Supplemental material to manuscript: Reviewing the environmental and human health knowledge base of carbon nanotubes.

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The supplemental material contains the following elements:

- Introduction to Carbon Nanotubes' (CNT) properties and applications.
- Results of expert interviews on environmental health risks of CNT
- Recommendation of precautionary measures when working with CNT
- Overview in Table form of i*n-vivo* and *in-vitro* studies summarized in model system, exposure conditions and key findings.

Carbon Nanotubes

Carbon nanotubes, first observed in 1991 by Sumio Iijima at NEC (Iijima 1991), are to be seen as one of the allotropes of elemental carbon. In addition to the amorphous state (occurring e.g. in soot) and the crystalline state (graphite, diamond) carbon can also occur in the form of tiny hollow cylinders with diameters in the range of 0.4 until 2.5 nm. Length knows no principle restriction and ranges typically from tens to hundreds of micrometers. An ideal CNT can be described as a rolled up graphene sheet consisting solely of carbon atoms arranged in hexagonal ring structure with aromatic bonds. With their graphene-like lattice, and nanoscale size, CNT are considered an intermediate between molecules and crystals.

Most of the technically produced CNT occur in multi-walled form where two or more SWCNT of different diameters are concentrically telescoped. MWCNT are reported to show diameters up to several hundreds of nanometers. The aspect ratio of MWCNT is not as high as for SWCNT which results in a higher number of chemically active tips per mass unit. Frequently, MWCNT exhibit disturbed wall texture and form, such as dangling edges of graphene lattice which are favoured for chemical reaction. These CNT may be chemically more active than ideal CNT. The morphology of the CNT stems from the synthesis method.

A multitude of approaches for CNT-synthesis is reported in the literature (Bhushan 2004). Most of them are enhancements of two basic techniques: solid carbon source sublimation and Chemical Vapor Deposition (CVD). Carbon sublimation is achieved by either electric-arc (arc discharge) (Iijima 1991; Keidar and Waas 2004) or laser ablation (Guo et al. 1995). Both processes take place by high temperatures (1200°C - 3000°C) enclosed in a furnace permeated with an inert gas (e.g. helium) (Kiang et al. 1995; Park et al. 2002). A key component of the production is transition metal particles (e.g. catalysts) thought to help nucleate the graphene structure. While the former technique produces SWCNT in mixtures of MWCNT and soot, the latter produces a high percentage of SWCNT (~70%) with the rest being catalyst particles and soot. The CNT precipitate on the cooler walls of the reactor downstream from the furnace. CVD synthesis relies on the thermal cracking of a precursor gas such as hydrocarbons or CO in presence of metal catalyst (Cassell et al. 1999). The synthesis takes place in an enclosed reactor at temperatures in the range of 700-900°C. A special type of CVD is the HiPCO process where catalyst nanoparticles grow by in-situ gas-phase reactions of a metal-organic precursor (iron carbonyl) in CO at high pressures (10-50 atm) and temperatures of 800-1200°C (Bronikowski et al. 2001; Nikolaev et al. 1999).

All synthesis approaches make use of transition metal catalysts such as Nickel (Ni), Cobalt (Co), Iron (Fe) or Yttrium (Y) (Moisala et al. 2003) which usually remain in the yield. The metal and the particle size determine

the diameter of the CNT growing on their surface. SWCNT grow on small catalyst particles in the nanometer range, whereas larger particles tend to produce MWCNT (Donaldson et al. 2006). Commercially available raw-CNT contain significant quantities of impurities such as amorphous carbon, nanographitic structures and carbon encapsulated catalytic metal nanoparticles (Giles 2006).

Currently a lot of research is conducted to improve the purity and structural quality of the CNT. In the literature there is a variety of purification approaches reported (Chiang et al. 2001). Usually amorphous carbon is removed through oxidation by high temperature treatment with gaseous oxidants or mineral acid flush. Wet treatment requires antecedent dispersion in water by ultrasonication. Since CNT tends to aggregate, surfactant can be added to support dispersion in water (Jiang et al. 2003; Wick et al. 2007). Oxidation causes structural defects of the CNT, which can be healed by subsequent annealing. Further refining steps may rely on solubilization, filtration, centrifugation, magnetic purification and chromatographic techniques.

