Carbon nanotubes - Exposure, toxicology and protective measures in the work environment

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– Exposure, toxicology and protective measures in the work environment

Kunskapsöversikt

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Preface

The Swedish Work Environment Authority (Arbetsmiljöverket) has been given an assignment by the Government to inform and disseminate knowledge on areas of importance for the work environment. Over the next few years, a number of summary reviews of current knowledge will be published in which renowned scientists summarise the current know-how within a number of themes. The manuscripts have been reviewed by external assessors and have been discussed in seminars at their respective universities.

The reports are available at no charge on Arbetsmiljöverket's website. There are also materials available from the seminars which Arbetsmiljöverket has arranged in connection with the publication of the reports.

The working group at the Work Environment Authority that has initiated and organized the production of the summary reviews was led by Professor Jan Ottosson. We would also like to thank other colleagues at Arbetsmiljöverket who have been helpful in producing the reports.

The views expressed in this report are the author's own and do not necessarily reflect the views of Arbetsmiljöverket.

Jan Ottosson

The authors

This report has been written on behalf of Arbetsmiljöverket by researchers working at Lund University in research collaborations between Metalund and Nano-Safety. This summary overview of current knowledge is a compilation of literature in the field of carbon nanotubes and deals with occupational exposure, toxicology and protective measures used in the work environment. Issues which in accordance with the assignment were to be investigated included - what the exposure situation was like during production, processing and handling of products containing carbon nanotubes. A compilation of advice and guidelines on safety and protection devices and personal protective equipment is also presented. The toxicological data available for carbon nanotubes has also been summarized. The purpose of the overview is to provide Arbetsmiljöverket with information and support for different types of measures. The overview may also provide support if special hygienic limit values within the area or other regulations are being considered. The report is based on original work and summary reviews identified in scientific databases based on systematic literature searches. For the section that deals with toxicology, a selection of relevant articles has been included. The remaining findings will be detailed in a document currently being presented to the Nordic Expert Group (NEG) for the production of criteria documents on chemical health risks.

The report was written by PhD Per Gustavsson, the Department of Biology, University of Lund, PhD Maria Hedmer, Division of Occupational and Environmental Medicine, Lund University and PhD Jenny Rissler, Department of Ergonomics and Aerosol Technology, Lund University.

A reference group of experts has been attached to this assignment and is made up of the following people: Associate Professor Maria Albin, the Department of Occupational and Environmental Medicine, Lund University; Professor Mats Bohgard, The Department of Ergonomics and Aerosol Technology, Lund University; Professor Martin Kanje, the Department of Biology, Lund University; and Professor Steffen Loft, Institut for Folkesundhedsvidenskab, University of Copenhagen.

Abbreviations

APS	aerodynamic particle sizer
CPC	condensation particle counter
CVD	chemical vapour deposition
DMA	differential mobility analyser
DNA	deoxyribonucleic acid
EDX	energy dispersive X-ray analyser
ELPI	electrical low-pressure impactor
ESP	electrostatic precipitator
FID	flame ionization detector
FMPS	fast mobility particle sizer
HEPA	high efficiency particulate air
HiPCO	high pressure carbon monoxide
HSE	Health and Safety Executive
ICP-MS	induktivt kopplad-plasma masspektrometri
IFA	Institute for Occupational Safety and Health of the German Social Accident Insurance
i.p.	intraperitoneally (into the abdominal cavity
i.v.	intravenously
LOEL	lowest observable effect level
LOD	limit of detection
MCE	mixed cellulose ester
MWCNT	multi-wall carbon nanotubes
N/A	not available, sample not taken
ND	not detected
NIOSH	National Institute for Occupational Safety and Health
NMAM	NIOSH Manual of Analytical Methods
NOEL	no observable effect limit
OPC	optical particle counter
PAS	photoelectric aerosol sensor
SEM	scanning electron microscope
SMPS	scanning mobility particle sizer
STEM	scanning transmission electron microscope
SWCNT	single-wall carbon nanotubes
TEM	transmission electronic microscope
TP	thermophoretic precipitator
UCPC	ultrafine condensation particle counter
WHO	World Health Organisation

Contents

Summary

The use of carbon nanotubes has increased substantially in recent years, and is expected to continue to increase strongly in the future. Increased production, handling and machining increase the risk of exposure in different work environments. Today, the production of carbon nanotubes takes place mainly in non-Nordic countries, but carbon nanotubes are used in the Nordic countries in research and development work. Carbon nanotubes are used mainly as a reinforcement material in various types of polymers, i.e. in composites, but there is considerable potential for use with other applications.

More recently people have become increasingly aware that the properties of nanoparticles may be substantially different than those of larger particles of the same material, parallels have also been drawn between the fibre-like shape of carbon nanotubes and asbestos.

It is therefore necessary to study the toxicity of carbon nanotubes to assess the risks associated with their handling and, if necessary, implement regulations, in the form of occupational exposure limits.

In this report, we have taken into account exposure via ingestion, inhalation and the skin. Inhalation appears to be the route of exposure that is associated with the greatest potential risk, since a carbon nanotube is a material which, in bulk form, has a very low density and produces a lot of dust during handling. Measurements also show that the highest occupational exposure takes place precisely when handling dry bulk material of carbon nanotubes.

Carbon nanotubes are a whole class of nanomaterials, not a single material, because there are many different types of carbon nanotubes regarding diameter, length, chiral angles, chemical functionalization, purity and bulk density (often related to dusting propensity).

Exposure to the working environment and measurement methods

Characteristic of a carbon nanotube is that its mass is small, but the number of nanoparticles is very large because of the low density. The majority of the exposure measurements at different workplaces where carbon nanotubes are produced or used have been traditional dust measurements, where mass concentration was determined. The measured levels have typically been 0.1 mg/m3 or less, but even higher concentrations have been reported. Results from this type of measurement are uncertain, because the method does not specifically measure the carbon nanotubes, but also includes other airborne particulates in the determined mass dose. To date, the measurement methodologies that have been used have mainly provided exposure data for emissions and possible exposure to carbon nanotubes at workplaces and supporting documentation for the evaluation of safety and protection devices. Measurements have been made over short periods and of a few number of work operations/exposure situations. Furthermore, different types of sampling equipment have been used, making it difficult to compare the results.

There is a need for a standardized measurement methodology for the quantification of occupational exposure to carbon nanotubes. In order to distinguish carbon nanotubes from other airborne particles, an analysis is required using scanning electron or transmission electron microscopy. The National Institute for Occupational Safety and Health, NIOSH, is now examining the effectiveness of their method for measuring diesel particulate matter (the analysis of elemental carbon) to test if it can also be used to determine the mass of carbon nanotubes, since they are also composed mainly of carbon. The sampling of carbon nanotubes could then be done with "open-face" filter cassettes with a quartz filter (to determine the total amount of dust) to be subsequently analyzed by thermal-optical analysis with a flame ionization detector (FID). Simultaneous sampling of "open-face" filter cassettes with an MCE filter (determination of total amount of dust) for the analysis of transmission electron microscopy would still be needed to ensure specificity.

When a reliable measurement methodology is available to determine the mass of carbon nanotubes, more workplaces must be examined and more full-day measurements must be carried out. Furthermore, personal sampling in the breathing zone must be carried out for more work operations/exposure situations. When reliable exposure data for carbon nanotubes is available only then will it be possible to carry out a risk assessment for carbon nanotubes.

Today, there is not enough knowledge about either the health effects or the exposure levels when handling carbon nanotubes; a precautionary principle should therefore prevail in the manufacture, handling and use of carbon nanotubes, and the machining of materials containing carbon nanotubes. In practice this means that you should use established safety and protection devices such as enclosures and process ventilation, together with personal protective equipment such as respiratory protective equipment, protective gloves and protective clothing.

Toxic effects

It is currently difficult to draw clear conclusions regarding the toxicity of carbon nanotubes. The fact that the physical properties of carbon nanotubes vary and that they contain pollutants from manufacturing processes such as metals complicates the interpretation of the results from completed toxicology tests. In some publications, a clear characterization of the physical properties and pollutants for the carbon nanotubes that have been used in experiments is lacking.

Several studies suggest that carbon nanotubes could pose a health risk, since effects on laboratory animals occur at relatively low concentrations. At the same time, there are studies that do not report any negative effects, or effects only at high doses. These studies fall short in some cases in their description of the experimental methodology or in how closely the effects have been studied. Relevant studies on humans are lacking.

Because of the physical similarities between asbestos and carbon nanotubes, there are suspicions that they might have similar biological effects, i.e. cause fibrosis of the lungs and respiratory passages, as well as lung cancer and mesothelioma (a malignant tumour originating from mesothelial cells, usually in the pleura). We believe that exposure through inhalation is a potential risk when working with carbon nanotubes,

since it has been observed that both single-walled and multi-walled carbon nanotubes may cause inflammation and fibrosis in the respiratory passages, lungs and pleura in relevant animal types. Some studies suggest that longer carbon nanotubes cause greater biological effects than shorter carbon nanotubes. There are not enough long-term studies with repeated exposure in order to draw safe conclusions about the capacity of carbon nanotubes to cause lung cancer or mesothelioma. Several findings have been made in which carbon nanotubes have caused damage to and mutations of DNA, indicating that repeated long-term exposure could increase the risk of developing cancer.

Based on the effects on laboratory animals, the lowest dose observed to cause adverse effects on the respiratory passages (inflammation and slight granuloma formation) from 0.2 to 0.3 mg/kg of body weight, the lowest air concentration where this has been observed is 0.1 mg/m3. At higher levels, more serious damage to the lungs can be observed as well with effects on the heart. After exposure to doses of 0.06 mg/kg body weight via tube-feeding, DNA damage occurred.

There is a risk that other organs other than the lungs may be exposed to carbon nanotubes if they pass into the bloodstream. A transition of this type could occur in the lung or gastrointestinal tract. Carbon nanotubes could end up in the gastrointestinal tract after being transported by the cilia in the respiratory passage up to the throat, where the nanotubes are then swallowed.

There are major gaps in knowledge regarding the health effects of carbon nanotubes. It is particularly important that more long-term studies are conducted with laboratory animals to measure the effects after inhalation, but more studies are needed of the reproductive effects and effects of skin exposure. The functionalization of carbon nanotubes, i.e. by binding chemical groups to the tubes strongly affects the half-life period in the blood and may influence their biological effects. More studies therefore are needed with respiratory exposure with functionalized carbon nanotubes. Human data is lacking.

1.1 Conclusions

An important issue for handling carbon nanotubes concerns their classification. Should they be classified as a separate substance or as carbon which is the main substance in the tubes, and should in that case, all tubes be classified as a substance or as single or multi-walled carbon nanotubes, with their respective functionalizations and be risk-assessed accordingly. Research indicates that the toxic properties of carbon nanotubes may be different from other types of nanoparticles that are made up of layers of graphene. It would suggest that carbon nanotubes should have a specific classification.

The risk assessment for carbon nanotubes is made more difficult by a lack of knowledge at several levels: 1) Consensus is lacking as to which dose-metric that is most relevant for health effects; 2) Carbon nanotubes can be found in a large number of variants that are likely to have different levels of toxicity; 3) The toxicological data is inadequate but indicates that there is a risk of inflammatory reaction and pulmonary fibrosis when inhaled at relatively low doses; there is also the risk of producing a DNA-damaging effect; 4) Exposure levels for the commercial handling of carbon nanotubes are incompletely characterized.

While waiting for the level of understanding to become clear, a strategy for the regulation of occupational exposure-related conditions may be to take note of the effects observed for the most toxic of carbon nanotubes. The proposed international occupational exposure limits are at very low levels. Airborne exposure arises from the manufacture, handling, and use of carbon nanotubes and in the machining of products containing carbon nanotubes. Established technical protective measures such as encapsulation and process ventilation should be applied in conjunction with personal protective equipment such as respiratory protective equipment, protective gloves and protective clothing.

2. Introduction

Observations of carbon nanotubes were first reported in scientific literature in 1991 (Iijima, 1991) and have since been the subject of much research. After the discovery and characterization of carbon nanotubes, numerous areas of use have been proposed and carbon nanotubes have been predicted as being able to contribute revolutionary properties to materials within areas such as mechanical strength, electrical properties, chemical stability, and medical applications.

Today, carbon nanotubes are used primarily in composites, for instance in plastics and rubber to make them lighter or stronger (Lam et al., 2006). Nanocomposite materials, which typically contain between 1-10% carbon nanotubes, are used for the manufacture of parts for automobiles and aircraft, wind power blades, and sports equipment (Hussain et al., 2006, Köhler et al., 2008, Sass et al., 2008, Thomas et al., 2009; KEMI 2009). The market also produces mobile phones and laptops with lithium-ion batteries that contain carbon nanotubes (Köhler et al., 2008; IRAP, 2011). Carbon nanotubes are also found in antifouling paints for boats (Nanocyl, 2010). The global production of carbon nanotubes is currently in excess of 2.5 tons/day.

Some future potential applications proposed for carbon nanotubes are textiles, building materials, electronics, energy systems, biomedicine, membrane technology and medical applications (Bhushan 2004, Aitken et al., 2006, Schneider et al., 2007, Endo et al., 2008, Köhler et al., 2008; Kostarelos et al., 2009; Aschberger et al., 2010; Barkauskas et al., 2010). Although it has been 20 years since the discovery, the actual areas of use for carbon nanotubes are currently very limited, mainly because of problems with well-controlled mass production. It is predicted that the largest field of application in the future will be composite materials, which do not require as high purity and as well-defined characteristics as with many electronics applications.

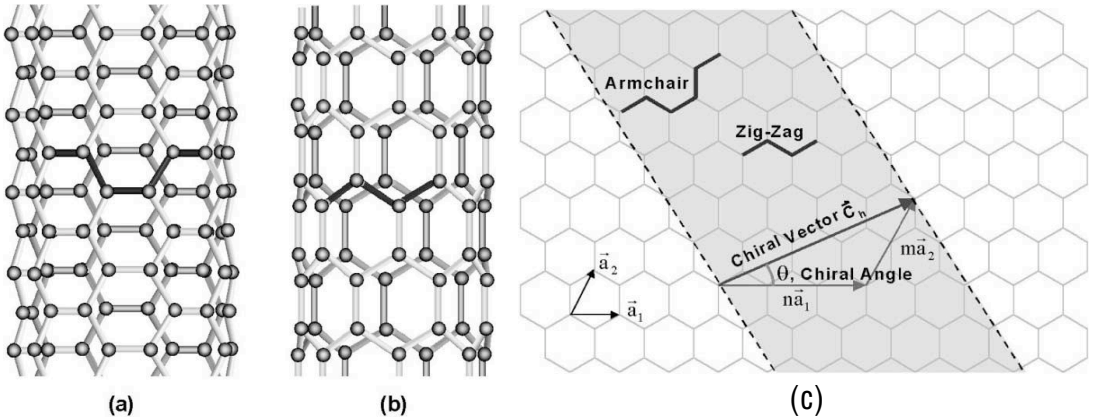
The use of carbon nanotubes is predicted to grow substantially in the future along with people's exposure to them at workplaces. In order to use carbon nanotubes to their full potential, one of the challenges is to deal with the tubes in a safe manner during manufacturing, processing, during use and during disposal. This report is a synopsis of the properties of carbon nanotubes, exposure situations that exist during production, processing and the handling of products containing carbon nanotubes and a summary as to what is known of the toxicology of carbon nanotubes.

3 Classification of carbon nanotubes

Carbon nanotubes consist of carbon atoms that are structured in layers of graphene rolled into the shape of a cylinder. Each carbon atom of graphene is symmetrical bound to three other carbon atoms, one-atom thick, which in turn form hexagonal rings (Figure 1). Carbon nanotubes can be open or closed at the ends. It is customary to categorize the carbon nanotubes into two groups – single-walled and multi-walled. A carbon nanotube that consists of only one layer of graphene is usually called a single-walled carbon nanotube or SWCNT (from Single-Walled Carbon NanoTube). The multi-walled nanotubes consist of several layers of graphene shaped into concentric cylinders, which are bound together by van der Waals forces. In this report we will to refer to multi-walled carbon nanotubes as MWCNT (from Multi-Walled Carbon NanoTube). As MWCNT consists of several layers of graphene, they will have a larger diameter than SWCNT. The most extreme MWCNT can be made up of up to several hundred concentric cylinders with a typical distance between the layers of graphene of ~ 0.34 nm (Popov, 2004). Even if carbon nanotubes are generally categorized into these two groups, each group can consist of a complex mixture of tubes of varying length, diameter, crystalline structure, surface chemistry, etc.

