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Carbon nanotubes

Types, products, market, and provisional assessment of the associated risks to man and the environment

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Title:

Carbon nanotubes

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Preface

This report was made to provide an overview of the CNT types and products on the current and near-future commercial market, the risk of CNT exposure and environmental release, the potential human and environmental hazard of CNT and finally to the extend possible, an integrated risk assessment of the product groups identified in the report.

The current project is one of the initiatives under the national action plan for Chemicals (2010-2013) and under the Agreement "Better Control of Nanomaterials" ("Bedre styr på nanomaterialer") (2012-2015).

The current work has been conducted in dialogue with a specialist steering group consisting of Flemming Ingerslev, Poul Bo Larsen and Anne Mette Zenner Boisen (Danish Environmental Protection Agency), Steffen Loft (Copenhagen University) Poul-Erik Andersen (The Danish Working Environment Authority), and Jesper de-Claville (University of Aalborg).

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Summary and conclusion

The aims of this report were:

1) To give a detailed introduction into the physico-chemical complexity of carbon nanotubes (CNT) and their variability depending in part on their synthesis conditions, purification, and potential coating and functionalization
2) To give an overview of the production volumes and capacities for producing the different types of commercial CNT, their cost and general application areas
3) To provide an overview of the current and near-market CNT-based down-stream and consumer products and the CNT loading in these.
4) To give an overview of reported occupational, consumer and environmental release and exposure to CNT and further discuss the likelihood of CNT exposure from major product groups.
5) To give a summary of the current understanding of human and eco-toxicity of CNT based on published toxicological studies and considering the different types of CNT that may be identified.
6) To perform a preliminary risk assessment based on current knowledge, including assessment of the release potential from major current and identified possible near-future consumer products

The report contains 6 chapters, in addition to the introduction and conclusions. Each chapter starts with an easy-to-read summary of the main findings and conclusions in the chapter. Further reading in each chapter offers more detailed and technical background information to enable the reader to achieve more advanced understanding of the topics.

Structure and physico-chemical characteristics of CNT
Carbon nanotubes (CNT) belong to a large group of hollow fiber-like carbon-based nanomaterials with diameters mostly below 100 nm and very high aspect ratios. CNT are of high technological interest because they can be produced or refined to possess specific properties such as high electrical conductivity, optical transmission, gas adsorption, or extremely high tensile strength. In their simplest categorization they are grouped into SW (Single-Walled), DW (Double-Walled) and MW (Multi-walled) CNT. However, other types such Bamboo (BB), Herring-Bone (HB) and Cup-Stacked (CS) CNT also exist on the market. More types are emerging and currently exist in the research and development stage. Most important new types may include branched and hybrid (mixed compounds) variations of the above-mentioned CNT types. Rolled-up graphene sheets (nanoscrolls) may also be considered a CNT.

The atomic structure of the CNT side-walls resembles a folded-up chicken-wire (graphite layer), but is rarely perfectly straight (graphitic). In many cases, the side-walls are kinked, branched, or irregular including other carbon allotrope impurities such as amorphous carbon, graphite, fullerenes. For SWCNT, the specific roll-up angle (chirality) of the graphene sheet is used for further categorization, which also categorize them after electrical properties. SWCNT at the extreme 0° and 30° chiral angles are called zig-zag and arm-chair SWCNT, respectively, while SWCNT with angles in between are called chiral. Arm-chair CNT is always metallic, whereas zig-zag and chiral can be either metallic, conducting, or insulating depending on chirality and tube diameter. Chirality is not yet extended to MWCNT.

The tube ends may be open or closed. The inner volume of the tube may be void, contain intergrowth structures and/or be filled with other materials. Finally, most CNT nanomaterials consist of CNT with variable degrees of homogeneity in CNT type, dimensions and associated amounts of impurities, which vary depending on the synthesis and purification methods applied. Initial impurities typically comprise other carbon allotropes such as amorphous carbon, graphite and fullerene as well as the metals or metal compounds used as catalysts. In the final material deliberate coatings or functionalizations may be present as part of the material in addition to residuals after purification.

Synthesis, purification, coating and functionalization

Most CNT appears to be produced by chemical vapour deposition using metal catalysts (e.g., Fe, Ni, Co) or nanopores in ceramic nanomaterials (e.g, SiC, Al_2O_3, TiO_2). Arc discharge is another often used synthesis technique where carbon is released from catalysts doped with catalyst source materials (e.g., Fe, Ni, Co, NiCo, Pt, Rh, RhPt). Laser ablation may also be used and enables production low-volume high-quality pure SWCNT. New procedures, including conversion of waste such as plastic and car-tires also exist. However, currently high-volume production of relatively good quality CNT is only feasible by chemical vapour deposition.

Defects in the atomic CNT structure play an important role for the mechanical and electronic transport properties of CNT as well as their chemical reactivity and ability to be functionalized. Defects may occur during both synthesis and after treatment (e.g., ball milling and chemical purification) and during modification for covalent functionalization. Defects can occur in both the side-wall and closed-ended tube tips, but is inevitable in open-ended CNT. Noteworthy, the tendency to have side-wall defects increases with decreasing CNT diameter due to higher lattice strain at small diameter. Defects may removed by annealing, also called graphitization of the CNT.

Coating or functionalization of CNT is usually required to achieve full dispersion and/or translation of the CNT properties in the intended application. One may consider: 1) non-covalent functionalization (specific CNT and molecule interaction), 2) defect-group functionalization (vacancy or SW-defects), 3) direct covalent side-wall functionalization, and 4) endohedral functionalization (tube-caged molecules). Non-covalent functionalization is suitable if the physical properties of the CNT should be affected as little as possible. Covalent defect-group and direct covalent functionalizations are possible at both tube ends and at tube side-walls. Despite covalent functionalization alters the CNT properties, it is still very attractive for e.g., composite materials because this may enable strong chemical bonds between the CNT and the material matrices.

CNT categorization

IUPAC classifies MWCNT as synthetic graphite in tubular shape and two types are currently registered as such in REACH. Another MWCNT is registered as graphite with notation of the IUPAC name. Chemists often consider all CNT as sub-classes of the fullerene group or as before roughly divided according to the general atomic structure, number of side-walls and chirality for SWCNT. The actual physico-chemical complexity of CNT may call for a more sophisticated categorization system which includes some of the important CNT properties and impurities. From an industrial application point of view, the dimensions, carbon structure, and purity are very important parameters and currently no systematic approach exists for this as well as for their reporting in chemicals registration.

Characteristics of commercial CNT

A meta-survey of commercially available CNT showed that they are mainly marketed as SW, DW, MW-type CNT. Most of the CNT nanomaterials were SWCNT (n = 76), DWCNT (n = 17), and MWCNT (n = 178), where the numbers in brackets are the number of different CNT found for each general type. Only one BB- and one CS-type CNT was found as commercial nanomaterials. However, the numbers of types within each of these CNT groups are not necessarily predictive for the production volume and width of application. CS-CNT has for example found great success in wide-spread high-end sports equipment. Lately, mixtures of CNT and other compounds (e.g., TiO2, clays, and carbon black) have also emerged with specific productions as a target.

The reported diameters varied from 0.7 to 2.5 nm for SWCNT, 1.3 to 5 nm for DWCNT, and 4 to 150 nm for MWCNT. The general tube lengths were comparable ranging from ca. 0.5 to 50 µm, but some MWCNT are reported to be up to 150 µm long. The purity levels of products marketed as CNT varied significantly. Overall, the full purity range was typically from more than 60 to more than 95 wt% CNT. The percentage of SWCNT and DWCNT with high impurity levels was higher than for MWCNT. The main impurities reported were typically other carbon materials and ash (inorganic catalyst material). Other CNT types in a product were also sometimes reported as impurities. The precise identity or composition of the impurities is usually not given.

Advertised ready-made CNT functionalizations were dominated by Li-ion, -OH, -COOH, and $-NH_2$. However, numerous types of functionalizations may be made on demand as well as delivery of CNT in master batches. The functionalization may constitute up to about 5 wt% of a CNT nanomaterial. Despite the survey did not cover all sources, the data are believed to be representative.

The market size and CNT-based consumer products

Reflecting the wide variety of CNT and their many interesting physicochemical properties, they have a wide range of existing and new potential technological applications ad well as commercial interest. The typically applied CNT properties are their high tensile strength, electrical conductivity, electrical charge capacity, gas and energy-storage as well as designed inertness to high chemical reactivity with applications within sensor technology and catalysis.

Production volumes

Company sources estimate a global production of 520 to 3000 metric tons CNT in 2014 with 1750 to 2500 as the intermediate estimate. The production capacity is many times larger and is estimated to be on the order of 20,000 tons in 2014. The broad group of MWCNT appears by far to be the most abundant product followed by SWCNT (ca. 5%). At ECHA, Graphistrength C100 (ARKEMA) is registered in the 1-10 tons/year category, whereas MWCNT from Bayer (now discontinued) and Nanocyl are registered with a production of 10 – 100 tons/year. The estimated global production volumes do not match entirely the CNT demand, which was found to be on the order of 3,300 – 3,700 tonnes already in 2012.

Market size and trade value of CNT and CNT-based products

The global trade value of CNT was on the order of $158.6 million in 2014 and is expected to have an annual growth rate of 33.4% until 2019. The most important applications are currently in various types of composites, electronics, and energy. The application volume and product diversity is growing. The CNT properties used is currently mainly their extraordinary tensile strength, their electrical conductivity and current carrying capacity, gas and energy storage capacity, and a tunable chemical reactivity from being inert to reactive with applications in sensors and catalysis.

Application areas and CNT-based products

The most important industrial sectors are the automotive, energy, paint and coatings, and electronics. Emerging application sectors appear to include textiles, sensor technology, diode light emitters. Near-future large tonnage applications of MWCNT is expected to be in various reinforced composites used for aerospace, automotive, marine and wind turbine industries where implementation of CNT is expected to result in weight reductions of approximately 10 %. SW- and DWCNT as well as the ‘exotic’ CNT types are not expected to reach the same levels of tonnages as MWCNT in the near future. The main application of SW- and DWCNT is in electronics

Batteries and conductive fillers. The oldest use may be in Li-ion batteries containing 1 - 3 weight percent CNT in the graphite electrodes. Another, apparently well-established use is the application of CNT as a conducting filler in base resins and thermoplastics where it can reduce the electrostatic charging of plastic parts and enabling electrostatic painting without further treatment of the materials.

Reinforcing composite fillers. The use of CNT as a strength-enhancing functional filler in base resins is well-known. Another application is as reinforcing agent in advanced polymer composites used for sports equipment, e.g. rackets, golf-clubs, and ice-hockey sticks, but also high performance bicycles, small boats and windmill blades containing CNT are on the market. There potential is, however, still not fully achieved due to technical challenges to be solved before the extreme strength and stiffness of CNT can be fully utilized in nanocomposites. Composite materials other than polymers are also emerging. These products include aluminum CNT composites and ceramic CNT composites, but only sparse information on these materials and their applications is currently available.

Paints and antifouling coatings. CNT-based epoxy-paints and antifouling coatings for the marine sector have been introduced more than five years back. The claim is that the CNT based coating has high strength and enables antifouling properties without releasing toxic substances into the sea water. The reduction of growing organisms on the ships translates directly into saved fuel and reduced CO_2 emissions. CNT-based high-durability epoxy paints are also suggested for other purposes and currently being developed for large windmill blades.

Conductive inks and layers. Following the line of CNT-containing coatings much research has been put into the development of conductive inks, which due to the dimensions of the CNT can be made transparent. This property is useful for electronic displays and touch screens, and products have most likely entered the market.

Textiles. CNT added to textile fibres have been demonstrated to imply electronically conducting properties to the finished textiles, and products utilizing this property is known on the market in Japan. If they are not already available in Europe they are likely to become so in the near future. This may be either as isolated products or as part of these. Electronically conducting textiles are used as extremely thin heating mats and has been proposed used for ‘intelligent’ clothing e.g. for winter sports applications.

Other uses. Many more applications for CNT have been suggested but it is difficult to determine exactly in which development stage they currently are. Applications include field emission devices (FED) for e.g. light sources and other electronic components, non-volatile RAM memory modules for computers etc. and heat management devices for microelectronics.

Human and environmental exposure

There is currently very limited data available on the exposure levels to CNT or their potential release associated with production and use of CNT-based products. The major route of release of free CNT to the environment (air-borne dust, as well as liquid and dry waste) appears to originate from synthesis and manufacturing of CNT-based products. Soil and sediments are the major sinks for accumulation of CNT arising from sedimentation of airborne dust, sludge treatment and release through waste-water, respectively. Discard of CNT waste and CNT products is estimated to mainly end up in landfill (ca. 70%) and waste incineration plants (ca. 17%). One major exception is recycling of Li-ion batteries, but re-use of CNT is not documented.

Occupational exposure

Occupational exposure to airborne CNT dust may reach up to at least a few hundred μg/m^3 air. Dermal exposure is also a consideration. One study revealed a worker glove-dose (whole hand) of 2-

6 g CNT in connection with handling crude CNT after production. De facto dermal exposure was not observed in another newer study using tape stripping. The nature of the CNT in reported occupational exposures and release studies mainly occurs in bundles, aggregates and agglomerates of CNT as well as in matrix bound fragments. However, there is a lack of studies documenting the nature of CNT in the exposure.

Consumer exposure

There appears to be no measurement data available on the actual consumer exposure and environmental release. The occupational exposure limits reported above serve as benchmark values for maximum typical achievable exposure levels for both consumer and direct contact exposure situations. Using logical conclusions, the overall risk of consumer exposure may be limited due to the general encapsulation or embedding of CNT in CNT-based products. However, cases have been demonstrated where exposure is possible, such as dermal exposure during use of CNT-coated textiles and release of CNT after weathering of composite materials.

Environmental release and exposure

Life cycle assessments suggest that there is a high potential of CNT release to the environment during production of CNT as well as production and discard of certain CNT-based products. Release to the environment may also occur in connection with some intended use and weathering of especially CNT-based nanocomposites. In our evaluation, the risk of CNT release to the environment appears to be highest during manufacturing, discard of process waste and ultimately discard of the final product. The modelled environmental concentrations in life-cycle analysis are still moderate, but hot spots and local accumulation is not considered in the first modelling attempts. Hot-spots around sources should be considered in future assessments.

Human toxicology of CNT

Results from the toxicological studies strongly indicate that exposure to certain CNTs may be associated with long-term adverse health effects in test-animals. The primary routes of exposures are by dermal contact, oral uptake and inhalation. Systemic effects partly depend in the ability of the CNT to translocate to other organs in the body. Therefore, the toxicological overview is presented according to current knowledge on CNT fate and translocation, pulmonary, oral and dermal toxicology.

Biological translocation and fate of CNT

For assessment of oral uptake, there is very little literature, but for MWCNT no ingested CNT has been detected to pass beyond the gastro-intestinal tract. One study report, transfer of short hydroxylated SWCNT.

After lung exposure, pulmonary dosed CNT is slowly cleared from the lungs when phagocytosed by macrophages. Phagocytosis seems to occur relatively fast. The macrophages also transport the CNT into the subplural regions of the lungs, which is a prerequisite if CNT are to cause mesotheliomas. Studies also suggest that a fraction of CNT may reach the sub-pleural space with assistance of the macrophages. Half-lives of up to 300 days have been reported.

If we assume that CNT do translocate at low rate, they will reach the blood stream. Once CNT reach the blood vessels, there is amble evidence that they will accumulate in Kupffer cells in the liver with a very low rate of elimination. Several different kinds of CNT have been observed in liver cells up to 1 year after exposure after intravenous injection. In agreement with this hypothesis, a 1-year study using aspired ^{14}C labelled MWCNT, showed accumulation of 0.75% of the deposited dose in the liver and 0.2 wt% in the spleen. Another study showed accumulation of 0.027% of the inhaled deposited dose in the liver after 1 year. A total of 0.037% was localized to extrapulmonary tissues.

Pulmonary toxicology
Numerous studies on pulmonary toxicity of CNT found adverse toxicological effects at realistic doses. Two key studies have been published on long-term inhalation following OECD guidelines. In both studies, rats were exposed to two different commercially available MWCNT, subclinical symptoms of inflammation was observed at a concentration of 0.1 mg/m^3 which was the lowest tested dose and therefore yielding a LOEL (Lowest Observed Effect Level). Both studies used aggregated or agglomerated MWCNT rather than single CNTs. It is not known whether inhalation of MWCNT that do not form aggregates will have effects at lower concentrations.

Suppressed T-cell response was found by two independent groups using inhalation or instillation, respectively, of two different MWCNT. Moreover, increased expression of TGF-β which initiates the immunesupression response was found both after inhalation of MWCNT and SWCNT.

Instillation of SWCNT induced single strand breaks in DNA in BAL cells after 24 hours. Oral dosing of the same SWCNT induced single strand breaks in DNA in liver cells. Mitsui MWCNT-7 has caused mesotheliomas in rat and in a susceptible mouse model. Mitsui MWCNT-7 consists of interconnected approximately 5 µm long individual MWCNT and has low Fe content. In a follow-up study, both fibrosis and patchy parietal mesothelial proliferation lesions was documented. A thinner and shorter MWCNT induced higher levels of pulmonary inflammation than the Mitsui MWCNT-7. Mesothelioma has not been shown by SWCNT in rats either.

Cardiovascular effects
There is evidence that pulmonary exposure to SWCNT in combination with a high-fat diet leads to plaque progression, and there is evidence that CNT present in the blood in high concentrations will promote platelet aggregation.

Reprotoxicity
In reprotoxicological tests, CNTs were found not to accumulate in testis cells after IP injection of CNTs, and injection of CNTs had no effects on male fertility. The significance of the found results is highly questionable, since there is little evidence that CNT will enter circulation at all. However, the results indicate that even if CNT would enter the body, there are no indications of direct effects of CNT on male fertility. However, reprotoxicological effects have been found for other NMs and ascribed to be most likely caused by indirect effects after pulmonary CNT exposure.

Dermal effects
Dermal inflammation was observed after exposure to unpurified SWCNT, but not after exposure to purified SWCNT and commercially available MWCNT. This might be caused by the high levels of impurities in the unpurified SWCNT rather than the CNT.

Ecotoxicity of CNT

The number of studies conclusive for the ecotoxicological effects of CNT is still limited. Here, we report only on results from key peer-reviewed scientific publications considering CNT biodistribution and effects in aquatic and terrestrial organisms. Overall, the lack of systematics and harmonization in ecotoxicological tests currently prevents generalization on the ecotoxicological effects of CNT.

Bioaccumulation
There does not seem to be passive uptake of CNT in the tested species, but several studies provide examples of uptake of CNT by filter feeders or on the gills of fish where water is flowing through. Further, CNT can adhere to surfaces of organisms or accumulate in the gut, and this way they induce several effects.

Effects on organisms in the aquatic environment
The toxicity of CNT seems to depend strongly on the level of dispersion in the tested media, since agglomerates have short residence time in water columns and will deposit. However, when dispersion of CNTs is maximized, they induce acute effects in fresh water and marine organisms with an effect concentration of about 0.1 mg/L (EC_{50} 96h mortality in fresh water crustacean *Daphnia magna*, inhibit growth at 96h LOEC of marine diatom *Thalassiosira pseudonania*, and EC50 inhibited growth of marine green algae *Dunaliella tertiolecta*) and therefore can be expected to induce chronic effects in water column at concentrations <0.1 mg/L. In the sediment, effects were observed at lowest effect concentrations above 30g/kg (mortality benthic amphipod *Leptocheirus plumulosus*).

Respiratory symptoms in rainbow trout *Oncorhynchus mykiss* at 0.1 mg/L were observed and the same concentration reduced population growth in marine copepod *Tigriopus japonicus*.
Toxic effects in second generation were observed for injected CNT 2 ng/zebrafish embryos *Danio rerio*, but the significance of this finding is unknown since there are no indications that CNT are taken up from the water. When CNT were dispersed in water, 120 mg/L exposure delayed hatching. No systematic genotoxic effects were observed.

Effect on terrestic organisms
DWCNTs affected reproduction (cocoon production) of earth worm *Eisenia veneta* at concentration 37 mg/kg dry food. For plants, growth reduction for Zucchini *Cucurbita pepo* plants occurred first at 1000 mg/L. However, enhanced germination followed by inhibition was observed for Mustard seeds. The germination stimulation LOEL 2.3 mg/L for oxidized MWCNT and 23 mg/L for pristine MWCNT, and germination inhibition LOEL 6.9 mg/L for oxidized MWCNT and 46 mg/L for pristine MWCNT for the mustard seeds. Interestingly, the oxidized MWCNT were less toxic than pristine CNT to mustard seeds.

Provisional integrated risk assessment

Evaluation of the observed hazard and the reported CNT properties in toxicological studies does not enable clear identification of certain CNT or properties which cause higher risk than others at this point. Yet, long and stiff (straight) CNT in general appears to have the ability to form serious pulmonary toxicity.

CNT hazard to humans
The results from current toxicological studies can only enable a first generation human exposure limits. Based on current experimental evidence, we derive a recommended respirable:

Occupational exposure limit	=	1	$\mu g/m^3$ (carbon or CNT)
Consumer exposure limit	=	0.25	$\mu g/m^3$ (CNT)

Specific dermal and oral exposure limits cannot be derived due to data limitations.

Occupational risk indication
The risk of human exposure is evident in the working environment. Reported exposures reach up to several hundred µg/m3 in average over one to several hours in connection with specific work processes. Despite being "whole dust" exposure, occupational exposure is potentially several hundred times higher than the recommended occupational exposure limits during specific tasks and appropriate protection must be emplaced. CNT recovery and powder handling are the most critical processes for release of airborne CNT. The reported occupational exposure levels also cause risk of environmental release of concern. Dermal exposure has been documented and may cause skin irritation. Oral exposure is likely if inhalation occurs and by accidental dermal to oral transfer. The knowledge on consequences of oral exposure to CNT is limited, but some studies have shown association with oxidative stress and DNA-damage.

Consumer risk indication
Assessment of consumer exposure risk is highly uncertain and no measurement data are currently available. To assess the risk associated with the identified major product groups, we therefore assessed the potential exposure risk and potential exposure levels based on different alternative procedures.

First an assessment was based simply on the type of product and where the CNT was located in the products. Thereafter, we established a procedure to assess the relative release potential by using a Margin of Nano Exposure (*MONE*) value and a Product Exposure Impact (*PEI*) considering the location of the CNT, the assumed amount of CNT in the product, and an assumed number of product objects.

The *MONE* values showed high release potential for several product groups including long-term degradation and expected in-use accidents (i.e. breaking a racket). However, the *PEI* values suggested that the impact of the potential consumer exposure currently is relatively low for the selected product groups.

Due to anticipated number of object, Li-ion batteries have the highest *PEI* despite of a low, but still present, consumer exposure potential. The second and third-most important product groups are thermoplastics and printed electronics (layer) and the fourth-most are sporting goods (layer), respectively.

Modelling demonstrated that it is generally difficult to excced the inhalatory consumer exposure limit. Reaching 0.25 $\mu g/m^3$ for 15 minutes would require that all CNT in a 3.5 to 3.96 m^2 and 5 µm thick layer with 1 wt% CNT in product was aerosolized within 1 to 15 minute. Such release areas and kinetics are only possible in connection with mechanical reworking, modification or “demolition” of products such as printed conductive coatings, antifouling coatings, epoxy paints, conductive textiles, and heating mats. Breaking layered CNT composites or unintended opening of Li-ion batteries may also cause relatively high acute exposure. However, these events will be very rare events for typical consumers.

We emphasize that our consumer risk assessment is purely theoretical and therefore not ultimately conclusive. It is impossible to perform a real risk assessment at the level of product groups due the complete lack of relevant data and the great variability within each product group. Indications from our different alternative approaches generally indicate low or rare risk of airway exposure to the consumer in the use-phase of un-altered products. However, if a consumer use could imply mechanical treatment of e.g, surface coatings and textiles with CNT, exposure limits could be significantly exceeded.

There appears to be a relatively high risk of dermal and accidental oral consumer exposure during intended use of specific consumer product groups as compared to inhalation exposure. The most potent product groups include CNT-textiles and various weathered or altered composite materials, antifouling paints, epoxy paint and their application if possible for consumers. It is, however, uncertain, whether the levels and durations of the consumer exposure situations will be sufficient to cause detrimental health effects in consumers.

CNT risk to the environment
There is currently no concentration limits for CNT in the environment and ecotoxicological testing is incomplete. Additionally, different test item preparation methods give different results. The most sensitive species to CNT exposures were *Daphnia magna* (0.05 mg/L); *Oncorhyncus mykiss* (0.1 mg/L), and *Tigriopus japanicus* (0.1 mg/L). For plants, seed germination is reported to be the most sensitive end-point. Mustard seed germination was inhibited at concentration 6.9 mg/L. However, the most sensitive species may not be relevant to the most critical exposure.

Evaluation of the most common product groups suggest that several products have the potential to cause environmental impact. As for consumer exposure, there is no measurement data available on CNT release and concentrations in the environment. However, the *MONE* values derived from the product characteristics and release potentials for consumers are also suitable for environmental risk assessment. From this assessment, layered printed electronics, coated textiles and heating mats, layered sporting goods and microscopy probes are those with highest release potential. The, second-rank group included most of the other products groups, such as Li-ion batteries, thermoplastics and nanocomposites with dispersed CNT.

However, considering the possibility of matrix deterioration of composite products, the exposure level at contact of aged products is a worst case scenario. In such cases matrix degradation may offer a fully CNT coated surface. From this view-point, basically all products have the potential to offer a high concentration exposure in long-term use and land-fill waste discard.

No measurements are currently available on the CNT concentration levels in the environment. Homogeneous dispersion modelling data from 2008 suggests that environmental concentrations are below the currently known ecotoxicological effect levels; also after adjusting the emission data to the 7 times higher production volumes today. However, it could be argued that the environmental concentrations locally may exceed the ecotoxicological effect levels, at expected hot spots at e.g., factories, landfill, waste recycling stations, and sludge-treated fields.

Sammenfatning og konklusion

Formålet med denne rapport var:

1) At give et detaljeret indblik i kulstof nanorørs (CNT; engelsk forkortelse for *Carbon NanoTubes*) fysisk-kemiske kompleksitet og variabilitet alt efter fremstillingssmetoder, oprensning, og overflade modifikation (coatning eller kemisk funktionalisering).
2) At give et overblik over produktions–voluminer og –kapacitet for de forskellige typer kommercielle CNT, deres prisniveau, og generelle anvendelsesområder.
3) At give indsigt i de kommercielle anvendelser og typen af eksisterende og kommende CNT-baserede produkter og koncentrationen af CNT i disse.
4) At give en gennemgang af situationer og processer, hvor der er arbejdsmiljø-, forbruger- eller miljø-eksponering for CNT eller processer hvorved de kan frigives og vurdere risikoen for frigivelse og eksponering for primære produktgrupper.
5) At give et overblik over eksisterende viden om de human- og økotoksikologiske effekter af CNT med de forskellige typer identificerede CNT taget i betragtning.
6) At lave en præliminær integreret risikovurdering for CNT og CNT-baserede produkter baseret på den eksisterende viden.

Rapporten indeholder 6 kapitler foruden introduktionen og konklusioner. Hvert kapitel indledes med en forholdsvis lettilgængelig opsummering af kapitlet og dets hovedkonklusioner. Mere detaljeret teknisk information fås ved videre læsning af kapitlet, som muliggør en dybere forståelse for de respektive emner.

CNT struktur og fysisk-kemiske karakteristika
CNT er en fælles betegnelse for en stor gruppe af rørformede kulstof-baserede nanomaterialer, som normalt har en diameter under 100 nm og meget høje længde-over-diameter forhold (aspekt ratio). Der er stor teknologisk interesse i CNT, idet de enten har eller kan modificeres, så de har overlegne egenskaber indenfor f.eks. elektrisk og optisk ledning, gas-adsorption, eller ekstrem høj trækstyrke.

CNT kategoriseres oftest i undergrupper efter antallet af vægge (atom-lag), i den rørformede atomstruktur: Dvs. enkeltvæggede (SW; *Single-Walled*), dobbeltvæggede (DW; *Double-Walled*) og flervæggede (MW; *Multi-walled*) CNT. Der findes også kommercielle CNT med andre atomstrukturer, såsom bambus (BB; *Bamboo*), sildeben (HB; *Herring-Bone*) og kop-stablede (CS; *Cup-Stacked*) CNT. Endnu flere typer CNT strukturer findes på forsknings- og udviklingsplan. Disse nye typer er bl.a. forgrenede (branched) og hybrid (to- eller multi-fase) varianter af de ovenstående traditionelle typer. Sammenrullede grafen flager (nanoscrolls), som er grafitiske kulstofflager bestående af ét atomlag, kan også betragtes som CNT.

SWCNT kan som den eneste CNT klassificeres yderligere alt efter kulstof-atomernes mønster (gitterstruktur) langs rørets længdeakse, som varierer med den såkaldte chirale vinkel for den hypotetiske foldning grafen-laget. Yderpunkterne findes ved en chiral vinkel på 0° and 30°, som resulterer i hhv. *zig-zag* og *arm-chair* (lænestol) SWCNT. Orienteringer mellem de to yderpunkter kaldes chiral. *Arm-chair* CNT er altid metallisk, hvorimod zig-zag og chiral CNT kan være enten metallisk, elektrisk ledende eller isolerende afhængig af chiraliten og rørenes diameter. Kategorisering efter chiralitet er endnu ikke opnået for de andre CNT strukturer.

De fysisk-kemiske karakteristika er meget vigtige for den eventuelle anvendelse af de forskellige CNT materialer. Udover orienteringen af kulstofgitteret, så er kvaliteten af væggene i CNT-strukturen også vigtig. Selvom idealbilledet af CNT væggene er perfekte lige (grafitisk) vægge, så kan de i virkeligheden have knæk (kinks), forgreninger, eller indeholde helt irregulære domæner med forskellige andre carbon faser (allotroper) såsom amorf kulstof, grafit, og fulleren (de såkaldte fodboldmolekyler).

CNT rørene kan også være enten åbne eller lukkede ved enderne og CNT røret kan være perfekt hult, men de kan også indeholde delvist sammenvoksede zoner og/eller være helt eller delvist udfyldt med andre materialer. I virkeligheden er CNT nanomaterialer oftest inhomogene med individuelle CNT-typer, dimensioner, og urenheder, som kan variere med de anvendte syntese- og efterbehandlingsmetoder. Råt syntetiseret CNT indeholder typisk metaller og metalforbindelser fra katalysatorermaterialer foruden andre carbon allotroper. De endelige kommercielle CNT nanomaterialer kan også være overflademodificeret (coated eller kemisk funktionaliseret) og dermed indeholde nye kemiske stoffer foruden de oprindelige urenheder og/eller kemiske rester efter eventuel oprensning.

Syntese, oprensning, coating og funktionalisering
De fleste CNT producenter laver CNT ved *chemical vapour deposition* (en slags gasfase syntese) katalyseret med metaller (f.eks. Fe, Ni, Co) eller nanoporer i keramiske materialer (f.eks. SiC, Al_2O_3, TiO_2). *Arc discharge* (glødetråd) metoden bruges også ofte til syntese af CNT. Her løsrives kulstof fra en grafitkilde tilsat katalysatorer (e.g., Fe, Ni, Co, NiCo, Pt, Rh, RhPt). *Laser ablation* kan også anvendes og gør det muligt at producere helt ren SWCNT af høj kvalitet, men kun i små mængder. Nye procedurer til massefremstilling inkluderer genanvendelse af plastik og bildæk. C*hemical vapour deposition* er dog den eneste metode, der lige nu kan anvendes til at producere tilstrækkelige mængder CNT af god kvalitet.

Andelen og typen af defekter i CNTs atomstruktur spiller en vigtig rolle for deres mekaniske og elektriske egenskaber, men også for deres kemiske reaktivitet og mulighederne for funktionalisering. Defekter kan opstå under syntesen, men også under efterbehandling (f.eks. formaling og kemisk oprensning) og modificering til kovalent funktionalisering. Defekterne kan introduceres i CNT side-væggene og i lukkede rør-ender, men er uundgåelige i CNT med åbne rør-ender. Det er værd at bemærke at der en tendens til at CNT får flere defekter jo mindre rør-diameteren er. Det skyldes at kulstofgitteret bliver stærkere og stærkere deformeret ved krumningen jo mindre rør-diameteren bliver. Defekter i sidevæggene kan fjernes ved termisk rekrystallisering; også kaldet grafitisering.

Det er typisk påkrævet at modificere CNT-overfladerne kemisk for at opnå god dispergering og/eller overførsel af CNT egenskaberne i den påtænkte anvendelse. Kemisk modifikation kan omfatte: 1) Non-kovalent funktionalisering (specifik interaktion mellem et molekyle og CNT); 2) defekt-gruppe funktionalisering (ved huller eller asymetrisk koordinering i atomstrukturen); 3) direkte kovalent side-vægs funktionalisering; og 4) endohedral functionalisering (molekyler i rørenes hulrum). Non-kovalent funktionalisering er at foretrække, hvis CNT egenskaberne skal bevares mest muligt. Kovalent defektgruppe og direkte kovalent funktionalisering er mulig både ved sidevæggene og CNT enderne. Selvom de kovalente funktionaliseringer ændrer CNT egenskaberne, så er denne form for funktionalsering meget attraktiv for f.eks. kompositmaterialer, fordi man på denne måde kan opnå meget stærke kemiske bindinger mellem CNT og kompositens matrice.

Klassifikation af CNT
IUPAC klassificerer MWCNT som syntetisk grafit i tubular form og der er allerede mindst to typer registeret i REACH. En anden MWCNT er registreret som grafit med bemærkning om dets IUPAC navn. Kemikere betragter ofte alle CNT som underklasser i fulleren gruppen eller tidligere omtalte grove opdeling efter overordnet atomstruktur, antal vægge og chiralitet for SWCNT. Det kan være

nødvendigt at etablere en mere sofistikeret kategorisering af CNT, som bygger mere på deres egenskaber og urenheder, hvis man skal muliggøre at tage de forskellige CNT typers faktiske fysisk-kemiske kompleksitet i betragtning. Fra industrielt synspunkt, så kan bl.a. dimensionerne, atomstrukturen, og renheden være meget vigtige parametre. Der foreligger endnu ingen systematisk metode for denne form for gruppering eller til kemisk registrering af CNT.

Karakteristik af kommercielle CNT
En gennemgang af kommercielt tilgængelige CNT viste at de hovedsageligt sælges som SW, DW, MW-type CNT. De fleste CNT materialer var SW-(n = 76), DW- (n = 17), og MWCNT (n = 178), hvor antallet i parentes er antallet af forskellige CNT nanomaterialer fundet i hver kategori. Udover disse blev kun fundet én BB- og én CS-type CNT blandt de kommercielle nanomaterialer. Det skal bemærkes at antallet af CNT produkter i hver kategori ikke er nødvendigvis hænger sammen med produktionsvoluminet og hvor meget det anvendes. Der er f.eks. kun fundet en type CS-CNT, men de anvendes f.eks. bredt anvendt i visse typer højkvalitets sportsudstyr.

De rapporterede fysisk-kemiske varierede for de forskellige CNT klasser. For SWCNT varierede diameteren mellem 0,7 og 2,5 nm, mens den for DWCNT og MWCNT varierede mellem hhv. 1,3 og 5 nm og 4 og 150 nm. Længderne var generelt sammenlignelige på tværs af typerne og varierede fra ca. 0,5 til 50 µm, men enkelte MWCNT var rapporteret med længder op til 150 µm. Renheden varierede meget og var rapporteret fra 60 til mere end 95 wt% CNT. Andelen af meget rene SW- og DWCNT var højere end for MWCNT. De rapporterede urenheder var typisk andre kulstof-baserede materialer og ”aske” (uorganisk katalysatormaterialer). Andre CNT typer i produktet blev i visse tilfælde rapporteret som urenheder. Urenhedernes kemiske sammensætning er sjældent rapporteret.

De umiddelbart kommercielt udbudte overflade-modifikationer var domineret at Li-ion, OH, COOH, og NH_2. De fleste forhandlere/producenter tilbyder dog at kunne lave andre specifikke overflademodifikationer ligesom levering af CNT i master batches ofte er muligt. Funktionaliseringen er rapporteret til at udgøre op til 5 wt% af hele CNT produktet. Selvom ikke alle potentielle kilder er gennemgået, så menes resultaterne fra denne analyse stadig at være repræsentative.

Markedsværdi og CNT-baserede produkter
I tråd med de mange CNT varianter og deres mange interessante fysisk-kemiske egenskaber, så har de også en række eksisterende og potentielt nye teknologiske anvendelser med stor kommerciel interesse. De mest anvendte egenskaber er deres høje tensile trækstyrke, elektriske ledningsevner, elektrisk ladning, gas- og energilagring, såvel som designet kemisk reaktivitet fra inert til høj-reaktiv med applikationer i sensorer og katalyse.

Produktionsvoluminer
Virksomhederne vurderede selv at de i 2009 havde en produktion på mellem 500 og 1000 tons CNT og i 2014 var tallet mellem 520 og 3000 tons med et intermediært estimat på 1750 til 2500 tons . Produktionskapaciteten er mange gange større og var forventet i størrelsesordenen 20.000 tons i 2014. MWCNT produktionen er klart den største efterfulgt af SWCNT (ca. 5%). Af Europæisk relevans, så er Graphistrength C100 (ARKEMA) registreret med en produktion på 1-10 tons/år hos ECHA, mens MWCNT fra Bayer og Nanocyl er registreret med en produktion på 10 – 100 tons/år. Bayer har dog stoppet deres produktion af CNT.

Handelsværdi
Den globale handelsværdi for CNT var i størrelsesordenen $156.6 millioner i 2014 og er forventet at vækste med 33.4% indtil 2019. Til sammenligning var den globale handelsværdi for CNT-baserede produkter vurderet til at være $5 milliarder i 2012. De vigtigste applikationer for nuværende er i forskellige typer kompositter, elektronik og i energi-området. Anvendelsesområderne vokser stadig

i antal og størrelse. F.eks. så stiger anvendelsen af CNT indenfor energi-lagring i disse år med ca. 8,5 % per år.

Anvendelsesområder og CNT-baserede produkter
De vigtigste industrielle anvendelser er indenfor automobil, energi, maling- og coatings-, og elektronik-branchen. Nye anvendelsesområder er på vej og omfatter tekstiler, sensorer, og lysdioder. MWCNT forventes i den nærmeste fremtid at blive anvendt i store mængder i CNT-forstærkede kompositter indenfor luft- og rumfart, automobil-, marin-, og vindmølle industrien, hvor implementering forventes at kunne reducere vægten på emnerne med ca. 10%. Ingen af de andre CNT typer er forventet at nå det samme anvendelsesniveau i den nærmeste fremtid. SW- og DWCNT anvendes primært i elektronik

Batterier og elektrisk ledende filler. Den ældste anvendelse af CNT formodes at være i Li-ion batterier, som indeholder 1 - 3 wt% CNT i grafit elektroderne. En anden tilsyneladende veletableret anvendelse er som elektrisk ledende filler i tilsætning til matrice materialer og termoplastik, hvor de kan reducere den elektrisk ladningsopbygning i plastikdelene og gøre det muligt at udføre elektrostatisk maling af emner uden videre forudgående behandling af materialerne.

Forstærkede kompositmaterialer. Det er velkendt at CNT anvendes som en forstærkende funktionel filler i plast og epoxy-dele. En anden anvendelse er som forstærkende tilsætning i avancerede polymer kompositer, som anvendes til sportsudstyr, f.eks. ketsjere, golfkøller, ishockey stave, men også i avancerede sportscykler, små både og vindmøllevinger med CNT er på markedet. Det fulde potentiale for disse anvendelser mangler generelt stadig, idet man endnu ikke er blevet i stand til fuldt ud at udnytte CNTs ekstreme trækstyrke. Andre kompositmaterialer er også på vej og omfatter aluminiums-CNT- og keramiske-CNT-kompositter, men der stadig kun lidt viden om dette anvendelsesområde.

Maling og bundmaling. CNT-baseret epoxymaling of bundmalinger blev introduceret i den marine sektor for nogle få år siden. Det hævdes at CNT-baseret overfladebelægninger har høj styrke og er begroningshindrende uden at frigive toksiske stoffer. Reduktion af vækst på skibe og både medfører direkte mindre brændstofbehov og reduktion af CO_2 emmission. CNT-baseret høj-kvalitets-epoxy-maling anvendes også til andre formål og er for tiden under udvikling til vindmøllevinger.

Elektrisk ledende blæk og coatings. Parallelt med udviklingen af CNT-baseret overfladebehandlinger, har der også været en del forskning angående udvikling af elektrisk ledende blæk, som grundet CNT-dimensionerne kan laves transparente. Denne egenskab er særdeles anvendelig i elektroniske displays og touch skærme. Sådanne produkter formodes at være på markedet.

Tekstiler. Det er vist at tekstilfibre med CNT giver stoffet elektrisk ledende egenskaber og produkter, der udnytter denne egenskab er allerede på markedet i Japan. Hvis de ikke allerede er på det Europæiske marked, så introduceres de sikkert i den nærmeste fremtid. Produkterne kan enten være tekstil eller tekstilet kan være en del af produktet. Elektrisk ledende tekstiler anvendes i meget tynde varmemåtter og er påtænkt anvendelse indenfor 'intelligent' tøj som i f.eks. vintersport.

Andre anvendelser. CNT er foreslået brugt til mange andre anvendelser, men det er svært at afgøre på hvilket udviklings niveau de er. Disse andre anvendelser omfatter *field emission devices* (FED), som anvendes til f.eks. lyskilder og andre elektroniske komponenter, non-volatile RAM memory moduler til computere og varme-kontrollerende enheder i mikro-elektronik.

Human og miljøeksponering

Der er begrænset viden om eksponeringsniveauerne og den mulige frigivelse af CNT under produktion og anvendelse af CNT-baserede produkter. De første livscyklus-analyser indikerer at

CNT overvejende vil slippe ud i miljøet (luftbårent støv, flydende og tør affald) i forbindelse med CNT produktion og fremstilling af CNT-baserede produkter. Hovedandelen af de frie CNT vil ende op i jord-zonen og sedimenter, pga. sedimenteret støv, spredning af slam, og transport med kloakvand og udslip fra rensningsanlæg. Det estimeres at 70% af affaldet fra CNT produktion og produkt-affald deponeres i jord-depoter og 17% håndteres på forbrændingsanstalter. En undtagelse er dog Li-ion batterier, hvor langt hovedandelen af batterierne forventes genanvendt. Der er dog ingen umiddelbar viden om genanvendelsen af CNT fra Li-ion batterier.

Eksponering i arbejdsmiljøet
Støveksponeringsniveauer for CNT i arbejdsmiljøet er vist at kunne nå op til mindst nogle få hundrede µg/m^3 luft. Hudeksponering skal tages i betragtning, men er hidtil kun dokumenteret i et studie, som viste at en arbejders totale handske-deponering var 2-6 g CNT efter høst og bearbejdning af CNT. I disse eksponeringssituationer bestod CNT overvejende i fiber aggregater og sammenfiltrede agglomerater og som partikler, hvor CNT var indlejret i en materiale- eller produkt-matrice. Et andet nyere studie, hvor hudeksponeringen blev undersøgt vha. tape stripping, viste ingen hudkontaminering af CNT.

De rapporterede eksponeringsniveauer i arbejdsmiljøet er anvendelige sammenligningsværdier for den typisk maksimalt opnåelige forbrugereksponering i situationer, hvor de anvendes eller er i direkte kontakt med CNT-baserede forbrugerprodukter og artikler. For alle eksponeringsscenarier mangler der studier, der specifikt undersøger og karakteriserer CNT eksponeringen.

Forbrugereksponering
Der er hverken fundet målinger af forbrugeres eksponering for CNT eller af udslippet til miljøet. Teoretisk set vil den generelle risiko for forbrugereksponering ifm. anvendelse af produkter, hvor CNT er indkapslet, være minimal. Der er dog specifikke situationer, såsom dermal eksponering ved anvendelse af CNT-coatede tekstiler og frigivelse af CNT fra ældede kompositmaterialer, hvor eksponering er mulig.

Eksponering i miljøet
Initiale livscyklusanalyser antyder at CNT udledes til miljøet under produktion af CNT og CNT-baserede produkter og fra det relaterede affald. CNT kan dog også ende miljøet i forbindelse med almen anvendelse af visse produkter og under deres ældning/nedbrydning af især nanokompositter. Raten for udslippene vil variere meget alt efter produktionsniveauet, typerne af udslip, det lokale miljø, anvendelsesforhold, og strategierne for affaldshåndteringen. Vi vurderer, at der er størst risiko for miljøudslip i forbindelse med industriel CNT produktion og anvendelse, afskaffelse af produktionsaffald, og endelig afskaffelse af de relaterede produkter. De modellerede miljøbelastninger er stadig moderate, men de hidtil anvendte modeller antager øjeblikkelig homogen opblanding i de forskellige dele af miljøet, hvilket er usandsynligt. Desuden inddrager modellerne hidtil heller ikke akkumulering i miljøet, selvom CNT bør betragtes som et unedbrydeligt (persistent) stof. Det er vigtigt at tage ”hot-spots” omkring kilderne og akkumulering tages med i betragtning i fremtidige analyser.

Toksiske effekter af CNT hos mennesker

Toksikologiske studier af dyremodeller viser at udsættelse for CNT kan medføre alvorlige sundhedseffekter. De primære eksponeringsveje er via huden (dermal), munden (oral), og luftvejene (inhalation). Hvorvidt der sker systemiske effekter afhænger til dels af om CNT kan passere biologiske membraner og transporteres til andre organer i kroppen. Derfor lægges der i den toksikologiske oversigt vægt på deres evne til at translokere og omdannes og efterfølgende gennemgås CNTs toksiske effekter i lungen, mave-tarmsystemet og huden.

Biologisk transport af CNT og dets persistens
Der findes adskillelige studier på dette område, men det er langt fra fuldt forstået hvordan CNT transporteres og eventuelt nedbrydes i det biologiske system samt hvilke mekanismer, der styrer CNTs toksiske egenskaber.

Der findes kun få studier, der har undersøgt translokation af CNT efter oral eksponering. Resultaterne viser generelt ingen eller meget lille transport af CNT til andre indre organer henover mave-tarm systemet.

I luftvejene sker der langsom fjernelse af CNT efter at de optages af makrofagceller. Fagocytosen ser dog ud til at foregår relativt hurtigt. Makrofagceller kan transportere CNT ud i de sub-pleurale dele af lungen (lungehinden), som er forudsætningen for at CNT eksponeringen kan medføre mesoteliom (lungehindekræft). Nyere studier tyder på, at en del af CNT også kan nå de sub-pleurale dele af lungen uden hjælp fra makrofagceller. Der er kun lille evidens for at CNT transporteres videre fra lungen og over i blodbanen, men de hidtil publicerede studier har ikke anvendt metoder der var sensitive nok til at detektere promille-koncentrationer af CNT, som er den forventede koncentration i blod, hvis translokationen skulle foregå. Derfor skal der nye studier til, som har den nødvendige sensitivitet for at afgøre om translokation fra lungen er en relevant mulighed. Halveringstider på op mod 300 dage er fundet for luftvejene.

Hvis CNT kommer ud i blodbanen, så er der stærke beviser på at de vil ophobe i leveren i såkaldte Kupffer celler, hvorfra de udskilles meget langsomt. Studier har vist adskillelige forskellige CNT materialer i lever celler op til 1 år efter intravenøs eksponering via blodbanen.

Lungeeffekter
Der er lavet mange studier af de potentielle toksikologiske effekter af CNT ved udsættelse via luftvejene. Mange af disse har fundet skadelige effekter ved rimelige doser. I denne oversigt har vi lagt vægt på to publicerede studier i rotter, som har fulgt OECD guiden for inhalationsstudier. Der blev eksponeret for to forskellige kommercielt tilgængelige MWCNT. Subklinisk inflammation blev observeret ved 0.1 mg/m^3, som var den laveste testede eksponering og gav dermed forsøgenes LOEL (Lowest Observed Effect Level). I begge studier blev dyrene udsat for aggregeret eller agglomereret MWCNT fremfor adskilte CNT fibre. Det vides ikke om udsættelse for adskilte CNT fibre vil resultere i en lavere LOEL.

