Status of characterization techniques for carbon nanotubes and suggestions towards standards suitable for toxicological assessment

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Status of characterization techniques for carbon nanotubes and suggestions towards standards suitable for toxicological assessment

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Abstract. Nanotechnologies promise to contribute significantly to major technological challenges of the upcoming century. Despite profound scientific progress in the last decades, only minor advances have been made in the field of nanomaterial toxicology. The International Team in Nanosafety (TITNT) is an international and multidisciplinary group of scientists, which aims at better understanding the risks of nanomaterials. Carbon nanotubes (CNT) account for one of the most promising nanomaterials and have therefore been chosen as representative material for nanoscaled particles. They are currently investigated by the different platforms of TITNT. As a starting point, the present report summarizes a literature-based study on the physico-chemical properties of CNT, as they are closely linked with toxicological properties. A brief introduction to synthesis, purification and material properties is given. Characterization methods for CNT are discussed with respect to their reliability and the information content on chemical properties. Recommendations for a set of standard characterizations mandatory for toxicological assessment are derived.

1. Introduction

Nanotechnologies are denoted to become key technologies of this century. Their cross-disciplinary character has the potential to change everyday life to a dramatic extent. Despite profound scientific and technological advances in the recent decades, only minor progress in understanding fundamental aspects of the toxicity of nanomaterials has been made [1-3]. Considering the large number of nanomaterial applications reaching the markets [4-6] significant progress in the field of nanomaterial release, environmental exposure and the toxicology [7] becomes more and more urgent.

TITNT (The International Team in Nanosafety) is an international and multidisciplinary team of scientists focusing on the investigation of potential risks of nanomaterials by assessing toxicity and exposure. Carbon nanotubes (CNT) were selected as representative class of nanoparticles (NP), as numerous applications in consumer products are under development and already surpassed the multi-ton production scale [8]. CNT are of particular interest as their toxicity is discussed very
controversially. On the one hand, there is the belief that CNT could behave toxicologically similar to asbestos [7,9,10] due to a comparable aspect ratio and physical resemblance, on the other hand, some studies indicate low toxicity [11-15].

The determination of characteristic physico-chemical properties with appropriate characterization techniques is generally accepted as a mandatory basis for any investigation on toxicological properties of nanomaterials. Due to an excess in surface-to-volume ratio for nano-scaled materials, biological interactions can also depend on physico-chemical properties, which are conventionally not considered in toxicological screenings of materials [16].

The present report summarizes results of a literature study on the physico-chemical properties of CNT and suitable characterization techniques. Both multi-walled and single-walled CNT were considered, as recommended in the literature [3,17]; however the study was limited to pristine CNT. Briefly an introduction to the synthesis and purification of CNT, as well as physico-chemical features limited to CNT are given. Applicable characterization techniques are ranked according to their information content. The report concludes with a recommendation for a set of standards and suitable characterization techniques for CNT with prospect towards later testing of toxicological properties.

2. Physico-chemical properties of CNT
Graphite nanofibres and nano-scaled filaments have long been known [18-19] before, in 1991, the observations of CNT by Iijima et al. raised interest in structural aspects of such materials [20]. Analysis by means of transmission electron microscopy (TEM) and electron diffraction allowed for structural characterization. Progress in large-scale synthesis of CNT and synthesis of SWCNT with well-defined morphology in 1993 [21] enabled further, more detailed investigations [22].

2.1. Synthesis methods

2.1.1. Carbon arc discharge (CA). Nanotubes were first observed in a DC electric arc discharge during the synthesis of fullerenes. The MWCNT grow at the cathode surface; the highest possible yield of CNT and other NPs is in the range of 25 % of the evaporated anode material. Obtaining samples with high quality is dependent on various parameters such as the current density, cooling or the stability of the formed plasma. The CNT are distributed bi-modal in bundles of 10 to 100 tubes perfectly aligned with nearly the same length [20,22-24]. Using additionally 1-10 % by weight of catalyst (i.e. Co, Ni), also SWCNT can be produced via arc discharge, providing yields of 75 % and more [21]. Occurring impurities of this process are carbon NPs, glassy carbon, amorphous carbon, and catalyst particles, making subsequent purification challenging and resulting in low yields (1%) [5,23].