Typically, the diameter of a carbon nanotube is in the range of 1-100 nm (10-9 m) – single-walled with a diameter of around 1-3 nm (Jorio et al., 2001) and multi-walled normally 10-200 nm (Hou et al., 2003). The diameter of the tubes depends on the method of production in which the diameter of the catalytic metal particles used plays a decisive role, particularly for single-walled tubes. Even if the length of carbon nanotubes may vary, they are typically in the order of tens of micrometers. The longest carbon nanotube reported measured 18 cm (Wang et al., 2009) and the shortest is the organic compound cycloparaphenylene, which was synthesized in early 2009 in Berkley (http://www.lbl.gov/msd/assets/docs/highlights/09-Bertozzi.pdf). The length of the tubes varies dependent on the manufacturing and purification process. During the purification process, when residues and impurities from the manufacturing process are removed; the nanotubes tend to be shorter due to the destructive conditions the tubes are subject to during purification (Liu et al., 1998).

The atomic structure of carbon nanotubes is described by the tube's chiral angle. The chiral angle determines how twisted the hexagons are in the graphene structure, as illustrated in Figure 1. Since the distance between the carbon atoms in the graphene structure is constant, tubes of different chiral angles will have different diameters. Multi-walled carbon nanotubes usually have adjacent concentric graphene layers hence the varying chiral angles.



Figur 1. a-b) The atomic structure of single-walled carbon nanotubes for two types of tubes: (a) shows a so-called "armchair" structure and (b) a zigzag structure. c) A diagram of different "twists" and chiral angles of a graphene sheet (source: Thostenson et al., 2001 Composites Science and Technology 61:1899-1912).

The chiral angle is often described by a vector (n, m) where n and m denote the integer of the unit vectors along the graphene structure (a1 and a2 in Figure 1). There are three main types of carbon nanotubes. If m=0 the nanotubes are of a zigzag type, if n=m they are of the armchair type, and any combination in between, they are referred to as of a chiral form.

1. The chiral angle of carbon nanotubes affects some of their properties such as mechanical properties, optical properties and, in particular, their electrical properties. Graphene itself is a semiconductor. Nanotubes in contrast, can be both metallic or semiconductors depending on the chiral angle. The mechanical properties are only affected minimally by the chiral angle (Thostenson et al., 2001).

4. Physical and other chemical properties

It is very rare that SWCNT (single-walled carbon nanotubes) are found as individual tubes; they are more often arranged in large bundles, 5-50 nm in diameter (Maynard et al., 2007), which are held together by van der Waals forces. These bundles in turn form themselves into larger agglomerates or clumps. MWCNT (multi-walled carbon nanotubes) appear more as individual tubes, but even these tend to form bundles (Lam et al., 2006). The cohesive van der Waals forces are however weaker for MWCNT than for SWCNT (Yu et al., 2000).

Due to their structure, carbon nanotubes have a very high surface area compared to their mass. The ratio of surface area and mass of the tubes depends on their diameter and the degree to which they form bundles. Individual SWCNT have a ratio (surface

area to mass) of about 1300 m2/g, while MWCNT typically have a ratio of around a few hundred m2/g (Peigney et al., 2001). Because SWCNT form themselves into bundles, the effective ratio of surface area and mass is often smaller or around ~ 300 m2/g (Ye et al., 1999).

The density of carbon nanotubes in powder form is very low and is dependent on the method of manufacture. In a study which compared the bulk densities of carbon nanotubes produced by laser ablation and HiPCO ® (the High-Pressure Carbon Monoxide process) it was found that the latter method gave a more porous material with bulk densities on the order of 1 mg/cm3 (Baron et al. 2003). No density was specified for laser ablation. According to the specification by Baytubes ® products, these are of a density of ~ 120-170 mg/cm3. Other manufacturers indicate bulk densities of around ~ 100 mg/cm3.

4.1 Mechanical properties

One of the carbon nanotubes' coveted properties is that they have extreme strength. Single-walled carbon nanotubes are said to be around 10 times stronger than steel and 1-2 times stiffer (axial) than diamond (Walters et al., 1999, Yu et al., 2000) and are considered to be one of the stiffest materials. Its excellent mechanical properties come from the strong covalent bonds (sp^2 hybrids) that connect the carbon atoms in the graphene structure. Radially, nanotubes are relatively soft and easily deformed. A study has shown that the van der Waals forces from adjacent carbon nanotubes are sufficient to deform the tubes (Ruoff et al., 1993). They are very flexible (radial flexibility) and can be bent several times up to 90 degrees without being damaged.

4.1.1. Carbon nanotubes as composite materials

Much research has been done on the use of carbon nanotubes as composite materials, especially for plastic composites. In this way, the primary intention would be to make use of the tubes' mechanically advantageous properties but also their ability to modify the thermal and electrical properties of polymers (Thostenson et al., 2001; Harris, 2004). This application dominates the use of carbon nanotubes today in industry from a quantity point of view. In addition to polymers, tubes have been proposed for use in various other types of composite materials. Two examples are ceramic materials and metal composites.

One problem with carbon nanotubes as composite materials has been that they do not quite fasten to the material but tend to crack and become detached from the material and get pulled out instead of breaking off. This means that the tubes' characteristics cannot be fully exploited (Harris, 2004).

Carbon nanotubes in polymers also change the polymer's thermal and electrical properties (Kymakis et al., 2002). According to Harris (2004) the greatest effort has previously focussed on exploiting the mechanical properties of carbon nanotubes rather than their electrical properties, but electrical properties are now viewed with greater interest. If we could fully exploit the tubes' unique properties in composite materials, this would provide us with a material that is light, strong, rigid and thermally and electrically conductive.

4.2 Electrical characteristics

Carbon nanotubes are both electrically conductive and semiconductors. The electrical characteristics of the tubes are primarily determined by their chiral angle, but for tubes with a small diameter also by their surface curvature (Lu and Chen, 2005). Theoretically, metallic carbon nanotubes can conduct electricity with a density that is 1,000 times greater than metals such as copper. The electrical resistance of the tubes is determined by quantum mechanical phenomena and is independent of the tubes' length.

There are many applications for the use of carbon nanotubes as electrical components. For example, diodes could be produced by merging SWCNT that have different electrical properties (Chico et al., 1996). It has also been shown that the electrical properties of the tubes change during deformation and stretching. This further increases the potential for the use of carbon nanotubes in electromechanical components such as sensors (Mahar et al., 2007).

4.3 Chemical properties

As described in previous chapters, carbon nanotubes consist of carbon atoms which are bound in a hexagonal pattern. These build up layers of graphene which are rolled into long tubes. These tubes are relatively stable and can be heated to above 500°C before they oxidize and burn up (Zhang et al., 2002). The smaller diameter, the higher the reactive. Bulk samples of carbon nanotubes include not only carbon nanotubes but also, depending on the manufacturing procedures, a number of impurities of varying amounts. These can be classified into five groups (Donaldson et al., 2006):

1) SWCNT	Carbon nanotubes
2) MWCNT	
3) Metas	residues
4) Support materials	
5) Carbon and organic matter	

When carbon nanotubes are manufactured, metals are often used as catalysts. The most common are iron, nickel, cobalt and molybdenum, of which nickel and cobalt are allergenic. In the production of SWCNT these catalytic metals are required, and the production of these often result in a material with a higher metal content than MWCNT. During production, support materials such as aluminium, manganese oxide or silica are also used.

The last group (Group 5) can be divided into two subgroups: i) organic molecules and ii) various forms of carbon such as soot particles, fullerenes or graphite (Lam et al., 2006). The degree and type of impurities depends on the production and purification method. Generally speaking, vapour phase methods tend to generate carbon

nanotubes with smaller amounts of impurities and are also considered to be easier to scale up. Production methods are discussed further in later chapters. Product specifications for Baytubes® carbon nanotubes indicate a purity of >95% (mass), whereas for other commercial products, a degree of purity of >60% and up to >99% is indicated (www.cheaptubes.com, 11-11-2011). In toxicological studies, the effect of impurities in the bulk samples used should be considered when certain impurities in such low quantities as 1% may in fact be toxic.

4.4 Purification processes

A critical point when it comes to scaling up the production of carbon nanotubes for certain applications with high demands on specification and homogeneity, is purity and the sorting of carbon nanotubes. This is important also when designing toxicological tests for a deeper understanding of the mechanism behind the toxicity of the tubes.

Today there are several different types of purification methods, but there still remains a lot of areas where the work can be improved, for instance, to scale up production (Liu et al., 1998, Barkauskas et al., 2010). Even if the metal catalyst, to a great extent, can be purified of the bulk material, significant amounts nevertheless remain (Donaldson et al., 2006). Because the purification process itself can damage the nanotubes, the extent of the purification must be balanced against the fact that the purification process also introduces impurities into the product in the form of damaged carbon nanotubes. For example, ultra-pure samples of carbon nanotubes often have defects in the form of carboxyl groups (Donaldson et al., 2006). Another reason why SWCNT to date are being used for relatively few commercial applications, despite the great potential that exists in the material, is that for many purposes the bulk material is not sufficiently uniform (Hersam, 2008). To overcome this, much effort has been devoted to developing purification/and sorting processes.

Basically, there are two main types of purification processes: 1) sorting out impurities, and 2) selective methods to provide a more homogeneous sample of the carbon nanotube with respect to for example diameter, length or electrical properties. Amorphous carbon, metallic residues and support materials are frequently washed away by ultrasonic bathing in combination with acids or bases. Certain types of impurities such as silica or aluminium, however, require strong acids. To avoid exposing the carbon nanotubes to these more destructive purification methods, support materials that are dissolved in mild acids are increasingly being used (such as magnesium oxide). Other types of treatment procedures include magnetic purification, functionalization and microfiltration. Combinations of different methods are often used.

To generate a more homogeneous material, chromatographic methods can be used to separate tubes of equal length and diameter. The method with the highest resolution can separate tubes of different lengths and provides a resolution of <10% (Huang et al., 2005). Carbon nanotubes of different diameters can also be separated by "density-gradient ultracentrifugation (DGU), (Hersam, 2008). Many types of applications require tubes, however, that are of an even greater uniformity than can be generated by

DGU, which places greater demands on sorting procedures. Examples of this can be various types of electronic components which require that the conductive carbon nanotubes are sorted from the semi-conductive (Hirsch and Vostrowsky, 2005). Other applications require the sorting of tubes based on their chiral angle.

Methods are available today that make it possible to sort carbon nanotubes by conductivity, but they are not yet available for mass production. A method that it is believed has great potential is based on DGU (Arnold et al., 2006). With this method, tubes of different chiral angles are sorted, and the method is based on the fact that tubes of various chiral angles have different densities. Another promising method for the separation of SWCNTs is based on the freezing of the sample, thawing, and compression of carbon nanotubes embedded in a gel of agarose (Tanaka et al., 2009a). Separation of SWCNTs can also be carried out using chromatographic methods (Tanaka et al., 2009b). Even the sorting of carbon nanotubes with respect to their chiral angle has been demonstrated (Tu et al., 2009).

4.5 Functionalization

Pure carbon nanotubes are very difficult to atomize in both water and other organic media. This has hampered the use of composite materials. Through functionalization, the tubes' properties may change and they can be made water-soluble (Hirsch and Vostrowsky, 2005). Even the mechanical and electrical properties of the tubes can be modified through functionalization.

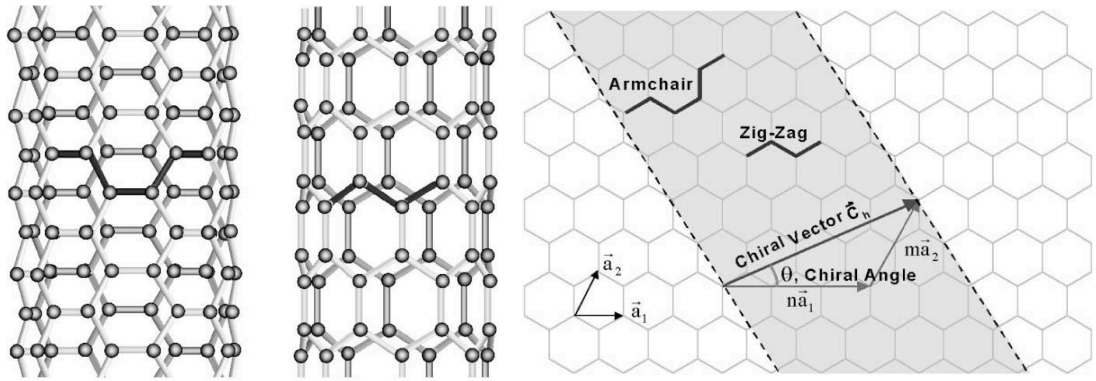
Functionalization is often divided into three main types: covalent, exohedral non-covalent and endohedral non-covalent. Covalent functionalization is based on the functional units by binding to the tubes via covalent linkage, while non-covalent functionalization is based on other types of bonds such as van der Waals forces or π-bindings. Non-covalent functionalization means that atoms or molecules may be linked to both the insides and outsides of the tubes. The advantage of non-covalent functionalization is that the stable structure of the tube is preserved. This type of functionalization has been proposed for non-destructive purification methods of the tubes and may be used to make the tubes easier to disperse in water.

As it is the surface of the nanotubes that mostly affects the biological interaction, the functionalization of the tubes probably has a major impact on the biological response produced by the exposure (Sayes et al., 2006). This is made use of, for instance, in medical applications such as diagnostic imaging or the administration of pharmaceutical preparations (Kostarelos et al., 2009). The interest in using carbon nanotubes for therapeutic purposes, for example, as carriers of pharmaceutical preparations or as a contrast medium for X-rays, has increased in recent years. Much of the research done on cells and animals is designed to do just that. This report does not take account of these applications to any great extent in the case of a special class of carbon nanotubes, which are often surface-modified and functionalized with molecules (pharmaceutical preparations) that do not have applications in other fields, such as surface coatings or composite materials. Section 8., entitled Toxicokinetics describes however, how certain types of these functionalized carbon nanotubes behave in the body after an intravenous injection

4.6 Challenges for toxicological studies

Carbon nanotubes can be produced using several different methods, which at first sight generate similar material. On closer examination, the materials however, vary considerably in terms of crystalline structure, morphology and the content of the residues from the production method (ENHRES, 2009) - properties that may underlie the tubes' toxicity. In order to interpret the observed biological effects, toxicological studies should include a detailed description of the physical and chemical properties that may be relevant to the biological response.

Another relevant issue in toxicological studies is whether the physical characteristics of the carbon nanotubes in the exposure study are the same as those we expect in an actual exposure situation. Examples of this are if the carbon nanotubes are inhaled as single tubes, and as bundles of tubes, or rolled into larger agglomerates. For example, agglomerates or aggregates often have a larger aerodynamic diameter than individual tubes and will therefore, to a greater extent, deposit in the upper respiratory airways, where other mechanisms remove or degrade the tubes compared with those in the alveoli. To suspend the carbon nanotubes to an airborne state in a controlled and reproducible way is a problem in toxicological inhalation studies. This means that other models of lung deposition are applied instead, such as intracheal instillation or aspiration. This is discussed further in later chapters.



The aggregation condition has not only an effect on where in the lung the carbon nanotubes are deposited but also on the biological response (Mercer et al., 2008; Shvedova et al., 2005). Only a few of the toxicological studies have been conducted as inhalation studies, and only a few methods for the suspension of carbon nanotubes into the air for this purpose have been developed. Two methods used in the suspension of carbon nanotubes into the air is to spray droplets of a solution of carbon nanotubes and to dry out the fluid so that only the tubes remain (Lee et al., 2010) or directly by suspending a powder (Maynard et al., 2004).

5. Inhalation and pulmonary deposition

We may be affected by the nanoparticles through several exposure routes such as inhalation, skin contact and ingestion. For workplace exposure to carbon nanotubes, inhalation is *the* exposure route that has been identified here as the most important. We are exposed daily to more or less high levels of ultrafine particles through the air we breathe. The fact that certain types of particles of this size can be harmful is not new, but has been shown in several studies of particle toxicity (Donaldson et al., 2005). Most people are aware of the harmful effects of exposure to asbestos (Donaldson et al., 2010).

The fiber-like structure of carbon nanotubes has meant that parallels have been drawn to asbestos fibres and their known toxicological effects. But it is still unclear how far this parallel is sustainable. The characteristics that, according to the fibre paradigm, are regarded as critical for asbestos fibres are i) that the dimensions and size of the fibres mean that they can follow air flows deep into the lungs when inhaled and are deposited in the alveolar region, ii) that they are sufficiently long and stiff so that macrophages fail to phagocytose (to be engulfed and digested), and iii) that they are biopersistent (Donaldson et al., 2006).

