Carbon nanotubes (CNT) are an important new class of technological materials that have numerous novel and useful properties. The forecast increase in manufacture makes it likely that increasing human exposure will occur, and as a result, CNT are beginning to come under toxicological scrutiny. This review seeks to set out the toxicological paradigms applicable to the toxicity of inhaled CNT, building on the toxicological database on nanoparticles (NP) and fibers. Relevant workplace regulation regarding exposure is also considered in the light of our knowledge of CNT. CNT could have features of both NP and conventional fibers, and so the current paradigm for fiber toxicology, which is based on mineral fibers and synthetic vitreous fibers, is discussed. The NP toxicology paradigm is also discussed in relation to CNT. The available peer-reviewed literature suggests that CNT may have unusual toxicity properties. In particular, CNT seem to have a special ability to stimulate mesenchymal cell growth and to cause granuloma formation and fibrogenesis. In several studies, CNT have more adverse effects than the same mass of NP carbon and quartz, the latter a commonly used benchmark of particle toxicity. There is, however, no definitive inhalation study available that would avoid the potential for artifactual effects due to large mats and aggregates forming during instillation exposure procedures. Studies also show that CNT may exhibit some of their effects through oxidative stress and inflammation. CNT represent a group of particles that are growing in production and use, and therefore, research into their toxicology and safe use is warranted.

Key Words: carbon nanotubes; graphite; inflammation; oxidative stress; nanoparticles; fibre; toxicity; asbestos; lungs; workplace.

INTRODUCTION TO CARBON NANOTUBES

Nanotechnology is a global cross-disciplinary undertaking that has extraordinary potential to change our lives by improving existing products and enabling new ones. Like most new technologies, the emphasis on benefits has been offset by considerable debate about the uses and safety of nanotechnologies (Brumfiel, 2003; Dreher, 2004; Giles, 2003; Hood, 2004; Maynard and Kuempel, 2005; The Royal Society and the Royal Academy of Engineering, 2004; Seaton and Donaldson, 2005). Occupational medicine is replete with examples of respirable dust particle exposures causing disease, and so matters of safety have been focused largely on nanoparticles (NP), which represent only one aspect of nanotechnology. NP, generally defined as particles less than 100 nm in any dimension and previously called ultrafine particles by inhalation toxicologists, are a concern because they may enter the body through the lungs, skin, or gut depending on the type of exposure. Several recent papers have highlighted this area of toxicology, its novelty, the gaps in research, and possible testing strategies for NP and nanotubes (Donaldson et al., 2004a; Oberdorster et al., 2005a,b; Seaton and Donaldson, 2005).

There is sufficient “ultrafine particle” toxicology literature to consider that the large and diverse group of manufactured NP arising from nanotechnology comprises an inhalation hazard of unknown potential. Most of the concern has flowed from this perception, enhanced by limited studies indicating that they may also be taken up through the skin or gut. The notion, for which some limited support exists, that NP may gain access to the blood poses a relatively new particle toxicological problem, i.e., particle effects at sites other than the lungs.

This paper examines the toxicology issues relating to carbon nanotubes (CNT), one of the major new NP that are set to be produced in bulk for many diverse purposes. CNT are discussed here exclusively as an inhalation hazard. Global revenues from CNT in 2006 are estimated at ~$230 million with a growth rate of ~170% (data from BCC Research, Norwalk, CT, United Kingdom; Oberdorster et al., 2005a,b).
This provides potential for workplace and even eventual general exposure, if there is attrition of materials that contain them. Concern has been raised over the safety of CNT because they have three properties that are clearly associated with pathogenicity in particles:

1. They are NP and so could have more toxicity than larger sized particles.
2. They are fiber shaped and so might behave like asbestos and other pathogenic fibers which have toxicity associated with their needle-like shape.
3. They are essentially graphitic and so are expected to be biologically biopersistent.

In this paper we describe the synthesis, structure, and composition of CNT and put this in the context of the known toxicology of NP and pathogenic fibers such as asbestos to draw conclusions regarding the toxicology issues relating to these materials. We also discuss the exposure measurement issue in relation to particles and fiber and highlight the difficulties that this presents for nanotubes.

### TYPES OF CNT

Comprised entirely of carbon, the structure of pure CNT can be visualized as a single sheet of graphite rolled to form a seamless cylinder. There are two classes of CNT: single-walled carbon nanotubes (SWCNT) and multiwalled carbon nanotubes (MWCNT; Fig. 1). MWCNT are larger and consist of many single-walled tubes stacked one inside the other. CNT are distinct from carbon fibers, which are not single molecules but strands of layered graphite sheets. CNT are reported to be physically strong and stiff and, e.g., SWCNT can be as much as 10 times as strong as steel and 1.2 times as stiff as diamond (Walters et al., 1999; Yu et al., 2000).

### SYNTHESIS OF CNT

There are three methods commonly used to synthesize CNT: arc discharge, chemical vapor deposition (CVD), and laser ablation (Fig. 2). The common feature of these methods is addition of energy to a carbon source to produce fragments (groups or single C atoms) that can recombine to generate CNT. The energy source may be electricity from an arc discharge, heat from a furnace (~ 900°C) for CVD, or the high-intensity light from a laser (laser ablation).

The mechanisms of CNT formation are widely debated (Cassell et al., 1999; Sinnott et al., 1999). Where metal catalysts are used, it is thought that carbon is deposited on a catalyst NP, from which the C network finally extrudes to form a graphite tube (Vinciguerra et al., 2003) (Fig. 3). This
growth has been observed using in situ electron microscopy (Helveg et al., 2004) (Fig. 3). The size of the metal particle determines the diameter of the tube; SWCNT grow from metal particles a few nanometers in diameter, while larger particles tend to produce wider MWCNT.

Commercially available CNT are often synthesized by CVD as this process is easily scaled up for industrial production. The annual global production of single-walled tubes was 9 tons in 2004 (Cientifica, 2004). Typically, a major producer of SWCNT produces ~450 kg/day (Borowiak-Palen et al., 2002; Bronikowski et al., 2001; Islam et al., 2003). The high-pressure carbon monoxide method, which uses iron as the catalyst and carbon monoxide as the carbon source (Bronikowski et al., 2001), is commonly employed. MWCNT are also in commercial production, and one company (see web site http://www.fibrils.com/) has been making plastic composites incorporating multiwalled nanotubes since 1983. The annual global production capacity for MWCNT was estimated at 100 tons in 2004 (Cientifica, 2004).

