve or whether they are electrically charged. There are also studies that suggest that nanoparticles can both agglomerate to form clusters and form aggregates directly. This can affect the subsequent fate of nanoparticles in the environment or in the body (Oberdörster, 2007).

The body can get rid of particles in the airways in two ways, either through mechanical removal of particles or through chemical processes. The chemical processes can take place everywhere in the airways, but to differing extents depending on different conditions inside and outside the cells. The mechanical movements, on the other hand, differ in the various parts of the airways.

According to Oberdörster and colleagues (2005; 2007) several studies indicate that nanoparticles that have ended up in the airways easily penetrate the epithelium and enter the interstitium. Nanoparticles being recovered around the airways instead of in the alveoli means that they can give rise to local effects there. Knowledge is lacking on whether this is also relevant to humans, but as interstitial transfer of small particles across the alveolar epithelium is more prominent in higher animals than in rodents it is reasonable to assume that the high transfer of nanoparticles that has been observed in rats also occurs in humans (Oberdörster *et al.,* 2005).

In a study in which lung flushing of rats exposed to polystyrene particles of varying size was performed, around 20% of particles 15-80 nm in size were recovered in the flushing fluid, while the majority (80%) were in the lung. The converse situation applied to polystyrene particles 0.5-10 µm in size, i.e. 80% were recovered in the flushing fluid (Kreyling *et al*., 2002; Oberdörster *et al.,* 1992, 2000; Semmler *et al.,* 2004). This is considered to indicate that the nanoparticles are either inside the epithelial cells or have moved to the interstitium and that the macrophages in the epithelium are ineffective in dealing with nanoparticles.

Spread from the lung to other parts of the body

Once a particle has reached the interstitium it can be taken up in the blood system, if size permits, as well as in the lymphatic system. The transfer of nanomaterials across the alveolar epithelium was shown for the first time by Berry *et al.* (1997), who instilled gold particles (30 nm) into the airways. After 30 min large quantities of the particles were recovered in the platelets in the lung capillaries. The authors suggested that this is a way for the body to get rid of inhaled particles. Several studies have subsequently confirmed the results with uptake of gold particles, 5-20 nm (Konig *et al*., 1993; Mehta *et al*., 2004; Heckel *et al*., 2004). Other particles for which transfer has been shown are iridium, 18-80 nm (Kreyling *et al*., 2002) and carbon, 35 nm (Oberdörster *et al*., 2002). Transfer of polystyrene particles, 60-400 nm, is more ambiguous and depends both on size and on surface-chemical properties (Nemmar *et al*., 2002; 2004; Kato *et al*., 2003). These studies, taken together, suggest that particle size, surface chemistry and charge are important factors for transfer across the cell membrane.

Evidence of transfer of inhaled nanomaterials to the blood circulation in humans is ambiguous. One study shows that inhaled nanoparticles of ^{99}Tc rapidly appears in the blood and accumulates in the liver (Nemmar *et al.*, 2002), while another study with the same particles did not show any accumulation (Brown *et al.*, 2002). Later studies of ^{99}Tc-labelled carbon nanoparticles (35 and 100 nm) also suggest slight or non-existent transfer from the lung to the blood circulation (Möller *et al*., 2006; Wiebert *et al*., 2006). In addition, there may be other mechanisms that mean that inhaled nanomaterials may indirectly be responsible for negative effects on other organs, for example cardiovascular effects following impact on cytokine signalling and platelet activation in the lung circulation (Li *et al.*, 2007). If knowledge from animals and humans is combined, however, it is not unlikely that transfer from the lung to the blood circulation also takes place in humans and the extent to which this happens might depend on the size of the particles, the properties of the surface and chemical composition. However, more research is needed on this area.

Once nanoparticles have been taken up in the blood they can spread throughout the body. Studies show that most of the particles are recovered in the liver, followed by the spleen. If the particles are surface-treated, their presence in the liver and spleen is prevented and they are recovered in other organs instead (Akerman *et al.*, 2002). It has been proposed that nanoparticles in the liver and spleen accumulate in the macrophages (Tran *et al.*, 2005). This is partly based on the knowledge that macrophages clean the blood of bacteria and other particles when it passes through these organs, and it has been observed for example that particles that have been injected into the blood accumulate in the macrophages in the liver and spleen and that bacteria are dealt with by the Kupfer cells (Stuart, 1970). Studies with polystyrene particles suggest, however, that the macrophages in the epithelium are ineffective in dealing with nanoparticles. It is unclear whether this applies in general to other nanomaterials.

The cardiovascular system, the nervous system and excretory organs have not previously been considered to be secondary target organs following inhalation of nanoparticles. Many recent studies on animals and humans have, however, pointed to the ability of ultrafine particles to move to the liver, heart and brain (Kreyling *et al*., 2002; Nemmar *et al*., 2002; Oberdörster *et al*., 2002). Examples of this are quantum dots (10 nm), metal fullerenes (<220 nm) and nanoparticles (90-250 nm) all of which were recovered in the bone marrow following intravenous injection in mice (Ballou *et al*., 2004; Cagle *et al*, 1999; Bazile *et al*., 1992). Although it is not known how the transfer takes place, these results show that nanomaterials may play an important role for example in inducing and promoting morbidity and mortality related to the heart and blood vessels.

To study whether nanoparticles are taken up and distributed from the lungs to the liver, heart, brain, olfactory lobe and kidneys, rats were exposed in an exposure chamber (6 hours) to ultrafine ^{13}carbon particles (18 and 180 μg particles/m^3, 20-29 nm) (Oberdörster *et al*., 2002). After five hours ^{13}carbon was found in the liver from animals in the high dose group, and after 24 hours the level of ^{13}carbon in the liver was approximately five times that in the lungs in all the exposed animals. As well as the liver, there was evidence that the olfactory lobe was a target organ for the particles that were inhaled.

Transfer to the brain

It has previously been shown that the olfactory nerve is an entry port to the brain for particles at nanoscale for poliovirus (30 nm) which was instilled into the noses of chimpanzees and rhesus monkeys (Bodian and Howe, 1941; 1941b; Howe and Bodian, 1940). In a study by de Lorenzo (1970) squirrel monkeys were exposed to gold particles surface-treated with silver which were instilled into the nose. The particles were transferred with the axons in the nasal

mucosa and reached the mitral cell dendrites within one hour. An interesting observation was that the nanoparticles in the olfactory lobe were no longer freely distributed in the cytoplasm but were to be found in the mitochondria. This may be significant for effects that may arise as a consequence of exposure to nanomaterials.

Recent studies confirm that these results are also relevant to inhaled particles at nanosize (Oberdörster *et al.*, 2005). Whole-body exposure to ^{13}carbon particles (37 nm) in an inhalation chamber resulted in a significant increase in the olfactory lobe on the first day and a continued increase for the following 7 days after exposure (Oberdörster *et al.*, 2004). A clear increase was also seen in the brain and cerebellum on the first day, but this increase did not persist and it is speculated that this may be due to transport across the blood-brain barrier in certain regions. The authors summarise by saying that the central nervous system may be a target organ for nanoparticles that are inhaled and that the most likely mechanism is uptake from the nasal mucosa and transport to the brain via the olfactory nerve.

Transfer from the olfactory lobe to the brain has also been reported in rats exposed to 500 µg/m^3 ultrafine particles (30 nm) of manganese oxide via the nose (Elder *et al.*, 2006). The authors' conclusion is that the olfactory nerve is an effective route for the transfer of manganese oxide at nanoscale to the brain, and that these can give rise to inflammatory changes. This result is backed up by a study in which rats were exposed to manganese oxide (30 nm) for 12 days. The increase in the olfactory lobe was 3.5 times, while the increase in the lung was only two-fold (Feikert *et al.*, 2004). This study can be compared with a study in which rats inhaled manganese particles (1.3-18 µm) for 15 days and in which no increase was observed in manganese in the olfactory lobe (Fechter *et al.*, 2002).

These studies together indicate that passage via the olfactory nerve should be considered with regard to human exposure to particles at nanoscale. However, there are important differences between humans and rodents: rodents always breathe through the nose, while humans can breathe through the nose and the mouth, and the olfactory mucosa in humans accounts for only 5% of the total nasal mucosa, compared with 50% in rats. This means that this route is important for animals with a well developed olfactory lobe, but that the significance for humans who lack this well developed system could be questioned. Models based on physiological parameters from rats and humans show, however, that the concentration of particles (20 nm) may be 1.6-10 times higher in the olfactory lobe of humans than that of rats (Oberdörster *et al*., 2005).

It has also been shown that nanoparticles can cross the blood-brain barrier (Kreuter *et al.*, 2002; Oberdörster *et al*., 2004) and that the charges on the surface of the nanoparticle can influence the properties of the blood-brain barrier (Lockman, 2004), which might mean a changed distribution and increased toxicity with regard to nanoparticles. Little attention has been paid to the significance of this among toxicologists and there is a lack of knowledge regarding the ability of nanoparticles to damage the nervous system.

Intravenous, intraperitoneal or intracerebral exposure to nanoparticles (50-60 µm) of copper, silver or aluminium opened the blood-brain barrier to Evans Blue stain in a highly selective and specific manner (Sharma *et al.*, 2006). Leakage was observed in the anterior surface of the brain and the adjacent posterior cortex after 24 hours.

Oral exposure

Intake of nanomaterials can take place directly via food, water or pharmaceuticals, or indirectly after they have been transported away from the airways by cilia.

There are few studies concerned with uptake and distribution of nanomaterials across the gastrointestinal tract, but most show that they are taken up and rapidly disappear from the

gastrointestinal tract (Oberdörster *et al*., 2005). According to Florence and Hussain (2001) transfer of particles across the gastrointestinal tract is well documented with regard to pharmaceuticals. There is also evidence that uptake is higher for nanoparticles than for larger particles (Tran *et al.*, 2005).

The difference in uptake is assumed to be due to factors such as the composition of the particle, its size and its surface chemistry. In a study by Jani and colleagues (1990) polystyrene particles (50-3,000 nm) were given to rats orally. A correlation was found between particle size and uptake via the gastrointestinal tract. Desai *et al*., (1996) reported that the uptake of 100 nm particles is 15-250 times greater than the uptake of microparticles. The effectiveness in uptake is stated as also being dependent on type of tissue. The results additionally show that the nanoparticles had diffused through the mucosa while the microparticles were in the epithelium. Ultrafine particles of ^{192}Ir, on the other hand, do not show any uptake via the gastrointestinal tract (Kreyling *et al*., 2002, Semmler *et al*., 2004).

In the oral exposure of rats to radioactively labelled C_{60} fullerenes 98% was excreted with the faeces within 48 hours, while the remainder was excreted in the urine, which suggests some uptake via the gastrointestinal tract (Yamago *et al*., 1995). When rats were exposed intravenously to the same C_{60} fullerenes in the same study, 90% remained in the body after one week, the greater part of this (73-80%) being recovered in the liver.

Dermal exposure

The skin may be an important uptake route for nanomaterials in occupational exposure but also as a result of use in cosmetics, sunscreens, shampoo etc.

The skin is the body's largest organ, and one of its tasks is to limit the intake of foreign substances. Most studies that relate to skin uptake have been made on particles larger than 1 µm. However, there are some studies that show how nanomaterials pass through or accumulate in the skin.

Damaged skin has been shown to present an important uptake route for slightly larger particles (0.5-0.7 µm), but a debate is also under way on the ability of the substances to penetrate healthy/intact skin (US EPA, 2007). It has been shown that stretched skin (as when the wrist is rotated) may make it possible for particles up to a size of 1 µm to be taken up (Tinkle *et al*., 2003). Various physiological properties that make the skin more permeable to nanomaterials have been reviewed by Hart (2004).

A study in which human skin was exposed to titanium dioxide at nanoscale shows that the nanoparticles penetrate the horny layer (the stratum corneum) (Lademan *et al.*, 1999). The largest share was recovered in the upper part of the horny layer, but a certain proportion was recovered further down. The authors suggest that it is most likely that the nanoparticles have entered through the hair follicles. It has also been shown that particles (50 and 500 nm) that are negatively charged pass through pig skin, unlike neutral or positively charged particles (Kohli and Alpar, 2004). Other authors do not regard themselves as at all convinced that nanoparticles permeate through intact skin (Nohynek, *et al.*, 2007).

Studies with mice and pigs show that quantum dots that have been injected into the skin find their way to the local lymph nodes (Kim *et al*., 2004). The likely transport mechanisms is via macrophages and Langerhans cells in the skin (Ohl *et al*., 2004; Sato *et al*., 1998). There are no studies on the transfer of nanomaterials from skin to blood, but transfer from lymph nodes to the blood system probably takes place as shown previously in studies with small asbestos fibres (Oberdörster *et al*., 1988). It is shown that particles can penetrate through the skin and reach the circulatory system by reports for instance on podoconiosis, where particles are taken up in the lymphatic system (Corachan *et al*., 1988; Blundell *et al*., 1989). Podoconiosis, or

elephantiasis, is a disease that is suggested to arise as a result of small silica particles from volcanic ash and aluminium silicates passing through the skin. These particles are cytotoxic to macrophages, resulting in the lymphatic vessels being obstructed. Depending on the size of the particles (often 2-20 µm) the lymphatic vessels in the foot or more central lymphatic vessels are obstructed. This can result in chronically obstructed lymphatic vessels and fibrosis.

