

# CARBON NANOTUBES

---

## 1. Exposure Data

The Working Group limited the scope of this *Monograph* on carbon nanotubes (CNT) to engineered/manufactured CNT, on the basis of three issues.

The Working Group recognized that co-exposure to CNT and carbon nanofibres (CNF) could arise, because CNF may be generated as impurities during the synthesis of CNT. However, CNT and CNF are usually produced separately. Of 11 studies in the workplace, only one reported the use of both CNT and CNF at one secondary manufacturing facility that produced composite materials.

The Working Group did not consider the use of CNT that are specifically designed for medical purposes, for which human exposures have not yet been described.

While the existence of naturally and incidentally occurring CNT has been acknowledged, the physico-chemical properties and biological reactivity of CNT in the general atmosphere are unknown.

### 1.1 Chemical and physical properties

#### 1.1.1 Nomenclature and general description

Although single-walled (SWCNT) and multiwalled (MWCNT) CNT were discovered in 1991 ([Iijima, 1991](#)), only one Chemical Abstracts

Service number, 308068-56-6, has been given to reference CNT to date; however, this number is not representative of all CNT because of great variations in the size and other characteristics of the tubes. In addition, with regard to nomenclature, CNT and CNF are often discussed together.

[Table 1.1](#) gives the most common definitions of CNT and other parameters related to nanomaterials. According to the International Organization for Standardization (ISO), CNT are defined as “nanotubes composed of carbon. CNT usually consist of curved graphene layers, including single-wall CNT and multiwall CNT” and CNF are defined as “nanofibres composed of carbon.” A nanofibre is described as a “nano-object with two similar external dimensions in the nanoscale and the third dimension significantly larger. A nanofibre can be flexible or rigid. The two similar external dimensions are considered to differ in size by less than three times and the significantly larger external dimension is considered to differ from the other two by more than three times. The largest external dimension is not necessarily in nanoscale” ([ISO, 2008](#)).

As a working definition for this *Monograph*, to counteract any potential confusion between the ISO definitions, we used the following differentiation between CNT and CNF proposed by [Kim et al. \(2013\)](#): “The geometry of CNF is different from the CNT containing an entire hollow core, because they can be visualized as regularly stacked truncated conical and planar layers along the filament length” (see [Fig. 1.1](#)).

**Table 1.1 Definitions relevant to nanomaterials and carbon nanotubes**

Term	Definition	Reference
Agglomerate	Collection of weakly bound particles or aggregates or mixtures of the two, in which the resulting external surface area is similar to the sum of the surface areas of the individual components Note 1: The forces holding an agglomerate together are weak, for example, van der Waals forces, or simple physical entanglement Note 2: Agglomerates are also termed secondary particles, and particles from the original source are termed primary particles	<a href="#">ISO (2008)</a>
Aggregate	Particle comprising strongly bonded or fused particles, in which the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components Note 1: The forces holding an aggregate together are strong, for example, covalent bonds, or those resulting from sintering or complex physical entanglement Note 2: Aggregates are also termed secondary particles and particles from the original source are termed primary particles	<a href="#">ISO (2008)</a>
CNF	Nanofibre composed of carbon	<a href="#">ISO (2010b)</a>
Carbon nanohorn	Short and irregular shaped CNT with a nanocone apex Note: In general, hundreds of carbon nanohorns constitute an aggregate nanoparticle	<a href="#">ISO (2010b)</a>
Carbon nanopeapod	Linear array of fullerenes enclosed in a CNT Note: This is an example of a composite nanofibre	<a href="#">ISO (2010b)</a>
Carbon nanoribbon	Nanoribbon composed of carbon Note: Carbon nanoribbons are often in the form of multiple layers of graphene. In the case of a single graphene layer, the term “graphene ribbon” is used	<a href="#">ISO (2010b)</a>
CNT	Nanotube composed of carbon Note: CNT are generally comprised of curved graphene layers, including single-walled (SWCNT) and multiwalled (MWCNT)	<a href="#">ISO (2010b)</a>
Chiral vector of SWCNT	Vector notation used to describe the helical structure of a SWCNT	<a href="#">ISO (2010b)</a>
Coating	Non-covalent surface modification Note: Coating includes Pluronic F108, Tween-80, or polyethylene glycol phospholipid	<a href="#">Ali-Boucetta &amp; Kostarelos (2013)</a>
Cup stack CNT	CNT composed of stacked truncated graphene nanocones Note: These are completely different from SWCNT or MWCNT in structure. The open top and bottom edges of truncated graphene nanocones appear on the inner and outer surfaces of the nanotube, respectively	<a href="#">ISO (2010b)</a>
Doping	Physical alteration of the surface of CNT with ions or molecules using weak forces such as van der Waals	
Double-walled carbon nanotube (DWCNT)	MWCNT composed of only two nested, concentric SWCNT Note: Although this is a type of MWCNT, its properties are rather closer to SWCNT	<a href="#">ISO (2010b)</a>
Engineered nanomaterial	Nanomaterial designed for a specific purpose or function	<a href="#">ISO (2010a)</a>
Fullerene	Molecule composed solely of an even number of carbon atoms, which form a closed cage-like fused-ring polycyclic system with 12 five-membered rings and the remainder as six-membered rings	<a href="#">ISO (2010b)</a>

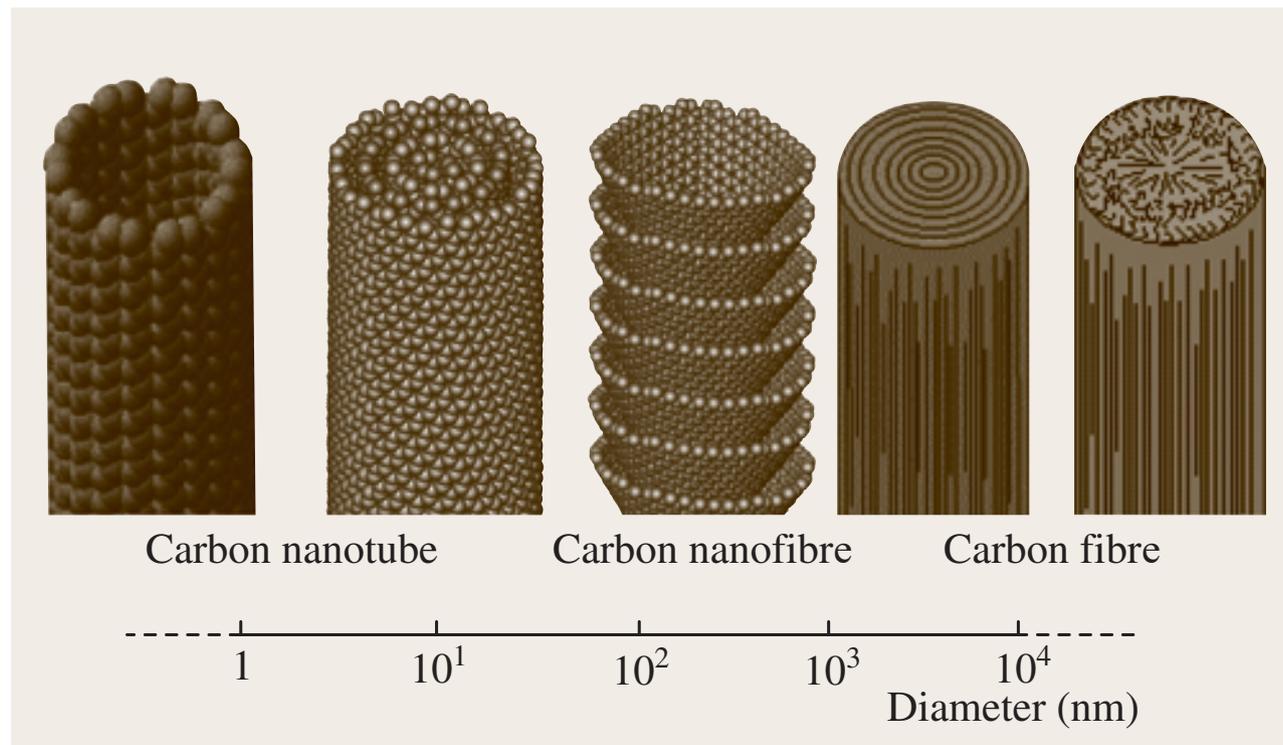
Table 1.1 (continued)

Term	Definition	Reference
Functionalization	Covalent modification of CNT Note: Covalent functional groups include hydroxyl, ammonium, glucosamine, taurine, diethylenetriaminepentaacetic acid, and 2-(4-isothiocyanoatobenzyl)-1,4,7,10-tetraazacyclodecane	<a href="#">Singh et al. (2006)</a> , <a href="#">Lacerda et al. (2008a, b)</a> , <a href="#">Ali-Boucetta &amp; Kostarelos (2013)</a>
Graphene	Single layer of carbon atoms with each atom bound to three neighbours in a honeycomb structure Note: This is an important building block of many carbon nano-objects	<a href="#">ISO (2010b)</a>
Graphite	Allotropic form of elemental carbon comprised of graphene layers stacked in parallel to each other in a three-dimensional, crystalline, long-range order Note 1: Adapted from the definition in the IUPAC <i>Compendium of Chemical Terminology</i> Note 2: Two allotropic forms exist with different stacking arrangements: hexagonal and rhombohedral	<a href="#">ISO (2010b)</a> , <a href="#">IUPAC (2014)</a>
Graphite nanofibre	Carbon nanofibre composed of multilayered graphene structures Note: Graphene layers can have any orientation with respect to the fibre axis without long-range order	<a href="#">ISO (2010b)</a>
Incidental nanomaterial	Nanomaterial generated as an unintentional by-product of a process Note: The process includes manufacturing, biotechnological, or other processes	<a href="#">ISO (2010a)</a>
Manufactured nanomaterial	Nanomaterial intentionally produced for commercial purposes with specific properties or composition	<a href="#">ISO (2010a)</a>
MWCNT	CNT composed of nested, concentric or near-concentric graphene sheets with interlayer distances similar to those of graphite Note: The structure is normally considered to be many SWCNT nesting within each other, and would be cylindrical for small diameters but tends to have a polygonal cross-section as the diameter increases	<a href="#">ISO (2010b)</a>
Nanocoone	Cone-shaped nanofibre or nanoparticle	<a href="#">ISO (2010b)</a>
Nanofibre	Nano-object with two similar external dimensions in the nanoscale and the third dimension significantly larger Note 1: A nanofibre can be flexible or rigid Note 2: The two similar external dimensions are considered to differ in size by less than threefold and the significantly larger external dimension is considered to differ from the other two by more than threefold Note 3: The largest external dimension is not necessarily in the nanoscale	<a href="#">ISO (2008)</a>
Nanomaterial	Material with any external dimension in the nanoscale or having an internal structure or surface structure in the nanoscale Note: This generic term is inclusive of nano-objects and nanostructured material	<a href="#">ISO (2010a)</a>

**Table 1.1 Definitions relevant to nanomaterials and carbon nanotubes (continued)**

Term	Definition	Reference
	<p>Definition of the Commission of the European Union:</p> <p>Point 1. “Nanomaterial” means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm. (In specific cases and where warranted by concerns for the environment, health, safety, or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%)</p> <p>Point 2. By derogation from point 1, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials</p> <p>Point 3. A material should be considered as falling under the definition in point 1 where the specific surface area by volume of the material is greater than 60 m<sup>2</sup>/cm<sup>3</sup>. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition in point 1 even if the material has a specific surface area lower than 60 m<sup>2</sup>/m<sup>3</sup></p>	<a href="#">EU Commission (2011)</a>
Nano-object	Material with one, two or three external dimensions in the nanoscale Note: Generic term for all discrete nanoscale objects	<a href="#">ISO (2008)</a>
Nano-onion	Spherical nanoparticle with concentric multiple shell structure	<a href="#">ISO (2010b)</a>
Nanoparticle	Nano-object with all three external dimensions in the nanoscale Note: If the lengths of the longest to the shortest axes of the nano-object differ significantly (typically by more than threefold), the terms nanorod or nanoplate are intended to be used instead of the term nanoparticle	<a href="#">ISO (2008)</a>
Nanoplate	Nano-object with one external dimension in the nanoscale and the two other external dimensions significantly larger Note 1: The smallest external dimension is the thickness of the nanoplate Note 2: The two significantly larger dimensions are considered to differ from the nanoscale dimension by more than threefold Note 3: The larger external dimensions are not necessarily in the nanoscale	<a href="#">ISO (2008)</a>
Nanoribbon	Nanoplate with one of its two larger dimensions in the nanoscale and the other significantly larger	<a href="#">ISO (2010b)</a>
Nanorod	Solid nanofibre	<a href="#">ISO (2010b)</a>
Nanoscale	Size range of approximately 1–100 nm	<a href="#">ISO (2008)</a>
Nanotube	Hollow nanofibre	<a href="#">ISO (2010b)</a>
Pristine	As-produced, as manufactured primarily	
SWCNT	CNT comprised of a single cylindrical graphene layer Note: The structure can be visualized as a graphene sheet rolled into a cylindrical honeycomb structure	<a href="#">ISO (2010b)</a>

CNF, carbon nanofibre; CNT, carbon nanotube; MWCNT, multiwalled carbon nanotube; SWCNT, single-walled carbon nanotube  
Compiled by the Working Group

**Fig. 1.1 Schematic comparison of various types of fibrous carbon by diameter on a log scale**

From [Kim et al. \(2013\)](#), with permission from Springer

While CNF may be produced as impurities during the synthesis of CNT (see Section 1.2), CNF are not the subject of this review.

CNT comprise a graphene sheet rolled into a cylinder which can, in some cases, be extremely long, occasionally reaching several hundreds of micrometres in length. In each of the carbon sheets, one carbon atom is bonded to three others in one place, and gives rise to hexagonal rings similar to those found in aromatic hydrocarbons. CNT may consist of a single graphene cylinder (SWCNT) or of many graphene cylinders inside one another in concentric layers kept together by van der Waals forces (MWCNT). The larger MWCNT can contain hundreds of concentric layers separated by a distance of 0.34 nm ([Popov, 2004](#)). The length of a C–C bond in a graphene sheet of SWCNT is 0.142 nm ([Wildoer et al., 1998](#)).

CNT have generally been categorized into two groups: SWCNT and MWCNT. However,

double-walled CNT (DWCNT) have also frequently been listed as a separate class. Depending on the production process, the physical and chemical characteristics (e.g. diameter and length) of CNT vary greatly ([Table 1.2](#)).

SWCNT do not normally exist as individual tubes ([Lam et al., 2006](#)). Due to the van der Waals forces, they are wont to form agglomerates or aggregates leading to the construction of microscopic bundles or ropes which can reach 5–50 nm in diameter ([Maynard et al., 2007](#)). These bundles tend to agglomerate loosely into small clumps. MWCNT are multiple graphene layers that surround one another and also tend to form bundles, but the van der Waals forces are usually weaker than those of SWCNT and MWCNT are therefore more likely to exist as single fibres ([Lam et al., 2006](#)).

**Table 1.2 Characteristic size and specific surface areas (surface area per mass) of carbon nanotubes**

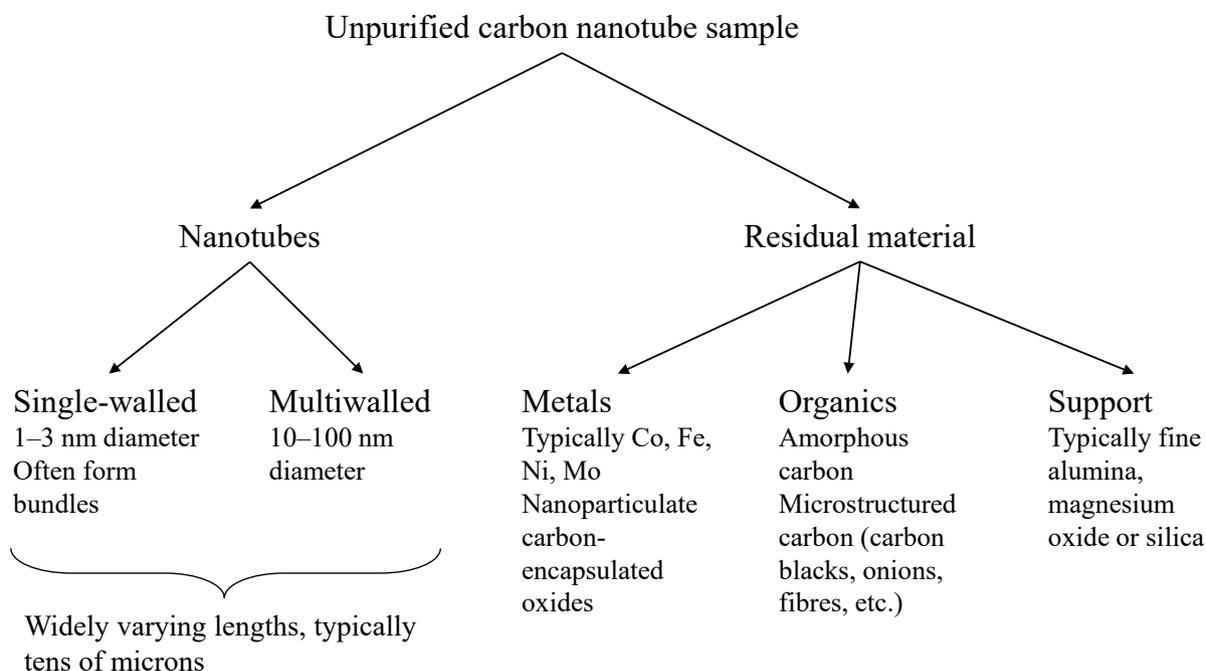
Material	Particle size: diameter (nm) × length (µm)	Specific surface area (m <sup>2</sup> /g)	Process	Reported purity (%)	Manufacturer	Reference
SWCNT	1–2 × 0.5–2	343	CVD	> 90 <sup>a</sup>	Cheap Tubes Inc., Brattleborough, VT, USA	<a href="#">Bello et al. (2009a)</a>
SWCNT	1–2 × 5–30	510	CVD	> 99 <sup>a</sup>	Cheap Tubes Inc., Brattleborough, VT, USA	<a href="#">Bello et al. (2009a)</a>
SWCNT	0.8–1.2 × 0.1–1	508	HiPCO	> 85–95 <sup>a</sup>	Carbon Nanotechnologies, Houston, TX, USA	<a href="#">Shvedova et al. (2008)</a>
SWCNT	0.9–1.7 × < 1	731	NR	NR	Thomas Swan, Consett, United Kingdom	<a href="#">Jacobsen et al. (2008)</a>
SWCNT	1–4 × 0.5–2	1040	HiPCO	> 85–95 <sup>a</sup>	Carbon Nanotechnologies, Houston, TX, USA	<a href="#">Witasp et al. (2009)</a>
SWCNT	12 × 0.32 (bundle in air)	1064	CVD	NR	National Institute of Advanced Industrial Science and Technology, Japan	<a href="#">Morimoto et al. (2012a)</a>
SWCNT	1.3–3.5	1700	CVD	50 <sup>a</sup>	SES Research, Houston, TX, USA	<a href="#">Hamilton et al. (2007)</a>
MWCNT	110–170 × 5–9	12.8	CVD	> 90 <sup>b</sup>	Sigma Aldrich, St Louis, MO, USA	<a href="#">Park et al. (2009)</a>
MWCNT (Mitsui-7)	49 × 3.9	26	CVD	99 <sup>a</sup>	Mitsui & Co., Ltd, Tokyo, Japan	<a href="#">Porter et al. (2010)</a> , <a href="#">Mercer et al. (2011)</a>
MWCNT	63 × 1.1 (in air)	69	CVD	NR	Nikkiso Co., Ltd, Tokyo, Japan	<a href="#">Morimoto et al. (2012b)</a>
MWCNT	10–20 × 5–15	100	CVD	> 95 <sup>a</sup>	Shenzhen Nanotech, Port, Shenzhen, China	<a href="#">Mitchell et al. (2007)</a>
MWCNT	11 × 1.1	130	CVD	75	SES Research, Houston, TX, USA	<a href="#">Hamilton et al. (2007)</a>
MWCNT (NC 7000)	5–15 × 0.1–10	250–300	CVD	> 90 <sup>a</sup>	Nanocyl S.A., Sambreville, Belgium	<a href="#">Ma-Hock et al. (2009)</a>
MWCNT (Baytubes)	10–15 × 0.2–1	257	CVD	> 95 <sup>a</sup> ; 1% cobalt	Bayer Material Science, Leverkusen, Germany	<a href="#">Ellinger-Ziegelbauer &amp; Pauluhn (2009)</a> , <a href="#">Pauluhn (2010a)</a>
MWCNT	50 × 10	280	CVD	> 95 <sup>a</sup>	Shenzhen Nanotech, Port, Shenzhen, China	<a href="#">Li et al. (2007a)</a>
MWCNT	20–40 × 0.5–5	300	CVD	80	Nanolab, Inc., USA	<a href="#">Sato et al. (2005)</a>
MWCNT	20–40 × 5–30	380	CVD	> 99	Nanotech Port, Shenzhen, China	<a href="#">Ye et al. (2009)</a>
MWCNT	10–20 × 0.5–2	140.6	CVD	> 96	Cheap Tubes Inc., Brattleborough, VT, USA	<a href="#">Hamilton et al. (2013a)</a>
MWCNT	10–20 × 10–30	204.9	CVD	> 95	Cheap Tubes Inc., Brattleborough, VT, USA	<a href="#">Hamilton et al. (2013a)</a>
MWCNT	30–50 × 0.5–2	217.3	CVD	> 98	Cheap Tubes Inc., Brattleborough, VT, USA	<a href="#">Hamilton et al. (2013a)</a>

<sup>a</sup> Purity reported in the producer's product catalogue

<sup>b</sup> Carbon content, based on [Ono-Ogasawara & Myojo \(2013\)](#)

CNT, carbon nanotubes; CVD, chemical vapour deposition; HiPCO, high-pressure carbon monoxide process; MWCNT, multiwalled carbon nanotubes; NR, not reported; SWCNT, single-walled carbon nanotubes  
Compiled by the Working Group

**Fig. 1.2 Possible components of a mixture of a sample of pristine carbon nanotubes (unpurified carbon nanotubes)**



From [Donaldson et al. \(2006\)](#) by permission of Oxford University Press

### 1.1.2 Chemical properties

#### (a) Elemental composition

Pure CNT consist of only one or several hexagonal graphene sheets of carbon atoms rolled into tubes and are considered to be rather non-reactive; for example, SWCNT have to be heated up to 500 °C to become oxidized and burned in the air ([Zhang et al., 2002](#)).

#### (b) Impurities

Because the synthesis of CNT frequently requires the presence of catalytic metals in the manufacturing process, CNT contain several residual impurities in addition to SWCNT and MWCNT, the concentrations of which may be relatively high in industrial-grade CNT ([Donaldson et al., 2006](#); [Fig. 1.2](#)).

CNT samples have various levels of purity and those of several market materials are reported in [Table 1.2](#). In the pristine [as-produced] samples,

residual materials include metals (i.e. iron and molybdenum), support substances (i.e. alumina and silica) and organics (i.e. carbon blacks and fibres). Metal catalysts are frequently used in the manufacture of SWCNT, the most common of which are iron, nickel, cobalt, and molybdenum ([Donaldson et al., 2006](#)). Hence, pristine SWCNT usually contain higher concentrations of trace metals ([Kitiyanan et al., 2000](#)) than MWCNT. Support materials, including fine alumina, magnesium oxide, or silica, are often included to support the catalyst or the region of growth of the tubes ([Donaldson et al., 2006](#)).

Residual organic materials can be divided into two categories: organic molecules and various forms – amorphous or micro-structured – of bulk carbon, such as soot particles, fullerenes, and graphene sheets ([Donaldson et al., 2006](#)). The levels and types of impurity depend on the production process. In general, gas-phase processes such as chemical vapour deposition (CVD) tend

to produce CNT with fewer impurities and are also suitable for large-scale production. The purity of commercial CNT preparations may vary considerably (50–99.9%), but post-production purification processes can be used to remove the remaining impurities and unwanted defects of graphene sheets. These involve harsh procedures such as mechanical handling and the use of strong acids and tend to shorten the CNT ([Lam et al., 2006](#); [Alexander, 2007](#)).

### 1.1.3 Physical properties

CNT preparations vary greatly in terms of diameter, length, atomic structure, surface chemistry, and defects (e.g. catalysts such as iron). Other important physical properties include mechanical, electrical, optical, and thermal characteristics and also the agglomeration and aggregation state, bulk density, and the specific surface area.

#### (a) Thickness

The thickness of CNT mainly depends on the number of graphene layers contained and the chirality of the tubes. In general, the outer diameter of SWCNT is 1–3 nm ([Jorio et al., 2001](#)) and that of MWCNT is 10–200 nm ([Hou et al., 2003](#)). Changes in the diameter depend on the synthetic process, in which the diameter of the catalytic metal plays an important role, especially in the case of SWCNT.

#### (b) Length

The length of typical CNT is a few micrometres, but this can vary between only a few hundred nanometres and several tens of micrometres. Tubes of 50  $\mu\text{m}$  in length are common and some are occasionally several hundreds of micrometres long. Variations in the length of the tubes are a rule rather than an exception in CNT preparations ([Lam et al., 2006](#)).

#### (c) Atomic structure

The atomic structure of CNT is described in terms of tube chirality, which is determined by the orientation of the graphene sheet when the tube is synthesized. Two common conformations include the so-called armchair and zig-zag conformations, which generally occur in a mixture of different conformations ([Fig. 1.2](#); [Thostenson et al., 2001](#)). The chiral axis, defined as the orientation of the axis of the carbon hexagon relative to the axis of the CNT, also has an effect on the diameter of the nanotube because the inter-atomic spacing of the carbon atoms is fixed as mentioned above (0.142 nm) ([Wildoer et al., 1998](#); [Hedmer et al., 2013](#)). In MWCNT, the adjacent graphene layers have different chiralities. Chirality also influences the optical and electrical properties of the CNT. Although graphene is a semi-metal, CNT can be either metallic or semi-conducting depending on the chiral angle. Chirality does not, however, modify the mechanical properties of CNT. Importantly, because SWCNT are frequently a mixture of single-walled tubes with different lengths and different chiralities, the existence of an aerosol, for example, consisting of SWCNT with only one type of chirality is not a viable assumption ([Thostenson et al., 2001](#)).

#### (d) Defects

During the synthesis of CNT, certain types of defect may occur, one example of which is the collapse of the tube that may arise from “bamboo-like” closures in the tube that can be detected by transmission electron microscopy (TEM) ([Saito & Zettl, 2008](#)). Such geometrical and typographical defects may be technologically important because they may dramatically alter the electrical properties of the CNT ([Ishigami et al., 2004](#); [Saito & Zettl, 2008](#)). For example, the pentagon–heptagon pair (5–7 pair), one of the simple and elegant topological defects ([Ishigami et al., 2004](#)), can be used to connect metallic and

semi-conducting tubes enabling the formation of semiconductor–semiconductor, semiconductor–metal, and metal–metal interfaces ([Bernholc et al., 1997](#)). For the above reasons, nanoscale devices comprised only of carbon can be produced. CNT are usually non-reactive, although defects in the structure, including missing carbon atoms, could increase their reactivity ([Bernholc et al., 1997](#)).

(e) *Surface-to-mass ratio or specific surface area*

Because of their small size and structure, each of the CNT exhibits extremely high surface-to-mass ratios, also referred to as the specific surface area. The specific surface area depends on the diameter, number of concentric graphene layers, and degree of bundling. The specific surface area of SWCNT is usually  $1300 \text{ m}^2/\text{g}$ , but the corresponding value for bundles of SWCNT is often six times lower (about  $300 \text{ m}^2/\text{g}$ ) ([Ye et al., 1999](#)).

(f) *Bulk density*

The bulk density of CNT is quite low and varies according to the production process. For example, a comparison of the powder resulting from laser ablation with that produced by the high-pressure carbon monoxide (HiPCO) process showed that the latter resulted in a bulk density as low as  $1 \text{ mg}/\text{cm}^3$ , whereas the bulk density of the MWCNT Baytubes was as great as  $120\text{--}170 \text{ mg}/\text{cm}^3$ . For example, the bulk density of pure graphite and graphene powder are  $2200$  and  $200\text{--}600 \text{ mg}/\text{cm}^3$ , respectively ([Chung et al., 1982](#); [Stankovich et al., 2007](#)).

(g) *Physical strength*

The physical strength of CNT is one their remarkable advantages. In terms of tensile strength and elastic modulus, CNT are the strongest and stiffest material to have been discovered to date, with an estimated tensile strength of  $200 \text{ GPa}$  ([Cheung et al., 2010](#)), and SWCNT can be 10 times stronger than high-strength stainless

steel ([Walters et al., 1999](#); [Yu et al., 2000](#)). Closely packed CNT ropes have shown tensile strength in excess of  $45 \text{ GPa}$ , more than 20 times higher than that of typical high-strength steel ( $2 \text{ GPa}$ ) ([Walters et al., 1999](#); [Thostenson et al., 2005](#)). With a tensile modulus of more than  $1 \text{ TPa}$ , CNT can also be 20% stiffer than diamonds ([Thostenson et al., 2005](#)). The remarkable strength of the CNT is due to the covalent bonds ( $\text{sp}^2$  hybridization) formed between the individual carbon atoms. High strength is thought to be a purely axial property of CNT because, in the radial direction, they are rather soft and can be deformed by van der Waals interactions between adjacent CNT ([Ruoff et al., 1993](#)). CNT are very flexible and can be bent more than  $110^\circ$  without damage ([Iijima et al., 1996](#)).

(h) *Electrical properties*

CNT can act as either semiconductors or conductors depending on their chirality ([Bernholc et al., 1997](#)), to which the electrical properties of the tubes are directly proportional; in the case of thin SWCNT, curvature is also a factor ([Lu & Chen, 2005](#)). In theory, metallic CNT can carry an electric current density of  $4 \times 10^9 \text{ A}/\text{cm}^2$ , which exceeds that of copper by a factor of 1000 ([Thostenson et al., 2005](#); [Cheung et al., 2010](#)). CNT have numerous potential applications in electric components and devices, and SWCNT with different electrical properties can be joined to make a diode ([Chico et al., 1996](#)). Furthermore, because CNT can be modified by deformation and stretching, they could, for example, be applied in sensors ([Mahar et al., 2007](#)).

(i) *Thermal and optical properties*

CNT also have remarkable optical and thermal properties. SWCNT readily absorb near infrared light ( $800\text{--}1600 \text{ nm}$ ) ([Cheung et al., 2010](#)) which covers the wavelength range that passes through biological tissues without remarkable scattering, absorption, heating, or damaging the

tissue. Hence, the optical properties of SWCNT can be used in photo-thermal therapy ([Kam et al., 2005](#); [Chakravarty et al., 2008](#); [Xiao et al., 2009](#)). CNT also exhibit remarkable thermal conductivity. SWCNT have thermal conductivities as high as 6000 W/(m.K) at room temperature, at which the corresponding value for diamonds is 3320 W/(m.K). SWCNT are also stable at temperatures as high as 2800 °C in a vacuum and 750 °C in air ([Thostenson et al., 2005](#)). These thermal properties could probably be used in the future in highly conducting components of integrated nanoscale circuits, such as transistors, and in thermal management ([Sinha et al., 2005](#); [Pop et al., 2006](#)).

#### (j) *Dustiness*

Dustiness corresponds to the propensity of a material to generate airborne dust during its handling ([Evans et al., 2013](#)) and, depending on the dustiness of the CNT/CNF material, the exposure of workers could vary significantly. A simulated workplace study of scooping/weighing/adding and cleaning/sweeping powders by [Brouwer et al. \(2006\)](#) found that dustiness was a major determinant of the exposure of workers and accounted for approximately 70% of variability in exposure. [Evans et al. \(2013\)](#) tested the dustiness of different materials including SWCNT, MWCNT, and CNF. Both the total and respirable dustiness of the dispersed powders spanned two orders of magnitude (0.3–37.9% and 0.1–31.8%, respectively). For many powders, significant respirable dustiness was observed, suggesting that workplace procedures may result in inhaled airborne dust, a large fraction of which may be capable of reaching the deep lung of a worker, and respirable dustiness accounted for approximately one-third of the total dustiness of most powders studied. The dustiest material examined was SWCNT manufactured by the HiPCO process, with  $37.9 \pm 3.4\%$  total dustiness and  $31.8 \pm 3.3\%$  respirable dustiness, which is of

particular concern because of the high respirable fraction ([Evans et al., 2013](#)).

#### 1.1.4 *Other properties*

Prototype or unchanged pristine preparations of all forms of CNT are extremely resistant to wetting and are exceedingly difficult to disperse or dissolve in aqueous solutions or organic media due to their high hydrophobicity and tendency to aggregate ([Kostarelos et al., 2009](#)). Hence, the use of CNT in composites is a challenge ([Sinnott, 2002](#); [Hirsch & Vostronowsky, 2005](#)).

Functionalization of CNT is a post-production process used to attach chemical groups to modify properties and handling and can be physical (non-covalent) and chemical (covalent). Attachments can occur outside or inside the tubes and can be used to increase the dispersibility of the tubes in surfactants or aqueous solutions because pristine CNT have a high tendency to interact in a hydrophobic manner and form aggregates or make nanoscale biosensors ([Thostenson et al., 2001](#); [Hirsch & Vostronowsky, 2005](#); [Alexander, 2007](#); [Kostarelos et al., 2009](#)). The functionalization can be divided into two categories: a direct attachment of functional groups to the graphitic surface; and the use of the nanotube-bound carboxylic acids ([Sun et al., 2002](#)). The ability to disperse CNT into water can be improved dramatically by their functionalization, which can also enhance their mechanical and electrical properties ([Kostarelos et al., 2009](#)). An alternative process to the functionalization of CNT – doping – has frequently been used in the nano-fabrication sector.

Doping is the physical alteration of the surface of CNT with ions or molecules using weak forces such as van der Waals. For example, DWCNT cables with iodine doping were shown to outperform copper and aluminium cables with regard to specific electrical conductivity as well as tensile strength ([Zhao et al., 2011](#)).

## 1.2 Sampling and analytical methods

The recommended physico-chemical characterization of nanomaterials includes the particle size and size distribution, aggregation/agglomeration state in relevant media, shape, surface area, composition, surface chemistry and solubility/dispersibility (ISO TR/13014, 2012). Selected methods of sampling and analysis in various matrices are given in [Table 1.3](#) and are discussed below.

### 1.2.1 Bulk samples

Several international standards are currently available to characterize CNT in powder form or liquid suspension (see also [Table 1.3](#)). Other methods that are not internationally standardized can be found in [ISO \(2012a\)](#).

Bulk samples of CNT are invariably analysed using scanning electron microscopy (SEM), TEM, near infrared photoluminescence spectroscopy, thermogravimetric analysis, and Raman spectroscopy. TEM has better resolution than SEM and allows electron diffraction with the use of an energy dispersive X-ray analyser to evaluate the morphology and aspect ratio, including the length and diameter of the tube structure. However, TEM generally needs more complicated and time-consuming sample preparation than SEM. Dynamic light scattering has been used to measure the hydrodynamic size of CNT in liquid media ([Kim et al., 2011](#)). In addition, the components of CNT can be analysed chemically by inductive coupled plasma-mass spectrometry, which is capable of detecting metals and many non-metals. Raman spectroscopy has been used to measure the diameter and crystallinity of CNT. Thermogravimetric analysis is commonly used to determine certain material characteristics that exhibit either a mass loss or gain due to decomposition, oxidation, or loss of volatile compounds (such as moisture) and to determine the mass

composition of CNT. Brunauer–Emmett–Teller analysis has been used to evaluate the surface area of CNT in powder form.

### 1.2.2 Air samples

No consensus has currently been reached on the best sampling method for characterizing exposure to CNT. Qualitative assessments comparing particle concentrations at the emission source with background particle concentrations are frequently used to identify emission sources of nanomaterials and implement measures for the mitigation of exposure ([Tsai et al., 2009](#); [Lee et al., 2010](#); [Methner et al., 2010](#); [Birch et al., 2011](#)). Various approaches can be applied to characterize exposure in an environment, and the monitoring devices generally used for nanoparticles or CNT have been described by [Yu et al. \(2014\)](#).

#### (a) Direct reading instruments

Direct reading instruments, such as the condensation particle counter and the optical particle counter, are non-specific devices that can be used to measure the particle number concentration directly (including CNT and their aggregates and agglomerates) ([Johnson et al., 2010](#); [Lee et al., 2010](#); [Dahm et al., 2011, 2012](#)). The number concentration, mass concentration, surface area concentration, and size distribution can be measured using a differential mobility analysing system and an electrical low-pressure impactor. These instruments do not give the CNT concentration, but give the airborne particle number concentration.

#### (b) Filter sampling

Area and personal filter-based samples are usually collected on appropriate filters for the measurement of mass concentration, electron microscopy combined with an energy dispersive X-ray analyser are used to estimate CNT morphology and count ([Han et al., 2008](#)).

**Table 1.3 Selected methods of sampling and analysis of carbon nanotubes in various matrices**

Sample matrix	Sample preparation	Assay method	Detection limit	Reference	
Bulk	TEM-grid	TEM-EDX		<a href="#">Birch et al. (2011)</a> , <a href="#">ISO (2012b)</a>	
	SEM-stub	SEM-EDX		<a href="#">Birch et al. (2011)</a> , <a href="#">ISO (2011a)</a>	
	Suspension in water, sodium dodecyl sulfate, sodium dodecylbenzene sulfonate, or sodium cholate	NIR			<a href="#">ISO (2010c)</a>
		TGA			<a href="#">ISO (2011b)</a>
		GC-MS			<a href="#">ISO (2010d)</a>
		BET			<a href="#">Birch et al. (2011)</a>
	Powder	ICP-AES		<a href="#">NIOSH 7300 (NIOSH, 2003c)</a>	
	Nitric acid/perchloric acid (4:1)			<a href="#">Maynard et al. (2004)</a>	
	Air	Direct-reading instruments	SMPS	4.2 nm < dm < 100 nm 69.8 nm < dm < 777 nm	<a href="#">Lee et al. (2010)</a> <a href="#">Han et al. (2008)</a>
			Dust monitor	5 nm < dm < 500 nm	<a href="#">Lee et al. (2010)</a>
		DustTrak	14 nm < dm < 630 nm	<a href="#">Bello et al. (2010)</a>	
		APS	250 nm < dm < 32 000 nm 10 nm < dm < 10 000 nm 500 nm < dae < 20 000 nm	<a href="#">Maynard et al. (2004)</a> , <a href="#">Han et al. (2008)</a> , <a href="#">Bello et al. (2010)</a>	
		CPC	10 nm < dm < 1000 nm	<a href="#">Bello et al. (2010)</a> , <a href="#">Johnson et al. (2010)</a>	
		FMPS	0–10 <sup>8</sup> particle/mL	<a href="#">Lee et al. (2010)</a>	
Personal and area sampling MCE filters (37 mm, 0.8 µm pore size)		TEM-EDX	5.6 nm < dm < 560 nm 0.04–0.5 fibres/mL for a 1000-L air sample	<a href="#">Tsai et al. (2009)</a> , <a href="#">Bello et al. (2010)</a> <a href="#">NIOSH 7402 (NIOSH, 1994a)</a> , <a href="#">Han et al. (2008)</a> , <a href="#">Methner et al. (2010)</a> , <a href="#">Johnson et al. (2010)</a> , <a href="#">Lee et al. (2010)</a>	
Cascade impactors, carbon-coated nickel grid, silicon monoxide-coated nickel grid, 25 mm MCE filter		TEM-EDX	0.04–0.5 fibres/mL for a 1000-L air sample	<a href="#">NIOSH 7402 (NIOSH, 1994a)</a> , <a href="#">Birch et al. (2011)</a>	
Electrostatic precipitator		TEM-EDX	0.04–0.5 fibres/mL for a 1000-L air sample	<a href="#">NIOSH 7402 (NIOSH, 1994a)</a> , <a href="#">Birch et al. (2011)</a>	
Personal and area sampling quartz-fibre filter (37 mm) with cyclone		EC/OC	0.3 µg per filter portion	<a href="#">NIOSH 5040 (NIOSH, 1999)</a> , <a href="#">Birch et al. (2011)</a> , <a href="#">Dahm et al. (2012)</a>	
MOUDI-quartz-fibre filter (47 mm)	EC/OC	0.3 µg per filter portion	<a href="#">NIOSH 5040 (NIOSH, 1999)</a> , <a href="#">Birch et al. (2011)</a>		
Personal and area sampling quartz-fibre filter (37 mm), 3:1 nitric/perchloric acid mixture extraction	ICP-AES	Depending on metal	<a href="#">NIOSH 7300 (NIOSH, 2003a)</a> , <a href="#">Birch et al. (2011)</a>		
Personal and area sampling MCE filters (37 mm, 0.8 µm pore size)	Gravimetry	0.03 mg per sample	<a href="#">NIOSH 0500 (NIOSH, 1994b)</a> , <a href="#">Han et al. (2008)</a> , <a href="#">Methner et al. (2010)</a> , <a href="#">Lee et al. (2010)</a>		

**Table 1.3 (continued)**

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Dermal	Cotton gloves	ICP-AES	Depending on metal	NIOSH 7300 (NIOSH, 2003a), <a href="#">Maynard et al. (2004)</a>

APS, aerodynamic particle sizer; BET, Brunauer-Emmett-Teller; CNT, carbon nanotubes; CPC, condensation particle counter; dae, aerodynamic diameter; dm, mobility diameter; EC/OC, elemental carbon/organic carbon; EDX, energy dispersive X-ray analyser; ELPI, electrical low-pressure impactor; ESP, electrostatic precipitator; FMPS, fast mobility particle sizer; GC-MS, gas chromatograph-mass spectrometry; ICP-AES, inductive coupled plasma-atomic emission spectroscopy; ICP-MS, inductive coupled plasma-mass spectroscopy; MCE, mixed cellulose ester; MOUDI, micro orifice uniform-deposit impactor; NIR, near infrared photoluminescence spectroscopy; PAS, photoelectric aerosol sensor; PBZ, personal breathing zone; SEM, scanning electron microscopy; SMPS, scanning mobility particle sizers; STEM, scanning transmission electron microscopy; TEM, transmission electron microscopy; TGA, thermogravimetric analysis; TP, thermal precipitator; TSP, total suspended particulate; WRASS, wide-range aerosol sampling system

Compiled by the Working Group

Chemical analysis involves the use of inductive coupled plasma-mass spectrometry and atomic absorption spectrometry. These filter-based air samples provide more specific information than direct reading instruments on the target CNT (e.g. size, shape, mass, and composition). Air samples allow an elemental mass analysis to determine the levels of metal (e.g. NIOSH Method 7303; [NIOSH, 2003b](#)) or elemental carbon (EC; e.g. NIOSH Method 5040; [NIOSH, 1999](#)), depending on the composition of the manufactured nanomaterials, plus particle characterization (e.g. size, shape, dimension, and degree of agglomeration) using TEM or SEM based on the measurement techniques specified in NIOSH Methods 7402 ([NIOSH, 1994a](#)) and 7404 ([NIOSH, 2003c](#)), respectively.

CNT in suspended particles can be measured in ambient particles separately from EC by selecting a specific oxidizing temperature ([Chow et al., 1993](#); [Hedmer et al., 2014](#))

Electrostatic precipitator or cascade impactor grid sampling ([Birch et al., 2011](#)) or filter sampling followed by grid mounting have been used to collect CNT structures from the air ([Han et al., 2008](#); [Lee et al., 2010](#)). These structures can be identified and counted using SEM or TEM or by combining these with an energy dispersive X-ray analyser to identify their constituents ([Han et al., 2008](#); [Lee et al., 2010](#); [Dahm et al., 2012](#)). [Chen et al. \(2012\)](#) published a protocol for counting MWCNT microscopically, geometrically sizing the particles, and collecting size-classified samples to determine the aerodynamic size distribution.

#### (c) *Dermal exposure*

To estimate the level of dermal exposure to CNT, cotton gloves are placed over rubber gloves, are removed immediately after handling the nanotubes, and are then sealed in separate plastic bags. The gloves are then analysed for catalyst metals as the surrogate total nanotube product mass. The samples are treated with nitric

acid and perchloric acid and analysed using inductive coupled plasma emission spectrometry ([NIOSH, 2003a](#); [Maynard et al., 2004](#)).

#### (d) *CNT in consumer products*

The process of the release of CNT from consumer products that contain them has been investigated using direct reading instruments to count airborne particle number concentrations, and TEM or SEM to identify whether free CNT or CNT composite structures are released during the life-cycle of such consumer products ([Bello et al., 2010](#)).

#### (e) *Limitations*

The assessment of exposure to CNT, such as SWCNT and MWCNT, remains a challenge in the field of occupational hygiene, because relatively few studies on CNT sampling have been carried out and the best sampling filters and methods have not yet been established. Most number-counting devices, such as the condensation particle counter and the optical particle counter, do not represent the exact exposure to CNT; measurements using a differential mobility analysing system (or a scanning mobility particle sizer) also do not always provide accurate information due to the arc charge caused by the charged CNT in the dynamic mechanical analysis ([Ku et al., 2007](#)). Although several groups have attempted to count the CNT structures using TEM or other microscopic methods ([Han et al., 2008](#); [Dahm et al., 2012](#)), no standard methods for CNT counting have yet been established. In addition, determining the mass concentration of CNT based on the measurement of EC remains a challenge due to the technical limitations of current analytical methods. Despite these limitations in assessing the exposure to nanomaterials or CNT, guidelines and reports have been published to guide and harmonize strategies for exposure measurement ([OECD, 2009a, b, c, d, 2010](#); [Brouwer et al., 2012](#)).

## 1.3 Production and use

### 1.3.1 Production levels

CNT preparations are not homogenous, but contain a diverse mixture of many different types of tube. The number of walls, diameters, lengths, chiral angles, chemical functionalization (i.e. surface modifications), purities, and bulk densities may all vary. In 2005, global production figures for MWCNT and SWCNT were estimated at 294 tons [~299 tonnes] and several hundred kilograms, respectively ([Köhler et al., 2008](#)). In 2006, the corresponding amounts were estimated to be ~300 tons [305 tonnes] and 7 tons [7.11 tonnes], respectively ([WTEC, 2007](#)). Today, the global production capacity, mainly of MWCNT, is probably much higher. However, an estimation of the production capacities for CNT at the country level is difficult because of the scarcity of governmental reports. The significant uncertainty in the estimation of global production is due to the continuously changing situation caused by new producers coming into the market; however, some data from the Republic of Korea and France are publicly available. For example, according to the nanomaterial inventory of the Ministry of Environment of the Republic of Korea, 3.0 tonnes of CNT were reported in 2009 ([NSTC, 2011](#)). In France, the nanomaterial inventory estimated that around 1 tonne of CNT was used by five industries involved in the production of inks, paints and plastics in 2009 ([Honnert & Grzebyk, 2014](#)). A public report on the use and production of nanomaterials in France in 2012 stated that several tens of tonnes of nanofibres and nanotubes were produced in 2011, but no precise figures were given regarding CNT ([DGCIS, 2012](#)).

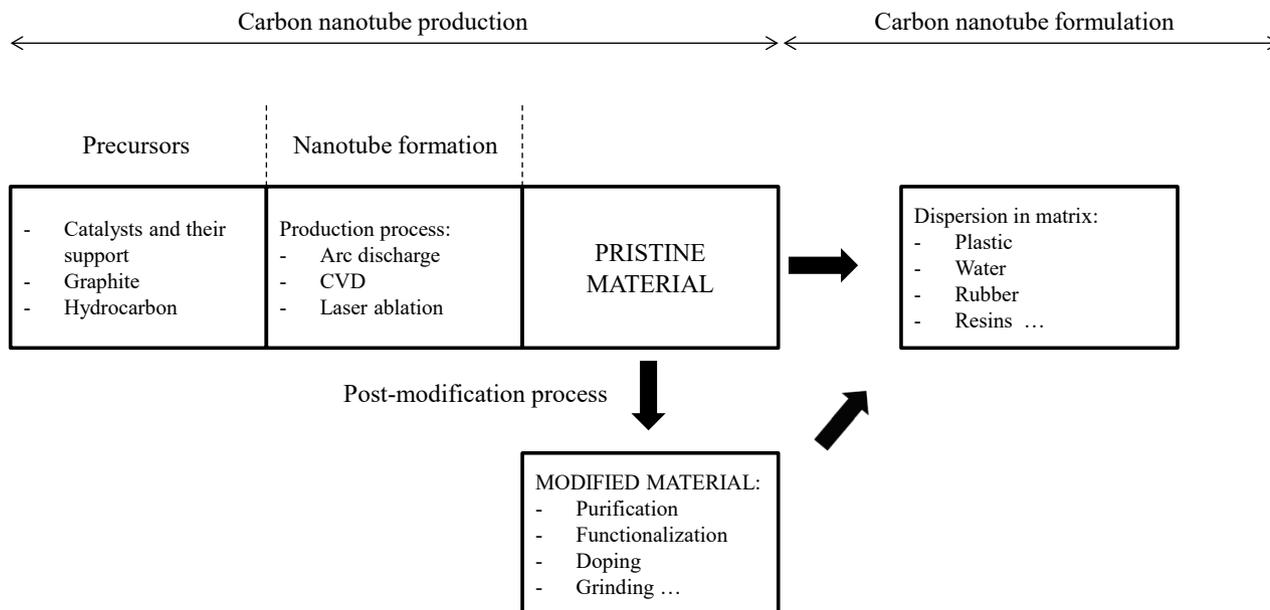
The data on global CNT production are also uncertain because the estimates made by the industry vary between 100 and 1000 tonnes annually ([Piccinno et al., 2012](#)).

### 1.3.2 Production methods

Several methods of CNT production have been described ([Bhushan, 2004](#); [Fig. 1.3](#)), one of the most commonly used of which involves the use of transition metals in the presence of atomic carbon at a high temperatures and/or pressure ([Maynard et al., 2004](#)). Both SWCNT and MWCNT are generally produced by one of the three principal techniques, i.e. CVD, arc discharge, or laser ablation. Depending on the production technique, various levels of impurities, such as metal catalysts, amorphous carbon, soot, graphite, and non-tubular fullerenes, may be present in the final preparation ([ENRHES, 2009](#); [Hedmer et al., 2013](#)). Removal of impurities requires the application of chemical purification processes, such as acid reflux, filtration, centrifugation, and repeated washing with solvents or water ([ENRHES, 2009](#)).

#### (a) Chemical vapour deposition

Thermal CVD, also called catalyst CVD, is most widely used in the production of CNT ([Kumar & Ando, 2010](#)). Low-temperature CVD (600–900 °C) yields MWCNT, whereas higher temperatures (900–1200 °C) promote SWCNT ([Karthikeyan et al., 2009](#)). CVD is based on the decomposition of hydrocarbon vapour in the presence of a metal catalyst. The precursor of CNT is a carbon-containing gas or vapour, such as carbon monoxide (HiPCO process), methane, acetylene, ethylene, benzene, or xylene. This is first heated with a plasma or coil and then allowed to react with the metal catalyst, which can be nickel, cobalt, or iron and acts as a seed for the growth of the tubes ([WTEC, 2007](#); [ENRHES, 2009](#); [Singh et al., 2009](#)). Although CNT can be produced in its absence, the use of catalysts is extremely helpful ([Lam et al., 2006](#); [Hedmer et al., 2013](#)).

**Fig. 1.3 Production process for carbon nanotubes**

CVD, chemical vapour deposition  
Compiled by the Working Group

### (b) Arc discharge

Arc discharge was the first technique reported to produce CNT (Iijima, 1991). The method usually included an anode and a cathode made of high-purity graphite. A voltage is applied across these rods until a stable arc is achieved in which the anode is consumed and the cathode is used to grow the tubes. The whole process takes place under a helium atmosphere. To obtain SWCNT, the electrodes are doped with a small amount of catalyst metal particles (Thostenson et al., 2001). This process produces a high yield of CNT but the levels of impurities in the final preparation are also high (Donaldson et al., 2006).

### (c) Laser ablation

Laser ablation, similarly to arc discharge, produces MWCNT (Guo et al., 1995a). More recently, this technique has been improved by using catalyst nanoparticles, notably a cobalt and nickel mixture, which also enables the synthesis of SWCNT (Guo et al., 1995b; Rinzler et al., 1998). A graphite target is maintained close to 1200 °C

while an inert gas, frequently argon, is bled into the chamber. Thereafter, pulses of high-intensity laser beam are used to vapourize the graphite target and CNT develop on the cooler surfaces of the reactor when the vapourized carbon condenses (Thostenson et al., 2001).

### 1.3.3 Use

CNT have a wide variety of applications, including their incorporation into fabrics in the textile industry, plastics, rubbers, reinforced structures, composite materials, and household commodities to reduce their weight and improve water- and wear-resistance (Lam et al., 2006). At present, CNT are also found in products made of nanocomposites such as polymers that contain up to 10% CNT by mass, e.g. sports articles such as jogging shoes and sportswear, tennis rackets, ice hockey sticks, bicycles (to strengthen and reduce weight), cycling shoes, golf clubs, skis, car parts, and wind power plants (to strengthen and reduce the weight of energy production wind mill wings)

([Hussain et al., 2006](#); [Köhler et al., 2008](#); [Thomas et al., 2009](#)). Lithium ion batteries used in mobile phones and laptops also contain CNT ([Köhler et al., 2008](#); [Zhang et al., 2010](#)). Other uses involve textiles made of fibres of CNT, including polymers with electrical antistatic, thermal conductive, flame retardant, and tear-proof properties ([Beyer, 2002](#); [Köhler et al., 2008](#)), and concrete reinforced with CNT ([Schneider et al., 2007](#); [Köhler et al., 2008](#); [Wohlleben et al., 2011](#)). CNT can also be used in car tyres to improve their strength ([Observatory Nano, 2011](#)). Research and development are still at the prototype stage in many cases, but development will be rapid and increasingly more products containing CNT are entering the market and being used in industrial processes ([Beyer, 2002](#); [Aitken et al., 2006](#); [Köhler et al., 2008](#)).

## 1.4 Occurrence and exposure

### 1.4.1 Environmental occurrence

Little is known about environmental exposure to CNT mainly because very few quantitative and specific trace analytical methods are available at present ([Gottschalk et al., 2013](#)).

Naturally occurring CNT have been found in 10 000-year-old ice core melt water in Greenland ([Murr et al., 2004a](#)), in smoke from wood combustion ([Murr & Guerrero, 2006](#)), and in a mixture of coal and petroleum ([Velasco-Santos et al., 2003](#)).

Sources of natural gas and propane gas, such as domestic (kitchen) stoves, were found to yield aggregates of silica nanocrystals intermixed with CNT and other carbon nanocrystals ([Murr et al., 2004b](#)). [Murr et al. \(2006\)](#) reported aggregate concentrations on outdoor sampling grids measured by TEM of about  $10^2$ – $10^3$  aggregates/ $m^3$ , while indoor aggregate concentrations in kitchens were found to be more variable, averaging  $10^3$ – $10^5$  aggregates/ $m^3$  above gas burners. According to [Murr & Soto \(2005\)](#), these aggregates contained

MWCNT of various sizes and aspect ratios and other concentric, fullerene polyhedra.

CNT may also enter the environment directly after unintentional release during the manufacture, use, and consumption of goods containing CNT or as waste from sewage-treatment plants, waste-incineration plants, and landfills ([Petersen et al., 2011](#); [Nowack et al., 2013](#); [Guseva Canu et al., 2016](#)). Modelling studies dealing with the environmental release of and exposure to nanomaterials have been published and provide estimates of predicted environmental concentrations ([Gottschalk et al., 2013](#)). [Gottschalk et al. \(2009, 2010\)](#) modelled concentrations of CNT for Europe, Switzerland, and the USA. The simulated modes (most frequent values) and range of the lower and upper quantiles for 2008 are reported in [Table 1.4](#).

### 1.4.2 Exposure of the general population

The main exposures of the general population probably result from the abrasion and weathering of consumer products that contain CNT embedded into a matrix. Exposure from medical devices (internal exposure through targeted drug delivery or contrast agents) is also possible. No quantitative data on the exposure of the general population to CNT have been identified. Although exposure from applications in which CNT are matrix-bound is expected to be very low, this may be increased when these consumer products are incinerated ([Aschberger et al., 2010](#)). CNT were found in the lung tissues of patients who were exposed to dust and smoke after the collapse of the World Trade Center on 11 September 2001 ([Wu et al., 2010](#)). Simulation studies have addressed the exposure of consumers ([Bello et al., 2009a, 2010](#)).

**Table 1.4 Predicted environmental concentrations of carbon nanotubes for Europe, Switzerland, and the USA in 2008<sup>a</sup>**

Environmental compartments	Europe	USA	Switzerland
Air (ng/m <sup>3</sup> )	0.003 [0.0025–0.007]	0.001 [0.00096–0.003]	0.008 [0.006–0.017]
Soil ( $\Delta$ ng/kg/year)	1.51 [1.07–3.22]	0.56 [0.43–1.34]	1.92 [1.44–3.83]
Sludge-treated soil ( $\Delta$ ng/kg per year)	73.6 [52.1–157]	31.4 [23.9–74.6]	Not reported
Surface water (ng/L)	0.004 [0.0035–0.021]	0.001 [0.0006–0.004]	0.003 [0.0028–0.025]
Sediment ( $\Delta$ ng/kg per year)	241 [215–1321]	46 [40–229]	229 [176–1557]
Sewage-treatment plant effluent (ng/L)	14.8 [11.4–31.5]	8.6 [6.6–18.4]	11.8 [7.6–19.1]
Sewage-treatment plant sludge (mg/kg)	0.062 [0.047–0.129]	0.068 [0.053–0.147]	0.069 [0.051–0.129]

<sup>a</sup> Based on estimations of both public sector expenditure to promote nanotechnology and the worldwide market value for products incorporating nano-sized materials for the period 2001–12, the modelled increase (base year 2008) in concentrations of engineered nanomaterials in sludge-treated soil and sediment was scaled to calculate annual increases of these concentrations for each year within the indicated period. Concentrations are expressed as mode (most frequent value) [lower quantile Q(0.15)–upper quantile Q(0.85)]. For air, surface water and sewage-treatment plant effluents, the data illustrate the 2008 concentrations of CNT; for soil, sludge-treated soil and sediments, the data illustrate the annual increase in the concentration of engineered nanomaterials (base year 2008).

Adapted with permission from [Gottschalk et al. \(2009\)](#). Copyright (2009) American Chemical Society

### 1.4.3 Occupational exposure

In occupational settings, exposure of workers to CNT could occur in principle at all phases of the generation and application of the material. Workers are not generally expected to be exposed during the synthesis phase of commercial production, which is performed in a closed reaction chamber; however, exposure is more liable to occur in subsequent phases when the reaction chamber is opened to recover the product, during the extraction and transport of the material produced, or when the system is cleaned. The highest exposures most probably occur when handling the dry powder, for example, during the collection, weighing, blending, transferral to containers and bagging of the material, and the maintenance of machinery. Various downstream applications of CNT may also result in occupational exposure, e.g. when the material containing CNT is machined or drilled, during wear and tear, and during disposal. The use and fabrication of CNT in drug delivery systems and imaging may also potentially give rise to occupational exposure to those who manufacture and administer the products as well as academic research staff ([Guseva Canu et al., 2016](#)).

The main routes of exposure in the occupational setting are anticipated to be inhalation and dermal contact. Ingestion may also occur as a consequence of swallowing inhaled material after mucociliary clearance or as a result of hand-to-mouth contact.

#### (a) General overview

The industrial production and use of CNT material is relatively recent and the size of the workforce in the CNT/CNF sector remains small; therefore, the currently available data on occupational exposure are still limited. Moreover, the available data are extremely heterogeneous due to the high variability of the methods and instruments used for sampling and analysis of exposure and that of the criteria used for interpreting the results.

Little consensus has been reached to date on the exposure metrics that would best correlate with adverse health outcomes ([Dahm et al., 2012](#); [Hedmer et al., 2014](#)). For instance, in early studies, gravimetric concentrations were measured for total suspended particles, then for the respirable or alveolar fraction of the aerosol or for particles smaller than 2.5  $\mu\text{m}$ . With the development of real-time aerosol monitoring

instruments, particle number concentration and specific or active surface area measurement results became available but without a common strategy (Guseva Canu et al., 2016). In the context of rapidly changing manufacturing technology, an additional difficulty arises from the diversity of the CNT material, the physical and chemical properties of which influence their potential release and dispersal (Guseva Canu et al., 2016).

Of the 19 studies that were reviewed, eight were simulation studies of exposure (i.e. carried out under well controlled laboratory conditions) and 11 were on-site studies conducted in real occupational settings. While the on-site studies were intended to evaluate the actual exposure of workers to CNT, simulation studies of exposure were performed to assess the release of CNT into the air under experimental or simulated industrial process conditions, often using a particular, well-characterized test material, to estimate the potential exposure of workers without protection. These two sets of data were considered separately.

(b) *Simulation studies of exposure to emissions*

(i) *Measurement of particle number concentration*

Tsai et al. (2009) characterized particle morphology and aerosol size during the synthesis of SWCNT by CVD (with and without a catalyst) and during the growth of MWCNT in the presence or absence of a substrate. Particle measurements made inside a fume hood during the synthesis of SWCNT were found to be as high as  $10^7$  particles/cm<sup>3</sup> with an average particle diameter of 50 nm; personal breathing zone (PBZ) samples collected from workers near the fume hood were considerably lower (< 2000 particles/cm<sup>3</sup>). The difference between the particle concentrations obtained during SWCNT growth using a catalyst and the control data (no catalyst) was small. Particle measurements made during the synthesis of MWCNT were found to peak at  $4 \times 10^6$  particles/cm<sup>3</sup> when

measured inside the fume hood; the particle size ranged from 25 to 100 nm when a substrate was used for MWCNT growth and from 20 to 200 nm when no substrate was present. PBZ samples collected from workers near the fume hood during MWCNT synthesis had particle concentrations similar to background. TEM analysis of MWCNT samples indicated the presence of individual particles as small as 20 nm with particle agglomerates as large as 300 nm. Some individual MWCNT were observed, but were often accompanied by clusters of carbon and iron particles. The diameter of the tubes was reported to be about 50 nm.

Bello et al. (2008) reported no increase in total airborne particle concentrations (compared with background) either during the removal of MWCNT from the reactor furnace or during the detachment of MWCNT from the growth substrate (with a razor blade), and no detectable amount of MWCNT, either as individual tubes or as agglomerates, in PBZ samples.

Johnson et al. (2010) investigated the release of airborne carbon-based nanomaterials (CNM) during the transfer and ultrasonic dispersion of MWCNT (diameter, 10–20 nm), fullerenes, and carbon black inside a laboratory fume hood (with the airflow turned off and the sash half open) during the weighing and transferral of dry CNM to beakers filled with reconstituted freshwater with and without natural organic matter that was then sonicated. Particle number concentrations for MWCNT and carbon black during the sonication of water samples were significantly greater than those found during the weighing and transferral of dry CNM. TEM analysis revealed agglomerates of all CNM and agglomerates of MWCNT 300–1000 nm in diameter.

Ogura et al. (2013a) investigated particle release during the grinding of polystyrene-based composites that contained 0 and 5% weight (wt) SWCNT (diameter, 3 nm) synthesized using a water-assisted CVD method. Considerable increases in the number concentration of

nano-sized aerosol particles were observed during the grinding of polystyrene containing CNT and CNT-free polystyrene. Nanoparticles were presumably volatile particles released by the friction heat produced by grinding the composite. In TEM analysis, micron-sized particles with protruding fibres (probably CNT) were observed, whereas free-standing CNT were not observed.

[Ji et al. \(2013\)](#) assessed the release of nano-materials during the preparation of conductive films by a spray-coating process using MWCNT. During a series of three processes, the number concentration, measured by an optical particle counter, increased from 0 to 290 particles/cm<sup>3</sup>, then dropped to 263 particles/cm<sup>3</sup>, and increased again to 724 particles/cm<sup>3</sup>. Using TEM, bundled CNT, long MWCNT with aggregations of other particles, and particle aggregations without MWCNT were observed.

(ii) *Measurement of particle number and/or respirable mass concentrations*

[Cena & Peters \(2011\)](#) evaluated the airborne release of CNT during the weighing of bulk CNT and the sanding of epoxy nanocomposite test samples. Particle number concentrations determined during the weighing process differed little from that observed in background samples (process to background ratio [P/B], 1.06), whereas the respirable mass concentration was increased (P/B, 1.79). The geometric mean (GM) respirable mass concentration inside the glove box was reported to be 0.03 µg/m<sup>3</sup> (background GM, 0.02 µg/m<sup>3</sup>). During the sanding process (with no local exhaust ventilation, in a fume hood, or in a biological safety cabinet), the PBZ nanoparticle number concentrations were negligible compared with background concentrations (average P/B, 1.04). Particles generated during sanding were reported to be predominantly micron-sized with protruding CNT and differed considerably from those of bulk CNT that tended to remain in large (> 1 µm) tangled agglomerates. Respirable mass concentrations

in the workers' breathing zones were elevated. However, the concentrations were lower when sanding was performed in the biological safety cabinet (GM, 0.2 µg/m<sup>3</sup>) than with no local exhaust ventilation (GM, 2.68 µg/m<sup>3</sup>;  $P < 0.0001$ ) or inside the fume hood (GM, 21.4 µg/m<sup>3</sup>;  $P < 0.0001$ ).

(iii) *Measurement of respirable mass concentrations and/or the count of structures containing CNT*

[Bello et al. \(2009b\)](#) investigated the release of CNT during the dry and wet cutting of a CNT-alumina composite (CNT diameter, 10–20 nm) using a band saw or rotary cutting wheel. Submicron and respirable fibres were both generated from dry cutting. Reported mean respirable mass concentrations were 2.11 and 8.38 mg/m<sup>3</sup> for area samples and 0.8 and 2.4 mg/m<sup>3</sup> for PBZ samples. TEM analysis found a concentration of 1.6 fibres/cm<sup>3</sup> in area samples and 0.2 fibres/cm<sup>3</sup> in PBZ samples. No data on fibre measurements were reported for the wet cutting of composite materials.

In a subsequent study, [Bello et al. \(2010\)](#) investigated the airborne release of CNT and other nano-sized fibres during the solid-core drilling of two types of advanced CNT-hybrid composite: (1) reinforced plastic hybrid laminates (alumina fibres and CNT); and (2) graphite-epoxy composites (carbon fibres and CNT). Airborne exposure to both alumina fibre and CNT structures were found to range in concentration from 1.0 fibres/cm<sup>3</sup> (alumina composite) to 1.9 fibres/cm<sup>3</sup> (carbon and CNT composite) for PBZ samples; similar concentrations were observed in area samples.

In summary, all but one simulation study of exposure identified either micron-sized particles with protruding CNT or bulk CNT in large tangled agglomerates by TEM analysis, providing evidence for potential exposure. The operations that may lead to exposure to CNT include CVD synthesis and sonication of MWCNT, and the

dry cutting, drilling, grinding, and sanding of composite materials containing CNT.

(c) *On-site studies*

Eleven on-site studies were carried out in different workplaces, including research and development laboratories, pilot small-scale production facilities, and, more rarely, large-scale primary or secondary manufacturer/user facilities. Most published studies were conducted in Japan, the Republic of Korea, and the USA, and only one study was recently carried out in Sweden. These data have been reviewed in detail ([Guseva Canu et al., 2016](#)) and the results are summarized in [Table 1.5](#).

In summary, taken together the available 11 on-site studies provided strong evidence that the exposure of workers to CNT/CNF material may occur, especially at workstations where no exposure control measures are implemented. In almost all situations where detectable amounts of either EC or the EC3 subfraction were found, the presence of CNT material was confirmed by TEM/SEM analyses of PBZ of area air samples. CNT were more frequently found to be attached to the soot or metal catalyst particles, embedded in other impurities or in the form of large entangled agglomerates. Individual CNT were rarely observed by SEM and TEM analysis and no validated protocol is available for counting such different structures, therefore only four studies attempted their quantification in PBZ samples. Nevertheless, different criteria for counting were applied which precluded any statistical treatment of the results. The reported values ranged between 0.003 and 0.01 structures/cm<sup>3</sup> for SWCNT and 0.008 and 193.6 structures/cm<sup>3</sup> for MWCNT, corresponding to the situation described in research and development laboratories compared with industrial settings. The operations yielding the highest release of CNT material/cm<sup>3</sup> included blending, transferral, sieving, pouring, and weighing as well as CNT production in both CVD and arc discharge processes, which encompasses

synthesis, harvesting from the reactor, and subsequent cleaning of the reactor. When the EC mass concentration was considered, values as high as 7.4–10 µg/m<sup>3</sup> and ranging from 0.68 to 38 µg/m<sup>3</sup> were measured in the PBZ samples of workers in MWCNT and SWCNT production. The operations in which the highest levels of EC were found included harvesting of CNT from the reactor, transferral of CNT and loading flasks with CNT, use of a batch mixer, CNT production, and cleaving of deposits. Only six studies measured the EC concentration and a comparison of the results is difficult because of the different EC protocols applied. Consequently, the reported values should be interpreted cautiously.

[Erdely et al. \(2013\)](#) adapted the results from the [Dahm et al. \(2012\)](#) study and those collected at three other facilities producing or using MWCNT in the USA to generate [Fig. 1.4](#). The average EC concentrations in the inhalable size fraction from the eight MWCNT sites were found to have an arithmetic mean of 10.6 µg/m<sup>3</sup> with a standard deviation (SD) of 17.2 (GM, 4.21 µg/m<sup>3</sup>; geometric SD [GSD], 4.15). In these eight MWCNT facilities, exposures ranged from non-detectable to 79.6 µg/m<sup>3</sup> and the exposure levels were log normally distributed (Shapiro-Wilk  $P = 0.97$ ).

(d) *Conclusion*

Uncertainty still exists regarding which exposure metrics should be used as indicators of potential exposure-related health effects. The results from studies using direct reading instruments are not appropriate for quantitative exposure assessment of CNT, because particle number concentration and active surface area are dominated by ultrafine particles, mostly of incidental or outdoor origin, and are not representative of engineered CNT release. The filter-based methods in combination with SEM/TEM analysis appear to be more selective and sensitive for the characterization of exposure to CNT. However, the results of studies that focused on

**Table 1.5 Occupational exposure to carbon nanotubes in different work settings**

Reference	Workplace, activity	Nanomaterial	Operation/ process	Sample	Total dust mass concentration ( $\mu\text{g}/\text{m}^3$ ) [range]	Proxy <sup>a</sup> of CNT/CNF gravimetric concentration ( $\mu\text{g}/\text{m}^3$ )	Evidence of CNT by TEM/SEM	Number of CNT (structures/ $\text{cm}^3$ )	Protective equipment
<i>Japan</i>									
<a href="#">Ogura et al. (2013b)</a>	Pilot-scale manufacture	SWCNT	Synthesis, harvesting, and package/water-assisted CVD	Area	1.6 (respirable)	from < 0.56 to < 2.3 (EC)	No	NM	Enclosure
<a href="#">Takaya et al. (2012)</a>	Polyester textile plant	MWCNT-coated yarn	Weaving a conductive fabric	Personal Area	92 (all); 66 (respirable) NR	4.8–35 (EC3') 5.3 (EC3')	Yes NM	NM NM	No
<i>Republic of Korea</i>									
<a href="#">Han et al. (2008)</a>	A-R&D laboratory	MWCNT produced by CVD	Thermal CVD	Personal Personal	ND ND	NM NM	No Yes	ND 0.08	No Fan installation, cleaning, and rearrangement
A-R&D laboratory			Al/CNT ball milling	Personal	ND	NM	No	ND	No
B-R&D laboratory			CNT solution spraying	Personal	193	NM	No	ND	No
C-R&D laboratory			Blending	Personal	30.9	NM	Yes	0.008	No
				Personal	331.7	NM	Yes	193.6	No
				Personal	ND	NM	Yes	0.018	Chiller delocalization and blender enclosure
				Area	434.5	NM	Yes	172.9	No
				Area	209	NM	No	NM	No
				Area	ND	NM	Yes	0.05	Chiller delocalisation and blender enclosure
				Area	ND	NM	No	ND	No

Table 1.5 (continued)

Reference	Workplace, activity	Nanomaterial	Operation/ process	Sample	Total dust mass concentration ( $\mu\text{g}/\text{m}^3$ ) [range]	Proxy <sup>a</sup> of CNT/CNF gravimetric concentration ( $\mu\text{g}/\text{m}^3$ )	Evidence of CNT by TEM/SEM	Number of CNT (structures/ $\text{cm}^3$ )	Protective equipment
<a href="#">Han et al. (2008)</a> (cont.)	B-R&D laboratory		Weighing	Area	113.3	NM	No	ND	No
			Weighing/spraying	Area	36.6	NM	No	ND	No
			Weighing	Area	ND	NM	Yes	1.997	
<a href="#">Lee et al. (2010)</a>	Industry	A-MWCNT	Catalyst manufacturing, MWCNT	Personal Area	[21.2–79.5]	NM	No	ND	Fume hood, enclosure, half-mask, skin protective gloves
			manufacturing (Bag cyclone, CVD)	Area	[17.7–124.1]	NM	No	ND	
Research institute		C-MWCNT	MWCNT manufacturing (CVD), spray and filtration of CNT solution	Personal Area	[42.2–285.9]	NM	No	ND	Natural ventilation
			MWCNT application (ultrasonic dispersion, addition of sulfuric acid in fume hood)	Personal Area	[7.8–145]	NM	No	ND	Fume hood
			MWCNT application, spraying CNT solution, and wafer heating	Personal Area	[87.7–160.9]	NM	No	ND	
			MWCNT application, spraying CNT solution, and wafer heating	Area	[31.1–120.4]	NM	No	0.00312	
Research institute		E-MWCNT	MWCNT application, spraying CNT solution, and wafer heating	Personal Area	[44.2–109.3]	NM	No	ND	Fume hood, enclosed, local exhaust system
			MWCNT application, spraying CNT solution, and wafer heating	Area	[80.4–127.5]	NM	No	ND	

**Table 1.5 Occupational exposure to carbon nanotubes in different work settings (continued)**

Reference	Workplace, activity	Nanomaterial	Operation/process	Sample	Total dust mass concentration ( $\mu\text{g}/\text{m}^3$ ) [range]	Proxy <sup>a</sup> of CNT/CNF gravimetric concentration ( $\mu\text{g}/\text{m}^3$ )	Evidence of CNT by TEM/SEM	Number of CNT (structures/ $\text{cm}^3$ )	Protective equipment
<a href="#">Lee et al. (2013)</a>	Two printed electronics facilities	CNT and silver nanoparticles	Roll to roll and roll to plate printing using nano-conductive ink	PBZ	[340–2930]	NM	No	NM	Fume hood, LEV
			Manufacture of CNT-nano-thin film solar cells/super capacitor	PBZ	[690–4240]	NM	No	NM	Fume hood, LEV
<i>Sweden</i>									
<a href="#">Hedmer et al. (2014)</a>	Small-scale factory, production and sieving	Unrefined MWCNT produced by arc discharge	Opening reactor and collecting the deposits	Area	< 2100 (respiratory fraction)	140 (EC); 91 (EC3)	No	ND	LEV/half-face respirator, nitrile gloves, and protecting overall
			Cleaving deposits	Area	< 1900	470 (EC); 460 (EC3)	Yes	1.6	None/same as above
			Harvesting MWCNT from deposits	Area	< 1300	< 1.4 (EC); < 1.4 (EC3)	No	ND	
			Sieving, mechanical work-up, pouring, weighing, and packaging	Area	< 430	250 (EC); 240 (EC3)	Yes	11	
			Lathe machining graphite electrode	Area	< 1700	NM	Yes	1.2	
			Reactor clean out Part I	Area	< 2800	NM	No	ND	As above
			Reactor clean out Part II	Area	6800	550 (EC); 190 (EC3)	No	ND	As above
			All operations	PBZ	79–93	7.4 (EC); 6.3 (EC3)	Yes	0.6–2.0	As above

Table 1.5 (continued)

Reference	Workplace, activity	Nanomaterial	Operation/process	Sample	Total dust mass concentration ( $\mu\text{g}/\text{m}^3$ ) [range]	Proxy <sup>a</sup> of CNT/CNF gravimetric concentration ( $\mu\text{g}/\text{m}^3$ )	Evidence of CNT by TEM/SEM	Number of CNT (structures/ $\text{cm}^3$ )	Protective equipment
<a href="#">Hedmer et al. (2014)</a> (cont.)	Small-scale factory, purification of MWCNT	Purified and functionalized MWCNT produced by arc discharge	Purification Part I Purification Part II Functionalization Part I Functionalization Part II Grinding All operations	Area Area Area Area Area PBZ*	< 360 < 320 < 1700 < 910 < 620 < 71 (LOD)	0.05 (EC); 0.05 (EC3) NM NM NM NM < 0.08 (EC); < 0.08 (EC3)	No Yes Yes No No Yes	ND 0.46 1.0 ND ND 0.04–0.1	Fume hood/ nitrile gloves, and protecting overall Fume hood/ nitrile gloves, and protecting overall As above As above
USA									
<a href="#">Maynard et al. (2004)</a>	R&D laboratory	Unprocessed SWCNT	Laser ablation CNT removal Pouring CNT generated by HiPCO Opening the chamber, removing CNT, cleaning Enclosure dismantling/ HiPCO	Personal Personal Personal Personal	NM NM NM NM	[0.70] (Ni) 36.29 (Fe) 9.86 (Ni)	NM NM NM	NM NM NM	Clean enclosure Clean enclosure Clean enclosure
<a href="#">Yeganeh et al. (2008)</a>	Primary manufacture	CNT, fullerenes	Synthesis/arc reaction	Area	[50–125] ( $\text{PM}_{2.5}$ )	NM	NM	NM	Fume hood
<a href="#">Methner et al. (2012)</a>	Industry production and handling of SWCNT	SWCNT	Harvesting material from reactor Wet wiping and mopping the room	PBZ Area PBZ Area	NR NR NR NR	38 (EC) 15 (EC) ND (EC) ND (EC)	Yes Yes Yes No	NM NM NM NM	General room ventilation, ceiling-mounted supply and exhaust

**Table 1.5 Occupational exposure to carbon nanotubes in different work settings (continued)**

Reference	Workplace, activity	Nanomaterial	Operation/process	Sample	Total dust mass concentration ( $\mu\text{g}/\text{m}^3$ ) [range]	Proxy <sup>a</sup> of CNT/CNF gravimetric concentration ( $\mu\text{g}/\text{m}^3$ )	Evidence of CNT by TEM/SEM	Number of CNT (structures/ $\text{cm}^3$ )	Protective equipment			
<a href="#">Methner et al. (2012)</a> (cont.)			Loading flasks with material	PBZ	NR	33 (EC)	Yes	NM				
			Loading trays with material	Area	NR	39 (EC)	Yes	NM				
				Area	NR	ND (EC)	Yes	NM				
				Area	NR	ND (EC)	Yes	NM				
<a href="#">Dahm et al. (2012)</a>	Primary CNT manufacture (CVD)	MWCNT, DWCNT	Outdoor background	AS	NR	0.33 (EC)	NM	NM	NA			
			MWCNT production	AS	NR	0.49 <sup>b</sup> (EC)	Yes	0.034	Enclosed process ventilated to roof/nitrile gloves			
			Harvesting MWCNT	ASC	NR	0.78 <sup>b</sup> (EC)	NM	NM	Enclosed process ventilated to roof/nitrile gloves			
				PBZ	NR	2.28 <sup>b</sup> (EC)	Yes	0.090	None/nitrile gloves, half-face respirator			
			Harvesting DWCNT	AS	NR	4.62 (EC)	Yes	0.026	NM			
				ASC	NR	PF	NM	NM				
			Harvesting MWCNT (second batch)	PBZ	NR	5.25 <sup>b</sup> (EC)	Yes	0.123	Yes	None/nitrile gloves		
				AS	NR	ND (EC)	Yes	0.047				
			Primary SWCNT manufacture	SWCNT manufacture	SWCNT	Outdoor background	PBZ	NR	2.74 <sup>b</sup> (EC)	Yes	0.399	None/nuisance dust mask
						Outdoor background	AS	NR	3.84 <sup>b</sup> (EC)	Yes	0.134	
						Outdoor background	AS	NR	0.76 (EC)	NM	NM	NA
						SWCNT production, harvesting, and reactor clean out (PBZ-reactor clean out only)	PBZ	NR	3.28 <sup>b</sup> (EC)	Yes	0.013	Reactor under vacuum/nitrile gloves, R-95 filtering face piece, and half-face respirator
Outdoor background (day 2)			Outdoor background (day 2)	AS	NR	1.13 (EC)	Yes	0.012				
			Outdoor background (day 2)	ASC	NR	ND (EC)	NM	NM				
			Outdoor background (day 2)	AS	NR	0.89 (EC)	NM	NM	NA			

Table 1.5 (continued)

Reference	Workplace, activity	Nanomaterial	Operation/ process	Sample	Total dust mass concentration ( $\mu\text{g}/\text{m}^3$ ) [range]	Proxy <sup>a</sup> of CNT/CNF gravimetric concentration ( $\mu\text{g}/\text{m}^3$ )	Evidence of CNT by TEM/SEM	Number of CNT (structures/ $\text{cm}^3$ )	Protective equipment
<a href="#">Dahm et al. (2012)</a> (cont.)			SWCNT production, harvesting, and reactor clean out (day 2)	PBZ AS ASC	NR NR NR	0.68 (EC) 1.02 (EC) 1.88 (EC)	Yes Yes NM	0.003 0.007 NM	Reactor under vacuum/nitrile gloves, R-95 filtering face piece, and half-face respirator NA
	Primary and secondary MWCNT manufacture	MWCNT	Outdoor background Production and harvesting of MWCNT	AS PBZ AS ASC	NR NR NR NR	0.76 <sup>b</sup> (EC) 1.6 (EC) 0.47 <sup>b</sup> (EC) 0.96 <sup>b</sup> (EC)	NM Yes No NM	NM 0.012 ND NM	NA Custom glove box/laboratory coat, latex gloves, surgical mask, and safety glasses None/ laboratory coat, latex gloves, and half-face respirator
			Sonication (day 2) (PBZ-sonication, sieving, and spray coating)	PBZ AS	NR NR	1.13 <sup>b</sup> (EC) ND (EC)	Yes Yes	0.010 0.002	None/ laboratory coat, latex gloves, and half-face respirator
			Sieving and spray coating (day 2)	AS ASC	NR NR	ND (EC) 0.7 <sup>b</sup> (EC)	Yes NM	0.002 NM	Chemical fume hood/ laboratory coat, latex gloves, and half-face respirator
	Secondary MWCNT manufacture for semiconductor devices	MWCNT	General office worker CNT waste collection and disposal	PBZ PBZ	NR NR	0.8 (EC) 1.06 <sup>b</sup> (EC)	Yes Yes	0.001 0.214	None/none None/full protective suit with booties, nitrile gloves

**Table 1.5 Occupational exposure to carbon nanotubes in different work settings (continued)**

Reference	Workplace, activity	Nanomaterial	Operation/process	Sample	Total dust mass concentration ( $\mu\text{g}/\text{m}^3$ ) [range]	Proxy <sup>a</sup> of CNT/CNF gravimetric concentration ( $\mu\text{g}/\text{m}^3$ )	Evidence of CNT by TEM/SEM	Number of CNT (structures/ $\text{cm}^3$ )	Protective equipment
<a href="#">Dahm et al. (2012)</a> (cont.)			Weighing	PBZ	NR	ND (EC)	No	ND	Ventilated glove box/full protective suit with booties, nitrile gloves, and full-face respirator
				AS	NR	ND (EC)	No	ND	
			Sonication	PBZ	NR	0.83 <sup>b</sup> (EC)	No	ND	Chemical fume hood/full protective suit with booties, gloves, acid smock, and face shield
				AS	NR	ND (EC)	Yes	0.003	
Secondary MWCNT manufacture/mixing with different resin formulations		MWCNT	Extrusion, weighing Batch mixer use	PBZ	NR	3.19 (EC)	Yes	0.067	Chemical fume hood and glove box/safety glasses, half-face respirator, Tyvek suit/laboratory coat, and nitrile gloves
				PBZ	NR	7.86 <sup>b</sup> (EC)	Yes	0.242	
				AS	NR	1.01 <sup>b</sup> (EC)	Yes	0.008	
				ASC	NR	1.89 <sup>b</sup> (EC)	NM	NM	
			Milling CNT composite	PBZ	NR	ND (EC)	No	ND	HEPA vacuum/safety glasses, half-face respirator, laboratory coat, and nitrile gloves
				AS	NR	ND (EC)	No	ND	

Table 1.5 (continued)

Reference	Workplace, activity	Nanomaterial	Operation/ process	Sample	Total dust mass concentration ( $\mu\text{g}/\text{m}^3$ ) [range]	Proxy <sup>a</sup> of CNT/CNF gravimetric concentration ( $\mu\text{g}/\text{m}^3$ )	Evidence of CNT by TEM/SEM	Number of CNT (structures/ $\text{cm}^3$ )	Protective equipment
<a href="#">Dahm et al. (2012)</a> (cont.)	Secondary MWCNT and CNF manufacture	MWCNT/ CNF	Transferral of nanofibres Weighing, mixing, and sonication of CNT and CNF	PBZ AS PBZ AS ASC	NR NR NR NR NR	4.15 <sup>b</sup> (EC) ND (EC) 7.54 (EC) ND (EC) 2.76 <sup>b</sup> (EC)	Yes Yes Yes Yes NM	1.1613 0.295 0.065 0.003 NM	Clean room, custom glove box/latex gloves, half-face respirator, and Tyvek smock

<sup>a</sup> The mass of total carbon (TC), total elemental carbon (EC), EC found at the highest oxidation temperature step (900 °C or 920 °C) (EC3 or EC3') or metal catalyser (nickel or iron) was used as the elemental marker for the presence of CNT/CNF

<sup>b</sup> Results were between the LOD and limit of quantification.