CNT are hydrophobic and therefore not soluble in water and, unlike closed cages such as C_{60}, most forms of CNT are also insoluble in organic solvents (Niyogi et al, 2001). In order to make them soluble, CNT are usually chemically altered by functionalisation, where submolecular structural motifs are covalently or non-covalently attached to the exterior of CNT (Rancan et al. 2002). For this purpose the CNT are first oxidised by a mixture of sulfuric and nitric acids in order to attach carboxylic acid or hydroxyl groups on wall (hydroxylation) as an intermediate for later reactions (Vast et al. 2004). Direct functionalisation is also possible by reaction with elemental fluorine (Touhara and Okino 2000). Functionalisation often significantly alters the overall properties of nanotubes, especially their chemical and electronic properties. For instance hydroxylated CNT are water soluble due to the polar hydroxyl groups on the exterior wall surface. Another way to make CNT soluble is through the noncovalent interactions with long molecules, adhering to the exterior wall (Arnold et al. 2005). That means attaching molecules at the CNTs' sidewall like paint by adhesive intermolecular forces which are weaker than covalent bonds. In this way, surfactants can be applied. CNT have been embedded in various organic (e.g. polymers) and inorganic materials (e.g. alumina, silica and titanium dioxide).

CNT exhibit excellent properties that can be customized in many ways and which are attractive for many technological applications (Ebbesen et al. 1996; Ruoff and Lorents 1995):

- Light weight, density = 2.6 g/cm^3 (steel: 7.8 g/cm^3).
- Elastic modulus (Young's modulus) may be up to 1 TPa and tensile strength is up to 150 GPa which is approximately 200 times stronger than steel (208 GPa and 0,4 GPa respectively).

- Novel electronic properties: CNT can be either metallic or semiconducting.
- High thermal conductivity.
- Excellent chemical and thermal stability.
- High storage capacity for chemical substances (e.g. hydrogen).

Table 1 summarizes potential future CNT applications reported in literature (Bachtold et al. 2001; Dillon et al. 1997; Hafner et al. 1999; Han 2005; Postma et al. 2001; Raffaelle et al. 2005; Robertson 2004).

Supporting Table 1 here

The different synthesis, purification and post processing methods produce CNT with different physical characteristics. For example, it is possible to customize the CNT by doping their carbon wall lattice with donor and acceptor dopants. Dopants substituted for carbon atoms within the graphitic network include nonmetals (such as nitrogen), alkali metals, transition metals (such as boron), and clusters (Golberg et al. 1999). Doping alters the physical and chemical properties of CNT significantly (Zhao et al. 2003).

The material characteristics of CNT are a key element in an assessment of the potential risks to human health or the environment, as different physical characteristics are likely to affect the risks of exposure.

Expert interviews

The published toxicological database of CNT presented in this article was found to be limited and with many open questions. Therefore, experts were identified and interviewed in order to present contemporary knowledge through assisting the assembling and organization of the findings given in the published studies, identify working hypothesis of the open questions, and present research questions from scientists in the field. The outcome of this combined methodology gives the current quantitative as well as qualitative knowledge base regarding the CNT impacts on human health and environment.

Selecting the Experts

We identified and contacted 17 leading experts based on the literature research as key authors of publications on environmental health impacts of CNT or as project leaders. The experts were selected with the intention of covering the topic from a multitude of scientific disciplines. In total were seven structured interviews conducted with scientists from USA, Europe and China.

Supporting Table 2 here

Preparing the interviews

The interview questions were developed through an extensive literature review where open questions posed by the scientists in the published literature were combined with open questions posed by people in the field in personal discussions. Questions related to the observed CNT toxicological/ ecotoxicological effects or mechanisms and more specifically how these effects relate to the specific properties of CNT were posed. However, depending on the background of the interviewees, not all questions could be answered by all the interviewees. Consequently, different questions were followed up in detail (see Table 2 for an excerpt of the questions). The questionnaire consisted of 25 questions in total.

Supporting Table 3 here

Conducting the Interviews

A time was scheduled with five of the experts for a phone interview. The questionnaire was sent prior to the interview. The interview lasted in average 50 min and were all recorded on tape and transcribed. Two of the experts answered the questionnaire in written form and sent it back electronically.

Data Analysis

All the answers to the questionnaire and the additional statements were compared. Thereafter, was the information structured and analyzed according to new insights given along the following themes:

1. present studies or present study designs
2. missing studies (missing knowledge) or missing study designs
3. concerns in terms of health effects / effects on environment

The information was then summarized and presented in the corresponding subchapters named “Results of expert interviews”. The statements given there may not be according to the opinion of all experts or may be mentioned only by one expert. In some cases the experts had contradicting opinions, but the information was nevertheless collected and presented.