2.1.2. Chemical vapour deposition (CVD). The classic CVD method is based on heating catalytic particles of transition metals (Co, Fe, Ni, etc.) in a furnace and flowing hydrocarbon gas through the reactor; thereby the catalytic decomposition of hydrocarbons as carbon sources towards production of MWCNT is achieved [3,25-26]. The uniformity in length and diameter is controlled by the catalyst particle size and its thermal stability. Presently, in most cases, CNT from CVD need to be size-selected and purified further, as the process may deposit substantial amounts of amorphous carbon on the surface of the tubes [4,27] and catalyst metals may be incorporated. The diversity of different CVD methods is well described in [28]. A modified process, the so called High-pressure CO (HiPCO) decomposition is based on the gas-phase growth from CO as carbon source (hereby the iron carbonyl complex Fe(CO)5 serves as catalytic precursor). It is reported to give CNT samples with highest yields and remarkable purity (97% for SWCNT) and even has been refined to a commercialized process [29-30].

2.1.3. Pulsed-laser vaporization (PLV). Another way to produce exclusively SWCNT in high yields (over 80%) is using a laser evaporation technique, where a laser evaporates graphite electrodes with
small amounts of transition metals (Co, Ni etc.) at temperatures of 1200 °C and under helium atmosphere [24]. The condensed material is collected at a water-cooled target (i.e. a copper rod) [27].

2.1.4. Comparison, limitations and potential of methods. The extreme sensitivity of the nanotube’s structure-property relations are a major challenge for the synthesis of CNT with controlled diameter, length, and chirality [26,31]. Gas phase techniques such as CVD possess the largest scale up potential for CNT production [27-28]. It can be concluded that depending on the requirements on the CNT properties, different methods must be balanced with respect to advantages and disadvantages since no optimal synthesis strategy for all types of CNT exists. With respect to toxicological studies, the most important aspects beside the CNT morphology and chemical functionality are the purity of the CNT sample and possible structural changes induced during purification.

2.2. Purification Methods
In general, all CNT materials prepared by the methods mentioned above contain impurities. Since both, amorphous carbon and metal impurities are of great toxicological concern [32], the purification of CNT materials is crucial for the acceptance of this new class of materials. Typical impurities consist of catalyst particles and of carbon in different modifications such as amorphous carbon and non tubular fullerenes [27,33-34]. The methods of purification can be divided into three categories, namely physical, chemical, and a combination of both as briefly summarized in the following as well as in reference [33].

2.2.1. Chemical purification. Chemical purification processes either focus on the removal of carbon or metal and metal oxide impurities. The carbon-removing techniques base on chemical oxidation by thermal or plasma gas phase oxidation [35], liquid phase oxidation [36], or electrochemical oxidation and rely on the fact that impurities like soot, fullerenes, of amorphous carbons [23] usually exhibit lower oxidation resistance than CNT. For effective removal of impurities, these oxidation techniques may be accompanied by considerable loss of CNT and oxidative opening of CNT endcaps [4,35]. Especially during liquid oxidation, functional groups may be created or oxidized debris may attach to CNT [37], which may affect the chemical reactivity and toxicological relevance of purified CNT [24]. Wet-chemical conversion of metallic impurities to water-soluble salts by mineral acids, which is the most important technique to remove metals and metal oxides from catalysts and their support, may profit from a preceding oxidative treatment to open the shell of carbon-encapsulated metallic particles that may prevent their dissolution. Since metal impurities, such as Co or Y are of great toxicological concern, effective purification processes are crucial to exclude possible effects of those metals.

2.2.2. Physical purification. Physical purification methods are based on a separation of CNT from impurities by means of physical property differences, both, among CNT, and between CNT and impurities, i.e. aspect ratio, size, solubility, gravity, magnetism, evaporation temperature etc. The methods include filtration, chromatography, centrifugation [24], field flow fractionation, electrophoresis, magnetophoresis [38], as well as high temperature annealing [31,39]. Physical purification methods may allow preserving the intrinsic structure of CNT. Unfortunately, most of the techniques, except high-temperature annealing, are not very effective and require the CNT to be in a well-dispersed form, which changes their degree of entanglement and may shorten them during mechanical disentangling.