Reduceret T-celle-respons blev fundet af to uafhængige grupper med inhalation eller lungedeponering af to forskellige MWCNT. Desuden blev forøget ekspression af TGF-β der initierer immunesupression respons fundet både efter indånding af MWCNT og SWCNT.

Lungedeponering af SWCNT resulterede i enkelt strengbrud i DNA i BAL-(lungeskyls)-celler efter 24 timer. Oral eksponering for den samme SWCNT inducerede enkelt strengbrud i DNA i leverceller. Mitsui MWCNT XNRI-7 har forårsaget mesoteliom i rotter og i en følsom musemodel. Mitsui MWCNT XNRI-7 består af forbundne ca. 5 µm lange individuelle MWCNT og har lavt Fe-indhold. I modsætning til Mitsui XNRI-7, medførte eksponering for en SWCNT og en kortere MWCNT ikke mesoteliom i rotter.

Hjerte-kar effekter
Der er bevis for, at lungeeksponering for SWCNT i kombination med kost med et højt fedtindhold fører til øget åreforkalkning, og der er tegn på, at hvis der er høje koncentrationer af CNT i blodet, så vil det fremme sammenklumpning af blodplader, hvilket kan lede til blodpropper.

Effekter på reproduktion
CNT ophobes ikke i testikelceller efter IP (intra peritonal) injektion, og injektion af CNT havde ingen effekt på mandlig fertilitet. Det er svært at vurdere betydningen af resultaterne, da meget lidt

tyder på, at CNT overhovedet vil translokeres over i kroppen fra de primære organer. Resultaterne viser imidlertid, at selvom CNT skulle komme over i blodbanen, så er der ingen tegn på direkte effekter af CNT på mandlig fertilitet. Imidlertid er der fundet reprotoksiske effekter efter udsættelse for andre NM og disse effekter forårsages formodentligt af indirekte påvirkninger efter eksponering i lungerne.

Effekter på huden
Inflammation blev observeret i huden efter udsættelse for et SWCNT råprodukt, men ikke efter eksponering for den oprensede SWCNT og en kommercielt tilgængelig MWCNT. Forskellen på disse effekter kan skyldes høje koncentrationer af forureninger af andre faser i SWCNT råproduktet og ikke en effekt af selve CNT.

Økotoksikologiske effekter af CNT

Det var ikke muligt at generalisere på tværs af studierne pga. et begrænset materialegrundlag og manglende systematik i undersøgelserne. De fleste studier er lavet på forskellige CNT og med anvendelse af forskellige dispergeringsprotokoller.Her fokuserer vi på videnskabelige publikationer der undersøger biodistribution og biologisk effekter af CNT i vand or jord organismer.

Bioakkumulering
Intet tyder på at der er passiv optag i de testede species. Der er dog adskillige studier der viser at CNT optages i fiskegæller og i organismer der filtrerer vand for at få føde. CNT kan desuden sætte sig på overfladen af organismer og akkumulere i deres maver, og på den måde medføre forskellige effekter.

Effekter på vandlevende organismer
Toksiciteten af CNT på vandlevende organismer ser ud til at afhænge af dispergeringen og stabiliteten af suspensionen i de testede væskemedier. Agglomerater har kort residenstid i vandkolonnen og sedimenterer ud. Når dispersionen af CNT er høj, så ses akutte effekter på i både ferskvand og marine organismer med effektkoncentrationer på ca. 0.1 mg/L (EC50 96 timer dødelighed i ferskvandskrebsen *Daphnia magna*, nedsat vækst med 96t LOEC for den marine kiselalge *Thalassiosira pseudonania*, og EC50 nedsat vækst for den marine grønalge *Dunaliella tertiolecta*). Derfor kan man forvente kroniske effekter som følge af CNT ved koncentrationer under 0.1 mg/L. I sedimenter blev der observeret effekter på organismer ved dosis større end 30 g/kg (dødelighed i den bundlevende organisme *Leptocheirus plumulosus*).

Der er desuden fundet negative respiratoriske symptomer i regnbue ørreden *Oncorhynchus mykiss* ved 0.1 mg/L. Den samme koncentration medførte nedsat vækst i den marine vandloppe *Tigriopus japonicus*. Toksiske effekter er fundet i anden generation af zebrafisken *Danio rerio* efter injektion af 2 ng CNT per embryo. Betydningen af denne observation er dog usikker, da der ikke er indikationer på at zebrafisk optager CNT fra vandkolonnen. I forsøg, hvor CNT var veldispergeret i vandet, er fundet forsinket klækning af zebrafisk ved 120 mg/L.

Der er ikke observeret systematiske genotoksiske effekter i marine organismer.

Effekter på terrestiske organismer
DWCNTs påvirkede reproduktion (larve produktion) af jordormen *Eisenia veneta* ved en koncentration på 37 mg/kg tørfoder. For planter er der observeret reduceret vækst for Zucchini *Cucurbita pepo* planten ved 1000 mg/L. Forbedret spiring fulgt af hæmning er observeret for sennepsfrø. Forbedret spiring blev set ved en LOEL på 2.3 mg/L for oksideret MWCNT, 23 mg/L for rå ubehandlet MWCNT, mens hæmning blev observeret ved en LOEL på hhv. 6.9 og 46 mg/L for oksideret og rå ubehandlet MWCNT. Det er interessant at oksideret MWCNT var mindre toksisk for sennepsfrø end de rå ubehandlede MWCNT.

Præliminær risikovurdering af CNT

Ved evaluering af resultaterne fra de toksikologiske undersøgelser og tilhørende informationer om de undersøgte CNT er det ikke er muligt klart at identificere om der er specifikke CNT typer har stærkere toksikologisk effekt end andre eller om toksiciteten skyldes specifikke CNT karakteristika. Dog er der en generel tendens til at CNT, der er rapporteret til at være lange og stive, kan medføre alvorlige toksikologiske effekter i lunger efter inhalation.

Humantoksikologisk farevurdering

De til dato publicerede toksikologiske studier giver kun grundlag for at anbefale nogle midlertidige grænseværdier for respirabel CNT støv:

Grænseværdi for arbejdsmiljøet = 1 µg/m^3 luft (carbon eller CNT)
En grænseværdi for forbrugere = 0.25 µg/m^3 luft (CNT)

Der er langtfra datagrundlag for at etablere grænseværdier for dermal og oral eksponering.

Risikovurdering for arbejdsmiljøet

Der er tydelig dokumentation for at der kan være eksponering for CNT i arbejdsmiljøet. Støvkoncentrationer på flere hundrede µg/m^3 over én til flere timer er rapporteret i forbindelse med forskellige arbejdsprocesser med CNT. Selvom eksponeringsmålingerne oftest er lavet på hele støvmængden, så når CNT koncentrationerne potentielt kritiske niveauer ved flere specifikke arbejdsprocesser og passende sikring mod eksponering bør etableres. De mest kritiske arbejdsprocesser er høstning af CNT og håndtering af pulver. Det skal bemærkes at eksponeringsniveauerne, der er observeret i arbejdsmiljøet, også medfører risiko for udslip af CNT til det ydre miljø.

Hudeksponering er blevet dokumenteret og kan medføre hudirritation. Oral eksponering er sandsynligt, hvis CNT inhaleres og det kan forekomme ved utilsigtet overførsel fra huden; f.eks. fra hånd til mund. Der er begrænset viden om toksiciteten af CNT i mave-tarm systemet, men nogle studier har vist en association mellem CNT eksponering og oksidativ stress og DNA-skade.

Risikovurdering for forbrugere

Der findes endnu ingen reelle eksponeringsmålinger for anvendelse af forbrugerprodukter. Vi lavede derfor nogle kvalitative til semi-kvantitative estimater af eksponerings-potentialer og -niveauer for specifikke produktgrupper ved hjælp af alternative tilgange. Vurderingen af risikoen for forbrugereksponeringen er dog under alle omstændigheder meget usikker.

Vi lavede først en simpel rangering af muligheden for eksponering baseret på lokaliseringen af CNT i produktet. Derefter etablerede vi en mere nuanceret procedure, hvor niveauet af potentiel frigivelse af CNT fra produktet estimeres vha. en systematisk beregnet skalering af *Margin Of Nano Exposure* (*MONE*) og den tilhørende potentielle effekt *Product Exposure Impact* (*PEI*). *MONE*-værdien er en skalering af eksponeringspotentialet for produktgruppen, hvor den arbitrære sandsynlighed for CNT frigivelse fra produktet, koncentrationen af CNT i overfladen og i den del af produktet, hvor den er højest, og 0.25 µg/m^3 (den foreslåede grænseværdi for forbrugereksponering). *PEI*-værdier blev beregnet ud fra *MONE*-værdierne koblet med en skaleret værdi for totale CNT mængde i produktet (µg, mg, g, og kg), og det vurderede antal af emner/mennesker potentielt eksponeret, sat i forhold til *PEI*-værdien for at alle danskere håndterer 1 kg CNT pulver én gang. I den sidste analyse anvendes modellering til at vurdere om det er sandsynligt at opnå en gennemsnitlig 15 minutters eksponering på 0.25 µg/m^3, i et 24.3 m^3 lokale med et luftskifte på 0,3 gange i timen.

MONE analysen viste at flere produktgrupper har et højt emissionspotentiale, når ældning, nedbrydning og forventelige uheld (f.eks. at knække en ketcher) tages med i betragtning.

PEI-værdierne viste, at den reelle påvirkning på forbrugernes sundhed formodentlig er lav for de udvalgte produktgrupper. Pga. det store forventede antal batterier og brugere, bliver Li-ion batterier produktgruppen med den højeste *PEI*, selvom, de har en lav (men stadig reel) forbruger eksponeringspotentiale. De næst- og tredje-mest vigtige produktgruppe er termoplast og elektronisk print, mens den fjerde-mest vigtige gruppe er sportsudstyr, hvor CNT forekommer i specifikke lag.

Modelberegningerne viste at det generelt er svært at nå op på forbrugernes grænseværdi for CNT på 0,25 $\mu g/m^3$. Det kræver 1 til 15 minutters aerosolisering af al CNT fra et 3,5 til 3,95 m^2 stort areal med en tykkelse på 5 µm og med 1 wt% CNT i et produkt kan medføre en gennemsnitlig 15 minutters luftkoncentration på 0,25 $\mu g/m^3$.Frigivelsesrater i den orden kan kun lade sig gøre i forbindelse med mekanisk slibning og skæring i CNT-baserede produkter som printede coatings, bundmaling, epoxymaling, elektrisk ledende tekstiler, og varmemåtter. Brud på lagdelte CNT kompositter eller Li-ion batterier, hvor CNT-koncentrationen lokalt kan være høj, kan også medføre høj akut eksponering. Disse situationer vil dog være særdeles sjældne for den almindelige forbruger.

Vi fremhæver, at det er umuligt at lave en reel risikovurdering for forbrugerne baseret på produktgrupper pga. mangel på relevante data og den store potentielle variabilitet indenfor produktgrupperne. De alternative metoder indikerer, at der generelt er en lav risiko for forbrugereksponering via luftvejene under forventet anvendelse af de CNT-baserede produktgrupper. Eksponeringsgrænseværdien kunne dog overskrides i de tilfælde forbrugeren kan udføre mekanisk bearbejdning af eksempelvis overflade coatings og CNT-tekstiler.

Der er relativ høj risiko for at forbrugere kan blive eksponeret på huden og oralt ved anvendelse af specifikke produktgrupper sammenlignet med risikoen for eksponering via luftvejene. Produktgrupperne inkluderer CNT-tekstiler og diverse ældede eller forvitrede kompositmaterialer, bundmaling og epoxymaling og påførsel af disse, hvis de er tilgængelige som forbrugerprodukter. Det er dog usikkert om eksponeringsniveauerne i normale brugssituationer er tilstrækkelige til at medføre toksikologiske effekter hos forbrugere.

Risikovurdering for miljøet
Der findes ingen grænseværdier for CNT i miljøet og de økotoksikologiske studier er langt fra komplette. Desuden har det vist sig, at de økotoksikologiske effekter varierer med den måde CNT er dispergeret på for at kunne udføre testene. De mest sensitive arter i vandmiljøet er hidtil *Daphnia magna* (LC_{50} = 0,05 mg/L); *Oncorhyncus mykiss* (LOEC = 0,1 mg/L), og *Tigriopus japanicus* (LOEC = 0,1 mg/L). For planter, er spiring hidtil fundet som værende det mest følsomme økotoksikologiske mål (LOEC på 6,9 mg/ml for sennepsfrø). Men, det er usikkert den mest følsomme art er relevant for den mest kritiske eksponering.

De fleste produktgrupper synes potentielt at kunne påvirke miljøet. Der er dog ingen målinger af CNT i miljøet. *MONE*-værdierne, som vi diskuterede ovenfor, er også relevante for vurderingen af risici for det ydre miljø. Fra denne vurdering har produktgrupper såsom printet elektronik, coatede tekstiler, varmemåtter, lagdelt sportsudstyr, og mikroskopi filamenter størst potentiale for at frigive CNT. Den sekundære gruppe inkluderede de fleste af de andre produktgrupper, såsom Li-ion batterier, termoplast og CNT-baserede nanokompositter med dispergeret CNT. Hvis vi antager at ældede og forvitrede produkter kan danne en CNT skorpe på produkterne som kan frigives. Set fra dette synspunkt kan stort set alle produkter potentielt medføre en eksponering af miljøet ved lang tids anvendelse og i affaldsdepoter.

1. Introduction

Carbon nanotubes (CNT) comprise a large group of nanometer-thin hollow fibrous nanomaterials with different physico-chemical characteristics (e.g., length to diameter ratios, atomic configuration, impurities, defects, and functionalization). In accord with their different physic-chemical characteristics, CNT also have a several interesting properties (e.g., electrical, optical, and thermal conductivity, tensile strength, and chemical reactivity). Therefore, CNT have a wide potential for industrial applications, which can enable several new materials and products, improve product performances, product lifetimes, energy savings etc. However, their properties and potential application varies with the type of CNT and quality of the CNT nanomaterial.

Currently, several companies have engaged into commercial scale production or utilization of CNT nanomaterials and they are available at numerous venders around the world. Today, several CNT manufacturers, have mega-ton annual production capacities and new low-cost synthesis methods are emerging, which can reduce the cost and increase the industrial interest considerably. This development is aligned with the still increasing demand and market growth. In 2009, the BCC Research reported that the global market for CNT nanomaterial grades reached $103 million.[10] In 2014, the market value had increased to $158.6 million and is expected to grow by 33.4% between 2014 and 2019.[12]

Most current products with CNT are thought to have been recently introduced onto the consumer market. However, a vast increase may occur within the next few years depending on the regulatory actions across the world. Some currently actual and potentially attractive applications are electronics, functional fillers in various types of composites (ceramics, polymer materials and textiles), chemical sensors, gas-storage etc.[10]. Other applications, such as the use of CNT in Li-ion batteries, may have existed for decades now.

Impediment to wide potential technological application of CNT, results from toxicological studies have indicated that at least some CNT may be able to induce serious human health effects. Most disturbing, is the observation of fibrosis-like reactions in test animals [118,198,199] and recent suggestion for occupational exposure limits for some types of CNT are in the range of a few µg/m^3 [4,166] as compared to mg/m^3 if CNT is regulated as graphite or carbon black.

Eco-toxicological studies also report high persistence and deleterious effects in some test animals [169,170]. While risk of occupational and environmental exposure to CNT is evident during their production, handling and application, exposure to the consumers may also occur in specific scenarios. Already in December 2011, these observations prompted the Environmental Protection Agency (US-EPA) in the USA to announce a SNUR (Significant New Use Rule) on the manufacture and use of CNT (http://chemicalwatch.com/9533/us-epa-issues-snur-for-20-substances). Because of a lack of established nomenclature for carbon nanotubes, the US-EPA currently identify the carbon nanotubes in generic form, e.g., carbon nanotube (CNT), single-walled (SW) CNT, double-walled (DW) CNT, and multi-walled (MW) CNT. However, other types and chemical derivatives exist and are on the commercial market.[67,149] In addition, high variability in impurity compositions and their concentrations may complicate a precise descriptive nomenclature for CNT. The apparent large variability in CNT types and purities makes it uncertain whether CNT, from a regulatory point of view, can be considered as one material at all or they should be grouped according to some of their many different characteristics.

The aim of this report is to:

- Provide an overview of the structure and chemistry of CNT nanomaterials with focus on the morphological types and derivatives, their atomic structure, basic properties, and manufacturing procedures

- Identify the types of commercial CNT nanomaterials and their main characteristics as well as their production volumes and main typical current and near-future industrial applications.

- Provide an overview and evaluation of the CNT exposure levels to humans and the environment from both production and use of CNT as chemicals, and their release from product use and discard as waste.

- Summarize the key results from human- and eco-toxicological studies on CNT nanomaterials and evaluate which exposures and end-points are of specific high concern
- Investigate whether data from the toxicological and exposure literature can be used to identify specific CNT nanomaterials, which may of high concern.

- Perform a preliminary integrated risk assessment of CNT considering the most critical route(s) of exposure.

Chapter 2 gives an introduction to the types and structures of the most common carbon nanotubes, their atomic structure and its importance for determination of the different properties of CNT. Special emphasis is made describe the variability of CNT and CNT nanomaterials, which may contain high amounts of carbon as well as inorganic impurities. The chapter is concluded with an overview of the types and general characteristics if commercially available CNT

Chapter 3 is an overview of the main current and near-future products containing or based on CNT. Again the range of applications is wide whereas the details on the specific CNT in use is often sparse. The overview is divided according to their main technical use categories. The chapter is concluded with an evaluation of the most important applications by volume.

Chapter 4 gives an overview over the published CNT exposure analysis to man and the environment. There is currently very limited data on potential consumer exposure as well as exposure measurements in the environment. Therefore, this chapter also includes an evaluation of the potential risk of CNT exposure to consumers and the environment. It is attempted to estimate the potential environmental release from productions based on data on exposure levels in workplaces and emissions from specific processes.

Chapter 5 summarizes the results from the most important toxicological studies relevant for human health. The chapter starts with an overview of the systemic biodistribution of CNT, followed by effects on the pulmonary, immune, and cardiovascular systems as well as their potential genotoxic, carcinogenic, and reprotoxicological effects. Finally, dermal effects are also briefly addressed.

Chapter 6 gives a brief summary of the results from ecotoxicological studies on CNT. First the biodistribution of CNT is discussed followed by an overview over a few select studies on specific aquatic and terrestrial species.

Chapter 7 gives an integrated risk assessment of CNT nanomaterials. First the role of specific CNT properties on CNT toxicity is discussed based on results from Chapters 2, 5 and 6 and suggested parameters relevant for CNT toxicity. Then an occupational and environmental exposure limit is derived based on key-literature findings. The release to the environment and potential human and consumer human exposure risk is discussed for the main product groups identified in Chapter 3 and assessed in Chapter 4. An integrated qualitative risk assessment is made using a simple control-banding principle. It should be noted that this risk assessment is very qualitative and can only be considered as an independent expert judgement.

Chapter 8 presents the general conclusions of the report.

For readers guide, each chapter starts with an easy-read summary of the main findings and conclusions for each chapter. Further reading in each chapter offers more detailed technical background information. It should be noted that other reports have also been published on these issues, but they rarely cover all the aspects at the same depth as herein. For briefer comparable overview, one may consult a previous Swedish report.[70] During development of this report, we found that the physico-chemical complexity of CNT nanomaterials, the current understanding of the exposure characteristics and levels, and finally the associated potential human and eco-toxicity requires an in-depth description to understand the complexity of CNT nanomaterials, their applications and not always easily interpreted in toxicity.

2. Types and characteristics of carbon nanotubes

This chapter describes the different principal types of carbon nanotubes (CNT), their characteristics, including their atomic structure and types and role of structural defects, as well as the types of typically abundant impurities. In addition, the common CNT production and purification methods applied to crude CNT, and their different main types of functionalization are briefly described. The chapter concludes with an overview of the main types and available characteristics found for commercial CNT nanomaterials.

2.1 Summary

Carbon nanotubes (CNT) are a group of nanomaterials consisting of cylindrical hollow carbon-based fibers (tubes). They have very high aspect ratios with diameters lower than about 100 nm (0.000001 mm) and lengths that can reach several hundred micrometers. They can be produced or refined to possess specific properties such as high electrical conductivity, optical transmission, or extremely high tensile strength. They are already used in several existing products in e.g., gas and energy storage, electronics, sensors, optics, and as additives in various nanocomposite materials.

It is important to note that crude CNT are usually inhomogeneous and have a high abundance of carbon and inorganic catalyst impurities. Due to technical challenges, commercial products are rarely fully purified. The actual purity and CNT quality depends on the manufacturing procedure and type of potential refinement of the products.

Types and atomic structure of CNT

CNT are typically grouped according to number of side-walls and the atomic structure in the side-walls. The simplest CNT are single-walled (SW), double-walled (DW) and multi-walled (MW) CNT. CNT are often considered as tubular fullerene materials. In REACH, the MWCNT are however, reported as “synthetic graphite in tubular shape”. Each tubular side-wall layer consists of carbon arranged in inter-connected hexagons, similar to the structure in graphene (the chicken wire pattern). In this structure, carbon is sp^2 hybridized, in which each carbon atom is bonded to three other carbon atoms in the same plane. A fourth remaining bonding electron is delocalized above and below the carbon plane. It is these delocalized electrons that causes the high electric conductivity in sp^2 hybridized carbon materials.

Besides SW, DW, and MWCNT, other CNT types include herring-bone (HB), bamboo (BB), and cup-stacked (SC)-CNT as well as branched CNT. Rolled-up graphene sheets (nanoscrolls) may also be considered CNT. In contrast to the simple types, the HB-, and BBCNT have internal structures in between the tube sidewalls. CSCNT is composed by stacks of open-ended cups or cones. Additional types are in the R&D stage and may involve tailored structures such as the nanobuds, which are CNT with fullerene-like protrusions at the sidewalls, tubes with endohedral (internal) fillings of specific molecules or phases making a transition from hybrid metal-CNT to nanowire materials with the tubular space completely filled with e.g., an electrical conducting metal.

For SWCNT, specific properties arise depending on the carbon bonding pattern. Relating the atomic CNT structure to the graphene sheet, a zig-zag pattern occurs when the sheet is rolled-up with no inclination (a chiral-angle of 0°) and a so-called arm-chair pattern appears at an orientation of 30°. In between these two extremes, carbon atoms produce a chiral (stair-case) pattern. In general, “armchair” SWCNT is metallic, whereas “zigzag” and “chiral” SWCNT can be metallic, semiconducting or insulating depending on tube diameter and roll-up orientation. The conductivity, however, also vary with the amount of structural defects in CNT lattice.

In reality, the CNT side-walls will rarely consist of perfect rolled-up graphene sheets containing only hexagons as described above. Vacancies (missing carbon atoms) or replacement of hexagons by pentagons and heptagons (so-called Stone-Wales defects) are the most common. Other common defects are dangling bonds at open-ended tube-ends and pentagon-heptagon defects in close-ended tubes. The Stone-Wales defects are especially favorable because they automatically give curvature and thereby lower the strain energy in the folded graphene sheets.

Synthesis methods for CNT

The structural quality and chemical purity of CNT vary with production technique and purification procedures applied after their synthesis. Most of the commercially available CNT appears to be produced by catalyst supported chemical vapor deposition (CVD). In conventional CVD, a carbon-rich feed gas is passed over the surface of a large population of nanopore or nanoparticle catalysts in a chamber with highly controlled atmospheric composition and pressure. At 350 to 1150°C the feed gas decomposes and re-deposits at the surface of the catalysts, where CNT nucleates and grows. In the floating catalyst method, nanoparticles are formed as part of the synthesis process. The dominant type of CNT, its structure and size are mainly controlled by size and type of catalyst, feed gas(ses) and temperature. CVD offers a wide possibility for growth of well-controlled tube dimensions, but the CNT may have many structural defects.

Carbon arc discharge synthesis is another important method where CNT is formed after evaporation of C from a graphite pole and deposited on the other pole. Arc discharge is a relatively simple and in-expensive method for large-scale production, but the purity is low due to co-formation of several different carbon phases. Carbon black, soot and fullerene are main by-products and for SWCNT, the raw product also has a high content of inorganic catalyst particles. The method is most suitable for production of short low-diameter CNT with low defect concentrations.

Other synthesis methods exist but are preferentially used for research work or synthesis of highly specific CNT types. One example of such methods is the pulsed laser ablation method, where a graphite target is evaporated together with metallic catalysts by a high intensity laser beam in an inert atmosphere. The vaporized carbon atoms start forming CNT in the vapor phase and the CNT material is deposited on a cooled substrate.

Purification and modification of CNT

Purification processes are typically employed to reduce the amount of impurities, to narrow size-distributions and purify the type of CNT. Impurity removal techniques include filtration with suitable solvents, oxidation by heat treatment in an oxygen atmosphere and treatment with acids which can dissolve metallic catalysts. Since the impurity levels of crude CNT can be as high as 80 % by weight depending on the synthesis method and –conditions, purification is a very important step to achieve high purity CNT. Size distributions are typically narrowed by chromatographic methods.

Chemical purification usually increases the concentration of sidewall defects, cut CNT in smaller segments and remove metal catalysts resulting also in open-ended CNT. Thermal annealing (graphitization) generally reduces the concentration of side-wall defects and results in higher electrical conductivity and improved tensile strength. A high concentration of defects on the other hand increases the chemical reactivity of CNT and is therefore also favorable for chemical functionalization. Knowledge on production methods and subsequent after-treatments can therefore give some indications on the CNT quality and impurity levels. In relation to this, chemically modified CNT (coated, functionalized, and filled) are by some considered a third generation of CNT called carbon meta-nanotubes.

Types and characteristics of commercial CNT
A survey over commercial CNT performed based on the typical distributors showed that detailed physicochemical descriptions were rarely available. Most CNT products were SW (n = 76), DW (n = 17), and MWCNT (n = 178). The tube diameters varied from 0.7 to 2.5 nm for SWCNT, 1.3 to 5 nm for DWCNT, and 4 to 150 nm for MWCNT. The general tube lengths were generally comparable ranging from ca. 0.5 to 50 µm, but some MWCNT are reported up to 150 µm long. Commercial bamboo-type and cup-stacked CNT products were also found, but these were minor in number of products. However, the frequency of a specific product does not reflect the production and use volume. Mixtures of CNT with TiO2, clays and carbon black also start to become available.

The purity levels varied significantly depending on the type of product. Overall, the full purity range was typically from at least 60 to at least 95 wt% CNT. The percentage of SW- and DWCNT with high impurity levels was higher than for MWCNT. The main impurities are normally other carbon materials and ash (inorganic catalyst material). In some cases other CNT types are also reported as impurities. The precise identity or composition of the impurities is usually not given.

Ready-made commercial CNT functionalizations are dominated by Li-ion, OH, COOH, and NH_2. However, numerous types of functionalizations can be made on demand as well as delivery of CNT in ready-made master batches. Functionalization may constitute up to about 5 wt% of a CNT nanomaterial. Despite the survey did not cover all sources, the data are believed to be representative.

2.2 Morphological and structural types of carbon nanotubes

Elemental carbon can exist in several amorphous, quasicrystalline to crystalline phases (allotropes) with different atomic structures. Fullerenes as well as single (SW) and multi-walled (MW) carbon nanotubes (CNT) and isolated graphite sheets (graphene) are the most recently discovered carbon materials [e.g., 1,48,86,191,209,249]. However, graphite and diamond are probably the most well-known carbon compounds in the general public (see Fig. 2-1 for structures of typical carbon allotropes).

The simplest ideal CNT can be depicted as a rolled-up-like graphene sheet that is welded into a seamless tube (Fig. 2-2). The truly rolled-up graphene sheets, however, also exists. They are called nanoscrolls [e.g.,226] and may be classified as one sub-class of CNT (Fig. 2.2a). By some chemists, CNT are considered a subgroup of fullerenes called tubular fullerene structures [217], but in REACH, using the IUPAC naming, CNT are however, currently reported as “synthetic graphite in tubular shape” (http://apps.echa.europa.eu/registered/data/ dossiers/DISS-b281d1a0-c6d8-5dcf-e044-00144f67d031/AGGR-721d9f57-fa89-40d0-9982-aaa951a89b80_DISS-b281d1a0-c6d8-5dcf-e044-00144f67d031.html).

In addition to the simple CNT types with straight sidewalls, additional morphological types may also occur. These include nanocones, cone- or cup-staked CNT, herringbone-bamboo CNT, normal and inverse bamboo CNT, and nanobuds. Especially, herring-bone and bamboo CNT have internal carbon structures partially filling the tubular space. The nanubuds on the other hand are CNT with build-in fullerene-like protrusions along the sidewalls. Finally, branched tubes varying from simple Y, L, T and H shaped branched morphologies to more complex multi- and network branched CNT can also be controlled in the laboratory now.[234]

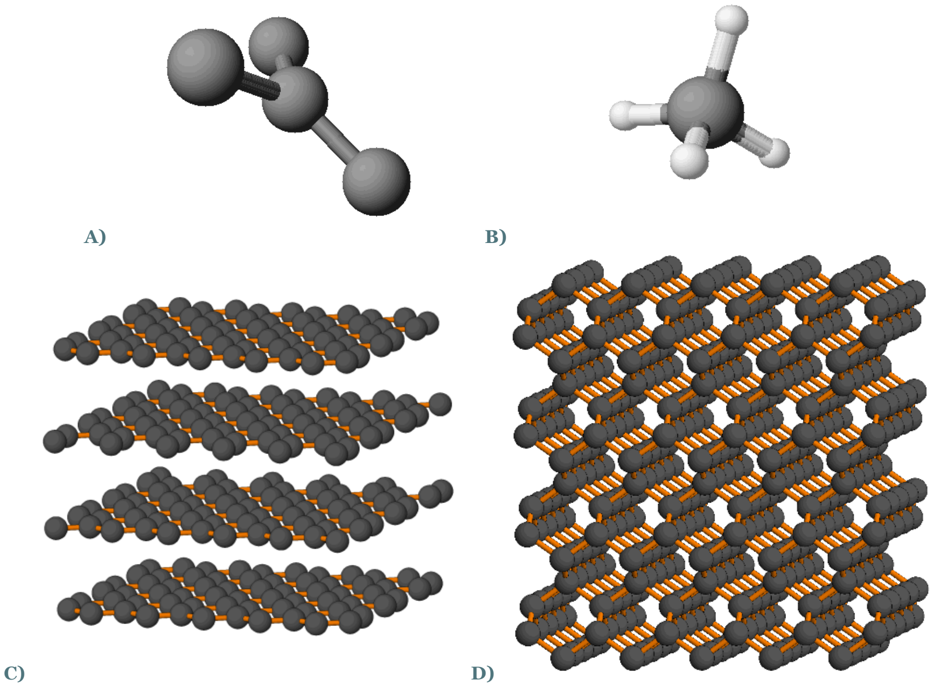


FIGURE 2-1

A) THE TRIANGULAR ARRANGEMENT OF THE THREE BONDS OF SP^2 HYBRIDIZED CARBON ATOMS. THE CENTRAL CARBON ATOM IS BONDED BY THREE OTHER CARBON ATOMS IN THE SAME PLANE AND THE FOURTH BONDING ELECTRON IS FREE. B) THE TETRAHEDRAL ARRANGEMENT OF THE FOUR BONDS OF SP^3 HYBRIDIZED CARBON ATOMS. EACH CARBON ATOM IS BONDED BY FOUR IDENTICAL BONDS TO FOUR CARBON ATOMS. C) PLANAR ATOMIC STRUCTURE OF GRAPHITE WITH STRONG SP^2 HYBRIDIZED CARBON IN EACH SHEET WITH EACH SHEET BONDED BY VAN DER WAAL FORCES. IT IS EVIDENT FROM THE BONDS AND THE STRUCTURE THAT THE GRAPHITE MATERIAL IS ANISOTROPIC. D) CUBIC DIAMOND STRUCTURE. NOTE THAT EACH CARBON ATOM BONDS TO 4 OTHER ATOMS IN A THREE DIMENSIONAL NETWORK. THIS MATERIAL IS GENERALLY CONSIDERED ISOTROPIC.

Consequently, including the nano-scolls, the CNT group may principally contain:

- rolled up graphene sheets (one or more spiral “layers”)
- open-ended seamless tubes (one or more concentric layers)
- regular close-ended seamless tubes (one or more concentric layers one)
- stacked seamless cones or cups (with one or more concentric layers)

Type 2 to 4, are grown in their length direction and typically from the surface of a catalyst particle or nanohole, whereas type 1 is formed after crystallization of graphene sheets or delaminating of graphite. The seamless tubes may also have the internal bamboo structures and protrusions mentioned above. All types may also be produced with branched structures which with time may be established as their own sub-group.

Closed-ended tubes may be end-capped by fullerene-like structures, build of hexagons and pentagons. This type of end-structure is shown in Fig. 2.2c. In structurally perfect, but open-ended tubes there will be chemically reactive “dangling bonds” (DB) at the extremities [240]. Reactive ends in un-capped CNT can be used for tip-functionalization. This end-structure can be seen in Fig. 2-2b.

Looking at the atomic structure, the imaginary graphene tube sheet can be rolled-up to form three different principal atomic structures in CNT; namely armchair, zig-zag and chiral CNT. The names arise from the carbon-carbon sidewall bond structure perpendicular to the CNT rotation axis. The “armchair” and the “zig-zag” “rolling” occurs at unique orientations, but the chiral CNT can occur within a 30°-range in orientation from the perfect zigzag to armchair. Imagining looking through the tube center in a chiral CNT, the carbon atoms will be arranged in a spiral stair-case.

The specific “roll-up” orientation or chirality (C_h) of a CNT is described by a geometric vector-system ($C_h = na_1 + ma_2$) related to a graphene sheet (Fig. 2-3). The vectors are a_1 and a_2 and n and m are their lengths in number of carbon atom steps. Therefore the chiral vector is simply described as (n,m). The vector system is used to classify the specific chiral types of CNT [36,93,149]. For zig-zag CNT n is an integer and m = 0, whereas n = m for “armchair” CNT. For chiral CNT both n and m are integers and $n > m$ and $m \neq 0$.

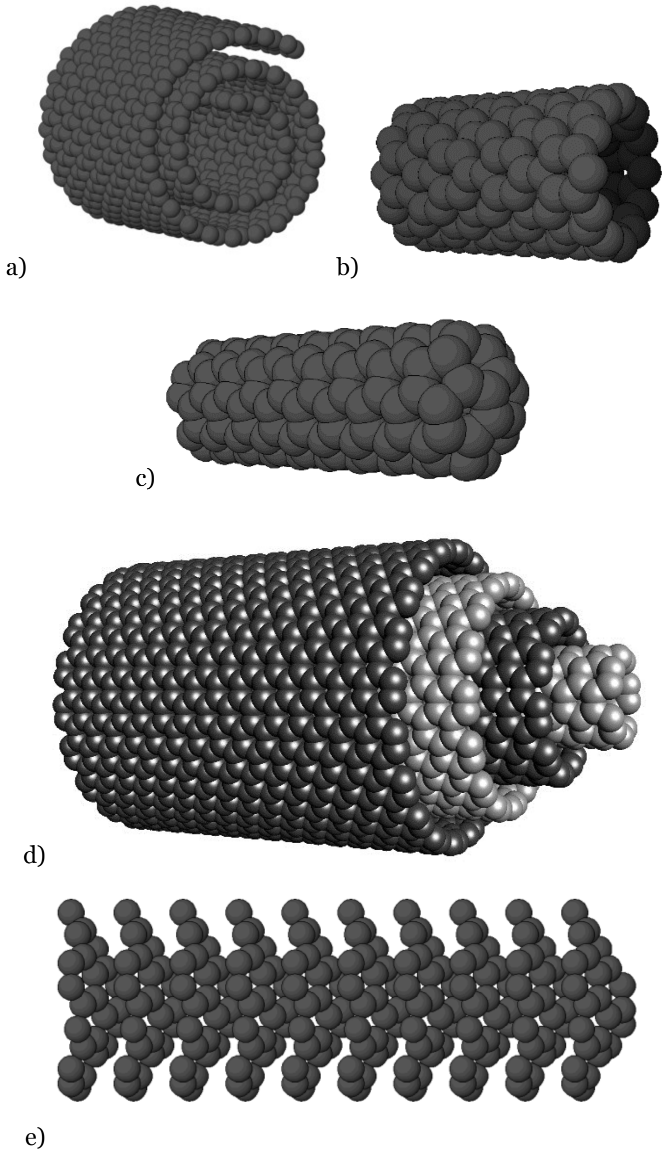


FIGURE 2-2

EXAMPLES OF PRIMARY CNT STRUCTURES: A) AN OPEN-ENDED GRAPHENE CNT. B) AN OPEN-ENDED SINGLE-WALLED ZIGZAG CNT. C) A CLOSE-ENDED SINGLE-WALLED CNT. D) AN OPEN-ENDED MULTI WALLED CNT CONSISTING OF FOUR CONCENTRIC TUBES, ALL BUILD WITH THE SAME HEXAGONAL LATTICE. E) A CONE-STACKED CNT, WHICH IS BUILT OF TUBULAR CONES WITH GRADUALLY CHANGING DIAMETER.

To illustrate the system, a SWCNT with a roll-up vector of (7, 3) is a chiral SWCNT. As seen in Figure 2-2, this SWCNT is 0.7 nm in diameter and can be constructed by theoretically “rolling-up” the graphene sheet in such a way that the carbon atom at O is superimposed on O'. A zigzag and an arm-chair SWCNT with similar diameter can be made with the chiral vectors (9,0) and (5,5), respectively. The "roll-up" vectors are also sometimes used to divide CNT into families with common properties. A family is described by a number that corresponds to 2n + m. This means that in, e.g. family 22, one finds SWCNT with the chiral vectors: (11,0), (10,2), (9,4), and (8,6).

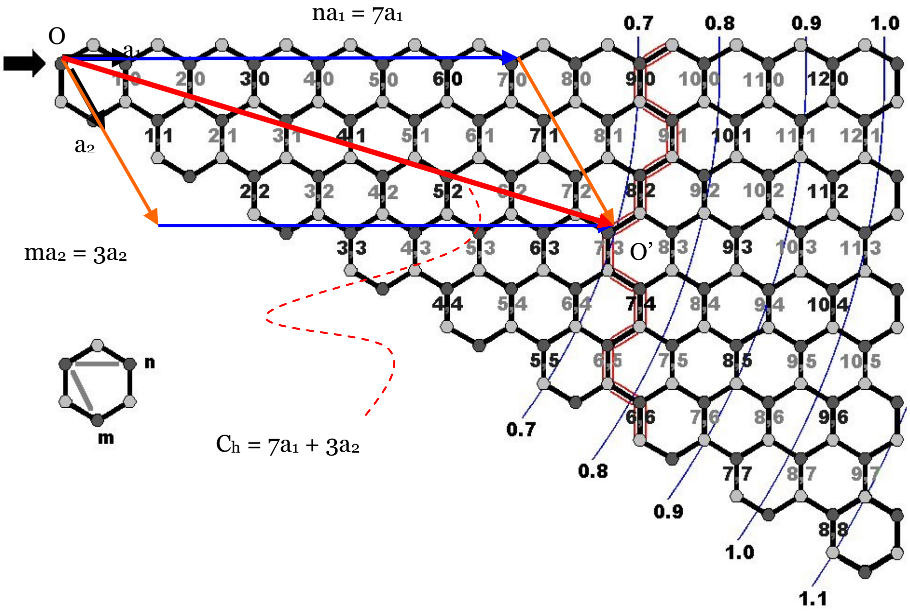


FIGURE 2-3

GRAPHENE SHEET WITH INDICATIONS OF THE POSSIBLE "ROLL-UP" (CHIRAL) VECTORS; CH = NA1 + MA2. THE ATOM MARKED WITH THE BLACK ARROW, CAN ONLY BE IDENTICAL, AND THEREFORE ROLL-UP, TO THE OTHER DARK ATOMS. THE CH (7,3) SWCNT IS EXEMPLIFIED WITH THE RED ARROW. THIS CNT CAN FORM SUPERIMPOSING THE CARBON ATOM AT O ONTO THE CARBON ATOM AT O'. DIAMETERS OF DIFFERENT CH'S (0.7 TO 1.1 NM ARE SHOWN) ARE INDICATED WITH THE BLUE LINES. THE RED-AND-BLACK LINE RUNNING FROM (9,0) TO (6,6) INDICATES THE SMALLEST STABLE SWCNT.

The smallest possible diameter of stable SWCNT is around 0.7 nm and found for two different tube orientations, namely the listed example at C_h(7,3) and another at C_h(9,0). However, structurally stable SWCNT appear from the vertical (black-and-red) thick line running from C_h(9,0) to (6,6) and higher chiral vectors.

The chirality of the graphene layer plays an important role on the physico-chemical properties of CNT. "Armchair" SWCNT is metallic, whereas "zigzag" and chiral SWCNT can be metallic, semiconducting or insulating depending on tube diameter and chirality. The larger the tube diameter, the higher number of tube configurations exist and potentially more different CNT's may be produced. This is supported by theoretical calculations, where phase diagrams for e.g., zigzag SWCNT suggest the presence of several different properties, including both repulsive SWCNT and different types of insulating and conducting phases [18]. However, one third of the tubes in crude SWCNT will always be metallic due to the purely statistical distribution of chirality. This is illustrated in Fig. 2-3, where the metallic tubes have numbers written in black and tubes with other properties are written in gray. It is important to also note that since the electronic structure influences on the chemical reactivity, the insulating, metallic and semi-conducting SWCNT will also have different chemical reactivity. Therefore chirality may also play a role on other properties of SWCNT.

2.2.1 Bonding of carbon

The type of bonding in carbon materials controls their electrical conductivity, general isotropi/anistropi as well as their mechanical (e.g, hardness, tensile strength) properties. It was already discussed above that CNT may have different conducting, metallic or insulating properties. This may be ascribed to both the atomic level and the "long-range" atomic nanostructure of the CNT.

At the atomic level, carbon in CNT and graphene belongs to the so-called sp^2 hybridized carbon materials, where *s* and *p* refers to two electronic orbitals of the carbon atom, respectively (Fig. 2-1a). This sp^2 group also includes graphite and fullerene (see more phases and common uses in Table 2-1). Diamond is a sp^3 material (Fig. 2-1b). A third type consisting of mixed sp^2-sp^3 hybridization occurs and is found in highly disordered (amorphous) carbon materials. See Appendix 1 for a more detailed description of the different types of carbon hybridization and typical compounds.

In sp^2 hybridized carbon materials, each atom is bonded to only 3 other atoms in a planar triangular configuration (Fig. 2-1a). If the triangle is perfect, the carbon atoms are arranged in a flat hexagonal lattice, which is the graphite or graphene sheet depicted in Figure 2-3. Imperfect triangles give rise to pentagons or heptagons (or other configurations) and the carbon sheet can no longer maintain being flat. In the hexagonal sheet, three of the four bonding electrons of carbon are used in the hexagonal lattice and the last electron is delocalized in so-called π-orbitals above and below the sheet. The delocalized electrons can move around on the graphite sheet, which is the reason for its electrical conductivity.

TABLE 2-1

EXAMPLES OF SP2 HYBRIDIZED PHASES

Phase	Comment	Use
Graphite		Lubricant, steel etc.
Graphene	New isolated material	In development
Fullerenes		Lubricant, cosmetics, electronics
Carbon nanotubes	Sometimes listed of a subgroup of fullerenes	See Chapter 3
Glassy carbon	Material with random orientation of graphene	Electrodes
Activated carbon	Very small particles. High surface area.	Absorbent of pollutants and noxious gasses
Carbon Black	Poorly defined structure, Typically planar stacks or multilayer balls	Pigment, printing ink and filler in rubber.
Carbon fibers	Graphite ribbons parallel to the fiber. Has a high tensile strength.	Used in high-strength plastics, e.g. for tennis rackets and aircraft components.

As understood from the triangular bonding arrangement, the sp^2 carbon materials are anisotropic and hence, do not have similar properties along the different crystallographic orientations. This is clear from Figure 2-1c showing the atomic structure of graphite, consisting of stacked graphene sheets, which can be in either hexagonal or rhombohedral symmetry. The bonds within a carbon sheet, cone or tube are very strong, but the bonding between the sheets is weak and maintained by Van der Wall forces.

Due to structural and bonding similarity, the sidewalls of MWCNT will have some of the same properties as graphite. However, it is important to note the curvature of the tubular and spherical carbon materials makes the *sp*^{2}-bonding protrude into the third dimension. Therefore these bend sheet structures can achieve some of the characteristics of the *sp*^{3} bonded materials (Fig. 2-1b), which are isotropi, high hardness high thermal conductivity, and no electrical conductivity. This is the reason that CNT can exhibit some mechanical and electrical properties in between that of graphite (Fig. 2-1c) and diamond (Fig. 2-1d).[240]

2.2.2 Types and roles of structural defects

In reality, CNT will rarely consist of perfect rolled-up graphene sheets containing only hexagons as described above. Point defects or replacement of hexagons by pentagon and heptagon “defects” can lower the strain energy in the graphene sheets because they automatically gives curvature as mentioned above (Figs. 2-5a-c shows these basic structures).[77,217] The importance of pentagons and heptagons in curved carbon structures is evident in the highly strained spherical fullerenes. For example C_{60} contains 20 hexagons and 12 pentagons and C_{70} contains 25 hexagons and 12 pentagons. In close-ended CNT, the end-cap consists of half a fullerene or a fullerene-like sphere exemplifying the structural link between fullerene and CNT. Other types of defects in the atomic structure may also occur at the end of tubes, and should be very pronounced where the CNT growth started from a catalyst.

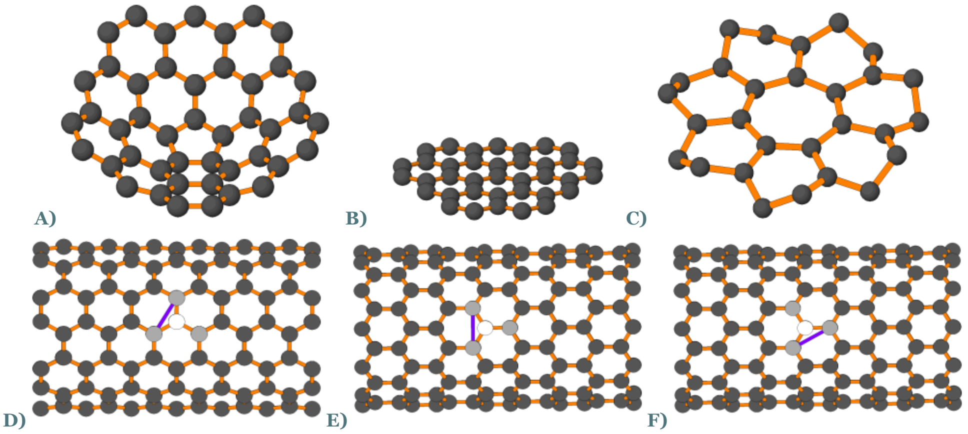


FIGURE 2-4

BASIC PERFECT AND DEFECT STRUCTURES IN CARBON SHEETS AND CNT A) PERFECT PENTAGON CARBON SHEET B) PERFECT HEXAGON CARBON SHEET, C) PERFECT HEPTAGON CARBON SHEET D) 5-1DB DEFECT (5 FOR PENTAGON AND 1DB FOR ONE DANGLING BOND), E) 5-1DB-T DEFECT IN CNT (1 PENTAGON, 1 DB; T = TILTED ALONG THE TUBE ROTATION AXIS), F) 5-1DB-P DEFECT IN CNT (1 PENTAGON, 1 DB; P = PERPENDICULAR TO THE TUBE ROTATION AXIS). IN D, E, AND F, THE WHITE-COLORED SPHERE IS THE VACANCY REPLACING THE CARBON ATOM. THE THREE GRAY-COLORED CARBON ATOMS HAVE BROKEN BONDS WHICH ARE COVALENTLY BONDED BY TWO OF THE THREE ATOMS.

Theory predicts that strain-induced structural defects should be most pronounced in CNT with small diameter sizes. [e.g., 77] Growth defects may also occur due to substitution of some C atoms by foreign atoms (e.g., He or B) in the CNT structure to form heterogeneous CNT. [149,150] Finally, end- and sidewall defects may also occur during purification and mechanical after-treatment of crude CNT (see section 2.5). Structural defects in CNT may therefore have quite different characteristics and form at different stages of the production and use. Still, the most common structural defects in CNT are single vacancies (missing atoms) and the so-called Stone-Wales (sw) defects, which are topological deviations from the hexagonal structure in the ideal graphene sheet.