In view of the fact that it has been shown that nanomaterials can reach the brain via the olfactory nerve, there has also been speculation that materials may be taken up and transported via the nerves in the skin (Oberdörster *et al*., 2005). Studies in which mice have been exposed on the tongue or through injection into facial muscles indicate that electrical charge is important to axonal transport (Arvidsson 1994; Malmgren *et al*., 1978; Olsson and Kristensson, 1981). Other studies in which ferritin (~112 nm) and iron dextran (11-21 nm) and gold protein (20-25 nm) have been injected show rapid transfer of nanoparticles smaller than 20 nm across the synapse between nerve cell and muscle (Oldfors and Fardeau, 1983). Transport along nerves of particles at nanosize has also been established for herpes virus (Kennedy and Chaudhuri, 2002).

Whether it can be considered that nanomaterials in general are taken up via the skin remains a grey area, and further research is needed in this area.

Eyes
The possibility of exposure via the eyes from powder or vapour, or from splashes, should also be considered (US EPA, 2007).

Knowledge is lacking on biotransformation and excretion from the body.

5. EFFECTS ON HEALTH AND ENVIRONMENT

At present, there are few ecotoxicological studies on nanomaterials. Therefore, it is not possible to draw any general conclusions on whether intentionally produced nanomaterials might pose a greater threat to the environment than intentionally produced substances in general. A general problem in assessment is that estimates of exposure are uncertain due to a lack of knowledge. Experimental problems in putting nanoparticles into suspension and what is toxicologically relevant exposure are two different aspects of the problem.

An area where effects might be expected is the use of nanoparticles of iron to decontaminate polluted soil as these reactive particles can change the soil chemistry and release other substances that have been bound.

Taken together, these data indicate that exposure to nanomaterials might harm human health under certain circumstances. However, not all nanomaterials lead to increased toxicity in comparison with the materials at larger size. Knowledge of effects and the mechanisms that govern them is very limited, and further research is therefore necessary in this area. It has not yet been clarified what properties are of greatest significance for the occurrence of toxic effects.

Exposure to nanoparticles appears to be associated with persistent inflammation in several different types of tissues and organs. It has been shown both *in vitro* and *in vivo* that reactive oxygen radicals are a large contributory factor to inflammation and toxicity caused by ultrafine particles. These are insights that can increase knowledge of the possible link between different nanomaterials and diseases, such as lung diseases, cardiovascular diseases and cancer.

It has been shown that nanoparticles can alter the properties of the blood-brain barrier, and that they may possibly damage the brain. The IARC has judged that titanium dioxide and carbon black are to be regarded as possibly carcinogenic in humans. Formation of DNA adducts has also been linked to nanoparticles, and there are studies that indicate that toxicity is higher for ultrafine particles than for fine particles. There is evidence that nanoparticles may be associated with cardiovascular disease and a change in immunological response. It has also been shown that nanoparticles can affect blood clotting, but the mechanism underlying this remains unclear. There are studies that show that nanoparticles can enter and affect the mitochondria and that they can enter the cell nucleus and induce enzymes. This might lead to increased oxidative stress or involvement in replication or repair of genes.

Experts do agree on that it is not possible to generalise and extrapolate knowledge on the toxicological properties of a bulk chemical to the substance at nanoscale. It is also unclear whether it is possible to extrapolate between species with regard to effects. The nanomaterials should therefore be assessed on a case-by-base basis for the time being.

5.1 Introduction

Studies concerned with the toxicology of nanomaterials are relatively limited. Some properties that may be of significance for the toxicity of nanomaterials are mentioned below.

Reduction in size to nanoscale means that the surface area increases in relation to volume. This means that the contact surface becomes much larger and the inherent properties of the substance are enhanced in this way (Donaldson *et al.*, 2004). It is considered that this may be one of the reasons why nanoparticles are generally more toxic than larger particles of the same material (SCENIHR, 2006). In a study with TiO_2 a greater inflammatory response was observed in the lung following exposure to ultrafine particles (20 nm) than if the particles were larger (250 nm). The difference disappeared when the dose was expressed per unit of surface area, which indicates that this is a better measure of dose than mass for exposure to particles (Oberdörster, 2000).

Form is also assumed to be important, although there is no evidence of this. With regard to fibres that are inhaled, their length and thickness are of decisive significance in determining where they end up in the airways and their inflammatory capacity. A special category of fibres is nanotubes, which may be a few nanometres wide and up to a few micrometres in length. The similarity to known carcinogenic asbestos fibres should be taken into account when judging the risks of nanotubes.

A free substance is assumed to be able to cause far more damage than one that is bound. It has been shown that if nanoparticles of iron oxide were inactivated by a surface treatment (Gupta and Gupta, 2005) or were derivatised (Berry *et al.*, 2003) the cell toxicity decreased *in vitro* for superparamagnetic nanoparticles of iron oxide. Chemicals that are adsorbed on the surface may also affect the reactivity of nanomaterials.

Chemical composition and inherent properties are also important to the toxicity of particles (Donaldson *et al.*, 2004). The effect of exposure to nanoparticles of carbon black was more severe than the effect of TiO_2, although both led to inflammation in the lungs and damage to the epithelium to a greater extent than their larger equivalents did. Among several different nanoparticles (polyvinyl chloride, TiO_2, SiO_2, Co and Ni) only Co led to toxicity in endothelial cells, accompanied by the formation of proinflammatory cytokine IL-8 (Peters *et al.*, 2004). This had previously only been shown for TiO_2 and SiO_2, and an explanation for the

difference might be that the particles are of varying size. The studied particles are between 14 and 120 nm and also contain aggregates up to 420 nm.

Toxic effects of nanomaterials may, in addition to the nanomaterial itself, be largely (or in some cases entirely) due to the contaminants that arise as part of the manufacturing process. This has been identified for both carbon nanotubes and nanoparticles of gold (Donaldson *et al*., 2006; Fadeel *et al*., 2007). As the level of contaminants may differ, this means that it is very important to be aware of the manufacturing process that has been used to produce a particular material.

Nanoparticles in the surrounding air consist of a mixture of substances that can interact with one another. Metallic iron can raise the effect of graphic nanoparticles, which leads to raised reactivity involving oxidative stress (Wilson *et al*., 2002), but an increased surface area can also lead to a weakening of cytotoxicity.

5.2 Effects in the environment

The sections below list information on toxicity which is available for the organisms that normally constitute the basis for assessment of risk to the environment. Information on toxicity for other organisms that may be used in assessments of environmental risk is summarised in the concluding section.

5.2.1 Crustacea (Daphnia)

Lover and Klaper (2006) have measured the toxicity of nanoparticles of TiO_2 and C_{60} fullerenes in Daphnia. The study showed that an effective solvent such as tetrahydrofuran was required to separate nanoparticles for an experimental solution. Ultrasound and suspension in water were not sufficient to separate the particles. Nanoparticles of TiO_2 expressed an LC_{50} value of 5.5 ppm, while C_{60} fullerenes had an LC_{50} of 0.86 ppm.

Particles which may have consisted of aggregated nanoparticles of titanium dioxide, silicon dioxide and zinc oxide proved to be toxic to Daphnia (Adams *et al*., 2006). Neither LC_{50} values nor NOEC, which are normally used in risk assessments, were established in this study, but 73% mortality was measured at 0.2 ppm for zinc oxide, which indicates that the LC_{50} is below 0.2 ppm. The bulk substance zinc oxide has a reported LC_{50} value of 24.5 ppm (Kemiska ämnen, 2007). The toxicity of titanium dioxide and silicon dioxide appeared to be two orders of magnitude below that of zinc oxide. The toxicity of the titanium dioxide particles in this study was also lower than in the study by Lover and Klaper (2006). Several different nominal sizes (i.e. the sizes stated by the manufacturers) of each substance were tested in this study, from 66 nm to 44 µm. Measurements on the test suspensions showed, however, that the actual particle sizes did not differ by more than a factor of 3. In addition, one of the particle suspensions in the experiments contains particles smaller than 100 nm. The average particle size was between 300 nm and 1 µm.

5.2.2 Fish

A study which attracted considerable attention showed that C_{60} fullerenes caused measurable oxidative stress (a sign of inflammation) on the brain of juvenile carp at a concentration of 0.5 ppm (Oberdörster, 2004). The results of this study indicated that the route of uptake to the brain may be through the fish's equivalent of the olfactory nerve, which it is to say that uptake took place through nerve cells. These results have, however, in part been questioned because the researchers used a substance which in itself is toxic (tetrahydrofuran) to dissolve the C_{60} fullerenes in water prior to the study. Results obtained by Henry *et al.* (2007) indicate among other things that residues of tetrahydrofuran in C_{60} fullerene solutions that had been prepared

using this solvent may explain the toxicity observed in these studies. Zhu *et al*. (2006) have, however, pointed to effects in the form of changes in gene expression in fish that had been exposed to C_{60} fullerene suspensions which had been prepared without solvent.

5.2.3 Higher organisms

General knowledge is still lacking on risks of nanomaterials being taken up via food in higher organisms such as birds, marine mammals and terrestrial predators, or whether nanomaterials could be imagined as leading to effects in these organisms. As health-related studies show that nanomaterials can be taken up and produce effects, there is reason to assume that this also applies to higher organisms in the environment.

5.2.4 Algae

There is no knowledge of how nanomaterials behave or what effects they may cause in existing standardised toxicity tests for algae.

5.2.5 Microorganisms

Certain materials are made to have antimicrobial effects, such as silver at nanoscale. However, it is unclear with regard to nanoparticles of silver whether the nanoscale contributes to the antimicrobial effect. Other nanomaterials that have an antimicrobial effect are titanium dioxide, silicon dioxide and zinc oxide (Adams *et al.*, 2006) and releases of these materials may affect bacterial degradation in sewage treatment plants. C_{60} fullerenes have also been found in laboratory studies to have antimicrobial activity and to inhibit bacterial growth at low concentrations (0.04 ppm, Fortner *et al*., 2005). The underlying mechanism of this effect may be that the C_{60} fullerenes dissolve in the cell membrane and cause oxidative processes (Fang *et al*., 2007). It is known that spherical fullerenes cause oxidative stress in biological systems and the bacteria in this study showed signs of defence against oxidative processes in the cell membrane. A study in which soil bacteria were exposed in soil did not, however, show any effects at concentrations of C_{60} fullerenes up to 1 g/kg soil, which is to be regarded as a high concentration (Tong *et al*., 2007). With regard to microorganisms it has also been shown that quantum dots of cadmium and selenium are taken up by bacteria (Kloepfer *et al*., 2005).

Possible effects of nanomaterials on microorganisms can also affect function in sewage treatment plants.

5.2.6 Effects in abiotic environment

The use of nanoparticles for the decontamination of polluted soil entails injecting nanoparticles directly into the soil. An effect that has been observed in experiments with nanoparticles of iron is that the soil-water chemistry is also affected by the oxygen in the soil being consumed (Zhang, 2003). This may lead to indirect effects, such as the substances that have previously been bound being liberated.

5.2.7 Other organisms and effects at cellular level

A large number of new results on the exotoxicological effects of nanomaterials have been presented at recent scientific conferences. Some examples are given here. Effects on earthworms have been observed for both C_{60} fullerenes and carbon nanotubes (Scott-Forsmand, 2006). Another terrestrial organism in which effects of intentionally produced nanoparticles of various metal oxides have been identified is the woodlouse (Drobne *et al*.,

2007). Prepared liver cells have shown cytotoxic effects on exposure to very low concentrations (0.4 µg/L) of quantum dots of cadmium telluride (Gagné *et al*., 2007).

5.3 Human health

The knowledge that is available on effects is largely based on knowledge relating to unintentionally formed particles in air pollutants, known as ultrafine particles (Oberdörster *et al*., 2005; 2007; Donaldson and Stone, 2003; Donaldson *et al*., 2001; 2004). There are also some data from studies of nanoparticles in pharmaceuticals (Baran *et al*., 2002; Cascone *et al*., 2002; Duncan, 2003; Kipp, 2004). The similarity of nanotubes to fibres has also raised concern that the effects observed following exposure to asbestos and some mineral fibres might also be relevant to nanotubes. Knowledge of the toxicology of fibres is principally based on experience with asbestos, but studies on mineral fibres have also contributed to increased knowledge of the significance of length and biopersistence in leading to damage.

An overview of existing knowledge of the effects of nanomaterials on some important organs/organ systems is presented below.

5.3.1 Lungs

With regard to the effects of nanoparticles on the organs of the body, it is effects on the lung that have been studied most. Macrophages (from both rats and humans) that were loaded with a particle quantity corresponding to “normal” air pollutant content in the form of aggregates of ultrafine particle showed impaired ability to phagocytize microorganisms (Lundborg *et al*., 2006; 2007). This can lead to increased susceptibility to infection and exacerbation of asthma and chronic obstructive pulmonary disease (COPD).

There is a well documented relationship between air pollution and health effects in vulnerable groups such as children and asthmatics (Nel, 2006). Increased respiratory symptoms, increased hospitalisations, impaired pulmonary function, changes in excretion, chronic obstructive pulmonary diseases and increased mortality are some of the effects that are linked to exposure to air pollutants (Gong *et al*., 2005; Pietropaoli *et al*., 2004). In asthmatics and patients with chronic obstructive pulmonary diseases exacerbation caused by inflammation in the lungs appears to be an important mechanism by which particles exercise their toxicity.