2.2.3. Multi-step purification. Only a combination of chemical and physical methods allows optimizing the purification in a way to obtain a high-purity, predominantly undamaged CNT sample under mild conditions. Since the morphology, content and structure of impurities is strongly related to the synthesis technique, no standardized method exists. Depending on material structure and purity, sample-specific optimization is necessary of efficient purification [33,40].
2.2.4. Conclusion and challenges. First of all, a precise definition of the term purity has to be made, since it may depend on the point of view. It can either relate to the impurity content or to the structure homogeneity of CNT samples [33]. Another common problem is that most purification methods known are tedious, low in yield, and result in damaged CNT [31]. Therefore the additional use of size exclusion chromatography and cascade filtration could be useful. However, such diameter-, length-, or chirality-related purifications steps are not easily scaled up yet [4]. Nevertheless, for some SWCNT, efficient multi-step purification methods leading to highly pure samples have been developed and are investigated for scale-up [41]. Summarizing, effective purification requires synthesis type or even batch dependent optimization of several purifications steps involving different techniques. There is a need for a CNT purity assessment standard for evaluating and systematically improving methods of purification [33].

2.3. Properties and particularities

2.3.1. General aspects. In a CNT each carbon atom is bonded to three neighbouring atoms through $sp^2$ hybridization to form a closed tubular shell of helical structure [23]. In SWCNT, every atom position is precisely determined and the periodicity in the lattice can be reduced to a unit cell type, allowing for predictions of its properties. CNT possess superior thermal properties with high thermal conductivity and the high stability of graphitic materials of up to 2800 °C in vacuum. From an electronic point of view, SWCNT can be considered as 1-dimensional conductive structure [23]. Due to the one-dimensional nature of the conduction electron states in CNT, well-spaced and symmetric structures, so called van Hove singularities appear in the density of states (DOS) of nanotubes [42]. They can be detected with scanning tunnelling microscopy (STM) and UV/optical spectroscopy. Doping of CNT allows controlling their electronic conductivity. Boron-doped CNT exhibit exclusively metallic conductivity, whereas the properties of un-doped CNT vary between metallic and semiconducting depending on chirality [43]. The length and diameter of CNT vary significantly depending on the synthesis method [34].

2.3.2. Chirality. CNT can be thought as being made of a sheet of graphite (graphene) wrapped into a cylinder, so that the long edges match and form a seamless structure. However, there are several directions of rolling of a sheet, which all lead to matching of edges. Translational shifts along the edges will lead to different helical orientation of carbon atoms along the tube and thus introduce the concept of chirality. The chirality parameters, called chirality indices, describe the deviation of from parallel to normal orientation of the ring arrays of the honeycomb structure with respect to the tube axis. Two extremes confirmations of CNT can be easily distinguished: The so-called «armchair» and «zigzag» confirmations with chiral angles of 30° and 0°, respectively [27]. Between these extremes, CNT with various degrees of helicity can be found [23]. A more detailed description of the concept of helicity including the definition by the Bravais lattice vectors is given in references [4,24,44]. A nanotube’s chirality characteristically determines the electronic, optical, and phononic properties of CNT [27]. For a single perfect SWCNT, the chirality indices ($n$, $m$) and the length completely determine the nanotube as a macromolecule. For MWCNT the situation is more involved since different layers may show different chirality. The determination of the chiralities of MWCNT is a complex problem [45-46].