Results of expert interviews on occupational settings

Most research thus far has indicated that CNT may cause serious health effects. Therefore the experts advocate application of the precautionary principle. If CNT are considered toxic, measures have to be taken to limit exposure to them. Understanding toxicological effects is important for understanding the origins of any observed toxicity, but it is less important for consumer and occupational safety. In the latter cases, it is,more important to characterize the exposure. It is vital to know what type of material workers or consumers are exposed to in order to design proper safety systems. Only in a second step is it important to understand whether, if so, why and how it is toxic, and at last to look for ways to modify the toxicity of the material.

For a sufficient occupational risk assessment the following must be known about the human-particle contact: 1) the type, level and duration of the real occupational exposure, 2) the health effects of such an exposure and 3) the number of exposed people.

Results of expert interviews on environmental impacts

Little is known about the influence of CNT on the environment because very few environmental studies have been conducted. Especially studies of the long term effects of CNTs’ presence in nature are lacking, such as effects on the food chain or reproduction. Furthermore the laboratory studies have so far not taken into account realistic biological settings, which are especially important, as the physical and chemical influences on the CNT change in the environment. For example, radiation breaks down both covalent modifications and

coatings, and therefore assumed 'non-toxic' forms may be dramatically and rapidly changed. Therefore, these studies could cover: the influences of UV, synergistic effects with toxins (absorption of contaminates in the environment), the half-life times or mobility in environmental compartments, the influence of agglomeration on the transport mechanisms.

Considering that many nanomaterials are currently manufactured in small scale, but have potential for market growth, it is necessary to know how much and where particles are used in industry in order to assess the life cycle effects properly. Therefore the material flow should be investigated from industrial systems to the environment.

Results of expert interviews on material properties and influence on toxicology

The present toxicological studies have often not attached sufficient importance to a detailed characterization of the material used (or to the verification of the properties indicated on the product purchased). The different practices of declaring the CNT material used in the studies can be seen in Table 2 and Table 3. This makes it very hard to compare the studies as CNT differ greatly. CNT are present in different spiral conformations, can be either metallic or semiconducting depending on their chirality and diameter, and have surface functionalities that may all have impacts on toxicity. Additionally may metal impurities, carbonaceous material, organic solvents, the distribution of perfect and not perfect built CNT, the "desirable" new properties like conductivity or hollowness, the reactivity in terms of oxido-reductions and the production of free radicals all be influencing factors.

There are also debates among researchers regarding study designs and the related toxicological effects of CNT (see for example Lam et al. (2006) or Wörle-Knirsch et al. (2006)). For example instillation studies have difficulties with CNT-agglomeration causing a mechanical blockage of the respiratory tract. They may furthermore not reflect the real respiratory mechanisms because they bypass the nose as a first filter. The absence of really good inhalation data causes over-interpretation of the scarce data and an important question raised was whether the effects of CNT reported in both *in vitro* and instillation models could be due to the CNTs' proclivity to agglomerate and thus whether these effects would be observed in an inhalation model.

The current studies (*in vivo* and *in vitro*) have often been performed within an inadequate time period and with unrealistically high concentrations. Consequently, establishment of standard protocols for nanotoxicology studies may be needed.

Much important knowledge is missing regarding the half-life of CNT in the body, their accumulation, degradation, elimination and bio persistency. However, there is an expectation that CNT accumulate quite significantly in fatty tissues and are very persistent due to the difficulty of modifying them even in laboratories.

In a complex water based system like the body or the environment, there is evidence that proteins, DNA and various biological polymers will stick to CNT extremely effectively (noncovalent binding). The effects of these interactions with other chemicals / proteins / particles are unknown, but could be very important as changing the quaternary structure of proteins could, for example, create an autoimmune response against the particles.

Few studies investigate biomarkers such as the TNF-α production as a key mediator in inflammation and lung fibrosis, and P450 induction as a key enzyme involved in xenobiotic bio transformation and cellular repair. In addition, studies about genotoxicity are also needed: an unpublished micronucleus assay has shown genotoxicity on the lung epithelial cells *in vivo* where MWCNT caused the formation of micronuclei and the complementary *in vitro* study confirming the direct formation of micronuclei in epithelial cells. Transport mechanisms in cell and body, effects on phagocytosis in lung, and toxicity for macrophages compared to other noxious substances are also areas that should be researched.