Deposition of nanoparticles in the lungs of susceptible groups was studied in humans. Individuals with mild to moderate asthma inhaled ultrafine carbon particles (median 23 nm, the smallest particles were 8.7 mm) for two hours, at rest and during exercise (Chalupa *et al*., 2004). Deposition increased when the particle size decreased and in exercise. It was also noted that deposition at rest appears to be greater for asthmatics compared to previous studies on healthy individuals. On the other hand, no difference was observed between the genders. When account has been taken of the fact that an increased proportion had been deposited and of cardiac output, the number of particles remaining in the lung was 74% higher in asthmatics than in healthy individuals. The conclusion was that individuals with asthma have a higher total dose of ultrafine particles at a given exposure, which may contribute to their increased susceptibility with regard to health effects related to air pollution.

It has been shown both *in vitro* and *in vivo* that reactive oxygen radicals are a large contributory factor to inflammation and toxicity caused by ultrafine particles. The ability of nanoparticles to give rise to reactive oxygen radicals that lead to oxidative stress, activation of signal pathways and cell death are relatively new insights which may increase our knowledge of the development of lung diseases and also other diseases. The relationship between oxidative stress and change in cell response caused by ultrafine particles is also illustrated in a model of oxidative stress (Nel *et al*., 2006; Brown *et al*., 2001; Donaldson *et al*., 2004,

Donaldson and Tran, 2002; Saldiva *et al*., 2002). Transcription factors translate the oxidative stress to pro-inflammatory proteins (Donaldson *et al*., 2004), and the inflammation can then give rise to respiratory diseases, cardiovascular diseases, fibrosis or cancer (Mauderly *et al*., 1994).

The harmful effects of particles are stated as being principally due to two things: the size of the surface area and the reactivity of the surface (Donaldson and Tran, 2002; Tran *et al*., 2000). The smaller the particles are the larger the surface area in relation to mass, which means that any reactivity per unit of surface area is enhanced (Duffin *et al*., 2002). Ultrafine particles of titanium dioxide showed a greater inflammatory response than fine particles of the same substance when they were instilled into rats or mice (Oberdörster *et al*., 1994). The difference disappeared, however, if the dose was expressed as surface area instead of mass, which showed that this may be a better measure of exposure with regard to nanoparticles. Ageing of material has been shown to lead to a decrease in the reactivity of the surface and indirectly in its toxicity (Oberdörster *et al*., 1995).

The significance of the surface for the development of acute inflammation in the lung has recently been confirmed for particles of the order of 10-50 nm (Stoeger *et al*., 2006). The interaction between ultrafine particles and transition metals leads to synergistic effects with regard to the formation of reactive oxygen radicals and inflammation (Brown *et al*., 2001; Donaldson *et al*., 2004). A relationship between the size and composition of particles and uptake in cell systems and ability to result in oxidative stress has been demonstrated for ultrafine particles and larger particles of polystyrene (Brown *et al*., 2001), and air pollutants (Kreyling *et al*., 2002).

Ultrafine particles have also been shown to be very potent with regard to giving rise to oxidative stress in macrophages and epithelial cells by inducing heme oxygenase-1 and consuming glutathione in the cells. Oxidative stress caused by ultrafine particles has also been reported to be involved in activation of mitogen-activating protein kinases (Oberdörster *et al*., 1995).

Nanotubes are made increasingly longer, and from the point where they are longer than 15-20 µm they satisfy the requirements to be counted as long fibres. The length of the fibres is important with regard to ability to lead to damage. If the fibres are substantially larger than the macrophages, this means that the macrophages in the alveoli will have difficulty to eliminate them by the mucocillary escalator in the airways. This means that they disappear more slowly from the lung (Searl *et al*., 1999), which sustains more marked inflammatory damage.

Additionally, the nanotubes are often based on graphite and thus not soluble at neutral or weakly acidic pH, which means that they may be biopersistent. Also, the nanotubes may be contaminated with metals, which may contribute to the development of inflammation and toxicity.

Knowledge of the toxicity of fibres is principally based on experience with asbestos, but studies on mineral fibres have also contributed to increased knowledge of the significance of biopersistence in causing damage (Bernstein *et al*., 2005). Other types of fibre such as carbon nanotubes have been mentioned as possible examples with regard to leading to diseases linked to fibrosis in the lung, i.e. asbestosis, lung cancer and mesothelioma. There are many proposed mechanisms underlying these diseases, and they include oxidative stress, inflammation and both direct and indirect genotoxicity. Oxidative stress mediated by metals appears to be significant with regard to the ability of asbestos fibres to lead to serious damage (Ghio *et al*., 1998). It is also likely that oxidative stress can arise in other ways, although these have not yet been entirely clarified (Donaldson *et al*., 2006).

There is an extensive literature that confirms increased mortality from lung cancer (mesothelioma) and morbidity in the airways following occupational exposure to asbestos and some other fibrous materials. Properties deciding that particles may be toxic are that they are longer than 10-15 µm, have a diameter of less than 3 µm and are soluble in the lung environment, with further contributions from the surface properties. This may also be relevant to some nanomaterials with a fibre structure, known as nanotubes (Tran *et al*., 2005; Donaldson *et al*., 2006).

In an experiment with inhalation of long amosite (also known as brown asbestos) and the same amosite ground down to shorter fibres, it was found that the long fibres led to more fibrosis and cancer in the rat (Davis *et al*., 1986). When the amosite was given intraperitoneally the long but not the short fibres led to mesothelioma (Donaldson *et al*., 1989). Long fibres have also been found to increase activation of a transcription factor which is involved in the initiation of inflammation (Ye *et al*., 1999a).

In three studies in which rodents were exposed on one occasion to carbon nanotubes in the airways by instillation, inflammation and granuloma were observed in the lung at both high and low dose. The effects in the rat decreased with time after exposure to 1 and 5 mg/kg body weight (Warheit, *et al*., 2004), while the effects remained for up to 90 days in mice exposed to 3.3-16.6 mg/kg body weight (Lam, 2004). The effects also persisted in mice which had been exposed to 10-40 µg per mouse (Shvedova *et al*., 2005). As the way of exposure differs markedly from continuous low-dose exposure by inhalation, further information is required from well controlled inhalation studies before the results from animal experiments can be transferred to humans.

5.3.2 Blood, liver and spleen

Nanoparticles taken up in the body and in the circulation are largely recovered in the liver, followed by the spleen. Studies of these organs are therefore highly relevant with regard to nanomaterials. As these organs have not previously been regarded as target organs, only limited information is available on effects of nanomaterials.

Nanoparticles are stated as possibly being capable of leading to thrombosis without any inflammation in the liver when they are injected into mice (Kandoga *et al.*, 2004). This is supported by studies by Schwartz (2001), who shows that nanoparticles are potent with regard to modifying clotting factors in blood.

Quantum dots of CdSe were found to be acutely toxic under certain conditions for primary hepatocytes. Cytotoxicity appears to be related to release of Cd^{2+} ions. The authors speculate that if the quantum dots are properly covered, non-toxic quantum dots of CdSe could be obtained (Derfus *et al*., 2004).

5.3.3 Skin

According to a review by Nohynek *et al.*, 2007, there is a lack of unambiguous evidence of the toxicological action of nanomaterials. This conclusion is principally based on knowledge of the most common nanoparticles in cosmetics, zinc oxide and titanium dioxide. However, more knowledge is required to be able to draw any general conclusion for nanomaterials.

In an *in vitro* study on human skin cells, Shvedovia and colleagues (2003) identified the ability of carbon nanotubes to lead to the formation of reactive oxygen radicals, which resulted in cell toxicity, lipid peroxidation, consumption of antioxidants and a decrease in the viability of the cells. The authors' conclusion from the study was that exposure to carbon nanotubes might lead to an increase in oxidative stress and marked effects in workers.

5.3.4 The cardiovascular system

As the cardiovascular system has not previously been regarded as a target organ, only limited information is available with regard to the effects of nanomaterials.

Exposure to different particles has been reported to cause oxidative stress, inflammation and cell death in the heart tissue of rats (Gurgueira *et al*., 2002; Rhoden *et al*., 2005; Kodavanti *et al*., 2003). Nanoparticles are also potent with regard to causing inflammation (Renwick *et al*., 2004), and particles from air pollutants can cause changes to clotting factors in blood (Schwartz, 2001), they readily reach the interstitium (Ferin *et al*.,1992) and can enter the blood (Nemmar *et al*., 2004). Blood-borne nanoparticles might reach the heart and directly affect myocardial cells and other tissues in the heart (Tran *et al*., 2005). Taken this together implies that exposure to nanoparticles is capable of leading to effects on the cardiovascular system (Tran *et al*., 2005).

Several studies indicate that an increase in airborne particles less than 10 µm in diameter (PM_{10}) leads to harmful effects on the cardiovascular system, for instance mortality, hospitalisations and myocardial infarction (Peters *et al*., 2001; Peters *et al*., 2004). Increased heart rate has also been observed (Peters *et al*., 1999), and when elderly persons were exposed to a concentrated quantity of particles from air pollution this led to decreased variation in heart rate (Devlin *et al*., 2003).

When rats were exposed to different substances, for example iron or nickel at the same time as ultrafine particles of carbon, this led to interaction and a more marked effect on heart rate than if the effects of the individual components were combined (Chang *et al*., 2007).

Following instillation of carbon nanotubes (SWCNTs) into the airways of C57BL/6 mice, damage to the DNA was observed in the mitochondria in the aorta 7, 28 and 60 days following exposure (Li *et al*., 2007). In addition, changed levels of glutathione and protein carbonyl were observed in the mitochondria. Repeated exposure of transgenic $ApoE^{-/-}$ mice to the same carbon nanotubes (once every two weeks for eight weeks) did not result in any change in the lipid profile but accelerated binding of plaque in the aorta. In addition, damage to the DNA in the mitochondria in the aorta was observed without any inflammation.

5.3.5 Nervous system

It has been shown in several studies that particles at nanoscale can enter the brain via the olfactory nerve (Bodian and Howe, 1941a; 1941b; Howe and Bodian, 1940; Hunter and Dey, 1998; Hunter and Undem, 1999; Oberdörster *et al*., 2004). It has also been shown that they can cross the blood-brain barrier (Kreuter *et al*., 2002; Oberdörster *et al*., 2004) and that the charges on the surface of the nanoparticle can influence the properties of the blood-brain barrier (Lockman, 2004), which might mean a modified distribution and increased toxicity for nanoparticles. Little attention has been paid to the significance of this among toxicologists and there is a lack of knowledge regarding the ability of nanomaterials to damage the nervous system.

There are, however, studies that indicate that nanoparticles might be capable of damaging the brain. Chen and Nadziejko (2005) reported that particles can alter the structure of the brain, and post-mortem examination of brains from individuals who had spent time in Mexico City, where traffic is very heavy, points to earlier development of Alzheimer's disease than is normally to be expected, which is assumed to be partly due to exposure to particulate matter (Calderon-Garciduenas *et al*., 2004). In both these studies there are particles of many different sizes, but a large proportion are regarded as being nanoparticles. In addition, epidemiological studies on welders show that this group develop Parkinson's disease 17 years earlier than the

general population (Racette *et al*., 2001), and welding gives rise to large quantities of fumes which contain ultrafine particles of manganese.

Nanoparticles (50-60 nm) of copper, silver or aluminium led to mild cognitive damage and cell changes in the brain in previously untreated rats exposed intraperitoneally once daily for one week (Sharma and Sharma, 2007). In rats which prior to exposure were exposed to whole-body hyperthermia the nanoparticles, depending on chemical composition, were found to be capable of exacerbating brain damage. The effects of silver and copper were most noticeable.

5.3.6 Immune system

It has been proposed that nanoparticles in bone marrow might lead to effects on the immune system and blood formation (Banjeree *et al*., 2002; Oberdörster *et al*., 2005).

Particles are known to be capable of facilitating sensitisation under certain conditions (De Haar *et al*., 2006; Granum and Løvik, 2002, Allessandrini, 2006). Smaller particles are additionally stated to have a greater effect. It has not, however, been entirely clarified that nanomaterials impair the immune system, and further research is required in this area (Nel *et al.*, 2006).

Particles of different sizes can act as adjuvants (Nygaard *et al*., 2005). An example of this is polystyrene particles with a diameter of 100 nm, which both enhanced the IgE sensitivity response to OVA (a model allergen) and, depending on genetic sensitivity, can lead to increased allergic inflammation and thus increase the allergic symptoms in certain individuals. The theory is that particles may play a role as carriers of allergens.

According to SCENIHR (2007) nanomaterials might also influence the development and degree of severity of other allergic diseases in the lungs, depending on the atopic status of different individuals.

Although the reticuloendothelial system cleans away nanoparticles, reactions between proteins and particles can change the formation of antigen and give rise to an autoimmune response. Composite nanoparticles and proteins can lead to an increase in immune response (Nel *et al.*, 2006). An example of this is that diesel exhaust gases and other particles in the surroundings may help to increase an individual's immune response to allergens in the environment. Finally there is also a possibility of the immune system to recognise nanomaterial directly, which is backed up by the results of a study in which mice which had received intraperitoneal injections of albumin-conjugated spherical fullerenes formed specific antibodies (Chen *et al.*, 1998).

Whether nanomaterials can induce hypersensitivity following skin exposure depends on whether they can be taken up via the skin and on their ability to bind to proteins. Nanoparticles might also facilitate sensitisation to other substances or proteins (Alessandrini *et al*., 2006).

Studies with mice and pigs show that quantum dots that have been injected into the skin find their way to the local lymph nodes (Kim *et al*., 2004). The likely transport mechanisms is via macrophages and Langerhans cells in the skin (Ohl *et al*., 2004; Sato *et al*., 1998). This has raised questions on whether adaptation of immune response takes place after macrophages and dendritic cells, that contain nanoparticles, interact with T-lymphocytes (Oberdörster *et al*., 2005).