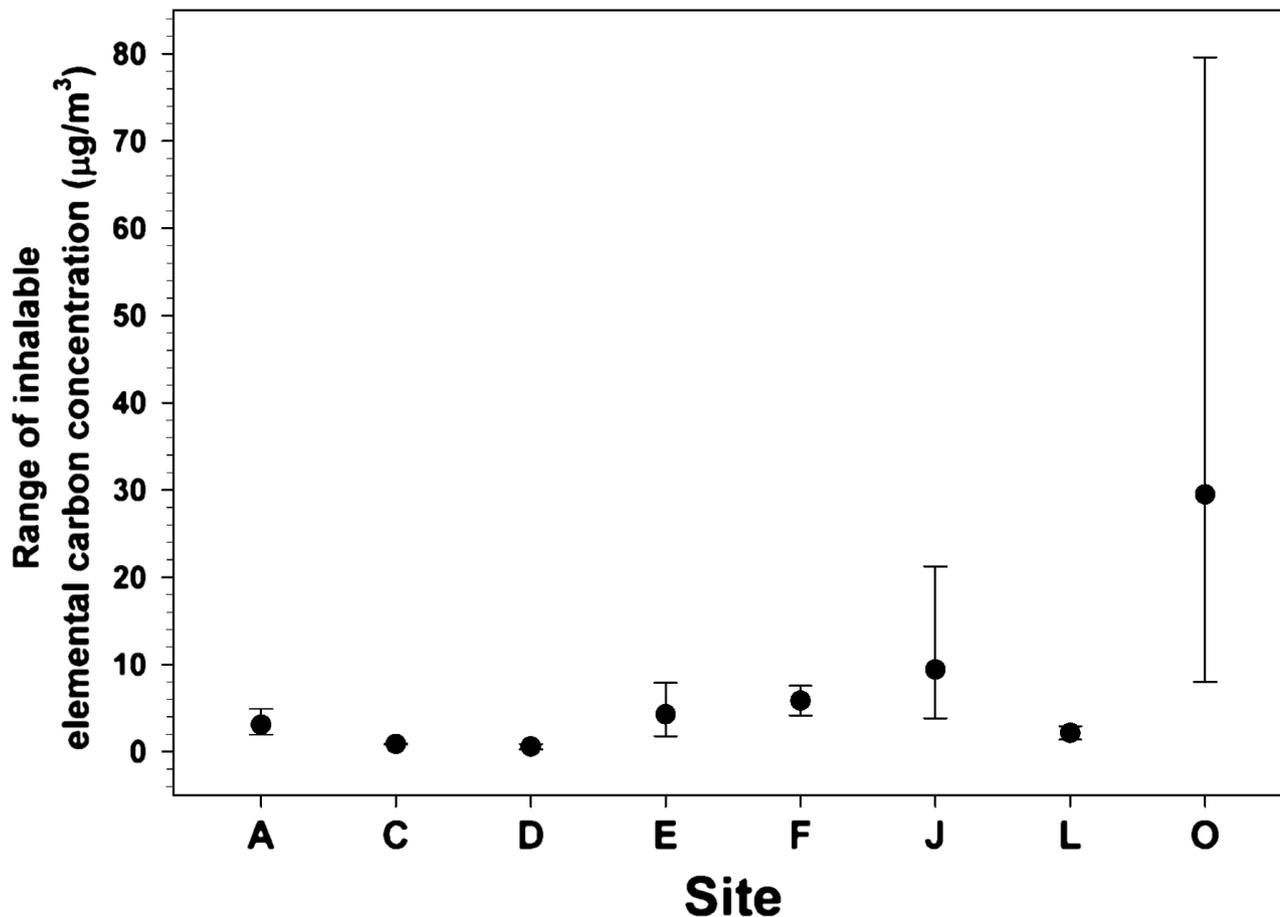
CNF, carbon nanofibres; CNT, carbon nanotubes; CVD, chemical vapour deposition; D, diameter; DWCNT, double-walled carbon nanotubes; Fe, iron; HEPA, high-efficiency particulate air; HIPCO, high-pressure carbon monoxide; LEV, local exhaust ventilation; LOD, limit of detection;  $\mu\text{g}/\text{m}^3$ , micrograms per cubic metre of air; MWCNT, multiwalled carbon nanotubes; NA, not applicable; ND, not detected (analytical limit of detection = 0.6  $\mu\text{g}/\text{sample}$ ); Ni, nickel; NM, not measured; NR, not reported; PBZ, personal breathing zone; PM<sub>2.5</sub>, particulate matter with particles of aerodynamic diameter < 2.5  $\mu\text{m}$ ; R&D, research and development; SEM, scanning electron microscopy; SWCNT, single-walled carbon nanotubes; TEM, transmission electron microscopy

In [Han et al. \(2008\)](#), total particle concentration ( $\mu\text{g}/\text{m}^3$ ) and number of MWCNT (tubes/ $\text{cm}^3$ ) were measured

In [Hedmer et al. \(2014\)](#), respiratory dust concentration was measured. \*The two workers in the purification laboratory only worked there for part of a shift and only occasionally worked there at the same time. Therefore, one sampler was used by both workers

In [Maynard et al. \(2004\)](#), nanotube concentrations were estimated assuming that the combined mass of nickel and iron constitute 30% of the SWCNT material. Measured amounts below the limit of quantification are shown in square brackets. Iron: limit of detection, 0.0643  $\mu\text{g}$ ; limit of quantification, 0.212  $\mu\text{g}$ ; nickel: limit of detection, 0.0182  $\mu\text{g}$ ; limit of quantification, 0.0601  $\mu\text{g}$

In [Dahm et al. \(2012\)](#), all EC concentrations were media blank corrected. PF, pump fault; AS, area sample (25-mm cassette collected at a fixed position); PBZ (25-mm cassette fixed to lapel of worker); ASC, area sample cyclone (37-mm cassette w/cyclone collected at a fixed position)

**Fig. 1.4 Concentrations of inhalable elemental carbon at eight facilities producing multiwalled carbon nanotubes**

The mean, with error bars representing the upper and lower range, of measured concentrations of elemental carbon ( $\mu\text{g}/\text{m}^3$ ) with background correction. The figure was adapted from previously published data: sites A, and C–F, from [Dahm et al. \(2012\)](#); and sites J, L, and O from [Dahm et al. \(2013\)](#) and [Erdely et al. \(2013\)](#). From [Erdely et al. \(2013\)](#). © Erdely et al.; licensee BioMed Central Ltd 2013

the collection of samples for the chemical-specific mass concentration analyses, such as EC mass concentration and TEM/SEM, were mostly based on original unvalidated methods and cannot be summarized appropriately. Consequently, the current available data do not allow the complete characterization of occupational exposure to CNT and only enable a limited description of some of occupational exposure situations.

## 1.5 Regulations and guidelines

No legal occupational exposure limit has been set for CNT.

In 2007, the British Standards Institution proposed a workplace exposure limit of 0.01 fibres/mL for fibrous nanomaterials with high aspect ratios ([BSI, 2007](#)) and published a “Guide to assessing airborne exposure in occupational settings relevant to nanomaterials” ([BSI, 2010](#)). The [Dutch Social and Economic Council \(2012\)](#) recommended an occupational exposure limit (OEL) of 0.01 fibres/cm<sup>3</sup> for SWCNT or MWCNT

**Table 1.6 Recommended occupational exposure limits for carbon nanotubes**

Institution	Concentration	Interpretation	Year
British Standards Institution (WEL)	0.01 fibres/mL	Fibrous nanomaterials with high aspect ratios (> 3:1) and length > 5000 nm	2007
Dutch Social and Economic Council (OEL)	0.01 fibres/cm <sup>3</sup>	SWCNT or MWCNT or metal oxide fibres for which asbestos-like effects are not excluded	2012
US NIOSH (REL)	1 µg/m <sup>3</sup> (EC)	8-hour TWA	2013
US OSHA (recommendation)	1 µg/m <sup>3</sup> (EC)	8-hour TWA	2013

EC, elemental carbon; NIOSH, National Institute for Occupational Safety and Health; OEL, occupational exposure limit; OSHA, Occupational Safety and Health Administration; REL, recommended exposure limit; TWA, time-weighted average; WEL, workplace exposure limit  
Compiled by the Working Group

or metal oxide fibres for which asbestos-like effects are not excluded ([van Broekhuizen et al., 2012](#)).

The United States National Institute for Occupational Safety and Health (NIOSH) has set a recommended exposure limit for CNT and CNF of 1 µg/m<sup>3</sup> as an 8-hour time-weighted average (TWA) of EC for the respirable range fraction ([NIOSH, 2013](#)). In 2013, the United States Occupational Safety and Health Administration published a Fact Sheet which recommends that exposure of workers to respirable CNT and CNF should not exceed 1 µg/m<sup>3</sup> as an 8-hour TWA, based on the NIOSH proposed recommended exposure limit ([OSHA, 2013](#)).

These recommended values are presented in [Table 1.6](#).

Other OELs have been proposed for CNT ranging from 1 to 50 µg/m<sup>3</sup> (8-hour TWA concentration) ([Nanocyl, 2009](#); [Aschberger et al., 2010](#); [Pauluhn, 2010a](#)). Despite the differences in risk assessment methods and assumptions, all of the derived OELs for CNT are low airborne mass concentrations relative to OELs for larger respirable carbon-based particles ([NIOSH, 2013](#)).

SWCNT and MWCNT are subject to regulation based on a US Toxic Substance Control Act (premanufacture notice) and Toxic Substance Control Act (significant new use rule) ([EPA, 2011, 2014](#)). Thus, a manufacturer or importer should submit a premanufacture notice to the US

Environmental Protection Agency (EPA) 90 days before manufacture or importation. In addition, SWCNT and MWCNT that are manufactured for uses other than those on the premanufacture notice are subject to a significant new use rule. A similar regulation applies to CNT in the [Canadian Environment Protection Act \(2014\)](#).

The [Australian National Industrial Chemical Notification and Assessment Scheme \(2010\)](#) also applies to substances containing 10% or more nanomaterials, including CNT. According to the implementation of the Globally Harmonized Classification and Labelling of Chemicals in Australia under the National Model Work Health and Safety Regulations, the classification of specific target organ toxicity applies to mixtures containing at least 10% SWCNT or MWCNT and the classification of carcinogenicity applies to mixtures containing at least 10% or more SWCNT or MWCNT ([Safe Work Australia, 2010](#)).

CNT are the subject of compulsory annual declarations of nanomaterials in France ([Journal Officiel, 2012](#)), Belgium (Arrêté Royal, 2014), and Denmark ([Danish Environment Protection, 2014](#)).

## 2. Cancer in Humans

No data were available to the Working Group.

## 3. Cancer in Experimental Animals

### 3.1 MWCNT

#### 3.1.1 Mouse

See [Table 3.1](#)

##### (a) Inhalation

In an initiation-promotion study, groups of 60 male B6C3F<sub>1</sub> mice (age, 6 weeks) were given a single intraperitoneal injection of vehicle (corn oil) or 10 µg/g body weight (bw) of 3-methylcholanthrene (3-MC) in corn oil to initiate carcinogenesis. One week after the injection, the mice were exposed to filtered air or 5 mg/m<sup>3</sup> of MWCNT-7 (Mitsui-7, Hodogaya Chemical Co.) for 5 hours per day for 15 days and were then observed for 17 months. MWCNT-7 particles ranged from single fibre-like nanotubes to tangled agglomerates with a mass median aerodynamic diameter (MMAD) of 1.59 µm and a GSD of 1.69. The count mode aerodynamic diameter of the MWCNT-7 fibres was 420 nm. Trace metal contamination was 1.32%, and iron was the major metal contaminant (1.06%). Tumour promotion activity was assessed by comparing the 3-MC plus MWCNT-7-exposed group with the 3-MC-exposed groups. The incidence of bronchiolo-alveolar lesions in mice receiving air, 3-MC, MWCNT-7, and 3-MC plus MWCNT-7 was: adenoma or carcinoma (combined) – 13 out of 56, 28 out of 54, 13 out of 49, and 38 out of 42, respectively; adenoma – 6 out of 56, 18 out of 54, 9 out of 49, and 32 out of 42, respectively; and carcinoma – 7 out of 56, 12 out of 54, 7 out of 49, and 26 out of 42, respectively. These results showed a significant difference between the 3-MC plus MWCNT-7 and 3-MC groups ( $P < 0.0001$ ),

indicating that MCWNT-7 promoted the induction of benign and malignant lung tumours. The incidence of lung tumours in animals treated with MCWNT-7 (in the absence of initiation with 3-MC) did not differ significantly from that in the filtered-air controls. However, the volume of lung tumours was significantly greater ( $P < 0.0001$ ) in the MWCNT-7-exposed group than in the air-exposed group ([Sargent et al., 2014](#)).

##### (b) Intratracheal instillation

Three groups of three male C57BL/6 mice (age, 6 weeks) were given a single intratracheal instillation of either a 50-µL aliquot of saline or MWCNT CM-95 (Hanwha Nanotech; as-produced; length, 7.71 µm; diameter, 13.5 nm) or acid-treated MWCNT CM-95 (length, 567.4 nm; diameter, 7.5 nm) in 50 µL saline (100 µg MWCNT/mouse) and were killed 6 months after the injection. None of the three mice given saline developed hyperplasia, adenoma, or adenocarcinoma of the lung. Of the three mice given MWCNT (as-produced), two developed peri-bronchial lymphoid hyperplasia, two developed adenomas, and one developed adenocarcinoma of the lung. Of the three mice given acid-treated MWCNT, two developed hyperplasia and one developed adenoma (described by the authors as a “slight” adenoma) of the lung ([Yu et al., 2013](#)). [The Working Group noted the short duration of the experiment, the very small number of animals that precluded a statistical assessment of the results, and the low susceptibility of this strain to lung carcinogenesis. The study was judged to be inadequate for an evaluation of carcinogenicity.]

##### (c) Intraperitoneal injection

Two groups of 19 male p53<sup>+/-</sup> mice (C57BL/6 background; age, 9–11 weeks) received a single intraperitoneal injection of either 1 mL of vehicle (control) or 3 mg (particle count,  $1 \times 10^9$ ) of MWCNT-7 (Mitsui; length, 1–19 (median, 2) µm; diameter, 70–170 (median, 90) nm; impurities:

Table 3.1 Studies on carcinogenicity with multiwalled carbon nanotubes in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
B6C3F <sub>1</sub> (M) 17 mo <a href="#">Sargent et al. (2014)</a>	Intraperitoneal injection of 0 or 10 µg/g bw of 3-MC in corn oil once (initiation) Inhalation exposure 0 or 5 mg/m <sup>3</sup> of MWCNT-7 (Mitsui-7, Hodgeya, Japan) in air, 5 h/day for 15 days, 1 wk later, then observed for 17 mo (promotion) 60/group	Bronchiolo-alveolar adenoma: Air, 6/56; 3-MC, 18/54; MWCNT-7, 9/49; 3-MC+MWCNT-7, 32/42 Bronchiolo-alveolar carcinoma: Air, 7/56; 3-MC, 12/54; MWCNT-7, 7/49; 3-MC+MWCNT-7, 26/42 Bronchiolo-alveolar adenoma or carcinoma (combined): Air, 13/56; 3-MC, 28/54; MWCNT-7, 13/49; 3-MC+MWCNT-7, 38/42	Air vs MWCNT-7, NS; 3-MC vs 3-MC+MWCNT-7, $P < 0.0001$ , Fisher exact test  Air vs MWCNT-7, NS; 3-MC vs 3-MC+MWCNT-7, $P < 0.0001$ , Fisher exact test  Air vs MWCNT-7, NS; 3-MC vs 3-MC+MWCNT-7, $P < 0.0001$ , Fisher exact test	Primary particles: MWCNT particles ranged from single fibre-like nanotubes to tangled agglomerates; MMAD, 1.59 µm; GSD, 1.69; count mode aerodynamic diameter, 420 nm; trace metal contamination, 1.32% (iron was the major metal contaminant, 1.06%) The lung tumour volume was significantly greater ( $P < 0.0001$ ) in the MWCNT-7-exposed groups compared with the air-exposed groups
C57BL/6 (M) 6 mo <a href="#">Yu et al. (2013)</a>	Intratracheal instillation 0 or 100 µg/mouse of as-produced or acid-treated MWCNT in 50 µL of saline once, then killed 6 mo later 3/group	Lung hyperplasia: 0/3, 2/3 (as-produced), 2/3 (acid-treated) Lung adenoma: 0/3, 2/3 (as-produced), 1/3 (acid-treated) Lung adenocarcinoma: 0/3, 1/3 (as-produced), 0/3 (acid-treated)	NA	MWCNT CM-95 (Hanwha Nanotech, Republic of Korea); primary particles: as-produced (length, 7.71 µm; diameter, 13.5 nm); acid-treated (treated with sulfuric/nitric acid (3:1, v/v); length, 567 nm; diameter, 7.5 nm) Small number of animals and short duration of the experiment; the very small number of animals precluded statistical assessment of the results; C57BL/6 mice have a low susceptibility to lung carcinogenesis
p53 <sup>-/-</sup> (C57BL/6 background) (M) 180 days <a href="#">Takagi et al. (2008)</a>	Intraperitoneal injection 0 (vehicle control) or 3 mg/mouse (particle count/mouse, $1 \times 10^9$ ) of MWCNT-7 (Mitsui) in 1 mL of 0.5% methyl cellulose + 1.0% Tween 80 once, then observed up to 180 days 19/group	Peritoneal mesothelioma (epithelial type): 0/19, 14/16*	* [ $P < 0.0001$ , Fisher's exact test]	Primary particles: length, 1–19 (median, 2) µm; diameter, 70–170 (median, 90) nm; iron, 3500 ppm (0.35%); sulfur, 470 ppm; chlorine, 20 ppm; fluorine, < 5 ppm; bromine, < 40 ppm

**Table 3.1 (continued)**

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
p53 <sup>-/-</sup> (C57BL/6 background) (M) 365 days <a href="#">Takagi et al. (2012)</a>	Intraperitoneal injection 1 mL of 0 (vehicle control), 3 (particle count, 1 × 10 <sup>6</sup> ), 30 (particle count, 1 × 10 <sup>7</sup> ), or 300 (particle count, 1 × 10 <sup>8</sup> ) µg/ mouse of MWCNT-7 (Mitsui) in 0.5% methyl cellulose + 1.0% Tween 80 once, then observed up to 365 days 20/group	Peritoneal mesothelioma (epithelial type): 0/20, 5/20*, 17/20**, 19/20**	* [P < 0.05, Fisher's exact test] ** [P < 0.0001, Fisher's exact test]	Primary particles: length, 1–19 (median, 2) µm; diameter, 70–170 (median, 90) nm; iron, 3500 ppm (0.35%); sulfur, 470 ppm; chlorine, 20 ppm; fluorine, < 5 ppm; bromine, < 40 ppm
rasH2 (C57BL/6 background) (M) 26 wk <a href="#">Takanashi et al. (2012)</a>	Subcutaneous injection 550 µL of 0 (control) or 75 mg/kg bw of MWCNT dispersed in saline containing 0.1% Tween 80 in the back, then killed after 26 wk 10/group	No increase in tumour incidence at any site in treated mice	NS	VGCF-S MWCNT (Showa Denko, Japan): mean length, 10 µm; mean diameter, 100 nm

bw, body weight; GSD, geometric standard deviation; M, male; 3-MC, 3-methylcholanthrene; MMAD, mass median aerodynamic diameter; mo, month; MWCNT, multiwalled carbon nanotubes; NA, not applicable; NS, not significant; ppm, parts per million; wk, week

iron, 3500 ppm (0.35%); sulfur, 470 ppm; chlorine, 20 ppm; fluorine, < 5 ppm; and bromine, < 40 ppm) in 1 mL of 0.5% methyl cellulose with 1.0% Tween 80, and were then observed for up to 180 days. The incidence of peritoneal mesothelioma was significantly increased in the MWCNT-7-exposed group (14 out of 16 versus 0 out of 19; [ $P < 0.0001$ ]) ([Takagi et al., 2008](#)).

Four groups of 20 male p53<sup>+/-</sup> mice (C57BL/6 background; age, 9–11 weeks) received a single intraperitoneal injection of 0 (vehicle control), 3, 30, or 300 µg/mouse of MWCNT-7 (Mitsui; length, 1–19 (median, 2) µm; diameter, 70–170 (median, 90) nm; impurities: iron, 3500 ppm (0.35%); sulfur, 470 ppm; chlorine, 20 ppm; fluorine, < 5 ppm; and bromine, < 40 ppm) (particle count: 0,  $1 \times 10^6$ ,  $1 \times 10^7$ , or  $1 \times 10^8$ , respectively) in 1 mL of 0.5% methyl cellulose with 1.0% Tween 80, and were then observed for up to 365 days. Survival of the mice in the dosed groups was shorter due to the high incidence of lethal mesothelioma. The incidence of peritoneal mesothelioma (0 out of 20 [control], 5 out of 20, 17 out of 20, and 19 out of 20, respectively) was significantly increased in all treated groups ([ $P < 0.05$ ,  $P < 0.0001$ , and  $P < 0.0001$ ], respectively) compared with controls ([Takagi et al., 2012](#)).

#### (d) Subcutaneous injection

Two groups of 10 male rasH2 (human c-Ha-ras proto-oncogene) transgenic mice (C57BL/6 background; age, 6 weeks) received a single subcutaneous injection in the back of 550 µL of vehicle or 75 mg/kg bw of MWCNT (VGCF-S; Showa Denko, Japan; mean length, 10 µm; mean diameter, 100 nm) dispersed in 550 µL saline containing 0.1% Tween 80, and were killed after 26 weeks. No significant increase in tumour incidence was observed at any site in the treated mice ([Takanashi et al., 2012](#)).

### 3.1.2 Rat

See [Table 3.2](#)

#### (a) Intraperitoneal injection/implantation

The carcinogenic potential of two different MWCNT (MWCNT+ and MWCNT–; University of Namur, Belgium) was compared. MWCNT+ had a length and diameter of about 0.7 µm and  $11.3 \pm 3.9$  nm, respectively, and a metal content (%) of: aluminium, 1.97; iron, 0.49; and cobalt, 0.48. MWCNT– had the same length and diameter, but a lower metal content (aluminium, 0.37%; iron, < 0.01%; and cobalt, < 0.01%) and fewer structural defects. Groups of 26 or 50 male Wistar rats (age, 10–13 weeks) received a single intraperitoneal injection of vehicle (control), MWCNT+ (2 mg or 20 mg/rat), MWCNT– (20 mg/rat), or crocidolite (2 mg/rat, positive control) in 2 mL phosphate-buffered saline (PBS) and were then observed for up to 24 months. The incidence of peritoneal mesothelioma was 1 out of 26, 2 out of 50, 0 out of 50, 3 out of 50, and 9 out of 26 ( $P < 0.01$ , crocidolite-treated group), respectively, and that of peritoneal lipoma, liposarcoma, or angiosarcoma (combined) was 0 out of 26, 1 out of 50, 3 out of 50, 3 out of 50, and 0 out of 26 (no significant difference), respectively ([Muller et al., 2009](#)). [The Working Group noted that peritoneal mesotheliomas are rare spontaneous neoplasms in rats. The Working Group also noted that the incidence of peritoneal mesothelioma in the vehicle-control group was unusually high (1 out of 26; 3.8%) and did not exclude the possibility that this tumour originated from the scrotum and spread into the peritoneal cavity.]

Two groups of six male Fischer 344 rats (weighing 400 g) [age and sex unspecified] received an intraperitoneal implant of a gelatin capsule containing either 10 mg/rat of MWCNT (Shenzhen Nanotech; length, 1–2 µm; diameter, 10–30 nm; 95–98% pure) or crystalline zinc oxide as a negative control. The experiment was terminated after 12 months. Mesotheliomas were not found but foreign body granulomatous lesions were observed in MWCNT-exposed rats ([Varga](#)

**Table 3.2 Studies of carcinogenicity with multiwalled carbon nanotubes in rats**

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Wistar (M) 24 mo <a href="#">Muller et al. (2009)</a>	Intraperitoneal injection 2 mL of 0 (vehicle control), 2 or 20 mg/rat of MWCNT+, 20 mg/rat of MWCNT-, or 2 mg/rat of crocidolite in PBS, then observed up to 24 mo Vehicle, 26 rats; 2 mg MWCNT+, 50 rats; 20 mg MWCNT+, 50 rats; 20 mg MWCNT-, 50 rats; 2 mg crocidolite, 26 rats	Peritoneal mesothelioma: 1/26, 2/50 (2 mg MWCNT+), 0/50 (20 mg MWCNT+), 3/50 (MWCNT-), 9/26* (crocidolite)  Peritoneal lipoma, liposarcoma or angiosarcoma (combined): 0/26, 1/50 (2 mg MWCNT+), 3/50 (20 mg MWCNT+), 3/50 (MWCNT-), 0/26 (crocidolite)	* $P < 0.01$ , Fisher's exact test  NS	MWCNT (University of Namur, Belgium); MWCNT+: length, ~0.7 µm; diameter, $11.3 \pm 3.9$ nm; metal content (%): aluminium, 1.97; iron, 0.49; cobalt, 0.48 MWCNT-: length, ~0.7 µm; diameter, $11.3 \pm 3.9$ nm; metal content (%): aluminium, 0.37; iron, < 0.01; cobalt, < 0.01
F344 (NR) 12 mo <a href="#">Varga &amp; Szendi (2010)</a>	Intraperitoneal implantation Gelatin capsule filled with crystalline zinc oxide (negative control) or 10 mg of MWCNT once, then observed for 12 mo 6/group	Mesothelioma: 0/6, 0/6	NS	MWCNT (length, 1–2 µm; diameter, 10–30 nm; 95–98% pure) produced by Shenzhen Nanotech Port Co. Ltd, China.  Study duration was short; no vehicle control; small number of animals per group; foreign body granulomatous lesions observed in MWCNT-exposed rats
F344/Brown Norway F <sub>1</sub> hybrid (M, F) 350 days or up to 3 yr <a href="#">Nagai et al. (2011, 2013)</a>	Intraperitoneal injection 1 mL of 0, 0.5 or 5 mg/mL of MWCNT in 0.5% BSA in saline, twice within a 1-wk period, then observed for up to 3 yr 6–43 M+F/group	Mesothelioma (M+F) at 350 days: 0/23 (0%), 12/15 (80%; NT50a(-agg*))*, 13/13 (100%; 1 mg/rat NT50a)*, 43/43 (100%; 10 mg/rat NT50a)*, 6/6 (100%; 10 mg/rat NT50b)*, 5/29 (17%; 1 mg/rat NT145)***, 28/30 (93%; 10 mg/rat NT145)*, 0/6 (0%; 10 mg/rat NTtngl)  Mesothelioma (M+F) at 3 yr: 0/6 (0%; 10 mg/rat NTtngl)	* [ $P < 0.0001$ ], Fisher's exact test; ** [ $P < 0.05$ ], Fisher's exact test  NS	MWCNT [NT50a (MWCNT-7) from Mitsui, others from Showa Denko] NT50a(-agg*): length, 5.29 µm; diameter, 49.95 nm; no aggregation; same number of fibres as in the NT145 suspension NT145 suspension NT50a: length, 5.29 µm; diameter, 49.95 nm; high level of aggregation NT50b: length, 4.60 µm; diameter, 52.40 nm; high level of aggregation NT145: length, 4.34 µm; diameter, 143.5 nm; low level of aggregation NTtngl: tangled conformation; diameter, 2–20 nm

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M) 52 wk <a href="#">Sakamoto et al. (2009)</a>	Intrascrotal injection 0 (vehicle control) in 2 mL/kg bw or 0.5 mg/mL (3.55 × 10 <sup>8</sup> particles/mg) of MWCNT-7 (Mitsui) in 2% carboxymethyl cellulose (0.24 mg/rat, 1.0 mg/kg bw) once, then observed up to 52 wk Vehicle, 5 rats; MWCNT-7, 7 rats.	Peritoneal mesothelioma hyperplasia: 0/5, 7/7* Peritoneal mesothelioma (epithelial and sarcomatoid mixed type): 0/5, 6/7*	* <i>P</i> < 0.05, Fisher's exact test	Primary particles: length, 1–19 (median, 2) μm; diameter, 70–170 (median, 90) nm; iron, 3500 ppm; sulfur, 470 ppm; chlorine, 20 ppm; fluorine, < 5 ppm; bromine, < 40 ppm Small number of animals per group

BSA, bovine serum albumin; F, female; M, male; mo, month; MWCNT, multiwalled carbon nanotubes; NR, not reported; NS, not significant; PBS, phosphate-buffered saline; wk, weeks; yr, year

& Szendi, 2010). [The Working Group noted the small number of animals, the short duration of the study, that the age and sex of the animals were not reported, and the lack of a vehicle control. The study was judged to be inadequate for an evaluation of carcinogenicity.]

Groups of male and female Fischer 344/Brown Norway F<sub>1</sub> hybrid rats (age, 6 weeks) received two intraperitoneal injections during a 1-week period of 1 mL of 0.5 or 5 mg/mL of NT50a or NT145 MWCNT, 0.5 mg/mL of NT50a(-agg\*) MWCNT, or 5 mg/mL NT50b or NTtngl MWCNT, and were then observed for up to 350 days. Control rats received two injections of the vehicle (0.5% bovine serum albumin in saline) alone. NT50a (MWCNT-7; length, 5.29 µm; diameter, 49.95 nm) was purchased from Mitsui; NT50b (length, 4.60 µm; diameter, 52.40 nm), NT145 (length, 4.34 µm; diameter, 143.5 nm), and NTtngl (diameter, 2–20 nm; tangled conformation, therefore length was not determined) were purchased from Showa Denko. NT50a(-agg\*) was obtained from the supernatant after the centrifugation of NT50a at 2200 g for 10 seconds, which was then concentrated to obtain the same fibre count as that of NT145. Aggregation was high in NT50a and NT50b suspensions, low in the NT145 suspension, and very high in the NTtngl suspension; no agglomerates were present in the NT50a(-agg\*) suspension. There was a significant increase in the incidence of mesothelioma in all MWCNT-treated groups, except in the NTtngl-treated group. The incidences of mesothelioma at 350 days were 0 out of 23 (control), 12 out of 15 (1 mg NT50a(-agg\*)), 13 out of 13 (1 mg NT50a), 43 out of 43 (10 mg NT50a), 6 out of 6 (10 mg NT50b), 5 out of 29 (1 mg NT145), 28 out of 30 (10 mg NT145), and 0 out of 6 (10 mg NTtngl) (Nagai et al., 2011). An additional six rats treated with 10 mg NTtngl were held for up to 3 years after treatment. Granulomas were induced but no mesotheliomas were observed (Nagai et al., 2013). [The Working Group noted the varying

number of animals per group and the small number of animals exposed to MWCNT with a tangled conformation. The authors stated that they found no evidence of mesothelioma induced by MWCNT with a tangled conformation, but the Working Group believed that the demonstration of a negative result requires a study with high statistical power which would not be reached with a sample size of six animals.]

#### (b) Intrascrotal injection

Two groups of male Fischer 344 rats (age, 12 weeks) received a single intrascrotal injection of vehicle (5 rats, 2 mL/kg bw) or 0.24 mg of 0.5 mg/mL MWCNT-7 (Mitsui; length, 1–19 (median, 2) µm; diameter, 70–170 (median, 90) nm; impurities: iron, 3500 ppm (0.35%); sulfur, 470 ppm; chlorine, 20 ppm; fluorine, < 5 ppm; and bromine, < 40 ppm) (7 rats, 1.0 mg/kg bw) in 2% carboxymethyl cellulose and were then observed for up to 52 weeks. The incidence of peritoneal mesothelial cell hyperplasia in the control and treated group was 0 out of 5 and 7 out of 7 ( $P < 0.05$ ), respectively, and that of peritoneal mesothelioma was 0 out of 5 and 6 out of 7 ( $P < 0.05$ ), respectively (Sakamoto et al., 2009). [The Working Group noted the small numbers of animals per group.]

## 3.2 SWCNT

See [Table 3.3](#)

### Rat

#### (a) Intratracheal instillation

Groups of male Crl: CD (SD) rats (age, 8 weeks) received intratracheal instillations with a type of SWCNT synthesized by the National Institute of Advanced Industrial Science and Technology, Japan (primary particle maximum length, 1200 µm; primary particle diameter, 3.0 nm; metal content: 145 ppm iron, 103 ppm

**Table 3.3 Studies of carcinogenicity with single-walled carbon nanotubes in rats**

Species, strain (sex)	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Crl: CD (SD) (M) Up to 26 wk <a href="#">Kobayashi et al. (2011)</a>	Experiment 1: Intratracheal instillation 1 mL/kg bw of 0 (vehicle), 0.2, or 2.0 mg/mL of SWCNT in Tween 80 in PBS once, then killed at 24 h, 3 days, 1 wk, 4 wk, or 13 wk Experiment 2: Intratracheal instillation 1 mL/kg bw of 0 (vehicle), 0.04, 0.2, or 1.0 mg/mL of SWCNT in Tween 80 in PBS once, then killed at 3 days, 1 wk, 4 wk, 13 wk, or 26 wk 6/group at each time-point	Lung tumour: None reported in any group	NS	Synthesized by National Institute of Advanced Industrial Science and Technology, Japan; primary particles: maximum length, 1200 µm; diameter, 3.0 ± 1.1 nm; iron, 145 ppm; nickel, 103 ppm; chromium, 34 ppm; manganese, 2 ppm; aluminium, 12 ppm; aggregates: length, 0.32 µm; diameter, 12.0 ± 6.5 nm Short duration of the study
Rat, F344 (NR) 12 mo <a href="#">Varga &amp; Szendi (2010)</a>	Intraperitoneal implantation Gelatin capsule filled with crystalline zinc oxide (negative control) or 10 mg of SWCNT once, then observed for 12 mo 6/group	Mesothelioma 0/6, 0/6	NS	SWCNT (length, 4–15 µm; diameter, < 2 nm; 90% pure) produced by Shenzhen Nanotech Port Co. Ltd, China Short duration of the study; no vehicle control; small number of animals per group; foreign body granulomatous lesions observed in SWCNT-exposed rats

bw, body weight; M, male; mo, month; NR, not reported; NS, not significant; PBS, phosphate-buffered saline; SWCNT, single-walled carbon nanotubes; wk, week

nickel, 34 ppm chromium, 2 ppm manganese, and 12 ppm aluminium; and aggregate length, 0.32  $\mu\text{m}$ ; aggregate diameter, 12.0 nm). In a first experiment, the rats were given a single dose of 1 mL/kg bw of a 0 (vehicle)-, 0.2-, or 2.0-mg/mL solution of SWCNT in Tween 80 in PBS (doses corresponding to 0.0, 0.2, or 2.0 mg/kg bw), and six rats per group were killed 24 hours, 3 days, 1 week, 4 weeks, or 13 weeks later. In a second experiment, the rats were given a single dose of 1 mL/kg bw of a 0 (vehicle)-, 0.04-, 0.2-, or 1.0-mg/mL solution of SWCNT in Tween 80 in PBS (doses corresponding to 0.0, 0.4, 0.2, or 1.0 mg/kg bw), and six rats per group were killed 3 days, 1 week, 4 weeks, 13 weeks, or 26 weeks later. No lung tumours were reported in any group ([Kobayashi et al., 2011](#)). [The Working Group noted the short duration of the experiments and judged the study to be inadequate for an evaluation of carcinogenicity.]

*(b) Intraperitoneal implantation*

Two groups of six Fischer 344 rats (weighing 400 g) [age and sex unspecified] received an intraperitoneal implant of a gelatin capsule containing either 10 mg/rat of SWCNT (Shenzhen Nanotech; diameter < 2 nm; length, 4–15  $\mu\text{m}$ ; 90% pure) or crystalline zinc oxide as a negative control, and the experiment was terminated after 12 months. Mesotheliomas were not found but foreign body granulomatous lesions were observed in SWCNT-exposed rats ([Varga & Szendi, 2010](#)). [The Working Group noted the small number of animals, the short duration of the study, that the age and sex of the animals were not reported, and the lack of a vehicle control. The study was judged to be inadequate for an evaluation of carcinogenicity.]

## 4. Mechanistic and Other Relevant Data

### 4.1 Deposition, phagocytosis, translocation, retention, and clearance

#### 4.1.1 Humans

No data were available to the Working Group.

#### 4.1.2 Experimental animals

##### *(a) Deposition*

In male Wistar rats exposed by whole-body inhalation for 6 hours per day on 5 days per week to 0.37 mg/m<sup>3</sup> of MWCNT (Nikkiso Co., Ltd; length, 1.1  $\mu\text{m}$ ; diameter, 63 nm) dispersed in an aqueous solution of 0.5 mg/mL Triton X-100 and atomized by a nebulizer into the exposure chamber (MWCNT aerosol comprised of approximately 70% of single fibres), lung deposition fractions of 0.18 or 0.2 were estimated from the measured mass of CNT in the lungs 3 days after the end of the 4-week experiment. The retained mass lung burdens were measured by X-ray diffraction or EC analysis at 3 days, 1 month, and 3 months after exposure, and the mass of MWCNT in the lungs 3 days after exposure was 68 and 76  $\mu\text{g}$ /lung, as measured by the two methods, respectively ([Oyabu et al., 2011](#)).

A deposition fraction of 5.7% MWCNT was estimated in rats by [Pauluhn \(2010b\)](#) using data on the airborne size distribution (e.g. MMAD,  $\sim 3 \mu\text{m}$ ; GSD,  $\sim 2$ ) and the Multiple-Path Particle Dosimetry (MPPD) model 2 software ([Anjilvel & Asgharian, 1995](#)). [NIOSH \(2013\)](#) provided a comparison of the rat alveolar deposition fraction estimates from [Pauluhn \(2010b\)](#) using two different versions of the MPPD software (v. 2.0 and 2.1) (CIIT & RIVM, 2006; [ARA, 2011](#)) and density values of either 1 or 0.2 g/mL. Estimated deposition fractions were 0.046, 0.027, or 0.023

from MPPD 2.0 (density 1 g/mL), MPPD 2.1 (density 1 g/mL), or MPPD 2.1 (density 0.2 g/mL), respectively. The aerodynamic particle size used was 2.74  $\mu\text{m}$  MMAD (GSD, 2.11) [middle of the three measures reported by [Pauluhn \(2010b\)](#)].

A 3-week inhalation study in male C57BL/6J mice exposed to 5  $\text{mg}/\text{m}^3$  of MWCNT (Mitsui-7 [MWCNT-7]; Hodogaya Chemical Co.) for 5 hours per day for 12 days provided information to estimate the lung deposition fraction ([Mercer et al., 2013a](#)). [The Working Group noted that, although a mouse lung deposition fraction was not reported in [Mercer et al. \(2013a\)](#), it can be estimated (as shown below) to provide additional information to and enable comparisons with estimates from other animal studies on the inhalation of CNT. The Working Group also noted that estimation of the deposition fraction from the measured lung burden at the end of inhalation exposure would be underestimated by the amount of CNT that was cleared from the lungs during the exposure period.]

The average lung burden measured 1 day after the end of the 3-week inhalation exposure was 28.1  $\mu\text{g}$  ([Mercer et al., 2013a](#)). The estimated deposition fraction can be estimated as:

Deposition fraction = total lung dose (mg)/  
exposure (h/d \* d \* min/h) \* L/min \*  $\text{m}^3/\text{L}$ , or

$$0.0095 = 28.1/5 \times (5 \times 12 \times 60) \times 0.165 \times (1/1000)$$

where the total lung dose was measured 1 day (d) after the end of the 12-day inhalation exposure ([Mercer et al., 2013a](#); [Table 4.1](#)) and the minute ventilation rate was 0.165 L/min ([Shvedova et al., 2008](#)). [Shvedova et al. \(2008\)](#) stated that mouse ventilation rates (including both tidal volumes and breathing rates) can be highly variable depending on how the values were measured. Using the [EPA \(1988, 2006\)](#) minute ventilation rate of 0.037 L/min in mice, the deposition fraction would be 0.042.

Expressed as a percentage, the mouse lung deposition fractions estimated above were approximately 1% or 4% for MWCNT in [Mercer et al. \(2013a\)](#) (using a minute ventilation rate

of either 0.165 or 0.037 L/min, respectively). In comparison, a mouse lung deposition fraction of 0.5% was reported for SWCNT by [Shvedova et al. \(2008\)](#), which was based on a mass mode aerodynamic diameter of 4.2  $\mu\text{m}$  and estimation of the deposition fraction from [Raabe et al. \(1988\)](#) [[Shvedova et al. \(2008\)](#) used the estimated deposition fraction in mice to estimate the deposited lung dose in mice and the worker-equivalent lung dose.] The mass mode aerodynamic diameter of MWCNT was 1.3  $\mu\text{m}$  ([Mercer et al., 2013a](#)) and the MMAD was 1.5  $\mu\text{m}$  (GSD, 1.67) ([Chen et al., 2012](#)). [This comparison shows reasonably consistent estimated deposition fractions in mice inhaling CNT, given the differences in the measures of aerodynamic diameter and the uncertainty about mouse ventilation rates.]

A study of MWCNT in male Sprague-Dawley rats (age, 9–10 weeks) provided a comparison of the lung responses to exposure to three different forms of MWCNT, including original (O), purified (P), and carboxylic acid-functionalized (F), at similar estimated lung doses by nose-only inhalation or tracheal instillation ([Silva et al., 2014](#)). The O-MWCNT contained 4.49% nickel and 0.76% iron residual catalysts; P-MWCNT contained 1.8% nickel and 0.08% iron; while F-MWCNT contained no detectable levels of nickel or iron. The dimensions of these MWCNT were: outer diameter, 20–30 nm; inner diameter, 5–10 nm; and length, 10–30  $\mu\text{m}$ . The MWCNT were aerosolized for inhalation by nebulization. The MMADs (GSD) for O-, P-, and F-MWCNT were 3.7 (2.5), 4.8 (2.9), and 3.3 (3.1)  $\mu\text{m}$ , respectively. Doses for tracheal instillation were 0, 10, 50, or 200  $\mu\text{g}$  in a biocompatible dispersion medium. The single (6-hour) inhalation exposure at a concentration of approximately 30  $\text{mg}/\text{m}^3$  was estimated to result in a deposited lung dose that was similar to or higher than that of the intratracheally administered dose of 200  $\mu\text{g}$  (estimated by assuming an alveolar and tracheobronchial deposition fraction of 0.14 and a ventilation rate of 0.15 L/min: 30  $\text{mg}/\text{m}^3 \times 0.15 \text{ L}/\text{min} \times 6 \text{ h} \times 60$

$\text{min/h} \times 1 \text{ m}^3 / 1000 \text{ L} \times 0.14 \times 1000 \mu\text{g} / 1 \text{ mg} = 227 \mu\text{g}$ . [The Working Group noted that the “Inhalation Exposure and Aerosol Characterization” section of the Methods in the publication reported a MWCNT aerosol concentration of  $38 \mu\text{g/L}$  (equal to  $38 \text{ mg/m}^3$ ), which would result in a deposition of  $287 \mu\text{g}$  MWCNT.]

### (b) Phagocytosis

CNT have been observed in cells using confocal Raman microscopy ([Romero et al., 2011](#)) or TEM ([Ryman-Rasmussen et al., 2009a](#)). The possible mechanisms by which CNT can enter cells include diffusion or penetration through cell membranes (passive internalization) or endocytosis (active internalization) ([Kunzmann et al., 2011](#); [Ye et al., 2013](#)), both of which may depend on the surface properties of the CNT and the activation state of the phagocytic cells. Four types of endocytosis have been reported ([Ye et al., 2013](#)): phagocytosis, clathrin-mediated endocytosis, caveola-mediated endocytosis, and macrophage pinocytosis. The first three types have been studied in relation to CNT. Phagocytosis is the engulfment of foreign materials by macrophages, monocytes, and neutrophils, the primary purpose of which is considered to be the elimination of larger pathogens (bacteria and yeast) or cell debris. Larger CNT structures (e.g.  $> 400 \text{ nm}$ ) or agglomerates were recognized by phagocytes, while individual structures evaded phagocytosis ([Antonelli et al., 2010](#); [Ali-Boucetta & Kostarelos, 2013](#)). Clathrin-mediated endocytosis involves the internalization of macromolecules by the inward budding of plasma membrane vesicles (with or without receptor- or ligand-specific binding) and many studies have reported the cell uptake of CNT by this mechanism ([Ye et al., 2013](#)). Caveola-mediated endocytosis involves caveolar vesicles that are composed of cholesterol and sphingolipids. A CNT radius of  $25 \text{ nm}$  was estimated to be associated with a maximal rate of endocytosis ([Jin et al., 2009](#)), while a maximum length of  $189 \text{ nm}$

of DNA-wrapped SWCNT was effectively endocytosed by various cell lines ([Becker et al., 2007](#)).

The mechanisms of cell uptake also depend on the cell type encountered by the CNT (which also depends on the route of exposure). Macrophages in the pulmonary or interstitial regions of the lungs are capable of phagocytosing CNT, although the size and surface properties of CNT influence their ability to be recognized and phagocytosed by these cells. In the liver, Kupffer cells are the primary cellular site where CNT are observed. Functionalizations/modifications to the surface of CNT (e.g. covalently bonded functional groups or non-covalently bonded coatings) can also influence the cell uptake of CNT ([Ali-Boucetta & Kostarelos, 2013](#)).

In rats exposed by pharyngeal aspiration, the alveolar macrophage uptake of SWCNT ( $< 0.23\%$  iron) was low ([Shvedova et al., 2005](#)). Morphological analysis showed that only  $10\%$  of the alveolar burden of SWCNT was located within the alveolar macrophages ([Shvedova et al., 2005](#)), while  $90\%$  of the dispersed SWCNT structures were observed to cross alveolar epithelial cells and enter the interstitium ([Mercer et al., 2008](#)). MWCNT were recognized more proficiently by alveolar macrophages; approximately  $70\%$  of MWCNT in the respiratory airways was taken up by alveolar macrophages,  $8\%$  migrated into the alveolar septa, and  $22\%$  was observed in granulomatous lesions ([Mercer et al., 2010, 2011](#)).

In an additional investigation of rats in a subchronic inhalation study ([Ma-Hock et al., 2009](#)), [Treumann et al. \(2013\)](#) examined ultrathin lung tissue sections from two rats by TEM at the end of the 13-week exposure to  $2.5 \text{ mg/m}^3$  of MWCNT. MWCNT structures were observed in the alveolar macrophages within the cytoplasm and membrane-bound organelles (phagosomes) in the form of “large ( $> 2 \mu\text{m}$ ) electron-dense clews of intermingled MWCNT” and irregularly shaped structures up to  $100 \text{ nm}$  in diameter; some MWCNT were observed free in the alveolar lumen. MWCNT were also observed in

**Table 4.1 Kinetics of carbon nanotubes in experimental animals**

Particle type	Particle dimensions and surface area	Species, strain (age; sex)	Route of exposure; dose/exposure concentration	Duration of study	Results	Comments	Reference
SWCNT (HiPCO purified); < 2 wt% of contaminants	SWCNT: max. area-equivalent diameter, 15.2 µm; dispersed SWCNT: mean diameter, 0.69 µm; max. area-equivalent diameter, 3.7 µm	Mouse, C57BL/6 (24 wk; M)	Pharyngeal aspiration; 10 µg/mouse (SWCNT or dispersed SWCNT)	1 h, 1–7 days, and 1 mo after exposure	Dispersed SWCNT were rapidly (1 day after exposure) incorporated into the alveolar interstitium (and produced increased collagen deposition); less dispersed SWCNT were concentrated near the proximal alveolar region (the main area of granulomatous lesion formation)	SWCNT labelled with gold particles	<a href="#">Mercer et al. (2008)</a>
SWCNT, raw (10% iron) or super purified	Diameter, 0.8–1.2 nm; length, 0.1–1 µm	Rat, Sprague-Dawley (6–8 wk; M)	Intratracheal or intravenous administration; 0.5 mg/animal (~2 µg/g bw)	2 wk after exposure	In intratracheal group, no CNT detected in liver, spleen, or kidneys; in intravenous group, decreased CNR at 6 and 24 h; similar to controls by 2 wk after exposure	CNR of MRI used to detect CNT	<a href="#">Al Faraj et al. (2009)</a>
SWCNT, raw (10% iron)	Diameter, 0.8–1.2 nm; length, 0.1–1 µm	Rat, Sprague-Dawley (6–8 wk; M)	Intratracheal administration; 0, 0.1, 0.5, or 1 mg/animal	1, 7, 30, or 90 days after exposure	Significant CNR detected in lungs after exposure to 0.5 and 1 mg	MRI and TEM used to detect CNT biodistribution (adverse lung effects)	<a href="#">Al Faraj et al. (2010)</a>
SWCNT, three types: pristine (raw and purified) CNT; (raw and purified) and functionalized (F-)	Pristine (raw and purified) CNT: diameter, 0.8–1.2 nm; length, 0.1–1 µm; F-CNT: diameter, 1–2 nm; length, 0.5–2 µm	Rat, Sprague-Dawley (6–8 wk; M)	Intravenous administration; 0.5 mg/animal	5 h, 1, 2, 7, or 14 days after exposure	Raw SWCNT detected in spleen (up to 7 days), liver (up to 2 days), and kidney (up to 5 h)	MRI, Raman spectroscopy and iron assays used to assess biodistribution; no significant detection of purified or F-CNT due to low iron	<a href="#">Al Faraj et al. (2011)</a>

**Table 4.1 Kinetics of carbon nanotubes in experimental animals (continued)**

Particle type	Particle dimensions and surface area	Species, strain (age; sex)	Route of exposure; dose/exposure concentration	Duration of study	Results	Comments	Reference
MWCNT	MMAD, ~3 µm; GSD, ~2	Rat, Wistar (2 mo; M)	Inhalation (nose-only); 0.1, 0.4, 1.5, and 6 mg/m <sup>3</sup>	13 wk (6 h/day, 5 days/wk) inhalation (nose-only); 1 day and 17, 26, and 39 wk after exposure	MWCNT lung burden (as ng cobalt) decreased slowly during the period after exposure, while the LN burden increased after 1.5 and 6 mg/m <sup>3</sup> ; retention t <sub>1/2</sub> estimates: 151, 350, 318, and 375 days after exposure to 0.1, 0.4, 1.5, and 6 mg/m <sup>3</sup> , respectively	Cobalt tracer used to measure MWCNT lung and LN burdens	<a href="#">Pauluhn (2010b)</a>
MWCNT-7 (Mitsui)	Median length, 3.86 µm; count mean diameter, 49 ± 13.4 (SD) nm	Mouse, C57BL/6J (7 wk; M)	Pharyngeal aspiration; 10, 20, 40, or 80 µg of MWCNT or vehicle control	1, 7, 28, and 56 days after exposure	MWCNT distribution at d 1 after exposure: 18% in airways; 81% in alveolar region; 0.6% in the subpleura; the density of MWCNT penetrations of subpleural tissue increased 28 and 56 days after exposure		<a href="#">Mercer et al. (2010)</a>
MWCNT-7 (Mitsui)	Median length, 3.86 µm; count mean diameter, 49 ± 13.4 (SD) nm; SA (BET): 26 m <sup>2</sup> /g	Mouse, C57BL/6J (7 wk; M)	Pharyngeal aspiration; 10, 20, 40, or 80 µg of MWCNT or vehicle control	1, 7, 28, and 56 days after exposure	MWCNT distribution at 56 days after exposure: 68.7% in alveolar macrophages; 7.5% in alveolar tissue; 22% in granulomatous lesions; 1.6% in subpleural tissues; no MWCNT found in the airways at 7, 28, or 56 days after exposure		<a href="#">Mercer et al. (2011)</a>
MWCNT-7 (Mitsui)	NR	Rat, F344 (13 wk; M)	Intratracheal administration; 40 or 160 µg/animal	1, 7, 28, or 91 days after exposure	Translocation to LN (right and left mediastinal and parathymus), which increased with dose and time after exposure; small aggregates of MWCNT-laden nodal macrophages on day 91	CNT observed in LN by TEM	<a href="#">Aiso et al. (2011)</a>

Table 4.1 (continued)

Particle type	Particle dimensions and surface area	Species, strain (age; sex)	Route of exposure; dose/exposure concentration	Duration of study	Results	Comments	Reference
MWCNT, well dispersed	GMD (GSD): 63 nm (1.5); GML (GSD): 1.1 $\mu\text{m}$ (2.7); SA: 69 $\text{m}^2/\text{g}$	Rat, Wistar (age NR; M)	Inhalation (whole-body); 0.37 $\text{mg}/\text{m}^3$ (~70% single fibres)	6 h/day, 5 days/wk for 4 wk; 3 days, 1 mo, and 3 mo after exposure	Dose of MWCNT in lungs 3 days after exposure: 68 $\pm$ 10 $\mu\text{g}/\text{lung}$ (by X-ray diffraction) or 76 $\pm$ 9 $\mu\text{g}/\text{lung}$ (by elemental chemical analysis); estimated deposition fractions: 18% or 2%, by respective methods; calculated $t_{1/2}$ : 51 or 54 days, respectively (assuming first-order clearance, i.e. the amount cleared is proportional to the amount deposited)	MWCNT in aqueous solution of 0.5 $\text{mg}/\text{mL}$ Triton X-100 atomized into exposure chambers by a nebulizer; no reduction in the clearance rate (due to overloading of clearance mechanisms) observed at the exposure concentration and duration used in this study; 10 rats per group	<a href="#">Oyabu et al. (2011)</a>
MWCNT-7 (Mitsui)	MMAD, 1.5 $\mu\text{m}$ ; GSD, 1.67; mean fibre length, 4.3 $\mu\text{m}$	Mouse, C57BL/6 (7 wk; M)	Inhalation (whole-body); 5 $\text{mg}/\text{m}^3$	3 wk (5 h/day for 12 days); 1, 14, 28, 84, 168, or 336 days after exposure	MWCNT lung burden measured 1 day after exposure: 28.1 $\mu\text{g}$ ; at day 336, 65% remained in the lungs; the number of MWCNT structures with multiple fibres decreased from day 1 to day 168, while the single fibre number remained the same		<a href="#">Mercer et al. (2013b)</a>

**Table 4.1 Kinetics of carbon nanotubes in experimental animals (continued)**

Particle type	Particle dimensions and surface area	Species, strain (age; sex)	Route of exposure; dose/exposure concentration	Duration of study	Results	Comments	Reference
MWCNT-7 (Mitsui)	MMAD, 1.5 µm; GSD, 1.67; mean fibre length, 4.3 µm	Mouse, C57BL/6 (7 wk; M)	Inhalation (whole-body); 5 mg/m <sup>3</sup>	3 wk (5 h/day, 4 times per week); 1, 14, 28, 84, 168, or 336 days after exposure	MWCNT observed in liver, kidney, heart, brain, diaphragm, and chest wall as single fibres only; lung burden included 54% agglomerated MWCNT; single fibre lengths were similar in lung and liver/kidney (7.5 and 8.2 µm, respectively); most of the extrapulmonary MWCNT were in the tracheobronchial LN (1.08% on day 1 and 7.34% after 336 days of exposure)		<a href="#">Mercer et al. (2013a)</a>
MWCNT, carboxyl or hydroxyl functional groups, hydrophilic	Diameter, 30 nm; length, 1–5 µm (80 nm agglom.); SA, NR	Mouse, JcL: ICR (8–12 wk; M)	Intravenous administration (tail vein); 0.25 mg/animal	28 days after exposure	Distinct colour change seen in lungs and liver 1 wk after injection; large number of CNT observed by TEM in lungs, small number in spleen and kidneys; amount of CNT decreased but remained in lungs and liver 4 wk after injection	Qualitative observations of organ dose only	<a href="#">Abe et al. (2012)</a>
MWCNT, <sup>14</sup> C-radiolabelled	Length, 3.9 µm (range, 0.5–12 µm); mean diameter, ~40 nm (range, 10–150 nm); 42 m <sup>2</sup> /g SA	Mouse, Balb/c (6 wk; F)	Pharyngeal aspiration; 20 µg/animal [10 µg in lungs on day 1 after exposure]	1 day, 7 days, 1, 3, 6, 9, and 12 mo after exposure	CNT decreased in the lungs to approximately 10% of administered dose at 3 mo and longer after exposure; CNT detected in liver and spleen on day 1 after exposure, and increased over time after exposure	Lung dose at day 1 after exposure was only 10 µg (suggesting the remainder was swallowed); CNT increased in the lungs at 9 and 12 mo after exposure	<a href="#">Czarny et al. (2014)</a>

Table 4.1 (continued)

Particle type	Particle dimensions and surface area	Species, strain (age; sex)	Route of exposure; dose/exposure concentration	Duration of study	Results	Comments	Reference
MWCNT-7 (Mitsui)	Diameter, 90.7 nm; length, 5.7 µm; MMAD (GSD), 1.4–1.6 µm (2.3–3.0); SA, 24–28 m <sup>2</sup> /g	Rat, F344/DuCr1Cr1j (4 wk; M and F)	Inhalation (whole-body); 0, 0.2, 1, and 5 mg/m <sup>3</sup>	6 h/day, 5 days/wk, 13 wk	Exposure-related increase in MWCNT lung dose at the end of the 13-wk exposure; mean lung dose of MWCNT at end of 13-wk exposure to 0.2, 1, and 5 mg/m <sup>3</sup> , respectively: 3.23, 21.2, and 120.3 µg/left lung (M); 2.30, 13.7, and 80.3 µg/left lung (F)	MWCNT quantified by a technique that uses a specific polycyclic aromatic hydrocarbon (benzo[ <i>g,h,i</i> ]perylene) as a marker of MWCNT	<a href="#">Kasai et al. (2015)</a>
MWCNT: original (O-), purified (P-), and carboxylic acid functionalized (F-), (Cheap Tubes, Inc., Brattleboro, VT, USA)	Outer diameter, 20–30 nm; length: 10–30 µm; SA: 182, 168, and 224 m <sup>2</sup> /g (O-, P-, and F- forms, respectively)	Rat, Sprague-Dawley (9–10 wk; M)	Inhalation (nose-only), ~30 mg/m <sup>3</sup> ; or intratracheal instillation, 0, 10, 50, or 200 µg (in dispersion medium)	1 day or 21 days after exposure	Uptake of MWCNT in alveolar macrophages greater for F-MWCNT than for O- or P-MWCNT; phagocytosed O- and P-MWCNT observed within phagolysosomes initially while F-MWCNT found in the cytosol or penetrating the cell membrane	Six rats/group	<a href="#">Silva et al. (2014)</a>

agglom., agglomerated; BET, Brunauer-Emmett-Teller method; bw, body weight; CNR, contrast-to-noise ratio; CNT, carbon nanotubes; F, female; F-CNT, functionalized CNT; GMD, geometric mean diameter; GML, geometric mean length; GSD, geometric standard deviation; HiPCO, high-pressure carbon monoxide production process; LN, lymph nodes; M, male; MMAD, mass median aerodynamic diameter; mo, month; MRI, magnetic resonance imaging; MWCNT, multiwalled carbon nanotubes; NR, not reported; SA, surface area; t<sub>1/2</sub>, retention half-life; SWCNT, single-walled carbon nanotubes; TEM, transmission electron microscopy; wk, week; wt, weight

focal accumulations of phagocytic cells within the subpleural connective tissue.

In the study by [Silva et al. \(2014\)](#), the physico-chemical properties of MWCNT influenced their uptake, location, and structure within the alveolar macrophages (as observed by TEM and bright-field microscopy). Rats that inhaled F-MWCNT had significantly more alveolar macrophages containing MWCNT structures than rats that inhaled O-MWCNT or P-MWCNT (as observed in the bronchoalveolar lavage fluid [BALF]) on days 1 and 21 after exposure (P-MWCNT were obtained after the treatment of O-MWCNT with nitric acid and ethyldiamine tetra-acetate in acetic acid at pH 4 to remove residual metals and amorphous carbon). On day 1 after exposure, O-MWCNT and P-MWCNT were observed within the phagolysosomes of macrophages, while F-MWCNT were seen in the cytosol and also protruding the cell membrane. On day 21 after exposure, O- and P-MWCNT were no longer compartmentalized but were observed in the cytosol as larger focal agglomerates; the F-MWCNT (obtained by adding the P-MWCNT to a reaction chamber containing nitric acid and sulfuric acid) in the cytosol were smaller, dispersed aggregates. The acidic functional groups brought about by increasing the pH and the resulting increase in hydrophilicity were thought to reduce the toxicity of F-MWCNT by preventing phagolysosome permeability – and the subsequent release of lysosomal contents into the cytosol with the downstream activation of the nucleotide-binding oligomerization domain receptor (NLRP3) inflammasome – after F-MWCNT were taken up by the alveolar macrophages. Thus the uptake of F-MWCNT into macrophages did not appear to cause cell toxicity at the doses and observation time-points in this study ([Silva et al., 2014](#)).

### (c) *Translocation*

Several studies have provided evidence that CNT can translocate from the lungs into the blood circulation. Adult CD-1 mice [sex unspecified] were exposed to untreated SWCNT (synthesized with an iron-cobalt/magnesium oxide catalyst) by nebulization. Acute exposures to a water aerosol containing CNT [concentration and dose unspecified] lasted 15 minutes. CNT structures were observed by Raman spectroscopy in blood samples taken from mice 24 hours after the inhalation of CNT. The quantity was not specified, but exceeded the detection limit of Raman spectroscopy. These CNT were observed as clusters with average diameters of several micrometres ([Ingle et al., 2013](#)). [The Working Group noted that smaller CNT clusters, if present, would have been below the detection threshold due to the qualitative nature of the Raman spectroscopy methods, which detect CNT in tissues but cannot provide quantitative dose measures.]

Evidence that CNT could translocate from the lungs of adult male Wistar albino rats (weighing 0.2–0.225 kg) after intratracheal administration of two types of MWCNT at a dose of 0.2, 1, or 5 mg/kg bw was reported by [Reddy et al. \(2010a\)](#). CNT were produced by electric arc (size, 90–150 nm; surface area, 197 mg<sup>2</sup>/g; crystallinity, hexagonal) or CVD (size, 60–80 nm; surface area, 252 mg<sup>2</sup>/g; crystallinity, cubic) and were dispersed in PBS plus Tween 80 solution then sonicated to prevent agglomeration before administration. Dose-dependent toxicity was observed in the liver and kidney of rats exposed to either type of MWCNT. Light micrographs of the liver tissue 1 day after instillation showed black pigments, but no quantitative data on CNT tissue doses were provided.

Inhaled MWCNT were observed in the subpleural wall and within the subpleural macrophages in groups of 10 male C57BL6 mice after a single 6-hour inhalation exposure

to 1 or 30 mg/m<sup>3</sup> of MWCNT (Helix, Inc.; MMAD, 164 or 183 nm, respectively; length, < 100 nm to > 10 µm; diameter, 10–50 nm) ([Ryman-Rasmussen et al., 2009a](#)). Carbon black (MMAD, 209 nm) at a concentration of 30 mg/m<sup>3</sup> was used as a comparison material. Lung tissues were collected 1 day, 2 weeks, 6 weeks, or 14 weeks after exposure. The calculated deposited doses of CNT were 0.2 or 4 mg/kg at concentrations of 1 or 30 mg/m<sup>3</sup> MWCNT, respectively (assuming a 10% deposition of the inhaled dose). The inhaled MWCNT were engulfed by macrophages, which migrated to the subpleural region. TEM images showed CNT within macrophages beneath the pleura. The authors hypothesized that activated macrophages containing MWCNT migrate through the pleural lymphatic drainage and stimulate the recruitment of mononuclear cells in the pleura (consistent with their previous finding ([Ryman-Rasmussen et al., 2009b](#)) that monocyte chemokine CCL2 was increased in mice after inhalation of MWCNT). Significant fibrosis (focal subpleural) was observed in mice 2 and 6 weeks after inhalation exposure to 30 mg/m<sup>3</sup> of MWCNT, but not in mice exposed to 1 mg/m<sup>3</sup> of MWCNT or 30 mg/m<sup>3</sup> of carbon black. Aggregates of MWCNT in lung tissues were significantly elevated in mice inhaling 30 mg/m<sup>3</sup> of MWCNT (but not carbon black or 1 mg/m<sup>3</sup> of MWCNT). No quantitative data on the dose of MWCNT in the lung tissues were reported ([Ryman-Rasmussen et al., 2009a](#)).

Translocation to the pleura was observed in a study of male Fischer 344 rats exposed five times to MWCNT (0.5 mL of 500 µg/mL) by intrapulmonary spraying over a 9-day period ([Xu et al., 2012](#)). The total mass dose was 1.25 mg/rat. Two types of MWCNT were studied – MWCNT-N (Nikkiso Co., Ltd) and MWCNT-M (Mitsui-7; Mitsui Chemicals, Inc.) – in addition to crocidolite as a control. Pleural cavity lavage was used to examine the presence of MWCNT or crocidolite in the pleural cavity, and SEM was used to confirm

the location of the MWCNT or crocidolite fibres in lung tissue sections. Both types of MWCNT and crocidolite fibres were found in the pleural cavity lavage cell pellets, mostly in macrophages. A few fibres were found in the intercellular space or on cell surfaces. In the tissue sections, both MWCNT and crocidolite were observed in the focal granulomatous lesions in alveoli and in alveolar macrophages. The MWCNT or crocidolite fibres were also found in the mediastinal lymph nodes, and a few were observed in liver sinusoid cells, blood vessel wall cells in the brain, renal tubular cells, and spleen sinus and macrophages. A few fibres were observed penetrating directly from the lungs to the pleural cavity through the visceral pleura, but no fibres were seen in the parietal pleura.

[Mercer et al. \(2013a\)](#) investigated the extrapulmonary transport of MWCNT in male C57BL/6 mice after inhalation exposure to 5 mg/m<sup>3</sup> of MWCNT (Mitsui-7) for 5 hours per day for 12 days in a 3-week study [the same study as that reported in [Mercer et al. \(2013b\)](#) for disposition in the lungs]. The lung burden of MWCNT on day 1 after exposure was 28.1 µg (47 × 10<sup>6</sup> MWCNT fibres/µg). Optical sectioning through serial sections of the lung, liver, and kidney was carried out to measure the length of the single MWCNT in those organs on days 1 and 336 after the end of inhalation exposure ([Mercer et al., 2013b](#)). The amount of MWCNT in the tracheobronchial lymph nodes was determined as the volume density of MWCNT in the lymph nodes relative to the volume density of MWCNT in the lungs 1 day after exposure. The numbers of MWCNT fibres in the extrapulmonary organs, diaphragm, and chest wall were counted per unit area and converted to number per organ using morphometric methods. Enhanced-darkfield light microscopy imaging of CNT was performed on sections of the exposed lungs to identify CNT that would not otherwise be detected. Most of the MWCNT that translocated from the lungs were found in the tracheobronchial lymph nodes

(1.08% on day 1 and 7.34% on day 336 after exposure, as a percentage of the lung burden on day 1 after exposure). The next highest extrapulmonary tissue burdens of MWCNT were reported in the liver (0.0028% on day 1 and 0.027% on day 336) and kidneys (0.0010% on day 1 and 0.0052% on day 336). Smaller amounts of MWCNT were detected in the heart, brain, chest wall, and diaphragm (with higher amounts at day 336 than at day 1 after exposure in all tissues except the chest wall). In the lungs, 54% of the MWCNT burden was agglomerated, while only singlet MWCNT were observed in the liver, kidney, heart, brain, chest wall, and diaphragm ([Mercer et al., 2013a](#)).

In an ex-vivo model, SWCNT (100 µg) were instilled into the airway of isolated perfused rat lung. The isolated perfused rat lung model retains the lung architecture but eliminates the systemic pharmacokinetics. The pulmonary translocation of SWCNT from the airways across the pulmonary barrier was less than 0.05% of the instilled dose after 90 minutes. A pharmacokinetic simulation estimated a cumulative pulmonary translocation from the rat lung of less than 0.15% over 14 days ([Matthews et al., 2013](#)).

The length of CNT that translocate from the lungs to the pleura (or were instilled therein in an experimental study) may influence their retention. Longer structures (> 5 µm) were retained in the pleura, while shorter structures were able to drain to the lymph nodes ([Poland et al., 2008](#); [Murphy et al., 2011](#)); however, [Kim et al. \(2014\)](#) found a persistent presence in the pleura and lung parenchyma 90 days after subacute (28 days) inhalation exposure to short-length ( $330.18 \pm 1.72$  nm) MWCNT. Stomata are outlets in the parietal pleura through which lymphatic drainage occurs ([Donaldson et al., 2010](#); [Murphy et al., 2011](#)). The maximum diameter of stomata in mice is 10 µm ([Murphy et al., 2011](#)). Using single-photon emission computed tomographic imaging, [Murphy et al. \(2011\)](#) reported that radiolabelled short CNT (length, 0.5–2 µm)

were observed in the cranial mediastinal lymph nodes (two bilateral lymph nodes located lateral to the thymus) within 1 hour of intrapleural injection of 5 µg/mouse, and increased up to the end of observation 24 hours after the injection. Qualitatively, fewer long (length, > 15 µm) than short CNT were observed in the lymph nodes ([Murphy et al., 2011](#)).

The translocation of <sup>14</sup>C-radiolabelled MWCNT from the lungs to other organs up to 1 year after pharyngeal aspiration of 20 µg CNT (suspended in 50 µL dispersion medium) was investigated in seven groups of 4 female Balb/c mice (age, 6 weeks). After dispersion, the mean length of the CNT was 3.9 µm (range, 500 nm–12 µm) and the mean diameter was approximately 40 nm (range, 10–150 nm). Time-points of examination were 1 and 7 days, and 1, 3, 6, 9, and 12 months after exposure. At 6 months after exposure, the average concentration of MWCNT in the lungs decreased to less than 10% of the administered dose, but increased at the last two time-points (to about 20% at 12 months after exposure). In contrast, the MWCNT concentration in the spleen and liver – which was detectable on day 1 after exposure – increased over time to approximately 0.1–0.2% of the administered dose in the spleen at 6–12 months after exposure and approximately 0.5–1% in the liver at the same time-points, although the liver had about half the mass concentration of MWCNT (µg/g) compared with the spleen ([Czarny et al., 2014](#)). [The Working Group noted that the authors reported that only half of the 20-µg dose administered was measured in the lungs on day 1 after exposure, and the initial lung dose was therefore adjusted to 10 µg; the remainder of the lung dose was considered to have probably been swallowed, thus reaching the stomach and gastrointestinal tract.]

A subsequent experiment on oral ingestion through the intra-oesophageal instillation of 50 µg of <sup>14</sup>C-MWCNT showed that approximately

95% of the ingested MWCNT dose was measured in the gastrointestinal tract and faeces after 24 hours; no MWCNT were detected (by radioactive signal) after 4 days; and no MWCNT were detected in the spleen or liver tissue sections on 1, 7, and 30 days after gavage with MWCNT. This finding was considered by the authors to support the evidence that translocation of the MWCNT after pharyngeal aspiration occurred through the air–blood barrier (including crossing the epithelial cells of the airways or the alveoli) and not across the intestinal lining ([Czarny et al., 2014](#)).

#### (d) Retention

Retention refers to the temporal distribution of uncleared particles in the respiratory tract ([Lioy et al., 1984](#)). This section focuses on retention in the lungs and lung-associated tissues (i.e. lung parenchyma, pleura, and lung-associated lymph nodes) (see also Section 4.1.2 (c) for data on doses of CNT in extrapulmonary organs). Retention (or biopersistence) in the lungs is higher for inhaled particles that are poorly soluble and poorly cleared from the lungs (e.g. due to size, shape, surface reactivity, and/or to a high dose that exceeds clearance capacity).

The lung burden of MWCNT (Baytubes, a proprietary product; Bayer MaterialScience, Germany) was measured in male Wistar rats 1 day, and 17, 26, and 39 weeks after 13 weeks of inhalation exposure to 0.1, 0.4, 1.5, and 6 mg/m<sup>3</sup> for 6 hours per day on 5 days per week. Tissue burdens of MWCNT (in the left lung lobe and in the lung-associated lymph nodes) were estimated from the measurements of residual cobalt tracer (0.115% matrix-bound). The dose deposited in the alveoli was calculated from the following information: concentration of cobalt (ng/L [air]) × minute ventilation rate (0.8 L/min/kg) [male rat control body weights: 231 and 369 g, at the beginning and end, respectively, of the 13-week exposure] × the alveolar deposition fraction (5.7%) × the cobalt fraction (%/100) (see also Section 4.1.2 (a) for more information on the estimated deposition

fraction). The retained MWCNT dose (measured as µg cobalt tracer/lung) in the lungs decreased slowly during the 39-week period after exposure, while the MWCNT in the lung-associated lymph nodes increased after exposure in mice exposed to 1.5 or 6 mg/m<sup>3</sup> ([Pauluhn, 2010a](#)).

The retention half-times at each concentration were calculated in [Pauluhn \(2010a\)](#) from the equation:  $dc/dt = a(1-kt)$  where  $k$  is the first-order elimination constant (calculated from the cobalt lung burden data 17, 26, and 39 weeks after exposure [although not reported]). The retention half-time (i.e. time to reduce the retained lung dose by half; also called the elimination half-time [ $t_{1/2}$ ]) was calculated from  $t_{1/2} = \ln(2)/k$ . The retention  $t_{1/2}$  was 151, 350, 318, and 375 days at exposure concentrations of 0.1, 0.4, 1.5, and 6 mg/m<sup>3</sup>, respectively. [From the retention half-times [ $t_{1/2}$ ], the first-order rate constant  $k$  can be estimated as approximately 0.002 d<sup>-1</sup> for the three higher concentrations and approximately 0.004 d<sup>-1</sup> for the lowest concentration.] [Pauluhn \(2010a\)](#) noted that the levels of cobalt measured in the lungs at the lowest concentration (0.1 mg/m<sup>3</sup>) were in the range of the limit of quantification, indicating possible imprecision in the  $t_{1/2}$  estimate at that concentration. In comparison, the retention  $t_{1/2}$  for respirable particles in rats at non-overloading doses was approximately 60 days, indicating that the rat lung clearance rates of MWCNT (Baytubes) were reduced by several fold at all exposure concentrations.

[Pauluhn \(2010a\)](#) estimated the MWCNT particle volume lung dose as 107–325, 466–1413, 1192–3917, and 3961–12 002 nL/g of lung in rats exposed to 0.1, 0.4, 1.5, and 6 mg/m<sup>3</sup>, respectively (at a density of MWCNT of 0.1–0.3 g/cm<sup>3</sup>, “corrected for void space volume which is 1.43 times greater than the volume of the MWCNT themselves” ([Brown et al., 2005](#))). In comparison, [Pauluhn \(2010a\)](#) quoted [Morrow \(1988, 1994\)](#), who found no significant difference in retention half-times between the control (unexposed) rats and the rats that had a particle volume lung dose

of 100 nL/g of rat lung, and [Oberdörster \(1995\)](#), who observed a doubling of the retention half-times in rats with a particle volume lung dose of 1400 nL/g of lung. [Pauluhn \(2010a\)](#) interpreted these comparisons as indicating that overloading of lung clearance was “minimal to moderate” in rats at 0.1 and 0.4 mg/m<sup>3</sup> of MWCNT while, at 1.5 and 6 mg/m<sup>3</sup>, clearance may have been completely impaired.

[Mercer et al. \(2010\)](#) reported the distribution of MWCNT-7 (Mitsui & Co.) (diameter, 49 nm; length, 3.9 µm) in the lungs of male C57BL/6J mice exposed by pharyngeal aspiration to 10, 20, 40, and 80 µg of MWCNT or the vehicle. The distribution of MWCNT was determined in fixed lung sections using morphometric methods at 1, 7, 28, and 56 days after exposure. Field-emission SEM was used to detect and count the number of MWCNT fibre penetrations of three biological tissue barriers: the alveolar epithelium (alveolar penetrations), the alveolar epithelium immediately adjacent to the pleura (subpleural tissue), and the visceral pleural surface (intrapleural space). The number of penetrations per lung (into the subpleural tissue and intrapleural space) increased with increasing dose administered. On day 1 after exposure to 80 µg, 18% of the MWCNT was observed in the airways, 81% in the alveolar region, and 0.6% in the subpleural tissue. Within the alveolar region, 62% of the dose was inside alveolar macrophages on day 1 after exposure. MWCNT penetrations were observed most frequently in the alveolar macrophages, followed by alveolar type I epithelial cells, and less frequently in alveolar interstitial cells (typically observed as fibres passing through adjacent epithelial cells). MWCNT inside the cells were not confined to phagolysosomes and were observed to extend from the cell surface through the nuclei and other organelles. Alveolar type II epithelial cells (2% of the normal epithelial surface) were rarely found to be penetrated by MWCNT. In the airways, MWCNT were observed in the mucous layer above airway epithelial cells and in airway

macrophages contained in the cilia-mucous lining layer; penetrations by MWCNT in the airways were rare. At the 20-µg dose, a total of  $15 \times 10^6$  MWCNT penetrations were observed in the  $11 \times 10^6$  alveolar type I epithelial cells in mouse lungs ([Mercer et al., 2010](#)).

The time course of this MWCNT in the intrapleural space showed a decrease from day 1 to day 7 after exposure ([Mercer et al., 2010](#)). This is consistent with a mechanism of shorter fibre clearance from the intrapleural space through the stomata (duct in the parietal pleura) to the lymphatic system ([Donaldson et al., 2010](#); [Murphy et al., 2011](#)). However, the amount of MWCNT in the intrapleural space increased again at day 28 after exposure and remained elevated at 56 days after exposure. The lung burden of MWCNT may act as a reservoir to replenish MWCNT in the intrapleural space or that even shorter fibres (length, 3.9 µm) could begin to clog the ducts if they reach a sufficient level within the intrapleural space ([Mercer et al., 2010](#)).

[Mercer et al. \(2013b\)](#) provided quantitative data on the retention and distribution of MWCNT in the lung and associated tissues of male C57BL/6 mice after a 3-week exposure by inhalation for 5 hours per day for 12 days to 5 mg/m<sup>3</sup> of MWCNT (Mitsui-7; mean length, 4.3 µm). The MWCNT lung burden was determined using a method reported by [Elder et al. \(2005\)](#). The lungs were removed after mice were killed on 1, 14, 28, 84, 168, or 336 days after exposure. Lung tissue was processed by digestion (in 25% potassium hydroxide/methanol (w/v)), centrifugation, and re-suspension of the pellet, and measurements of the optical density of the solution were compared with MWCNT standards that were processed in parallel with the lung samples. The mass of MWCNT in the lungs was determined from a standard curve. [[Elder et al. \(2005\)](#) reported that the limit of detection of this assay was 0.1 µg/mL of suspended solution.] Several imaging techniques (light microscopy, field emission SEM, and enhanced-darkfield

microscopy) were used to observe and quantify the distribution of the MWCNT fibres in tissue sections of the lungs. MWCNT were counted in lung tissue sections using an enhanced-darkfield optical system; eight animals were analysed per group and counting was accomplished using an  $11 \times 11$  (121 point) overlay grid pattern to ensure uniform sampling of the section. The number of fibres per MWCNT structure was also determined by enhanced-darkfield microscopy. MWCNT fibres were observed in the alveolar macrophages and alveolar interstitium, and penetrated the visceral pleura (Mercer et al., 2013b). On day 14 and later time-points after exposure, clusters of MWCNT were observed within the ridge of the first alveolar duct bifurcation [which is the primary site of particle deposition after inhalation exposures to particles and fibres, as reported previously by Brody & Roe (1983) and Chang et al. (1988)]. The MWCNT lung burden in mice measured on day 1 after exposure was  $28.1 \mu\text{g}$  ( $1321 \times 10^9$ ; total fibre number estimate based on 47 million MWCNT fibres/ $\mu\text{g}$  [conversion reported in Chen et al. (2012)]) (Mercer et al., 2013b). Of this lung burden, 84% ( $23.6 \mu\text{g}$ ) was found in the alveolar (pulmonary) region of the lungs and 16% ( $4.5 \mu\text{g}$ ) in the airways. Similar distributions of MWCNT were observed in two previous studies of MWCNT by Porter et al. (2010, 2013) in mice exposed by pharyngeal aspiration or acute inhalation.

Within the alveolar region, 56% of the MWCNT lung burden was in alveolar macrophages on day 1 after exposure, 7% was in the alveolar airways, and 20% was in the alveolar tissue. These findings indicated a fairly rapid and substantial distribution of inhaled MWCNT to the lung interstitium. By day 1 after exposure,  $\sim 1.2\%$  ( $0.34 \mu\text{g}$ ) of the MWCNT lung burden was observed as single fibres in the pleural compartment (including the subpleural tissue and visceral pleura) (Mercer et al., 2013b).

At 336 days after exposure, 65% of the MWCNT lung burden ( $28.1 \mu\text{g}$ ) on day 1 after

exposure was retained in the lungs ( $18.2 \mu\text{g}$ ), most of which (96%) was retained in the alveolar region (including 4.8% in subpleural tissue) and 4% ( $0.73 \mu\text{g}$ ) of which was retained in the airways. The distribution of MWCNT in the lungs shifted from alveolar macrophages (3 times more than in lung tissue on day 1 after exposure) to the alveolar tissue, where the dose increased from 5.8 to  $9.5 \mu\text{g}$  on days 1 and 168 after exposure, respectively. Thus, the alveolar interstitial lung burden increased as the MWCNT in the alveoli were cleared (Mercer et al., 2013b).

The number of larger or agglomerated MWCNT structures ( $> 4$  fibres/MWCNT) decreased over time (from 53 to 25% of the lung burden on days 1 and 168 after exposure, respectively). The number of structures with 2, 3, or 4 fibres also decreased significantly. However, the percentage of single fibres in the MWCNT lung burden did not change significantly from days 1 to 168 after exposure. Thus, the MWCNT structures decreased in size, resulting in a relatively constant number of single MWCNT fibres in the lungs over time (Mercer et al., 2013b).

The mouse lung response on day 1 of this study after exposure to a lung dose of  $28.1 \mu\text{g}$  MWCNT was an increase in the thickness (fibrillar collagen) of the alveolar connective tissue over time, with a 70% increase on day 336 after exposure (Mercer et al., 2013b). The translocation of MWCNT to extrapulmonary organs were described in Mercer et al. (2013a) (see also Section 4.1.2 (c)).

The lung burden of MWCNT (diameter, 90.7 nm; length,  $5.7 \mu\text{m}$ ; MMAD (GSD),  $1.4\text{--}1.6 \mu\text{m}$  (2.3–3.0)) was measured in male and female Fischer 344 rats after 13 weeks of whole-body inhalation exposure for 6 hours per day on 5 days per week to concentrations of 0, 0.2, 1, and  $5 \text{ mg}/\text{m}^3$ . Left lung tissues ( $0.18\text{--}0.36 \text{ g}$ ) were sampled from five rats in each MWCNT-exposed group. MWCNT was quantified using a technique in which a specific polycyclic aromatic hydrocarbon (benzo[*g,h,i*]perylene) serves as a

marker of these MWCNT. The mass of MWCNT in the lungs of male and female rats increased in relation to the exposure concentration (Table 4.1) and was reported to be 1.4–1.6 times greater in the left lungs of males than in those of females (Kasai et al., 2015). [The Working Group noted that, when the measured MWCNT lung doses are normalized to the average control left lung weight (0.43 g, males; 0.32 g, females), the retained lung doses in males were similar (1.0–1.2 times the lung doses in females).]

In the study by Silva et al. (2014), the physico-chemical properties (metal content, hydrophilicity, and carboxylic acid functionalization) or route of exposure did not significantly influence the retention of MWCNT in the lungs of male Sprague-Dawley rats (as measured in the right caudal lung lobe by programmed thermal analysis). However, the findings suggested that instilled F-MWCNT were retained in the lungs to a greater extent than the same instilled dose of O-MWCNT or P-MWCNT. The retention of instilled F-MWCNT in the lungs was also greater, although not significantly, than the retention of a similar deposited dose of inhaled F-MWCNT (Silva et al., 2014).

#### (e) Clearance

The mechanisms of clearance depend on the initial site of particle deposition within the respiratory tract and on the physico-chemical properties of the particle (e.g. solubility and functionalization). Soluble particles can dissolve in alveolar lining fluid and then enter the blood or lymph (Dahl et al., 1991; ICRP, 1994; Schlesinger, 1995). Dissolution rates do not vary widely across species, because they are primarily determined by the physico-chemical properties of the material (Dahl et al., 1991). Clearance rates of poorly soluble particles, however, can fluctuate among species due to differences in the macrophage-mediated clearance from the alveolar region and the rates of mucociliary transport in the conducting airways (Snipes, 1989).

Inhaled CNT may be phagocytosed by macrophages and cleared from the lungs by the mucociliary escalator and swallowed (entering the gut). CNT that are not cleared from the lungs by macrophages may enter the epithelial cells that line the alveolar region of the lungs, where their fibres can be retained in the lung interstitium or pass into the lymph or blood circulation (Mercer et al., 2008; Ryman-Rasmussen et al., 2009a; Mercer et al., 2013a, b).

CNT that reach the blood circulation (either by translocation from the lungs or through direct intravenous administration) may be excreted from the body through either the renal (urine) or biliary pathway. Many SWCNT or MWCNT exceed the particle size threshold for renal excretion, particularly if agglomerated (Liu et al., 2008a), and thus accumulate in the liver where they undergo biliary excretion (Cherukuri et al., 2006). The type of surface functionalization can also strongly influence the biodistribution and elimination pathways (see also Section 4.1.2 (g)).

In a study of male Wistar rats that inhaled 0.37 mg/m<sup>3</sup> of MWCNT for 6 hours per day on 5 days per week for 4 weeks, lung clearance was reported to be proportional to the amount in the lungs. The retention half-time ( $t_{1/2}$ ), defined as the time for the retained dose to be reduced by half, was estimated to be 51 or 54 days (based on the lung dose measured by X-ray diffraction or EC analysis, respectively) (Oyabu et al., 2011). [These retention half-time estimates are consistent with normal rat clearance rates reported in other studies, indicating that no reduction in the clearance rate (due to overloading of clearance mechanisms) occurred at the concentration and duration of exposure used in this study.]

In a study of the intratracheal instillation of 10 or 100 µg/mouse of pristine [as-produced] (mean length, 7.5 nm; mean diameter, 13.5 nm) or acid-treated (mean length, 400 nm; mean diameter, 15 µm) MWCNT in male C57BL/6 mice, both types of MWCNT were seen in the lymphatic system in the mediastinal lymph nodes. The

acid-treated MWCNT (that contained fewer metal contaminants and were more hydrophilic) induced less severe acute lung inflammation than the pristine MWCNT ([Kim et al., 2010](#)). [No quantitative data on the dose were provided.]

No significant lung clearance was observed from day 1 to day 21 after intratracheal administration of O-, P-, or F-MWCNT to male Sprague-Dawley rats. MWCNT structures were observed inside the alveolar macrophages and polymorphonuclear leukocytes in BALF and in the airway cilia, suggesting some, but insignificant, MWCNT clearance ([Silva et al. 2014](#)).

#### (f) Biodegradation

The size, structure (wall number), and functionalization of CNT may influence their distribution in the body and their ability to pass into cells through cell membranes ([Bianco et al., 2011](#)). Much of the literature on the kinetics of CNT is motivated by the potential use of CNT as targeted medical delivery systems to specific tissues for therapeutic purposes ([Ali-Boucetta & Kostarelos, 2013](#)). SWCNT functionalized with polyethylene glycol (PEG) were more hydrophilic, had greater dispersibility in aqueous media than unfunctionalized SWCNT, and were excreted through biliary and renal pathways ([Bhirde et al., 2010](#)).

##### (i) *In vitro and ex vivo*

Pulmonary eosinophils from humans (*in vitro*) and mice (activated *ex vivo*) were shown to degrade SWCNT through an enzyme (eosinophil peroxidase, EPO) that is exocytosed when cells are activated (e.g. by the presence of CNT) and is one of the major oxidant-generating enzymes in the human lung. The EPO-catalysed oxidative biodegradation was assessed by TEM, ultraviolet-visible-near-infrared absorption spectroscopy, and Raman spectroscopy and was found to occur extracellularly ([Andón et al., 2013](#)).

Another study by [Kagan et al. \(2010\)](#) showed that polymorphonuclear leukocyte (neutrophil)

myeloperoxidase (MPO) also catalysed the biodegradation of SWCNT, although the SWCNT in this study were pre-opsonized with immunoglobulins to increase the internalization efficiency by neutrophils. The difference in the mechanisms of degradation of the two cell types is due to neutrophils using MPO to kill bacteria inside the phagolysosome, while eosinophils use secreted EPO to kill larger extracellular organisms, such as parasites.

##### (ii) *In vivo*

The role of neutrophils or eosinophils in the biodegradation of CNT *in vivo* is unclear. In the lungs, CNT that are not cleared by alveolar macrophages may translocate into the lung interstitium and stimulate the development of fibrosis or translocate to distant sites and elicit systemic inflammatory and/or immunological responses ([Mercer et al., 2008](#); [Ryman-Rasmussen et al., 2009a](#)).

[Shvedova et al. \(2012a\)](#) demonstrated the role of MPO, an abundant enzyme in inflammatory cells such as polymorphonuclear leukocytes (or neutrophils), in the clearance and retention of CNT in the lungs of mice by comparing the clearance of SWCNT in wild-type and MPO-deficient (knockout) C57Bl/6 mice given 40 µg/mouse by pharyngeal aspiration. The MPO-mediated biodegradation of SWCNT occurs through the oxidative modification or “cutting” of the SWCNT (resulting in oxidative defects in CNT that are detectable by Raman spectroscopy). A significant difference was observed in the clearance of SWCNT from the lungs in wild-type compared with MPO-knockout mice. The degradation of SWCNT (assessed by Raman spectroscopy) was significantly greater in wild-type mice and the volume of SWCNT aggregates per total lung volume (quantified in lung tissues by light microscopic imaging analysis) was significantly greater in MPO-knockout mice than in wild-type mice 28 days after exposure. [The Working Group noted that, consistent with the higher dose

of SWCNT retained, the MPO-deficient mice showed a greater degree of fibrosis, as measured by higher collagen content, and a greater average thickness of the alveolar connective tissue in the lungs than wild-type mice; however, wild-type mice also showed significant fibrosis.]

(g) *Biokinetics of bioengineered CNT administered by intravenous administration*

Much of the literature on the biokinetics of CNT in the body involves studies on their potential use in biomedical applications. The route of exposure has been shown to influence the biodistribution of CNT in the body ([Ali-Boucetta & Kostarelos, 2013](#)), with the highest initial dose observed at the site of administration. In medical imaging or therapeutical applications, the route of exposure is typically the intravenous injection.

CNT injected intravenously accumulate in the liver and spleen, while CNT administered orally are found primarily in the stomach and intestines ([Ali-Boucetta & Kostarelos, 2013](#)).

Well dispersed short MWCNT (length < 500 nm) injected intravenously into mice were excreted rapidly through the kidneys (no nephrotoxicity observed), while longer MWCNT were retained in the spleen, lungs, and liver (resulting in hepatotoxicity) ([Jain et al., 2011](#)).

Surface modification was considered to be the most important factor influencing the biodistribution of CNT ([Ali-Boucetta & Kostarelos, 2013](#)). The two main types of functionalization are the coating (i.e. non-covalent surface modification) of SWCNT and the covalent functionalization of SWCNT or MWCNT.

The coatings that have been studied include surfactant Pluronic F108, Tween 80, and PEG phospholipid. The blood clearance of Pluronic F108-coated CNT injected intravenously into rabbits was rapid (half-life  $t_{1/2} < 1$  h), which was attributed to the formation of SWCNT–protein complexes or SWCNT aggregates that accumulated primarily in the liver ([Cherukuri et al.,](#)

[2006](#)). Tween 80-coated SWCNT were retained (for up to 28 days) in the liver, lungs, and spleen in injected mice (the CNT had a  $^{13}\text{C}$ -enriched backbone) ([Yang et al., 2007](#)). The circulation of PEGylated CNT in the blood was longer (half-life  $t_{1/2}$ , 5 h) and was further extended (half-life  $t_{1/2}$ , 12–22 hours) by increasing the branching of the PEG and liver uptake was reduced in injected mice ([Liu et al., 2008a, 2011a](#); [Prencipe et al., 2009](#)). PEGylated CNT were eliminated through biliary excretion (over 2 months). Pristine, non-covalently functionalized SWCNT mostly accumulated in the liver.