After synthesis, CNT are usually purified to remove amorphous carbon ("soot"), free residual catalyst (metal from which nanotubes failed to grow), and any support material. This is typically achieved by washing or ultrasonincating with dilute acid. Highly purified tubes may have additionally undergone some form of oxidation. Removal of some supports like silica and alumina necessitates a stronger treatment process (e.g., with concentrated acid; Borowiak-Palen et al., 2002; Chiang et al., 2001). Since purification also destroys CNT, the removal of impurities must be balanced against the introduction of defects into tubes.

**COMPOSITION AND REACTIVITY OF CNT**

The chemistry of pure CNT is surprisingly uninteresting. They are significantly unreactive; e.g., SWCNT must be heated to 500°C before they burn in air (Zhang et al., 2002). However, there are points in the structure of CNT which are more reactive than others, such as defects due to missing carbon atoms and the more strained curved-end caps (Lin et al., 2003). Smaller nanotubes are more "strained" because they deviate further from the ideal planar structure of graphite. Purified nanotubes are likely to contain additional defects in the form of carboxylic acid (-COOH) residues.

A sample of CNT invariably contains a variety of residual impurities. There are three classes of impurities that may remain from the synthesis process: metals, organics, and support material.

Co, Fe, Ni, and Mo are by far the most commonly used metals in CNT synthesis, and mixtures of these are also widely employed; Mo is often used as an additive to promote SWCNT growth (Kittiyanan et al., 2000). Postproduction processing removes the majority of metal catalyst; however, Carbon Nanotechnologies Incorporated (CNI, Houston, TX) purified grade tubes, e.g., may still contain up to 15% residual metal by mass (Brelsford, personal communication, CNI). Residual metal is usually encapsulated in a layer of carbon, either amorphous soot or layers of graphite. Metal NP that fail to grow tubes often end up encapsulated in this way. Additionally, it is observed that metal NP often become incorporated into the ends of nanotubes; in this case, it would appear that the metal is inaccessible to the outside environment (Dai, 2002). Most residual metal is nanoparticulate, although aggregates may be formed. Any metal NP not coated in carbon will be coated with a metal oxide layer.

Residual organics can be divided into two subclasses: various forms of bulk (amorphous or microstructured) carbon and residual organic molecules. Bulk carbon structures formed include amorphous soot particles and a variety of structures built up from graphite sheets of different sizes; e.g., sheets can linearly stack to make carbon fibers or they can nest in spheres. Carbon fibers may consist of planar or "paper cup"-shaped graphite sheets stacked together (Ertl et al., 1997).

CNT synthesis involves the use of a material to support the catalyst or growth region. Aluminates and silicates (e.g., zeolites) are common supports but require strong acids or bases to remove them later. Magnesium oxide is increasingly used as a CNT growth support as it can be dissolved in milder acids. A high surface area is a desirable property for these
supports and helps prevent coalescence of metal NP during synthesis; for this reason, nanopowders are often used as supports.

SIZE OF CNT

CNT vary significantly in length and diameter depending on the synthetic procedure. Lengths are generally dependent on synthesis time but are typically tens of microns, although significantly shorter and longer nanotubes have been made (Motta et al., 2005; Puretzky et al., 2002). Cleaned and processed CNT are usually shorter than as-produced nanotubes due to the destructive conditions used in purification (Liu et al., 1998).

The diameters of SWCNT are controlled by the sizes of the metal NP from which they are grown, which vary between about 0.7 and 3 nm (Jorio et al., 2001). MWCNT generally range from 10 to 200 nm in diameter (Hou et al., 2003). More important, however, is the strong tendency of both SWCNT and MWCNT to bundle together in ropes as a consequence of attractive van der Waals forces analogous to forces that bind sheets of graphite (Thess et al., 1996). Bundles typically contain many tens of nanotubes and can be considerably longer and wider than the nanotubes from which they are formed. This could have important toxicological consequences.

Much research has focused on creating homogeneous dispersions of CNT in various solvents. CNT are slightly dispersed in some organic solvents such as dichlorobenzene (95 mg/l), chloroform (31 mg/l), and carbon disulphide (2.6 mg/l; Bahr et al., 2001). More complete dispersion is generally achieved by prolonged sonication, but dispersions of CNT tend to aggregate over time. CNT are not readily dispersible in water without the aid of surfactant molecules to exfoliate individual or small bundles of nanotubes from larger aggregates, enclosing them in a hydrophilic coating. Common surfactants used for this purpose are sodium dodecylbenzenesulfonate, Triton X-100, sodium dodecyl sulfate, and Cetyl Trimethyl Ammonium Bromide (Islam et al., 2003; Moore et al., 2003; O’Connell et al., 2002).

As a consequence of their dimensions, CNT have very high surface areas. The available surface area is dependent on the length, diameter, and degree of bundling. Theoretically, discrete SWCNT have surface areas of ~1300 m²/g, whereas MWCNT generally have surface areas of a few hundred m²/g (Peigney et al., 2001). As a result of bundling, the surface area of most samples of SWCNT is dramatically lowered to ~300 m²/g, or less, which is still a very high value (Ye et al., 1999b).

Functionalization of CNT Surfaces

Much research on CNT focuses on modifying these as-produced tubes by the addition of different chemical groups, leading to a significant change in many of their properties (Banerjee et al., 2003). Many companies sell these “derivatized” or “functionalized” nanotubes including fluorine groups as a starting point for various other chemical modifications. Functionalization with polymer groups is used to enhance solubility. Functionalization with different groups is likely to result in different toxicity (Sayes et al., 2005) since the particle surface is important in interacting with biological systems.

PARTICLE TOXICOLOGY OF RELEVANCE TO CNT

Nanoparticles

Compact particles have their harmful effects as a consequence of two factors that act together to contribute to their potential to cause harm: their surface area and the reactivity or intrinsic toxicity of that surface (Donaldson and Tran, 2002; Tran et al., 2000). The smaller the particles are, the more surface area they have per unit mass; therefore, any intrinsic toxicity of the particle surface will be emphasized (Duffin et al., 2002). Therefore, as particles become generally smaller, their likelihood of causing harm to the lung increases. Thus, particles with a relatively inert surface can exert their effects on cells by having a large surface area. Alternatively, particles with a highly reactive surface can affect cells without necessarily having a large surface area. NP are currently available in a variety of compositions that range from very simple—almost pure carbon or titanium dioxide (TiO₂)—to very complex structures, where surface modifications or coatings are applied. The nanotechnology industry is also developing new NP for use in a variety of purposes (The Royal Society and the Royal Academy of Engineering, 2004), and the toxicology of these materials is largely unknown.