5.3.7 Damage to the reproductive system and foetuses

Knowledge is lacking on the ability of nanomaterials to damage the reproductive system or to affect the hormonal system.

It has not been clarified whether nanomaterials are capable of damaging the placenta or foetus. The possible capability of nanomaterials to reach the blood means that they might be transferred via the placenta to the foetus. Toxicity in foetuses was detected after the mothers had inhaled diesel exhaust (Fujimoto *et al.*, 2005). It is unclear, however, whether it is an indirect effect of inflammation in the lungs or the presence of nanoparticles in the placenta, or direct action of nanoparticles on the foetus.

5.3.8 Mutagenicity

Little is known about the genotoxic properties of nanomaterials. The *in vivo* studies that exist on nanoparticles have mostly been carried out at a high concentration and/or with long-term exposure, and they are often associated with inflammation and proliferation, which might conceal or modify the genotoxic response. It is therefore important to be cautious in interpreting and extrapolating genotoxicity data from nanoparticles, particularly where *in vitro* data are concerned.

The formation of DNA adducts has been linked to nanoparticles which are unintentionally formed in combustion, diesel exhausts or carbon black (Borm *et al*., 2004).

The formation of reactive oxygen radicals is important with regard to genotoxicity (Nel *et al*., 2006). There are studies that show that ultrafine nanoparticles can enter and affect the mitochondria (Li, *et al*., 2003) and that particles of SiO_2 can enter the cell nucleus and induce gene transcription (Chen and von Mickecz, 2005).

Both dose-dependent and time-dependent increase in DNA damage were observed in a COMET test when breast cancer cells were exposed to silica colloids or fullerenes, which are stated as being capable of leading to mutations and possible increased risk of cancer (Pacheco *et al*., (2007), presented at the annual meeting of the American Association for Cancer Research.

5.3.9 Cancer

With regard to the carcinogenic properties of particles it is very difficult to interpret the results of experimental studies with respect to dose-response relationship and the possibility of extrapolating between species (SCENIHR; 2007). Various interpretations, for instance with respect to dose-response relationships, have been made for example of the results of a study in which rats were exposed various types of particles by inhalation (fine and ultrafine particles of titanium dioxide and to carbon black) without a consensus being reached (Borm *et al.*, 2004; Morfeld *et al*., 2006).

The IARC has classified the substance titanium dioxide, which comprises the substance both at nanosize and in larger particle sizes, as a possible carcinogen in humans based on an increased number of tumours in rats exposed by inhalation or intratracheal instillation. Experiments in which animals have been exposed orally, subcutaneously or intraperitoneally did not lead to any increase in the frequency of tumours. Carbon black is also classified as a possible carcinogen to humans by the IARC. None of these substances is classified with respect to cancer under the EU classification system. In a preliminary statement the EU's Scientific Committee on Consumer Products (SCCP) mentions that it is considered necessary to re-assess risks associated with the nanoform of titanium dioxide in cosmetic products.

It is likely that non-toxic nanoparticles of low solubility which are inhaled, may lead to lung tumours in rodents through the same mechanisms as have been found for fine particles. These mechanisms include DNA damage and increase in cell proliferation and are associated with persistent inflammation in the lung. It remains unclear what leads to this response, but from the toxicological perspective, there is greatest support for the surface.

As surface reactivity also affects inflammation (Duffin *et al*., 2002), the ability of particles to lead to chronic inflammation and fibrosis, and thus to be carcinogenic, is considered to depend on the surface area of the particle and its reactivity. This is important with regard to manufactured nanoparticles, which have a large surface area per unit of mass, and the potential to have a reactive surface. The greater surface area of nanoparticles might therefore mean that the nanoparticle have a greater ability to lead to cancer than larger particles. It has also been found that chronic toxicity correlates better with the surface area of particles and fibres than their mass (Driscoll, 1996; Tran 2000).

In studies of ultrafine particles of carbon black and titanium dioxide it was shown that the lung is overloaded at a far lower level than previously thought (Oberdörster, 1996).

No increase in tumours outside the lung has been observed in inhalation studies.

5.3.10 Other toxicity

Interaction between nanoparticles and biomolecules and microorganisms is an area that is being increasingly expanded, but there has been no research at all on how nanoparticles of metals can interact with viruses. A paper by Elechiguerra and colleagues (2005) reported that interaction between silver nanoparticles and HIV-1 was size-dependent, and only particles between 1-10 nm were attached to the virus. The underlying mechanism proposed is that the silver nanoparticles bind to a glycoprotein and thus prevent the HIV-1 virus from binding to the host cells.

6. METHODS OF MEASUREMENT, TEST METHODS AND RISK ASSESSMENT

It is unclear today whether existing test methods are also applicable to nanomaterials. To ensure that what is tested and how it is tested is also applicable to nanomaterials, existing test methods should therefore be evaluated. Certain existing test methods may be relevant while others should be adapted so that they can also apply to nanomaterials. In addition there may be a need to develop completely new test methods.

Owing to a lack of data it is not possible at present to make satisfactory risk assessments with regard to manufactured substances at nanoscale. As well as fundamental knowledge of physico-chemical, toxicological and ecotoxicological properties it is important, among other things, to take account of the unique physico-chemical properties and new biological activity of nanomaterials, new target organs and the routes by which they can enter the body and be dispersed in the environment.

Account should also be taken of sensitive groups such as people with chronic diseases related to pulmonary impairment, the cardiovascular system and the immune system. These groups appear to be particularly susceptible with regard to exposure to nanoparticles, and account must be taken of this in a risk assessment.

A problem that necessitates caution is that detecting nanomaterials in the environment will probably pose great technical difficulties.

6.1 Measuring and sampling methods

6.1.1 Methods for characterisation of nanomaterials

A number of different parameters can be used to characterise the nanomaterials themselves. Examples are chemical composition, crystal structure, particle size and size distribution, specific surface area and surface charge. A number of instruments and methods used for larger particles can also be used for nanomaterials. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are the most common methods used to characterise the actual manufactured products. These methods can measure and visualise size and shape in structures down to ~0.1 nm or a few nanometres in size. Like all microscopy techniques, however, they are based on analysis of individual nanoparticles, so that an analysis of many thousand particles is required in order to be able to measure a size distribution with these microscopy techniques. In order to be able to measure the same particles in an environmental sample with a high background of natural nanoparticles, on the other hand, screening of millions of particles may perhaps be required to obtain statistically acceptable measurement of the synthetic nanoparticles. TEM and SEM are therefore chiefly suitable for the characterisation of starting materials rather than in environmental samples or in toxicological experiments.

The size distribution of airborne nanoparticles can be measured by what is known as impactors (see below). The size distribution of nanoparticles in emulsions or in solutions can be measured by light scattering. X-ray diffraction is used to characterise crystal structures and BET (Brunauer, Emmet, Teller) surface adsorption to measure specific surface area. Atomic absorption spectrometry (AAS) and mass spectrometry (ICP-MS) can be used to measure chemical composition. It should be mentioned that the latter methods only can be used on larger quantities of nanoparticles and thus provide a mean value of all the particles in the sample. Field-Flow Fractionation linked to ICP-MS is a more recent method that analyses the elemental composition specifically in the nanoparticles as a function of size. Electron diffraction and electron spectroscopy can be used in combination with TEM to characterise the crystal structure and elemental composition of individual nanoparticles. There is a lack of methods today to measure the chemical (molecular) composition of individual nanoparticles, with regard to both the total composition of nanoparticles and their composition on the surface (which may be assumed to be of great relevance to toxicological effects).

With regard to methods of measurement for the materials it is important that methods that are relevant to risk assessments are also developed. Adams *et al*. (2006) made use of commercially available nanoparticles of titanium dioxide, silicon dioxide and zinc oxide for ecotoxicological tests but did not succeed in creating suspensions that contained particles at nanoscale from these materials. If the measured size is greater than indicated, there may be several different reasons: the properties of the material may change in storage or it may be that the method used to sample and separate the particles prior to measurement does not work. It may also happen that the material already clumps together when suspensions of the material are to be created. In some cases smaller particles than indicated have been measured, which is difficult to explain other than that the material does not comply with the manufacturer's own specification. In order to be able to make predictions about effects based on properties of the materials and for it to be possible for toxicological and ecotoxicological test methods to provide satisfactory results, it is important that knowledge of relevant exposure is fed into work on standardising test methods for materials.

6.1.2 Methods for sampling and measurements of exposure

Methods for sampling and analysis are needed in several different contexts. In testing the effects of a nanomaterial there is a need to measure the actual exposure so that it is possible for example to establish a dose-response relationship. There is also a need to measure exposure in the working environment and in the external environment. There are certain ways of obtaining a measurement of levels of exposure in the air in the working environment at present, at least expressed as the number of particles, while major technical challenges remain, and it may therefore take some time before specific nanomaterials in the external environment can be collected and analysed.

There are a number of different sampling methods for particles in air. Most have been used to collect larger particles in the micrometre range, known as PM_{10} and $PM_{2.5}$, which means particulate matter smaller than 10 and 2.5 µm respectively. There are also methods today for the collection of nanoparticles in air, a size fraction which is usually referred to in these contexts as ultrafine particles and means particles smaller than 150 nm. Techniques that can be used are multi-stage impactors and DMAs (differential mobility analysers). Impactors collect the particles for further analysis, while DMA calculates the number of particles in different size fractions (McMurry, 2000). The background presence of nanoparticles from natural sources and human sources such as road traffic can also pose a problem in the working environment in measuring exposure to nanoparticles by known techniques (DEFRA, 2006).

Methods of analysing exposure to nanoparticles in air and water have been highlighted as two of the five greatest challenges to safe treatment of nanotechnology in the future (Maynard *et al*., 2006). Methods of measurement for nanomaterials are also discussed in papers by Maynard and Aitken (2007) and the US EPA, (2007). Methods specifically designated for air are personal particle counters or sensors that measure potential for oxidative stress (Maynard *et al*., 2006).

There are ultrafiltration techniques to collect very small size fractions for sampling in water.

When nanoparticles reach the environment, they will mix with particles of other origin, and it is reasonable to assume that in many cases it will be difficult to separate the particles to be analysed. Isolating and concentrating the particles intended to be analysed for example with respect to size distribution and molecular composition is associated with great technical difficulties. There is consequently a need to develop analytical methods with the capacity to screen a very large number of particles as well as selective detection. Various fractionation methods (impactors in air and ultrafiltration and Field-Flow Fractionation in water) linked to selective spectroscopic methods may be a route to follow.

Tracing with the aid of stable isotopes has been proposed for a number of metal-based nanomaterials as a complementary method to study exposure in the environment (Gulson and Wong, 2006). This could be applied to substances that have natural variation in isotope composition (due to natural occurrence of a radioactive mother isotope) and where the raw material for the substance at nanoscale would therefore have a different isotope composition. Another option is intentional production of nanomaterials with an enriched composition of stable isotopes as a test material for exposure and effect studies. Methodology based on stable isotopes necessitates the subsequent analysis being done by mass-spectrometric methods such as ICP-MS.

6.2 Test methods for health and environmental hazard

Access to test methods is of key significance to gain knowledge of the properties of chemical substances hazardous to health and the environment, in order to be able to make a risk

assessment and be able to devise classification criteria. This work is largely undertaken at international level. Swedish work on test methods is principally focused on the OECD, as the OECD guidelines for testing are globally harmonised and because the principle of "Mutual Acceptance of Data (MAD") covers data obtained using the OECD test methods. This makes international harmonisation possible in the assessment of chemicals, limits repetition of animal experiments and promotes free movement of goods in the EU and globally. The OECD test methods are incorporated, essentially unchanged, into EU regulatory framework[1].

Many gaps in knowledge must be plugged before it will be possible to decide to what extent existing test methods can be used to test nanomaterials, whether it is sufficient to modify existing ones and to what extent new methods need to be developed.

It is clear that the traditionally used units to express dose/concentration-response/effect relationships based for example on mass per unit of volume are not sufficient. Additional account needs to be taken of surface area and/or the number of particles per unit of volume (SCENHIR, 2007). Other properties of the particles may need to be considered, such as morphology. Dosimetry is probably the most important area where present-day test methods need to be updated.

When physico-chemical test methods are developed it is particularly important that they also take account of the physico-chemical properties that are significant for exposure and biological effects.

At a workshop earlier this year arranged by the Royal Society with participants from the research community and the OECD working party for nanomaterials it was proposed that the OECD can play an important role by coordinating the research on health and environmental risks with manufactured nanomaterials. This is to ensure international exchange of knowledge and avoid unnecessary duplication of effort.

A Working Party has been set up within the OECD to assist the OECD chemicals committee. Its work covers the description, identification and definitions of nanomaterials, the need for and development of guidance documents for test methods and risk assessment, and dissemination of information and knowledge on nanomaterials. Working groups have been set up to cover these different areas and drive the work forward.

The work has been divided into a number of working groups. Three of these are of particular importance to enable suitable test methods to be identified/developed:

- EHS Research Strategies on Manufactured Nanomaterials (SG2);
- Safety Testing of a Representative Set of Manufactured Nanomaterials (SG3);
- Manufactured Nanomaterials and Test Guidelines (SG4).