Precautionary measures

Although normal handling operations may not generate high concentrations of CNT at the workplace, the degree of CNT toxicity is still uncertain and workers should therefore be systematically screened for health effects. Interesting indicators to screen include lung inflammation markers, allergy test outcomes or cardiovascular effects (lung, skin, heart and potentially mucosa). These indicators are better adapted for a survey of the exposed population, as the medical procedure is not invasive and therefore easier to monitor systematically than inner organs like spleen, liver and kidney, endothelium, blood or nervous system etc. This type of studies should go over longer time periods to pick up long term effects and include characterizations of the type, level and duration of CNT exposure. However, for medical screening it is important to have a sufficient prevalence of preclinical effects in the screened population and sensitive, valid tests. These tests are still to be developed for CNT effects.

The studies reviewed suggest that CNT will produce a toxic response in a time and dose dependent manner once they reach the lungs in sufficient quantity. Therefore, we recommend taking a precautionary approach in order to prevent or minimize exposure to the human body and the environment. The studies suggest that CNT are in general more toxic than carbon black and quartz, and therefore Lam et al. (2006) have recommended that the occupational exposure limit for CNT should be no greater than 0.1mg/m^3, the same as the U.S. Occupational Safety & Health Administration's permissible exposure limit (OSHA PEL) for quartz. However, considering the unique shape and aspect ratio of CNT, it remains questionable whether a mass-value based exposure limit will be sufficient. Some scientists recommend developing a new volume/particle number based indicator.

Precautionary measures should also be implemented in other stages of the CNT life cycle. Exposure may be reduced in occupational settings through redesigning the production processes or change the handling and transportation procedures. During product design it is important to consider whether the product function can be obtained through alternatives to CNT which are less uncertain (i.e. more is known about their capacity to cause harm, their novel effects, persistency and mobility). If not, can CNT be integrated into the product in a way that makes it possible to control the size and number of CNT containing particles released into the surroundings due to wear, tear and corrosion? Finally, can the CNT-based product in the last stages of the life cycle be disposed of in a way that minimizes potential release? If not, there may be a need for targeted schemes (reuse, recycle or disposal) that ensure proper end-of-life treatment.

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Supplemental Material Table S1: Application areas of CNT

Industry sector	Application / material
Materials & Chemistry	
	- Ceramic and metallic CNT composites
	- Polymer CNT composites (heat conducting polymers)
	- Coatings (e.g. conductive surfaces)
	- Membranes and catalysis
	- Tips of Scanning Probe Microscopes (SPM)
Medicine & Life Science	
	- Medical diagnosis (e.g. Lab on a Chip (LOC))
	- Medical applications (e.g. drug delivery)
	- Chemical sensors
	- Filters for water and food treatment
Electronics & ICT	
	- Lighting elements, CNT based field emission displays
	- Microelectronic: Single electron transistor
	- Molecular computing and data storage
	- Ultra-sensitive electromechanical sensors
	- Micro-Electro-Mechanical Systems (MEMS)
Energy	
	- Hydrogen storage, energy storage (super capacitors)
	- Solar cells
	- Fuel cells
	- Superconductive materials

Supplemental Material Table S2: List of experts interviewed

Field of speciality	Name	Institution
Nanotoxicity		
Chemistry	Yuliang Zhao	Lab. for Bio-Environmental Effects of Nanomaterials and nanosafety, Chinese Academy of Sciences, and National Center for Nanoscience and Nanotechnology of China.
Pulmonary toxicology	David B. Warheit	DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, USA
Pulmonary toxicology	Dominique Lison	Industrial Toxicology and Occupational Medicine unit Catholic University of Louvain, Brussels, Belgium
Environmental Impacts		
Ecotoxicity	Mary L. Haasch	USEPA Mid-Continent Ecology Division Molecular and Cellular Mechanisms Research
Ecotoxicity	Eva Oberdörster	Department of Biology, Southern Methodist University, Dallas, Texas, USA
Environmental chemistry	Kevin Ausman	Department of Chemistry, Oklahoma State University, USA
Colloidal science & risk assessment	Mark R. Wiesner	Pratt School of Engineering, Duke University, USA

Supplemental Material Table S3: Excerpt of the questions used in the interview

(2) What are the most important/relevant (eco-)toxicological effects or mechanisms observed caused by different types of CNT?

(2a) Are any of these observed toxic effects and mechanisms caused by CNT considered as “new” or “specific” to CNT?

(2b) Which physical or chemical characteristics/properties (length, diameter, relation: length to diameter, SW or MW, stability) of CNT may be relevant for these observed effects?