2.3.3. Tube ends. Depending on synthesis and purification method, CNT can exhibit closed tube ends [20,22]. For a fullerene-like hemisphere, 6 pentagons are necessary to close the tube [24], however defects and faceted CNT walls allow for alternative configurations of the tube caps. The curvature of the caps reduces their thermal stability and enhances the reactivity of CNT. Closed SWCNT can, for instance, be opened easily by heating in air or oxygen at 750 °C.
2.3.4. **Material Variability.** Variation of the chirality indices $n, m$ together with CNT length lead to an infinite manifold of different SWCNT [26]. Coaxial stacking of SWCNT to MWCNT further enhances the spectrum of ideal CNT structures, whereas real-world materials exhibit defects, structure irregularities, and impurities, which make the material spectrum even broader. As a consequence, defective, i.e. real-world CNT require many parameters for a comprehensive characterization. For ensembles of defective CNT, such characterization requires determination of particle property distributions. For toxicological studies of CNT materials, it has to be considered that structural complexity of ensembles of defective CNT cannot be comprehensively investigated by contemporary analytical techniques.

2.3.5. **Solubility and dispersibility.** Both pristine SWCNT and MWCNT show poor dispersibility in polar media like water and many organic solvents, imposing limitations towards their applications [47]. CNT exhibit a strong tendency to aggregate in bundles or ropes, due to easy polarizability and high molecular weights which promote dispersive interaction forces [48]. The different strategies to overcome the problem of poor dispersibility can be divided into covalent and non-covalent approaches. The non-covalent approach uses surfactants which only weakly interfere with the electronic CNT structure [49]. Also wrapping of CNT by polymers, peptides, or DNA can help to suspend CNT [49-50]. The covalent approaches involve the functionalization of CNT, for instance by controlling the oxidation degree of the CNT, and affect their electronic properties. Many different functionalities like peptides, acid or amino groups, or polymers have been successfully bound covalently to the sidewalls of CNT [48]. Uniform dispersion of entangled or agglomerated CNT in solvents generally requires techniques that allow exerting high shear forces to the mixture. If disentangling is unavoidable, the method will have to break and shorten tubes. The energy required for CNT dispersion can for instance be provided by ultra sonic baths or probes, high pressure-spraying, and roller mills. Solvents adapted to the CNT functionalization or surfactant chemistry are required to obtain stable suspensions [7,48,51].

3. Conclusion and recommendation
Based on the previous sections, materials selection and characterization standards are suggested that should be applied to CNT materials that are subjected to toxicological testing. For this, the different techniques of production, purification, and characterization will be reviewed to derive guidelines for method selection.

3.1. General aspects on sample selection and testing requirements
For toxicological studies aiming at investigating effects characteristic for CNT, specially tailored samples of high purity, structural uniformity, and ensemble homogeneity should be chosen and be characterized physico-chemically as comprehensively as possible.

The alternative aim to generally assess the toxicology of “real-world” CNT materials should be based on materials from industrial mass production that should likewise be characterized as comprehensively as possible. It is essential that all available information on producer, production process, batch number, storing conditions, and sample age should be compiled.

A part of the sample should be stored under dark, dry and inert conditions for possible future studies.

3.2. Production
For the sake of relevance and comparability of toxicological testing, CNT materials from well-established or highly reproducible production processes should be selected. In order to facilitate the identification of CNT-related characteristics, emphasis should be put on sample uniformity and material purity. For SWCNT, the HiPCO process evolved to a well-established, commercialized process of high structural uniformity and mature purification strategies for remaining catalyst metals [28-30]. Commercial HiPCO materials may be of environmental relevance. However, CVD synthesis
techniques for SWCNT are rapidly evolving in structure and chirality control. They show a general advantage in growth efficiency, leading to lower metal impurity content, especially for fluidized-bed and for vertically-aligned CVD synthesis of SW- and MWCNT with subtracted catalysts [33]. CNT from fluidized bed synthesis contemporarily suffer from curling and entangling due to CNT by agglomerate collisions, whereas CNT vertically-aligned CVD synthesis are straight and more facile dispersed. MWCNT from CVD meanwhile can reach good uniformity in size and very high length but may suffer from amorphous carbon adsorbents [4,16,21]. MWCNT from CA may show a high degree of crystallinity and straightness that can generally be obtained by CVD synthesis only after a graphitization step [26-27]. Since the CVD method is highly suitable for mass production, the relevance of CNT from CVD for emission scenarios is higher and may justify the costs of toxicological investigations [31]. For an extended toxicological study it could be interesting to study effects of different synthesis methods, which highly affects type and amount of impurities as well as length and diameter distributions etc. [52].