NP in the lungs. We are already exposed to large numbers of ambient NP in environmental air pollution (Maynard and Howard, 1999). In this regard, combustion-derived NP have been the focus of much research as a likely driver of adverse health effects, such as exacerbations of airways disease as well as deaths and hospitalization from cardiovascular disease (Donaldson et al., 2005). There is a large existing database in the lung particle toxicology literature that shows NP of various sorts to have extra toxicity (Donaldson et al., 2002) by which we mean that the same material in the form of NP is more toxic than in the form of larger, still respirable particles. The mechanism of lung injury caused by combustion-derived NP is predominantly via oxidative stress, produced at the particle surface that leads to cell injury and lung inflammation. The oxidative stress is produced as a consequence of the properties of the combustion-derived NP, principally the large surface area, metals, and organic molecules (reviewed in Donaldson et al., 2004). Oxidative stress–responsive transcription factors such as nuclear factor kB (NF-kB) and Activator Protein-1 then translate this oxidative stress into proinflammatory proteins, a sequence of events described for a number of pathogenic particle types (Donaldson et al., 2004a; Schins and Donaldson, 2000). Inflammation can then impact a number of pathological processes such as airways disease, cardiovascular disease, fibrosis, or cancer (Mauderly et al., 1994).
Earlier carcinogenesis studies with ultrafine carbon black and TiO₂ were complicated by rat lung overload, a nonspecific response of the rat lung to high levels of even low-toxicity particles that probably does not operate in humans (Lee et al., 1986, 1985a,b; Mauderly et al., 1990). However, it has recently been shown that low (1000 mg/m³) short-term (7 h) exposures to NP carbon black cause mild inflammatory effects in rat lungs (Gilmour et al., 2004b). Proinflammatory effects of NP have been described in a number of in vitro models (Brown et al., 2004; Gilmour et al., 2004a), as has their ability to generate reactive oxygen species and oxidative stress (Stone et al., 1998; Wilson et al., 2002). Additionally NP have been seen to inhibit phagocytosis (Renwick et al., 2001) and increase macrophage sensitivity to chemotactic complement components (Renwick et al., 2004).

Very small particles and structures could have a range of other effects that are not seen with conventional particles. For instance, they may not be detected by the normal phagocytic defenses allowing them to gain access to the blood or the nervous system. Very small particles are smaller than some molecules and could act like haptons to modify protein structures: either altering their function or rendering them antigenic, raising the potential for autoimmune effects (Borm and Kreyling, 2004).

NP and extrapulmonary responses. Of special concern is the apparent ability of NP to redistribute from their site of deposition. Thus, following inhalation exposure, NP have been reported to travel via the nasal nerves to the brain (Kreuter et al., 2002; Oberdorster et al., 2004), as has been described for poliovirus (Bodian and Howe, 1941), and to gain access to the blood and other organs (Kreyling et al., 2004). Thus, adverse effects of NP are likely to occur in highly different scenarios, dependent on particle type and portal of entry. For NP that are made and handled in bulk, there is potential for lung exposure. For some NP, such as those in sunblock creams, dermal exposure is already occurring, and the range of different NP in cosmetics is likely to increase (Pinnell et al., 2000; Tan et al., 1996). NP in food are reported to cross into the gut lymphatics and redistribute to other organs more readily than larger particles (Hillery et al., 1994; Jani et al., 1990). Furthermore, a huge class of NP is designed to be introduced directly into the body for diagnostic and therapeutic reasons (Duncan, 2003). However, it is worth noting that the chemistry and properties of these different classes of NP are very diverse, and it is likely that while some are likely to be toxic, others such as those screened extensively for drug delivery may be relatively low in their toxic potential.

Fibers

The vast majority of information on fiber toxicology is derived from asbestos, but the synthetic vitreous fibers (SVF) have contributed greatly to our current understanding of the roles of length and biopersistence in mediating pathogenicity. Consequently, the fiber toxicology paradigm discussed here is based on mineral fibers and SVF (Bernstein et al., 2005); other paradigms, as yet unknown, could be involved in pathogenicity of other fibers types such as CNT. Inhalation of pathogenic fibers is associated with fibrosis of the lung, i.e., asbestosis, lung cancer, and mesothelioma (i.e., cancer of the mesothelium lining the pleural and peritoneal cavities). Despite the decline in use of asbestos in many countries, the number of deaths attributed to mesothelioma, a disease often attributed solely to asbestos exposure, continues to rise, and in the United Kingdom it is predicted to peak between 2011 and 2015 at somewhere between 1950 and 2450 deaths/year. The proposed mechanisms of lung disease caused by fibers are numerous and include oxidative stress, inflammation, and both direct and indirect genotoxicity (Bernstein et al., 2005; Kane, 1996).

Fiber deposition. If they are sufficiently thin, even very long fibers can readily penetrate to the distal lung, traveling into the lung by aligning their longitudinal axis with the airstream. For such particles, deposition is dictated by aerodynamic diameter, which, for fibers with an actual diameter that is very small, is not unduly influenced by length (Kennedy and Kelly, 1993). There is substantial penetration of long thin fibers (for a qualification of the term “long” see below) beyond the ciliated airways. Brody et al. (1984) described the deposition of long chrysotile fibers at and around the alveolar ducts, where the net flow of air becomes zero, and so the potential for fibers settling out is great (Lippmann, 1993). The ability of long and thin fibers to reach the gas-exchange region of the lungs is important because a failure to clear these fibers during ongoing exposure leads to a build up of dose. The deposition of CNT in the lung is expected to depend on the form that the CNT take when they are aerosolized. In an aggregated form, the CNT are likely to be deposited in the lung like a particle with a similar aerodynamic diameter. However, if CNT can be aerosolized as single fibers, their deposition will be conventional, i.e., based on aerodynamic diameter and the possibility of aligning with the airstream.