In order to be able to assess which test methods are suitable for nanomaterials very similar strategies are gradually being proposed by the OECD and in several national plans (for example in the United States, the UK). The following are identified, or devised within the OECD:

1. The most important physico-chemical properties relevant to nanomaterials (e.g. surface area, surface reactivity, particle size, shape), the areas of effect that are particularly relevant to nanomaterials (e.g. inhalatory toxicity, aquatic toxicity, cardiovascular effects) and other relevant criteria such as likely exposure routes and type of exposure as well as frequency;

[1] Annex V to 67/548/EEC

2. The need for updating of existing test methods or new test methods for the assessment of physico-chemical properties;
3. Test methods from other sources (non-OECD) which are used or under development and could be recast as OECD test methods or guidance documents or complement the OECD test methods. Cooperation is taking place here for instance with the International Standardisation Organisation (ISO).
4. Present-day test methods to assess health and environmental effects on the basis of the criteria developed in the above stages. The methods are to be reviewed in particular on the basis of whether they can be used to assess possible new effects relevant to nanomaterials such as inflammation and cardiovascular effects.
5. Guidance documents are to be developed to indicate how test materials are to be prepared and dosed in *in vivo/in vitro* studies to examine health and environmental effects and in studies to study dispersion in the environment (fate and behaviour).

   New, and probably complex, processes must be taken into account when test methods are developed for these materials. An important aspect may be the relevance of the test results. An example of this is that the method used to dissolve, or actually create a suspension of, spherical fullerenes for exposure has been found to be of decisive significance for the size, morphology, charge and hydrophobicity of the fullerenes (Brant *et al*., 2006). The expected toxicity is thus also markedly affected, as has been shown in toxicity studies (Zhu *et al*., 2006; Dhawan *et al*., 2006).
6. Other test methods *(in vivo/in vitro)* that can be used either in combination with present-day OECD test methods or in a test strategy.

   In vitro studies on tissues that can be predicted to be exposed first might be usable to determine mechanisms of action (cell toxicity/cell death, inflammation, oxidative stress and effects on the circulatory system, for example modulation of blood clotting, activation of complements). *In vitro* methods may also be usable to measure permeability for example via gastric mucosa, the respiratory tract etc.

Several review articles that have examined the usability of *in-vitro* test methods to assess preliminary effects of nanomaterials have come to the conclusion these are usable (NRCG, 2006). It has not been considered possible to use any method alone, except possibly for mutagenicity. Obtaining *in-vitro* test methods that are validated is, however, a great problem.

6.3 Risk assessment

The principal reason for manufacturing a substance at nanoscale is to devise new, unique properties. There is reason to suspect that these materials are also unique with regard to the way in which they are taken up, distributed and disappear from the body or nature, or with regard to their toxicological properties. It is therefore important to take account of new biological activity, new target organs and what routes nanomaterials can follow into the body, or transport routes in the environment.

New toxicological data on the substance at nanoscale might result in a completely different assessment of the risk posed by the substance than if the assessment was based on the toxicological properties of the substance in macroform or as a bulk chemical. To clarify that the toxicological profile of the substance at nanoscale is not necessarily the same as for the material at larger scale, manufactured substances at nanoscale could be allocated some form of unique identification. Some authorities or organisations have nevertheless chosen to regard manufactured nanomaterials as an ordinary substance that does not differ from the

substance/material at larger size. This might mean that they are regulated as a variant of a substance and that the risk assessment is largely based on knowledge about the substance at macro size, or that special and complementary information is required for “nanovariants” and their use so that these too are covered by the description/hazard/risk assessment of the parent substance.

The fact that all individuals in the population do not respond in the same way to exposure to particles should be considered when assessing the risks of nanomaterials. Account should for example be taken of sensitive groups such as children, the elderly or people with chronic diseases related to pulmonary impairment, the cardiovascular system and the immune system.

Nanotechnological products already occur in areas such as cosmetics, automotive tyres, textiles, fuel cells, optics and electronics, and it can be assumed that their use will increase sharply over the next few years. Although the attention paid to nanoengineering is not yet so great, it is advisable to examine and tackle risks to health and the environment before their dispersion becomes too great. For manufacturers of nanomaterials it will be important to show that their products have a low risk profile. If these preventive measures are not taken, the public and authorities may regard nanomaterials as hazardous. This might lead to unnecessarily heavy regulation and hamper the use of nanomaterials.

Environmental risk assessment at present lacks adequate methods to estimate levels of exposure to substances in particle form in the environment. There is adequate knowledge in other disciplines to start the development of an integrated model for particle substances. As the mechanisms may be more complex than for the substances in molecular form and the applicability of the models from adjacent disciplines is not well known, the interval of uncertainty in the results will be considerably greater than today. With the approach applied in risk assessments, this should mean that the assessments are conservative and will more often indicate risk than they would need to do if knowledge was better.

A problem that calls for caution is that it will probably be difficult to detect nanomaterials in the environment. It has been retrospectively possible to find persistent and bioaccumulative substances that occur in molecular form in the environment and to deal with them afterwards. Examples of this are classical environmental toxins such as PCBs and brominated flame retardants. If certain forms of nanomaterials accumulate in the environment and exert a toxic action on organisms the possibility of detecting that these particular forms are the cause of the problem will probably be smaller than in the case of the substances in molecular form (see also 6.1.1 Methods of characterising nanomaterials).

Increased knowledge of health and environmental risks and enhanced handling of risk assessment in the regulatory systems might also lead to improved handling of substances which today are already manufactured in and occur at nanoscale and are in widespread use, such as carbon black in automotive tyres and fumed silica for concrete production.

7. HANDLING OF NANOMATERIALS IN DIFFERENT LEGISLATIONS

The use of nanomaterials is broad and therefore concerns many different areas of legislation, such as chemicals, biocides, plant production products, cosmetics, pharmaceuticals and foods. In all areas, nanomaterials are covered by applicable legislation for the area in which they are used but nothing is mentioned specifically about nanomaterials in the actual wording of the legislation.

With regard to the classification and labelling of health and environmental hazard it is already possible to classify nanomaterials based on available test methods and classification criteria today, although there is no specific identification for the substance at nanoscale. On the other hand, there is uncertainty on the validity of negative results in tests.

It may become necessary to develop the legislation for nanomaterials when more knowledge is obtained. It will also become important for guidance documents to describe in what cases there is a need to adapt, change or expand the information requirements as it is possible that the tests that exist today do not work or do not provide relevant results. Differing views have been put forward on whether existing materials at nanoscale should be treated as new unique substances or as an application of already existing substances in the new chemicals legislation, Reach, and this may have a great impact on the assessment of chemical safety.

In addition, many nanomaterials will presumably be produced or imported in low volumes, that is to say less than 10 tonnes per year, which means that the information requirements that exist in Reach are low and alternative information requirements should be considered. If the substance is manufactured or imported in quantities of less than 1 tonne, no registration is required at all. Nor are the nanomaterials covered by the rules on dangerous substances in articles if they are present in concentrations of less than 0.1%, even if they would have very dangerous properties.

7.1 Existing technology

7.1.1 Swedish chemicals legislation

Relevant Swedish legislation includes Chapters 2 and 14 of the Environmental Code, that is to say the rules on consideration contained in Chapter 2 and rules on chemical products and biotechnical organisms contained in Chapter 14. Chapter 15, which relates to waste, may also be relevant in the context when chemical products and articles containing chemical products become waste.

According to the definition given in Chapter 14, the term “chemical products” means substances and preparations of chemical products. This implies that since chemical substances and preparations at nanoscale are chemical products, even if the product has different properties, nanomaterials should also be covered by Chapter 14. Chapter 14, like all the other provisions of the Code relating to chemical products, according to Chapter 14(1) second paragraph can also be made applicable to articles.

Chapter 14 (in Section 7) contains an obligation on manufacturers and importers of chemical products to ensure that there is a satisfactory investigation for the damage to health and the environment that the product may cause to be assessed. Section 8 contains requirements for product information, which means that anyone who manufactures or imports a chemical product has to provide the information required for the protection of human health or the environment by labelling or in some other way. This information rule is also applicable to articles, but without specific regulation, under the second paragraph of Section 3 of the Chemical Products and Biotechnical Organisms Ordinance (1998:941).

Chapter 2 contains an example of how the precautionary principle can be worded, which in principle means that precautions must be taken as soon as there is cause to assume that an activity or measure may cause damage or detriment to human health or the environment. The precautionary principle essentially means that full proof for example that something is dangerous from the health or environmental point of view is not needed before a measure can be taken (for example prohibition), and that it is sufficient for science to be well on the way to

identifying the risks, cf. the expression “cause to assume”. The precautionary principle is thus a kind of alleviation of the burden of proof rule. Where there are great gaps in knowledge for nanomaterials, the precautionary principle could therefore be a way of preserving a high level of protection in the individual case. It is important to state in general precisely where the boundaries lie. This must be examined in each individual case. There are also other formulations of the precautionary principle, which is also an important principle in European Community law. Article 174(2) of the Treaty Establishing the Community states that Community environmental policy has to be based on the precautionary principle. This principle is also expressed in several pieces of Community law, for example Directive 90/219/EEC on the Contained Use of Genetically Modified Micro-organisms[2] or Reach[3] (1907/2006/EC), Article 1(3), which describes aim and scope. Reference is also made with regard to the precautionary principle to the Commission Communication on the Precautionary Principle (COM (2000) 1 final), in which the Commission has stated that the precautionary principle is also applicable to harmful effects in the long term.

Another Swedish statutory instrument in which risk assessment of nanomaterials may be applicable is the Product Safety Act (2004:451), which implements the Product Safety Directive (2001/95/EC). The Product Safety Act is aimed at ensuring that articles supplied to consumers do not cause personal injury.

The Product Safety Act lies outside the scope of the Environmental Code, but is relevant to chemical products if the latter can cause harm to the health of consumers. The Act applies to both acute and longer-term harm. According to the Government Bill[4] adverse effects on health that are revealed after a long period of time are also to be considered in assessing whether an article is safe. Harm that may arise as a result of a hazardous chemical substance is mentioned in the Bill as an example of longer-term risks.

7.1.2 EU legislation for chemicals (Swedish Chemicals Agency)

In the European Community there is the directly applicable Reach Regulation (1907/2006) for chemicals. As it is a European Community Regulation it is not “transposed” into Swedish law (unlike directives and decisions) and is applicable alongside the Environmental Code. The first parts of Reach entered into force on 1 June 2007.

Reach entails expanded responsibility for those who manufacture and import chemical substances into the EU to analyse and report whether their substances pose risks and what safety measures are required. Those who use chemical substances further down the chain (known as downstream users) also have responsibility for assessing risks in their use of them.

Nanomaterials and nanoparticles are not mentioned anywhere in the legislation but are covered in the same way as other substances if they fulfil certain criteria. Aspects such as production volume and what is to be regarded as a separate substance will play a role in determining how nanomaterials are handled in Reach. It is not yet clear how nanomaterials will be handled in Reach, whether the substance at nanoscale is a separate substance due to its specific properties or whether it is be treated as an area of use of a substance.

If a substance that is produced in or imported into the EU is identified as particularly hazardous to health or the environment, it is included in a candidate list for inclusion in the authorisation procedure, which means that importers of articles containing this substance have a special duty of notification to the European Chemicals Agency (ECHA). This only applies if

[2] OJ L 117, 8.5.1990, p.1
[3] OJ L 397, 30.12.2006, p.1
[4] See Government Bill 2003/04:121 New Product Safety Act p. 96 ff

the concentration of the substance is at least 0.1% and the annual volume of the substance for the importer is at least 1 tonne. Difficulties in testing and assessing the risk of substances in nanoform which have been described in this report might lead to problems in clearly identifying them as candidates for the authorisation procedure. This, together with the requirements relating to concentrations in the article and total annual volume, may mean that chemical safety assessments in Reach in many cases will not be made for nanomaterials that reach the European market through the importing of articles. The Product Safety Act (see 7.1.1 above) also applies to imported articles.

Activities
The Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has been asked on two occasions to assess how suitable existing risk assessment methods are in the guidance documents of current chemicals legislation (SCENIHR 2006; 2007). The committee's remit included proposing improvements with regard to the risk assessment of nanomaterials. The latest report has recently been the subject of an external consultation exercise.

The report proposes a number of different measures, for instance a need for more test methods to characterise nanoparticles with regard to parameters that may be important from the toxicological and ecotoxicological points of view, and states that knowledge is needed on test methods that take account of biological processes that concern nanoparticles such as uptake in cells and toxicological mechanisms. Existing methods with regard to testing and risk assessment are regarded as particularly deficient with regard to effects on the environment.

A working group consisting of experts from the EU Member States has been formed to deal with issues linked to new and existing substances such as the development of nanomaterials and the need for guidance documents for testing and risk assessment.

7.1.3 Pesticides (Swedish Chemicals Agency)

The legislation for pesticides comprises the Plant Protection Products Directive (91/414/EEC) and the Biocidal Products Directive (98/8/EC), as well as regulations linked to these directives. Both the directives are harmonised, which means that the Member States are obliged to implement them in full. There is thus no scope for more liberal or stricter national legislation and/or implementation.

The two directives indicate in detail what is to be assessed for active substances and products, as well as the extent of the data which companies must submit when applying for authorisation to use their active substances in the products they wish to put on the market, along with authorisation to place these on the market. The data requirements for and assessment of active substances are in the main identical regardless of whether they are contained in plant protection products or biocidal products. The data to be assessed comprise the chemical and physical properties of the active substance, how the substance is converted and dispersed in nature and its toxicological and exotoxicological properties. These data underlie the assessment of risk to health and the environment in the use of a product that contains the substance concerned. Evaluation of the data which, under the appropriate directive, are required to assess an active substance, as well as assessment of the risk posed by use of this substance, are made in a joint process by the Member States of the EU. In cases concerned with a plant protection product the European Food Safety Agency (EFSA) also takes part. The product approvals are made at Member State level, but apply mutually to all Member States.