The covalent functional groups that have been studied include hydroxyl, ammonium, glucosamine, and taurine ([Ali-Boucetta & Kostarelos, 2013](#)). Many of these studies used radiolabelled CNT. Higher degrees of functionalization facilitated the dispersion of individual CNT that led to predominantly urinary excretion ([Singh et al., 2006](#); [Lacerda et al., 2008a, b, c](#)). However, MWCNT–taurine accumulated in the liver, heart, and lung ([Deng et al., 2007](#)).

Many of these studies reported qualitative estimates of the amount of CNT in various organs (e.g. by whole-body imaging). The techniques used showed the relative amount of CNT, although small amounts may have been missed due to limits of sensitivity. The most typically reported quantitative measure was the half-life  $t_{1/2}$  in blood circulation. While these studies provide valuable insights into the factors that influence the biodistribution of CNT, they focused on medical applications of CNT and thus have limited direct relevance to occupational or environmental exposures.

## 4.2 Physico-chemical properties associated with toxicity

The physico-chemical properties of CNT may be modulated by varying the method of synthesis, by applying modification processes

after synthesis, and/or by the covalent functionalization of their external surface. A large variety of CNT forms may thus be produced that exhibit different features that influence their pathogenicity. CNT cannot be considered as a single well defined substance but as families of different materials, the number of which is growing dramatically.

Evidence has been found that the responses of cells to CNT are modulated by their physico-chemical properties. The variability of the CNT employed in different studies gives rise to the discrepancies observed in biological outcomes ([Muller et al., 2005](#); [Kagan et al., 2006](#); [Elgrabli et al., 2008](#); [Poland et al., 2008](#); [Takagi et al., 2008](#); [Ma-Hock et al., 2009](#); [Sakamoto et al., 2009](#); [Fubini et al., 2010, 2011](#)).

Major studies on the effects of relevant physico-chemical characteristics on the adverse responses to CNT in various experimental models are summarized in [Table 4.2](#).

#### 4.2.1 Crystal structure and defects

Purification and functionalization can induce defects in CNT and may modify or increase their toxicity. Nitric acid, which is involved in purification and functionalization, destroys SWCNT, resulting in the production of amorphous carbon and a reduction in the amount of the transition metal catalyst used in their production ([Hu et al., 2003](#)).

Perfectly crystalline CNT are formed only by hexagonal rings of  $sp^2$  hybridized carbons. However, the graphene layers contain a variable number and degree of defects that may arise directly from the process of synthesis or may be introduced or eliminated during treatments after synthesis ([Galano et al., 2010](#)). The CNT that are currently produced are far from perfect and may include various numbers and types of defect, such as non-hexagonal rings, atom vacancies (topological defects), carbon with  $sp^3$  hybridization, incomplete bonding, and oxygenated

groups ([Ebbesen & Takada, 1995](#); [Charlier, 2002](#); [Galano, 2010](#)). After systematic variation of the physical and chemical features of a given MWCNT specimen with or without defects, genotoxicity in vitro and inflammogenicity and fibrogenicity in vivo ([Muller et al., 2008a](#)), but not carcinogenicity ([Muller et al., 2009](#); see Section 3), were correlated with the presence of defects. [The Working Group noted that only a single type of defect (i.e. broken C–C bonding generated by grinding) was evaluated in these studies.]

Defects impart the potential to quench free radicals to both MWCNT and SWCNT ([Galano, 2010](#)). CNT retard the oxidation of polystyrene, polyethylene, polypropylene, and poly(vinylidene) fluoride due to their strong ability to accept radicals, which may interrupt chain propagation, leading to antioxidant effects in polymeric material ([Watts et al., 2003](#)). Pristine SWCNT were demonstrated to be powerful antioxidants ([Lucente-Schultz et al., 2009](#)) and a variety of modified CNT exhibited different defective sites.

#### 4.2.2 Form and size

A fibre shape associated with high durability has been proposed as a critical factor in CNT-induced pleural toxicity and carcinogenicity ([Donaldson et al., 2011](#) and references therein). [Poland et al. \(2008\)](#) reported an “asbestos-like” pathogenicity of long, rigid CNT in the induction of inflammation while tangled CNT were less potent. Similarly to short amphibole asbestos fibres, shorter CNT induced less inflammation. In addition to dimensions and shape, other physico-chemical features are involved in fibre toxicity, suggesting that the fibre paradigm is not the only mechanism ([Jaurand et al., 2009](#); [Sanchez et al., 2009](#); [Fubini et al., 2011](#)). The physico-chemical properties of asbestos fibres and CNT differ substantially, correlating with the marked differences in their chemical

**Table 4.2 Physico-chemical properties of carbon nanotubes that are relevant to toxicity: summary of the most pertinent studies**

Property	Type of CNT	End-points/models	Effects	References	Characteristics
Diameter	MWCNT	Cellular viability, phagocytosis, apoptosis/guinea-pig alveolar macrophages	Greater toxicity of thick CNT	<a href="#">Wang et al. (2009)</a>	Length, 1–5 µm; diameter: 10–20 nm, 40–60 nm, and 60–100 nm; purity: different nickel content
	MWCNT	Cytotoxicity, uptake/human mesothelial cells; inflammation, cellular viability, carcinogenicity/rats	Greater toxicity of 50-nm CNT compared with thicker and thin/tangled	<a href="#">Nagai et al. (2011)</a>	Length, ~5 µm; diameter: 15 nm (tangled), 50 nm, and 100–250 nm; purity: iron and copper (amounts not reported)
	MWCNT	Cytotoxicity, uptake, oxidative stress induction/murine alveolar macrophages; lung inflammation/rats	Greater toxicity of thin CNT	<a href="#">Fenoglio et al. (2012)</a>	Length, < 5 µm; diameter, 9.4 and 70 nm; purity: metal contaminants < 0.08%
Length	MWCNT/ SWCNT	Cytotoxicity/human EAhy926, human A549, human HepG2, mouse DMBM-2, hamster V79, and human TK-6 cells	Thin CNT more cytotoxic than thick CNT	<a href="#">Fröhlich et al. (2013)</a>	Length, 0.5–2 µm; diameter of SWCNT, 1–2 µm; diameter of MWCNT, < 8, 20–30, and > 50 nm; purity: SWCNT > 90% and MWCNT > 95%
	MWCNT	Lung inflammation, granuloma formation/mice	Long CNT induced inflammatory and granulomatous response	<a href="#">Poland et al. (2008)</a>	Length, 1–5 and 5–20 µm (tangled), 13 µm, and 56 µm; diameter: 15 nm, 10 nm, 85 nm, and 165 nm; purity: iron, copper, vanadium, nickel, zinc, and cobalt content
	MWCNT	Cytotoxicity, DNA damage/A459 cells; lung inflammation/mice	Long and thick CNT, but not short and thin CNT, caused DNA damage/inflammation	<a href="#">Yamashita et al. (2010)</a>	Length, 5–15 µm and 1–2 µm; diameter: 20–100 nm and < 10 nm
Defects	SWCNT	Lung inflammation, granuloma formation/mice	CNT > 10 µm induced granuloma formation; CNT < 300 nm excreted from the body	<a href="#">Kolosnjaj-Tabi et al. (2010)</a>	Length, > 1–2 µm and 20–80 nm; diameter, ~1 nm; purity: different iron content (1.5–25%)
	MWCNT	Pleural inflammation, cellular viability in the mesothelium/mice	Greater toxicity of longer CNT	<a href="#">Murphy et al. (2011)</a>	Length: 0.5–2 µm (short), 5–20, and 1–5 µm (tangled), 13 and ≤ 56 µm (long); diameter: 20–30 nm, 15 and 15 nm, 40–50, and 20–100 nm
	MWCNT	Genotoxicity/rat lung epithelial cells; lung inflammation, fibrosis/rats	Defects increased the inflammatory and genotoxic potential	<a href="#">Fenoglio et al. (2008)</a> , <a href="#">Müller et al. (2008a)</a>	Length, 0.7 µm; diameter, 11 nm; purity: aluminium, cobalt, and iron < 0.08%
	MWCNT	Carcinogenicity/rats	Carcinogenicity not affected by defects	<a href="#">Müller et al. (2009)</a>	Length, 0.7 µm; diameter, 11 nm; purity: aluminium, cobalt, and iron < 0.08%

Table 4.2 (continued)

Property	Type of CNT	End-points/models	Effects	References	Characteristics
Surface functionalities: oxidation/functionalization/carboxylation	MWCNT	Cytotoxicity/human T-cells	Surface functionalities (oxidized) increased the toxicity	<a href="#">Bottini et al. (2006)</a>	Length, 1–5 µm; diameter, 20–40 nm; purity: > 95%
	MWCNT	Cytotoxicity, oxidative stress/human neuroblastoma cells	Surface functionalities (oxidized) and impurities increased the toxicity	<a href="#">Vitorio et al. (2009)</a>	Length, 0.5–2 µm; diameter, > 35–40 nm and 20–40 nm; purity: 97–99%
	SWCNT	Embryotoxicity/mice, mouse embryonic stem cells, NIH3T3 cells	Surface functionalities (oxidized) increased the embryotoxicity	<a href="#">Pietrojusti et al. (2011)</a>	Length, 0.37–0.85 µm; diameter, ~2 nm; purity: calcium, chromium, iron, and cobalt < 4% (higher in pristine)
	MWCNT	Profibrogenic markers/BEAS-2B cells, human monocyelocytic leukaemia THP-1 cells; fibrosis/mice	Carboxylation of MWCNT decreased the fibrogenicity	<a href="#">Wang et al. (2011d)</a>	Length, 10–30 µm; diameter, 20–30 nm; purity: ~99%; nickel and iron not detected
Metals from residual catalyst	SWCNT/MWCNT	Cytotoxicity/mammalian CHO cells, human HeLa cells	No cytotoxicity with F-SWCNT; DNA complexes	<a href="#">Pantarotto et al. (2004)</a>	No characterization
	SWCNT/MWCNT	Cytotoxicity/human EAhy926, human A549, human HepG2, mouse DMBM-2, hamster V79, and human TK-6 cells	Carboxylated CNT more cytotoxic than pristine CNT	<a href="#">Fröhlich et al. (2013)</a>	Length, 0.5–2 µm; diameter of SWCNT, 1–2 µm; diameter of MWCNT, < 8, 20–30, and > 50 nm; purity: SWCNT > 90% and MWCNT > 95%
	SWCNT	Osteoblast proliferation/osteosarcoma rat ROS 17/2.8 cells	Cells growth depended on the type of functionalization	<a href="#">Zanello et al. (2006)</a>	Length, from nm to µm; diameter, 1.5 nm
Metals from residual catalyst	MWCNT, raw and metal-purified	Cell viability, inflammasome activation/freshly isolated alveolar macrophages from C57Bl/6 mice	Carboxylation after surface oxidation reduced cytotoxicity and inflammasome activation	<a href="#">Hamilton et al. (2013b)</a>	Dimensions not reported
	MWCNT	Inflammation, fibrosis in vivo, inflammasome activation/C57Bl/6 mice	Carboxylation after surface oxidation decreased inflammation, fibrosis, and inflammasome activation	<a href="#">Sager et al. (2014)</a>	Dimensions not reported
	SWCNT	Oxidative stress/mouse RAW 264.7 macrophages	Iron increased oxidative stress	<a href="#">Kagan et al. (2006)</a>	Diameter: 1–4 nm; purity: iron, 0.23–26%
Metals from residual catalyst	SWCNT/MWCNT	Cytotoxicity, release of inflammatory mediators, oxidative stress/rat NR8383 cells, human A549 lung cells	Metals increased oxidative stress	<a href="#">Pulskamp et al. (2007)</a>	Diameter: 1–2 nm, 10–20 nm, and 30–50 nm; purity: different content of cobalt, iron, nickel, copper, and molybdenum

**Table 4.2 Physico-chemical properties of carbon nanotubes that are relevant to toxicity: summary of the most pertinent studies (continued)**

Property	Type of CNT	End-points/models	Effects	References	Characteristics
	MWCNT	Genotoxicity/rat lung epithelial cells; lung inflammation, fibrosis/rats	Metals residues contributed to an increase in toxicity	<a href="#">Muller et al. (2008a)</a>	Length, 0.7 µm; diameter, 11 nm; purity: cobalt (~0.50%), and iron (0.48%)
	SWCNT	Oxidative stress, inflammation/ EpiDerm FT engineered human skin; collagen accumulation/ mice	Metals residues contributed to an increase in toxicity	<a href="#">Murray et al. (2009)</a>	Iron, 30% and 0.23%
	MWCNT	Cell viability, inflammasome activation/freshly isolated alveolar macrophages from C57Bl/6 mice	Slight reduction in cytotoxicity and inflammasome activation after purification	<a href="#">Hamilton et al. (2013b)</a>	Dimensions not reported
Residual catalyst support/ amorphous carbon	MWCNT	Cell viability, inflammasome activation/freshly isolated alveolar macrophages from C57Bl/6 mice	Slight reduction in cytotoxicity and inflammasome activation after removal of amorphous carbon	<a href="#">Hamilton et al. (2013b)</a>	Dimensions not reported
Chirality		No studies			

CNT, carbon nanotubes; F-SWCNT, functionalized SWCNT; MWCNT, multiwalled carbon nanotubes; SWCNT, single-walled carbon nanotubes  
Adapted with permission from [Ghiazza et al. \(2014\)](#). Copyright (2014), with permission from Elsevier

composition and structure ([Fubini et al., 2010, 2011](#)) which are illustrated in [Table 4.3](#) ([Fubini et al., 2011](#)).

(a) *Length*

[Schinwald et al. \(2012\)](#) reported that CNT over 4  $\mu\text{m}$  in length are pathogenic to the pleura in mice and proposed a threshold length value (4–5  $\mu\text{m}$ ) for the induction of an acute inflammatory response in a mouse model. Pleural inflammation and fibrosis are induced only by long (> 10  $\mu\text{m}$ ) CNT after intraperitoneal ([Kolosnjaj-Tabi et al., 2010](#)) or intrapleural ([Murphy et al., 2011](#)) injection. The adverse effects of long (> 10  $\mu\text{m}$ ), rigid CNT were related to their physical interaction with cells resulting in incomplete internalization and “frustrated phagocytosis”, which activate an inflammatory response. Stomata (diameter, 3–10  $\mu\text{m}$ ) in the parietal pleura act as a “sieve” in drainage from the pleural space and fail to clear the long CNT ([Murphy et al., 2011](#)).

[Manshian et al. \(2013\)](#) investigated the role of the length of SWCNT in the induction of genotoxicity in human bronchial epithelial BEAS-2B and lymphoblastoid MCL-5 cells. SWCNT induced significant levels of chromosomal damage at subcytotoxic concentrations, the potency of which, according to the length of the SWCNT, was 400–800 nm > 5–30  $\mu\text{m}$  > 1–3  $\mu\text{m}$ . The authors hypothesized that surface area is an important determinant in cellular response, as well as the secondary structure of CNT under experimental conditions. In contrast, only SWCNT 1–3  $\mu\text{m}$  in length were found to be mutagenic in mammalian cells (see Section 4.3).

(b) *Thickness*

A study of two MWCNT of similar length (< 5  $\mu\text{m}$ ) and surface reactivity but different diameter (9.4 and 70 nm) showed that thinner MWCNT appeared to be significantly more toxic than their thicker counterparts in vivo (rat lung)

and in vitro (murine alveolar macrophages) ([Fenoglio et al., 2012](#)). [Nagai et al. \(2011\)](#) also reported an effect of the diameter of CNT on mesothelial toxicity and carcinogenicity in rats (see also Section 3). Short CNT with different diameters that had or had not been subjected to carboxyl surface functionalization were assessed for cytotoxicity in phagocytic and non-phagocytic cells. The role of oxidative stress was evaluated by assessing the intracellular glutathione (GSH) levels and protection by *N*-acetyl cysteine (NAC). CNT < 8 nm in diameter were more cytotoxic than CNT  $\geq$  20 nm in diameter and carboxylated CNT were more toxic than as-produced CNT. Protection by NAC was maximal for larger-diameter as-produced CNT and minimal for small-diameter carboxylated CNT. Thinner (diameter < 8 nm) CNT acted mainly through the disruption of membrane integrity, and CNT with a larger diameter mainly induced apoptotic changes ([Fröhlich et al., 2013](#)).

#### 4.2.3 Surface reactivity

The variability in the toxicity elicited by CNT can mostly be ascribed to both differences in shape and modifications to the chemical composition/structure of the CNT employed in the different studies ([Fubini et al., 2011](#) and references therein). Differences in surface state between asbestos and CNT (in contrast to asbestos, CNT quench radicals, are hydrophobic, and may be fully freed from metal impurities) suggest that these two fibrous materials might induce toxicity by different mechanisms ([Fubini et al., 2011](#))

Physical and chemical properties are generally accepted to modulate the cell responses to CNT. The introduction of surface oxygenated functionalities increased the toxicity of CNT in some models ([Bottini et al., 2006](#); [Vittorio et al., 2009](#); [Pietroiusti et al., 2011](#)). In contrast, [Cheng et al. \(2008\)](#) reported that purified PEGylated SWCNT, although reversibly internalized and translocated into the nucleus, were

**Table 4.3 Comparison of the major physico-chemical features of carbon nanotubes and asbestos**

Feature	Asbestos	
	Carbon nanotubes	Chrysotile serpentine
Origin	Manufactured	Natural
Chemical composition	Carbon	Silicate framework including Mg <sup>2+</sup> , Fe <sup>2+/3+</sup> , Na <sup>+</sup> , and Ca <sup>2+</sup> as structural or substitution unit
Structure	Single or multiple rolled graphene sheets	Octahedrally coordinated cation layers sandwiched between tetrahedral silicate layers
Nature of the chemical bond	Fully covalent	Mixed, covalent-polar and ionic
Structural defects	Ring shapes other than hexagon, sp <sup>3</sup> hybridized carbon, dangling bonds at lattice defects, and end caps	Framework defects, absence or substitution of metal ions
Agglomeration	High	Naturally in bundles
Durability in water	High	High in all media
Hydrophilicity/hydrophobicity	Highly hydrophobic if not functionalized	Highly hydrophilic
Bio-available metals	Highly variable (cobalt, nickel, iron – metallic or ionic)	Stoichiometric Fe <sup>2+</sup> and Fe <sup>3+</sup> ions in crystal structure
Surface charge (physiological pH)	Very low, negative if not functionalized	High, negative
Free radicals	Scavenging of free radicals and reactive oxygen species	Generation of free radicals and reactive oxygen species
Dissolution/degradation	Enzymatic degradation in neutrophils (SWCNT) and degradation in phagolysosomal fluid (carboxylated SWCNT)	Selective leaching of iron ions only in the presence of strong chelators

Ca, calcium; Fe, iron; Mg, magnesium; Na, sodium; SWCNT, single-walled carbon nanotubes  
 Created by the Working Group with data from [Fubini et al. \(2011\)](#)

non-genotoxic in mammalian cells in terms of cell-cycle distribution and mitosis after 5 days of continuous exposure, suggesting that intensive purification and functionalization improves the biocompatibility of CNT.

[Li et al. \(2013\)](#) reported the role of surface charge in determining the pulmonary fibrogenic effects of MWCNT. Anionic functionalization with carboxylate and PEG decreased pulmonary fibrogenic potential compared with as-prepared MWCNT; strong cationic functionalization with polyetherimide induced a greater degree of pulmonary fibrosis. Neutral and weakly cationic (sidewall amine) functionalized CNT had similar fibrogenic potential to as-produced CNT. The mechanism of these effects involves differences in the cellular uptake of MWCNT, lysosomal damage, and cathepsin B release in macrophages, associated with the activation of NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome ([Li et al., 2013](#)).

[Hamilton et al. \(2013b\)](#) examined the consequences of surface carboxylation of MWCNT on bioactivity. Hydrochloric acid refluxing was used to purify raw “as-received” MWCNT by removing the amorphous carbon layer on their surface and reducing the metal impurities (e.g. nickel). The sidewall of raw and hydrochloric acid-purified MWCNT was further functionalized with the carboxyl moiety using nitric acid oxidation, a common approach that imparts the carboxyl functional group to the MWCNT. No structural damage was observed. Four distinct MWCNT were compared for their bioactivity: raw “as-received”, purified, carboxyl-terminated raw MWCNT, and carboxyl-terminated purified MWCNT. Raw and hydrochloric acid-purified MWCNT are poorly soluble in water. In contrast, after nitric acid oxidation, both carboxylated forms of MWCNT showed very good water solubility. Freshly isolated alveolar macrophages from C57Bl/6 mice were exposed to these nanomaterials to determine the effects

of these modifications on cell viability and inflammasome activation, which was confirmed using inhibitors of cathepsin B and caspase-1. Purification slightly reduced cell toxicity and inflammasome activation compared with raw MWCNT. In contrast, functionalization of MWCNT with carboxyl groups dramatically reduced cytotoxicity and inflammasome activation. Similar results were seen in human monocytic THP-1 cells. All nanomaterials, regardless of modification, were taken up by alveolar macrophages. However, the manner in which the nanomaterials were processed within the cells differed. Purified MWCNT were taken up in large vacuoles or phagolysosomes and did not appear to be free in the cytoplasm. In contrast, the two functionalized MWCNT did not appear to be incorporated in large vacuoles, but were more evenly distributed in smaller phagolysosomal structures or free in the cytoplasm. The results confirmed that MWCNT activate NLRP3 inflammasome through a process that involves phagolysosomal permeabilization, the release of cathepsin B, and the activation of caspase-1 ([Hamilton et al., 2013b](#)).

[Sager et al. \(2014\)](#) investigated whether MWCNT (same nanomaterial as that used in [Hamilton et al., 2013b](#)) with different surface functionalities would exhibit different bioactivity profiles in vivo. Unmodified (bare) MWCNT and MWCNT that were surface functionalized with the carboxyl group (F-MWCNT) were instilled intratracheally into C57BL/6 mice. Mice were then examined for biomarkers of inflammation and injury, as well as histologically for the development of pulmonary disease as a function of dose and time. Biomarkers for pulmonary inflammation included cytokines (interleukin [IL]-1 $\beta$ , IL-18, and IL-33), profibrotic mediators, the presence of inflammatory cells (neutrophils), lysosomal release of cathepsin B, and markers of injury (albumin and lactate dehydrogenase [LDH]). The results showed that surface modification of the MWCNT by the addition of the

carboxyl group significantly reduced bioactivity and pathogenicity. Bare MWCNT were more bioactive, causing more inflammation, lung pathology, and fibrosis than the F-MWCNT. This difference in bioactivity correlated with the activation of NLRP3 inflammasome ([Sager et al., 2014](#)).

#### (a) *Generation of free radicals*

Unlike other toxic particulates (e.g. asbestos), CNT modified by grinding to introduce structural defects have been reported not to generate but to quench free radicals in cell-free systems. This scavenging activity was eliminated in CNT that were fully divested of their defects (i.e. by heating at 2400 °C) ([Fenoglio et al., 2008](#)). CNT in composites (CNT-polymer) have been employed to preserve the polymeric matrix from oxidative degradation by their radical scavenging ability ([Watts et al., 2003](#)). The susceptibility of CNT to attack by radicals has been exploited to introduce functionalities at their surface ([Ghiazza et al., 2014](#) and references therein). However, SWCNT with different iron contents displayed different redox activity in a cell-free model system, as revealed by the formation of ascorbate radicals resulting from ascorbate oxidation detected by electron paramagnetic resonance ([Kagan et al., 2006](#)). In the presence of zymosan-stimulated RAW 264.7 macrophages, non-purified iron-rich SWCNT were more effective in generating hydroxyl radicals (documented by electron paramagnetic resonance spin-trapping with 5,5-dimethyl-1-pyrroline-*N*-oxide) than purified SWCNT ([Kagan et al., 2006](#)). Exposure of immortalized human epidermal HaCaT keratinocytes to SWCNT induced oxidative stress, which was confirmed by the formation of free radical species, the accumulation of peroxidation products and thiobarbituric acid-reactive substances, the reduction of low-molecular-weight thiols and protein sulfhydryls, and a decrease in vitamin E and total antioxidant reserves in the cells. As-produced unrefined SWCNT contain up to

30% iron, and the authors hypothesized a Fenton-like reaction resulting in HO· generation, which increased in the presence of hydrogen peroxide and decreased in the presence of catalase (a hydrogen peroxide scavenger) or desferoxamine (a strong iron chelator) ([Shvedova et al., 2003](#)).

Whether CNT in cell-free media do not generate hydroxyl radicals and/or other reactive oxygen species (ROS) per se or whether what is hypothetically generated would immediately be quenched by defects is not clear ([Fenoglio et al., 2006, 2008](#)). Purified MWCNT scavenge hydroxyl radicals generated by different sources ([Fenoglio et al., 2006](#)). A local decrease in ROS was observed in vivo after intratracheal instillation of DWCNT in mice ([Crouzier et al., 2010](#)). However, CNT and other graphene materials have been reported to deplete the cellular antioxidant defences of cells by oxidizing GSH through a reaction with oxygen at the surface ([Liu et al., 2011b](#)). Therefore, CNT interact with the cellular antioxidant defence system in several ways. [The overall effect of CNT on the homeostasis of ROS in cells still needs to be clarified.]

#### (b) *Bioavailability and biodeposition of metals*

After synthesis, CNT generally contain amorphous carbon and metals – iron and other different redox active metals (e.g. cobalt, nickel, and molybdenum) – as a residue of the catalyst employed in their synthesis. The amounts are highly variable and may reach 20% in unpurified, as-produced CNT (see also Section 1). Metals may be present in different oxidation states as ions, clusters, or even organized in metal nanoparticles. The iron in CNT has been reported to be a mixture of  $\alpha$ -Fe<sup>0</sup>,  $\gamma$ -Fe<sup>0</sup>, and carbide phases; much of the metal appears by TEM to be at least superficially encapsulated by carbon ([Guo et al., 2007](#)). Most of the iron is located within the tube, and is thus not readily accessible to target cells. The metal residues may be extracted from the CNT, e.g. by acid treatment, but often a fraction remains inside. Toxicologically significant

amounts of iron can be mobilized from a diverse set of commercial nanotube samples in the presence of ascorbate and the chelating agent, ferrozine. This mobilized iron is redox active and induces single-strand breaks in plasmid DNA in the presence of ascorbate. Iron bioavailability is not fully suppressed by vendor “purification” and is sensitive to partial oxidation, mechanical stress, sample ageing, and intentional chelation (Guo et al., 2007). Because iron sealed within the graphene layers cannot be released in physiological media, the amount of bioavailable iron in CNT varies greatly from sample to sample and cannot be predicted from the total iron content (Guo et al., 2007, Fubini et al., 2011 and references therein).

Redox active metals associated with CNT (e.g. iron) have been reported to induce oxidative stress and toxicity (Kagan et al., 2006; Pulskamp et al., 2007). Clear evidence on the role of iron in the toxicity of CNT was obtained by showing that simple removal of most of the iron residues caused a remarkable decrease in the toxicity of SWCNT (Kagan et al., 2006) and MWCNT (Aldieri et al., 2013). Iron-rich SWCNT caused a significant loss of intracellular low-molecular-mass thiols (predominantly GSH) and accumulation of lipid hydroperoxides in both zymosan- and phorbol myristate acetate-stimulated RAW 264.7 macrophages (Kagan et al., 2006). Two MWCNT differing only in the presence or absence of iron were compared at dose ranges of 25–100  $\mu\text{g}/\text{cm}^2$ . While iron-rich MWCNT (50  $\mu\text{g}/\text{cm}^2$ ) were significantly cytotoxic and genotoxic and induced a potent cellular oxidative stress response, iron-free MWCNT (50  $\mu\text{g}/\text{cm}^2$ ) did not exert any of these adverse effects (Aldieri et al., 2013).

Complete elimination of any metal trace can be achieved only by heating CNT to a very high temperature (2400 °C) at which metal vapourizes. Lung toxicity in vivo but not genotoxicity in vitro induced by MWCNT was decreased, but not completely eliminated, by heating at 600 °C, when metals are fully vapourized but

defects remain (Fenoglio et al., 2008; Muller et al., 2008a).

#### 4.2.4 Fibre durability (leaching, dissolution, and breakage)

CNT are highly insoluble due to their graphitic structure (Lam et al., 2004) and they have been suggested to be as biopersistent as amphiboles (Sanchez et al., 2009). However, several studies reported that the carbon structure may be attacked and degraded, mainly by endogenous oxidants in biological simulation fluids or in vivo (Kagan et al., 2010; Shvedova et al., 2012a, b).

##### (a) *In vivo*

Using MPO-deficient mice, Shvedova et al. (2012a) showed that MPO contributes to the pulmonary oxidative biodegradation of SWCNT in vivo (see also Section 4.1.2 (f)).

##### (b) *In vitro*

Two different routes of attack and degradation of CNT by endogenous oxidants were reported in studies in vitro (see Section 4.1.2 (f)). One enzymatic route is through degradation by several peroxidases, such as MPO (Kagan et al., 2010), lactoperoxidase, and EPO (Shvedova et al., 2012b), while the second route follows non-enzymatic degradation when CNT are in contact with simulated phagolysosomal fluid (Liu et al., 2010). Degradation of SWCNT after incubation with human EPO and hydrogen peroxide has been reported; the biodegradation was greater in the presence of sodium bromide. However, neither EPO nor hydrogen peroxide alone caused SWCNT degradation (Andón et al., 2013).

Surface functionalization affects the biodegradability of CNT (Liu et al., 2010; Bianco et al., 2011). The rate of degradation is associated with both the degree of surface functional groups and the type of CNT; MWCNT are more resistant

than SWCNT and thus require a longer time for degradation ([Bianco et al., 2011](#)).

#### 4.2.5 Physico-chemical determinants of defined biological end-points

Because of the extreme variability of the features of CNT, the method to be adopted to associate a physico-chemical feature to a given effect in vivo is to modify one single property at a time of a well defined specimen of CNT and test all modified specimens using exactly the same procedure. Two typical examples of this type of procedure taken from [Table 4.2](#) are highlighted below.

This approach showed clearly that a slight modification in cytotoxicity and inflammogenicity occurred after purification, while acute inflammogenicity (demonstrated by inflammatory activation in MWCNT in vitro and in vivo) was dramatically reduced, with a consequent reduction in pathogenicity after functionalization of the surface with carboxyl ([Hamilton et al., 2013b](#); [Sager et al., 2014](#)).

Modification of MWCNT by progressive heating during which metals and defects are gradually eliminated (see [Table 4.4](#)) enabled an association of genotoxicity in vitro with defects and respiratory toxicity in vivo with both metals and defects ([Fenoglio et al., 2008](#); [Muller et al., 2008a](#)).

### 4.3 Genetic and related effects

#### 4.3.1 Humans

##### (a) Exposed humans

No data were available to the Working Group.

##### (b) Human cells in vitro

See Section 4.3.2 (b)

#### 4.3.2 Experimental systems

##### (a) In vivo

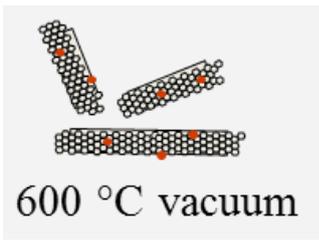
##### (i) DNA damage

Investigations on the direct genotoxicity of CNT have focused on end-points measured by the comet assay and oxidatively generated DNA lesions. [Table 4.5](#) lists the in-vivo studies that have assessed levels of DNA damage in rodent tissues after exposure to CNT.

Intratracheal instillation of SWCNT into mice (54 µg/animal) increased the levels of DNA strand breaks in BALF cells 3 hours after exposure ([Jacobsen et al., 2009](#)). Another study showed that a single intratracheal instillation of MWCNT (50 or 200 µg/mouse) was associated with increased levels of DNA strand breaks in lung tissues of mice 3 hours after exposure, and also documented increased levels of 8-oxodeoxyguanosine (8-oxodG) and lipid peroxidation-derived DNA lesions in lung tissues of mice 3–168 hours after exposure. However, the baseline level of 8-oxodG was 4.8 lesions/10<sup>6</sup> nucleotides (corresponding to 22 lesions/10<sup>6</sup> deoxyguanosine [dG]), indicating spurious oxidation of DNA during the processing or analysis of samples ([Kato et al., 2013](#)). Another study showed that pulmonary exposure to MWCNT once every 2 weeks for 24 weeks was associated with an increased level of 8-oxodG in lung tissues of rats [the detection method was not described and the basal level of 8-oxodG was very high (1.3 ng/µg DNA, corresponding to 7600 lesions/10<sup>6</sup> dG)] ([Xu et al., 2014](#)).

Nose-only inhalation of 0.17–0.96 mg/m<sup>3</sup> of MWCNT for 6 hours per day on 5 days per week for 28 days was associated with increased levels of DNA strand breaks in the lung tissues of rats ([Kim et al., 2014](#)). A similar study by the same authors in which rats were exposed by whole-body inhalation of 0.16–0.94 mg/m<sup>3</sup> of MWCNT for 6 hours per day for 5 days also showed increased levels of DNA strand breaks in lung tissues ([Kim et al., 2012a](#)). Weekly intratracheal

**Table 4.4 Example of an experimental mechanistic approach to evaluate specific physico-chemical determinants of biological activity for ground multiwalled carbon nanotubes**

Multiwalled carbon nanotubes	Defects	Metals	Quenching activity	In-vivo respiratory toxicity		In-vitro genotoxicity
				Lung response	Cytokines	
 <p>Ground</p>	Yes	Yes (in oxidized form)	Positive	Positive	Positive	Positive
 <p>600 °C vacuum</p>	Yes (less)	Yes (in reduced form)	Positive	Reduced	Negative	Positive
 <p>Heated 2400 °C air</p>	No	No	Negative	Negative	Negative	Negative
 <p>Heated 2400 °C ground</p>	Yes	No	Positive	Positive	Negative	Positive

Created by the Working Group with data from [Fenoglio et al. \(2008\)](#) and [Muller et al. \(2008a\)](#)

**Table 4.5 Studies of DNA damage and mutation in tissues of experimental animals exposed to carbon nanotubes in vivo**

Material tested <sup>a</sup>	Species, strain	End-point, test system	Exposure	Result <sup>b</sup>	Reference
SWCNT (diameter, 0.9–1.7 nm; length, < 1 µm; SSA, 731 m <sup>2</sup> /g)	Mouse, C57BL/6 <i>ApoE</i> <sup>-/-</sup> knockout	DNA strand breaks in BALF, comet assay	54 µg/mouse, intratracheal instillation	+	<a href="#">Jacobsen et al. (2009)</a>
SWCNT (diameter, 0.9–1.7 nm; length, < 1 µm; SSA, 731 m <sup>2</sup> /g; iron, 2%)	Mouse, C57BL/6 <i>ApoE</i> <sup>-/-</sup> knockout	DNA strand breaks and FPG-sensitive sites in lung, comet assay	0.5 mg/kg bw twice (24 h interval) (total dose, 1 mg/kg), intratracheal instillation	- DSB - FPG	<a href="#">Yesterdal et al. (2014a)</a>
SWCNT (diameter, 1.8 nm; length, 4.4 µm; SSA, 878 m <sup>2</sup> /g; iron, 4.4%)	Rat, Cri: CD (SD)	DNA strand breaks in lung, comet assay	0.2 or 1 mg/kg bw (single dose), or 0.04 or 0.2 mg/kg bw once/wk, 5 wk, intratracheal instillation	-	<a href="#">Naya et al. (2012)</a>
MWCNT (diameter, 90 nm; length, 2 µm)	Mouse, ICR	DNA strand breaks in lung, comet assay	50 or 200 µg/mouse, intratracheal instillation	+	<a href="#">Kato et al. (2013)</a>
MWCNT (NM400; diameter, 5–35 nm; length, 0.7–3.0 µm; SSA, 298 m <sup>2</sup> /g; NM402; diameter, 6–20 nm; length, 0.7–4.0 µm; SSA, 225 m <sup>2</sup> /g)	Mouse, C57BL/6 <i>ApoE</i> <sup>-/-</sup> knockout	DNA strand breaks and FPG-sensitive sites in lung, comet assay	25.6 µg/mouse once/wk, 5 wk, intratracheal instillation	+ DSB - FPG	<a href="#">Cao et al. (2014)</a>
MWCNT (diameter, 12 nm; length, up to 12 µm; SSA, 41–42 m <sup>2</sup> /g), non-functionalized or functionalized (acid-treated)	Mouse, Swiss-Webster	DNA strand breaks in peripheral blood leukocytes, comet assay	0.25–0.75 mg/kg bw once/day, 5 days, intraperitoneal injection	+	<a href="#">Patlolla et al. (2010)</a>
MWCNT (diameter, 7–15 nm; length, 0.5–200 µm)	Mouse, Swiss albino	DNA strand breaks in bone marrow cells, comet assay	2, 5, or 10 mg/kg bw, intraperitoneal injection	+	<a href="#">Ghosh et al. (2011)</a>
MWCNT (diameter, 44 nm; length, 2.7 µm; SAA, 69 m <sup>2</sup> /g; iron, 5.3%)	Rat, Cri/CD (SD)	DNA strand breaks in lung, comet assay	0.2 or 1 mg/kg bw (single dose), or 0.04 or 0.2 mg/kg bw once/wk, 5 wk, intratracheal instillation	-	<a href="#">Ema et al. (2013a)</a>
MWCNT (diameter, 10–15 nm; length, 20 µm; SSA, 225 m <sup>2</sup> /g; iron, 2%)	Rat, Sprague-Dawley	DNA strand breaks in lung immediately and 1 mo after the last exposure, comet assay	0.16, 0.34, or 0.94 mg/m <sup>3</sup> , 6 h/day, 5 days, whole-body inhalation	+	<a href="#">Kim et al. (2012a)</a>
MWCNT (diameter, 10–15 nm; length, 330 nm; SSA, 225 m <sup>2</sup> /g; iron, 2%)	Rat, F344	DNA strand breaks in lung immediately and 90 days after the last exposure, comet assay	0.17, 0.49, or 0.96 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 6 wk, nose-only inhalation	+	<a href="#">Kim et al. (2014)</a>
MWCNT (diameter, 90 nm; length, 2 µm)	Mouse, ICR	DNA adduct HedC in lung, LC-MS/MS	200 µg/mouse, intratracheal instillation	+	<a href="#">Kato et al. (2013)</a>
SWCNT (diameter, 0.8–1.2 nm; length, 0.1–1 µm; iron < 23%; diameter, 1.2–2 nm; length, 1–15 µm; iron, 0.05%)	Mouse, ICR [with allergic pulmonary inflammation (ovalbumin-sensitized) or normal counterparts]	DNA adduct 8-oxodG in lung, immunohistochemistry	50 µg/mouse, once/wk, 6 wk, intratracheal instillation	+	<a href="#">Inoue et al. (2010)</a>

Table 4.5 (continued)

Material tested <sup>a</sup>	Species, strain	End-point, test system	Exposure	Result <sup>b</sup>	Reference
SWCNT (diameter, 0.9–1.7 nm; length, < 1 µm; SSA, 731 m <sup>2</sup> /g; iron, 2%)	Rat, F344	DNA adduct 8-oxodG in lung, liver, and colon mucosa, HPLC-ECD	0.064 or 0.64 mg/kg bw, gavage	+ lung and liver - colon mucosa	<a href="#">Folkmann et al. (2009)</a>
MWCNT (diameter, 90 nm; length, 2 µm)	Mouse, ICR	DNA adduct 8-oxodG in lung, LC-MS/MS	200 µg/mouse, intratracheal instillation	+ <sup>c</sup>	<a href="#">Kato et al. (2013)</a>
MWCNT described as “short” (diameter, 15 nm; length, 3 µm) or “long” (diameter, 150 nm; length, 8 µm)	Rat, F344	DNA adduct 8-oxodG in lung	0.125 mg/rat once every other wk, 24 wk (total dose, 1.6 mg/kg), trans-tracheal intrapulmonary spraying	(+) <sup>d</sup>	<a href="#">Xu et al. (2014)</a>
SWCNT (diameter, 0.8–1.2 nm; length, 0.1–1 µm; iron, 17.7%)	Mouse, C57BL/6	K- <i>ras</i> gene mutation in lung, PCR	5 mg/m <sup>3</sup> , 5 h/day, 4 days, inhalation	+	<a href="#">Shvedova et al. (2008)</a>
SWCNT (diameter, 0.8–1.2 nm; length, 0.1–1 µm; iron, 17.7%)	Mouse, C57BL/6	K- <i>ras</i> gene mutation in lung, PCR	5–20 µg/mouse, pharyngeal aspiration	-	<a href="#">Shvedova et al. (2008)</a>
MWCNT (diameter, 90 nm; length, 2 µm)	Mouse, <i>Gpt</i> delta transgenic	<i>Gpt</i> gene mutant frequency in lung	0.2 mg/mouse once/wk, 4 wk, intratracheal instillation	+	<a href="#">Kato et al. (2013)</a>

<sup>a</sup> Nanomaterial characteristics include diameter; length, specific surface area (SSA), and content of transition metals

<sup>b</sup> +, positive; -, negative; (+), weakly positive

<sup>c</sup> The baseline levels of DNA lesions were rather high (approximately 4.8 lesions/10<sup>6</sup> nucleotides, corresponding to 22 lesions/10<sup>6</sup> dG)

<sup>d</sup> The detection method was not described and the basal levels of 8-oxodG (1.3 ng/mg DNA) corresponded to 7600 lesions/10<sup>6</sup> dG, assuming that the molecular weight of 8-oxodG is 283 g/mol and 1 fmol/µg DNA is equal to 1.64 lesions/10<sup>6</sup> dG

BALE, bronchoalveolar lavage fluid; bw, body weight; CNT, carbon nanotubes; dG, deoxyguanosine; DSB, DNA strand breaks; FPG, formamidopyrimidine glycosylase; *Gpt*, guanine phosphoribosyltransferase; HsdC, heptanone etheno-deoxyribocytosine (DNA adduct, lipid peroxidation product-derived); HPLC-ECD, high-performance liquid chromatography-electrochemical detection; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MWCNT, multiwalled carbon nanotubes; 8-oxodG, 8-oxodeoxyguanosine; PCR, polymerase chain reaction; SWCNT, single-walled carbon nanotubes; wk, week

instillations of 25.6 µg of MWCNT for 5 weeks were associated with elevated levels of DNA strand breaks in the lung tissues of mice, whereas unaltered levels of formamidopyrimidine glycosylase (FPG)-sensitive sites were found in the same tissues (Cao et al., 2014). Two intratracheal instillations of 0.5 mg/kg bw of SWCNT at an interval of 24 hours did not increase the level of DNA strand breaks or FPG-sensitive sites in mice 2 hours after the last injection (Vesterdal et al., 2014a). No difference in the levels of DNA strand breaks was observed in the lung tissues of rats after intratracheal instillation of a single dose of 0.2 or 1 mg/kg bw or of 0.04 or 0.2 mg/kg bw once per week for 5 weeks of MWCNT (Ema et al., 2013a) or SWCNT (Naya et al., 2012). Increased immunostaining of 8-oxodG was seen in the lung tissues of mice exposed to SWCNT by intratracheal instillation of 50 µg/mouse per week for 6 weeks (Inoue et al., 2010).

Intraperitoneal injection of 0.25–0.75 mg/kg bw of MWCNT once per day for 5 days resulted in increased levels of DNA strand breaks in the peripheral blood leukocytes of mice 24 hours after the last exposure (Patlolla et al., 2010). A single intraperitoneal injection of 2–10 mg/kg bw of MWCNT was also associated with an increased level of DNA strand breaks in the bone marrow cells of mice 3 hours after exposure (Ghosh et al., 2011).

Gastrointestinal exposure by gavage to 0.064 or 0.64 mg/kg bw of SWCNT in either saline suspension or corn oil was associated with increased levels of 8-oxodG in the liver and lung tissues of rats, whereas the same doses did not affect the level of 8-oxodG in colon mucosa cells (Folkmann et al., 2009).

## (ii) Gene mutation

See Table 4.5

Inhalation exposure to 5 mg/m<sup>3</sup> of SWCNT for 5 hours per day for 4 days enhanced mutation of the proto-oncogene *K-ras* in the lung

of C57BL/6 mice. Mutations were found 1 day after the end of inhalation and progressed at 28 days (compared with sham-exposed controls,  $P = 0.045$ ), but mutations were not increased after a single pharyngeal aspiration of 5–20 µg/mouse (Shvedova et al., 2008). One year after exposure, karyotypic changes were shown by micronuclei and multinucleated cells in type II pneumocytes (Shvedova et al., 2014). A study of the intratracheal instillation of 0.2 mg/mouse of MWCNT once per week for 4 weeks showed enhanced guanine phosphoribosyltransferase *Gpt* gene mutation frequencies in the lungs (Kato et al., 2013).

## (iii) Chromosomal alterations

Table 4.6 lists the studies that have assessed chromosomal alterations (micronucleus formation and chromosomal aberration) in rodents after exposure to CNT.

Only one investigation examined CNT-induced chromosomal aberrations in rodents. In this study, Swiss-Webster mice (age, 6 weeks) received intraperitoneal injections of 0.25–0.75 mg/kg bw of native and acid-washed MWCNT (diameter, 12 nm; length, < 12 µm) once per day for 5 days. The bone marrow cells were prepared for cytogenetic analysis 24 hours after the exposure, which was associated with a dose-dependent increase in the levels of chromosome gaps, chromatid and isochromatid breaks, fragments, and structural rearrangements, including centromeric fusions and dicentric chromosomes (Patlolla et al., 2010).

Studies on the formation of micronuclei in experimental animals have mainly explored effects after non-pulmonary exposures, although one study in Wistar rats showed an increased frequency of micronuclei in type II pneumocytes isolated 3 days after intratracheal instillation of 0.5–2 mg/rat of MWCNT (Muller et al., 2008b). Oral exposure to 60–200 or 5–20 mg/kg bw of SWCNT once per day for 2 days did not affect the frequency of micronucleated polychromatic

**Table 4.6 Studies of micronucleus frequency and chromosomal aberrations in cells of experimental animals exposed to carbon nanotubes in vivo**

Material <sup>a</sup>	Species, strain	End-point, test system	Exposure	Results <sup>b</sup>	Reference
SWCNT (diameter, 1.8 nm; SSA, 878 m <sup>2</sup> /g; iron, 4.4%)	Mouse, ICR	Micronucleus formation in immature erythrocytes in bone marrow cells	5–20 mg/kg bw once/day, 2 days, oral gavage	–	<a href="#">Ema et al. (2013b)</a>
SWCNT (diameter, 3 nm; length, 1.2 μm; SSA, 1064 m <sup>2</sup> /g)	Mouse, CD-1	Micronucleus formation in polychromatic erythrocytes in bone marrow cells	60 or 200 mg/kg bw once/day, 2 days, oral gavage	–	<a href="#">Nava et al. (2011)</a>
MWCNT (diameter, 11 nm; length, 0.7 μm; 2% impurities)	Rat, Wistar	Micronucleus formation in type II pneumocytes	0.5 and 2 mg/rat, intratracheal instillation	+ <sup>c</sup>	<a href="#">Muller et al. (2008b)</a>
MWCNT (diameter, 12 nm; length, up to 12 μm; SSA, 41–42 m <sup>2</sup> /g, non-functionalized or functionalized (acid-treated))	Mouse, Swiss-Webster	Micronucleus formation in femoral bone marrow cells	0.25–0.75 mg/kg bw once/day, 5 days, intraperitoneal injection	+ <sup>d</sup>	<a href="#">Patlolla et al. (2010)</a>
MWCNT (diameter, 7–15 nm; length, 0.5–200 μm)	Mouse, Swiss albino	Micronucleus formation in polychromatic erythrocytes in bone marrow cells	2, 5, or 10 mg/kg bw, intraperitoneal injection	+	<a href="#">Ghosh et al. 2011</a>
MWCNT (diameter, 10–15 nm; length, 0.15 or 10 μm; SSA, 178 or 195 m <sup>2</sup> /g; iron, 1% or 5%)	Mouse, ICR	Micronucleus formation in polychromatic erythrocytes in femoral bone marrow cells	50 mg/kg bw, intraperitoneal injection	–	<a href="#">Kim et al. (2011)</a>
MWCNT (diameter, 12 nm; length, up to 12 μm; SSA, 41–42 m <sup>2</sup> /g), non-functionalized or functionalized (acid-treated)	Mouse, Swiss-Webster	Chromosomal aberrations in femoral bone marrow cells	0.25–0.75 mg/kg bw once/day, 5 days, intraperitoneal injection	+ <sup>e</sup>	<a href="#">Patlolla et al. (2010)</a>

<sup>a</sup> Nanomaterial characteristics include diameter, length, specific surface area (SSA), and content of transition metals

<sup>b</sup> +, positive; –, negative

<sup>c</sup> Occurring concurrently with pulmonary inflammation (assessed as increased number of macrophages and neutrophils in bronchoalveolar lavage fluid)

<sup>d</sup> Dose-dependent increase in micronucleus frequency

<sup>e</sup> Dose-dependent increase in structural chromosomal aberrations

bw, body weight; CNT, carbon nanotubes; MWCNT, multiwalled carbon nanotubes; SWCNT, single-walled carbon nanotubes

or immature erythrocytes in the bone marrow cells of ICR or CD-1 mice ([Naya et al., 2011](#); [Ema et al., 2013b](#)). Intraperitoneal injection of 0.25–0.75 mg/kg bw of MWCNT once per day for 5 days was associated with an increased frequency of micronuclei in bone marrow cells in one study in Swiss-Webster mice ([Patlolla et al., 2010](#)). Intraperitoneal injection of 2–10 mg/kg bw of MWCNT in Swiss albino mice increased the frequency of micronuclei in bone marrow cells, whereas the percentage of polychromatic erythrocytes was unaltered ([Ghosh et al., 2011](#)). Another study showed no increase in the frequency of micronuclei and no alteration in the frequency of polychromatic erythrocytes in the bone marrow cells of ICR mice after a single intraperitoneal injection of 12.5–50 mg/kg bw of MWCNT ([Kim et al., 2011](#)).

(b) *In vitro*

(i) *DNA damage*

Studies that have assessed the levels of DNA damage in cell cultures after exposure to CNT are presented in [Table 4.7](#). The neutral version of the comet assay showed unaltered levels of double-strand breaks in human alveolar basal epithelial adenocarcinoma A549 cells after exposure to MWCNT ([Ju et al., 2014](#)).

Several studies have documented that exposure to SWCNT or MWCNT increased the levels of DNA strand breaks in human colon carcinoma tissue HT29 cells ([Pelka et al., 2013](#)), bronchial epithelial BEAS-2B cells ([Lindberg et al., 2009, 2013](#)), lung adenocarcinoma A549 cells ([Karlsson et al., 2008](#); [Cavallo et al., 2012](#)), mesothelial cells ([Pacurari et al., 2008b](#); [Lindberg et al., 2013](#)), human gingival fibroblasts ([Cicchetti et al., 2011](#)), Chinese hamster V79 fibroblasts and primary mouse embryo fibroblasts ([Kisin et al., 2007, 2011](#); [Yang et al., 2009](#)), human lymphocytes ([Ghosh et al., 2011](#)), phytohaemagglutinin-stimulated human lymphocytes ([Kim & Yu, 2014](#)), murine macrophages ([Migliore et al.,](#)

[2010](#); [Di Giorgio et al., 2011](#); [Aldieri et al., 2013](#)), human and rat kidney epithelial cells ([Barillet et al., 2010](#); [Kermanizadeh et al., 2013](#)), and human hepatocytes ([Kermanizadeh et al., 2012](#); [Alarifi et al., 2014](#); [Vesterdal et al., 2014b](#)). Increased levels of DNA strand breaks were also observed in rat aortic endothelial and human lung adenocarcinoma A549 cells after exposure to CNT, but the statistical analysis appeared to have been based on the total number of comets from a single experiment rather than the mean values from independent experiments ([Yamashita et al., 2010](#); [Cheng et al., 2012](#)). However, another study used all comets in the statistical analysis and showed no alteration in DNA strand breaks in human peripheral lymphocytes exposed to SWCNT ([Zeni et al., 2008](#)). [The Working Group noted the uncertainty that replicates were independent experiments.] Other studies have shown no alterations in the levels of DNA strand breaks in human lung adenocarcinoma A549 cells and human HaCaT keratinocytes after exposure to MWCNT ([Thurnherr et al., 2011](#); [McShan & Yu, 2014](#)) or in FE1 MML mouse lung epithelial cells exposed to SWCNT ([Jacobsen et al., 2008](#)).

The protocol of the alkaline comet assay that measures DNA strand breaks can be extended using an additional DNA digestion step with DNA repair enzymes from bacterial or human cells. The bacterial enzymes include FPG and endonuclease III (ENDOIII). The FPG enzyme also cleaves DNA at ring-opened formamidopyrimidine lesions, including 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine. ENDOIII lesions comprise oxidized pyrimidines, such as uracil glycol, thymine glycol, 5-hydroxycytosine, and 5-hydroxyuracil. Results from these enzyme-modified comet assay measurements have been reported either as total sites (DNA strand breaks plus extra breaks generated by the enzyme) or enzyme-sensitive sites (breaks generated by the enzyme minus the basal level of DNA strand breaks).

**Table 4.7 Studies of DNA damage and mutation in experimental systems after exposure to carbon nanotubes in vitro**

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Results <sup>c</sup>	Comments	Reference
MWCNT (diameter, 10–20 nm; length, 0.3–0.7 µm; iron, 0.06%)	Human lung adenocarcinoma A549 cells	DNA strand breaks, neutral comet assay	30 µg/mL	–		<a href="#">Ju et al. (2014)</a>
SWCNT (diameter, 1.8 nm; length, 0.5–2 µm)	Human colon carcinoma HT29 cells	DNA strand breaks and FPG-sensitive sites, comet assay	0.00001 µg/mL	+ DSB – FPG	Incubation with FPG did not increase the level of FPG-sensitive sites above that of DNA strand breaks over the whole range (0.00001–0.2 µg/mL)	<a href="#">Pelka et al. (2013)</a>
Mixed CNT (more than 50% SWCNT; diameter, 1.1 nm; length, 0.5–100 µm)	Human bronchial epithelial BEAS-2B cells	DNA strand breaks, comet assay	1 µg/cm <sup>2</sup>	+	Dose-dependent increase in DNA strand breaks over the whole range (1–100 µg/cm <sup>2</sup> )	<a href="#">Lindberg et al. (2009)</a>
SWCNT (diameter < 2 nm; length, 1–5 µm)	Human bronchial epithelial BEAS-2B or mesothelial MeT-5A cells	DNA strand breaks, comet assay	5–200 µg/cm <sup>2</sup>	+		<a href="#">Lindberg et al. (2013)</a>
MWCNT (diameter, 10–30 nm; length, 1–2 µm)	Human bronchial epithelial BEAS-2B or mesothelial MeT-5A cells	DNA strand breaks, comet assay	5–200 µg/cm <sup>2</sup>	– BEAS-2B + MeT-5A		<a href="#">Lindberg et al. (2013)</a>
MWCNT (diameter, 20–40 nm; length, 0.5–200 µm; iron, 0.55%)	Human lung adenocarcinoma A549 cells	DNA strand breaks and FPG-sensitive sites, comet assay	5–100 µg/mL	+ DSB – FPG	No change in FPG-sensitive sites; uncertainty about the result because of lack of positive control	<a href="#">Cavallo et al. (2012)</a>
MWCNT (diameter, 100–200 nm; length, few µm)	Human lung adenocarcinoma A549 cells	DNA strand breaks and FPG-sensitive sites, comet assay	1–40 µg/cm <sup>2</sup>	+ DSB – FPG	Dose–response trend for DNA strand breaks; no change in FPG-sensitive sites	<a href="#">Karlsson et al. (2008)</a>
SWCNT (diameter, 1.4 nm; length, 2–5 µm; SSA, 293 m <sup>2</sup> /g; iron, 0.07%)	Human mesothelial cells	DNA strand breaks, comet assay	25–50 mg/cm <sup>2</sup>	+		<a href="#">Pacurari et al. (2008a)</a>
SWCNT (diameter, 1.6 nm; length, 0.8 µm; SSA, 407 m <sup>2</sup> /g; purity > 90%)	Human gingival fibroblasts	DNA strand breaks, comet assay	50–150 µg/mL	+		<a href="#">Cicchetti et al. (2011)</a>

**Table 4.7 Studies of DNA damage and mutation in experimental systems after exposure to carbon nanotubes in vitro (continued)**

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Results <sup>c</sup>	Comments	Reference
MWCNT (diameter, 20–40 nm; length, 1–5 µm; 1% impurities), and pristine or amide-functionalized SWCNT (30% impurities)	Human fibroblasts	DNA strand breaks, γH2AX foci	25–150 µg/mL	+	Increased DNA double-strand breaks determined by γH2AX foci	<a href="#">Cveticanin et al. (2010)</a>
MWCNT (diameter, 7–15 nm; length, 0.5–200 µm)	Human lymphocytes	DNA strand breaks, comet assay	2 µg/mL	+	Increased levels at 2 µg/mL, but not at 1, 5, or 10 µg/mL	<a href="#">Ghosh et al. (2011)</a>
SWCNT (diameter, 1–1.2 nm; length, 20 µm)	Human lymphocytes	DNA strand breaks, comet assay	25–100 µg/mL	+		<a href="#">Kim &amp; Yu (2014)</a>
MWCNT (NM400: diameter, 5–35 nm; length, 0.7–3.0 µm; SSA, 298 m <sup>2</sup> /g; NM402: diameter, 6–20 nm; length, 0.7–4.0 µm; SSA, 225 m <sup>2</sup> /g)	Human renal proximal tubule epithelial HK-2 cells	DNA strand breaks and FPG-sensitive sites, comet assay	1.25–5 µg/cm <sup>2</sup>	+ DSB – FPG	Increased levels of DNA lesions; subtraction of DNA strand break levels from the total sites after treatment with FPG indicated negative values of FPG-sensitive sites	<a href="#">Kermanizadeh et al. (2013)</a>
SWCNT (diameter, 1.2–1.7 nm; length, 0.1–4 µm)	Human hepatocyte HepG2 cells	DNA strand breaks, comet assay	5–20 µg/mL	+		<a href="#">Akarifi et al. (2014)</a>
MWCNT (NM400: diameter, 5–35 nm; length, 0.7–3.0 µm; SSA, 298 m <sup>2</sup> /g; NM402: diameter, 6–20 nm; length, 0.7–4.0 µm; SSA, 225 m <sup>2</sup> /g)	Human hepatoblastoma C3A cells	DNA strand breaks and FPG-sensitive sites, comet assay	5–20 µg/cm <sup>2</sup>	+ DSB + FPG with NM402 – FPG with NM400	Levels of FPG-sensitive sites increased with NM402; subtraction of DNA strand break levels from the total sites after treatment with FPG indicated negative values for FPG-sensitive sites with NM400	<a href="#">Kermanizadeh et al. (2012)</a>
SWCNT (diameter, 0.9–1.7 nm; length < 1 µm; SSA, 731 m <sup>2</sup> /g; iron, 2%)	Human hepatocyte HepG2 cells	DNA strand breaks and FPG-sensitive sites, comet assay	25 µg/mL	+ DSB + FPG	Increased levels of both DNA strand breaks and FPG-sensitive sites	<a href="#">Vesterdal et al. (2014b)</a>
MWCNT (M1: diameter, 20–60 nm; length, 5–15 µm; M2: diameter, 60–100 nm; length, 1–2 µm; M3: diameter < 10 nm; length, 1–2 µm)	Human lung adenocarcinoma A549 cells	DNA strand breaks, comet assay	50 µg/mL	+ with M1 + with M2 – with M3	Uncertainty about replicates being independent experiments	<a href="#">Yamashita et al. (2010)</a>
SWCNT (diameter < 2 nm; length, 5–15 µm)	Human lung adenocarcinoma A549 cells	DNA strand breaks, comet assay	50 µg/mL	–	Uncertainty about replicates being independent experiments	<a href="#">Yamashita et al. (2010)</a>

Table 4.7 (continued)

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Results <sup>c</sup>	Comments	Reference
MWCNT (diameter, 6–24 nm; length, 2–5 µm; < 0.4% impurities)	Human lung adenocarcinoma A549 cells	DNA strand breaks, comet assay	7.5–30 µg/mL	–		<a href="#">Thurnherr et al. (2011)</a>
SWCNT (diameter < 2 nm; length, 5–15 µm) and MWCNT (diameter, 10–30 nm; length, 5–15 µm)	Human pleural mesothelial Met-5A cells	DNA strand breaks, comet assay	20 µg/mL	+	Statistical analysis based on the total number of comets	<a href="#">Ogasawara et al. (2012)</a>
SWCNT (diameter, 1.1 nm; length, 50 µm; 3.7% impurities)	Human peripheral lymphocytes	DNA strand breaks, comet assay	1–10 µg/mL	–	Statistical analysis based on the total number of comets	<a href="#">Zeni et al. (2008)</a>
MWCNT (characteristics not reported, used as pristine, purified and carboxyl-functionalized samples)	Human HaCaT keratinocytes	DNA strand breaks and FPG-sensitive sites, comet assay	20 µg/mL	– DSB + FPG	Unaltered level of DNA strand breaks; increased levels of FPG-sensitive sites with all MWCNT tested	<a href="#">McShan &amp; Yu (2014)</a>
SWCNT (diameter < 2 nm; length, 5–15 µm) and MWCNT (diameter, 10–30 nm; length, 5–15 µm)	Human pleural mesothelial Met-5A cells	DNA adduct 8-oxodG, HPLC-ECD	20 µg/mL	–	Uncertainty about replicates being independent experiments; high baseline level of 8-oxodG	<a href="#">Ogasawara et al. (2012)</a>
SWCNT (diameter < 2 nm; length, 1–5 µm)	Human bronchial epithelial BEAS-2B or mesothelial MeT-5A cells	DNA adduct M1dG, immune-slot blot	1–160 µg/cm <sup>2</sup>	+	Increase in adducts in both cell lines after 48-h treatment; decrease in adducts in MeT-5A cells after 72-h treatment	<a href="#">Lindberg et al. (2013)</a>
MWCNT (diameter, 10–30 nm; length: 1–2 µm)	Human bronchial epithelial BEAS-2B or mesothelial MeT-5A cells	DNA adducts M1dG, immune-slot blot	1–160 µg/cm <sup>2</sup>	–	Decrease in adducts in both cell lines after 72-h treatment	<a href="#">Lindberg et al. (2013)</a>
SWCNT (diameter, 1–2 nm; length, 0.4–0.8 µm or 5–30 µm)	Human lymphoblastoid MCL-5 cells	HGPRT locus gene mutation frequency (forward mutation)	25–100 µg/mL	+ with short CNT – with long CNT		<a href="#">Manshian et al. (2013)</a>
SWCNT (diameter, 12 nm; length < 5 µm; < 1% impurities)	Primary mouse embryo fibroblasts	DNA strand breaks, comet assay	5 µg/mL	+		<a href="#">Yang et al. (2009)</a>
MWCNT (diameter, 67–70 nm; length, 1.1–1.2 µm; SSA, 52–60 m <sup>2</sup> /g) either pristine (iron, 0.5%) or purified (iron, 0.002%)	Mouse alveolar macrophage MH-S cells	DNA strand breaks, comet assay	50 µg/cm <sup>2</sup>	+ with pristine – with purified		<a href="#">Aldieri et al. (2013)</a>

**Table 4.7 Studies of DNA damage and mutation in experimental systems after exposure to carbon nanotubes in vitro (continued)**

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Results <sup>c</sup>	Comments	Reference
SWCNT (diameter, 0.9–1.7 nm; length < 1 µm; SSA, 731 m <sup>2</sup> /g; iron, 2%)	Mouse lung epithelial FE1-MML cells	DNA strand breaks and FPG-sensitive sites, comet assay	100 µg/mL	- DSB + FPG	Unaltered level of DNA strand breaks; increased levels of FPG-sensitive sites	<a href="#">Jacobsen et al. (2008)</a>
SWCNT (diameter, 1.2–1.5 nm; length, 2–5 µm; nickel, 1.5%)	Mouse macrophage RAW 264.7 cells	DNA strand breaks, comet assay	3–50 µg/mL	+	Bell-shaped concentration–response relationship	<a href="#">Di Giorgio et al. (2011)</a>
MWCNT (diameter, 10–25 nm; length, 0.5–50 µm; SSA, 400 m <sup>2</sup> /g; nickel, 1.5%)	Mouse macrophage RAW 264.7 cells	DNA strand breaks, comet assay	3–50 µg/mL	+	Bell-shaped concentration–response relationship	<a href="#">Di Giorgio et al. (2011)</a>
SWCNT (diameter, 0.7–1.2 nm; length, 0.5–100 µm; SSA, 400 m <sup>2</sup> /g) and MWCNT (diameter, 110–170 nm; length, 5–9 µm; SSA, 22 m <sup>2</sup> /g; iron < 0.1%)	Mouse macrophage RAW 264.7 cells	DNA strand breaks, ENDOIII- and FPG-sensitive sites, comet assay	1–100 µg/mL	+ DSB + FPG + ENDOIII	Increased levels of DNA strand breaks and ENDOIII- and FPG-sensitive sites (increase in oxidized purines and pyrimidines) with both CNT	<a href="#">Migliore et al. (2010)</a>
MWCNT defined as “short” (diameter, 7–180 nm; length, 0.1–5 µm) and “long” (diameter, 8–177 nm; length, 0.1–20 µm)	Rat kidney epithelial NRK-52E cells	DNA strand breaks, comet assay	20–200 µg/mL	+ with long CNT - with short CNT	Levels of DNA strand breaks in unexposed cells not reported	<a href="#">Barillet et al. (2010)</a>
SWCNT (diameter, 0.8–12 nm; length, several µm; iron, 0.62%)	Rat aortic endothelial cells	DNA strand breaks, comet assay	50–200 µg/mL	+	Dose–response increase; uncertainty about replicates being independent experiments	<a href="#">Cheng et al. (2012)</a>
SWCNT (diameter, 0.4–1.2 nm; length, 1–3 µm; SSA, 1040 m <sup>2</sup> /g; iron, 0.23%)	Chinese hamster lung V79 fibroblasts	DNA strand breaks, comet assay	24–96 µg/cm <sup>2</sup>	+		<a href="#">Kisin et al. (2007; 2011)</a>
MWCNT (length, 7.4 µm)	Chicken DT40 lymphoid cells	DNA adduct 8-oxodG, immunostaining	5 µg/mL	+		<a href="#">Mohiuddin et al. (2014)</a>
MWCNT (characteristics not given)	Mouse embryonic stem cells	<i>Aprt</i> locus, gene mutation frequency	5 µg/mL	+		<a href="#">Zhu et al. (2007)</a>
SWCNT (diameter, 0.9–1.7 nm; length < 1 µm)	FE1-Muta™ mouse lung epithelial cells	<i>cH1</i> locus, gene mutation frequency	100 µg/mL	-		<a href="#">Jacobsen et al. (2008)</a>

**Table 4.7 (continued)**

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Results <sup>c</sup>	Comments	Reference
MWCNT (diameter, 88 nm; length, 5 µm)	Chinese hamster lung CHL/UI cells	<i>Hgprrt</i> locus, gene mutation frequency	3.3–100 µg/mL	–		<a href="#">Asakura et al. (2010)</a>

<sup>a</sup> Nanomaterial characteristics include diameter, length, specific surface area (SSA), and content of transition metals

<sup>b</sup> LEC, lowest effective concentration; HIC, highest ineffective concentration

<sup>c</sup> +, positive; –, negative

*Aprt*, adenine phosphoribosyltransferase; CNT, carbon nanotubes; DSB, DNA strand breaks; ENDO-III, endonuclease-III; FPG, formamidopyrimidine glycosylase; *Hgprrt*, hypoxanthine-guanine phosphoribosyltransferase; HPLC-ECD, high-performance liquid chromatography-electrochemical detection; M1dG, N1-N<sup>2</sup>malondialdehyde-2'-deoxyguanosine; MWCNT, multiwalled carbon nanotubes; 8-oxodG, 8-oxodeoxyguanosine; SWCNT, single-walled carbon nanotubes

Exposure to SWCNT increased the level of FPG-sensitive sites in FE1-MML mouse lung epithelial and human hepatoblastoma HepG2 cells (Jacobsen et al., 2008; Vesterdal et al., 2014b). Both SWCNT and MWCNT increased the level of ENDOIII- and FPG-sensitive sites in rat RAW 264.7 macrophages (Migliore et al., 2010), but the levels of FPG-sensitive sites in human lung adenocarcinoma A549 cells were unaltered after exposure to MWCNT, although the validity of this observation is questionable due to the lack of a positive control (Cavallo et al., 2012). Another study also found unaltered levels of FPG-sensitive sites in A549 cells after exposure to MWCNT (Karlsson et al., 2008). [The Working Group noted that the increased levels of FPG-sensitive sites were observed in cells after exposure to zinc oxide and copper oxide nanoparticles, indicating a reliable methodology for measuring oxidative damage to DNA.]

Exposure of human colon carcinoma cells to SWCNT did not increase the level of extra FPG sites (Pelka et al., 2013). Exposure to two different types of MWCNT increased the levels of total sites in human hepatoblastoma cells after treatment with FPG, whereas the FPG-modified assay generated fewer lesions compared with DNA strand breaks with one type of MWCNT (NM 402) but not with the other (NM 400) (Kermanizadeh et al., 2012). The same authors also showed increased levels of total sites in renal cells exposed to the same types of MWCNT, but the net level of FPG-sensitive sites did not appear to differ between exposed and unexposed cells (Kermanizadeh et al., 2013), indicating that the exposure to MWCNT was not associated with specific oxidative damage to DNA nucleobases but did seem to generate DNA strand breaks. Exposure of human HaCaT keratinocytes to MWCNT was associated with increased levels of total FPG sites (McShan & Yu, 2014).

Increased levels of lipid peroxidation product-derived 3-(2'-deoxy- $\beta$ -D-erythropentofuranosyl)-pyrimido[1,2- $\alpha$ ]-purin-10(3H)-one] adducts [M1dG

or N1N<sup>2</sup>malondialdehyde-2'-deoxyguanosine] were detected by immunoblot in human bronchial epithelial BEAS-2B and human pleural mesothelial Met-5A cells, after 48 hours of exposure to SWCNT but decreased levels 72 hours after exposure (Lindberg et al., 2013). No increase in the levels of 8-oxodG, measured by high-performance liquid chromatography with electrochemical detection, were observed in human pleural mesothelial Met-5A cells after exposure to SWCNT or MWCNT (Ogasawara et al., 2012). [The Working Group noted the uncertainty that replicates were independent experiments.] However, the baseline level of 8-oxodG (8 lesions/10<sup>6</sup> dG) was high, indicating spurious oxidation of DNA during the processing or analysis of samples (Ogasawara et al., 2012). [In keeping with the recommendations of the European Committee on Oxidative DNA Damage, reports with baseline levels of 8-oxodG higher than 5 lesions/10<sup>6</sup> dG in unexposed cells or animals should be interpreted with caution because of the risk of flawed methodology (ESCODD, 2003).] One study investigated oxidative damage to DNA in cells using antibody-based techniques, and showed increased levels of 8-oxodG by immunostaining in chicken lymphoid cells after exposure to MWCNT (Mohiuddin et al., 2014).

#### (ii) Gene mutations

Exposure of mouse embryonic stem cells to MWCNT increased the mutation frequency in the adenine phosphoribosyltransferase (*Aprt*) gene (Zhu et al., 2007). However, mutation frequency in the hypoxanthine-guanine phosphoribosyltransferase (*Hgppt*) gene was unaltered after Chinese hamster lung cells were exposed to MWCNT (Asakura et al., 2010). Increased levels of mutations in the *Hgppt* gene were observed in human lymphoblastic MCL-5 cells after exposure to SWCNT with a length of 1–3  $\mu$ m, whereas shorter (0.4–0.8  $\mu$ m) and longer (5–30  $\mu$ m) nanotubes were not associated with mutagenicity (Manshian et al., 2013). Long-term

exposure (24 days) of FE1-Muta<sup>TM</sup>Mouse lung epithelial cells to SWCNT (length < 1  $\mu\text{m}$ ) did not increase the frequency of mutation in the *cII* gene (Jacobsen et al., 2008).

### (iii) Micronucleus formation

The results on the induction of micronuclei in cultured cells after exposure to CNT have been conflicting. No difference between the distribution of studies showing an increased formation of micronuclei or a null effect was apparent, with regard to the use of the cytokinesis-block micronucleus protocol or other protocols to score micronuclei. Specific assay protocols have therefore not been highlighted in the descriptions of the findings in cell cultures. Table 4.8 lists the studies that have assessed chromosomal alterations in cell cultures after exposure to CNT.

The assessment of micronucleus frequency in human lymphocytes after exposure to six different types of MWCNT did not show a monotonic concentration–response relationship, although one sample with a short fibre length (0.4  $\mu\text{m}$ ) gave statistically significant results at all concentrations tested and one other sample yielded increased micronucleus frequencies at a low concentration of 2.5  $\mu\text{g/mL}$ . The diameter and length of the tubes could not explain the observed results and other structural differences, including surface area and transition metal content, might be implicated (Tavares et al., 2014). Observations from cultured lymphocytes indicated no effect on micronucleus formation after exposure to MWCNT (Szendi & Varga, 2008), whereas both MWCNT and SWCNT increased the frequency of micronuclei in another study in lymphocytes (Cveticanin et al., 2010). Exposure to SWCNT was also associated with an increased frequency of micronuclei in phytohaemagglutinin-stimulated human lymphocytes (Kim & Yu, 2014). Increased frequencies of micronuclei (Kato et al., 2013) or no increase in micronuclei (Thurnherr et al., 2011) were observed in human lung adenocarcinoma A549 cells after exposure

to MWCNT. Exposure of human immortalized bronchial epithelial BEAS-2B cells to SWCNT yielded either a null effect (Lindberg et al., 2009, 2013) or an increased frequency of micronuclei (Manshian et al., 2013). Similarly, hamster lung V79 fibroblasts responded with unaltered micronucleus frequency (Kisin et al., 2007; Pelka et al., 2013) or increased micronucleus frequency (Asakura et al., 2010; Cicchetti et al., 2011; Kisin et al., 2011) after exposure to either SWCNT or MWCNT. Increased micronucleus frequencies were also observed in human breast epithelial MCF-7 and lung adenocarcinoma A549 cells, rat lung epithelial cells, mouse RAW 264.7 macrophages, and human B-lymphoblastoid MCL-5 cells after exposure to either MWCNT or SWCNT (Muller et al., 2008b; Di Giorgio et al., 2011; Kato et al., 2013; Manshian et al., 2013). A sample of MWCNT with a relatively short fibre length (0.7  $\mu\text{m}$ ) and low transition metal content (iron, 0.48%; cobalt, 0.49%) was used to study the impact of structural defects and metals content on the formation of micronuclei in rat lung epithelial cells. Ground MWCNT (producing structural defects) increased the micronucleus frequency, whereas heated (2400  $^{\circ}\text{C}$ ) ground MWCNT (which ablates the structural defects and eliminates metals) did not (Muller et al., 2008a).

[Collectively, cell culture studies document the ability of MWCNT and SWCNT to increase the frequency of micronuclei in proliferating cells, although substantial differences in effects were seen between studies, possibly originating from differences in cell types, characteristics of the CNT, dispersion protocols, and assay conditions.]

### (iv) Chromosomal aberrations

Table 4.8 lists in-vitro investigations in which established cell lines were exposed to SWCNT and MWCNT.

Increased chromosome breakage and aneuploid cells were demonstrated in mouse

**Table 4.8 Studies of micronucleus frequency, chromosomal aberrations, and sister-chromatid exchange in experimental systems after exposure to carbon nanotubes in vitro**

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Result <sup>c</sup>	Comments	Reference
MWCNT (six different samples): NM400 (diameter, 11 nm; length, 0.7 µm; SSA, 280 m <sup>2</sup> /g), NM401 (diameter, 63 nm; length, 3.4 µm; SSA, 300 m <sup>2</sup> /g), NM402 (diameter, 11 nm; length, 1.1 µm; SSA, 250 m <sup>2</sup> /g), NM403 (diameter, 11 nm; length, 0.4 µm), NRCWE (diameter, 69 nm; length, 44 µm; SSA, 24–28 m <sup>2</sup> /g), and NRCWE-007 (diameter, 15 nm; length, 0.4 µm; SSA, 233 m <sup>2</sup> /g)	Human lymphocytes	Micronucleus, CBMN assay	2.5–250 µg/mL	+ with NM403 + with NRCWE (+) with NM402 – with NM400, NM401, and NRCWE-007	Increased frequency of micronuclei at all concentrations of NM403; NRCWE associated with increased micronucleus frequency at two low concentrations (2.5 and 15 µg/mL); micronucleus frequency increased at one concentration of NM402 (15 µg/mL), but regarded as an equivocal result	<a href="#">Tavares et al. (2014)</a>
MWCNT (diameter, 10–30 nm; length, 1–2 µm)	Human lymphocytes	Micronucleus formation, CBMN assay	1000 µg/mL	–	No changes in micronucleus frequency (CBMN on samples from three different donors)	<a href="#">Szendi &amp; Varga (2008)</a>
MWCNT (diameter, 20–40 nm; length, 1–5 µm; 1% impurities), and pristine or amide-functionalized SWCNT (30% impurities)	Human lymphocytes	Micronucleus formation, CBMN assay	25–150 µg/mL	+	Increased micronucleus frequency by MWCNT and SWCNT (both pristine and functionalized form); decreased CBPI at high concentrations	<a href="#">Cveticanin et al. (2010)</a>
SWCNT (diameter, 1–1.2 nm; length, 20 µm)	Human lymphocytes	Micronucleus formation	25–100 µg/mL	+		<a href="#">Kim &amp; Yu (2014)</a>
MWCNT (diameter, 6–24 nm; length, 2–5 µm; < 0.4% impurities)	Human lung adenocarcinoma A549 cells	Micronucleus formation	2.8–11.3 µg/mL	–		<a href="#">Thurnherr et al. (2011)</a>
MWCNT (diameter, 90 nm; length, 2 µm)	Human lung adenocarcinoma A549 cells	Micronucleus formation	20–200 µg/mL	+		<a href="#">Kato et al. (2013)</a>
Mixed CNT (more than 50% SWCNT; diameter, 1.1 nm; length, 0.5–100 µm)	Human bronchial epithelial BEAS-2B cells	Micronucleus formation	1–100 µg/cm <sup>2</sup>	–	Decreased CBPI at high concentrations	<a href="#">Lindberg et al. (2009)</a>

Table 4.8 (continued)

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Result <sup>c</sup>	Comments	Reference
SWCNT (diameter < 2 nm; length, 1–5 µm) and MWCNT (diameter, 10–30 nm; length, 1–2 µm)	Human bronchial epithelial BEAS-2B cells	Micronucleus formation	5–200 µg/cm <sup>2</sup>	–	Decreased CBPI at high concentrations with MWCNT	<a href="#">Lindberg et al. (2013)</a>
SWCNT: short (diameter, 1–2 nm; length, 0.4–0.8 µm; SSA, 585 m <sup>2</sup> /g), medium (diameter, 1–2 nm; length, 1–3 µm; SSA, 337 m <sup>2</sup> /g), or long (diameter, 1–2 nm; length, 5–30 µm; SSA, 310 m <sup>2</sup> /g); with few impurities	Human bronchial epithelial BEAS-2B or MCL-5 cells	Micronucleus formation	1–100 µg/mL	+	Increased micronucleus frequency in both BEAS-2B and MCL-5; unaltered CBPI	<a href="#">Manshian et al. (2013)</a>
MWCNT (diameter, 11 nm; length, 0.7 µm; 2% impurities)	Human breast epithelial MCF-7 cells	Micronucleus formation, CBMN and FISH using a pancentromeric probe	10–50 µg/mL	+	Dose-dependent increase in micronucleus frequency, induction of both centromere-positive and -negative micronuclei; no clear dose-dependent effect on CBPI	<a href="#">Muller et al. (2008b)</a>
MWCNT (diameter, 1.6 nm; length, 0.8 µm; SSA, 407 m <sup>2</sup> /g; 10% impurities)	Human gingival fibroblasts	Micronucleus formation, CBMN assay	50–150 µg/mL	+	Bell-shaped dose–response curve (maximal micronucleus frequency at 100 µg/mL); decreased CBPI at high concentrations	<a href="#">Cicchetti et al. (2011)</a>
SWCNT (diameter, 1–4 nm; length, 0.5–1.0 µm; SSA, 1040 m <sup>2</sup> /g; iron, 0.23%)	Primary human respiratory epithelial SAEC or human bronchial epithelial BEAS-2B cells	Chromosomal aberrations, aneuploidy (FISH), and multipolar mitotic spindles (dual-label immunofluorescence)	24–96 µg/cm <sup>2</sup>	+	Significant dose–response relationship for aneuploidy and multipolar mitotic spindles	<a href="#">Sargent et al. (2009)</a>
SWCNT (diameter, 1.0 nm; length, 0.5–1.0 µm; SSA, 1040 m <sup>2</sup> /g; iron, 0.23%)	Primary human respiratory epithelial SAEC or human bronchial epithelial BEAS-2B cells	Chromosomal aberrations, aneuploidy (FISH), and multipolar mitotic spindles (dual-label immunofluorescence)	0.024–24 µg/cm <sup>2</sup>	+	Significant dose–response relationship for aneuploidy including an equal number of chromosome losses and gains; a dose–response relationship for multipolar mitotic spindles	<a href="#">Sargent et al. (2012a)</a>

**Table 4.8 Studies of micronucleus frequency, chromosomal aberrations, and sister-chromatid exchange in experimental systems after exposure to carbon nanotubes in vitro (continued)**

Material <sup>a</sup>	Cells	End-point; test system	Concentration (LEC or HIC) <sup>b</sup>	Result	Comments	Reference
MWCNT (diameter, 10–20 nm; length, 1 µm; iron, 0.03%; no cobalt or nickel content)	Human bronchial epithelial BEAS-2B cells	Chromosomal aberrations, disrupted mitotic spindle	0.024–24 µg/mL	+	Dose-response relationship for aneuploidy and polyploidy; errors in chromosome number included more than losses; dose-response relationship for disrupted mitotic spindle predominantly with a single pole	<a href="#">Sargent et al. (2012b)</a> , <a href="#">Siegrist et al. (2014)</a>
SWCNT (diameter < 2 nm; length, 1.5 µm; SSA, 436 m <sup>2</sup> /g) and MWCNT (diameter, 10–30 nm; length, 1–2 µm; SSA, 60 m <sup>2</sup> /g)	Human lymphocytes	Chromosomal aberrations	6.25–300 µg/mL	+	Increased chromatid and chromosome-type breakage with both CNT	<a href="#">Catalán et al. 2012</a>
MWCNT (diameter, 10–30 nm; length, 1–2 µm)	Human lymphocytes	Sister-chromatid exchange, 5-BrdU incorporation	1000 µg/mL	-		<a href="#">Szendi &amp; Varga (2008)</a>
MWCNT (diameter, 10–25 nm; length, 0.5–50 µm; SSA, 400 m <sup>2</sup> /g; nickel, 1.5%) and SWCNT (diameter, 1.2–1.5 nm; length, 2–5 µm; nickel, 1.5%)	Mouse macrophage RAW 264.7 cells	Micronucleus formation	1–10 µg/mL	+	Dose-dependent increase; same effect with both CNT	<a href="#">Di Giorgio et al. (2011)</a>
MWCNT (diameter, 11 nm; length, 0.7 µm; 2% impurities)	Rat lung epithelial cells	Micronucleus formation, CBMN assay	10–50 µg/mL	+	Dose-dependent increase	<a href="#">Muller et al. (2008b)</a>
MWCNT (diameter, 20–50 nm; length, 0.7 µm; iron, 0.48%; cobalt, 0.49%) ground to produce structural defects and/or heated (2400 °C) to eliminate metals and defects	Rat lung epithelial cells	Micronucleus formation, CBMN assay	25 µg/mL	+ with CNTg - with CNTg before heating + with heating before CNTg	Ground CNT (CNTg) increased micronucleus frequency, whereas ground and subsequently heated (2400 °C) sample did not; MWCNT that were heated (2400 °C) and subsequently ground increased the micronucleus frequency	<a href="#">Muller et al. (2008a)</a>

Table 4.8 (continued)

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Result <sup>c</sup>	Comments	Reference
SWCNT (diameter, 0.4–1.2 nm; length, 1–3 µm; SSA, 1040 m <sup>2</sup> /g; iron, 0.23%)	Hamster lung V79 fibroblasts	Micronucleus formation	12–96 µg/cm <sup>2</sup>	–	No increase in micronucleus formation (cytotoxicity up to ~70%)	<a href="#">Kisin et al. (2007)</a>
SWCNT (diameter, 0.4–1.2 nm; length, 1–3 µm; SSA, 1040 m <sup>2</sup> /g; iron, 0.23%)	Hamster lung V79 fibroblasts	Micronucleus formation	12–48 µg/cm <sup>2</sup>	+		<a href="#">Kisin et al. (2011)</a>
SWCNT (diameter, 1.8 nm; length, 0.5 µm)	Hamster lung V79 fibroblasts	Micronucleus formation	10 pg/mL–0.2 µg/mL	–	Unaltered micronucleus frequency, assessed by similar number of kinetochore-negative cells in SWCNT-treated and control groups	<a href="#">Pelka et al. (2013)</a>
MWCNT (diameter, 88 nm; length, 5 µm)	Chinese hamster lung CHL/IU cells	Micronucleus formation	0.02–5 µg/mL	+	Significant dose-dependent increase in the number of micronuclei, especially related to bi- and multinucleated cells	<a href="#">Asakura et al. (2010)</a>
MWCNT (diameter, 10–25 nm; length, 0.5–50 µm; SSA, 400 m <sup>2</sup> /g; nickel, 1.5%) and SWCNT (diameter, 1.2–1.5 nm; length, 2–5 µm; nickel, 1.5%)	Mouse macrophage RAW 264.7 cells	Chromosomal aberrations	1–10 µg/mL	+	Increased percentage of cells with chromosomal aberrations: acentric fragments, centromeric fusion, breaks, chromatid separation, and polyploidy with both CNT	<a href="#">Di Giorgio et al. (2011)</a>
MWCNT (diameter, 88 nm; length, 5 µm)	Chinese hamster lung CHL/IU cells	Chromosomal aberrations	0.8–5 µg/mL	+	Dose-dependent increase in polyploidy (numerical chromosomal aberrations); no structural chromosomal changes	<a href="#">Asakura et al. (2010)</a>
SWCNT (diameter, 1.8 nm; SSA, 878 m <sup>2</sup> /g; iron, 4.4%)	Chinese hamster lung fibroblast CHL/IU cells	Chromosomal aberrations with and without S9	6.25–100 µg/mL	–	No increase in the number of structural or numerical chromosomal aberrations (polyploidy) with or without S9	<a href="#">Ema et al. (2013b)</a>

**Table 4.8 Studies of micronucleus frequency, chromosomal aberrations, and sister-chromatid exchange in experimental systems after exposure to carbon nanotubes in vitro (continued)**

Material <sup>a</sup>	Cells	End-point, test system	Concentration (LEC or HIC) <sup>b</sup>	Result	Comments	Reference
MWCNT (high- or low-aspect-ratio: diameter, 10–15 or ~10 nm; length, 0.15 or 10 µm; SSA, 178 or 195 m <sup>2</sup> /g; iron, 1% or 5%; respectively)	Chinese hamster ovary CHO-k1 cells	Chromosomal aberrations with and without S9	1.6–12.5 µg/mL	–	No increase in chromatid-type breakage or exchange with or without S9	<a href="#">Kim et al. (2011)</a>
SWCNT (diameter, 3 nm; length, 1.2 µm; SSA, 1064 m <sup>2</sup> /g)	Chinese hamster lung fibroblast CHL/IU cells	Chromosomal aberrations with and without S9	300–1000 µg/mL	–	No increase in chromosomal gaps or polyploidy with or without S9	<a href="#">Naya et al. (2011)</a>
MWCNT (diameter, 90 nm; length, 2 µm)	Chinese hamster ovary CHO AA8 cells	Sister-chromatid exchange	0.1–2 µg/mL	+		<a href="#">Kato et al. (2013)</a>

<sup>a</sup> Nanomaterial characteristics include diameter, length, specific surface area (SSA), and content of transition metals

<sup>b</sup> LEC, lowest effective concentration; HIC, highest ineffective concentration

<sup>c</sup> +, positive; –, negative; (+), weakly positive

5-BrdU, 5-bromo-2'-deoxyuridine; CBMN; cytokinesis-block micronucleus assay; CBPI, cytokinesis-block proliferation index; CNT, carbon nanotubes; FISH, fluorescence in situ hybridization; MWCNT, multiwalled carbon nanotubes; S9, metabolic activation system; SWCNT, single-walled carbon nanotubes

macrophage RAW 264.7 cell lines, Chinese hamster lung (CHL/IU) cell lines, primary human respiratory epithelial SAEC cell lines, and human bronchial epithelial BEAS-2B cells ([Sargent et al., 2009, 2012a](#); [Asakura et al., 2010](#); [Di Giorgio et al., 2011](#); [Siegrist et al., 2014](#)). Other investigations in immortalized Chinese hamster lung fibroblasts and Chinese hamster ovary cells did not show increased aneuploidy or chromosomal aberrations after exposure to SWCNT ([Naya et al., 2011](#); [Ema et al., 2013b](#)) or MWCNT ([Kim et al., 2011](#)). Both SWCNT and MWCNT increased chromosome and chromatid breakage in phytohaemagglutinin-stimulated human lymphocytes ([Catalán et al., 2012](#)).