The principal mechanisms by which particles are deposited in the lungs are impaction, sedimentation, diffusion and interception. Fibres that have a diameter ≤100 nm often have an aerodynamic diameter ≤400 nm (Gross, 1981) and since fibre-like particles tend to align themselves parallel to an airflow, they can, despite their length, be transported far down into the lungs where they are finally deposited. The deposit is due to interception which increases with increasing fibre length (Lippmann, 1990). In the lower part of the lung, macrophages are responsible for the principal removal of deposited non-soluble particles. Macrophages are in the order of 10-20 μm and have difficulty in removing fibres that are of and above this size (Donaldson et al., 2010).

6. Occurrence, manufacture and use

6.1 Occurrence

Carbon nanotubes occur in nature and are formed for example naturally when burning, and can also be found in soot. Since the discovery of carbon nanotubes, they are produced and mixed in plastics, rubber, composite materials to make them lighter or stronger (Lam et al., 2006). Nanocomposite materials that contain between 1-10% carbon nanotubes are used in the manufacture of automobiles and aircraft, wind power blades, and sports equipment such as tennis rackets, hockey and golf clubs, bicycles, cycling shoes, skis, darts and baseball bats (Hussain et al., 2006, Köhler et al ., 2008, Sass et al., 2008, Thomas et al., 2009; KEMI, 2009). The market also produces mobile phones and laptops with lithium-ion batteries that contain carbon nanotubes (Köhler et al., 2008; IRAP, 2011). Carbon nanotubes are also found in antifouling paints for boats

(Nanocyl, 2010). In the future, carbon nanotubes will be used in many more areas such as in textiles, building materials, electronics, energy and medical applications (Bhushan, 2004; Aitken et al., 2006; Schneider et al., 2007; Endo et al., 2008; Köhler et al., 2008; Kostarelos et al., 2009). Today, research and development is still being carried on in these areas. In terms of value, it has been predicted that electronics will be the most important carbon nanotube product group, while the use of composite materials in cars will account for the largest volumes (KEMI, 2009).

6.2 Manufacture

The global production of carbon nanotubes in 2006 was estimated at 300 tonnes of MWCNT and seven tons of SWCNT (WTEC, 2007). In 2007 there were about 35 producers of MWCNT in the world (KEMI, 2009). At present, there are at least 42 suppliers of carbon nanotubes according to an Internet site (www.nanotube-suppliers.com), which has been jointly established by manufacturers and sellers of carbon nanotubes. The global production capacity of carbon nanotubes today is over 2.5 tonnes/day (ENHRES, 2009) and approximately 710 tonnes of carbon nanotubes was expected to have been produced in 2010, representing about 17% of production capacity (IRAP, 2011). The actual production of carbon nanotubes is predicted to be more than 9,300 tons in 2015 (IRAP, 2011). Production capacity for both SWCNT and MWCNT is 2-3 times higher in Asia compared with Europe and the U.S. together, see Table 1. (WTEC, 2007; iRAP, 2011). Japan leads the world in production of MWCNT, but China and Korea will soon catch up (IRAP, 2011). In Japan, the use of MWCNT in lithium-ion battery electrodes has driven up production volumes (IRAP, 2011). North America seems to focus more on research and the production of SWCNT (WTEC, 2007). Table 2 shows which countries have companies that produce carbon nanotubes and their production capacity. In Scandinavia there is only one Norwegian company producing MWCNT (Schneider et al., 2007; KEMI, 2009). At least three companies in Sweden conduct research and development of carbon nanotubes in composite materials primarily for the aircaft industry (Schneider et al., 2007: personal communication with P Fernberg).

Carbon nanotubes are usually made with three different techniques, chemical vapour deposition (CVD), arch discharge and laser ablation.

Table 1. The global production capacity for SWCNT and MWCNT (WTEC, 2007).

Continent	**Production capacity**	
	SWCNT (kg/year)	**MWCNT (tpa)**
Norh America	1457	74
Europe	100	27
Asia	5302	170
Total capacity	6859	271

Table 2. Manufacturers of carbon nanotubes and their production capacity (according to www.nanotube-suppliers.com and WTEC, 2007).

Continent	Country	No. of compa-nies	Type of carbon nanotubes produced		Production capacity	
			SWCNT	MWCNT	SWCNT (kg/year)	MWCNT (tpa)
Europe	Belgium	1	Yes	Yes		400
Europe	Cyprus	1	No	Yes		0,2[a]
Europe	France	2	No	Yes		
Europe	Greece	1	Yes	Yes		1
Europé	United Kingdom	1	Yes	Yes		
Europe	Germany	2	Yes (1)	Yes (2)	~25[b]	~5[b]-200
Europe	Austria	1	Yes	Yes		5-10
North America	USA	21	Yes (17)	Yes (16)	12-600[c]	10-50[c]
North America	Canada	4	Yes (4)	Yes (2)	720[d]	
Asia	China	5	Yes (4)	Yes (5)	~25-500[b]	~0,6-10[b]
Asia	Korea	1	Yes	Yes	2,4	10
Asia	Japan	5	Yes (3)	Yes (2)	600-1500	30-100
Asia	India	1	Yes	Yes		
Asia	Taiwan	1	No	Yes		

[a] Production capacity to be increased soon to about 10 tons/year

[b] The production capacity has been recalculated as it was specified per day

[c] Figures for production are incomplete as they were missing for some manufacturers

[d] Only one manufacturer has specified production capacity

6.2.1 Chemical vapour deposition

CVD is the most common and widely used manufacturing technique due to its low start-up cost, high yield, purity, and because it can easily be scaled-up. CVD is based on the thermal decomposition of gaseous hydrocarbon in the presence of a metal catalyst such as iron, cobalt, nickel or yttrium. Gases containing carbon such as carbon dioxide, methane, acetylene are led into an oven to be heated up. The heated gas reacts with the metal catalyst, which also acts as a "growth seeds". Ethylene, benzene or xylene can also be used in the manufacturing process. With CVD, both MWCNT and SWCNT are produced, but the quality is better with MWCNT. An industrial variant of CVD is HiPCO and is used to mass-produce carbon nanotubes. Commercially available raw materials of carbon nanotubes can contain large amounts of pollutants, up to 40%, and these consist of amorphous carbon, nano-structured graphite and carbon encapsulated metal particles from the catalyst (Köhler et al., 2008).

6.2.2 Arc discharge

The arc discharge technique was the technique used when carbon nanotubes were first discovered (Iijima, 1991). The technology is based on the application of a sufficiently high voltage field over two graphite rods - an anode and a cathode - for it to form a stable arc discharge between the rods. The carbon nanotubes grow on the cathode while the anode is consumed. The distance between cathode and anode is kept constant while the anode's position is adjusted. The entire process takes place in helium gas. In order to produce SWCNT, the electrodes are doped with a small amount of metal particles which act as a catalyst (Thostenson et al., 2001; Journet et al., 1997, Shi et al., 2000). The diameter of the nanotubes generated is determined by the metal catalyst (Shi et al., 2000; Popov 2004). The exact process varies with the size and shape of the graphite rods, doping etc. In general, this method generates carbon nanotubes of high quality and has the advantage that it is a relatively cheap method, but a major disadvantage is that the amount of impurities is high (Donald et al., 2006).

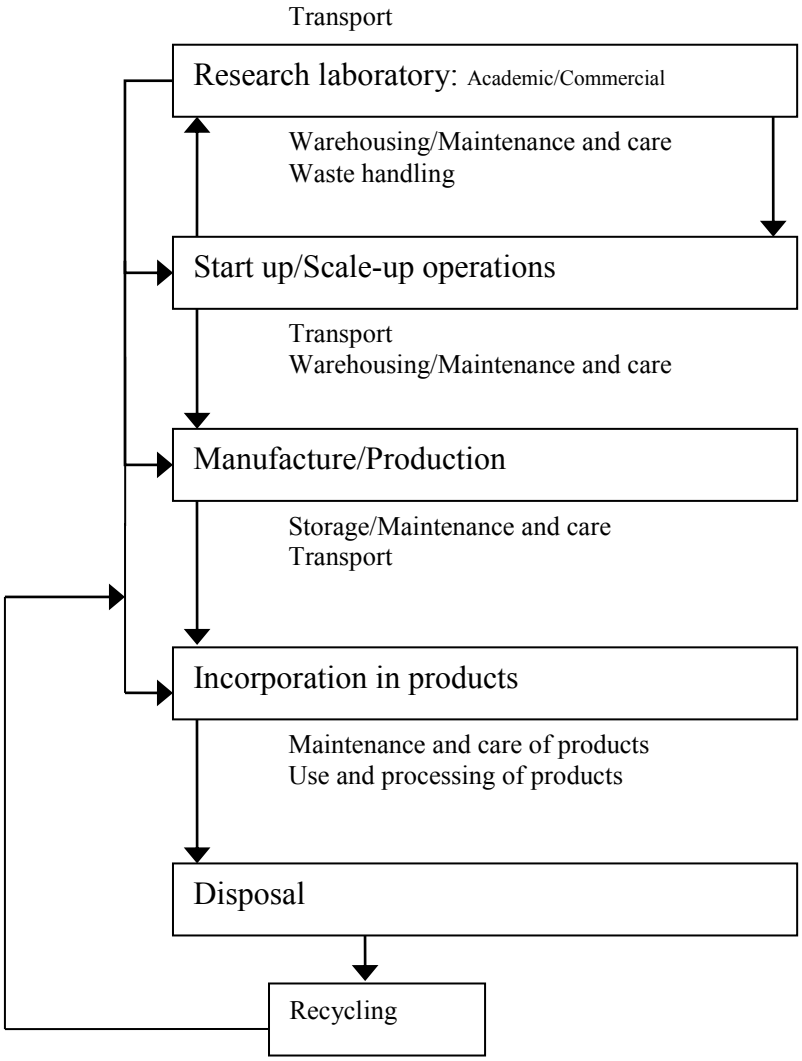
6.2.3 Laser ablation

Laser ablation is a technique that was introduced for the production of carbon nanotubes by Smalley and co-workers (Guo et al., 1995a). As with the arc discharge technique, laser ablation was used first only to generate MWCNT, but has since been refined through for example, the introduction of catalytic particles (cobalt and nickel compounds) and can now also be used for production of SWCNT (Guo et al., 1995b; Rinzler et al., 1998; Thostenson et al., 2001). In laser ablation a graphite target is kept at 1200 °C in a chamber. Laser pulses of high energy are used to vaporize the graphite target, and carbon nanotubes grow on a cooled object in the chamber as the vaporized carbon condenses. Everything is done in an atmosphere of an inert gas, usually argon, which slowly flows through the chamber. If the object consists of pure graphite, MWCNT is generated, and to produce SWCNT, the graphite is doped with cobalt or nickel (Thostenson et al., 2001, Dai et al., 2002). The diameter of the SWCNT is controlled by the reaction temperature.

6.3 Occurrence in the work environment

Occupational exposure to carbon nanotubes can in principle occur at all stages in its life cycle, see Figure 2. Today, occupational exposure to carbon nanotubes may occur during the production of raw materials for carbon nanotubes, research and development work at laboratories, the manufacture and processing of products

containing carbon nanotubes, and probably also in the recycling and disposal of these materials. Future areas of use for carbon nanotubes, for example in health care – when administering medication or diagnostic imaging, can also potentially provide occupational exposure to those who manufacture and administer this (Kostarelos et al., 2009; Aschberg et al., 2010).



Figur 2. Potential life-cycle for carbon nanotubes with defined steps in which occupational exposure may occur.

6.3.1 Occupational exposure in the manufacture of carbon nanotubes

In the manufacture of carbon nanotubes, typically closed production systems are usually employed. This means that no occupational exposure to carbon nanotubes is expected during the actual synthesization phase in commercial production, but exposure is likely in subsequent phases (Aschberg et al., 2010). During the development phase of carbon nanotubes, it is likely that the material is produced under strictly controlled conditions and is usually in very small volumes (Aschberg et al. 2010). The various operations required to produce carbon nanotubes are described in Figure 3. A number of production cycles can be made every day, up to 5 are described in the literature (Lee et al., 2010).

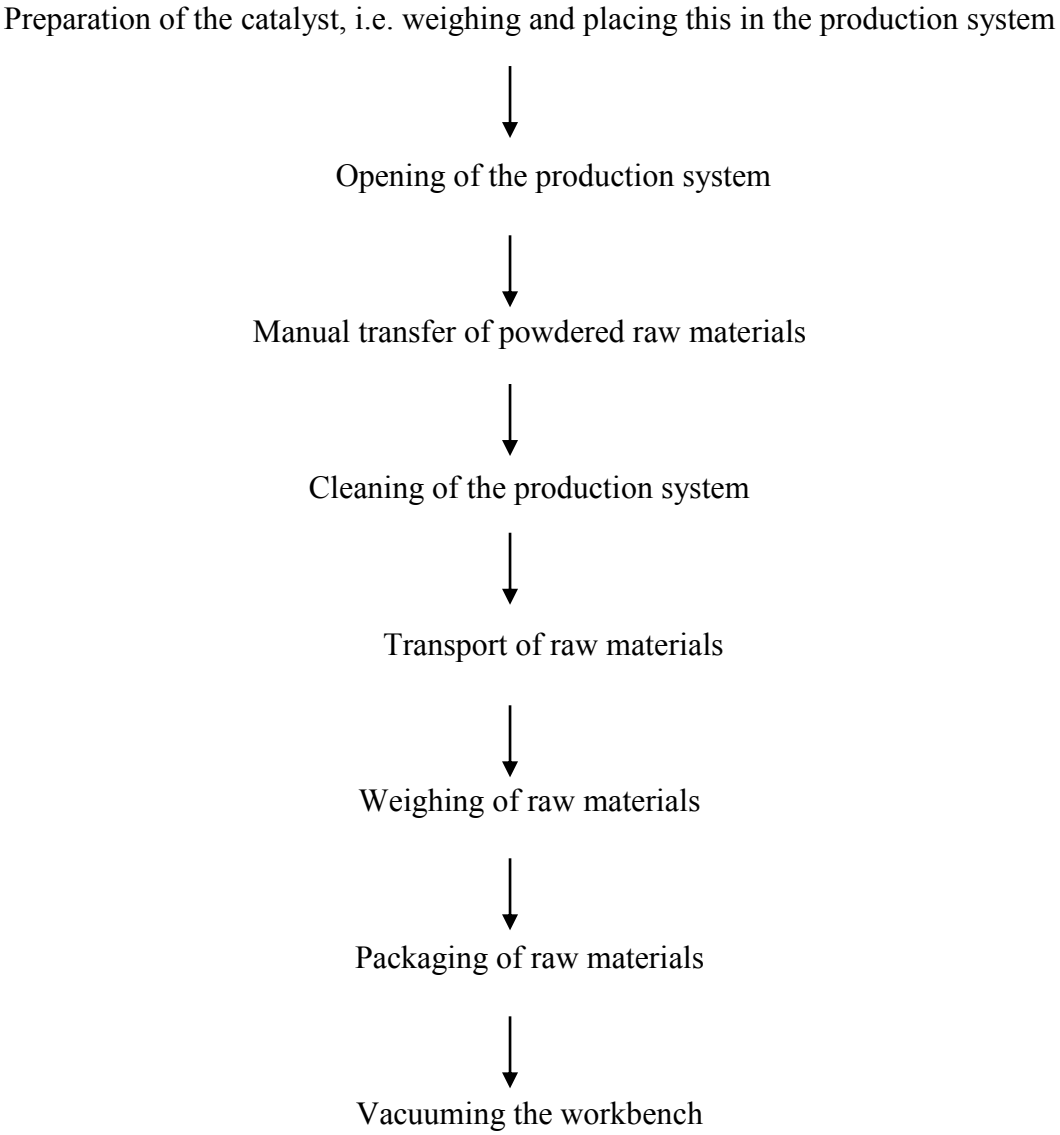


Figure 3. Work operations in the manufacture of carbon nanotube

Emissions of carbon nanotubes and therefore exposure to carbon nanotubes may occur when you open the cover to the closed production vessel (Lee et al., 2010). Exposure may also occur when handling the manufactured carbon nanotube powder through for example manual transfer from the production vessel (Figure 4), (Maynard et al., 2004; Aschberg et al., 2010). The production container should be enclosed in some form of clean-air enclosure when you conduct the manual transfer of the raw material (Maynard et al., 2004). Furthermore, handling such as weighing (Figure 5) and the packaging of raw materials may also lead to exposure to carbon nanotubes. The highest exposure is likely when handling dry, carbon nanotube materials in powdered form (Aschberg et al., 2010). Cleaning operations and maintenance of equipment and the workplace may also lead to exposure to carbon nanotubes (Helland et al. 2007, Köhler et al. 2008; Aschberg et al., 2010). Waste management, for example materials from the ventilation system may also lead to a potential exposure to carbon nanotubes (Köhler et al., 2008).