In this report the defects are groups into the following main types:

- as-produced structural and impurity defects
- secondary structural defects caused by mechanical after-treatment
- structural defects caused during chemical purification and covalent
- functionalization.

Concerning CNT quality, it is important to note that CNT loose some of the graphitic properties with increase defect concentrations. On the other hand they may become chemically more reactive. Hence, the imperfections opens up for a higher degree of functionalization than for perfect graphitized (hexagonal) CNT. The characteristics of the defects and the type of CNT may therefore ultimately determine the type of functionalization that can be made and hence the different uses of CNT. In fact functionalization is usually required to disperse and or chemically bind the CNT in the different commercial applications.

2.2.2.1 Vacancy defects

As can be understood from the sp^2 atom bonding (Fig. 2-1a), a single vacancy results in three dangling bonds. Similar dangling bonds also occur for each carbon atom at the extremities of open-ended CNT. Dangling bonds enable chemical bridging between individual tubes and is also a site for covalent functionalization.[219] Vacancies result in chemically much more reactive sites than topological defects and are also important for the gas adsorption and radical scavenging capacity of CNT.[77]

Vacancy defects may result in different types of topological re-structuring depending on tube chirality (Figures 2-5d-f). In a (n,n) armchair CNT, the ideal single vacancy results in a single dangling bond and a pentagon, denoted a 5-1DB defect (5 for pentagon and 1DB for one dangling bond). In (n,0) tubes (zig-zag), the ideal vacancy results in a 5-1DB-T defect (1 pentagon, 1 DB; T = tilted along the tube rotation axis) or a 5-1DB-P defect (1 pentagon, 1 DB; P = Perpendicular to the tube rotation axis). Calculations suggest that the 5-DB and 5-1DB-P defects are energetically much more favorable than the 5-1DB-T type defect.[77] However, the defect energies vary with tube diameter and the hexagonal structure overall becomes more stable at increasing size.[135]

2.2.2.2 Stone-wales defects

The topological sw-defects result in a change in hybridization at specific sites in the CNT lattice and formation of local regions covalently interconnected arrangements of two heptagons and two pentagons organized in pairs opposite each other (Fig. 2-6). Therefore, the sw-defect is also known as the 5-77-5 defect. This structural sub-unit can be incorporated into the hexagonal sheet by rotating one of the C-C bonds by $\pi/2$. The sw-defects are typically observed both at sidewalls and closed-end tube tips, which strongly affects the electronic, mechanical, and transport properties as well as the chemical reactivity of the CNT. sw-defects are also most pronounced in CNT with small diameters and can be considered as strain-relaxing topological adjustments. Therefore the likelihood of sw-defects formation also varies greatly with chirality. They are energetically most favorable at high chiral angles (i.e. the metallic armchair (n,n) CNT) and least in the zigzag (n,0) CNT.[77]

The covalent connection of the heptagons and pentagons in the sw-defect occupy the free electron present in the hexagonal sheet. This results in chemical reactivity of the sw-defects. Acid treatment will normally induce a number of sw-defects along the tubular structure as well as at the caps of the CNT (Section 2.5). Acid treatment, which can be used as a means of CNT purification, is therefore a deliberate or accidental means of increasing the chemical reactivity and sorption capacity of CNT.

Incorporation of pentagons and heptagons do not only cause defects, which are only visible at the atomic scale. Pentagon and heptagon defects can also induce kinks in the CNT structure, which can result in a change of growth direction in parts of the tube wall. They are also fundamental for development of the branched types of CNT, which currently has received increasing interest due to potential applications in optics and sensor technology and nanoelectronics, not least as nano-size circuits.[207,221,234]

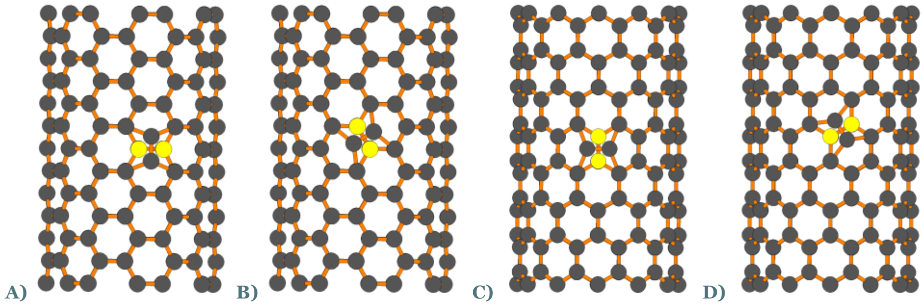


FIGURE 2-5

DIFFERENT CONFIGURATIONS OF THE STONE-WALES (5-77-5) DEFECTS IN ARM-CHAIR AND ZIG-ZAG TYPE CNT. A) (N,N)-I; B) (N,N)-II; C) (N,0)-I; AND D) (N,0)-II. (AFTER [77]). THE YELLOW SPHERES REPRESENT CARBON ATOM VACANCIES IN THE HEXAGONAL STRUCTURE RESULTING IN RECONFIGURATION INTO THE STONE-WALES (SW) DEFECTS.

2.2.2.3 Additional defects in "atypical" CNT structures

There is less information on the defect structures of other "atypical" morphological types of CNT. It is known that vacancy defects are also the main defect type in carbon nanocones.[66] It is inferred that the so-called cup-stack and cone-stacked nanotubes have some structural similarities with singular nanocones. Calculations suggest that the tip-region of a single nanocone may contain 1-5 atom vacancies, which can be adjusted by structural relaxation by formation of pentagons and other polygons. The most stable tip re-configuation consists of two pentagons and one heptagon around the defect.[66] The defects in the fullerene-like protrusions in nanobuds may consist of both nanocone- and tip-type defects observed in close-ended CNT.

The chemical reactivity at the sidewalls of the "atypical" CNT may however, be quite different. It is assumed that true side-wall defects in the various stacked CNT structures are generally comparable to those present in single nanocones and straight-wall CNT. However, special for the herring-bone-bamboo and stacked CNT, is that they are build-up by many segments (stacks). In addition to internal tip-defects and side-wall defects, these CNT types should also have a high concentration of dangling bonds at the top of each cone (or at each fullerene-like protrusion in case of the nanobud). Therefore, these structural types may be chemically more reactive, which opens up for a higher degree of functionalization than for straight-wall CNT. The cup-stacked-CNT has also been found to act more as a semi-conducter than a metallic material due to the graphite-like structure induced by the stacking.[128] They are also found to be more elastic than CNT while still possessing higher strength than graphite if the cone tilting angles between the sidewalls and the tube rotation axis are sufficiently low.[233] This is applied in recent sporting goods (Chapter 3).

2.3 Synthesis Methods

Several techniques have been developed to produce CNT in high quantities and other for production of specific high-quality CNT. Chemical vapor deposition (CVD), and arc discharge are the most frequently applied methods, whereas laser ablation can be used for well-controlled high-purity CNT. [e.g, 180]. The general characteristics of these procedures and their products are summarized in Table 2-2. Most of synthesis processes take place in vacuum with controlled process gases, but both carbon arc-discharge and CVD growth of CNT can be done at both vacuum and at atmospheric pressure.[87] High production rates can be achieved by CVD and carbon arc-discharge. Advances in catalysis and continuous growth processes are still being further developed to enable cheaper high-volume CNT production. On-going developments in manufacturing techniques may even result in new large-scale manufacturing techniques in the years to come.[248] For example, CNT production is possible in waste recycling of plastic bags [172] and large-scale production of CNT has started using recycled car-tires as a source (http://www.nanocarbontechnology.com/)

Current CNT production may be based on either floating or supported catalyst routes. Floating catalysts are nucleation seeds that are generated during the process; either by co-formation from the same source, different sources or parallel injection of a nanocluster or small nanoparticles. Supported catalysts are either fixed nanoparticles or nanoholes from which the CNT can nucleate and grow. Different CNT structures (and other carbon allotropes) appear depending on process gas, temperature, types of catalyst particle and production technique. Figure 2-6 illustrates the different CNT types appearing with increasing production temperature using metal-particle catalyzed CVD growth.[239]

2.3.1 Chemical Vapor Deposition

The CVD technique is the most widely used technique for large-scale production of CNT. The method involves growth of CNT from volatile carbonaceous precursers (e.g., acetylene, ethylene, ethanol or methane) at 350 to 1150°C using either a nanoparticle or a nanopore as a catalyst.[87,180] CVD has the advantage of being very flexible for modifications and easy to scale. Rümmeli et al. [180] specifically report eleven different variations of the CVD process of which the thermochemical and high-pressure CO disproportionation (HiPCO) techniques probably are the most widespread in industry. Other methods include plasma-enhanced CVD, Aerosol-Assisted CVD (AA-CVD), aerogel supported CVD, Alcohol Catalytic CVD at atmospheric pressure or in vacuum, and hybrid laser-assisted thermal CVD.

The thermochemical CVD process can be carried out in a fluidized bed reactor where the feed gas (such Ar, H_2, N_2 and NH_4 or mixtures thereof) is blown into the heated reactor together with the carbon source (Fig. 2-8a). Some of the carbon source condenses on the catalysts where it starts to decompose followed by CNT nucleation and growth. The catalyst can be either floating or supported as shown in Figure 2-8a. Floating catalysts are used in the HiPCO process where the catalyst particle forms after volatilization of a pre-curser. In the HiPCO process, SWCNT is synthesized by a high pressure gas phase reaction of CO with typically iron as a catalyst.

In general, the choice of catalyst determines the type of CNT and the temperature and reaction time can be used to control the quality and length of the tubes. Recent development in CVD has enabled faster growth rate and improved CNT quality (Table 2-2). E.g., water-assisted chemical vapour deposition has enabled growth of densely aligned SWCNT on substrates with mm-long tubes within 10 minutes.[75] Figure 2-8b shows an example of this material.

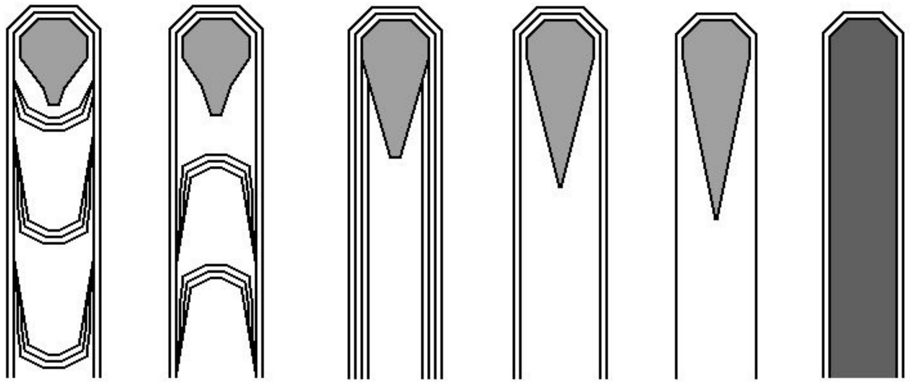


FIGURE 2-6

SCHEMATIC ILLUSTRATION OF DIFFERENT CARBON NANOTUBE SPECIES OBSERVED AT INCREASING CVD OPERATION TEMPERATURE. FROM LEFT TO RIGHT, INVERSE BAMBOO MWCNT, BAMBOO MWCNT, STRAIGHT-

WALLED MWCNT, DWCNT, SWCNT AND METALLIC NANOWIRE SWCNT APPEAR WITH INCREASING TEMPERATURE. IN THIS CASE THE NANOPARTICLE CATALYST IS A LOOSE SUBSTRATE, WHICH MEANS THAT THE NANOPARTICLE CATALYST (GRAY PARTICLE INSIDE THE CNT) CONTINUOUSLY MOVES UP AT THE TOP NANOTUBE DURING GROWTH (MODIFIED FROM [239]). MWCNT TYPES ARE PRODUCED AT CA. 300 TO 800°C AND SWCNT CA. 600 TO 1150°C.[87]

TABLE 2-2

SUMMARY OF THE MOST IMPORTANT CNT PRODUCTION METHODS COUPLED WITH INFORMATION ON PRODUCTION YIELD, TYPES OF CNT AND ADVANTAGES/DISADVANTAGES. (COMPILED FROM OVERVIEW BY DAENEN AT EL.[27]; ISMAIL ET AL.[87]; AND RÜMMELI ET AL.[180])

Method	Typical catalysts	Typical CNT Yield	Typical SWCNT	Typical MWCNT	Advantage	Disadvantage	Origin
Chemical vapour deposition (CVD) **(Many different variants)**	Floating/supported Fe, Ni, Co Support e.g., Al_2O_3 "nanopores as catalysts" SiC, Al_2O_3, TiO_2	20 to 100 %	Length: Up to cm's possible OD: 0.6-4 nm	Length: Up to cm's OD: 10-240 nm	Long tube length possible, simple process, SWCNT diameter can be controlled well	Depending on method there may be high impurity concentrations. Especially MWCNT are riddled with defects	Endo, Shinshu University, Nagano, Japan, 1995 [49]
Arc discharge	For SWCNT, the anode is doped with Co, Ni, Fe (Fe requires H_2 in gas), or [RhPt]	30 to 90 %	Length: Not reported OD: 0.6 - 1.4 nm	Length: ~ 20 µm ID: 1-3 nm OD: ~ 10 nm	Easy production of SWCNT and MWCNT. SWCNT have few structural defects. MWCNT are without catalyst. Relatively in-expensive and open air synthesis is possible.	CNT in random sizes, bundles and aggregates; co-production of fullerene, as well as amorphous and crystalline carbon increase needs for purification	Ebbesen and Ajayan, NEC, Japan 1992 [41]

Method	Typical catalysts	Typical CNT Yield	Typical SWCNT	Typical MWCNT	Advantage	Disadvantage	Origin
Laser ablation (vaporization)	Catalysts mandatory Metal-doped carbon source [Fe+O_2], [Fe+H_2], Ni, Co, Pt, Rh, [NiCo]	Up to 70 %	Length: 5 – 20 µm OD: 1 - 2 nm.	Synthesis is possible. Little information available	Suited for low-volume high-quality CNT (primarily SWCNT), relatively high purity, high size control and few defects.	CNT in bundles and robes; Costly and low production volume. May need purification of fullerene, amorphous carbon and metal catalyst	Guo, Rice, 1995 [69]

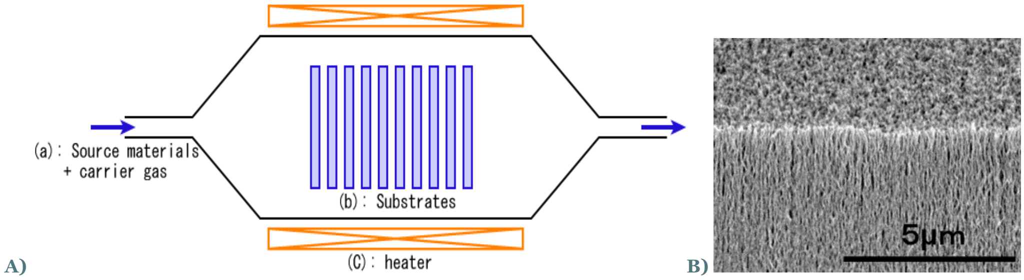


FIGURE 2-7

PRINCIPAL SKETCH OF TYPICAL CVD METHODS FOR HIGH-VOLUME PRODUCTION OF CNT. A) SUPPORTED CATALYST THERMOCHEMICAL CVD. THE CATALYSTS ARE PLACED AT (B), THE SUBSTRATES, FROM WHICH THE CNT GROW AT TEMPERATURES CONTROLLED BY THE HEATING COIL SURROUNDING THE FLUID BED CHAMBER. B) SCANNING ELECTRON MICROSCOPY IMAGE OF ALIGNED SWCNT GROWN BY ENHANCED GROWTH WATER-ASSISTED CVD (SOURCE: HTTP://EN.WIKIPEDIA.ORG/WIKI/CARBON_NANOTUBE#CITE_NOTE-63).

2.3.2 Carbon arc discharge

The carbon arc-discharge method is a high temperature process, which originally was used for mass production of fullerene.[180] However, CNT also form in the process and doping the carbon anode with a metal catalyst (typically Ni or Co) enables growth of SWCNT. Other catalysts are Fe, which requires H_2 in the reactor atmosphere, or [RhPt] alloys (Table 2-2).

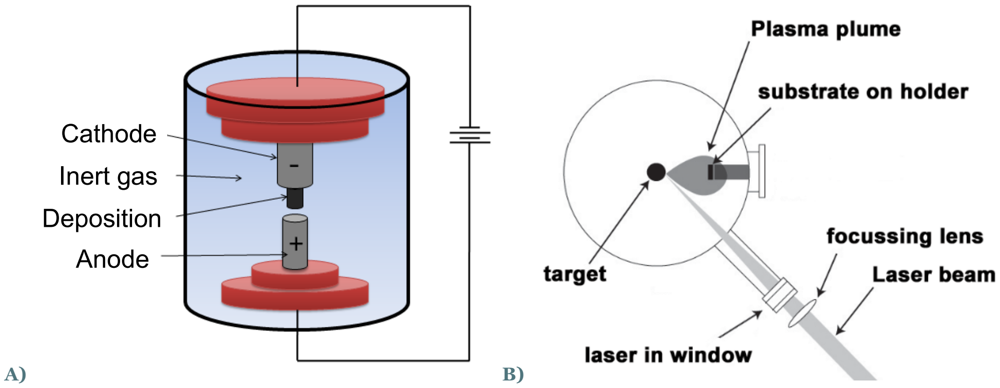
Arc discharge is a relatively simple and in-expensive method for large-scale production, but the purity is low due to co-formation of several different carbon allotropes (See Appendix 1 for examples of different carbon allotropes). Carbon black, soot and fullerene are main by-products and for SWCNT, the raw product also has a high content of catalyst particles (Table 7-4). Therefore several purification steps are necessary to achieve a product rich in CNT.

In the arc discharge process, two graphite electrodes are placed ca. 1 mm apart in a reaction chamber (Figure 2-8a). Then a low-pressure atmosphere (typically an Ar/H_2 mixture) is established in the reactor and a 50 to 100A current is established by a differential voltage of about 20 V over the electrodes. This creates a high-temperature discharge during which the surface of the anode is vaporized followed by air-borne nucleation and growth of CNT and collection on the cathode (Figure 2-8a). A high CNT yield depends on the uniformity of the plasma arc, and the temperature of the deposit forming on the carbon electrode.

2.3.3 Laser Ablation

Pulsed laser ablation is a Physical Vapor Desorption (PVD) method, where carbon atoms are evaporated from a heated graphite specimen (the target) in an inert atmosphere by intense pulsed laser irradiation (Figure 2-8b). The product is deposited on a cooled substrate positioned adjacent to the target. The method was originally used to produce fullerene [115], but doping the graphite target with catalyst materials enable the formation of SWCNT with a narrow diameter size distribution. The catalysts may be Fe, Co, Ni, Pt, Rh or NiCo alloy.[180] If using Fe as a catalyst, the reactor atmosphere must contain either O_2 or H_2 to activate the catalytic process. It is also possible to produce MWCNT with this technique, but currently this procedure is not competitive for MWCNT.[87]

Due to the high energy consumption, laser ablation process is expensive. However, the method is still attractive due to the capability to yield very well defined materials of high purity. Consequently, based on current techniques, the laser ablation method is not likely to become a procedure for major CNT production.



A)

B)

FIGURE 2-8

A) SKETCH OF THE CARBON ARC DISCHARGE METHOD FOR CNT PRODUCTION. CARBON IS EVAPORIZED FROM THE CARBON ROD ATTACHED TO THE ANODE AT CA. 20 V AND MAY CONDENSATE AND GROW ON THE CATHODE AS VARIOUS CARBON ALLOTROPES, INCLUDING CNT DEPENDING ON THE PRESENCE OF CATALYST MATERIALS AND THE PROCESS GAS. B) SKETCH OF THE LASER ABLATION METHOD FOR PRODUCTION OF SWCNT. CARBON IS EVAPORATED AFTER LASER IRRADIATION AND CONDENSATES AND GROWS FROM A CATALYST TEMPLATE. (SOURCE: WIKEPEDIA).

2.4 Chemical and Structural Purification

Purification of CNT is often required to reduce the concentrations catalysts and other carbon allotropes in the final CNT nanomaterial. Figure 2-9 shows a principle purification and sorting hierarchy for CNT and some of the specific purification procedures are summarized in Table 2-3 to Table 2-5. Overview and more in-depth descriptions of these methods can be found in e.g., Daenen et al.[27], SeO et al.[193], and Chaturvedi et al.[21]

Understood from the section above, the types and extend of purification required to produce a certain CNT material quality depends on the method of CNT synthesis, the type of catalyst used, and the purpose of the material. Especially purification of catalyst-based CNT often requires several steps addressing removal of each type of impurity. [193,21] Specific purification and filtration may even be used to separate out SWCNT and even specific SWCNT chiral types from a batch or CNT with a specific range in diameters and lengths.[3,21] Final purification or quality improvement of the atomic structure in the CNT sidewalls may be done by annealing (graphitization).

2.4.1.1 Purification of carbon allotropes

Purification is mainly performed following a chemical route for either removal of specific carbon allotropes such as filtration after solubilization in CS_2 (CS_2 filtration) which enable removal or retrieval of fullerene and/or oxidation in pure oxygen, which will preferably decompose SWCNT (Table 2-3). Other processes aim at removing carbon impurities as well as to expose the catalysts for subsequent treatment for removal of catalyst material (Table 2-4). Typical methods for removal of other carbon allotropes include sonication in alcohol, oxidation or microwave treatment. Finally, procedures are also applied to shorten CNT to achieve more uniform materials. Breaking up of CNT aggregates and CNT shortening may be done mechanically by ball-milling, but this method also increases the concentration of structural defects and increases the content of carbon impurities. Other and more frequently used methods include oxidation, acid treatment or functionalization, sometimes followed by centrifugal, Gel Permeation Chromatography (GPC) or High-Performance Liquid Chromatography (HPLC) size fractionation (Table 2-3).

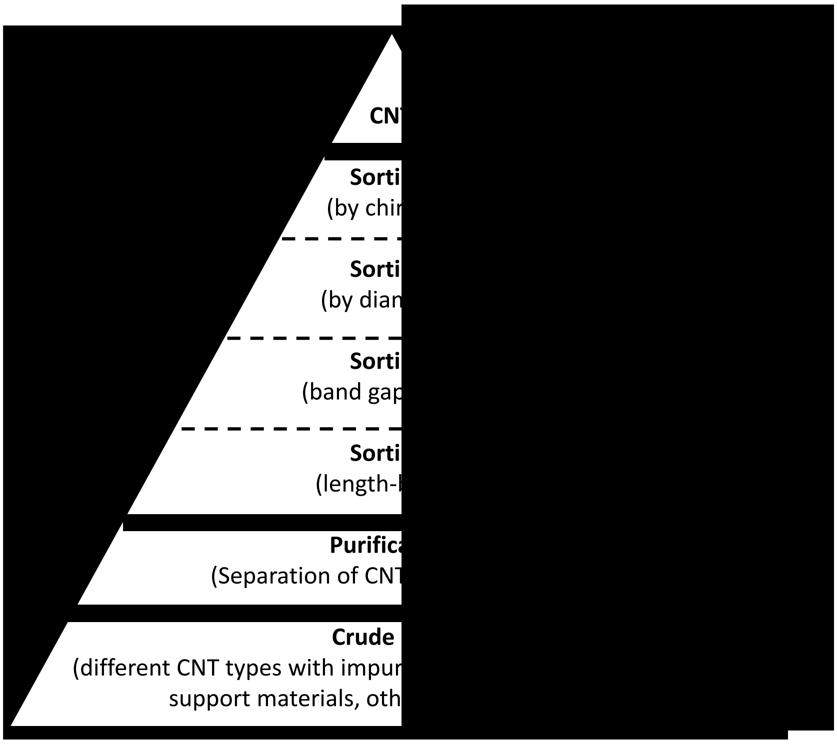


FIGURE 2-9
PURIFICATION AND SORTING HIERACHI FOR A CRUDE CNT. THE PURIFICATION AND REFINEMENT PROCEDURES RESULTS IN HIGHER AND HIGHER PURITY APPROACHING THE TOP OF THE PYRAMID (MODIFIED AFTER CHATURVEDI ET AL. [21]).

2.4.1.2 Purification for inorganic compounds

Reduction of metal or ceramic impurities is another major issue to reach high purity of all particle catalyst-assisted production of CNT. The impurity content of crude particle-catalyst-formed CNT may reach up to ca. 80 wt% (Table 2-2). Table 2-4 summarizes a range of methods applied for removal of catalyst impurities, which may follow pre-treatments listed in Table 2-3. Acid treatment is a typical purification process, which dissolves the metal impurities. However, acid purification usually introduces vacancy defects in the CNT structure, even-though a reduction in defect concentration in some cases can be observed during the early phases of purification. Acid treatment may also result in attachment of carboxylic acid functionalities (COOH) directly onto the CNT. Other “metal-cleaning” techniques involve sequential steps of dispersion, (ultra)centrifugation and reclaim of the CNT. Finally magnetic separation of catalyst particles is possible for some materials and results in chemically very pure materials.

2.4.1.3 Graphitization

Annealing (or graphitization) is a procedure for structural quality-enhancement of CNT, where structural defects are removed or reduced by recrystallization of the graphene sheet. Therefore, annealed CNT are often referred to as graphitized CNT. Graphitized MWCNT have very high electrical conductivities and are good stability in oxidizing environments. The electrical conductivity of graphitized MWCNTs is on the same order as that of graphite. Different methods exist for partial or complete CNT graphitization (Table 2-5). These include the use of high temperatures and or vacuum with pressures below 10^{-3} bar or mild oxidation. Sonication in acid may also decrease defect densities, but the effect is time-dependent and the method is mainly applied for removal of metallic catalysts.

Based on the observations above, combined information on synthesis procedures and subsequent purification and annealing, can enable general indications on the CNT types and qualities of the specific CNT products.

TABLE 2-3

DIFFERENT CARBON PURIFICATION AND TUBE LENGTH MODIFICATION TECHNIQUES APPLIED FOR CNT. SYMBOLS ARE - = NO EFFECT ON ITEM; M = MINOR EFFECT ON ITEM; M = MAJOR EFFECT ON ITEM. (MODIFIED AFTER DAENEN ET AL. [27]).

Technique	SWCNT	Carbon particles/ graphite	Fullerenes	Metal catalyst	Effect on carbon	Comment
CS_2 Filtration	-	-	M	-	Specific removal of fullerenes	Easy way to separate fullerenes without harming them.
(Ultra)sonication (in alcohol)	-	-	-	-	Enhances purification techniques	Enlarges reactive surface, often in combination with other techniques. Suspends particles enabling mass separation.
Fixed Air Oxidation	M	M	M	-	Low carbon impurities; expose catalyst particles	The metal catalyst will be exposed. Process makes it easier to remove metal catalyst
Wet Air Oxidation (used for HiPCO)	M	M	M	-	Low carbon impurities, metal exposed	Removes carbon allotrope impurities and oxidizes exposed metal.
Wet Oxidation (H_2O_2)	M	M	M	-	Remove carbon coating on metal catalysts	Reflux in hydrogen peroxide. Process makes it easier to remove metal catalyst
Microwave Treatment	M	M	M	M	Low carbon impurities, metal exposed	Oxidation and rupturing of the carbon passivation layer.
Ball-milling or Grinding	M	M	M	-	Shorter SWCNTs; more carbon impurities	The SWCNTs are mechanically shortened and disordered carbon is formed.

Technique	SWCNT	Carbon particles/ graphite	Fullerene s	Metal catalyst	Effect on carbon	Comment
Ultrasonication (in acid)	M	-	-	M	Cuts CNT after certain time	Longer duration, cuts the tubes.
Cutting through Fluorization	M	-	-	-	Shorter tubes, good purity	Cutting SWCNTs with initial length of 1 µm. Average length: less then 50 um.
Pure Oxygen Oxidation (size refinement)	M	M	M	-	Low carbon impurities	Preferentially burns SWCNT with smaller diameter. $P = 1.5 - 5 \cdot 10^{-6}$ mbar; $T = 450 - 600\ ^oC$
GPC (size separation)	-	-	-	-	To obtain very pure SWCNT	CNT is functionalized with octade-cyamine. Size separation by GPC.
HPLC (Size separation)	-	-	-	-	Very pure	Size separation. SWCNTs must be cut and purified in advance.

Table 2-4

DIFFERENT METAL PURIFICATION TECHNIQUES APPLIED FOR CNT. SYMBOLS ARE - = NO EFFECT ON ITEM; M = MINOR EFFECT ON ITEM; M = MAJOR EFFECT ON ITEM. (MODIFIED AFTER DAENEN ET AL. [27]).

Technique	SWCNT	Carbon particles/ Graphite	Fullerenes	Metal catalyst	Effect on metal	Comment
Ultrasonication (in acid)	M	-	-	M	Time-dependent, may remove metals	Short duration, solvates metal.
Thermal Oxidation	M	M	M	-	not reported	Heating to 350 – 600 °C. Metal used as oxidation catalyst.
HNO_3 Treatment	-	-	-	M	< 0.1 wt % Metal	Reflux or sonication in nitric acid. Catalysts will solvate.
HCl Treatment	M	M	m	M	< 0.1 wt % metal	Reflux or sonication in HCl. Catalysts will solvate.
Mild Acid Treatment	-	-	-	M	< 0.2 wt% Metal	Most metal removed, when their surfaces are exposed to the acid. (4 M HCl reflux)
Functionalization (In diethylether)	-	-	-	-	~ 0,4 % Metal	Functionalized with 1.3 dipolar cycloaddition and solvated. Is used as preparation step for separation techniques
Magnetic Purification	-	-	-	M	Metal hardly detectable	Magnetic particles are trapped with magnetic poles.

TABLE 2-5

DIFFERENT TREATMENTS FOR ANNEALING CNT. SYMBOLS ARE - = NO EFFECT ON ITEM; M = MINOR EFFECT ON ITEM; M = MAJOR EFFECT ON ITEM. (MODIFIED AFTER DAENEN ET AL. [27]).

Technique	SWCNT	Carbon particles/ graphite	Fullerenes	Metal catalyst	Effect on CNT	Comment
Mild Oxidation (H_2SO_4 and H_2O_2)	M	M	M	-	Decrease defects	Cuts tubes and consume sidewall defects.
High Temperature Annealing	-	M	M	-	Decrease defects	Recrystallize CNT. Annealing in Ar at T = up to 800 ^oC.
High Temperature Vacuum Treatment	-	M	M	M	Decrease defects	Recrystallize SWCNT walls, removal of graphite and metals $P = 10^{-3}$ Pa; T = 1600 ^oC
Vacuum Annealing	-	M	M	-	Decrease defects	Recrystallize CNT and consumes defects. $P = 5 \cdot 10^{-6}$ mbar; T = 600 ^oC

2.5 Functionalization

Much effort has been put into finding methods to alter the surface chemistry of CNT to increase their solubility in liquids or chemical binding in solid composite systems in order to use or modify their physico-chemical properties. Functionalization is also very important in the potential biological applications of CNT. One may consult Tasis et al. [216]; Herranz and Martin [81]; Hauke & Hirsch [76]; Monthioux et al. [150] for advanced reviews on the functionalization of CNT. Some recent work categorize the functionalized CNT, following the naming in this report, to be a third generation CNT, called carbon meta-nanotubes.[149]

As explained above, the ability for functionalization depends on the CNT type and applied purification and annealing processes, if any. The pure graphitized straight-wall CNT surface is smooth and polar, which makes them almost insoluble in both aqueous and organic solvents. This is because half of the electrons in the π-orbitals occupy the outside surface, which enables bonding with other molecules with π-orbitals.[76] The presence of these surface electrons also partly explains the agglomeration behavior of CNT. Especially SWCNT may form bundles or "ropes", where several carbon nanotubes are aligned and bond by Van der Waals forces, in which the π-orbital interaction is an important component. As mentioned in section 2.2, vacancy defects may also contribute to π-orbital electrons and aggregation by bonding between CNT. However all tube-end and sidewall defects, which will be abundant in most non-annealed purified CNT, are also favorable sites for chemical functionalization. It was noted above that structural defects generally are more abundant and the chemical reactivity higher in small- than large-diameter CNT. This is due to the intimate association between side-wall defect concentrations and the strain energy in the sp^2 hybridizised carbon atoms positioned perpendicular to the tube rotation axis.

Different types of functionalization may be applied depending on the designated use. One may discriminate between:

- non-covalent functionalization (specific CNT and molecule interaction)
- defect-group functionalization (bonding at vacancy or sw-defects)
- direct covalent side-wall functionalization (bonding forming topological defects)
- endohedral functionalization (tube-caged molecules, elements or metals)

Non-covalent functionalization is highly attractive when the physical properties of the CNT should be affected as little as possible. In non-covalent functionalization, one takes advantage of the high adsorption capacity of the CNTs, which is facilitated by their high surface energy. The procedure includes wrapping of CNT by polymers, polypeptides, and DNA.[76,189] Interaction of the π-orbitals of CNT with aromatic moieties such as pyrene has also been widely used for non-covalent functionalization. The larger the π-orbital system on the aromatic molecule, the better they are attached. In toxicological studies, non-covalent functionalization using proteins is also often used for making steric stabilization of CNT in dispersions.[80,84,179,228]

Defect-group and direct covalent functionalizations are possible at the tube ends and the tube walls. Despite the fact that defect-group and covalent CNT will have reduced strength and conductivity as compared to the starting material, this type of functionalization is widely explored and used.[76] As mentioned above, treatment of CNT with oxidizing acids typically forms carboxylic acid groups (COOH) at the tube ends and/or at carbon atom vacancies or sw-defects in the hexagonal structure. The carboxylic acid groups (and other minor oxygen containing groups) are starting points for many different further chemical modifications. Fluorination and hydrogenation has also been widely applied and it has been shown that many of the well-known reactions in organic chemistry can be used on CNT.[122] These include Grignard (an organo-metallic chemical reaction), radical addition, electrophile addition and many others. A quick overview of these chemical reactions can be found at http://www.organic-chemistry.org/namedreactions/.

Direct covalent attachment of a molecule (covalent or sidewall functionalization) to a carbon atom in the CNT lattice, changes the hybridization of this carbon atom from sp^2 to sp^3. Therefore, if not already formed, the covalent bonding involves the introduction of a defect in the hexagonal CNT sheet(s). By this, the external π-orbital layer will be altered and lower the mechanical and electrical performance of the CNT significantly.[76] Nevertheless, covalent functionalization is still very attractive in e.g., composite materials due to interest in strong chemical bonds between the CNT and the material matrices. Especially the covalent attachment of polymer chains to the CNT has attracted much attention because of the possibility to create polymer-CNT composite materials with strong interfaces by this approach. The list of polymers that can be coupled to CNT in this way is very long and still growing. One example is Kevlar functionalization for uniform dispersion in polymers.[187] Also endohedral functionalization deserves mentioning. This is the possibility to place molecules inside the CNT which is pursued by some research groups.

Finally, the “atypical” CNT structures comprise or enable specific types of functionalization. E.g, the carbon nanobud is a special derivative with fullerenes covalently bonded to the outside walls of the underlying nanotube. Consequently, nanobuds exhibit properties of both CNT and fullerenes.[157] The fullerenes also help to keep the CNT apart from each other, and this may help to make them more soluble. The fullerenes are also much more chemically reactive, and they may be further used when producing functionalized CNT. Information on the use of functionalization of cup-stack, cone-stacked, herring-bone and bamboo nanotubes has not been recovered. However, composite material products do exist based on, at least some of these materials, already.

2.6 Types and characteristics of commercial CNT

The previous sections in this chapter focused on the principal structures and basic characteristics of CNT. In this section an overview of the CNT nanomaterials currently available on the commercial market is provided together with physico-chemical information and general applications given. The information was collected from material datasheets, presentations, websites from various major and/or well-known producers, and information acquired during visits to two major European CNT producers. A list of selected information is collected in Appendix 2.

It should be noted that the list of producers is not complete and only covers CNT on the market until ultimo 2014. Therefore some size ranges and CNT types may not appear. However, the information is believed to be generally representative of the types and general characteristics of commercially available CNT.

Due to the available information, the commercial CNT nanomaterials could only be categorized depending on the general number of CNT side-walls and - morphology; i.e. whether they are SW, DW, or MW CNT. A total of 76 SWCNT, 17 DWCNT, and 178 MWCNT nanomaterials were found. In addition two products were found listed as CS-CNT and Herring-Bone (HB) CNT, respectively. Table 2-6 shows the typical dimensions and purity ranges of these main CNT types along with their general cost and application areas.

2.6.1 SWCNT

Prices are typically in the range € 21.500 - € 61.600 per kg andfor some reach €264.000 per kg (2015 prices) and SWCNT are hence still relatively expensive materials. Prices are generally approximately 25% higher for functionalized qualities, and the most abundant functionalizations are acidic (-COOH), hydroxylic (-OH), and (-NH_2), which makes the functionalized SWCNT dispersible in water. The functionalization typically constitute less than 3 wt% of SWCNT product, but it may reach up to 5 wt% (Appendix 2).

TABLE 2-6

GENERAL PROPERTIES OF THE MOST COMMON CNT MATERIALS. THE FIGURES GIVEN IN THE TABLE ARE GENERAL FIGURES, AND SPECIFIC PRODUCT CAN BE FOUND OUTSIDE THE RANGES GIVEN.

CNT Type	Diameter [nm]	Length [µm]	CNT Content	Reported types of impurities$	Listed Functionalizations	Price [€/kg]	Typical Main Applications
SWCNT	0.7-2.5	0.5-50	50 – 90	MWCNT, fullerene, amorphous carbon, graphite, ash (Nil)	-COOH -OH -NH2	21.500 – 264.000	Research, microelectronics and others
DWCNT	1.3-5	0.5-40	60 – 90	Metal oxide, ash, amorhous carbon	-COOH -OH	21.500 – 264.000	Research, FEDs, transparent conductive films and others
MWCNT	4-150	0.5-150	70 – 99.9	SWCNT, amorphous carbon, ash, Fe, Al_2O_3	-COOH -OH $-NH_4$ -Li	300 – 22.000	Li-ion batteries, antistatic plastics, textiles, heating elements, anti-fouling coatings and others
CSCNT£	80-100	5; on demand	highly pure	no information	on demand	unknown	Badminton, tennis rackets, golf clubs, bicycle, microelectronics and others

SWCNT typically have minimum diameters between 0.7 and 1 nm and the upper diameter is almost always 2 nm. The reported SWCNT lengths range from 0.5 to 50 µm. Only 25% have a minimum length of 5 µm or larger and about 25% have a maximum length of 5 µm or smaller. Most SWCNT are between 5 and 30 µm long.

The purity is typically in the range of 60-90 percent by weight and the typical impurities are MWCNT, fullerenes, amorphous carbon and unspecified catalyst residues referred to as ash. About 30 percent of the SWCNT material contains only 50 to 70% structural SWCNT.

The SWCNT materials are usually supplied in suspensions or as powders in an agglomerated or aggregated state with specific surface areas in the range 300-500 m^2/g. The typical application areas quoted by the producers are research and development, microelectronics and others.

2.6.2 DWCNT

DWCNT is an emerging product with prices typically in the same price-range as SWCNT. DWCNT is currently supplied by fewer producers than SWCNT and MWCNT. Typical functionalizations of DWCNT are acidic (-COOH), hydroxylic (-OH) and amino (NH_2) for water dispersible grades. The diameter of DWCNT is typically in the range of 2 - 4 nm and. The lengths vary from 0.5 to 40 µm and are typically in the 5 – 15 µm range.

The purity of typical DWCNT materials is in the range 60 – 90 percent by weight and typical reported impurities are metal catalysts and amorphous carbon. A single product only consisted of 15 percent DWCNT with the rest being mainly catalyst material and other carbon allotropes. As for SWCNT, DWCNT are also usually delivered in suspensions or as powders in agglomerated or aggregated state. Their specific surface areas in the range of 300 – 500 m^2/g. Typical applications are in research and development, FEDs, transparent conducting films, surface chemistry and others.

2.6.3 MWCNT

MWCNT is the CNT material that by far is produced in the highest quantities and with the highest diversity. Most of the individual CNT materials are defined by variations in lengths, but also in their variation in purity and functionalization (Appendix 2). Figure 2-10 shows an example of two different MWCNT products, with different dimensions, degree of entanglement, different types of catalysts and catalyst concentrations, as well as differences in the association between the catalyst and the CNT.

The total range in reported MWCNT diameters is 3 to 170 nm, but it is only a few materials that exceed the 100 nm-range. More than 60% of the MWCNT have reported maximum diameters smaller than 30 nm. Total reported range in length is 0.5 to 150 µm, but the materials appear to occur in several length groups. Slightly more than half of the MWCNT had minimum lengths shorter than 5 µm, whereas about 20% had a maximum length of 2 µm or less. The rest (80%) had maximum lengths exceeding 5 µm.

The reported purity of MWCNT products is typically about 95 percent or higher by weight and typical impurities are amorphous carbon, alumina, catalyst material referred to as ash or in single cases Fe or metal oxides. About 15 percent of the identified CNT materials had CNT contents between 70 and 90 percent. A small fraction of these had more detailed information revealing that the amount of the MWCNT was even lower than that (min 50 to 70 percent depending on the product) due to mixtures with other CNT types. It is therefore uncertain whether the listed purities in all cases are regarding the MWCNT or just CNT.

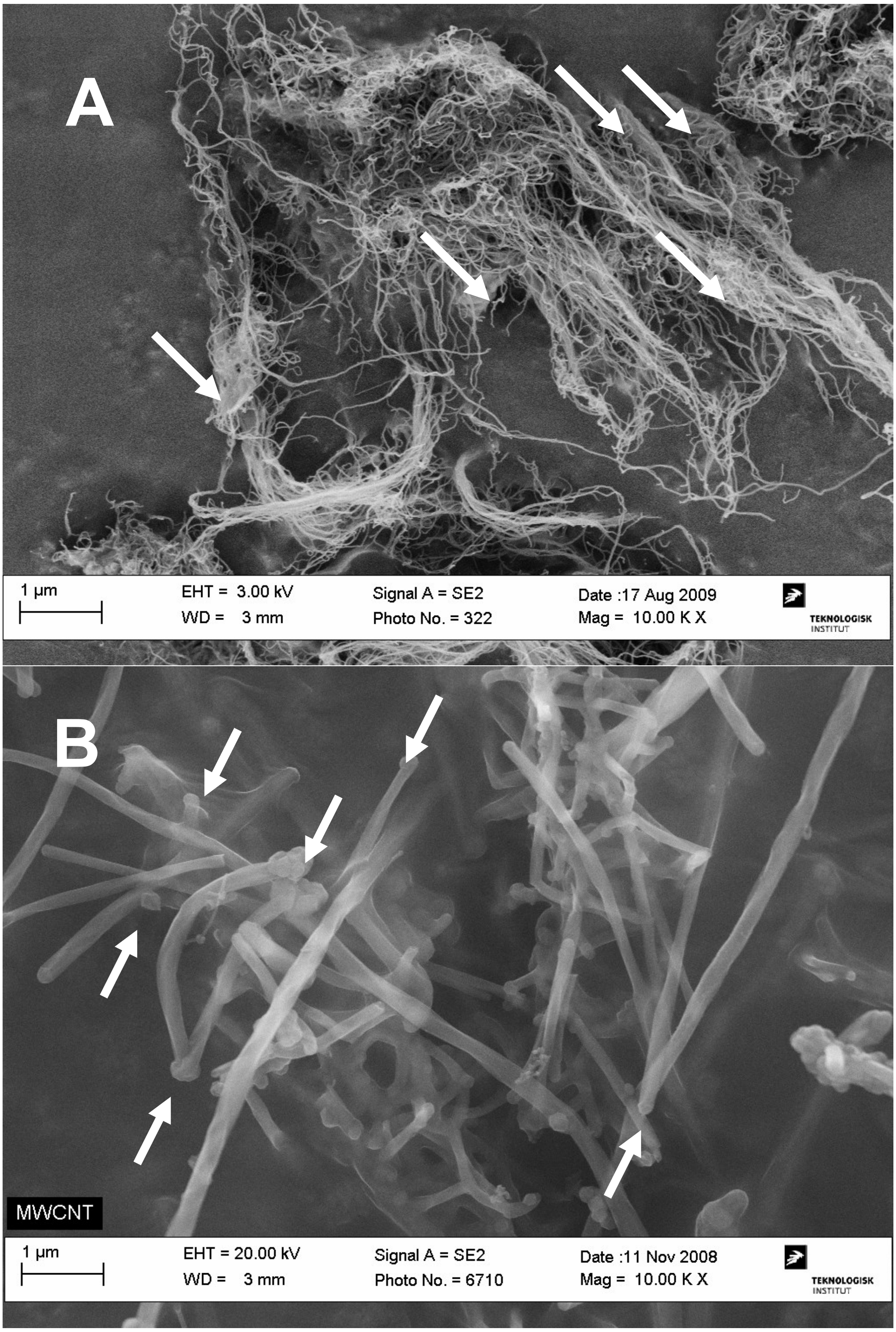


FIGURE 2-10

SCANNING ELECTRON MICROSCOPY IMAGES SHOWING DIFFERENT MORPHOLOGIES OF TWO DIFFERENT MWCNT. A) EXAMPLE OF INDUSTRIAL GRADE MWCNT WITH TYPICAL DIAMETERS IN THE RANGE OF 10-30 NM AND LENGTHS OF ONE TO SEVERAL TENS OF MICROMETERS. SEVERAL PLATY CRYSTALS OF A CERAMIC A CATALYST SUPPORT PARTICLE ARE OBSERVED AS INDICATED AT THE ARROWS. THE TENDENCY OF CNT TO FORM ROPES AND BUNDLES IS TYPICAL FOR CNT OF THIS QUALITY. B) EXAMPLE OF A RESEARCH GRADE MWCNT CONSISTING OF MAINLY INDIVIDUAL 100-200 NM DIAMETER SIZE MWCNT. THE LENGTH OF THESE MWCNT RANGED FROM CA. ONE TO SEVERAL TENS OF MICROMETERS. ARROWS INDICATE GROWTH CATALYST PARTICLES, WHICH ARE SEEN AS THE BULKY PARTS AT THE ENDS OF THE TUBES.

When MWCNT are supplied as powder they are usually in a loose powder or granulated form with specific surface areas of 50-350 m^2/g, but according to manufactures' much MWCNT is sold pre-dispersed in thermoplastics as master batches or as dispersions in various epoxy formulations. The applications of MWCNT reflect their good electrical conductivity and low percolation threshold for which reason they are used in anti-static plastic parts, textiles and epoxy composites. Other applications are as additives in graphite electrodes for Li-ion batteries, as reinforcing agent in epoxy composites, in anti-fouling coatings, as current carriers in electrical heating elements and others.

MWCNT is generally the cheapest group of all the CNT materials. Prices typically range within € 300 - € 21,000 per kg and the prices for functionalized materials is typically 3-10 times that of the pristine qualities. Typical functionalizations are oxidized derivates with acid (-COOH) and hydroxyl (-OH) groups and for more specialized applications amino ($-NH_2$) functionalization.

2.6.4 Other CNT structures

As discussed in the previous chapter, several additional CNT types exist in addition to SW, DW, and MW. Despite the number of different types found in research publications, only Cup-Stacked (CS) and Herringbone-Bamboo (HB) CNT were found or at least indicated as specific commercial products at the time of writing this report. Both products may be obtained as powders, slurries or in master-batches.

The HB-CNT, was 5-15 µm long, 10-20 nm in diameter and had a wide range in specific surface area of 40 to 300 m^2/g. No specific information on applications was available on this product.

The CS-CNT is currently used in composite sport equipments, but can also be used in microelectronics and field emitters. The reported dimension is 80 – 100 nm in diameter and different lengths depending on the costumer requirements. The surface area is ca. 50 m^2/g. The CS-CNT is reported to have a large capacity for functionalization, but none appears to be offered as a shelf material.