Fiber length and inflammation. Length is an important feature of fibers that modulates their pathogenicity. In a seminal study (Davis et al., 1986), rats were exposed to both long amosite and the same amosite shortened by milling. Following inhalation exposure to equal airborne mass concentrations, there was substantially more fibrosis and cancer with the long fibers. Following ip instillation, mesothelioma was caused by the long amosite but not the short amosite. The same group (Donaldson et al., 1989) instilled the same fiber samples into the mouse peritoneal cavity and reported intense inflammation with the long amosite and virtually no inflammation with the short amosite. The role of fiber length was further emphasized when the length of glass fibers was found to be critical in NF-kB activation (Ye et al., 1999a) in macrophages, leading to the production of TNF-α. Using different size classes, the activation of this transcription factor, vital in the initiation of inflammation, was far greater with the longest fraction.
Fiber length, clearance, and accumulated dose. Long fibers can be defined as fibers that significantly exceed the size of macrophages and are usually taken to be 10- to 20-mm long. Such fibers present problems to alveolar macrophages, which will have difficulty in effectively phagocytosing and “clearing” them to the mucociliary escalator. Long fibers are, therefore, more slowly cleared from the deep lung than shorter fibers, as demonstrated in several studies (e.g., Coin et al., 1994; Searl et al., 1999). Therefore, with ongoing exposure, the dose of long fibers will accumulate more than for the more rapidly clearing short fibers. Clearly, however, dose is not the only explanation for the greater pathogenicity of long fibers. In studies using the mouse peritoneal cavity (Donaldson et al., 1989) and in vitro (Donaldson et al., 1992; Ye et al., 1999a), where failed clearance is not the explanation, long fibers have much more pronounced proinflammatory effects than short fibers. There is, therefore, a “double hit” caused by long fibers—firstly, they are more likely to be retained, so building up the dose available to make contact with cells; secondly, they are more stimulatory and biologically active than short fibers when they make contact with cells.

Composition and Biopersistence

Biopersistence effects arise when fibers do not undergo chemical dissolution in the lung tissue, weaken, break, or dissolve away and therefore persist in the lungs. Asbestos fibers are crystalline silicates with a number of other elements present in the crystal structure that cause them to be identifiable by their elemental composition into the amphiboles and chrysotile. Among the SVF there is also considerable variation in chemical composition, but all are basically amorphous and noncrystalline. Typically, these are made from melted rock or slag or by melting together a mixture of components of which the most common is SiO with varying amounts of other oxides, such as BaO₂, TiO₂, and CaO, to produce fibers with different properties (WHO IARC I.L.F., 2002). There are some crystalline man-made fibers such as SiC.

These differences in structure provide for differences in the ability of fibers to resist chemical attack in the lungs. When fibers are treated in vitro with salt solutions that are typical of those found in biological systems, the fibers differ in their “durability” (Eastes et al., 2000), i.e., some types of fibers undergo dissolution and either break up into smaller fragments or dissolve entirely. Other types of fibers, like amphibole asbestos, resist dissolution. In the lung, these differences are reflected in a tendency for long fibers of nonbiopersistent material to be cleared, as they dissolve or break up into shorter fibers that can be removed (Hesterberg et al., 1998; Searl et al., 1999). Thus, the harmful doses of fibers in the lungs are long biopersistent fibers (Miller et al., 1999). This has lead to the paradigm shown in Figure 5.

There was evidence in the earliest studies that chrysotile fibers disappear much faster from the lungs of asbestos-exposed workers, and differential losses of chrysotile from the lungs of Canadian chrysotile miners, with resulting dramatic increases in the tremolite (amphibole) chrysotile ratio, were well documented (McDonald, 1998). Recent studies have shown chrysotile to be relatively nonbiopersistent in rat lungs (Bernstein et al., 2003a,b). Based on extensive studies with SVF, biopersistence is now seen as the most important factor dictating pathogenicity of a fiber, and industries are manipulating the composition of their products to obtain less biopersistent fibers.

Composition and Pathogenicity

Proinflammatory effects may arise from fibers as a result of their reactive surface or the release of biologically active ions such as transition metals. The central hypothesis of the proinflammatory effects of fibers implies an important role for oxidative stress. The oxidative stress/NF-κB pathway has great potential importance for the production of fiber-mediated inflammation. Transition metal–mediated oxidative stress appears to be important in the adverse effects of asbestos fibers, advanced in the early 1980’s (Ghio et al., 1998; Hardy and Aust, 1995; Weitzman and Graceffa, 1984). However, although
bioavailable transition metals may play a role in the genesis of inflammation and DNA damage by crocidolite, which is the most extensively tested fiber, a study of a range of pathogenic and nonpathogenic fibers found that bioavailable iron does not represent a generic pathway for the adverse health effects of fibers (Fisher et al., 1998). It is possible that oxidative stress at fiber surfaces might be important in leading to inflammation by pathways other than via transition metals. These pathways, although postulated (Fubini, 1996), are not well understood but include the reduction of oxygen directly by electrons to form hydrogen peroxide and its further reduction to hydroxyl radical or hydrogen abstraction from an organic molecule by a free radical to produce an organic radical (Fubini, 1996).

The relevance of the above to nanotubes relates to the fact that nanotubes are being made increasingly longer, and so they definitively satisfy the requirement for length in the above paradigm when they become greater than ~15 μm. Likewise, being essentially graphitic, they will not be soluble in a neutral or mild acid pH and so are potentially biopersistent. Furthermore, their tendency to be contaminated with metals may contribute to oxidative-induced inflammation and toxicity.

TOXICOLOGICAL CONSIDERATIONS OF NANOTUBES

Based on the above description of CNT synthesis size and composition, coupled with the known toxicology of NP and fibers, we can address the toxicological paradigms that could be relevant to CNT.

Length—The Fiber Issue

If CNT are longer than about 20 μm then, analogous to mineral and SVF fibers, if inhaled we can expect them to cause the same types of pathology that long biopersistent fibers cause in rats and humans if the fiber number is sufficient. These pathologies are fibrosis, cancer, pleural changes, and mesothelioma. This current paradigm of fiber pathogenicity does not discriminate between different compositions of long biopersistent fibers, except where composition can determine biopersistence. There are, however, two cases where long biopersistent fiber types—erionite (Wagner et al., 1985) and SiC (Davis et al., 1996)—showed special proclivity to cause mesothelioma for reasons that were not easily explained, since they were not especially long or more biopersistent than amphiboles. The chemical basis of the extra pathogenicity of these two fibers has not been elucidated. This suggests that some fiber types may possess surface reactivity that imparts added pathogenicity; we do not know if CNT have this potential. Carbon particles would not normally be anticipated to have specially active surfaces; however, due to their small size, NP carbon black, composed of degenerated graphitic crystallites, is able to generate more oxidative stress in vitro than fine carbon black (Wilson et al., 2002). The role of nanodimensions in determining CNT toxicity is yet to be investigated.

SWCNT are thin, but MWCNT are not much thinner than chrysotile asbestos fibrils, which are 20 nm in diameter (Roggli and Coin, 2003). Chrysotile fibers are considered to be nonbiopersistent in human lungs (Bernstein et al., 2003a,b), and so there is no experience of a workforce being potentially exposed to a biopersistent fiber of this degree of thinness.