3.3. Purification
For both SWCNT and MWCNT a purification procedure should be used which is tailored to the later requirements of the sample. It should be based on a multi-step purification protocol, taking advantage of both chemical and physical methods [40,33]. A few aspects have to be taken into account while choosing a purification protocol. It is important to obtain samples with lowest possible metal content, since in many cases due to inadequate assessment, the observed toxicity indices may reflect these byproducts or residues rather than the primary material structure [53,15-16]. Furthermore, the following characteristics of CNT have been reported to be altered due to modifications induced by post synthesis treatments, including: absorptive nature, aspect ratio, surface reactivity, hydrophilic properties and surface functionalization [53]. Thus, since purification also destroys CNT, the removal of impurities must be balanced against the introduction of defects into tubes [34].

3.4. Metal and amorphous carbon impurities
As mentioned previously, metal and amorphous carbon impurities can be of higher toxicological relevance than CNT-related properties. Frustrated phagocytosis in macrophages resulting from the physical CNT properties may be of higher relevance than chemical composition of fibres [9]. The relevance of radicals, oxidative potential, as well as the chemical functionality of the CNT on the reactivity as well as on the dispersibility must not be underestimated since both affect the bioavailability of CNT. Especially, the role of CNT surface area, surface functionality and polarity in immobilization of nutrients and biomolecules deserves special attention in cell tests, since serum depletion by contact to nanoparticles of large surface area has been reported. Cross checks with supernatants of centrifuged serum-CNT dispersions are considered indispensible.

3.5. Dispersion
Dispersion of CNT materials for toxicological testing is an almost philosophic problem: Are fundamental interactions of high-purity CNT and cells or organs in focus, which may require fully-dispersed CNT; or is a realistic exposure scenario planned, which may require un-purified industrially-produced CNT with a dispersion degree adapted to the emission source?

3.6. Commonly applied characterization techniques
Description of analytical capabilities of characterization techniques for CNT would exceed the scope of this work, therefore the reader is referred to the literature.
Figure 1 shows a statistic analysis of methods for CNT mentioned in 58 articles consulted for this work, excluding books and reviews. The presented characterization techniques can also be sorted according to their information content on the physico-chemical properties of CNT. The following metrics, covering the information from all techniques, can be extracted: size (aspect ratio, distribution), shape, surface area (area/ mass ratio), composition (element analysis), surface chemistry
(functionalization), crystallinity, agglomeration (dispersion), porosity, heterogeneity, stability (thermal), impurities, defects, solubility, chirality and conductivity.

![Figure 1](image)

**Figure 1.** Number of mentions of technique per reviewed literature, including 58 consulted papers with mentions of at least one of the stated techniques

3.7. Literature recommendations for characterization methods

Literature suggestions on minimum requirements for the characterization of CNT samples are compiled in Table 1.

<table>
<thead>
<tr>
<th>Source</th>
<th>Metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safadi et al. [54]</td>
<td>density, diameter, length, aspect/ratio, modulus.</td>
</tr>
<tr>
<td>Müller et al. [53]</td>
<td>surface area, size, composition, shape, porosity, surface, dispersion.</td>
</tr>
<tr>
<td>Yang et al. [55]</td>
<td>surface area, pore volume, surface chemistry, structural defects, fraction of open ends.</td>
</tr>
<tr>
<td>Hurt et al. [15]</td>
<td>element analysis, surface area, morphology (aspect ratio, secondary carbon forms, metals location), functionalization, crystallinity, defects.</td>
</tr>
<tr>
<td>Arepalli et al. [52]</td>
<td>homogeneity, purity, thermal stability.</td>
</tr>
<tr>
<td>Oberdörster et al. [16]</td>
<td>size (distribution), agglomeration, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, porosity, particle number</td>
</tr>
</tbody>
</table>

3.8. Suggestions for combinations of characterization methods

Considering the fact that previously acquired data on CNT material properties must be comparable at least with reference to the measurement method, the following recommendations can be made. Raman spectroscopy, SEM, and particularly TEM can be considered as base techniques for CNT characterization. Additionally, TGA, UV-Vis/NIR, and XPS are necessary assets for a complete characterization. ED and EDX/EDS are usually included in modern TEM devices and may thus be available without additional instrumentation. Local probe techniques that characterize CNT on an individual basis such as AFM, STM, and STS have not been included here, as they are not applicable for a routine characterization of CNT.