Figure 4. The manual transfer of carbon nanotubes from a production vessel (courtesy of PA Schulte, National Institute for Occupational Safety and Health [NIOSH]).

6.3.2 Occupational exposure in laboratory work

Below is a description of typical work operations that nanotube raw materials undergo and that can cause occupational exposure. The raw materials must be purified and these purification processes include operations such as weighing, sonication with an ultrasonic bath and chemical treatments with acids. Even after-treatments such as mixing, shaking, drying, packaging, dispersion (the mixture of carbon nanotubes in liquid) and functionalization, which includes several stages of sonication, are carried out (Helland et al., 2007; ENRHES, 2009; Aschberg et al., 2010; Methner et al., 2010). It is common that carbon nanotubes are in suspension to be more easily administered in experimental models (Johnson et al., 2010). Other operations which take place in laboratories may for example include the production of a thin carbon nanotube film by spraying the dispersed carbon nanotubes on silica wafers, which are then heated for 10 minutes (Han et al., 2008, Lee et al., 2010). The spraying can be done in some form of

protective enclosure from which carbon nanotubes may be emitted when the protective enclosure is opened (Lee et al., 2010).

There are studies that indicate that the laboratory personnel may be subjected to an increased risk of exposure to carbon nanotubes, especially to functionalized (Johnson et al., 2010).

Figure 5. Weighing MWCNT (courtesy of PA Schulte, NIOSH)

6.3.3 Occupational exposure during the machining of products containing carbon nanotubes

In the literature only the manufacture of lithium-ion batteries containing carbon nanotubes has been described as of yet (Köhler et al. 2008) and the machining of composite materials containing carbon nanotubes with a saw and a drill (Bello et al., 2009, 2010). In battery manufacturing, there is a potential risk of emissions of carbon nanotubes into the work environment during each stage of manufacturing (mechanical milling of carbon nanotubes, preparation of the electrode material, the assembly of electrodes, the unwinding of the electrodes) until the lithium-ion cell is sealed. Other potential sources of emissions of carbon nanotubes can be the waste from the production process or contaminated surfaces in the workplace (Köhler et al., 2008). Machining by sawing or drilling in composite laminates containing carbon nanotubes may also release carbon nanotubes. So far it has only been demonstrated that of aggregates of carbon nanotubes are emitted, thus providing airborne exposure (Bello et al., 2009, 2010).

7. Possibilities of measuring exposure in a work environment

7.1 Airborne Exposure

Traditional occupational hygiene measurements of particulates in the air are based on whether the particles are fibrous or non-fibrous. Fibrous particles are measured as the number per unit volume (fibre/cm^3), for non-fibrous particles mass is determined per unit volume (mg/m^3). For carbon nanotubes it is not known today which unit of measurement that best correlates to the toxicological effects when taking air measurements (Savolainen et al., 2010). Below is a description of the exposure measurements carried out in areas where carbon nanotubes were manufactured, processed and used. To determine occupational exposure to carbon nanotubes, total dust, respirable dust, respirable fibre concentration or the number of carbon nanotubes in the air was measured.

7.1.1 Determination of total dust

In order to sample carbon nanotubes, "open-face" filter cassettes with "mixed cellulose ester" (MCE) filters were used (Han et al., 2008, Lee et al., 2010) or "methyl cellulose ester" filters for metals (Maynard et al., 2004). Both stationary and personal sampling were performed for total dust. The concentration of carbon nanotubes in air was determined by gravimetric analysis. No detection limits (limit of detection, LOD) are reported. In one study the iron and nickel content was determined for the filters by inductively coupled plasma mass spectrometry (ICP-MS) as a surrogate for the carbon nanotubes mass (Maynard et al., 2004). LOD for iron and nickel were 0.064 µg an 0.018 µg respectively.

7.1.2 Determination of respirable dust

One study has also carried out personal sampling of both respirable dust and total dust, but the sampling methods are not specified (Takaya et al., 2010).

7.1.3 Determination of respirable fibre concentrations

Sampling of respirable fibre concentration was carried out by sucking air through a filter cassette (equipped with an electrically conductive 50 mm cowl) for asbestos with an MCE filter (Bello et al., 2008, 2009, 2010). In these studies, the analysis of the respirable fibre concentration was carried out (length > 5µm and the length-width ratio > 3:1) with a phase-contrast microscope in accordance with the NIOSH Manual of Analytical Methods (NMAM) Method 7400 (LOD ~ 250 nm in diameter; Schulte et al., 2010), and also a determination of the respirable carbon nanotubes with a scanning electron microscope (SEM).

7.1.4 Determination of the number concentration of carbon nanotubes

Sampling of the number concentration of carbon nanotubes was carried out by sucking air through a filter cassette (equipped with an electrically conductive 50 mm cowl) for asbestos with an MCE filter (Han et al., 2008). The filters were analyzed by transmission electron microscopy (TEM) according to NIOSH NMAM method 7402 (Han et al., 2008). The LOD of the method was <0.01 fibre/cm^3.

7.1.5 Characterization by aerosol instruments

Occupational exposure to carbon nanotubes has also been characterized by measuring other parameters than the mass or the number concentration of respirable fibres. Workplaces with the presence of carbon nanotubes (manufacturers, laboratories for research and development) have measured the number concentration, size distribution, surface area, morphology, size and chemical composition (Maynard et al., 2004, Bello et al., 2008, 2009, 2010; Yeganeh et al., 2008; Bello et al., 2009; Methner et al., 2010; Johnson et al., 2010; Lee et al. 2010). The measurements were usually stationary, but in two studies samples were also collcetedof the air in the breathing zone (Bello et al., 2009, 2010). Table 3 presents a summary of the various direct-reading aerosol instruments that have been used to monitor carbon nanotubes in air at different workplaces.

7.2 Dermal exposure

So far, the potential dermal exposure to carbon nanotubes has been measured by using glove contamination as a surrogate for real skin contamination (Maynard et al., 2004). By placing a pair of cotton gloves over the protective gloves normally worn, the potential skin exposure could be measured. The cotton gloves were removed immediately after handling the carbon nanotubes and placed in a sealed plastic bag. The gloves were analyzed by ICP-MS, and iron and nickel were determined as a surrogate for the carbon nanotube mass. The detection limit for iron and nickel was 0.016 μg and 0.046 μg respectively.

7.3 Summary and discussion

Characteristic of carbon nanotube exposure is that the mass is small, but the number concentration of nanoparticles is very high. The majority of the exposure measurements at various workplaces where carbon nanotubes are produced or used have been traditional dust measurements. When conducting dust measurements, there is a degree of uncertainty with regard to the results because you can also sample other particles (lack of specificity). Even when sampling respirable fibres, there is a lack of specificity as all types of fibres are sampled. The error can however be estimated through the characterization of fibres with TEM/SEM/STEM. In two studies, in which exposure measurements were carried out in the machining of composite materials containing carbon nanotubes, the respirable fibre concentration was determined using a method (NIOSH NMAM 7400) that can only analyze respirable fibres that are of the micron-size (Bello et al., 2009, 2010). Therefore, no individual carbon nanotube or agglomerated carbon nanotube can be determined. You need to agree on the measurement method to be used to measure occupational exposure to carbon nanotubes in order to achieve reliable exposure data. So far, different methods have been used, which have resulted in exposure data with different units, which complicates comparisons. Exposure data based on the respirable fibre concentration or the number concentration of carbon nanotubes has been determined by

TEM/SEM/STEM analysis, such as the NIOSH NMAM method 7402, which is regarded as most relevant to carbon nanotubes, because you can identify the nanotubes and in this way ensure specificity.
Today, NIOSH is working to validate whether the NIOSH NMAM method 5040 for diesel particulate matter (analysis of elemental carbon) can also be used to determine the mass of carbon nanotubes, as they are composed mainly of carbon (Methner et al., 2010a, 2010b). The sampling of carbon nanotubes could then be done with "open-face" filter cassettes with a quartz filter (to determine the total dust) to be subsequently analyzed by thermal-optical analysis with a flame ionization detector (FID). Simultaneous sampling of "open-face" filter cassettes with an MCE filter (determination of total dust) for TEM analysis (NIOSH NMAM 7402) would still be needed to ensure specificity (Methner et al., 2010a).

Table 3. Summary of the techniques used to characterize airborne carbon nanotube exposure in the workplace

Parameter	Technique	Measuring range	Detection limits	References
Number-concentration	Fast Mobility Particle Sizer (FMPS)	5,6-560 nm	Lower: ~100 prticles/cm^3 vid 10 nm for ~ 10 particles/cm^3 at 100 nm Upper: ~1000000 particles/cm^3	Bello et al., 2008, 2009, 2010
	Scanning Mobility Particle Sizer (SMPS)	14-630 nm		Han et al., 2008; Yeganeh et al., 2008; Methner et al., 2010
	Aerodynamic particel Counter (APS)	0,5-20 µm	Upper: 10000 particles/cm^3	Bello et al., 2008, 2009, 2010; Han et al., 2008
	Condensation particle counter (CPC)	10-1000 nm 14-500 nm	1-100000 particles/cm^3	Maynard et al., 2004; Bello et al., 2008, 2009; Methner et al., 2010; Johnson et al., 2010; Lee et al., 2010
	Ultrafine condensation particle counter (UCPC)	>3 nm	0-100000 particles/cm^3	Lee et al., 2010
	Electrical low-pressure impactor (ELPI)			Methner et al., 2010
	Optical particle counter (OPC)	300-10000 nm	Upper: 70000 particles/cm^3	Maynard et al., 2004; Methner et al., 2010; Johnson et al., 2010
Size distribution	SMPS and differential mobility analyser (DMA)	4-673 nm 14-673 nm 5-500 nm		Yeganeh et al., 2008; Han et al., 2008; Methner et al., 2010; Lee et al. 2010
	FMPS and APS			Bello et al., 2008, 2009, 2010
	UCPC	14-630 nm		Han et al., 2008

	Aersolspectro-meter (Dust monitor)	0,25-32 µm		Lee et al., 2010
Surface area	APS	0,5-20 µm		Han et al., 2008
Mass-concentration	OPC	300-10000 nm		Maynard et al., 2004
	Aerosol-photometer (Dust Trak)	<2.5 µm	Upper: 100 mg/m^3	Yeganeh et al., 2008; Bello et al., 2009
	Aethalometer (measures carbon black)			Han et al., 2008; Lee et al., 2010

Parameter	Technique	Measuring range	Detection limits	References
Morphology and size	Thermophoretic precipitator (TP)	1- >100 nm		Bello et al., 2008, 2009, 2010; Methner et al., 2010; Johnson et al., 2010
	Electric precipitator (ESP)	1- >100 nm		Bello et al., 2008, 2009, 2010; Methner et al., 2010; Johnson et al., 2010
	Transmission electron microscopy (TEM)		Lower: 1 nm	Bello et al., 2008, 2009, 2010; Methner et al., 2010; Johnson et al., 2010
	Scanning electron microscopy (SEM)			Maynard et al., 2004, Bello et al., 2008, 2009, 2010
	Scanning Transmission electron microscope (STEM)			Han et al., 2008
Chemical composition	Phtotelectric aerosol sensor (PAS)		Upper: 1000 ng/m^3	Yeganeh et al., 2008
	Energy-dispersive X-ray (EDX)			Han et al., 2008; Bello et al., 2009

8. Occupational exposure data

As of today, there is limited occupational exposure data available for airborne carbon nanotubes, see Table 4. In a few studies, air measurements have been conducted during specific work tasks, such as the growth of carbon nanotubes by CVD techniques or the removal of raw materials from a production vessel, therefore the sampling time is short (0.5 to 1.5 h; Maynard et al. 2004 ; Bello et al., 2008). Air sampling of total dust from carbon nanotubes has so far been made at seven production units, two packaging units and eight laboratories. Air sampling of respirable dust has been conducted at two packaging units. Respirable fibre concentration has been measured at four laboratories.

8.1 Mass concentration

8.1.1 SWCNT

Only one study has investigated occupational exposure to SWCNT (Maynard et al., 2004). At four U.S. companies that manufactured SWCNT, personal sampling of total dust was conducted. Production vessels were placed in a clean-air enclosure and personal sampling were made for a period of 30 minutes as the worker emptied the production vessel. Concentrations of SWCNT were measured at between 0.7 and 53 µg/m^3. The highest peak value from the measurements with direct reading instruments (OPC) was estimated at approximately 1,600 µg/m^3 and was measured when cleaning with a vacuum cleaner inside the clean-air enclosure (only the tube was inserted into the clean-air enclosure).

8.1.2 MWCNT

Most exposure measurements have been made to date in the workplace where MWCNT are produced and handled. Several measurements showed total dust concentrations of around 100 µg/m^3 or less (Table 4). The first exposure measurements were made at three research laboratories (Han et al., 2008). The measurements were taken before and after the installation of technical safety and protection devices. Before installation, personal exposure ranged from undetectable (ND - Not Detected) to 332 µg/m^3. After installing the fans and encapsulation, personal exposure was measured at between ND and 31 µg/m^3. Stationary sampling was also carried out with the sampler placed 3-4 m from the workstation. The stationary air concentration was ND-435 µg/m^3 before, and ND-39 µg/m^3 after the installation of the technical safety and protection equipment.

Another study conducted exposure measurements at 7 workplaces that manufactured or handled MWCNT (Lee et al., 2010). The measured average concentration of total dust was determined as 106 µg/m^3 and 81 µg/m^3 for personal and stationary samples performed. The emission of nanoparticles and fine particles occurred mainly at the opening of the cover to the growth chamber. Other work processes that could produce particle emissions include preparing the catalyst, spraying, the preparation of carbon nanotubes, dispersion with ultrasound, heating, and opening the cover to the water bath. Personal sampling of the MWCNT was carried out on workers in two packaging units, of which one was manual and the other one was automated (Takaya et al., 2010). The measured background concentration of total dust was almost the same (240

µg/m^3) in the two units. Those who worked with manual packaging of carbon nanotubes were exposed to air concentrations of 2,390 µg/m^3 (total dust) and 390 µg/m^3 (respirable dust). At the automated packaging process, concentrations of 290 µg/m^3 (total dust) and 80 µg/m^3 (respirable dust) were measured.

From the exposure data in Table 4, it is possible to assess the personal exposure to a specific operation or exposure situation, see Table 5. Operations with the highest exposure included manual packing and mixing in an open container. Machining with a bandsaw also produced a high exposure, but probably the majority of the measured particles are comprised of other components in the composite material, such as epoxy, carbon fibre and aluminium.

8.2 Respirable fibre concentration

The respirable fibre concentration was determined through the machining of composite materials containing carbon nanotubes to 1.6 fibre/cm^3 when sawing and 0.7 to 1.0 fibre/cm^3 when drilling (Bello et al., 2009, 2010).

8.3 Number of carbon nanotubes

In a research laboratory personal sampling of respirable fibre content were conducted for the manufacture of carbon nanotubes (CVD technology) and during the subsequent handling of carbon nanotube materials, but no individual fibres or bundles of nano-size fibers could be detected in the analysis (Bello et al., 2008). In a previously described study that measured the personal exposure before, and after, the installation of technical safety and protection equipment, levels were measured at 194 fibre/cm^3 (before) and 0.02 fibre/cm^3 (after, Han et al., 2008). For mechanical processing by sawing or drilling in composites containing carbon nanotubes, no individual, or bundles of carbon nanotubes could be determined (Bello et al., 2009, 2010).