The strategy for assessing the pathogenicity of “long CNT” would, therefore, identify end points recognized from classical fiber pathogenicity, such as durability, biopersistence, persistent inflammogenicity, and proliferation (Donaldson and Tran, 2004; Warheit et al., 1995). Chronic studies should emphasize carcinogenic and mesothelioma end points in the lungs, and positive controls would include amphibole asbestos. Pleural mesothelioma is a key end point to study but is a rare tumor

FIG. 5. Paradigm for the role of long fibers and biopersistence in the pathogenic effects of fibers.
even in animal experiments; however, hamsters have been shown to be a species especially susceptible to mesothelioma (Mcconnell et al., 1999) and so may have some utility. Since some types of CNT appear to be flexible and have the ability to bend or curl up to form a more compact particle (Maynard et al., 2004; Shvedova et al., 2005), the rigidity of CNT may be a factor in driving toxicity, since less rigid particles would be more easily cleared by phagocytic cells.

Surface Area—The NP Issue

If CNT are appreciably shorter than 15–20 µm, then we may expect them to be adequately phagocytosed by macrophages and to act like NP in that their dominant adverse effect is likely by virtue of their small size and large surface area. However, it is important to also bear in mind that very small nanotubes may be essentially “stealth particles” that avoid the phagocytic defenses (Borm and Kreyling, 2004). It should be noted that the surface area has been used as a metric for describing asbestos fiber toxicity (Lippmann, 1988). The overall surface area of CNT is, of course, much greater than that of a NP of similar diameter. Therefore, on a per-particle basis, we would expect even a “short” nanotube of 20 × 2000 nm to deliver 100 times more surface area than a spherical 20-nm diameter NP. Equal masses of the same 20-nm NP and the 20 × 2000-nm nanotubes would be composed of about a hundred times more compact NP, and so on a mass basis, the surface area dose would be roughly similar. It seems likely that because of the difficulty of detecting nanotubes by phase-contrast optical microscopy (PCOM), the current standard technique for fibers, they may be regulated on the basis of mass. Given the argument relating to surface area, CNT number alone may not be a suitable metric, and a surface area metric might be more appropriate taking into account the extra surface area of short nanotubes compared to NP.

The mechanism of the direct local adverse effects of NP in the lungs will likely depend on the nature of the NP surface, and the ability to cause oxidative stress and inflammation appear to be important. The NP that are most analogous to CNT are NP of carbon black (as opposed to diesel soot, welding fumes, etc.). Nanoparticulate carbon black (NPCB) is composed of disordered graphite sheets and so differs from the continuous graphitic sheet nature of the nanotube surface. As described previously, NPCB cause oxidative stress in cells (Stone et al., 1998) and cell-free systems (Wilson et al., 2002) and oxidative stress–mediated cell signaling (Beck-Speier et al., 2005; Tamaoki et al., 2004) and cause inflammation after short-term inhalation (Gilmour et al., 2004b) and instillation exposure (Renwick et al., 2004). These effects are highly likely driven through surface area effects as carbon black has the same proinflammatory surface properties as a range of low-toxicity, low-solubility (LTLS) particles that have their acute inflammatory effects through surface area alone (Duffin et al., 2002). Soluble leachates of NPCB had no cellular or proinflammatory effects, again suggesting that no metals or soluble organics are involved (Brown et al., 2000).

Therefore, we can anticipate that the CNT “skeleton” will have proinflammatory potential that is driven by their surface area, as found for other graphitic NP.

The toxicology strategy for “short CNT” could, therefore, include comparison with NPCB and other LTLS particles for their ability to cause inflammation. Studies should benchmark CNT against the same surface area dose of LTLS to determine whether there is extra toxicity. Oxidative stress potential and proinflammatory signaling should be assessed.

Several in vivo instillation studies (Lam et al., 2004; Muller et al., 2005; Shvedova et al., 2005; Warheit et al., 2004) have compared NPCB with CNT and found the CNT to be more pathogenic, especially with regard to granuloma formation. It is not yet clear whether this is a result of chemical difference in the graphitic surface or physical size of the aggregates produced in the studies, a factor suggested by the authors of one study (Warheit et al., 2004).

Metals

Depending on the mode of synthesis, CNT may contain a number of toxic metals that can be viewed as contaminants in that they are not required for the function of the nanotubes. These metals include Co, Fe, Ni, and Mo, all of which have documented toxic effects. Transition metals such as Fe can be singled out as metals that are important in the toxicity of a range of pathogenic dusts through their ability to redox-cycle and cause oxidative stress (Donaldson et al., 1996; Ghio et al., 1999; Kennedy et al., 1989). The potential role of these soluble metals and matrix-bound metals should be a key strategy in determining the overall toxicity of the nanotubes and their mechanism(s).

The strategy for determining the role of metals should include testing of soluble fractions to determine the role of metals. This can be coupled with the use of leached and chelator-treated nanotubes, depleted of metals, to determine the effect of metal depletion on nanotube toxicity. The role of translocation and redistribution of metals should also be considered.

Organics

The argument for a potential role for organics in the toxicity of nanotubes comes from PM<sub>10</sub> where there is a large organic component in the NP fraction. Many of these organics are associated with diesel soot and other combustion NP and have the ability to generate free radicals and cause oxidative stress (Kendall et al., 2001). The organics in PM<sub>10</sub> (the mass of airborne particulate material per unit volume collected by a sampling convention with 50% efficiency for particles with an aerodynamic diameter of 10 µm) are complex and include combustion-derived molecules and also unburnt fuel and lube oil (Kendall et al., 2001; Squadrito et al., 2001). The latter will, of course, be absent in the combustion mix used for nanotube synthesis, but airborne organics could be present along with
contaminating organics on the reactants. If organics are produced in appreciable quantities, they can interact with enzyme systems in the lungs to result in oxidative stress (Hiura et al., 2000) via the production of superoxide (Kumagai et al., 1997), etc. Oxidative stress may then activate oxidative stress-responsive signaling pathways in cells, such as mitogen-activated protein kinase and NF-kB (Bonvallot et al., 2001), and deplete antioxidant defenses (Abe et al., 2000), leading to release of proinflammatory cytokines (Abe et al., 2000).

Strategies for assessing the role of organics would include solvent extraction and testing of the extracts and the organics-depleted CNT.

Aggregation

As described earlier, although MWCNT range from 10 to 100 nm in diameter, they have a strong tendency to bundle together in “ropes” due to van der Waals forces. Bundles typically contain many tens of nanotubes and can be considerably longer and wider than the nanotubes from which they are formed. This would be a very important factor modifying toxicity.

Deposition. Aggregates might have a much larger aerodynamic diameter than singlet CNT and so could be less respirable and could be large enough to deposit with a different anatomic pattern in the lungs compared to the singlet CNT.