Additionally, the capabilities of the different analytical techniques and the requirements from the literature regarding the minimum necessary metrics from Table 1 have to be matched. A complete characterization will be impractical, therefore a consensus on the minimum set of characteristics is
necessary. The set should also comply with the condition imposed by Oberdörster et al. that the set has to comprise information on all potentially significant material characteristics of CNT. This should enable even a retrospective interpretation of toxicological datasets in the light of new findings [16]. In the following Tab. 2, a recommendation for a set of characterization techniques which balances the different requirements and the feasibility of the method [16,33] is given, sorted by relevance of the method.

Table 2. Recommendation for a set of CNT characterization techniques for toxicological studies. Quantitative information is marked by *, important characteristics are typeset in bold.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM (EDS/EDX; ED)</td>
<td>diameter*, length*, agglomeration, composition, impurities, surface contaminations, uniformity, crystallinity, chirality</td>
</tr>
<tr>
<td>SEM (EDX)</td>
<td>diameter*, length*, agglomeration, composition, impurities, surface contaminations, uniformity</td>
</tr>
<tr>
<td>Raman</td>
<td>chirality, diameter*, crystallinity*; graphitization degree vs. structure defects</td>
</tr>
<tr>
<td>TGA</td>
<td>stability*, composition*, uniformity*, purity*, amorphous carbon surface contaminations*, surface chemistry</td>
</tr>
<tr>
<td>BET</td>
<td>porosity*, surface area*</td>
</tr>
<tr>
<td>XPS</td>
<td>composition*, surface chemistry*, impurities*, stability</td>
</tr>
<tr>
<td>UV/Vis-NIR</td>
<td>agglomeration, size, uniformity, conductivity</td>
</tr>
</tbody>
</table>

3.9. Aging of CNT
The characterization of CNT is additionally complicated by aging phenomena. It has been reported that aging of CNT occurs with respect to surface area and pore volume, surface oxygen, and structural defects. It has therefore been suggested that physico-chemical properties can be reliably characterized only after a quasi-stable conditions were obtained, which typically occurs after storing for 9-15 month [55]. Consequently, the post-synthesis stability, details of storage conditions like humidity, temperature, exposure to light, and atmosphere conditions, and aging of materials under non equilibrium and under ambient conditions [15] is an important issue, which has to be addressed and investigated prior to subjecting CNT samples extended toxicological studies [16].

4. Sample proposition and summary
Concluding the above mentioned, the following set of CNT samples should be promoted to a standard set of materials for toxicological studies. The aim is to allow correlation of toxicological data generated from future studies [48]. The ideal CNT samples should exhibit a mono-modal distribution in length and diameter, a uniform chemical functionalization and crystal structure [56].

The following set of standard CNT should be prepared, fulfilling the aforementioned requirements:
1. MWCNT from CVD process; as prepared
2. MWCNT from CVD process; optimized purification and uniform aspect-ratio (length, diameter)
3. SWCNT from HiPCO process; as prepared
4. SWCNT from HiPCO process; optimized purification and uniform aspect-ratio

Prior to toxicological testing, the samples should additionally be characterized with respect to
1. Aging behaviour of samples, until the samples remain stable (9-15 month)
2. Dispersion quality and stability of samples in aqueous solutions [51].
Minimum characterization requirements for of material properties have been presented in table 2 and include TEM, EDX, ED, Raman, SEM, TGA, BET, UV/Vis-NIR and XPS.
Additionally, for a complete assessment of the toxicological effects of CNT, tubes from different processes and different manufacturers could be investigated [48], however, this will most certainly go beyond the scope of capabilities and possibilities.

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