Table 4. Exposure data from workplaces where they manufactured and handled carbon nanotubes and in the machining of composite materials containing carbon nanotubes

Workplace	Operation	Type of carbon nanotubes	Sampling	No. Of samples	Sampling time (h)	Dust fraction	Air content		Sampling method	Reference
							Mass konc., µg/m^3 median (range)	Number CNT, #/cm^3 median (range)		
4 manufacturing units	Empty of carbon nanotubes from production containers	SWCNT	Personal	4	0,5	Total	23 (0,7-53)	N/A	Open-face filter-kassett[a] with methyl cellulose ester filter (25 mm)	Maynard et al., 2004
1 research laboratory	Manufacturing	Carbon nanotubes	Personal	1	1,5		N/A	ND	Filter cassette for asbestos with MCE- filter (25 mm)[b,c]	Bello et al., 2008
1 research unit (with 3 laboratories)	Manufacturing, blending, grinding, weighing, spraying	MWCNT	Personal Stationary	8 10	4-6	Total	ND (ND-332) 37 (ND-435)	0,005 (ND-194) ND (ND-173)	Filter cassette with MCE- filter (35 mm)[c]	Han et al., 2008
1 research laboratory	Mechanical processing of carbon nanotube composites (sawing)	carbon nanotubes	Stationary	4			N/A	ND	Filter cassette for asbest with MCE-filter (25 mm)[b,c]	Bello et al., 2009
3 manufacturing units 4 forsknings-laboratorium	Manufacturing, handling	MWCNT	Personal Stationary		3-6,8	Total	106 (7,8-321) 81 (12,6-187) medel	N/A	Open-face filter cassette with MCE-filter (37 mm)[b]	Lee et al., 2010
2 packaging units	Packaging	MWCNT	Personal	2 2		Total Respirabel	1340 (290-2390) 235 (80-390) average	N/A	Sampler $PM_{4,0}$	Takaya M et al., 2010

N/A: Not available, sample not taken; ND: Not detectable; [a]Made of conductive material [b]MCE- mixed cellulose ester [c]Electrically conductive 50 mm cowl

Table 5. Specific operations and personal exposure levels to carbon nanotubes (max. levels are presented)

Exposure Situation/Operation		Personal exposure to total dust ($\mu g/m^3$)	References
Emptying the production vessel	HiPCO manufacturing	53	Maynard et al., 2004
	Laser ablation production	9,9	
Manufacturing	Before the installation of safety and protection devices	ND[a]	Han et al., 2008
Milling	Before the installation of safety and protection devices	ND	
Spraying with carbon nanotube-containing solution	Before the installation of safety and protection devices	193	
	After the installation of safety and protection devices	31	
Blending	Open blender (before the installation of safety and protective devices)	332	
	Encapsulated blender (after the installation of safety and protective devices)	ND	
Manual packaging		2390	Takaya et al., 2010
Automated packaging		290	
Machining (band saw)		2400	Bello et al., 2009

[a]ND = Not detectable

8.4 Characterization

8.4.1 Number concentration and size distribution

8.4.1.1 Manufacturing units
When opening the cover to the growth chamber following the manufacturing of carbon nanotubes, concentrations of particulate matter were measured at 11,039 particles/cm^3 (geometric mean). The particles had a diameter of about 20 and 50 nm (Lee et al., 2010). Another study showed the significance of ventilation on opening the growth chamber, which measured 300 particles/cm^3 with ventilation and 42,400 particles/cm^3 without ventilation (Methner et al., 2010). Even when cleaning inside the clean-air enclosures used for the emptying of the production vessel, high particle concentrations were measured, max. ~760,000 particles/cm^3 (Maynard et al., 2004). There is also a study that could not detect any elevated levels of nanoparticles either in the manufacture of carbon nanotubes or their handling (Bello et al., 2008).

8.4.1.2 Research laboratories
When mixing carbon nanotube materials, concentrations of > 12,000 particles/cm^3 with a size range between 14-630 nm (Han et al., 2008). The handling of carbon nanotubes and fullerenes in a commercial laboratory resulted in a short-term elevated number concentration (Yeganeh et al., 2008). Weighing and functionalization of carbon nanotubes and sonication of water which contained carbon nanotubes also led to an increased number of particles in the air, up to 2,776 particles/cm^3 (Johnson et al., 2010). Weighing carbon nanotubes without ventilation, and the sonication of raw carbon nanotube materials resulted in number concentrations up to 1,580 and 2,800 particles/cm^3 (Methner et al., 2010). For the machining of carbon nanotube composite materials the highest concentrations of particulates was measured (Bello et al., 2009, 2010). The number of measured particles stationary (10 cm from the source) and in the breathing zone when sawing was done, were 294,000 and 153,000 particles/cm^3. For stationary sampling near the source (10 cm from the source) when drilling with high and low speed in carbon nanotube composites, 11 million and 3.9 million particles/cm^3 (max) were measured. The corresponding particle concentrations in the breathing zone were 1.3 million (high speed) and 2.9 million (low speed) particles/cm^3. Size distributions were polydispersive when sawing with maximum at 12, 20, 230 ± 20 nm and 1 ± 0.1 µm (Bello et al. 2009).

8.4.2 Particle morphology
The analysis of filter samples collected in the manufacturing of SWCNT indicated that the particles appeared to have a compact structure (Maynard et al., 2004). On the analysis of MWCNT a varied form was found, i.e. they occur both as single carbon nanotubes, multiple structures (agglomerated) and as clumps (Han et al., 2008). A maximum fibre length of 1.5 µm was also observed in this study. Filter samples from a manufacturing facility showed a lack of carbon nanotubes (Bello et al., 2008). Also filter samples from a laboratory where the raw materials of MWCNT were weighed and sonicated lacked typical carbon nanotubes structures (Johnson et al., 2010). When composite materials containing carbon nanotubes where machined, neither individual carbon nanotubes (when sawn), bundles of these, or carbon nanotubes that were attached to larger particles were found (Bello et al., 2009). When drilling in the same material it was found, however, that clusters of carbon nanotube aggregates were emitted (Bello et al., 2010). The size of these aggregates was in the respirable size range (a few microns).

8.5 Dermal exposure

In the manufacture and handling of carbon nanotubes, carbon nanotubes may be deposited on the skin if you do not have protective clothing (Lam et al., 2006). There is no literature on skin absorption of carbon nanotubes (Crosera et al., 2006). To date, one study examined the potential dermal exposure to carbon nanotubes by analyzing protective gloves (Maynard et al., 2004). Carbon nanotube deposits were determined to be 0.2 to 6 mg/hand. In most cases, contamination could be seen on the gloves at the end of the sampling period, see Figure 6. Most of the contamination of carbon nanotubes was on the inside of the hand, on the palm and fingers. Based on this study, the maximum skin exposure would be 12 mg/person (both hands) if you did not use gloves. By using gloves, dermal uptake via the hands may be reduced by 90% (ENHRES, 2009; Aschberg et al., 2010). The maximum daily skin exposure was judged to be 14.3 µg/cm^3 and day (ENRHES, 2009) and 1.2 mg/person (Aschberg et al., 2010). Carbon nanotubes produced with the HiPCO technology caused higher levels of contamination on the gloves (Maynard et al., 2004). Unprotected skin areas could also be exposed due to larger clumps of carbon nanotubes may become airborne and stay in the air for long periods (Maynard et al., 2004). These clumps can be deposited on surfaces, skin and protective clothing. Dermal exposure can be minimized by using protective gloves in combination with other personal protective equipment such as protective clothing and respiratory protective equipment.

Figure 6. Protective gloves contaminated with very fine carbon nanotube dust (courtesy of A Maynard, NIOSH, Baron et al., 2003)

8.6 Summary and discussion

A limited number of exposure measurements have been made to date in the workplace where carbon nanotubes are produced and used. Both stationary and personal sampling in the breathing zone have been made. The level of exposure depends primarily on work operations and on how effective the occupational safety and protective equipment is. The highest levels of occupational exposure to carbon nanotubes have been measured while handling dry carbon nanotube materials. It is important to note that occupational exposure to carbon nanotubes was higher at some workplaces in the absence of safety and protection devices where they also had poorer industrial hygiene. In order to conduct risk assessments for carbon nanotubes, exposure data based on personal sampling where the concentration in the breathing zone is determined is needed, partly throughout the working day (8 h) and partly on more working operations/exposure situations.

Measurement data suggests that the production of carbon nanotubes with HiPCO gives higher concentrations in the air and higher levels of contamination on gloves (Maynard et al. 2004). This may be due to HiPCO-manufactured carbon nanotube materials being more porous and having a lower density compared to other techniques described as providing more compact carbon nanotubes. Two studies also showed the importance of installing and using safety and protection devices to reduce exposure when handling and packing carbon nanotubes (Han et al., 2008; Takaya et al., 2010). Safety and protection devices can reduce exposure by 90% (Han et al., 2008).

According to WHO, the minimum length of a fibre is defined as 5 μm (WHO 1997). One study measured the maximum fibre length at 1.5 μm, which cannot be regarded as fibre according to WHO. For the machining of carbon nanotube composites rather high respirable fibre concentrations were measured compared to the occupational exposure limit for asbestos (0.1 fibre/cm^3). What was measured in these two studies are different types of respirable fibrous structures from composite materials. These fibres may consist for instance of carbon fibre epoxy and aluminium, which was found in the material. Furthermore, no forms of carbon nanotube structures could be detected, which may be because they were encapsulated in epoxy plastic. Perhaps it is that carbon nanotubes, which are mixed into various materials are not emitted as individual carbon nanotubes but either form large aggregates or are embedded in larger particles and fibres. However, Bello et al. (2010) has demonstrated that there is a potential for carbon nanotubes being released during drilling.

9. Toxicokinetics

9.1 Absorption and distribution

There are three major paths by which carbon nanotubes could get into the body: through inhalation, absorption through the skin and absorption through the gastrointestinal tract. In addition to this, nanoparticles may be injected into the bloodstream in future medical applications. In experimental studies of how carbon nanotubes behave in the body, carbon nanotubes have been given to laboratory animals through inhalation, instillation in the trachea (carbon nanotubes in a small volume of liquid), intravenous injection or intraperitoneal injection.

Water-soluble carbon nanotubes injected into the abdomen enter into the bloodstream and reach individual organs and the same applies to intravenously injected carbon nanotubes (Deng et al., 2007, Singh et al., 2006; Wang et al., 2008, Guo et al., 2007).

After tube feeding laboratory animals with carbon nanotubes, it was in one case no demonstrated absorption from the gastrointestinal tract into the blood (Deng et al., 2007; Kolosnjaj-Tabi et al., 2010). Two other studies show, however, actual absorption or systemic effects that indicate absorption from the gastrointestinal tract. One study found that SWCNT after tube feeding was absorbed in the intestinal cells and that they also were found in several internal organs, including the brain (Yang et al., 2010). In this study, very short carbon nanotubes with a length of 50-300 nm were used. The relevance of carbon nanotubes at this length for industrial applications is unclear. Genotoxic (DNA-damaging) effects on cells in rats' livers were seen after tube feeding them carbon nanotubes, suggesting that the

nanotubes in this study may have been passed from the intestines to the blood (Folkmann et al., 2009).

The injection of MWCNT into the bloodstream of experimental animals leads to the nanotubes quickly spreading to major organs such as the liver, spleen and kidneys. (Georgina et al., 2009, Yang et al., 2007, Keren et al., 2008, Kang et al., 2009, McDevitt et al., 2007; Cherukuri et al., 2006). Radioactively labelled SWCNT carrying hydroxyl groups (-OH groups) on their surface were taken up in in a manner similar to carbon nanotubes without functionalization in the liver and kidneys, but were accumulated in the stomach and the intestines (Wang et al., 2008). In some cases carbon nanotubes were also discovered in the urine and faeces (Georgina et al., 2009), as well as in bone tissue (McDevitt et al., 2007). Similar distribution patterns were observed after injection of carbon nanotubes into the abdominal cavity (Guo et al., 2007; Wang et al., 2008, McDevitt et al., 2007). Other surface modifications of carbon nanotubes lead to a different distribution. Polyethylene glycol (PEG) leads, for example, to SWCNTs remaining in some organs for up to 90 days (Yang et al., 2008a, Liu et al., 2008), while other molecular modifications lead to rapid excretion within a few hours (Singh et al., 2006).

Inhaled carbon nanotubes remain either in the lungs for a long time or are transported out through the airways via macrophages and mucociliary elevator. As mentioned earlier, it has not been shown that carbon nanotubes are transferred from the respiratory passages and out into the blood (Deng et al., 2007). However, in some cases it has been observed that MWCNT has crossed over to the pleura and adjacent lung tissue and has been transported with macrophages to the local lymph nodes (Mercer et al. 2010Porter et al., 2010; Ryman-Rasmussen et al., 2009a; Elgrabli et al., 2008).

The information on how carbon nanotubes behave after they have entered the digestive tract is limited. When mice are tube-fed with SWCNT of different lengths, particles were observed only in the stomach, small intestine, large intestine and faeces, and no adverse effects were observed (Deng et al., 2007). In contrast, in another study it was found that SWCNT given to mice via tube feeding, were taken up by the intestinal cells and were found in cells of internal organs, including the brain (Yang et al., 2010). Oxidative DNA damage was detected in the liver and the lungs of rats that were tube fed with carbon nanotubes, which further indicates that the passage of carbon nanotubes from the gastrointestinal tract into the circulation is possible (Folkmann et al., 2009).

The skin is the body's primary protection against foreign substances and has different levels of permeability for different substances depending on their physical and chemical properties. It has so far not been demonstrated that carbon nanotubes pass from the skin into the blood. MWCNTs, when injected under the skin (subcutaneously) however, were taken up in the blood and were found in the heart, liver, spleen, lungs, kidney and subcutaneous tissue (Jia et al., 2009).

9.2 Biotransformation

Carbon nanotubes are usually described as resistant to biological modifications which has been observed when laboratory animals were exposed (Deng et al., 2007; Yang et al., 2008b). However, physically and chemically modified MWCNT have been observed in the lungs of laboratory animals and carbon nanotubes in the blood bound to plasma proteins (Elgrabli et al., 2008; Cherukuri et al., 2006).

In *in vitro* systems, it has been seen that carbon nanotubes can bind to complement factors from plasma and serum which could explain some of the biological effects of carbon nanotubes (Hamad et al., 2008, Salvador-Morales et al., 2006). In addition, it has been found that functionalized carbon nanotubes can be defunctionalized in the liver (Yang et al., 2009) and that enzymatic degradation of carbon nanotubes that have been functionalized with antibodies can take place in white blood cells (neutrophils granulocytes) (Kagan et al., 2010) . Similar enzymatic degradation has been observed by carbon nanotubes in cell-free systems with peroxidases as the active enzyme (Allen et al., 2008).

9.3 Excretion and elimination

Carbon nanotubes can be eliminated from the airways by being taken up by macrophages and are expelled through ciliary movements of the mucociliary elevator. Approximately 16% of instilled MWCNT was left in the lungs of rats after 6 months according to one study (Elgrabli et al., 2008). Another study found that 81% of instilled MWCNT was left after 60 days (Muller et al., 2005). If an organism is exposed to carbon nanotubes through diet, the nanotubes are likely to pass through the digestive tract and be excreted with the faeces (Deng et al., 2007). Studies have shown that certain types of carbon nanotubes can be eliminated by the kidneys or faeces (Georgina et al., 2009, Lacerda et al. 2008b; McDevitt et al., 2007; Kolosnjaj-Tabi et al., 2010). Size and surface chemistry affect the rate at which carbon nanotubes are excreted, and the half-life period in blood varies widely among different kinds of carbon nanotubes, see Table 6 (Lacerda et al., 2008a; Wang et al., 2008; Yang et al., 2008a; Liu et al., 2008; Guo et al., 2007; Kang et al., 2009; Singh et al., 2006; Yang et al., 2007; Cherukuri et al., 2006).

Table 6. Half lives of carbon nanotubes in the blood (i.v. = intravenous, i.p. = intraperitoneal, PEG = polyethylene glycol, DTPA = diethylenetriamine pentaacetic acid).

Carbon nanotubes	Functionalization	Half-life	Administration	Species	Reference
MWCNT	-	3 h	i.v.	Mouse	Lacerda et al. 2008a
MWCNT	glucosamin	5.5 h	i.p.	Mouse	Guo et al. 2007
SWCNT	Hydroxyl	50 min	i.v.	Mouse	Wang et al. 2008
SWCNT	Pluronics F108	1 h	i.v.	Rabbit	Cherukuri et al. 2006
SWCNT	DTPA	3-3.5 h	i.v.	Mouse	Singh et al. 2006
SWCNT	chitosan	3-4 h	i.v.	Mouse	Kang et al. 2009
SWCNT	PEG	15 h	i.v.	Mouse	Liu et al. 2008
SWCNT	PEG	15.4 h	i.v.	Mouse	Yang et al. 2008

10. Toxic mechanisms

There are various theories as to why and how carbon nanotubes cause toxic effects, see details below. A common interpretation is that carbon nanotubes, due to certain physical and chemical properties, can cause oxidative stress. Oxidative stress means that the cells are subjected to stress from free radicals, specifically reactive oxygen radicals. In addition, carbon nanotubes can cause frustrated phagocytosis in macrophages and genotoxic effects. The different mechanisms are to some extent dependent on each other as frustrated phagocytosis may lead to inflammation which in turn can lead to oxidative stress. For those who wish further information on the mechanisms underlying the toxicity of carbon nanotubes in cell and animal testing, a recent paper by Johnston et al. (2010) is recommended reading.