Clearance and cell activation. Macrophages may very well be able to phagocytose a large aggregate of nanotubes as a single entity and clear it when the same amount of singlet CNT would be more difficult to handle. This would be evident as a different oxidative burst or gene expression response to a large aggregate than to singlet particles as a result of the surface area or volume of the aggregate (Duffin et al., 2002).

All deposited particles enter into the mucus or epithelial lining fluid, and so strategically, the role of these fluids is important and should be addressed in toxicity studies. The epithelial lining fluid of the centriacinar region contains surfactant and protein that could promote dispersion of CNT, including exfoliation or breakup of larger CNT aggregates.

Strategies for investigating the role of aggregation in in vitro studies and studies where CNT are presented to rat lungs should mimic the degree of aggregation seen in CNT in workplace air. Preliminary hygiene studies suggest that there is a considerable degree of aggregation of CNT in the air in workplace situations (Maynard et al., 2004). However, experimental studies should fully explore the role of aggregation status where possible. In this regard, the use of dispersants is key and this is discussed below for published studies.

Pathogenic Processes

The dominant mechanism for particle toxicity is oxidative stress, which plays a role in the adverse effects of all pathogenic particles and fibers so far studied (Donaldson and Tran, 2002). Both NP and fibers fit this paradigm, and it is likely that CNT will also cause oxidative stress, and there are some data to this effect. Oxidative stress triggers inflammation via oxidative stress–responsive transcription factors, and inflammation is central to the pathogenic effects of other particles (Donaldson and Tran, 2002). Oxidative stress from particles and inflammatory cells also causes the guanine adduct 8-hydroxy deoxyguanosine (8-OH-dG) that may be important in carcinogenesis by particles (Schins, 2002; Schins et al., 2002). Chronic inflammation and oxidative stress caused by ongoing exposure lead to a number of particle-specific effects. Fibers such as asbestos cause fibrosis and cancer (Mossman and Churg, 1998) that could be due to the direct effects of fibers on cells or as a result of oxidative stress from the fibers or the inflammatory response causing 8-OH-dG adducts leading to mutation (Kane, 1996). Acute increases in ambient air pollution particles (PM10) are linked to increases in exacerbations of airways disease and deaths and hospitalizations for cardiovascular disease (Pope and Dockery, 1999) that are considered to be driven by combustion-derived NP (Donaldson et al., 2005; Lighty et al., 2000; Neumann, 2001; Pope, 1995; Pope, III et al., 1999; Schwartz, 2000). In chronic exposure studies, increased incidence of lung cancer is associated with living in an area with increased PM10 (Pope, III et al., 2002), an effect that is likely driven by combustion-derived NP (Dybdahl et al., 2004). These same mechanisms are likely to be important in the pathogenic effects of CNT and are the end points that should be studied. The proclivity of asbestos to cause pleural pathology should be borne in mind in any strategy for assessing nanotube toxicology. The sensitivity of mesothelial cells to asbestos fibers is well known (Lechner et al., 1985), and so the consequences of nanotube-mediated effects on mesothelial cells is warranted.

Translocation

Some NP are characterized by their ability to translocate, from their site of deposition in the lungs to the blood (Nemmar et al., 2001, 2002) and the brain (Oberdorster et al., 2004). Chrysotile fibrils, which are in the order of thickness of MWCNT (20 nm), have been detected in the blood after gut ingestion (Weinzweig and Richards, 1983). Also, fibers have been reported to translocate to the pleural space where their presence is linked to the development of mesothelioma (Gelzleichter et al., 1999; Viallat et al., 1986). There are no data on this property for CNT, but this is clearly a potentially important process that should be included in any CNT research strategy, especially translocation to the pleura.

Figure 6 summarizes the likely toxicopathological pathways that could be important in the adverse effects of CNT.

PUBLISHED STUDIES ON THE EFFECTS OF SWCNT AND MWCNT IN TOXICOLOGICAL MODELS

Several existing studies address the potential pulmonary and cellular toxicity of nanotubes, and yet, none have addressed
whether the particle or fiber paradigm is dominant. Of the in vivo studies, none has used inhalation, and all use instillation where the high dose and dose rate raise questions about physiological relevance; no study has addressed the role of length, in the sense that none has compared long (>20 μm) to short (<10 μm) nanotubes. Although there are several in vitro studies in various models and end points, no biopersistence study has been carried out. Lam et al. (2004) demonstrated that a single intratracheal instillation in mice with three different types of SWCNT resulted in dose-dependent “epithelioid granulomas” and some evidence of interstitial inflammation. No size distribution was given for the CNT. Quartz and carbon NP at equal mass dose were used as controls, and the authors concluded that, on an equal mass basis, SWCNT in the lungs were far more toxic than carbon black and even quartz. The relative toxicity compared to quartz was evident from histological images where the nanotubes caused extensive granulomas and fibrous lesions, whereas the controls did not. Based on histological assessment, inflammation was variable with the different samples of nanotubes. However, bronchoalveolar lavage would have provided a more accurate quantitation of inflammation. The different samples of nanotubes had different metal contents, e.g., Fe and Ni, but this did not result in differences in their ability to produce granulomas, suggesting that metals could not explain this effect.

Warheit et al., evaluated the acute lung toxicity of intratracheally instilled SWCNT that were 1.4 nm wide × >1 μm long and contained appreciable amounts of Ni, Co, and amorphous carbon. In their study, the authors showed that exposure to SWCNT produced a transient inflammation and a non–dose-dependent accumulation of multifocal granulomas. The toxicological significance of these granulomas was, however, questioned as their formation was possibly due to the instillation of a bolus of agglomerated nanotubes (Warheit et al., 2004).

Muller et al. (2005) exposed rats intratracheally to whole or ground MWCNT of 0.7 or 5.9 μm length, respectively, and characterized their biological activity. These workers also measured biopersistence at two time points. The longer, unground nanotubes were more biopersistent than the short nanotubes at 60 days. Although this is in keeping with the greater biopersistence of long fibers seen in studies with asbestos and other mineral fibers, it should be noted that these long nanotubes were much shorter than those mineral fibers defined as long, which approach 20 μm (Donaldson and Tran, 2004). The CNT were highly fibrogenic and inflammogenic, being roughly equivalent to a chrysotile asbestos control (no size distribution given), and more active than NPCB (Muller et al., 2005). Ground nanotubes were on the whole more active than unground nanotubes, and this may have been a result of greater dispersion of the dose within the lungs, or grinding may release metals previously trapped within CNT structure, allowing them to be biologically active. An in vitro study generally supported the in vivo study with ground being more potent than unground in stimulating TNF-α production. Shvedova et al. (2005) studied mice exposed to SWCNT of 99.7% weight elemental carbon and 0.23% weight iron. The primary nanotubes were 1–4 nm in diameter, but, as delivered by pharyngeal aspiration, two distinct particle morphologies were observed: compact aggregates and dispersed structures. These two morphologies were linked to two distinct responses with dense SWCNT aggregates being associated with foci of granulomatous inflammation, including discrete granulomas with hypertrophic epithelial cells. In areas that did not have

FIG. 6. Important characteristics of nanotubes and their potential effects on processes that could impact on adverse effects.
these aggregates, diffuse interstitial fibrosis dominated, assumed to have been caused by exposure to the dispersed SWCNT. Control mice received NPCB or quartz, but these treatments did not cause thickening of alveolar walls or induce the formation of granulomas.