Chromosome breakage and translocations between chromosomes were observed in an immortalized mouse macrophage RAW 264.7 cell line after exposure to 10 µg/mL of SWCNT or MWCNT. The modal number of the macrophage cell line karyotype was 40 chromosomes; however, the mean number of chromosomes per cell after exposure to either SWCNT or MWCNT was 20–60 with no distinct modal number, indicating a high degree of aneuploidy in the original cell line ([Di Giorgio et al., 2011](#)). [Asakura et al. \(2010\)](#) demonstrated an 8–34-fold increase in polyploidy in Chinese hamster lung cells treated with MWCNT (diameter, 88 nm; length, 5 µm). The authors of both studies attributed the increase in polyploid cells to a failure of cytokinesis ([Asakura et al., 2010](#); [Di Giorgio et al., 2011](#)).

Chromosome breakage and errors in chromosome number were observed in cultured primary human respiratory epithelial cells after exposure to either SWCNT or MWCNT determined by analysis of chromosomes spreads or fluorescence in situ hybridization. The analysis of cultured primary human respiratory cells exposed to SWCNT demonstrated significantly increased aneuploidy, which was due to an equal number of gains and losses of chromosomes, while MWCNT-exposed cells had a significantly greater number of chromosomal gains than

losses, indicating polyploidy ([Sargent et al., 2009, 2012a, b](#); [Siegrist et al., 2014](#)).

[Collectively, in-vitro investigations in immortalized and primary cells documented the ability of CNT to increase the frequency of chromosomal damage and aneuploidy in proliferating cells. Similar to the results from studies of micronucleus frequency after exposure to CNT, substantial effect differences between studies were found, possibly originating from differences in cell types, characteristics of CNT, dispersion protocols, and assay conditions.]

(v) *Alterations in the mitotic spindle, cell cycle, and sister-chromatid exchange*

The data that demonstrated chromosomal damage and errors in chromosomes after in-vitro exposure to either SWCNT or MWCNT (see [Table 4.8](#)) suggested an alteration in the integrity of the mitotic spindle, which was investigated by exposure to SWCNT (diameter, 1.0 nm) or MWCNT (diameter, 10–20 nm). The exposure to 1-nm SWCNT resulted in mitotic spindles with multiple poles ([Sargent et al., 2009, 2012a](#)), while cells treated with 10–20-nm MWCNT had mitotic spindles with one pole ([Sargent et al., 2012b](#); [Siegrist et al., 2014](#)). Three-dimensional reconstructions of 0.1-µm optical sections showed CNT integrated with microtubules, DNA and within the centrosome structure. Further analysis by confocal microscopy and TEM demonstrated fragmented centrosomes after exposure to either SWCNT or MWCNT ([Sargent et al., 2009, 2012a, b](#); [Siegrist et al., 2014](#)). The mitotic disruption associated with SWCNT treatment resulted in a G2/M block in the cell cycle while MWCNT treatment was associated with a block in G1/S ([Sargent et al., 2009, 2012b](#); [Siegrist et al., 2014](#)). [When mammalian cells are exposed to agents that cause a block in the S-phase, the DNA is repaired by homologous recombination. The increased recombination between sister chromatids can be observed by the incorporation of 5-bromodeoxyuridine. The

increase in sister chromatid exchange suggests genotoxicity ([Pfuhler et al., 2013](#).)] The observation of increased sister-chromatid exchange in Chinese hamster ovary AA8 cells after exposure to MWCNT 90 nm in diameter further suggests a block in the S-phase ([Kato et al., 2013](#)).

[To date, four studies have shown CNT-induced mitotic spindle and cell-cycle disruption and three investigations demonstrated CNT-mediated centrosome disruption. These investigations documented the ability of CNT to disrupt the mitotic spindle, fragment the centrosome, and cause a block in the cell cycle in cultured cells.]

#### (vi) Mutation in bacteria

See [Table 4.9](#)

The mutagenic effect of MWCNT was evaluated in the bacterial reverse mutation assay (Ames test) in *Salmonella typhimurium* TA98 and TA100 and in *Escherichia coli* WP2uvrA in the presence and in absence of a metabolic activation system. MWCNT did not produce mutagenic effects at any concentration tested. In *S. typhimurium* TA98 in absence of metabolic activation, a reduction in the number of spontaneous revertant colonies was observed at concentrations ranging from 0.13 to 9.0 µg/plate, which was not concentration-dependent. In this bacterial strain, spontaneous mutational DNA damage is reverted to wild-type by specific mechanisms of frameshift ([Di Sotto et al., 2009](#)).

[Kim et al. \(2011\)](#) studied high-aspect-ratio (diameter, 10–15 nm; length, ≈10 nm) and low-aspect-ratio (diameter, 10–15 nm; length, ≈150 nm) MWCNT. Neither the high- nor the low-aspect-ratio MWCNT induced genotoxicity in the bacterial reverse mutation test in *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537, and in *Escherichia coli* WP2uvrA in the presence and in absence of a metabolic activation system.

## 4.4 Other mechanisms of carcinogenesis

No published studies concerning the health effects of CNT in exposed humans were available to the Working Group. The body of relevant literature primarily comprises in-vivo studies in experimental animals, in-vitro studies using human cell lines, and a limited number of studies of occupational exposure (see Section 1, [Table 1.5](#)).

There were no studies in humans exposed to CNT only. However, four studies have been published in which several biological end-points of an occupational cohort (in Taiwan, China) exposed to engineered nanomaterials ( $n = 241$ ) were compared with those of an unexposed control group ( $n = 196$ ). Among the population exposed to engineered nanomaterials, a subgroup of workers ( $n = 57$ ) was exposed to CNT originating from three facilities that used CNT and one facility that used and produced CNT. [The Working Group noted that the number of subjects in each individual study varied, probably due to missing data on specific end-points or follow-up. With the exception of the study describing the results on fractional exhaled nitric oxide, the studies did not report results separately for the CNT-exposed population.] [Wu et al. \(2014\)](#) described an increase in fractional exhaled nitric oxide in workers exposed to nanomaterials, that was limited to the population exposed to titanium dioxide ( $\beta = 0.351$ , SE = 0.166;  $P = 0.035$ ). Results among CNT-exposed workers ( $n = 57$ ) were null ( $\beta = 0.045$ , SE = 0.124;  $P = 0.715$ ). [Liou et al. \(2012\)](#) studied approximately the same population and measured antioxidant enzyme activities, markers of inflammation and oxidative damage, cardiovascular biomarkers, genotoxicity, lung function, and neurobehavioural functions. In a cross-sectional evaluation, associations were found with significantly lower antioxidant enzyme activity (i.e. superoxide dismutase [SOD]), elevated markers of

**Table 4.9 Studies of mutation in bacteria exposed to carbon nanotubes**

Material <sup>a</sup>	Cells	End-point	Concentration (LEC or HIC) <sup>b</sup>	Results <sup>c</sup>		Reference
				Without metabolic activation system	With metabolic activation system	
MWCNT (diameter, 110–170 nm; length, 5–9 µm)	<i>Salmonella typhimurium</i> TA98 and TA100 and <i>Escherichia coli</i> WP2 uvrA	Reverse mutation	9.0 µg/plate	–	–	<a href="#">Di Sotto et al. (2009)</a>
MWCNT (high-aspect-ratio: diameter, 10–15 nm; length, ≈10 µm; low-aspect-ratio: diameter, 10–15 nm; length, ≈150 nm)	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, and TA1537	Reverse mutation	1000 µg/plate	–	–	<a href="#">Kim et al. (2011)</a>
MWCNT (diameter, 110–170 nm; length, 5–9 µm)	<i>Escherichia coli</i> WP2 uvrA	Reverse mutation	9.0 µg/plate	–	–	<a href="#">Di Sotto et al. (2009)</a>
MWCNT (high-aspect-ratio: diameter, 10–15 nm; length, ≈10 µm; low-aspect-ratio: diameter, 10–15 nm; length, ≈150 nm)	<i>Escherichia coli</i> WP2 uvrA	Reverse mutation	1000 µg/plate	–	–	<a href="#">Kim et al. (2011)</a>

<sup>a</sup> Nanomaterial characteristics include diameter and length

<sup>b</sup> LEC, lowest effective concentration; HIC, highest ineffective concentration

<sup>c</sup> +, positive; –, negative

MWCNT, multiwalled carbon nanotubes

cardiovascular disease (i.e. fibrinogen and intercellular adhesion molecule-1), and reduced neurobehavioural function. No specific analyses for the CNT-exposed population ( $n = 52$ ) were presented. In a longitudinal analysis of a subpopulation of the same population with a follow-up of 6 months, [Liao et al. \(2014a\)](#) reported a significant association between exposure to engineered nanomaterials and an increase in antioxidant enzymes (SOD and GSH peroxidase [GPx]) and cardiovascular markers (vascular cell adhesion molecule and paraoxonase) among exposed workers compared with control workers over the follow-up period. The results were not presented by subtype of engineered nanomaterials. [Liao et al. \(2014b\)](#) studied the same exposed population for work-related symptoms and diseases and reported a significant worsening of allergic dermatitis among workers exposed to engineered

nanomaterial. No specific results for workers exposed to CNT were presented.

[The Working Group noted that the exposure assessments of [Liao et al. \(2014a, b\)](#), [Liou et al. \(2012\)](#), and [Wu et al. \(2014\)](#) were based on the control-banding approach of [Paik et al. \(2008\)](#). The exposure scores were based on both an estimate of nano-toxicity and the expected probability of exposure. The selection of controls for the above studies was not clearly described, although confounding factors did not seem to differ between the exposed and unexposed workers except for sex and level of education.]

#### 4.4.1 Inflammasome activation

##### (a) Human cells *in vitro*

An interlaboratory validation study confirmed the extracellular release of IL-1 $\beta$  from the human THP-1 macrophage cell line exposed to as-produced MWCNT (Cheap Tubes Inc., Brattleboro, VT, USA) at non-cytotoxic doses ([Xia et al., 2013](#)). A comprehensive analysis of surface functionalization of the as-produced MWCNT ([Li et al., 2013](#)), and the dispersion of MWCNT in bovine serum albumin or by the triblock copolymer Pluronic F108 ([Wang et al., 2012a](#)) showed that surface charge, chemical functionalization, and dispersal state were important determinants of inflammasome activation and release of IL-1 $\beta$  from THP-1 macrophages. Anionic functionalization (carboxylate or PEG) decreased, cationic functionalization (polyetherimide) increased ([Li et al., 2013](#)), and dispersion (using Pluronic F108) prevented ([Wang et al., 2012a](#)) the release of IL-1 $\beta$  from THP-1 macrophages.

Platelet-derived growth factor (PDGF), in combination with transforming growth factor (TGF)- $\beta$ , activates the “epithelial-mesenchymal trophic unit” in the lungs resulting in collagen deposition and fibrosis (reviewed in [Bonner et al., 2013](#)). These reciprocal interactions of cytokines and growth factors, initiated by inflammasome activation and the release of IL-1 $\beta$  from macrophages, were repeated in transwell co-cultures of human THP-1 macrophages and immortalized human BEAS-2B lung epithelial cells exposed to as-produced or cationic functionalized MWCNT ([Wang et al., 2012a](#); [Li et al., 2013](#)).

[Hamilton et al. \(2013b\)](#) used the same as-produced MWCNT with variable diameters and lengths to assess the role of dimensions in the release of IL-1 $\beta$  from human THP-1 macrophages or murine primary alveolar macrophages. MWCNT with a greater diameter (30–50 nm) or length (10–30  $\mu$ m) were more potent in inducing

the release of IL-1 $\beta$  than shorter (length < 2  $\mu$ m) or thinner (diameter, 10–20 nm) MWCNT. These investigators also studied a panel of nine MWCNT (including the as-produced sample from Cheap Tubes) and compared their potency for inflammasome activation and IL-1 $\beta$  and IL-18 release in primary murine alveolar macrophages co-stimulated with 20 ng/mL of lipopolysaccharide (LPS). Linear regression analysis demonstrated a significant correlation between the nickel content of the MWCNT samples and the release of IL-1 $\beta$  ([Hamilton et al., 2012](#)). Removal of nickel contaminants from the as-produced MWCNT sample slightly decreased the release of IL-1 $\beta$  in this *in-vitro* assay ([Hamilton et al., 2013a](#)). [Haniu et al. \(2011\)](#) confirmed that another commercial MWCNT sample (VGCF; Showa Denko, Tokyo, Japan) also induced IL-1 $\beta$  as well as the release of tumour necrosis factor (TNF)- $\alpha$  from human THP-1 macrophages. Inflammasome activation and IL-1 $\beta$  and IL-18 release was also induced by CNT (80% DWCNT; DWCNT, 0.1–100  $\mu$ m in length) synthesized by CVD in human peripheral blood monocytes primed with LPS ([Meunier et al., 2012](#)).

##### (b) Experimental systems *in vivo*

See [Table 4.10](#)

##### (i) Inhalation

Three 13-week studies of two MWCNT and one CNF in Wistar rats showed that exposure to MWCNT induced persistent inflammation. In the studies of [Ma-Hock et al. \(2009\)](#) and [Pauluhn \(2010b\)](#), these inflammatory responses to MWCNT were observed in the lungs of males and females. The response to CNF was observed at high concentration ([Delorme et al., 2012](#)). The minimum concentrations that induced persistent or moderate inflammation were 2.5 mg/m<sup>3</sup> and 1.5 mg/m<sup>3</sup> of MWCNT and 25 mg/m<sup>3</sup> of CNF ([Ma-Hock et al., 2009](#); [Pauluhn, 2010b](#)).

Histopathological analysis of the 13-week inhalation study of MWCNT of [Ma-Hock et al.](#)

**Table 4.10 Studies of persistent inflammation, granulomatosis, and fibrosis in experimental animals after exposure to carbon nanotubes**

Route of administration	Species, strain (sex)	Type of CNT	Exposure concentration or dose, duration	Recovery	Characterization of CNT	Inflammation	Fibrosis or granulomatosis	Reference
<i>Inhalation</i>								
Head/nose-only	Rat, Wistar (M, F)	MWCNT	0.1, 0.5, or 2.5 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 13 wk	None	Metal oxide, 10%; diameter, 5–15 nm; length, 0.1–10 µm; BET, 250–300 m <sup>2</sup> /g; MMAD, 0.5–1.3 µm	0.1 mg/m <sup>3</sup> (minimal), 0.5 mg/m <sup>3</sup> (slight), 2.5 mg/m <sup>3</sup> (moderate)	Granulomatous inflammation: 0.1 mg/m <sup>3</sup> (minimal), 0.5 mg/m <sup>3</sup> (slight), 2.5 mg/m <sup>3</sup> (moderate)	<a href="#">Ma-Hock et al. (2009)</a>
Nose-only	Rat, Wistar (M, F)	MWCNT	0.1, 0.4, 1.5, or 6 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 13 wk	6 mo	Cobalt, 0.46–0.53%; BET, 253 m <sup>2</sup> /g; length, 200–300 nm	0.1 mg/m <sup>3</sup> (none), 0.4 mg/m <sup>3</sup> (transient), 1.5 mg/m <sup>3</sup> (persistent), 6 mg/m <sup>3</sup> (persistent)	0.1 mg/m <sup>3</sup> (none), 0.4 mg/m <sup>3</sup> (persistent), 1.5 mg/m <sup>3</sup> (persistent), 6 mg/m <sup>3</sup> (persistent)	<a href="#">Pauluhn (2010b)</a>
Nose-only	Rat, Sprague-Dawley (M, F)	VGCF-H CNF	0.54 mg/m <sup>3</sup> (4.9 f/mL), 2.5 mg/m <sup>3</sup> (56 f/mL), or 25 mg/m <sup>3</sup> (252 f/mL), 6 h/day, 5 days/wk, 13 wk	90 days	Carbon > 99.5%; diameter, 158 nm; length, 5.8 µm; BET, 13.8 m <sup>2</sup> /g	25 mg/m <sup>3</sup> (persistent)		<a href="#">Delorme et al. (2012)</a>
Whole-body	Rat, Wistar (M)	SWCNT	0.03 mg/m <sup>3</sup> (5 × 10 <sup>4</sup> particles/cm <sup>3</sup> ) or 0.13 mg/m <sup>3</sup> (6.6 × 10 <sup>4</sup> particles/cm <sup>3</sup> ), 6 h/day, 5 days/wk, 4 wk	3 mo	Diameter, 3 nm; BET, 1064 m <sup>2</sup> /g; 0.03% impurities	No persistent inflammation	No fibrosis	<a href="#">Morimoto et al. (2012a)</a>
Whole-body	Rat, Wistar (M)	MWCNT	0.37 mg/m <sup>3</sup> (> 70% individual fibres), 6 h/day, 5 days/wk, 4 wk	1 or 3 mo	Diameter, 44 nm; length, 1.1 µm; BET, 69 m <sup>2</sup> /g; iron, 0.0005%	No persistent inflammation	No fibrosis	<a href="#">Morimoto et al. (2012b)</a>
Nose-only	Rat, Sprague-Dawley (M, F)	MWCNT	0.17, 0.49, or 0.96 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 4 wk	28 or 90 days	Cobalt < wt 2%; diameter, 10–15 nm; length, 330.18 µm; BET, 224.9 m <sup>2</sup> /g	No change in the inflammatory cytokines levels in BALF	CNT were located in the lung and pleura after 28-day exposure and up to 90 days after exposure	<a href="#">Kim et al. (2014)</a>

**Table 4.10 Studies of persistent inflammation, granulomatosis, and fibrosis in experimental animals after exposure to carbon nanotubes (continued)**

Route of administration	Species, strain (sex)	Type of CNT	Exposure concentration or dose, duration	Recovery	Characterization of CNT	Inflammation	Fibrosis or granulomatosis	Reference
Nose-only	Rat, Wistar (M)	MWCNT	11 or 241 mg/m <sup>3</sup> , 6 h	90 days	Cobalt, 0.53%; BET, 253 m <sup>2</sup> /g; length, 200–300 nm	241 mg/m <sup>3</sup> (persistent)	241 mg/m <sup>3</sup> (persistent or slight)	<a href="#">Ellinger-Ziegelbauer &amp; Pauluhn (2009)</a>
Whole-body	Rat, F344 (M, F)	MWCNT	0.2, 1, or 5 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 13 wk	None	Fibre-like BET, 24–28 m <sup>2</sup> /g; length, 5.7 µm	From 0.2 mg/m <sup>3</sup>	Granulomatous changes: M, from 0.2 mg/m <sup>3</sup> ; F, from 1 mg/m <sup>3</sup>	<a href="#">Kasai et al. (2015)</a>
Nose-only	Mouse, C57BL/6 (M)	MWCNT	1 or 30 mg/m <sup>3</sup> , 6 h	1 day, 2, 6, or 14 wk	Diameter, 10–30 nm; length, 0.5–40 µm; BET, 40–300 m <sup>2</sup> /g		Subpleural fibrosis	<a href="#">Ryman-Rasmussen et al. (2009a)</a>
<i>Intratracheal administration</i>								
Instillation	Rat, F344 (M)	MWCNT	40 or 160 µg	Up to 91 days	Diameter, 88 nm; length, 5 µm; iron, 0.44%	Persistent	Persistent	<a href="#">Aiso et al. (2010)</a>
Instillation	Rat, Sprague-Dawley (M)	MWCNT	0.04, 0.2, or 1 mg/kg bw	Up to 6 mo	BET, 23.0 m <sup>2</sup> /g; length < 20 µm	Transient	Minimal (1 mg/kg)	<a href="#">Kobayashi et al. (2010)</a>
Instillation	Rat, Wistar (M)	SWCNT	0.04, 0.2, 1, or 2 mg/kg bw	6 mo (3 mo for 2 mg/kg)	BET, 1064 m <sup>2</sup> /g; 0.03% impurities	0.04 mg/kg (transient), 1 mg/kg (persistent), 2 mg/kg (persistent)	Minimal (1 mg/kg)	<a href="#">Kobayashi et al. (2011)</a>
Instillation	Rat, Wistar (M)	MWCNT	0.66 or 3.3 mg/kg bw	Up to 6 mo	Diameter, 44 nm; length, 1.1 µm; BET, 69 m <sup>2</sup> /g; iron, 0.0005%	0.66 mg/kg (transient), 3.3 mg/kg (persistent)	Transient	<a href="#">Morimoto et al. (2012b)</a>
Instillation	Rat, Wistar (M)	SWCNT	0.66 or 1.32 mg/kg bw	Up to 6 mo	Diameter, 1.8 nm; BET, 878 m <sup>2</sup> /g	Persistent	Minimal	<a href="#">Morimoto et al. (2012c)</a>
Instillation	Rat, CD (M)	SWCNT	1 or 5 mg/kg bw	Up to 3 mo	Nominal diameter, 1.4 nm; length, > 1 µm; agglomerated ropes, ~30 nm	Transient	Multifocal granulomas	<a href="#">Warheit et al. (2004)</a>

Table 4.10 (continued)

Route of administration	Species, strain (sex)	Type of CNT	Exposure concentration or dose, duration	Recovery	Characterization of CNT	Inflammation	Fibrosis or granulomatosis	Reference
Instillation	Rat, Wistar (M)	SWCNT	0.2 or 0.4 mg	Up to 754 days	BET, 877.7 m <sup>2</sup> /g; diameter, 44 nm; length, 0.69 µm		Granuloma (disappeared at 365 days post-instillation)	<a href="#">Fujita et al. (2015)</a>
Instillation	Rat, Sprague-Dawley (M)	MWCNT	4 mg/kg bw	21 days	BET, 109 m <sup>2</sup> /g; length, 0.3–50 µm; diameter, 10–30 nm		Slight fibrosis	<a href="#">Cesta et al. (2010)</a>
Instillation	Rat, Sprague-Dawley (F)	MWCNT	0.5, 2, or 5 mg	60 days	Length, 5.9 µm; ground: length, 0.7 µm	Inflammation (until 15 days)	Granuloma, fibrosis	<a href="#">Muller et al. (2005)</a>
<i>Pharyngeal aspiration</i>								
	Mouse, C57Bl (F)	MWCNT	20 or 40 µg	7 days	Diameter, 31 nm; length, 20 µm; BET, 50 m <sup>2</sup> /g; 3.5 wt% impurities	Transient		<a href="#">Han et al. (2010)</a>
	Mouse, C57Bl/6J (M)	MWCNT	10, 20, 40, or 80 µg	Up to 56 days	GML, 3.86 µm; CMD, 49 nm; 0.78% impurities	Persistent	80 µg (progressive)	<a href="#">Porter et al. (2010)</a> , <a href="#">Mercer et al. (2011)</a>
	Mouse, C57Bl/6 (M)	DWCNT	1, 10, or 40 µg	Up to 56 days	Diameter, 1–2 nm; length < 5 µm	10 and 40 µg (persistent alveolitis)	10 and 40 µg (interstitial fibrosis)	<a href="#">Sager et al. (2013)</a>
	Mouse, C57Bl/6 (F)	SWCNT	10, 20, or 40 µg	Up to 60 days	Diameter, 1–4 nm; BET, 1024 m <sup>2</sup> /g; EC, 99.7%; iron, 0.23%	Persistent	Fibrosis	<a href="#">Shvedova et al. (2005)</a>

**Table 4.10 Studies of persistent inflammation, granulomatosis, and fibrosis in experimental animals after exposure to carbon nanotubes (continued)**

Route of administration	Species, strain (sex)	Type of CNT	Exposure concentration or dose, duration	Recovery	Characterization of CNT	Inflammation	Fibrosis or granulomatosis	Reference
	Mouse, C57BL/6 (F)	SWCNT	40 µg	Up to 28 days	Diameter, 1–4 nm; length, 1–3 µm; BET, 1040 m <sup>2</sup> /g; iron, 0.23%	Persistent inflammation	Granuloma	<a href="#">Murray et al. (2012)</a>
	Mouse, C57BL/6 J (M)	MWCNT	10, 20, 40, or 80 µg	Up to 56 days	GML, 3.86 µm; CMD, 49 nm; BET, 26 m <sup>2</sup> /g	Transient	Progressive fibrosis (granuloma)	<a href="#">Mercer et al. (2011)</a>
<i>Intraperitoneal administration</i>								
	Rat, Wistar (M)	MWCNT	2 or 20 mg	24 mo	With and without defects; diameter, 11.3 nm; length, ~0.7 µm	Transient		<a href="#">Muller et al. (2009)</a>
	Mouse, C57BL/6 (F)	Long MWCNT	50 µg	7 days	Mean length, 13 µm; maximal length, 56 µm		Granulomatous inflammation	<a href="#">Poland et al. (2008)</a>
	Mouse, C57BL/6 (F)	Tangled MWCNT	50 µg	7 days	Length, 1–5 µm; length, 5–20 µm		Minimum	<a href="#">Poland et al. (2008)</a>

BALF, bronchoalveolar lavage fluid; BET, Brunauer-Emmett-Teller surface area analysis; bw, body weight; CMD, count mean diameter; CNT, carbon nanotubes; DWCNT, double-walled carbon nanotubes; EC, elemental carbon; F, female; f/mL, fibres per millilitre; GML, geometric median length; M, male; MMAD, mass median aerodynamic diameter; mo, month; MWCNT, multiwalled carbon nanotubes; SWCNT, single-walled carbon nanotubes; VGCF-H CNF, vapour grown carbon nanofibres; wk, week

(2009) demonstrated focal granuloma formation and the accumulation of subpleural cells in a dose-dependent manner. Masson's stain established the presence of collagen within the sites of granuloma formation, while a reticulin fibre index further confirmed a dose-dependent increase in collagen within the alveolar walls (Treumann et al., 2013).

Two 4-week studies of SWCNT and MWCNT in Wistar rats showed evidence of transient (not persistent) inflammation. The maximum concentration that did not induce significant inflammation was 0.13 mg/m<sup>3</sup> of SWCNT and 0.37 mg/m<sup>3</sup> of MWCNT (Morimoto et al., 2012a, b).

A 4-week study of MWCNT (length, 330.18 nm; diameter, 10–15 nm) in Sprague-Dawley rats with a 90-day recovery period showed no statistically significant difference in the levels of inflammatory cytokines, bronchoalveolar cell distribution, or markers in the BALF or in histopathology (Kim et al., 2014).

Exposure to MWCNT for 6 hours provided evidence of persistent inflammation at high concentrations in male rats (Ellinger-Ziegelbauer & Pauluhn, 2009).

Kasai et al. (2015) reported a dose-independent increase in inflammatory parameters after exposure of male rats to a lower dose of MWCNT (0.2 mg/m<sup>3</sup>). In male mice, subpleural fibrosis increased 2 and 6 weeks after inhalation of MWCNT (Ryman-Rasmussen et al., 2009a).

#### (ii) Intratracheal instillation

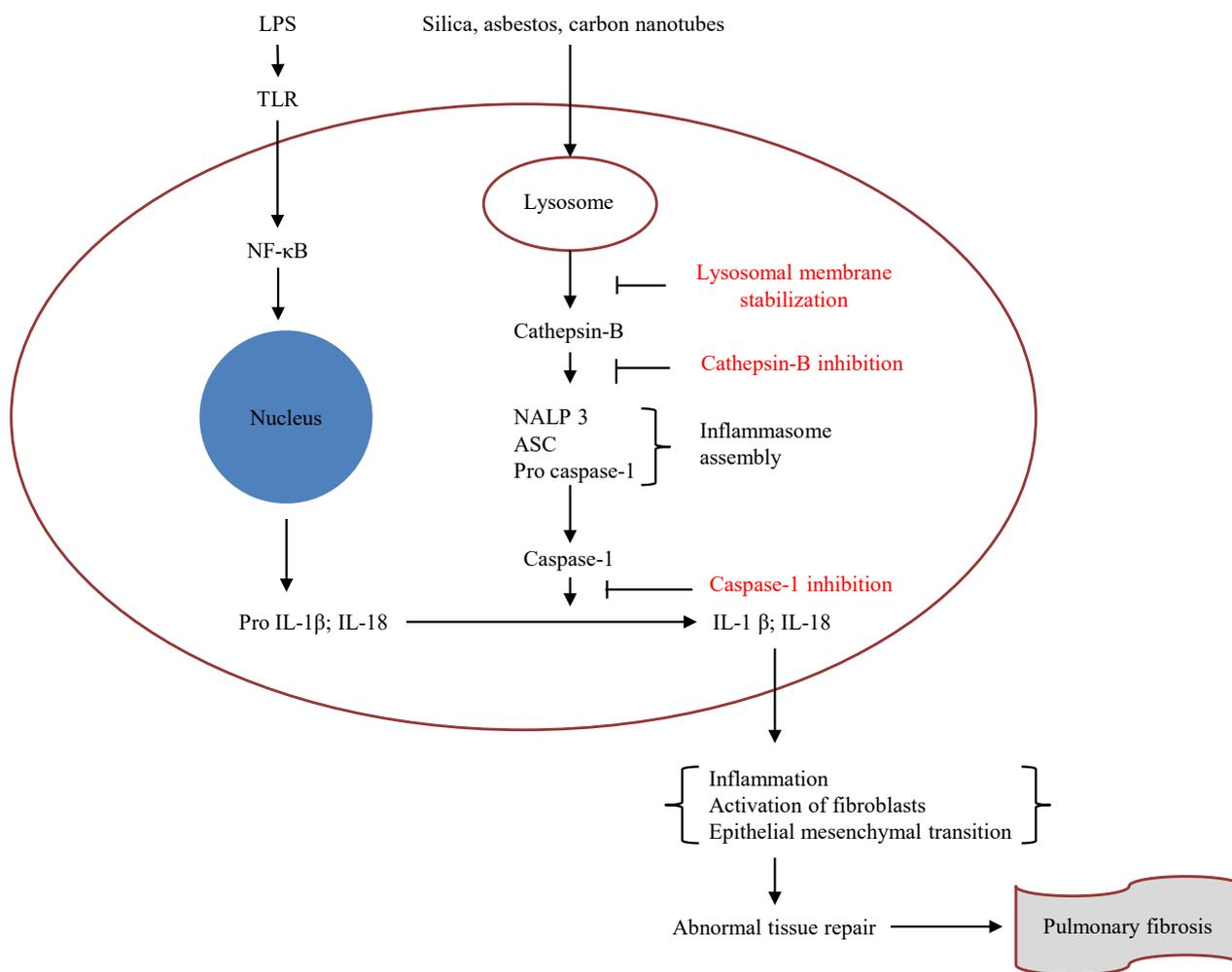
Fifteen studies of intratracheal instillation in rats and pharyngeal aspiration in mice have been reported (Lam et al., 2004; Warheit et al., 2004; Muller et al., 2005, 2008b; Shvedova et al., 2005; Aiso et al., 2010; Cesta et al., 2010; Han et al., 2010; Kobayashi et al., 2010, 2011; Mercer et al., 2011; Porter et al., 2010; Morimoto et al., 2012b, c; Murray et al., 2012; Sager et al., 2013; Fujita et al., 2015), most of which revealed that exposure to SWCNT and MWCNT led to persistent inflammation in the lung. In contrast, several studies in

rats and mice revealed that exposure to SWCNT and MWCNT led to transient responses in the lung. [From the above studies, the Working Group considered that the pulmonary responses of rats and mice to SWCNT and MWCNT did not differ significantly.]

#### (c) Experimental systems in vitro

CNT, as well as asbestos fibres and poorly soluble crystalline particles (IARC, 2012), have been shown to induce inflammation, as assessed by the release of pro-inflammatory mediators (reviewed in Boyles et al., 2014). Two hypotheses have been proposed for the pro-inflammatory effects of high-aspect-ratio nanoparticles, including CNT and asbestos fibres: (1) frustrated phagocytosis; and (2) inflammasome activation (see Fig. 4.1). Frustrated phagocytosis is elicited in response to high-aspect-ratio, fibrous nanoparticles longer than ~15 µm that cannot be completely phagocytized by macrophages, resulting in their impaired clearance from the lungs and pleural linings and persistent inflammation accompanied by the prolonged release of ROS, pro-inflammatory mediators, and proteases (Johnston et al., 2010). Inflammasome activation triggered by lysosomal damage after the phagocytosis of crystalline minerals (e.g. silica, asbestos fibres) or CNT is the second mechanism that leads to the secretion of the pro-inflammatory mediators, IL-1β and IL-18 (Biswas et al., 2011; Palomäki et al., 2011). These two mechanisms are not exclusive and Hamilton et al. (2009) proposed that all high-aspect-ratio nanomaterials can induce frustrated phagocytosis and inflammasome activation similarly to asbestos fibres. The experimental evidence for inflammasome activation and the release of pro-inflammatory mediators based on in-vivo and in-vitro studies is summarized below.

Functionalized SWCNT produced by the HiPCO procedure (Unidym Inc., Sunnyvale, CA, USA) were evaluated for inflammasome activation and the release of IL-1β in LPS-primed,

**Fig. 4.1 Inflammasome activation and inflammation induced by nanoparticles, including carbon nanotubes**

Adapted from [Biswas et al. \(2011\)](#). Copyright © 2011

ASC, apoptosis-associated specific-like protein containing a caspase recruitment domain (CARD); IL, interleukin; LPS, lipopolysaccharide; NALP3, a member of the NOD-like receptor family; NF-κB, nuclear factor-kappa B; TLR, toll-like receptor

immortalized, bone marrow-derived murine macrophages ([Yang et al., 2013](#)). Oxidized SWCNT increased the release of IL-1β while benzoic acid functionalization decreased the release of pro-inflammatory cytokines.

See also [Hamilton et al. \(2013a\)](#); discussed in Section 4.4.1 (b).

#### 4.4.2 Release of cytokines, chemokines, and growth factors

##### (a) Exposed humans

Markers of inflammation and oxidative stress were monitored among workers handling engineered nanomaterials ([Liao et al., 2014a, b](#)). No effects were reported for IL-6 and IL-6 receptors, but depression of antioxidant enzymes was found among these workers ([Liou et al., 2012](#)).

*(b) Human cells in vitro*

A panel of well-characterized MWCNT was investigated for their ability to stimulate cytokine release from human THP-1 macrophages and the immortalized human mesothelial Met-5A cell line. Only long CNT samples (mean length, 13–36  $\mu\text{m}$ ) at a sublethal dose of 5  $\mu\text{g}/\text{mL}$  induced the release of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 from macrophages but not from mesothelial cells after 24 hours ([Murphy et al., 2012](#)). Immortalized human lung epithelial BEAS-2B cells were exposed to 30  $\mu\text{g}/\text{mL}$  of highly purified (10 minutes at 37  $^{\circ}\text{C}$ ) MWCNT (HTT2800 (see [Haniu et al., 2010](#)); diameter, 100–150 nm; length, 10–20  $\mu\text{m}$ ) and assessed for the release of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10, and IL-12 after 24 hours; only the release of IL-6 and IL-8 was detected ([Tsukahara & Haniu, 2011](#)). A dynamic cell growth model designed to mimic expansion and contraction during normal breathing was established using human lung adenocarcinoma A549 cells. Two samples of SWCNT (Cheap Tubes) were tested for the release of the cytokine, IL-8: a short CNT (diameter, 1–2 nm; length, 0.5–2  $\mu\text{m}$ ) and a long CNT (diameter, 1–2 nm; length, 5–30  $\mu\text{m}$ ). Only the long CNT induced the release of IL-8 after 24–72 hours and the levels were significantly higher in the dynamic cell growth model compared with static growth conditions ([Patel & Kwon, 2013](#)). A triple co-culture model of human lung epithelial 16HBE14o cells, primary human blood-derived dendritic cells, and primary human blood-derived macrophages in transwell cultures was used to evaluate the release of TNF- $\alpha$  and IL-8 after exposure to MWCNT (3 or 30  $\mu\text{g}/\text{mL}$ ) for 24 hours. As-produced and carboxylated MWCNT synthesized by CVD (Chengdu Carbon Nanomaterials R&D Center, Sichuan, China) were pre-coated with Curosurf 120 (porcine lung surfactant). Pre-coated, as-produced, and carboxylated MWCNT elicited the release of both TNF- $\alpha$  and IL-8 in this model system ([Gasser et al., 2012](#)). Transwell

co-cultures of human THP-1 macrophages and immortalized human lung epithelial BEAS-2B cells exposed to MWCNT released the profibrotic mediators, TGF- $\beta$ 1 and PDGF ([Wang et al., 2012a](#); [Li et al., 2013](#)).

[The use of in-vitro human or animal cell systems does not represent physiological routes of exposure for humans. The doses used in the in-vitro studies should be relevant to those to which humans are exposed, and the doses used in in-vitro studies may lead to mechanisms that differ from those that arise from the actual exposure concentrations of humans. Thus, interpretation of the data, including those on cytotoxicity and mechanisms, obtained from in-vitro studies should be evaluated cautiously.]

*(c) Experimental systems in vivo*

Several studies have investigated the pulmonary effects of CNT after inhalation, intratracheal or intranasal instillation, and pharyngeal aspiration. The results of these studies are summarized and the characteristics of the CNT investigated are described in [Table 4.11](#).

Inflammatory responses were assessed after the inhalation of MWCNT ([Ma-Hock et al., 2009](#); [Kim et al., 2014](#); [Kasai et al., 2015](#)). [Ma-Hock et al. \(2009\)](#) performed a 5-day range-finding inhalation study to select test concentrations for a 90-day inhalation toxicity study with MWCNT (Nanocyl NC 7000). Groups of male Wistar rats were exposed by head/nose-only inhalation to an aerosol of MWCNT dust for 6 hours per day on 5 consecutive days at target concentrations of 0, 2, 8, and 32  $\text{mg}/\text{m}^3$ . Treatment-related increases in BALF total cell counts (due to a significant increase in polymorphonuclear neutrophils), total protein content, and enzyme activities were observed in all treated groups 3 days after the last exposure. At the end of the 24-day recovery period, the same pattern of BALF findings was found. In the animals exposed to 2  $\text{mg}/\text{m}^3$  (the lowest concentration), slight recovery was observed; protein

**Table 4.11 Studies of pulmonary effects in experimental animals exposed to carbon nanotubes in vivo**

Route of administration	Species, strain (sex)	Type of CNT	Exposure concentration or dose, duration	Recovery	Characterization of CNT	Effects	Reference
<i>Inhalation</i>							
Head/nose-only	Rat, Wistar (M)	MWCNT	0, 2, 8, or 32 mg/m <sup>3</sup> , 6 h/day, 5 days/wk	3 and 24 days	Diameter, 5–15 nm; length, 0.1–10 µm; metal oxide, 10%; BET, 250–300 m <sup>2</sup> /g; MMAD, 0.5–1.3 µm	Increases in BALF total cell counts, total protein content, and enzyme activities in all treated groups	<a href="#">Ma-Hock et al. (2009)</a>
Whole-body	Rat, F344 (M, F)	MWCNT, fibre-like	0, 0.2, 1, or 5 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 13 wk	None	Diameter, 94.1–98 nm; length, 5.53–6.19 µm; purity > 99.6–99.8%; BET, 24–28 m <sup>2</sup> /g; MMAD, 1.4–1.6 µm	Increases in lung weights and inflammatory parameters in BALF, granulomatous changes, focal fibrosis of the alveolar wall, inflammatory infiltration in the visceral pleural and subpleural areas observed	<a href="#">Kasai et al. (2015)</a>
Nose-only	Rat, Sprague-Dawley (M, F)	MWCNT, short	0, 0.17, 0.49, or 0.96 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 4 wk	28 and 90 days	Length, 330.18 m; diameter, 10–15 nm; BET, 224.9 m <sup>2</sup> /g	No increase in inflammatory cytokines levels, inflammatory cells, or inflammatory proteins	<a href="#">Kim et al. (2014)</a>
Whole-body	Mouse, C57BL/6 (M)	MWCNT (mixture of MWCNT and graphitic nanofibres)	0.3, 1, or 5.3 mg/m <sup>3</sup> , 6 h/day, 7 or 14 days	None	Diameter, 10–20 nm; length, 5–15 µm; nickel and iron, 0.5%; BET, 100 m <sup>2</sup> /g; MMAD, 700–1000 nm/1800 nm	No local pulmonary effects but non-monotonic systemic immune suppression	<a href="#">Mitchell et al. (2007)</a>
Whole-body	Mouse, C57BL/6 (M)	MWCNT	0.3 or 1 mg/m <sup>3</sup> , 6 h/day, 14 days	None	Mean diameter (based on particle mass), 146.3 nm <sup>2</sup> /cm <sup>3</sup>	Systemic immune suppression, not due to systemic uptake of MWCNT but to release of immune suppressing signals from the lung	<a href="#">Mitchell et al. (2009)</a>
<i>Intratracheal instillation</i>							
	Rat, Sprague-Dawley (M)	MWCNT	0.04, 0.2, or 1 mg/kg bw	3, 7, 28, or 91 days	Diameter, 60 nm; length, 1.5 µm; 99.79% impurities; carbon, 7–8% (carbon soot); BET, 23.0 m <sup>2</sup> /g	Increase in BALF neutrophils and eosinophils, increased LDH and total protein levels; no change in levels of BALF cytokines	<a href="#">Kobayashi et al. (2010)</a>
	Rat, Sprague-Dawley (M)	SWCNT	0.04, 0.2, 1, or 2 mg/kg bw	1, 3, 7, 28, 91, or 182 days	Diameter, 12 nm; length, 0.32 µm; total metal, 0.05%; BET, 1064 m <sup>2</sup> /g	Increases in BALF neutrophils, macrophages, lymphocytes, eosinophils, LDH, protein, IL-1β, and IL-6	<a href="#">Kobayashi et al. (2011)</a>

Table 4.11 (continued)

Route of administration	Species, strain (sex)	Type of CNT	Exposure concentration or dose, duration	Recovery	Characterization of CNT	Effects	Reference
	Rat, Sprague-Dawley (M)	MWCNT	1, 10, or 100 µg	1, 7, 30, 90, or 180 days	Diameter, 20–50 nm; length, 0.5–2 µm; purity > 95%; BET, 280 m <sup>2</sup> /g	No inflammation; apoptosis of macrophages having phagocytosed MWCNT (elimination)	<a href="#">Elgrabli et al. (2008)</a>
	Mouse, ICR (M)	SWCNT	0.5 mg/kg bw	3 or 14 days	Diameter, 1–2 nm; length, several µm (NR)	Release of cytokines (NF-κB)	<a href="#">Chou et al. (2008)</a>
	Mouse, ICR (M)	MWCNT	5, 20, or 50 mg/kg bw	1, 3, 7, or 14 days	Diameter, 11–170 nm; length, 5–9 µm; carbon content > 90%; BET, 12.83 m <sup>2</sup> /g	Increase in immune cells and pro-inflammatory cytokines (IL-1, TNF-α, IL-6, IL-4, IL-5, IL-10, IL-12, and IFN-γ) and IgE; distribution of B-cells in the spleen	<a href="#">Park et al. (2009)</a>
<i>Intranasal instillation</i>							
	Mouse, Swiss (M)	Purified DWCNT (80%)	1.5 mg/kg bw	6, 24, or 48 h	Diameter, 1.2–3.2 nm; length, 1–10 µm (bundles up to 100 µm)	Local and systemic inflammation; no increase in TNF-α; decrease in local oxidative stress	<a href="#">Crouzier et al. (2010)</a>
		DWCNT, 20%					
		SWCNT)					
<i>Pharyngeal aspiration</i>							
	Mouse, C57BL/6j (M)	MWCNT	10, 20, 40, or 80 µg	1, 7, 28, or 56 days	Diameter, 49 nm; length, 3.86 µm; total metals, 0.78%	Increase in PMN, LDH, and albumin in BALF	<a href="#">Porter et al. (2010)</a>
	Mouse, C57BL/6j (F)	Purified SWCNT	10, 20, or 40 µg (0.5, 1, or 2 mg/kg bw)	1, 3, 7, 28, or 60 days	Diameter, 1–4 nm; iron, 0.23%; BET, 1040 m <sup>2</sup> /g	Inflammation (TNF-α and IL-1β increased)	<a href="#">Shvedova et al. (2005)</a>
	Mouse, C57BL/6j (F)	Purified SWCNT	40 µg (1.9 mg/kg bw)	1, 7, or 28 days	Diameter, 1–4 nm; EC, 99.7%; iron, 0.23%; BET, 1040 m <sup>2</sup> /g	Robust, acute inflammation (PMN, TNF-α, IL-6, and LDH increased)	<a href="#">Shvedova et al. (2007)</a>
	Mouse, C57BL/6j (F)	SWCNT	5, 10, or 20 µg (0.25, 0.5, or 1 mg/kg bw)	1, 7, or 28 days	Diameter, 0.8–1.2 nm; length, 100–1000 nm; EC, 82% wt; iron, 17.7%; BET, 508 m <sup>2</sup> /g	Inflammation (TNF-α, IL-6, and TGF-β increased), GSH depletion, lipid peroxidation, and oxidized proteins	<a href="#">Shvedova et al. (2008)</a>

BALF, bronchoalveolar lavage fluid; BET, Brunauer-Emmett-Teller surface area analysis; bw, body weight; CNT, carbon nanotubes; DWCNT, double-walled carbon nanotubes; EC, elemental carbon; F, female; GSH, glutathione; IgE, immunoglobulin E; IL, interleukin; IFN, interferon; LDH, lactate dehydrogenase; M, male; MMAD, mass median aerodynamic diameter; MWCNT, multiwalled carbon nanotubes; NF-κB, nuclear factor kappa B; NR, not reported; PMN, polymorphonuclear leukocytes; SWCNT, single-walled carbon nanotubes; TGF-β, transforming growth factor β; TNF-α, tumour necrosis factor α; wk, week

content and *N*-acetyl glucosaminidase activity returned to control levels, but the other parameters were still significantly increased. [Kasai et al. \(2015\)](#) conducted a 13-week study in male and female Fischer 344 rats exposed by whole-body inhalation to MWCNT (Hodogaya Chemical Co., Ltd, Tokyo, Japan) at concentrations of 0, 0.2, 1, and 5 mg/m<sup>3</sup> using a generation and exposure system based on the cyclone sieve method. In the BALF analyses, inflammatory parameters were increased in a concentration-dependent manner in both sexes from the lowest dose upwards ([Kasai et al., 2015](#)). A 4-week inhalation study of MWCNT (length, 330.18 nm; diameter, 10–15 nm) in Sprague-Dawley rats with a recovery period of up to 90 days showed that the levels of inflammatory cytokines (TNF- $\alpha$ , TGF- $\beta$ , IL-1, IL-2, IL-4, IL-5, IL-10, IL-12, and interferon (IFN)- $\gamma$ ) and inflammatory proteins (albumin, total protein, and LDH) in the BALF did not differ significantly ([Kim et al., 2014](#)). No local pulmonary effects were observed in C57BL/6 mice exposed to a mixture of MWCNT and graphitic nanofibres ([Mitchell et al., 2007](#)).

Intratracheal or intranasal instillation and pharyngeal aspiration are not physiological routes of exposure for humans but have been used in mice and rats to investigate the potential pulmonary and systemic toxicity of high concentrations of CNT.

Biological responses to MWCNT (Mitsui & Co. Ltd) were assessed in male rats after a single intratracheal instillation (0.04–1 mg/kg bw) ([Kobayashi et al., 2010](#)). Transient pulmonary inflammatory responses were observed in the lungs of the rats exposed to 1 mg/kg bw of MWCNT. However, the levels of cytokines in BALF did not change significantly at any time-point (3, 7, 28, or 91 days after exposure).

Pulmonary and systemic responses were assessed in male rats after the intratracheal instillation of highly pure, well dispersed, and well-characterized SWCNT. The numbers of BALF inflammatory cells (neutrophils, macrophages,

lymphocytes, and eosinophils) were increased in a dose-dependent manner. LDH values and the protein contents in BALF were significantly greater in the groups exposed to doses of SWCNT of 0.2 mg/kg bw and higher compared with those in the control group up to 3 months after instillation. Only small differences were observed between the SWCNT-exposed groups and the control group for the cytokines IL-1 $\alpha$ , IL-2, IL-4, IL-10, granulocyte macrophage colony-stimulating factor, IFN- $\gamma$ , and TNF- $\alpha$  at any of the time-points, but significant increases were observed for IL-1 $\beta$  and IL-6 at several time-points ([Kobayashi et al., 2011](#)).

Intratracheal instillation of 0.5 mg of SWCNT into male ICR mice induced alveolar macrophage activation, various chronic inflammatory responses, and severe pulmonary granuloma formation. Affymetrix microarrays were used to investigate the molecular effects on the macrophages exposed to SWCNT. A biological pathway analysis, a literature survey, and experimental validation suggested that the uptake of SWCNT into the macrophages can activate various transcription factors, such as nuclear factor kappa B (NF- $\kappa$ B) and activator protein 1 (AP-1), and that this leads to oxidative stress, the release of pro-inflammatory cytokines, the recruitment of leukocytes, the induction of protective and anti-apoptotic gene expression, and the activation of T-cells. [The resulting innate and adaptive immune responses may explain the chronic pulmonary inflammation and granuloma formation in vivo caused by SWCNT ([Chou et al., 2008](#)).]

Pulmonary and systemic immune responses induced by intratracheal instillation of 5, 20, and 50 mg/kg bw of MWCNT (Sigma-Aldrich, St. Louis, MO, USA (Cat. No. 659258)) into male mice were investigated ([Park et al., 2009](#)). Total numbers of immune cells in BALF were significantly increased in the treated groups and the distribution of neutrophils was elevated on day 1 after instillation. Pro-inflammatory cytokines

(IL-1, TNF- $\alpha$ , IL-6, IL-4, IL-5, IL-10, IL-12, and IFN- $\gamma$ ) were also increased in a dose-dependent manner in both BALF and blood. The highest levels of the cytokines were seen on day 1 after instillation and then decreased. The levels of T-helper (Th) 2-type cytokines (IL-4, IL-5, and IL-10) in the treated group were higher than those of the Th1-type cytokines (IL-12 and IFN- $\gamma$ ).

Male Swiss mice were intranasally instilled with 1.5 mg/kg bw of CNT (DWCNT, 80%; SWCNT, 20%). Local oxidative perturbations were investigated using electronic spin resonance (ESR) spin trapping experiments, and systemic inflammation was assessed by measuring the plasma concentrations of TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, insulin-like growth factor 1, leptin, granulocyte-colony stimulating factor, and vascular endothelial growth factor (Crouzier et al., 2010). Examination of the lungs and the elevation of pro-inflammatory cytokines in the plasma (leptin and IL-6 at 6 h) confirmed the induction of an inflammatory response, which was accompanied by a decrease in the local oxidative stress.

A dose-response and time-course study of MWCNT (Mitsui & Co. Ltd) was conducted in male mice exposed by pharyngeal aspiration. Examination of the BALF demonstrated that pulmonary inflammation and damage were dose-dependent and peaked at 7 days after exposure. By 56 days after exposure, markers of pulmonary inflammation and damage began returning to control levels, except in mice exposed to the 40- $\mu$ g MWCNT dose which still had significantly higher levels than vehicle controls (Porter et al., 2010).

Pharyngeal aspiration of SWCNT (CNI, Inc.) by female C57BL/6 mice elicited unusual pulmonary effects that combined robust but acute inflammation with early-onset, progressive fibrosis and granulomas. A dose-dependent increase in LDH and  $\gamma$ -glutamyl transferase activities was found in the BALF, together with an accumulation of 4-hydroxynonenal [an

oxidative biomarker] and the depletion of GSH in the lungs. An early accumulation of neutrophils, followed by lymphocyte and macrophage influx, was accompanied by an early elevation of pro-inflammatory cytokines (TNF- $\alpha$  and IL-1 $\beta$ ) followed by fibrogenic TGF- $\beta$ 1 (Shvedova et al., 2005).

Female C57BL/6 mice were maintained on vitamin E-sufficient or vitamin E-deficient diets and were exposed by aspiration to SWCNT (CNI, Inc.) to explore and compare their pulmonary inflammatory reactions. The vitamin E-deficient diet caused a 90-fold depletion of  $\alpha$ -tocopherol in the lung tissue and resulted in a significant decline of other antioxidants (reduced GSH and ascorbate) as well as an accumulation of lipid peroxidation products. A greater decrease in pulmonary antioxidants was detected in SWCNT-treated vitamin E-deficient mice compared with controls. The lower levels of antioxidants in vitamin E-deficient mice were associated with a higher sensitivity to SWCNT-induced acute inflammation (increases in the total number of inflammatory cells, the number of polymorphonuclear leukocytes, the release of LDH, total protein content, and the levels of pro-inflammatory cytokines, TNF- $\alpha$  and IL-6) and enhanced profibrotic responses (elevation of TGF- $\beta$  and collagen deposition). Exposure to SWCNT also markedly shifted the ratio of cleaved to full-length extracellular SOD (Shvedova et al., 2007).

In female C57BL/6 mice, the inhalation of stable and uniform dispersions of 5 mg/m<sup>3</sup> of unpurified SWCNT (CNI, Inc.; iron, 17.7% wt) for 5 hours per day for 4 days was compared with the pharyngeal aspiration of varying doses (5–20 g/mouse) of the same SWCNT. Overall, the outcomes of inhalation exposure to respirable SWCNT were very similar to those of pharyngeal exposure, both of which led to pulmonary toxicity. However, inhalation of SWCNT was more effective than aspiration in causing inflammatory response, oxidative stress, collagen

deposition, and fibrosis, as well as mutations at the *K-ras* gene locus in the lung ([Shvedova et al., 2008](#)).

(d) *Experimental systems in vitro*

No data were available to the Working Group

#### 4.4.3 Immune effects

(a) *Exposed humans*

No data were available to the Working Group.

(b) *Human cells in vitro*

In in-vitro test systems, macrophages and other relevant mammalian cells are frequently used as test cells for nanomaterials because they are primarily responsible for surveillance in the body. However, they are highly reactive with endotoxins and the distinction between the response to endotoxins and that to nanomaterials is difficult to make. Consequently, contamination with endotoxins confounds the result of tests in vitro. A preliminary examination for endotoxins is therefore required to minimize contamination or confirm an insignificant level in the test sample. Their quantification is also important for an adequate interpretation of data obtained in in-vitro biological test systems ([ISO, 2010e](#)).

Exposure to CNT may alter innate immune responses by triggering the complement system, the clearance of apoptotic cells by macrophages, and the induction of adaptive immune responses (reviewed by [Andersen et al., 2012](#)). Different responses have been reported for as-produced versus functionalized or coated CNT, and deliberate surface modifications have been attempted to enhance biocompatibility for drug delivery applications ([Hamad et al., 2010](#); [Moghimi et al., 2010](#)). The complement system is present in the lining fluid of the lung, and inhaled particles and fibres have been shown to induce complement-generating chemotactic activity that correlates with macrophage accumulation in vivo

([Warheit et al., 1985, 1988](#)). Direct binding of as-produced CNT to Clq protein, which leads to the classical pathway of complement activation, has been described in some studies while other investigators reported that complement proteins were bound to CNT but were not activated ([Ling et al., 2011](#)). The macrophage-mediated clearance of apoptotic cells is important for the regulation of immune responses and the suppression of macrophage function, and may lead to impaired clearance of particle-laden neutrophils from the lungs ([Wiethoff et al., 2003](#)) and chronic inflammation ([Witasp et al., 2008](#)). Human peripheral blood monocyte-derived macrophages exposed to purified SWCNT (CNI, Inc.) at non-toxic doses impaired the chemotaxis and phagocytosis of apoptotic target cells – human Jurkat T lymphoblastic leukaemia cells ([Witasp et al., 2009](#)). In an in-vitro three-dimensional model of granuloma formation, three commercial samples of MWCNT (MWCNT-7, Mitsui & Co. Ltd; other MWCNT, MER Corp., AZ, USA) or crocidolite asbestos fibres (UICC) altered the phenotype with the co-expression of pro-inflammatory (M1) and profibrotic (M2) markers of murine bone marrow-derived macrophages after 7–14 days ([Sanchez et al., 2011](#)).

Exposure of innate immune cells or lymphocytes to CNT in vitro may also impair the presentation of antigens and the activation of lymphocytes with variable results depending on the physical properties and surface functionalization of the CNT tested ([Andersen et al., 2012](#)). For example, carboxylated MWCNT have been reported to enhance cytokine secretion by purified human peripheral blood lymphocytes and stimulate lymphocyte-mediated tumour cell cytotoxicity ([Sun et al., 2011](#)), while amino-functionalized or oxidized MWCNT activated human monocytes and natural killer cells ([Delogu et al., 2012](#)). Purified samples (SES Research) of short SWCNT (length, 1–5 µm) and short MWCNT (length, 1–2 µm) caused minimal activation of antigen-presenting cells in vitro in contrast to

titanium dioxide (rutile) or zinc nanoparticles ([Palomäki et al., 2010](#)).

(c) *Experimental systems in vivo*

See [Table 4.11](#)

[The Working Group noted that, as in the case of in-vitro experiments on immune effects, the number of published in-vivo studies on CNT is too limited to draw any general conclusions, and the wide variation in the CNT used impedes any comparison of the reports from different studies.]

The pulmonary and systemic immune responses of male C57BL/6 mice to the inhalation of MWCNT were assessed ([Mitchell et al., 2007](#)). Analysis by TEM revealed that the material used was a mixture of MWCNT and graphitic nanofibres ([Lison & Muller, 2008](#); [McDonald & Mitchell, 2008](#)). After whole-body inhalation for 14 days, MWCNT were engulfed by alveolar macrophages and were distributed throughout the lung. However, no increases in inflammatory cell infiltration were found and no inflammation, granuloma formation, fibrosis, or tissue injury occurred up to the highest dose (5.3 mg/m<sup>3</sup>) tested. Despite the lack of local pulmonary effects, systemic immunity was affected at all concentrations tested. The measurement of immune function in spleen-derived cells showed a suppressed T-cell-dependent antibody response, a decreased proliferation of T-cells after mitogen stimulation, and altered natural killer cell killing. These results were accompanied by increased gene expression of indicators of oxidative stress and altered immune function [nicotinamide adenine dinucleotide phosphate (NADPH) dehydrogenase quinone 1 and IL-10] in the spleen, but not in the lung. Immune suppression persisted for up to 30 days after exposure ([Mitchell et al., 2007](#)). A follow-up study investigated the mechanism of the suppressed systemic immune function. Mice exposed to a dose of 1 mg/m<sup>3</sup> of MWCNT by whole-body inhalation presented suppressed immune function, which involved

the activation of cyclooxygenase enzymes in the spleen in response to a signal from the lung. Inhaled MWCNT were shown to activate the release of TGF- $\beta$  in the lung, which was postulated to have a direct effect on prostaglandin production in spleen cells, leading to immune suppression. However, to induce this altered systemic immunity, an additional [yet unknown] signalling mechanism from the lung would be necessary because not all observed systemic effects could be explained by this pathway ([Mitchell et al., 2009](#)).

Based on the results from these two studies ([Mitchell et al., 2007, 2009](#)), [Aschberger et al. \(2010\)](#) concluded that systemic immune effects are related to relatively short-term exposures to MWCNT. [The Working Group noted that the translocation of CNT from the lung does not appear to be necessary for such effects, although further investigation is required to confirm this hypothesis.]

In the study conducted by [Park et al. \(2009\)](#), distributions of B-cells in the spleen and blood were significantly increased on day 1 after intratracheal instillation of MWCNT into ICR mice, indicating that Th2-type cytokines had activated B-cells and caused them to proliferate. Together with the increased number of B-cells, granuloma formation in the lung tissue and the production of immunoglobulin (Ig) E were also observed with an intensity that was dependent on the dose of MWCNT instilled. [The Working Group noted that this study suggested that MWCNT may induce allergic responses in mice through B-cell activation and the production of IgE.]

(d) *Experimental systems in vitro*

Murine bone marrow-derived dendritic cells exposed to purified SWCNT (CNI, Inc.) in vitro and co-cultured with splenic T lymphocytes suppressed T-cell proliferation ([Tkach et al., 2011](#)). SWCNT (Chengdu Organic Chemicals Co., Ltd) also suppressed lymphocyte proliferation

in co-cultures of primary murine peritoneal macrophages and T lymphocytes activated by concanavalin A ([Dong et al., 2012](#)).

#### 4.4.4 Apoptosis

##### (a) Exposed humans

No data were available to the Working Group.

##### (b) Human cells in vitro

Several mechanisms have been shown to cause cell death in target cells exposed to engineered nanoparticles in vitro, including apoptosis, necrosis, and autophagic cell death (reviewed in [Andón & Fadeel, 2013](#)). In general, acute exposure to high concentrations (~30–400 µg/mL) of engineered nanoparticles can cause mitochondrial injury, increased intracellular generation of ROS, impaired adenosine triphosphate synthesis, and lysosomal damage leading to cell death. Therefore, the selection of doses of CNT for short-term in-vitro toxicity testing is problematic. Ideally, the doses should reflect the mass dose retained in workers exposed to CNT expressed as dose per alveolar epithelial cell surface area ([Gangwal et al., 2011](#)).

For a lifetime exposure to an airborne concentration of 1 mg/m<sup>3</sup> over 45 years, the relevant in-vitro dose would be ~50–70 µg/mL. However, short-term in-vitro toxicity testing is usually conducted after 24 hours of exposure and the equivalent dose for a 24-hour exposure of workers to an airborne concentration of 1 mg/m<sup>3</sup> would be ~0.2–0.6 µg/mL, which is two orders of magnitude lower, and doses > 50 µg/mL have been considered to be an “extraordinary high concentration” for use as a bolus dose in short-term in-vitro toxicity assays ([Oberdörster, 2012](#)).

The mechanistic pathways leading to cell death vary depending on the dose as well as the physical and chemical characteristics of the nanoparticles and the target cell type (see Section 4.2). Additional caveats in short-term in-vitro toxicity studies include other variables in experimental

design ([Table 4.12](#)) and the variability and purity of the sample ([Table 4.13](#)).

Various mechanisms have been proposed for the induction of cell death by CNT, including direct membrane damage, intracellular generation of ROS, and destabilization of lysosomal membranes. Thin, rigid MWCNT (Mitsui & Co., Ltd) directly penetrated human mesothelial cells and induced the depletion of adenosine triphosphate and cell death at a dose of 5 µg/cm<sup>2</sup> after 4 days ([Nagai et al., 2011](#)). In normal and malignant human mesothelial cell lines, exposure to MWCNT prepared by the CVD process (Mitsui & Co., Ltd) induced low levels of intracellular ROS and induced apoptosis at doses ≥ 50 µg/cm<sup>2</sup> after 24 hours ([Pacurari et al., 2008a, b](#)). A commercial sample of carboxylated SWCNT (Sigma-Aldrich) induced autophagic cell death in the human lung adenocarcinoma A549 cell line at a dose of 1 mg/mL after 24 hours ([Liu et al., 2011](#)).

##### (c) Experimental systems in vivo

After the intratracheal instillation of 0, 1, 10, or 100 µg of MWCNT (dispersed with albumin) into rats, inflammation, apoptosis, fibrosis, respiratory function, and granuloma formation were assessed after 1, 7, 30, 90, and 180 days. The results were obtained by plethysmography, soluble collagen quantification, quantitative real-time polymerase chain reaction (qRT-PCR), luminex measurement of cytokine expression, and histopathological examination. Only evidence of apoptosis of the alveolar macrophages was shown ([Elgrabli et al., 2008](#)).

##### (d) Experimental systems in vitro

High-aspect-ratio fibrous nanomaterials, including MWCNT, have been shown to cause direct plasma membrane penetration and increased permeability in the murine J774.1 ([Hirano et al., 2008](#)) and RAW 264 ([Shimizu et al., 2013](#)) macrophage cell lines after exposure to ~100 µg/mL. Acid-functionalized SWCNT synthesized by the CVD process (Chengdu Organic

**Table 4.12 Limitations of in-vitro assays for nanoparticle-induced toxicity**

Experimental design	Examples and selected references
Target cells: primary, immortalized or malignant cell lines	Stress induced by isolation of primary cells ( <a href="#">Stone et al., 2009</a> )
Culture format: monolayer, suspension, transwell, or three-dimensional (3-D) culture	Transwell models ( <a href="#">Snyder-Talkington et al., 2012</a> ) 3-D models ( <a href="#">Movia et al., 2011</a> ; <a href="#">Sanchez et al., 2011</a> )
single-cell, multicellular	Co-cultures more predictive for in-vivo end-points ( <a href="#">Müller et al., 2010</a> ; <a href="#">Clift et al., 2014</a> )
Use of dispersants to prevent agglomeration in cell culture medium: influence on cell uptake	Inhibition of cell uptake after dispersal with pluronic F127 ( <a href="#">Ali-Boucetta et al., 2011</a> ) or pluronic F108 ( <a href="#">Wang et al., 2012a</a> )
Secondary modification of nanoparticles during dispersion	Sonication-induced damage ( <a href="#">Bussy et al., 2012</a> )
Failure to confirm toxicity through morphology or different assays	<a href="#">Monteiro-Riviere et al. (2009)</a>
Physical or chemical interference	Examples and selected references
Physical adsorption to probes or reaction products	Neutral red assay ( <a href="#">Davoren et al., 2007</a> ) Formazan product of the MTT assay ( <a href="#">Wörle-Knirsch et al., 2006</a> )
Blocking or quenching of transmitted or emitted light	Thiobarbituric acid assay ( <a href="#">Fenoglio et al., 2006</a> ; <a href="#">Creighton et al., 2013</a> )
Direct oxidation of probed or substrates by nanoparticles	Surface generation of radicals ( <a href="#">Fenoglio et al., 2008</a> ; <a href="#">Tournebize et al., 2013</a> )
Adsorption or inactivation of cellular enzymes	Inactivation of lactate dehydrogenase ( <a href="#">Karajanagi et al., 2004</a> ; <a href="#">Zhang et al., 2011</a> )
Adsorption of secreted cytokines	<a href="#">Val et al. (2009)</a> , <a href="#">Horie et al. (2013)</a>
Contamination with endotoxin	Adsorption to carbon surfaces ( <a href="#">Delogu et al., 2010</a> )
Adsorption of micronutrients from cell culture media	Medium depletion ( <a href="#">Casey et al., 2008</a> ) Micronutrient depletion ( <a href="#">Guo et al., 2012</a> )

Compiled by the Working Group with data from [Stone et al. \(2009\)](#) and [Tournebize et al. \(2013\)](#)

Chemicals Co.) induced autophagy and cell death in primary murine peritoneal macrophages at doses of 10–50 µg/mL after 24 hours ([Wan et al., 2013](#)).

#### 4.4.5 Activation of intracellular signalling pathways

##### (a) Exposed humans

No data were available to the Working Group.

##### (b) Human cells in vitro

In addition to inflammasome activation, other intracellular signalling pathways have been shown to be activated by the exposure of macrophages in vitro to CNT.

In human THP-1 macrophages, MWCNT (XNRI, Bussan Nanotech Research, Ibaraki,

Japan) at a dose of 10 µg/mL for 24 hours induced the release of IL-1β via the activation of the Rho-kinase pathway ([Kanno et al., 2014](#)). In human lung fibroblast (IMR-90 or CRL-1490) cell lines, exposure to MWCNT prepared by the CVD method or SWCNT (CNI, Inc.) prepared by the HiPCO method activated the mitogen-activated protein kinase (MAPK)/p38 pathway at low doses ≤ 5 µg/mL, leading to the upregulation of pro-inflammatory gene expression and collagen deposition ([Ding et al., 2005](#); [Azad et al., 2013](#)). In normal and malignant human mesothelial cell lines, MWCNT (Mitsui & Co., Ltd) induced the activation of the MAPK/p38 pathway at a dose of 25 µg/cm<sup>2</sup> after 30–120 minutes ([Pacurari et al., 2008a](#)). Exposure of normal and malignant human mesothelial cell lines to unpurified SWCNT (National Institute

**Table 4.13 Minimal criteria required for the interpretation of in-vitro nanotoxicology assays**

Criteria	Selected references
Complete physico-chemical characterization of nanoparticles	<a href="#">Warheit (2008)</a>
Sample variability depending on batch, synthesis technique, and later processing	<a href="#">Fubini et al. (2011)</a>
Consideration of contaminants (e.g. amorphous carbon)	<a href="#">Wang et al. (2011c)</a>
Bioavailability of metal catalyst residues	<a href="#">Kagan et al. (2006)</a> , <a href="#">Pulskamp et al. (2007)</a> , <a href="#">Liu et al. (2008b)</a> , <a href="#">Aldieri et al. (2013)</a>
Agglomeration state	<a href="#">Wick et al. (2007)</a> , <a href="#">Murray et al. (2012)</a>
Comparison with positive and negative reference particles	<a href="#">Stone et al. (2009)</a>
Positive controls for toxicity assays	<a href="#">Stone et al. (2009)</a>
Use of doses that reflect potential human inhalation exposures	<a href="#">Oberdörster et al. (2013)</a>
Consideration of dose-dependent mechanisms of adaptation and toxicity	<a href="#">Slikker et al. (2004)</a> , <a href="#">Bhattacharya et al. (2011)</a>

Compiled by the Working Group with data from [Warheit \(2008\)](#) and [Stone et al. \(2009\)](#)

of Standards and Technology, Gaithersburg, MD, USA) induced the intracellular generation of ROS and activation of the AP-1 and NF- $\kappa$ B pathways at a dose of 25  $\mu$ g/cm<sup>2</sup> after 1–4 hours ([Pacurari et al., 2008b](#)).

#### (c) *Experimental systems in vivo*

Some studies revealed that a Th2-associated response to CNT is activated through both adaptive and innate immune responses. In studies of MWCNT (NanoTechLabs, Inc., Yadkinville, USA)-exposed mice, the expression of IL-33 was accompanied by lung dysfunction and the upregulation of Th2-associated cytokines, such as IL-5 and eotaxin ([Katwa et al., 2012](#); [Beamer et al., 2013](#)). The exposure of mice to SWCNT or MWCNT produced a dose-dependent increase in ovalbumin (OVA)-specific IgE and IgG1 in the serum ([Nygaard et al., 2009](#)).

With regard to signal transduction, the expression of four genes (coiled-coil domain containing-99, muscle segment homeobox gene-2, nitric oxide synthase-2, and wingless-type inhibitory factor-1) among 63 genes in the lung of mice exposed to MWCNT (Mitsui & Co. Ltd) was altered at two time-points, as determined by a quantitative PCR assay ([Pacurari et al., 2011](#)). In a study in mice, exposure to semi-SWCNT [semi-conductive components of SWCNT] (metal

content approximately 10% wt, selectively separated from a mixture of metallic and semi-metallic SWCNT) induced phosphorylation of the signal transducer and activator of transcription-3, which forms part of the Janus kinase/signal transducers and activators of transcription signalling cascade, at two time-points ([Park et al., 2011a](#)). With regard to transcription factors, cFos mRNA levels in whole blood were increased in SWCNT-exposed mice ([Erdely et al., 2009](#)). In another study in mice, although the dose used for intratracheal instillation was excessive (1 mg/mouse, 10 mg/kg bw), NF- $\kappa$ B and AP-1 transcription factors were activated in the lung after exposure to SWCNT ([Chou et al., 2008](#); [Zhang et al., 2013](#)).

#### (d) *Experimental systems in vitro*

The MAPK/extracellular signal-regulated kinase 1,2 pathway was upregulated in the murine macrophage RAW 264.7 cell line exposed to MWCNT (Helix Material Solutions, Inc., Richardson, TX, USA) at doses  $\geq$  50  $\mu$ g/mL for 24 hours, resulting in an increased expression of cyclooxygenase-2 and inducible nitric oxide synthase ([Lee et al., 2012](#)). The exposure of murine epidermal JB6P+ cells to partially purified SWCNT produced by the HiPCO process (CNI, Inc.) at doses  $>$  60  $\mu$ g/mL for 24 hours, activated the NF- $\kappa$ B pathway dose-dependently ([Murray et al., 2009](#)).

Exposure of Chinese hamster ovary K1 cells (transfected with an NF- $\kappa$ B reporter gene construct) to MWCNT (XNRI, Bussan Nanotech Research) prepared by the CVD process at doses between 1 and 10  $\mu$ g/mL for 20 hours upregulated the NF- $\kappa$ B pathway ([Hirano et al., 2010](#)).

#### 4.4.6 Resistance to apoptosis

##### (a) Exposed humans

No data were available to the Working Group.

##### (b) Human cells in vitro

No data were available to the Working Group.

##### (c) Experimental systems in vivo

The formation of large tumours from injected SWCNT-transformed cells (which were also reported to be resistant to apoptosis due to a low level of p53 phosphorylation in an in-vitro study) was observed in immunodeficient mice ([Wang et al., 2011a](#)). The expression of LC3B and the autophagy-related proteins p62 and Beclin-1 was upregulated and the expression of proliferating cell nuclear antigen was also elevated in mice exposed intratracheally to 100  $\mu$ g of MWCNT ([Yu et al., 2013](#)). In another study, the expression of anti-apoptotic genes, such as cIAP2, SOD2, and A20, was induced in mouse lung by an [excessive] dose of SWCNT (1 mg/mouse) ([Chou et al., 2008](#)).

##### (d) Experimental systems in vitro

No data were available to the Working Group.

#### 4.4.7 Cell proliferation

##### (a) Exposed humans

No data were available to the Working Group.

##### (b) Human cells in vitro

Human cell lines were exposed to titanium dioxide nanobelts or purified MWCNT (Cheap Tubes, Inc.) at doses of 10 or 100  $\mu$ g/mL for 1 and

24 hours. The target cell lines included human THP-1-derived macrophages and primary cultures of small airway epithelial cells. Total RNA was isolated and used for microarray analysis using Human Genome U133A 2.0 GeneChips (Affymetrix, Santa Clara, CA, USA). An analysis of the Global proteomics was conducted using liquid chromatography tandem mass spectrometry on tryptic digested cell lysates. The short exposure elicited similar proteomic responses to titanium dioxide nanobelts and MWCNT with different patterns of expression in various cell types. THP-1 macrophages showed the most significant transcriptional responses in 272 genes after 24 hours of exposure to MWCNT with unique patterns of gene expression in pathways related to cell-cycle regulation and cell proliferation (MYC and CDK1), as well as anti-apoptosis (survivin). Genes involved in the Sp1/AhR-dependent stress response were down-regulated by exposure to MWCNT ([Tilton et al., 2014](#)).

In a study in human lung small airway epithelial cells exposed to SWCNT or MWCNT, occupationally relevant concentrations of CNT produced a neoplastic-like transformation phenotype depicted by increased cell proliferation, anchorage-independent growth, invasion, and angiogenesis ([Wang et al., 2014](#)).

##### (c) Experimental systems in vivo

##### (i) Bronchiolar and alveolar epithelial cells

Some, but not all, studies provided evidence of the proliferation of bronchiolar and alveolar epithelial cells.

In a 13-week inhalation study, exposure to CNF (VGCF-H) induced cell proliferation in the terminal bronchioles, alveolar ducts, and subpleural region of the respiratory tract in the lungs of male and female rats; however, this proliferation was not persistent and was absent in the subpleural region in females 3 months after exposure ([Delorme et al., 2012](#)).

Two studies of exposure by intratracheal instillation of rats to MWCNT ([Roda et al., 2011](#)) and mice to SWCNT ([Murray et al., 2012](#)) were performed. Exposure to as-produced or functionalized MWCNT induced proliferation of the alveolar and bronchiolar epithelial cells and alveolar macrophages in rats ([Roda et al., 2011](#)). Exposure to SWCNT decreased the proliferation of splenic T cells in mice ([Murray et al., 2012](#)). In another study in rats ([Warheit et al., 2004](#)), intratracheal instillation of SWCNT did not induce the proliferation of lung parenchymal cells assessed by 5-bromo-2-deoxyuridine.

(ii) *Other cells*

The proliferation of T-cells was induced in mice exposed to MWCNT in one study ([Grecco et al., 2011](#)) but was decreased in another study ([Murray et al., 2012](#)).

Exposure of mice to SWCNT increased the occurrence of epithelial-derived fibroblasts. The aberrant activation of TGF- $\beta$ /p-Smad2 or  $\beta$ -catenin was postulated to induce epithelial-mesenchymal transition during SWCNT-induced fibrosis ([Chang et al., 2012](#)). In rats, MWCNT induced visceral mesothelial cell proliferation, (assessed by proliferating cell nuclear antigen immunostaining), accompanied by increases in the number of macrophages and of the concentration of protein in the pleural lavage ([Xu et al., 2012](#)).

(d) *Experimental systems in vitro*

Murine lung epithelial FE1 cells are a spontaneously immortalized cell line isolated from a Muta<sup>TM</sup> Mouse ([Søs Poulsen et al., 2013](#)). These cells were exposed to 12.5, 25, or 100  $\mu\text{g}/\text{mL}$  of MWCNT (Mitsui-7; MWCNT-XNRI-7, lot 05072001K28, Hadoga Chemical Industry, Japan) suspended by sonication in cell culture medium. After 24 hours, the cells were harvested and total RNA was extracted for microarray analysis (Agilent 8  $\times$  66K oligonucleotide microarrays); selected genes were verified using qRT-PCR. A

total of 565 genes were differentially expressed at all concentrations. Classification of gene ontology revealed that most of the differentially expressed genes were involved in cell proliferation. At the highest exposure concentration, differentially expressed genes were related to cell death, cell-cycle arrest, oxidation reduction, and other metabolic pathways. Genes involved in fibrosis, cholesterol biosynthesis, GSH-mediated detoxification, and aryl hydrocarbon receptor signalling molecular canonical pathways were downregulated.

#### 4.4.8 Granuloma formation and fibrosis

(a) *Exposed humans*

No data were available to the Working Group.

(b) *Human cells in vitro*

No data were available to the Working Group.

(c) *Experimental systems in vivo*

See [Table 4.10](#)

(i) *Inhalation*

Two 13-week studies of MWCNT in Wistar rats provided evidence of granulomatous inflammation and fibrosis in the lungs of male and female rats. The minimum concentrations of MWCNT that induced the persistent or slight fibrotic responses were 0.5  $\text{mg}/\text{m}^3$  ([Pauluhn, 2010b](#)) and 0.4  $\text{mg}/\text{m}^3$  ([Treumann et al., 2013](#)), respectively.

Two 4-week studies of MWCNT and SWCNT in Wistar rats showed no evidence of fibrosis in the lung at maximal concentrations of 0.37  $\text{mg}/\text{m}^3$  of MWCNT and 0.13  $\text{mg}/\text{m}^3$  of SWCNT ([Morimoto et al., 2012a, b](#)).

Two 6-hour exposure studies to MWCNT provided evidence of persistent fibrosis at high concentrations (241 and 30  $\text{mg}/\text{m}^3$ ) in male rats ([Ellinger-Ziegelbauer & Pauluhn, 2009](#)) and mice ([Ryman-Rasmussen et al., 2009a](#)).

*(ii) Intratracheal instillation*

Fifteen studies of intratracheal instillation in rats and pharyngeal aspiration in mice have been reported ([Lam et al., 2004](#); [Muller et al., 2005](#); [Warheit et al., 2004](#); [Shvedova et al., 2005](#); [Aiso et al., 2010](#); [Cesta et al., 2010](#); [Han et al., 2010](#); [Kobayashi et al., 2010, 2011](#); [Porter et al., 2010](#); [Mercer et al., 2011](#); [Morimoto et al., 2012b, c](#); [Murray et al., 2012](#); [Sager et al., 2013](#); [Fujita et al., 2015](#)). Most of the studies revealed that exposure to SWCNT and MWCNT resulted in persistent or progressive fibrosis in the lung ([Muller et al., 2005](#); [Shvedova et al., 2005](#); [Aiso et al., 2010](#); [Cesta et al., 2010](#); [Porter et al., 2010](#); [Mercer et al., 2011](#); [Murray et al., 2012](#); [Sager et al., 2013](#)), whereas some studies in rats and one study in mice demonstrated transient or minimal fibrosis in the lung ([Kobayashi et al., 2010, 2011](#); [Morimoto et al., 2012b, c](#)). In a long-term study, the formation of granuloma in the lungs disappeared over time ([Fujita et al., 2015](#)). [No significant differences in pulmonary responses were observed between rats and mice.]

*(iii) Intraperitoneal injection*

Exposure to long MWCNT led to granulomatous inflammation in the peritoneal cavity but exposure to tangled MWCNT induced weak or slight responses ([Poland et al., 2008](#)). In another study, MWCNT did not induce sustained inflammatory responses ([Muller et al., 2009](#)).

*(d) Experimental systems in vitro*

No data were available to the Working Group.

#### 4.4.9 Alterations in DNA damage-induced response pathways

*(a) Exposed humans*

No data were available to the Working Group.

*(b) Human cells in vitro*

See [Table 4.14](#)

Human cells were used to study the effect of SWCNT on the expression of stress-response genes. The target cells included primary normal human bronchial epithelial cells, diseased human bronchial epithelial cells from asthma patients or from patients with chronic obstructive pulmonary disease, lung adenocarcinoma A549 cells and pharyngeal carcinoma FaDu cells. Cells were exposed to 0.1 or 1.0 mg/mL of SWCNT (Meijo Nano Carbon Co., Ltd, Nagoya, Japan) for 6 hours. A PCR array was conducted to examine 84 stress-response genes. Expression levels of 11 stress-response genes, including ERCC1 encoding a DNA repair enzyme, were downregulated more than twofold after exposure to SWCNT. Other genes belonging to the inflammatory responses, IL-6 and TNF- $\alpha$ , were significantly downregulated in normal human bronchial epithelial cells indicating that inflammatory cytokines were not activated under these conditions ([Hitoshi et al., 2012](#)).

Protein expression was investigated in human monoblastic leukaemia U937 cells exposed to MWCNT (100  $\mu$ g/mL) that had been thermally treated at 1800 °C or 2800 °C. An analysis of proteomics was performed after two-dimensional electrophoresis and protein identification by matrix-assisted laser desorption/ionization-time of flight mass spectrometry. The expression of proteins involved in stress responses and DNA repair (such as DNA mismatch repair protein Msh2 and DNA damage-binding protein 1) was enhanced, suggesting the induction of DNA repair; however, the efficiency of repair was not evaluated ([Haniu et al., 2010](#)).

Gene and protein expression was studied in three human cell lines exposed to two types of high-aspect-ratio nanoparticles: MWCNT and titanium dioxide nanobelts that are known to exert low and high toxicity, respectively, in other cell systems. The sizes of the MWCNT and titanium dioxide nanobelts were  $375 \pm 23$  nm and  $1590 \pm 126$  nm in RPMI medium and  $458 \pm 16$  nm and  $634 \pm 86$  nm in DMEM medium,

**Table 4.14 Studies of impaired DNA repair in human cells exposed to carbon nanotubes in vitro**

Cell types	Type of carbon nanotube, treatment, duration	Assay/result	Reference
NHBE cells, and DHBE cells from asthma and COPD lung adenocarcinoma A549 and pharynx carcinoma FaDu cell lines	SWCNT, 0.1 or 1.0 mg/mL for 6 h	Measurement of gene expression using PCR array evaluating 84 stress-response genes Downregulation of 11 genes, including ERCC1 encoding a DNA repair enzyme	<a href="#">Hitoshi et al. (2012)</a>
Human monoblastic leukaemia U937 cells	MWCNT, 50 µL (10 mg/mL) added to 5 mL of culture medium that contained $2.5 \times 10^5$ cells	Analysis of proteomics performed by two-dimensional electrophoresis and protein identification by MALDI-TOF MS Enhancement of the expression of proteins in response to stress and DNA repair (such as DNA mismatch repair protein Msh2 and DNA damage-binding protein 1)	<a href="#">Haniu et al. (2010)</a>
Macrophage-like THP-1 cells, primary small airway epithelial cells, and Caco-2/HT29-MTX co-cultures	Cells exposed to 10 or 100 µg/mL MWCNT or titanium oxide nanobelts (TiO <sub>2</sub> -NB) for 1 h and 24 h or 3 h and 24 h MWCNT and TiO <sub>2</sub> -NB sizes: 375 ± 23 nm and 1590 ± 126 nm, respectively, in RPMI medium and 458 ± 16 nm and 634 ± 86 nm, respectively, in DMEM medium	Transcriptomic and proteomic analyses: comparison of elicited signatures induced by TiO <sub>2</sub> -NB and MWCNT THP-1 cells were the most responsive cells. Three pathways were upregulated specifically by MWCNT, but not by TiO <sub>2</sub> -NB: DNA damage checkpoint, DNA damage (double-strand breakage) repair and cytoskeleton spindle microtubules, consistent with DNA damage and mitotic perturbations induced by MWCNT	<a href="#">Tilton et al. (2014)</a>

COPD, chronic obstructive pulmonary disease; DHBE, diseased human bronchial epithelial; ERCC1, excision repair cross-complementary rodent repair deficiency complementation group 1; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MWCNT, multiwalled carbon nanotubes; NHBE, primary normal human bronchial epithelial; PCR, polymerase chain reaction; SWCNT, single-walled carbon nanotubes

respectively. The cell lines were human THP-1 cells, primary small airway epithelial cells, and Caco-2/HT29-MTX co-cultures. The Caco-2 is a malignant human intestinal epithelial cell line and HT29-MTX is a goblet cell line. This co-culture mimics the intestinal epithelium. The cells were exposed to 10 or 100  $\mu\text{g/mL}$  for 1 and 24 hours and 3 and 24 hours for transcriptomic and proteomic analyses, respectively. Titanium dioxide nanobelts were more cytotoxic than MWCNT, but the toxicity was low at early time-points. THP-1 cells were the most responsive and showed time-, concentration-, and particle type-dependent responses; the highest responses occurred at 100  $\mu\text{g/mL}$ . Fewer genes were differentially expressed between untreated and treated cells in the other cell types. Cells responded to these nanoparticles by differentially regulating both common and unique sets of biological processes. In this study, the results were analysed by a comparison of the changes in regulatory pathways. When comparing signatures induced by titanium dioxide nanobelts and MWCNT in THP-1 cells, three pathways were upregulated specifically by MWCNT and not by titanium dioxide nanobelts: DNA damage checkpoint, DNA strand break repair, and cytoskeleton spindle microtubules. These results were consistent with others that demonstrated DNA damage and mitotic perturbations caused by MWCNT (Tilton et al., 2014). [Globally, these data showed common differential expression across the cell types and common pathways in response to exposure to titanium dioxide nanobelts or MWCNT, possibly linked to a common mechanism. However, they also showed cell-specific responses and particle-specific effects, thus addressing the question of target cell specificity.]

(c) *Experimental systems in vivo and in vitro*

See [Table 4.15](#)

Female Fischer 344 rats received a single intragastric dose of SWCNT (Thomas Swan and Co. Ltd, Consett, United Kingdom) or  $\text{C}_{60}$

fullerenes by gavage (0.064 or 0.64 mg/kg bw). Both  $\text{C}_{60}$  fullerenes (highest dose only) and SWCNT significantly enhanced the 8-oxodG level in the liver and lung tissues of rats in comparison with controls. SWCNT did not produce a significant increase in the gene expression of the DNA repair enzyme 8-oxoguanine DNA glycosylase in the liver. DNA repair activity was assessed in the liver using substrate nuclei containing 8-oxodG, and the level of 8-oxoguanine DNA glycosylase was not significantly altered (Folkmann et al., 2009).

In RAW 264.7 macrophages, SWCNT (Chengdu Organic Chemicals Co., Ltd) down-regulated several genes involved in the DNA repair process (Dong et al., 2012).

The assessment of MWCNT genotoxicity in rats (Kim et al., 2012a, 2014) and mice (Ghosh et al., 2011) showed a persistence of DNA damage up to 90 days after exposure. [The findings may suggest a low or lack of DNA repair.]

Pregnant heterozygous p53 mice ( $p53^{+/-}$ ) received an intravenous injection of MWCNT (200  $\mu\text{L}$ , 2 mg/kg bw). DNA integrity was assessed using a long PCR assay. In general, DNA damage was found in the fetuses and in placental cells, with an enhancement of the mRNA expression of bax and p21. Nonetheless, DNA damage was higher in  $p53^{-/-}$  and  $p53^{+/-}$  fetuses than in  $p53^{+/+}$  fetuses and neither *Bax* nor *p21* expression levels were modified in  $p53^{-/-}$  fetuses, in agreement with a defective DNA repair system in these mice cells due to the absence of p53 (Huang et al., 2014). [These results were consistent with the induction of p53-dependent apoptosis in this test system, and showed that the repair of DNA damage and apoptosis are dependent on the p53 status resulting from the intravenous injection of MWCNT into mice. They did not directly demonstrate that DNA repair was impaired by MWCNT, but showed an impaired response in p53-deficient mice.]