10.1 Oxidative stress

The cause of the oxidative stress caused by carbon nanotubes is still being debated. Many studies however, assign the toxicity to the pollution that comes with carbon nanotubes following the manufacturing process (Shvedova et al., 2003). The toxic effects of oxidative stress could therefore be due to impurities and not the nanotubes themselves.

10.1.1 Oxidative stress can lead to DNA and protein damage

Reactive oxygen radicals can cause DNA damage in the form of mutations that have implications for cell division. DNA damage of this type has for example been observed in macrophages treated with carbon nanotubes (Migliore et al., 2010). Oxidative DNA damage has been found in the liver and the lungs of rats tube fed with carbon nanotubes (Folkmann et al., 2009). Similar observations were made in cardiac muscle cells after carbon nanotubes were instilled (sprayed) into the lungs of mice (Li et al., 2007b). The application of carbon nanotubes on the skin of mice resulted in oxidative stress and the subsequent modification of proteins in skin cells (Murray et al., 2009). It was found that carbon nanotubes can cause genotoxic effects of faulty chromosomes and so-called micronuclei by acting directly on DNA during cell division (Sargent et al 2009).

10.2 Frustrated phagocytosis

Macrophages are a type of white blood cells in our innate immune system whose job it is to eat up (phagocytose), envelop and destroy foreign objects in the body. It could be bacteria or small non-biological particles such as carbon nanotubes for example. If nanotubes are too long and rigid, macrophages cannot absorb them and destroy them andare constantly trying to break them down instead. This phenomenon is called “frustrated phagocytosis” (Brown et al., 2007). In the end, it can lead to the macrophage undergoing apoptosis, i.e.programmed cell death.

10.2.1 Frustrated phagocytosis can lead to inflammation and oxidative stress

Frustrated phagocytosis can result in an increased production of inflammatory proteins in macrophages. These inflammatory proteins, in turn, make other white blood cells become attracted to the tissue which can produce substances that lead to oxidative stress (Johnston et al. 2010) If inflammation occurs, there is a risk that granulomas will form, which is a prolonged accumulation of phagocytic inflammatory cells. Some results suggest that longer and thicker carbon nanotubes could cause greater effects than shorter and narrower tubes

with respect to, among other things, DNA damage and granuloma formation, both *in vivo* and *in vitro* (Poland et al., 2008, Yamashita et al., 2010).

11. Effects on cells and animal experiments

In many cases it is difficult to compare the results from different studies. There is, for example, no consensus on how to report the dose of carbon nanotubes. Some argue that the conventional amount measured in milligrams per kilogram (mg/kg) body weight is not sufficiently informative, but that the surface area of nanotubes must be taken into account as well as an indication of the surface that is dosed. Another aspect is that it the physical and chemical properties of the carbon nanotubes used are not always fully described. Diameter, length, surface area/unit weight and degree of purity are characteristics that could determine whether a particular type of carbon nanotube is toxic or not. How the laboratory animals are exposed to carbon nanotubes is also important. Experimental respiratory exposure may therefore produce different results. Instillation means a small drop containing carbon nanotubes is placed into the trachea of the animals and after that the carbon nanotubes start to spread. On inhalation, an aerosol is produced of the carbon nanotubes which the animals may inhale. Either the whole animal is exposed, or the head or just the nose. These different methods have different advantages and disadvantages. The deposition pattern, i.e. how the carbon nanotubes spread out throughout the respiratory passages, differs between the methods and can therefore produce different results (Li et al., 2007a). Such effects may be reflected in the subsequent toxicity evaluation in the study. In the following paragraphs, a selection of the most relevant animal and cellular studies are presented and summarized; they were found in systematic literature searches in scientific, medical and toxicological databases. The relevance assessment includes, among other things, that the purpose of the study was to investigate toxicity, that the control experiments carried out were sufficient, that the particles have been sufficiently characterized, and that the published studies were examined according to the peer-review model.

11.1 Mortality

The dose of carbon nanotubes that can be described as lethal is unknown because no studies have attempted to determine the minimum lethal dose. For mice it has been found that an instilled dose of 0.5 mg of SWCNT in the respiratory passages caused the deaths of 5 out of 9 animals (Lam et al., 2004). A single oral dose of 1,000 mg of carbon nanotubes/kg body weight did not cause death in any of the mice treated, therefore, the lethal dose is above this value according to the writers of the article (Kolosnjaj-Tabi et al., 2010).

11.2 Cardiovascular effects

Animal experiments have shown that carbon nanotubes may affect the heart and blood vessels after they have been dosed via the respiratory passages. The effects include increased expression of stress-related genes and the genes involved in the recruitment of immune cells as well as an increase in markers for oxidative stress. The latter increased in both the lungs, aorta and cardiac tissue (Erdely et al., 2009, Li et al., 2007b). Other effects are a negative impact on the regulation of blood pressure in the arteries and reduced blood flow to the coronary arteries and the degeneration of the heart tissue (Legramante et al., 2009, Tong et al., 2009). The effects on the cardio-vascular system were most likely not due to the nanotubes being transferred from the respiratory passages, but on the indirect effects for which the exact mechanism is unknown. However, it has been suggested that an increased

production of cytokines induced by carbon nanotubes can be a reason for the onset of systemic effects. (Erdely et al. 2009).

11.3 Effects on the liver and the spleen

If the carbon nanotubes come into the blood, they will reach the liver and spleen. The toxic effects of clean non-functionalized MWCNT on the liver include increased levels of inflammatory cells, increases in injury markers and the death of liver tissue (Ji et al., 2009, Zhang et al., 2010). The effects were dependent on concentration, which means that low doses were not toxic. For the liver, adverse effects (inflammation and fibrosis of the liver) were observed in mice after administration of 250 mg/kg MWCNT in the abdominal cavity. However, the dose, 250 mg/kg, is relatively high compared with other studies. MWCNT that has been functionalized with PEG had much less significant effects, suggesting that the toxicity depends on what the surface of the carbon nanotubes look like (Zhang et al., 2010). Following oral exposure, oxidative DNA damage was found in the rats' livers (Folkmann et al., 2009). The spleen is one of the organs where injected carbon nanotubes were taken up the most, but the toxic effects previously seen in animal studies are limited, with a transient increase in spleen weight (Deng et al., 2009, Schipper et al., 2008).

11.4 Effects on the airways

The effect of carbon nanotubes on the airways in laboratory animals has been studied through the instillation and inhalation of carbon nanotubes. After inhalation of MWCNT, increases in inflammatory cells have been observed as well as increased levels of injury markers (Ellinger-Ziegelbauer and Pauluhn, 2009). In another inhalation study it was seen that MWCNT could pass through the lung to its outer casing, the pleura, when treating mice with 30 mg/m^3 for a six-hour exposure. At the same time, pleural fibrosis was observed, which means that the connective tissue in the outer portion of the lung becomes covered with a thick layer of non-expansible fibrous tissue and increases in size (Ryman-Rasmussen et al., 2009a). Similar observations have been made after MWCNTs were instilled in the airways of mice. Carbon nanotubes were also found in the pleura. The penetration of the lung barriers was dependent on dose, and increased after treating the animals with 10 to 80 µg carbon nanotubes per animal. (Mercer et al., 2010). Fibrosis and carbon nanotubes that had translocated over to the pleura after treatment with MWCNT have been observed in other studies as well (Porter et al., 2010). The presence of granulomas has been reported in several studies of carbon nanotube treatment of the airways, both after inhalation and instillation of SWCNT or MWCNT (Muller et al., 2005, Mercer et al. 2010; Ma-Hock et al., 2009, Chou et al., 2008; Lam et al., 2004). One study compared the effect of instillation and inhalation, and concluded that inhalation produced worse effects than instillation (Shvedova et al., 2008b). This comparison is interesting since most studies use instillation to study exposure to carbon nanotubes and inhalation is a more relevant model.

In two studies on cultured lung epithelial cells, researchers have demonstrated that MWCNT and SWCNT can affect permeability between the cells. This means that the permeability barrier that the epithelial cells form against contaminants might be harmed and that harmful substances could penetrate between the cells and reach other parts of the lung tissue. Increased permeability was observed only for long carbon nanotubes (5-9 µm for MWCNT and 0.5-100 µm for SWCNT), while shorter carbon nanotubes had no effect. It is not known whether similar events can take place in live animals, but it has been observed that asbestos can cause similar effects in cell culture (Rotoli et al., 2009; Rotoli et al., 2008).

11.4.1 Co-exposure of the airways

There is evidence that carbon nanotubes can aggravate various allergic conditions in the respiratory passages. Laboratory animals which had induced asthma or allergy and which were co-exposed to carbon nanotubes have, in several cases, demonstrated an exacerbated allergic condition. Conditions deteriorated regardless of whether the animals were exposed to SWCNT or MWCNT (Nygaard et al., 2009, Inoue et al., 2009, Inoue et al. 2010; Ryman-Rasmussen et al., 2009b). Co-exposure of airways of mice to carbon nanotubes and then bacteria led to an exacerbation of the inflammatory response and a slower phagocytosis of bacteria (Shvedova et al., 2008a).

11.5 Irritation

Whether the carbon nanotubes give rise to irritation is not clear. In a study of eye irritation and carbon nanotubes, a so-called Draize test revealed no irritation in the eyes of rabbits after 72 hours (Huczko and Lange, 2001), while in another study, transient reddening of the eyes was observed (Kishore et al., 2009). There is a need for research in this area.

11.6 The nervous system

The effects of carbon nanotubes on the nervous system have been studied through cell culture and by using laboratory animals exposed by direct injection into the brain or by intragastric tube feeding. In some cases, there were no adverse effects of carbon nanotubes on the nervous system, for instance after direct injection (Bardi et al., 2009; Gaillard et al., 2009; Yang et al., 2010) whereas disturbances in electrical activity of neurons and effects on cell survival were observed (Xu et al., 2009; Belyanskaya et al., 2009). Ultrastructural studies using transmission electron microscopy on neurons in the brain showed that following tube feeding, carbon nanotubes were found mainly in lysosomes and to some extent in the mitochondria (Yang et al., 2010).

11.7 Skin

Today, it is unknown whether carbon nanotubes can penetrate the stratum corneum of the skin and reach further down the skin. Inflammation of the epidermis and dermis and increased collagen production has been observed after the carbon nanotubes were applied to the skin of laboratory animals. The reason for this type of damage is believed to be an increase in reactive oxygen radicals, causing what is known as oxidative stress (Murray et al., 2009). Studies of cultured cells have shown that carbon nanotubes can induce cell death, inflammatory proteins and oxidative stress (Shvedova et al., 2003). Results that contradict these negative effects are also available, where no effects were found on either laboratory animals or on cultured cells (Huczko and Lange, 2001, Kishore et al., 2009).

11.8 Reproduction

The knowledge of the effects of carbon nanotubes on reproduction is very limited. Repeated intravenous injection of water-soluble MWCNT resulted in absorption in the testicles and temporarily decreased fertility in male mice (Bai et al., 2010). Carbon nanotubes may affect embryonic development in zebra fish. MWCNT causes defects at low concentrations and cell death, embryonic death, delayed hatching and improper spinal cord development at high

concentrations (Asharani et al., 2008). Low concentrations of MWCNT caused no effects on embryos in another study. On the other hand, survival of their offspring was affected, the reason for this is unknown. (Cheng et al., 2009).

11.9 Genotoxic effects

The genetic effects of carbon nanotubes have mainly been studied in experiments on cultured cells. In several cases, the genetic effects have been studied and so-called micronuclei, breaks in the double-stranded DNA, chromosomal abnormalities, mutations and activation of enzymes that repair DNA have all been found. Such effects have been observed for both MWCNT and SWCNT and the cause is not clear, but some speculate that it may be metal contaminants in the carbon nanotubes that are the cause or that nanotubes have the same dimension as the cytoskeleton of the cell and therefore may affect chromosome separation during cell division. (Cveticanin et al., 2010; Zhu et al., 2007; Ochoa-Olmos et al., 2009; Migliore et al., 2010; Sargent et al., 2009; Patlolla et al., 2010; Lindberg et al., 2009; Jacobsen et al., 2008). Some studies showed no genotoxicity at all, which could be due to the nature of the carbon nanotubes used (SWCNT), but also on the purity and how well the particles were separated from each other in solution (Kisin et al., 2007; Zeni et al., 2008).

11.10 Carcinogenicity

The formation of mesothelioma or granuloma after MWCNT in the abdominal cavity has been demonstrated in some studies (Poland et al., 2008, Takagi et al., 2008, Sakamoto et al., 2009). Such findings suggest that carbon nanotubes could produce the same fibre effects as asbestos in the event that carbon nanotubes come in contact with the mesothelium, the tissue layer that lines the outside of the body's organs. The discovery of mesothelioma in the abdominal cavity was questioned in some studies, in which only granulomas were found (Varga and Szendi, 2010). One of the studies which showed formation of mesotheliomas used mice which were mutants for p53, a protein that controls cell division, and therefore more likely to develop cancer (Takagi et al., 2008). A long-term study over a period of two years after a single dose of MWCNT did not demonstrate the presence of cancer after the injection of carbon nanotubes into the abdominal cavity. The researchers pointed out that the study should be interpreted with caution as the absence of a reaction does not mean that carbon nanotubes cannot cause cancer. A possible explanation was that the nanotubes were too short (0.7 μm) to have any carcinogenic effect (Muller et al., 2009).

11.11 Short-term and long-term effects after repeated exposure (up to 90 days)

There are few long-term studies as to how carbon nanotubes affect animals and cells after repeated exposure. Most studies have been done using a single dose exposure whereupon laboratory animals were studied for different lengths of time, the longest so far being two years. For repeated exposure, laboratory animals have been studied for periods of up to 3 months. In one study rats inhaled MWCNT at doses ranging from 0.1 to 6 mg/m^3 for 6 hours a day, 5 days a week for 13 weeks. No effects were found at 0.1 mg/m^3 , but progressive effects such as inflammation and epithelial damage at 0.4 to 6 mg/m^3 (Pauluhn, 2010). In a similar study in which rats inhaled aerosolized MWCNT on 65 occasions over a three month period, the animals developed granulomas and exhibited inflammation in the

airways. If we calculate the dose to mg/kg body weight, the LOEL (Lowest observable effect level) occurs already at 0.23 mg/kg body weight for males and 0.3 mg/kg for female rats. The strongest effects were found at 0.5 to 2.5 mg/m^3, but because effects had already been found at 0.1 mg/m^3 it was not possible to determine a NOEL (No observable effect level). On the basis of using a relevant methodology and the low repeated doses which had an effect, this study is important for the risk assessment of carbon nanotubes (Ma-Hock et al., 2009). When mice were exposed repeatedly for a period of 7-14 days by inhaling similar doses of MWCNT, no effects on the lungs were observed. However an inhibition of the systemic immune defence was found (Mitchell et al., 2007).

11.12 Summary and discussion

In several studies, a clear correlation has been seen between the dose of carbon nanotubes and the biological effects that occur. Tables 7-11 summarize the relationship between the dose given to laboratory animals or cell cultures and the observed effects. A selection of representative studies have been made based on information found in the systematic literature searches. Some studies on animals and cells that are not included, but will be described in a document in preparation for the Nordic Expert Group for criteria documentation on chemical health risks, a document which is a further development of this report. In a number of studies, it was observed that respiratory exposure to carbon nanotubes may lead to the formation of granulomas, fibrosis and the transition of carbon nanotubes to the pleura. This occurs even at such low concentrations in the air as 0.1 mg/m^3. Progressive fibrosis occurs at 0.25 mg/kg body weight SWCNT on repeated exposure of mice, for example, for MWCNT mild granulomatous inflammation of the respiratory passages of rats was seen following repeated exposure for a total of 0.23 to 0.3 mg/kg body weight. Increased dosage leads in most studies to a worsened condition, see Tables 7 and 8 for further effects.

As for skin exposure, the effects on laboratory animals can be seen at 4.7 mg/kg body weight or 0.6 mg/kg bodyweight following subcutaneous injection of SWCNT and MWCNT. For superficial dermal exposure to SWCNT, there is a dose-response relationship, see Table 9.