All the four studies had a similar design with quartz and NPCB as controls. All showed a similar florid fibrotic response to nanotubes that was absent with NPCB and quartz. Both CNT and carbon black are predominantly forms of graphite. This graphite is a rolled seamless sheet in the case of nanotubes plus various metals, amorphous carbon, and contaminants. In the case of carbon black, the graphite has a degenerated graphitic crystallite structure with little in the way of contaminants (Donaldson et al., 2005). The extended form of the graphite in nanotubes could, therefore, be important in encouraging fibroblast proliferation or activation of local cells to release stimulants of fibroblast proliferation and growth; the role of the contaminants of the nanotubes remains undetermined. None of these studies looked at translocation to the pleura or to extrapulmonary tissues.

No inhalation studies with nanotubes are yet available, and the extent of the necessary investment in such an undertaking cannot be overemphasized. A large and, therefore, expensive quantity of CNT is needed, a representative CNT type must be chosen, and the target concentration and the aggregations status and the relevance to the actual workplace cloud needs also to be considered. The inflammatory and fibrogenic effects seen so far may be a result of large nanotube complexes being formed during the instillation or pharyngeal aspiration procedure and that would not be present in a respirable cloud. Such aggregates, while pathogenic after instillation, might never reach the periphery of the lung under normal inhalation exposure because of their large aerodynamic size. However, large “feathery” aggregates may have a large physical diameter, but, since their density is low, they may have a small aerodynamic diameter and be respirable.

In in vitro studies, CNT have been tested in a range of different cell types to assess their potential toxicity. Treatments of human keratinocytes, mimicking potential dermal exposure, have shown that both SWCNT and MWCNT are capable of localizing within and causing cellular toxicity (Monteiro-Riviere et al., 2005; Shvedova et al., 2003). Human T lymphocytes, a possible target cell for nanotubes that gain access to lymphoid tissue following interstitialization, exposed to specifically oxidized MWCNT were found to be killed in a time- and dose-dependent manner, with higher concentrations inducing a marked decrease in cellular viability via an apoptotic mechanism (Bottini et al., 2006). Inhibition of cell function and the induction of apoptosis in vitro were reported in kidney cells treated with SWCNT (Cui et al., 2005). In a study with alveolar macrophages, SWCNT were more cytotoxic than MWCNT at equal mass dose using an assay that measures mitochondrial metabolism through the production of a colored formazan molecule (the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (1)bromide assay). However, this assay is potentially complicated in macrophages, as they release reactive oxygen species during phagocytosis that could potentially interfere with the oxidative process that generates the formazan product in the assay (Jia et al., 2005). Manna et al. (2005) demonstrated a clear dose-dependent activation of NF-κB and oxidative stress in human keratinocytes along with IkB depletion and MAPK phosphorylation. The ability to cause oxidative stress and inflammation is in clear concordance with other proinflammatory particles such as asbestos, PM10, and quartz that activate these pathways (Donaldson and Tran, 2002) and suggests that SWCNT may have some similarities to other pathogenic particles.

The extended repetitive structure of long asbestos fibers has been implicated in causing aggregation of the epidermal growth factor receptor (EGF-R) and initiating signaling cascades (Pache et al., 1998). The EGF-R is implicated in the induction of inflammation and fibrosis. The ordered graphitic structure of a long CNT might also have some ability to cause receptor aggregation. The idea that the graphitic structure could be important is supported by the finding that the more the surface of SWCNT is derivatized/functionalyzed, the lesser is its in vitro toxicity (Sayes et al., 2005).

Dispersion of CNT in Toxicology Studies

As discussed above, dispersion of CNT is problematic, and a number of different surfactants have been used for this purpose. Because of the importance of the particle surface in toxicology, the use of surfactants needs careful consideration and control. A number of different treatments have been used to disperse CNT in toxicological studies. In in vivo studies, Tween 80 has been used (Muller et al., 2005; Warheit et al., 2004) as well as sonication in phosphate-buffered saline (Shvedova et al., 2005) and shearing in a glass homogenizing tube plus sonication in heat-inactivated mouse serum (Lam et al., 2004). In in vitro studies, water or medium have been used as the dispersant in some studies (Bottini et al., 2006; Cui et al., 2005; Monteiro-Riviere et al., 2005), while one study used a Dounce homogenizer, sonication, and vortexing of particles in culture medium (Jia et al., 2005). One study dissolved the CNT first in dimethylformamide, and control treatments received an equivalent volume of dimethylformamide (Manna et al., 2005). Sayes et al. (2005) used “water-dispersible SWCNT” in their studies of functionalized CNT. With such a wide spectrum of different pretreatments of CNT in different assays, care needs to be taken in relating such toxicity data to the actual toxicity of CNT in the lungs. In the lungs, all particles encounter lung-lining fluid, which is likely to bind to the particles and can modify the toxicity of some particles (Brown et al., 1998; Fisher et al., 1998). No recommendations can presently be made as to the best dispersant to use since they have not been systematically compared as to effectiveness in causing dispersion or their toxicity.

Dispersion in air for inhalation studies is also potentially problematic in view of the well-documented tendency of the
nanotubes to aggregate together. However, if the workplace cloud is similarly aggregated, then the use of aggregated CNT in animal inhalation experiments may be warranted as the most relevant exposure.

REGULATION OF EXPOSURE IN WORKPLACES

Particles

Regulation intended to limit workplace exposure to chemicals, including particles, has been in place for many years in most industrialized countries. The required approach is highly similar in most cases. For example, in the United Kingdom, the legislation dealing with the control of exposure to harmful chemicals is the Control of Substances Hazardous to Health Regulations 2002. These requirements and associated guidance provide a step-by-step approach for employers to assess risks, implement measures needed to control exposure, assess the efficacy of these measures, and to establish good and safe working practices. In the United States, the Occupational Safety and Health Administration is responsible for developing and enforcing workplace safety and health regulations based on the same principles. The regulations specify control limits for workplace air expressed in terms of mass concentration per unit volume. Where it is appropriate to carry out assessment of exposure, the current best practice for airborne particles is to use a personal sampling device to collect a sample of the most appropriate, biologically relevant fraction (HSE, 2000) of the aerosol. It is common practice to use samplers conforming to the inhalable or respirable convention (see below), and samples collected via a personal sampling device are subsequently assessed either gravimetrically or via chemical analysis to determine the mass and hence average concentration over the defined period. These samplers provide an estimate of time-weighted mass concentration, from which personal exposure may be derived.