**Table 4.15 Studies of impaired DNA repair in tissues of experimental animals exposed to carbon nanotubes in vivo or in vitro**

Species, strain (sex), or cell types (in vitro)	Route of administration (in vivo)/exposure concentration or dose, duration	Recovery	Result	Reference
Rat, F344 (F)	Intragastric (gavage) SWCNT or C <sub>60</sub> fullerenes at 0.064 or 0.64 mg/kg bw Diameter, 0.9–1.7 nm; length < 1 µm (SWCNT); diameter, 0.7 nm (fullerenes)	24 h	Enhancement of 8-oxodG levels in the liver and lung tissues Increased gene expression of <i>OGGI</i> in the liver by C <sub>60</sub> fullerenes (not significant with SWCNT) DNA repair activity assessed in the liver, using substrate nuclei containing 8-oxodG: no altered level of <i>OGGI</i> repair activity	<a href="#">Folkmann et al. (2009)</a>
Rat, Sprague-Dawley (M)	Inhalation (in inhalation chambers) MWCNT: 0.16, 0.34, and 0.94 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 5 days	1 mo or none	Comet assay in lung cells Significant DNA damage at 0.94 mg/m <sup>3</sup> Similar patterns of DNA damage at both time-points after exposure The persistence of DNA damage at 1 mo may suggest a lack of DNA repair.	<a href="#">Kim et al. (2012a)</a>
Rat, Sprague-Dawley (M, F)	Nose-only inhalation MWCNT: 0.17, 0.49, and 0.96 mg/m <sup>3</sup> , 6 h/day, 5 days/wk, 28 days Diameter, 10–15 nm	28 or 90 days	Comet assay in lung cells Significant DNA damage at 0.94 mg/m <sup>3</sup> Similar patterns of DNA damage at 28 and 90 days after exposure may suggest a lack of DNA repair	<a href="#">Kim et al. (2014)</a>
Mouse, Swiss albino (M)	Intraperitoneal injection MWCNT: 2, 5, and 10 mg/kg bw at 0 and 24 h	3 h (comet assay) or 24 h (micronucleus)	Micronucleus and comet assay in bone marrow cells Results suggested low or lack of DNA repair during the recovery period	<a href="#">Ghosh et al. (2011)</a>
Mouse, pregnant heterozygous <i>p53</i> ( <i>p53</i> <sup>+/-</sup> )	Intravenous injection MWCNT: 2 mg/kg bw (single injection)		Studies of fetuses and placenta cells; measurement of DNA integrity using the L-PCR assay and mRNA expression of <i>Bax</i> and <i>p21</i> DNA damage greater in <i>p53</i> <sup>-/-</sup> and <i>p53</i> <sup>+/-</sup> fetuses than in <i>p53</i> <sup>+/+</sup> fetuses Neither <i>Bax</i> nor <i>p21</i> expression levels were modified in <i>p53</i> <sup>-/-</sup> fetuses, in agreement with an absence of DNA repair in the cells of these mice	<a href="#">Huang et al. (2014)</a>

**Table 4.15 (continued)**

Species, strain (sex), or cell types (in vitro)	Route of administration (in vivo)/exposure concentration or dose, duration	Recovery	Result	Reference
Murine RAW 264.7 macrophages	Acid-functionalized SWCNT: 1, 10, and 50 µg/mL for 24 h	None	Gene expression profiles analysed by cDNA microarray Several genes from the DNA repair processes were downregulated (e.g. <i>XPA</i> , <i>XRCC1</i> , and <i>XRCC4</i> ) Results suggest that acid-functionalized SWCNT may induce resistance to apoptosis and DNA damage repair deficiencies in these cells	<a href="#">Dong et al. (2012)</a>

bw, body weight; F, female; L-PCR, long polymerase chain reaction; M, male; mo, month; MWCNT, multiwalled carbon nanotubes; OGG1, 8-oxoguanine glycosylase; 8-oxo-dG, 8-oxo-2'-deoxyguanosine; SWCNT, single-walled carbon nanotubes; wk, week

#### 4.4.10 Depletion of antioxidants

As described previously, CNT have been shown to generate (or catalyse the formation of) ROS directly; however, this does not exclude a secondary generation of ROS by target cells *in vitro* or *in vivo* after exposure to CNT ([Stone et al., 2009](#); [Fubini et al., 2010, 2011](#)).

##### (a) Exposed humans

No data were available to the Working Group.

##### (b) Human cells *in vitro*

See [Table 4.16](#)

##### (i) SWCNT

The effects of SWCNT (NASA-JSC, TX, USA) were determined in cell cultures of immortalized human epidermal HaCaT keratinocytes. The generation of HO• was observed in HaCaT keratinocytes exposed to SWCNT at 0.24 mg/mL, using the ESR spin trapping technique. Both GSH and the antioxidant levels of the HaCaT keratinocytes were decreased at doses of 0.06, 0.12, and 0.24 mg/mL. In parallel, a significant increase in the accumulation of lipid peroxidation products (thiobarbituric acid-reactive substances) was seen in cells exposed to SWCNT ([Shvedova et al., 2003](#)).

Human BJ foreskin cells were exposed to SWCNT (Sigma) dispersed in dimethyl formamide. ROS production was determined using the 2,7-dichlorofluorescein diacetate assay, and was induced by exposure to SWCNT at doses of 6, 8, and 10 µg/mL after 3 hours of incubation. Antioxidant defences were assessed in BJ cells co-incubated with exogenous antioxidants, NAC and GSH, in the presence or absence of SWCNT (6 µg/mL). Exogenous NAC and GSH decreased the induction of ROS by SWCNT approximately two- and 2.5-fold, respectively ([Sarkar et al., 2007](#)). [These results suggested that compromised cellular antioxidant defences may be responsible

for the generation of excess ROS in response to SWCNT in BJ foreskin cells.]

The effects of SWCNT were investigated in human macrophage-like cells differentiated from a human monocytic leukaemia THP-1 cell line. Affymetrix microarrays were performed to investigate the changes in gene expression after exposure to SWCNT. Cells were exposed to 0.05 µg/mL of SWCNT for 24 hours, which resulted in an increased expression of SOD2; the levels of catalase, GPx1, GSH reductase, GSH synthetase, and NADPH-dependent oxidase were not altered ([Chou et al., 2008](#)).

Human hepatoma HepG2 cells were exposed to purified HiPCO SWCNT (Unidym) produced by the CVD process or to graphene. Proteins were extracted and their profile analysed using the iTRAQ-coupled two-dimensional liquid chromatography tandem mass spectrometry approach. Peptides and proteins were identified automatically using the Spectrum Proteomics Workbench software. Protein ratios were determined in treated and untreated control HepG2 cells. Only quantification data on proteins with relative changes in expression of > 1.25 or < 0.8 were considered. After exposure to 1 µg/mL for 48 hours, 37 differentially expressed proteins were found in cells exposed to SWCNT or to graphene. Differentially expressed proteins involved in metabolic pathways, redox regulation, and cytoskeletal dynamics were identified. The antioxidant protein SOD2 was downregulated ([Yuan et al., 2011](#)).

Human colon adenocarcinoma Caco-2 cells were exposed to 0, 5, 10, 50, 100, 500, and 1000 µg/mL of F-SWCNT (Sigma-Aldrich, Madrid, Spain). The production of ROS and biomarkers of oxidation were quantified, including lipid peroxidation, generation of ROS, GSH levels, and SOD, GPx, GSH reductase, and catalase activities. ROS generation was increased at a dose of 100 µg/mL and lipid peroxidation at a dose of 50 µg/mL. Catalase activity increased at doses up to 500 µg/mL, then significantly

**Table 4.16 Depletion of antioxidants in cultured cells exposed to carbon nanotubes**

Cell type	Type of carbon nanotube; treatment; duration	Assay/result	Reference
Human HaCaT keratinocytes	SWCNT; 0.24 mg/mL (ESR assay), and 0.06, 0.12, and 0.24 µg/mL (oxidation assay); 2, 4, 6, 8, and 18 h	Generation of HO• using ESR spin trapping technique; measurement of GSH, antioxidant levels and LPO products (TBARS) GSH and antioxidant levels decreased versus control cells, and the accumulation of LPO products significantly increased	<a href="#">Shvedova et al. (2003)</a>
Human BJ foreskin cells	SWCNT; 6, 8, and 10 µg/mL; 3 h	Induction of ROS by SWCNT compared with control cells (dispersion medium) using DCF Antioxidants (NAC and GSH) reduced ROS production by SWCNT (6 µg/mL)	<a href="#">Sarkar et al. (2007)</a>
Human macrophage-like cells differentiated from a human monocytic leukaemia THP-1 cell line	SWCNT; 0.05 µg/mL; 24 h	Molecular effects investigated using Affymetrix microarrays Expression of SOD2 increased, but levels of catalase, GPx1, GR, GS, and NOX not modified	<a href="#">Chou et al. (2008)</a>
Human hepatoma HepG2 cells	SWCNT; 1 µg/mL; 48 h	Protein expression profile using the iTRAQ-coupled 2D LC-MS/MS approach; protein ratios determined for treated and untreated control HepG2 cells Differential expression of 37 proteins observed in treated cells, among which SOD2 was downregulated	<a href="#">Yuan et al. (2011)</a>
Human colon adenocarcinoma Caco-2 cells	Carboxylic acid-functionalized SWCNT (F-SWCNT); 0, 5, 10, 50, 100, 500, and 1000 µg/mL; 48 h	Determination of ROS, LPO, and GSH levels, and SOD, GPx, GR, and catalase activities Generation of ROS increased from 100 µg/mL and that of LPO from 50 µg/mL; catalase activity increased up to 500 µg/mL, but significantly decreased at 1000 µg/mL; SOD also enhanced (maximum up to 100 µg/mL); GPx activity enhanced at the highest concentrations and GR at 1000 µg/mL; level of GSH depleted (significant at 1000 µg/mL)	<a href="#">Pichardo et al. (2012)</a>
Human bronchial epithelial BEAS-2B cells	Three samples of SWCNT (diameter, 1–2 nm; lengths, 400–800 nm, 1–3 µm, or 5–30 µm); 1 and 100 µg/mL; 24 or 48 h	Investigation of intracellular production of ROS and gene expression profiling using a pathway-specific RT-PCR array of 84 oxidative stress and antioxidant defence pathway genes ROS detected in cells treated with the two shortest SWCNT samples; with the 1–3-µm sample, several genes strongly upregulated, including EPHX2; expression level of few genes modified by the other SWCNT samples, but 400–800-nm SWCNT produced a low level of upregulation of CCS, MT3, and NOS2 All samples induced downregulation of GPx4, GPx7, and the peroxidase cytoglobin, and upregulation of NCF1	<a href="#">Manshian et al. (2013)</a>

**Table 4.16 Depletion of antioxidants in cultured cells exposed to carbon nanotubes (continued)**

Cell type	Type of carbon nanotube; treatment; duration	Assay/result	Reference
Human colon carcinoma HT29 cell line	SWCNT; nine concentrations from 0.01 ng to 0.2 µg/mL; 3 or 24h	Determination of total GSH levels The range of 0.0001–0.01 µg/mL increased intracellular total GSH levels in comparison with control cells (significant at 0.001 and 0.01 µg/mL) at 24 h	<a href="#">Pelka et al. (2013)</a>
Rat lung epithelial cells	SWCNT; 2.5, 5, and 10 µg/mL; up to 72 h	Determination of ROS (using the DCF assay), GSH, SOD-1, and SOD-2 levels ROS production was enhanced in a dose-dependent manner; GSH levels decreased in cells treated with 10 µg/mL for 6 h; SOD-1 and SOD-2 protein expression decreased after treatment for 24 h in comparison with control cells	<a href="#">Sharma et al. (2007)</a>
Rat PC12 cells (adrenal gland pheochromocytoma)	SWCNT; 5–600 µg/mL; 24 and 48 h	Induction of mitochondrial membrane damage and the formation of increased levels of ROS and MDA Decreased levels of GSH and decreased activities of SOD, GPx, and catalase were observed at cytotoxic concentrations in a concentration-dependent manner	<a href="#">Wang et al. (2011b)</a>
Rat PC12 cells (adrenal gland pheochromocytoma)	SWCNT; 50 µg/mL; 24 and 48 h	Determination of SOD, catalase, and GPx activities and of GSH content ROS generation enhanced in comparison with control cells, but MDA levels not modified; activities of SOD, catalase, and GPx were decreased	<a href="#">Wang et al. (2012b)</a>
RAW 264.7 macrophages	Unpurified (iron, 26.0 wt %) or purified (iron, 0.23 wt %) SWCNT; 0.12–0.5 mg/mL without or with zymosan (0.25 µg/mL); 1–2 h	Determination of specific free radical intermediates by EPR spectroscopy Neither purified nor unpurified SWCNT generated intracellular ROS In the presence of zymosan, production of HO• more effective with unpurified than with purified SWCNT; LPO enhanced and GSH content decreased; both levels decreased by the addition of SWCNT in comparison with macrophages stimulated with zymosan only	<a href="#">Kagan et al. (2006)</a>
Murine epidermal JB6 P+ cells	SWCNT; 0.06, 0.12, and 0.24 mg/mL; 24 h	A significant dose-dependent decrease in GSH observed after incubation of cells with partially purified SWCNT for 24 h; exposure to unpurified (iron, 30 wt%) SWCNT induced a greater reduction in GSH than exposure to partially purified (iron, 0.23 wt%) SWCNT	<a href="#">Murray et al. (2009)</a>

Table 4.16 (continued)

Cell type	Type of carbon nanotube; treatment; duration	Assay/result	Reference
Primary mouse embryo fibroblasts	SWCNT (or carbon black, silicon dioxide, and zinc oxide); 5, 10, 20, 50, and 100 µg/mL; 24 h	Measurement of intracellular ROS, GSH and MDA levels, and SOD activity ROS production enhanced with all particles, in a dose-dependent manner up to 50 µg/mL; dose-dependent decrease in intracellular GSH level and SOD activity observed in comparison with control cells; with SWCNT, LPO was significantly enhanced only at 100 µg/mL	<a href="#">Yang et al. (2009)</a>
Human epidermal keratinocytes	MWCNT; 0.4 mg/mL; 24 and 48h	Protein expression analysed by two-dimensional gel electrophoresis and mass spectrometry Significant differential expression of 152 proteins, among which the expression of SOD2 was decreased by 1.4- and 1.9-fold at 24 and 48 h, respectively, in comparison with untreated cells	<a href="#">Witzmann &amp; Monteiro-Riviere (2006)</a>
Human embryonic kidney HEK 293 cell line	MWCNT produced by electric arc process using graphite as a source (MWCNT1) and chemical vapour deposition using methane as hydrocarbon (MWCNT2); 10–100 µg/mL; for 48 h	Measurement of cellular levels of reduced GSH and MDA content The level of MDA increased and that of intracellular GSH decreased	<a href="#">Reddy et al. (2010b)</a>
Human monocyte THP-1 cells differentiated into macrophages with phorbol myristate acetate	Three types of MWCNT: long (approximately 50 µm) straight MWCNT (diameter, 20–100 nm; NT1), micron-sized aggregated relatively straight MWCNT (diameter, approximately 150 nm; NT2), and MWCNT with an aggregated entangled structure (individual diameter, approximately 20 nm; NT3); 62.5 µg/mL; 4 h Control particles of carbon black (diameter, 260 nm or 14 nm) and LFA (length, > 5 mm; diameter, < 200 nm); 62.5 µg/mL; 4 h	Determination of <i>GSTpi</i> and <i>HO-1</i> expression (RT-PCR), <i>HO-1</i> protein expression (ELISA), and GST activity <i>GSTpi</i> expression not modified after any treatment; <i>HO-1</i> expression enhanced by NT1 (significant) and NT2 (not significant), and reduced by NT3, 260-nm carbon black and LFA; <i>HO-1</i> protein and GST activity (no significant difference)	<a href="#">Brown et al. (2010)</a>
Human umbilical vein endothelial cells	MWCNT: 0, 5, and 20 µg/mL, 2 h	Measurement of LPO and antioxidant levels: MDA, SOD and GPx; the antioxidant NAC was used to explore the involvement of ROS in cell injury ROS levels significantly increased by 20 µg/mL MWCNT; SOD and GPx activities enhanced at the lowest doses, but reduced at 20 µg/mL; pretreatment with NAC reduced ROS in comparison with exposure to MWCNT only	<a href="#">Guo et al. (2011)</a>

**Table 4.16 Depletion of antioxidants in cultured cells exposed to carbon nanotubes (continued)**

Cell type	Type of carbon nanotube; treatment; duration	Assay/result	Reference
Human embryonic kidney HEK 293 cell line	Four different MWCNT (CNM1, CNM2, CNM4, and CNM3; size: 100–800, 200–500, 150–750, and 230–1700 nm, respectively); 3, 10, 30, 100, and 300 µg/mL; 48 h	Measurement of cellular levels of GSH (colorimetric assay) and LPO (MDA content) Cytotoxicity and oxidative stress induced in a concentration-dependent manner; increased MDA content and decreased intracellular GSH levels observed with 30 and 100 µg/mL	<a href="#">Rama Narsimha Reddy et al. (2011)</a>
Human lung adenocarcinoma epithelial A549 cells	MWCNT; 0.5, 1, 5, 10, 50, and 100 µg/mL; 6–72 h	Measurement of cellular levels of GSH (colorimetric assay), LPO (Lipid Peroxidation Assay Kit), and catalase activity (commercial kit) Significant ROS production at 10 and 50 µg/mL; enhancement of LPO significant at all MWCNT concentrations (for 24 h); significant decreases in intracellular GSH level and catalase activity at 50 µg/mL	<a href="#">Srivastava et al. (2011)</a>
Human hepatoblastoma C3A cell line	Two types of MWCNT (NM 400, Nanocyl; diameter, 30 nm; length, 5 µm; and NM 402, Arkema Graphistrength C100; diameter, 30 nm; length, 20 µm); 0.5–256 µg/mL; 2–24 h	Determination of intracellular ROS level (using the DCF assay) and GSH (using OPT) Generation of ROS with both MWCNT and a decrease in total GSH and GSH cell content at 24 h; pretreatment with an antioxidant (Trolox) prevented MWCNT-induced ROS production	<a href="#">Kermanizadeh et al. (2012)</a>
Telomerase-immortalized human keratinocytes (N-hTERT)	MWCNT; 100 µg/mL; 30 min–24 h	Determination of intracellular ROS level and oxidative stress (using DCF oxidation), and reduced GSH (commercial kit) Increased DCF oxidation induced by MWCNT stirred or sonicated in water; in contrast, no effects seen with MWCNT sonicated in dispersive agents (HPC, Fagron or Pluronic FI08); addition of the antioxidant Trolox prevented DCF oxidation; significant reduction in GSH level compared with controls	<a href="#">Yankomingsloo et al. (2012)</a>
Rat glioma C6 cell line	MWCNT1 (diameter, 10–20 nm; average length, 2 µm) and MWCNT2 (diameter, 40–100 nm; average length, 10 µm); 25–400 µg/mL; 24 h	Determination of cytotoxicity, MDA levels and SOD activity MWCNT1 more cytotoxic than MWCNT2; increased levels of oxidative stress observed; both CNT induced a significant increase in MDA level (at 100 µg/mL) compared with that in controls and lowered the activity of SOD	<a href="#">Han et al. (2012)</a>

CCS, copper chaperone for superoxide dismutase; DCF, 2,2'-dichlorofluorescein; ELISA, enzyme-linked immunosorbent assay; EPHX2, epoxide hydrolase 2; ESR, electronic spin resonance; GPx, glutathione peroxidase; GR, glutathione reductase; GS, glutathione synthetase; GSH, reduced glutathione; GST, glutathione S-transferase; HO-1, haeme-oxygenase-1; HPC, hydroxypropylcellulose; iTRAQ-coupled 2D LC-MS/MS, iTRAQ-coupled two-dimensional liquid chromatography tandem mass spectrometry; LFA, long fibre amosite asbestos; LPO, lipid peroxidation; MDA, malondialdehyde; MT3, metallothionein 3; MWCNT, multiwalled carbon nanotubes; NAC, N-acetylcysteine; NOS2, nitric oxide synthase 2; NOX, NADPH-dependent oxidase; OPT, fluorescent substrate *o*-phthalaldehyde; ROS, reactive oxygen species; RT-PCR, real-time polymerase chain reaction; SOD, superoxide dismutase; SWCNT, single-walled carbon nanotubes; TBARS, thiobarbituric acid-reactive substance

decreased at 1000  $\mu\text{g/mL}$ . SOD activity was also increased at doses up to 100  $\mu\text{g/mL}$ . GPx activity was enhanced at the highest doses and GSH reductase at a dose of 1000  $\mu\text{g/mL}$ . GSH level was depleted at all doses tested (significant at 1000  $\mu\text{g/mL}$ ) ([Pichardo et al., 2012](#)). [These results showed the induction of antioxidant defences in response to exposure to F-SWCNT and an increase in lipid peroxidation products, possibly causing toxic effects.]

Intracellular generation of ROS and expression profiles of oxidative stress response genes were assessed in human bronchial epithelial BEAS-2B cells exposed to SWCNT. Three SWCNT samples were tested (diameter, 1–2 nm ; length, 400–800 nm, 1–3  $\mu\text{m}$  or 5–30  $\mu\text{m}$ ). Crocidolite asbestos fibres were used as positive control. Gene expression profiles were studied using a pathway-specific RT-PCR array (SuperArray) comprising primers for 84 oxidative stress and antioxidant defence pathway genes. All SWCNT samples and crocidolite asbestos fibres induced the formation of micronuclei; the generation of ROS was detected in cells exposed to the two shortest SWCNT samples and crocidolite asbestos fibres. The 1–3- $\mu\text{m}$  SWCNT sample upregulated the expression of several genes: epoxide hydrolase 2, surfactant protein D, and neutrophil cytosolic factor 1. Few genes showed differential expression with the other SWCNT samples; however, 400–800-nm SWCNT induced the upregulation of three proteins: copper chaperone for SOD, metallothionein 3, and nitric oxide synthase 2. Differentially expressed genes common to all SWCNT included the upregulation of titin and copper chaperone for SOD and downregulation of the GPxs, *GPX4* and *GPX7*, and the peroxidase cytoglobin that may be involved in protection against oxidative stress; neutrophil cytosolic factor 1 (required for the activation of latent NADPH oxidase, which is necessary for superoxide anion production) was also upregulated by all samples ([Manshian et al., 2013](#)).

The effects of SWCNT dispersed in sodium chololate on total GSH levels was evaluated in the human colon carcinoma HT29 cell line. Nine doses of SWCNT were tested (from 0.01 ng/mL to 0.2  $\mu\text{g/mL}$ ). Exposure to SWCNT in a dose range of 0.0001  $\mu\text{g/mL}$  to 0.01  $\mu\text{g/mL}$  increased the intracellular level of total GSH (significant at 0.001 and 0.01  $\mu\text{g/mL}$ ) ([Pelka et al., 2013](#)).

### (ii) MWCNT

Human epidermal keratinocytes were exposed to MWCNT manufactured using a microwave plasma-enhanced CVD system. After exposure to 0.4 mg/mL for 24 and 48 hours, proteins were extracted and analysed by two-dimensional gel electrophoresis and mass spectrometry. Of these, 152 were observed to be differentially expressed and to be associated with several pathways: metabolism, cell signalling, stress, the expression of cytoskeletal components, and vesicular trafficking. Among them, SOD2 protein was decreased by 1.4- and 1.9-fold at 24 and 48 hours, respectively, in comparison with untreated cells ([Witzmann & Monteiro-Riviere, 2006](#)).

MWCNT produced by an electric arc process using graphite as a source (MWCNT1) and by CVD using methane as the hydrocarbon (MWCNT2) were obtained from the Centre for Environment, Institute of Science and Technology, JNTU, Hyderabad, India. Cytotoxicity and oxidative stress were studied in the human embryonic kidney HEK 293 cell line. Cellular levels of reduced GSH and MDA content were measured to assess lipid peroxidation. Exposure of HEK 293 cells to MWCNT (10–100  $\mu\text{g/mL}$ ) for 48 hours resulted in concentration-dependent cytotoxicity, increased levels of MDA, and decreased intracellular levels of GSH ([Reddy et al., 2010b](#)). [These findings suggested that MWCNT induced oxidative stress and cytotoxicity in these target cells.]

Oxidative stress was studied after exposure to MWCNT of the human monocytic

THP-1 cell line differentiated into macrophages using phorbol myristate acetate. Three types of MWCNT were used: long straight MWCNT (length, approximately 50  $\mu\text{m}$ ; diameter, 20–100 nm; NT1); micron-sized aggregated MWCNT (relatively straight MWCNT; diameter, approximately 150 nm; NT2); and MWCNT with an aggregated entangled structure (individual diameter, approximately 20 nm; NT3). Control particles were carbon black 260 nm in diameter, carbon black 14 nm in diameter and long fibre amosite asbestos (length > 5  $\mu\text{m}$ ; diameter < 200 nm). Investigations included the assessment of gene expression using RT-PCR (GSH S-transferase [GST]pi and haeme oxidase 1[HO-1]), expression of HO-1 protein (enzyme-linked immunosorbent assay) and GST activity. After exposure of THP-1 cells to 62.5  $\mu\text{g}/\text{mL}$  of particles for 4 hours, GSTpi expression was not modified after any treatment; HO-1 expression was enhanced in cells exposed to NT1 (statistically significant) and NT2 (not statistically significant), and reduced in cells exposed to NT3, 260-nm carbon black and long fibre amosite asbestos. No significant difference from the control was observed with any of the treatments for either GST activity or HO-1 protein expression. However, an enhancement of the Nrf2 protein expression was found in the nucleus of cells treated with NT1, which was eradicated by the addition of antioxidants (Brown et al., 2010). [This study suggested that the activation of the antioxidant response pathway is mediated by the antioxidant response element and Nrf2.]

Human umbilical vein endothelial cells were exposed for 2 hours to 0, 5, and 20  $\mu\text{g}/\text{mL}$  of MWCNT (from Dr F. Chen, Lawrence Berkeley National Laboratory, Berkeley, CA) synthesized using a CVD method. Lipid peroxidation and antioxidant defences were determined by the quantification of MDA, and the activity of SOD and GPx. These cells were also incubated with the antioxidant NAC to explore the role of ROS in the induction of cell injury. Cellular ROS

levels were significantly increased by exposure to 20  $\mu\text{g}/\text{mL}$  of MWCNT. The activities of SOD and GPx were enhanced at the lowest concentrations but reduced at 20  $\mu\text{g}/\text{mL}$ . When human umbilical vein endothelial cells were pretreated with NAC before exposure to MWCNT, ROS production was reduced compared with the groups exposed to MWCNT only, and cytotoxicity and DNA damage (DNA breakage by quantification of  $\gamma\text{H2AX}$  foci) were also reduced (Guo et al., 2011). [These results showed that depletion of antioxidants was associated, at least partially, with cytotoxicity and DNA damage.]

Cellular levels of GSH were determined in the human embryonic kidney HEK 293 cell line exposed to four MWCNT with different dimensions (CNM1, CNM2, CNM4, and CNM3; size: 100–800, 200–500, 150–750, and 230–1700 nm, respectively) from the Centre for Environment (Institute of Science and Technology, JNTU, Hyderabad). In-vitro exposure of HEK 293 cells to 3–300  $\mu\text{g}/\text{mL}$  of these MWCNT for 48 hours produced cytotoxicity and oxidative stress in a concentration-dependent manner. Increased lipid peroxidation (measured by MDA content) and decreased intracellular GSH levels were observed at concentrations of 30 and 100  $\mu\text{g}/\text{mL}$  (Rama Narsimha Reddy et al., 2011).

Human lung adenocarcinoma epithelial A549 cells were exposed to MWCNT (provided by Professor D.G. Weiss, Department of Biological Sciences, Institute of Cell Biology and Biosystems Technology, Rostock University, Germany) at 0.5–100  $\mu\text{g}/\text{mL}$  for 6–72 hours. Apoptotic cells were detected after exposure to 50  $\mu\text{g}/\text{mL}$  for 72 hours. Significant ROS production was found at doses of 10 and 50  $\mu\text{g}/\text{mL}$ , which was not due to mitochondrial activity. Lipid peroxidation was determined using a Lipid Peroxidation Assay Kit, and GSH levels determined using a colorimetric assay. Increased lipid peroxidation was significant at all concentrations of MWCNT after 24 hours of treatment, and both intracellular GSH levels

and catalase activity were significantly reduced at a dose of 50 µg/mL ([Srivastava et al., 2011](#)).

The effects of two types of MWCNT – NM 400 (Nanocyl; diameter, 30 nm; length, 5 µm) and NM 402 (Arkema Graphistrength C100 ; diameter, 30 nm; length, 20 µm) – were assessed in the human hepatoblastoma C3A cell line. Intracellular ROS generation was measured using the 2,7-dichlorofluorescein diacetate assay, and GSH was quantified by the reaction of sulfhydryl groups with the fluorescent substrate ortho-phthalaldehyde. Both samples of MWCNT induced the generation of ROS and decreased total GSH and cellular GSH content after 24 hours. Pretreatment with the antioxidant, Trolox, prevented MWCNT-induced production of ROS. Cytotoxicity was also reduced in C3A cells pretreated with Trolox before exposure to MWCNT ([Kermanizadeh et al., 2012](#)).

The intracellular level of GSH was quantified in telomerase-immortalized human keratinocytes (N-hTERT) exposed to 100 µg/mL of MWCNT (Nanocyl™ NC7000 MWCNT from Nanocyl, produced by catalytic CVD) for 30 minutes or 24 hours. Oxidative stress was assessed using 2,7-dichlorofluorescein oxidation. MWCNT stirred or sonicated in water were marked by increased dichlorofluorescein fluorescence, suggesting an increased intracellular generation of ROS. In contrast, MWCNT sonicated in dispersants (HPC, Fagron, or Pluronic F108) showed no significant effects. The addition of the antioxidant, Trolox, a hydrophilic analogue of the lipophilic antioxidant vitamin E, prevented 2,7-dichlorofluorescein oxidation. Moreover, GSH was significantly decreased compared with untreated controls ([Vankoningsloo et al., 2012](#)).

(c) *Experimental systems in vivo*

See [Table 4.17](#)

(i) *SWCNT*

C57BL/6 mice were maintained on vitamin E-sufficient or vitamin E-deficient diets and were exposed to SWCNT (CNI, Inc.) by pharyngeal aspiration. Antioxidant levels were determined in lung homogenates 28 days after exposure to 40 µg/mouse of SWCNT. Treatment with SWCNT induced greater increase in lipid peroxidation products and greater decrease in GSH levels in mice fed a vitamin E-deficient diet than in those fed basal diet, showing that SWCNT produced antioxidant depletion which was associated with a higher sensitivity to SWCNT-induced acute inflammation ([Shvedova et al., 2007](#)).

C57BL/6 mice were exposed to 5 mg/m<sup>3</sup> of SWCNT in inhalation chambers for 5 hours per day for 4 days. The level of oxidative damage produced was measured in the lung homogenates. GSH levels were significantly depleted, and the level of lipid peroxidation products – measured as malondialdehyde (MDA) – showed a significant accumulation compared with controls 7 and 28 days after exposure. Total antioxidant capacity was reduced 1 and 7 days after treatment, but returned to the control level by 28 days after exposure ([Shvedova et al., 2008](#)).

SKH-1 immune competent hairless mice were exposed to SWCNT (CNI, Inc.) by daily skin application at doses of 40, 80, or 160 µg/mouse for 5 days. A reduction in GSH levels was found in skin homogenates of mice treated with the highest dose, but no change was found with the other doses ([Murray et al., 2009](#)).

Male BALB/c mice were exposed to 5 µg/g bw of aerosolized SWCNT (diameter, 1–2 nm; length, 0.5–2.0 µm; from Aldrich) in PBS or PBS only, for 20 minutes per day on 7 consecutive days in a nose-only exposure system. The animals were killed at the end of the exposure period and lung tissues were collected. The intracellular levels of MDA and ROS, and the activities of SOD, catalase, and GPx were measured. Apoptosis was assessed by the measurement of

**Table 4.17 Depletion of antioxidants in experimental animals exposed to carbon nanotubes**

Species, strain (sex)	Route of administration exposure concentration or dose, duration	Recovery	Assay/result	Reference
Mouse, C57BL/6 fed vitamin E-sufficient or vitamin E-deficient diets (F)	Pharyngeal aspiration SWCNT: single dose of 40 µg/mouse	28 days	Measurement of lipid peroxidation products and GSH levels in lung homogenates SWCNT produced antioxidant depletion; higher increase in lipid peroxidation products and greater decrease in GSH levels found in mice fed vitamin E-deficient diets	<a href="#">Shvedova et al. (2007)</a>
Mouse, C57BL/6 (F)	Inhalation (in inhalation chambers) SWCNT: 5 mg/m <sup>3</sup> , 5 h/day, 4 days	1, 7 or 28 days	Level of oxidative damage (lipid peroxidation measured as MDA and GSH levels) measured in lung homogenates Significant accumulation of lipid peroxidation products compared with controls 7 and 28 days after exposure; GSH levels significantly depleted; total antioxidant capacity reduced 1 and 7 days after treatment, but returned to the control level by 28 days after exposure	<a href="#">Shvedova et al. (2008)</a>
Mouse, SKH-1 immune competent hairless (NR)	Skin application SWCNT: 40, 80, or 160 µg/mouse, once/day, 5 days	24 h	Measurement of GSH levels in skin homogenates Reduction of GSH levels in mice treated with the highest dose; no change with the other doses	<a href="#">Murray et al. (2009)</a>
Mouse, BALB/c (M)	Inhalation (nose-only exposure) SWCNT: 5 µg/g bw, 20 min/day, 7 days	None	Measurement of intracellular levels of MDA and ROS, and activities of SOD, catalase, and GPx in the lung; caspase-3 and -8 activities for apoptosis in lung homogenates; and MPO activity in BALF ROS and MDA levels significantly higher in the lungs of SWCNT-exposed mice versus controls; reduced activities of SOD, catalase, and GPx; apoptosis induction; MPO activity higher in the BALF from SWCNT-exposed than control animals	<a href="#">Ravichandran et al. (2011)</a>
Rat, Wistar albino (M)	Intratracheal instillation Two MWCNT samples: single dose of 0.2, 1, or 5 mg/kg bw or quartz-crystalline silica particles (positive control)	1, 7, 30, and 90 days	Antioxidant capacity determined in blood samples: measurement of GSH, lipid peroxidation product (MDA), and SOD and catalase activities Total antioxidant capacity assessed by the ability to scavenge the free radical, α,α-diphenyl-β-picryl hydrazyl With both MWCNT and quartz: significant dose-dependent depletion of GSH levels and decrease in SOD activity; transient dose-dependent decrease in catalase activity; dose-dependent increase in amount of MDA 1 day after instillation, that decreased later; decreased total antioxidant capacity	<a href="#">Reddy et al. (2011)</a>
Rat, Wistar (M)	Intraperitoneal injection MWCNT functionalized with single-strand DNA: single dose of 270 mg/L		Levels of GSH measured in the plasma and liver after 1, 3, 6, 24, 48, or 144 h Significant decrease in the levels of GSH in plasma at all time points after exposure, and in the liver after 3 and 24 h, but not at 48 or 144 h; GSH level returned to normal within 6 days; decreased activity of MnSOD (SOD2) in the liver at 1, 24, and 48 h, but not at 144 h	<a href="#">Clichici et al. (2012)</a>

Table 4.17 (continued)

Species, strain (sex)	Route of administration exposure concentration or dose, duration	Recovery	Assay/result	Reference
Mouse, BALB/c (M)	Inhalation (nose-only exposure) MWCNT: 5 µg/g bw, 20 min/day, 7 days	None	Measurement of intracellular levels of MDA and ROS, and activities of SOD, catalase and GPx in the lung; caspase-3 and -8 activities for apoptosis in lung homogenates; and MPO activity in BALF ROS and MDA levels significantly higher in lungs of MWCNT-exposed mice; reduced activities of SOD, catalase, and GPx; apoptosis induction; MPO activity higher in the BALF from MWCNT-exposed mice	<a href="#">Ravichandran et al. (2011)</a>
Mouse, pregnant heterozygous <i>p53</i> ( <i>p53<sup>+/-</sup></i> )	Intravenous injection MWCNT: 2 mg/kg bw on gestational days 10.5, 12.5, and 15.5		Investigation of MEF Treatment of dams with MWCNT and an antioxidant, <i>N</i> -acetylcysteine, abolished MWCNT-only induced DNA breakage observed in MEF	<a href="#">Huang et al. (2014)</a>

BALF, bronchoalveolar lavage fluid; bw, body weight; GPx, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; MEF, mouse embryo fibroblasts; MnsOD, manganese superoxide dismutase; MPO, myeloperoxidase; MWCNT, multiwalled carbon nanotubes; NR, not reported; ROS, reactive oxygen species; SOD, superoxide dismutase; SWCNT, single-walled carbon nanotubes

caspace-3 and -8 activities in lung homogenates. MPO activity was measured in the BALF. MPO activity was higher in the BALF from SWCNT-exposed mice compared with controls. ROS and MDA levels were significantly higher in the lungs of SWCNT-exposed mice compared with controls, and SOD, catalase, and GPx were reduced. In parallel, apoptosis was demonstrated by the enhancement of caspace-3 and -8 activities ([Ravichandran et al., 2011](#)).

(ii) *MWCNT*

The antioxidant status of rat serum was evaluated after intratracheal instillation of MWCNT into male Wistar albino rats. Two MWCNT samples were used (from the Centre for Environment, Institute of Science and Technology, JNTU, Hyderabad), produced either by the electric arc process using graphite as a source or CVD using methane as the hydrocarbon. The rats received a single dose of 0.2, 1, or 5 mg/kg bw of MWCNT or quartz-crystalline silica particles (positive control). Blood samples were collected at 1, 7, 30, and 90 days after the instillation. Antioxidant capacity was determined by the measurement of GSH, a lipid peroxidation product (MDA), and SOD and catalase activities. Both MWCNT induced a significant dose-dependent depletion of GSH levels, decrease in SOD activity, and a transient dose-dependent decrease in catalase activity. Similarly, the amount of MDA was increased by both MWCNT in a dose-dependent manner 1 day after instillation and later decreased. Total antioxidant capacity, assessed by the ability to scavenge the free radical  $\alpha,\alpha$ -diphenyl- $\beta$ -picryl hydrazyl, was decreased after exposure to MWCNT ([Reddy et al., 2011](#)). [These results indicated a reduction in antioxidant defence mechanisms after an instillation of MWCNT.]

Wistar rats received a single intraperitoneal injection of 270 mg/L of MWCNT (exterior diameter, 15–25 nm; interior diameter, 10–15 nm; surface, 88 m<sup>2</sup>/g) synthesized by the

CVD technique and functionalized with single-strand DNA. The level of GSH was measured in the plasma and liver 1, 3, 6, 24, 48, and 144 hours later. A significant decrease in the level of GSH was observed in the plasma at all timepoints after exposure, and after 3 and 24 hours (but not after 48 or 144 hours) in the liver. This result was consistent with a depletion of antioxidants by this single-strand DNA–MWCNT sample. The GSH level returned to normal within 6 days. The activity of manganese SOD (*SOD2*) in the liver was decreased after 1, 24, and 48 hours, but not after 144 hours ([Clichici et al., 2012](#)). [These results could be consistent with a decrease in antioxidant defence after exposure to this single strand DNA–MWCNT sample.]

In a transplacental study carried out on mouse embryo fibroblasts from fetuses of p53<sup>+/-</sup> heterozygous mice, the treatment of dams with MWCNT and an antioxidant, NAC, abolished the MWCNT-only induced DNA breakage observed in these cells ([Huang et al., 2014](#)). [This result was consistent with the generation of ROS in cells exposed to MWCNT.]

Male BALB/c mice were exposed daily to 5  $\mu$ g/g bw of aerosolized MWCNT (diameter, 20–50 nm; length, 6–13 nm; from Sigma) in PBS or to PBS only for 20 minutes on 7 consecutive days, in a nose-only exposure system. Animals were killed at the end of the exposure period and lung tissues were collected. The intracellular levels of MDA and ROS, and the activities of SOD, catalase, and GPx were measured. Apoptosis was assessed by the measurement of caspace-3 and -8 activities in lung homogenates. MPO activity was measured in the BALF. MPO activity was higher in the BALF from MWCNT-exposed mice compared with controls. ROS and MDA levels were significantly higher in the lungs of MWCNT-exposed mice compared with controls, and SOD, catalase, and GPx were reduced. In parallel, apoptosis was demonstrated by the enhancement of caspace-3 and -8 activities ([Ravichandran et al., 2011](#)).

(c) *Experimental systems in vitro*

See [Table 4.16](#)

(i) *SWCNT*

Rat lung epithelial cell cultures were exposed to SWCNT (Sigma Chemical Co.). The levels of ROS (2,7-dichlorofluorescein diacetate assay), GSH content, and the levels of SOD1 and SOD2 antioxidant enzymes were quantified. The results showed the production of ROS in a concentration-dependent manner. GSH levels were decreased in cells treated with 10  $\mu\text{g}/\text{mL}$  for 6 hours. Expression of SOD1 and SOD2 proteins was decreased after 24 hours in comparison with control cells ([Sharma et al., 2007](#)). [Globally, exposure to SWCNT induced oxidative stress and depletion of antioxidants.]

Rat adrenal gland pheochromocytoma PC12 cells were exposed to SWCNT (diameter, 1–2 nm; length,  $\sim 20 \mu\text{m}$ ) (Beijing Nachen Technology & Development Co. Ltd, Beijing, China) at concentrations of 5–600  $\mu\text{g}/\text{mL}$  for 24 and 48 hours. Exposure to SWCNT induced mitochondrial membrane damage, the formation of ROS, and increased levels of the lipid peroxidation product MDA. GSH levels, and activities of SOD, GPx, and catalase were decreased at cytotoxic concentrations in a concentration-dependent manner ([Wang et al., 2011b](#)). [These findings revealed that SWCNT induced oxidative stress in these target cells.]

The effects of SWCNT (diameter, 1–2 nm; length,  $\sim 20 \mu\text{m}$ ; Beijing Nachen Technology & Development Co. Ltd) were studied in rat adrenal gland pheochromocytoma PC12 cells. The activities of catalase, SOD, and GPx and the GSH content were determined 24 and 48 hours after exposure to 50  $\mu\text{g}/\text{mL}$ . The generation of ROS was enhanced in SWCNT-treated PC12 cells, but the level of the lipid peroxidation product, MDA, did not appear to be elevated. The activities of SOD, catalase, and GPx were all decreased ([Wang et al., 2012b](#)).

Oxidative stress was assessed in RAW 264.7 macrophages exposed to SWCNT produced by the HiPCO disproportionation technique, with iron carbonyl as the iron-containing catalyst precursor (CNI, Inc.). The SWCNT used were unpurified (iron, 26.0 wt%) or purified (iron, 0.23 wt%) to determine the effects of iron. Specific free radical intermediates produced by RAW 264.7 cells exposed to 0.12–0.5  $\text{mg}/\text{mL}$  for 1–2 hours were determined using electron paramagnetic resonance spectroscopy. Neither purified nor unpurified SWCNT induced the intracellular production of superoxide radicals or nitric oxide in RAW 264.7 macrophages. The production of radicals was observed when RAW 264.7 cells were stimulated with zymosan (0.25  $\text{mg}/\text{mL}$ ), an agent known to activate the generation of ROS in macrophages. Under these conditions, HO $\bullet$  production was enhanced in zymosan-treated cells, and unpurified iron-rich SWCNT were more potent than purified SWCNT. Lipid peroxidation assessed by MDA levels was enhanced and GSH content was decreased in zymosan-stimulated RAW 264.7 macrophages. The addition of SWCNT lowered both lipid peroxidation and GSH content in comparison with zymosan-stimulated macrophages ([Kagan et al., 2006](#)).

Murine epidermal JB6 P+ cells were exposed to SWCNT (CNI, Inc.) produced by the HiPCO disproportionation process. A significant concentration-dependent decrease in GSH content was observed after a 24-hour incubation of JB6 P+ cells with 0.06  $\text{mg}/\text{mL}$ , 0.12  $\text{mg}/\text{mL}$ , and 0.24  $\text{mg}/\text{mL}$  of partially purified SWCNT. Exposure to unpurified (iron, 30 wt%) SWCNT induced a greater reduction in GSH than exposure to partially purified (iron, 0.23 wt%) SWCNT ([Murray et al., 2009](#)).

Primary mouse embryo fibroblasts were exposed to various manufactured nanoparticles: SWCNT (diameter, 8 nm; length  $< 5 \mu\text{m}$ ), carbon black, silicon dioxide, and zinc oxide. Intracellular generation of ROS, GSH and MDA levels, and SOD activity were determined after exposure to

particle suspensions at doses of 5, 10, 20, 50, and 100 µg/mL for 24 hours. ROS production was enhanced by all particles in a concentration-dependent manner up to 50 µg/mL. Intracellular GSH levels decreased dose-dependently and the activity of SOD was decreased in treated fibroblasts in comparison with untreated control cells. Lipid peroxidation was significantly enhanced in cells exposed to SWCNT at a dose of 100 µg/mL only. In this study, SWCNT exhibited greater genotoxicity than zinc oxide nanoparticles, although zinc oxide induced more oxidative stress ([Yang et al., 2009](#)).

(ii) *MWCNT*

Two types of MWCNT – MWCNT1 (diameter, 10–20 nm; average length, 2 µm) and MWCNT2 (diameter, 40–100 nm; average length, 10 µm) – were studied in the C6 rat glioma cell line with regard to effects on MDA levels and SOD activity. Exposure of C6 rat glioma cells to MWCNT (25–400 µg/mL) for 24 hours resulted in an increased level of oxidative stress, and MWCNT1 was more cytotoxic than MWCNT2. MDA levels increased significantly after treatment with 100 µg/mL of both MWCNT1 and MWCNT2 compared with those in untreated controls. SOD activity was decreased by both MWCNTs ([Han et al., 2012](#)).

#### 4.4.11 *Activation of oncogenes and inactivation of tumour-suppressor genes*

The expression of an important number of oncogenes and tumour-suppressor genes has been analysed in CNT-exposed experimental animals. Overall, most of these genes had different expression levels compared with unexposed control animals.

Lists of oncogenes, tumour-suppressor genes and cancer genes are available in the supplementary tables in [Vogelstein et al. \(2013\)](#).

(a) *Exposed humans*

No data were available to the Working Group.

(b) *Human cells in vitro*

See [Table 4.18](#)

(i) *SWCNT*

Cell-cycle regulation was investigated using microarray analysis in human embryo kidney HEK 293 cells exposed to 25 µg/mL of SWCNT (Carbon Nanotechnologies, Inc.) for 48 hours. Under these experimental conditions, cell viability was approximately 84%. Exposure to SWCNT was associated with the induction of apoptosis and cell-cycle control genes, and several oncogenes or tumour-suppressor genes were either downregulated (e.g. *CDK2*, *CDK4*, *CDK6*), or upregulated (e.g. *CDKN2A*, *TP53*), consistent with the activation of an apoptotic response and cell-cycle arrest ([Cui et al., 2005](#)). [A discrepancy was noted by the Working Group between the text and Table 2 in [Cui et al. \(2005\)](#) regarding the up- or downregulation of *TP53*.]

Human BJ foreskin CRL-2522 cells were exposed to 0 (control) or 6 µg/mL of SWCNT (Sigma) in dimethylformamide vehicle for 24 hours. Gene expression was assessed using a Stress and Toxicity Array (Super Array, Frederick, MD, USA) and was altered in 96 genes in SWCNT-treated cells compared with controls; 28 of these genes showed significant upregulation, with a ratio ranging from 1.5 to 3. The altered genes were involved in several pathways – apoptosis, xenobiotic metabolism, DNA repair, and oxidative stress – and may represent potential oncogenes or tumour-suppressor genes (i.e. *DNAJB4*, *ATM*, *CCNC*) ([Sarkar et al., 2007](#)). [These genes play a role in the response to stress, DNA repair and apoptosis, and cell-cycle progression. Their activation in SWCNT-exposed cells was not indicative of damage to these cells but signified the activation of defence mechanisms by BJ cells.]

**Table 4.18 Expression of genes and proteins in human cells and experimental systems exposed to carbon nanotubes in vitro**

Cell types	Type of carbon nanotube: treatment	Assay/result	Reference
Human embryo kidney HEK 293 cells	Unpurified SWCNT: 25 µg/mL, 48 h	Cell cycle regulation investigated by microarray analysis Several oncogenes or tumour-suppressor genes such as <i>CDK2</i> , <i>CDK4</i> , and <i>CDK6</i> , downregulated; <i>CDKN2A</i> and <i>TP53</i> , upregulated [discrepancy between the text and Table 2 in <a href="#">Cui et al. (2005)</a> in which <i>TP53</i> was downregulated]	<a href="#">Cui et al. (2005)</a>
Human BJ foreskin CRL-2522 cells	Unpurified SWCNT: 6 µg/mL, 24 h	Gene expression studied using Stress and Toxicity Array Modification of the expression of 96 genes in SWCNT-treated cells compared with controls, of which 28 showed significant upregulation; some genes may play a role as potential oncogenes or tumour-suppressor genes: <i>DNAJB4</i> (Dna) [Hsp40 homologue), <i>ATM</i> (ataxia telangiectasia mutated), and <i>CCNC</i> (cyclin C)	<a href="#">Sarkar et al. (2007)</a>
Human bronchial epithelial BEAS-2B cells	Purified SWCNT: 0.02 µg/cm <sup>2</sup> continuously, passaged weekly, 24 wk	Determination of p53 status by immunoblotting, using human apoptosis array After 24 wk of exposure, SWCNT-treated cells showed morphological features of malignant transformation Different expression profiles of apoptosis proteins in transformed SWCNT-treated cells compared with controls: differential expression of the phosphorylated forms of p53, which was lower in transformed SWCNT-treated BEAS-2B cells than in controls	<a href="#">Wang et al. (2011a)</a>
Human colon carcinoma HT29 cell line	Purified SWCNT: eight concentrations from 0.05 ng/mL to 0.2 µg/mL, 3 or 24 h	Investigation of p53 phosphorylation by Western blot Induction of phospho-p53 observed at concentrations ≥ 5 ng/mL but declined at higher concentrations (0.1 and 0.2 µg/mL)	<a href="#">Pelka et al. (2013)</a>
Human pleural mesothelial MeT-5A and peritoneal mesothelial LP-9 cells	Unpurified SWCNT: 0.02, 0.06, and 0.2 µg/cm <sup>2</sup> continuously, 2 mo	Protein expression of HRAS by Western blot, and activation of downstream signalling of the <i>HRAS</i> pathway Phenotypical changes characteristic of neoplastic transformation (cell growth in soft agar and invasion capability) Enhancement of HRAS protein expression and activation of the ERK1/2 pathway associated with more neoplastic phenotypes Exposure to SWCNT enhanced the expression of AKT (potential oncogenic protein) and lowered the expression level of genes involved in the epithelial-mesenchymal transition process ( <i>TWIST</i> and <i>SNAI1</i> , [discrepancy between the text and Figure 5])	<a href="#">Lohcharoenkai et al. (2014)</a>
Murine monocytic RAW 264.7 macrophages	AF-SWCNT: 0, 1, 10, and 50 µg/mL, 24 h	Determination of gene expression profiles by cDNA microarray A total of 130 genes differentially expressed; 126 were underexpressed and 4 were overexpressed, and <i>CHEK1</i> (potential tumour-suppressor gene) was downregulated	<a href="#">Dong et al. (2012)</a>
Human skin HSF42 and human embryonic lung IMR-90 fibroblasts	MWCNO and unpurified MWCNT: 0.6 and 6 µg/mL, and 0.06 and 0.6 µg/mL, respectively, 48 h	Whole genome expression array Modification of the expression of numerous genes with both particles; no evidence of oncogene activation or tumour-suppressor gene inactivation seen with MWCNT	<a href="#">Ding et al. (2005)</a>

**Table 4.18 Expression of genes and proteins in human cells and experimental systems exposed to carbon nanotubes in vitro (continued)**

Cell types	Type of carbon nanotube: treatment	Assay/result	Reference
Human lung adenocarcinoma A549 cells	Unpurified MWCNT: 1–50 µg/mL, 3, 6, 12, 24, and 48 h	Transcriptional gene expression determined by semiquantitative PCR analysis; protein expression by Western blot Increase in mRNA expression of <i>TP53</i> , <i>CDKN1A</i> , and <i>BAX</i> , in comparison with untreated cells at 10 and 50 µg/mL and decreased levels of <i>BCL2</i> ; protein levels were similarly modified	<a href="#">Srivastava et al. (2011)</a>
Normal human bronchial epithelial cell line	Unpurified MWCNT or crocidolite: 0.01–0.1%, 24 or 48 h	Gene expression analysis with whole human genome microarray (44 K) Upregulation of 1201 and 1252 genes by both asbestos and MWCNT observed after 6 and 24 h of exposure, respectively, and downregulation of 1977 and 1542 genes, respectively When compared with a list of genes known to be deregulated in human malignant mesothelioma or lung cancer, 12 and 22 genes were modulated by exposure to both MWCNT and crocidolite in malignant mesothelioma and lung cancers, respectively. Genes included oncogenes, tumour-suppressor genes or potential tumour-suppressor genes; <i>CDKN2A</i> , was downregulated, <i>CITGF</i> was upregulated, and <i>BCL2</i> oncogene expression was enhanced, similarly to both malignant mesothelioma and lung cancer	<a href="#">Kim et al. (2012b)</a>
Human embryonic kidney epithelial HEK 293 cells, mouse mesenchymal C2C12 stem cells, and human neuroblastoma NB1691 cells	Purified carboxylated MWCNT: 100 µg/mL, 24 h	Expression of proteins by Western blot and mRNA expression by RT-PCR Expression of p21 protein enhanced in MWCNT-treated proliferating C2C12, HEK 293, and NB1691 cells; no change in p53 protein expression observed; enhancement of the unphosphorylated form of pRb and of <i>CDKN1A</i> gene expression found	<a href="#">Zhang &amp; Yan (2012)</a>
Telomerase-immortalized human keratinocytes (N-hTERT)	Unpurified MWCNT: 100 µg/mL, 24 h	Small-scale transcriptomic TLDA profiling Different expression levels of 46 mRNAs observed in treated cells compared with untreated cells; enhancement of <i>BCL2</i> expression	<a href="#">Vankoningsloo et al. (2012)</a>
Rat PC12 cells (adrenal gland pheochromocytoma)	Unpurified SWCNT: 50 µg/mL, 24 or 48 h	Determination of the expression of proteins involved in apoptosis by flow cytometry, and of caspase activation Decreased expression of Bcl-2 observed in comparison with control cells	<a href="#">Wang et al. (2012b)</a>
Rat lung epithelial RL 65 cells	Unpurified MWCNT: 5 µg/mL, 12 h	Analysis of protein expression levels by Western blot Increased levels of p53, p21 <sup>Cip1/Waf1</sup> , and bax proteins found in MWCNT-treated cells	<a href="#">Ravichandran et al. (2010)</a>
Mouse embryonic J11 stem cells	Purified MWCNT: 5 and 100 µg/mL, 2, 4, and 24 h	Analysis of p53 protein expression levels by Western blot Expression of p53 protein observed within 2 h of exposure; p53 expression levels increased proportionally with the amount of MWCNT	<a href="#">Zhu et al. (2007)</a>

Table 4.18 (continued)

Cell types	Type of carbon nanotube: treatment	Assay/result	Reference
Co-culture of mouse leukaemic monocyte RAW 264.7 macrophage and mouse embryonic fibroblast NIH 3T3 cell lines	Short or long unpurified MWCNT: 15 µg/mL, 24 h; RAW 264.7 cells seeded in the well below were treated first for 24 h and then co-cultured with NIH 3T3 cells attached on the top of the insert for another 24 h	Expression of <i>TGFβ1</i> measured by RT-PCR analysis Upregulation of mRNA levels of <i>TGFβ1</i> greater with long MWCNT than with short MWCNT; in parallel, long MWCNT induced more TGF-β1 protein than short MWCNT	<a href="#">Wang et al. (2013)</a>
Lung epithelial FE1 cells	Unpurified MWCNT (Mitsui 7): 12.5, 25, or 100 µg/mL, 24 h	Microarray and pathway analyses Among the upregulated genes, several were known or potential oncogenes, or genes involved in cancer, such as <i>Jun</i> , <i>Ddit3</i> , and <i>Hmga2</i> , or <i>Ctgf</i> , <i>Runx1</i> , and <i>Fosl1</i> , some of which may also have suppressor functions in specific systems Among the downregulated genes, several were known or potential tumour-suppressor genes, or genes involved in cancer ( <i>Pdgfr1</i> , <i>Id4</i> , <i>Cdkn2c</i> , <i>Cdkn2d</i> , or <i>Tgfb2</i> , <i>Gstm2</i> , and <i>Gstt1</i> ), some of which may also have oncogenic functions in specific systems	<a href="#">Søs Poulsen et al. (2013)</a>

AF-SWCNT, acid-functionalized, further purified SWCNT; mo, month; MWCNO, multiwalled carbon nano-onions; MWCNT, multiwalled carbon nanotubes; RT-PCR, real-time polymerase chain reaction; SWCNT, single-walled carbon nanotubes; TGF, tumour growth factor; TLDA, Taghmon low density array; wk, week

The effects of SWCNT (CNI, Inc.) produced by the HiPCO technique were evaluated in human bronchial BEAS-2B cells using the Human Apoptosis Array (R&D Biosystems) which detects the 35 most common apoptosis-regulatory proteins by immunoblotting. The cells were continuously exposed to a subcytotoxic concentration ( $0.02 \mu\text{g}/\text{cm}^2$ ) of SWCNT in culture and were passaged weekly. After 24 weeks of exposure, SWCNT-treated cells showed the morphological features of malignant transformation. Transformed SWCNT-exposed cells exhibited differential expression of apoptosis-related proteins compared with controls. A differential expression of the phosphorylated forms of p53 was observed which was lower in SWCNT-treated cells than in untreated cells. [Because the phosphorylation of p53 is an indicator of the activation of the p53 tumour suppressor, these results suggested a loss of p53 activity.] (Wang et al., 2011a). [This SWCNT sample can be assumed to have impaired the apoptotic potential of p53. However, these results should be interpreted with caution because BEAS-2B cells are immortalized with SV40 viral oncoproteins and express large SV40 T-antigen, a protein that binds to and inactivates p53 protein.]

Toxicity (cell growth, ROS production, DNA damage assessed by the comet and micronucleus assays, and p53 induction) of SWCNT dispersed in sodium cholate was studied in the human colon carcinoma HT29 cell line. Eight doses from  $0.05 \text{ ng}/\text{mL}$  to  $0.2 \mu\text{g}/\text{mL}$  were tested. Phosphorylation of the tumour-suppressor protein p53 was investigated in exposed and untreated control cells using Western blot analysis. After 3 and 24 hours of exposure, the phospho-p53 protein was induced at concentrations  $\geq 5 \text{ ng}/\text{mL}$ . A decline was observed at higher concentrations ( $0.1$  and  $0.2 \mu\text{g}/\text{mL}$ ) (Pelka et al., 2013). [These results are consistent with the activation of DNA repair at subcytotoxic doses.]

Human mesothelial cells were continuously exposed to SWCNT synthesized by HiPCO (CNI,

Inc.) at concentrations of  $0.02$ ,  $0.06$ , or  $0.2 \mu\text{g}/\text{cm}^2$  for 2 months. Expression of the *HRAS* oncogene was assessed using HRAS protein analysis by Western blot and the activation of downstream signalling of the *HRAS* pathway. In parallel, phenotypical changes characteristic of neoplastic transformation were studied (cell growth in soft agar and invasion capability). Increased HRAS protein expression and activation of the ERK1/2 pathway were found to be associated with more neoplastic phenotypes. SWCNT enhanced the expression of a potential oncogenic protein (AKT) and downregulated expression of genes (*TWIST* and *SNAI1*) known to be involved in the epithelial–mesenchymal transition process (Lohcharoenkal et al., 2014).

#### (ii) MWCNT

A whole genome expression array (GeneChip® assay) was performed using human skin HSF42 fibroblasts and human embryonic lung IMR-90 fibroblasts exposed for 48 hours to  $0.6$  and  $6 \mu\text{g}/\text{mL}$  of multiwalled carbon nano-onions and  $0.06$  and  $0.6 \mu\text{g}/\text{mL}$  of MWCNT synthesized by the CVD method. Numerous genes showed changes in expression after treatment with the different particles. Similar to multiwalled carbon nano-onions, exposure to MWCNT upregulated the expression of genes involved in pathways related to cellular transport, metabolism, cell-cycle regulation, and stress response, but no evidence of oncogene activation or tumour-suppressor gene inactivation was found (Ding et al., 2005).

Human lung adenocarcinoma A549 cells were exposed to  $1$ – $50 \mu\text{g}/\text{mL}$  of MWCNT (provided by Professor D.G. Weiss, Department of Biological Sciences, Institute of Cell Biology and Biosystems Technology, Rostock University, Germany) for 3, 6, 12, 24, and 48 hours. Gene expression was analysed using semiquantitative PCR (RT-PCR). mRNA expression of *TP53* and *CDKN1A* (that encode p53 and p21<sup>Cip1/Waf1</sup>, respectively) and the apoptotic gene *BAX*, was

increased in comparison with untreated cells at doses of 10 and 50 µg/mL, and the expression of the anti-apoptotic and potential oncogene *BCL2* was decreased. Protein levels were determined using Western blot analysis and confirmed differential mRNA expression. Apoptotic cells were detected after exposure to a dose of 50 µg/mL of MWCNT for 72 hours ([Srivastava et al., 2011](#)). [These results were consistent with a change in the expression of tumour-suppressor genes/ oncogenes related to the induction of apoptosis.]

Normal human bronchial epithelial cells were exposed to MWCNT or crocidolite asbestos fibres at doses of 0.01–0.1% for 24 or 48 hours. Gene expression was investigated using the Whole Human Genome Microarray (44 K) (Agilent Technology). A total of 1201 and 1252 genes were upregulated and 1977 and 1542 genes were downregulated by both asbestos and MWCNT after 6 and 24 hours of exposure, respectively. These lists were compared with a list of genes known to be deregulated in human malignant mesothelioma or human lung cancers, using a data mining database (GeneCards). The authors found 12 and 22 genes modulated by exposure to both MWCNT and crocidolite in malignant mesothelioma and lung cancers, respectively, some of which were oncogenes and known or potential tumour-suppressor genes. One tumour-suppressor gene – *CDKN2A* – was downregulated 24 hours after exposure to each of the particles; and *CTGF* was upregulated similarly to human lung cancers. In addition, the expression of the *BCL2* oncogene was enhanced in comparison with control cells, similarly to both malignant mesothelioma and lung cancers ([Kim et al., 2012b](#)). [These results demonstrated that exposure to MWCNT in vitro (i) downregulated or upregulated some tumour-suppressor genes and oncogenes, respectively, and (ii) modified the expression of cancer genes also found to be deregulated in human lung cancers and malignant mesotheliomas with similar effects produced by exposure to crocidolite asbestos.]

A transcriptomic analysis was performed in telomerase-immortalized human keratinocytes (N-hTERT) exposed to MWCNT (Nanocyl™ NC7000 MWCNT; from Nanocyl) produced by catalytic CVD. Cells were exposed to a dose of 100 µg/mL for 24 hours and mRNA expression was investigated using small-scale transcriptomic TaqMan low density array profiling (TLDA, Applied Biosystems). The relative expression levels of 46 mRNAs in treated cells compared with untreated cells were reported. Expression of the *BCL2* oncogene was increased after exposure to water-sonicated MWCNT ([Vankoningsloo et al., 2012](#)). [The authors did not discuss the statistical analysis of these results.]

Carboxylated MWCNT were studied in human embryonic kidney epithelial HEK 293 cells, mouse mesenchymal stem C2C12 cells, and human neuroblastoma NB1691 cells. The expression of cell-cycle regulatory proteins was analysed using Western blot and mRNA expression was assessed using RT-PCR after exposure to 100 µg/mL of MWCNT for 24 hours. Expression of the protein p21, encoded by *CDKN1A* (a potential tumour-suppressor gene), was enhanced in MWCNT-exposed proliferating C2C12, HEK 293, and NB1691 cells. This was associated with an increased expression of the unphosphorylated form of pRb, concordant with cell-cycle downregulation. The expression of *CDKN1A* (an inhibitor of cell-cycle progression) was also enhanced at the transcriptional level as assessed using RT-PCR. Interestingly, expression of the p53 protein was not found to be enhanced, consistent with the absence of apoptosis ([Zhang & Yan, 2012](#)). [These results suggested the p53-independent induction of p21 in this experimental model.]

(c) *Experimental systems in vivo*

See [Table 4.19](#)

**Table 4.19 Activation of oncogenes and inactivation of tumour-suppressor genes in experimental animals exposed to carbon nanotubes**

Species, strain (sex)	Route of administration/dose, duration	Recovery	Assay/result	Reference
Mouse, C57BL/6 (F)	Inhalation, in inhalation chambers, or pharyngeal aspiration Unpurified (iron, 17.7%) SWCNT: 5 mg/m <sup>3</sup> , 5 h/day, 4 days (inhalation) or 10 µg (pharyngeal aspiration)	1, 7, and 27 days	Investigation of <i>K-ras</i> mutations in DNA from lung sections Three different types of mutation detected in <i>K-ras</i> after inhalation: two at codon 12 and one double mutation at codons 12 and 8; pharyngeal aspiration did not significantly enhance the mutation rate in <i>K-ras</i>	<a href="#">Shvedova et al. (2008)</a>
Mouse, ICR (NR)	Intratracheal instillation Purified SWCNT: 100 µg/kg bw	1, 7, 14, and 28 days	Proteins extracted from lung tissue; protein analyses performed by Western blot Expression of p53 was enhanced as early as 1 day after exposure	<a href="#">Park et al. (2011a)</a>
Mouse, ICR (M)	Intratracheal instillation Unpurified SWCNT: 100 µg/kg bw	1, 7, and 14 days	Determination of the expression of several proteins by Western blot in lung tissue Expression of p53, Cox-2 and caspase-3 enhanced in the lungs of exposed mice in comparison with controls, then decreased at 28 days, consistent with an increase in p53 tumour-suppressor gene expression related to apoptosis	<a href="#">Park et al. (2011b)</a>
Mouse, CCR5 <sup>+/+</sup> (wild-type) and CCR5 <sup>-/-</sup> (knockout)	Intratracheal instillation Unpurified SWCNT: 100 µg/kg	7 days	Expression of apoptosis-related proteins, caspase-9, caspase-3, and cleaved PARP, and p21 <sup>Cip1/Waf1</sup> , cyclin D1, and TGF-β by cell-cycle analysis in the lungs Expression of caspase-9, caspase-3, and cleaved PARP increased in the lung tissue of knockout mice relative to wild-type mice; phospho-p53, p21 <sup>Cip1/Waf1</sup> , and cyclinD1 expression was also increased in knockout mice	<a href="#">Park et al. (2013)</a>
Mouse, C57BL/6J (M)	Pharyngeal aspiration Unpurified MWCNT: 0, 10, 20, 40, or 80 µg	7 and 56 days	Extraction of total RNA from frozen lung; quantification of 63 genes by qRT-PCR, including 47 signature genes of human non-small cell lung cancer and 16 hallmarks genes of cancer signalling pathways Differential expression of seven genes (by day 7) and 11 genes (by day 56) was observed in the exposed and control groups, including the downregulation of both <i>Wif1</i> and <i>Bcl2</i> ; among these, four were differentially expressed at both time-points: <i>Cdce99</i> , <i>Msx2</i> , and <i>Nos2</i> (upregulated), and <i>Wif1</i> (downregulated)	<a href="#">Pacurari et al. (2011)</a>
Mouse, C57BL/6J (M)	Pharyngeal aspiration Unpurified MWCNT: 0, 10, 20, 40, or 80 µg	1, 7, 28, and 56 days	Genome-wide mRNA expression profiles in lungs analysed through Ingenuity Pathway Twenty-four genes consistently differentially expressed; after 56 days, 38 genes were associated with cancer; when matched in human genomes, 16 genes (all time-points) and 35 genes (56 days) were predictive of human lung cancer risk and prognosis. The MWCNT signature was represented by 35 genes; two were potential oncogenes ( <i>BCL3</i> and <i>EGFR</i> ) involved in lung cancer development, but were downregulated	<a href="#">Guo et al. (2012)</a>

Table 4.19 (continued)

Species, strain (sex)	Route of administration/dose, duration	Recovery	Assay/result	Reference
Mouse, C57BL/6J (M)	Pharyngeal aspiration Unpurified MWCNT: 0, 10, 20, 40, or 80 µg	1, 7, 28, and 56 days	Genome-wide mRNA expression profiles and pathological analysis of lungs, referring to inflammatory and fibrotic pathways analysed through Ingenuity Pathway Differential expression of 67 genes involved in the inflammatory process and 69 involved in fibrosis was observed; 23 genes were involved in both processes; two genes were potential oncogenes: <i>egfr</i> (downregulated) and <i>jumb</i> (overexpressed at all time-points at most doses)	<a href="#">Snyder-Talkington et al. (2013a)</a>
Rat, spontaneously hypertensive [derived from WKY rat] (M)	Intratracheal instillation Short (0.5–2 µm) and long (20–50 µm) unpurified MWCNT particles: 0.6 mg/rat, once/day, 2 days	1, 7, and 30 days	Investigation of TGF-β1 protein expression (immunohistochemistry in the lungs) and by qRT-PCR analysis; mRNA expression of genes involved in the TGF-β/Smad signalling pathway In MWCNT-exposed rats, TGF-β1 protein expressed in macrophages and near the bronchiolar epithelium; gene expression of <i>Tgfb1</i> and <i>Tgbr2</i> enhanced at day 7; stimulation of the TGF-β/Smad signalling pathway only observed in rats treated with long MWCNT	<a href="#">Wang et al. (2013)</a>
Mouse, pregnant heterozygous <i>p53</i> ( <i>p53<sup>+/-</sup></i> )	Intravenous injection Unpurified MWCNT: 2 mg/kg bw (single injection)		RNA isolated from mouse fetal liver and mouse embryo fibroblasts with different <i>p53</i> -status; <i>Cdkn1a</i> (encoding p21 <sup>Cip1</sup> ) and <i>Bax</i> gene expression quantified by RT-PCR; protein expression determined in mouse embryo fibroblasts with the different types of <i>p53</i> -status MWCNT induced the mRNA transcription of two tumour-suppressor genes ( <i>Cdkn1a</i> and <i>Bax</i> ) in <i>p53<sup>+/-</sup></i> fetuses but to a lesser extent in <i>p53<sup>-/-</sup></i> and <i>p53<sup>+/-</sup></i> mice; expression of <i>p21</i> and <i>Bax</i> triggered in a <i>p53</i> status-dependent manner	<a href="#">Huang et al. (2014)</a>
Mouse, C57BL/6J (F)	Intratracheal instillation Unpurified MWCNT (Mitsui 7): 18, 54, or 162 µg/mouse	24 h	Microarray analyses of gene expression in the lungs Referring to human orthologous genes, some oncogenes were upregulated ( <i>Aurka</i> and <i>Bcl3</i> ); downregulated genes also included known or potential oncogenes ( <i>Wnt1</i> , <i>Myb</i> , and <i>Dnajd4</i> )	<a href="#">Søs Poulsen et al. (2013)</a>

bw, body weight; Cox-2, cyclooxygenase 2; F, female; M, male; MWCNT, multiwalled carbon nanotubes; NR, not reported; PARP, poly(ADP-ribose) polymerase; qRT-PCR, quantitative real time-polymerase chain reaction; SWCNT, single-walled carbon nanotubes; TGF, tumour growth factor

## (i) SWCNT

C57BL/6 mice were exposed to 5 mg/m<sup>3</sup> of SWCNT by inhalation for 5 hours per day for 4 days, or to 10 µg of SWCNT delivered by pharyngeal aspiration, after which DNA was isolated from lung sections. Three different types of mutation were detected in the *K-ras* gene after inhalation; two at codon 12, one of the most common mutation sites in human lung cancer, and one double mutation at codons 12 and 8. Pharyngeal aspiration did not significantly enhance *K-ras* gene mutations ([Shvedova et al., 2008](#)).

ICR mice were exposed to 100 µg/kg bw of SWCNT (ASP-100F from Hanhwa Nanotech, Republic of Korea) delivered by intratracheal instillation. The lungs were harvested 1, 7, 14, and 28 days after injection, and proteins were extracted from the lung tissue and analysed using Western blots. The expression of p53 protein was enhanced as early as 1 day after exposure ([Park et al., 2011a](#)). [The authors did not discuss the origin of the cell or the mechanism responsible for increased p53 protein expression.]

Exposure of male ICR mice to 100 µg/kg bw of SWCNT (metal content, approximately 10% wt; diameter, 1.2 nm; length, 2–10 µm; ASP-100F, Hanhwa Nanotech) by intratracheal instillation resulted in the modification of the expression of several proteins assessed by Western blot. One, 7, and 14 days after exposure, the expression of p53, cyclooxygenase 2, and caspase-3 proteins was increased in the lungs of exposed mice in comparison with controls, then decreased after 28 days ([Park et al., 2011b](#)). [These findings were consistent with an increase in the expression of the p53 tumour-suppressor gene that is related to the induction of apoptosis.]

The effects of SWCNT (diameter, 1.2 nm; length, 2–10 µm; ASP-100 F, Hanhwa Nanotech) were studied in CCR5<sup>+/+</sup> (wild-type) and CCR5<sup>-/-</sup> (knockout) mice exposed to a dose of 100 µg/kg bw delivered by intratracheal instillation. CCR5

is a chemokine receptor that plays a role in inflammatory responses. The cell cycle was analysed to determine the expression of apoptosis-related proteins, and p21<sup>Cip1/Waf1</sup>, cyclin D1 (*cc1*), and TGF-β in the lungs 7 days after instillation. The expression of apoptosis-related proteins – caspase-9, caspase-3, and cleaved poly(ADP-ribose) polymerase – and phospho-p53 protein was more markedly increased in the lung tissue of knockout mice than in that of wild-type mice. The expression of other proteins – p21<sup>Cip1/Waf1</sup> and *cc1* (both potential oncogenes) – was also increased in knockout mice ([Park et al., 2013](#)). [These results were consistent with SWCNT-induced apoptosis, but also showed that the expression of some known or potential oncogenes (*Cdkn1a* and *Ccnd1* encoding p21<sup>Cip1/Waf1</sup> and *cc1*, respectively) can be altered in the lungs of mice exposed to SWCNT.]

## (ii) MWCNT

Male C57BL/6J mice were exposed to 10, 20, 40, or 80 µg of MWCNT (MWCNT-7, lot # 05072001K28, from Mitsui & Co.) or vehicle by pharyngeal aspiration for 7 or 56 days. Total RNA was extracted from frozen lung and quantified using qRT-PCR. A total of 63 genes were investigated, 47 of which were selected from previous studies that had identified gene expression signatures of human non-small cell lung cancers, determined using genome-wide DNA microarray analyses as being potentially associated with lung cancer risk, and 16 of which were hallmarks of cancer signalling pathways. At 7 and 56 days after exposure, a set of seven and 11 genes, respectively, showed differential expression in the lungs of mice exposed to MWCNT compared with the vehicle-treated control group. Among these, *Wif1* (a gene functioning as a tumour-suppression gene that has been found to be epigenetically silenced in various cancers) was downregulated and an oncogene, *Bcl-2*, was also downregulated. Four genes from these two subsets of genes showing significant differential

mRNA expression at both time-points were either upregulated (*Ccdc99*, *Msx2*, and *Nos2*) or downregulated (*Wif1*) ([Pacurari et al., 2011](#)). [These results demonstrated that exposure to this sample of MWCNT could modify the expression of genes that have been shown to be prognostic biomarkers in human lung cancers, including persistent downregulation of a putative tumour-suppressor gene.]

C57BL/6J mice were exposed to 0 (vehicle control), 10, 20, 40, or 80 µg of MWCNT (MWCNT-7, lot # 05072001K28; from Mitsui & Co.) by pharyngeal aspiration. RNA extracted 1, 7, 28, and 56 days after exposure was analysed for gene expression profiling using Agilent Mouse Whole Genome Arrays (Agilent, Santa Clara, CA). Selected genes showed significant changes at a minimum of two time-points and with a more than 1.5-fold change at all doses, and were significant in the linear model for dose or interaction of time and dose. The authors compared the list of differentially expressed genes from the microarray gene expression data with two published studies on microarray profiles in human lung carcinomas. In treated mice, 24 genes were consistently differentially expressed. From data at 56 days after exposure, 38 genes were selected as being associated with cancer. When matched in human genomes using gene symbols, 16 and 35 genes were found to predict the risk and prognosis of human lung cancer from data obtained at all time-points and at 56 days, respectively. Among the proteins encoded by the list of 35 genes with differential expression induced by exposure to MWCNT, several were implicated in lung cancer development, including two potential oncogenes – *BCL3* and *EGFR*. However, both genes were downregulated after exposure to MWCNT ([Guo et al., 2012](#)).

Microarray gene expression profiling was investigated using RNA isolated from the lungs of male C57BL/6J mice exposed to 0 (vehicle control), 10, 20, 40, or 80 µg of MWCNT (MWCNT-7, lot #05072001K28; from Mitsui &

Co.) delivered by pharyngeal aspiration for 1, 7, 28, or 56 days. The authors applied a novel computational model to generate genome-wide mRNA expression profiles that correlated with histopathological analysis of mouse lungs, focusing on inflammatory and fibrosis pathways identified using Ingenuity Pathway identification. Twenty-three genes were found to be involved in both MWCNT-induced inflammation and fibrosis – 67 in inflammation and 69 in fibrosis. Two of these genes are potential oncogenes; *egfr* was downregulated and *junb* was overexpressed across all days at most doses, possibly in relation to persistent inflammation ([Snyder-Talkington et al., 2013a](#)).

The expression of *Tgfb1* was measured in spontaneously hypertensive male rats exposed to PBS (control) or 0.6 mg/rat of short (0.5–2 µm) or long (20–50 µm) unpurified MWCNT (Nanotech Port, Chengdu, China) suspended in PBS by non-surgical intratracheal instillation once per day for two consecutive days. *Tgfb1* expression was evaluated by immunohistochemistry on the lung tissue sections and by qRT-PCR analysis. mRNA expression of other genes involved in the TGF-β Smad signalling pathway was also measured. [Several genes – *Tgfbr2*, *Smad2*, and *Smad3* – are potential tumour-suppressor genes.] TGF-β1 protein expression was detected in lung macrophages and near the bronchiolar epithelium in response to MWCNT; the expression of both *Tgfb1* and *Tgfbr2* genes was increased after 7 days of exposure (other times tested: 1 and 30 days). Additional results suggested that the TGF-β/Smad signalling pathway was upregulated only in rats exposed to long MWCNT ([Wang et al., 2013](#)).

Pregnant heterozygous *p53*<sup>+/-</sup> mice received an intravenous injection of 2 mg/kg bw of MWCNT. Exposure to MWCNT induced mRNA expression of two tumour-suppressor genes – *Cdkn1a* (encoding p21<sup>Cip1</sup>) and *Bax* – in *p53*<sup>+/+</sup> fetuses, but to a lesser extent in *p53*<sup>+/-</sup> and *p53*<sup>-/-</sup> mice ([Huang et al., 2014](#)). [These results

suggested that exposure to MWCNT triggers apoptosis in mice, a process decreased or inactivated in p53-deficient mice, depending on their p53 status.]

Pulmonary responses of C57BL/6 mice after exposure to MWCNT (Mitsui 7) were compared with in-vitro studies using cultured lung epithelial FE1 cells at the global transcriptomic level. Mice were exposed by intratracheal instillation to doses of 18, 54, and 162 µg/mouse of MWCNT, and lung samples were collected 24 hours after exposure. Microarray analyses were performed using Agilent 8 × 66K oligonucleotide microarrays, and gene expression was analysed using the gene ontology classifications of all of the differentially expressed genes. After in-vivo exposure, several pathways were commonly (more than one dose) or uniquely (one dose) affected. Referring to human orthologous genes, expression of some oncogenes was upregulated (*Aurka* and *Bcl3*). Downregulated genes also included known or potential oncogenes (*Wnt1*, *Myb*, and *Dnaja4*) (Søs Poulsen et al., 2013). [When comparing in-vivo and in-vitro models, most of the genes associated with exposure to MWCNT involved the same pathways, but the number of differentially expressed genes, in comparison with untreated mice, was higher in vivo than in vitro, which was at least partly linked to the multicellular versus unicellular nature of these model systems.]

#### (d) Experimental systems in vitro

See [Table 4.18](#)

##### (i) SWCNT

Murine monocytic RAW 264.7 cells were exposed to 0, 1, 10 or 50 µg/mL of acid-functionalized SWCNT (AF-SWCNT) for 24 hours. Gene expression profiles were analysed using cDNA microarrays. Based on the criteria of significance ( $P < 0.001$  and fold change  $> 2$ ), differentially expressed genes were identified at a dose of 10 µg/mL. A total of 130 genes were differentially

expressed: 126 were underexpressed and four were overexpressed. Among these genes, *MYC* (oncogene) mRNA expression was upregulated in AF-SWCNT-treated RAW 264.7 cells in comparison with controls, confirmed using RT-PCR analyses. Several genes involved in DNA repair were downregulated, including *XPA*, *XRCCI*, *XRCC4*, and *CHEK1* (potential tumour-suppressor genes). Globally, AF-SWCNT altered the expression of genes related to ribosome function, mitochondrial function, inflammatory response, cell cycle/apoptosis, and the proteasome pathway (Dong et al., 2012). [These results showed that AF-SWCNT may downregulate tumour-suppressor genes involved in the repair of DNA damage and stimulate the expression of oncogenes in RAW 264.7 cells.]

##### (ii) MWCNT

Rat lung epithelial RL 65 cells exposed to MWCNT (diameter, 6–13 nm; length, 2.5–20 µm; Sigma-Aldrich) showed increased levels of p53, p21<sup>Cip1/Waf1</sup>, and bax protein expression after 12 hours of exposure to 5 µg/mL, probably related to the induction of apoptosis (Ravichandran et al., 2010).

SWCNT (outside diameter, 1–2 nm; length, ~20 µm; Beijing Nachen Technology & Development Co. Ltd) were studied in rat adrenal gland pheochromocytoma PC12 cells. After 24 and 48 hours of exposure to 50 µg/mL of SWCNT, the expression of proteins involved in apoptosis – Bcl-2, an oncogenic protein, and bax – was determined using flow cytometry. Bcl-2 expression was decreased and bax protein and caspase-3 activity were increased in comparison with control cells, consistent with the induction of apoptosis in SWCNT-treated PC12 cells (Wang et al., 2012b).

Mouse embryonic J11 stem cells were exposed to MWCNT (Tsinghua and Nanfeng Chemical Group Cooperation, China) and the DNA damage response induced was analysed by measuring p53 protein expression levels. The expression

of p53 protein was observed within 2 hours of exposure, and increased proportionally with the dose (5 and 100  $\mu\text{g}/\text{mL}$ ). Phosphorylation of p53 protein was assessed using the phospho-specific antibody to p53-Ser-23 and confirmed the activation of the p53 DNA damage-induced response pathway (Zhu et al., 2007). [Increased p53 protein expression suggests that MWCNT could cause DNA damage.]

The expression of *TGF $\beta$ 1*, a tumour-suppressor gene that might also be an oncogene, was assessed in a co-culture of the mouse leukaemic monocyte macrophage RAW 264.7 cell line and the mouse embryonic fibroblast NIH 3T3 cell line, using RT-PCR analysis. RAW 264.7 cells seeded in the bottom well were first exposed to short (length, 0.5–2  $\mu\text{m}$ ) or long (20–50  $\mu\text{m}$ ) MWCNT (15  $\mu\text{g}/\text{mL}$ ) for 24 hours, and then NIH 3T3 cells that had attached on the top of the insert for 24 hours were co-cultured with RAW 264.7 for another 24 hours. mRNA expression of *TGF- $\beta$ 1* was more upregulated by exposure to long MWCNT in comparison with short MWCNT. In parallel, more TGF- $\beta$ 1 protein was expressed in co-cultures exposed to long MWCNT than those exposed to short MWCNT (Wang et al., 2013).

A comparison of the in-vivo pulmonary responses of C57BL/6 mice to MWCNT (Mitsui 7) with the in-vitro response of lung epithelial FE1 cells (a spontaneously immortalized lung epithelial cell line derived from a normal healthy MutaTM Mouse) was made at the global transcriptomic level (Søs Poulsen et al., 2013). This cell line retains key endogenous metabolic capacity and intact p53 signalling pathways, and expresses both type I and type II alveolar phenotypes (Berndt-Weis et al., 2009). FE1 cells were exposed to 12.5, 25, or 100  $\mu\text{g}/\text{mL}$  of MWCNT for 24 hours. Microarray analyses were performed using Agilent 8  $\times$  66K oligonucleotide microarrays and gene expression was analysed using the gene ontology classification of differentially expressed genes. After in-vivo exposure, several pathways were commonly

(several doses) or uniquely (one dose) affected. In FE1 cells in vitro, genes commonly affected included pathways involving aryl hydrocarbon receptor signalling, GSH-mediated detoxification, acute phase response signalling, and the nuclear factor (erythroid-derived 2)-like 2-mediated oxidative stress response. Among the upregulated genes, several were known or potential oncogenes or genes involved in cancer, including *Jun*, *Ddit3*, *Hmga2*, *Ctgf*, *Runx1*, and *Fosl1*, some of which may also have tumour-suppressor functions in specific models. Among the downregulated genes, several were also known or potential tumour-suppressor genes or genes involved in cancer, such as *Pdgfrl*, *Id4*, *Cdkn2c*, *Cdkn2d* (p19), *Tgf $\beta$ 2*, *Gstm2*, and *Gstt1*, some of which may also have oncogenic functions in specific systems (Pdgfra and Cdkn2d). In-vitro data showed a high degree of overlap across the exposure groups, with some exceptions at the highest concentration (Søs Poulsen et al., 2013). [When comparing the two in-vitro and in-vivo models after exposure to MWCNT, most of the genes were associated with the same pathways, but the number of differentially expressed genes was lower in vitro and in vivo in comparison with untreated mice, which was at least partly linked to the multicellular versus unicellular nature of the systems.]

#### (e) Acellular systems in vitro

The generation of radicals by MWCNT was studied in an acellular system. MWCNT were synthesized by the decomposition of ethylene on an alumina support doped with a cobalt-iron catalyst mixture and purified by subsequent treatment with sodium hydroxide. The potential of MWCNT to release free radicals in aqueous suspensions was thus monitored by ESR spectroscopy, using 5,5-dimethyl-1-pyrroline-*N*-oxide as a trapping agent. A suspension of 5 mg of MWCNT did not generate oxygen or carbon-centred free radicals in the presence of hydrogen peroxide or formate, respectively. In

contrast, MWCNT were able to scavenge radicals in the presence of an external source of hydroxyl radicals,  $\bullet\text{OH}$ , or superoxide radicals,  $\text{O}_2^{\bullet-}$  (Fenoglio et al., 2006). [Although not formally demonstrated, it is possible that MWCNT might protect against antioxidant depletion.]

The ability of various types of MWCNT to generate/scavenge radical formation was studied in both cell-free systems and human bronchial BEAS-2B cells. Printex 90 carbon black, crocidolite asbestos, and glass wool were also used. Hydrogen peroxide-induced free radical formation was determined by ESR. All CNM were found to scavenge the induction of  $\bullet\text{OH}$ , but the presence of bovine serum albumin abolished  $\bullet\text{OH}$  production in some samples. In addition to a scavenging effect, two types of long, needle-like MWCNT (average diameter, > 74 and 64.2 nm; average length, 5.7 and 4.0  $\mu\text{m}$ , respectively) induced the dose-dependent formation of a unique, as yet unidentified radical in both the absence and presence of cells, which also coincided with cytotoxicity. The ability of MWCNT to protect against oxidant formation also depended on the composition of the medium (Nymark et al., 2014).

## 4.5 Susceptible populations

See [Table 4.20](#)

No data on human populations were available to the Working Group. One study was carried out in transgenic animals with increased susceptibility to carcinogenic substances (Takanashi et al., 2012). Several experimental studies focused on the possible aggravation of airway disease and the effects of CNT on pulmonary vessels using models of asthma in mice.

Studies of genes related to inflammation in genetically deficient mice are also reported below. Although not related to cancer, these studies are summarized in relation to their pertinence to inflammatory processes.