2.7 Concluding remarks

It is found that CNT comprise a diverse group of nanomaterials consisting of cylindrical hollow carbon-based fibers (tubes) with diameters typically lower than 100 nm and lengths that can reach several hundred micrometers; even millimeters if needed. The simplest CNT are single-walled (SW), double-walled (DW) and multi-walled (MW) CNT. Other and less common CNT types include herring-bone (HB), bamboo (BB)- and cup-stacked (SC)-CNT as well as branched morphological derivates of most types of CNT. Truly rolled-up graphene sheets (nanoscrolls) may also be considered a CNT. In addition to these primary types, a class of functionalized CNT also occurs and adds to the complexity.

Functionalization is a procedure by which metals or organic molecules are associated with the CNT structure. The ability for functionalization depends on the CNT type and applied purification and annealing processes, if any. One may discriminate between:

- non-covalent functionalization (specific CNT and molecule interaction)
- defect-group functionalization (bonding at vacancy or SW-defects)
- direct covalent side-wall functionalization (bonding forming topological defects)
- endohedral functionalization (tube-caged molecules, elements or metals)

A total of 273 different CNT nanomaterials were found at various distributors. MWCNT types (n = 178), was more abundant than SWCNT (n = 76), and DWCNT (n = 17). The functionalized CNT were dominated by Li-ion, OH, COOH, and NH_2 derivatives, but not abundant. However, functionalizations can be made on demand as well as delivery of CNT in ready-made masterbatches. Only one bamboo and one cup-stacked CNT was also found. The annual global production volume of CNT is very uncertain. It is estimated to be in the range of 1000 tonnes and is heavily dominated by MWCNT.

The lengths of commercial CNT do usually not exceed 50 µm and limited differences were found in reported length ranges of the three major CNT types. A large fraction of the different CNT types were between 0.5 and 5 µm long. As expected, SWCNT and DWCNT are the thinnest with diameters from 0.7 to 2.5 nm and from 1.3 to 5 nm, respectively. The reported diameters of MWCNT range from 4 to 150 nm.

Commercial CNT nanomaterials are rarely pure and usually have a high abundance of different CNT types, other carbon compounds and inorganic catalyst impurities. The quality of the CNT nanomaterial can be greatly improved by purification and separation of specific structures or dimensions. By mass, the CNT purity is lowest for SWCNT (typically 50 – 90 percent) and DWCNT (typically 60 – 90 percent). The purity of MWCNT is typically 70 to 99.5 percent. This picture is both regarding catalyst materials and other carbon allotropes.

The primary structural quality of CNT and the chemical purity of the CNT nanomaterials vary with production technique and purification procedures applied after their synthesis. High-volume production is possible using chemical vapor deposition (CVD) and carbon arc discharge. Many different CNT types with and without catalysts can be produced by these methods. However, a larger variety in tube diameters and tunable lengths can be made with CVD, but these CNT on the other hand may contain many defects. Carbon arc discharge is most suitable for production of shorter tubes with thinner diameters (less than ca. 10 nm) and few structural defects. Laser ablation may be used for relatively well-controlled high-purity synthesis of SWCNT.

Chemical and/or structural purification and/or CNT sorting may be required to produce commercially attractive CNT nanomaterials. Chemical purification is usually done using acids, which increases the abundance of structural defects in the CNT. It can also be used to cut CNT into smaller segments and remove metal catalysts, which also results in open-ended CNT. Increased defect concentrations increase the chemical reactivity of CNT and are favorable for chemical functionalization. Annealing (graphitization) (oxidation, thermal or vacuum treatment) reduces the concentration of side-wall defects. Annealing increases electrical conductivity and tensile strength. Full purification of impure crude CNT is hindered by technical challenges and their purity usually does not exceed 90 to 95% CNT.

Compared to the structural and compositional complexity of CNT nanomaterials, the readily available information on CNT types, as well as the amounts of impurities from is currently very sparse. The products are normally only described according to the primary sidewall topology, diameter-range, and length range and generic descriptions of impurities. Detailed information is very rarely given. This makes it a challenge for down-stream users to immediately identify CNT materials suitable for their purposes. Regulators potentially face similar difficulties. Knowledge on applied production methods and subsequent purification and sorting procedures can give some, but still incomplete information on the CNT quality. A systematic procedure for reporting and categorizing CNT nanomaterial qualities is needed to enable easy identification of technically suitable materials and specific material characteristics for regulation. A proposal for such a system is shown in Appendix 3.

3. Main current and near-future applications of CNT

This chapter gives an overview of the current and potential near-market commercial production and use of CNT. The chapter first summarizes an evaluation of the annual production volume and commercial value of CNT grades and CNT-based products followed by a short overview on the main industrial sectors utilizing CNT. Finally, there is a summary with more detailed examples of current and near-future commercial products encountered in major application areas. The chapter is concluded with an evaluation of the timeline for different products, which may appear on the market in the near future. The information for this chapter is gathered from scientific papers, published professional market analyses as well as internet searches, and information gathered during communication with a few main CNT suppliers.

3.1 Summary

The great diversity in CNT is also is reflected in a wide range of technological applications. The production volume in 2014 was estimated to be on the order of 520 to 3,000 tons and by far dominated by MWCNT. However, the production capacity is many times larger and reached around 20,000 tons in 2014. At ECHA, MWCNT from Bayer (terminated production in 2013) and Nanocyl are registered with a production of 10 – 100 tons/year whereas Graphistrength C100 from ARKEMA is registered in the 1-10 ton/year category.

The global trade value of all CNT nanomaterials was $158.6 million in 2014 and is in solid growth. The market potential for CNT-based products is much higher and estimated to be on the order to $20 billion in 2015. The most important applications are in various types of composites, electronics, and energy. The CNT properties used is currently mainly their extraordinary tensile strength, their electrical conductivity and current carrying capacity, gas and energy storage capacity, as well as well as tunable chemical reactivity from being inert to reactive with applications in sensors and catalysis.

The most important industrial sectors are the automotive, energy, paint and coatings, and electronics. The oldest use may be in Li-ion batteries where they constitute 1 - 3 weight percent of the graphite electrodes. Another, apparently well-established use is the application of CNT is as conducting filler in base resins and thermoplastics where it may reduce the tendency of the plastic parts to become charged by static electricity and enabling electrostatic painting without further treatment of the materials. The use of CNT in base resins due to their strength-enhancing properties is also well-known.

Another more application is as reinforcing agent in advanced polymer composites used for sports equipment, e.g. rackets, golf-clubs, and ice-hockey sticks, but also high performance bicycles, small boats and windmill blades containing CNT are on the market. There is however still technical challenges to be solved before the extreme strength and stiffness of CNT can be fully utilized in composites and this is one of the reasons for the still relatively limited use of CNT for specific enhancement of mechanical properties of materials. Composite materials other than polymers are also being investigated, e.g. aluminium CNT composites and ceramic CNT composites. Only sparse information on these materials and their applications is available, and most likely their actual use is currently very limited.

CNT-based epoxy-paints and antifouling coatings for the marine sector have been introduced few years back. The claim is that the CNT based coating has high strength and enables antifouling properties without releasing toxic substances into the sea water. The reduction of growing organisms on the ships translates directly into saved fuel and reduced CO_2 emissions. CNT-based high-durability epoxy paints are also suggested for other purposes and currently being developed for large windmill blades.

Following the line of CNT containing coatings much research has been put into the development of conductive inks, which due to the dimensions of the CNT can be made transparent. This property is useful for electronic displays and touch screens, and products are most likely on the market.

Furthermore adding CNT to textile fibres have been demonstrated to imply electronically conducting properties to the finished textiles, and products utilizing this property is known on the market in Japan. If they are not already available in Europe they are likely to become so in the near future. This may be either as CNT heating textiles or as part of final products. Electronically conducting textiles are used as extremely thin heating mats and has been proposed used for 'intelligent' clothing e.g. for winter sports applications.

Many more applications for CNT have been suggested but it is difficult to determine exactly in which development stage they currently are. Applications include field emission devices (FED) for e.g. light sources and other electronic components, non-volatile RAM memory modules for computers etc. and heat management devices for microelectronics.

Existing and near-future large tonnage applications of MWCNT is expected to be in various reinforced composites used for aerospace, automotive, marine and wind turbine industries where implementation of CNT is expected to result in weight reductions of approximately 10 %. SW- and DW-CNT as well as the 'exotic' CNT types are not expected to reach the same levels of tonnages as MWCNT in the near future. The main application of SW- and DW-CNT is in electronics.

3.2 Global CNT production volume and application areas

It is evident that the commercial interest in CNT has increased significantly over the last decade. The global market for CNT nanomaterial grades reached $103 million in 2009 [10].and reached 158.6 million in 2014.[12]. The projected annual growth is 33.4% and the still rapid increase may in part be explained by the increasing ability to mass-produce a variety of applicable CNT nanomaterial grades at commercially interesting prizes. It is naturally difficult to assess what the global market value corresponds to in volume of CNT production, because there is at least 250 times difference in cost of the cheapest and the most expensive CNT grades.

3.3 Global market value and production volume

The first global survey on the CNT production volume was made in 2004 and concluded from the 54 interviewed producers an annual production of 65 tons of CNT and carbon nanofibres.[25] Between 2004 and 2008, the annual growth rate was 30%, but then dropped in 2009 and 2010 due to the global market crisis.[56] Using the 65 tons CNT estimated by Cientifica in 2004 and the growth rates observed by Future Markets Inc., resulted in an estimated global production of around 250 tons CNT in 2010. This is slightly lower than the 271 and 7 tons MWCNT and SWCNT annual production estimated for 2007 by Rakov [177,177]. In 2014, the production volume is estimated to be between 520 and 3000 tons with 1750 – 2500 tons as the intermediate estimate.[58]

It is evident that the production capacity is even much higher than the annual production. Future Markets Inc. estimated the global production capacity to be on the order of 10,000 tons per year in 2011 and 20.000 in 2014.[57] The largest potential producers in the world are the Chinese companies CNano and Showa Denko, who each could already produce 500 tons per year in 2011.[57] The largest European producers were listed with 400 (Arkema), 250 (Bayer who terminated CNT production in

2013; http://www.press.bayer.com/baynews/baynews.nsf/id/Bayer-MaterialScience-brings-nano-projects-to-a-close), and 400 (Nanocyl) tons MWCNT in annual production capacity. For comparison, at ECHA Bayer and Nanocyl are registered with a production of 10 – 100 tons/year whereas Graphistrength C100 from ARKEMA is registered in the 1-10 ton/year category (http://apps.echa.europa.eu/registered/data/dossiers/ DISS-abda73cd-a2cd-3801-e044-00144f67d249/DISS-abda73cd-a2cd-3801-e044-00144f67d249_DISS-abda73cd-a2cd-3801-e044-00144f67d249.html; and http://apps.echa.europa.eu/ registered/data/dossiers/ DISS-b281d1a0-c6d8-5dcf-e044-00144f67d031/DISS-b281d1a0-c6d8-5dcf-e044-00144f67d031_DISS-b281d1a0-c6d8-5dcf-e044-00144f67d031.html).

Parallel to the growth in production volume, there is also and increased demand for CNT raw materials. Forecasts made by BCC Research predicted an annual growth of 58.9% of the CNT trade between 2010 and 2014.[10] However, the growth rate was not nearly as dramatic as expected and is considered to be in part due to the impact of the financial crisis.[11] The currently estimated annual growth rate from BCC Research is 33.4% between 2014 and 2019.[12] According to an anonymous European CNT producer, the majority of their production is currently exported to Asian countries, especially Korea, where it is used in thermoplastic materials. The overall application areas are currently dominated by electronics (28%), energy (22%), plastics and composites (17%), and academic research (12%).[57]

New producers and alternative production methods also appear. Klean Industries is one of these new producers, who in collaboration with Mitsubishi Corporation produces 10 – 30 tons CNT per year from recycled car-tires (http://www.kleanindustries.com/s/tire_plastic__recycling_feasibility_studies.asp?ReportID=128033). One million tires is used to produce 1 ton CNT, therefore the potential capacity using this technioque is also very large and potentially competitive considering the recycling aspects.

3.3.1 Global product trade value and main industrial sectors

CNT-based products have much higher value than CNT nanomaterial grades and were expected to reach a global trade value of $5 billion in 2012.[165] Some actual applications are in various types of composites, electronics, and energy with the use in different types of composites still being the most important-[10,11] The use of CNT in Li-ion batteries may have existed for decades now and the application of CNT in energy storage increased at a rate of about 8.5% in 2010.[165] and the demand is still increasing.[58] BCC Research, however, suggested that the greatest growth will be in the single (149.1%) and few-wall (131.6%) CNT between 2012 and 2016.[11] In this respect, the use of CNT in e.g., paints, pigments and coatings (6% of the CNT application by volume in 2012 [57]) may also steadily grow more important, because some of the market may be hidden in the electronic (display), automotive and aerospace applications.

By volume, MWCNT is still by far the most applied CNT material. This may in part be explained by their lower cost and available high-tonnage synthesis techniques, but also that their range of application is wide, covering energy, coatings, composite materials, electronic and more. As reported in chapter 2, SWCNT and DWCNT are still much more costly materials than MWCNT and they are expected to reach more general application within electronics within the current decade.[95]

Table 3-1 shows a list of the industrial sectors where significant use has been or may soon be established. Due to the high production volumes and the use of CNT in bulk materials, the automotive and energy sectors are known to use relatively high volumes of CNT materials. A short description of the use in these sectors is given hereafter.

TABLE 3-1

INDUSTRIAL SECTORS WHERE CNT ARE EXPECTED TO GAIN SIGNIFICANT USES.

Primary sectors	Secondary sectors
Automotive	Biotech, research etc.
Energy	Biomedical
Electronics	
Paint and coatings	

3.3.1.1 The automotive sector

The automotive sector has a very high consumption of thermoplastic parts. Addition of MWCNT into thermoplastic material parts enables electrostatic painting without any use of conductive primers on the surfaces. The CNT replace the former use of carbon black, where less CNT is required to achieve the anti-static effect.

CNT in thermoplastics also reduce brittleness and tendency to give off dust particles as compared to carbon black. CNT addition in thermoplastic base resins also increases their toughness, which is critical in many automotive applications. Adding CNT into resins also eliminates the need for impact modifiers (e.g., rubber) and thereby also results in preservation of heat resistance in critical body parts. CNT addition to a polymer may even result in retarded combustion.[14] Such properties may also be useful for application in aviation, public transport, construction, electrical wiring, and electronics.

New and improved materials for load-bearing applications are constantly being developed by the automotive sector. For this reason CNT nanocomposites of epoxy thermosets and aluminium metal are also being developed. These new nanocomposites will initially be used in expensive race cars, but when such materials finds their way into series produced vehicles the required amounts of CNT is going to become significant (Nanocyl, personal communication).

3.3.1.2 The energy sector

The energy sector has a long tradition for using carbon materials in electrodes or as conductive fillers in electrochemical systems. For this reason it has been a natural development to utilize CNT for optimizing such systems. Carbon nanofibers and MWCNT are currently used as anode and cathode materials in commercial Li-ion batteries. In this application the CNT increase the durability of the battery compared to traditional graphite materials.[47]

Electrically conductive coatings based on CNT have potential applications in devices such as solar cells, touch sensitive displays and others. In a future energy system with a higher share of sustainable energy production, such applications are likely to result in considerable global consumption of CNT.[8,47,98]

CNT may be able to improve the structural properties of fiber-reinforced composites used for e.g. windmill blades. General implementation will result in a very high use of CNT in this sector. Furthermore CNT have potential application in fuel cells for electricity generation, which may already have be realized. In the fuel cell, CNT is used as catalyst supports where they serve as high surface area materials for the support of the expensive platinum particles and at the same time they help dissipating the heat generated by the chemical reaction in the fuel cell.[47,67]

3.3.1.3 The electronics sector

In the electronics sector, CNT have many different potential applications – some are already used and others are very close to commercialization. Yet, due to the way CNT are exploited in electronics, the total use-tonnages are expected to be lower than in the automotive and energy sectors. On the other hand the quality requirements are expected to be higher in electronics, and many applications in microelectronics will require high purity of both individualized SWCNT and DWCNT.[47] New developments in branched CNT (chapter 2) also show high potential, but their application may not occur in the very near future. Application of MWCNT may however also be relevant as in the previously discussed fire-retardent in wiring etc.

CNT application in various types of electronics is potentially at the frontier of fast growth. In 2010, four out of the five top assignees of CNT patents under "chemistry, electrical current producing apparatus, product, and process" were Samsung Group, NEC Corporation, Sony Corporation, and Panasonic Corporation who has main products in the area of electronics and screens.[165] Samsung grew to be the biggest actor with about 400 granted patents in just five years.

3.3.1.4 The paint and coatings sector

In addition to the conductive coatings, the more conventional coating areas are also a market with potential high-tonnage use of CNT. The paint and coatings sector is broad and comprise manufactures' of architectural coatings, industrial coatings, and special-purpose coatings, which include marine paints, high-performance maintenance coatings, automotive refinish paints, transportation markings. Development into "non-toxic" CNT-doped bottom paints for the marine sector have been commercialized as well as high-durability primers and paints (http://www.nanocyl.com/Products-Solutions/Products/BIOCYL; http://www.amcoat.no/contact-amc.html). Research and developments is currently ongoing for enabling application of these types of coatings in the wind-mill industry as well (see section 3.3.2.3). One may imagine many potential uses for high-durability surface coatings and the use of CNT in the paint and coatings sector is generally in its early stage.

3.3.1.5 Other sectors

Secondary industry sectors with respect to the tonnages required include the medico sector, where CNT enhanced materials may find applications in improved medical devices.[196,211,223] CNT would be used to improve the mechanical properties of the materials making size and weight reductions feasible. Also applications in metal-free skin electrodes for e.g. electrocardiography have been proposed.[47]

Highly specialized CNT products are predicted quite widespread use in microscopy applications in the biotech and research sectors. This includes e.g. SWCNT labeled with fluorophores for UV-microscopy of cells and tissue or scanning probe tips for materials studies. However, also infra-red spectroscopy probes have been shown to be feasible.[123] The usability of SWCNT as carrier particles in drug delivery applications have attracted much attention in the academia since it was observed that SWCNT possess a unique ability in some cases to penetrate cells. It is however still too early to assess whether drugs containing CNT will ever be developed for everyday use.[47,67]

3.4 Examples of CNT-based products in specific application areas

In this section, more detail is given on products and near-market CNT-based products sorted according to their applications in electronics, coatings, energy, materials, and the biomedical area (see overview In Table 3-2).

TABLE 3-2

APPLICATION AREAS OF CURRENT AND NEAR-FUTURE INDUSTRIAL USES OF CNT

Electronics				Coatings		Energy			Materials		Biomedical		
Micro electronics	Bulk electronics	Sensors	Actuators	Conductive coatings	Anti-fouling coatings	Electrode materials	Catalyst support	Current carrier	Antistatic materials	Structural composites	Medical devices	Drug delivery	Microscopy

3.4.1 Electronics

3.4.1.1 Microelectronics

A new type of non-volatile RAM memory based on is being developed by Nantero Inc. By incorporating CNT into well-established microelectronics manufacturing processes the new NRAMs are expected to have higher storage density compared to existing technologies. CNT also hold great promises for applications in other electronic components e.g. in integrated circuits where SWCNT are seen as the 'ultimate' nanowire for connecting the transistors. Even transistors based on SWCNT have been demonstrated by several groups. Due to the small dimensions of SWCNT, it has been proposed to use this technology to create invisible electronic circuits with transparent transistors etc. This has many potential applications within consumer electronics, packaging, military and industrial electronic systems, and apart from being transparent the circuits also have the advantage that they can be placed onto flexible substrates e.g. thin plastic films, which has been demonstrated.[47] Use of branched CNT may also be interesting for these applications in the future.

Another application of CNT in electronics, which is emerging or likely to reach the commercial stage within the near future, is in the area of on-chip thermal management. The heat generated by microprocessors today is considerable and the problem of heat dissipation from the very narrow spaces involved is a big issue. Studies have reported the axial thermal conductivity of individual CNT to be very high, and efficient cooling via CNT on silicon chips has been demonstrated.[47,55,112]

In a more distant future CNT and nanotechnology in general has potential to revolutionize electronics.[19,52] Electrons can have different spin depending on which way they rotate. This property is utilized in spintronics, a special kind of electronics where the digital bits (one and zero) are translated into the two different spin states (spin-up and spin-down). Due to their electronic structure SWCNT are able to act as spin-valves where the conductivity depends on the actual spin of the electron. Recently this has been achieved at room temperature, which is a major step towards realistic applications.[210]

In photonics the photons (light particles) are playing the role of the electrons in electronics and CNT have been suggested as building blocks of nonlinear optical devices which are needed in order to create the photonic transistor.[5] This would enable creation of all-optical circuits with the potential to replace much of the current micro-electronics used in computers. Currently both spintronics and photonics are in a very early stage of development and still require much basic research. Microelectronics entirely based on CNT is not likely to reach the commercialization stage within the near future.[47]

3.4.1.2 Bulk electronics

Field emission (FED) light sources for e.g. displays based on aligned arrays of SWCNT have been demonstrated, but stability issues have so far impeded the commercial break-through. Various patents describe different ways to create light sources based on CNT containing emitters. CNT-based FED screens may have reached the market before 2010 (https://en.wikipedia.org/wiki/Field_emission_display; http://www.appliednanotech.net/tech/cnt_displays.php).

3.4.1.3 Sensors

Gas sensors based on CNT have been commercialized by e.g. Nanomix and are used to monitor toxic and flammable gasses like ammonia (NH_4), nitrogen oxides (NO_x) and carbon monoxide (CO). The advantage according to Nanomix is increased sensitivity and reduced power consumption of the sensor enabling the development of small portable sensors, which even could be integrated into clothing.[47]

Another example of a CNT-based sensor has been developed by Applied Nanotech Holdings Inc., Their biosensor is based on a three-dimensional enzyme-coated CNT network can be used for medical, environmental and chemical analysis. The claim is that this type of sensor for example can analyze a large number of analytes from a single drop of blood, urine or saliva for metabolic analysis as well as be configured for detection of toxic gases and chemical warfare (http://www.appliednanotech.net/tech/ecnt.php).

The number of industries, where CNT-based sensors may become important is very large. Sinha et al. discussed in a review, the application of CNT-based sensors in the manufacturing, biomedical, automotive, food, environmental monitoring, agriculture and fishing industries, as well as in defense and homeland security monitoring.[203] The types of CNT's used in this area appear to vary depending on the type of sensors developed.

3.4.1.4 Actuators

CNT based actuators are mentioned in both patent and scientific literature.[51,60] In this connection actuators cover all sorts of MEMS devices with moving parts e.g. rotating nanomotors or artificial muscles created by incorporating the CNT in a flexible matrix in such a way that the material can change shape in response to an external stimulus.[47,138]

3.4.2 Coatings

3.4.2.1 Conductive coatings

By adding only a few percent CNT in a resin, it is possible to achieve an electrically conductive coating with a conductivity suited for electrostatic dissipation. This requires a conductivity which is sufficient to dissipate the charge in a controlled way. With higher loadings it is possible to make conducting ink, which can be used for printing electronics. Due to the size of the CNT it is also possible to create electrically conducting films with good optical transparency, usually only with a slight grey color (Figure 3-1). Such transparent conducting films are typically used for touch screens, solar cells, OLED displays etc.[47,83] Conductive coatings are already on the market and probably accounts for a significant part of the SWCNT and possibly also some of the MWCNT materials currently produced. Large growth rates are furthermore expected for CNT based conductive coatings due to high expectations to the global demand for solar cells, and consumer electronics with touch screens etc. Several examples of use can be seen at Eikos Inc. (http://www.eikos.com/conductive-coatings.html)

FIGURE 3-1

TRANSPARENT CONDUCTIVE FILM. SOURCE: NANOCYL

3.4.2.2 Anti-fouling coatings, primers and topcoats

Apart from electric conductivity, the incorporation of CNT into coatings has also shown to add anti-fouling properties. Such coatings have passed testing stage and are in use on vessels from racing boats and yachts to large container ships. Different antifouling coating manufacturers claim to obtain the effect in different ways. In one product the CNT is said to cause a nano-scale roughening of the surface which impedes the adhesion of the marine organisms to the surface (http://www.nanocyl.com/Products-Solutions/Products/BIOCYL).

In another product, called GOC (Green Ocean Coatings) AFS H, the surface of the product is said to be extremely smooth and hydrophobic, which also hampers the growth of marine organisms on the surface (http://www.amcoat.no/contact-amc.html). The GOC AFS H products was applied on a,o, a larger marine vessel (Berge Arzew; http://www.motorship.com/news101/ships-and-shipyards/coating-uses-carbon-nano-technology-for-durability-and-performance) (Figure 3-2). However, the current state of the product and Amcoat is currently unclear.

In addition to anti-foul coatings, primers and topcoat coatings are available at e.g. Tesla Nanocoatings (http://www.teslanano.com/). These Tesla primers are designed for application on steel and other metal surfaces while the topcoats are developed for protection against corrosion and wear in many oil and gas exploration and production applications like drilling rigs, offshore platforms, storage tanks and process vessels, pipelines and other equipment.

Overall, antifouling, primer and paint coatings have very high potential for becoming relatively high tonnage products. New developments in antifouling coatings are under development and may persue materials with embedded functionalized CNT as investigated in recent work.[30]

FIGURE 3-2

RED CNT-BASED ANTI-FOULING PAINT APPLIED TO THE MARINE VESSEL, BERGE ARZEW (SOURCE: HTTP://WWW.AMCOAT.NO

3.4.3 Energy

3.4.3.1 Electrode materials

Li-ion batteries utilizing the high surface area and thermal and mechanical stability of CNT are commercially available and considered as a mature technology.[54] Yet new developments are still made to improve the performance of the batteries.[129] Products may be added 2-3 wt% in the anode and 1-2 wt% in the cathode. The CNT used in Li-ion batteries is normally MWCNT. A few examples of Li-doped CNT are listed in Appendix 2. An additional example is from NanoAmor, where the material is a 95% pure MWCNT with a diameter of 50-80 nm and a length of 5 – 20 µm. In this example, the impurity is carbon black. A presentation of the general application principle and some developments is available from a presentation from Showa Denko (http://www.oecd.org/science/nanosafety/44028808.pdf). According to this presentation the MWCNT coats and interconnects $LiCoO_2$ and graphite particles in the cathode and anode, respectively. Continuous development in design and materials increased the power of these Li-ion batteries from 900 mAh in 1991 to 2800 mAh already in 2008 along with improved durability and shorter charging time.

CNT are also of interest for supercapacitors.[2,54] Supercapacitors are electronic components used for storage of electrical energy able of very fast charging and discharging. Supercapacitors are typically used for stabilization of voltages in smaller systems and for devices with high momentary loads such as e.g. electrical vehicles, home solar energy systems etc.[47]

In low temperature fuel cells e.g. proton exchange membrane (PEM) fuel cells CNT can be used as catalyst supports for the active platinum particles. In order to maximize the activity of the catalytic particles it is preferential to have as small particles as possible, but since the fuel cells develops heat during operation and heat causes fusion of the platinum (nano)particles it is important to remove the heat from the platinum particles. In this case the high thermal conductivity of the CNT increases the durability of the catalyst in the PEM cell.[47]

3.4.4 Materials

Currently, there are two main reasons to incorporate CNT in materials: one is to exploit the electrical properties of the tubes and the other is to utilize the extraordinary mechanical properties. Where the first use from a technical point of view is fairly straight forward, the latter has probably not yet fully realized its potential implications. The reason for this is that in order to exploit the electrical properties of CNT in a polymer material the CNT only need to be sufficiently well dispersed to create a network structure through the polymer matrix. It is not critical to have obtained complete dispersion or a strong coupling between the CNT and the polymer matrix. As long as the CNT just touch each other, the nanocomposite will show the wanted electrical properties. Also the requirements for structural perfection of the CNT are less, when the goal is to achieve electrical conductivity.[47,67]

3.4.4.1 Plastics

As mentioned above antistatic and electro-paintable materials such as thermoplastic parts in cars, antistatic textiles etc. are most likely accounting for the majority of the CNT used today (Figure 3-3). For these applications industrial grade MWCNT with a purity of about 90% or higher are well suited.

Inclusion of MWCNT into thermoplastics makes them paintable by electrostatic painting. This process is used for e.g. automotive exterior parts like fenders, bumpers, mirror housings, door handles etc. Some CNT producers expect the MWCNT containing materials also to extend into interior automotive plastic parts like dashboards and cabin door handles etc. in the near future. Traditionally statics was prevented by addition of low-molecular agents, but they are lost by washing or simply slowly evaporate from the plastic. Carbon black has also traditionally been used for enabling electrical conductivity, but much higher loadings of carbon black are required as compared to CNT and carbon black doping has a tendency to cause the plastic to erode and give off small particles. CNT do not increase the 'dustiness' of a plastic and the anti-static property is permanent with gain in performance.

FIGURE 3-3

ANTISTATIC PLASTIC PARTS USED IN THE MANUFACTURE OF HARD DISK DRIVES. SOURCE: NANOCYL

For automotive parts that are in contact with fuel, electrical charge build-up is prevented by the dissipative properties of the CNT. Therefore fuel lines, o-rings, filter housings, pump modules etc. are increasingly being made of plastics containing MWCNT (http://www.nanocyl.com/). CNT is also applied to increase strength and toughness in fenders, body panels, and fuel lines (http://hyperioncatalysis.com/automotive2.htm).

CNT containing plastics are also used in electronic packaging, hard disk cassettes, silicon wafer carriers and similar critical high-cost parts where anti-static, cleanliness and surface properties are important (Figure 3-3). Furthermore MWCNT may be used in elastomers for e.g. conveyor belts and cable jackets where they add antistatic and in some cases even electromagnetic shielding properties to the polymer, at the same time as they are claimed to increase the wear resistance (http://www.nanocyl.com/). CNT use in care-tires is debated for achieving higher strength, elasticity, and sticky properties but whether general application has been launched is unclear at this point in time. Certainly patents exist (e.g., http://www.google.com/patents/US20110146859) and several product examples can be found on the internet (e.g., http://www.examiner.com/article/bike-tires-go-high-tech-with-carbon-nanotubes; http://www.youtube.com/watch?v=dUotWMkZ_mk)

3.4.4.2 Textiles

Synthetic textiles are typically made from thermoplastic materials spun into thin fibers. CNT can be added to the fibers either before the spinning process or as an external coating on the fibers after spinning. Both processes have been used to produce strength-enhanced and electrically conducting yarns for textiles and many different uses of such textiles have been proposed. CNT may also be used in non-woven textiles and different types of CNT may apply. The production is currently moving to large-scale. As an example NanoComp Technologies has announced that their production of yarns and non-woven textiles will reach 4 tons in 2012 (http://www.techbriefs.com/component/content/article/5608). The strength of these textiles is 0.2 to 1 GPa and the electrical conductivity exceed 2×10^6 S/m). Figure 3-4 shows an assembly of images of textiles and yarns with CNT from two producers.

Besides the use of CNT yarns for strength and wiring (Fig. 3-4a), CNT textiles can are also be used for electrical heating. Heat is generated when electrical current is passed through the textile due the resistance (ohmic) loss in the fibers. Current uses of this type of materials include heating mats for water heating appliances. Thin woven heating mats have also been tested successfully in Japan for the purpose of de-icing outdoor walking areas and personal heating pads.

Other uses of CNT textiles may be electromagnetic shielding and microwave reflectors as well as armor (body and structural composites). Future uses have been envisioned within ‘intelligent’ clothing e.g. for work, medical and winter sports applications. Textiles containing CNT can also be strain sensitive such that the electrical conductivity changes when the textile fibers are stretched. This is used in some cars to monitor which seats are occupied.

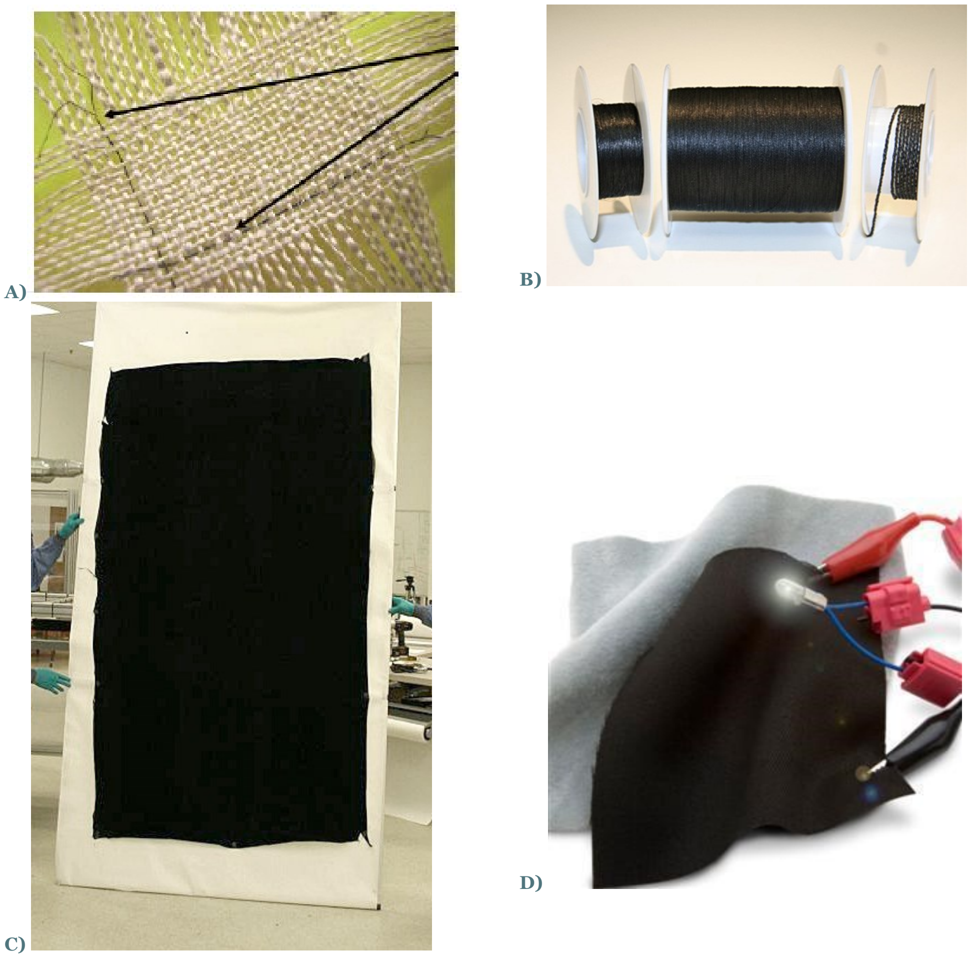


FIGURE 3-4

A) CONDUCTIVE CNT FIBERS INSERTED IN A WOVEN TEXTILE (NANOCYL). B) THREE DIFFERENT TYPES OF YARNS USED FOR PRODUCTION OF WOVEN TEXTILE SHEETS (HTTP://WWW.NANOCOMPTECH.COM/). C) A 4 BY 8 INCH WOVEN CNT SHEET. (HTTP://WWW.NANOCOMPTECH.COM). D) WOVEN CONDUCTIVE CNT TEXTILE (HTTPS://WWW.INVENTABLES.COM/TECHNOLOGIES/SOFT-FEELING-CONDUCTIVE-TEXTILES).

3.4.4.3 Structural composites

Typical application areas are as light-weight load-bearing parts within aerospace, automotive, marine and sports equipment. With a tensile strength on the order of 15 times that of steel and a density which is only one sixth it is easy to imagine composites with extremely high strength to weight ratios. In order to fully realize the potential improvements in mechanical properties – typically strength, stiffness and toughness – of the matrix material, the requirements to the nanoscale structure of the composite materials are very high. They include requirements for the dispersion of the CNT throughout the matrix, a low ‘waviness’ of the CNT (the more straight and ‘fiberlike’ the better, from an engineering point of view), a high degree of alignment of the CNT and a strong interface ensuring an effective stress transfer from matrix to CNT.[17,50]

These requirements are based on the long experience with traditional fiber reinforced composites. But when it comes to nanocomposites new issues emerge:

First the CNT need to be perfectly dispersed in the polymer matrix because agglomerates will lead to stress concentrations in the material causing premature failure when the composite is subjected to load. When visualizing this perfect dispersion it is important to remember the extremely high specific surface area of CNT, which easily can be in the order of hundreds of square meters per gram of material. This means that as the CNT loading is increased the thickness of an imaginary layer of polymer covering the surface of each of the CNT fiber rapidly decreases, but since the size of a polymer molecule typically is in the order of 10 – 100 nm, there is a lower limit to how thin this layer can become. This is the reason that nanocomposites of CNT usually only contain less than 3-5 percent by weight of CNT.

Second there must be a very strong coupling between the CNT and the polymer matrix in order for the mechanical forces acting on the composite part to get transferred from the matrix to the CNT. In traditional fiber composites this strong coupling is achieved through mechanical locking of the matrix material into the surface roughness of the reinforcing fibers, but a similar roughness is not present on CNT. With suitable chemical modifications of the CNT it is possible to achieve a covalent coupling to an epoxy resin and this has been pursued by several companies. Similar coupling to other polymers have so far not been demonstrated in significant scales impeding the use of CNT as structural reinforcing agents in other polymers. When this problem is solved however, a significant increase in the use of CNT as mechanical reinforcing fibers for polymers is expected.

These requirements for mixing and chemical bonding suggest the most suitable CNT-types for disperse structural composites are end-functionalized MWCNT, Cup-stack or other complex CNT grades.

Several examples of property enhancement of fiber-reinforced polymer composites have been demonstrated. Already in 2010, the American Zyvex unveiled a CNT reinforced boat in which CNT enhanced carbon fibers are enabling a significant weight reduction compared to materials used previously (Figure 3-5a). This translates into reduced fuel consumption and increased action radius for the vessel (http://www.zyvextech.com/).

Various windmill producers have conducted development projects aiming at reducing the weight of wind mill blades by incorporation of CNT in the resin with success. Especially a former collaboration between Bayer and Amroy Oy in Finland (company terminated) developed a CNT re-enforced epoxy resin, Hyptonite® useful for construction of light-weight wind turbine blades. The technology has been demonstrated in small household windmills (Figure 3-5b). However, it is uncertain whether wide-spread application in larger in larger (megawatt) windmills has begun. The application in windmill wings is very advantageous giving lighter wings and increased performance. The weight of a typical 50-60 m blade for a MW windmill is about 20 ton, which could be reduced to 14 – 18 ton by changing to epoxy based composites, the addition of 1 percent by weight of the resin (excluding glass or carbon fibers) would correspond to app. 100 kg of CNT per windmill. Assuming an outbuilding of the Danish wind energy sector of about 400 MegaWatt per year this would correspond to an estimated consumption of 10 ton of CNT per year.

FIGURE 3-5

A) LIGHT-WEIGHT BOAT BUILD WITH CNT-REINFORCED COMPOSITE (ZYVEX). B) HOUSEHOLD WIND MILL. (FORMER AMROY WEB-SITE); C) EASTON ARROW (ACADEMY.COM; SPORTS WEBSHOP), TOUGHLEX FRAME IN YONEX TENNIS RACKET BUILD OF SYNTHETIC RESIN WITH CUP-STACK CNT (YONEX).

CNT-enhanced sports equipment is an important consumer market. Badminton and tennis rackets as well as golf clubs and ice hockey sticks are thought to be the major product lines with CNT. Other products are fishing poles and arrows as depicted Figure 3-5c. Among these products, Yonex uses the Carbere® CSCNT in the head of the racket of the ArcSaber badminton as well as the VCore and S-Fit tennis rackets and new rackets swith Toughlex frames (Figure 3-4d). A “Dual Nanoscience” concept of mixed CS-CNT and nano-Ti is used in the head and shaft certain golf clubs. Each cup in the CSCNT stack is said to have the ability to move and flex independently at the point of impact, generating extra power and increasing ball dwell time which gives improved directional accuracy.

This property is also used in CNT reinforced epoxy composite hockey stick from Montreal Hockey Company, which was developed in collaboration by Montreal Hockey Company, Amroy and the MW Baytubes (Montreal Hockey Company). The "CNT Bicycle" from the Swiss bike manufacturer BMC is another example of high-end sports equipment: By replacing a small amount of the epoxy resin the specific density of the composite is reduced slightly without loss of mechanical properties.

Some examples of high end parts of aluminum nano-composites can also be found on the market today, e.g. forks, chain rings, wheel rims etc. for bikes. The market is difficult to assess because words like "nano" and "carbon" are used to cause associations with high strength, stiffness and low weight, which justifies high cost. It is however uncertain to which extent these products actually contain CNT. Materials development projects aiming at realizing true nanocomposites of aluminum and CNT are ongoing. The aim is reduced weight high performance load bearing parts. (Nanocyl, personal comm.).

3.4.5 Biomedical

3.4.5.1 Medical devices

In a study of the thrombogenicity and blood coagulability of CNT containing polyamide microcatheters it was observed that the nanocomposite materials showed better performance than the neat polymer. The mechanism for the improved biocompatibility of the CNT nanocomposite materials is unclear, but the results of the study indicate that medical devices might be improved if they are produced from CNT nanocomposites.[47,195]

The possibility to create a metal-free skin electrode based on CNT have also been proposed and studied. With traditional microelectronics fabrication techniques it is possible to create an array of free standing CNT of a suitable length. The CNT can penetrate the outermost layer of the skin providing a superior electrical contact without touching the nerve cells in deeper skin layers. Both of these products examples appear to be still in development, but could reach the commercial market within the near future.[47,125]

3.4.5.2 Gene therapy, drug delivery

The potential for SWCNT to act as carriers for small molecules in biological systems is one application that has attracted much attention from academia and industrial research departments for a number of years.[139] It has been found that SWCNT can have low cytotoxicity in some cases and due to their large specific surface area they can carry more active drug molecules per weight unit compared to vesicles and other vehicles typically used. In a scenario where SWCNT are used to carry genes they have been observed to be able to pass the cell membrane and deliver the gene in the interior of the cell and in the nucleus. Much of the research in different variations of CNT based drug delivery systems is targeted at cancer therapy.[97,121] Since much remains unknown about the transport mechanisms, fate and adverse effects of CNT at the cell level applications in the biomedical area are believed not to reach the market in the near future.[67]

3.4.5.3 Microscopy

In biological imaging the optical properties of SWCNT can be exploited to observe their presence in blood, cells and thick tissue.[100,242] Because of the photostability of the SWCNT photoluminescence longer excitation times and higher laser power can be used compared to other fluorophores, without problems due to photobleaching. CNT can show extremely large resonance enhancement of Raman signals which is useful in studies of cells and tissues in Raman microscopy.[127]

In scanning probe microscopes (SPM) single CNT are used as extremely sharp tips for topological studies of surfaces. The working principle of an SPM resembles that of a record player: A thin needle is moved across a surface and the movements of the needle are detected and translated into a profile of the sample surface. By the use of extremely sharp tips it is possible to obtain atomic resolution, and SPM can provide valuable information about the nanoscale and sub-nanoscale structure of materials.[47] Current developments goes in the direction of using these abilities coupled with other measurement principles.[136]

3.5 Concluding remarks

It is evident that many different products with CNT are commercially available and several more are near-to-market. However, it is very difficult to get a complete overview based on the readily available information. It appears that the number of products steadily increases with increased time for searching and following different search paths. In addition many products are likely to contain CNT without declaration. By volume the major applications appears to be in the automotive industry, energy and electronics and many emergent uses are appearing. Table 3-3 lists the main different CNT product groups observed with indication of their anticipated time of introduction to the commercial market. It is realized from the table that only a relatively few number of products are expected in the near-future and farther future. This is probably due to lack of knowledge.

TABLE 3-3

ANTICIPATED TIME FOR MARKET INTRODUCTION OF CNT-BASED PRODUCTS.

Application area	Now	Near future	Future
Electronics	Printed electronics (conductive ink)	FEDs CPU heat sinks	Spintronics, photonics
Coatings	Conductive coatings (displays and touch screens) Anti-fouling coatings High-durability epoxy-paints	Solar cells	
Energy	Li-ion batteries	Fuel cells Super capacitors	
Materials	Antistatic thermoplastics Textiles (conducting and reflecting) Thin heating mats High performance sporting goods Windmill blades (small and large at least in test)	Al-CNT nanocomposites Thermoplastic parts for E-painting Large windmill blades	Load-bearing thermoplastic nanocomposites
Application area	Now	Medical devices, skin electrodes	Gene therapy

4. Exposure to man and the environment

This chapter summarizes the current knowledge on human and environmental exposure to CNT. Results from a life-cycle assessment are presented to illustrate the potential mass-flow of CNT from manufacturing to disposal. Hereafter, we discuss the potential exposure and environmental release during specific CNT and product manufacturing processes. We also perform a qualitative assessment of the risk of occupational and consumer exposure to CNT as well as environmental release for the major product groups identified in Chapter 3. Even though exposure measurement data are emerging, data are still scarce, and the overall assessment should generally be understood as expert judgment.

4.1 Summary

There is currently very limited data available on the exposure levels or potential release associated with production and use of CNT-based products. Life-cycle analysis suggests that the major route of release of free CNT to the environment (air-borne dust, as well as liquid and dry waste) originates from synthesis and manufacturing of CNT-based products. Soil and sediments are the major sinks for accumulation of CNT arising from sedimentation of airborne dust, sludge treatment and release through waste-water, respectively. Discard of CNT waste and CNT products mainly ends up in landfill (ca. 70%) and waste incineration plants (ca. 17%). One major exception is recycling of Li-ion batteries, but re-use of CNT is not documented.

Occupational exposure to airborne CNT dust may reach up to at least a few hundred µg/m^3 air and very few measurements show no detectable exposure. A single study showed that wide-spread surface contamination by CNT at a small scale producer as a critical source for secondary exposure. Dermal exposure is also a consideration where one study showed worker glove-dose of 2-6 g CNT associated with harvesting and handling crude CNT after production. These concentrations serve as a benchmark values for maximum typical achievable exposure levels. The nature of the CNT in reported occupational exposures and release studies mainly occurs in bundles, aggregates and agglomerates of CNT as well as in matrix bound fragments.

There appears to be no measurement data available on the actual consumer exposure and to CNT and environmental release. From logical conclusions, the risk of consumer exposure may be limited due to the general incapsulation or embedding of CNT in CNT-based products. However, possible cases have been presented, such as dermal exposure during use of CNT-coated textiles and release of CNT after weathering of composite materials.

Life cycle assessment and our own evaluation suggest that there is a high potential of CNT release to the environment during production of CNT as well as production and discard of certain CNT-based products. Release to the environment may also occur in connection with some intended use and weathering of CNT-based nanocomposites. The environmental release rate will obviously strongly depend on the occupational activity, route of release, the local environment, use conditions, and waste handling strategies. In our evaluation, CNT release to the environment appears to be most potential during manufacturing, discard of process waste and ultimately discard of the final product. The modelled environmental concentrations in life-cycle analysis are still moderate, but the models only consider homogeneous mixing in the environmental compartments, which is unlikely. Hot-spots around sources should be considered in future assessments.

More detailed assessment of consumer exposure and environmental release is performed in Chapter 8, which attempts to perform a more detailed risk assessment for the major product groups identified in this report.

4.2 Exposure and environmental release of CNT

The potential inhalation, dermal, and accidental oral exposure to CNT is obvious in occupational settings, where CNT is produced, harvested, purified and functionalized as well as during subsequent manufacturing of CNT-based products. Currently, data only exist on occupational exposure and specific process-related emissions.[13,28,72,120] Exposure assessments and experimental data are slowly emerging on the in-use potential release and potential consumer exposure from CNT-based products.[16,158,238] No measurement data were found on the industrial release of CNT to the environment. However, assessment of environmental release has been made using both qualitative assessments and quantitative simulation including the most important life-cycle stages.[42,64,65,108] A recent qualitative assessment of worker and consumer exposure as well as environmental release is given by Nowack et al. [158]

Figures 4.1 and 4.2 illustrate the major pathways to environmental exposure from industrial release and transport accidents, respectively. Direct worker exposure is likely along several of these pathways, whereas the environmental exposure mainly occurs after transfer through air, soil or water. However, the nature and potential hazard of aged CNT is not known. Even-though CNT is considered a highly durable material; interaction with the environment is expected to change the physico-chemical properties and agglomeration state of the CNT. [168] This may also influence on their ecotoxicological properties (Chapter 6). As long as the fate of CNT as well as wear/weathering debris with CNT has not yet been clearly understood, there is additional uncertainty on the nature and hazard of the environmental exposure. [42] It is normally assumed that there are no changes in properties and hazard during transition through the environment.