(2c) Could you think of further effects and mechanisms not yet observed?

(6) Can the surface of CNT be modified by interaction with the living environment (i.e. proteins, hormones, macrophages)?

(6a) Do you think it is possible to control the toxicity of CNT?

(9) How sure is it that the observed effects and mechanisms are not caused by impurities?

(11) Can CNT be degraded by bacteria, plants, abiotic processes (i.e. UV) in the environment?

(11a) If yes, are the intermediates bio-persistent? Is something known about the half-life time and the risk potential of intermediates?

(13) Are CNT bio-accumulative?

(14) What do we know about the ability of CNT to translocate in the body/ in the environment?

(15) What type of NP uptake could potentially involve the most risks (e.g. Inhalation, dermal, ingestion of food or digestion of mucus)?

(15a) For environmentally relevant species?

(15b) For humans?

(18) Based on the current scientific knowledge, in your opinion, is a long term exposure to CNT related to high risks?

(20) Which type of applications for CNT would you expect involve high risks? Why?

Supplemental Material Table S4: The collection of in vivo studies reviewed summarized in model system, exposure conditions, material and key results.

Model system	Exposure / Endpoint	Material	Key results	Reference
Intratracheal instillation in guinea pigs.	- 6.25 mg per guinea pig (250g) - 4 weeks	- Soot containing CNT from a catalyst doped (Co/Ni) graphite anode. - Arc discharge sublimation method.	- No change in pulmonary function - No abnormalities - No inflammatory reactions	(Huczko et al. 2001)
Intratracheal instillation in guinea pigs	- 12.5 mg per guinea pig (males 200-250g) - 90d (days)	- NanoLab CNT, 80% purity (Fe<0.01ppm) - NanoLab CNT 95 % purity (Fe=0) - Pyrograf PR-1 nanofibres (Fe=0) - Showa Denko nanofibres (Fe=0) - CVD-CNT - Carbon arc CNT	- Multifocal granuloma, inflammatory reaction - Without or with mild peribronchial fibrosis in alveolar septa.	(Grubek-Jaworska et al. 2006; Huczko et al. 2005)
Intratracheal instillation in rats.	- 0.25, 1.25 mg per rat - BAL assessment after 1, 7, 30 and 90 days	- SWCNT soot by laser ablation process (5% Ni, 5% Co, 30-40 % C). - 1.4 nm diameter, >1 µm length	- Mechanical blockage of upper airways - Non-dose dependant inflammation - Cell injury effects - Multifocal granuloma formation.	(Warheit et al. 2004)

Intratracheal instillation in mice	- 0.1, 0.5 mg per mouse - 7 and 90 days	- Raw HiPCO SWCNT (26.9% Fe, 0.78% Ni) - Purified raw HiPCO SWCNT (2.14% Fe) - Electric arc SWCNT (0.53% Fe, 25.99% Ni, 5.01% Y)	- Dose-dependent granulomas - Interstitial inflammation - SWCNT more toxic then carbon black.	(Lam et al. 2004)
Intratracheal instillation in rats	- 0.5, 2, 5 mg per rat. - 3d, 15d inflammation - 1h, 28d and 60 days biopersistency - 60 days fibrosis	- Ground and intact MWCNT with ~15 layers - 0.95% Co, 98% C) synthesized by the de-composition of ethylene on an aluminia support doped with a Co-Fe catalyst mixture. - Outer diameter 11.3 ground and 9.7 nm intact - Length 0.7 ground and 5.9 µm intact - Surface area 307 ground and 378 m2/g intact	- MWCNT present in the lung after 60 days - Dose dependent inflammatory - Fibrotic reactions - MWCNT agglomeration in airways - TNFα stimulation	(Muller et al. 2005)
Pharyngeal aspiration in mice	- 0, 10, 20 and 40 µg per mouse - 1, 3, 7, 28 and 60 d	- HiPCO SWCNT (0.23% Fe, 99.7% C) - Diameter 1-4 nm - Surface area 1040 m2/g	- Dose dependent inflammation - Progressive fibrosis - Granulomas and alveolar wall thickening	(Shvedova et al. 2005)
Human patch test on skin and rabbit eye test	- 0.2 ml per eye - 96 h patch test - 24h, 48h and 72 h rabbit eye test;	- Soot containing CNT from a catalyst doped (Co/Ni) graphite anode.	- No signs of health hazard in dermatological trials	(Huczko and Lange 2001)