### 4.5.1 Modification of risks for cancer of the lung

#### (a) MWCNT

The effects of MWCNT on allergic airway inflammation were studied in four groups of ICR mice that received intratracheal injections of vehicle, MWCNT (50  $\mu\text{g}/\text{animal}$ ; one of two types: Bussan Nanotech Research and SES Research), OVA, and OVA+MWCNT. Biological parameters were measured in the BALF (cellularity), lungs (histology, protein levels of cytokines related to allergic inflammation in lung homogenates and BALF), and serum (Ig levels). MWCNT exacerbated murine allergic airway inflammation, as demonstrated by an aggravation of allergen-induced airway inflammation and an increased number of goblet cells in the bronchial epithelium, and exhibited adjuvant activity for allergen-specific IgG1 and IgE. OVA+MWCNT amplified the lung levels of Th2 cytokines (e.g. IL-4, IL-5, and IL-13) and chemokines (e.g. thymus- and activation-regulated chemokine and macrophage-derived chemokine) compared with OVA (Inoue et al., 2009).

The effects of the inhalation of MWCNT on airway fibrosis were investigated in normal and OVA-sensitized mice with allergic asthma. Quantitative morphometry showed significant airway fibrosis in OVA-sensitized mice 14 days after exposure to MWCNT but not in mice treated with OVA or MWCNT alone. The levels of inflammatory factors in the BALF differed according to the exposure: IL-13 and TGF- $\beta$ 1 were elevated in OVA-sensitized mice while PDGF-AA was elevated in MWCNT-treated mice, suggesting that the airway fibrosis resulting from the combined effect of OVA and MWCNT required PDGF (a fibroblast mitogen) and TGF- $\beta$ 1 that stimulates collagen production (Ryman-Rasmussen et al., 2009b). [These findings indicated that individuals with pre-existing

**Table 4.20 Susceptibility to cancer in experimental animals exposed to carbon nanotubes**

Species, strain (sex)	Route of administration/treatment	Observation time	Result	Reference
Mouse, ICR (M)	Pharyngeal aspiration Vehicle control (PBS), once/wk, 6 wk MWCNT-treated group, 50 µg once/wk, 6 wk OVA-treated group, 1 µg every 2 wk then PBS every 2 wk, 6 wk OVA+MWCNT-treated group: same protocol as OVA- and MWCNT-treated groups	24 h	BALF: number of total cells significantly greater in MWCNT-, OVA-, and OVA+MWCNT-treated groups than in the vehicle controls Lung homogenates: moderate infiltration of neutrophils seen in the MWCNT- and OVA-treated groups, more marked in the OVA+MWCNT-treated group; significant induction of goblet cell hyperplasia in the airways of OVA- and MWCNT-treated groups compared with vehicle controls, with a marked progression in the OVA+MWCNT-treated group; a significant increase in the number of eosinophils, lymphocytes, and neutrophils in the MWCNT-, OVA-, and OVA+MWCNT-treated groups compared with vehicle controls; protein levels of cytokines and chemokines related to allergic inflammation greater in the OVA+MWCNT-treated group compared with the MWCNT- or OVA-treated groups Serum: allergen-specific IgG1 and IgE levels significantly greater in the OVA-treated or OVA+MWCNT-treated groups compared with controls, and higher in the OVA+MWCNT-treated group than in the OVA- or MWCNT-treated groups	<a href="#">Inoue et al. (2009)</a>
Mouse, C57BL/6 (M)	Inhalation MWCNT: ~100 mg/m <sup>3</sup> , 6 h (10 mg/kg bw range) Prior sensitization to OVA: intraperitoneal injection of 10 µg, once/wk, 2 wk, then intranasal challenge with 100 µL of 1% OVA 1 day before exposure to MWCNT Intraperitoneal injection of 50 mg/kg bw 5'-bromodeoxyuridine 1 hour before euthanasia	1 or 14 days after exposure	OV+MWCNT: trend of increased neutrophils in BALF compared with a group treated with MWCNT alone; airway fibrosis noted at 14 days, but not in mice that received OVA or MWCNT alone OVA+MWCNT: increased IL-13 protein levels in BALF and of mRNA in lung tissue, compared with unsensitized or MWCNT-treated animals but not with animals treated with OVA alone OVA+MWCNT: both PDGF-AA and TGF-β1 increased (MWCNT alone: PDGF-AA increased) No focal areas of 5'-bromodeoxyuridine staining observed in the lung tissue of controls or MWCNT-treated animals	<a href="#">Ryman-Rasmussen et al. (2009a)</a>
Mouse, BALB/c (M)	Intranasal instillation Sensitization with 30 µL of 0.01, 0.1, or 1 mg/mL MWCNT with or without 2.5 mg/mL OVA at 0, 1, 2, 14, 15, and 16 days Challenges by intratracheal administration of 20 µL of 1% OVA at 28, 29, 30, and 35 days		Enhancement of the degree of airway resistance in MWCNT+OVA-treated group compared with vehicle-, MWCNT-, and OVA-treated groups OVA+MWCNT (1 mg/mL): significant enhancement of IL-4, IL-5, IL-13, and IL-17 in the lung tissue, and of C3a in the BALF in comparison with control, MWCNT-treated, and OVA-treated groups; goblet cell hyperplasia observed OVA + MWCNT: increased levels of OVA-specific IgE, IgG1, and IgG2a in serum	<a href="#">Mizutani et al. (2012)</a>

**Table 4.20 Susceptibility to cancer in experimental animals exposed to carbon nanotubes (continued)**

Species, strain (sex)	Route of administration/treatment	Observation time	Result	Reference
Mouse, BALB/cByJ (M)	Intranasal instillation Vehicle control group HDM (a <i>Dematophagoides pteromyssinus</i> extract): 2 µg Der p1 on days 0, 7, 14, and 21 MWCNT (75 µg) or MWCNT (75 µg) + HDM on days 0, 7, and 14 MWCNT (225 µg) or MWCNT (225 µg) + HDM on days 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 Analyses of serum, BALF, and lung	day 23	Effects of MWCNT + HDM compared with HDM alone or control: Serum: increased level of specific and total IgG1 BALF: enhancement of allergen-induced airway inflammation Lungs: allergic and epithelium-derived cytokines significantly increased (TSLP, IL-33, and IL-25); tissue remodelling	<a href="#">Ronzani et al. (2014)</a>
Mouse, C57BL/6J wild-type and macrophage-specific PPAR $\gamma$ knockout (NR)	Subcutaneous implantation MWCNT: 100 µg Analyses in BALF (cell counts; transcripts for IFN- $\gamma$ , osteopontin, and CCL2; IFN- $\gamma$ and CCL2 proteins) and lung histology: granulomas	60 days	Decreased PPAR $\gamma$ expression and activity in alveolar macrophages from MWCNT-instilled wild-type C57BL/6 mice bearing granulomas Granuloma formation in macrophage-specific PPAR $\gamma$ knockout mice exceeded that in wild-type animals Pro-inflammatory cytokines elevated in lung, granulomas and BALF from MWCNT-instilled macrophage-specific PPAR $\gamma$ knockout mice Minimal staining of CD3+ (T-cells), monocytes and macrophages in tissue sections from sham-treated C57BL/6 or PPAR $\gamma$ knockout control mice, but major staining in MWCNT-instilled mice	<a href="#">Huizar et al. (2013)</a>
Mouse, C57BL/6J $\times$ 129/Ola wild-type or homozygous knockout ( <i>Cox-2</i> <sup>-/-</sup> ) (M + F)	Oropharyngeal aspiration Intranasal challenge with 20 mg OVA in saline on days 26, 28, and 32 MWCNT: 4 mg/kg bw on day 34	1 or 14 days after exposure	Exacerbation of OVA-induced airway inflammation Enhancement of OVA-induced mucus-cell metaplasia in <i>Cox-2</i> knockout mice Amplification of OVA-induced Th2, Th1, and Th17 mediators in <i>Cox-2</i> knockout mice	<a href="#">Sayers et al. (2013)</a>
Mouse, transgenic rasH2 which express the <i>c-Ha-ras</i> proto-oncogene derived from humans (M)	Subcutaneous implantation MWCNT (VGCF-S; Showa Denko, Japan) produced by CVD (mean diameter, 100 nm; mean length, 10 µm; 99.9% carbon content), sterilized by autoclaving at 121 °C for 15 min, and dispersed in Tween 80 Black tattoo ink (diameter, 30–50 nm; carbon, 99.57 wt%; sodium, 0.43 wt%) Particles: 75 mg/kg bw injected into the tissue of the back of each mouse	26 wk	No tumour developed in animals treated with MWCNT Some neoplasms in carbon black-treated mice Tumours in all positive control mice treated with <i>N</i> -methyl- <i>N</i> -nitrosourea	<a href="#">Takanashi et al. (2012)</a>

Table 4.20 (continued)

Species, strain (sex)	Route of administration/treatment	Observation time	Result	Reference
Rat, Wistar (M)	Intratracheal instillation SWCNT: 0.02, 0.2, or 2.0 mg/kg bw every 3 days (total 13 instillations) Vitamin E: 100 mg/kg bw 3 h after 0.2 or 2.0 mg/kg bw SWCNT Sensitization by subcutaneous injection of 200 mg OVA on days 6, 20, and 27; challenge with 1% OVA on days 33–39	24 h after final challenge	Exacerbation of OVA-induced allergic asthma by SWCNT Exacerbation was counteracted by concurrent administration vitamin E	<a href="#">Li et al. (2014)</a>
Mouse, ICR (M)	Intratracheal instillation Vehicle control SWCNT: 50 µg/mice once/wk, 6 wk OVA: 1 µg/mice every 2 wk, 6 wk OVA+SWCNT: same protocol as that for OVA and SWCNT alone	24 h after the final administration	BALF cellularity and infiltration of inflammatory leukocytes in the lung significantly greater in OVA+SWCNT-treated group compared with SWCNT- or OVA-treated groups; SWCNT potentiated mucus hyperplasia Measurement of protein levels of allergic response-related cytokines and chemokines in lung tissue homogenates, and inflammatory cytokines in BALF: higher levels of almost all of the cytokines tested in the OVA+SWCNT-treated group relative to the other three groups Serum: significantly higher levels of allergen-specific IgG1, IgG2a, and IgE in OVA- and OVA+SWCNT-treated groups compared with vehicle controls; both IgG1 and IgE higher in OVA+SWCNT-treated compared with OVA- or SWCNT-treated mice SWCNT potentiated allergen-induced oxidative stress in the lung as measured by MPO, NO, and lipid hydroperoxide levels in the BALF/lung homogenates, HO-1 transcript level, and 8-hydroxy-dG staining, compared with SWCNT or OVA alone	<a href="#">Inoue et al. (2010)</a>
Mouse, <i>Ccr5</i> <sup>+/+</sup> (wild-type) and <i>Ccr5</i> <sup>-/-</sup> (knockout) (NR)	Intratracheal instillation SWCNT: 100 µg/kg bw Analyses in BALF (cell count, cell-cycle analysis, and cell distribution), lung histology, and expression of apoptotic and pro-inflammatory response-related proteins	7 days	Factors modified in BALF of <i>Ccr5</i> knockout mice relative to wild-type mice: % of macrophages (% of neutrophils lower) % of cells in G0/G1 (higher, cell cycle arrest) Enhanced expression of apoptosis- and inflammatory response-related proteins CCR4 expression more markedly increased in lung tissue Factors enhanced in lungs of <i>Ccr5</i> knockout mice relative to wild-type mice: Expression of the G1 arrest-related proteins, Cdk2, Ccnd1, and p21 Expression of TGF-β and mesothelin	<a href="#">Park et al. (2013)</a>

**Table 4.20 Susceptibility to cancer in experimental animals exposed to carbon nanotubes (continued)**

Species, strain (sex)	Route of administration/treatment	Observation time	Result	Reference
Mouse, B6.129P2- <i>ApoE</i> <sup>tm1Unc</sup> ( <i>ApoE</i> <sup>-/-</sup> ) (M)	Intrapharyngeal instillation SWCNT: 20 µg/mouse once/2 wk, 8 wk Regular chow diet (regimen 1) or a high fat chow diet (regimen 2), 4 wk, followed by regular chow, 4 wk Controls exposed to vehicle Measurement of plasma levels of cholesterol, triglycerides, glucose, LDH, and inflammatory cytokines Quantification of plaque formation in brachiocephalic arteries Evaluation of mitochondrial DNA damage (quantitative polymerase chain reaction)		Plasma levels of cholesterol, glucose, and LDH comparable in SWCNT-treated and vehicle control mice regardless of diet regimens Plasma levels of inflammatory cytokines: no significant difference in <i>ApoE</i> <sup>-/-</sup> mice treated with vehicle or SWCNT Exposure to SWCNT associated with considerably larger areas of atherosclerotic lesions; significant enhancement in <i>ApoE</i> <sup>-/-</sup> mice fed regimen 2 compared with those fed regimen 1 SWCNT-exposed <i>ApoE</i> <sup>-/-</sup> mice demonstrated significantly increased mitochondrial DNA damage	<a href="#">Li et al. (2007b)</a>

*ApoE*, apolipoproteinase 2; BALF, bronchoalveolar lavage fluid; bw, body weight; CCL2, C-C motif chemokine ligand 2; Ccr5, chemokine (C-C motif) receptor 5; Cdk2, cyclin dependent-kinase 2; Ccnd1, cyclin D1; CVD, chemical vapour deposition; dG, deoxyguanosine; F, female; HMD, house dust mites; HO-1, haeme oxygenase 1; IFN, interferon; Ig, immunoglobulin; IL, interleukin; LDH, lactate dehydrogenase; M, male; min, minute; MPO, myeloperoxidase; MWCNT, multiwalled carbon nanotubes; NO, nitric oxide; NR, not reported; OVA, ovalbumin; p21, p21<sup>Waf1/Cip1</sup> protein; PBS, phosphate-buffered saline; PDGF, platelet-derived growth factor; PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma; SWCNT, single-walled carbon nanotubes; TGF- $\beta$ , transforming growth factor  $\beta$ ; Th, T helper; TSLP, thymic stromal lymphopoietin; wk, week

allergic inflammation may be susceptible to airway fibrosis from inhaled MWCNT.]

Whether sensitization by MWCNT (30  $\mu$ L of 0.01, 0.1, or 1 mg/mL) and OVA (30  $\mu$ L of 2.5 mg/mL) (combined) promotes an allergic asthmatic response was examined in mice. An increase in airway resistance was observed in the groups treated with OVA + 0.1 or 1 mg/mL of MWCNT compared with controls and those treated with OVA or MWCNT alone. In OVA + 1-mg/mL MWCNT-treated mice, the concentration of pro-inflammatory cytokines (IL-4, IL-5, IL-13, and IL-17) was increased in lung tissues and that of the anaphylatoxin C3a in the BALF. OVA-specific IgE, IgG1, and IgG2a were increased in the serum of mice sensitized with OVA and MWCNT ([Mizutani et al., 2012](#)).

The effects of MWCNT on the systemic immune response, airway inflammation, and remodelling induced by house dust mites (HDM) was investigated in BALB/cByJ mice. MWCNT increased the systemic immune response (significantly enhanced levels of specific and total IgG1 in the serum of HDM+MWCNT-treated mice compared with control mice and mice treated with the highest dose of HDM), airway inflammation (significantly enhanced number of eosinophils, neutrophils, and lymphocytes in the BALF of HDM+MWCNT-treated mice compared with control mice and mice treated with the highest dose of HDM), mucus production, and fibrotic response in a dose-dependent manner, as demonstrated by histological analyses of the lungs ([Ronzani et al., 2014](#)). [HDM are the most frequent allergens associated with asthma to date; using this model of asthma in mice, exposure to MWCNT was found to aggravate allergen-induced systemic immune responses, as well as airway inflammation and remodelling.]

The instillation of CNT has been shown to induce granulomatous changes and a study was performed to determine whether peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) deficiency would enhance granuloma formation

after exposure to MWCNT ([Huizar et al., 2013](#)). PPAR $\gamma$  is a transcription factor that acts as a negative regulator of genes linked to inflammatory events. The alveolar macrophages of healthy individuals constitutively express PPAR $\gamma$  but PPAR $\gamma$  is deficient in the alveolar macrophages of patients with severe sarcoidosis, a granulomatous disease. PPAR $\gamma$  was therefore hypothesized to play a role in the formation of MWCNT-induced granulomas. Wild-type and macrophage-specific PPAR $\gamma$  knockout C57BL/6 mice received oropharyngeal instillations of 100  $\mu$ g of MWCNT. The expression and activity of PPAR $\gamma$  by alveolar macrophages were significantly reduced in MWCNT-treated wild-type mice bearing granulomas. Granuloma formation was more extensive in MWCNT-treated macrophage-specific PPAR $\gamma$  knockout mice than in wild-type mice. PPAR $\gamma$  knockout mice exposed to MWCNT also demonstrated an elevated expression of pro-inflammatory cytokines in the lung tissues, laser-microdissected lung granulomas, and BALF cells. [These data suggested that PPAR $\gamma$  deficiency may promote inflammation and granuloma formation.]

Wild-type or cyclooxygenase 2 knockout mice were sensitized to OVA to induce allergic airway inflammation before exposure to 4 mg/kg bw of MWCNT by oropharyngeal aspiration. Exposure to MWCNT significantly increased OVA-induced lung inflammation and mucus-cell metaplasia in knockout mice compared with wild-type mice. Allergen-induced cytokines involved in Th2, Th1, and Th17 inflammatory responses were significantly enhanced in MWCNT-treated knockout but not in wild-type mice ([Sayers et al., 2013](#)).

MWCNT were implanted subcutaneously into transgenic *rash2* mice that overexpress the *c-Ha-ras* oncogene and are highly sensitive to carcinogens. Carbon black and *N*-methyl-*N*-nitrosourea were used as controls. No tumour developed in MWCNT-treated mice. In the carbon black-treated group, one mouse had a

haemangioma in the spleen and another had an adenoma in the lung. Neoplasms developed in all mice in the *N*-methyl-*N*-nitrosourea-treated group but in none of the solvent-treated group (Takanashi et al., 2012). [These results showed that carcinogen-sensitive rasH2 mice did not develop neoplasms after subcutaneous implantation of MWCNT under these experimental conditions.]

(b) SWCNT

OVA-sensitized rats were exposed to SWCNT by intratracheal instillation. SWCNT exacerbated OVA-induced allergic asthma and this exacerbation was counteracted by the concurrent administration of vitamin E (Li et al., 2014).

The effects of SWCNT on allergic airway inflammation was studied in four groups of ICR mice that received intratracheal instillations of vehicle, SWCNT (50 µg/animal), OVA, and OVA+SWCNT. Two types of SWCNT were administered: one type ranged from 0.8 to 1.2 nm in diameter and 100 to 1000 nm in length and contained < 35% (by weight) iron; the other type (SES Research) was formed in the arc process and ranged from 1.2 to 2 nm in diameter and 1 to 15 µm in length. Both types of SWCNT contained up to 75% nanotubes (the remaining material consisted of amorphous carbon and other carbon nanoparticles) and were autoclaved at 250 °C for 2 hours before use. SWCNT aggravated allergen-induced pulmonary inflammation with mucus hyperplasia. OVA+SWCNT enhanced the protein levels of Th cytokines and chemokines related to allergy in the lung and exhibited adjuvant activity for allergen-specific IgG1 and IgE compared with OVA alone. OVA+SWCNT-treated mice also had enhanced oxidative stress-related biomarkers in the airways (Inoue et al., 2010). [These results were consistent with an exacerbation of allergic airway inflammation in mice via the enhanced activation of Th immunity and increased oxidative stress.]

The effects of SWCNT were investigated in wild-type and *Ccr5* (a C-C chemokine receptor predominantly expressed on T-cells, macrophages, dendritic cells, and microglia, which plays an important role in inflammatory responses to infections) knockout mice. A comparison of wild-type and knockout mice exposed to SWCNT showed a significant decrease in the levels of neutrophils and an increase in the expression of apoptosis-related proteins, TGF-β1, and mesothelin in knockout mice. Histopathological lesions were also observed more frequently in knockout mice. The concentrations of the pro-inflammatory cytokines IL-6, IL-13, and IL-17 in BALF were significantly higher in knockout than in wild-type mice, but the levels of IL-1β, IL-10, and IFN-γ were similar in both models. *Ccr5* deficiency may delay the resolution of inflammatory responses triggered by SWCNT and shifts the inflammatory response for SWCNT clearance from a Th1-type to a Th2-type (Park et al., 2013).

Nanoparticles have been reported to produce respiratory damage associated with adverse cardiovascular effects. To evaluate the effects of SWCNT on the progression of atherosclerosis, apolipoprotein E knockout (*ApoE*<sup>-/-</sup>) C57BL/6 mice were fed normal or atherogenic diets and were exposed by intrapharyngeal instillation to SWCNT. *ApoE*<sup>-/-</sup> mice lack ApoE, a high-affinity ligand for lipoprotein receptors, and consequently have elevated plasma levels of cholesterol and triglycerides and develop atherosclerotic plaques. Exposure to SWCNT did not modify the lipid profiles of *ApoE*<sup>-/-</sup> mice but induced accelerated plaque formation in mice fed an atherogenic diet. This response was accompanied by increased mitochondrial DNA damage but not inflammation (Li et al., 2007b). [These findings suggested that ApoE deficiency may enhance sensitivity to SWCNT.]

## 4.6 Mechanistic considerations

### 4.6.1 *Physical and chemical properties associated with biological activity*

See [Fig. 4.2](#)

The physico-chemical properties of CNT may be modulated by their production method, by applying post-synthesis modification (purification), and/or by covalent functionalization of their external surface. The resulting large variety of CNT, their different features and their impact on biological activity and pathogenicity are reviewed in Section 4.2 and summarized in [Table 4.2](#) and [Fig. 4.2](#).

### 4.6.2 *Deposition, biopersistence, translocation, and associated endpoints*

See [Table 4.21](#)

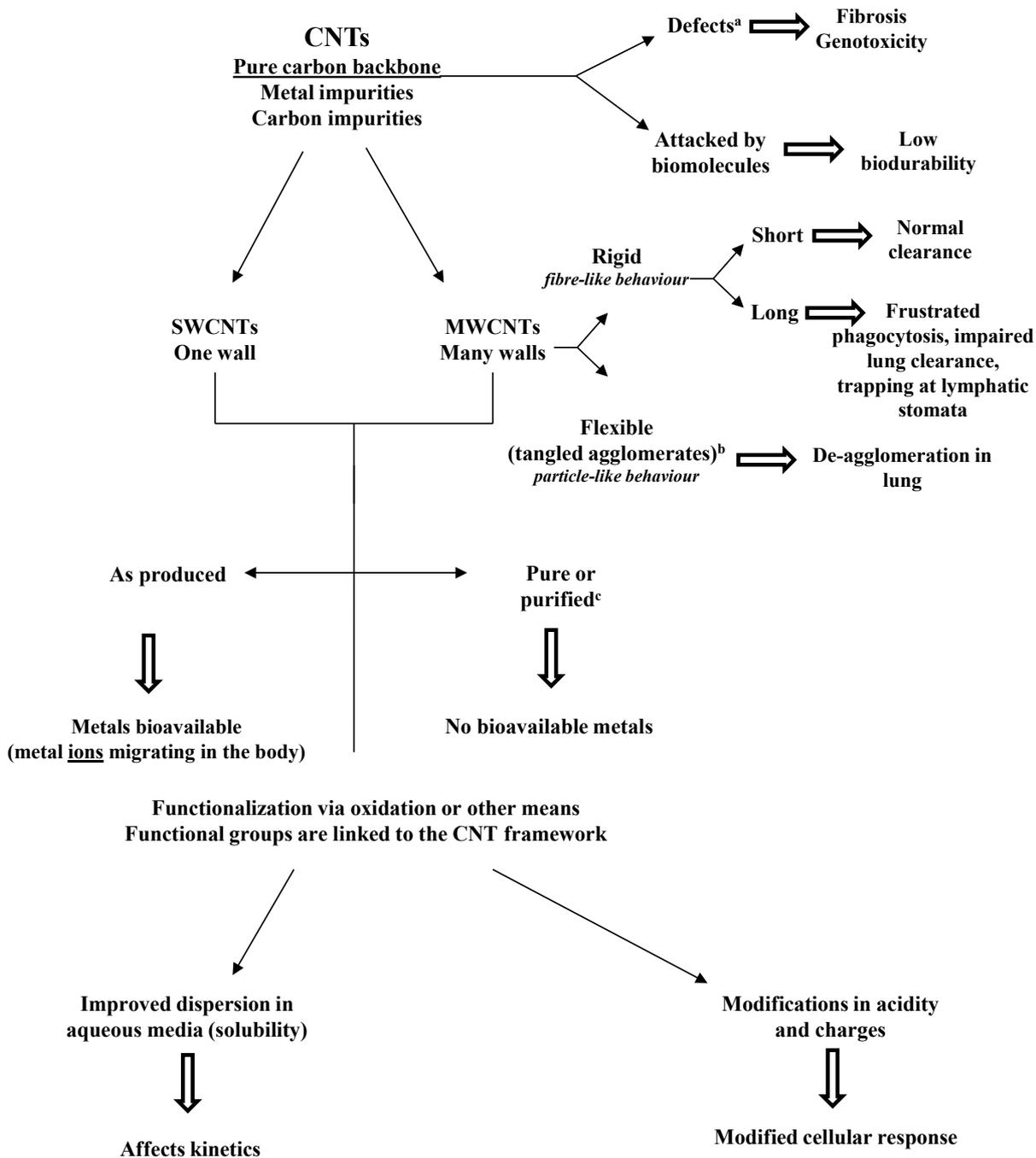
The lung interstitium and pleura were the target tissues for the carcinogenic (see Section 3), inflammogenic, and fibrotic effects that have been reported to be associated with exposure to MWCNT in rats and mice.

The biokinetic factors that relate to the mechanisms of carcinogenicity are those that influence the dose to the target tissue. These factors include the particle characteristics that determine the efficiency of their deposition in the respiratory tract, their clearance or retention, and their potential for translocation to distal sites. Airborne CNT include inhalable (capable of depositing in any region of the respiratory tract; 50% cut size, 10  $\mu\text{m}$ ) or respirable size particles (capable of depositing in the pulmonary or alveolar region of the lungs where gas exchange occurs; 3 and 5  $\mu\text{m}$  for adults and children, respectively) ([Brown et al., 2013](#)). Particles that are deposited in the pulmonary region can be cleared from the lungs by alveolar macrophages, and those that are not cleared have the potential to translocate beyond the lungs.

CNT of respirable size have been shown to be deposited in the lungs of rats and mice exposed by inhalation, with estimated pulmonary deposition fractions of approximately 1–4% for SWCNT or MWCNT in mice ([Shvedova et al., 2008](#); [Mercer et al., 2013a](#)) and approximately 5–20% for MWCNT in rats ([Pauluhn, 2010b](#); [Oyabu et al., 2011](#)). Estimated human pulmonary deposition fractions for MWCNT or SWCNT studied in rodents were approximately 8 to 10% ([NIOSH, 2013](#)).

CNT can enter cells by passive internalization (diffusion or penetration of the cell membrane) or active internalization (phagocytosis or other types of endocytosis) ([Kunzmann et al., 2011](#); [Ye et al., 2013](#)). The mechanisms of cell uptake depend on the surface properties of the CNT, the cell type encountered and its activation state. SWCNT uptake into alveolar macrophages was low (10% of alveolar burden in mice) ([Shvedova et al., 2005](#)) and 90% of dispersed SWCNT structures were observed in the lung interstitium ([Mercer et al., 2008](#)). More effective uptake of MWCNT has been observed ([Mercer et al., 2010, 2011](#); [Treumann et al., 2013](#)). F-MWCNT significantly increased the alveolar macrophage uptake in comparison with O- or P-MWCNT ([Silva et al., 2014](#)).

CNT translocated from the lungs of mice and were observed in blood samples ([Ingle et al., 2013](#)). Two sizes of MWCNT (diameter, 60–80 nm or 90–150 nm) were observed as black pigments in liver tissue 1 day after intratracheal administration; dose-dependent toxicity and necrosis were observed in the liver and kidney ([Reddy et al., 2010a](#)). MWCNT seen by TEM were located in alveolar macrophages in the subpleural region, where focal subpleural fibrosis was also observed 2 weeks after inhalation exposure of 30  $\text{mg}/\text{m}^3$  in mice ([Ryman-Rasmussen et al., 2009a](#)). MWCNT administered to rats by intrapulmonary spraying were observed to penetrate directly from the lungs to the pleural cavity through the visceral pleura, where visceral

**Fig. 4.2 Physical and chemical properties of carbon nanotubes associated with biological activity**

<sup>a</sup> CNTs do not generate radicals/reactive oxygen species (ROS) per se, but act as quenchers of radicals/ROS. When in contact with cells, an oxidative stress response may take place, but the radicals/ROS generated by cells may be totally or partially quenched by CNT. A balance between radicals/ROS generated or quenched depends on cell activity and the quenching potential of CNT, and is strictly related to defects

<sup>b</sup> Tangled or less dense material may lead to volumetric overload mechanism. Reagglomeration may occur due to hydrophobic interaction

<sup>c</sup> Nitric acid purification may also lead to defects

CNT, carbon nanotube; MWCNT, multiwalled carbon nanotube; SWCNT, single-walled carbon nanotube

Prepared by the Working Group

**Table 4.21 Studies of the kinetics of MWCNT or SWCNT<sup>a</sup> in vivo: deposition, biopersistence, translocation, and associated end-points**

Target tissue	MWCNT <sup>b</sup>	SWCNT
Penetration of the lung interstitium	(+) MWCNT (Mitsui-7) penetrated the alveolar epithelium and visceral pleura at a high frequency: > 20 million alveolar epithelial cell penetrations throughout the lungs at doses $\geq 40 \mu\text{g}$ in mice ( <a href="#">Mercer et al., 2010</a> )	(+) Rapid translocation (1 day after PA) of dispersed SWCNT (HiPCO) (10 $\mu\text{g}$ ) and increased collagen in mice ( <a href="#">Mercer et al., 2008</a> ) ( $\pm$ ) Of the poorly dispersed SWCNT (HiPCO), 80% remained in airspaces/granulomas; 20% of the SWCNT material was sufficiently small to enter the alveolar wall and stimulate collagen production in mice ( <a href="#">Mercer et al., 2008</a> )
Translocation to the pleura	(+) MWCNT (Mitsui-7): 0.6% in subpleura on day 1 after PA in mice; MWCNT-loaded pleura ( <a href="#">Mercer et al., 2010</a> ) (+) MWCNT (Helix Material Solutions) was seen in the subpleural lung tissue 1 day after inhalation in mice (30 $\text{mg}/\text{m}^3$ ); focal pleural inflammation and fibrosis were seen 2 and 6 wk after exposure ( <a href="#">Kymann-Rasmussen et al., 2009a</a> ) (+) MWCNT (Mitsui-7) penetrated the visceral pleura in the intrapleural space after PA (10 $\mu\text{g}$ ) in mice ( <a href="#">Porter et al., 2010</a> ) (+) MWCNT (Mitsui-7): 1.6% of dose was observed in the subpleura 56 days after exposure (PA) (10–80 $\mu\text{g}$ ) in mice ( <a href="#">Mercer et al., 2011</a> ) (+) MWCNT (diameter, 10–15 nm; length, 20 $\mu\text{m}$ ) caused persistent DNA damage in the rat lung 90 days after inhalation ( <a href="#">Kim et al., 2014</a> ) [note: short length (330 $\pm$ 1.72 nm) administered]	( $\pm$ ) Dispersed SWCNT (HiPCO) observed “in the vicinity of the pleura” in mice ( <a href="#">Mercer et al., 2008</a> )
Translocation to the pleura and other organs	(+) MWCNT (Mitsui-7): inhaled particles (5 $\text{mg}/\text{m}^3$ , 12 days) penetrated the visceral pleura and were found in the pleural lavage as singlets with an average length of 6.9 $\mu\text{m}$ , in close contact with or penetrating into the cytoplasm and/or nucleus of monocytes in mice; translocation to other organs was also observed: 15 371 and 109 885 fibres/g in the liver, kidney, heart, or brain 1 and 336 days after exposure, respectively; the number of singlet MWCNT in the lymph nodes, diaphragm, chest wall, and extrapulmonary organs was significantly higher 336 days after exposure compared with 1 day after exposure ( <a href="#">Mercer et al., 2013a, b</a> ) (+) Translocation of MWCNT-N (Nikkiso Co.), MWCNT-M (Mitsui-7), or crocidolite asbestos was observed in rats after intrapulmonary spraying (5 times over 9 days; total dose, 1.25 $\text{mg}/\text{rat}$ ); MWCNT or crocidolite fibres were found in the mediastinal lymph nodes, liver sinusoid cells, blood vessel wall cells in the brain, renal tubular cells, spleen sinus, and macrophages; a few fibres penetrated directly from the lungs into the pleural cavity through the visceral pleura; no fibres were seen in the parietal pleura; visceral pleural cell proliferation was observed at the end of exposure on day 9 ( <a href="#">Xu et al., 2012</a> )	

**Table 4.21 (continued)**

Target tissue	MWCNT <sup>b</sup>	SWCNT
Biopersistence/ increased retention half- time	(+) MWCNT (Baytubes) had significantly increased retention half-times (at $\geq 0.4 \text{ mg/m}^3$ ) in rats ( <a href="#">Pauluhn, 2010a</a> ) (+) MWCNT (Mitsui-7): 65% of the dose remained in the lungs 336 days after inhalation ( $5 \text{ mg/m}^3$ ), and the dispersion of multiple structures to single fibres over time was seen in mice ( <a href="#">Mercer et al., 2013b</a> ) (+) MWCNT (Mitsui-7): 8% of a PA-administered dose ( $10\text{--}80 \text{ }\mu\text{g}$ ) was seen in the alveolar septa ( <a href="#">Mercer et al., 2011</a> )	

<sup>a</sup> The metal catalyst content in all CNT materials in these studies was reported to be  $< 2\%$  (as-produced). No studies of the disposition of purified CNT in the lungs were found; no studies of CNT other than SWNT and MWCNT were found

<sup>b</sup> Studies of intrapleural and intraperitoneal injection in mice showed that short ( $\sim 5 \text{ }\mu\text{m}$ ) or tangled MWCNT were effectively cleared through the lymphatic stomata, while long, rigid MWCNT were trapped in the stomata and induced inflammation and granuloma formation ([Poland et al., 2008](#); [Murphy et al., 2011](#)).  
(+) positive finding; (±) equivocal finding; CNT, carbon nanotubes; HiPCO, high-pressure carbon monoxide process; MWCNT, multiwalled carbon nanotubes; PA, pharyngeal aspiration; SWCNT, single-walled carbon nanotubes

pleural cell proliferation was apparent at the end of the 9-day exposure ([Xu et al., 2012](#)). MWCNT (as-produced, CM-100; diameter, ~10–15 nm; length, ~20  $\mu\text{m}$ ) were observed in the pleura 28 days after a 90-day exposure by inhalation in rats, and DNA damage was observed (by the comet assay) up to 90 days after exposure ([Kim et al., 2014](#)). [The Working Group noted the short length of the aerosol generated.]

The numbers of MWCNT in the lungs and other organs were quantified after a 12-day exposure of mice to 5 mg/m<sup>3</sup> for 5 hours per day; most of the MWCNT in the lungs were agglomerated, but only singlet MWCNT structures (average length, 6.9  $\mu\text{m}$ ) were observed in the liver, kidney, heart, brain, chest wall, and diaphragm ([Mercer et al., 2013a, b](#)). Rapid translocation of MWCNT occurred and 0.6% of the dose administered by pharyngeal aspiration was seen in the subpleura of mice 1 day after exposure ([Mercer et al., 2010](#)). <sup>14</sup>C-Radiolabelled MWCNT administered to mice by pharyngeal aspiration was detected in the spleen and liver 1 day after exposure, increasing to 0.1–1% of the administered dose by 6–12 months after exposure, while the lung dose decreased to 10–20% of the administered dose over that time ([Czarny et al., 2014](#)).

The length and rigidity of the MWCNT influenced their clearance from the pleura after intrapleural injection; mice given the longer structures (mean length, 13  $\mu\text{m}$ ) developed significant inflammation and fibrosis of the parietal pleura compared with those given the shorter MWCNT (length, 0.5–5  $\mu\text{m}$ ) ([Murphy et al., 2011](#)).

The rat lung retention rate of short MWCNT (geometric mean length, 1.1  $\mu\text{m}$ ; GSD, 2.7) was similar to that for respirable poorly soluble spherical particles, with a retention half-time of approximately 50 days after inhalation exposure to 0.37 mg/m<sup>3</sup> of MWCNT ([Oyabu et al., 2011](#)). The rat lung retention half-times were greater for another MWCNT (Baytubes; MMAD, ~3  $\mu\text{m}$ ; GSD, ~2), ranging from 151 to 375 days in rats

exposed to inhalation concentrations ranging from 0.1 to 6 mg/m<sup>3</sup> ([Pauluhn, 2010a](#)).

#### 4.6.3 Persistent inflammation, granuloma formation, fibrosis, and pleural end-points

The studies on the toxicity of CNT in vivo are summarized in [Table 4.22](#), in which the types of CNT and biological end-points are identified. Acute or persistent pulmonary inflammation ([Fig. 4.3](#)), pulmonary granuloma, fibrosis, and pleural end-points with well-defined effects were observed in the studies of MWCNT, SWCNT, and other CNT. Regardless of the number of walls or extent of purification, significant dose–response relationships were observed for these pulmonary end-points.

Occupational exposures to CNT may be to various types and forms of CNT that vary with respect to purity, especially in the content and bioavailability of metal catalyst residues. In general, MWCNT and SWCNT used in their “as-produced” or pure or purified forms produce a marked acute inflammatory response in the lungs after inhalation/aspiration. There is some evidence that “fully purified” MWCNT showed less severe responses than “as-produced” or partially purified MWCNT. Repeated exposure to CNT by inhalation/aspiration induces a persistent inflammatory response with concomitant focal granuloma formation and co-localization of fibrosis in a dose-dependent fashion. Even acute exposure to MWCNT can lead to their translocation to the pleura with subpleural cellular infiltration, collagen deposition, and pleural (mesothelial) cell hyperplasia.

#### 4.6.4 Genotoxicity

See [Table 4.23](#), [Table 4.24](#), and [Table 4.25](#)

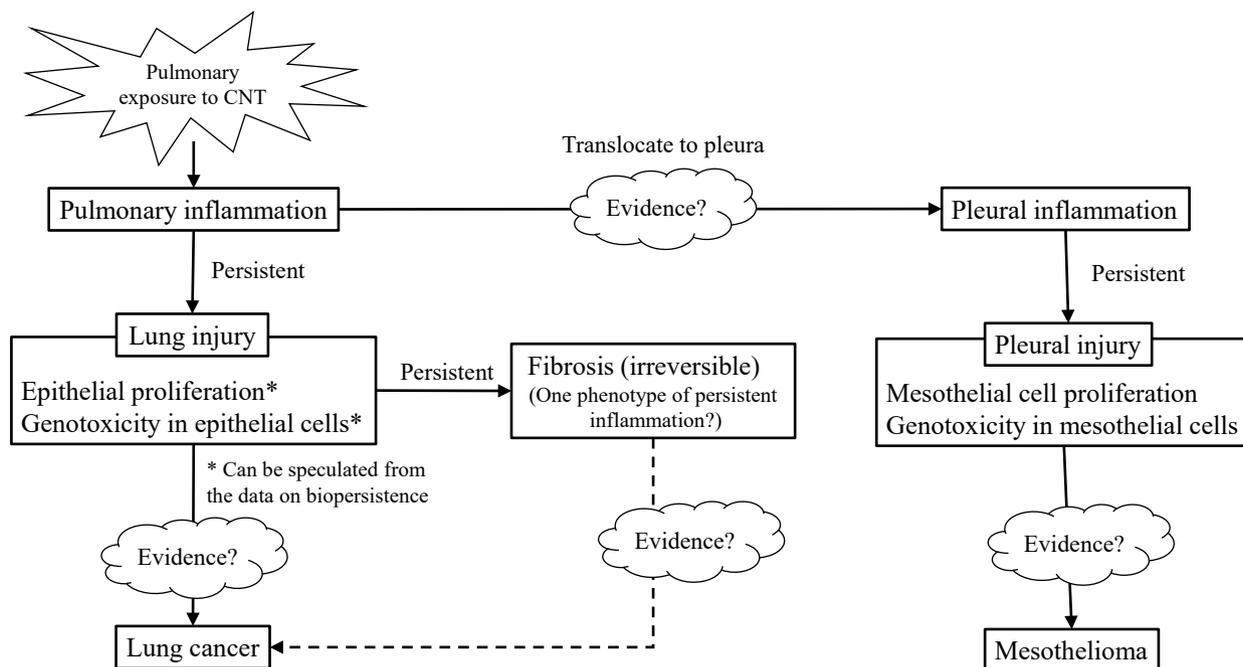
[The Working Group recognized the difficulties in evaluating the results of studies of genotoxicity due to the lack of standardized methods

**Table 4.22 Summary of results for end-points related to persistent inflammation, granuloma formation, fibrosis, and pleural end-points after exposure to carbon nanotubes in vivo**

End-point	MWCNT			SWCNT			Other CNT
	As-produced	Pure or purified	Fully purified <sup>a</sup>	As-produced	Pure or purified	Other CNT	
Acute pulmonary inflammation	(+) <a href="#">Muller et al. (2008a)</a>	(+) <a href="#">Muller et al. (2005)</a>	(-) <a href="#">Muller et al. (2008a)</a>	(+) <a href="#">Warheit et al. (2004)</a>	(-) <a href="#">Morimoto et al. (2012a)</a>	(+) <a href="#">Crouzier et al. (2010)</a>	(+) <a href="#">Crouzier et al. (2010)</a>
	(+) <a href="#">Ma-Hock et al. (2009)</a>	(+) <a href="#">Han et al. (2010)</a>				(80% DWCNT/20% SWCNT)	
	(+) <a href="#">Park et al. (2009)</a>	(+) <a href="#">Kasai et al. (2015)</a>					
	(-) <a href="#">Mitchell et al. (2007)</a>	(+) <a href="#">Muller et al. (2008a)</a>					
	(-) <a href="#">Elgrabi et al. (2008)</a>	(-) <a href="#">Morimoto et al. (2012b)</a>					
	(-) <a href="#">Mitchell et al. (2009)</a>	(inhalation exposure)					
Persistent pulmonary inflammation	(+) <a href="#">Ma-Hock et al. (2009)</a>	(+) <a href="#">Ellinger-Ziegelbauer &amp; Pauluhn (2009)</a>	(+) <a href="#">Shvedova et al. (2005)</a>	(+) <a href="#">Shvedova et al. (2008)</a>	(+) <a href="#">Shvedova et al. (2005)</a>	(+) <a href="#">Delorme et al. (2012)</a>	(+) <a href="#">Delorme et al. (2012)</a>
	(+) <a href="#">Ellinger-Ziegelbauer &amp; Pauluhn (2009)</a>	(+) <a href="#">Kobayashi et al. (2010)</a>			(+) <a href="#">Shvedova et al. (2007)</a>	(CNF)	(+) <a href="#">Shvedova et al. (2007)</a>
	(+) <a href="#">Aiso et al. (2010)</a>	(+) <a href="#">Porter et al. (2010)</a>			(+) <a href="#">Kobayashi et al. (2011)</a>	(CNF)	(+) <a href="#">Murray et al. (2012)</a>
	(+) <a href="#">Treumann et al. (2013)</a>	(+) <a href="#">Mercer et al. (2011)</a>			(+) <a href="#">Morimoto et al. (2012c)</a>	(CNF)	(+) <a href="#">Murray et al. (2012)</a>
	(+) <a href="#">Mercer et al. (2013a)</a>	(+) <a href="#">Morimoto et al. (2012b)</a>			(+) <a href="#">Murray et al. (2012)</a>	(CNF)	(+) <a href="#">Murray et al. (2012)</a>
	(-) <a href="#">Elgrabi et al. (2008)</a>	(intratracheal instillation)			(-) <a href="#">Morimoto et al. (2012a)</a>	(CNF)	(+) <a href="#">Sager et al. (2013)</a>
Pulmonary granuloma or fibrosis	(+) <a href="#">Muller et al. (2008a)</a>	(+) <a href="#">Muller et al. (2005)</a>	(±) <a href="#">Muller et al. (2008a)</a>		(+) <a href="#">Shvedova et al. (2005)</a>	(+) <a href="#">Delorme et al. (2012)</a>	(+) <a href="#">Delorme et al. (2012)</a>
	(+) <a href="#">Ma-Hock et al. (2009)</a>	(+) <a href="#">Ellinger-Ziegelbauer &amp; Pauluhn (2009)</a>			(+) <a href="#">Kobayashi et al. (2011)</a>	(CNF)	(+) <a href="#">Kobayashi et al. (2011)</a>
	(+) <a href="#">Ryman-Rasmussen et al. (2009a)</a>	(+) <a href="#">Porter et al. (2010)</a>			(+) <a href="#">Morimoto et al. (2012c)</a>	(CNF)	(+) <a href="#">Morimoto et al. (2012c)</a>
	(+) <a href="#">Aiso et al. (2010)</a>	(+) <a href="#">Mercer et al. (2011)</a>			(+) <a href="#">Murray et al. (2012)</a>	(CNF)	(+) <a href="#">Murray et al. (2012)</a>
	(+) <a href="#">Cesta et al. (2010)</a>	(+) <a href="#">Morimoto et al. (2012b)</a>			(-) <a href="#">Morimoto et al. (2012a)</a>	(CNF)	(+) <a href="#">Sager et al. (2013)</a>
	(+) <a href="#">Pauluhn (2010b)</a>	(intratracheal instillation; bolus)			(-) <a href="#">Fujita et al. (2015)</a>	(DWCNT)	(-) <a href="#">Fujita et al. (2015)</a>
	(+) <a href="#">Treumann et al. (2013)</a>	(+) <a href="#">Kasai et al. (2015)</a>					
	(+) <a href="#">Mercer et al. (2013a)</a>	(+) <a href="#">Muller et al. (2008a)</a>					
		(-) <a href="#">Morimoto et al. (2012b)</a>					
		(inhalation exposure)					
Other biological effects	(+) <a href="#">Poland et al. (2008)</a>	(-) <a href="#">Muller et al. (2009)</a>					
	(peritoneal granuloma)						
	(+) <a href="#">Murphy et al. (2011)</a>						
	(pleural inflammation)						
	(+) <a href="#">Schinwald et al. (2012)</a>						
	(pleural inflammation)						

<sup>a</sup> Metal impurities and defects were eliminated by heating at high temperatures (2400 °C)

(+) positive findings were observed at any tested dose; (±) equivocal findings were observed at the highest dose; (-) no positive findings were observed at the highest dose; (+D), positive dose-response relationship was observed; CNF, carbon nanofibres; CNT, carbon nanotubes; DWCNT, double-walled carbon nanotubes; MWCNT, multiwalled carbon nanotubes; SWCNT, single-walled carbon nanotubes

**Fig. 4.3 Persistent inflammation and exposure to carbon nanotubes**

CNT, carbon nanotubes

Compiled by the Working Group with data from [Morimoto et al. \(2014\)](#)

for genotoxicity testing, and variations in sample preparations and characterization of CNT.]

The Working Group did not identify any studies on genotoxicity end-points in presumed target tissues, surrogate cells (peripheral blood leukocytes), or matrices (e.g. urine) in humans with well-defined exposure to CNT and therefore regarded the observations in cultured human cells as being the most relevant with regard to supporting mechanistic evidence for carcinogenicity. In particular, both MWCNT and SWCNT induced aneuploidy in primary or immortalized human airway epithelial cells ([Sargent et al., 2009, 2012a](#)). This mechanism, which is described as a physical interference between CNT and the mitotic apparatus or fragmentation of the centrosome, is considered to be relevant for (airway) exposure of humans in vivo. These observations of chromosomal damage are supported by positive findings for SWCNT in cultured primary human lymphocytes ([Catalán et al., 2012](#)) and for MWCNT in the bronchial

epithelial BEAS-2B cell line ([Siegrist et al., 2014](#)). Further supporting evidence in six out of eight studies showed an increased frequency of micronuclei in human cell lines after exposure to either SWCNT or MWCNT ([Muller et al., 2008b](#); [Cveticanin et al., 2010](#); [Cicchetti et al., 2011](#); [Thurnherr et al., 2011](#); [Lindberg et al., 2013](#); [Manshian et al., 2013](#); [Kim & Yu, 2014](#); [Tavares et al., 2014](#)). Studies that gave negative results investigated the effects of pure MWCNT (length, 2–5 µm; diameter, 6–26 nm; 0.4% iron) ([Thurnherr et al., 2011](#)) and SWCNT (length, 1–5 µm; diameter, < 2 nm; impurities not reported) ([Lindberg et al., 2013](#)) that did not appear to differ from samples that caused the formation of micronuclei. In addition, one study showed that only two out of six MWCNT samples generated micronuclei, although they did not have overtly different physico-chemical characteristics compared with non-genotoxic samples ([Tavares et al., 2014](#)).

The strongest evidence of mutagenesis derives from animal studies that showed increased

**Table 4.23 Summary of results for end-points related to genotoxicity, gene expression, and cellular transformation after exposure to carbon nanotubes in vitro**

End-point	MWCNT	SWCNT
DNA oxidation products	± (FPG) <a href="#">Kermanizadeh et al. (2012, 2013)</a> <sup>a</sup> + (FPG) <a href="#">McShan &amp; Yu (2014)</a> <sup>a</sup> + (FPG, ENDIII) (iron, 0.1%) <a href="#">Migliore et al. (2010)</a> – (FPG) <a href="#">Cavallo et al. (2012)</a> <sup>a</sup> – (FPG) <a href="#">Karlisson et al. (2008)</a>	+ (FPG) <a href="#">Vesterdal et al. (2014b)</a> <sup>a</sup> + (FPG) (iron, 2%) <a href="#">Jacobsen et al. (2008)</a> + (FPG, ENDIII) <a href="#">Migliore et al. (2010)</a> – (FPG) <a href="#">Pelka et al. (2013)</a> <sup>a</sup>
DNA strand breaks	+ <a href="#">Lindberg et al. (2013)</a> <sup>b</sup> + (iron, 0.55%) <a href="#">Cavallo et al. (2012)</a> <sup>a</sup> + <a href="#">Karlisson et al. (2008)</a> <sup>a</sup> + <a href="#">Cicchetti et al. (2011)</a> <sup>a</sup> + <a href="#">Kermanizadeh et al. (2012, 2013)</a> <sup>a</sup> + <a href="#">Alarif et al. (2014)</a> <sup>a</sup> + (iron, 0.420%) <a href="#">Aldieri et al. (2013)</a> + (nickel, 1.5%) <a href="#">Di Giorgio et al. (2011)</a> + (iron, 0.1%) <a href="#">Migliore et al. (2010)</a> + (1% impurities) <a href="#">Cveticanin et al. (2010)</a> <sup>a</sup> + <a href="#">Barillet et al. (2010)</a> <sup>c</sup> – <a href="#">Ju et al. (2014)</a> (neutral comet assay) <sup>a</sup> – <a href="#">Thurnherr et al. (2011)</a> – <a href="#">McShan &amp; Yu (2014)</a> – <a href="#">Barillet et al. (2010)</a> <sup>d</sup> – <a href="#">Lindberg et al. (2013)</a> <sup>a,e</sup> – (iron, 0.002%) <a href="#">Aldieri et al. (2013)</a>	+ <a href="#">Pelka et al. (2013)</a> <sup>a</sup> + <a href="#">Lindberg et al. (2013)</a> <sup>a,b</sup> + <a href="#">Kim &amp; Yu (2014)</a> <sup>a</sup> + <a href="#">Vesterdal et al. (2014b)</a> <sup>a</sup> + (iron, 0.07%) <a href="#">Pacurari et al. (2008a)</a> <sup>a,b</sup> + (> 50% SWCNT) <a href="#">Lindberg et al. (2009)</a> <sup>a</sup> + (iron, 0.23%) <a href="#">Kisin et al. (2007)</a> + (iron, 0.23%) <a href="#">Kisin et al. (2011)</a> + (1% impurities) <a href="#">Yang et al. (2009)</a> + (30% impurities, amide functionalized) <a href="#">Cveticanin et al. (2010)</a> <sup>a</sup> + (nickel, 1.5%) <a href="#">Di Giorgio et al. (2011)</a> + <a href="#">Migliore et al. (2010)</a> – (iron, 2%) <a href="#">Jacobsen et al. (2008)</a>
Chromosomal aberrations	+ <a href="#">Siegrist et al. (2014)</a> <sup>a</sup> + <a href="#">Asakura et al. (2010)</a> – (iron, 1 or 5%) <a href="#">Kim et al. (2011)</a>	+ (iron, 0.23%) <a href="#">Sargent et al. (2009)</a> <sup>a</sup> + (iron, 0.23%) <a href="#">Sargent et al. (2012a)</a> <sup>a</sup> + <a href="#">Catalán et al. (2012)</a> <sup>a</sup> – (iron, 4.4%) <a href="#">Ema et al. (2013b)</a> – <a href="#">Naya et al. (2011)</a>
Micronucleus formation	+ (1% impurities) <a href="#">Cveticanin et al. (2010)</a> <sup>a</sup> + (1% impurities, amide functionalized) <a href="#">Cveticanin et al. (2010)</a> <sup>a</sup> + (10% impurities) <a href="#">Cicchetti et al. (2011)</a> <sup>a</sup> + <a href="#">Tavares et al. (2014)</a> <sup>a</sup> + <a href="#">Asakura et al. (2010)</a> + (nickel, 1.5%) <a href="#">Di Giorgio et al. (2011)</a> + (2% impurities, ground sample) <a href="#">Muller et al. (2008b)</a> + (iron, 0.48%; cobalt, 0.49%) <a href="#">Muller et al. (2008a)</a> <sup>f</sup> + <a href="#">Kato et al. (2013)</a> <sup>a</sup> – <a href="#">Szendi &amp; Varga (2008)</a> <sup>a</sup> – (0.4% impurities) <a href="#">Thurnherr et al. (2011)</a> <sup>a</sup>	+ (30% impurities) <a href="#">Cveticanin et al. (2010)</a> <sup>a</sup> + (30% impurities, amide-functionalized) <a href="#">Cveticanin et al. (2010)</a> <sup>a</sup> + <a href="#">Kim &amp; Yu (2014)</a> <sup>a</sup> + <a href="#">Manshian et al. (2013)</a> <sup>a</sup> + <a href="#">Kato et al. (2013)</a> + (iron, 0.23%) <a href="#">Kisin et al. (2011)</a> + (nickel, 1.5%) <a href="#">Di Giorgio et al. (2011)</a> – (> 50% SWCNT) <a href="#">Lindberg et al. (2009)</a> <sup>a</sup> – <a href="#">Lindberg et al. (2013)</a> <sup>a</sup> – (iron, 0.23%) <a href="#">Kisin et al. (2007)</a> – <a href="#">Pelka et al. (2013)</a>

Table 4.23 (continued)

End-point	MWCNT	SWCNT
Sister-chromatid exchange	+ <a href="#">Kato et al. (2013)</a>	
Mutations	+ <a href="#">Zhu et al. (2007)</a> <i>Aprt</i> - <a href="#">Asakura et al. (2010)</a> <i>Hgprt</i> - <a href="#">Di Sotito et al. (2009)</a> <i>Escherichia coli</i> - <a href="#">Kim et al. (2011)</a> <i>Salmonella</i> and <i>Escherichia coli</i>	- <a href="#">Manshian et al. (2013)</a> <i>HGPRT</i> <sup>a</sup> - <a href="#">Jacobsen et al. (2008)</a> <i>cII</i>
Gene expression <sup>g</sup>	+ <a href="#">Ravichandran et al. (2010)</a> p53, p21 (up) <sup>a</sup> + <a href="#">Srivastava et al. (2011)</a> P53, CDKN1A (up); BCL2 (down) <sup>a</sup> + <a href="#">Kim et al. (2012b)</a> CDKN2A (down), BCL2 (up) <sup>a</sup> + <a href="#">Yankoningsloo et al. (2012)</a> <i>Bcl2</i> (up) + <a href="#">Søs Poulsen et al. (2013)</a> <i>Jun</i> (up), <i>Cdkn2c</i> (down) + <a href="#">Zhu et al. (2007)</a> p53 (up) + <a href="#">Zhang &amp; Yan (2012)</a> <i>Cdkn1a</i> (up), <i>pRb</i> unphosphorylated (up) - <a href="#">Zhang &amp; Yan (2012)</a> P53 <sup>a</sup>	+ <a href="#">Sarkar et al. (2007)</a> ATM (up) <sup>a</sup> + <a href="#">Wang et al. (2011a)</a> P53 phosphorylated (down) <sup>a</sup> + <a href="#">Pelka et al. (2013)</a> P53 phosphorylated (up) <sup>a</sup> + <a href="#">Wang et al. (2012b)</a> Bcl2 (down) + <a href="#">Lohcharoenkal et al. (2014)</a> H-RAS (up) <sup>a,b</sup> + <a href="#">Wang et al. (2014)</a> MYC (up), P53 (down) <sup>a</sup>
In-vitro cellular transformation	+ <a href="#">Wang et al. (2014)</a> <sup>a</sup>	+ <a href="#">Wang et al. (2011a)</a> <sup>a</sup> + <a href="#">Lohcharoenkal et al. (2014)</a> <sup>a</sup> + <a href="#">Wang et al. (2014)</a> <sup>a</sup>

<sup>a</sup> Measurements in human cells [the Working Group noted that the studies reported by [Sargent et al. \(2009, 2012a\)](#) were carried out on primary or immortalized human airway epithelial cells; the proposed mechanisms were supported by observations under acellular conditions ([Li et al., 2006](#); [Dinu et al., 2009](#)), leading to physical interference with the mitotic apparatus or fragmentation of the centrosome in response to both SWCNT and MWCNT (approximately 1 µm); these interactions led to aneuploidy in the daughter cells.]

<sup>b</sup> Studied in mesothelial cells

<sup>c</sup> Defined as “long” MWCNT (0.1–20 µm)

<sup>d</sup> Defined as “short” MWCNT (0.1–5 µm)

<sup>e</sup> Studied in bronchial epithelial BEAS-2B cells

<sup>f</sup> Heating (2400 °C) of the ground sample abolished genotoxicity; samples that were heated and subsequently ground increased the formation of micronuclei

<sup>g</sup> Gene expression included oncogenes, tumour-suppressor genes, and genes involved in DNA repair and cell-cycle regulation; +, differential expression between control (untreated) and treated cells; -, unaltered

NNT, carbon nanotubes; ENDOIII, endonuclease III; FPG, formamidopyrimidine glycosylase; MWCNT, multiwalled carbon nanotubes; SWCNT, single-walled carbon nanotubes  
Levels of DNA damage, mutations, chromosome damage and cellular transformation were increased (+) or unaltered (-) in exposed cells compared with unexposed controls; (±), equivocal result

**Table 4.24 Summary of results for end-points related to genotoxicity and gene expression after exposure to carbon nanotubes in vivo**

End-point	MWCNT	SWCNT
DNA oxidation products (lung)	- (FPG) <a href="#">Cao et al. (2014)</a>	- (FPG) (iron, 2%) <a href="#">Vesterdal et al. (2014a)</a>
DNA breaks (lung)	+ <a href="#">Kato et al. (2013)</a> + <a href="#">Cao et al. (2014)</a> + (iron, 2%) <a href="#">Kim et al. (2012a)</a> + (iron, 2%) <a href="#">Kim et al. (2014)</a> - <a href="#">Ema et al. (2013a)</a> + <a href="#">Kato et al. (2013)</a> <i>Gpt</i>	- (iron, 2%) <a href="#">Vesterdal et al. (2014a)</a> - (iron, 4.4%) <a href="#">Naya et al. (2012)</a>
Mutations (lung)	+ <a href="#">Kato et al. (2013)</a> <i>Gpt</i>	+ (iron, 17.7%) <a href="#">Shvedova et al. (2008)</a> <i>K-Ras</i> (inhalation exposure) - (iron, 17.7%) <a href="#">Shvedova et al. (2008)</a> <i>K-Ras</i> (pharyngeal aspiration)
Micronuclei (lung)	+ (2% impurities) <a href="#">Muller et al. (2008b)</a>	
Gene expression <sup>a</sup>	+ <a href="#">Snyder-Talkington et al. (2013b)</a> <i>Vegfa</i> (down), <i>Iumb</i> (up) + <a href="#">Guo et al. (2012)</a> <i>Bcl3</i> (down), <i>EgFr</i> (down) + <a href="#">Huang et al. (2014)</a> <i>Cdkn1</i> , <i>Bax</i> (up) + <a href="#">Søs Poulsen et al. (2013)</a> <i>Bcl3</i> , <i>Aurka</i> (up); <i>Myb</i> (down)	+ (Unpurified sample) <a href="#">Park et al. (2011a)</a> p53 (up) + (Purified sample) <a href="#">Park et al. (2011b)</a> p53 (up) + <a href="#">Park et al. (2013)</a> p53 phosphorylated (up)
DNA oxidation products (gavage study)		+ (8-oxodG) (iron, 2%) <a href="#">Folkmann et al. (2009)</a> liver, lung - (8-oxodG) (iron, 2%) <a href="#">Folkmann et al. (2009)</a> colon mucosa
Micronucleus formation (gavage study)		- (iron, 4.4%) <a href="#">Ema et al. (2013b)</a> immature bone marrow erythrocytes - <a href="#">Naya et al. (2011)</a> polychromatic bone marrow erythrocytes
DNA breaks (intraperitoneal injection)	+ (Both pristine and acid-functionalized samples) <a href="#">Patlolla et al. (2010)</a> leukocytes + <a href="#">Ghosh et al. (2011)</a> bone marrow cells	
Micronucleus formation (intraperitoneal injection)	+ (Both pristine and acid-functionalized samples) <a href="#">Patlolla et al. (2010)</a> femoral bone marrow cells + <a href="#">Ghosh et al. (2011)</a> polychromatic erythrocytes, femoral bone marrow cells - <a href="#">Kim et al. (2011)</a> polychromatic erythrocytes	
Chromosomal aberrations (intraperitoneal injection)	+ (Both pristine and acid-functionalized samples) <a href="#">Patlolla et al. (2010)</a> femoral bone marrow cells	

<sup>a</sup> Gene expression included oncogenes, tumour-suppressor genes, and genes involved in DNA repair and cell-cycle regulation; +, differential expression between control (untreated) and treated cells.

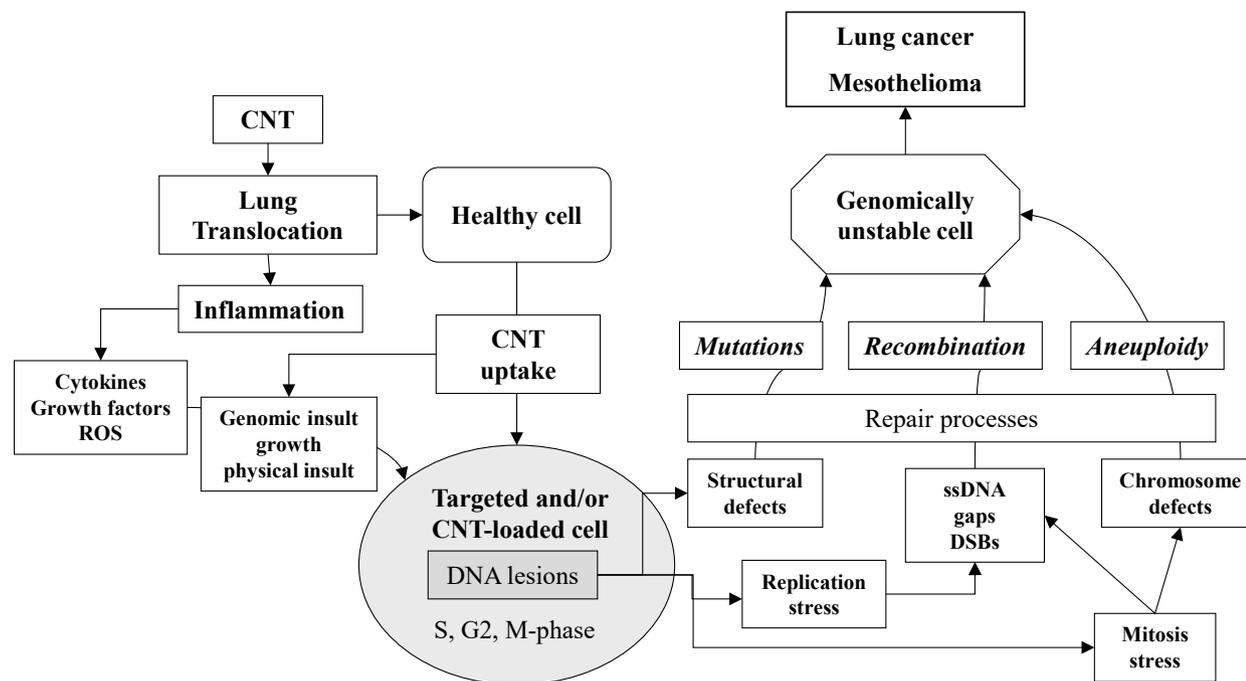
Levels of DNA damage, mutations, chromosome damage and cellular transformation were increased (+) or unaltered (-) in exposed cells compared with unexposed controls.

CNT, carbon nanotubes; FPG, formamidopyrimidine glycosylase; MWCNT, multiwalled carbon nanotubes; SWCNT, single-walled carbon nanotubes

**Table 4.25 Overall summary of results for genetic and related end-points in studies of exposure to carbon nanotubes in vivo and in vitro**

End-point	In vivo (studies in experimental animals only)		In vitro (studies in cells of humans and experimental animals only)	
	MWCNT	SWCNT	MWCNT	SWCNT
DNA oxidation products	1 negative (FPG, lung) at low-dose	1 negative (FPG, lung) at low doses 1 positive (8-oxodG) (gavage exposure)	2/5 positive (FPG)	3/4 positive (FPG)
DNA strand breaks	4/5 positive (lung), 2 positive (intraperitoneal injection)	2/2 negative (FPG, lung)	11/15 positive	11/13 positive
Mutations	1 positive (lung)	1 positive (lung)	2/2 negative	2/2 negative
Chromosomal aberrations	1 positive (intraperitoneal injection)	No information	2/3 positive	3/5 positive
Micronucleus formation	1 positive (lung), 2/3 positive (intraperitoneal injection)	2/2 negative	9/11 positive	7/9 positive
Sister-chromatid exchange	1 positive	No information	No information	No information
Gene expression	Modulation of genes involved in DNA repair, apoptosis, and cell-cycle control	Modulation of p53 expression (up)	Modulation of genes involved in DNA repair, apoptosis, and cell-cycle control	Modulation of p53 expression (up) and genes involved in DNA repair
Transformation	No information	No information	1 positive	3/3 positive

FPG, formamidopyrimidine glycosylase; MWCNT, multiwalled carbon nanotubes; 8-oxodG, 8-oxodeoxyguanosine; SWCNT, single-walled carbon nanotubes

**Fig. 4.4 Mechanisms of genomic instability generated by carbon nanotubes**

CNT, carbon nanotubes; ssDNA, single-strand DNA; DSB, double-strand break; ROS, reactive oxygen species

Cancer arises from genomic instability (GIN), and the genotoxic effects of carbon nanotubes (CNT) are consistent with an ability to generate GIN. Inhaled CNT may induce local inflammation associated with the production of cytokines, growth factors, and reactive oxygen species, which can induce genomic insult and stimulate cell growth. Alternatively, fibres can be internalized by many cell types, resulting in a physical insult due to fibre load. In these “targeted and/or fibre-loaded” cells, DNA lesions produce defects in DNA structure. DNA breakage is generated by replication stress, and mitosis stress generates both DNA breaks and chromosome defects. Various repair mechanisms and cell-cycle checkpoints are then activated to control genome integrity. Unrepaired or error-prone repair processes can entail mutations, chromosomal rearrangements and variations in chromosome number or morphology, which are the causes of GIN. Selection and amplification of genomically unstable cells can progress to lung cancer and mesothelioma.

Compiled by the Working Group

levels of guanine phosphoribosyltransferase (*Gpt*) mutations in the lung tissues of mice after intratracheal exposure to MWCNT (Kato et al., 2013) and of *K-Ras* mutations after inhalation exposure to SWCNT (Shvedova et al., 2008, 2014). The results for mutagenesis in cultured cells have been negative, including one study in human lymphoblastoid MCL-5 cells (Manshian et al., 2013). Genotoxicity studies have provided information on the mechanisms of genomic instability generated by CNT (Fig. 4.4). Studies of DNA damage – essentially DNA strand breaks and oxidatively damaged DNA measured by the comet assay – in cultured human cells have shown genotoxicity after exposure to either MWCNT or SWCNT. Increased levels of DNA strand breaks

in the lungs of rodents after pulmonary exposure to either MWCNT or SWCNT were found in four studies (Kim et al., 2012a; Kato et al., 2013; Cao et al., 2014; Kim et al., 2014) while no increase was found in three studies (Naya et al., 2012; Ema et al., 2013a; Vesterdal et al., 2014a); intraperitoneal injection of MWCNT yielded positive results in two studies (Patlolla et al., 2010; Ghosh et al., 2011). No data were available regarding the relationship between the characteristics of CNT and their ability to generate DNA damage in human cultured cells and organs of exposed animals. [These observations indicate that the mechanisms of genotoxicity involve chromosomal aberrations and oxidative stress, although a formal assessment of the inhibition of DNA damage

through supplementation with antioxidants in CNT-exposed cells has not been pursued.] This mechanism of DNA damage is known to occur in human cells after exposure to particulate matter. Two human mesothelial (pleural Met-5A and peritoneal LP-9) cell lines showed features of morphological transformation and *H-RAS* expression after continuous exposure to SWCNT ([Lohcharoenkal et al., 2014](#)).

Pulmonary exposure to MWCNT and SWCNT had no effect on oxidative DNA damage (i.e. FPG-sensitive sites) in studies that mainly focused on cardiovascular effects in atherosclerosis-prone (*ApoE*<sup>-/-</sup> knockout) mice, but the administered doses were low (maximal dose of 1 mg/kg bw as two intratracheal instillations ([Vesterdal et al., 2014a](#)) and 25.6 µg/mouse per week ([Cao et al., 2014](#))). [Therefore, these studies cannot rule out the possibility that DNA damage is generated by oxidative stress in pulmonary tissues after airway exposure to MWCNT and SWCNT.] One study showed increased levels of pro-mutagenic 8-oxodG lesions in both lung and liver tissues after gastrointestinal administration of low doses (0.064 and 0.64 mg/kg bw) of SWCNT ([Folkmann et al., 2009](#)). [This study suggests the involvement of a genotoxic mechanism arising as a consequence of oxidative stress, although it is impossible to distinguish between direct and indirect genotoxic mechanisms.]

The MWCNT and SWCNT investigated originated from different manufacturing processes, leading to substantial differences in dimensions and residual transition metal content. The available literature supports the conclusion that exposure to a range of different MWCNT (including Mitsui-7) and SWCNT can generate DNA strand breaks, oxidized DNA nucleobases, micronuclei, and chromosomal aberrations in animal and human cells through various mechanisms according to the type of CNT material. Overall, there is strong evidence that a genotoxic mechanism in human cells leads to carcinogenesis after exposure to both MWCNT and SWCNT.

## 5. Summary of Data Reported

### 5.1 Exposure data

Carbon nanotubes (CNT) are comprised of graphene sheets rolled into cylinders, some of which may be hundreds of micrometres in length and be composed of either a single graphene cylinder (single-walled carbon nanotube; SWCNT) or many graphene cylinders inside one another in concentric layers (multiwalled carbon nanotubes; MWCNT). The outer diameter of SWCNT is generally 1–3 nm and that of MWCNT is 10–200 nm. The thickness of CNT mainly depends on the number of graphene layers contained therein and on the chirality of the tubes. The length of a typical CNT is a few micrometres, but their length can vary from only a few hundreds of nanometres to several tens of micrometres.

The production of CNT involves the use of a carbon source at a high temperature and/or pressure in the presence of transition metals. Both SWCNT and MWCNT are normally produced by one of three principal techniques: chemical vapour deposition, arc discharge, or laser ablation. Chemical vapour deposition is the most common production method. Depending on the production technique, the physical and chemical characteristics (e.g. diameter, length, atomic structure, surface chemistry, and defects) and the levels of impurities (such as metal catalysts, amorphous carbon, carbon black, fibres, soot, graphite, and non-tubular fullerenes) present in the final preparation may vary greatly.

Industrial-scale commercial production of CNT began in the twenty-first century. In 2006, global production of MWCNT and SWCNT was estimated at 300 and 7 tonnes, respectively. Because the industrial production and use of CNT material are relatively recent and the size of the workforce in CNT is still small, currently available data on occupational exposure are

limited. The main route of exposure in occupational settings is anticipated to be inhalation.

Due to the limitations of exposure assessment methods and the lack of a consensus on the most relevant exposure metrics, the available data do not allow complete characterization of occupational exposure to SWCNT and MWCNT and only permit a limited description of some occupational exposure situations. The operations that yield the highest release of CNT material include production, blending, transferral, sieving, pouring, weighing, and cleaning. CNT were more frequently found in the form of large entangled agglomerates; individual CNT were rarely observed and may be dependent on the work process or task.

CNT have a wide variety of applications, including incorporation into fabrics, plastics, rubbers, electronics, reinforced structures, composite materials, and other household products to improve their strength and water- and wear-resistance and reduce their weight. No quantitative data on consumer exposure to CNT have been identified, but exposure can occur in principle at all phases of the life-cycle of CNT, ranging from production to waste treatment. Several studies describing the release of CNT from consumer products have been conducted to estimate exposures from abrasion and weathering, but quantitative values of CNT or CNT composites released from the products have not been specified.

## 5.2 Human carcinogenicity data

No relevant data were available to the Working Group.

## 5.3 Animal carcinogenicity data

MWCNT-7 significantly increased the incidence of peritoneal mesothelioma in one study by intrascrotal injection in male rats, of

mesothelioma in one study by intraperitoneal injection in male and female rats (combined), and of peritoneal mesothelioma in two studies by intraperitoneal administration in male p53<sup>+/-</sup> mice. In one inhalation study in male mice, MWCNT-7 was a promoter of 3-methylcholanthrene-initiated bronchiolo-alveolar adenoma and carcinoma.

In one study, intraperitoneal administration of two types of MWCNT with physical dimensions similar to those of MWCNT-7 (length, 1–19 µm; diameter, 40–170 nm) significantly increased the incidence of mesothelioma in male and female rats (combined).

One study of intraperitoneal administration of MWCNT in male rats and one study of subcutaneous administration of MWCNT in male mice gave negative results. One study of intratracheal instillation of MWCNT in male mice and one study of intraperitoneal implantation of MWCNT in rats were inadequate for an evaluation.

One study of intratracheal instillation and one study of intraperitoneal implantation of SWCNT in rats were inadequate for an evaluation.

## 5.4 Mechanistic and other relevant data

### 5.4.1 *Biopersistence, pleural translocation, and injury*

MWCNT have been associated with increased retention half-times in rat lungs at lower mass doses than those observed for other poorly soluble, respirable particles. MWCNT and SWCNT enter the lung interstitium in rodents exposed by inhalation.

In rodents, three types of long, rigid MWCNT (MWCNT-7, MWCNT-N, and Helix) reached the subpleural tissues after inhalation, and one of these (Helix) induced subpleural fibrosis in mice. The three types of MWCNT also translocated to lung-associated lymph nodes and distal

organs at increasing concentrations after inhalation or intrapulmonary spraying into the lungs. MWCNT-7 rapidly translocated to the pleura and intrapleural space in mice (small percentage of the dose within 1 day of administration).

No studies evaluated the pleural penetration, translocation, or injury of other types of CNT administered by lung instillation or inhalation.

#### 5.4.2 Lung inflammation and fibrosis

Acute exposure to CNT was associated with transient inflammation that resolved over time, although CNT were able to persist in the tissues. Long-term exposure to CNT induced a sustained inflammatory response associated with granuloma formation, fibrosis, and subpleural thickening. Acute or persistent pulmonary inflammation, pulmonary granuloma or fibrosis, and bronchiolar or bronchoalveolar hyperplasia were observed in most of the studies with MWCNT, SWCNT, and other CNT. Regardless of the number of walls or extent of purification, statistically significant dose-response relationships were observed for these pulmonary end-points.

Lung epithelial cell proliferation was observed in one study in rats exposed to as-produced or functionalized MWCNT by pharyngeal aspiration.

#### 5.4.3 Genotoxicity in vivo and in vitro

SWCNT and MWCNT induced genetic lesions in experimental animals and similar genetic injuries (end-points) in cultured human and animal cells. Positive and negative results were observed in human primary and immortalized lung and mesothelial cells in short-term assays in vitro. DNA strand breaks, oxidized DNA bases, mutations, micronucleus formation, and numerical and structural chromosomal abnormalities have been reported. SWCNT and MWCNT interacted with and perturbed the

cellular mitotic apparatus, including microtubules and centrosomes, in human lung epithelial cells. *K-Ras* point mutations were observed in the lung tissues of mice 1 and 28 days and 1 year after a 4-day inhalation exposure to one type of SWCNT; 1 year after exposure, karyotypic changes were shown by micronuclei and multinucleated cells in type II pneumocytes. In two studies, MWCNT was genotoxic (by the comet assay) in rats after inhalation exposure. Overall, experimental studies are too limited to link the specific physical and chemical properties of SWCNT or MWCNT with genotoxicity. Consistent evidence indicated that SWCNT and MWCNT are genotoxic in vitro to relevant human target cells in the lungs and pleura.

#### 5.4.4 Conclusion

The results of studies of genotoxicity in vivo and in vitro were positive for SWCNT and MWCNT. Lung inflammation, granuloma formation, and fibrosis were observed in rats and mice exposed by inhalation, intratracheal instillation, or pharyngeal aspiration to SWCNT, double-walled CNT, or MWCNT. Pleural inflammation or proliferation was observed in mice or rats exposed by inhalation or intrapulmonary spraying to three types of MWCNT (MWCNT-7, MWCNT-N, and Helix).

For end-points related to mesothelioma, the mechanistic evidence is *moderate* for MWCNT and is *weak* for SWCNT due to the lack of data.

For end-points related to cancer of the lung, the mechanistic evidence is *moderate* for MWCNT and *equivocal* for SWCNT.

The mechanistic evidence for other CNT is *weak* due to limited data.

The mechanistic events relevant to genotoxicity, lung inflammation, and fibrosis as well as translocation to the pleura, are liable to occur in humans exposed to CNT by inhalation.

Due to the heterogeneity of CNT and the limited long-term studies, significant data

gaps remain with regard to understanding the mechanisms of carcinogenicity.

## 6. Evaluation

### 6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of carbon nanotubes.

### 6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of MWCNT-7 multiwalled carbon nanotubes.

There is *limited evidence* in experimental animals for the carcinogenicity of two types of multiwalled carbon nanotube with dimensions similar to MWCNT-7.

There is *inadequate evidence* in experimental animals for the carcinogenicity of multiwalled carbon nanotubes other than MWCNT-7.

There is *inadequate evidence* in experimental animals for the carcinogenicity of single-walled carbon nanotubes.

### 6.3 Overall evaluation

MWCNT-7 multiwalled carbon nanotubes are *possibly carcinogenic to humans* (Group 2B).

Multiwalled carbon nanotubes other than MWCNT-7 are *not classifiable* as to their carcinogenicity to humans (Group 3).

Single-walled carbon nanotubes are *not classifiable* as to their carcinogenicity to humans (Group 3).

## References

Abe S, Itoh S, Hayashi D, Kobayashi T, Kiba T, Akasaka T, et al. (2012). Biodistribution of aqueous suspensions of carbon nanotubes in mice and their biocompatibility.