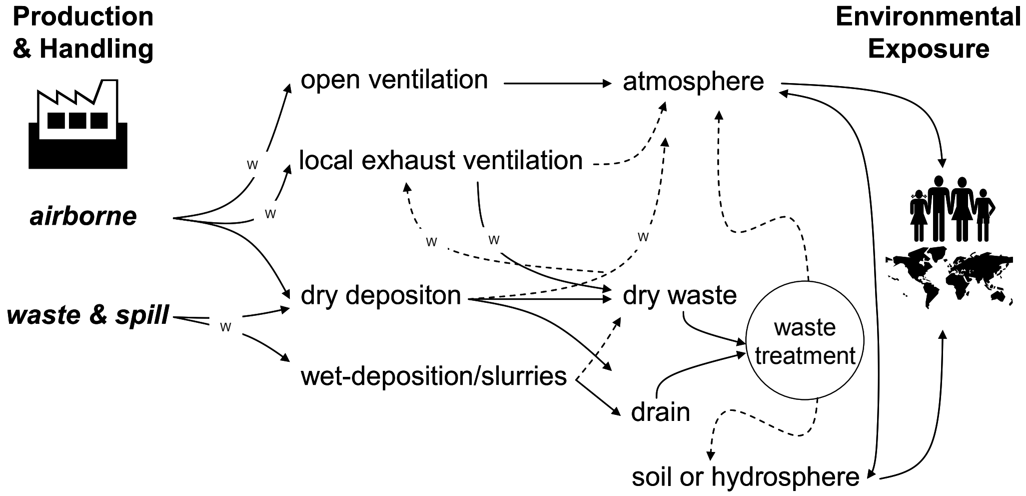


FIGURE 4-1
SCHEMATIC ILLUSTRATION WORKER EXPOSURE SITUATIONS DURING CNT PRODUCTION AND HANDLING AND MATERIAL FLOW, WHICH CAN LEAD TO ENVIRONMENTAL EXPOSURE. PROCESSES AND PATHWAYS MARKED WITH "W" ARE ASSUMED TO BE WITH HIGH RISK OF WORKER EXPOSURE.

Table 4-1 shows the simulated material flow from the CNT PMC (Production, Manufacture and Consumption) using the waste treatment strategy in the USA and resulting concentrations in the environment for the USA and the EU, respectively.[64] The assessment was based on a global annual production of 500 metric ton CNT, and downstream production of CNT based composites, consumer electronics, and R&D. The production and product volumes were allocated regionally per capita between USA, Asia and the EU. About 90% of the CNT-based products were allocated to composites and consumer electronics. Therefore most of the product waste goes to landfill and incineration.

The model results suggested that about 0.6% of the release is airborne and is originating almost entirely from CNT synthesis and manufacturing. Importantly, the absolute pollution in the EU exceeds that in the USA, which is mainly due to a smaller area and higher population density in the EU. The lower and upper 15% percentile dust concentrations were estimated to be 0.0025 and 0.007 ng/m^3 per 500 tons CNT produced per year The greatest exposure concentrations and annual doses 500 tons CNT produced were found in sediment (215 to 1,321 ng/kg/year) and sludge treated soil (52.1 to 157 ng/kg/year) of which the latter is due to a rather high concentration modelled to go into sludge (> 0.1 mg/kg). Outlet concentrations from sewage treatment plants (SWT) were also relatively high with lower and upper 15% percentiles being 11.4 and 31.5 ng/L.

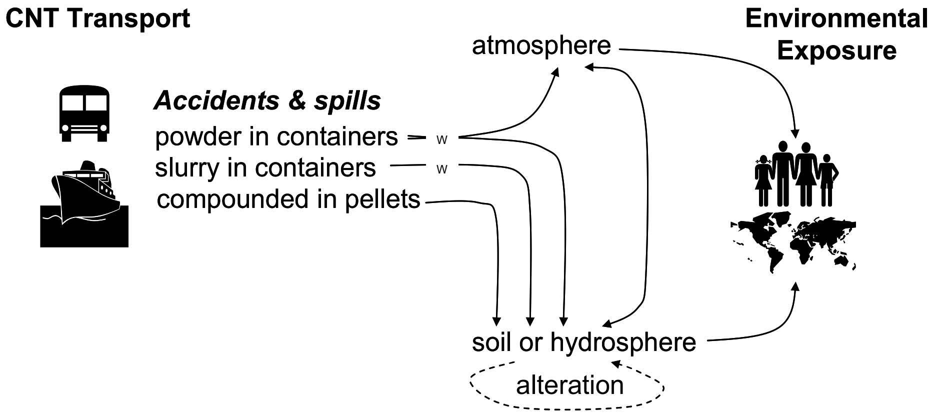


FIGURE 4-2

SCHEMATIC ILLUSTRATION OF THE POTENTIAL EXPOSURES DURING TRANSPORT OF TYPICAL RAW CNT PRODUCTS AND MATERIAL FLOW WHICH CAN LEAD TO ENVIRONMENTAL EXPOSURE. ENVIRONMENTAL EXPOSURE TO COMPOUNDED CNT REQUIRES PRECEDING ALTERATION OF THE PELLETS TO LIBERATE CNT FROM THE MATRIX. PROCESSES AND PATHWAYS MARKED WITH “W” ARE ASSUMED TO BE WITH HIGH RISK OF WORKER EXPOSURE.

At first glance, the estimated environmental accumulation rates estimated by Gottscalk et al. [64] may not be dramatic, but considering that the CNT production – or at least the production capacity - is rapidly increasing combined with the apparent high persistence of CNT, the accumulation rates, as well as absolute concentrations, will increase. Another important issue is the authors’ assumption of instant homogeneous mixing in the different model compartments. Practically, no compound will disperse uniformly in the environment. Therefore “hot-spots” is expected and should be considered. To truly make this scientific step, a significant increase in experimental data is required.[170]

4.2.1 Exposure and release during synthesis and product manufacturing

To identify potential “hot-spots” it is relevant to consider the most potent processes in greater detail. The life-cycle assessment by Gottschalk et al. [64] showed that industrial activities are the most potent sources to direct environmental release of CNT and exposure. However, all activities are not conducted at the same scale in every country and will include different manufacturing processes at different scales and different contextual conditions. Therefore regional assessments may not be adequate for specific national assessments of industrial release to the environment. Further understanding may be achieved by assessing the exposure and release potentials of specific occupational activities in the synthesis and manufacturing phase.

TABLE 4-1

CNT RELEASE FROM PMC (PRODUCTION, MANUFACTURE AND CONSUMPTION) COMBINED WITH ACCUMULATION RATES AND YEAR 2008 CONCENTRATIONS IN THE US AND EU ENVIRONMENTS ACCORDING TO GOTTSCHALK ET AL.[64]

	USA			USA			EU			
CNT Material flow	t/year	%	Compartment	Mean	$Q_{0.15}$	$Q_{0.85}$	Mean	$Q_{0.15}$	$Q_{0.85}$	unit
Loss from PMC	4.11	5.5	Air (1 km mixing height)	0.001	0.00096	0.003	0.003	0.0025	0.007	ng/m^3
Atmosphere	0.48	0.6	Soil (0.2 m agri$^{\#}$ / 0.05 m other)	0.56	0.43	1.34	1.51	1.07	3.22	ng/kg/year
Seweage treatment plant	0.98	1.3	Sludge treated soil (0.2 m agr$^{\#}$)	31.4	23.9	74.6	73.6	52.1	157	ng/kg/year
Waste Incineration Plant	12.5	16.7	Sediment (0.03 m)*	46	40	229	241	215	1321	ng/kg/year
Recycling	2.63	3.5	Surface water (3 m)	0.001	0.0006	0.004	0.004	0.0035	0.021	ng/L
Landfill	54.2	72.4	STP effluent	8.6	6.6	18.4	14.8	11.4	31.5	ng/L
			STP sludge	0.068	0.053	0.147	0.062	0.047	0.129	mg/kg
Total	74.9	100.0								

$Q_{0.15}$ and $Q_{0.85}$, reperesent the lower and upper 15 percentiles. $^{\#}$ agricultural; * only sediment under water.

It is worth noting that each CNT batch may lead to both worker exposure and environmental release during several steps of the manufacturing process starting at manufacturing of the crude CNT, its purification and functionalization to the subsequent handling for their application in CNT-based products and product finishing. In addition, transport of CNT is also associated with a risk of environmental relase (Figure 4-2). Special concerns are spills and accidents, which can result in larger local exposure and difficult clean-up situations.

Table 4-2 lists an evaluation of 15 major work situations with CNT that could result in environmental release of CNT in dust, spills, and waste (see Appendix for a summary of key exposure measurement data and literature). Each activity is given a color code (or increasing gray scale with severity) grouping our judgment of 1) the exposure level and 2) risk of release into low, intermediate and high. These scales are partly arbitrary.

TABLE 4-2

RANKING OF EXPOSURE AND ENVIRONMENTAL RELEASE POTENTIALS IN SPECIFIC MANUFACTURING STEPS. SEE TEXT AND FOOTNOTES FOR COLOR CODES AND INFORMATION.

Process	Transfer route	CNT mass in process[§]	Highest reported dust[#] level [$\mu g/m^3$]	Major route for environmental release
Synthesis	Air[£]	mg – kg	N.D.	Only accidents
Harvesting	Air	mg – kg	≤ 286,TSP[$]	LEV & room air
Normal cleaning	Air	µg – g	≤ 286TSP[$]	LEV & room air
	Liquid	µg – g	N.A.	drain and waste
Purification/fractionation	Air	mg – kg	≤ 93 PM_4	LEV & room air
	Liquid	mg – kg	N.A.	drain and waste
Packaging	Air	mg – kg	N.A.	LEV & room air
	Liquid	mg – kg	N.A.	drain and waste
Storage	Sealed	mg – t	None	Only accidents
Shipping	Sealed	mg – kg	None	Only accidents
Unpacking	Air	mg – kg	N.A.	LEV & room air, accidents
Pouring/transfer of CNT	Air	µg – kg	≥ 212 PM_{inh}[$]	LEV & room air
	Liquid	µg – g	N.A.	drain and waste
Powder mixing dry	Air	µg – kg	≤ 332 TSP[$] ≤ 200 black carbon	LEV & room air, waste
Powder mixing in liquid	Liquid	µg – kg	N.A.[€]	LEV, room air, drain,waste
Spraying	Air	ng – µg	≤ 193 TSP[$]	LEV, room air, drain,waste
	Liquid	µg – mg	N.A.	drain and waste
Embedding/encapsulation	Air	µg – mg	N.A.	LEV, room air, drain,waste
Weaving CNT yarns	Air	g - kg	≤ 159 TSP[$]	LEV, room air, waste
Mechanical wear of CNT nanocomposites	Air	mg/kg	≤ 2,410 PM_{10}[#]	LEV, room air, drain,waste

N.D.: Not detected; N.A.: Not analyzed; LEV: Local exhaust control; [§]Estimated mass ranges; [&]Performed together with harvesting from CVD; [$]TSP: Total Suspended Particulates; PM_{inh}: Inhalable dust; [£]Release from inspection doors may occur; [€] Increase in particle number concentration observed during ultrasound bath treatment. [#]CNT-bearing composite particles from dry-cutting composites with no LEV (use of wet-cutting and LEV reduced the particle concentration to background levels).

The color code indicates the level of exposure risk from unlikely/very low exposure level (green), possible/moderate exposure level (yellow) or likely/relatively high exposure level (red).

First, we ranked the exposure potential based on the amount of material produced or used in the activity. The amount and type of work are determinants for the exposure and release potential. For the ranking of airborne release potential, critical mass-ranges were based on Near-Field (NF) particle concentration calculated using the exposure potential algorithm in the NanoSafer 1.1 control-banding tool.[92] In defining the limits, we assumed a high dustiness level of the CNT (375 mg respirable dust per kg powder); NanoSafers default value for materials with unknown dustiness; and a work room of 5 by 5 and 3 m high; and a ventilation rate of 5 h^{-1}. No local exhaust ventilation control was used.

Using these conditions, careful scooping of 2.5 g CNT over 15 minutes with low energy (handling energy factor 0.1 of the dustiness index) is predicted to result in 1 $\mu g/m^3$ exposure to respirable CNT dust, but 10 $\mu g/m^3$ if the 2.5 g CNT would be subject to high energy handling (e.g, dry mixing; handling energy factor = 1). Considering the proposed chronic exposure limit of 1 $\mu g/m^3$ by Asberger et al. [4], a critical acute airborne exposure (2 times the chronic exposure limit) may be reached during 15 min handling or processing of between ca. 0.5 g ("high energy process and no ventilation") and 5.5 g CNT (low handling energy and a work-room ventilation rate of 5 per hour) if no local exhaust ventilation protection or enclosure is applied. The actual exposure potential will strongly depend on the activity handling energy and the true dustiness level of the CNT powder. If the dustiness levels are low, the amounts that can be handled will increase considerably to be on the order of and 25 and 275 g for the theoretical handling situations described above.

As seen in Table 4-2, many occupational activities may imply use or handling more than gram-levels of CNT powder and could potentially be associated with a very high exposure level. These activities are marked red in table 4-2. Processes using less than 1 g may be categorized as activities with intermediate to moderate potential exposure and are labeled in yellow. Processes assumed to be matrix bound, fully closed or to release low amounts of CNT were assumed to be associated with low risk of exposure to CNT liberated from the matrix and has been labeled green.

A second exposure risk categorization was for the reported airborne occupational exposure or particle concentration levels. These values were grouped into three exposure categories or qualitative evaluations of the release potentials, when no data were available. In this the case the suggested chronic exposure limit of 1 $\mu g/m^3$ [4] was used as a benchmark:

- CNT not detected and < 0.05 $\mu g/m^3$ increase in dust = low CNT exposure: green
- CNT detected and 0.05 to 0.5 $\mu g/m^3$ increase in dust = intermediate CNT exposure: yellow
- CNT detected and > 0.5 $\mu g/m^3$ increase in dust = high risk of CNT exposure: red

Finally, the potential environmental release associated with the industrial process was assessed depending on a simple qualitative assessment of the likelihood of release:

No or very low likelihood of environmental release:	green
Possible or minor release to the environment:	yellow
Known or high likelihood of environmental release:	red

As shown in Table 4-2 most of the occupational activities are associated with either documented or evaluated important occupational exposure and/or environmental release potentials. The backgrounds for each of these evaluations are given hereafter.

4.2.1.1 Synthesis

Particle release during the CNT synthesis has been measured.[29,72,78,120,144], In most of these studies, CNT was produced by CVD, which probably is the most dominant production method (Chapter 2). None of the studies report increased particle number concentrations in the air during the specific synthesis process or on gravimtric samples (Table 4-2). It is anticipated that risk of CNT exposure is negligible in the closed-reactor synthesis procedures. Hence, exposure and environmental release is only expected in case of accidents.

4.2.1.2 Harvesting and cleaning of reactor area

Several studies have shown exposure risk during harvesting of CNT from the production reactors (Table 4-2). Even operation in fume hoods does not always prevent worker exposure. Particle dust concentrations up to 286 μg/m³ have been reported during harvesting MWCNT after industrial production in a fixed catalyst CVD process.[120] However, this mass concentration was not due to MWCNT exposure alone. TEM analysis showed presence of catalyst particles and free single CNT fibers were not observed. However, online analysis showed that most of the exposure occurred immediately after opening the reactor door and sometimes can be associated with prolonged increased airborne black carbon concentrations.

Harvesting is the first manufacturing process with serious risk of exposure. Free, bundled and agglomerated SWCNT and MWCNT have been observed during harvesting from both HiPCO, Arc discharge, and pulsed laser ablation CVD methods.[72,78,140,144] Methner et al. also tested the work zone concentration with the fume hood switched of and found a quick increase in airborne fine particle (10 – 1000 nm) concentrations from < 5000 n/cm³ to 42,400 n/cm³ during harvest of 1-2 mg short MWCNT. However, Maynard et al. [140] reported a big difference in particle release and nature of raw CNT materials depending on synthesis method. In their study HiPCO materials released at least five times more dust, measuredas TSP, than laser ablation materials and was retrieved as larger (typically > 100 μm) clumps of nanorobes and aggregates as compared to μm-size nanorobes in laser ablation. These differences will have great influence on the CNT aerosol behavior, capture and fixation in ventilation systems as well of their migration in case of accidental environmental release.

Harvesting is also associated with cleaning, which appears to be mainly done by vacuuming. This may be followed by dry and wet-wiping. Finally work suits and gloves will be contaminated during all the manufacturing and subsequent handling operations and may be discarded of as regular dry waste if no special procedure has been established. The contamination levels are generally unknown, but Maynard et al. [140] estimated glove contamination on the order of 2-6 μg per hand after approximately 30 min of CNT handling.

All in all, the published studies indicate that there is a significant CNT load on the filters in the engineered control units at CNT production facilities. Filter-damage and accidents are prone to cause significant exposure to both workers and the environment following the pathways shown in Figure 4-1. Additionally, waste will be produced in the form of contaminated filters, dust bags, gloves, paper-tissue, and residual CNT powders or suspended in liquid. The extent of wet-cleaning and its CNT exposure potential is unknown, but inevitably results in either wet-wipe waste (dry) or liquid cleaning agents led to drains.

4.2.1.3 Purification and functionalization

After harvesting, CNT’s may undergo one or more handling activities as powders, suspensions or slurries for purification and functionalization, if made. No information on exposure risk or environmental exposure levels is available for the specific steps in industrial scale purification processes. Tables 2-5 to 2-6 listed several purification methods, which are mainly wet-chemical processes. These processes will result in liquid chemical waste enriched in CNT or other carbon allotropes, or dissolved catalyst material. Functionalization will additionally add to this waste pool by clean-out of residual material in containers and reactors, which probably will be done with water- or chemicals-based cleaning. The potential worker and environmental exposure risk is assumed to be likely and related to handling of powder and dispersions as well as liquid waste. Emissions from CNT handling is discussed below.

4.2.1.4 Handling and mixing of powders and dispersions

Handling of CNT powders and dispersions occur in several steps after harvesting. Most CNT producers also sell their materials as powders, liquid dispersions or more rarely as CNT embedded in polymer master batches. Handling of powders and dispersions are therefore considered the most common activities at both the CNT producer and at the first industrial downstream users. Yet, exposure data have only been found in studies of laboratory scale powder handling activities in the literature.

Up to 332 µg/m^3 (200 µg/m^3 black carbon) have been observed during handling of CNT in workplaces and was found after opening a blender after mixing CNT for composites in a un-ventilated area.[72] Clear evidence of CNT aerosolization also was found during 20 min probe-sonication of 100 mg of both pure and hydroxylated long MWCNT per liter natural organic water with not local exhaust control.[94,144] In both cases, the emission was greatly reduced after enclosure in fume hoods. The highest concentrations reported during powder handling is 212 µg/m^3 inhalable dust and measured during transferring large spoonfuls MWCNT during transfer of 0.5 and 1 kg CNT powder and funneling it into small flasks in a highly ventilated (60 – 550 m^3/h) and 3.6 m^3 size temporary work enclosure [114].

Clearly, both powder handling, mixing and sonication of liquid CNT dispersions can cause aerosolization of CNT. The amounts of CNT handled in the reported studies rarely exceed 100 g. Despite working with these relatively low masses, notable dust concentrations are observed. Model calculations made to define the dust exposure risk ranges above also indicated that potentially critical exposure levels are easily achieved in the workplace. Clearly both ventilation systems, filters, dry and wet waste, dust bags and transfer through room air are potential routes for environmental release.

4.2.1.5 Spraying of dispersions

CNT's are sometimes applied onto a surface in the final down-stream professional user stage. Two studies report the personal exposure and workplace particle concentrations during spray-application of MWCNT dispersions onto wafers. This work was conducted in fume hoods or in a ventilated enclosure. Han et al. [72] reported personal exposure and workplace TSP concentrations of up to 193 µg/m^3, which were reduced to 31 µg/m^3 after an unspecified intervention. Lee et al. [120] reported small scale application of CNT by spraying in a ventilated enclosure. Even-though both TSP concentrations and particle concentrations often increased in the work area (44-128 µg/m^3) as compared to the background (80 µg/m^3) immediately after opening the chamber, CNT was not observed in the collected air-samples by electron microscopy. This may be due to short-term acute exposure, which may be difficult to catch by filter sampling.

Indoor spraying of CNT dispersions involves aerosolization of CNT and in the reported studies, the majority of excess airborne CNT is expected to be removed by the chamber ventilation and local exhaust control. Again, the exhaust filtration system is the immediate location of CNT accumulation and environmental exposure will be related to filtration efficiency and disposal of filters, dust bags as well as liquid and dry waste. Outdoor spraying of CNT-based products is also a possible large-scale activity and was documented in the application of anti-fouling paint on a larger vessel. In this case the paint was sprayed onto the ship using a high-pressure spray paint procedure in open air (https://www.youtube.com/watch?v=TlI1VIkizrY). In such cases it is clear that release of CNT-epoxy paint droplets occurs directly to environment and bystander (worker and public) exposure is likely.

4.2.1.6 Transport of powders and slurries

Transport and shipping of CNT powders and dispersions is also an issue and accidental release can lead to release of relatively large amounts to the environment (Figure 4-2). Naturally, the potential exposures and releases are considered rare, but can reach all compartments of the environment. The shipped masses are currently unknown, but a European producer informed us that most of the European CNT production is currently shipped to Asia. Considering the amounts of synthesized CNT, shipments of up to at least a few hundred kg may be realistic. Accidental release of this amount of material will lead to local very high concentrations of either highly mobile or dusty material (powders), highly dispersed and soluble individual CNT in slurries/dispersions, or less mobile CNT in compounded master batch pellets (Fig. 4.2). Compounded material must be considered a relatively safe transport medium, which enables time for clean-up, before the matrix starts to degrade and liberate CNT. However, such materials are not always applicable for the user.

4.2.1.7 Exposure and environmental release during manufacturing of CNT-based products

Production of CNT-based downstream professional and consumer products may also be associated with a high number of exposure and environmental release scenarios. In the final manufacturing stage, the potential exposure risks and risks of environmental releases is related to the specific product type and selected manufacturing process, needs for mechanically modifying and polishing the materials. So far only limited information is available on these topics. Below are examples of weaving with CNT-coated yarns, cutting alumina and epoxy CNT-based nanocomposites, and sanding of CNT-reinforced thermoplastics and concrete.

CNT-reinforced and/or conductive textiles are an emerging product group. One study has investigated the exposure conditions during weaving of CNT-coated yarns for heating mat textiles [215]. Electron microscopy showed that CNT fibers coated the yarns and some tube ends were protruding from the surface, which enables direct skin contact. Free CNT fibers were not observed in air-samples from the weaving factory, but the airborne concentration of μm-size yarn fragments was significant with dust concentrations of 0.159 mg TSP/m^3 and 0.093 mg respirable dust/m^3, respectively). The results indicate that there is a potential consumer exposure and environmental release, which might be enhanced over time due to coating and not embedding the CNT in the yarns. However, another group of authors have evaluated that the realistic direct exposure is too low to cause detrimental effects from CNT textiles.[205] However, environmental release and exposure could become important with waste-water as an important route.

Intermediate stages and finishing of products can result in both worker and environmental release. Bello et al. [13] reported an analysis of particle release during cutting in MWCNT-reinforced alumina (0.5 – 4.5% MWCNT) and epoxy nano-composites (0.05% MWCNT). The authors observed high particle release with PM_{10} concentrations up to 2.4 mg/m^3 (15.3e4 particles/cm^3 air) during dry-cutting Alumina CNT nanocomposite. However, in both cases the airborne emission dropped below the detection limit using wet-cutting. Bello's work indicates that wet-cutting CNT composites mainly lead to cutting debris of coarser particles with embedded CNT. The main risk of environmental release from this process is consequently associated with the debris waste consisting of CNT-doped nanocomposite fragments.

Finally, grinding and sanding is another major activity associated with high dust release potentials.[110,111,184,185] Cena and Peters [20] published results on sanding CNT-reinforced epoxy whereas Wohlleben et al. [238] studied the release from a CNT reinforced thermoplastic called polyoxymethlane (POM) and cement using taber abrasion, sanding, and UV-exposure, respectively.

The study by Cena and Peters showed the risk of airborne exposure to CNT and CNT-loaded composite debris.[20] They found that respirable dust concentrations increased with less than 3 μg/m^3 (5.9 times the background) during sanding for 15 to 30 minutes. Work in a costum hood with a 0.2 – 5.0 m/sec air-velocity increased the exposure level to 21.4 µg/m^3 whereas work in a biosafety cabinet reduced the personal exposure to 0.2 µg/m^3, which was 5.9 and 0.7 times the background concentration, respectively. Debris with protruding CNT, but no free CNT fibers, were observed in this study. A later study suggested that CNT may be liberated from a polymer matrix if the product is aged.[82,238]

The study by Wohlleben et al. [238] was focused on characterization of the release potentials in the three scenarios. Taber abrasion tests simulated both consumer and occupational use (e.g., abrasion by sliding an office chair; renovation and repair by sanding) and environmental release after long-term weathering by UV-irradiation without washing by rain. Here we focus on the sanding experiment, which showed release of particles with a wide size-distribution and a high number of 10-80 µm size particles. CNT was not found to be liberated from either of the two matrices. The high concentration of coarse particles in the cement study is partly due to sampling of all wear particles and release of refractory particles from the sanding paper. Only about 1 and 3 wt% of the particles passed a 2.7 µm filter for the thermoplastic and cement products, respectively. The absolute fine particle concentrations were 10^3 cm^{-1} and 10^4-10^6 cm^{-1} for the thermoplastic and cement products, respectively.

4.2.2 Exposure and release during consumer use and disposal

As mentioned above, there is very limited knowledge on the levels of consumer exposure to CNT and environmental release from products and articles. Naturally, the exposure potential is strongly depending on the type of product and there is a major gap in knowledge on the in-use and long-term performance of the wide range of CNT-based materials. In the assessment made by Gottschalk et al. (Table 4-1), most of the products, by far, went to landfill and incineration where the release is somewhat controlled.[64] Human exposure and degradation of CNT nanocomposites were not explicitly assessed.

From logical thinking one would expect high release and exposure potentials when CNT is applied to, bound or embedded in surface treatments. No or very low release and consumer exposure potentials are expected when CNT are embedded in matrices. However, the release potentials depends on both the strength and durability of the composite matrix in the given environmental and use conditions. In that context, studies have shown that CNT-based epoxy and polymer products may undergo matrix alteration and liberate CNT. First matrix alteration results in formation of an enriched CNT crust (sometimes photo-oxidized) at the surface, which may protect the matrix for further alteration.[6,82,170] Friction and use of a product would however, be likely to result in removal of this layer and result in a possibility for human and/or environmental exposure. This type of alteration is probably most severe in the case of products and articles in direct contact with the environment such as composite materials for the transport sector, ship-coatings, windmills etc. The total environmental load, however, still strongly depends on the national waste handling strategies and consumer behavior.

Due to the nature of most of the consumer products identified in Chapter 3, the risk of direct consumer exposure to CNT at first sight generally appears to be very low; at least when the products are un-damaged and used as intended (Table 4-3). If direct application of epoxy paints etc. could be done by unprofessional end-users, this would be a case for concern. Similar concerns immediately arise for use or wear of CNT textiles, but the airborne release potential may not be sufficient to be any hazard for the consumer.

The risk of environmental release related to consumer use, range from low to possible. Based on qualitative evaluation of the likely availability of the CNT in the products, the highest exposure risks would be expected for products such as printed electronics, anti-fouling and epoxy paints, conductive textiles and heating mats where CNT occur in the surface layer of the product (Table 4-3). Köhler et al [109] evaluated that the use of CNT-based Li-ion batteries would normally not involve a serious risk of personal exposure, even-though rare battery fires and accidental damage should be considered. Yet, they considered that there is a risk of CNT residues in bottom ash or slag in waste incineration plants, which can result in both human exposure and environmental release.

TABLE 4-3

QUALITATIVE EXPOSURE RANKING FOR PRODUCTION, USE AND DISPOSAL OF CURRENT HIGH-VOLUME CNT-BASED PRODUCTS

Current Product Group	Workplace€	Consumer (intended use)	Environment		
			Production/Application €	Intended Use	Product Waste
Printed electronics (conductive ink)	powder, slurries, printing	Unlikely. Only possible if user can have direct contact	See Table 4-2	Unlikely. Only in case if direct contact with the surrounding environment	Release likely. Special waste handling strategy necessary iin recycling of electronics.
Anti-fouling coatings	powder, slurries, rolling spraying	Possible. Long-term degradation of coating	See Table 4-2	Possible. Weathering/ Abrasion	Release likely; Removal as paert of article (e.g. ship) waste
High-durability epoxy-paints	powder, slurries, rolling, spraying	Possible. Long-term degradation of paint	See Table 4-2	Possible. Weathering, abrasion, in-use damage	Release likely. Removal as paert of article (e.g. ship) waste
Li-ion batteries	Powder, packing, encasing	Unlikely. Enclosed in battery casing	See Table 4-2	Encased	Uncertainty if recycling strategy in place for CNT
Antistatic thermoplastics	Powder, slurries, extrusion	Possible. Damage and slow long-term wear	See Table 4-2	Possible. Abrasion, weathering, in-use damage	Release possibler to likely. Incineration may be incomplete, CNT eventually released in landfill.
Conductive textiles	Powder, slurries, coating/extrusion, weaving	Possible. Direct contact, long-term alteration	See Table 4-2	Possible. Weathering and in-use damage	Release possibler to likely. Incineration may be incomplete, CNT eventually released in landfill
Thin heating mats	Powder, slurries, coating, extrusion, weaving	Possible if direct contact, long-term alteration	See Table 4-2	Possible. Weathering	Release possibler to likely. Disposal may be difficult if used in construcvtions, pavement heating, and as part of other articles
High performance sporting goods	Powder, slurries, layer composite/ embedded	Possible. Damage, slow long-term wear	See Table 4-2	Possible. Weathering and in-use damage	Release possibler to likely. Incineration complete?, Landfill release CNT with time

Microscopy probes	In situ synthesis / handling	Unlikely. Filament encased	See Table 4-2	Unlikely. Filament encased	Release unlikely to possible

[c] All processes involved synthesis and recovery, which potentially result in exposure and environmental release.

Dermal consumer exposure may also be an issue. In the EU FP7 project NaNex, a theoretical worst-case assessment of the potential dermal consumer exposure from textiles has also been made using three different models.[145] Assuming daily use, full-body dressing in a textile with 20% CNT textile and release of 10% CNT, the dermal exposure was 1.7 10^2 to 8.3 10^2 mg/kg body weight/day depending on the model used. However, as the authors state, this assessment is very conservative considering worst case exposure.

If the end-of-use results in waste disposal by burial, all products are evaluated as being associated with high risk of environmental release and subsequent exposure. However, due to the amount of CNT in e.g., microscopy probes, the associated environmental release may not possess a true risk in reality. If incineration is used for waste treatment, one may expect complete or near-complete incineration of the CNT in most of the products. The incineration temperature in Danish municipal waste incinerators is on the order of 950°C and the duration in the incineration furnace may be on the order of hours. This prolonged combustion should ensure a relatively high degree of CNT combustion; even in durable composite materials. From thermogravimetric analysis, pure MWCNT combusts at temperatures up to ca. 650°C at oxidized conditions. However, this temperature may not be sufficient for all CNT materials. Studies have reported delayed and highly slowed combustion of certain polymer composites added CNT alone and in combination with organoclays.[14] The reason for this is not fully understood at this time.

However, incineration may not be suitable or ensured for all products. E.g., if heating mats are used for outdoor heating under tiles and asphalt they may be disposed of together with construction debris rather than for incineration. Heating mats and conductive textiles may also be used in car seats. Car seats, bumpers, fenders, door-handles etc. may not be separated from cars before the vehicles are disposed of, unless each article has been labeled to ensure proper disposal. Finally, incineration may not be complete due to encapsulation and protection of the CNT by the material matrices. In case of batteries, the Danish procedure ensures a high degree of Li-ion battery recycling, which should reduce the risk of environmental release to a minimum. However, it is unclear how the CNT is treated in this process and whether there is a specific waste handling or recycling procedure for the carbonaceous material in the batteries.

4.3 Concluding remarks

There are currently no experimental data available on actual exposure levels to consumers and release to the environment from consumer products. Scarce data are available on worker exposure, but the amounts of CNT in these studies have only been partially quantified in a few cases. Therefore, we made a qualitative risk assessment from the scarce knowledge about worker exposures and process-related emissions supplemented with identification of some potential release situations during CNT and product manufacturing.

We conclude that there is a risk of workplace exposure and potential environmental release associated with the CNT production and manufacturing of all CNT-based products. Manufacture of most products; involve the use of probably grams to kilograms of CNT powder or slurries per work cycle. In some products the CNT is applied to or bound or embedded in surface treatments. We demonstrated that the handling of powders and slurries and spraying CNT is certainly associated with risk of exposure above.

Consumer exposure seems possible in some cases. However, the exposure levels, duration and frequency are thought to be relatively low. Low level chronic or brief accidental exposure may occur during use of CNT-bearing textiles, printed electronics, anti-fouling and epoxy paints, as well as heating mats. Accidental rare exposures may also occur in general when products are damaged. These products could include CNT-based sports equipment, Li-ion batteries, touch screens, composites for automotive sector.

Environmental release and exposure appears to be very likely in connection with production, some uses, but is inevitable during discard of waste and spills. It is important to gather knowledge on the exact life-cycle and fate of CNT and CNT products and their stability in the environment. In this regard, discard of thermoplastics, epoxy, antistatic coatings, Li-ion batteries, and other CNT-based nanocomposites, which often are part of an article, may be responsible for a larger release to the environment than anticipated hitherto. Published modelling results suggests about 0.6% of the CNT is released into the atmosphere as CNT dust and the rest is lost and discarded of following different direct or indirect (through a product) waste routes of which about 17% of the CNT is proposed to end up in a waste incineration plant.

Production waste will include dry waste due to residuals and CNT powders collected by dry and wet-wiping and filter bags from vacuum cleaning, as well as contaminated gloves and textiles. There will be liquid waste, which may be generated during multiple steps of purification, characterization, testing, functionalization, and their application. There will also be a large contribution from local exhaust and general ventilation filters in synthesis and CNT-based product manufacturing companies. Finally environmental release will also include CNT in various debris from modifying and finishing CNT nanocomposites, as well as debris or even direct release from material weathering.

Finally, there are large uncertainties in all of these exposure results and assessments made. Life-cycle modelling results suggests low regional concentrations of CNT in the environment. Occupational exposure studies and assessment of potential environmental release from production suggests that important release and contaminations may occur. Especially considering that there is currently no special regulation on production and use of CNT and that local high-concentration (hot-spots) is likely to form around sources, which is not considered in current scientific publications.

5. Human health effects of carbon nanotubes

The number of toxicological studies on CNT relevant for human health is currently increasing at fast pace and there are several very recent reviews on CNT toxicology. None of these reviews suggest that CNT exposure may cause serious acute toxicity.[4,33,34,101,118,159-161,174,197] However, potential long-term effects are apparent and some of the observations are of great concern.

It is currently our opinion that the hazards of CNT are best assessed from in vivo studies and that both inhalation, dermal and oral exposure should be considered. Therefore, this chapter has focused on studies of in vivo toxicological effects after ingestion, inhalation and dermal exposure to CNT. We have focused the overview based on apparent best key studies with prioritization on studies that have used commercially available CNT or CNT that have sufficient characterization of dimensions and functionalization. The results are described systematically starting with studies on biodistribution followed by pulmonary, cardiovascular, reprotoxicity and dermal effects.

5.1 Summary

The toxicological studies strongly indicate that exposure to certain CNTs may be associated with serious long-term health effects in test-animals. However, there are many different observations and the fate of CNT in biological systems as well as the toxicological mechanisms is far from fully understood. To summarize:

For assessment of oral uptake, a recent well-performed study with a low detection limit shows no ingested CNT beyond the GI tract. Thus, there is no evidence to suggest that CNT are taken up from the GI tract.

After lung exposure, pulmonary dosed CNT on the other hand is slowly cleared from the lungs after being phagocytosed by macrophages. Phagocytosis seems to occur relatively fast. The macrophages also transport the CNT into the subplural regions of the lungs, which is a prerequisite if CNT are to cause mesotheliomas. Half-lives of up to 300 days have been reported. There is evidence that CNT translocate from the lungs via the blood vessels to secondary organs. Thus, 1% of inhaled CNTs were found in the tracheobronchial lymph nodes in murine lungs after 1 day and 7% after 336 days, whereas 0.01% and 0.04% were localised in extra-pulmonary tissues after 1 day and after 336 days, respectively.

Once CNT reach the blood vessels, there is amble evidence that they will accumulate in Kupffer cells in the liver with a very low rate of elimination. Several different kinds of CNT have been observed in liver cells up to 1 year after exposure. In a quantitative study, 0.03% of the inhaled MWCNT localised to the liver after 336 days. In another quantitative study 0.75% of radioactively labelled MWCNT dosed by aspiration localised to the liver and 0.2% localised to the spleen after 1 year.

Regarding pulmonary toxicology, we have put weight on the two OECD guideline studies because they were long-term inhalation studies. In both studies of rats using two different commercially available MWCNT, subclinical symptoms of inflammation was observed at a concentration of 0.1 mg/m^3 which was the lowest tested dose. Both studies used MWCNT that form aggregates rather than being aerosolised as single CNT fibres. It is not known whether inhalation of MWCNT that do not form aggregates will have effects at lower concentrations.

Suppressed T-cell response was found by two independent groups using inhalation or instillation, respectively, of two different MWCNT. Moreover, increased expression of TGF-□ which initiates the immunesupression response was found both after inhalation of MWCNT and SWCNT.

Instillation of SWCNT induced single strand breaks in DNA in BAL cells after 24 hours. Oral dosing of the same SWCNT induced single strand breaks in DNA in liver cells. Mitsui MWCNT-7 MWCNT has caused mesotheliomas in rat and in a susceptible mouse model in a dose-dependent manner. Mitsui MWCNT-7 MWCNT are long MWCNT with a low Fe content. In contrast, SWCNT and a shorter MWCNT did not cause mesothelioma in rats. Thus, this evidence suggests that long and straight MWCNT like Mitsui MWCNT-7 may be carcinogenic.

There is evidence that pulmonary exposure to SWCNT in combination with a high-fat diet leads to plaque progression, and there is evidence that CNT present in the blood in high concentrations will promote platelet aggregation. It was recently shown that inhalation of TiO_2 caused a strong induction of the acute phase response. Acute phase response proteins in blood are risk factors for cardiovascular disease. It is not yet known whether pulmonary exposure to CNT results in induction of the acute phase response.

In reprotoxicological tests, were CNTs found not to accumulate in testis cells after IP injection of CNTs, and injection of CNTs had no effects on male fertility. The significance of the found results is highly questionable, since there is little evidence that CNT will enter circulation at all. However, the results indicate that even if CNT would enter the body, there are no indications of direct effects of CNT on male fertility. However, the indirect effects of pulmonary CNT exposure have not been assessed.

The dermal inflammation was observed after exposure to unpurified SWCNT, but not after exposure to purified SWCNT and commercially available MWCNT. This might be caused by the high levels of impurities in the unpurified SWCNT rather than the CNT.

As a general conclusion from all studies, there is still a far way to go before we will reach a good understanding of the mechanisms behind CNT toxicity and whether the different types and chemical derivatives have similar or different hazards. Currently several different exposure strategies and test systems have been applied on a relatively modest number of different CNT materials and the CNT types tested are mainly pristine SWCNT and MWCNT and a minor amount of various functionalizations thereof. The in-use CNT and derivatives made in the final production stage may be rarely disclosed.

5.2 Biodistribution

In this section, the translocation of CNT from primary deposition in airways, gastro-intrestinal tract and the blood-stream is reported.

5.2.1 Inhalation

Inhalation is a major exposure route for particles. It has been shown that inhaled carbon nanotubes has a very long pulmonary half-life (5-12 months) and thus persists in alveoli and lung tissue long after exposure.[45,71,167,181] Consistent with this, translocation to circulation may persist for months or years and extra pulmonary effects may increase or even arise long after exposure has ended.

Ryman Rasmussen and co-workers showed that inhaled MWCNT (Helix Material Solutions L: 0.5-40 µm, D: 10-50nm, 1 or 30 mg/m^3 for 6 hours) could translocate to the subpleural region of the lung. The nanotubes translocated rapidly (1 day) and were observed in the subpleura until the experiment ended 14 weeks after exposure. Nanotubes were observed both within macrophages, within subpleural mesenchymal cells and within the collagen matrix of the subpleura and a suggested mechanism were migration as engulfed cargo within macrophages.[71]

In line with this, it has been estimated that 0.6% of the pulmonary deposited dose following pharyngeal aspiration had translocated to the subpleura by day 1. The authors documented that MWCNT (Mitsui, XNRI MWNT-7, L:3.9 µm, D: 49 µm) penetrations of alveolar macrophages, the alveolar wall, and visceral pleura are both very frequent and sustained. They estimated that a pulmonary dose of 40 µg caused in excess of 20 million cellular penetrations throughout the lung and that 56 days post exposure 1 of 400 fibre penetrations will be in the subpleural or intrapleural space.[142]

Another study by the same group identified MWCNT (Mitsui, XNRI MWNT-7, L:3.9 µm, D: 49 µm) inside as well as protruding from alveolar macrophages (penetrations). One hour after aspiration, MWCNTs were engulfed by macrophages and by type 1 alveolar epithelial cells. At later time points, MWCNTs were not observed on the surface of epithelial cells but were located within the alveolar interstitium, interstitial cells or alveolar macrophages. MWCNT were detected within the pleura 56 days post pharyngeal aspiration.[175]

The findings strongly support the finding by Ryman-Rasmussen and co-workers [181], and demonstrate that MWCNT are transported from the alveolar air exposed lumen into the subpleural region of the lung. A recent review by Donaldson and co-workers argues that this migration into the intrapleural space is common for a fraction of all particles deposited in the distal lung. They additionally hypothesized a mechanism of particle clearance from the pleura. Particles follow lymph fluid through narrow holes (stomata) in the parietal pleura. However long fibres can not pass, and this will lead to retention causing inflammation and pleural pathology including mesothelioma.[34]

It should be underlined that above publications only focused on inter-pulmonary translocation, and made no attempts to measure a further translocation to extra pulmonary organs.

Extra pulmonary translocation has been addressed in a number of studies. The first was following instillation of pristine MWCNT (cont. 0.53 % Ni w/w). Rats were instilled with 1, 10, 100 µg/animal (Sigma, L: 0.5-2 L:3.9 µm, D: 49 µm D: 20-50 nm).[44] The lung-burden of MWCNT was assessed by ICP-OES of the associated Ni catalyst. From 63 to 78% of the instilled Ni was detected in the lung at days 1, 7 and 30. After 3 months, 38% of the Ni was detected in the lung and after 6 months, 16% was still detected in the lung. The detection limit was 0.1 µg MWCNT/g tissue. This allowed for the detection of Ni associated with the MWCNT product in the lungs, but would not be sensitive enough to detect a possible translocation to the liver, which would be expected to be in the order of promille. The authors also state that they would only be able to detect a translocation if this was above 2% of the doses given. However, they did detect MWCNT in lymph nodes by TEM, 7 days after rats were instilled with 2 mg/animal.[44]

In the second study, translocation of 10 µg taurine ($H_2NCH_2CH_2SO_3H$)-functionalised-MWCNT labelled with ^{14}C (L: 10-600 nm, D: 10-15 nm), was followed after instillation in mice.[31] The instilled ^{14}C-tau-MWCNT was only detected in the lungs, declining from 78% of the administered dose at day 1 to 20% at day 28. No ^{14}C activity was detected in blood or other key organs such as liver, spleen, kidneys and more at any of the studied time point. However, the specific activity of the dosed ^{14}C-tau-MWCNT was 2700 cpm/µg and 10 µg were dosed, thus the level of radioactive labelling was likely too low to detect translocation in the order of a few per mille which would be an expected level.

In a third study, mice were exposed to MWCNT (XNRI MWNT-7, L:4.3 µm, D: 49 µm), by inhalation 5 hr/day for 12 days to 5 mg/m3. Total lung burden was given as 28 ug/lung.[143]

MWCNTs were detected by enhanced dark field microscopy and quantified by counting fibres 1 day and 336 days post-exposure. After 1 day, 1.08% of the MWCNTs were localised to tracheobronchial lymph nodes and 0.0028% were localised to the liver. In total 0.009% localised to extra-pulmonary tissues. After 336 days, 7.3% of the MWCNTs were localised to tracheobronchial lymph nodes and 0.027% were localised to the liver. In total 0.037% localised to extra-pulmonary tissues. In both cases, the majority of the extra-pulmonary MWCNTs localised to the liver.

Czarny et al labelled MWCNT with ^{14}C during synthesis with very high specific activity, giving a detection limit of only 0.2 pg MWCNT.[26] In this study, 20 ug of radioactively labelled MWCNTs (3.9 um long and 40 nm in diameter) were dosed to mice by aspiration. After 1 year, 0.75% of the deposited dose was found in liver and 0.2% was found in the spleen.

Biodistribution may also be altered due to degradation and biodurability data are slowly emerging. In one study, SWCNT (origin not specified) were chemically cut.[96] The chemical cutting seems to be sonication in H_2SO_4 and H_2O_2 to create short SWCNT that are carboxylated at both ends. The chemically cut SWCNT could be degraded in vitro by a combination of human myeloperoxidase and H_2O_2. While aspiration of 40 µg/mice of the chemically cut SWCNT induced inflammation 24 hours after aspiration, degraded SWCNT did not cause inflammation. This strongly indicates that the extent to which the CNT are biodegradable is a major determinant of the inflammatory response.

5.2.2 Oral exposure to CNT

It is well known that most particles deposited in the pulmonary region are cleared via the mucociliary escalator into the gastrointestinal tract and excretion via faeces.[113] However, as particles pass from the lung and the larynx to the pharynx and the gastrointestinal tract there is a second chance for uptake and translocation through the intestine.

Wang and colleagues showed that small hydroxylated SWCNT (KOH-SWCNT, L: 300 nm, D: 1.4 nm) could easily pass through organs and compartments and basically moved freely and rapidly around the body despite a large molecular weight of 600,000 u. Three hours after oral administration of 1.5 µg the SWCNT tubes were detected in all twelve analysed organs with highest concentrations in stomach, kidney, lungs and bone and with lowest concentrations in brain, heart and muscle.[230]

Deng et al., studied distribution following orally dosed ^{14}C-tau-MWCNT (10 µg. L: 10-600 nm, D: 10-15 nm). These were transiently detected in stomach, small intestine and large intestine, ending up in faeces. Radioactivity passed downstream over time. About 74% of the exposed dose is directly excreted in faeces within 12 hours after-exposure. No labelling was detected in blood, indicating that ^{14}C-tau-MWCNT can neither be absorbed from the gastrointestinal tract nor, enter the blood circulation in detectable concentrations.[31]

Czarny et al labelled MWCNT with ^{14}C during synthesis with very high specific activity, giving a detection limit of only 0.2 pg MWCNT.[26] Mice were dosed 50 ug MWCNT (3.9 um long and 40 nm in diameter) by intraesophageal instillation. Radioactivity was assessed in faeces, liver and spleen. After 24 hr, 95%+/- 15% of the ingested MWCNTs were found in the gastrointestinal tract and feces. After 4 days, no radioactivity was found in liver and spleen. Thus, there was no evidence of uptake of the MWCNT from the gastrointestinal tract.

5.2.3 Biological distribution from blood.

Numerous experiments have been published regarding the bio-distribution of nanotubes entered in the blood stream. Traditional physiology would suggest that once particles are in the blood circulation, small particles can be excreted in the urine. This requires passage through the renal corpuscle with the 4-7 nm large perforations (fenestrations) of the endothelium. Larger particles would accumulate within the body, e.g. in Kupffer cells of the liver. Given the size of used CNT and their tendency to agglomerate, one would expect that CNTs could accumulate in the liver, specifically in the Kupffer cells.