Carbon nanotubes have so far not been shown to cause mesothelioma (cancer) of the pleura in laboratory animals. However mesothelioma arose after intraperitoneal injection in mice and in the scrotum of rats. The effects occurred at doses of 111-121 mg/kg body weight in mice and 1 mg/kg body weight in rats. Mesothelioma can occur in humans after exposure to asbestos. Both carbon nanotubes and asbestos fibres demonstrate similar characteristics: they have a length-width ratio greater than 3:1, some asbestos fibres have similar diameters of MWCNT (40 nm). They differ however in terms of their chemistry and structure. Both materials can be considered biopersistent, even if it is not entirely clear to what extent the carbon nanotubes can be broken down. Carbon nanotubes have a tendency to form aggregates in the form of balls or rope-like structures and this is due to their intrinsic hydrophobic properties, which make them difficult to dissolve in liquids without the use of detergents for example.

Table 7. Effects of airway exposure to SWCNT

Absolute dose	Dose (mg/kg)	Ani-mal	Num-ber	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference
5 µg (5mg/m^3)	0,25	Mouse	12	0,8-1,2 nm	100-1000 nm	Unmodified, 17,7% Fe-content	Inhalation	5 h/day i 4 days	Increase in inflammatory cells and proteins, progressive fibrosis	Shvedova et al. 2008b
5 µg	0,25	Mouse	12	0,8-1,2 nm	100-1000 nm	Unmodified, 17,7% Fe-content	Instillation	1 dose	Increased number of inflammatory cells, increase of inflammatory proteins	Shvedova et al. 2008b
10 µg	0,3	Mouse	4-6	-	-	Well separated	Instillation	1 dose	Transient inflammatory response, no granulomas observed. Increase of connective tissue thickness.	Mercer et al. 2008
10 µg	0,5	Mouse	12	0,8-1,2 nm	100-1000 nm	Unmodified, 17,7% Fe-content	Instillation	1 dose	Increased number of inflammatory cells, increase of inflammatory proteins	Shvedova et al. 2008b
10µg	0,5	Mouse	6	1-4 nm	1-3 µm	Unmodified	Aspiration	1 dose + bacterial/10 days	Increase in inflammatory cells	Shvedova et al. 2008a
10µg	0,5	Mouse	6	1-4 nm	1-3 µm	Unmodified	Aspiration	1 dose /10 days	Increase in inflammatory cells	Shvedova et al. 2008a
20 µg	1	Mouse	12	0,8-1,2 nm	100-1000 nm	Unmodified, 17,7% Fe-content	Instillation	1 dose	Increased number of inflammatory cells, increase of inflammatory proteins	Shvedova et al. 2008b
40 µg	1,3	Mouse	4	-	Aggregates approx 25-150 nm	Treated with acid or not	Aspiration	1 dose	Increased number of inflammatory cells, increase of inflammatory proteins	Saxena et al. 2007
40 µg	1,6	Mouse	4-8	1-2 nm	100-2000 nm	Unmodified, dissolved in PBS	Instillation	1 dose	After 30 days, inflammation and granuloma	Mutlu et al. 2010
40 µg	1,6	Mouse	4-8	1-2 nm	100-2000 nm	Unmodified, dissolved in Pluronics	Instillation	1 dose	Well separated CNTs caused no effects. Excretion via macrophages	Mutlu et al. 2010
40µg	2	Mous e	6	1-4 nm	1-3 µm	Unmodified	Aspiration	1 dose + bacterial/10 days	Increase in inflammatory cells, synergistic increase in collagen after CNT bacterial exposure, impaired phagocytosis of bacteria	Shvedova et al. 2008a

Absolute dose	Dose (mg/kg)	Animal	Number	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference
40μg	2	Mouse	6	1-4 nm	1-3 μm	Unmodified	Aspiration	1 dose/10 days	Increase in inflammatory cells, increase in collagen	Shvedova et al. 2008a
0,1 mg	3,3	Mouse	5	-	-	High levels of metal contaminants	Instillation	1 dose	Slight inflammation and granulomas in animals treated with contaminated CNT	Lam et al. 2004
-	4	Mouse	8	2-20 nm	100 nm - several μm	Unmodified	Instillation	1 dose	Treatment with CNT and 33 μg/kg LPS led to worsening pneumonia. Proinflammatory proteins increased in the blood.	Inoue et al. 2008
0,5 mg	16,5	Mouse	5	-	-	High levels of metal contaminants	Instillation	1 dose	More severe inflammation and granuloma, mortality 5/9 in a group	Lam et al. 2004
0,5 mg	-	Mouse	-	-	-	-	Instillation	1 dose	Activation of macrophages, the presence of granulomas, oxidative stress	Chou et al. 2008
50 μg × 7	1,6 × 7	Mouse	12-13	0,8-2 nm	100 nm -15 μm	-	Instillation	1 dose	Carbon nanotubes exacerbated allergic response in mice. T-helper chemo-and cytokines increased	Inoue et al. 2010

Table 8. Effects of airway exposure to MWCNT.

Absolute dose	Dose (mg/kg)	Animal	Number	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference
1 µg	0,005	Rat	6	20-50 nm	0,5-2 µm	Dispersed with BSA	Instillation	1 dose	Significant increase in phagocytosis 1 and 7 days after exposure	Elgrabli et al. 2008
-	0,04	Rat	-	60 nm	1,5 µm	Unmodified	Instillation	1 dose	No effects after 6 months	Kobayashi et al. 2010
10 µg	0,05	Rat	6	20-50 nm	0,5-2 µm	Dispersed with BSA	Instillation	1 dose	Significant increase in phagocytosis 1-90 days after exposure	Elgrabli et al. 2008
0,3 mg/m^3	0,2	Mouse	6	10-20 nm	5-15 µm	Unmodified	Inhalation	6 h/day	Repeated inhalation 7-14 days. Carbon nanotubes in the lung macrophages, no increase in white blood cells. Systemic immunosuppression	Mitchell et al. 2007
-	0,2	Rat	-	60 nm	1,5 µm	Unmodified	Instillation	1 dose	Observations 1 day - 6 months after dosing. No effects	Kobayashi et al. 2010
1 mg/m^3	0.2	Mouse	10	10-50 nm	1-10 µm	-	Inhalation	En 6 h exposure	Observed 1-14 days. No effects	Ryman-Rasmussen et al. 2009a
0,1 mg/m3 (46,8 µg/animal)	0,23-0,3	Rat	10	5-15 nm	0,1 -10 µm	9,6% AlO	Inhalation	6h/day on 65 occasions for 90 days	Mild granulomatous inflammation. No systemic effects. (LOEC)	Ma-Hock et al. 2009
1 mg/m^3	0,5	Mouse	6	10-20 nm	5-15 µm	Unmodified	Inhalation	6 h/day	Repeated inhalation 7-14 days. Carbon nanotubes in the lung macrophages, no increase in white blood cells. Systemic immunosuppression	Mitchell et al. 2007
10 µg	0,5	Mouse	6-8	49 nm	3,9 µm	Unmodified	Aspiration	1 dose	Observed 1-56 days. CNTs, which penetrated the alveolar epithelium	Mercer et al. 2010
100 µg	0,5	Rat	6	20-50 nm	0,5-2 µm	Dispersed with BSA	Instillation	1 dose	Increase in inflammatory cells after 7 and 180 days, significant phagocytosis 1-180 days	Elgrabli et al. 2008

20 µg	0,9	Mouse	6-8	49 nm	3,9 µm	Unmodified	Aspiration	1 dose	Observed 1-56 days. Granulomatous ulcers, CNT, which penetrated the alveolar epithelium	Mercer et al. 2010
-	1	Rat	-	40-60 nm	0,5-500 µm	Unmodified	Instillation	1 dose	Mild inflammation	Liu et al. 2008

Absolute dose	Dose (mg/kg)	Animal	Number	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference
-	1	Rat	-	60 nm	1,5 µm	Unmodified	Instillation	1 dose	Observations 1 day - 6 months after dosing. Transient inflammation. MWCNT in macrophages. Slight granuloma formation	Kobayashi et al. 2010
0,5 mg/m^3 (243 µg/djur)	1,2-1,6	Rat	10	5-15 nm	0,1 -10 µm	9,6% AlO	Inhalation	6h/day on 65 occasions for 90 days	Granulomatous inflammation, inflammation, lipoproteinosis. No systemic effects	Ma-Hock et al. 2009
0,05 mg	1,6	Mouse	5-6	50 nm	10 µm	Unmodified	Instillation	1 dose	Inflammation of the bronchi, damage to the alveoli, immune response	Li et al. 2007a
40 µg	1,8	Mouse	7-8	49 nm	3,9 µm	Unmodified	Aspiration	1 dose	Observed 1-56 days. Granulomatous ulcers CNT which penetreated the aveolar epithelium	Mercer et al. 2010
0,5 mg	2,2	Rat	4-6	9,7 nm	5,9 µm	Unmodified	Instillation	1 dose	No pathological findings, 81% of the dose remaining after 60 days	Muller et al. 2005
0,5 mg	2,2	Rat	4-6	11,3 nm	0,7 µm	Ground	Instillation	1 dose	Increase of TNF-alpha and soluble type I collagen, 36% in a dose remaining after 60 days	Muller et al. 2005
0,07 mg	2,3	Mouse	5-6	50 nm	10 µm	Unmodified	Inhalation	-	Repeated inhalation for 8 days. Proliferation and increase in the thickness of the alveoli	Li et al. 2007a
5 mg/m^3	2,7	Mouse	6	10-20 nm	5-15 µm	Unmodified	Inhalation	6 h/day	Repeated inhalation 7-14 days. Carbon nanotubes in the lung macrophages, no increase in white blood cells. Systemic	Mitchell et al. 2007

									immunosuppression	
	3	Rat	-	40-60 nm	0,5-500 µm	Unmodified	Instillation	1 dose	Severe inflammation, carbon nanotubes remaining after 3 months	Liu et al. 2008
80 µg	3,6	Mouse	6-8	49 nm	3,9 µm	Unmodified	Instillation	1 dose	Observed 1-56 days. Granulomatous ulcers, CNT, which penetrated the alveolar epithelium	Mercer et al. 2010
-	4	Mouse	8	2-20 nm	100 nm - serveral µm	Unmodified	Instillation	1 dose	Treatment with CNT and 33 µg/kg LPS led to worsening pneumonia. Proinflammatory proteins increased in the blood.	Inoue et al. 2008

Absolute dose	Dose (mg/kg)	Animal	Number	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference
30 mg/m^3	4	Mouse	10	10-50 nm	1-10 µm	-	Inhalation	6hr exposure	Observed 1-14 days. Focal subpleural fibrosis, inflammation	Ryman-Rasmussen et al. 2009a
100 mg/m^3	4, trakeobronkialt	Mouse	40	10-30/30-50 nm	0,5-40 µm	Unmodified, 5% Ni	Inhalation	6hr exposure	Airway fibrosis after 14 days in mice that have been co-exposed to ovalbumin, but not for mice that only received CNTs.	Ryman-Rasmussen et al. 2009b
0,14 mg	4,6	Mouse	5-6	50 nm	10 µm	Unmodified	Inhalation	-	Repeated inhalation for 16 days. Proliferation and increase in the thickness of the alveoli	Li et al. 2007a
-	5	Rat	-	40-60 nm	0,5-500 µm	Unmodified	Instillation	1 dose	Severe inflammation, carbon nanotubes remaining after 3 months	Liu et al. 2008
2,5 mg/m^3 (1170 µg/djur)	5,7-7,5	Rat	10	5-15 nm	0,1 -10 µm	9,6% AlO	Inhalation	6h/day on 65 occasions for 90 days	Granulomatous inflammation, inflammation, lipoproteinos. No systemic effects	Ma-Hock et al. 2009
-	7	Rat	-	40-60 nm	0,5-500 µm	Unmodified	Instillation	1 dose	Severe inflammation, carbon nanotubes remaining after 3 months	Liu et al. 2008

0,21 mg	7	Mouse	5-6	50 nm	10 µm	Unmodified	Inhalation	-	Repeated inhalation for 24 days. Proliferation and increase in the thickness of the alveoli	Li et al. 2007a
2 mg	8,9	Rat	4-6	9,7 nm	5,9 µm	Unmodified	Instillation	1 dose	Granuloma, alveolitis, fibrosis and other pathological changes after 2 months	Muller et al. 2005
2 mg	8,9	Rat	4-6	11,3 nm	0,7 µm	Ground	Instillation	1 dose	Granuloma, alveolitis, fibrosis and other pathological changes after 2 months, TNF-alpha	Muller et al. 2005
100 mg/m^3	12, alveolärt	Mouse	40	10-30/30-50 nm	0,5-40 µm	Unmodified, 5% Ni	Inhalation	6hr exposure	Airway fibrosis after 14 days in mice that have been co-exposed to ovalbumin, but not for mice that only received CNTs.	Ryman-Rasmussen et al. 2009b
5 mg	22,2	Rat	4-6	9,7 nm	5,9 µm	Unmodified	Instillation	1 dose	Granuloma, alveolitis, fibrosis and other pathological changes after 2 months	Muller et al. 2005

Absolute dose	Dose (mg/kg)	Animal	Number	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference
5 mg	22,2	Rat	4-6	11,3 nm	0,7 µm	Ground	Instillation	1 dose	Granuloma, alveolitis, fibrosis and other pathological changes after 2 months, TNF-alpha	Muller et al. 2005
20	-	Mouse	4	49 nm	3,86 µm	Unmodified	Aspiration	1 dose	Inflammatory response, some inflammation of the pleura, fibrosis.	Porter et al 2010
40	-	Mouse	4	49 nm	3,86 µm	Unmodified	Aspiration	1 dose	Inflammatory response, fibrosis	Porter et al 2010
80	-	Mouse	4	49 nm	3,86 µm	Unmodified	Aspiration	1 dose	Inflammatory response, some inflammation of the pleura, fibrosis. Carbon nanotubes in the pleura	Porter et al 2010
10	-	Mouse	4	49 nm	3,86 µm	Unmodified	Aspiration	-	Inflammatory response	Porter et al 2010
0,1 mg/m^3	-	Rat	50	10 nm	200-300 nm	-	Inhalation	6 h/day×5 days for 13 weeks	No effects (NOEL)	Pauluhn 2010
0,4 mg/m^3	-	Rat	50	10 nm	200-300 nm	-	Inhalation	6 h/day×5 days for 13 weeks	Damage to the epithelium. Increase in inflammatory cells	Pauluhn 2010

1,5 mg/m^3	-	Rat	50	10 nm	200-300 nm	-	Inhalation	6 h/day×5 days for 13 weeks	Damage to the epithelium, increase in inflammatory cells, translocation of the CNT to the lymph nodes	Pauluhn 2010
6 mg/m^3	-	Rat	50	10 nm	200-300 nm	-	Inhalation	6 h/day×5 days for 13 weeks	Increase in inflammatory cells, granulomatous effects, increases in bronchoalveolar hyperplasia, transport of the CNT to the lymph nodes	Pauluhn 2010
11 mg/m^3	-	Rat	-	10-16 nm	-	0,53% Co	Inhalation	1×6h, 3 months post exposure	Dose-dependent increase in inflammatory cells and cytotoxic markers, increased collagen production in the BAL	Ellinger-Ziegelbauer & Pauluhn 2009
241 mg/m^3	-	Rat	-	10-16 nm	-	0,53% Co	Inhalation	1x6h, 3 months post exposure	Dose-dependent increase in inflammatory cells and cytotoxic markers, increased collagen production in the BAL	Ellinger-Ziegelbauer & Pauluhn 2009
11 mg/m^3	-	Rat	-	10-16 nm	-	0,12% Co	Inhalation	1x6h, 3 months post exposure	Dose-dependent increase in inflammatory cells and cytotoxic markers, increased collagen production in BAL	Ellinger-Ziegelbauer & Pauluhn 2009

Table 9. Effects after skin exposure to MWCNT and SWCNT.

Absolute dose	Dose (mg/kg)	Animal	Number	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference	Type
0,5 mg	-	Rabbit	-	-	-	Dimension: 901 nm	Dermal	4 h	None	Kishore et al. 2009	MWCNT
0,5 mg	-	Rabbit	-	-	-	Dimension 554 nm	Dermal	4 h	None	Kishore et al. 2009	MWCNT
0,1 mg	0,6	Rat	-	20-50 nm	220 nm	Unmodified	Subcutaneous	1-4 weeks	Slight inflammation, no neutrophils, no necrosis	Sato et al. 2005	MWCNT
0,1 mg	0,6	Rat	-	20-50 nm	825 nm	Unmodified	Subcutaneous	1-4 weeks	Slight inflammation, no neutrophils, no necrosis	Sato et al. 2005	MWCNT
40 µg	2,35	Mouse	3	-	-	30% Iron	Dermal	1 dose/day, 5 days	No observed effects	Murray et al. 2009	SWCNT
80 µg	4,7	Mouse	3	-	-	30% Iron	Dermal	1 dose/day, 5 days	Several cells in the epidermis, oxidative damage	Murray et al. 2009	SWCNT
160 µg	9,4	Mouse	3	-	-	30% Iron	Dermal	1 dose/day, 5 days	More cells in the epidermis, thickening of the skin, collagen production, oxidative damage	Murray et al. 2009	SWCNT

Table 10. Cardiovascular effects after exposure to MWCNT, and SWCNT.