- J Nanosci Nanotechnol*, 12(1):700–6. doi:[10.1166/jnn.2012.5391](https://doi.org/10.1166/jnn.2012.5391) PMID:[22524043](https://pubmed.ncbi.nlm.nih.gov/22524043/)
- Aiso S, Kubota H, Umeda Y, Kasai T, Takaya M, Yamazaki K, et al. (2011). Translocation of intratracheally instilled multiwall carbon nanotubes to lung-associated lymph nodes in rats. *Ind Health*, 49(2):215–20. doi:[10.2486/indhealth.MS1213](https://doi.org/10.2486/indhealth.MS1213) PMID:[21173528](https://pubmed.ncbi.nlm.nih.gov/21173528/)
- Aiso S, Yamazaki K, Umeda Y, Asakura M, Kasai T, Takaya M, et al. (2010). Pulmonary toxicity of intratracheally instilled multiwall carbon nanotubes in male Fischer 344 rats. *Ind Health*, 48(6):783–95. doi:[10.2486/indhealth.MS1129](https://doi.org/10.2486/indhealth.MS1129) PMID:[20616469](https://pubmed.ncbi.nlm.nih.gov/20616469/)
- Aitken RJ, Chaudhry MQ, Boxall AB, Hull M (2006). Manufacture and use of nanomaterials: current status in the UK and global trends. *Occup Med (Lond)*, 56(5):300–6. doi:[10.1093/occmed/kql051](https://doi.org/10.1093/occmed/kql051) PMID:[16868127](https://pubmed.ncbi.nlm.nih.gov/16868127/)
- Al Faraj A, Bessaad A, Cieslar K, Lacroix G, Canet-Soulas E, Crémillieux Y (2010). Long-term follow-up of lung biodistribution and effect of instilled SWCNTs using multiscale imaging techniques. *Nanotechnology*, 21(17):175103. doi:[10.1088/0957-4484/21/17/175103](https://doi.org/10.1088/0957-4484/21/17/175103) PMID:[20368681](https://pubmed.ncbi.nlm.nih.gov/20368681/)
- Al Faraj A, Cieslar K, Lacroix G, Gaillard S, Canet-Soulas E, Crémillieux Y (2009). In vivo imaging of carbon nanotube biodistribution using magnetic resonance imaging. *Nano Lett*, 9(3):1023–7. doi:[10.1021/nl8032608](https://doi.org/10.1021/nl8032608) PMID:[19199447](https://pubmed.ncbi.nlm.nih.gov/19199447/)
- Al Faraj A, Fauvelle F, Luciani N, Lacroix G, Levy M, Crémillieux Y, et al. (2011). In vivo biodistribution and biological impact of injected carbon nanotubes using magnetic resonance techniques. *Int J Nanomedicine*, 6:351–61. doi:[10.2147/IJN.S16653](https://doi.org/10.2147/IJN.S16653) PMID:[21499425](https://pubmed.ncbi.nlm.nih.gov/21499425/)
- Alarifi S, Ali D, Verma A, Almajhdi FN, Al-Qahtani AA (2014). Single-walled carbon nanotubes induce cytotoxicity and DNA damage via reactive oxygen species in human hepatocarcinoma cells. *In Vitro Cell Dev Biol Anim*, 50(8):714–22. doi:[10.1007/s11626-014-9760-3](https://doi.org/10.1007/s11626-014-9760-3) PMID:[24789727](https://pubmed.ncbi.nlm.nih.gov/24789727/)
- Aldieri E, Fenoglio I, Cesano F, Gazzano E, Gulino G, Scarano D, et al. (2013). The role of iron impurities in the toxic effects exerted by short multiwalled carbon nanotubes (MWCNT) in murine alveolar macrophages. *J Toxicol Environ Health A*, 76(18):1056–71. doi:[10.1080/15287394.2013.834855](https://doi.org/10.1080/15287394.2013.834855) PMID:[24188191](https://pubmed.ncbi.nlm.nih.gov/24188191/)
- Alexander AJ (2007). Carbon Nanotube Structures and Composition: Implications for Toxicological Studies. In: Monteiro-Riviere NA, Tran CL, editors. *Nanotoxicology: Characterization, Dosing and Health Effects*. New York (NY), USA: Informa Healthcare USA; pp. 7–18.
- Ali-Boucetta H, Al-Jamal KT, Müller KH, Li S, Porter AE, Eddaoudi A, et al. (2011). Cellular uptake and cytotoxic impact of chemically functionalized and polymer-coated carbon nanotubes. *Small*, 7(22):3230–8. doi:[10.1002/smll.201101004](https://doi.org/10.1002/smll.201101004) PMID:[21919194](https://pubmed.ncbi.nlm.nih.gov/21919194/)

- Ali-Boucetta H, Kostarelos K (2013). Pharmacology of carbon nanotubes: toxicokinetics, excretion and tissue accumulation. *Adv Drug Deliv Rev*, 65(15):2111–9. doi:[10.1016/j.addr.2013.10.004](https://doi.org/10.1016/j.addr.2013.10.004) PMID:[24184372](https://pubmed.ncbi.nlm.nih.gov/24184372/)
- Andersen AJ, Wibroe PP, Moghimi SM (2012). Perspectives on carbon nanotube-mediated adverse immune effects. *Adv Drug Deliv Rev*, 64(15):1700–5. doi:[10.1016/j.addr.2012.05.005](https://doi.org/10.1016/j.addr.2012.05.005) PMID:[22634159](https://pubmed.ncbi.nlm.nih.gov/22634159/)
- Andón FT, Fadeel B (2013). Programmed cell death: molecular mechanisms and implications for safety assessment of nanomaterials. *Acc Chem Res*, 46(3):733–42. doi:[10.1021/ar300020b](https://doi.org/10.1021/ar300020b) PMID:[22720979](https://pubmed.ncbi.nlm.nih.gov/22720979/)
- Andón FT, Kapralov AA, Yanamala N, Feng W, Baygan A, Chambers BJ, et al. (2013). Biodegradation of single-walled carbon nanotubes by eosinophil peroxidase. *Small*, 9(16):2721–9, 2720. doi:[10.1002/smll.201202508](https://doi.org/10.1002/smll.201202508) PMID:[23447468](https://pubmed.ncbi.nlm.nih.gov/23447468/)
- Anjilvel S, Asgharian B (1995). A multiple-path model of particle deposition in the rat lung. *Fundam Appl Toxicol*, 28(1):41–50. doi:[10.1006/faat.1995.1144](https://doi.org/10.1006/faat.1995.1144) PMID:[8566482](https://pubmed.ncbi.nlm.nih.gov/8566482/)
- Antonelli A, Serafini S, Menotta M, Sfara C, Pierigé F, Giorgi L, et al. (2010). Improved cellular uptake of functionalized single-walled carbon nanotubes. *Nanotechnology*, 21(42):425101 doi:[10.1088/0957-4484/21/42/425101](https://doi.org/10.1088/0957-4484/21/42/425101) PMID:[20858931](https://pubmed.ncbi.nlm.nih.gov/20858931/)
- ARA(2011). Multiple-path particle deposition (MPPD 2.1): a model for human and rat airway particle dosimetry. Raleigh (NC), USA: Applied Research Associates, Inc.
- Arrêté Royal (2014). [Arrêté royal relatif à la mise sur le marché des substances manufacturées à l'état nanoparticulaire.] C-2014/24329, 27 May 2014. Bruxelles, Belgium: Service Public Fédéral Santé Publique, Sécurité de la Chaîne Alimentaire et Environnement. Available from: [http://www.nanotechia.org/sites/default/files/files/20140924\\_belgian\\_register\\_fr\\_nl.pdf](http://www.nanotechia.org/sites/default/files/files/20140924_belgian_register_fr_nl.pdf).
- Asakura M, Sasaki T, Sugiyama T, Takaya M, Koda S, Nagano K, et al. (2010). Genotoxicity and cytotoxicity of multi-wall carbon nanotubes in cultured Chinese hamster lung cells in comparison with chrysotile A fibers. *J Occup Health*, 52(3):155–66. doi:[10.1539/joh.L9150](https://doi.org/10.1539/joh.L9150) PMID:[20379079](https://pubmed.ncbi.nlm.nih.gov/20379079/)
- Aschberger K, Johnston HJ, Stone V, Aitken RJ, Hankin SM, Peters SA, et al. (2010). Review of carbon nanotubes toxicity and exposure–appraisal of human health risk assessment based on open literature. *Crit Rev Toxicol*, 40(9):759–90. doi:[10.3109/10408444.2010.506638](https://doi.org/10.3109/10408444.2010.506638) PMID:[20860524](https://pubmed.ncbi.nlm.nih.gov/20860524/)
- Australian National Industrial Chemical Notification and Assessment Scheme (NICNAS) (2010). NICNAS working definition for ‘industrial nanomaterial’. Available from: <http://www.nicnas.gov.au/communications/issues/nanomaterials-nanotechnology/nicnas-working-definition-for-industrial-nanomaterial>, accessed 1 October 2014.
- Azad N, Iyer AKV, Wang L, Liu Y, Lu Y, Rojanasakul Y (2013). Reactive oxygen species-mediated p38 MAPK regulates carbon nanotube-induced fibrogenic and angiogenic responses. *Nanotoxicology*, 7(2):157–68. doi:[10.3109/17435390.2011.647929](https://doi.org/10.3109/17435390.2011.647929) PMID:[22263913](https://pubmed.ncbi.nlm.nih.gov/22263913/)
- Barillet S, Simon-Deckers A, Herlin-Boime N, Mayne-L’Hermitte M, Reynaud C, Cassio D, et al. (2010). Toxicological consequences of TiO<sub>2</sub>, SiC nanoparticles and multi-walled carbon nanotubes exposure in several mammalian cell types: an in vitro study. *J Nanopart Res*, 12(1):61–73. doi:[10.1007/s11051-009-9694-y](https://doi.org/10.1007/s11051-009-9694-y)
- Beamer CA, Girtsman TA, Seaver BP, Finsaas KJ, Migliaccio CT, Perry VK, et al. (2013). IL-33 mediates multi-walled carbon nanotube (MWCNT)-induced airway hyper-reactivity via the mobilization of innate helper cells in the lung. *Nanotoxicology*, 7(6):1070–81. doi:[10.3109/17435390.2012.702230](https://doi.org/10.3109/17435390.2012.702230) PMID:[22686327](https://pubmed.ncbi.nlm.nih.gov/22686327/)
- Becker ML, Fagan JA, Gallant ND, Bauer BJ, Bajpai V, Hobbie EK, et al. (2007). Length-dependent uptake of DNA-wrapped single-walled carbon nanotubes. *Adv Mater*, 19(7):939–45. doi:[10.1002/adma.200602667](https://doi.org/10.1002/adma.200602667)
- Bello D, Hart AJ, Ahn K, Hallock M, Yamamoto N, Garcia EJ, et al. (2008). Particle exposure levels during CVD growth and subsequent handling of vertically-aligned carbon nanotube films. *Carbon*, 46(6):974–7. doi:[10.1016/j.carbon.2008.03.003](https://doi.org/10.1016/j.carbon.2008.03.003)
- Bello D, Hsieh SF, Schmidt DF, Rogers EJ (2009a). Nanomaterials properties vs. biological oxidative damage: Implications for toxicity screening and exposure assessment. *Nanotoxicology*, 3(3):249–61. doi:[10.1080/17435390902989270](https://doi.org/10.1080/17435390902989270)
- Bello D, Wardle BL, Yamamoto N, Guzman de Villoria R, Garcia EJ, Hart AJ, et al. (2009b). Exposure to nanoscale particles and fibers during machining of hybrid advanced composites containing carbon nanotubes. *J Nanopart Res*, 11(1):231–49. doi:[10.1007/s11051-008-9499-4](https://doi.org/10.1007/s11051-008-9499-4)
- Bello D, Wardle BL, Zhang J, Yamamoto N, Santeufemio C, Hallock M, et al. (2010). Characterization of exposures to nanoscale particles and fibers during solid core drilling of hybrid carbon nanotube advanced composites. *Int J Occup Environ Health*, 16(4):434–50. doi:[10.1179/oeh.2010.16.4.434](https://doi.org/10.1179/oeh.2010.16.4.434) PMID:[21222387](https://pubmed.ncbi.nlm.nih.gov/21222387/)
- Berndt-Weis ML, Kauri LM, Williams A, White P, Douglas G, Yauk C (2009). Global transcriptional characterization of a mouse pulmonary epithelial cell line for use in genetic toxicology. *Toxicol In Vitro*, 23(5):816–33. doi:[10.1016/j.tiv.2009.04.008](https://doi.org/10.1016/j.tiv.2009.04.008) PMID:[19406224](https://pubmed.ncbi.nlm.nih.gov/19406224/)
- Bernholc J, Roland C, Yakobson BI (1997). Nanotubes. *Curr Opin Solid State Mater Sci*, 2(6):706–15. doi:[10.1016/S1359-0286\(97\)80014-9](https://doi.org/10.1016/S1359-0286(97)80014-9)
- Beyer G (2002). Short communication: carbon nanotubes and flame retardants for polymers. *Fire Mater*, 26(6):291–3. doi:[10.1002/fam.805](https://doi.org/10.1002/fam.805)
- Bhattacharya S, Zhang Q, Carmichael PL, Boekelheide K, Andersen ME (2011). Toxicity testing in the 21 century: defining new risk assessment approaches based on perturbation of intracellular toxicity pathways. *PLoS*

- One, 6(6):e20887 doi:[10.1371/journal.pone.0020887](https://doi.org/10.1371/journal.pone.0020887) PMID:[21701582](https://pubmed.ncbi.nlm.nih.gov/21701582/)
- Bhirde AA, Patel S, Sousa AA, Patel V, Molinolo AA, Ji Y, et al. (2010). Distribution and clearance of PEG-single-walled carbon nanotube cancer drug delivery vehicles in mice. *Nanomedicine (Lond)*, 5(10):1535–46. doi:[10.2217/nnm.10.90](https://doi.org/10.2217/nnm.10.90) PMID:[21143032](https://pubmed.ncbi.nlm.nih.gov/21143032/)
- Bhushan B (2004). Springer handbook of nanotechnology. Berlin, Germany: Springer.
- Bianco A, Kostarelos K, Prato M (2011). Making carbon nanotubes biocompatible and biodegradable. *Chem Commun (Camb)*, 47(37):10182–8. doi:[10.1039/c1cc13011k](https://doi.org/10.1039/c1cc13011k) PMID:[21776531](https://pubmed.ncbi.nlm.nih.gov/21776531/)
- Birch ME, Ku BK, Evans DE, Ruda-Eberenz TA (2011). Exposure and emissions monitoring during carbon nanofiber production—Part I: elemental carbon and iron-soot aerosols. *Ann Occup Hyg*, 55(9):1016–36. doi:[10.1093/annhyg/mer073](https://doi.org/10.1093/annhyg/mer073) PMID:[21965464](https://pubmed.ncbi.nlm.nih.gov/21965464/)
- Biswas R, Bunderson-Schelvan M, Holian A (2011). Potential role of the inflammasome-derived inflammatory cytokines in pulmonary fibrosis. *Pulm Med*, 2011:105707. doi:[10.1155/2011/105707](https://doi.org/10.1155/2011/105707) PMID:[21660282](https://pubmed.ncbi.nlm.nih.gov/21660282/)
- Bonner JC, Silva RM, Taylor AJ, Brown JM, Hilderbrand SC, Castranova V, et al. (2013). Interlaboratory evaluation of rodent pulmonary responses to engineered nanomaterials: the NIEHS Nano GO Consortium. *Environ Health Perspect*, 121(6):676–82. doi:[10.1289/ehp.1205693](https://doi.org/10.1289/ehp.1205693) PMID:[23649427](https://pubmed.ncbi.nlm.nih.gov/23649427/)
- Bottini M, Bruckner S, Nika K, Bottini N, Bellucci S, Magrini A, et al. (2006). Multi-walled carbon nanotubes induce T lymphocyte apoptosis. *Toxicol Lett*, 160(2):121–6. doi:[10.1016/j.toxlet.2005.06.020](https://doi.org/10.1016/j.toxlet.2005.06.020) PMID:[16125885](https://pubmed.ncbi.nlm.nih.gov/16125885/)
- Boyles MSP, Stoehr LC, Schlinkert P, Himly M, Duschl A (2014). The significance and insignificance of carbon nanotube-induced inflammation. *Fibers*, 2(1):45–74. doi:[10.3390/fib2010045](https://doi.org/10.3390/fib2010045)
- Brody AR, Roe MW (1983). Deposition pattern of inorganic particles at the alveolar level in the lungs of rats and mice. *Am Rev Respir Dis*, 128(4):724–9. PMID:[6625350](https://pubmed.ncbi.nlm.nih.gov/6625350/)
- Brouwer D, Berges M, Virji MA, Fransman W, Bello D, Hodson L, et al. (2012). Harmonization of measurement strategies for exposure to manufactured nano-objects; report of a workshop. *Ann Occup Hyg*, 56(1):1–9. doi:[10.1093/annhyg/mer099](https://doi.org/10.1093/annhyg/mer099) PMID:[22156566](https://pubmed.ncbi.nlm.nih.gov/22156566/)
- Brouwer DH, Links IH, De Vreede SA, Christopher Y (2006). Size selective dustiness and exposure; simulated workplace comparisons. *Ann Occup Hyg*, 50(5):445–52. doi:[10.1093/annhyg/mel015](https://doi.org/10.1093/annhyg/mel015) PMID:[16524926](https://pubmed.ncbi.nlm.nih.gov/16524926/)
- Brown DM, Donaldson K, Stone V (2010). Nuclear translocation of Nrf2 and expression of antioxidant defence genes in THP-1 cells exposed to carbon nanotubes. *J Biomed Nanotechnol*, 6(3):224–33. doi:[10.1166/jbn.2010.1117](https://doi.org/10.1166/jbn.2010.1117) PMID:[21179939](https://pubmed.ncbi.nlm.nih.gov/21179939/)
- Brown JS, Gordon T, Price O, Asgharian B (2013). Thoracic and respirable particle definitions for human health risk assessment. *Part Fibre Toxicol*, 10(1):12. doi:[10.1186/1743-8977-10-12](https://doi.org/10.1186/1743-8977-10-12) PMID:[23575443](https://pubmed.ncbi.nlm.nih.gov/23575443/)
- Brown JS, Wilson WE, Grant LD (2005). Dosimetric comparisons of particle deposition and retention in rats and humans. *Inhal Toxicol*, 17(7–8):355–85. doi:[10.1080/08958370590929475](https://doi.org/10.1080/08958370590929475) PMID:[16020034](https://pubmed.ncbi.nlm.nih.gov/16020034/)
- BSI (2007). Nanotechnologies – Part 2: Guide to safe handling and disposal of manufactured nanomaterials. PD 6699–2:2008. London, United Kingdom: British Standardization Institution.
- BSI (2010). Nanotechnologies – Part 3: Guide to assessing airborne exposure in occupational settings relevant to nanomaterials. London, United Kingdom: British Standardization Institution.
- Bussy C, Pinault M, Cambedouzou J, Landry MJ, Jegou P, Mayne-L'hermite M, et al. (2012). Critical role of surface chemical modifications induced by length shortening on multi-walled carbon nanotubes-induced toxicity. *Part Fibre Toxicol*, 9(46):46 doi:[10.1186/1743-8977-9-46](https://doi.org/10.1186/1743-8977-9-46) PMID:[23181604](https://pubmed.ncbi.nlm.nih.gov/23181604/)
- Canadian Environment Protection Act (2014). Proposed Regulatory Framework for nanomaterials under the Canadian Environmental Protection Act, 1999. Available from: <http://www.ec.gc.ca/subsnouvelles-news/subs/default.asp?lang=En&n=FD117B60-1>, accessed 9 April 2014.
- Cao Y, Jacobsen NR, Danielsen PH, Lenz AG, Stoeger T, Loft S, et al. (2014). Vascular effects of multiwalled carbon nanotubes in dyslipidemic ApoE<sup>−/−</sup> mice and cultured endothelial cells. *Toxicol Sci*, 138(1):104–16. doi:[10.1093/toxsci/kft328](https://doi.org/10.1093/toxsci/kft328) PMID:[24431218](https://pubmed.ncbi.nlm.nih.gov/24431218/)
- Casey A, Herzog E, Lyng FM, Byrne HJ, Chambers G, Davoren M (2008). Single walled carbon nanotubes induce indirect cytotoxicity by medium depletion in A549 lung cells. *Toxicol Lett*, 179(2):78–84. doi:[10.1016/j.toxlet.2008.04.006](https://doi.org/10.1016/j.toxlet.2008.04.006) PMID:[18502058](https://pubmed.ncbi.nlm.nih.gov/18502058/)
- Catalán J, Järventaus H, Vippola M, Savolainen K, Norppa H (2012). Induction of chromosomal aberrations by carbon nanotubes and titanium dioxide nanoparticles in human lymphocytes in vitro. *Nanotoxicology*, 6(8):825–36. doi:[10.3109/17435390.2011.625130](https://doi.org/10.3109/17435390.2011.625130) PMID:[21995283](https://pubmed.ncbi.nlm.nih.gov/21995283/)
- Cavallo D, Fanizza C, Ursini CL, Casciardi S, Paba E, Ciervo A, et al. (2012). Multi-walled carbon nanotubes induce cytotoxicity and genotoxicity in human lung epithelial cells. *J Appl Toxicol*, 32(6):454–64. doi:[10.1002/jat.2711](https://doi.org/10.1002/jat.2711) PMID:[22271384](https://pubmed.ncbi.nlm.nih.gov/22271384/)
- Cena LG, Peters TM (2011). Characterization and control of airborne particles emitted during production of epoxy/carbon nanotube nanocomposites. *J Occup Environ Hyg*, 8(2):86–92. doi:[10.1080/15459624.2011.545943](https://doi.org/10.1080/15459624.2011.545943) PMID:[21253981](https://pubmed.ncbi.nlm.nih.gov/21253981/)
- Cesta MF, Ryman-Rasmussen JP, Wallace DG, Masinde T, Hurlburt G, Taylor AJ, et al. (2010). Bacterial lipopolysaccharide enhances PDGF signalling and pulmonary fibrosis in rats exposed to carbon nanotubes. *Am*

- J Respir Cell Mol Biol*, 43(2):142–51. doi:[10.1165/rcmb.2009-0113OC](https://doi.org/10.1165/rcmb.2009-0113OC) PMID:[19738159](https://pubmed.ncbi.nlm.nih.gov/19738159/)
- Chakravarty P, Marches R, Zimmerman NS, Swafford AD, Bajaj P, Musselman IH, et al. (2008). Thermal ablation of tumor cells with antibody-functionalized single-walled carbon nanotubes. *Proc Natl Acad Sci USA*, 105(25):8697–702. doi:[10.1073/pnas.0803557105](https://doi.org/10.1073/pnas.0803557105) PMID:[18559847](https://pubmed.ncbi.nlm.nih.gov/18559847/)
- Chang CC, Tsai ML, Huang HC, Chen CY, Dai SX (2012). Epithelial-mesenchymal transition contributes to SWCNT-induced pulmonary fibrosis. *Nanotoxicology*, 6(6):600–10. doi:[10.3109/17435390.2011.594913](https://doi.org/10.3109/17435390.2011.594913) PMID:[21711127](https://pubmed.ncbi.nlm.nih.gov/21711127/)
- Chang LY, Overby LH, Brody AR, Crapo JD (1988). Progressive lung cell reactions and extracellular matrix production after a brief exposure to asbestos. *Am J Pathol*, 131(1):156–70. PMID:[2833103](https://pubmed.ncbi.nlm.nih.gov/2833103/)
- Charlier JC (2002). Defects in carbon nanotubes. *Acc Chem Res*, 35(12):1063–9. doi:[10.1021/ar010166k](https://doi.org/10.1021/ar010166k) PMID:[12484794](https://pubmed.ncbi.nlm.nih.gov/12484794/)
- Chen BT, Schwegler-Berry D, McKinney W, Stone S, Cumpston JL, Friend S, et al. (2012). Multi-walled carbon nanotubes: sampling criteria and aerosol characterization. *Inhal Toxicol*, 24(12):798–820. doi:[10.3109/08958378.2012.720741](https://doi.org/10.3109/08958378.2012.720741) PMID:[23033994](https://pubmed.ncbi.nlm.nih.gov/23033994/)
- Cheng J, Fernando KA, Veca LM, Sun YP, Lamond AI, Lam YW, et al. (2008). Reversible accumulation of PEGylated single-walled carbon nanotubes in the mammalian nucleus. *ACS Nano*, 2(10):2085–94. doi:[10.1021/nn800461u](https://doi.org/10.1021/nn800461u) PMID:[19206455](https://pubmed.ncbi.nlm.nih.gov/19206455/)
- Cheng WW, Lin ZQ, Ceng Q, Wei BF, Fan XJ, Zhang HS, et al. (2012). Single-wall carbon nanotubes induce oxidative stress in rat aortic endothelial cells. *Toxicol Mech Methods*, 22(4):268–76. doi:[10.3109/15376516.2011.647112](https://doi.org/10.3109/15376516.2011.647112) PMID:[22500782](https://pubmed.ncbi.nlm.nih.gov/22500782/)
- Cherukuri P, Gannon CJ, Leeuw TK, Schmidt HK, Smalley RE, Curley SA, et al. (2006). Mammalian pharmacokinetics of carbon nanotubes using intrinsic near-infrared fluorescence. *Proc Natl Acad Sci USA*, 103(50):18882–6. doi:[10.1073/pnas.0609265103](https://doi.org/10.1073/pnas.0609265103) PMID:[17135351](https://pubmed.ncbi.nlm.nih.gov/17135351/)
- Cheung W, Pontoriero F, Taratula O, Chen AM, He H (2010). DNA and carbon nanotubes as medicine. *Adv Drug Deliv Rev*, 62(6):633–49. doi:[10.1016/j.addr.2010.03.007](https://doi.org/10.1016/j.addr.2010.03.007) PMID:[20338203](https://pubmed.ncbi.nlm.nih.gov/20338203/)
- Chico L, Crespi VH, Benedict LX, Louie SG, Cohen ML (1996). Pure carbon nanoscale devices: Nanotube heterojunctions. *Phys Rev Lett*, 76(6):971–4. doi:[10.1103/PhysRevLett.76.971](https://doi.org/10.1103/PhysRevLett.76.971) PMID:[10061598](https://pubmed.ncbi.nlm.nih.gov/10061598/)
- Chou CC, Hsiao HY, Hong QS, Chen CH, Peng YW, Chen HW, et al. (2008). Single-walled carbon nanotubes can induce pulmonary injury in mouse model. *Nano Lett*, 8(2):437–45. doi:[10.1021/nl0723634](https://doi.org/10.1021/nl0723634) PMID:[18225938](https://pubmed.ncbi.nlm.nih.gov/18225938/)
- Chow JC, Watson JG, Pritchett LC, Pierson WR, Frazier CA, Purcell RG (1993). The DRI thermal/optical reflectance carbon analysis system: description, evaluation and applicaiton in US Ari quality studies. *Atmos Environ*, 27A(8):1185–201. doi:[10.1016/0960-1686\(93\)90245-T](https://doi.org/10.1016/0960-1686(93)90245-T)
- Chung KT, Sabo A, Pica AP (1982). Electrical permittivity and conductivity of carbon black-polyvinyl chloride composites. *J Appl Phys*, 53(10):6867–79. doi:[10.1063/1.330027](https://doi.org/10.1063/1.330027)
- Cicchetti R, Divizia M, Valentini F, Argentin G (2011). Effects of single-wall carbon nanotubes in human cells of the oral cavity: geno-cytotoxic risk. *Toxicol In Vitro*, 25(8):1811–9. doi:[10.1016/j.tiv.2011.09.017](https://doi.org/10.1016/j.tiv.2011.09.017) PMID:[21968257](https://pubmed.ncbi.nlm.nih.gov/21968257/)
- CIIT and RIVM (2006). Multiple-path particle dosimetry (MPPD, version 2.0): a model for human and rat airway particle dosimetry. Research Triangle Park (NC), USA: Centers for Health Research (CIIT) and the Netherlands: National Institute for Public Health and the Environment (RIVM).
- Clichici S, Biris AR, Tabaran F, Filip A (2012). Transient oxidative stress and inflammation after intraperitoneal administration of multiwalled carbon nanotubes functionalized with single strand DNA in rats. *Toxicol Appl Pharmacol*, 259(3):281–92. doi:[10.1016/j.taap.2012.01.004](https://doi.org/10.1016/j.taap.2012.01.004) PMID:[22280989](https://pubmed.ncbi.nlm.nih.gov/22280989/)
- Clift MJ, Endes C, Vanhecke D, Wick P, Gehr P, Schins RP, et al. (2014). A comparative study of different in vitro lung cell culture systems to assess the most beneficial tool for screening the potential adverse effects of carbon nanotubes. *Toxicol Sci*, 137(1):55–64. doi:[10.1093/toxsci/kft216](https://doi.org/10.1093/toxsci/kft216) PMID:[24284789](https://pubmed.ncbi.nlm.nih.gov/24284789/)
- Creighton MA, Rangel-Mendez JR, Huang J, Kane AB, Hurt RH (2013). Graphene-induced adsorptive and optical artifacts during in vitro toxicology assays. *Small*, 9(11):1921–7. doi:[10.1002/smll.201202625](https://doi.org/10.1002/smll.201202625) PMID:[25018686](https://pubmed.ncbi.nlm.nih.gov/25018686/)
- Crouzier D, Follot S, Gentilhomme E, Flahaut E, Arnaud R, Dabouis V, et al. (2010). Carbon nanotubes induce inflammation but decrease the production of reactive oxygen species in lung. *Toxicology*, 272(1–3):39–45. doi:[10.1016/j.tox.2010.04.001](https://doi.org/10.1016/j.tox.2010.04.001) PMID:[20381574](https://pubmed.ncbi.nlm.nih.gov/20381574/)
- Cui D, Tian F, Ozkan CS, Wang M, Gao H (2005). Effect of single wall carbon nanotubes on human HEK293 cells. *Toxicol Lett*, 155(1):73–85. doi:[10.1016/j.toxlet.2004.08.015](https://doi.org/10.1016/j.toxlet.2004.08.015) PMID:[15585362](https://pubmed.ncbi.nlm.nih.gov/15585362/)
- Cveticanin J, Joksic G, Leskovic A, Petrovic S, Sobot AV, Neskovic O (2010). Using carbon nanotubes to induce micronuclei and double strand breaks of the DNA in human cells. *Nanotechnology*, 21(1):015102. doi:[10.1088/0957-4484/21/1/015102](https://doi.org/10.1088/0957-4484/21/1/015102) PMID:[19946169](https://pubmed.ncbi.nlm.nih.gov/19946169/)
- Czarny B, Georgin D, Berthon F, Plastow G, Pinault M, Patriarche G, et al. (2014). Carbon nanotube translocation to distant organs after pulmonary exposure: insights from in situ (14)C-radiolabeling and tissue radioimaging. *ACS Nano*, 8(6):5715–24. doi:[10.1021/nl500475u](https://doi.org/10.1021/nl500475u) PMID:[24853551](https://pubmed.ncbi.nlm.nih.gov/24853551/)
- Dahl AR, Schlesinger RB, Heck HD, Medinsky MA, Lucier GW (1991). Comparative dosimetry of inhaled

- materials: differences among animal species and extrapolation to man. *Fundam Appl Toxicol*, 16(1):1–13. doi:[10.1016/0272-0590\(91\)90125-N](https://doi.org/10.1016/0272-0590(91)90125-N) PMID:[2019334](https://pubmed.ncbi.nlm.nih.gov/2019334/)
- Dahm MM, Evans DE, Schubauer-Berigan MK, Birch ME, Deddens JA (2013). Occupational exposure assessment in carbon nanotube and nanofiber primary and secondary manufacturers: mobile direct-reading sampling. *Ann Occup Hyg*, 57(3):328–44. doi:[10.1093/annhyg/mes079](https://doi.org/10.1093/annhyg/mes079) PMID:[23100605](https://pubmed.ncbi.nlm.nih.gov/23100605/)
- Dahm MM, Evans DE, Schubauer-Berigan MK, Birch ME, Fernback JE (2012). Occupational exposure assessment in carbon nanotube and nanofiber primary and secondary manufacturers. *Ann Occup Hyg*, 56(5):542–56. PMID:[22156567](https://pubmed.ncbi.nlm.nih.gov/22156567/)
- Dahm MM, Yencken MS, Schubauer-Berigan MK (2011). Exposure control strategies in the carbonaceous nanomaterial industry. *J Occup Environ Med*, 53(6):Suppl: S68–73. doi:[10.1097/JOM.0b013e31821b1d3b](https://doi.org/10.1097/JOM.0b013e31821b1d3b) PMID:[21654421](https://pubmed.ncbi.nlm.nih.gov/21654421/)
- Danish Environment Protection (2014). Danish Mandatory Nano-Register. Available from: <https://www.retsinformation.dk/Forms/R0710.aspx?id=163367>, accessed 10 January 2014.
- Davoren M, Herzog E, Casey A, Cottineau B, Chambers G, Byrne HJ, et al. (2007). In vitro toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. *Toxicol In Vitro*, 21(3):438–48. doi:[10.1016/j.tiv.2006.10.007](https://doi.org/10.1016/j.tiv.2006.10.007) PMID:[17125965](https://pubmed.ncbi.nlm.nih.gov/17125965/)
- Delogu LG, Stanford SM, Santelli E, Magrini A, Bergamaschi A, Motamedchaboki K, et al. (2010). Carbon nanotube-based nanocarriers: the importance of keeping it clean. *J Nanosci Nanotechnol*, 10(8):5293–301. doi:[10.1166/jnn.2010.3083](https://doi.org/10.1166/jnn.2010.3083) PMID:[21125885](https://pubmed.ncbi.nlm.nih.gov/21125885/)
- Delogu LG, Venturelli E, Manetti R, Pinna GA, Carru C, Madeddu R, et al. (2012). Ex vivo impact of functionalized carbon nanotubes on human immune cells. *Nanomedicine (Lond)*, 7(2):231–43. doi:[10.2217/nnm.11.101](https://doi.org/10.2217/nnm.11.101) PMID:[22106855](https://pubmed.ncbi.nlm.nih.gov/22106855/)
- Delorme MP, Muro Y, Arai T, Banas DA, Frame SR, Reed KL, et al. (2012). Ninety-day inhalation toxicity study with a vapor grown carbon nanofiber in rats. *Toxicol Sci*, 128(2):449–60. doi:[10.1093/toxsci/kfs172](https://doi.org/10.1093/toxsci/kfs172) PMID:[22581831](https://pubmed.ncbi.nlm.nih.gov/22581831/)
- Deng X, Jia G, Wang H, Sun H, Wang X, Yang S, et al. (2007). Translocation and fate of multi-walled carbon nanotubes in vivo. *Carbon*, 45(7):1419–24. doi:[10.1016/j.carbon.2007.03.035](https://doi.org/10.1016/j.carbon.2007.03.035)
- DGCIS (2012). [Les réalités industrielles dans le domaine des nanomatériaux en France – Analyse de la réalité du poids des nanomatériaux dans la filière industrielle concernée.] Paris, France: Direction générale de la compétitivité, de l'industrie et des services. Available from: <http://www.entreprises.gouv.fr/files/files/guides/realites-industrielles-nanomateriaux-france.pdf>. [French]
- Di Giorgio ML, Di Bucchianico S, Ragnelli AM, Aimola P, Santucci S, Poma A (2011). Effects of single and multi walled carbon nanotubes on macrophages: cyto and genotoxicity and electron microscopy. *Mutat Res*, 722(1):20–31. doi:[10.1016/j.mrgentox.2011.02.008](https://doi.org/10.1016/j.mrgentox.2011.02.008) PMID:[21382506](https://pubmed.ncbi.nlm.nih.gov/21382506/)
- Di Sotto A, Chiaretti M, Carru GA, Bellucci S, Mazzanti G (2009). Multi-walled carbon nanotubes: Lack of mutagenic activity in the bacterial reverse mutation assay. *Toxicol Lett*, 184(3):192–7. doi:[10.1016/j.toxlet.2008.11.007](https://doi.org/10.1016/j.toxlet.2008.11.007) PMID:[19063954](https://pubmed.ncbi.nlm.nih.gov/19063954/)
- Ding L, Stilwell J, Zhang T, Elboudwarej O, Jiang H, Selegue JP, et al. (2005). Molecular characterization of the cytotoxic mechanism of multiwall carbon nanotubes and nano-onions on human skin fibroblast. *Nano Lett*, 5(12):2448–64. doi:[10.1021/nl051748o](https://doi.org/10.1021/nl051748o) PMID:[16351195](https://pubmed.ncbi.nlm.nih.gov/16351195/)
- Dinu CZ, Bale SS, Zhu G, Dordick JS (2009). Tubulin encapsulation of carbon nanotubes into functional hybrid assemblies. *Small*, 5(3):310–5. doi:[10.1002/smll.200801434](https://doi.org/10.1002/smll.200801434) PMID:[19148890](https://pubmed.ncbi.nlm.nih.gov/19148890/)
- Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G, et al. (2006). Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicol Sci*, 92(1):5–22. doi:[10.1093/toxsci/kfj130](https://doi.org/10.1093/toxsci/kfj130) PMID:[16484287](https://pubmed.ncbi.nlm.nih.gov/16484287/)
- Donaldson K, Murphy F, Schinwald A, Duffin R, Poland CA (2011). Identifying the pulmonary hazard of high aspect ratio nanoparticles to enable their safety-by-design. *Nanomedicine (Lond)*, 6(1):143–56. doi:[10.2217/nnm.10.139](https://doi.org/10.2217/nnm.10.139) PMID:[21182425](https://pubmed.ncbi.nlm.nih.gov/21182425/)
- Donaldson K, Murphy FA, Duffin R, Poland CA (2010). Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Part Fibre Toxicol*, 7(1):5. doi:[10.1186/1743-8977-7-5](https://doi.org/10.1186/1743-8977-7-5) PMID:[20307263](https://pubmed.ncbi.nlm.nih.gov/20307263/)
- Dong PX, Wan B, Guo LH (2012). In vitro toxicity of acid-functionalized single-walled carbon nanotubes: effects on murine macrophages and gene expression profiling. *Nanotoxicology*, 6(3):288–303. doi:[10.3109/17435390.2011.573101](https://doi.org/10.3109/17435390.2011.573101) PMID:[21486190](https://pubmed.ncbi.nlm.nih.gov/21486190/)
- Dutch Social and Economic Council (2012). Provisional nano reference values for engineered nanomaterials. The Hague, the Netherlands: Social Economic Council.
- Ebbesen TW, Takada T (1995). Topological and sp<sup>3</sup> defect structures in nanotubes. *Carbon*, 33(7):973–8. doi:[10.1016/0008-6223\(95\)00025-9](https://doi.org/10.1016/0008-6223(95)00025-9)
- Elder A, Gelein R, Finkelstein JN, Driscoll KE, Harkema J, Oberdörster G (2005). Effects of subchronically inhaled carbon black in three species. I. Retention kinetics, lung inflammation, and histopathology. *Toxicol Sci*, 88(2):614–29. doi:[10.1093/toxsci/kfi327](https://doi.org/10.1093/toxsci/kfi327) PMID:[16177241](https://pubmed.ncbi.nlm.nih.gov/16177241/)
- Elgrabli D, Abella-Gallart S, Robidel F, Rogerieux F, Boczkowski J, Lacroix G (2008). Induction of apoptosis and absence of inflammation in rat lung after

- intratracheal instillation of multiwalled carbon nanotubes. *Toxicology*, 253(1–3):131–6. doi:[10.1016/j.tox.2008.09.004](https://doi.org/10.1016/j.tox.2008.09.004) PMID:[18834917](https://pubmed.ncbi.nlm.nih.gov/18834917/)
- Ellinger-Ziegelbauer H, Pauluhn J (2009). Pulmonary toxicity of multi-walled carbon nanotubes (Baytubes) relative to alpha-quartz following a single 6h inhalation exposure of rats and a 3 months post-exposure period. *Toxicology*, 266(1–3):16–29. doi:[10.1016/j.tox.2009.10.007](https://doi.org/10.1016/j.tox.2009.10.007) PMID:[19836432](https://pubmed.ncbi.nlm.nih.gov/19836432/)
- Ema M, Imamura T, Suzuki H, Kobayashi N, Naya M, Nakanishi J (2013b). Genotoxicity evaluation for single-walled carbon nanotubes in a battery of in vitro and in vivo assays. *J Appl Toxicol*, 33(9):933–9. doi:[10.1002/jat.2772](https://doi.org/10.1002/jat.2772) PMID:[22763644](https://pubmed.ncbi.nlm.nih.gov/22763644/)
- Ema M, Masumori S, Kobayashi N, Naya M, Endoh S, Maru J, et al. (2013a). In vivo comet assay of multi-walled carbon nanotubes using lung cells of rats intratracheally instilled. *J Appl Toxicol*, 33(10):1053–60. doi:[10.1002/jat.2810](https://doi.org/10.1002/jat.2810) PMID:[22936419](https://pubmed.ncbi.nlm.nih.gov/22936419/)
- ENRHES (2009). Engineered nanoparticles – Review of health and environmental safety (ENRHES). ENRHES project. ENRHES. Available from: <http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report>, accessed 30 May 2013.
- EPA (1988). Reference physiological parameters in pharmacokinetic modeling. Washington (DC), USA: Office of Health and Environment Assessment, Exposure Assessment Group, United States Environmental Protection Agency. EPA report no. EPA/600/6–88/004.
- EPA (2006). Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment. Washington (DC), USA: National Center for Environmental Assessment, Office of Research and Development, United States Environmental Protection Agency. EPA/600/R.05/043F.
- EPA (2011). Multi-walled carbon nanotubes: significant new use rule, 40 CFR Parts 9 and 721 [EPA–HQ–OPPT–2009–0686; FRL–8865–4] RIN 2070–AB27. *Fed Regist*, 76(88). Washington (DC), USA: United States Environmental Protection Agency.
- EPA (2014). Control of nanoscale materials under the Toxic Substance Control Act. Washington (DC), USA: United States Environmental Protection Agency. Available from: <http://www.epa.gov/opptintr/nano/>, accessed 9 April 2014.
- Erdely A, Dahm M, Chen BT, Zeidler-Erdely PC, Fernback JE, Birch ME, et al. (2013). Carbon nanotube dosimetry: from workplace exposure assessment to inhalation toxicology. *Part Fibre Toxicol*, 10(1):53. doi:[10.1186/1743-8977-10-53](https://doi.org/10.1186/1743-8977-10-53) PMID:[24144386](https://pubmed.ncbi.nlm.nih.gov/24144386/)
- Erdely A, Hulderman T, Salmen R, Liston A, Zeidler-Erdely PC, Schwegler-Berry D, et al. (2009). Cross-talk between lung and systemic circulation during carbon nanotube respiratory exposure. Potential biomarkers. *Nano Lett*, 9(1):36–43. doi:[10.1021/nl801828z](https://doi.org/10.1021/nl801828z) PMID:[19049393](https://pubmed.ncbi.nlm.nih.gov/19049393/)
- EU Commission (2011). EU Commission recommendation on the definition of nanomaterial (2011/696/EU). Official Journal of the European Union. 20.10.2011: L 275/38–40. Available from: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011H0696&from=EN>, accessed 1 October 2014.
- European Standards Committee on Oxidative DNA Damage (ESCODD) (2003). Measurement of DNA oxidation in human cells by chromatographic and enzymic methods. *Free Radic Biol Med*, 34(8):1089–99. doi:[10.1016/S0891-5849\(03\)00041-8](https://doi.org/10.1016/S0891-5849(03)00041-8) PMID:[12684094](https://pubmed.ncbi.nlm.nih.gov/12684094/)
- Evans DE, Turkevich LA, Roettgers CT, Deye GJ, Baron PA (2013). Dustiness of fine and nanoscale powders. *Ann Occup Hyg*, 57(2):261–77. doi:[10.1093/annhyg/mes060](https://doi.org/10.1093/annhyg/mes060) PMID:[23065675](https://pubmed.ncbi.nlm.nih.gov/23065675/)
- Fenoglio I, Aldieri E, Gazzano E, Cesano F, Colonna M, Scarano D, et al. (2012). Thickness of multiwalled carbon nanotubes affects their lung toxicity. *Chem Res Toxicol*, 25(1):74–82. doi:[10.1021/tx200255h](https://doi.org/10.1021/tx200255h) PMID:[22128750](https://pubmed.ncbi.nlm.nih.gov/22128750/)
- Fenoglio I, Greco G, Tomatis M, Muller J, Raymundo-Piñero E, Béguin F, et al. (2008). Structural defects play a major role in the acute lung toxicity of multi-wall carbon nanotubes: physicochemical aspects. *Chem Res Toxicol*, 21(9):1690–7. doi:[10.1021/tx800100s](https://doi.org/10.1021/tx800100s) PMID:[18636755](https://pubmed.ncbi.nlm.nih.gov/18636755/)
- Fenoglio I, Tomatis M, Lison D, Muller J, Fonseca A, Nagy JB, et al. (2006). Reactivity of carbon nanotubes: free radical generation or scavenging activity? *Free Radic Biol Med*, 40(7):1227–33. doi:[10.1016/j.freeradbiomed.2005.11.010](https://doi.org/10.1016/j.freeradbiomed.2005.11.010) PMID:[16545691](https://pubmed.ncbi.nlm.nih.gov/16545691/)
- Folkmann JK, Risom L, Jacobsen NR, Wallin H, Loft S, Møller P (2009). Oxidatively damaged DNA in rats exposed by oral gavage to C60 fullerenes and single-walled carbon nanotubes. *Environ Health Perspect*, 117(5):703–8. doi:[10.1289/ehp.11922](https://doi.org/10.1289/ehp.11922) PMID:[19479010](https://pubmed.ncbi.nlm.nih.gov/19479010/)
- Fröhlich E, Meindl C, Höfler A, Leitinger G, Roblegg E (2013). Combination of small size and carboxyl functionalisation causes cytotoxicity of short carbon nanotubes. *Nanotoxicology*, 7(7):1211–24. doi:[10.3109/17435390.2012.729274](https://doi.org/10.3109/17435390.2012.729274) PMID:[22963691](https://pubmed.ncbi.nlm.nih.gov/22963691/)
- Fubini B, Fenoglio I, Tomatis M, Turci F (2011). Effect of chemical composition and state of the surface on the toxic response to high aspect ratio nanomaterials. *Nanomedicine (Lond)*, 6(5):899–920. doi:[10.2217/nnm.11.80](https://doi.org/10.2217/nnm.11.80) PMID:[21793679](https://pubmed.ncbi.nlm.nih.gov/21793679/)
- Fubini B, Ghiazza M, Fenoglio I (2010). Physico-chemical features of engineered nanoparticles relevant to their toxicity. *Nanotoxicology*, 4(4):347–63. doi:[10.3109/17435390.2010.509519](https://doi.org/10.3109/17435390.2010.509519) PMID:[20858045](https://pubmed.ncbi.nlm.nih.gov/20858045/)
- Fujita K, Fukuda M, Fukui H, Horie M, Endoh S, Uchida K, et al. (2015). Intratracheal instillation of single-wall carbon nanotubes in the rat lung induces time-dependent changes in gene expression. *Nanotoxicology*,

- 9(3):290–301. doi:[10.3109/17435390.2014.921737](https://doi.org/10.3109/17435390.2014.921737) PMID:[24911292](https://pubmed.ncbi.nlm.nih.gov/24911292/)
- Galano A (2010). Carbon nanotubes: promising agents against free radicals. *Nanoscale*, 2(3):373–80. doi:[10.1039/b9nr00364a](https://doi.org/10.1039/b9nr00364a) PMID:[20644818](https://pubmed.ncbi.nlm.nih.gov/20644818/)
- Galano A, Francisco-Marquez M, Martinez A (2010). Influence of point defects on the free-radical scavenging capability of single-walled carbon nanotubes. *J Phys Chem C*, 114(18):8302–8. doi:[10.1021/jp101544u](https://doi.org/10.1021/jp101544u)
- Gangwal S, Brown JS, Wang A, Houck KA, Dix DJ, Kavlock RJ, et al. (2011). Informing selection of nanomaterial concentrations for ToxCast in vitro testing based on occupational exposure potential. *Environ Health Perspect*, 119(11):1539–46. doi:[10.1289/ehp.1103750](https://doi.org/10.1289/ehp.1103750) PMID:[21788197](https://pubmed.ncbi.nlm.nih.gov/21788197/)
- Gasser M, Wick P, Clift MJD, Blank F, Diener L, Yan B, et al. (2012). Pulmonary surfactant coating of multi-walled carbon nanotubes (MWCNTs) influences their oxidative and pro-inflammatory potential in vitro. *Part Fibre Toxicol*, 9(1):17. doi:[10.1186/1743-8977-9-17](https://doi.org/10.1186/1743-8977-9-17) PMID:[22624622](https://pubmed.ncbi.nlm.nih.gov/22624622/)
- Ghiazza M, Vietti G, Fenoglio I (2014). Carbon nanotubes: properties, applications and toxicity. In: Pielichowski K, Zhu H, editors. *Health and environmental risks of nanomaterials: Polymer nanocomposites and other materials containing nanoparticles* Woodhead Publishing Series in Composites Science and Engineering No. 49 Njuguna J. Woodhead Publishing U.K.; Chapter 8, pp. 147–74. doi:[10.1533/9780857096678.3.147](https://doi.org/10.1533/9780857096678.3.147)
- Ghosh M, Chakraborty A, Bandyopadhyay M, Mukherjee A (2011). Multi-walled carbon nanotubes (MWCNT): induction of DNA damage in plant and mammalian cells. *J Hazard Mater*, 197:327–36. doi:[10.1016/j.jhazmat.2011.09.090](https://doi.org/10.1016/j.jhazmat.2011.09.090) PMID:[21999988](https://pubmed.ncbi.nlm.nih.gov/21999988/)
- Gottschalk F, Kost E, Nowack B (2013). Engineered nanomaterials in water and soils: a risk quantification based on probabilistic exposure and effect modeling. *Environ Toxicol Chem*, 32(6):1278–87. doi:[10.1002/etc.2177](https://doi.org/10.1002/etc.2177) PMID:[23418073](https://pubmed.ncbi.nlm.nih.gov/23418073/)
- Gottschalk F, Sonderer T, Scholz RW, Nowack B (2009). Modeled environmental concentrations of engineered nanomaterials (TiO<sub>2</sub>), ZnO, Ag, CNT, Fullerenes) for different regions. *Environ Sci Technol*, 43(24):9216–22. doi:[10.1021/es9015553](https://doi.org/10.1021/es9015553) PMID:[20000512](https://pubmed.ncbi.nlm.nih.gov/20000512/)
- Gottschalk F, Sonderer T, Scholz RW, Nowack B (2010). Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. *Environ Toxicol Chem*, 29(5):1036–48. PMID:[20821538](https://pubmed.ncbi.nlm.nih.gov/20821538/)
- Grecco ACP, Paula RFO, Mizutani E, Sartorelli JC, Milani AM, Longhini AL, et al. (2011). Up-regulation of T lymphocyte and antibody production by inflammatory cytokines released by macrophage exposure to multi-walled carbon nanotubes. *Nanotechnology*, 22(26):265103. doi:[10.1088/0957-4484/22/26/265103](https://doi.org/10.1088/0957-4484/22/26/265103) PMID:[21576788](https://pubmed.ncbi.nlm.nih.gov/21576788/)
- Guo L, Morris DG, Liu X, Vaslet C, Hurt RH, Kane AB (2007). Iron bioavailability and redox activity in diverse carbon nanotube samples. *Chem Mater*, 19(14):3472–8. doi:[10.1021/cm062691p](https://doi.org/10.1021/cm062691p)
- Guo NL, Wan YW, Denvir J, Porter DW, Pacurari M, Wolfarth MG, et al. (2012). Multiwalled carbon nanotube-induced gene signatures in the mouse lung: potential predictive value for human lung cancer risk and prognosis. *J Toxicol Environ Health A*, 75(18):1129–53. doi:[10.1080/15287394.2012.699852](https://doi.org/10.1080/15287394.2012.699852) PMID:[22891886](https://pubmed.ncbi.nlm.nih.gov/22891886/)
- Guo T, Nikolaev P, Rinzler AG, Tomanek D, Colbert DT, Smalley RE (1995a). Self-assembly of tubular fullerenes. *J Phys Chem B*, 99(27):10694–7. doi:[10.1021/j100027a002](https://doi.org/10.1021/j100027a002)
- Guo T, Nikolaev P, Thess A, Colbert DT, Smalley RE (1995b). Catalytic growth of single-walled carbon nanotubes by laser vaporization. *Chem Phys Lett*, 243(1–2):49–54. doi:[10.1016/0009-2614\(95\)00825-O](https://doi.org/10.1016/0009-2614(95)00825-O)
- Guo YY, Zhang J, Zheng YF, Yang J, Zhu XQ (2011). Cytotoxic and genotoxic effects of multi-wall carbon nanotubes on human umbilical vein endothelial cells in vitro. *Mutat Res*, 721(2):184–91. doi:[10.1016/j.mrgentox.2011.01.014](https://doi.org/10.1016/j.mrgentox.2011.01.014) PMID:[21296185](https://pubmed.ncbi.nlm.nih.gov/21296185/)
- Guseva Canu I, Bateson TF, Bouvard V, Debia M, Dion C, Savolainen K, et al. (2016). Human exposure to carbon-based fibrous nanomaterials: A review. *Int J Hyg Environ Health*, 219(2):166–75. doi:[10.1016/j.ijheh.2015.12.005](https://doi.org/10.1016/j.ijheh.2015.12.005) PMID:[26752069](https://pubmed.ncbi.nlm.nih.gov/26752069/)
- Hamad I, Al-Hanbali O, Hunter AC, Rutt KJ, Andresen TL, Moghimi SM (2010). Distinct polymer architecture mediates switching of complement activation pathways at the nanosphere-serum interface: implications for stealth nanoparticle engineering. *ACS Nano*, 4(11):6629–38. doi:[10.1021/nn101990a](https://doi.org/10.1021/nn101990a) PMID:[21028845](https://pubmed.ncbi.nlm.nih.gov/21028845/)
- Hamilton RF Jr, Buford M, Xiang C, Wu N, Holian A (2012). NLRP3 inflammasome activation in murine alveolar macrophages and related lung pathology is associated with MWCNT nickel contamination. *Inhal Toxicol*, 24(14):995–1008. doi:[10.3109/08958378.2012.745633](https://doi.org/10.3109/08958378.2012.745633) PMID:[23216160](https://pubmed.ncbi.nlm.nih.gov/23216160/)
- Hamilton RF Jr, Buford MC, Wood MB, Arnone B, Morandi M, Holian A (2007). Engineered carbon nanoparticles alter macrophage immune function and initiate airway hyper-responsiveness in the BALB/c mouse model. *Nanotoxicology*, 1(2):104–17. doi:[10.1080/17435390600926939](https://doi.org/10.1080/17435390600926939)
- Hamilton RF Jr, Wu N, Porter D, Buford M, Wolfarth M, Holian A (2009). Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity. *Part Fibre Toxicol*, 6(1):35. doi:[10.1186/1743-8977-6-35](https://doi.org/10.1186/1743-8977-6-35) PMID:[20043844](https://pubmed.ncbi.nlm.nih.gov/20043844/)
- Hamilton RF Jr, Wu Z, Mitra S, Shaw PK, Holian A (2013b). Effect of MWCNT size, carboxylation, and purification on in vitro and in vivo toxicity, inflammation and lung pathology. *Part Fibre Toxicol*, 10(1):57. doi:[10.1186/1743-8977-10-57](https://doi.org/10.1186/1743-8977-10-57) PMID:[24225053](https://pubmed.ncbi.nlm.nih.gov/24225053/)

- Hamilton RF Jr, Xiang C, Li M, Ka I, Yang F, Ma D, et al. (2013a). Purification and sidewall functionalization of multiwalled carbon nanotubes and resulting bioactivity in two macrophage models. *Inhal Toxicol*, 25(4):199–210. doi:[10.3109/08958378.2013.775197](https://doi.org/10.3109/08958378.2013.775197) PMID:[23480196](https://pubmed.ncbi.nlm.nih.gov/23480196/)
- Han JH, Lee EJ, Lee JH, So KP, Lee YH, Bae GN, et al. (2008). Monitoring multiwalled carbon nanotube exposure in carbon nanotube research facility. *Inhal Toxicol*, 20(8):741–9. doi:[10.1080/08958370801942238](https://doi.org/10.1080/08958370801942238) PMID:[18569096](https://pubmed.ncbi.nlm.nih.gov/18569096/)
- Han SG, Andrews R, Gairola CG (2010). Acute pulmonary response of mice to multi-wall carbon nanotubes. *Inhal Toxicol*, 22(4):340–7. doi:[10.3109/08958370903359984](https://doi.org/10.3109/08958370903359984) PMID:[20064106](https://pubmed.ncbi.nlm.nih.gov/20064106/)
- Han YG, Xu J, Li ZG, Ren GG, Yang Z (2012). In vitro toxicity of multi-walled carbon nanotubes in C6 rat glioma cells. *Neurotoxicology*, 33(5):1128–34. doi:[10.1016/j.neuro.2012.06.004](https://doi.org/10.1016/j.neuro.2012.06.004) PMID:[22728153](https://pubmed.ncbi.nlm.nih.gov/22728153/)
- Haniu H, Matsuda Y, Takeuchi K, Kim YA, Hayashi T, Endo M (2010). Proteomics-based safety evaluation of multi-walled carbon nanotubes. *Toxicol Appl Pharmacol*, 242(3):256–62. doi:[10.1016/j.taap.2009.10.015](https://doi.org/10.1016/j.taap.2009.10.015) PMID:[19874835](https://pubmed.ncbi.nlm.nih.gov/19874835/)
- Haniu H, Saito N, Matsuda Y, Kim YA, Park KC, Tsukahara T, et al. (2011). Elucidation mechanism of different biological responses to multi-walled carbon nanotubes using four cell lines. *Int J Nanomedicine*, 6:3487–97. doi:[10.2147/IJN.S26689](https://doi.org/10.2147/IJN.S26689) PMID:[22267932](https://pubmed.ncbi.nlm.nih.gov/22267932/)
- Hedmer M, Isaxon C, Nilsson PT, Ludvigsson L, Messing ME, Genberg J, et al. (2014). Exposure and emission measurements during production, purification, and functionalization of arc-discharge-produced multi-walled carbon nanotubes. *Ann Occup Hyg*, 58(3):355–79. doi:[10.1093/annhyg/met072](https://doi.org/10.1093/annhyg/met072) PMID:[24389082](https://pubmed.ncbi.nlm.nih.gov/24389082/)
- Hedmer M, Kåredal M, Gustavsson P, Rissler J (2013). 148 Carbon nanotubes. Report - The Nordic Expert Group for Criteria Documentation of Health Risks of Chemicals. *Arbete och hälsa*. 47(5):1-252. Available from: [https://gupea.ub.gu.se/bitstream/2077/34499/1/gupea\\_2077\\_34499\\_1.pdf](https://gupea.ub.gu.se/bitstream/2077/34499/1/gupea_2077_34499_1.pdf).
- Hirano S, Fujitani Y, Furuyama A, Kanno S (2010). Uptake and cytotoxic effects of multi-walled carbon nanotubes in human bronchial epithelial cells. *Toxicol Appl Pharmacol*, 249(1):8–15. doi:[10.1016/j.taap.2010.08.019](https://doi.org/10.1016/j.taap.2010.08.019) PMID:[20800606](https://pubmed.ncbi.nlm.nih.gov/20800606/)
- Hirano S, Kanno S, Furuyama A (2008). Multi-walled carbon nanotubes injure the plasma membrane of macrophages. *Toxicol Appl Pharmacol*, 232(2):244–51. doi:[10.1016/j.taap.2008.06.016](https://doi.org/10.1016/j.taap.2008.06.016) PMID:[18655803](https://pubmed.ncbi.nlm.nih.gov/18655803/)
- Hirsch A, Vostronowsky O (2005). Functionalization of carbon nanotubes. In: Schluter AD, editor. *Functional molecular nanostructures. Topics in current chemistry*. Volume 245. Berlin, Heidelberg: Springer-Verlag; pp. 193–237.
- Hitoshi K, Katoh M, Suzuki T, Ando Y, Nadai M (2012). Single-walled carbon nanotubes downregulate stress-responsive genes in human respiratory tract cells. *Biol Pharm Bull*, 35(4):455–63. doi:[10.1248/bpb.35.455](https://doi.org/10.1248/bpb.35.455) PMID:[22466547](https://pubmed.ncbi.nlm.nih.gov/22466547/)
- Honnert B, Grzebyk M (2014). Manufactured nano-objects: an occupational survey in five industries in France. *Ann Occup Hyg*, 58(1):121–35. doi:[10.1093/annhyg/met058](https://doi.org/10.1093/annhyg/met058) PMID:[24142930](https://pubmed.ncbi.nlm.nih.gov/24142930/)
- Horie M, Kato H, Iwahashi H (2013). Cellular effects of manufactured nanoparticles: effect of adsorption ability of nanoparticles. *Arch Toxicol*, 87(5):771–81. doi:[10.1007/s00204-013-1033-5](https://doi.org/10.1007/s00204-013-1033-5) PMID:[23503611](https://pubmed.ncbi.nlm.nih.gov/23503611/)
- Hou P-X, Xu S-T, Ying Z, Yang Q-H, Liu C, Cheng H-M (2003). Hydrogen adsorption/desorption behavior of multi-walled carbon nanotubes with different diameters. *Carbon*, 41(13):2471–6. doi:[10.1016/S0008-6223\(03\)00271-9](https://doi.org/10.1016/S0008-6223(03)00271-9)
- Hu H, Zhao B, Itkis ME, Haddon RC (2003). Nitric acid purification of single-walled carbon nanotubes *J Phys Chem B*, 107(50):13838–42. doi:[10.1021/jp035719i](https://doi.org/10.1021/jp035719i)
- Huang X, Zhang F, Sun X, Choi KY, Niu G, Zhang G, et al. (2014). The genotype-dependent influence of functionalized multiwalled carbon nanotubes on fetal development. *Biomaterials*, 35(2):856–65. doi:[10.1016/j.biomaterials.2013.10.027](https://doi.org/10.1016/j.biomaterials.2013.10.027) PMID:[24344357](https://pubmed.ncbi.nlm.nih.gov/24344357/)
- Huizar I, Malur A, Patel J, McPeck M, Dobbs L, Wingard C, et al. (2013). The role of PPAR $\gamma$  in carbon nanotube-elicited granulomatous lung inflammation. *Respir Res*, 14(1):7. doi:[10.1186/1465-9921-14-7](https://doi.org/10.1186/1465-9921-14-7) PMID:[23343389](https://pubmed.ncbi.nlm.nih.gov/23343389/)
- Hussain F, Hojjati M, Okomoto M, Gorga RE (2006). Review article: Polymer-matrix nanocomposites, processing, manufacturing, and applications: An overview. *J Compos Mater*, 40(17):1511–75. doi:[10.1177/0021998306067321](https://doi.org/10.1177/0021998306067321)
- IARC (2012). Arsenic, metals, fibres, and dusts. *IARC Monogr Eval Carcinog Risks Hum*, 100C:1–499. Available from: <http://monographs.iarc.fr/ENG/Monographs/vol100C/index.php>. PMID:[23189751](https://pubmed.ncbi.nlm.nih.gov/23189751/)
- ICRP (International Commission on Radiological Protection) (1994). Human respiratory tract model for radiological protection. *Annals of the ICRP, Publication 66*. Tarrytown (NY), USA: Elsevier Science Ltd.
- Iijima S (1991). Helical microtubules of graphite carbon. *Nature*, 354(6348):56–8. doi:[10.1038/354056a0](https://doi.org/10.1038/354056a0)
- Iijima S, Brabec C, Maiti A, Bernholc C (1996). Structural flexibility of carbon nanotubes. *J Chem Phys*, 104(5):2089–92. doi:[10.1063/1.470966](https://doi.org/10.1063/1.470966)
- Ingle T, Dervishi E, Biris AR, Mustafa T, Buchanan RA, Biris AS (2013). Raman spectroscopy analysis and mapping the biodistribution of inhaled carbon nanotubes in the lungs and blood of mice. *J Appl Toxicol*, 33(10):1044–52. doi:[10.1002/jat.2796](https://doi.org/10.1002/jat.2796) PMID:[23047664](https://pubmed.ncbi.nlm.nih.gov/23047664/)
- Inoue K, Koike E, Yanagisawa R, Hirano S, Nishikawa M, Takano H (2009). Effects of multi-walled carbon nanotubes on a murine allergic airway inflammation model.

- Toxicol Appl Pharmacol*, 237(3):306–16. doi:[10.1016/j.taap.2009.04.003](https://doi.org/10.1016/j.taap.2009.04.003) PMID:[19371758](https://pubmed.ncbi.nlm.nih.gov/19371758/)
- Inoue K, Yanagisawa R, Koike E, Nishikawa M, Takano H (2010). Repeated pulmonary exposure to single-walled carbon nanotubes exacerbates allergic inflammation of the airway: Possible role of oxidative stress. *Free Radic Biol Med*, 48(7):924–34. doi:[10.1016/j.freeradbiomed.2010.01.013](https://doi.org/10.1016/j.freeradbiomed.2010.01.013) PMID:[20093178](https://pubmed.ncbi.nlm.nih.gov/20093178/)
- Ishigami M, Choi HJ, Aloni S, Louie SG, Cohen ML, Zettl A (2004). Identifying defects in nanoscale materials. *Phys Rev Lett*, 93(19):196803. doi:[10.1103/PhysRevLett.93.196803](https://doi.org/10.1103/PhysRevLett.93.196803) PMID:[15600863](https://pubmed.ncbi.nlm.nih.gov/15600863/)
- ISO(2008).ISO/TS27687.Nanotechnologies–Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate. Geneva, Switzerland: International Organization for Standardization.
- ISO (2010a). ISO/TS 80004–1. Nanotechnologies – Vocabulary Part 1: Core terms. Geneva, Switzerland: International Organization for Standardization.
- ISO (2010b). ISO/TS 80004–3. Nanotechnologies – Vocabulary Part 3: Carbon nano-objects. Geneva, Switzerland: International Organization for Standardization.
- ISO (2010c). ISO/TS 10867. Nanotechnologies – Characterization of single-wall carbon nanotubes using near infrared photoluminescence spectroscopy. Geneva, Switzerland: International Organization for Standardization.
- ISO (2010d). ISO/TS 11251. Nanotechnologies – Characterization of volatile components in single-wall carbon nanotube samples using evolved gas analysis/gas chromatograph-mass spectrometry. Geneva, Switzerland: International Organization for Standardization.
- ISO (2010e). ISO 29701. Nanotechnologies – Endotoxin test on nanomaterial samples in vitro systems-Liu-mulus amebocyte lysis (LAL) test. Geneva, Switzerland: International Organization for Standardization.
- ISO (2011a). ISO/TS 10798. Nanotechnologies – Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis. Geneva, Switzerland: International Organization for Standardization.
- ISO (2011b). ISO/TS 11308. Nanotechnologies – Characterization of single-wall carbon nanotubes using thermogravimetric analysis. Geneva, Switzerland: International Organization for Standardization.
- ISO (2012a). ISO/TR 13014. Nanotechnologies – Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment. Geneva, Switzerland: International Organization for Standardization.
- ISO (2012b). ISO/TS 10797. Nanotechnologies – Characterization of single-wall carbon nanotubes using transmission electron microscopy. Geneva, Switzerland: International Organization for Standardization.
- Switzerland: International Organization for Standardization.
- IUPAC (2014). Compendium of Chemical Terminology – Gold book. 1622 pp. Research Triangle Park (NC), USA: International Union of Pure and Applied Chemistry. Available from: <http://goldbook.iupac.org/PDF/goldbook.pdf>.
- Jacobsen NR, Møller P, Jensen KA, Vogel U, Ladefoged O, Loft S, et al. (2009). Lung inflammation and genotoxicity following pulmonary exposure to nanoparticles in ApoE–/– mice. *Part Fibre Toxicol*, 6(1):2. doi:[10.1186/1743-8977-6-2](https://doi.org/10.1186/1743-8977-6-2) PMID:[19138394](https://pubmed.ncbi.nlm.nih.gov/19138394/)
- Jacobsen NR, Pojana G, White P, Møller P, Cohn CA, Korsholm KS, et al. (2008). Genotoxicity, cytotoxicity, and reactive oxygen species induced by single-walled carbon nanotubes and C(60) fullerenes in the FE1-Mutatrade markMouse lung epithelial cells. *Environ Mol Mutagen*, 49(6):476–87. doi:[10.1002/em.20406](https://doi.org/10.1002/em.20406) PMID:[18618583](https://pubmed.ncbi.nlm.nih.gov/18618583/)
- Jain S, Thakare VS, Das M, Godugu C, Jain AK, Mathur R, et al. (2011). Toxicity of multiwalled carbon nanotubes with end defects critically depends on their functionalization density. *Chem Res Toxicol*, 24(11):2028–39. doi:[10.1021/tx2003728](https://doi.org/10.1021/tx2003728) PMID:[21978239](https://pubmed.ncbi.nlm.nih.gov/21978239/)
- Jaurand MCF, Renier A, Daubriac J (2009). Mesothelioma: Do asbestos and carbon nanotubes pose the same health risk? *Part Fibre Toxicol*, 6(1):16. doi:[10.1186/1743-8977-6-16](https://doi.org/10.1186/1743-8977-6-16) PMID:[19523217](https://pubmed.ncbi.nlm.nih.gov/19523217/)
- Ji JH, Woo D, Lee SB, Kim T, Kim D, Kim JH, et al. (2013). Detection and characterization of nanomaterials released in low concentrations during multi-walled carbon nanotube spraying process in a cleanroom. *Inhal Toxicol*, 25(14):759–65. doi:[10.3109/08958378.2013.846951](https://doi.org/10.3109/08958378.2013.846951) PMID:[24304302](https://pubmed.ncbi.nlm.nih.gov/24304302/)
- Jin H, Heller DA, Sharma R, Strano MS (2009). Size-dependent cellular uptake and expulsion of single-walled carbon nanotubes: single particle tracking and a generic uptake model for nanoparticles. *ACS Nano*, 3(1):149–58. doi:[10.1021/nn800532m](https://doi.org/10.1021/nn800532m) PMID:[19206261](https://pubmed.ncbi.nlm.nih.gov/19206261/)
- Johnson DR, Methner MM, Kennedy AJ, Steevens JA (2010). Potential for occupational exposure to engineered carbon-based nanomaterials in environmental laboratory studies. *Environ Health Perspect*, 118(1):49–54. PMID:[20056572](https://pubmed.ncbi.nlm.nih.gov/20056572/)
- Johnston HJ, Hutchison GR, Christensen FM, Peters S, Hankin S, Aschberger K, et al. (2010). A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes: The contribution of physico-chemical characteristics. *Nanotoxicology*, 4(2):207–46. doi:[10.3109/17435390903569639](https://doi.org/10.3109/17435390903569639) PMID:[20795897](https://pubmed.ncbi.nlm.nih.gov/20795897/)
- Jorio A, Saito R, Hafner JH, Lieber CM, Hunter M, McClure T, et al. (2001). Structural (n, m) determination of isolated single-wall carbon nanotubes by resonant Raman scattering. *Phys Rev Lett*, 86(6):1118–21. doi:[10.1103/PhysRevLett.86.1118](https://doi.org/10.1103/PhysRevLett.86.1118) PMID:[11178024](https://pubmed.ncbi.nlm.nih.gov/11178024/)

- Journal Officiel (2012). [Décret No 2012\_232 du 17 février 2012 relatif à la déclaration annuelle des substances à l'état nanoparticulaire pris en application de l'article L. 523-4 du code de l'environnement.] Paris, France: Journal officiel de la République Française. [French]
- Ju L, Zhang G, Zhang X, Jia Z, Gao X, Jiang Y, et al. (2014). Proteomic analysis of cellular response induced by multi-walled carbon nanotubes exposure in A549 cells. *PLoS One*, 9(1):e84974. doi:[10.1371/journal.pone.0084974](https://doi.org/10.1371/journal.pone.0084974) PMID:[24454774](https://pubmed.ncbi.nlm.nih.gov/24454774/)
- Kagan VE, Konduru NV, Feng W, Allen BL, Conroy J, Volkov Y, et al. (2010). Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation. *Nat Nanotechnol*, 5(5):354-9. doi:[10.1038/nnano.2010.44](https://doi.org/10.1038/nnano.2010.44) PMID:[20364135](https://pubmed.ncbi.nlm.nih.gov/20364135/)
- Kagan VE, Tyurina YY, Tyurin VA, Konduru NV, Potapovich AI, Osipov AN, et al. (2006). Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: role of iron. *Toxicol Lett*, 165(1):88-100. doi:[10.1016/j.toxlet.2006.02.001](https://doi.org/10.1016/j.toxlet.2006.02.001) PMID:[16527436](https://pubmed.ncbi.nlm.nih.gov/16527436/)
- Kam NW, O'Connell M, Wisdom JA, Dai H (2005). Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc Natl Acad Sci USA*, 102(33):11600-5. doi:[10.1073/pnas.0502680102](https://doi.org/10.1073/pnas.0502680102) PMID:[16087878](https://pubmed.ncbi.nlm.nih.gov/16087878/)
- Kanno S, Hirano S, Chiba S, Takeshita H, Nagai T, Takada M, et al. (2014). The role of Rho-kinases in IL-1 $\beta$  release through phagocytosis of fibrous particles in human monocytes *Arch Toxicol*. PMID:[24760326](https://pubmed.ncbi.nlm.nih.gov/24760326/)
- Karajanagi SS, Vertegel AA, Kane RS, Dordick JS (2004). Structure and function of enzymes adsorbed onto single-walled carbon nanotubes. *Langmuir*, 20(26):11594-9. doi:[10.1021/la047994h](https://doi.org/10.1021/la047994h) PMID:[15595788](https://pubmed.ncbi.nlm.nih.gov/15595788/)
- Karlsson HL, Cronholm P, Gustafsson J, Möller L (2008). Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chem Res Toxicol*, 21(9):1726-32. doi:[10.1021/tx800064j](https://doi.org/10.1021/tx800064j) PMID:[18710264](https://pubmed.ncbi.nlm.nih.gov/18710264/)
- Karthikeyan S, Mahalingam P, Karthik M (2009). Large scale synthesis of carbon nanotubes. *E-J Chem*, 6(1):1-12. doi:[10.1155/2009/756410](https://doi.org/10.1155/2009/756410)
- Kasai T, Umeda Y, Ohnishi M, Kondo H, Takeuchi T, Aiso S, et al. (2015). Thirteen-week study of toxicity of fiber-like multi-walled carbon nanotubes with whole-body inhalation exposure in rats. *Nanotoxicology*, 9(4):413-22. doi:[10.3109/17435390.2014.933903](https://doi.org/10.3109/17435390.2014.933903) PMID:[25030099](https://pubmed.ncbi.nlm.nih.gov/25030099/)
- Kato T, Totsuka Y, Ishino K, Matsumoto Y, Tada Y, Nakae D, et al. (2013). Genotoxicity of multi-walled carbon nanotubes in both in vitro and in vivo assay systems. *Nanotoxicology*, 7(4):452-61. doi:[10.3109/17435390.2012.674571](https://doi.org/10.3109/17435390.2012.674571) PMID:[22397533](https://pubmed.ncbi.nlm.nih.gov/22397533/)
- Katwa P, Wang X, Urankar RN, Podila R, Hilderbrand SC, Fick RB, et al. (2012). A carbon nanotube toxicity paradigm driven by mast cells and the IL-<sub>33</sub>/ST<sub>2</sub> axis. *Small*, 8(18):2904-12. doi:[10.1002/smll.201200873](https://doi.org/10.1002/smll.201200873) PMID:[22777948](https://pubmed.ncbi.nlm.nih.gov/22777948/)
- Kermanizadeh A, Gaiser BK, Hutchison GR, Stone V (2012). An in vitro liver model-assessing oxidative stress and genotoxicity following exposure of hepatocytes to a panel of engineered nanomaterials. *Part Fibre Toxicol*, 9(1):28. doi:[10.1186/1743-8977-9-28](https://doi.org/10.1186/1743-8977-9-28) PMID:[22812506](https://pubmed.ncbi.nlm.nih.gov/22812506/)
- Kermanizadeh A, Vranic S, Boland S, Moreau K, Baeza-Squiban A, Gaiser BK, et al. (2013). An in vitro assessment of panel of engineered nanomaterials using a human renal cell line: cytotoxicity, pro-inflammatory response, oxidative stress and genotoxicity. *BMC Nephrol*, 14(1):96. doi:[10.1186/1471-2369-14-96](https://doi.org/10.1186/1471-2369-14-96) PMID:[23617532](https://pubmed.ncbi.nlm.nih.gov/23617532/)
- Kim JE, Lim HT, Minai-Tehrani A, Kwon JT, Shin JY, Woo CG, et al. (2010). Toxicity and clearance of intratracheally administered multiwalled carbon nanotubes from murine lung. *J Toxicol Environ Health A*, 73(21-22):1530-43. doi:[10.1080/15287394.2010.511578](https://doi.org/10.1080/15287394.2010.511578) PMID:[20954079](https://pubmed.ncbi.nlm.nih.gov/20954079/)
- Kim JS, Lee K, Lee YH, Cho HS, Kim KH, Choi KH, et al. (2011). Aspect ratio has no effect on genotoxicity of multi-wall carbon nanotubes. *Arch Toxicol*, 85(7):775-86. doi:[10.1007/s00204-010-0574-0](https://doi.org/10.1007/s00204-010-0574-0) PMID:[20617304](https://pubmed.ncbi.nlm.nih.gov/20617304/)
- Kim JS, Song KS, Lee JK, Choi YC, Bang IS, Kang CS, et al. (2012b). Toxicogenomic comparison of multi-wall carbon nanotubes (MWCNTs) and asbestos. *Arch Toxicol*, 86(4):553-62. doi:[10.1007/s00204-011-0770-6](https://doi.org/10.1007/s00204-011-0770-6) PMID:[22076105](https://pubmed.ncbi.nlm.nih.gov/22076105/)
- Kim JS, Sung JH, Choi BG, Ryu HY, Song KS, Shin JH, et al. (2014). In vivo genotoxicity evaluation of lung cells from Fischer 344 rats following 28 days of inhalation exposure to MWCNTs, plus 28 days and 90 days post-exposure. *Inhal Toxicol*, 26(4):222-34. doi:[10.3109/08958378.2013.878006](https://doi.org/10.3109/08958378.2013.878006) PMID:[24568578](https://pubmed.ncbi.nlm.nih.gov/24568578/)
- Kim JS, Sung JH, Song KS, Lee JH, Kim SM, Lee GH, et al. (2012a). Persistent DNA damage measured by comet assay of Sprague Dawley rat lung cells after five days of inhalation exposure and 1 month post-exposure to dispersed multi-wall carbon nanotubes (MWCNTs) generated by new MWCNT aerosol generation system. *Toxicol Sci*, 128(2):439-48. doi:[10.1093/toxsci/kfs161](https://doi.org/10.1093/toxsci/kfs161) PMID:[22543278](https://pubmed.ncbi.nlm.nih.gov/22543278/)
- Kim JS, Yu IJ (2014). Single-wall carbon nanotubes (SWCNT) induce cytotoxicity and genotoxicity produced by reactive oxygen species (ROS) generation in phytohemagglutinin (PHA)-stimulated male human peripheral blood lymphocytes. *J Toxicol Environ Health A*, 77(19):1141-53. doi:[10.1080/15287394.2014.917062](https://doi.org/10.1080/15287394.2014.917062) PMID:[25119736](https://pubmed.ncbi.nlm.nih.gov/25119736/)
- Kim YA, Hayashi T, Endo M, Dresselhaus MS (2013). 7. Carbon Nanofibers. In: Vajtai R editor. *Springer handbook of nanomaterials*. doi:[10.1007/978-3-642-20595-8\\_7](https://doi.org/10.1007/978-3-642-20595-8_7)

- Kisin ER, Murray AR, Keane MJ, Shi XC, Schwegler-Berry D, Gorelik O, et al. (2007). Single-walled carbon nanotubes: geno- and cytotoxic effects in lung fibroblast V79 cells. *J Toxicol Environ Health A*, 70(24):2071–9. doi:[10.1080/15287390701601251](https://doi.org/10.1080/15287390701601251) PMID:[18049996](https://pubmed.ncbi.nlm.nih.gov/18049996/)
- Kisin ER, Murray AR, Sargent L, Lowry D, Chirila M, Siegrist KJ, et al. (2011). Genotoxicity of carbon nanofibers: are they potentially more or less dangerous than carbon nanotubes or asbestos? *Toxicol Appl Pharmacol*, 252(1):1–10. doi:[10.1016/j.taap.2011.02.001](https://doi.org/10.1016/j.taap.2011.02.001) PMID:[21310169](https://pubmed.ncbi.nlm.nih.gov/21310169/)
- Kitiyanan B, Alvarez WE, Harwell JH, Resasco DE (2000). Controlled production of single-wall carbon nanotubes by catalytic decomposition of CO on bimetallic Co-Mo catalysts. *Chem Phys Lett*, 317(3–5):497–503. doi:[10.1016/S0009-2614\(99\)01379-2](https://doi.org/10.1016/S0009-2614(99)01379-2)
- Kobayashi N, Naya M, Ema M, Endoh S, Maru J, Mizuno K, et al. (2010). Biological response and morphological assessment of individually dispersed multi-wall carbon nanotubes in the lung after intratracheal instillation in rats. *Toxicology*, 276(3):143–53. doi:[10.1016/j.tox.2010.07.021](https://doi.org/10.1016/j.tox.2010.07.021) PMID:[20696199](https://pubmed.ncbi.nlm.nih.gov/20696199/)
- Kobayashi N, Naya M, Mizuno K, Yamamoto K, Ema M, Nakanishi J (2011). Pulmonary and systemic responses of highly pure and well-dispersed single-wall carbon nanotubes after intratracheal instillation in rats. *Inhal Toxicol*, 23(13):814–28. doi:[10.3109/08958378.2011.614968](https://doi.org/10.3109/08958378.2011.614968) PMID:[22004357](https://pubmed.ncbi.nlm.nih.gov/22004357/)
- Köhler AR, Som C, Helland A, Gottschalk F (2008). Studying the potential release of carbon nanotubes throughout the application life cycle. *J Clean Prod*, 16(8–9):927–37. doi:[10.1016/j.jclepro.2007.04.007](https://doi.org/10.1016/j.jclepro.2007.04.007)
- Kolosnjaj-Tabi J, Hartman KB, Boudjemaa S, Ananta JS, Morgant G, Szwarc H, et al. (2010). In vivo behavior of large doses of ultrashort and full-length single-walled carbon nanotubes after oral and intraperitoneal administration to Swiss mice. *ACS Nano*, 4(3):1481–92. doi:[10.1021/nn901573w](https://doi.org/10.1021/nn901573w) PMID:[20175510](https://pubmed.ncbi.nlm.nih.gov/20175510/)
- Kostarelos K, Bianco A, Prato M (2009). Promises, facts and challenges for carbon nanotubes in imaging and therapeutics. *Nat Nanotechnol*, 4(10):627–33. doi:[10.1038/nnano.2009.241](https://doi.org/10.1038/nnano.2009.241) PMID:[19809452](https://pubmed.ncbi.nlm.nih.gov/19809452/)
- Ku BK, Maynard AD, Baron OA, Deye GJ (2007). Observation and measurement of anomalous responses in a differential mobility analyser caused by ultrafine fibrous carbon aerosols. *J Electrostat*, 65(8):542–8. doi:[10.1016/j.elstat.2006.10.012](https://doi.org/10.1016/j.elstat.2006.10.012)
- Kumar M, Ando Y (2010). Chemical vapor deposition of carbon nanotubes: a review on growth mechanism and mass production. *J Nanosci Nanotechnol*, 10(6):3739–58. doi:[10.1166/jnn.2010.2939](https://doi.org/10.1166/jnn.2010.2939) PMID:[20355365](https://pubmed.ncbi.nlm.nih.gov/20355365/)
- Kunzmann A, Andersson B, Thurnherr T, Krug H, Scheynius A, Fadeel B (2011). Toxicology of engineered nanomaterials: focus on biocompatibility, biodistribution and biodegradation. *Biochim Biophys Acta*, 1810(3):361–73. doi:[10.1016/j.bbagen.2010.04.007](https://doi.org/10.1016/j.bbagen.2010.04.007) PMID:[20435096](https://pubmed.ncbi.nlm.nih.gov/20435096/)
- Lacerda L, Ali-Boucetta H, Herrero MA, Pastorin G, Bianco A, Prato M, et al. (2008b). Tissue histology and physiology following intravenous administration of different types of functionalized multiwalled carbon nanotubes. *Nanomedicine (Lond)*, 3(2):149–61. doi:[10.2217/17435889.3.2.149](https://doi.org/10.2217/17435889.3.2.149) PMID:[18373422](https://pubmed.ncbi.nlm.nih.gov/18373422/)
- Lacerda L, Herrero MA, Venner K, Bianco A, Prato M, Kostarelos K (2008c). Carbon-nanotube shape and individualization critical for renal excretion. *Small*, 4(8):1130–2. doi:[10.1002/smll.200800323](https://doi.org/10.1002/smll.200800323) PMID:[18666166](https://pubmed.ncbi.nlm.nih.gov/18666166/)
- Lacerda L, Soundararajan A, Singh R, Pastorin G, Al-Jamal KT, Turton J, et al. (2008a). Dynamic imaging of functionalized multi-walled carbon nanotube systemic circulation and urinary excretion. *Adv Mater*, 20(2):225–30. doi:[10.1002/adma.200702334](https://doi.org/10.1002/adma.200702334)
- Lam CW, James JT, McCluskey R, Arepalli S, Hunter RL (2006). A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit Rev Toxicol*, 36(3):189–217. doi:[10.1080/10408440600570233](https://doi.org/10.1080/10408440600570233) PMID:[16686422](https://pubmed.ncbi.nlm.nih.gov/16686422/)
- Lam CW, James JT, McCluskey R, Hunter RL (2004). Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol Sci*, 77(1):126–34. doi:[10.1093/toxsci/kfg243](https://doi.org/10.1093/toxsci/kfg243) PMID:[14514958](https://pubmed.ncbi.nlm.nih.gov/14514958/)
- Lee JH, Lee SB, Bae GN, Jeon KS, Yoon JU, Ji JH, et al. (2010). Exposure assessment of carbon nanotube manufacturing workplaces. *Inhal Toxicol*, 22(5):369–81. doi:[10.3109/08958370903367359](https://doi.org/10.3109/08958370903367359) PMID:[20121582](https://pubmed.ncbi.nlm.nih.gov/20121582/)
- Lee JH, Sohn EK, Ahn JS, Ahn K, Kim KS, Lee JH, et al. (2013). Exposure assessment of workers in printed electronics workplace. *Inhal Toxicol*, 25(8):426–34. doi:[10.3109/08958378.2013.800617](https://doi.org/10.3109/08958378.2013.800617) PMID:[23808635](https://pubmed.ncbi.nlm.nih.gov/23808635/)
- Lee JK, Sayers BC, Chun KS, Lao HC, Shipley-Phillips JK, Bonner JC, et al. (2012). Multi-walled carbon nanotubes induce COX-2 and iNOS expression via MAP kinase-dependent and -independent mechanisms in mouse RAW264.7 macrophages. *Part Fibre Toxicol*, 9(1):14. doi:[10.1186/1743-8977-9-14](https://doi.org/10.1186/1743-8977-9-14) PMID:[22571318](https://pubmed.ncbi.nlm.nih.gov/22571318/)
- Li J, Li L, Chen H, Chang Q, Liu X, Wu Y, et al. (2014). Application of vitamin E to antagonize SWCNTs-induced exacerbation of allergic asthma. *Sci Rep*, 4:4275. PMID:[24589727](https://pubmed.ncbi.nlm.nih.gov/24589727/)
- Li JG, Li WX, Xu JY, Cai XQ, Liu RL, Li YJ, et al. (2007a). Comparative study of pathological lesions induced by multiwalled carbon nanotubes in lungs of mice by intratracheal instillation and inhalation. *Environ Toxicol*, 22(4):415–21. doi:[10.1002/tox.20270](https://doi.org/10.1002/tox.20270) PMID:[17607736](https://pubmed.ncbi.nlm.nih.gov/17607736/)
- Li R, Wang X, Ji Z, Sun B, Zhang H, Chang CH, et al. (2013). Surface charge and cellular processing of covalently functionalized multiwall carbon nanotubes

- determine pulmonary toxicity. *ACS Nano*, 7(3):2352–68. doi:[10.1021/nn305567s](https://doi.org/10.1021/nn305567s) PMID:[23414138](https://pubmed.ncbi.nlm.nih.gov/23414138/)
- Li X, Peng Y, Qu X (2006). Carbon nanotubes selective destabilization of duplex and triplex DNA and inducing B-A transition in solution. *Nucleic Acids Res*, 34(13):3670–6. doi:[10.1093/nar/gkl513](https://doi.org/10.1093/nar/gkl513) PMID:[16885240](https://pubmed.ncbi.nlm.nih.gov/16885240/)
- Li Z, Hulderman T, Salmen R, Chapman R, Leonard SS, Young SH, et al. (2007b). Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. *Environ Health Perspect*, 115(3):377–82. doi:[10.1289/ehp.9688](https://doi.org/10.1289/ehp.9688) PMID:[17431486](https://pubmed.ncbi.nlm.nih.gov/17431486/)
- Liao HY, Chung YT, Lai CH, Lin MH, Liou SH (2014b). Sneezing and allergic dermatitis were increased in engineered nanomaterial handling workers. *Ind Health*, 52(3):199–215. doi:[10.2486/indhealth.2013-0100](https://doi.org/10.2486/indhealth.2013-0100) PMID:[24492762](https://pubmed.ncbi.nlm.nih.gov/24492762/)
- Liao HY, Chung YT, Lai CH, Wang SL, Chiang HC, Li LA, et al. (2014a). Six-month follow-up study of health markers of nanomaterials among workers handling engineered nanomaterials. *Nanotoxicology*, 8(S1):Suppl 1: 100–10. doi:[10.3109/17435390.2013.858793](https://doi.org/10.3109/17435390.2013.858793) PMID:[24295335](https://pubmed.ncbi.nlm.nih.gov/24295335/)
- Lindberg HK, Falck GC, Singh R, Suhonen S, Järventaus H, Vanhala E, et al. (2013). Genotoxicity of short single-wall and multi-wall carbon nanotubes in human bronchial epithelial and mesothelial cells in vitro. *Toxicology*, 313(1):24–37. doi:[10.1016/j.tox.2012.12.008](https://doi.org/10.1016/j.tox.2012.12.008) PMID:[23266321](https://pubmed.ncbi.nlm.nih.gov/23266321/)
- Lindberg HK, Falck GC, Suhonen S, Vippola M, Vanhala E, Catalán J, et al. (2009). Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells in vitro. *Toxicol Lett*, 186(3):166–73. doi:[10.1016/j.toxlet.2008.11.019](https://doi.org/10.1016/j.toxlet.2008.11.019) PMID:[19114091](https://pubmed.ncbi.nlm.nih.gov/19114091/)
- Ling WL, Biro A, Bally I, Tacnet P, Deniaud A, Doris E, et al. (2011). Proteins of the innate immune system crystallize on carbon nanotubes but are not activated. *ACS Nano*, 5(2):730–7. doi:[10.1021/nn102400w](https://doi.org/10.1021/nn102400w) PMID:[21214219](https://pubmed.ncbi.nlm.nih.gov/21214219/)
- Liou SH, Tsou TC, Wang SL, Li LA, Chiang HC, Li WF, et al. (2012). Epidemiological study of health hazards among workers handling engineered nanomaterials. *J Nanopart Res*, 14(8):878. doi:[10.1007/s11051-012-0878-5](https://doi.org/10.1007/s11051-012-0878-5)
- Liou PJ, Lippmann M, Phalen RF (1984). Rationale for particle size-selective air sampling. In Particle size-selective sampling in the workplace: report of the ACGIH Technical Committee on Air Sampling Procedures *Ann Am Conf Ind Hyg*, 11:27–34.
- Lison D, Muller J (2008). Lung and systemic responses to carbon nanotubes (CNT) in mice. *Toxicol Sci*, 101(1):179–80, author reply 181–2. doi:[10.1093/toxsci/kfm249](https://doi.org/10.1093/toxsci/kfm249) PMID:[17897971](https://pubmed.ncbi.nlm.nih.gov/17897971/)
- Liu HL, Zhang YL, Yang N, Zhang YX, Liu XQ, Li CG, et al. (2011). A functionalized single-walled carbon nanotube-induced autophagic cell death in human lung cells through Akt-TSC2-mTOR signalling. *Cell Death Dis*, 2(5):e159. doi:[10.1038/cddis.2011.27](https://doi.org/10.1038/cddis.2011.27) PMID:[21593791](https://pubmed.ncbi.nlm.nih.gov/21593791/)
- Liu X, Guo L, Morris D, Kane AB, Hurt RH (2008b). Targeted removal of bioavailable metal as a detoxification strategy for carbon nanotubes. *Carbon N Y*, 46(3):489–500. doi:[10.1016/j.carbon.2007.12.018](https://doi.org/10.1016/j.carbon.2007.12.018) PMID:[19255622](https://pubmed.ncbi.nlm.nih.gov/19255622/)
- Liu X, Hurt RH, Kane AB (2010). Biodurability of single-walled carbon nanotubes depends on surface functionalization. *Carbon N Y*, 48(7):1961–9. doi:[10.1016/j.carbon.2010.02.002](https://doi.org/10.1016/j.carbon.2010.02.002) PMID:[20352066](https://pubmed.ncbi.nlm.nih.gov/20352066/)
- Liu X, Sen S, Liu J, Kulaots I, Geohagan D, Kane A, et al. (2011b). Antioxidant deactivation on graphenic nanocarbon surfaces. *Small*, 7(19):2775–85. doi:[10.1002/sml.201100651](https://doi.org/10.1002/sml.201100651) PMID:[21818846](https://pubmed.ncbi.nlm.nih.gov/21818846/)
- Liu X, Tao H, Yang K, Zhang S, Lee S-T, Liu Z (2011a). Optimization of surface chemistry on single-walled carbon nanotubes for in vivo photothermal ablation of tumors. *Biomaterials*, 32(1):144–51. doi:[10.1016/j.biomaterials.2010.08.096](https://doi.org/10.1016/j.biomaterials.2010.08.096) PMID:[20888630](https://pubmed.ncbi.nlm.nih.gov/20888630/)
- Liu Z, Davis C, Cai W, He L, Chen X, Dai H (2008a). Circulation and long-term fate of functionalized, biocompatible single-walled carbon nanotubes in mice probed by Raman spectroscopy. *Proc Natl Acad Sci USA*, 105(5):1410–5. doi:[10.1073/pnas.0707654105](https://doi.org/10.1073/pnas.0707654105) PMID:[18230737](https://pubmed.ncbi.nlm.nih.gov/18230737/)
- Lohcharoenkal W, Wang L, Stueckle TA, Park J, Tse W, Dinu CZ, et al. (2014). Role of H-Ras/ERK signalling in carbon nanotube-induced neoplastic-like transformation of human mesothelial cells. *Front Physiol*, 5:222. doi:[10.3389/fphys.2014.00222](https://doi.org/10.3389/fphys.2014.00222) PMID:[24971065](https://pubmed.ncbi.nlm.nih.gov/24971065/)
- Lu X, Chen Z (2005). Curved pi-conjugation, aromaticity, and the related chemistry of small fullerenes (< C60) and single-walled carbon nanotubes. *Chem Rev*, 105(10):3643–96. doi:[10.1021/cr030093d](https://doi.org/10.1021/cr030093d) PMID:[16218563](https://pubmed.ncbi.nlm.nih.gov/16218563/)
- Lucente-Schultz RM, Moore VC, Leonard AD, Price BK, Kosynkin DV, Lu M, et al. (2009). Antioxidant single-walled carbon nanotubes. *J Am Chem Soc*, 131(11):3934–41. doi:[10.1021/ja805721p](https://doi.org/10.1021/ja805721p) PMID:[19243186](https://pubmed.ncbi.nlm.nih.gov/19243186/)
- Ma-Hock L, Treumann S, Strauss V, Brill S, Luizi F, Mertler M, et al. (2009). Inhalation toxicity of multi-wall carbon nanotubes in rats exposed for 3 months. *Toxicol Sci*, 112(2):468–81. doi:[10.1093/toxsci/kfp146](https://doi.org/10.1093/toxsci/kfp146) PMID:[19584127](https://pubmed.ncbi.nlm.nih.gov/19584127/)
- Mahar B, Laslau C, Yip R, Sun Y (2007). Development of carbon nanotube-based sensors – A review. *IEEE Sens J*, 7(2):266–84. doi:[10.1109/JSEN.2006.886863](https://doi.org/10.1109/JSEN.2006.886863)
- Manshian BB, Jenkins GJS, Williams PM, Wright C, Barron AR, Brown AP, et al. (2013). Single-walled carbon nanotubes: differential genotoxic potential associated with physico-chemical properties. *Nanotoxicology*, 7(2):144–56. doi:[10.3109/17435390.2011.647928](https://doi.org/10.3109/17435390.2011.647928) PMID:[22263934](https://pubmed.ncbi.nlm.nih.gov/22263934/)
- Matthews IP, Gregory CJ, Aljayyousi G, Morris CJ, McDonald I, Hoogendoorn B, et al. (2013). Maximal