- Intraperitonally -, subcutaneous -, and intravenous injection, - Stomach incubation - Mice	- 100 µl per mouse of 15 µg/ml ^{125}I-SWCNTols concentration	- Raw soot arc-charge SWCNT - Purified to 90% carbon contents - Water soluble hydroxilated - Diameter 1.4 nm	- Distribution of the SWCNTols in stomach, kidneys and bone. - Excretion of SWCNTols by urine	(Wang et al. 2004)
Tail vein injection in mice	A. Biodistribution - 60 µg per mouse - 30min, 3h and 24h B. Urine excretion - 400 µg per mouse - Collected over 18 h	- Ammonium f-SWCNT: diameter ±1 nm, lengths: 300-1000 nm). - Ammonium f-MWCNT (94% pure): diameter: 20-30nm, lengths: 0.5-2µm.	- Rapid removal from blood by urine of intact CNT (within 3 hours). - No observed accumulation in organs	(Singh et al. 2006)
Subcutaneous implantation in rats	- 0.1 mg per rat. - 4 weeks	- CVD MWCNT (purity: ~80 wt%, impurities: amorphous carbon, Fe, Mo, Cr and Al) - Diameter: 20-40 nm. - Two sample lengths of 220nm and 825nm.	- Activation of THP-1 cells *in vitro* - Length dependent inflammatory response *in vivo*. - No severe inflammatory response	(Sato et al. 2005)
Subcutaneous implantation in mice	- 2 mg per mouse. - 1, 2, 3 weeks and 1, 2, 3 months	- CVD-SWCNT diameter: 0.8-2.0 nm, Fe content 1-1.5 wt%. - CVD-MWCNT diameter 20-70 nm Fe content 3-5 wt%.	- No change in body weight of animals - After 1 week only SWCNT activated major histo-compatibility complex (MHC) class I pathway of antigen-antibody response system.	(Koyama et al. 2006)

- CVD-MWCNT, diameter 50-150nm, Fe <300 ppm, thermal post treatment 2800°C
- Cup-stacked CNT by floating reactant method diameter 50-150 nm, Fe content 1 wt%.

- After 2 weeks all CNT types activated MHC class II.

Supplemental Material Table S5: The collection of *in vitro* studies reviewed is grouped by tissue and summarized in model system, exposure conditions, material and key results

Model system	Exposure / Endpoint	Material	Key results	Reference
Lung				
Murine macrophage cell line J 774 human macrophage	- 15, 30, 60 µg/ml - 24, 48 and 72h	- Ni / Co based SWCNT - C_{60} - Synthetic graphite	- Time dependent NO production - All applied Np were taken up over 72h - Increase of apoptotic / necrotic cells after 48h graphite exposition	(Fiorito et al. 2006)
RAW 246.7 macrophages of mice	- 0.12 mg/ml - 1 – 2 h at 37°C	- SWCNT purified (Fe 0.23 wt %) - SWCNT raw material (Fe 26.0 wt %)	- Neither purified nor raw SWCNT generated superoxide radicals or nitric oxide - Activated RAW macrophages generated hydroxyl radicals after SWCNT treatment	(Kagan et al. 2006)
Alveolar macrophages of guinea pigs	- 1.41 – 28.25 µg/cm2 cell culture - 3, 6, and 12h	- C_{60}, SWCNT and MWCNT were suspended in cell culture medium by sonication and homogenization - No free tubes, bundles of 10 to 100 SWCNT - Bundles of 4 to 6 MWCNT	- Cytotoxicity followed with equal mass basis: SWCNT > MWCNT > quartz > C_{60}	(Jia et al. 2005)