Absolute dose	Dose (mg/kg)	Animal	Number	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference	Type
-	0,064	Rat	8-10	0,9-1,7 nm	<1µm	-	Tube feed	1 dose, 24 hrs	Modified nucleotides in the DNA of the lung and liver	Folkmann et al. 2009	SWCNT
10 µg	0,3	Mouse	-	-	-	Unfunctionalized	Intratracheal	1 dose	No significant effects	Tong et al. 2009	SWCNT
10 µg	0,4	Mouse	-	0,7-1,5 nm	1 µm	-	Pharyngeal instillation	1 dose, 60 days	Damage to mitochondrial DNA in the aorta	Li et al. 2007b	SWCNT
-	0,64	Rat	8-10	0,9-1,7 nm	<1µm	-	Tube feed	1 dose, 24hrs	Modified nucleotides in the DNA of the lung and liver	Folkmann et al. 2009	SWCNT
-	1	Rat	8	1,2-1,6 nm	2-5 nm (sic!)	-	Intratracheal	2 does over 4 weeks	Modified baroreflex, the influence of autonomic cardiovascular control	Legramante et al. 2009	SWCNT
40 µg	1,3	Mouse	-	-	-	Unfunctionalized	Intratracheal	1 dose	Increase of creatine kinase, cardiac fibre degeneration	Tong et al. 2009	SWCNT
40 µg	1,3	Mouse	-	-	-	Acid-functionalized	Intratracheal	1 dose	Significant increase in size of infarction, creatine kinase, cardiac fibre degeneration	Tong et al. 2009	SWCNT
10 µg	1,3	Mouse	-	-	-	Acid-functionalized	Intratracheal	1 dose	No significant effects	Tong et al. 2009	SWCNT
40 µg	1,4	Mouse	-	0,7-1,5 nm	1 µm	-	Pharyngeal instillation	1 dose, 60 days	Damage to mitochondrial DNA	Li et al. 2007b	SWCNT
40 µg	1,5	Mouse	-	0,8-1,2 nm	0,1-1 µm	8,8 wt% Iron	Pharyngeal aspiration	4 h	Local and systemic effect on inflammatory parameters	Ederly et al. 2009	SWCNT
40 µg	1,5	Mouse	-	80 nm	10-20 µm	0,2% wt% Iron	Pharyngeal aspiration	4 h	Local and systemic effect on inflammatory parameters	Ederly et al. 2009	MWCNT
20 µg x 4	2,8	Mouse	-	0,7-1,5 nm	1 µm	-	Pharyngeal instillation	4 does over 8 weeks	Atherosclerosis was increased in ApoE-/- transgenic mice. Plaque formation in aorta, mtDNA damage	Li et al. 2007b	SWCNT

Table 11. Carcinogenic effects after exposure to MWCNT or SWCNT.

Absolute dose	Dose (mg/kg)	Animal	Number	Diameter	Length	Characteristics	Exposure form	Exposure time	Effects	Reference	Type
-	1	Rat	7	70-110 nm	2 µm	Unmodified	Intrascotal	1 dose, 1 year	Mesoteliom, 6/7 animals died	Sakamoto et al. 2009	MWCNT
0,5 mg	2-2,5	Rat	5	11 ,3 nm	0,7 µm	Ground	Intratracheal	1 dose, 3 days	Inflammation, neutrophils and macrophages	Muller et al. 2008	MWCNT
50 µg	2,7	Mouse	3-6	15 nm	1-5 µm	Short	Intraperitoneal	1 dose, 7 days	non significant inflammation, no granulomas	Poland et al. 2008	MWCNT
50 µg	2,7	Mouse	3-6	10,4 nm	5-20 µm	Short	Intraperitoneal	1 dose, 7 days	no significant inflammation, 1 granuloma in 1 animal	Poland et al. 2008	MWCNT
50 µg	2,7	Mouse	3-6	85 nm	13 µm	Long	Intraperitoneal	1 dose, 7 days	significant inflammation, granuloma in abdominal cavity mesotel	Poland et al. 2008	MWCNT
50 µg	2,7	Mouse	3-6	165 nm	56 µm	Long	Intraperitoneal	1 dose, 7 days	significant inflammation, granuloma in abdominal cavity mesotel	Poland et al. 2008	MWCNT
2 mg	5,6-7,1	Rat	50	11,3 nm	0,7 µm	Unmodified	Intraperitoneal	1 dose, 2 years	None	Muller et al. 2009	MWCNT
2 mg	5,6-7,1	Rat	50	11,3 nm	0,7 µm	Structural defects	Intraperitoneal	1 dose, 2 years	None	Muller et al. 2009	MWCNT
2 mg	8-10	Rat	5	11 ,3 nm	0,7 µm	Ground	Intratracheal	1 dose, 3 days	Inflammation, neutrophils and macrophages. Induction of micronuclei in pneumocyte	Muller et al. 2008	MWCNT
5 mg	20-25	Rat	5	11 ,3 nm	0,7 µm	Ground	Intratracheal	1 dose, 3 days	Inflammation, neutrophils and macrophages.	Muller et al. 2008	MWCNT
20 mg	55-71	Rat	50	11,3 nm	0,7 µm	Structural defects	Intraperitoneal	1 dose, 2 years	None	Muller et al. 2009	MWCNT
20 mg	55-71	Rat	50	11,3 nm	0,7 µm	Unmodified	Intraperitoneal	1 dose, 2 years	None	Muller et al. 2009	MWCNT
3 mg	111-121	Mouse	19	100 nm	5 µm	Unmodified	Intraperitoneal	1 dose, 180 days	Mesothelioma, 14/16 animals died	Takagi et al. 2008	MWCNT

12. Effects on human

The effects on humans after exposure to carbon nanotubes are more or less unknown. In one study, tests were made to find out whether carbon nanotubes caused skin irritation in human volunteers, but no effects were observed (Huczko and Lange, 2001). This study is flawed because it applied a mixture of soot with a high content of unspecified carbon nanotubes. The concentration of carbon nanotubes in the soot was not stated. In a study of deceased patients who participated in the dereliction work at Ground Zero after the terrorist attacks of 11 September 2001 carbon nanotubes were found in their lungs. However, there is no correlation between the discovery of carbon nanotubes and causes of death (Wu et al. 2010). There are major gaps in the knowledge with respect to the effects of carbon nanotubes on human beings. The growing use of carbon nanotubes in industrial applications makes such studies urgent, see further discussion under the section "Future research needs."

13. Occupational exposure limits

To date, two manufacturers of MWCNT (Bayer, Nanocyl) have proposed their own occpational exposure limits on their products, see Table 12 (Schulte et al., 2010). NIOSH has proposed an occupational exposure limit for MWCNT at 7 µg/m^3 (Schulte et al., 2010). Even a benchmark limit for carbon nanotubes based on the number of fibres per unit volume has been suggested, 0.01 fibre/cm^3 (IFA, 2009). This guideline is based on observed health effects, therefore, a health risk to workers may still exist even though the value is followed.

Tabel 12. Proposed occupational exposure limit values for carbon nanotubes

Type of carbon nanotubes	Occupational exposure limit (8 hr time-weighted average)	References
MWCNT	2.5 µg/m^3	Nanocyl, 2009
MWCNT	50 µg/m^3	Bayer, 2010
MWCNT	7 µg/m^3	Schulte et al., 2010
Carbon nanotubes	0,01 fibre/cm^{3a}	Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), 2009

[a]Benchmark limit, not health-based

14. Safety and protection devices and personal protective equipment

Below is a summary of the advice and guidelines that various national organizations such as NIOSH, Health and Safety Executive (HSE) and the IFA, have for work with carbon nanotubes/nanoparticles. The precautionary principle should prevail when working with carbon nanotubes/nanoparticles until we know more about their toxicity and until occupational exposure limits have been established (HSE, 2009). If the use of carbon nanotubes cannot be avoided, a high level of elimination measures should be used so that occupational exposure is reduced to the lowest possible level.

Airborne nanoparticles behave like gas molecules, therefore established safety and protection devices for gases should also work in order to protect workers from exposure to nanoparticles. Traditionally when it comes to limiting and controlling the various types of air pollution through elimination controls, a step by step approach is used, see Table 13. This should also be used on exposure to airborne nanoparticles (Schulte et al.., 2008; NIOSH, 2009).

Table 13. The hierachy of elimination controls with regard to limiting and controlling air pollutants of nanoparticles based on traditional industrial hygiene

Control method/Elimination control	Process, equipment, or work task
1. Elimination	Change the composition to eliminate the risk
2. Substitution	Exchange of substance with a high risk to low risk
3. Safety and protection devices	Isolation/enclosure, ventilation (process, general)
4. Administrative controls	Handling and safety instructions, the correct work methodology, training, education, shift planning, medical checks, regular cleaning, labelling of products that contain nanomaterials, safety data sheets about nanomaterials
5. Personal protective equipment	Respiratory protective equipment, protective clothing, protective gloves, protective goggles, ear defenders

It is also important, with regard to risk management when handling carbon nanotubes, to limit the number of workers potentially exposed to carbon nanotubes and minimize (HSE, 2009):

- the level and duration of exposure (through training and handling instructions)
- the volumes used
- the handling of the carbon nanotubes

If possible, the carbon nanotube materials should be kept wet or damp to reduce the risk of them becoming airborne.

14.1 Control banding

"Control banding" has its origins in the pharmaceutical industry and is a useful tool for making exposure assessments in the workplace. Control banding is based on the appropriate control technology is recommended to a chemical that falls within a given hazardous group (based on risk phrases from safety data sheets and handling). Control banding has the potential to be a useful concept for workplaces that handle nanomaterials (Maynard et al., 2007, Schulte et al., 2008).

14.2 Safety and protection devices

To limit worker exposure to carbon nanotubes, it is important to avoid any free air flowing nanoparticles (Nanosafe, 2008). For most work processes and handling operations the emission of carbon nanotubes into the air can probably be controlled through different types of safety and protection devices (enclosures, fume cupboards, drawbenches, local extraction, etc. (Methner et al. 2010b). One recommendation is that all processes that use nanoparticles are enclosed and that these enclosures are designed to fit gaseous substances. Another recommendation is that safety and protection devices that are effective against dust in general are also suitable for the elimination of nanoparticles and ultrafine particles (IFA, 2009). According to NIOSH, conventional safety and protection devices, such as equipment for the encapsulation of emission sources and process ventilation, should be efficient enough to also capture airborne nanoparticles (2009). Current knowledge suggests that the use of HEPA filters in ventilation systems are likely to capture nanoparticles effectively (Hinds, 1999), cyclones however, do not. When exposure measurements at workplaces are conducted where carbon nanotubes were produced and handled, it was reported that the established safety and protection devices seemed to be effective (Han et al. (2008, Yeganeh et al., 2008). If you cannot enclose a process that uses nanoparticles, local process ventilation should be used, such as local exhaust ventilation with a particulate filter (e.g. HEPA) along with extensive personal protective equipment (respiratory equipment, protective gloves, protective clothing, safety shoes, Nanosafe, 2008; Methner, 2008).

14.3 Personal protective equipment

Use of personal protective equipment such as respiratory protective equipment, protective gloves and protective clothing is the last step in the previously described elimination controls and is the last step among the controls to be taken to protect workers from the harmful effects of carbon nanotubes (Schulte et al., 2008). Because there is insufficient knowledge about the effects on health with regard to carbon nanotubes/nanoparticles, and there is limited data available in order to assess how effective occupational exposure can be controlled, the use of personal protective equipment may need to be used.

14.3.1 Respiratory protective equipment

Power assisted respiratory protective equipment provide the best protection and are the most comfortable (Nanosafe, 2008). A half mask with particle filter class FFP3 can also be used (Nanosafe, 2008). Respiratory protective equipment should only be used in conjunction with other safety and protection devices. HSE recommends respiratory protective equipment with a protection factor of 40 or higher, and that all employees who use such equipment have undergone training and have had face fit testing.

14.3.2 Protective glove

Disposable gloves made of nitrile appear to be particularly suitable for work with nanoparticles (Nanosafe, 2008). Attention must be paid to the gloves so that they have

adequate mechanical stability to prevent skin contact (IFA, 2009). The manner in which the gloves are put on and taken off, as well as their overlap with protective clothing is more relevant for possible skin exposure than the glove material's permeability (IFA, 2009).

14.3.3 Protective clothing

Protective clothing should prevent dermal exposure, and such clothing should preferably be made of membrane materials, as woven or knitted fabrics and fabrics made of cotton or wool provide less protection (IFA, 2009). This is confirmed by a recent study which showed that the non-woven fabrics of polypropylene or polyethylene (airtight material) offer more effective protection to nanoparticles than cotton (Golanski et al., 2009). It is not known at present how effective protective clothing is in preventing the skin from being exposed to different nanoparticles (Schulte et al., 2008). However, there are already some standards for test methods for protective clothing or results for nanometre-sized particles, and this may provide an indication of the effectiveness of protective clothing (Schulte et al., 2008; ASTM, 2003).

14.4 Cleaning

Carbon nanotubes are stable compounds and have no natural biodegradability. Carbon nanotubes that have been emitted into the work environment are still there unless you eliminate them by cleaning operations. Regular cleaning in the workplace is an important operation in the manufacturing and use of nanoparticles (Nanosafe, 2008). It is important to have easily cleaned surfaces when handling nanoparticles. Vacuuming seems to be an effective cleaning method, however, some consideration should be taken when working with nanoparticles to the risk of a dust explosion (carbon nanotubes have a low risk of this; Bouillard et al., 2009; Nanosafe, 2008). In addition, it is important that you immediately clean up any spills and leakages of nanoparticles in the work areas. Adequate personal protective equipment should always be used during cleaning and maintenance work.

14.5 Waste disposal of carbon nanotubes

According to the HSE (2009), waste containing carbon nanotubes must be classified and labelled as hazardous waste. Carbon nanotubes waste must be sealed carefully using double layers of polyethylene bags. This should be done in a fume cupboard which has a HEPA filter or by using process ventilation such as local exhaust ventilation with HEPA filters. Combustion of waste containing carbon nanotubes is preferred as pyrolysis above 500 °C oxidizes carbon nanotubes completely.

14.6 Summary

When working with carbon nanotubes/nanoparticles, a precautionary principle should prevail. If working with carbon nanotubes, a high level of elimination controls should be used so that occupational exposure is reduced to the lowest possible level. For most work processes and handling operations, the emission of carbon nanotubes into the air can probably be controlled through different types of safety and protection devices such as enclosures, fume cupboards, drawbenches, local extraction, etc. Personal protective equipment in the form of respiratory protection equipment, gloves and protective clothing may also need to be used when there is limited capabilities of measuring and assessing occupational exposure to carbon nanotubes.

15. Future research needs

Below is a summary of future research needs within the area identified when this report was written.

- There is a need for a standardized measurement methodology for the quantification of occupational exposure to carbon nanotubes.
- More exposure measurements must be conducted at workplaces where carbon nanotubes are produced, used, handled or where products containing carbon nanotubes are machined. Both the number of personal day measurements with sampling in the breathing zone must be done, but also more personal exposure measurements in specific situations/exposure situations must also be done.
- More workplaces where carbon nanotubes are produced, used, handled or where products containing carbon nanotubes are processed must also be characterized by measurements with direct-reading aerosol instruments.
- There is a need for personal sampling equipment to be developed to measure parameters other than mass concentration.
- The risk assessment of carbon nanotubes must be done when reliable exposure data for carbon nanotubes is available.
- A consensus must be achieved in terms of dosmetric and the parameters that affect the toxicity of carbon nanotubes.
- More studies on reproductive toxicology are required.
- The bio-persistency of carbon nanotubes needs to be examined more, i.e. how long they remain in the body after exposure.
- Inhalation or instillation studies with repeated dosing over periods greater than 90 days are needed to evaluate the long-term effects of carbon nanotubes in a way that reflects true exposure and its effects.
- Studies as to whether carbon nanotubes can cause mesothelioma or not in the airways.
- Toxicity in composite materials consisting of the carbon nanotubes.
- Development of biomarkers to evaluate and detect exposure to carbon nanotubes.

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