- extent of translocation of single-walled carbon nanotubes from lung airways of the rat. *Environ Toxicol Pharmacol*, 35(3):461–4. doi:[10.1016/j.etap.2013.02.002](https://doi.org/10.1016/j.etap.2013.02.002) PMID:[23501606](https://pubmed.ncbi.nlm.nih.gov/23501606/)
- Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER, Castranova V (2004). Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material. *J Toxicol Environ Health A*, 67(1):87–107. doi:[10.1080/15287390490253688](https://doi.org/10.1080/15287390490253688) PMID:[14668113](https://pubmed.ncbi.nlm.nih.gov/14668113/)
- Maynard AD, Ku BK, Emery M, Stolzenburg M, McMurry PH (2007). Measuring particle size-dependent physicochemical structure in airborne single walled carbon nanotube agglomerates. *J Nanopart Res*, 9(1):85–92. doi:[10.1007/s11051-006-9178-2](https://doi.org/10.1007/s11051-006-9178-2)
- McDonald J, Mitchell L (2008). Lung and systemic responses to carbon nanotubes (CNT) in mice. *Toxicol Sci*, 101(1):181–2. doi:[10.1093/toxsci/kfm250](https://doi.org/10.1093/toxsci/kfm250) PMID:[18281258](https://pubmed.ncbi.nlm.nih.gov/18281258/)
- McShan D, Yu H (2014). DNA damage in human skin keratinocytes caused by multiwalled carbon nanotubes with carboxylate functionalization. *Toxicol Ind Health*, 30(6):489–98. doi:[10.1177/0748233712459914](https://doi.org/10.1177/0748233712459914) PMID:[23012341](https://pubmed.ncbi.nlm.nih.gov/23012341/)
- Mercer RR, Hubbs AF, Scabilloni JF, Wang L, Battelli LA, Friend S, et al. (2011). Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes. *Part Fibre Toxicol*, 8(1):21. doi:[10.1186/1743-8977-8-21](https://doi.org/10.1186/1743-8977-8-21) PMID:[21781304](https://pubmed.ncbi.nlm.nih.gov/21781304/)
- Mercer RR, Hubbs AF, Scabilloni JF, Wang L, Battelli LA, Schwegler-Berry D, et al. (2010). Distribution and persistence of pleural penetrations by multi-walled carbon nanotubes. *Part Fibre Toxicol*, 7(1):28. doi:[10.1186/1743-8977-7-28](https://doi.org/10.1186/1743-8977-7-28) PMID:[20920331](https://pubmed.ncbi.nlm.nih.gov/20920331/)
- Mercer RR, Scabilloni J, Wang L, Kisin E, Murray AR, Schwegler-Berry D, et al. (2008). Alteration of deposition pattern and pulmonary response as a result of improved dispersion of aspirated single-walled carbon nanotubes in a mouse model. *Am J Physiol Lung Cell Mol Physiol*, 294(1):L87–97. doi:[10.1152/ajplung.00186.2007](https://doi.org/10.1152/ajplung.00186.2007) PMID:[18024722](https://pubmed.ncbi.nlm.nih.gov/18024722/)
- Mercer RR, Scabilloni JF, Hubbs AF, Battelli LA, McKinney W, Friend S, et al. (2013b). Distribution and fibrotic response following inhalation exposure to multi-walled carbon nanotubes. *Part Fibre Toxicol*, 10(1):33. doi:[10.1186/1743-8977-10-33](https://doi.org/10.1186/1743-8977-10-33) PMID:[23895460](https://pubmed.ncbi.nlm.nih.gov/23895460/)
- Mercer RR, Scabilloni JF, Hubbs AF, Wang L, Battelli LA, McKinney W, et al. (2013a). Extrapulmonary transport of MWCNT following inhalation exposure. *Part Fibre Toxicol*, 10(1):38. doi:[10.1186/1743-8977-10-38](https://doi.org/10.1186/1743-8977-10-38) PMID:[23927530](https://pubmed.ncbi.nlm.nih.gov/23927530/)
- Methner M, Beaucham C, Crawford C, Hodson L, Geraci C (2012). Field application of the Nanoparticle Emission Assessment Technique (NEAT): task-based air monitoring during the processing of engineered nanomaterials (ENM) at four facilities. *J Occup Environ Hyg*, 9(9):543–55. doi:[10.1080/15459624.2012.699388](https://doi.org/10.1080/15459624.2012.699388) PMID:[22816668](https://pubmed.ncbi.nlm.nih.gov/22816668/)
- Methner M, Hodson L, Dames A, Geraci C (2010). Nanoparticle Emission Assessment Technique (NEAT) for the identification and measurement of potential inhalation exposure to engineered nanomaterials—Part B: Results from 12 field studies. *J Occup Environ Hyg*, 7(3):163–76. doi:[10.1080/15459620903508066](https://doi.org/10.1080/15459620903508066) PMID:[20063229](https://pubmed.ncbi.nlm.nih.gov/20063229/)
- Meunier E, Coste A, Olganier D, Authier H, Lefèvre L, Dardenne C, et al. (2012). Double-walled carbon nanotubes trigger IL-1 $\beta$  release in human monocytes through Nlrp3 inflammasome activation. *Nanomedicine*, 8(6):987–95. doi:[10.1016/j.nano.2011.11.004](https://doi.org/10.1016/j.nano.2011.11.004) PMID:[22100755](https://pubmed.ncbi.nlm.nih.gov/22100755/)
- Migliore L, Saracino D, Bonelli A, Colognato R, D’Errico MR, Magrini A, et al. (2010). Carbon nanotubes induce oxidative DNA damage in RAW 264.7 cells. *Environ Mol Mutagen*, 51(4):294–303. PMID:[20091701](https://pubmed.ncbi.nlm.nih.gov/20091701/)
- Mitchell LA, Gao J, Wal RV, Gigliotti A, Burchiel SW, McDonald JD (2007). Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. *Toxicol Sci*, 100(1):203–14. doi:[10.1093/toxsci/kfm196](https://doi.org/10.1093/toxsci/kfm196) PMID:[17660506](https://pubmed.ncbi.nlm.nih.gov/17660506/)
- Mitchell LA, Lauer FT, Burchiel SW, McDonald JD (2009). Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice. *Nat Nanotechnol*, 4(7):451–6. doi:[10.1038/nnano.2009.151](https://doi.org/10.1038/nnano.2009.151) PMID:[19581899](https://pubmed.ncbi.nlm.nih.gov/19581899/)
- Mizutani N, Nabe T, Yoshino S (2012). Exposure to multi-walled carbon nanotubes and allergen promotes early- and late-phase increases in airway resistance in mice. *Biol Pharm Bull*, 35(12):2133–40. doi:[10.1248/bpb.b12-00357](https://doi.org/10.1248/bpb.b12-00357) PMID:[23207765](https://pubmed.ncbi.nlm.nih.gov/23207765/)
- Moghimi SM, Andersen AJ, Hashemi SH, Lettiero B, Ahmadvand D, Hunter AC, et al. (2010). Complement activation cascade triggered by PEG-PL engineered nanomedicines and carbon nanotubes: the challenges ahead. *J Control Release*, 146(2):175–81. doi:[10.1016/j.jconrel.2010.04.003](https://doi.org/10.1016/j.jconrel.2010.04.003) PMID:[20388529](https://pubmed.ncbi.nlm.nih.gov/20388529/)
- Mohiuddin, Keka IS, Evans TJ, Hirota K, Shimizu H, Kono K, et al. (2014). A novel genotoxicity assay of carbon nanotubes using functional macrophage receptor with collagenous structure (MARCO)-expressing chicken B lymphocytes. *Arch Toxicol*, 88(1):145–60. doi:[10.1007/s00204-013-1084-7](https://doi.org/10.1007/s00204-013-1084-7) PMID:[23963510](https://pubmed.ncbi.nlm.nih.gov/23963510/)
- Monteiro-Riviere NA, Inman AO, Zhang LW (2009). Limitations and relative utility of screening assays to assess engineered nanoparticle toxicity in a human cell line. *Toxicol Appl Pharmacol*, 234(2):222–35. doi:[10.1016/j.taap.2008.09.030](https://doi.org/10.1016/j.taap.2008.09.030) PMID:[18983864](https://pubmed.ncbi.nlm.nih.gov/18983864/)
- Morimoto Y, Hirohashi M, Horie M, Ogami A, Oyabu T, Myojo T, et al. (2012c). Pulmonary toxicity of well-dispersed single-wall carbon nanotubes following intratracheal instillation. *J Nano Res*, 18–19:9–25. doi:[10.4028/www.scientific.net/JNanoR.18-19.9](https://doi.org/10.4028/www.scientific.net/JNanoR.18-19.9)

- Morimoto Y, Hirohashi M, Kobayashi N, Ogami A, Horie M, Oyabu T, et al. (2012a). Pulmonary toxicity of well-dispersed single-wall carbon nanotubes after inhalation. *Nanotoxicology*, 6(7):766–75. doi:[10.3109/17435390.2011.620719](https://doi.org/10.3109/17435390.2011.620719) PMID:[21942532](https://pubmed.ncbi.nlm.nih.gov/21942532/)
- Morimoto Y, Hirohashi M, Ogami A, Oyabu T, Myojo T, Todoroki M, et al. (2012b). Pulmonary toxicity of well-dispersed multi-wall carbon nanotubes following inhalation and intratracheal instillation. *Nanotoxicology*, 6(6):587–99. doi:[10.3109/17435390.2011.594912](https://doi.org/10.3109/17435390.2011.594912) PMID:[21714591](https://pubmed.ncbi.nlm.nih.gov/21714591/)
- Morimoto Y, Izumi H, Kuroda E (2014). Significance of persistent inflammation in respiratory disorders induced by nanoparticles. *J Immunol Res*, 2014(962871):962871. PMID:[25097864](https://pubmed.ncbi.nlm.nih.gov/25097864/)
- Morrow PE (1988). Possible mechanisms to explain dust overloading of the lungs. *Fundam Appl Toxicol*, 10(3):369–84. doi:[10.1016/0272-0590\(88\)90284-9](https://doi.org/10.1016/0272-0590(88)90284-9) PMID:[3286345](https://pubmed.ncbi.nlm.nih.gov/3286345/)
- Morrow PE (1994). Mechanisms and significance of “particle overload”. In: *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract*. Proceedings of the 4th International Inhalation Symposium, March 1993, Hanover, Germany, pp. 17–25. Washington (DC), USA: International Life Sciences Institute Press.
- Movia D, Prina-Mello A, Bazou D, Volkov Y, Giordani S (2011). Screening the cytotoxicity of single-walled carbon nanotubes using novel 3D tissue-mimetic models. *ACS Nano*, 5(11):9278–90. doi:[10.1021/nn203659m](https://doi.org/10.1021/nn203659m) PMID:[22017733](https://pubmed.ncbi.nlm.nih.gov/22017733/)
- Muller J, Decordier I, Hoet PH, Lombaert N, Thomassen L, Huaux F, et al. (2008b). Clastogenic and aneugenic effects of multi-wall carbon nanotubes in epithelial cells. *Carcinogenesis*, 29(2):427–33. doi:[10.1093/carcin/bgm243](https://doi.org/10.1093/carcin/bgm243) PMID:[18174261](https://pubmed.ncbi.nlm.nih.gov/18174261/)
- Muller J, Delos M, Panin N, Rabolli V, Huaux F, Lison D (2009). Absence of carcinogenic response to multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat. *Toxicol Sci*, 110(2):442–8. doi:[10.1093/toxsci/kfp100](https://doi.org/10.1093/toxsci/kfp100) PMID:[19429663](https://pubmed.ncbi.nlm.nih.gov/19429663/)
- Muller J, Huaux F, Fonseca A, Nagy JB, Moreau N, Delos M, et al. (2008a). Structural defects play a major role in the acute lung toxicity of multiwall carbon nanotubes: toxicological aspects. *Chem Res Toxicol*, 21(9):1698–705. doi:[10.1021/tx800101p](https://doi.org/10.1021/tx800101p) PMID:[18636756](https://pubmed.ncbi.nlm.nih.gov/18636756/)
- Muller J, Huaux F, Moreau N, Misson P, Heilier JF, Delos M, et al. (2005). Respiratory toxicity of multi-wall carbon nanotubes. *Toxicol Appl Pharmacol*, 207(3):221–31. doi:[10.1016/j.taap.2005.01.008](https://doi.org/10.1016/j.taap.2005.01.008) PMID:[16129115](https://pubmed.ncbi.nlm.nih.gov/16129115/)
- Müller L, Riediker M, Wick P, Mohr M, Gehr P, Rothen-Rutishauser B (2010). Oxidative stress and inflammation response after nanoparticle exposure: differences between human lung cell monocultures and an advanced three-dimensional model of the human epithelial airways. *J R Soc Interface*, 7:Suppl 1: S27–40. doi:[10.1098/rsif.2009.0161.focus](https://doi.org/10.1098/rsif.2009.0161.focus) PMID:[19586954](https://pubmed.ncbi.nlm.nih.gov/19586954/)
- Murphy FA, Poland CA, Duffin R, Al-Jamal KT, Ali-Boucetta H, Nunes A, et al. (2011). Length-dependent retention of carbon nanotubes in the pleural space of mice initiates sustained inflammation and progressive fibrosis on the parietal pleura. *Am J Pathol*, 178(6):2587–600. doi:[10.1016/j.ajpath.2011.02.040](https://doi.org/10.1016/j.ajpath.2011.02.040) PMID:[21641383](https://pubmed.ncbi.nlm.nih.gov/21641383/)
- Murphy FA, Schinwald A, Poland CA, Donaldson K (2012). The mechanism of pleural inflammation by long carbon nanotubes: interaction of long fibres with macrophages stimulates them to amplify pro-inflammatory responses in mesothelial cells. *Part Fibre Toxicol*, 9(1):8. doi:[10.1186/1743-8977-9-8](https://doi.org/10.1186/1743-8977-9-8) PMID:[22472194](https://pubmed.ncbi.nlm.nih.gov/22472194/)
- Murr LE, Bang JJ, Esquivel EV, Guerrero PA, Lopez A (2004b). Carbon nanotubes, nanocrystal forms, and complex nanoparticle aggregates in common fuel-gas combustion sources and the ambient air. *J Nanopart Res*, 6(2/3):241–51. doi:[10.1023/B:NANO.0000034651.91325.40](https://doi.org/10.1023/B:NANO.0000034651.91325.40)
- Murr LE, Esquivel EV, Bang JJ, de la Rosa G, Gardea-Torresdey JL (2004a). Chemistry and nanoparticulate compositions of a 10,000 year-old ice core melt water. *Water Res*, 38(19):4282–96. doi:[10.1016/j.watres.2004.08.010](https://doi.org/10.1016/j.watres.2004.08.010) PMID:[15491674](https://pubmed.ncbi.nlm.nih.gov/15491674/)
- Murr LE, Guerrero PA (2006). Carbon nanotubes in wood soot *Atmosph Sci Lett*, 793–95.
- Murr LE, Soto KF (2005). A TEM study of soot, carbon nanotubes, and related fullerene nanopolyhedra in common fuel-gas combustion sources. *Mater Charact*, 55(1):50–65. doi:[10.1016/j.matchar.2005.02.008](https://doi.org/10.1016/j.matchar.2005.02.008)
- Murr LE, Soto KF, Garza KM, Guerrero PA, Martinez F, Esquivel EV, et al. (2006). Combustion-generated nanoparticulates in the El Paso, TX, USA / Juarez, Mexico Metroplex: their comparative characterization and potential for adverse health effects. *Int J Environ Res Public Health*, 3(1):48–66. doi:[10.3390/ijerph2006030007](https://doi.org/10.3390/ijerph2006030007) PMID:[16823077](https://pubmed.ncbi.nlm.nih.gov/16823077/)
- Murray AR, Kisin E, Leonard SS, Young SH, Kommineni C, Kagan VE, et al. (2009). Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes. *Toxicology*, 257(3):161–71. doi:[10.1016/j.tox.2008.12.023](https://doi.org/10.1016/j.tox.2008.12.023) PMID:[19150385](https://pubmed.ncbi.nlm.nih.gov/19150385/)
- Murray AR, Kisin ER, Tkach AV, Yanamala N, Mercer R, Young SH, et al. (2012). Factoring-in agglomeration of carbon nanotubes and nanofibers for better prediction of their toxicity versus asbestos. *Part Fibre Toxicol*, 9(1):10. doi:[10.1186/1743-8977-9-10](https://doi.org/10.1186/1743-8977-9-10) PMID:[22490147](https://pubmed.ncbi.nlm.nih.gov/22490147/)
- Nagai H, Okazaki Y, Chew SH, Misawa N, Miyata Y, Shinohara H, et al. (2013). Intraperitoneal administration of tangled multiwalled carbon nanotubes of 15 nm in diameter does not induce mesothelial carcinogenesis in rats. *Pathol Int*, 63(9):457–62. doi:[10.1111/pin.12093](https://doi.org/10.1111/pin.12093) PMID:[24200157](https://pubmed.ncbi.nlm.nih.gov/24200157/)
- Nagai H, Okazaki Y, Chew SH, Misawa N, Yamashita Y, Akatsuka S, et al. (2011). Diameter and rigidity of multiwalled carbon nanotubes are critical factors in

- mesothelial injury and carcinogenesis. *Proc Natl Acad Sci USA*, 108(49):E1330–8. doi:[10.1073/pnas.1110013108](https://doi.org/10.1073/pnas.1110013108) PMID:[22084097](https://pubmed.ncbi.nlm.nih.gov/22084097/)
- Nanocyl (2009). Responsible care and nanomaterials case study Nanocyl. Presentation at European Responsible Care Conference, 21–23 October 2009, Prague, Czech Republic. Brussels, Belgium: The European Chemical Industry Council (CEFIC). Available from: [http://www.cefic.org/Documents/ResponsibleCare/04\\_Nanocyl.pdf](http://www.cefic.org/Documents/ResponsibleCare/04_Nanocyl.pdf).
- Naya M, Kobayashi N, Endoh S, Maru J, Honda K, Ema M, et al. (2012). In vivo genotoxicity study of single-wall carbon nanotubes using comet assay following intratracheal instillation in rats. *Regul Toxicol Pharmacol*, 64(1):124–9. doi:[10.1016/j.yrtph.2012.05.020](https://doi.org/10.1016/j.yrtph.2012.05.020) PMID:[22735368](https://pubmed.ncbi.nlm.nih.gov/22735368/)
- Naya M, Kobayashi N, Mizuno K, Matsumoto K, Ema M, Nakanishi J (2011). Evaluation of the genotoxic potential of single-wall carbon nanotubes by using a battery of in vitro and in vivo genotoxicity assays. *Regul Toxicol Pharmacol*, 61(2):192–8. doi:[10.1016/j.yrtph.2011.07.008](https://doi.org/10.1016/j.yrtph.2011.07.008) PMID:[21821090](https://pubmed.ncbi.nlm.nih.gov/21821090/)
- NIOSH (1994a). NIOSH Manual of Analytical Methods. 4th Edition, Method No. 7402. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.
- NIOSH (1994b). NIOSH Manual of Analytical Methods. 4th Edition, Method No. 0500. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.
- NIOSH (1999). NIOSH Manual of Analytical Methods. 4th Edition, Method No. 5040. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.
- NIOSH (2003a). NIOSH Manual of Analytical Methods. 4th Edition, Method No. 7300. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.
- NIOSH (2003b). NIOSH Manual of Analytical Methods. 4th Edition, Method No. 7303. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.
- NIOSH (2003c). NIOSH Manual of Analytical Methods. 4th Edition, Method No. 7404. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.
- NIOSH (2013). Current Intelligence Bulletin 65: Occupational Exposure to Carbon Nanotubes and Nanofibers. Publication No. 2013–145. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. Available from: <http://www.cdc.gov/niosh/review/peer/HISA/nano-pr.html>.
- Nowack B, David RM, Fissan H, Morris H, Shatkin JA, Stintz M, et al. (2013). Potential release scenarios for carbon nanotubes used in composites. *Environ Int*, 59:1–11. doi:[10.1016/j.envint.2013.04.003](https://doi.org/10.1016/j.envint.2013.04.003) PMID:[23708563](https://pubmed.ncbi.nlm.nih.gov/23708563/)
- NSTC (2011). The First Nanosafety Management Master Plan (2012–2016), National Science and Technology Commission. [in Korean]. Seoul, Republic of Korea: National Science and Technology Commission. Available from: [http://www.nnpc.re.kr/knowledge/nano\\_policy/19/view?p\\_page=1&p\\_pagesize=10&ofield=&direction=&sttype=&keyword=&category](http://www.nnpc.re.kr/knowledge/nano_policy/19/view?p_page=1&p_pagesize=10&ofield=&direction=&sttype=&keyword=&category), accessed 23 January 2016.
- Nygaard UC, Hansen JS, Samuelsen M, Alberg T, Marioara CD, Løvik M (2009). Single-walled and multi-walled carbon nanotubes promote allergic immune responses in mice. *Toxicol Sci*, 109(1):113–23. doi:[10.1093/toxsci/kfp057](https://doi.org/10.1093/toxsci/kfp057) PMID:[19293371](https://pubmed.ncbi.nlm.nih.gov/19293371/)
- Nymark P, Jensen KA, Suhonen S, Kembouche Y, Vippola M, Kleinjans J, et al. (2014). Free radical scavenging and formation by multi-walled carbon nanotubes in cell free conditions and in human bronchial epithelial cells. *Part Fibre Toxicol*, 11(1):4. doi:[10.1186/1743-8977-11-4](https://doi.org/10.1186/1743-8977-11-4) PMID:[24438343](https://pubmed.ncbi.nlm.nih.gov/24438343/)
- Oberdörster G (1995). Lung particle overload: implications for occupational exposures to particles. *Regul Toxicol Pharmacol*, 21(1):123–35. doi:[10.1006/rtph.1995.1017](https://doi.org/10.1006/rtph.1995.1017) PMID:[7784625](https://pubmed.ncbi.nlm.nih.gov/7784625/)
- Oberdörster G (2012). Nanotoxicology: in vitro-in vivo dosimetry. *Environ Health Perspect*, 120(1):A13–, author reply A13. doi:[10.1289/ehp.1104320](https://doi.org/10.1289/ehp.1104320) PMID:[22214547](https://pubmed.ncbi.nlm.nih.gov/22214547/)
- Oberdörster G, Kane AB, Klaper RD, Hurt RH (2013). Nanotoxicology. In: Casarett and Doull's toxicology. Columbus (OH), USA: McGraw-Hill Companies, Inc.
- Observatory Nano (2011). Briefing No. 23: Transport — Nanotechnology in automotive tyres. European Commission. Available from: [http://nanopinion.eu/sites/default/files/briefing\\_no.23\\_nanotechnology\\_in\\_automotive\\_tyres.pdf](http://nanopinion.eu/sites/default/files/briefing_no.23_nanotechnology_in_automotive_tyres.pdf).
- OECD (2009a). Series on the safety of manufactured nanomaterials, preliminary analysis of exposure measurement and exposure mitigation in occupational settings: manufactured nanomaterials. Paris, France: Organisation for Economic Co-operation and Development (OECD).
- OECD (2009b). Emission assessment for identification of sources and release of airborne manufactured

- nanomaterials in the workplace: compilation of existing guidance. Paris, France: Organisation for Economic Co-operation and Development.
- OECD (2009c). Identification, compilation and analysis of guidance information for exposure measurement and exposure mitigation: manufactured nanomaterials. Paris, France: Organisation for Economic Co-operation and Development.
- OECD (2009d). Report of an OECD Workshop on Exposure Assessment and Exposure Mitigation: Manufactured Nanomaterials. Paris, France: Organisation for Economic Co-operation and Development.
- OECD (2010). Compilation and Comparison of Guidelines Related to Exposure to Nanomaterials in Laboratories. Paris, France: Organisation for Economic Co-operation and Development.
- Ogasawara Y, Umezumi N, Ishii K (2012). [DNA damage in human pleural mesothelial cells induced by exposure to carbon nanotubes] *Nippon Eiseigaku Zasshi*, 67(1):76–83. doi:[10.1265/jjh.67.76](https://doi.org/10.1265/jjh.67.76) PMID:[22449827](https://pubmed.ncbi.nlm.nih.gov/22449827/)
- Ogura I, Kotake M, Hashimoto N, Gotoh K, Kishimoto A (2013b). Release characteristics of single-wall carbon nanotubes during manufacturing and handling. *J Phys Conf Ser*, 429:012057. doi:[10.1088/1742-6596/429/1/012057](https://doi.org/10.1088/1742-6596/429/1/012057)
- Ogura I, Kotake M, Shigeta M, Uejima M, Saito K, Hashimoto N, et al. (2013a). Potential release of carbon nanotubes from their composites during grinding. *J Phys Conf Ser*, 429:012049. doi:[10.1088/1742-6596/429/1/012049](https://doi.org/10.1088/1742-6596/429/1/012049)
- Ono-Ogasawara M, Myojo T (2013). Characteristics of multi-walled carbon nanotubes and background aerosols by carbon analysis; particle size and oxidation temperature. *Adv Powder Technol*, 24(1):263–9. doi:[10.1016/j.apt.2012.06.013](https://doi.org/10.1016/j.apt.2012.06.013)
- OSHA (2013). Fact Sheet: Working Safely with Nanomaterials. Washington (DC), USA: Occupational Safety and Health Administration, United States Department of Labor. Available from: [https://www.osha.gov/Publications/OSHA\\_FS-3634.pdf](https://www.osha.gov/Publications/OSHA_FS-3634.pdf), accessed 9 September 2014.
- Oyabu T, Myojo T, Morimoto Y, Ogami A, Hirohashi M, Yamamoto M, et al. (2011). Biopersistence of inhaled MWCNT in rat lungs in a 4-week well-characterized exposure. *Inhal Toxicol*, 23(13):784–91. doi:[10.3109/08958378.2011.608096](https://doi.org/10.3109/08958378.2011.608096) PMID:[22035120](https://pubmed.ncbi.nlm.nih.gov/22035120/)
- Pacurari M, Qian Y, Porter DW, Wolfarth M, Wan Y, Luo D, et al. (2011). Multi-walled carbon nanotube-induced gene expression in the mouse lung: association with lung pathology. *Toxicol Appl Pharmacol*, 255(1):18–31. doi:[10.1016/j.taap.2011.05.012](https://doi.org/10.1016/j.taap.2011.05.012) PMID:[21624382](https://pubmed.ncbi.nlm.nih.gov/21624382/)
- Pacurari M, Yin XJ, Ding M, Leonard SS, Schwegler-Berry D, Ducatman BS, et al. (2008a). Oxidative and molecular interactions of multi-wall carbon nanotubes (MWCNT) in normal and malignant human mesothelial cells. *Nanotoxicology*, 2(3):155–70. doi:[10.1080/17435390802318356](https://doi.org/10.1080/17435390802318356)
- Pacurari M, Yin XJ, Zhao J, Ding M, Leonard SS, Schwegler-Berry D, et al. (2008b). Raw single-wall carbon nanotubes induce oxidative stress and activate MAPKs, AP-1, NF-kappaB, and Akt in normal and malignant human mesothelial cells. *Environ Health Perspect*, 116(9):1211–7. doi:[10.1289/ehp.10924](https://doi.org/10.1289/ehp.10924) PMID:[18795165](https://pubmed.ncbi.nlm.nih.gov/18795165/)
- Paik SY, Zalk DM, Swuste P (2008). Application of a pilot control banding tool for risk level assessment and control of nanoparticle exposures. *Ann Occup Hyg*, 52(6):419–28. doi:[10.1093/annhyg/men041](https://doi.org/10.1093/annhyg/men041) PMID:[18632731](https://pubmed.ncbi.nlm.nih.gov/18632731/)
- Palomäki J, Karisola P, Pylkkänen L, Savolainen K, Alenius H (2010). Engineered nanomaterials cause cytotoxicity and activation on mouse antigen presenting cells. *Toxicology*, 267(1–3):125–31. doi:[10.1016/j.tox.2009.10.034](https://doi.org/10.1016/j.tox.2009.10.034) PMID:[19897006](https://pubmed.ncbi.nlm.nih.gov/19897006/)
- Palomäki J, Välimäki E, Sund J, Vippola M, Clausen PA, Jensen KA, et al. (2011). Long, needle-like carbon nanotubes and asbestos activate the NLRP3 inflammasome through a similar mechanism. *ACS Nano*, 5(9):6861–70. doi:[10.1021/nn200595c](https://doi.org/10.1021/nn200595c) PMID:[21800904](https://pubmed.ncbi.nlm.nih.gov/21800904/)
- Pantarotto D, Singh R, McCarthy D, Erhardt M, Briand JP, Prato M, et al. (2004). Functionalized carbon nanotubes for plasmid DNA gene delivery. *Angew Chem Int Ed Engl*, 43(39):5242–6. doi:[10.1002/anie.200460437](https://doi.org/10.1002/anie.200460437) PMID:[15455428](https://pubmed.ncbi.nlm.nih.gov/15455428/)
- Park EJ, Cho WS, Jeong J, Yi J, Choi K, Park K (2009). Pro-inflammatory and potential allergic responses resulting from B cell activation in mice treated with multi-walled carbon nanotubes by intratracheal instillation. *Toxicology*, 259(3):113–21. doi:[10.1016/j.tox.2009.02.009](https://doi.org/10.1016/j.tox.2009.02.009) PMID:[19428951](https://pubmed.ncbi.nlm.nih.gov/19428951/)
- Park EJ, Roh J, Kim SN, Kang MS, Han YA, Kim Y, et al. (2011b). A single intratracheal instillation of single-walled carbon nanotubes induced early lung fibrosis and subchronic tissue damage in mice. *Arch Toxicol*, 85(9):1121–31. doi:[10.1007/s00204-011-0655-8](https://doi.org/10.1007/s00204-011-0655-8) PMID:[21472445](https://pubmed.ncbi.nlm.nih.gov/21472445/)
- Park EJ, Roh J, Kim SN, Kang MS, Lee BS, Kim Y, et al. (2011a). Biological toxicity and inflammatory response of semi-single-walled carbon nanotubes. *PLoS One*, 6(10):e25892. doi:[10.1371/journal.pone.0025892](https://doi.org/10.1371/journal.pone.0025892) PMID:[22016783](https://pubmed.ncbi.nlm.nih.gov/22016783/)
- Park EJ, Roh J, Kim SN, Kim Y, Han SB, Hong JT (2013). CCR5 plays an important role in resolving an inflammatory response to single-walled carbon nanotubes. *J Appl Toxicol*, 33(8):845–53. doi:[10.1002/jat.2744](https://doi.org/10.1002/jat.2744) PMID:[22438032](https://pubmed.ncbi.nlm.nih.gov/22438032/)
- Patel HJ, Kwon S (2013). Length-dependent effect of single-walled carbon nanotube exposure in a dynamic cell growth environment of human alveolar epithelial cells. *J Expo Sci Environ Epidemiol*, 23(1):101–8. doi:[10.1038/jes.2012.75](https://doi.org/10.1038/jes.2012.75) PMID:[22854519](https://pubmed.ncbi.nlm.nih.gov/22854519/)
- Patlolla AK, Hussain SM, Schlager JJ, Patlolla S, Tchounwou PB (2010). Comparative study of the clastogenicity of functionalized and nonfunctionalized

- multiwalled carbon nanotubes in bone marrow cells of Swiss-Webster mice. *Environ Toxicol*, 25(6):608–21. doi:[10.1002/tox.20621](https://doi.org/10.1002/tox.20621) PMID:[20549644](https://pubmed.ncbi.nlm.nih.gov/20549644/)
- Pauluhn J (2010a). Multi-walled carbon nanotubes (Baytubes): approach for derivation of occupational exposure limit. *Regul Toxicol Pharmacol*, 57(1):78–89. doi:[10.1016/j.yrtph.2009.12.012](https://doi.org/10.1016/j.yrtph.2009.12.012) PMID:[20074606](https://pubmed.ncbi.nlm.nih.gov/20074606/)
- Pauluhn J (2010b). Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: toxic effects are determined by density of agglomerate structures, not fibrillar structures. *Toxicol Sci*, 113(1):226–42. doi:[10.1093/toxsci/kfp247](https://doi.org/10.1093/toxsci/kfp247) PMID:[19822600](https://pubmed.ncbi.nlm.nih.gov/19822600/)
- Pelka J, Gehrke H, Rechel A, Kappes M, Hennrich F, Hartinger CG, et al. (2013). DNA damaging properties of single walled carbon nanotubes in human colon carcinoma cells. *Nanotoxicology*, 7(1):2–20. doi:[10.3109/17435390.2011.626536](https://doi.org/10.3109/17435390.2011.626536) PMID:[22007624](https://pubmed.ncbi.nlm.nih.gov/22007624/)
- Petersen EJ, Zhang L, Mattison NT, O'Carroll DM, Whelton AJ, Uddin N, et al. (2011). Potential release pathways, environmental fate, and ecological risks of carbon nanotubes. *Environ Sci Technol*, 45(23):9837–56. doi:[10.1021/es201579y](https://doi.org/10.1021/es201579y) PMID:[21988187](https://pubmed.ncbi.nlm.nih.gov/21988187/)
- Pfuhler S, Elespuru R, Aardema MJ, Doak SH, Maria Donner E, Honma M, et al. (2013). Genotoxicity of nanomaterials: refining strategies and tests for hazard identification. *Environ Mol Mutagen*, 54(4):229–39. doi:[10.1002/em.21770](https://doi.org/10.1002/em.21770) PMID:[23519787](https://pubmed.ncbi.nlm.nih.gov/23519787/)
- Piccinno F, Gottschalk F, Seeger S, Nowack B (2012). Industrial production quantities and uses of ten engineered nanomaterials in Europe and in the world. *J Nanopart Res*, 14(9):1109. doi:[10.1007/s11051-012-1109-9](https://doi.org/10.1007/s11051-012-1109-9)
- Pichardo S, Gutiérrez-Praena D, Puerto M, Sánchez E, Grilo A, Cameán AM, et al. (2012). Oxidative stress responses to carboxylic acid functionalized single wall carbon nanotubes on the human intestinal cell line Caco-2. *Toxicol In Vitro*, 26(5):672–7. doi:[10.1016/j.tiv.2012.03.007](https://doi.org/10.1016/j.tiv.2012.03.007) PMID:[22449549](https://pubmed.ncbi.nlm.nih.gov/22449549/)
- Pietroiusti A, Massimiani M, Fenoglio I, Colonna M, Valentini F, Palleschi G, et al. (2011). Low doses of pristine and oxidized single-wall carbon nanotubes affect mammalian embryonic development. *ACS Nano*, 5(6):4624–33. doi:[10.1021/nn200372g](https://doi.org/10.1021/nn200372g) PMID:[21615177](https://pubmed.ncbi.nlm.nih.gov/21615177/)
- Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WAH, Seaton A, et al. (2008). Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nanotechnol*, 3(7):423–8. doi:[10.1038/nnano.2008.111](https://doi.org/10.1038/nnano.2008.111) PMID:[18654567](https://pubmed.ncbi.nlm.nih.gov/18654567/)
- Pop E, Mann D, Wang Q, Goodson K, Dai H (2006). Thermal conductance of an individual single-wall carbon nanotube above room temperature. *Nano Lett*, 6(1):96–100. doi:[10.1021/nl052145f](https://doi.org/10.1021/nl052145f) PMID:[16402794](https://pubmed.ncbi.nlm.nih.gov/16402794/)
- Popov VN (2004). Carbon nanotubes: properties and applications. *Mater Sci Eng Rep*, 43(3):61–102. doi:[10.1016/j.msere.2003.10.001](https://doi.org/10.1016/j.msere.2003.10.001)
- Porter DW, Hubbs AF, Chen BT, McKinney W, Mercer RR, Wolfarth MG, et al. (2013). Acute pulmonary dose-responses to inhaled multi-walled carbon nanotubes. *Nanotoxicology*, 7(7):1179–94. doi:[10.3109/17435390.2012.719649](https://doi.org/10.3109/17435390.2012.719649) PMID:[22881873](https://pubmed.ncbi.nlm.nih.gov/22881873/)
- Porter DW, Hubbs AF, Mercer RR, Wu N, Wolfarth MG, Sriram K, et al. (2010). Mouse pulmonary dose- and time course-responses induced by exposure to multi-walled carbon nanotubes. *Toxicology*, 269(2–3):136–47. doi:[10.1016/j.tox.2009.10.017](https://doi.org/10.1016/j.tox.2009.10.017) PMID:[19857541](https://pubmed.ncbi.nlm.nih.gov/19857541/)
- Prencipe G, Tabakman SM, Welscher K, Liu Z, Goodwin AP, Zhang L, et al. (2009). PEG branched polymer for functionalization of nanomaterials with ultralong blood circulation. *J Am Chem Soc*, 131(13):4783–7. doi:[10.1021/ja809086q](https://doi.org/10.1021/ja809086q) PMID:[19173646](https://pubmed.ncbi.nlm.nih.gov/19173646/)
- Pulskamp K, Diabaté S, Krug HF (2007). Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicol Lett*, 168(1):58–74. doi:[10.1016/j.toxlet.2006.11.001](https://doi.org/10.1016/j.toxlet.2006.11.001) PMID:[17141434](https://pubmed.ncbi.nlm.nih.gov/17141434/)
- Raabe OG, Al-Bayati MA, Teague SV, Rasolt A (1988). Regional deposition of inhaled monodisperse coarse and fine aerosol particles in small laboratory animals. *Ann Occup Hyg*, 32: inhaled particles VI: 53–63. doi:[10.1093/annhyg/32.inhaled\\_particles\\_VI.53](https://doi.org/10.1093/annhyg/32.inhaled_particles_VI.53)
- Rama Narsimha Reddy A, Narsimha Reddy Y, Himabindu V, Rama Krishna D (2011). Induction of oxidative stress and cytotoxicity by carbon nanomaterials is dependent on physical properties. *Toxicol Ind Health*, 27(1):3–10. doi:[10.1177/0748233710377780](https://doi.org/10.1177/0748233710377780) PMID:[20639279](https://pubmed.ncbi.nlm.nih.gov/20639279/)
- Ravichandran P, Baluchamy S, Gopikrishnan R, Biradar S, Ramesh V, Goornavar V, et al. (2011). Pulmonary biocompatibility assessment of inhaled single-wall and multiwall carbon nanotubes in BALB/c mice. *J Biol Chem*, 286(34):29725–33. doi:[10.1074/jbc.M111.251884](https://doi.org/10.1074/jbc.M111.251884) PMID:[21705330](https://pubmed.ncbi.nlm.nih.gov/21705330/)
- Ravichandran P, Baluchamy S, Sadanandan B, Gopikrishnan R, Biradar S, Ramesh V, et al. (2010). Multiwalled carbon nanotubes activate NF- $\kappa$ B and AP-1 signalling pathways to induce apoptosis in rat lung epithelial cells. *Apoptosis*, 15(12):1507–16. doi:[10.1007/s10495-010-0532-6](https://doi.org/10.1007/s10495-010-0532-6) PMID:[20694747](https://pubmed.ncbi.nlm.nih.gov/20694747/)
- Reddy AR, Rao MV, Krishna DR, Himabindu V, Reddy YN (2011). Evaluation of oxidative stress and anti-oxidant status in rat serum following exposure of carbon nanotubes. *Regul Toxicol Pharmacol*, 59(2):251–7. doi:[10.1016/j.yrtph.2010.10.007](https://doi.org/10.1016/j.yrtph.2010.10.007) PMID:[20955749](https://pubmed.ncbi.nlm.nih.gov/20955749/)
- Reddy AR, Reddy YN, Krishna DR, Himabindu V (2010b). Multi wall carbon nanotubes induce oxidative stress and cytotoxicity in human embryonic kidney (HEK293) cells. *Toxicology*, 272(1–3):11–6. doi:[10.1016/j.tox.2010.03.017](https://doi.org/10.1016/j.tox.2010.03.017) PMID:[20371264](https://pubmed.ncbi.nlm.nih.gov/20371264/)
- Reddy ARN, Krishna DR, Reddy YN, Himabindu V (2010a). Translocation and extra pulmonary toxicities of multi wall carbon nanotubes in rats. *Toxicol Mech*

- Methods*, 20(5):267–72. doi:[10.3109/15376516.2010.484077](https://doi.org/10.3109/15376516.2010.484077) PMID:[20482408](https://pubmed.ncbi.nlm.nih.gov/20482408/)
- Rinzler AG, Liu J, Dai H, Nikolaev P, Huffman CB, Rodriguez-Macias FJ, et al. (1998). Large-scale purification of single-wall carbon nanotubes: process, product, and characterization. *Appl Phys, A Mater Sci Process*, 67(1):29–37. doi:[10.1007/s003390050734](https://doi.org/10.1007/s003390050734)
- Roda E, Coccini T, Acerbi D, Barni S, Vaccarone R, Manzo L (2011). Comparative pulmonary toxicity assessment of pristine and functionalized multi-walled carbon nanotubes intratracheally instilled in rats: morphohistochemical evaluations. *Histol Histopathol*, 26(3):357–67. PMID:[21210349](https://pubmed.ncbi.nlm.nih.gov/21210349/)
- Romero G, Rojas E, Estrela-Lopis I, Donath E, Moya SE (2011). Spontaneous confocal Raman microscopy—a tool to study the uptake of nanoparticles and carbon nanotubes into cells. *Nanoscale Res Lett*, 6(1):429. doi:[10.1186/1556-276X-6-429](https://doi.org/10.1186/1556-276X-6-429) PMID:[21711493](https://pubmed.ncbi.nlm.nih.gov/21711493/)
- Ronzani C, Casset A, Pons F (2014). Exposure to multi-walled carbon nanotubes results in aggravation of airway inflammation and remodeling and in increased production of epithelium-derived innate cytokines in a mouse model of asthma. *Arch Toxicol*, 88(2):489–99. doi:[10.1007/s00204-013-1116-3](https://doi.org/10.1007/s00204-013-1116-3) PMID:[23948970](https://pubmed.ncbi.nlm.nih.gov/23948970/)
- Ruoff RS, Thersoff J, Lorents DC, Subramoney S, Chan B (1993). Radial deformation of carbon nanotubes by van-der-Waals forces. *Nature*, 364(6437):514–6. doi:[10.1038/364514a0](https://doi.org/10.1038/364514a0)
- Ryman-Rasmussen JP, Cesta MF, Brody AR, Shipley-Phillips JK, Everitt JI, Tewksbury EW, et al. (2009a). Inhaled carbon nanotubes reach the subpleural tissue in mice. *Nat Nanotechnol*, 4(11):747–51. doi:[10.1038/nnano.2009.305](https://doi.org/10.1038/nnano.2009.305) PMID:[19893520](https://pubmed.ncbi.nlm.nih.gov/19893520/)
- Ryman-Rasmussen JP, Tewksbury EW, Moss OR, Cesta MF, Wong BA, Bonner JC (2009b). Inhaled multiwalled carbon nanotubes potentiate airway fibrosis in murine allergic asthma. *Am J Respir Cell Mol Biol*, 40(3):349–58. doi:[10.1165/rcmb.2008-0276OC](https://doi.org/10.1165/rcmb.2008-0276OC) PMID:[18787175](https://pubmed.ncbi.nlm.nih.gov/18787175/)
- Safe Work Australia (2010). Human health hazard assessment and classification of carbon nanotubes. Canberra, Australia: Safe Work Australia.
- Sager TM, Wolfarth MW, Andrew M, Hubbs A, Friend S, Chen TH, et al. (2014). Effect of multi-walled carbon nanotube surface modification on bioactivity in the C57BL/6 mouse model. *Nanotoxicology*, 8(3):317–27. doi:[10.3109/17435390.2013.779757](https://doi.org/10.3109/17435390.2013.779757) PMID:[23432020](https://pubmed.ncbi.nlm.nih.gov/23432020/)
- Sager TM, Wolfarth MW, Battelli LA, Leonard SS, Andrew M, Steinbach T, et al. (2013). Investigation of the pulmonary bioactivity of double-walled carbon nanotubes. *J Toxicol Environ Health A*, 76(15):922–36. doi:[10.1080/15287394.2013.825571](https://doi.org/10.1080/15287394.2013.825571) PMID:[24156695](https://pubmed.ncbi.nlm.nih.gov/24156695/)
- Saito S, Zettl A (2008). Carbon nanotubes: Quantum cylinders and graphene. In: Burstein E, Cohen ML, Mills DL, Stiles PJ editors. Series: Contemporary concepts of condensed matter science. Volume 3: Oxford, United Kingdom: Elsevier; pp. 1–215.
- Sakamoto Y, Nakae D, Fukumori N, Tayama K, Maekawa A, Imai K, et al. (2009). Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. *J Toxicol Sci*, 34(1):65–76. doi:[10.2131/jts.34.65](https://doi.org/10.2131/jts.34.65) PMID:[19182436](https://pubmed.ncbi.nlm.nih.gov/19182436/)
- Sanchez VC, Pietruska JR, Miselis NR, Hurt RH, Kane AB (2009). Biopersistence and potential adverse health impacts of fibrous nanomaterials: what have we learned from asbestos? *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 1(5):511–29. doi:[10.1002/wnan.41](https://doi.org/10.1002/wnan.41) PMID:[20049814](https://pubmed.ncbi.nlm.nih.gov/20049814/)
- Sanchez VC, Weston P, Yan A, Hurt RH, Kane AB (2011). A 3-dimensional in vitro model of epithelioid granulomas induced by high aspect ratio nanomaterials. *Part Fibre Toxicol*, 8(1):17. doi:[10.1186/1743-8977-8-17](https://doi.org/10.1186/1743-8977-8-17) PMID:[21592387](https://pubmed.ncbi.nlm.nih.gov/21592387/)
- Sargent LM, Hubbs AF, Young SH, Kashon ML, Dinu CZ, Salisbury JL, et al. (2012a). Single-walled carbon nanotube-induced mitotic disruption. *Mutat Res*, 745(1–2):28–37. doi:[10.1016/j.mrgentox.2011.11.017](https://doi.org/10.1016/j.mrgentox.2011.11.017) PMID:[22178868](https://pubmed.ncbi.nlm.nih.gov/22178868/)
- Sargent LM, Porter DW, Staska LM, Hubbs AF, Lowry DT, Battelli L, et al. (2014). Promotion of lung adenocarcinoma following inhalation exposure to multi-walled carbon nanotubes. *Part Fibre Toxicol*, 11(1):3 doi:[10.1186/1743-8977-11-3](https://doi.org/10.1186/1743-8977-11-3) PMID:[24405760](https://pubmed.ncbi.nlm.nih.gov/24405760/)
- Sargent LM, Reynolds SH, Lowry D, Kashon ML, Benkovic SA, Salisbury JL, et al. (2012b). Abstract 5464. Genotoxicity of multi-walled carbon nanotubes at occupationally relevant doses *Cancer Res*, 72(8):Suppl 1. doi:[10.1158/1538-7445.AM2012-5464](https://doi.org/10.1158/1538-7445.AM2012-5464)
- Sargent LM, Shvedova AA, Hubbs AF, Salisbury JL, Benkovic SA, Kashon ML, et al. (2009). Induction of aneuploidy by single-walled carbon nanotubes. *Environ Mol Mutagen*, 50(8):708–17. doi:[10.1002/em.20529](https://doi.org/10.1002/em.20529) PMID:[19774611](https://pubmed.ncbi.nlm.nih.gov/19774611/)
- Sarkar S, Sharma C, Yog R, Periakaruppan A, Jejelowo O, Thomas R, et al. (2007). Analysis of stress responsive genes induced by single-walled carbon nanotubes in BJ Foreskin cells. *J Nanosci Nanotechnol*, 7(2):584–92. PMID:[17450800](https://pubmed.ncbi.nlm.nih.gov/17450800/)
- Sato Y, Yokoyama A, Shibata K, Akimoto Y, Ogino S, Nodasaka Y, et al. (2005). Influence of length on cytotoxicity of multi-walled carbon nanotubes against human acute monocytic leukemia cell line THP-1 in vitro and subcutaneous tissue of rats in vivo. *Mol Biosyst*, 1(2):176–82. doi:[10.1039/b502429c](https://doi.org/10.1039/b502429c) PMID:[16880981](https://pubmed.ncbi.nlm.nih.gov/16880981/)
- Sayers BC, Taylor AJ, Glista-Baker EE, Shipley-Phillips JK, Dackor RT, Edin ML, et al. (2013). Role of cyclooxygenase-2 in exacerbation of allergen-induced airway remodeling by multiwalled carbon nanotubes. *Am J Respir Cell Mol Biol*, 49(4):525–35. doi:[10.1165/rcmb.2013-0019OC](https://doi.org/10.1165/rcmb.2013-0019OC) PMID:[23642096](https://pubmed.ncbi.nlm.nih.gov/23642096/)
- Schinwald A, Murphy FA, Prina-Mello A, Poland CA, Byrne F, Movia D, et al. (2012). The threshold length for fiber-induced acute pleural inflammation: shedding

- light on the early events in asbestos-induced mesothelioma. *Toxicol Sci*, 128(2):461–70. doi:[10.1093/toxsci/kfs171](https://doi.org/10.1093/toxsci/kfs171) PMID:[22584686](https://pubmed.ncbi.nlm.nih.gov/22584686/)
- Schlesinger RB (1995). Interaction of gaseous and particulate pollutants in the respiratory tract: mechanisms and modulators. *Toxicology*, 105(2–3):315–25. doi:[10.1016/0300-483X\(95\)03228-8](https://doi.org/10.1016/0300-483X(95)03228-8) PMID:[8571368](https://pubmed.ncbi.nlm.nih.gov/8571368/)
- Schneider T, Jansson A, Alstrup JK, Kristjanson V, Luotamo M, Nygren O, et al. (2007). Evaluation and control of occupational health risks from nanoparticles. TemaNord: 581. Copenhagen, Denmark: Nordic Council of Ministers. 10.6027/tn2007-581 doi:[10.6027/tn2007-581](https://doi.org/10.6027/tn2007-581)
- Sharma CS, Sarkar S, Periyakaruppan A, Barr J, Wise K, Thomas R, et al. (2007). Single-walled carbon nanotubes induces oxidative stress in rat lung epithelial cells. *J Nanosci Nanotechnol*, 7(7):2466–72. doi:[10.1166/jnn.2007.431](https://doi.org/10.1166/jnn.2007.431) PMID:[17663266](https://pubmed.ncbi.nlm.nih.gov/17663266/)
- Shimizu K, Uchiyama A, Yamashita M, Hirose A, Nishimura T, Oku N (2013). Biomembrane damage caused by exposure to multi-walled carbon nanotubes. *J Toxicol Sci*, 38(1):7–12. doi:[10.2131/jts.38.7](https://doi.org/10.2131/jts.38.7) PMID:[23358135](https://pubmed.ncbi.nlm.nih.gov/23358135/)
- Shvedova AA, Castranova V, Kisin ER, Schwegler-Berry D, Murray AR, Gandelsman VZ, et al. (2003). Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. *J Toxicol Environ Health A*, 66(20):1909–26. doi:[10.1080/713853956](https://doi.org/10.1080/713853956) PMID:[14514433](https://pubmed.ncbi.nlm.nih.gov/14514433/)
- Shvedova AA, Kapralov AA, Feng WH, Kisin ER, Murray AR, Mercer RR, et al. (2012a). Impaired clearance and enhanced pulmonary inflammatory/fibrotic response to carbon nanotubes in myeloperoxidase-deficient mice. *PLoS One*, 7(3):e30923. doi:[10.1371/journal.pone.0030923](https://doi.org/10.1371/journal.pone.0030923) PMID:[22479306](https://pubmed.ncbi.nlm.nih.gov/22479306/)
- Shvedova AA, Kisin E, Murray AR, Johnson VJ, Gorelik O, Arepalli S, et al. (2008). Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis. *Am J Physiol Lung Cell Mol Physiol*, 295(4):L552–65. doi:[10.1152/ajplung.90287.2008](https://doi.org/10.1152/ajplung.90287.2008) PMID:[18658273](https://pubmed.ncbi.nlm.nih.gov/18658273/)
- Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, et al. (2005). Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *Am J Physiol Lung Cell Mol Physiol*, 289(5):L698–708. doi:[10.1152/ajplung.00084.2005](https://doi.org/10.1152/ajplung.00084.2005) PMID:[15951334](https://pubmed.ncbi.nlm.nih.gov/15951334/)
- Shvedova AA, Kisin ER, Murray AR, Gorelik O, Arepalli S, Castranova V, et al. (2007). Vitamin E deficiency enhances pulmonary inflammatory response and oxidative stress induced by single-walled carbon nanotubes in C57BL/6 mice. *Toxicol Appl Pharmacol*, 221(3):339–48. doi:[10.1016/j.taap.2007.03.018](https://doi.org/10.1016/j.taap.2007.03.018) PMID:[17482224](https://pubmed.ncbi.nlm.nih.gov/17482224/)
- Shvedova AA, Pietrojusti A, Fadeel B, Kagan VE (2012b). Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. *Toxicol Appl Pharmacol*, 261(2):121–33. doi:[10.1016/j.taap.2012.03.023](https://doi.org/10.1016/j.taap.2012.03.023) PMID:[22513272](https://pubmed.ncbi.nlm.nih.gov/22513272/)
- Shvedova AA, Yanamala N, Kisin ER, Tkach AV, Murray AR, Hubbs A, et al. (2014). Long-term effects of carbon containing engineered nanomaterials and asbestos in the lung: one year postexposure comparisons. *Am J Physiol Lung Cell Mol Physiol*, 306(2):L170–82. doi:[10.1152/ajplung.00167.2013](https://doi.org/10.1152/ajplung.00167.2013) PMID:[24213921](https://pubmed.ncbi.nlm.nih.gov/24213921/)
- Siegrist KJ, Reynolds SH, Kashon ML, Lowry DT, Dong C, Hubbs AF, et al. (2014). Genotoxicity of multi-walled carbon nanotubes at occupationally relevant doses. *Part Fibre Toxicol*, 11(6):6. doi:[10.1186/1743-8977-11-6](https://doi.org/10.1186/1743-8977-11-6) PMID:[24479647](https://pubmed.ncbi.nlm.nih.gov/24479647/)
- Silva RM, Doudrick K, Franzi LM, TeeSy C, Anderson DS, Wu Z, et al. (2014). Instillation versus inhalation of multiwalled carbon nanotubes: exposure-related health effects, clearance, and the role of particle characteristics. *ACS Nano*, 8(9):8911–31. doi:[10.1021/nn503887r](https://doi.org/10.1021/nn503887r) PMID:[25144856](https://pubmed.ncbi.nlm.nih.gov/25144856/)
- Singh N, Manshian B, Jenkins GJ, Griffiths SM, Williams PM, Maffei TG, et al. (2009). NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. *Biomaterials*, 30(23–24):3891–914. doi:[10.1016/j.biomaterials.2009.04.009](https://doi.org/10.1016/j.biomaterials.2009.04.009) PMID:[19427031](https://pubmed.ncbi.nlm.nih.gov/19427031/)
- Singh R, Pantarotto D, Lacerda L, Pastorin G, Klumpp C, Prato M, et al. (2006). Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. *Proc Natl Acad Sci USA*, 103(9):3357–62. doi:[10.1073/pnas.0509009103](https://doi.org/10.1073/pnas.0509009103) PMID:[16492781](https://pubmed.ncbi.nlm.nih.gov/16492781/)
- Sinha S, Barjami S, Iannacchione G, Schwab A, Muench G (2005). Off-axis thermal properties of carbon nanotube films. *J Nanopart Res*, 7(6):651–7. doi:[10.1007/s11051-005-8382-9](https://doi.org/10.1007/s11051-005-8382-9)
- Sinnott SB (2002). Chemical functionalization of carbon nanotubes. *J Nanosci Nanotechnol*, 2(2):113–23. doi:[10.1166/jnn.2002.107](https://doi.org/10.1166/jnn.2002.107) PMID:[12908295](https://pubmed.ncbi.nlm.nih.gov/12908295/)
- Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, et al. (2004). Dose-dependent transitions in mechanisms of toxicity: case studies. *Toxicol Appl Pharmacol*, 201(3):226–94. doi:[10.1016/j.taap.2004.06.027](https://doi.org/10.1016/j.taap.2004.06.027) PMID:[15582646](https://pubmed.ncbi.nlm.nih.gov/15582646/)
- Snipes MB (1989). Long-term retention and clearance of particles inhaled by mammalian species. *Crit Rev Toxicol*, 20(3):175–211. doi:[10.3109/10408448909017909](https://doi.org/10.3109/10408448909017909) PMID:[2692607](https://pubmed.ncbi.nlm.nih.gov/2692607/)
- Snyder-Talkington BN, Dymacek J, Porter DW, Wolfarth MG, Mercer RR, Pacurari M, et al. (2013a). System-based identification of toxicity pathways associated with multi-walled carbon nanotube-induced pathological responses. *Toxicol Appl Pharmacol*, 272(2):476–89. doi:[10.1016/j.taap.2013.06.026](https://doi.org/10.1016/j.taap.2013.06.026) PMID:[23845593](https://pubmed.ncbi.nlm.nih.gov/23845593/)
- Snyder-Talkington BN, Pacurari M, Dong C, Leonard SS, Schwegler-Berry D, Castranova V, et al. (2013b). Systematic analysis of multiwalled carbon nanotube-induced cellular signalling and gene expression in human

- small airway epithelial cells. *Toxicol Sci*, 133(1):79–89. doi:[10.1093/toxsci/kft019](https://doi.org/10.1093/toxsci/kft019) PMID:[23377615](https://pubmed.ncbi.nlm.nih.gov/23377615/)
- Snyder-Talkington BN, Qian Y, Castranova V, Guo NL (2012). New perspectives for in vitro risk assessment of multiwalled carbon nanotubes: application of coculture and bioinformatics. *J Toxicol Environ Health B Crit Rev*, 15(7):468–92. doi:[10.1080/10937404.2012.736856](https://doi.org/10.1080/10937404.2012.736856) PMID:[23190270](https://pubmed.ncbi.nlm.nih.gov/23190270/)
- Søs Poulsen S, Jacobsen NR, Labib S, Wu D, Husain M, Williams A, et al. (2013). Transcriptomic analysis reveals novel mechanistic insight into murine biological responses to multi-walled carbon nanotubes in lungs and cultured lung epithelial cells. *PLoS One*, 8(11):e80452. doi:[10.1371/journal.pone.0080452](https://doi.org/10.1371/journal.pone.0080452) PMID:[24260392](https://pubmed.ncbi.nlm.nih.gov/24260392/)
- Srivastava RK, Pant AB, Kashyap MP, Kumar V, Lohani M, Jonas L, et al. (2011). Multi-walled carbon nanotubes induce oxidative stress and apoptosis in human lung cancer cell line-A549. *Nanotoxicology*, 5(2):195–207. doi:[10.3109/17435390.2010.503944](https://doi.org/10.3109/17435390.2010.503944) PMID:[20804439](https://pubmed.ncbi.nlm.nih.gov/20804439/)
- Stankovich S, Dikin DA, Piner RD, Kohlhaas KA, Kleinhammes A, Jia Y, et al. (2007). Wu y, Nguyen ST, Ruoff RS. Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide. *Carbon*, 45(7):1558–65. doi:[10.1016/j.carbon.2007.02.034](https://doi.org/10.1016/j.carbon.2007.02.034)
- Stone V, Johnston H, Schins RPF (2009). Development of in vitro systems for nanotoxicology: methodological considerations. *Crit Rev Toxicol*, 39(7):613–26. doi:[10.1080/10408440903120975](https://doi.org/10.1080/10408440903120975) PMID:[19650720](https://pubmed.ncbi.nlm.nih.gov/19650720/)
- Sun YP, Fu K, Lin Y, Huang W (2002). Functionalized carbon nanotubes: properties and applications. *Acc Chem Res*, 35(12):1096–104. doi:[10.1021/ar010160v](https://doi.org/10.1021/ar010160v) PMID:[12484798](https://pubmed.ncbi.nlm.nih.gov/12484798/)
- Sun Z, Liu Z, Meng J, Meng J, Duan J, Xie S, et al. (2011). Carbon nanotubes enhance cytotoxicity mediated by human lymphocytes in vitro. *PLoS One*, 6(6):e21073. doi:[10.1371/journal.pone.0021073](https://doi.org/10.1371/journal.pone.0021073) PMID:[21731651](https://pubmed.ncbi.nlm.nih.gov/21731651/)
- Szendi K, Varga C (2008). Lack of genotoxicity of carbon nanotubes in a pilot study. *Anticancer Res*, 28:1A:349–52. PMID:[18383868](https://pubmed.ncbi.nlm.nih.gov/18383868/)
- Takagi A, Hirose A, Futakuchi M, Tsuda H, Kanno J (2012). Dose-dependent mesothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice. *Cancer Sci*, 103(8):1440–4. doi:[10.1111/j.1349-7006.2012.02318.x](https://doi.org/10.1111/j.1349-7006.2012.02318.x) PMID:[22537085](https://pubmed.ncbi.nlm.nih.gov/22537085/)
- Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, et al. (2008). Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. *J Toxicol Sci*, 33(1):105–16. doi:[10.2131/jts.33.105](https://doi.org/10.2131/jts.33.105) PMID:[18303189](https://pubmed.ncbi.nlm.nih.gov/18303189/)
- Takanashi S, Hara K, Aoki K, Usui Y, Shimizu M, Haniu H, et al. (2012). Carcinogenicity evaluation for the application of carbon nanotubes as biomaterials in rasH2 mice. *Sci Rep*, 2:498. doi:[10.1038/srep00498](https://doi.org/10.1038/srep00498) PMID:[22787556](https://pubmed.ncbi.nlm.nih.gov/22787556/)
- Takaya M, Ono-Ogasawara M, Shinohara Y, Kubota H, Tsuruoka S, Koda S (2012). Evaluation of exposure risk in the weaving process of MWCNT-coated yarn with real-time particle concentration measurements and characterization of dust particles. *Ind Health*, 50(2):147–55. doi:[10.2486/indhealth.MS1312](https://doi.org/10.2486/indhealth.MS1312) PMID:[22293727](https://pubmed.ncbi.nlm.nih.gov/22293727/)
- Tavares AM, Louro H, Antunes S, Quarré S, Simar S, De Temmerman PJ, et al. (2014). Genotoxicity evaluation of nanosized titanium dioxide, synthetic amorphous silica and multi-walled carbon nanotubes in human lymphocytes. *Toxicol In Vitro*, 28(1):60–9. doi:[10.1016/j.tiv.2013.06.009](https://doi.org/10.1016/j.tiv.2013.06.009) PMID:[23811260](https://pubmed.ncbi.nlm.nih.gov/23811260/)
- Thomas T, Bahadori T, Savage N, Thomas K (2009). Moving toward exposure and risk evaluation of nanomaterials: challenges and future directions. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 1(4):426–33. doi:[10.1002/wnan.34](https://doi.org/10.1002/wnan.34) PMID:[20049808](https://pubmed.ncbi.nlm.nih.gov/20049808/)
- Thostenson ET, Li CY, Chou TW (2005). Nanocomposites in context. *Compos Sci Technol*, 65(3–4):491–516. doi:[10.1016/j.compscitech.2004.11.003](https://doi.org/10.1016/j.compscitech.2004.11.003)
- Thostenson ET, Ren ZF, Chou TW (2001). Advances in the science and technology of carbon nanotubes and human fibroblasts and their composites: a review. *Compos Sci Technol*, 61:1899–912. doi:[10.1016/S0266-3538\(01\)00094-X](https://doi.org/10.1016/S0266-3538(01)00094-X)
- Thurnherr T, Brandenberger C, Fischer K, Diener L, Manser P, Maeder-Althaus X, et al. (2011). A comparison of acute and long-term effects of industrial multi-walled carbon nanotubes on human lung and immune cells in vitro. *Toxicol Lett*, 200(3):176–86. doi:[10.1016/j.toxlet.2010.11.012](https://doi.org/10.1016/j.toxlet.2010.11.012) PMID:[21112381](https://pubmed.ncbi.nlm.nih.gov/21112381/)
- Tilton SC, Karin NJ, Tolic A, Xie Y, Lai X, Hamilton RF Jr, et al. (2014). Three human cell types respond to multi-walled carbon nanotubes and titanium dioxide nanobelts with cell-specific transcriptomic and proteomic expression patterns. *Nanotoxicology*, 8(5):533–48. doi:[10.3109/17435390.2013.803624](https://doi.org/10.3109/17435390.2013.803624) PMID:[23659652](https://pubmed.ncbi.nlm.nih.gov/23659652/)
- Tkach AV, Shurin GV, Shurin MR, Kisin ER, Murray AR, Young SH, et al. (2011). Direct effects of carbon nanotubes on dendritic cells induce immune suppression upon pulmonary exposure. *ACS Nano*, 5(7):5755–62. doi:[10.1021/nn2014479](https://doi.org/10.1021/nn2014479) PMID:[21657201](https://pubmed.ncbi.nlm.nih.gov/21657201/)
- Tournebize J, Sapin-Minet A, Bartosz G, Leroy P, Boudier A (2013). Pitfalls of assays devoted to evaluation of oxidative stress induced by inorganic nanoparticles. *Talanta*, 116:753–63. doi:[10.1016/j.talanta.2013.07.077](https://doi.org/10.1016/j.talanta.2013.07.077) PMID:[24148470](https://pubmed.ncbi.nlm.nih.gov/24148470/)
- Treumann S, Ma-Hock L, Gröters S, Landsiedel R, van Ravenzwaay B (2013). Additional histopathologic examination of the lungs from a 3-month inhalation toxicity study with multiwall carbon nanotubes in rats. *Toxicol Sci*, 134(1):103–10. doi:[10.1093/toxsci/kft089](https://doi.org/10.1093/toxsci/kft089) PMID:[23570993](https://pubmed.ncbi.nlm.nih.gov/23570993/)
- Tsai SJ, Hofmann M, Hallock M, Ada E, Kong J, Ellenbecker M (2009). Characterization and evaluation of nanoparticle release during the synthesis of single-walled

- and multiwalled carbon nanotubes by chemical vapor deposition. *Environ Sci Technol*, 43(15):6017–23. doi:[10.1021/es900486y](https://doi.org/10.1021/es900486y) PMID:[19731712](https://pubmed.ncbi.nlm.nih.gov/19731712/)
- Tsukahara T, Haniu H (2011). Cellular cytotoxic response induced by highly purified multi-wall carbon nanotube in human lung cells. *Mol Cell Biochem*, 352(1–2):57–63. doi:[10.1007/s11010-011-0739-z](https://doi.org/10.1007/s11010-011-0739-z) PMID:[21298324](https://pubmed.ncbi.nlm.nih.gov/21298324/)
- Val S, Hussain S, Boland S, Hamel R, Baeza-Squiban A, Marano F (2009). Carbon black and titanium dioxide nanoparticles induce pro-inflammatory responses in bronchial epithelial cells: need for multiparametric evaluation due to adsorption artifacts. *Inhal Toxicol*, 21(s1):Suppl 1: 115–22. doi:[10.1080/08958370902942533](https://doi.org/10.1080/08958370902942533) PMID:[19558243](https://pubmed.ncbi.nlm.nih.gov/19558243/)
- van Broekhuizen P, van Veelen W, Streekstra WH, Schulte P, Reijnders L (2012). Exposure limits for nanoparticles: report of an international workshop on nano reference values. *Ann Occup Hyg*, 56(5):515–24. PMID:[22752096](https://pubmed.ncbi.nlm.nih.gov/22752096/)
- Vankoningsloo S, Piret JP, Saout C, Noel F, Mejia J, Coquette A, et al. (2012). Pro-inflammatory effects of different MWCNTs dispersions in p16(INK4A)-deficient telomerase-expressing human keratinocytes but not in human SV-40 immortalized sebocytes. *Nanotoxicology*, 6(1):77–93. doi:[10.3109/17435390.2011.558642](https://doi.org/10.3109/17435390.2011.558642) PMID:[21352087](https://pubmed.ncbi.nlm.nih.gov/21352087/)
- Varga C, Szendi K (2010). Carbon nanotubes induce granulomas but not mesotheliomas. *In Vivo*, 24(2):153–6. PMID:[20363987](https://pubmed.ncbi.nlm.nih.gov/20363987/)
- Velasco-Santos C, Martinez-Hernandez AL, Consultchi A, Rodriguez R, Castano VM (2003). Naturally produced carbon nanotubes. *Chem Phys Lett*, 373(3–4):272–6. doi:[10.1016/S0009-2614\(03\)00615-8](https://doi.org/10.1016/S0009-2614(03)00615-8)
- Vesterdal LK, Danielsen PH, Folkmann JK, Jespersen LF, Aguilar-Pelaez K, Roursgaard M, et al. (2014b). Accumulation of lipids and oxidatively damaged DNA in hepatocytes exposed to particles. *Toxicol Appl Pharmacol*, 274(2):350–60. doi:[10.1016/j.taap.2013.10.001](https://doi.org/10.1016/j.taap.2013.10.001) PMID:[24121055](https://pubmed.ncbi.nlm.nih.gov/24121055/)
- Vesterdal LK, Jantzen K, Sheykhzade M, Roursgaard M, Folkmann JK, Loft S, et al. (2014a). Pulmonary exposure to particles from diesel exhaust, urban dust or single-walled carbon nanotubes and oxidatively damaged DNA and vascular function in apoE(–/–) mice. *Nanotoxicology*, 8(1):61–71. doi:[10.3109/17435390.0.2012.750385](https://doi.org/10.3109/17435390.0.2012.750385) PMID:[23148895](https://pubmed.ncbi.nlm.nih.gov/23148895/)
- Vittorio O, Raffa V, Cuschieri A (2009). Influence of purity and surface oxidation on cytotoxicity of multi-walled carbon nanotubes with human neuroblastoma cells. *Nanomedicine*, 5(4):424–31. doi:[10.1016/j.nano.2009.02.006](https://doi.org/10.1016/j.nano.2009.02.006) PMID:[19341817](https://pubmed.ncbi.nlm.nih.gov/19341817/)
- Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW (2013). Cancer genome landscapes. *Science*, 339(6127):1546–58. doi:[10.1126/science.1235122](https://doi.org/10.1126/science.1235122) PMID:[23539594](https://pubmed.ncbi.nlm.nih.gov/23539594/)
- Walters DA, Ericson LM, Casavant MJ, Liu J, Colbert DT, Smith KA, et al. (1999). m Smalley RE. Elastic strain of freely suspended single-wall carbon nanotube ropes. *Appl Phys Lett*, 74(25):3803–5. doi:[10.1063/1.124185](https://doi.org/10.1063/1.124185)
- Wan B, Wang ZX, Lv QY, Dong PX, Zhao LX, Yang Y, et al. (2013). Single-walled carbon nanotubes and graphene oxides induce autophagosomal accumulation and lysosome impairment in primarily cultured murine peritoneal macrophages. *Toxicol Lett*, 221(2):118–27. doi:[10.1016/j.toxlet.2013.06.208](https://doi.org/10.1016/j.toxlet.2013.06.208) PMID:[23769962](https://pubmed.ncbi.nlm.nih.gov/23769962/)
- Wang J, Sun P, Bao Y, Dou B, Song D, Li Y (2012b). Vitamin E renders protection to PC12 cells against oxidative damage and apoptosis induced by single-walled carbon nanotubes. *Toxicol In Vitro*, 26(1):32–41. doi:[10.1016/j.tiv.2011.10.004](https://doi.org/10.1016/j.tiv.2011.10.004) PMID:[22020378](https://pubmed.ncbi.nlm.nih.gov/22020378/)
- Wang J, Sun P, Bao Y, Liu J, An L (2011b). Cytotoxicity of single-walled carbon nanotubes on PC12 cells. *Toxicol In Vitro*, 25(1):242–50. doi:[10.1016/j.tiv.2010.11.010](https://doi.org/10.1016/j.tiv.2010.11.010) PMID:[21094249](https://pubmed.ncbi.nlm.nih.gov/21094249/)
- Wang L, Luanpitpong S, Castranova V, Tse W, Lu Y, Pongrakhananon V, et al. (2011a). Carbon nanotubes induce malignant transformation and tumorigenesis of human lung epithelial cells. *Nano Lett*, 11(7):2796–803. doi:[10.1021/nl2011214](https://doi.org/10.1021/nl2011214) PMID:[21657258](https://pubmed.ncbi.nlm.nih.gov/21657258/)
- Wang L, Stueckle TA, Mishra A, Derk R, Meighan T, Castranova V, et al. (2014). Neoplastic-like transformation effect of single-walled and multi-walled carbon nanotubes compared to asbestos on human lung small airway epithelial cells. *Nanotoxicology*, 8(5):485–507. doi:[10.3109/17435390.2013.801089](https://doi.org/10.3109/17435390.2013.801089) PMID:[23634900](https://pubmed.ncbi.nlm.nih.gov/23634900/)
- Wang P, Nie X, Wang Y, Li Y, Ge C, Zhang L, et al. (2013). Multiwall carbon nanotubes mediate macrophage activation and promote pulmonary fibrosis through TGF- $\beta$ /Smad signalling pathway. *Small*, 9(22):3799–811. doi:[10.1002/smll.201300607](https://doi.org/10.1002/smll.201300607) PMID:[23650105](https://pubmed.ncbi.nlm.nih.gov/23650105/)
- Wang R, Mikoryak C, Li S, Bushdiecker D 2nd, Musselman IH, Pantano P, et al. (2011c). Cytotoxicity screening of single-walled carbon nanotubes: detection and removal of cytotoxic contaminants from carboxylated carbon nanotubes. *Mol Pharm*, 8(4):1351–61. doi:[10.1021/mp2001439](https://doi.org/10.1021/mp2001439) PMID:[21688794](https://pubmed.ncbi.nlm.nih.gov/21688794/)
- Wang X, Jia G, Wang H, Nie H, Yan L, Deng XY, et al. (2009). Diameter effects on cytotoxicity of multi-walled carbon nanotubes. *J Nanosci Nanotechnol*, 9(5):3025–33. doi:[10.1166/jnn.2009.025](https://doi.org/10.1166/jnn.2009.025) PMID:[19452965](https://pubmed.ncbi.nlm.nih.gov/19452965/)
- Wang X, Xia T, Duch MC, Ji Z, Zhang H, Li R, et al. (2012a). Pluronic F108 coating decreases the lung fibrosis potential of multiwall carbon nanotubes by reducing lysosomal injury. *Nano Lett*, 12(6):3050–61. doi:[10.1021/nl300895y](https://doi.org/10.1021/nl300895y) PMID:[22546002](https://pubmed.ncbi.nlm.nih.gov/22546002/)
- Wang X, Xia T, Ntim SA, Ji Z, Lin S, Meng H, et al. (2011d). Dispersal state of multiwalled carbon nanotubes elicits profibrogenic cellular responses that correlate with fibrogenesis biomarkers and fibrosis in the murine lung. *ACS Nano*, 5(12):9772–87. doi:[10.1021/nn2033055](https://doi.org/10.1021/nn2033055) PMID:[22047207](https://pubmed.ncbi.nlm.nih.gov/22047207/)
- Warheit DB (2008). How meaningful are the results of nanotoxicity studies in the absence of adequate material

- characterization? *Toxicol Sci*, 101(2):183–5. doi:[10.1093/toxsci/kfm279](https://doi.org/10.1093/toxsci/kfm279) PMID:[18300382](https://pubmed.ncbi.nlm.nih.gov/18300382/)
- Warheit DB, George G, Hill LH, Snyderman R, Brody AR (1985). Inhaled asbestos activates a complement-dependent chemoattractant for macrophages. *Lab Invest*, 52(5):505–14. PMID:[3990243](https://pubmed.ncbi.nlm.nih.gov/3990243/)
- Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GAM, Webb TR (2004). Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol Sci*, 77(1):117–25. doi:[10.1093/toxsci/kfg228](https://doi.org/10.1093/toxsci/kfg228) PMID:[14514968](https://pubmed.ncbi.nlm.nih.gov/14514968/)
- Warheit DB, Overby LH, George G, Brody AR (1988). Pulmonary macrophages are attracted to inhaled particles through complement activation. *Exp Lung Res*, 14(1):51–66. doi:[10.3109/01902148809062850](https://doi.org/10.3109/01902148809062850) PMID:[2830106](https://pubmed.ncbi.nlm.nih.gov/2830106/)
- Watts PCP, Fearon PK, Hsu WK, Billingham NC, Kroto HW, Walton DRM (2003). Carbon nanotubes as polymer antioxidants. *J Mater Chem*, 13(3):491–5. doi:[10.1039/b211328g](https://doi.org/10.1039/b211328g)
- Wick P, Manser P, Limbach LK, Dettlaff-Weglikowska U, Krumeich F, Roth S, et al. (2007). The degree and kind of agglomeration affect carbon nanotube cytotoxicity. *Toxicol Lett*, 168(2):121–31. doi:[10.1016/j.toxlet.2006.08.019](https://doi.org/10.1016/j.toxlet.2006.08.019) PMID:[17169512](https://pubmed.ncbi.nlm.nih.gov/17169512/)
- Wiethoff AJ, Reed KL, Webb TR, Warheit DB (2003). Assessing the role of neutrophil apoptosis in the resolution of particle-induced pulmonary inflammation. *Inhal Toxicol*, 15(12):1231–46. doi:[10.1080/08958370390229898](https://doi.org/10.1080/08958370390229898) PMID:[14515224](https://pubmed.ncbi.nlm.nih.gov/14515224/)
- Wildoer JWG, Venema LC, Rinzler AG, Smalley RE, Decker C (1998). Electronic structure of atomically resolved carbon nanotubes. *Nature*, 391(6662):59–62. doi:[10.1038/34139](https://doi.org/10.1038/34139)
- Witasp E, Kagan V, Fadeel B (2008). Programmed cell clearance: molecular mechanisms and role in autoimmune disease, chronic inflammation, and anti-cancer immune responses. *Curr Immunol Rev*, 4(2):53–69. doi:[10.2174/157339508784325064](https://doi.org/10.2174/157339508784325064)
- Witasp E, Shvedova AA, Kagan VE, Fadeel B (2009). Single-walled carbon nanotubes impair human macrophage engulfment of apoptotic cell corpses. *Inhal Toxicol*, 21(s1):Suppl 1: 131–6. doi:[10.1080/08958370902942574](https://doi.org/10.1080/08958370902942574) PMID:[19558245](https://pubmed.ncbi.nlm.nih.gov/19558245/)
- Witzmann FA, Monteiro-Riviere NA (2006). Multi-walled carbon nanotube exposure alters protein expression in human keratinocytes. *Nanomedicine*, 2(3):158–68. [Lond Print] doi:[10.1016/j.nano.2006.07.005](https://doi.org/10.1016/j.nano.2006.07.005) PMID:[17292138](https://pubmed.ncbi.nlm.nih.gov/17292138/)
- Wohlleben W, Brill S, Meier MW, Mertler M, Cox G, Hirth S, et al. (2011). On the lifecycle of nanocomposites: comparing released fragments and their in-vivo hazards from three release mechanisms and four nanocomposites. *Small*, 7(16):2384–95. doi:[10.1002/smll.201002054](https://doi.org/10.1002/smll.201002054) PMID:[21671434](https://pubmed.ncbi.nlm.nih.gov/21671434/)
- Wörle-Knirsch JM, Pulskamp K, Krug HF (2006). Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Lett*, 6(6):1261–8. doi:[10.1021/nl060177c](https://doi.org/10.1021/nl060177c) PMID:[16771591](https://pubmed.ncbi.nlm.nih.gov/16771591/)
- WTEC (2007). Panel report on international assessment of carbon nanotube manufacturing and applications. Baltimore (MD), USA: World Technology Evaluation Center, Inc.
- Wu M, Gordon RE, Herbert R, Padilla M, Moline J, Mendelson D, et al. (2010). Case report: Lung disease in World Trade Center responders exposed to dust and smoke: carbon nanotubes found in the lungs of World Trade Center patients and dust samples. *Environ Health Perspect*, 118(4):499–504. doi:[10.1289/ehp.0901159](https://doi.org/10.1289/ehp.0901159) PMID:[20368128](https://pubmed.ncbi.nlm.nih.gov/20368128/)
- Wu WT, Liao HY, Chung YT, Li WF, Tsou TC, Li LA, et al. (2014). Effect of nanoparticles exposure on fractional exhaled nitric oxide (FENO) in workers exposed to nanomaterials. *Int J Mol Sci*, 15(1):878–94. doi:[10.3390/ijms15010878](https://doi.org/10.3390/ijms15010878) PMID:[24413755](https://pubmed.ncbi.nlm.nih.gov/24413755/)
- Xia T, Hamilton RF Jr, Bonner JC, Crandall ED, Elder A, Fazlollahi F, et al. (2013). Interlaboratory evaluation of in vitro cytotoxicity and inflammatory responses to engineered nanomaterials: the NIEHS Nano GO Consortium. *Environ Health Perspect*, 121(6):683–90. doi:[10.1289/ehp.1306561](https://doi.org/10.1289/ehp.1306561) PMID:[23649538](https://pubmed.ncbi.nlm.nih.gov/23649538/)
- Xiao Y, Gao X, Taratula O, Treado S, Urbas A, Holbrook RD, et al. (2009). Anti-HER2 IgY antibody-functionalized single-walled carbon nanotubes for detection of breast cancer cells. *BMC Cancer*, 9:351. doi:[10.1186/1471-2407-9-351](https://doi.org/10.1186/1471-2407-9-351) PMID:[19799784](https://pubmed.ncbi.nlm.nih.gov/19799784/)
- Xu J, Alexander DB, Futakuchi M, Numano T, Fukamachi K, Suzui M, et al. (2014). Size- and shape-dependent pleural translocation, deposition, fibrogenesis, and mesothelial proliferation by multiwalled carbon nanotubes. *Cancer Sci*, 105(7):763–9. doi:[10.1111/cas.12437](https://doi.org/10.1111/cas.12437) PMID:[24815191](https://pubmed.ncbi.nlm.nih.gov/24815191/)
- Xu J, Futakuchi M, Shimizu H, Alexander DB, Yanagihara K, Fukamachi K, et al. (2012). Multi-walled carbon nanotubes translocate into the pleural cavity and induce visceral mesothelial proliferation in rats. *Cancer Sci*, 103(12):2045–50. doi:[10.1111/cas.12005](https://doi.org/10.1111/cas.12005) PMID:[22938569](https://pubmed.ncbi.nlm.nih.gov/22938569/)
- Yamashita K, Yoshioka Y, Higashisaka K, Morishita Y, Yoshida T, Fujimura M, et al. (2010). Carbon nanotubes elicit DNA damage and inflammatory response relative to their size and shape. *Inflammation*, 33(4):276–80. doi:[10.1007/s10753-010-9182-7](https://doi.org/10.1007/s10753-010-9182-7) PMID:[20174859](https://pubmed.ncbi.nlm.nih.gov/20174859/)
- Yang H, Liu C, Yang D, Zhang H, Xi Z (2009). Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. *J Appl Toxicol*, 29(1):69–78. doi:[10.1002/jat.1385](https://doi.org/10.1002/jat.1385) PMID:[18756589](https://pubmed.ncbi.nlm.nih.gov/18756589/)
- Yang M, Flavin K, Kopf I, Radics G, Hearnden CH, McManus GJ, et al. (2013). Functionalization of carbon nanoparticles modulates inflammatory cell

- recruitment and NLRP3 inflammasome activation. *Small*, 9(24):4194–206. doi:[10.1002/smll.201300481](https://doi.org/10.1002/smll.201300481) PMID:[23839951](https://pubmed.ncbi.nlm.nih.gov/23839951/)
- Yang ST, Guo W, Lin Y, Deng XY, Wang HF, Sun HF, et al. (2007). Biodistribution of pristine single-walled carbon nanotubes in vivo. *J Phys Chem C*, 111(48):17761–4. doi:[10.1021/jp070712c](https://doi.org/10.1021/jp070712c)
- Ye R, Wang S, Wang J, Luo Z, Peng Q, Cai X, et al. (2013). Pharmacokinetics of CNT-based drug delivery systems. *Curr Drug Metab*, 14(8):910–20. doi:[10.2174/138920021131400113](https://doi.org/10.2174/138920021131400113) PMID:[24016105](https://pubmed.ncbi.nlm.nih.gov/24016105/)
- Ye SF, Wu YH, Hou ZQ, Zhang QQ (2009). ROS and NF-kappaB are involved in upregulation of IL-8 in A549 cells exposed to multi-walled carbon nanotubes. *Biochem Biophys Res Commun*, 379(2):643–8. doi:[10.1016/j.bbrc.2008.12.137](https://doi.org/10.1016/j.bbrc.2008.12.137) PMID:[19121628](https://pubmed.ncbi.nlm.nih.gov/19121628/)
- Ye Y, Ahn CC, Witham C, Fultz B, Liu J, Rintzer AG, et al. (1999). Hydrogen absorption and cohesive energy of single-walled carbon nanotubes. *Appl Phys Lett*, 74(16):2307–9. doi:[10.1063/1.123833](https://doi.org/10.1063/1.123833)
- Yeganeh B, Kull CM, Hull MS, Marr LC (2008). Characterization of airborne particles during production of carbonaceous nanomaterials. *Environ Sci Technol*, 42(12):4600–6. doi:[10.1021/es703043c](https://doi.org/10.1021/es703043c) PMID:[18605593](https://pubmed.ncbi.nlm.nih.gov/18605593/)
- Yu IJ, Ichihara G, Ahn K (2014). In: Njuguna J, Pielichowski K, Zhu H, editors. Health and Environmental Safety of Nanomaterials: Nanoparticle exposure assessment: methods, sampling techniques, and data analysis. Salt Lake City (UT), USA: Woodland Publishing; pp. 47–62. doi:[10.1533/9780857096678.2.47](https://doi.org/10.1533/9780857096678.2.47)
- Yu KN, Kim JE, Seo HW, Chae C, Cho MH (2013). Differential toxic responses between pristine and functionalized multiwall nanotubes involve induction of autophagy accumulation in murine lung. *J Toxicol Environ Health A*, 76(23):1282–92. doi:[10.1080/15287394.2013.850137](https://doi.org/10.1080/15287394.2013.850137) PMID:[24283420](https://pubmed.ncbi.nlm.nih.gov/24283420/)
- Yu MF, Files BS, Arepalli S, Ruoff RS (2000). Tensile loading of ropes of single wall carbon nanotubes and their mechanical properties. *Phys Rev Lett*, 84(24):5552–5. doi:[10.1103/PhysRevLett.84.5552](https://doi.org/10.1103/PhysRevLett.84.5552) PMID:[10990992](https://pubmed.ncbi.nlm.nih.gov/10990992/)
- Yuan J, Gao H, Ching CB (2011). Comparative protein profile of human hepatoma HepG2 cells treated with graphene and single-walled carbon nanotubes: an iTRAQ-coupled 2D LC-MS/MS proteome analysis. *Toxicol Lett*, 207(3):213–21. doi:[10.1016/j.toxlet.2011.09.014](https://doi.org/10.1016/j.toxlet.2011.09.014) PMID:[21963432](https://pubmed.ncbi.nlm.nih.gov/21963432/)
- Zanello LP, Zhao B, Hu H, Haddon RC (2006). Bone cell proliferation on carbon nanotubes. *Nano Lett*, 6(3):562–7. doi:[10.1021/nl051861e](https://doi.org/10.1021/nl051861e) PMID:[16522063](https://pubmed.ncbi.nlm.nih.gov/16522063/)
- Zeni O, Palumbo R, Bernini R, Zeni L, Sarti M, Scarfi MR (2008). Cytotoxicity investigation on cultured human blood cells treated with single-wall carbon nanotubes. *Sensors (Basel Switzerland)*, 8(1):488–99. doi:[10.3390/s8010488](https://doi.org/10.3390/s8010488)
- Zhang F, Wang N, Kong J, Dai J, Chang F, Feng G, et al. (2011). Multi-walled carbon nanotubes decrease lactate dehydrogenase activity in enzymatic reaction. *Bioelectrochemistry*, 82(1):74–8. doi:[10.1016/j.bioelechem.2011.04.007](https://doi.org/10.1016/j.bioelechem.2011.04.007) PMID:[21612987](https://pubmed.ncbi.nlm.nih.gov/21612987/)
- Zhang H, Cao CP, Wang ZY, Yang YS, Shi ZJ, Gu ZA (2010). Carbon nanotube array anodes for high-rate Li-ion batteries. *Electrochim Acta*, 55(8):2873–7. doi:[10.1016/j.electacta.2010.01.028](https://doi.org/10.1016/j.electacta.2010.01.028)
- Zhang MF, Yudasaka M, Koshio A, Iijima S (2002). Thermogravimetric analysis of single-wall carbon nanotubes ultrasonicated in monochlorobenzene. *Chem Phys Lett*, 364(3–4):420–6. doi:[10.1016/S0009-2614\(02\)01347-7](https://doi.org/10.1016/S0009-2614(02)01347-7)
- Zhang Y, Deng J, Zhang Y, Guo F, Li C, Zou Z, et al. (2013). Functionalized single-walled carbon nanotubes cause reversible acute lung injury and induce fibrosis in mice. *J Mol Med (Berl)*, 91(1):117–28. doi:[10.1007/s00109-012-0940-x](https://doi.org/10.1007/s00109-012-0940-x) PMID:[22878607](https://pubmed.ncbi.nlm.nih.gov/22878607/)
- Zhang Y, Yan B (2012). Cell cycle regulation by carboxylated multiwalled carbon nanotubes through p53-independent induction of p21 under the control of the BMP signalling pathway. *Chem Res Toxicol*, 25(6):1212–21. doi:[10.1021/tx300059m](https://doi.org/10.1021/tx300059m) PMID:[22428663](https://pubmed.ncbi.nlm.nih.gov/22428663/)
- Zhao Y, Wei J, Vajtai R, Ajayan PM, Barrera EV (2011). Iodine doped carbon nanotube cables exceeding specific electrical conductivity of metals. *Sci Rep*, 1:83. doi:[10.1038/srep00083](https://doi.org/10.1038/srep00083) PMID:[22355602](https://pubmed.ncbi.nlm.nih.gov/22355602/)
- Zhu L, Chang DW, Dai L, Hong Y (2007). DNA damage induced by multiwalled carbon nanotubes in mouse embryonic stem cells. *Nano Lett*, 7(12):3592–7. doi:[10.1021/nl071303v](https://doi.org/10.1021/nl071303v) PMID:[18044946](https://pubmed.ncbi.nlm.nih.gov/18044946/)