5.2.3.1 Unmodified particles

^{13}C - enriched SWCNT (L: 2-3 µm, D: 10-30 nm) were injected in the tail vein and biodistribution was studied up to 28 days [243]. Very little excretion could be determined in urine and feces. Therefore, the pristine SWCNTs were suggested to mainly be distributed internally. ^{13}C was detected in a variety of organs, with highest concentrations in the liver (21, 18 and 21% of the injected dose, respectively after 1, 7 and 28 days), lung (15, 13 and 9% of injected dose, respectively) and spleen (1, 2 and 2% of the injected dose). In the liver, SWCNT were localised in Kupffer cells as shown by electron microscopy. SWCNTs were also detected in the lungs by TEM.[243]

In another study from the same group from Peking University [245], SWCNT (L: 2-3 µm , D: 10-30 nm) was injected i.v. in mice. SWCNT were detected by Raman spectroscopy, in lung, liver and spleen 90 days post injection. The intensity was strongest in the lung, and although the analysis was qualitative, the authors noted that the accumulated amounts in the lung were remarkable. SWCNT were also visible in light microscopy as black spots and by TEM in lysates of liver and lung from the high dose (1 mg/animal) animals after 90 days.[245]

Unmodified non-commercially available SWCNT (L: 300 nm, D: 1 nm) were injected i.v. into rabbits (75 µg/animal, 20 µg/kg) and the SWCNTs were detected using near-infrared fluorescence [24]. Injected SWCNTs were quickly covered with blood proteins and the blood concentration had a half-life of an hour. The experiment was terminated after 24 hours, and SWCNTs were clearly detected in the liver as the only organ.[24]

CNT may be considered as physically large entities, and the overall tendency that unmodified particles are not excreted but stored in liver tissue is thus expected. However, the reason for nanotubes to move from the blood stream to lung tissue as observed in one of the publications is unknown. A possible hypothesis could be formation of CNT agglomerates which can be caught in the many fine blood vessels of the lung.

5.2.3.2 Modified nanotubes (SWCNT and MWCNT)

CNT have been proposed as possible carriers for drugs, proteins, peptides and nucleic acids (for gene transfer or gene silencing), and as stable bio-molecules in diagnostic applications. For such, chemical modifications of the CNT surface may be necessary to direct its translocation. However, the modification may also change the natural clearance or storage mechanisms of the body, and thus give a completely different image of the distribution/clearance. Several groups have actively explored this area and several papers have been published.

Schipper and co-workers injected oxidized or non-oxidized non-covalently pegylated SWCNT (L: 100-300 µm, D: 1-5 nm, oxidised CNT: L: 0.05-0.2 µm) and determined by histology and Raman that nanotubes persisted within liver and spleen macrophages at the termination of the experiment 4 months post exposure without apparent toxicity. There were no changes in survival and clinical parameters of toxicity over the 4 months.[190]

Another study investigated ^{64}Cu-radio-labelled-DOTA-PEG-SWCNT (L: 100-300 nm, D: 1-5 nm). It was found that small and large PEG chains-SWCNT had blood circulation half-times of 0.5 and 2 hours, respectively. There was a prominent uptake in the liver and to a much lesser extent spleen and kidney. Larger PEG chain caused a slower blood clearance and lower concentration in liver.[131]

A third study using water-soluble and ^{111}In labelled SWCNT (L: 0.1-1 µm, D: 1.4 nm) for in vivo biodistribution found a fast blood clearance with only 3% and 0.4% injected dose/gram tissue left in the blood after 1 and 20 hours, respectively. Tissue analysis indicated that the major sites of ^{111}In

accumulation were the kidney followed by liver, spleen, and, to a much lesser extent, bone. ^{111}In cleared the kidneys more rapidly than the spleen and liver. Urine samples were collected from mice 1 hour post injection and some radioactivity was noted indicating a possible urine excretion.[141]

Carlos Villa and colleagues used SWCNT-^{111}In-(DOTA)-coupled to modified DNA oligonucleotides. The biodistribution was quantified using the radiolabelling. The results suggested a relatively fast clearance from the blood with 0.4% injected dose/gram tissue remaining at 24 hours. Nanotubes were mainly located in liver, kidney and spleen. Lower concentration was detected in seven additional organs. The organ radioactivity was relatively similar throughout the experiment (1 to 96 hours).[227]

Hydroxylated and ^{131}I labelled SWCNT (L: 0.05-0.2 µm, D: 1-3 nm) were used in a short term (2 minute to 1 hour) bio-distribution studies. Radioactivity was found all over the body (except brain) within 2 minutes. Kidneys, liver, bone, spleen, stomach and lungs were the major target organs in mentioned order. Content in heart, lungs, skin and muscle tissue was positively correlated with the content in the blood, illustrating clearly that the blood stream brings the modified SWCNT to the whole body. The CNT did not accumulate in these tissues. Blood half-life was about 4 and 50 minutes in the beginning and end of the experiment, respectively.[231]

Kang and co-workers showed blood clearance with a half-life of 3 to 4 hours, with a concomitant rapid uptake of high levels in liver of mice using 488F-chitosan-functionalized SWCNT (before functionalization L: 0.05-0.2 µm, D: 1-3 nm). The accumulated CNT in livers did not change during the 24 hours, and led to pathological changes such as macrophages injury, cellular swelling, and inflammation and blood coagulation. A similar uptake was observed in spleen and kidney; however, the authors did not observe obvious pathological changes in these organs.[99]

PEG1500N-functionalized acid-treated and thermally annealed SWCNT (L: 0.3-1 µm; D not reported) were administrated to mice in order to clarify distribution and biological defunctionalization using Raman and photoluminescence measurements. Only two organs were analyzed (liver/spleen) and SWCNT were detected at both 1 week, 4 weeks and 8 weeks post exposure. Additionally, results suggest that a de-functionalization of the amide linkages between the PEG occurred in the liver (4 weeks and 8 weeks) but not in the spleen.[244]

Using Raman spectroscopy of SWCNT non-covalently functionalized by three different lengths of PEG chains (2, 5 and 7 kDa) it was again shown that liver (30-65% injected dose/gram tissue) and spleen (19-30% injected dose/gram tissue) are the major sites of accumulation (24 hours) followed by bone, kidney, intestine, stomach and lung. This detection pattern suggests excretion and possible clearance via biliary and/or renal pathways. However the authors do suggest that the majority of CNT accumulate in the liver and renal excretion only occur for the shortest CNT. Six other tested organs were below detection limit. Blood circulation time, defined as the time until the blood SWCNT level had dropped to 5% of the injected dose/gram, was between 1 and 15 hours. The decay rate depended on the modification and size of PEG chains in such a way that functionalization with larger PEG chains increased circulation time. Additionally, the authors detected a reduced uptake in liver and spleen for largest PEG chain modified SWCNT compared with the small 2 kDa modified SWCNT.[133] The results were in line with a previous publication by the same group [132] in which a larger sized PEG chain caused a slower blood clearance and lower concentration in liver.

Wang and co-workers used hydroxylated SWCNT labelled with ^{125}I (L: 300 nm, D: 1.4 nm). The distribution pattern of the i.v. injected nanotubes mimicked that following the oral exposure described above. The nanotubes moved easily to all compartments and tissues of the body. Highest amounts detected in stomach, kidney, lungs and bone and very small amounts detected in brain. An analysis of the excretion over 11 days demonstrated that about 80% of the total injected radiolabel was collected within feces and urine (94% in urine and 6% in feces).[230]

Water-soluble, SWCNT (L: 0.3-1 µm, D: 1nm) and MWCNT (L: 0.5-2 µm, D: 20-30nm) functionalized with diethylentriaminepentaacetic (DTPA) and labelled with indium (^{111}In) were used for distribution and imaging analyses.[202] Results indicated that the CNT were not retained in organs such as liver and spleen but are cleared from blood circulation with a half-life of about 3.5 hours through the renal excretion route. No labeling was observed in the livers. Additionally, urine excretion studies of 400 µg DTPA-SWCNT and DTPA-MWCNT identified both nanotubes by transmission electron microscopy in the urine. In these analysis it was found that SWCNT primarily existed as bundles of 10-40 tubes (D: 13-40 nm) and the MWCNT primarily as individual tubes (D: 30-38 nm).

Diethylentriaminepentaacetic dianhydride (DTPA-MWNT) and ^{111}In-labelled MWCNT were tracked in rats (before functionalisation: L: 0.5-2 µm, D: 20-30 nm).[117] Imaging showed that within a minute the CNT began to accumulate in the kidneys and bladder. At 30 minutes most of the detected activity was in the kidneys/bladder. At 6 hours almost all CNT eliminated via renal excretion route. At 24 hours only residual levels were detected in liver, spleen as well as in the kidneys and bladder. Urinary excretion of the vast majority of radio-labelling (nanotubes) was confirmed at 24 hours, were it was shown that 11.5% of the injected dose per gram tissue was in the urine compared to liver, spleen, bladder and kidneys all being well below 1%. Kidney histology (24 hours) showed normal renal morphology without MWCNT accumulation. The rapid urinary clearance of these nanotubes is in contrast to the many other studies that reported rapid blood clearance profiles predominantly leading to hepatic accumulation.

The distribution of ^{14}C-tau-MWCNT (10 µg, L: 10-600 nm, D: 10-15 nm) has also been investigated following intravenous injection. Within 10 minutes ca. 80% of the exposed dose had accumulated in the liver. The detected values in liver then varied between 75 and 85% through the first month. After 90 days, 20% of the dose was still localized to the liver. TEM images clearly showed entrapment of MWCNT in Kupffer cells. Smaller amounts 1-5% was detected in spleen and lung. The results indicated elimination from lung tissue but a constant or slight build up in spleen during the 90 days.[31]

Taurine covalently functionalized and Tween-80 wrapped nanotubes labelled with ^{125}I (^{125}I-tau-MWCNT and ^{125}I-Tween-MWCNT, 10 µg) were dosed i.v. into mice.[32] After 5 minutes about 80% of ^{125}I-tau-MWCNT was in the liver and smaller amounts in spleen and temporary in the lung. Liver concentration remained high and ended at 75% after 6 hours. ^{125}I-Tween-MWCNT distribute to the liver, spleen and lung, but also into stomach, kidney, large and small intestine. There were indications that Tween-MWCNT partly evades liver capture, possible due to dispersion properties of Tween It should be noted that these result are very similar as those mentioned above by the same group using ^{14}C-tau-MWCNT.[31]

^{14}C-MWCNT (L: 3-4 µm, D: ca. 100 nm) was sonicated in pure rat serum until the formation of a stable suspension which was delivered i.v. in rats. The MWNTs were rapidly cleared from blood and distributed into mainly liver. Lower amounts were detected in lungs, spleen, and kidneys, whereas no radioactivity was detected in brain, heart, bones, stomach, and muscle (i.e. less than 10 pg in each organ). Optical microscopy revealed dark clusters in lung and liver that coincided with radioactive hot spots. Decreasing radioactivities were observed in all organs over the course of the experiment.[61]

5.2.4 Biological distribution following peritoneal deposition.

Intraperitoneal injection (i.p.) is an injection into the body cavity below the diaphragm. I.p. is rarely used in humans but is frequently used in veterinary medicine and in scientific experiments. A few papers have described the biological distribution of nanotubes following a peritoneal deposition.

Wang and co-workers used hydroxylated SWCNT labelled with ^{125}I. The distribution pattern of the i.p. injected nanotubes at 3 hours was similar to that observed following oral and i.v. exposure described above. Highest amounts detected in stomach, kidney, lungs and bone and very small amounts detected in brain. At 6 hours amounts were highest in stomach, kidney and bone. At 3 and 6 days nanotubes were only detected in kidney and bone. Very low radio-labelling was detected in all other organs.[230]

Hydroxylated and ^{131}I labelled SWCNT were used in a short term (2 minutes to 1 hour) bio-distribution. Radioactivity was found all over the body (except brain) within 2 minutes. Bone, kidney, stomach, blood, spleen, liver are in mentioned order the major target organs. Lower concentrations were detected in all other organs. Blood half-life was about 4 and 55 minutes in the beginning and end of the experiment, respectively.[231]

When using water-soluble MWCNT (L: tens of µm, D: 20-40 nm) functionalized with glucosamine and labeled with radioactive technetium (^{99m}Tc-MWNT-G) Guo and co-workers showed that blood half-time was 5.5 hours and that CNT moved easily throughout the compartments and tissues of the body. The major organs were the stomach and the enterogastric area. A subsequent excretion experiment (24 hours) showed that about 70% of the activity was collected in urine/feces. Results indicated that a vast majority of ^{99m}Tc was bound to MWNT-G in urine after 24 hours and that there was no free ^{99m}Tc.[68]

Unmodified Mitsui MWCNT XNRI-7 (1 mg/kg) (L: 3-4 µm, D: ca. 100 nm) were injected into the scrotum of male Fischer rats.[188] The scrotum of rats is in direct connection to the peritoneal cavity. MWCNT moved to the peritoneum but were also detected in portal macrophages and Kupffer cells in the liver and in mesenteric lymph nodes by TEM.

5.2.5 Biodistribution summary

One high-quality study found no uptake in spleen and liver following oral dose of 50 ug radioactively labelled MWCNT . The study had a very low detection limit of 0.2 pg MWCNT. Thus, there is no evidence of uptake of MWCNT from the gastro-intestinal tract.

There is evidence that CNT translocate from the lungs via the blood vessels to secondary organs. Thus, 1% of inhaled CNTs were found in the tracheobronchial lymph nodes in murine lungs after 1 day and 7% after 1 year, whereas 0.01% and 0.04% were localised in extra-pulmonary tissues after 1 day and 336 days, respectively. In another study using aspiration of MWCNT radioactively labelled with ^{14}C 0.75% of the deposited dose was found in liver and 0.2% in spleen 1 year after exposure. Thus, deposited CNT appears to be slowly cleared away from the lungs after being phagocytosed by macrophages. Phagocytosis seems to occur relatively fast. The macrophages also transport the CNT into the subplural regions of the lungs, which is a prerequisite if CNT are to cause mesothelioma [35]. Hence, studies with adequate sensitivity are required to disclose whether CNT translocate at the expected low rate. The possible ability CNT biodegradation is a critical end-point. One study has indicated that SWCNT may be biodegradable. Further experimental evidence is needed.

Within biodistribution of CNT most studies have focused on kinetics following intraveneous delivery. Additional, most of these studies used relatively short SWCNT with lengths ranging from 0.01 to 3 µm. If CNT does translocate, which may be important in case of long-term exposure, they will reach the blood stream. Once CNT reach the circulation, there is amble evidence that they are cleared from the blood-stream relatively fast. The vast majority of the abovementioned publications give the same impression that modified SWCNT have a blood half-life of a few minutes to hours. The primary target organs for accumulation are mainly liver and to a much lesser extent kidney and spleen. Some studies also report accumulation in lung (both pristine, hydroxylated, and some PEG-functionalized SWCNT; plus ^{125}I-tau- and ^{125}I-tween80-MWCNT) as well as other loci such as stomach and intestine, bladder, bone and very low trace amounts in the brain (several types of derivatives). In the liver, CNT will most likely accumulate in Kupffer cells with a very low rate of elimination. Several different kinds of CNT have been observed in liver cells up to one year after exposure.

The accumulation in kidney is interesting as it suggests excretion via urine. Some of the above publications suggest this excretion route as an option for their smallest nanotubes. Three publications found clear renal excretion and attempted to quantify this. All detected the nanotubes indirectly via radiolabelling (^{125}I and ^{111}In). One of the publications used short SWCNT (D:1.4nm, L:300nm) [230], another used both short SWCNT (D:1nm, L:300-1000nm) and longer MWCNT (D:20-30nm, L:500-2000nm).[202] whereas the last one used MWCNT (Before functionalization D: 20-30nm, L: 500-2000nm).[117] It is a surprising observation that the MWCNT and bundles of SWCNT detected by TEM in urine was of a size (D: 13-40 nm) that should not be able to pass glomerular filtration. A point also noted by others.[134] However it is possible that besides the CNT dimensions also the type of functionalization plays important roles on the CNT distribution and accumulation kinetics.

5.3 Toxicological effects of pulmonary exposure to CNT

5.3.1 Instillation of SWCNT

Rats were instilled with 1 or 5 mg/kg SWCNT (L: > 1 µm, D: 1.4 nm) and followed for 1 day, 1 week, 1 month or 3 months.[232] The SWCNT were non-commercially available from DuPont Central Research and contained 30-40% amorphous carbon (w/w), 5% Ni and 5% Co. Inflammation and tissue damage were detected 24 hours after instillation of 5 mg/kg, but not at the lower dose and not at later time points.

In one of the first studies of pulmonary response to CNT, mice were instilled with 100 or 500 µg/animal SWCNT corresponding to ca. 5 or 25 mg/kg.[119] Three different SWCNT were used, a raw unpurified SWCNT with 27% w/w Fe (donated by Rice University), the same partially purified to contain 2% Fe and a SWCNT (CarboLex, Inc) containing 26% w/w Ni. Five of nine mice died 4-7 days after instillation of the high dose of Ni-containing SWCNT, most likely as a result of toxic effects of the Ni. The rest of the animals were followed for 7 or 90 days. Inflammation was assessed by histopathological examination of lung tissue. Dose-dependent epithelioid granulomas and interstitial inflammation was seen at both 7 and 90 days.

5.3.2 Inhalation and aspiration exposure to SWCNT

Mice inhaled SWCNT (L: 0.1-1 µm, D: 0.8-1.2 nm) (Carbon Nanotechnology, CNI) which contained 18% w/w iron.[198] The mice were exposed by whole body inhalation to 5 mg/m^3 for 5 hour/day for 4 days and were killed after 1, 7 and 28 days. The mass mode aerodynamic diameter was about 4.2 µm, whereas the count mode aerodynamic diameter was about 240 nm. The particle number size-distribution was asymmetrical towards smaller particles. It was calculated that the deposited dose was 5 µg, assuming 0.5% deposition and a ventilation rate of 165 cm^3/min.

A strong inflammatory response was seen after 1 and 7 days and inflammation was still evident after 28 days. Inflammation was characterized by increased numbers of PMN and increased levels of cytokine *IL6* and *TNF* in bronchoalveolar lavage fluid. Changes in all examined markers of oxidative stress were observed, including decreased glutathione (GSH), increased protein thiols and malondialdehyde. Furthermore, collagen deposition and pulmonary fibrosis were observed at all investigated time points.

Aspiration of 5, 10 and 20 µg SWCNT yielded the same response in a dose- and time dependent manner. When pulmonary responses to exposure by inhalation and aspiration were compared, stronger responses were observed for inhalation of the same calculated mass dose. When the response to aspiration of unpurified SWCNT with very high iron content (18% w/w) was compared to the response to aspiration of highly purified SWCNT (also from Carbon Nanotechnology, CNI) [96,200], exposure to unpurified SWCNT resulted in more oxidative stress and stronger pulmonary response, including more release of LDH and higher levels of collagen deposition.

5.3.3 Instillation of MWCNT

Rats were instilled with 0.5, 2 or 5 mg non-commercially available MWCNT (Facultés Universitaires Notre-Dame de al Paix, Namur) dispersed in 0.9% Saline with 1% Tween 80.[155] The doses corresponded to ca. 2, 8 or 20 mg/kg and the animals were followed for 3, 15 or 60 days. Intact MWCNT contained 1% Co and ground (milled) MWCNT were administered intratracheally to rats. Instilled MWCNT were found to induce an inflammatory response that was more marked when ground MWCNT was used. However, intact MWCNT caused a stronger fibrotic response after 60 days. The stronger fibrotic response for the longer intact MWCNT is consistent with the fibre paradigm.

Low amounts of MWCNT (Sigma-Aldrich, L: 0.5-2 µm, D: 20-50 nm) (0.001, 0.01, and 0.1 mg/animal) were dispersed in BSA-saline mixture and instilled i.t. in rats corresponding to 0.005, 0.05 or 0.5 mg/kg). The treatment did not induce inflammation after 1, 7, 30, 90 or 180 days.[43]

5.3.4 Aspiration of MWCNT

Mice were exposed to MWCNT (MWCNT-7, Mitsui, L: 3.86 µm, D: 49 nm) by aspiration at 10, 20, 40 or 80 µg/animal and killed after 1, 7, 28 and 56 days.[175] MWCNT had a low content of Na (0.78%) and Fe (0.32%) and TEM pictures showed individual fibers that were 49 nm in diameter and a median length of 3.86 µm. Strong inflammatory responses were observed in a dose and time-dependent manner. Increased numbers of PNM were observed at all time points for 20 µg and above, whereas the number of PMNs had returned to background level for the 10 µg dosed animals at day 56. Lung damage was assessed by LDH leakage and the presence of albumin in BAL. These biomarkers were increased at all time points for all doses. Fibrosis was observed at 7 days post exposure and persisted throughout the experiment until day 56 days.

5.3.5 Inhalation exposure to MWCNT

Mice inhaled MWCNT (Baytubes, Bayer MaterialScience, L: 0.1-1 µm, D: 0.8-1.2 nm) by a single nose-only inhalation for 6 hours at 247 mg/m^3 or 11 mg/m^3 and were followed for 1, 4 or 13 weeks.[198] MWCNT were depleted for cobalt or not depleted. Based on the size of the inhaled particles, it was assumed that 6% of the inhaled mass was deposited in the alveolar region.

Extensive and long lasting inflammation evidenced by increased cell number in BAL, increased relative and absolute numbers of PMN, increased amounts of protein and LDH in BAL was found in mice exposed to 247 mg/m^3 MWCNT. The inflammatory response was strongest after 1 week and decreased over time. Much less inflammation was observed at 11 mg/m^3, but the pattern was the same. There was little difference in inflammation between MWCNT and cobalt depleted MWCNT suggesting that the observed effects were caused by the CNT rather than by the associated metal. Gene expression assessed by gene expression arrays revealed up-regulation of a number of genes involved in inflammatory response, including several cytokines and chemokines and complement factors. Furthermore genes involved in response to oxidative stress, general stress mechanical stress and cell injury were expressed at high levels in MWCNT exposed lungs.

5.3.6 Studies performed according to OECD guidelines

Two inhalation studies were performed according to OECD guidelines. Rats in groups of 50 were exposed by nose-only inhalation to 0, 0.1, 0.4, 1.5 or 6 mg/m^3 MWCNT (Baytubes, Bayer Material Science, 0.2-1 µm) for 6 hours/day for 13 weeks and were followed for 0, 4, 13 and 26 weeks.[167] The MWCNT formed agglomerates (described as coiled, tangled assemblages) with a median particle size by mass of 1.8 µm and by particle number of 0.9 µm. The individual tubes had a diameter of approximately 10 nm and an average length of 2-300 nm although fibres with lengths up to 1500 nm were observed. Cobalt associated with MWCNT was cleared away from the lungs with a half-life of 375 days for the highest dose. Inflammation was observed in an exposure dose-dependent manner. Increased numbers of PMN (neutrophilic granulocytes) were significantly increased at 0.4 mg/m^3 and higher doses. The number of macrophages with 'vacuolated or foamy appearance' was increased at 1.5 mg/m^3 and 6 mg/m^3. Increase in a number of toxicological endpoints was observed at some time points at 0.1 mg/m^3 including alkaline phosphotase activity, lactate dehydrogenase activity, protein and soluble collagen content in lung, and gamma-glutamyl transpeptidase activity. However, the authors conclude that 'all end-points examined were unremarkable at 0.1 mg/m^3, which is noted as the NOAEL (no-observed-adverse-effect-level).

Wistar rats were exposed to Nanocyl NC 7000 (Nanocyl S.A., L: 0.1-10 µm, D: 5-15 nm) MWCNT by head-nose inhalation 6 hours/day, 5 days/week for 13 weeks at concentrations of 0.1, 0.5 or 2 mg/m^3.[137] Characterization of the aerosolized particles indicated that MWCNT were aerosolized as agglomerates with an aerodynamic diameter between 0.7 and 2 µm. The total pulmonary dose was estimated to be 47, 243 and 1170 µg pr animal after the 13 weeks of exposure to 0.1, 0.5 and 2.5 mg/m^3 MWCNT assuming 10% deposition. Dose dependent pulmonary inflammation was found. At the lowest concentration of 0.1 mg/m^3, low levels of granulomatous inflammation were observed. The authors conclude that although these findings at low concentration are subclinical and unlikely to be associated with functional effects, they mean that a no-observed-effect-concentration (NOEC) could not be established; 0.1 mg/m^3 is the lowest-observed-effect-concentration (LOEC). Furthermore, no pronounced diffuse fibrosis was observed in exposed lungs. Conversely, alveolar lipoproteinosis was observed.

Thus, in both OECD guideline studies of rats using two different commercially available MWCNT, subclinical symptoms of inflammation was observed at a concentration of 0.1 mg/m^3. Both studies used MWCNT that form aggregates rather than being aerosolized as single CNT fibers.

5.4 Genotoxicity and Cancer

The key issue has been to clarify whether CNT have properties similar to asbestos. They are long, thin and biopersistent but are they able to cause mesothelioma and lung cancer after pulmonary exposure.

In an attempt to establish whether MWCNT cause chronic inflammation, the peritoneal (abdominal) cavity was used as a model for the pleural cavity where mesotheliomas develop in response to asbestos exposure. Four different MWCNT were studied; two that formed spherical aggregates in the respirable range (<5 µm) with and without protruding longer fibres (NanoLab inc), two MWCNT that were bundles, robes or singlets that formed long, thin fibres (Mitsui and a non-commercially available from Manchester University).[173] Mitsui MWCNT had a diameter of 85 nm and a mean length of 13 µm, the non-commercially available MWCNT had a diameter of 165 nm and a max length of 56 nm (no mean length given). IP injection of 50 µg of the two long MWCNT but not of the two shorter MWCNT products caused extensive inflammation after 24 hours measured as PMNs and total protein in lavage fluid, and after 7 days measured as the presence of foreign body giant cells and the formation of granuloma at the peritoneal surface of the diaphragm. This indicates that injection of long MWCNT fibres causes long lasting inflammation, whereas this is not observed using MWCNT that form respirable bundles.

In a study from a Japanese group, two different MWCNTs, one thick and long, MWCNT-L and one thin and shorter, MWCNT-S were dosed to rats by transtracheal intrapulmonary spraying.[241] The rats were dosed 13 times 125 ug/rat over a 24 week period. Lung inflammation and translocation to the plural cavity were assessed. MWCNT-S gave more pulmonary inflammation in terms of inflammatory cells and cytokine production in lung tissue. In contrast, only MWCNT-L was reported to translocate to the pleural cavity, deposit in the parietal pleura and induce fibrosis and patchy parietal mesothelial proliferation lesions. Thus, the results from this study agrees well with the previously mentioned work and suggest that only long and straight MWCNTs become trapped in the pleural cavity in a similar manner as has been observed for asbestos.

ApoE-/- mice were instilled with 54 µg/animal SWCNT (Thomas Swan et Co Ltd., L: < 1 µm, D: 1 nm) and DNA damage in BAL cells was assessed 3 hours after instillation.[89] Mice instilled with SWCNT had increased numbers of DNA single strand breaks, indicating the presence of DNA damage.

Rats exposed by gavage to 0.064 or 0.64 mg/kg SWCNT (Thomas Swan, L: < 1 µm, D: 1 nm) in saline or in corn oil had increased levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) in liver and lung tissue.[53]

Unmodified MWCNT (Mitsui XNRI-7, L: 3-4 µm, D: ca. 100 nm, 3 mg/animal corresponding to approximately 150 mg/kg) was injected i.p. in male p53 heterozygous (+/-) mice [212]. Additional groups were dosed with fullerenes, crocidolite asbestos and with vehicle solution. The mice were followed until one of the groups reached 100% mortality which occurred after 180 days. Mesothelioma incidence was 14/16 in the MWCNT group, 14/18 in the crocidolite group and none in the groups dosed with fullerenes or vehicle control. The study was criticized for using very high dose and for the lack of proper characterization of fibre size [85,213]. However, several papers have been published using Mitsui XNRI-7 as MWCNT with amble size characterization. In a follow-up study, where 10 times, 100 times and 1000 times lower doses of Mitsui XNRI-7 were used in the same experimental setup dose-dependent induction of mesothelioma was observed.[213,214]

Unmodified MWCNT (Mitsui XNRI-7, L: 3-4 µm, D: ca. 100 nm, 0.24 mg/rat, 1 mg/kg) were injected into the scrotum of male Fischer rats [188]. The MWCNT had a low content of Fe (3500 ppm), no Ni and were approximately90 nm in diameter and 2 µm long with 72 % being 1-4 µm long. Cancer incidence was evaluated after 52 weeks. 6/7 rats developed intraperitoneally disseminated mesothelioma before week 52 and 1/7 had mesothelial hyperplasia whereas 0/3 unexposed rats had any signs of malignancy.

Unmodified SWCNT (< 2 nm in diameter and 4-15 µm long) and MWCNT (10-30 nm in diameter and 1-2 µm long) (Shenzhen Nanotech) were encapsulated in hard gelatine capsules (10 mg) and implanted into the peritoneal envelope in groups of 6 rats weighing 400 g.[224] The negative control was capsules containing crystalline zink oxide. The rats were followed for 12 months. None of the rats developed mesothelioma or signs of malignancy. No positive control was included.

Unmodified non-commercially available MWCNT were heated to 2400°C under argon to decrease the level of structural defects. Both the treated and the untreated MWCNT had a diameter of 11 nm and were approximately 0.7 µm long. Rats were injected intraperiotneally with 2 or 20 mg/animal MWCNT with defects or 20 mg/animal MWCNT without defects.[154] 1/26 developed mesothelioma in the control group as compared to 2/50 and 0/50 in the 2 and 20 mg MWCNT with defects and 3/50 in the group injected with 20 mg/animal without defects. Thus, no statistically significant differences in cancer incidence were found. Mesotheliomas were found in 35% of the positive control group receiving 2 mg/animal crocidolite asbestos.

In summary, instillation of SWCNT induced single strand breaks in DNA in BAL cells after 24 hours. Oral dosing of the same SWCNT induced pre-mutagenic lesions in DNA in liver and lung tissue in a dose-dependent manner. MWCNT (Mitsui XNRI-7) has caused mesotheliomas in rat and in a susceptible mouse model. Mitsui XNRI-7 is considered relatively long MWCNT with a low Fe content. In contrast, SWCNT and a shorter MWCNT did not cause mesothelioma in rats. This indicates that there is evidence that long and straight MWCNTs as represented by Mitsui XNRI-7 may be carcinogenic, whereas there is no evidence that shorter and/or more entangled MWCNT are carcinogenic.

5.5 Cardiovascular effects of CNT

5.5.1 Pulmonary exposure to CNT

Transgenic mice expressing luciferase under control of the heme oxygenase promoter and ApoE knockout mice were exposed to 10 or 40 µg/mouse SWCNT (CNI, Houston, Texas) by intrapharyngeal aspiration and followed for 7, 28 or 60 days.[124] 60% increased plaque-formation in the aorta was observed in ApoE knock-out mice fed with a western-type diet exposed to four times 20 µg CNT/mouse over 8 weeks. In mice fed normal diet, plaque formation was too limited to allow quantification.

Female mice were exposed by oropharyngeal aspiration to 10 or 40 µg/animal SWCNT (Sigma) or acid functionalised SWCNT.[222] Cardiac function was assessed on isolated perfused hearts 24 hours after exposure. Hearts from mice exposed to 40 µg/animal acid-functionalised SWCNT had lower cardiac functional recovery, greater infarct size and higher coronary flow rate than controls. No effects were observed for hearts of mice exposed to unmodified SWCNT.

Saber et al showed that intratracheal instillation of Mitsui XNRI-7 and two different SWCNT as well as a number of nanoparticles and diesel exhaust particles all cause pulmonary acute phase response, leading to systemic circulation of acute phase proteins.[186] Acute phase proteins are well established risk factors for cardiovascular effects. The acute phase response induced by the MWCNT and SWCNT were strong and long-lasting, but the MWCNT induced a stronger acute phase response compared to SWCNT. The acute phase response (measured as Saa3 mRNA levels) correlated closely with neutrophil influx in bronchial lavage fluid. This indicates that inhalation of CNT may increase risk of cardiovascular through induction of a pulmonary acute phase response.

5.5.2 Systemic administration of CNT

Injected MWCNT and SWCNT (SES Research, Houston TX) at 20 µg/animal stimulated rat carotid thrombosis.[176] In vitro studies also demonstrated increased platelet aggregation. However, the chosen dose in both assays was very high in relation to assessing the effect of translocated particles on platelet aggregation.

Another group has essentially confirmed the results also using SWCNT from SES Research.[15] They reported increased platelet activation in vitro and decreased thrombosis time in small mesenteric arteries of mice injected with 0.1 mg/kg, and 1 mg/kg SWCNT.

In summary, there is evidence that pulmonary exposure to SWCNT in combination with a high-fat diet leads to plaque progression, and there is evidence that CNT present in the blood in high concentrations will promote platelet aggregation. It was recently shown that inhalation of TiO_2 caused a strong induction of the acute phase response [71]. Acute phase response proteins in blood are risk factors for cardiovascular disease [178]. It is not yet known whether pulmonary exposure to CNT results in induction of the acute phase response.

5.6 Immunotoxic effects of CNT

Male mice were exposed to MWCNT (Shenzhen Nanotech Port Co) by inhalation in whole-body exposure chamber for 6 hours/day for 7 or 14 consecutive days at 0.3, 1.0 and 5 mg/m^3 [146]. The aerodynamic particle diameter was 0.7-1.0 µm for 0.3 and 1.0 mg/m^3 and 1.8 µm for 5 mg/m^3. No lung inflammation was observed. In the spleen of mice exposed to 1.0 or 5 mg/m^3 for 14 days, increased expression of *IL10* and *NQO1* was found. No changes were found in the lung. Lison and Muller critizised that the used MWCNT was a nanorobe rather than a nanotube and proposed that this was the reason for the absence of inflammation [126]. Immunosupression was found in mice exposed for 14 days but in mice exposed for 7 days. Immunosuppression included lowered T-cell dependent antibody response to sheep erythrocytes, lowered T-cell profilerative ability in presence of mitogen and lowered natural killer cell function. B-cell response was not affected by treatment. The same group later demonstrated that immunosuppression was caused by activation of *TGF-α* in macrophages in the lungs, which in turn, caused transcription activation of *COX-2* and subsequent prostaglandin synthesis in spleen cells [147]. *COX-2* knock-out mice did not exhibit immunesuppression, demonstrating a crucial role of prostaglandin synthesis in the immunesuppression.

Increased protein levels of *TGF* were also observed in BAL fluid 1, 7 and 28 days after inhalation exposure to 5 mg/m³ SWCNT (CNI, Houston, TX, L: 0.1-1 µm, D: 0.8-1.2 nm) 5 hours/day for 4 days and 1 and 7 days after aspiration of 10 µg/animal of the same SWCNT [198].

Instillation of 5, 20, or 50 mg/kg MWCNT (Sigma-Aldrich, L: 5-9 µm, D: 110-170 nm) resulted in strong and long-lasting lung inflammation and at the highest dose, decreased numbers of T-cells and natural killer cells in blood and spleen [163], thus confirming the previous results. Moreover, an increased number of B-cells were found in blood and spleen, and this was accompanied by increased IgE concentrations in BAL fluid and blood.

In summary, suppressed T-cell response was found by two independent groups using inhalation or instillation, respectively, of two different MWCNT. Moreover, increased expression of *TGF-α* which initiates the immunesupression response was found both after inhalation of MWCNT and SWCNT.

5.7 Reprotoxicological effects

MWCNT were carboxylated or amine-functionalised to obtain negatively or positively charged MWCNT [7]. Male mice were intra peritoneal injected with 5 mg/kg or 25 mg/kg dosed over 5 injections over 13 days. MWCNT were detected in testis using ^{64}Cu-labelled carboxylated MWCNT. Doses of 41, 61 and 151 ng/kg MWCNT were detected after 10 min, 1 and 24 h after a single injection, respectively. Thus the observed MWCNT concentration in testes was 100.000 times lower than would be expected if the distribution was evenly throughout the body. No effects were observed on fertility. Gestation and delivery of female mice mated with exposed males was also unchanged by exposure.

The results indicate that even if these CNT enter the body, there are no indications of direct effects of CNT on male fertility. However, the indirect effects of CNT exposure on testis are not assessed. There are indications that exposure to air pollution affects male fertility [206,246] but it is not known whether CNT have similar effects. This would be possible if the effects of air pollution are caused by induction of pulmonary inflammation

5.8 Dermal toxicity of CNT

Only few studies have addressed dermal toxicity of CNT. Unmodified SWCNT (CNI, Houston, TX) were either not purified or purified [156]. The unpurified SWCNT contained 30% w/w iron, whereas the purified SWCNT contained 0.23% iron. Hairless SKH-1 mice were exposed dermally to 40, 80 or 160 µg/mouse/day for 5 consecutive days and killed 24 hours after last exposure. Skin thickening, mast cell infiltration, collagen accumulation and oxidative stress in terms of increased expression of cytokines, increased myelperoxidase activity, gluthathione depletion and protein oxidation was observed. The purified SWCNT was only tested using in vitro systems, and the purified SWCNT was found to cause much less dermal toxicity using engineered human skin and epidermal cells in culture. This suggests that the very high iron content was the main cause of the observed extensive dermal toxicity but this was not specifically addressed experimentally.

Dermal application of two different MWCNT (Sigma-Aldrich) caused no effects on rabbits [106]. No information was provided about the metal content of these MWCNT.

Finally, a dermal and eye irritation and skin sensitization study was made following OECD Guideline 405 and 406 using Male Kbl:NZW rabbits and male Slc:Hartley guinea pigs exposed to Nikkiso-SWCNTs (N-SWCNTs), Super-growth SWCNTs (SG-SWCNTs), Nikkiso-MWCNTs (N-MWCNTs), and Mitsui product of MWCNTs (MWNT-7), respectively [46]. N-SWCNT was 1.8 nm in diameter and had a BET surface area of 878 m^2/g, contained 43,700 ppm Fe, 56 ppm Rb, 22 ppm Zn, 12 ppm Ga, 10 ppm Cu, 9 ppm Ni, and 6 ppm Pb. SG-SWCNTs, had a mean diameter of 3.0 nm and a BET surface area of 1064 m^2/g, and contained 145 ppm Fe, 103 ppm Ni, 34 ppm Cr, 15 ppm Mn, 12 ppm Al. N-MWCNTs had a mean diameter of 44 nm and a BET surface area of 69 m^2/g, and contained 176 ppm Ga, 80 ppm Al, 53 ppm Fe, 16 ppm Cd, and 0.5 ppm Li. Mitsui MWNT-7 was found to have a mean diameter of 60 nm and a BET surface area of only 23 m^2/g, and contained 3600 ppm Fe, 14 ppm Cr, 6 ppm Bi, and 4 ppm Ni. The experiment involved analysis of erythema, eschar formation and edema formation at 1, 24, 48 and 72 h after 4-hour exposure to patches with 2 wt% (MWNT-7) and 1 wt% (all other) CNT dispersed in olive oil. The results showed that only N-MWCNT was a very weak acute irritant to the skin and eyes. None of the tested CNT exhibited skin-sensitization effects.

In summary, the observed dermal inflammation observed after exposure to unpurified SWCNT, but not after exposure to purified SWCNT and commercially available MWCNT, may be caused by the high iron content of the unpurified SWCNT rather than the CNT itself. However, other factors may be at play, because the role of Fe could not immediately be confirmed in an OECD study on two SWCNT and two MWCNT where only visual analyses were made.

5.9 Concluding remarks

Chapter 4 showed that inhalation of CNT is the expected major human exposure route of CNTs. Inhalation is also currently considered the potentially most hazardous human exposure to CNT. Dermal and oral exposure is also possible, but less focus and therefore less knowledge is currently available on these exposure routes.

Despite the fact that numerous studies have already been conducted to assess the toxicity of CNT, the information level on the tested CNT is generally still insufficient to enable a mechanistic understanding. Extensive characterization of CNT is especially important considering the complexity of this material class, which has been discussed in great detail in Chapter 2. However, some important preliminary conclusions may already be drawn at this point:

In a well-designed study with low detection limit, orally dosed CNT were not detected beyond the gastrointestinal tract. Thus, there is no evidence to suggest that CNT are taken up from the gastrointestinal tract. However, there is limited literature on the subject.

In lungs, there is evidence that pulmonary dosed CNT are slowly cleared from the lungs after being phagocytized by macrophages. Phagocytosis seems to occur relatively fast. The macrophages also transport the CNT into the subplural regions of the lungs, which is a prerequisite if CNT are to cause mesotheliomas. Only long and straight CNTs have been detected in the pleural cavity. Half-lives of CNT presence in lug of up to 300 days have been reported. There is evidence that CNT translocate from the lungs via the blood vessels to secondary organs. Thus, 1% of inhaled CNTs were found in the tracheobronchial lymph nodes in murine lungs after 1 day and 7% after 336 days, whereas 0.01% and 0.04% were localised in extra-pulmonary tissues after 1 day and after 336 days, respectively.

Once CNT reach the blood vessels, there is amble evidence that they will accumulate in Kupffer cells in the liver with a very low rate of elimination. Several different kinds of CNT have been observed in liver cells up to 1 year after exposure. In a quantitative study, 0.03% of the inhaled MWCNT localised to the liver after 1 year. In another study 0.75% of radioactively labelled MWCNT dosed by aspiration localised to the liver and 0.2% localised to the spleen.

In lungs, there is evidence that pulmonary dosed CNT are slowly cleared from the lungs after being phagocytized by macrophages. Phagocytosis seems to occur relatively fast. The macrophages also transport the CNT into the subplural regions of the lungs, which is a prerequisite if CNT are to cause mesotheliomas. Half-lives of up to 375 days have been reported. On the other hand, there is little evidence that CNT translocate from the lungs to the blood vessels, but the studies did not have sufficient sensitivity to detect a translocation from lung to blood in the order of promille, which would be the expected rate. So studies with adequate sensitivity are required to disclose whether CNT translocate at low rate. If we assume that CNT do translocate at low rate, they will reach the blood stream. Once CNT reach the blood vessels, there is amble evidence that they will accumulate in Kupffer cells in the liver with a very low rate of elimination. Several different kinds of CNT have been observed in liver cells up to 1 year after exposure.

After inhalation biological effects may occur at various doses depending on the types of CNT and type of administration. Most importantly, two OECD long-term inhalation guideline studies of two different MWCNT showed subclinical symptoms of inflammation at the lowest concentration tested (0.1 mg/m^3). Noteworthy, in this study, the MWCNT occurred in aggregates and agglomerates rather single CNT fibres. It is not known whether inhalation of MWCNT that do not form aggregates will have effects at lower concentrations.

Suppressed T-cell response was found by two independent groups using inhalation or instillation, respectively, of two different MWCNT. Moreover, increased expression of *TGF* which initiates the immunesupression response was found both after inhalation of MWCNT and SWCNT.

Instillation of SWCNT induced single strand breaks in DNA in BAL cells after 24 hours. Oral dosing of the same SWCNT induced pre mutagenic lesions in DNA in liver and lung tissue. MWCNT (Mitsui XNRI-7) has caused peritoneal mesotheliomas in rat and in a susceptible mouse model. Mitsui XNRI-7 consists of relatively long MWCNT with a low Fe content. In contrast, SWCNT and a shorter MWCNT did not cause mesothelioma in rats.

There is also evidence that pulmonary exposure to SWCNT in combination with a high-fat diet leads to plaque progression, and there is evidence that CNT present in the blood in high concentrations will promote platelet aggregation. Acute phase response proteins in blood are risk factors for cardiovascular disease. Pulmonary exposure to CNT has been shown to induce the acute phase response.

The results indicate that even if CNT enter the body, there is no evidence of direct effects of CNT on male fertility. However, the indirect effects of CNT exposure have not been assessed.

In one study dermal inflammation was observed after exposure to unpurified SWCNT, but not after exposure to purified SWCNT and commercially available MWCNT. Another study showed low-level visual skin-irritation in guinea-pigs after exposure to a MWCNT with low impurity concentrations. It has been suggested that skin irritation was related to high levels of impurities, but this is not confirmed from the presented studies.

To summarize, the major effect of CNTs irrespectively of type and surface-modifications is the induction of long-lasting pulmonary inflammation. Long-lasting inflammation is linked to both cancer and cardiovascular risk and is therefore an important toxicological endpoint. CNT length is major determinant of clearance and therefore of pulmonary inflammation. However, both OECD guideline sub-chronic inhalation studies which used relatively short CNTs both found pulmonary effect at the lowest examined dose of 0.1 mg/m^3. The effects of surface modifications etc. on pulmonary inflammation are not clear.

A second very important finding is the carcinogenic effects of some CNTs. The thick, long and straight Mitsui-7 MWCNT has been shown to induce cancer in a dose-dependent manner in a susceptible mouse model. There is therefore evidence that this specific type of MWCNT is mutagenic in rodents. There is also strong evidence that another MWCNT did not cause cancer in a two-year study in rats. Furthermore, the role of surface modifications in relation to induction of DNA damage has not been addressed adequately.

Future work should include testing of well-characterized CNT and analysis of the agglomeration behavior and fate of the CNT in the exposure mediums. As shown in Chapter 2, several types of CNT and CNT qualities exist and their different characteristics may play a role regarding their toxicological effects. Moreover, great effort should be made to improve the understanding of chemical and physical interactions between CNT, the mediums, nutrients, and tissue to establish a better mechanistic understanding of the potential toxicity of CNT.

6. Ecotoxicology

Numerous exposure pathways are possible for the uptake of engineered nanoparticles (including CNT) in the aquatic species.[9] For example, CNT could adhere to algae that may then be ingested by filter-feeders, possibly leading to transfer of nanoparticles to higher trophic levels, which in combination with the high biopersistence of CNT could trigger biomagnification. In the water column, significant sedimentation can be expected, and sediment will be an important sink for CNT (see Chapter 4). However, due to deposition of airborne CNT dust and sludge treatment life-cycle-assessments indicate that soil is the largest sink for CNT in the terrestrial environment [63-65]. These estimates are however uncertain. More detailed understanding on the CNT applications and their life cycle as well as more robust data on production volumes are needed, to give better predictions on environmental concentrations.

In this chapter, we summarize the most important knowledge presented in peer-reviewed scientific literature about carbon nanotube biodistribution and effects in aquatic and terrestrial organisms. The results are presented systematically focusing on experiments on biodistribution, aquatic and terrestic ecotoxicology.

6.1 Summary

As a start, the reader should be aware that is is not yet possible to make a general conclusion on the ecotixity of all CNT. This is in part due to the fact that different CNT and different dispersion protocols were used in the different ecotoxicity assays performed. Therefore the knowledge summarized are conclusions based on current knowledge and could change in the future.

There does not seem to be a passive uptake of CNT, but several studies provide examples of uptake of CNT by filter feeders or on the gills of fish where water is flowing through. Further, CNT can adhere to surfaces of organisms or agglomerate in the gut, and this way they induce several effects.

The toxicity of CNT seems to depend strongly on the stability of the dispersions in the test medium. Agglomerates have short residence time in the water column and rapidly settle out whereas highly dispersed CNT can stay in suspension for extensive lengths of time. Dispersed CNT induce acute effects in fresh water and marine organisms with an effect concentration of about 0.1 mg/L (EC50 96h mortality in fresh water crustacean *Daphnia magna*, inhibit growth at 96h LOEC of marine diatom *Thalassiosira pseudonania*, and EC50 inhibited growth of marine green algae *Dunaliella tertiolecta*) and therefore can be expected to induce chronic effects in water column at concentrations <0.1 mg/L. In the sediment, effects were observed at lowest effect concentrations above 30g/kg (mortality benthic amphipod *Leptocheirus plumulosus*).

Respiratory symptoms in rainbow trout *Oncorhynchus mykiss* at 0.1 mg/L were observed and the same concentration reduced population growth in marine copepod *Tigriopus japonicus*. Toxic effects in second generation were observed for injected CNT 2 ng/zebrafish embryos *Danio rerio*, but the significance of this finding is unknown since there are no indications that CNT are taken up from the water. When CNTs were dispersed in water, 120 mg/L exposure delayed hatching. Moreover, DWCNTs affected reproduction (cocoon production) of earth worm *Eisenia veneta* at concentration 37 mg/kg dry food. No systematic genotoxic effects were observed. Effects in sediment appear at high concentrations.