Neither of the directives mentioned above has specific data requirements for nanomaterials or products that contain nanomaterials. Nor is it stated in either of the directives that the risk in

the use of nano-formulations has to be assessed in a different way than for other products. No guidance documents have yet been drawn up for the assessment of nanomaterials in plant protection products or biocidal products.

There is no active substance at nanoscale at present that has been the subject of risk assessment or approval in the Union under either of the directives. It is likely, however, that such formulations may come into question for both plant protection products and biocides. The Commission has recently designated Sweden as 'reporting Member State' for silver as an active substance in antibacterial products, and the documentation on silver contains a formulation at nanoscale. The task of the reporting Member State is to assess the data which companies attach to their applications for approval of the substance in products in the EU on behalf of all the Member States, and to present a risk assessment and submit a recommendation to the Commission to approve or prohibit use of the substance concerned.

7.1.4 Classification and labelling of hazardousness to health and the environment

In principle, if there is information that leads to a substance being classified on the basis of information from larger particles the classification also has to apply to smaller particles, including nanoforms. Relevant examples are zinc oxide which is classified as hazardous to the environment and cadmium, which is classified as hazardous to health and the environment.

The general rule for chemicals is that if new information emerges that a substance fulfils the criteria for classification and this substance has not yet been classified, this substance has to be self-classified by the companies. An example of a substance for which this has happened is carbon black, which has been self-classified by certain companies since the IARC judged that it is a possible carcinogen for humans.

Nanomaterials are not mentioned in either the Dangerous Preparations Directive (1999/45/EEC) or in Directive 2006/121/2006 of the European Parliament and of the Council. Nor are nanomaterials mentioned in the future Global Harmonised System of Classification and Labelling of Chemicals (GHS) which is under development (UN, 2005). An important reason is that knowledge is lacking on the relevance of available test methods to nanomaterials, whether new test methods are required and if so how these should be designed.

No new classification criteria are under discussion at present, either under the GHS cooperation or in the run-up to its introduction in the EU through the new classification regulation. New criteria cannot be devised until relevant test methods are ready in the OECD and other internationally recognised organisations.

It is in principle already possible today to classify nanomaterials on the basis of available test methods and classification criteria, even if there is no unique identification number for this form. On the other hand, there is particular uncertainty over the validity of negative results in tests. Specific classification of a substance at nanoscale is possible today.

7.1.5 Medicinal products(Swedish Medical Products Agency)

All the requirements for toxicological testing are governed by ICH guidelines: ICH Safety I-VII, plus the multidisciplinary ICH M3 (preclinical in relation to timing of clinical trials (ICH, 2007). These guidelines have been jointly drawn up by authorities and industry in the United States, Japan and the EU. No specific tests for nanomaterials have yet been developed, but a Safety Working Party in the EU has just started discussing the nano area.

7.1.6 Cosmetics (Swedish Medical Products Agency)

Cosmetics legislation
Virtually all legislation on cosmetics and hygiene products has now been harmonised throughout the EU, through the Council Directive concerning Cosmetic Products (76/768/EEC) which has to be transposed into the law of all Member States.

The Cosmetics Directive is incorporated into Swedish legislation through the Swedish Medical Products Agency Regulations on Cosmetic and Hygiene Products, Control of Cosmetic and Hygiene Products LVFS 2004:12 and Prohibition of and Restrictions on the Inclusion of Certain Substances in Cosmetic or Hygiene Products (LVFS 2007:4).

The Environmental Code (1998:808) and the Cosmetic and Hygiene Products Ordinance (1993:1283) indicate the opportunities the Swedish Medical Products Agency has to legislate in this area.

Nanomaterials are not specifically regulated under cosmetics legislation at present and are instead covered by the general requirements for ingredients, safety documentation etc.

Activities in the European Commission
The Scientific Committee on Consumer Products, which covers cosmetics, has been asked to examine whether there is a need to amend the existing guidelines on safety assessment in view of development in the area of nanomaterials. In a preliminary statement, which has recently been the subject of an open consultation procedure, the Committee has weighed up existing knowledge on uptake via the skin and the toxicity of titanium dioxide and zinc oxide at nanoscale in cosmetic products, including the IARC's classification of titanium dioxide as possibly carcinogenic to humans (SCCP, 2007). The conclusion drawn is that it is necessary to re-assess the safety of use of titanium dioxide in cosmetic products.

Discussions are currently under way in the EU to revise how account should be taken of the specific properties of nanomaterials in cosmetics legislation.

7.1.7 Food (Swedish National Food Administration)

Food legislation
Virtually all legislation on food today is common throughout the EU. Sweden can only stipulate its own rules in the area of food in exceptional cases if it can be shown for example that there is a health risk.

Most of the rules on food are contained in EU regulations (list of EU regulations to which the Food Act has to apply: SFS 2006: 1032) and in National Food Administration regulations (published in LIVSFS). The EU Regulations apply directly and are not transposed into National Food Administration regulations.

The Food Act (2006:804) and the Food Ordinance (SFS 2006:813) indicates what possibilities the National Food Administration has to legislate (published in Swedish Code of Statutes, SFS).

Nanomaterials or nanosubstances are not specifically regulated in food legislation today, although substances such as titanium dioxide occur in materials in contact with foods (framework regulation 1935/2004/EC, the framework directive on plastics 2002/72/EC) or as food additives (framework directive 89/107/EEC).

Activities in the National Food Administration
The National Food Administration today monitors the area on the basis of activities nationally and internationally since the issue has not been prioritised.

Activities at the European Food Safety Agency (EFSA)
A broad presentation of nanomaterials was made to the EFSA's Advisory Forum in 2005. This has not, however, led to any visible activity. Nanomaterials have also received attention from the working group that forms part of the EFSA's panel on food additives, flavourings, processing aids and materials in contact with food.

It is also mentioned in the EFSA Management Plan 2008 that work relating to nanoparticles is due to start.

The EFSA and the University of Parma will be organising a scientific symposium on nanotechnology under the title of "Scientific Symposium on Food Safety, Nutrition and Nanotechnology" in October 2007 in Parma (EFSA, 2007).

7.1.8 Working environment (Swedish Work Environment Authority)

Working environment legislation
The Swedish Work Environment Authority has issued a number of regulations (AFS) aimed at reducing the risk of ill-health due to exposure to chemical substances in the working environment. The terms nanomaterials and nanoparticles do not appear in any of these regulations.

The regulations on chemical hazards in the working environment (AFS 2000:4) include rules on risk assessment and risk-reduction measures. Exposure to hazardous chemical substances has to be minimised. A hazardous substance primarily has to be replaced by another one which is less hazardous, if possible.

The regulations on Hygiene Limit Values and measures against air pollution (AFS 2005:17) stipulate the maximum acceptable mean concentration (time-weighted average, mg/m^3) in the inhaled air of around 400 air pollutants in the form of dust, fume, mist, gas or vapour. Limit values are specified for various types of dust as total dust, respirable dust or inhalable dust. These dust fractions are defined in the regulations, and the procedure to be followed in measurement is also stated.

Many particles in the nano range, <0.1 µm, are formed in welding, and there are requirements on monitoring air pollutant levels in the regulations on fusion welding and thermal cutting (AFS 1992:9). However, there is no "limit value for welding fume", and individual components, for example various metals, are referred to instead.

The Swedish Work Environment Authority has also issued regulations on synthetic inorganic fibres (AFS 2004:1). Fibres are defined as particles with a length-to-breadth ratio of more than 3:1. Microfibres are fibres with a maximum diameter of 1 µm. Graphic fibres are mentioned as an example of synthetic inorganic crystalline fibres. Carbon nanotubes could be another example, but are not mentioned.
The regulations on medical checks (AFS 2005:6) contain provisions on offering employees regular spirometric tests if a risk assessment has shown that there is a risk of damage to lungs and airways due to exposure for instance to dust/particulates.

Activities in the area of the working environment
The Swedish Work Environment Authority has played an active part in the work of drawing up the technical standardisation report ISO/TR 27628 (Workplace atmospheres – Ultrafine, nanoparticle and nano-structured aerosols – Inhalation exposure characterisation and assessment) and is continuing with its work on several sampling standards under ISO/TC 146/SC 2 (Air quality; Workplace atmospheres) and CEN/TC 137 (Assessment of workplace exposure). No active work on health effects is in progress at present.

The National Institute for Occupational Safety and Health (NIOSH) in the United States has recently produced a report, Progress Toward Safe Nanotechnology in the Workplace, which can be downloaded from the NIOSH website (http://www.cdc.gov/niosh/docs/2007-123/).

7.1.9 External environment (Swedish Environmental Protection Agency)

The Government issued an ordinance (1998:897) on environmental quality standards in 1998. The substances regulated were nitrogen dioxide, sulphur dioxide and lead. This decision was based on a proposal from the Swedish Environmental Protection Agency (Environmental quality standards – a new tool in environmental policy, Swedish EPA report 4793) and meant that Sweden implemented the greater part of the 1st daughter directive (Directive 99/30/EC) of the EU framework directive on air quality (Directive 96/62/EC).

In the spring of 2001 the Government also took decisions on environmental quality standards for airborne particles less than 10 µm in diameter (PM_{10}) and nitrogen oxides in outdoor air. The Environmental Quality Standards Ordinance (1998:897) was then replaced by the Environmental Quality Standards for Outdoor Air Ordinance (2001:527). There is no regulation of the presence of nanoparticles in air. The Environmental Quality Standards for Outdoor Air Ordinance (2001:527) includes environmental quality standards for airborne particles less than 10 µm in diameter (PM_{10}).

Waste legislation is another area of law that may affect nanomaterials. The EU is revising its framework directive on waste. The primary aim is to make the provisions clearer and simpler. The framework directive provides the basis for Swedish regulations on waste.

7.2 Handling of nanomaterials in legislation, with examples from Reach

Reach does not explicitly deal with the possibility of there being several different forms of a single substance. With regard to nanomaterials that may be regarded as a new form of a substance that already exists (e.g. TiO_2) the problem arises on what form of the substance the information requirements are to apply to. Guidance documents specifically aimed at substances at nanoscale that come under Reach could fulfil an important function here. Further requirements in the guidance on Reach are changed or expanded information requirements as it is possible that the tests that exist today do not work or do not provide relevant results. For comparison it could be mentioned that safety data sheets for the bulk material are used for most nanomaterials in the United States (Colvin, 2003). Additions to and further guidance on Reach must reflect the work of developing and updating test methods for nanomaterials that is being done in OECD.

New substance or different forms of a substance

The differentiated information requirements in Reach based on manufactured or imported volume represent a rough tool for prioritisation, for instance with respect to risk. Depending on the volume in which the nanomaterial is produced, there are different consequences for how the substances are treated in Reach. Aspects such as volume of production and what is to be regarded as a separate substance will therefore play a role in how nanomaterials are handled in Reach. There is still insufficient information for it to be possible to generalise and categorise in the same way as for the substances in molecular form or to establish whether new information requirements should be considered for nanomaterials.

Various views have been put forward on whether existing chemicals at nanoscale should be treated as new or existing substances.

In 2004 the UK Royal Society and the Royal Academy of Engineering published a report which said that as harmful effects of nanomaterials cannot be predicted on the basis of knowledge of the substance at larger scale, substances in the form of nanoparticles or nanotubes should be treated as new substances under the Notification of New Substances and in Reach. (Royal Society, 2004).

The European Parliament also considers that nanoforms of existing materials should be treated as new substances, because of their unique properties. “In particular, the question of whether the threshold levels for production and import laid down within that framework are also adequate for nanoparticles should be investigated.” (European Parliament, 2006).

The Commission’s position was expressed ahead of a meeting of authorities affected by the chemicals legislation then existing: it was stated that the chemicals legislation differentiates between new and existing substances. A nanomaterial was to be regarded as an existing substance if the substance from which it originates or is created from is listed in EINECS (the list of existing substances). If it is not listed in EINECS it is to be regarded as a new substance and is covered by legislation on new substances.

If a nanomaterial is regarded as a new substance, the information requirements in Reach will be based on this material. However, the volumes are assumed to be low for nanomaterials in most cases, which means low information requirements. If the nanomaterial is regarded instead as an application of an existing substance, the total volume, and indirectly the information requirements, will be greater. Guidance documents for how information is to be produced for different variants becomes important here so that the risk assessment is not based solely on information concerning the bulk chemical.

A decision to regard all substances at nanoscale as unique substances in Reach might also mean that they are no longer counted among the “phase-in substances”. These nanomaterials would not then be covered by the timetable for registration that exists for phase-in substances, and registration would instead have to take place before the substance can be placed on the market. Such a decision would probably need to be supplemented by transitional rules for phase-in substances, or a notified substance under the Dangerous Substances Directive (67/548/EEC), if it occurs at nanoscale.