RAW 267.9 Murine (lung) alveolar macrophage cell line	- Start 10 µg/ml with 11 doubling dilutions - 48h	- SWCNT powder intermixed with robes/ bundles, Fe catalyst - MWCNT powder mix (50% tubes) - MWCNT powder aggregated (85% tubes)	- Cell viability EC50 values all around 5.5 – 6.0 µg/ml, identical for asbestos	(Soto et al. 2005)
RAW 267.9 Murine (lung) alveolar macrophage cell line	- Start 10 µg/ml with 11 doubling dilutions - 48h	- SWCNT powder intermixed with robes/ bundles, Fe iron catalyst - MWCNT powder mix 50% tubes - MWCNT powder aggregated 85% tubes - CNT analyzed with TEM and SAED - Suspended in 5µg/ml DMSO	- 50% decrease of cell viability at 5 µg/ml tubes - No expression of IL-10 nor IL-12	(Shvedova et al. 2005)
Peritoneal macrophage culture of rats	- 20, 50, 100 µg/well - 6h, 24h and 3 days	- Ground MWCNT (15 layers, outer diameter 9.7-11.3 nm, length 0.7 to 5.9 µm, surface area 307–378 m2/kg, 0.95% Co residue)	- TNFα mRNA increased after 6h - TNFα increase after 24h - LDH release dose dependent	(Muller et al. 2005)
Mouse peritoneal macrophage-like cells	- 0 – 7.3 µg/ml - 24h	- SWCNT suspended in Pluronic F108	- 7.3 µg/ml applied during 24h linear uptake - Within 24h, 1.65 µg/ml was taken up	(Cherukuri et al. 2004)
Human mesothelioma	- 7.5, 15, 30 µg/ml	- Arc-discharged SWCNT of different	- SWCNT-bundles less cytotoxic then SWCNT µm-	(Wick et al. 2007)

cell line MSTO-211H	- 3 days	agglomerations - SWCNT-bundles of 10-20 single tubes vs rope-like agglomerates of µm size	sized agglomerates based on cell activity and cell proliferation.	
Skin				
Human skin fibroblast	- 0.6 and 0.06 µg/ml - 48h	- MWCNT and MW onion	- Induced cell cycle arrest - Increased apoptosis/necrosis - MWCNT activated: cellular transport, metabolism, cell cycle regulation, and stress response genes. - Strong immune and inflammatory response: interferon and p38/ERK-MAPK cascades induced	(Ding et al. 2005)
Human keratinocytes HaCaT	- 0.1 -20 µg/ml - 24, 48 and 72h	- SWCNT dissolved in DMF	- Dose dependent activation of NF-kB due to the activation of stress related kinases - SWCNT increased free radicals - Decreased cell activity - Increased cell apoptosis	(Manna et al. 2005)
Human epidermal Keratinocytes	- 100, 200, 400 µg/ml - 2, 4, 6, 8, 12, and 24h	- Chemically unmodified MWCNT - TEM analysis	- Release of IL-8 in a time dependent manner - Vacuoles uptake of MWCNT with up to 3.6 µm length	(Monteiro-Riviere et al. 2005)
Human dermal	- 0.2 – 2000 µg/ml	- SWCNT-phenyl-SO_3H	- With an increasing degree of sidewall functionali-	(Sayes et al.

Fibroblast	- 48h	- SWCNT-phenyl-SO_3Na - SWCNT-phenyl-$(COOH)_2$ - SWCNT suspended in 1% Pluronic F108 - SWCNT-phenyl-SO_3H with increasing degree of functionalization	zation, a decrease in SWCNT sample cytotoxicity was observed	2006)
Keratinocyte epithelial cell line HaCaT	- 60, 120, 240 µg/ml - 2, 4, 6, 8, and 18h	- Nitric and perchloric acid purified SWCNT - Suspended in KGM basal medium	- Dose dependent decrease of the cell viability - Oxidative stress, antioxidant depletion - Morphological changes of the cells	(Shvedova et al. 2003)
Neuron				
Hippocampal neurons	- Coated glass coverslips - 3 days	- Polyethyleneimine f-SWCNT - Unmodified MWCNT	- SWCNT – PEI promoted neurite outgrowth and branching	(Hu et al. 2005)
Hippocampal neurons	- Coated glass substrate - 8, 9, 10 days	- DMF suspended MWCNT (coated on glass and incubated at 350°C under nitrogen atmosphere)	- Growth of neuronal circuits on a MWCNT grid was accompanied by a significant increase in network activity	(Lovat et al. 2005)
Hippocampal neurons	- Coated glass coverslips - 8, 9, 10 days	- MWCNT	- Spontaneous synaptic activity and firing of hippo-campal neurons were increased by MWCNT substrate - Number of neurons and neurites per cell not affected	(Ni et al. 2005)