First enhanced growth and then inhibition have been observed for Mustard seeds and growth reduction for Zucchini *Cucurbita pepo* plants. The growth stimulation for the mustard seeds was LOEL 2.3 mg/L for oxidized MWCNT and 23 mg/L for pristine MWCNT, and growth inhibition LOEL 6.9 mg/L for oxidized MWCNT and 46 mg/L for pristine MWCNT. Interestingly, the pristine MWCNT were less toxic than pristine CNT to mustard plants.

Future work should include testing of well-characterized CNT and description of the state and fate of the CNT in the exposure mediums. There is great gaps from the knowledge on physicochemical properties and characteristics of CNT and the information typically given in (eco-)toxicological paper. Moreover, great effort should be made to improve the understanding of chemical and physical interactions between CNT, the mediums, food and nutrients, and other particles therein. These mechanisms may be playing a greater role on the ecotoxicological effects than previously anticipated.

6.2 Bioaccumulation

SWCNT and MWCNT (Shenzhen, China) were suspended in reconstituted water, shaken at room temperature and evaluated for 48-hour acute toxicity to common zooplankton *Daphnia magna* (OECD Guideline 202 with slight modifications).[250] *Daphnia* was observed to ingest the CNT.

Uptake and depuration of ^{14}C labelled CNT was studied in aquatic worms *Lumbriculus variegates* [169]. SWCNT and MWCNT (not commercially available) were dispersed by sonication and mixed with natural sediment at concentrations 0.003 or 0.03 mg/g dry sediment for SWCNT and 0.037 or 0.37 mg/g dry sediment for MWCNT. CNT did not absorb in *L. Variegates* tissues and worms purged all CNT after intake.

Similarly, infaunal lugworm *Arenicola marina* exposed to SWCNTs (Sigma–Aldrich #652512) for 10 days.[59] SWCNT were sonicated in ultrapure water and added to sediment/artificial sea water mixture at concentrations 0.003-0.03 g/kg. No uptake of SWCNT into tissues was observed, suggesting that these particles either remain in the sediment or pass through the gut and are excreted.

Amphibian species *Xenopus laevis* larvae at developmental stage 50 were exposed under standard conditions for 12 days to MWCNT (Graphistrength C100) or double-walled CNT (non-commercially available).[151-153] Both CNT were suspended in reconstituted water at nominal concentrations 0.1 to 50 or 100 mg/L respectively. CNT were detected in the lumen of the intestine, but not inside intestinal cells or in circulating blood of exposed amphibian larvae Xenopus laevis. This suggests that CNT do not cross the intestinal cells.

In another study, the fluorescent-labelled MWCNT (Nanostructured and Amorphous Materials), carboxylised and FITC-BSA-conjugated by amidation, were injected in 1-cell stage zebrafish *Danio rerio* embrios 2 ng/embryo at 1-cell stage and at 72 hours post fertilization through microinjection.[22] The injected FITC-BSA-MWCNT was allocated to all blastoderm cells of the embryos through proliferation, and was distinctively excluded from the yolk cell. When introduced into the circulation system, FITC-BSA-MWCNT moved easily in the compartments and finally was cleaned out by the body at 96 hours after the loading.

6.3 Aquatic toxicity

6.3.1 Fresh water species

SWCNT were assessed for ecotoxic effects on bacteria (Microtox test), algae (PAM test), crustacean (Chydotox test) and soil enzymes (Biolog test).[225] The SWCNT suspended in ultrapure water or natural pond-water was observed to form large particles that settled fast. No appreciable effects were observed at nominal concentrations up to 100 mg/L. The absence of toxic effects was assumed to be due to low (short-term) bioavailability of CNT in the water.

SWCNT and MWCNT (both from Shenzhen, China) were evaluated for 48-hour acute toxicity to common zooplankton *Daphnia magna* (OECD Guideline 202 with slight modifications).[250] CNT were suspended in reconstituted water, shaken at room temperature in final concentration 1000 mg/L. *D. magna* was exposed in water solution to concentrations ranging from 0.1 to 100 mg/L. The EC_{50} (effect concentration) was calculated to 1.3 and 8.7 mg/L, and LC_{50} (lethal concentration) was calculated to 2.4 and 22.8 mg/L for SWCNT and MWCNT respectively. For comparison, a study of Lysophosphatidylcholine-coated SWCNT showed 48-h mortality in *D. magna* at LC50 6.1 mg/L and 96-h mortality LC50 at 0.05 mg/L.[105]

In two studies, the variation in 48-hour acute *Ceriodaphnia dubia* mortality after exposure to MWCNT dispersed in NOM or functionalized MWCNT dispersed in water was studied.[102,103] The LOEC was 16 mg/L and 48 mg/L for the raw and hydroxylated-carboxylated MWCNT, respectively. However, alkylated and aminated MWCNT were more toxic to *C. dubia* as compared to the raw MWCNT, causing 48-hour increased mortality at LOEC 15 mg/L and 2 mg/L, respectively. Dispersion by stirring or sonication did not result in major changes in toxicity of the *C. dubia*

Juvenile rainbow trout *Oncorhynchus mykiss* was exposed for 10 days to SWCNT (Cheaptubes Inc.) at nominal concentrations 0.1, 0.25 and 0.5 mg/L.[204] SWCNT stock solutions were dispersed in solvent, sonicated and added to fresh water tanks under semi-static conditions. Gill irritation and increased mucus secretion was observed in the presence of SWCNT. All SWCNT fish had increased ventilation rates in a dose dependent manner. SWCNT were found on the surface of gills from SWCNT treated fish. SWCNT exposure caused significant increase in Na^+K^+-ATPase activity in the gills and intestine and significant increase in glutathione levels in gills and liver compared to controls. The authors conclude that SWCNT were respiratory toxicants in trout. No dose-response relationship was established.

Life cycle effects of MWCNT were assessed in zebrafish *Danio rerio*.[22] MWCNT (Nanostructured and Amorphous Materials), carboxylised and FITC-BSA-conjugated by amidation, were injected in 1-cell stage zebrafish embryos at 2 ng/embryo. The exposed fish developed normally and produced second generation later on. The survival of the second generation was lower compared to untreated groups. However, since there is little indication that CNT are internalized in fish from water, the significance of the findings are unknown.

Another study focused on zebrafish (Danio rerio) embryos (4 to 96-h post fertilization) exposed to SWCNT with 10% nickel and cobalt impurities dispersed into tap water (Cheng [23]. At 120 mg/L, the authors observed delayed hatching, but no effect was seen on embryonic development until 360 mg/L. In the same study, DWCNT dispersed in tap water delayed hatching at the LOEC of 240 mg/L.

Amphibian species *Xenopus laevis* larvae at stage 50 were exposed under standard conditions for 12 days to MWCNT (Graphistrength C100, France) or double-walled CNT (non-commercially available) both dispersed in reconstituted water. MWNT inhibited growth of *Xenopus* larvae starting at 50 mg/L.[152] Growth retardation and increased mortality was also observed for double-walled CNT in concentrations starting at 10 mg/L.[151,153] Toxicity was related to blockage of gills and/or digestive tract. Differences in effect concentration between the two CNT were assumed to be due size differences. Studies also evaluated the genotoxicity of CNT by micronucleus assay in *Xenopus* larvae erythrocytes. No genotoxicity was found for MWCNT in at concentrations of 0.1-50 mg/L [152] or for double-walled CNT in concentrations of 10-500 mg/L.[151,153] However, the chosen biomarker for genotoxicity (micronucleus) was challenged by the authors, since CNT elsewhere has been reported to be genotoxic.

MWCNT dispersed by stirring, sonication or sonication with organic material were assessed for the effects on benthic amphipod *Hyalella azteca*. In a whole-sediment assay the sonicated MWCNT induced *H. azteca* mortality at 300 g/kg.[102]

6.3.2 Marine species

Oxidised MWCNT (Cheap Tubes Inc,) were assessed for effects on growth and photosynthesis function of unicellular marine green algae *Dunaliella tertiolecta*.[235] MWCNT were oxidized to improve solubility and were suspended in artificial seawater. Oxidized MWCNT inhibited growth of *D. tertiolecta* with an EC_{50} (effect concentration) of 0.82 mg/L. No growth inhibition was observed when the MWCNT were filtered using 0.2 µm filtration, indicating that the MWCNT were present as agglomerates. At nominal concentration 10 mg/L significant oxidative stress and inhibition of photosynthesis was observed.

Acute and chronic toxicity of SWCNT was evaluated in full life-cycle assay with estuarine copepod *Amphiascus tenuiremis* (ASTM method E-2317-04).[218] SWCNT dispersed in sea water were tested “as prepared” (AP) or electrophoretically purified or as the fluorescent fraction nanocarbon synthetic byproducts. AP-SWCNT induced mortality, reduced reproduction and molting success at 10 mg/L. Purified SWCNT did not affect mortality, development or reproduction. Synthetic byproducts increased mortality and reduced molting at 10 mg/L.

A chronic toxicity of DWCNT (non-commercially available) were assessed in marine species diatom *Thalassiosira pseudonana*, copepod *Tigriopus japonicus* and medaka fish *Oryzias melastigma*.[116] DWCNT suspended in artificial seawater caused growth inhibition of *T. pseudonana* with an EC_{50} (effect concentration) of 1.86 mg/L, while stirred double-walled CNT had same effect first at EC_{50} (effect concentration) 22.7 mg/L. Sonicated double-walled CNT caused population growth inhibition of *T. japonicus* at nominal concentrations 0.1 mg/L, while stirred double-walled CNT reduced populations at 10 mg/L. Finally, sonicated DWCNT caused a growth inhibition of *O. melastigma* at 10 mg/L, while stirred DWCNT had effect above 10 mg/L.

MWCNT dispersed by stirring, sonication or sonication with organic material were assessed for effects on bentic marine amphipod *Leptocheirus plumulosus*. In a whole-sediment assay MWCNT treated by all dispersion protocols induced mortality of L. plumulosus at 30 g/kg.[102]

To assess the toxicity in estuarine environments, in faunal lugworm *Arenicola marina* was exposed to SWCNT (Sigma–Aldrich, #652512) using 10-day OECD/ASTM acute toxicity test.[59] SWCNT were sonicated in MilliQ water and added to sediment/artificial sea water mixture at concentrations 0.003-0.03 g/kg. No significant effects were seen for SWCNT up to 0.03 g/kg, possibly due to limited bioavailability at current test.

6.4 Terrestrial toxicity

Two soil bacteria models (*Escherichia coli,* sensitive strain and *Cupriavidus metallidurans,* non-sensitive strain) were exposed to raw and Fe-coated MWCNT (non-commercially available).[201] MWCNT were dispersed in ultrapure sterile water with solvent gum Arabic (0.25 wt.%), sonicated and bacteria were exposed to this liquid solution. Toxicity of both MWCNT was similar. MWCNT at concentration 100 mg/L were bactericidal to *E.coli*, while *C.metallidurans* was unaffected. MWCNT could eliminate sensitive environmental bacteria, while it could be mobilized and transported through environment via resistant strains.

The lethal and sub-lethal toxicity of DWCNTs was assessed in soil-dwelling earthworm *Eisenia veneta*.[192] DWCNT (SES research) were suspended in deionized water, steered in ultrasonic bath and added to dry food at concentration ranging from 50 – 495 mg/ kg dry food. Equivalent of 10 g of dry food was spiked directly to a hole in loamy sand soil every 7 days during 28 day experiment. DWCNT did not induce mortality or significantly reduce worm growth. However, reproduction was affected in concentrations above 37 mg/kg dry food (cocoon production). Hatchability, survival and mortality not affected.

SWCNT (70% purity, Ni:Y-catalyst, CSI) and MWCNT (Materials and Electrochemical Research Corporation, MER) were tested for toxicity in *Drosophila melanogaster*.[130] CNT were placed in solvent and sonicated, before they were stirred in the larval food, gels with CNT dose 0.1 and 1 mg/kg. Drosophila was exposed from egg to adulthood. CNT had no effect on egg hatching, general survival or fecundity, even though CNT were sequestered into *Drosophila* tissue. Adult flies were also exposed to CNT dry powder. Depending on the prior treatment, CNT could adhere to fly surfaces and overwhelm natural grooming, impair locomotor function and induce mortality.

MWCNT (not commercially available) were tested for plant toxicity with zucchini *Cucurbita pepo* grown in hydroponic solutions.[208] Bulk material solution was prepared at 1000 mg/L in 25% Hoagland solutions and sonicated. *C. pepo* germination and hydroponic biomass was assessed. MWCNT did not affect germination, root elongation, but reduced plant growth (15-day trial) by reducing biomass by 60%.

When tomato seeds were germinated in the presence of 10, 20 or 40 mg/L MWCNT dispersed in a growth medium. Seed germination and biomass production were enhanced by the presence of CNT and the authors suggested that CNT promote water transport inside the seeds by penetration of the seed coat or by the regulation of water channels.[104] This study and other studies with plants was perfomed as hydropinic culure and therefore describe exposure as mass per volume water and not mass per kg soil. The ecotoxicological data can be concerted by diluting 1 L mass dose into 1 m^2 of the soil (depth: 0.2 m agricultural and 0.03 m sediment and density = 2 g/cm^3).

In another study effects of 60% purity 30 nm MWCNTs obtained from Arry International (Germany) were studied on on mustard seeds.[148] Both as delivered and purified oxidized MWCNT were studied. Purification caused water-solubilization due to carboxylation and hydroxylation during purification. Important MWCNT modification including thinning to 20 nm, shortening and severe structural damage (amorphization) was evident after purification. Both MWCNT and oxidized MWCNT were dispersed in distilled water by ultrasound treatment and enhanced at 23 or 2.3 mg/L or inhibited at 46 or 6.9 mg/L germination and growth of the mustard seeds, respectively. It was suggested that the positive effect was due to increased water intake, while the higher dose was phytotoxic.

6.5 Concluding remarks

Ecotoxicological data on CNT is currently emerging with great pace. This review and others agree that due to a great lack of systematic testing, it is currently difficult to provide general conclusions in relation to the ecotoxicological effects of specific types of carbon nanotubes.[88,38] Moreover, it is not possible to generalize across studies, since different CNTs, preparatory modification of the CNT, as well as application of different dispersion protocols were used to assess ecotoxicity. It is increasingly recognized that some study results may also be related to artefacts and insufficient understanding of the test materials, contaminants, role of metals, as well as interference and interaction between the tests materials and the medium nutrients.[171]

There does not seem to be a passive uptake of CNT in the tested aquatic species, but several studies provide examples of uptake of CNT by filter feeders or on the gills of fish where water is flowing through. Further, CNT can adhere to surfaces of organisms, and this way they induce several effects.

The toxicity of CNT seems to depend strongly on the dispersion stability in the tested media, since agglomerates have short residence time in water columns and sediment. When CNTs remain suspended for longer durations of time, they induce acute effects in fresh water and marine organisms with an effect concentration of about 0.1 mg/L (EC50 96h mortality in fresh water crustacean *Daphnia magna*, inhibit growth at 96h LOEC of marine diatom *Thalassiosira pseudonania*, and EC50 inhibited growth of marine green algae *Dunaliella tertiolecta*) and therefore can be expected to induce chronic effects in the water column at concentrations <0.1 mg/L. In the sediment, effects were observed at lowest effect concentrations above 30g/kg (mortality benthic amphipod *Leptocheirus plumulosus*).

Respiratory symptoms in rainbow trout *Oncorhynchus mykiss* at 0.1 mg/L were observed and the same concentration reduced population growth in marine copepod *Tigriopus japonicus*.
Toxic effects in second generation were observed for injected CNT 2 ng/zebrafish embryos *Danio rerio*, but the significance of this finding is unknown since there are no indications that CNT are taken up from the water. When CNT were dispersed in water, 120 mg/L exposure delayed hatching.

No systematic genotoxic effects were observed.

DWCNTs affected reproduction (cocoon production) of earth worm *Eisenia veneta* at 37 mg/kg dry food. For plants, growth reduction for Zucchini *Cucurbita pepo* plants occurred first at 1000 mg/L., However, enhanced germination followed by inhibition was observed for Mustard seeds and The germination stimulation LOEL 2.3 mg/L for oxidized MWCNT and 23 mg/L for pristine MWCNT, and germination inhibition LOEL 6.9 mg/L for oxidized MWCNT and 46 mg/L for pristine MWCNT for the mustard seeds. Interestingly, the oxidized MWCNT were less toxic than pristine CNT to mustard seeds.

In conclusion, even though exotoxicologival data on CNT is rapidly emerging, large gaps are still present. Especially, the early studies had poor particle and exposure characterization, making it difficult to fully understand the presented toxic effects of the studied CNT. Now, there is general consensus that ecotoxicology studies should include nanomaterial characterization such as information on particle size, dissolution, surface area, surface charge, and surface composition/surface chemistry; unified protocols have to be followed to allow comparison of generated data; and reference materials should be developed. However, especially for CNT, the great complexity in physical state, atomic structures, and chemical composition and impurities should be considered to potentially establish links between properties and effects. Future work should also be aware of the interaction between CNT, nutrients and the mediums, which may be a very important factor controlling the observed effect to a greater degree than previously anticipated.

7. Provisional integrated risk assessment

As it has been presented in the previous chapters CNTs are highly inhomogeneous with several different structural, chemical and physical properties (Chapter 2). It is therefore not possible to perform risk assessment of CNTs as a group in the same way as for more homogeneous substance-groups with a higher level of knowledge.

In this chapter, we therefore first assess whether it is possible to establish a link between CNT characteristics and properties. Based on this analysis, we perform a preliminary hazard assessment based on current knowledge. Thereafter, we present an exposure assessment for the worker, the consumer and the environment based on published exposure data and simple model considerations. Finally, we the combine the findings into an integrated preliminary risk assessment for the major product groups identified in Chapter 3.

7.1 Summary

Evaluation of the observed hazard and the reported CNT properties in toxicological studies does not enable clear identification of certain CNT or properties which cause higher risk than others at this point. Yet, long and stiff CNT in general appears to have the ability to form serious pulmonary toxicity.

The toxicological studies can only enable a first generation of human exposure limits. Based on current experimental evidence, we derive an:

Occupational Exposure Limit = 1 $\mu g/m^3$ (as carbon or CNT)
Consumer exposure limit = 0.25 $\mu g/m^3$ (as CNT)

Dermal and oral exposure limits cannot be derived.

The risk of human exposure is evident in the working environment. Reported average exposure levels reach up to several hundred $\mu g/m^3$ over one to several hours. Despite being “whole dust” exposure, occupational exposure is potentially reaching critical levels during specific tasks and appropriate protection must be emplaced. CNT recovery and powder handling are the most critical processes for release of airborne CNT. The reported occupational exposure levels also cause risk of environmental release of concern

Assessment of consumer exposure risk is highly uncertain and no measurement data are currently available. To assess the risk associated with the identified major product groups, we therefore assessed the potential exposure risk and potential exposure levels based on different alternative procedures. First an assessment was simply based on the type of product and where the CNT was located in the product. Thereafter, we established a procedure where assessment of the release potential is done by rating using a Margin Of Nano Exposure (*MONE*) value and Product Exposure Impact (*PEI*) considering the location of the CNT, assumed the amount of CNT in the product and assumed number of product objects. Finally, we estimated the actual release potential and likelihood of exceeding our derived consumer inhalation exposure limit for 15 min in a simple twobox instant dispersion model assuming that all CNT at the surface of the product would be released over a period ranging from 1 second to 1 day.

The *MONE* values showed high release potential for several product groups including long-term degradation and expected in-use accidents (i.e. breaking a racket). However, the *PEI* values suggested that the impact of the potential consumer exposure currently is relatively low for the selected product groups. Due to anticipated number of object, Li-ion batteries have the highest *PEI* despite of a low, but still present, consumer exposure potential. The second and third-most important product groups are thermoplastics and printed electronics (layer) and the fourthmost are sporting goods (layer), respectively.

In conclusion, the lack of real exposure data and high variety of products within each product group makes it impossible to perform a fully reliable risk assessment for consumers. However, indications from our different approaches generally indicate possible consumer exposure, but the consumer risk generally appears low in the intended use-phase of un-altered products. However, if consumer use scenarios imply mechanical treatment of e.g, surface coatings and textiles with CNT, the derived exposure limts could be exceeded significantly. At least it could lead to acute and relatively high exposure.

There is currently no proposed concentration limits for CNT in the environment and ecotoxicological testing is incomplete. Additionally, different test item preparation methods give different results. The most sensitive aquatic species appear to be *Daphnia magna* (96-h mortality at 0.05 mg MWCNT/L) as well *Oncorhyncus mykiss* (0.1 mg SWCNT/L) suffering effect to the respiratory system, and *Tigriopus japanicus* (0.1 mg MWCNT/L). However, as we show below, the most sensitive species may not be the most critical when exposure is taken into consideration.

Published environmental model annual release and deposition concentrations estimated using a complete dispersion model was adjusted by a factor of 7 to comply with current production volume and material demand of 3200 to 3700 tonnes per year. The modelled concentration estimates do not exceed any of the critical exposure levels observed in the ecotoxicological studies used in this report. However, the model does not take hot spots into account. Hot spots would be highly relevant at production sites and at landfills. Accumulation in soil after distribution via sludge should also be of concern. Secondarily, the risk of local accumulation and hot spots in the fresh- and marine waters should also be of concern.

Evaluation of the most common products groups suggest that several products have the potential to cause environmental impact. As for consumer exposure, there is no measurement data available on CNT release and concentrations in the environment. However, the *MONE* values derived from the product characteristics and release potentials for consumers are also suitable for environmental risk assessment. From this assessment, layered printed electronics, coated textiles and heating mats, layered sporting goods and microscopy probes are those with highest release potential. The, second-rank group included most of the other products groups, such as Li-ion batteries, thermoplastics and nanocomposites with dispersed CNT.

Considering the possibility of matrix deterioration of composite products, the exposure level at contact of aged products is a worst case scenario. In such cases matrix degradation may offer a fully CNT coated surface. From this view-point, basically all products have the potential to offer a high concentration exposure in long-term use and land-fill waste discard.

7.2 Effects of CNT types and characteristics

As discussed in chapter 2, CNT are not homogenous materials. They vary in structural types and chirality, the number of tube-walls, tube homogeneity, impurity concentrations, defect types and concentration, covalent and non-covalent functionalization etc. We already mentioned the need for a more detailed systematic categorization system and proposed one based on physicochemical properties and characteristics in Appendix 3. But this system may not answer the needs from toxicological and regulatory points of view. Therefore, it is important to evaluate the toxicological literature for identification of particular characteristics that could indicate the hazard or safety of specific types of CNT.

Donaldson et al.[33], identified a number of potential CNT properties relevant for their pulmonary toxicology. The characteristics are summarized in Fig. 7-1. The properties can be grouped into four types of characteristics:

- CNT side-wall topology
- CNT characteristics
- Biodurability
- Reactivity

In addition to direct characteristics, the importance of CNT morphology (aggregation and agglomeration) was pointed out and may have strong influence on their air-way deposition, biological fate and the pathological mechanisms.

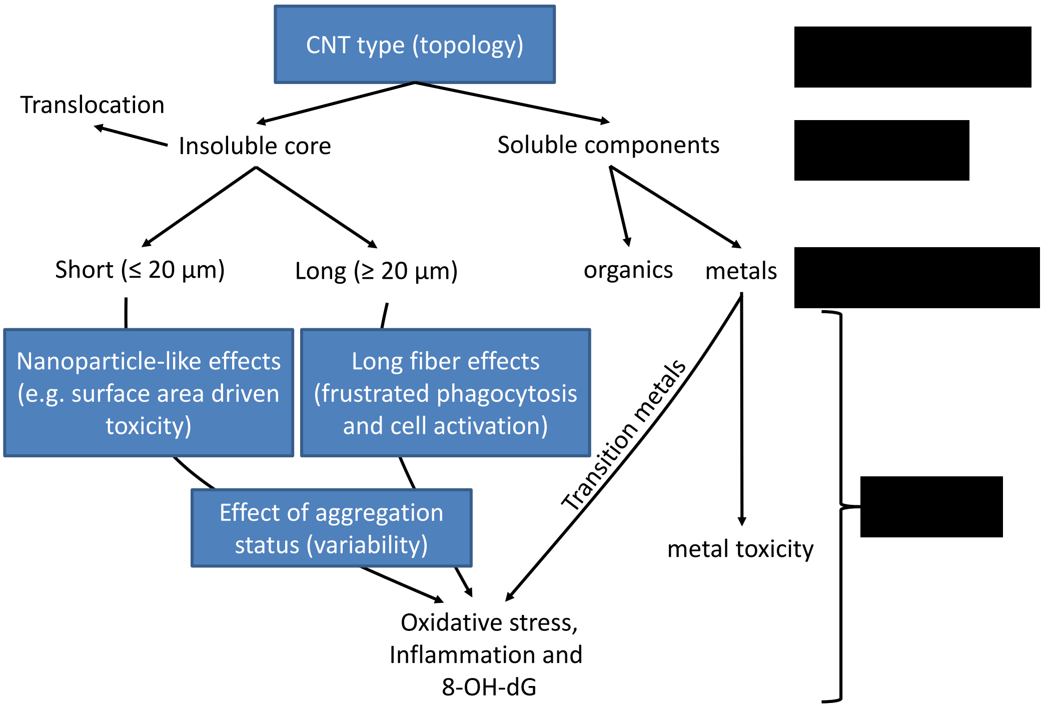


FIGURE 7-1

IMPORTANT PHYSICOCHEMICAL PROPERTIES AND PARAMETERS RELATED TO CNT TOXICOLOGY MODIFIED FROM DONALDSON ET AL.[33]

Below, it is attempted to evaluate the paradigm developed by Donaldson et al. [33] based on current toxicological studies. In this evaluation, we decided that conclusions could not be drawn on isolated studies. Based on the findings of Chapter 2, we demonstrate that when coating, labelling or purification for carbon allotropes or catalyst impurities, and annealing is done on a CNT nanomaterial, the characteristics and properties of the carbon nanotubes itself may also be affected in each of these steps.

7.2.1 Role of side-wall topology

Sidewall topology describes the CNT type by the number and atomic structure of the CNT walls. Except from some studies investigating thin and thick CNT or SWCNT vs. MWCNT, we did not indentify any systematic investigations addressing the role of the various side-wall topologies identified in Chapter 2.

7.2.2 Role of CNT diameter and length

SWCNT are thinner and more flexible than MWCNT. This can be expected to be a determinant of aerosolized particle size and thus pulmonary deposition. Rigid MWCNTs with many walls could be expected to behave more like asbestos fibers in air than thinner and more flexible tubes (see Figure 2-9). However there is limited scientific evidence to address the issue. The CNT length is an important parameter in relation to fiber-like hazard properties, in combination with rigidness of the CNT, since the asbestos-like effects are caused by frustrated phagocytosis when macrophages try to engulf fibres which are longer than the macrophage itself (approximately 15 μm).[33,34,173,174] However, the exact cut-off in size which causes frustrated phagocytosis has not been clearly established. On the other hand some evidence is emerging that rigid fibers may be hazardous by similar mechanisms as asbestos.[162]

7.2.3 Role of carbon impurities

Industrial CNT are usually reported to contain a fraction of carbon impurities, which may be amorphous carbon, fullerenes, graphite, and other CNT structures. Some of the impurities may be carbon black-like particles. The carbon black nanoparticle Printex 90 has been extensively studied as a model of both nanoparticles in general and of air pollution particles and is an efficient generator of reactive oxygen species in both acelluar systems and intracellularly.[90] Printex 90 induces DNA damage in vitro and in vivo [89,91,182] and mutations in vitro [91] which are likely caused by reactive oxygen species [229]. Test of a larger, but still nanoparticulate carbon black, Flammrüss 101 did not show any adverse effects at relevant in vivo doses.[184] In comparison to CNTs, Printex 90 appears to be ca. 10 times less potent in inducing lung toxicity in sub-chronic inhalation studies in rats.[137] Hence, as Printex 90 is one of the most toxic of the other carbon materials, a high content of carbon impurities would normally be required to substantially influence the toxicity of the CNTs. We therefore believe that carbon impurities play a minor role in the evaluation of CNT toxicity.

7.2.4 Role of catalyst impurities in CNT nanomaterials

It has been much debated in the scientific literature whether inflammation and other adverse effects mainly are caused by the CNT or by metal catalyst or other compounds. There are examples where e.g. lethal effects of instillation of CNT were ascribed to a very high content of Ni.[119] Moreover, when unpurified and purified SWCNT were compared, the inflammatory responses were noted by the authors to be higher in animals exposed to unpurified SWCNT with a high Fe (18%) content.[198] It has been demonstrated that degraded CNT do not cause inflammation, indicating that the CNT structure causes inflammation.[96] However, based on the reported findings, there is evidence that high levels of metal impurities in CNTs participates in the induction of inflammation. On the other hand, strong and long-lasting inflammation is observed after inhalation of relatively pure CNTs at relatively low concentrations. This indicates that pulmonary inflammation is an important toxicological effect following pulmonary exposure to CNTs.[137,167] Moreover, considering the topological consequences of chemical CNT purification, it is unclear whether the CNT and the effects would be comparable at any time between unpurified and purified CNT of the same batch.

7.2.5 Role of CNT agglomeration

Different CNT behave very differently when aerosolized or dispersed in various mediums. As reported in Chapter 4, most exposure analyses have reported low abundance of free single CNT in dusts from handling CNT powders, but quantitative data are still scarce. In dispersions, some CNT form large agglomerates with apparently very few single fibres present, whereas others seem to form a combination of agglomerates and single fibres, and yet others can be dominated by small agglomerates and single fibres. Since aggregate size and agglomeration determines the aerodynamic size of the inhaled CNT particle, these mechanisms are predictors of pulmonary dose, and therefore finally they will also control of inflammation. There is a strong need for systematic investigations of how different CNT behave in air and in suspensions of toxicological experiments, and furthermore knowledge on whether the toxicological properties of agglomerated CNT can predict the toxicological properties of matrix-bound CNT.

7.2.6 Findings of CNT-type and hazard link analysis

In brief, the analysis shows that a link between physico-chemical properties and biological effects cannot be established at this point in time. We find that no study has systematically tested the influence of specific isolated physico-chemical properties of a large suite of well-characterized CNT and their toxicological effects in vivo. There are only a few inhalation studies and the dust particles have not been characterized in sufficient detail to know what fraction of dust is of greater relevance if any. Liquid dispersion may result in highly dispersed CNT, but using liquid dispersion normally involves use of dispersants that may modify the surface reactivity of the CNT as compared to the pristine powder CNT. Consequently, except from some indications on the fiber-like properties, there is still great uncertainty on which characteristics of CNT that is linked to their toxicological properties.

7.3 Hazard evaluation for humans

Inhalation of CNT is expected to be the most critical exposure route of CNT for humans.:The major effect of CNTs irrespectively of type and surface-modifications is the induction of long-lasting pulmonary inflammation. Long-lasting inflammation is linked to both cancer and cardiovasucular risk and is therefore an important toxicological endpoint. In general, the major overarching determinant of lung inflammation appears to be the surface area of the deposited particles.[39] Consequently factors determining the reactivity and interaction with the surface area and the lung clearance rate will be determinants of the duration of pulmonary inflammation. CNT length is major determinant of clearance and therefore also of pulmonary inflammation. However, two OECD guideline sub-chronic inhalation studies which used relatively short CNTs both found pulmonary effect at the lowest examined dose of 0.1 mg/m^3. The effects of surface modifications on pulmonary inflammation are not clear.

A second very important finding is the carcinogenic effects of some CNTs. The thick, long and straight Mitsui-7 MWCNT has been shown to induce cancer in a dose-dependent manner in a susceptible mouse model. There is therefore evidence that this specific type of MWCNT is mutagenic in rodents. There is also strong evidence that another, more entangled MWCNT did not cause cancer in a two-year study in rats. These studies were of high quality, leading to het conclusion that CNTs differ in their carcinogenic potential. In addition, the role of surface modifications in relation to induction of DNA damage has not been addressed adequately.
This is a major issue, because numerous toxicological studies have used functionalized or coated CNT to enable exposure (e.g., instillation and aspiration) of CNT dispersed in a hydrous medium. We propose to derive exposure limits on the basis of pulmonary inflammation rather than the carcinogenic effect because this is the critical endpoint with the lowest threshold. In addition, the established link between pulmonary inflammation, pulmonary acute phase response and cardiovascular risk underscores the relevance of pulmonary inflammation as an adverse endpoint.

Below, we discuss our considerations about the doses and specific toxicological responses observed in published studies. Based on these results, we derive exposure limits for the human environmental and occupational exposure. The result is compared to recent published exposure limits using the same literature values.

7.3.1 Deposited dose

Comparisons between inhalation exposure and instillation or aspiration are strongly dependent on the assumed inhaled and deposited dose. When calculating the dose deposited in the airways (PM_{dose}), it is usually done as follows:

$$PM_{dose} = \frac{C_i \cdot V_{inhaled} \cdot D_{efficiency}}{hour},$$

Where C_i is the airborne mass-concentration, $V_{inhaled}$ is the inhaled volume per hour, and $D_{efficiency}$ is the deposition efficiency (deposited fraction) of the inhaled fraction. In this calculation it is assumed that all dust mass is inhaled and it is therefore imperative that the exposure is not dominated by large particles.

Different groups have used different inhalation volumes for mice. Schvedova et al.[198] used a mean ventilation rate of 165 cm^3/min corresponding to 9900 cm^3/hour (i.e. 9.9 L/hour), whereas Dybing and Sanner [40] recommend a standard value of 1.8 L/hour for mice. Our own experimental measurements have suggested that mice have a single inhalation volume of 0.15-0.2 ml, and breathe 250 times pr minute. This corresponds to 2.25 to 3 L/hour (Gunnar Damgaard Nielsen, personal communication). Hence, differences in used inhalation volumes and consequently deposited dose vary up to a factor of 5.5.

The deposition efficiency is of course a very important determinant of the deposited mass, and it depends strongly on the aerodynamic size of the inhaled particle. The assumed deposition fraction of CNT varied in the literature reviewed from 0.5% [198] to 30% [175] and will of course depend strongly on the observed aerodynamic size distribution of the studied which in turn depends on the degree of agglomeration. In some studies, the assumptions underlying the deposition fraction are not given [200]. Information on the size-distribution of the exposure and the model used to establish the deposition efficiency is extremely important and may be associated with large error. Only limited data exist for calculating the deposition efficiency of particles in mice.[247,89] Therefore direct quantification of lung-deposition is currently the most optimal.

7.3.2 Biopersistence

There are several indications that CNTs are removed very slowly from the lungs. Pauluhn used CNT-associated cobalt (Co) as a measure of CNT biopersistence in lung, and found that Co was cleared away from the lungs of rats with a half-life of 375 days for the highest dose (6 mg/m^3 6 hours/day 5 days/week for 13 weeks).[167] Similar estimates were reported for lower doses. One could argue that at the highest inhaled dose, overload occurred, leading to impaired clearance. However, as half-lives were similar at lower doses, we think that the half-life at the highest dose represents the best estimate, as there were most time points above the detection limit. Furthermore, if Co was loosely associated with CNT, Co clearance would be assumed to be faster than clearance of the much larger CNT, therefore, Co clearance should be a minimum estimate of the retention time. It is assumed that a small fraction of CNT will translocate from the lung to systemic circulation were they mainly deposit in the liver. Several studies have noted that internalised CNT appear to be removed very slowly from liver. Thus both Yang [245] and Schipper [190] detected CNT in mouse livers throughout their experiments (3 and 4 months, respectively). This indicates that CNT are cleared away very slowly, and this should be a strong concern in risk assessment. A recent study, however, has indicated that acid-treated and functionalized CNT may have a faster degradability than non-functionalized CNT.[164] This finding has yet not been confirmed in vivo.

7.3.3 Cancer

We did not identify any long term (over 2 years) cancer study that allows assessment of the carcinogenic effects of inhaled or ingested CNT. The National Toxicology Programme of the US EPA is currently planning a 2-year toxicity/carcinogenicity study in rats exposed to long (5-15 μm) MWCNT. Since short term animal studies suggest that exposure to some CNTs may introduce DNA damage or even mutations, such studies are urgently needed. Additionally, it should be mentioned that pulmonary exposure to CNT may cause fibrosis [119,175,198] and a linkage between fibrosis and lung cancer in general has been hypothesized.[194] The available long term cancer studies are diverging and all made using IP injected CNT. However, there is a tendency that short CNT (0.7 µm) do not cause peritoneal mesothelioma whereas the investigated longer tubes (<19 µm, mean=3-4 µm) cause cancer in a dose-dependent manner. The rigidness of the tubes may be an important predictor of carcinogenicity.

7.3.4 Exposure limits

A number of scientists have proposed exposure limits for CNT exposure.[4,107,166] A review by Aschberger and co-workers used two inhalation studies to estimate a LOAEC.[4] Both of these were GLP guideline studies (OECD TG 413), which are reliable and of high quality. When using inhalation studies it eliminates the differences and concerns regarding inhalation vs. instillation.[37] However, the concern regarding agglomerated vs. individual CNT-fibres needs to be addressed experimentally and due to great difficulties was not or at least not fully quantified in previous studies.

Aschberger et al. [4] propose the following strategy:

The lowest tested subchronic exposure in both studies was 0.1 mg/m^3 6 hours/day, 5 days/week, 13 weeks. One study defined this to be LOAEC (lowest observed adverse effect concentration) [137] and the other to be NOAEC (no observed adverse effect concentration).[167] In our opinion the lowest inhalation concentration in the study by Pauluhn (0.1 mg/m^3) should not have been defined as a NOAEC but as a LOAEC or as a LOEC (lowest observed effect level) since significant differences as well as marginal differences were observed. These two studies are therefore combined to one sub-chronic LOAEC for rats of 0.1 mg/m^3.

Previous experiments have shown that exposure duration is relevant. Rats were exposed 6 hours/day whereas consumers or the general public can be exposed to ambient air for 24 hours/day. Therefore a corrected LOAEC for a 0.1 mg/m^3, 24 hours/day, 5 days/week, 13 weeks is presumed to be:

$$0.1 \text{ mg/m}^3 \text{ x } 6 \text{ hours/24hours} = 0.025 \text{ mg/m}^3$$

In order to convert this into safe exposure levels for humans, a number of assessment factors are added. These are explicitly described in our following assessment:

Inhalation at 0.025 mg/m^3 is defined as LOAEC. An assessment factor of 2 is needed to derive a NAEC (no adverse effect concentration). Normally a standard default value of 3 is used but here this is reduced to 2 since the effects of both studies are determined to be minimal and the LOAEC or LOEC is therefore suggested to be close to the NOAEC.

An interspecies variation factor of 2.5 is used since it is not assumed that local pulmonary effects depend on specific metabolism or systemic absorption.

The default intraspecies variation factor of 10 is used to meet differences in susceptibility in the general public,including especially susceptible groups such as people with disease, children and elderly.

Extrapolation of the duration from sub-chronic (13 weeks) to chronic gives a factor of 2.

This totals into a safety factor of 100 (2 x 2.5 x 10 x 2). Hereby, the rat inhalation LOAEC of 0.1 mg/m^3 is converted to a suggested NEAC exposure limit of 0.25 µg/m^3 for the general public. The 0.25 µg/m^3 corresponds to 3.18 CNT per cm^3 (100 nm diameter; 5 µm long; density 2 g/cm^3).

In this risk assessment, the low clearance rate of CNT from lung (at least 5-12 months) and the possible carcinogenic effects of CNTs mentioned above are not taken into account. We would suggest that additional safety factors are added especially for the environmental exposure limit to take these factors into account.

In relation to the **occupational exposure limits (OEL)** the calculation would be as follows:

Rats were exposed 6 hours/day whereas workers are exposed to ambient air 8 hours/day. The corrected LOAEC is therefore:

$0.1 \text{ mg/m}^3 \times 6 \text{ hours/8hours} = 0.075 \text{ mg/m}^3$

Additionally a correction is performed to take into account differences in respiratory volume between animals at rest and workers at light activity:

$0.025 \text{ mg/m}^3 \times 6.7 \text{ m}^3/10\text{m}^3 = 0.05 \text{ mg/m}^3$

In order to convert this into safe exposure levels for occupational setting for humans, a number of assessment factors are added:

An assessment factor of 2 to derive a NAEC (no adverse effect concentration) from the LOAEC.

An interspecies variation factor of 2.5

An intraspecies variation factor of 5 instead of 10 for the general public. It is presumed that the intraspecies variation among workers is less than for general public.

Extrapolation of the duration from subchronic (13 weeks) to chronic gives a factor of 2. This totals a safety factor of 50 (2 x 2.5 x 5 x 2).

Thus the suggested rat LOAEC of 0.1 mg/m^3 (0.05 mg/m^3 light activity 8 hours/day) is converted to an occupational exposure limit of 1 µg/m^3. The current Danish TWA is 3.5 mg/m^3 for respirable carbon black, which is the OEL often referred to in Safety Data Sheets. It should be noted that if we had accepted 0.1 mg/m^3 as a NOAEL for rats as suggested by Pauluhn this would have meant the elimination of the first conversion factor and thus to an occupational exposure limit of 2 µg/m^3.

Pauluhn has suggested an occupational exposure limit of 50 µg/m^3 [166] based on his own OECD guideline inhalation experiment [167], which also forms the basis of the risk assessment above. Kobayashi et al. [107] previously, on a different material, suggested that an acceptable exposure concentration to be 210 µg/m^3 for working 8 hours/day 5 days a week.

Recently, NIOSH proposed a recommended exposure limit (REL) of 1 µg/m^3 carbon of CNT and carbon nanofibers in air as an 8-hour, time-weighted average, respirable mass concentration (http://www.cdc.gov/niosh/docs/2013-145/). The draft document states, " *The NIOSH REL is expected to reduce the risk for pulmonary inflammation and fibrosis. However, because of some residual risk at the REL and uncertainty concerning chronic health effects, including whether some types of CNTs may be carcinogenic, continued efforts should be made to reduce exposures as much as possible*".

We are in favour of the lower OEL of 1 µg/m^3 proposed by Aschberger et al [4] and the latest REL bulleting from NIOSH due to the long pulmonary retention times of CNT. Rapid developments in proposed occupational exposure limits have been observed since 2009 (Table 7.1). Increased knowledge is probably, the strongest driver for the decreasing proposed exposure limits with time.

TABLE 7-1

RELEVANT OCCUPATIONAL EXPOSURE LIMIT (OEL) FOR RESPIRABLE CNT COMPARED TO OELS SUGGESTED IN THE LITERATURE FOR CHRONIC EXPOSURE (8-HR/DAY, 5 DAY/WEEK),

Reference	Suggested limit values	Month-Year
Inhalable mineral dust	10.000 mg/m3	Danish OEL
Respirable mineral dust	5.000 mg/m3	Danish OEL
Respirable carbon black	3.500 mg/m3	Danish OEL
Respirable graphite (natural)	2.500 mg/m3	Danish OEL
Kobayashi et al. (2009)[107]	0.210 mg/m3	10-2009
Pauluhn (2010)[166]	0.050 mg/m3	01-2010
Aschberger et al. (2010)[4]	0.001 mg/m3	07-2010
NIOSH/CDC (2010) http://www.cdc.gov/niosh/updates/upd-12-02-10.html	0.007 mg/m3	12-2010
NIOSH/CDC (2013) http://www.cdc.gov/niosh/docs/2013-145/).	0.001 mg/m3 as elemental carbon	04-2013

7.4 Assessment of human exposure risk

Exposure data (measured or modelled) or alternative assessments of exposure are key parameters for identification of a true risk to health. In this section we conclude from Chapter 4 on the observed and assessed occupational and direct consumer exposure as well as the risk of environmental release. To enable risk assessment from consumer products where no measured data were found, we attempt to further assess the releative exposure risks that could arise for the specific product groups identified in Chapter 3. This was completed using a combination of previously used strategies for qualitative risk assessment [73,220,237], and quantitative release potential estimates for consumer products and evaluation of the general characteristics of the exposure.

7.4.1 Occupational exposure during synthesis and manufacturing

Chapter 4 demonstrated that there are several observations of exposure and hence a high risk of exposure to CNT in the working environment. Unfortunately, the precise characteristic of the CNT (type, dimensions, catalyst impurity concentrations etc) was rarely known. On the other hand, the hazard assessment above was not able to identify CNT types or state of the exposure, which clearly are more toxic than others, either at this point. Consequently, we allow ourselves not to discriminate between exposure levels observed for the different types of CNT if such information should have been given. The information is used for final integrated risk assessment in section 7.6.

7.4.1.1 Inhalation risk

CNT exposure is occasionally observed to be high during handling of CNT powders and dispersions. In these cases the airborne exposure appears to be dominated by aggregated or agglomerated CNT with a relatively low number of free CNT fibers, if any. Most studies have measured the mass concentration of total suspended dust and/or the particle number concentration of fine dust and they are rarely corrected or accompanied by simultaneous measurements of background concentrations (See Chapter 4). Therefore, the reported exposure concentrations may overestimate the true CNT exposure. On the other hand, some of the studies report the number of free CNT fibers in the air following the paradigm for asbestos counting. Yet, following the conclusions from the hazard assessment the fiber-paradigm may be insufficient to assess the risk of CNT dust.

The occupational exposure concentrations reported in the literature are when working with powder or suspended CNT as the only particle compound were:

CNT harvesting	0.7 to 286	µg TSP/m^3	[120,140]
Powder handling	36 to 212	µg TSP/m^3	[114,140]
Sonication	88 to 332	µg TSP/m^3	[72,120]
Spraying	31 to 193	µg TSP/m^3	[72]
Downstream use	0.15 to 2.92	µg EC/m^3	[29]

In a study where particle release was measured during cutting CNT-based nanocomposite of CNT-alumina and CNT-epoxy composites. This resulted in 800 to 2400 µg/m^3 airborne PM_{10} (particles smaller than 10 µm) if dry-cutting was applied.[13] The highest concentrations were found for CNT-epoxy composites, but the particle release was about half of the emission from cutting CNT-free epoxy composite. Wet-sawing reduced the airborne particle concentration to background levels (20 to 40 µg/m^3) (See Chapter 4). No free CNT was observed in the dust measurements. However, the risk of CNT exposure in connection with modification of CNT-based products will vary with the specific product and at this point we are not able to conclude from one product to the other.

No clear measurement data are currently available on processes such as purification and functionalization. It may be assumed that the exposure risk is comparable to that of handling of powders and sonication of dispersions. Other exposures may occur at both the professional and consumer down-stream user. These will vary with the products. In Table 4-2, we evaluated that professional end-user to generally have high risk of exposure. The risk of exposure may even be very high in case of application of CNT-based antifouling and surface epoxy paints as well as subsequent repair and removal.

7.4.1.2 Dermal exposure risk

It is evident that there is a risk of dermal contact in connection with handling of all powders and liquid dispersions as well as during cleaning and waste handling. However, dermal exposure has only been reported in one study by Maynard et al. [140] In this case workers harvested and poured powder CNT. By analysis of the gloves used in the work, the authors found a dermal concentration of up to 6 mg/hand. We assume that the handling reported in this study represent cases with most intense physical contact with powder CNT. In a more recent study from 2015, no dermal CNT exposure was detected on workers in a small CNT synthesis facility.[79] There is no data available for dermal exposure in connection with work with CNT dispersions.

7.4.1.3 Oral exposure risk

It has not been possible to identify any references reporting on the oral exposure risk to CNT. The risk of oral exposure is considered low. However, accidental oral exposure may occur in the workplace. It is critical that high hygiene standards are enforced in workplaces where handling of powders and dispersions occur. Oral exposure may also occur as a consequence of inhalation. Due to the apparent large size of the particle aggregates and agglomerates and small size of free CNT, upper air-way deposition may be significant as well as entry from particles cleared by the mucociliary escalator.

7.4.2 Consumer exposure to products

Clearly, the risk of consumer exposure depends on the type of product, the location of the CNT and the intended use. As described in Chapter 4, there are no in-use measurements of consumer exposure available. Therefore, consumer exposure can only be assessed qualitatively or inferred from occupational exposure data or release studies.