Need for tools for prediction of effects on health and the environment

For substances in volumes of production of 1-10 tonnes, if nanomaterials are regarded as new substances, information is required from the whole of Annex VII in accordance with Article 12 in Reach. If, on the other hand, a nanomaterial is regarded as a variant of an existing substance, Annex III can decide, i.e. any further testing only has to be done if it is assumed that the substance will fulfil the criteria to be classified in accordance with Directive 67/548/EEC. This means that there must be some tool, on the basis of physico-chemical properties or other knowledge of the particle, to make predictions about their effects on health and the environment so that an assessment of chemical safety can be made. This may have the consequence for substances in volumes of production between 1 and 10 tonnes that there will be fewer fulfilments of information requirements in accordance with Annex VII for as long as no such tools exist. Among substances with higher volumes of production it partially has the opposite effect as there will probably be fewer categories and quantitative structure-activity relationships to use in order to abandon testing.

Different ways of looking at substances

Nanotechnology should not entail any new problems with regard to defining substances. It should be possible also to adapt the procedures used to date to define substances for nanomaterials. Three different ways of looking at substances stand out with regard to the principal groups of nanomaterials:

– Substances such as titanium dioxide, silicon dioxide and zinc oxide at nanoscale, which are often what are referred to as top-down substances that are ground down to nanoscale, are to be regarded as forms of the same substances in the same way as different size fractions of these substances are already to be regarded today as the same substance.

– Materials such as carbon black, spherical fullerenes and carbon nanotubes consist of elemental carbon but have molecular structures that are unique and the manufacturing process for the particular use differs from other forms of carbon, which means that they can be regarded as unique substances. The precise division should be decided by what is practically possible and defined in guidance documents on Reach..

– Quantum dots which consist of condensed metals and other “bottom-up” particles require a special approach. As these particles have unique properties due to the combination of two or more elements, they could be regarded as unique substances.

More complex nanoparticles could be likened to macromolecules, for example purified proteins which are also regarded as separate substances. The particular properties of combinations of metals have also been emphasised previously with regard to alloys. Information on the metals contained must, however, be included in the assessment of these unique substances.

8. OTHER COUNTRIES

Many countries commit large resources to the research and development of nanotechnology. In addition to this, important international cooperation has started, for example in the OECD, to prevent duplication and speed up the process of getting more information.

There is an awareness in many cases that account should be taken of safety for health and the environment at an early stage in the development of this area of technology, for example in the EU's Seventh Framework Programme for research and technological development but also in individual countries around the world. Several countries have, for example, drawn up strategies for the development of nanotechnology that contain challenges regarding effects on health and the environment, and some have developed voluntary reporting systems for industry.

Descriptions of activities relating to nanotechnology and risks to humans and the environment follow below. The descriptions are examples and are not claimed to be exhaustive.

8.1 EU level

8.1.1 COM

In March 2004 the Directorate General for Health and Consumer Protection held a workshop on the risks associated with nanotechnology, and a preliminary risk analysis was produced. In May of the same year the Commission adopted a strategy for nanotechnology in which a strengthening of the EU’s position with regard to research and development in nanoscience and nanotechnology was proposed (COM, 2004). This strategy emphasises the environmental and health risks associated with nanoparticles as an important area to tackle.

In June 2005 the Commission presented an action plan for nanoscience and nanotechnology 2005-2009 (COM, 2005). The plan proposes a doubling of the grants for nano-research with

increases in particular for interdisciplinary research and development. With regard to environmental and health risks collaboration on toxicological research on nanoparticles is to be encouraged, as well as the development of appropriate methods. The Commission also stresses that all use of nanotechnology must comply with the high level of protection that applies in the Union and that risks assessments are to be made at all stages in the product's life cycle.

The Nanologue project (www.nanologue.net) started in March 2005. This project, which has now been completed, was financed by the Commission and was intended to promote an open and honest dialogue between researchers, businesses and civil society on the benefits and risks of nanotechnology in order to avoid an overheated debate on the risks of nanotechnology similar to the one on genetic engineering and genetically modified organisms.

The Commission also has a website containing information on activities and projects related to nanotechnology (COM, 2007).

Effects on health, safety and the environment have been identified as a special area of research in Theme 4, Nanosciences, Nanotechnologies, Materials and New Production Technologies in the EU's Seventh Framework Programme (COM, 2007).

Nanotechnology has been identified as a cross-thematic approach in the Seventh Framework Programme. This means that particular attention is paid to research activities that contribute to the aims and expectations in other prioritised areas. In the research theme of Environment (Theme 6) and Health (Theme 1), however, no areas for general knowledge on risks associated with nanotechnology are identified. In the area of health, only toxicology for nanoparticles used in medical diagnostics is prioritised. With regard to the theme of environment no risks associated with nanoparticles are identified at all.

8.1.2 United Kingdom

The Royal Society and the Royal Academy of Engineering in 2004 published a report that discussed the opportunities and risks presented by nanotechnology. In accordance with the UK Government's response to the report, a group (Nanotechnology Research Co-ordination Group) was set up, consisting of individuals from several different authorities to coordinate research needs with regard to risks to health and the environment caused by nanomaterials. A decision was taken to restrict the group's work to dealing with engineered nanomaterials that are of nanosize in at least two dimensions.

In its first report, the group presented a programme of nineteen research objectives which describe possible risks posed by nanotechnology (Nanotechnology Research Co-ordination Group, 2005). The research objectives encompass metrology, characterisation, standardisation and reference materials, exposures, human health hazard and environmental hazard including risk assessment, as well as social and economic dimensions. The report also discusses activities in progress and funding mechanisms that cover these objectives.

One year later the group published a follow-up report which among other things notes that it is not possible for all the gaps in knowledge identified to be plugged by the United Kingdom itself (Nanotechnology Research Co-ordination Group, 2006). The report instead encourages cooperation at international level so that the gaps in knowledge that have been identified can be plugged more quickly and better.

A report was recently published by the Council for Science and Technology which reviewed what has been done in this area in the UK (Council for Science and Technology, 2007). It is also noted that although progress has been made in a number of areas not enough has been done to plug the gaps identified in the report by the Royal Society and the Royal Academy of

Engineering. A research programme to deal with any risks to human health and the environment has not been formed sufficiently quickly, for instance. An introductory comment on the report from the Royal Society and the Royal Academy of Engineering welcomes the review and shares the concern expressed in the report (Royal Society and Royal Academy of Engineering, 2007).

8.1.3 Germany

German authorities have published a national research strategy with 25 prioritised areas of research (Nanotechnology: Health and Environmental Risks of Nanoparticles – Research strategy, BAuA, BfR, UBA, draft August 2006). This strategy identified the need for toxicological test strategies and in particular the link to relevant physico-chemical test methods. Other important areas that were identified were measuring methods for all imaginable matrices linked to risk assessments (everything from food to air and sediment).

The German action plan (Nano-initiative—Action Plan 2010) consists of a number of proposals for measures to meet the challenges that may conceivably arise when attempts are made to exploit the advantages of nanotechnology (Federal Minister of Education and Research, 2007). An important element of the action plan is to behave in a responsible manner. This includes developing research strategy and studying effects on human health and the environment.

8.1.4 Nordic countries

With the objective of Denmark being among the world leaders in developing nanotechnology for industrial use in 2020, the Danish Ministry of Science drew up a nanotechnology action plan in 2004 (Videnskabsministeriet, 2004). This action plan contains proposals for priority areas and focuses principally on research and development, but it is mentioned as a secondary objective that attention should be paid to the hazard to human health and the environment, as well as ethical considerations.

The Danish Board of Technology has commissioned a report that discusses legislation on nanotechnological products from the environmental and health points of view (Teknologirådet, 2006). The report puts forward a number of recommendations, for instance that the legislation needs to be expanded and that a special research programme should be established.

NanoDTU (2007) is an interdisciplinary centre for nanotechnology in which research groups from 11 institutes in Denmark are taking part. More than 200 research scientists at the Technical University of Denmark are involved in the nine research themes of NanoDTU, which also encompass environmental risks. The Danish National Research Centre for the Working Environment has prioritised nanoparticles as a strategic research area in research on the working environment (http://www.arbejdsmiljoforskningdk/forskningsresultater/Nye%teknologier.aspx)

The Finnish Academy of Science and Letters has started a research programme (FinNano) to promote the development of nanotechnology nationally. The objectives of the programme also include promoting responsible development of nanotechnology which includes safety, health and the environment.

8.2 International level

8.2.1 United States

The United States has long been a world leader in the area of nanotechnology, but only a small part of its effort has been devoted to studying possible risks posed by nanotechnology.

In 2001 the National Nanotechnology Initiative (NNI) was established by the Clinton Administration to coordinate nanotechnological research and development between federal agencies (NNI, 2007). At present there are twenty-five agencies in the initiative, of which 13 have their own budget relating to nanotechnology. The NNI supports research and development in a broad area, from research and development of technology, instrumentation, measurement and standards to research on risks to health and the environment. Investments in nanotechnology-related activities coordinated by the NNI have increased from 464 million tonnes in 2001 to 1.3 billion dollars in 2006. An increase to 1.5 billion dollars is proposed in the 2008 budget. In addition, there is 2 billion dollars invested annually by other sectors such as states, academia and private industry (NNI, 2007).

The US Environmental Protection Agency recently published a white paper on nanotechnology (US EPA, 2007). One of the aims was to identify research needs linked to nanotechnology and to communicate scientific issues to stakeholders and the general public. The US EPA plays an active part in work on the development and evaluation of nanotechnology in the United States. These activities include active participation in NNI, cooperation with international research scientists, carrying out regional nanotechnological research, developing voluntary programmes for the collection of information from industry, studying the use of nanomaterials and examining problems relating to information and analysis. The US EPA is also developing a programme for chemicals at nanoscale as a complement and support for the programmes for new and existing substances under the Toxic Substances Act (TSCA).

The National Institute for Occupational Safety and Health (NIOSH), a leading authority in several high-priority areas of nanotechnology, has compiled a report on development towards safe nanotechnology at the workplace (NIOSH, 2007). There is a research centre (Nanotechnology Research Center, NTRC), with a limited budget of 4.6 million dollars for 2007, which is to investigate whether nanomaterials pose a risk of work-related injuries, conduct research on how injuries can be prevented and improve safety and health at workplaces through national and international cooperation on research and guidance.

Note that these figures do not include defence-related expenditure, nor a few other areas, which at present are estimated to account for around a third of the federal budget for nanotechnology.

8.2.2 Other countries, particularly in Asia

In 2001 Japan made nanotechnology one of its high-priority areas of research. Funding rose dramatically from 400 million dollars in 2001 to around 800 million dollars in 2003, and was expected to rise by a further 20% by 2004.

South Korea has initiated an ambitious ten-year programme with around two billion dollars in public funding, while Taiwan has earmarked around 600 million dollars for public funding in six years.

China is putting ever greater resources into nanotechnology, which is particularly significant in view of Chinese purchasing power. China’s share of research articles published around the

world has increased rapidly, with a rate of increase of 200% at the end of the 20th century. It is now starting to catch up with the EU and the United States.

The Russian Federation is well established in nanotechnology, and the same applies to several of the newly independent states. Several other regions and countries are showing increasing interest in nanotechnology, including Australia, Canada, India, Israel, Latin America, Malaysia, New Zealand, the Philippines, Singapore, South Africa and Thailand.

However, it is unclear at present what proportion of these initiatives is concerned with the risks posed by nanotechnology.

8.2.3 OECD

A special session on the health and environmental aspects of nanomaterials was held ahead of the OECD Joint Meeting in June 2005. Many people at the meeting felt that in addition to the great effort being made to develop nanotechnology there is a need for research on possible risks, to prevent possible risks or alarms which would be a setback for the technology. A workshop was then held in December 2005 under the auspices of the OECD to identify areas which are important to develop with regard to risks posed by nanotechnology.

These two activities led to the formation of a working party to assist the OECD's Chemicals Committee. The Working Party's activity includes building up a database of scientific studies on risks posed by nanomaterials, description, identification and definitions of nanomaterials, development of guidance documents for test methods, risk assessment of representative nanomaterials, coordination of voluntary reporting programmes for industry on manufacturing and the dissemination of information and knowledge on nanomaterials. Working groups have been formed within the Working Party to cover these different areas and drive the work forward.

8.2.4 Other aspects

Environmental movements around the world have warned that nanomaterials might have serious effects on human health and the environment, and have therefore demanded a ban on manufacturing until more knowledge has been acquired (Greenpeace, 2003; ICTA, 2006; FOE, 2006).

In a cooperation project between industry and environmental movements a proposal has been drawn up to identify and tackle any risks to health and the environment from nanomaterials (Environmental defense-DuPont Nano partnership, 2007).

In 2005 industry formed the "Nanoparticle Occupational Safety and Health Consortium" (2007) to study occupational health and safety problems related to nanoparticles in aerosol form and measurements of exposure at workplaces. The consortium is led by DuPont with more than 14 participants from industry, academia and governmental agencies.

8.3 Standardisation

With regard to standardisation work the European standisation organisation CEN (http://www.cen.eu/cenorm/index.htm) devised a strategy in the spring of 2005 for European standardisation in the area of nanotechnology. Future work will probably be structured in four working groups:

- Terminology, classification and nomenclature
- Metrology and characterisation
- Health, safety the environment

– Process and product standards

The International Organisation for Standardisation (ISO) (http://www.iso.org/iso/en/ISOOnline.frontpage) is examining interest in starting standardisation in the area of nanotechnology. It is likely that standards for terminology and vocabulary will primarily be drawn up, but in the longer term there may also be standards for health and environmental risk assessment. A British Standards document on vocabulary (BSI PAS 71:2005) has recently been elevated to a technical specification at ISO level (ISO/TS 27 687).

A new area of work that has been approved in ISO is “Nanotechnologies - Endotoxin Test on Nanomaterial Samples for In Vitro Systems”, which means that at present there are two areas of work in ISO.