Hippocampal neurons;	- Coated glass coverslips - 3 days	- MWCNT–COOH - MWCNT-ethylendiamine - MWCNT-poly-m-aminobenzene sulfonic acid	- Branching of neurites showed graded dependency of MWCNT charge in the order: MWCNT-EN > MWCNT-PABS > MWCNT-COOH	(Hu et al. 2004)
Diverse				
- Human osteoblastic cell line hFOB 1.19, - Human fibroblastic line HS-5 - Mouse peritoneum macrophages	- Cells cultivated on polysulfone embedded MWCNT - 24h, 48h and 7 days	- CVD MWCNT catalysed by CoO/MgO - Co = 2 wt%	- No decrease in fibroblast, and osteoblastic cell viability - No release of IL-6 by fibroblasts, - No increase of osteocalcin by osteoblastic cells - No activation of the macrophages - No release of free radicals	(Chlopek et al. 2006)
Human umbilical vein endothelial cells (HU)	- 5 cm2 surface equals 0.5 – 0.9 µg/ml - 3 days	- 3 different DoubleWalled CNT - Co and W catalysts	- No cytotoxicity found for any sample	(Flahaut et al. 2006)
Human T cells Jurkat and peripheral blood lymphocytes	- 40 and 400 µg/ml (corresponds to 1 ng/cell, 10 ng/cell) - 24, 48, 72, 96, 120h	- MWCNT of > 95% purity (Newton, MA, USA) - Nitric acid oxidized MWCNT (Newton) - Carbon black (Cynthiana, KY, USA)	- Oxidized MWCNT killed > 80% of cells within 5 days - Pristine MWCNT killed less than 50% of cells	(Bottini et al. 2006)

Chinese hamster ovary (CHO) cells	- 100 µg/ml - 3 days	- SWCNT coated with poly(methyl) vinyl ketone as backbone and decorated with α-N-acetylgalactosamine residues - Unmodified SWCNT	- Coated SWCNT were non-toxic, - Unmodified SWCNT induced cell death	(Chen X et al. 2006)
Human embryo kidney cells (HEK293)	- 0.78 – 200 µg/ml - 1, 2, and 5 days	- SWCNT from Carbon Nanotechnologies Inc. dissolved in 0.5% DMSO	- Concentration and time dependent decrease in cell proliferation and cell adhesion - Provoked secretion of 20-30kDa proteins - 25 µg / ml SWCNT induced G1 arrest - Cell apoptosis - Up-regulation of cell cycle associated genes - Down regulation of signal transduction-associated genes and adhesion associated proteins	(Cui et al. 2005)
Mammalian HeLa cells	- 1.5 – 2.0 µg/ml - 24h	- SWCNT-LC-SPDP - SWCNT-PL-PEG with DNA or siRNA1 - SWCNT-PL-PEG-SS with DNA 15mer or siRNA	- f-SWCNT transported various biological molecules inside living cells with no-ill effect on cell viability and proliferation - Uptake was mediated by endocytosis	(Kam and Dai 2005)
HeLa cells	- 2.5 -5.0 µg / ml	- Purified Hipco SWCNT	- Cy3-DNA-SWCNT were internalized and accumulated	(Kam et al.

	- 1 – 5h	- Cy3-DNA-SWCNT - PL-PEG-FA-SWCNT	in the cells around the nucleus - Only folate receptor+ cells incorporated PL-PEG-FA-SWCNT	2005)
Chinese hamster ovary (CHO) cells	- 5mM - immediate analysis	- f-SWCNT (2-aminoethylmethanethiosulfonate) - unfunctionalized SWCNT (u-SWCNT)	- Inhibiting the current flow of HCN2 channels - Reversible blocking of the channel by u-SWCNT - Irreversible blocking by f-SWCNT	(Chhowalla et al. 2005)
Non activated mouse splenocytes	- 0.005, 0.05, 0.5 and 5 µg/ml - 3 days	- Cationic f-SWCNT with one specific ODN immunostimulatory CpG motif	- No toxic effects of SWCNT on mitogen activated and nonactivated mouse splenocytes observed	(Bianco 2004)
- Human promyelocytic leukemia (HL60) cells - Human T cells Jurkat	- 10 – 200 µg/ml - 24h, 48h	- SWCNT refluxed in 2.5 M HNO3 and separated by cup-horn sonication - Carboxylated SWCNT functionalized with EDC fluorescein, EDC biotin LC-PEO-amine, and fluoresceinated streptavidin. - Zeta potential measured	- Uptake: time (0.05 mg/ml over 5h) and concentration dependent	(Kam et al. 2004)
- Mammalian HeLa cells - Chinese hamster	- 2500 µg/ml - 1h – 3h	- Ammonium f-SWCNT and f-MWCNT	- Both f-CNT traversed cell membranes - 1h incubation then epoxy resin fixed. - No toxic effect described	(Pantarotto et al. 2004)

ovary cells (CHO)