7.4.2.1 Inhalation

In table 4.2, we identified potential consumer exposure for several products such as conductive textiles, high performance sporting goods, and antistatic thermoplastics. We also found that continuous release of CNT may be related to natural long-term weathering and degradation of e.g., anti-fouling, high-durability epoxy paints and nanocomposite materials.[6,82,170,238]

Potential unprofessional mechanical removal of coatings and modification of nanocomposites by sanding, grinding, drilling and cutting may also lead to consumer exposure. Such emissions have been demonstrated by e.g., Koponen et al. [110,111], Göhler et al. [62], and Wohlleben et al. [238]. This type of exposure is expected to be limited to a few exposure incidents during a life-time of a consumer. Sanding debris from CNT-reinforced cement was, however, found to have similar toxicity as reference cement. Similar results have been observed in analysis if paint dust [183-185]. When aged and weathered, it is anticipated that CNT will be more readily released during sanding and in this case increase the hazard profile of similar products is prone to increase with time. On the other hand, real CNT composites might be protected by a coating of paint or lacquer. This might very well reduce the risk of e.g, UV-oxidation–related matrix deterioration to initially concern the "protective" coating.

7.4.2.2 Dermal

Skin contact is inevitable in case conductive textiles become part of regular clothing and may also occur in contact with altered or weathered surface coatings and nanocomposites as discussed above. The dermal consumer exposure risk and exposure levels are generally evaluated negligible to very low for the product types currently identified on the market. In a conservative assessment Micheletti et al. [145] calculated dermal exposure of $1.7\ 10^2$ to $8.3\ 10^2$ mg/kg body weight/day depending on the model used and assuming daily use, full-body dressing in a textile with 20% CNT textile and release of 10% CNT. Based on aging effects in different products, including textiles, it is our opinion, that at least long-term exposure should be considered. This would include touching surfaces of aged composites with available CNT, which may result in both dermal and accidental oral exposure.

7.4.2.3 Oral

Oral exposure is mainly expected to occur in connection with accidental uptake. The main possible pathways would be hand-to-mouth exposure, clearance from upper and thoracic airways. Another possibility is digestion of potentially contaminated food and beverages where CNT-based technology is or might be used for packaging and heating. The absolute potential exposure levels are difficult to ascertain, but should be considered as closely linked to both dermal contact and clearance of inhaled CNT.

7.4.2.4 Ranking of consumer inhalation exposure potential and impact

As inhalation is currently considered the most important route of CNT exposure, we wish to further evaluate the potential consumer exposure levels and rank these for possible prioritization. Before, we only assessed whether there could potentially be an exposure to CNT and ranked the products groups according to unlikely, possible, likely, depending on location of the CNT and type of material (product matrix). However, it is not possible to make a more detailed assessment of the consumer exposure using conventional methods, because in addition to the lack of exposure data, there is too great uncertainty or lack of knowledge on the duration and frequency of use as well as on the size of the consumer groups, which are used in traditional assessments. Moreover, in this report we wish to assess broad product groups. Therefore, we cannot use the e.g, NanoRiskCat categorization system developed by Hansen et al.[73,74], which is intended for specific products, which at best has detailed information.

To overcome the conceptual- and information knowledge-gaps, we established an alternative approach considering the exposure potential (product surface relaease potential for normal use (including normal use worst case "accidents"), the material characteristics (e.g., concentration of CNT, location of the CNT (matrix, surface, composite /functional layer, powder, dispersion) in combination with evaluation of the apparent durability of the matrix), and the suggested consumer exposure limit. From these data, we derive a Margin Of Nano-Exposure (MONE) inspired by the MOE (Margin Of Exposure) used by the US-EPA and the US Soap and Detergent Association.[220] The MONE value may be considered a risk categorization of potential exposure. For inhalation exposure the MONE is simply derived as:

$$MONE = \frac{EP \cdot IC}{CEL_{air}},$$

Where: CEL_{air} is our derived consumer exposure limit (0.25 µg/m^3), EP is the exposure potential (0 when unlikely, 0.05 when possible, 0.25 when likely) of CNT from the product and IC is the ingredient concentration (wt%) at the point of highest concentration in the product (IC_{max}) and at the surface ($IC_{surface}$). EP is based on the availability of the CNT in the product according to the location as mentioned above.

The results from the MONE calculations are listed in Table 7-2 and shows that five consumer product groups are associated with maximum worst case potential for exposure. These are printed electronics with conductive layers, coated conductive textiles, coated heating mats, sporting goods with layered CNT composite, and the reference powder CNT. However, in normal consumer user scenarios, only three of the products (conductive textiles, heating mats, microscopy probes) are associated with a high MONE value. The additional products, which appear in the worst case consumer use scenarios, are due to considerations of accidental contact to layered composites and condictive inks used in electronics. These incidents are probably mostly hypothetical consumer exposures.

To further attempt to set the exposure assessment into perspective, the *MONE*-values are subsequently used to assess the Product Exposure Impact (*PEI*). In this assessment, we consider the total amount of CNT (TOT_{CNT}) in the product and the number of items / people exposed (N; proposed or estimated) and *PEI* for the total Danish population handling 1 kg of CNT powder once during their life-time as a maximum risk reference. The *PEI* is simply derived as described below, where TOT_{CNT} is categorized into four arbitrary scales: 1 for µg, 10 for mg, 100 for g; 1000 for kg CNT in the product:

$$PEI = \frac{MONE \cdot TOT_{CNT} \cdot N}{PEI_{CNT_{powder}}}$$

Critical for this assessment is knowledge of the product size and the number of people in the consumer group or number of items present. Table 7-2 lists suggested values, but it should be emphasized that these values are best guesses and highly uncertain.

The calculated PEI values are plotted in Figure 7-2 together with the MONE-values for comparison. The result from the PEI assessments suggests that the impact of the potential consumer exposure currently is relatively low for the selected product groups. Due to the number of estimated items, Li-ion batteries have the highest PEI despite of a low, but still present, consumer exposure potential. The second and third-most important product groups are thermoplastics and printed electronics (layer) and the fourthmost are sporting goods (layer), respectively. As seen the estimated impact levels are from a promille to a few percent levels as compared to the PEI of consumers handling 1 kg of CNT powder once.

TABLE 7-2

INPUT PARAMETERS AND MONE VALUES FOR LISTED PRODUCT GROUPS

Product	EP	IC_{max}	$IC_{surface}$	$MONE_{max}$	$MONE_{surface}$	Product size	N€
Printed electronics (layer)	0.25	100	0	100	-	1	3,000,000
Printed electronics (dispersed)	-	1	-	-	-	1	3,000,000
Anti-fouling coatings	0.05	1	1	0.2	0.2	1000	100
high-durability epoxy paints	0.05	1	1	0.2	0.2	1000	100
Li-ion batteries (product)	0.25	5	0	5.0	-	100	25,000,000
Thermoplast and nanocomposites	0.05	5	5	1.0	1	100	5,000,000
Conductive textiles (coated)	0.25	100	100	100	100	100	10
Conductive textiles (dispersed)	0.05	5	5	1	1	100	10
Heating mats (coated)	0.25	100	100	100	100	100	10
Heating mats (dispersed)	0.05	5	5	1	1.0	100	10
Sporting goods (layer)	0.25	100	0	100	-	10	10,000
Sporting goods (dispersed)	0.05	5	1	1	0.2	10	10,000
Microscopy probes	0.25	100	100	100	100	1	10
CNT powder (DK pop. at CEL)$	0.25	100	100	100	100	1000	5,543,453

€ Proposed or estimated number of items/exposed for impact assessment; $ Data used for normalization of impact.

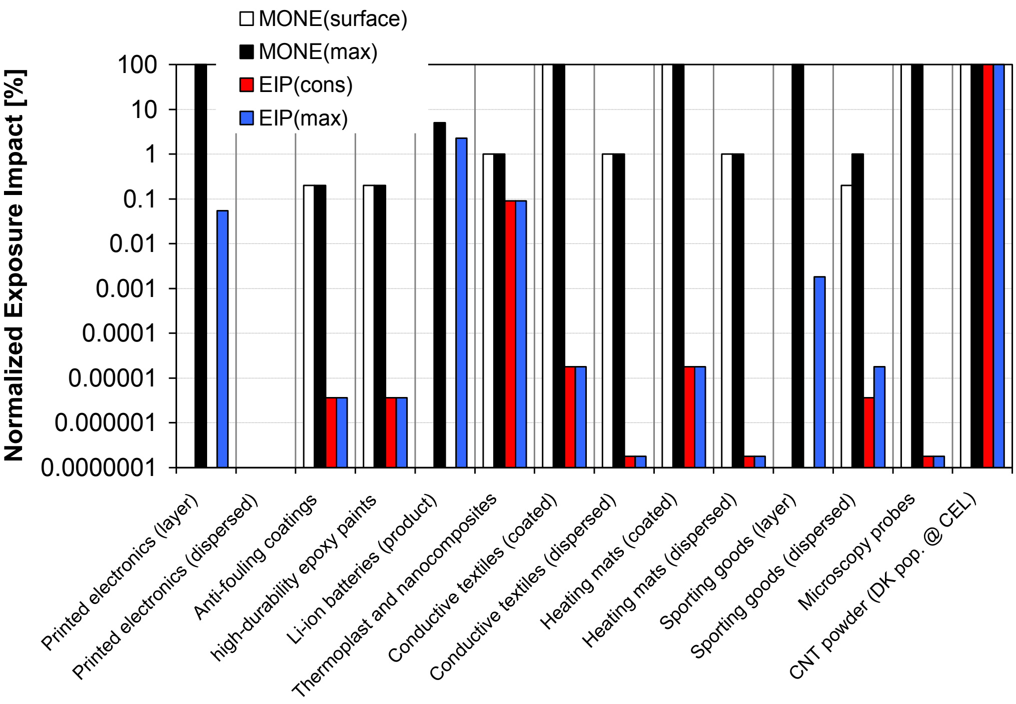


FIGURE 7-2

RELATIVE PRODUCT EXPOSURE IMPACT (PEI) OF THE LISTED PRODUCT GROUPS COMPARED TO THE DERIVED MONE-VALUES. ALL DATA ARE NORMALIZED TO HANDLING OF KG-LEVELS OF CNT POWDER BY THE TOTAL DANISH POPULATION.

7.4.2.5 Likelihood of reaching critical consumer exposure levels

In addition to the number of items, the significance of the *MONE* and *PEI* results depends highly on the exposure characteristics and whether sufficient CNT can really be released from a product. Therefore, we finally consider the potential nature of the release and the likelihood of reaching the consumer exposure limit currently defined at 0.25 $\mu g/m^3$.

The assessment of CNT release requirements was completed using the NanoSafer two-compartment instant mixing model, which is used to make exposure estimates in the NanoSafer 1.1 control banding tool,[92,114] to estimate the acute critical release rate from a product to achieve a 15-min average near-field concentration of 0.25 $\mu g/m^3$. For the calculation we assumed different percentages of CNT at the surface of a product and estimated the critical area of release to reach the exposure limit. The calculations were made in a worst case scenario with a room size of 5 x 5 x 2.7 m, a low air-exchange rate (0.3 h^{-1}). The nature of the potential exposure was evaluated based on the physical nature of the CNT in the product and current understanding of matrix behavior during aging and weathering.

Considering the characteristics of the potential release from the selected product groups, free CNT may be expected during normal use of conductive textiles, heating mats, high performance sporting goods, and microscopy probes (Table 7-3). In sporting goods, the release may be most relevant, if the sporting goods are constructed by layered CNT composites with pure or high-concentration CNT layers. Textiles and heating mats may currently be produced with CNT bound to the surfaces and microscopy probes are freely accessible filaments. Therefore these materials are high risk from their exposure characteristics (red in Table 7-3). However, the level of exposure may vary greatly.

For the products with CNT dispersed in a matrix, the first exposure is thought to be limited to material fragments with CNT, fragments with partially embedded CNT. However, with time free CNT may become available in anti-fouling coatings, high-durability epoxy paints, thermoplastics

TABLE 7-3

ASSESSMENT OF CONSUMER EXPOSURE DURING INTENDED CONSUMER USE FOR CURRENT HIGH-VOLUME CNT-BASED PRODUCTS

Product group	Qualitative evaluation of consumer exposure	CNT concentration (in layer / product (wt%)	Anticipated characteristics of exposure from final product (in use)	Single product size [cm^2]
Printed electronics (conductive ink)	Unlikely. Only possible if user can have direct contact	≤ 100 wt% in the conducting layer	Free CNT possible if the consumer access direct contact (opening of device)	From cm^2 to m^2-size depending on use
Anti-fouling coatings	Possible. Long-term degradation of coating	Guess 1 wt%, which results in 0.4 mg/m^2 kg per solid layer (several layers possible)	CNT-composite debris possible in the early phase, aging may uncover and release free CNT	Up to several hundred m^2
High-durability epoxy-paints	Possible. Long-term degradation of paint	Guess 1 wt%	CNT-composite debris possible in the early phase, aging may uncover and release free CNT	Up to several hundred m^2
Li-ion batteries	Unlikely. Enclosed in battery casing	ca. 2 wt%	Enclosed. Exposure only possible in case of accidents or if the user breaks the casing.	Mostly ca. 5 – 100 g but larger high power battery systems are possible
Antistatic thermoplastics	Possible. Damage and slow long-term wear	< 3-5 wt%	CNT-composite debris possible in the early phase, aging may uncover and release free CNT	From cm^2 size to several hundred m^2 in e.g., vessel bodies
Conductive textiles	Possible. Direct contact, long-term alteration	100% if surface coated yarn / Guess 3-5 wt% if embedded	Free CNT exposure is likely in textiles with CNT coating. There is direct dermal, and possible inhalation and ingestion exposure	Up to m^2 size in wearable textiles, and potential uses in car seats etc.
Thin heating mats	Possible if direct contact, long-term alteration	100% if surface coated yarn / Guess 3-5 wt% if embedded	Free CNT is possible, but depends on the location of the CNT. Free CNT release and exposure is likely during renovation etc.	Depends on use and probably do not currently exceed 20 m^2
High performance sporting goods	Possible. Damage, slow long-term wear	100 wt% in specific layers < 3-5 wt% of composte	Free CNT exposure is possible in case of wear damage and failure of product (e.g., breaking a racket)	Up to ca. 0.25 m^2 Expected to restricted to frames, rods, or frames bicycles etc.

Microscopy probes	Unlikely to very low.	Probe filament is 100 wt%	No exposure in general use. Exposure only possible in case of accidents.	Single CNT fiber or CNT rod devises

€ * model room may not apply; sanding/modifying aged surface may be critical.

Colour code: Green: low potential for CNT exposure; Yellow: intermediate potential for CNT exposure; Red: High potential for CNT exposure due to aging, weathering and alteration. Finally free CNT may become available in printed electronics due to necessity of opening an electronic device (e.g., home repair). However, CNT in other printed electronics could be printed on accessible surfaces or in low-durability materials (flexible displays, smart labels, decorative and animated posters, and active clothing (http://en.wikipedia.org/wiki/Printed_electronics). Due to these exposure characteristics, these products are considered to be associated with intermediate potential for exposure and marked with yellow in Table 7-3 to indicate possibility for exposure to free CNT.

Release of free CNT from Li-ion batteries is only possible in case of accidents or non-intended consumer use, such as deliberate breaking of the battery casing. We have assessed this product to be associated with negligible to very low possibility for exposure to free CNT and marked it green in Table 7-3. However, there is still a critical latent exposure in Li-ion batteries due to the presence of g level of CNT in the core of the batteries.

Considering the release rate required to reach the consumer exposure limit, the NanoSafer model calculations with the conditions above, showed that 2.75 g CNT release in 1 minute or 0.208 µg/min (3.115 g total CNT release) for 15 min would result in a 15 minute average exposure of 0.25 µg/m^3 if the consumer is next to the source. This corresponds to aerosolization of all CNT from an approximately 350 and 396 cm^2 5 µm thick surface consisting of vertically aligned CNT with a diameter of 100 nm and a length of 5 µm and a density of 2 g/cm^3, respectively. This scenario could be considered as a worst case scenario for a textile fully coated with CNT at the surface. In case of nanocomposites, which typically contain up to 1 wt% CNT, the release rates required to exceed the acute 0.25 µg/m^3 exposure would be immediate release of 3.50 to 3.96 m^2 layer of similar 5 µm thicknes.

Combining the exposure characteristics, the critical area release rate requirements, and product sizes, it appears generally challenging for a product to result in sufficient release to exceed 0.25 µg/m^3 acute CNT exposure. It could occur during sanding and grinding surfaces and would consequently be an episodic event. For some product groups (e.g., abrasion on anti-fouling coatings and epoxy paints), the work may likely occur outdoors with much greater levels of dust dispersion. However, since we use near-field (activity) exposure levels for the calculations, the critical area release rate may still be indicative for precautionary reasons.

7.5 Assessment of environmental release and exposure

In chapter 4 we demonstrated that there is a potential environmental release during especially CNT production and handling as well as discard of waste and accidents during transport (Tables 4-1 and 4-2). Air and waste stream pathways were both important for the release to the environment. However, no monitoring data were available on the environmental release and exposure to the general public.

Gottschalk[64] made a life-cycle analysis for a scenario assuming instant distribution of CNT in 1 km atmosphere, 5 - 20 cm soil, 3 cm sediment, and 3 m water column in an area corresponding to that of Europe and assuming that the CNT stay dispersed and fully accessible. This scenario was calculated using production and use of 500 metric ton CNT (corresponding to the worldwide production of CNT in year 2008). Under these conditions, upper and lower 15% percentile concentration of 0.0025 – 0.007 ng/m^3 air, 1.07 – 3.22 ng/kg soil/year, 215 – 1321 ng/kg sediment/year, 0.0035 – 0.021 ng/L surface water. For comparison, it is worth noting that the entire CNT demand has been found be on the order of 3,300 – 3,700 tonnes in 2012 and the estimated range of production for 2014 was between 520 and 3000 tons with 1750 – 2500 tons as an intermediate estimate.[58] Consequently the estimated values by Gottschalk are current minimum values and the current rates may be up to ca. 7 times higher considering the global scale production in 2015. These data are shown in Table 7-4.

One of the major critical issues regarding the life-cycle assessment model by Gottschalk et al. [63,64], is the instant homogeneous dilution of CNT. In particular, effects at product interfaces and environmental hot spots should also be considered. Hot spots are where the emissions of CNT would be expected to accumulate in the local environment around the source or in environmental traps along the environmental migration pathways. This is a fundamentally different way of assessing the potential worst case environmental exposure risk to CNT. For ambient air-concentrations a link between environmental modelling and process-specific workplace measurement data as reported in Table 4-2 could be valuable. In addition many different types of environmental interaction are expected, but very difficult to take into account.

TABLE 7-4

MODELLED ENVIRONMENTAL ANNUAL ENRICHMENT OF CNT IN EUROPE ADJUSTED TO A PRODUCTION VOLUME OF 3,500 METRIC TONS (MODIFIED FROM GOTTSCHALK ET AL[64]).

Compartment	Mean	$Q_{0.15}$	$Q_{0.85}$
Air [ng/m^3] **(1 km mixing height)**	0.021	0.0175	0.0490
Soil [ng/kg/year] **(0.2 m agri# / 0.05 m other)**	10.57	7.490	22.54
Sludge treated soil [ng/kg/year] **(0.2 m agri#)**	515.2	364.7	10.99
Sediment [ng/kg/year] **(0.03 m)***	1505	850	9247
Surface water [ng/L] **(3 m)**	0.028	0.0245	0.147

agricultural

Considering the specific major product groups in this report, the MONE values (Margin Of Nanomaterial Exposure) derived in this report (see section 7.4.2.4; Table 7-2 and Figure 7-2) also apply for ranking the potential environmental exposures according to the likelihood that could be significant. This is because the MONE values are only ranking the absolute release potential from the product. As seen, the maximum MONE values were reached for layered printed electronics, coated textiles and heating mats, layered sporting goods and microscopy probes. The second-rank MONE group included most of the other products groups, such as Li-ion batteries, thermoplastics and nanocomposites with dispersed CNT.

For the final environmental release and exposure assessment, it is crucial to have good understanding of the product size and waste handling strategy. This does currently not exist. However, the exposure level at the product surface is a worst case scenario. As mentioned before, products with CNT in the surface may due to matrix degradation offer a fully CNT coated surface. From this view-point, basically all products have the potential of environmental exposure during long-term use and land-fill waste discard.

7.6 Provisional Integrated Risk assessment

Due to the still very limited knowledge on the CNT exposure to workers and consumers, an integrated risk assessment is highly uncertain. We are aware of no measured data on the environmental release and exposure in environment. Similarly, the human and environmental toxicity of CNT is far from being fully investigated. Therefore, combining the uncertain data-sets into a valid integrated risk assessment will be associated with even greater uncertainty.

7.6.1 Human risk

Combination of the exposure assessment with the derived chronic occupational (1 µg/m^3) as carbon or CNT and consumer (0.25 µg/m^3) as CNT exposure limits leads to a final risk assessment of the production, handling and use of CNT nanomaterials and CNT-based based products.

Occupational risk

Almost all measurements in work situations show potential exposure to CNT and the exposure levels are often prone to be at siginificant levels or even exceed the OELs derived above. Also the acute exposure levels are often exceeded if we follow the conventional rule of the 15 minutes exposure limit being two times the 8-hour OEL. It should be noted that these measurements are most frequently made of whole-dust and CNT makes a fraction of this dust. The publications, however, usually do demonstrate that CNT is an important fraction of the dust concentration. Some of the more recent publications quantify the CNT exposure by the concentration of elemental carbon where exposure levels reach ranges around the exposure limits proposed for respirable CNT.[29,78,79]

Consumer risk assessment

It was found that it was difficult to achieve the critical inhalation exposure limit in case of use of consumer products. However, as illustrated in Table 7-3, CNT release is possible for all the products marked yellow and red. If the proposed CNT consumer exposure limit remains at the 0.25 µg/m^3 level or lower, specific incidents may still be critical and could be exceeded during e.g., removal of surface coatings for repair and repainting as well as deterioration of textiles. Experimental work and exposure measurements are required to document the realistic exposure levels achievable.

7.6.2 Environmental risk

Chapter 6 listed the results from a series of ecotoxicological studies identified to be important for the purpose of this report. The key results on observed effect concentrations and the doses causing 50% lethality are summarized in Table 7-5. Clearly, there are several missing data points as well as open issues and uncertainties regarding potential differences between different CNT types, as well as on how the sample preparation methods and dosimetry may influence on the test results. Moreover, the environmental release estimates are highly uncertain. Also, more robust data on actual production and down-stream use-volumes with defined geographic locations would give much better predictions on environmental concentrations. The early models assuming full dispersion of CNT do not indicate that CNT's may pose a risk in the environment. This is even after considering the 7 times higher CNT demand (use) in 2012 than estimated in the 2008 assessment by Gotschalk et al. However, the potential for accumulation of persistent CNT over time has not been included, and is another issue. Further, hot spots (i.e. production and landfill sites) have not been considered.

To shortly summarize the key studies, the most sensitive fresh-water species was a lower pelagic organism a Crustacean *Daphnia magna*, for which was calculated 96-h mortality LC50 at 0.05 mg/L [105]. Toxicity of CNT to aquatic vertebrates ranged from 10-240 mg/L. Though, SWCNT were found to be a respiratory toxicant to trout *Oncorhynchus mykiss* and reproductive toxicant to the copepod *Tigriopus japonicus* starting at concentrations 0.1 mg/L. In the benthos, MWCNT treated by all dispersion protocols induced mortality of *L. plumulosus* at 30 g/kg.

In terrestrial organism results are negative or in hydroponic cultures with concentrations above 6.9 mg/L. Noteworthy, our assessment suggests that plant toxicity or disturbance of vegetative growth may the most critical end-point at this time. Especially potential distribution via sludge should be of concern. This is because if the CNT are not degraded or are highly resistant, fields would first have a risk of growth inhibition and deleterious effects in worms and bacteria. Uptake in plant stems would keep the CNT in the upper soil-layer and potentially cause indirect exposure via feed and food.

TABLE 7-5

SUMMARY OF CURRENTLY IDENTIFIED KEY ECOTOXICOLOGICAL DATA ON NOEC, LOEC, EC50 AND LC50 FOR AQUATIC, BENTHIC AND SOIL SPECIES.

		mg/L			
	AQUATIC SPECIES	NOEC	LOEC	EC_{50}	LC_{50}
SWCNT	Trout fish *Oncorhynchus mykiss* resp. tox.[204]		0.1		
	Zebrafish *Danio rerio* hatching delay[23]		120		
	Copepod *Amphiascus tenuiremis* reproduction[218]		10		
	Crustacean *Daphnia magna* 48-h mortality[250]				2.4
	Crustacean *Daphnia magna* immobilization[250]			1.3	
	Algae *Dunaliella teriolecta* growth[236]			0.82	
DWCNT	Zebrafish *Danio rerio* hatching delay[23]		240		
	Copepod *Tigriopus japonicus* reproduction[116]		0.1		
	Copepod *Tigriopus japonicus* mortality[116]		30		
	Diatom *Thalassiosira pseudonana* 96-h growth[116]		0.1	1.86	
MWCNT	Frog larvae *Xenopus laevis* mortality[153]		100		
	Frog larvae *Xenopus laevis* growth[152]		50		
	Frog larvae *Xenopus laevis* mortality[152]	50			
	Crustacean *Daphnia magna* 48-h mortality[250]				23
	Crustacean *Daphnia magna* 48-h immobilization[250]			9	
	Crustacean *Daphnia magna* 48-h mortality[105]				6
	Crustacean *Daphnia magna* 96-h mortality[105]				0.05
	Crustacean *Ceriodaphnia dubia* 48-h mortality[102]		16		17
	Crustacean *Ceriodaphnia dubia* 48-h mortality[103]		48	51	
		g/kg			
	BENTHIC SPECIES	NOEC	LOEC	EC50	LC50
MWCNT	Amphipod *Leptocheirus plumulosus* mortality[103]		99		68
	Amphipod *Leptocheirus plumulosus* mortality[102]		30		
	Crustacean *Hyalella azteca* mortality[102]		300		
	Crustacean *Hydra attenuata* mortality[103]		264		
	Polychaeta *Arenicola marina* behavior & genotoxicity[59]	0.03			
	TERRESTIAL SPECIES	NOEC	LOEC	EC50	LC50
SWCNT	Tomato plant *Solanum lycopersicum* germination ↑↑ (mg/L)[104]		10		
	Fruit fly *Drosophila melanogaster* life-cycle (mg/kg food)[130]	100			
DWCNT	Earthworm *Eisenia veneta* mortality (mg/kg food)[192]	500			
	Earthworm *Eisenia veneta* reproduction (mg/kg food)[192]			37	
MWCNT	Mustard plant Brassica germination ↑↑ (mg/L)[148]		2.3		
	Mustard plant Brassica germination ↓↓ (mg/L)[148]		6.9		
	Zucchini *Cucurbita pepo* 15-day growth (mg/L)[208]		1000		

Data in bold are for the species currently setting the ecotoxicologicsal threshold values.

In agreement with the final conclusions on the ecotoxicological risk assessment in this report, other reviews conclude that risk assessment of CNT in aquatic and terrestrial organisms is currently not possible without reaching to highly speculative conclusions.[88,170]

For example, Jackson et al.[88] analyzes the quality of the material and exposure characterization in the different published papers: e.g., CNT lengths, impurity concentrations, surface treatments, and the fate in the wide variety of dispersion protocols. Often only the nominal exposure concentrations are presented and the behaviour and fate (e.g. sedimentation) of the tested CNT is unknown. In future studies, inclusion of benchmark materials with well-known biological effects could aid in proper inter and intra interpretation of the assessed toxicity. While better material characterization per se is essential for increasing the understanding of the exposure/effect relationship, the lack of knowledge about CNT mode-of-action hampers firm conclusions on the cause-effect relationships between inherent properties and toxicity.[88]

In summary, current simplified estimations assuming full dispersion of CNT does not indicate that CNT's do not pose general a risk in the environment. This exposure assessment of CNT is on one hand expected to underestimate the real concentrations in inevitable hots spots (e.g. production sites and accumulation) while they on the other hand does not take the binding and incorporation CNT's to natural solid matter (particles) into account.

7.7 Concluding remarks

It is evident that there is still a lack of publically available systematic toxicological studies on CNT that can be applied for establishment of exposure limits and applied for regulatory purposes. Hypotheses have been established proposing that the toxicity of CNT should vary with the side-wall topology (i.e. type of CNT), their persistence in biological compartments, their length and diameter, contents of impurities, and (bio-)chemical reactivity. There is clearly a need to improve the material and exposure characterization for all of these endpoints in a harmonized manner and implement such information in toxicological studies. Especially, it may be of crucial importance to improve our understanding of the biological interaction, both for CNT within and outside the scope of high-aspect ratio nanomaterials.

Based on scientifically derived occupational exposure limits and results from reviews, the proposed inhalation exposure limits are one to three orders of magnitude lower than the currently regulatory enforced exposure limits for CNT. The highest exposure limit was derived by Kobayashi et al [107] who suggested that an exposure yo be 210 µg /m^3 was acceptable for working 8 hours/day 5 days a week. Pauluhn has suggested an occupational exposure limit of 50 µg/m^3 [166] based on his own OECD guideline inhalation experiment [167]. In 2010, NIOSH proposed a recommended exposure limit (REL) of 7 µg/m^3 of CNT or carbon nanofibers in air as an eight-hour, time-weighted average, respirable mass concentration (www.cdc.gov/niosh/docket/review/docket161A/). This value was re-adjusted to be 1 µg carbon/m^3 in April, 2013 (http://www.cdc.gov/niosh/docs/2013-145).

Previously, Aschberger proposed a comparable occupational exposure limit (1 µg/m^3) [4] and an exposure limit of 0.25 µg/m^3 for the general public (including consumers). The earliest exposure limits were based on a smaller data set, and also reflect results from tests on different CNT. It is unclear whether the differences are fulle related to different procedures for establishment of exposure limits or differences in materials. From a precautionary principle, we favour the lowest derived exposure limits due to the long pulmonary retention times of CNT and the fact that none of the risk assessments are based on the most critical endpoint, mesothelial cancer.

The specific exposure levels to CNT are largely unknown. So far, the exposure studies have focused on inhalation and, as far as we know, dermal exposure has only been measured in one case. It is clear that exposure measurement studies need to be improved, which also is identified within the occupational exposure assessment communities. However, there will still be uncertainty for some years on the applicability and accuracy of the different old and new quantification methods currently considered. Simple mass-based measurements show excessive exposure to CNT in many industrial workplaces where average concentrations of up to 286 µg/m^3 are reported during harvesting and handling powder. Almost similar airborne concentration levels may be reached during spraying and sonication. It should be noted that these reported levels include both CNT and background dust, so the true levels of CNT exposure may be lower as indicated in studies including analysis of elemental carbon.

Considering the recently recommended occupational inhalation exposure limits (RELs), workers are often prone to be exposured to excessive concentrations of CNT. The levels may be several hundred times higher than the latest derived REL of 1 µg carbon/m³. Dermal exposure may be inevitable and may cause skin irritation (see Chapter 5). Oral exposure is likely if inhalation occurs and by accidental dermal to oral transfer. The knowledge on consequences of oral exposure to CNT is limited, but some studies have shown association with oxidative stress and DNA-damage (Chapter 5).

There are no measurement data available on consumer exposure. A qualitative assessment suggest that exposure to CNT is possible in several cases. Especially when products contain CNT at the surface or in concentrated layers, such as in layer composites, surface treatments and functional surfaces. However, by applying the Margin of Nanomaterial Exposure (MONE) and Product Emmision Impact (PEI) assessment procedures developed within this work, it appears that it is generally difficult to achieve a critical consumer exposure level during intended use. This is further supported from modelling, where it is demonstrated that if a product contains 1 wt% CNT in 5 µm surface layer, at least 3.5 m² of the product or more has to be aerosolized within 1 minute to reach a potential CNT exposure exceeding 0.25 µg/m³ in a 5 x 5 x 2.7 m³ room with a room ventilation rate of 0.3 per hour. Such large areas could be aerosolized during modification processes such as sanding and grinding. It is found that critical acute consumer exposure levels may be possible during mechanical reworking, modification or "demolition" of products such as printed conductive coatings, antifouling coatings, epoxy paints, conductive textiles, and heating mats. Accidents, such as breaking layered CNT composites or unintended opening of Li-ion batteries may also cause relatively high acute exposure. However, these events will be vey rare events for typical consumers.

Despite lack of empirical and experimental data, there appears to be a relatively high risk of dermal and accidental oral consumer exposure during intended use of specific consumer product groups as compared to inhalation exposure. Inhalation exposure, however, is likely for specific scenarios (e.g., mechanical reworking such as grinding and sanding and demolition) where CNT occur at the surface or in surface layers. It is, however, uncertain, whether the levels and durations of the consumer exposure situations will be sufficient to cause detrimental health effects in consumers.

Data on the ecotoxicty of CNT are still too uncertain to make certain conclusions on the environmental impact. Method developments are still required to harmonize test item preparation and test procedures to ensure comparability of data. At this point it appears as though a good dispersion of CNT in the exposure medium results in higher toxicity in aquatic environments than when the test material is aggregated. The lowest acute effect levels observed are currently around 0.1 mg/L in fresh water and marine organisms (e.g., EC50 96h mortality in fresh water crustacean and respiratory symptoms in rainbow trout *Oncorhynchus mykiss*). In the sediment, LOEL is currently above 30g/kg (mortality benthic amphipod *Leptocheirus plumulosus*). In soil, reproduction (cocoon production) of earth worm *Eisenia veneta* was affected at 37 mg DWCNT/kg dry food. For plants, enhanced germination was observed in Mustard seeds at LOEL 2.3 mg/L for oxidized MWCNT and 23 mg/L for pristine MWCNT, and germination inhibition LOEL 6.9 mg/L for oxidized MWCNT and 46 mg/L for pristine MWCNT.

There are currently are no measurements available on the CNT concentration levels in the environment. Homogeneous dispersion modelling data are available for Europe using data from 2008. Multipying the entire CNT-production by 7 times to adjust the data to the current upper level production volumes, still shows that the expected concentrations are below the current ecotoxicological effect levels. It is, however, likely that the environmental concentrations locally may be excessive, because hot spots such as those that can be expected at factories, landfill, waste recycling stations, and sludge-teated fields. On the other hand, the interaction between the CNT and the environment has not been considered, which may reduces environmental migration.

The lack of enough and highly reliable data on the ecotoxicology of CNT and environmental concentrations are currently too severe to discuss actual risk assessment. Modelled environmental concentrations do not exceed current effect levels. However, environmental CNT concentrations may locally exceed relevant effect concentrations levels.

8. Conclusions

Based on our report we can draw some major conclusions and identify recommendations for improving future knowledge on CNT characteristics, its hazards and exposure risk:

Carbon nanotubes

Carbon nanotubes (CNT) comprise a group of manufactured high aspect ratio nanomaterials. They consist of cylindrical hollow carbon fibers (tubes) and as a product often contain impurities of other carbon phases and inorganic catalyst nanomaterials. CNT have many technologically interesting properties (e.g., thermal, electrical and optical, tensile strength, specific chemical reactivity, gas and energy storage capacity, tunable chemical reactivity).

The cylindrical part of an ideal CNT can be depicted as a rolled-up graphene sheet, which resembles a chickenwire network. Each cylinder may contain one, two or more layers of such graphene-like side-walls, which leads to their typical division into single-walled (SW), double-walled (DW), and multi-walled (MW) CNT. The en of the carbon tube may be either closed or open. Other CNT types also exist of which cup-stacked CNT (stacked cones with graphite sidewall) and bamboo-type (internal structures resulting in non-continuous tube) products were also identified as commercial products. The minor CNT types may have wide application as observed for cup-stack CNT in sports equipment.

Commercial CNT products (273 types included in our assessment) were typically reported to have diameters ranging from 0.7 nm (SWCNT) to about 100 nm (MWCNT) and lengths ranging from 0.5 to 50 µm. The commercial CNT products were found to have CNT purities from 60% to more than 95% by weight. The typical impurities are various carbon compounds and inorganic catalyst materials (often transition elements). About 85% of the identified products had less than 10% impurities by weight. Crude CNT may contain negligible to more than 80% impurities by weight depending on synthesis procedure.

A minor number of commercial CNT types are readily available for purchase as functionalized CNT to improve dispersion and enable further chemical modification. Alternatively, CNT can be delivered in ready-made master batches. The most common functionalizations were Li-ion doping or covalent binding of hydroxyl, carboxyl, or nitrite at sidewall and tube-end defect sites made by chemical purification. If needed, perfection of the side-wall structures can be achieved by graphitization (annealing) after purification.

Based on the observed types and structural complexity of CNT, the typically applied categorization of CNT into number of sidewalls may not be sufficient for neither technical categorization and quality assessment nor regulatory categorization and reporting for risk assessment. A more advanced systematic procedure appears to be needed for reporting and categorizing CNT qualities to enable easy identification of technically suitable materials and specific material characteristics for regulation. A proposal for such a system is shown in Appendix 3.

Production and application

The CNT production volume has been estimated to be between 520 and 3000 metric ton in 2014 and is dominated by MWCNT. The production capacity is many times larger than the demand and was estimated to be on the order of 20,000 metric tons in 2014.

The CNT business is still interesting. The global trade value of CNT nanomaterials was $158.6 million in 2014. For comparison, the global trade value of CNT-based products reached $5 billion in 2012 and is expected to reach $20 billion in 2015. The automotive, energy, paint and coatings, and electronics sectors were found to be the most important users of CNT by volume.

Due to the different types and properties of CNT and use in different sectors, they may be applied in a wide range of products. SWCNT and DWCNT are mainly used in electronics and optics, whereas MWCNT is mainly used in the various composites. The oldest use may be in Li-ion batteries where CNT constitute 1 - 3 weight percent of the graphite electrodes. Application of CNT as a conducting and strength-enhancing filler in base resins and thermoplastics was also found to be well-established. Many other uses exist and comprise: Polymer composites (in e.g. rackets, golf-clubs, and ice-hockey sticks, high performance bicycles, small boats and windmill blades); epoxy-paints and antifouling coatings for the marine sector and other high performance surfaces such as windmill blades; conductive inks for electronic displays and touch screens; textiles for 'intelligent' clothing, heating mats and thermal control; and electronic components, non-volatile RAM memory modules for computers etc. and heat management devices for microelectronics.

Emerging and near-future large tonnage applications of MWCNT is expected to be in various reinforced composites used for aerospace, automotive, marine and wind turbine industries where implementation of CNT is expected to result in weight reductions of approximately 10 %. SWCNT and DW CNT as well as the 'exotic' CNT types are not expected to reach the same levels of tonnages as MWCNT in the near future.

Exposure

There appears to be no measurement data available on consumer exposure and environmental release. Inferred from product types, consumer exposure to CNT may be generally low due to the general incapsulation or embedding of CNT. But some direct dermal exposure during use of CNT-coated textiles and release of CNT after weathering of composite materials is expected.

Occupational exposure to airborne CNT dust has been documented with concentrations reaching at least a few hundred µg/m^3 air. Dermal exposure is also a consideration and a worker glove-dose of 2-6 g CNT has been documented in a single study of harvesting and handling crude CNT after production. The CNT in the reported occupational exposures and release studies mainly occurs in bundles, aggregates and agglomerates as well as in matrix bound fragments.

Current life-cycle analysis suggest that the major route of CNT release to the environment are expected from synthesis and manufacturing of CNT-based products. Soil and sediment appears to be the major sinks for accumulation of CNT arising from sedimentation of airborne dust, sludge treatment and release through waste-water, respectively. Discard of CNT waste and CNT products are expected to mainly end up in landfill (ca. 70%) and waste incineration plants (ca. 17%).

The modelled environmental CNT concentrations in life-cycle analysis are moderate, but the models only consider homogeneous mixing in the environmental compartments, which is unlikely. Hot-spots around production and manufacturing sources as well as landfills etc. should be considered and are proposed to be included in surveillance. In addition, it should be noted that the estimates does not consider the interaction between CNT and the environmental constituents either, which may also prevent wide-spread distribution of CNT.

Human Hazard

Exposure to certain CNTs may be associated with serious long-term health effects in test-animals, but the effects are strongly depending on the exposure route.

Most literature exists on lung exposure. Biokinetic studies show phagocytosis and transport of macrophages into the subplural regions of the lungs. Residence times with half-lives of up to 300 days have been reported. Translocation from the lung is limited, but when CNT reach the blood vessels, there is amble evidence that they will accumulate in Kupffer cells in the liver with a very low rate of elimination. Different kinds of CNT have been observed to remain in liver cells up to 1 year after exposure. Evidence suggests that CNT are not taken up from the GI tract in a significant amount, and there is no evidence to suggest that CNTs are taken up from the skin.

Long-term OECD guideline inhalation studies on rats exposed to agglomerates of two different commercially available MWCNT showed subclinical symptoms of inflammation at the lowest dose; 0.1 mg/m^3. It is not known whether inhalation of dispersed free MWCNT have effects at lower concentrations.

Instillation of SWCNT induced single strand breaks in DNA in BAL cells after 24 hours. Oral dosing of the same SWCNT induced single strand breaks in DNA in liver cells. Mitsui XNRI-7 MWCNT has caused mesothelioma in rat and in a susceptible mouse model in a dose-dependent manner. Mitsui XNRI-7 is reported as relatively straight and long MWCNT with a low Fe content. In contrast, SWCNT and a shorter MWCNT did not cause mesothelioma in rats.

Pulmonary exposure to SWCNT in combination with a high-fat diet has shown the possibility to lead to plaque progression, and there is evidence that CNT present in the blood in high concentrations will promote platelet aggregation.

In reprotoxicological tests, no accumulation of CNTs was found in testis cells after IP injection, and injection of CNTs had no effects on male fertility. The relevance of the findings is unclear, since there is little evidence that CNT will enter circulation at all. However, the results indicate that even if CNT would enter the body, there are no indications of direct effects of CNT on male fertility.

Dermal inflammation was observed after exposure to unpurified SWCNT, but not after exposure to purified SWCNT and commercially available MWCNT. The finding for the SWCNT may be caused by the high levels of impurities in the unpurified SWCNT rather than the SWCNT.

Overall, there is still by far insufficient knowledge to reach a good understanding of the mechanisms behind the CNT toxicity and of whether the different types and chemical derivatives of CNT have comparable or significantly different levels of hazard. More relevant dose-response studies following the same dispersion and exposure protocol(s), using a range of different well-characterized CNT types and derivatives, are needed to reach this understanding.

Ecotoxicity

The data on ecotoxicological effects of CNT is scarce and data may not be directly comparable, since also in ecotoxicological studies, different CNT and different dispersion protocols were used to assess the toxicities in the different environmental model species. It is therefore not fully certain whether the toxicity of CNT vary with CNT type and impurity levels.

Several studies provide examples of CNT deposition on organisms or in their gut. Uptake was observed by filter feeders and on the gills of fish. This uptake and accumulation may cause toxicological effects.

In the aquatic environment, the toxicity of CNT seems to depend strongly on the level of dispersion and stability in the test media. Well-dispersed CNT induce acute effects in fresh water and marine organisms with an effect concentration of about 0.1 mg/L (e.g., EC50 96h mortality in fresh water crustacean *Daphnia magna*, EC50 inhibited growth of marine green algae *Dunaliella tertiolecta*, and respiratory symptoms in rainbow trout *Oncorhynchus mykiss*).

In sediment and soil organisms the toxicological effects appear only to occur at relatively high concentrations. More than 30 g MWCNT/kg was required to induce mortality in the benthic amphipod *Leptocheirus plumulosus*. Cocoon production of earth worm *Eisenia veneta* was affected at a concentration of 37 mg/kg dry food. No systematic genotoxic effects were observed.

Vegetation may also suffer from effects from CNT exposure. Enhanced growth followed by inhibition with increasing dose has been observed for Mustard seeds, whereas growth reduction was observed for Zucchini *Cucurbita pepo* plants. The growth stimulation LOEL 2.3 mg/L for oxidized MWCNT and 23 mg/L for pristine MWCNT, and growth inhibition LOEL 6.9 mg/L for oxidized MWCNT and 46 mg/L for pristine MWCNT for the mustard seeds.

As for human hazard studies, future work should include testing of a range of well-characterized CNT types using harmonized procedures for dispersion and description of the state and fate of the CNT in the exposure. Moreover, great effort should be made to improve the understanding of chemical and physical interactions between CNT, the mediums, food and nutrients, and other particles therein. These mechanisms may be playing a greater role on the ecotoxicological effects than previously anticipated.

Integrated Risk Assessment

There is still insufficient (eco-)toxicological studies available to establish whether it is specific physico-chemical properties that control the hazard of CNT. Consequently, the different types of CNT are currently still considered as one material group with a few proposed airway exposure limits. Limit values have not been set for the environment.

The recommended occupational exposure limits (REL) is at the end of year 2013, 1 µg/m^3 (carbon or carbon nanotube depending on the source) and the consumer exposure limit is 0.25 µg/m^3. This REL is much reduced from that of carbon black (3.5 mg respirable dust/m^3), which is the material often referred to in safety data sheets for CNT. The first exposure limit set for CNT was 210 µg /m^3 in 2008. From a precautionary principle, we agree with the lowest derived exposure limits for CNT due to observed long pulmonary retention times and the fact that none of the risk assessments are based on the most critical endpoint, mesothelial cancer.

Based on the exposure measurement data in Chapter 4, workers are prone to experience excessive exposure to CNT at many industrial workplaces. Concentrations of up to 286 µg/m^3 have been reported during harvesting and handling of CNT powders. It should be noted that these concentration levels include both CNT and background dust, so the true levels of CNT exposure may be slightly lower. In any case the occupational exposure levels may be several hundred times higher than the recent REL for CNT.

There are no measurement data available on consumer exposure. There appears to be a relatively high risk of dermal and accidental oral exposure during intended use of specific product groups; especially when products contain CNT at the surface or in concentrated layers, such as in layer composites, surface treatments and functional surfaces. Inhalation exposure is likely for specific scenarios (e.g., mechanical reworking such as grinding and sanding and demolition) where CNT occur at the surface or in surface layers. However, by applying the Margin Of Nanomaterial Exposure (MONE) and Product Emmision Impact (PEI) assessment procedures developed within this work, it appears that it is generally difficult to achieve a critical consumer exposure level during intended use. This is further supported from modelling.

The ecotoxicology of CNT is still too uncertain and too scarce to make conclusions on the environmental impact. The lowest acute effect levels currently observed are around 0.1 mg/L in fresh water and marine organisms (e.g., EC50 96h mortality in fresh water crustacean and respiratory symptoms in rainbow trout *Oncorhynchus mykiss*). In the sediment, LOEL is currently above 30g/kg (mortality benthic amphipod *Leptocheirus plumulosus*). In soil, reproduction (cocoon production) of earth worm *Eisenia veneta* was affected at 37 mg DWCNT/kg dry food. For plants, enhanced germination was observed in Mustard seeds at LOEL 2.3 mg/L for oxidized MWCNT and 23 mg/L for pristine MWCNT, and germination inhibition LOEL 6.9 mg/L for oxidized MWCNT and 46 mg/L for pristine MWCNT.

There are currently are no measurements available on the CNT concentration levels in the environment. Homogeneous dispersion modelling data are available for Europe using data from 2008 and were adjusted to match the current CNT-production. The model data suggest that the environmental concentrations are still much lower than the effect levels. However, the existing modelling data do not consider (potential) local accumulation and hot spots at e.g., at factories, landfill; waste recycling stations, and sludge-treated fields, as well as the potential CNT interaction with the environmental constituents, which may prevent further distribution into the environment.

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Carbon nanotubes

This report provides a detailed overview of the variability and physico-chemical complexity of carbon nanotubes (CNT) and how they are produced, purified and surface modified. In addition the report gives an overview of the different commercial uses of CNT now and in the future. Finally the report gives an overview of CNT-exposure in relation to consumers, environment and workers.
Last part of the report is providing an overview of the existing knowledge of effects on humans and the environment and this information is used in a preliminary risk assessment on basis of existing knowledge.

Rapporten giver et detaljeret indblik i kulstof nanorørs (CNT) fysisk-kemiske kompleksitet og variabilitet samt i hvordan de fremstilles, oprenses og modificeres på overfladen.
Endvidere giver den et overblik over hvad forskellige typer kommercielle CNT anvendes til nu og i fremtiden. Endelige gennemgås det hvor arbejdsmiljø-, forbruger- eller miljø-eksponering for CNT kan forekomme.
Afsluttende giver rapporten et overblik over eksisterende viden om effekter på miljø- og mennesker og det vurderes præliminært om der på grundlag er eksisterende viden er risiko forbundet med anvendelse af CNT.

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