The Swedish Standards Institute (SIS) (http://www.sis.se/DefaultMain.aspx) plans to set up projects through which Swedish stakeholders could influence European and international standardisation work. A national network has also been formed.

9 COMPILATION OF GAPS IN KNOWLEDGE

Knowledge on the risks posed by nanotechnology to health and the environment is generally limited, and the existing data is of variable quality. There is therefore a great need for more knowledge on the risks of nanotechnology in order to be able to attain the environmental objective of A Non-Toxic Environment.

This lack of knowledge also means that it is difficult at present to inform and communicate on the risks of nanotechnology. It is even more difficult to speculate on future use.

As the gaps in knowledge in several cases are linked to several different sections in the text above, a different breakdown of chapter headings has been chosen in describing gaps in knowledge. Areas in which important gaps in knowledge have been identified are listed below without being ranked.

Presence of nanomaterials in chemical products and articles

General knowledge of the presence and use of nanomaterials is lacking in Sweden. This can be regarded as a cause for concern as reports from the United States, the United Kingdom and the OECD, for instance, anticipate a sharp increase in the use of nanotechnology in the near future, as well as a change of emphasis in objective over the next few years.

Exposure to nanomaterials and effects on biological systems

There is a great need for more knowledge on the effects of exposure to nanomaterials on human health and the environment.

Several studies have shown that materials at nanoscale have properties that differ from their larger equivalents, but knowledge is inadequate and the existing data is of variable quality. Nanomaterials can cross biological barriers, including the blood-brain barrier, and enter the cell nucleus or mitochondria. Uptake of nanoparticles in the brain via the olfactory nerve has also been demonstrated. This, together with studies indicating that nanomaterials can cause damage to the brain, lungs, cardiovascular system and immune system, means that there may be risks to human health after exposure to nanomaterials.

There have been few studies on how nanomaterials affect the environment. A problem that is mentioned with regard to carrying out environmental risk assessments is that the nanomaterials will probably be difficult to detect in the environment.

Methods to predict exposure and effects

Knowledge is lacking on what chemical and physical properties are of decisive significance to the ability of nanomaterials to cause damage. For this reason there are no tools at present that permit categorisation and make it possible to predict exposure and effect. These tools should be developed as soon as the scientific basis is available..

There will be a need to categorise nanomaterials with respect to the potential danger to health and the environment. Depending on what directive or regulation governs the risk/safety assessment of nanoparticles, however, the need to be able to make good predictions of exposure and effects based on the properties of particles varies.

Test methods and risk assessment methods

The methods normally used to test hazard to health and the environment are probably only partly relevant to the testing of nanomaterials. New methods therefore need to be developed, while also adapting existing methods. There is also a need for biological exposure models to get relevant information on effects on human health. This requires more knowledge on the uptake of nanomaterials and how they interact with viable cells and micro-organisms.

Experience is very limited with regard to risk assessment. In KemI, human health and environmental risk assessments of silver as a nanomaterial is ongoing and several authorities and countries consider that they will be compelled to respond to risk assessment issues regarding nanomaterials in the near future.

In 2006, the OECD began work aimed at developing test methods and risk assessment methodology for nanomaterials, in which Sweden is taking part.

Application of legislation in the EU

Nanomaterials are covered by current legislation for the area in which they are used. However, nanomaterials are not explicitly mentioned in any legislation concerned with the hazards posed by the substances to health and the environment. An analysis of how current legislation can and should be applied, where the state of knowledge on risks posed by nanomaterials is taken into account, will therefore be required. A build-up of knowledge in the competent authorities is also required to make such an analysis possible.

The information requirements in Reach are small for chemical substances that are manufactured in or imported into the EU in low volumes, that is to say <10 tonnes per year, which is expected to be the case for many nanomaterials. Future guidance will therefore become important to maintain a high level of safety and be able to utilise and apply knowledge that is continuously gained in research.

It is unclear whether a particular nanomaterial according to the new chemicals legislation Reach is to be regarded as a new substance or as a substance that already exists in a special physical form. This may have a great impact on the assessment of chemical safety.

In addition, nanomaterials in articles imported into the EU will in many cases not be covered by the safety assessment made in the framework of Reach.

10. PROPOSALS FOR MEASURES

Rapid development is currently taking place in nanotechnology, but knowledge of risks posed by nanotechnology to health and the environment is deficient. The legislation imposes the same responsibility on companies with regard to nanomaterials as for chemicals, chemical products and articles in general. The scientific limitations that exist today with regard to assessing the risks mean, however, that companies in developing and using nanomaterials should take special safety measures to limit human and environmental exposure. This applies in particular to the risks of inhalation of nanoparticles.

We propose the following measures to fill up the gaps in knowledge:

Research and development

It is proposed that the Swedish Government commissions the Swedish Government Agency for Innovation Systems (VINNOVA) to formulate a Swedish strategy for safe research and development of nanotechnology. The objective of a Swedish strategy for nanotechnological development should be to coordinate efforts and in so doing strengthen Swedish nanotechnology research and also contribute to Swedish research scientists taking leading roles in projects relating to nanotechnology in the EU's framework programmes for research. The initiative should include research on nanotechnology and its applications as well as research on the risks posed by nanomaterials to health and the environment and integration of such knowledge in the development and commercialisation of nanomaterials.

It is proposed that the Swedish Government grants special research funds for the area of Health and environmental risks of nanoparticles to the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), which has coordinating responsibility for environmental toxicological research. This includes research on exposure and effects on humans and the environment. This area of research is poorly developed in Sweden at present, but there are strong research mileus that have what is required for a targeted effort in the area to initiate high-quality research.

Swedish research of high-quality is essential for safe nanotechnology in the country, both with regard to research and development and with regard to the hazardousness of substances to health and the environment. Swedish experts in the area are significant both to companies and with regard to the possibility of Swedish government agencies playing an active part in the development of test methods and risk assessment methods in the EU and other relevant international forums. It is judged that high-class Swedish research on the risks posed by nanomaterials to health and the environment can continue in the longer term to contribute to strengthening the competitiveness of Swedish companies that are active in the area of nanotechnology.

Continued monitoring and development of test methods

The Swedish Chemicals Agency should be commissioned to continuously cover development in the area and propose measures where justified. It should work to build up knowledge on and adopt common principles for the assessment of risks posed by nanomaterials under its Toxicological Council.

The Swedish Chemicals Agency should also be instructed to play an active part in the development of new or modified test methods in OECD cooperation. Test methods fall within the area of responsibility of the Swedish Chemicals Agency, and activities have recently commenced to develop and validate test methods for nanotechnological substances under the OECD in which the Swedish Chemicals Agency plays an active part. The programme is at an

early stage, and when it becomes possible, depending on the outcome of Swedish research, there may be a need for this activity to increase.

There are also other organisations/agencies with responsibility for test methods that can be linked to risks posed by nanotechnology and lie outside the framework of OECD and/or the area of responsibility of the Swedish Chemicals Agency. Examples of this are tests in the area of medicinal products (EMEA/ICH/Swedish Medical Products Agency) and standardisation (ISO/CEN/SIS). Further assignments may therefore become relevant, equivalent to that proposed for the Swedish Chemicals Agency, for government agencies with other areas of responsibility.

Presence and use

The Swedish Chemicals Agency should be commissioned, following consultation with industry organisations, to make an in-depth analysis with regard to the presence and use of nanomaterials in chemical products and articles. The analysis should include a forecast of future use.

Legislation

The Government should commission agencies concerned to review the need for additions to existing legislation in Sweden and the EU, for instance on how companies are to test the health and environmental hazards posed by nanomaterials. The assignment should also include reviewing the need for special rules for nanomaterials in the area of chemicals.

A further task for governmental agencies is to coordinate principles of assessment in developing guidance documents and applying existing legislation, as well as in international cooperation on devising new test methods.

Although evidence that the development and use of nanomaterials poses risks to health and the environment is deficient at present, the rapid development taking place in this area and the widespread ignorance of risks to health and the environment call for safety measures. The Government, and governmental agencies, should therefore aim for a high level of protection with regard to requirements for knowledge and if necessary take measures relating to the hazards posed by substances to health and the environment in legislation.

Workshop during the forthcoming Swedish EU presidency

Finally it is proposed that the Government takes the initiative to hold a workshop during the Swedish presidency of the EU in 2009 on how risks posed by nanotechnology to health and the environment should be dealt with in legislation. The workshop should discuss the need for additions to existing legislation and any needs for new rules. Swedish positions on the need for additions or amendments to existing rules and any proposed for new regulations should have been prepared ahead of the workshop.

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GLOSSARY

Antibodies	Proteins produced by the body's immune system for the purpose of detecting foreign substances in the body and rendering them harmless. Because of their unique ability to bind exclusively to a particular type of molecular structure, antibodies also have many medical applications.
Blood-brain barrier	The blood-brain barrier is a physiological barrier that separates the brain from the rest of the body. The principal task of the blood-brain barrier is to protect the brain from exposure to harmful substances in the blood.
Bottom-up	Creating material at nanoscale from atoms or molecules (relates to use of the term in nanotechnology)
DNA	The molecules that make up the genetic material of living cells
Dendrimes	Spherical polymer molecules (see polymer)
Ecotoxicology	The study of the toxic action of substances in and on ecosystems. Includes studies of the flows of substances in the environment and their effects on organisms and ecosystems.
Exposure	Contact of a chemical with the environment, an organism or target organ in organisms.
Fluorescence	Certain substances absorb energy in light of a particular colour (wavelength range) effectively and immediately emit energy again in the form of light (they fluoresce). This phenomenon can be used in fluorescence microscopy where the object under examination is exposed to short-wave light and fluorescent substances at low concentrations then emit light and can be studied.
Fullerenes	A new form of carbon called fullerenes or C_{60} was discovered in the mid-eighties (Kroto et al, 1985). These are spherical molecules which are approximately 1 nm in diameter and consist of carbon 60 arranged as hexagons and pentagons, which together look like a football.
Physico-chemical properties	Relates to properties in the substance itself, such as density, boiling point, solubility and explosiveness.
Graphite	A form of carbon in which the carbon atoms are arranged in hexagonal structures that form very large discs or sheets which in turn are stacked one on top of the other.
Humus	Natural organic substances, often very large molecules, which are breakdown products of living matter. Present in soil and water.
in vitro	Experiments performed in an artificial system outside an organism, such as a test tube or culture medium.

in vivo	Refers to biological processes in living cells and tissues when they are at their natural place in whole organisms, particularly processes which are used in scientific experiments and clinical tests.
Catalytic	A property of a substance that means that it speeds up chemical reactions without itself being consumed.
Composite (composite material).	A mixture of two or more materials which have properties that differ markedly from the components contained.
Quantum dot	A small volume of semiconductor material which is embedded in another material, for example a metal.
Quantum effect	The effect of quantum mechanics when relatively few atoms are combined into a particle which is smaller than approximately 10 nm and the electrical, optical or magnetic properties may differ markedly in relation to equivalent larger particles.
Quantum mechanics	(quantum physics, quantum theory) is an overarching theory in modern physics which describes the behaviour of matter and energy in the microcosm. Quantum-mechanical effects are usually not noticed at microscopic level, but atomic and subatomic systems are quantum-mechanical. Quantum mechanics differs from classical mechanics on a number of crucial points. The most important is that its predictions are statistical, in the sense that it is not possible to predict what result a measurement will produce, only the probabilities of possible outcomes.
Model enzyme system	Method based on proteins with specific function having isolated from cells and the activity of these proteins being tested in relation to the substances to be studied. The results of such tests thus represent a piece of a model of a function in a whole organism.
Nano	A prefix that means a billionth part and comes from the Greek word for dwarf, nanos.
Nanometre	One billionth of a metre or 10^{-9} metre, compared with a typical atom which is just over 0.1 nm in diameter.
Nanoengineering	Engineering with a size suitable for measurement in nanometres. It can be used in electronics and materials technology, but also in chemical and biological applications.
Nanotechnology	Nanotechnology entails manufacturing or manipulation of materials by physical or chemical processes. Nanotechnology can be defined as research and development at atomic, nanometric or macromolecular level if the size of the nanostructures is less than 100 nanometres in at least one dimension.

Nanomaterial	Material which is either nanostructured or made up of nanoaparticles or other materials which have one or two dimensions at nanoscale.
Nanostructured	Intentionally created structures at nanoscale. Principally relates to surface structures on materials at larger scale.
Nanoparticles	Particles that are less than 100 nanometres in diameter, i.e. are at nanoscale in three dimensions.
Nanotube	A carbon nanotube can be likened to a graphite disc that has been rolled up into a tube. Some tubes are open at the ends while others are closed. Nanotubes with a single wall (SWCNT = Single Wall Carbon NanoTube) are the simplest form. There are also nanotubes with several walls (MWCNT = Multi Wall Carbon NanoTube), i.e. several SWCNTs one inside the other.
PM_{10}	Airborne particles less than 10 µm in diameter
$PM_{2.5}$	Airborne particles less than 2.5 µm diameter
Polymer	Natural or synthetic substance, consisting of smaller units, which are linked together in the form of a chain. Examples of natural polymers are proteins, DNA and cellulose. Synthetic polymers may be plastics and fibres.
Top-down	Grinding material down at macroscale to material at nanoscale (relates to use of the term in nanotechnology)
Toxicology	Toxicology is the study of toxic substances and how they affect living organisms: plants, animals and humans. Relates primarily to toxic effect in humans (see ecotoxicology).
Suspension	Fine particles suspended in liquid

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