Currently, biological relevance is determined as a function of particle size, with the appropriate metric being aerodynamic diameter (Jones et al., 1988).

The two biologically relevant size fractions currently used are the inhalable (< 10-μm aerodynamic diameter) and respirable fractions (< 4.5-μm aerodynamic diameter). These are considered to represent the fraction of airborne material that can enter the human respiratory tract (inhalable) and can penetrate beyond the ciliated airways (respirable). Definitions for these fractions are well established and have international agreement (ACGIH, 1993; CEN, 1993; ISO, 1993). Mostly, occupational exposure limit values are defined either in terms of inhalable or respirable, although some substances have values for both. These well-established conventions represent size fractions which are much larger than those normally considered relevant to NP. One recent development, which reflects increasing concerns about the potential risks of NP, is the publication for comment, of a draft-recommended exposure limit for TiO₂ by NIOSH (2005). They recommend exposure limits of 1.5 mg/m³ for fine TiO₂ and 0.1 mg/m³ for ultrafine TiO₂. “Fine” is defined in this document as equivalent to the respirable fraction, and “ultrafine” is defined as the fraction of respirable particles with primary particle diameter < 100 nm. Determination of the proportion of ultrafine particles in the sample is based on transmission electron microscopy (TEM). Potentially, this approach may be used as a basis for and assessing compliance with standards for other NP aerosols.

Other than their use in conventions, there is little requirement to collect information about particle size of workplace aerosols. With the exception of fibers (see below), there is no requirement to collect information about particle number for workplace aerosols nor is there any requirement to collect information about particle surface area. In the case of NP, the surface area is likely to be the most relevant metric (Oberdorster et al., 2005a).

Fibers

In contrast to other workplace aerosols, where mass is the metric which is used to determine regulatory compliance, fibers are regulated in terms of particle number. The two main groups of fibers that have been of interest for regulation in the workplace are various types of asbestos fibers and various types of SVF. Many countries have established personal exposure limits for airborne fibers in workplace atmospheres expressed in terms of fiber number concentration. The method typically used to determine these concentrations is the so-called membrane filter method. Various specifications for the membrane filter method have been published, and while there are differences in detail between these specifications, the broad thrust of each method is the same.

Publication of the World Health Organization (WHO) method for determination of airborne fiber number concentrations (WHO, 1997) was an important attempt to harmonize these various methods. This WHO method is expected to be the basis of current and new regulation in most countries.

A key issue in the method is the definition of a fiber. WHO methods define a fiber as an object with length greater than 5 μm, a width less than 3 μm, and a length to width ratio (aspect ratio) greater than 3:1. The method is based on PCOM determination of the airborne fiber number concentrations, collected on a membrane filter by means of sampling pump. The analysis relies on manual fiber counting of a relatively small number of fibers, and the method is widely recognized as one of the least precise analytical techniques used in the occupational environment, being subject to a number of systematic and random errors as well as individual counter bias including limit of detection issues. The minimum visible width according to the WHO method is 0.13–0.15 × 10⁻⁶ m. However, in practice the smallest visible fibers will be about 0.2–0.25 μm wide. Since some fibers will fall below the limit...
of visibility, the count represents only a certain proportion of the total number of fibers present. Thus, the count represents only an index of the numerical concentration of fibers and is not an absolute measure of the number of fibers present. Fibers falling within a graticule within the eyepiece of the microscope are counted, and various counting rules are specified by the WHO for fibers partially out of the field of view, for fibers attached to particles, and for aggregates. Neither standard error of the mean (SEM) nor TEM are routinely used for fiber counting but can be used where more information, particularly on fiber type, is required, as recommended by the U.K. Health and Safety Executive (HSE, 1998).

CNT will only be visible by this method if they have sufficient contrast and are greater than 0.2–0.25 μm wide; in fact, this means that singlet CNT will not be countable by this methodology. As described earlier, CNT tend to aggregate into ropes, and these may be visible but they may not be greater than 5 μm in length, especially if, as has been observed for some CNT, they are not rigid and so they curl up (Maynard et al., 2004; Shvedova et al., 2005). It is clear that conventional fiber counting, as it applies to mineral fibers, is not currently practical for the determination of airborne CNT.

Implications for Regulation of CNT in Workplaces

Current regulations for the control of fibers in the workplace do provide a framework by which exposure to CNT in the workplace may be regulated. However, the use of these regulations in controlling CNT exposure presents several challenges. At this stage we cannot be sure how these materials will present in the workplace. It will depend on the specific material, the process of this manufacture or use, type of fiber, i.e., single walled or multiwalled, and the scenario.

From what we know about MWCNT, we might expect that these would have diameter from 20 to greater than 200 nm and that lengths could vary from less than 1000 nm to more than 10⁶ nm. They may be straight and partly rigid, or they may be bent or curled and partly flexible. They could appear as single particles or entangled as clumps or ropes. As single particles, those which were greater than 5 μm in length would certainly comply with the WHO definition of a fiber, having an aspect ratio greater than 3:1. However, those with diameters less than 200 nm would not be easily detected by PCOM due to the limit of optical detection. It is well recognized that fibers such as asbestos have a distribution of diameters. For this reason, while the count obtained in a PCOM analysis would also exclude fibers less than 200 nm, it is considered that the count would produce an acceptable index of the fibers which are present. It is clearly the case, however, that MWCNT will have a highly different distribution and may well be designed to have a very narrow distribution of diameters. So, e.g., a sample of MWCNT having a diameter of 30 nm would not be detected by PCOM.

MWCNT may also appear as clumps or ropes. In this case, if the individual fibers were not “easily distinguishable,” the rope would count as a single fiber provided that its characteristics lay within the definition of the fiber. If the rope did not lie within that definition, it would not be counted. This implies that in health terms fibers within the rope will continue to behave as a single entity as they enter the respiratory tract, which may or may not be the case.

It is not clear whether SWCNT can appear as single entities in workplace exposures. Exposures in scenarios for the production and use of SWCNT (and MWCNT) have not been well investigated. In the one study of SWCNT production that has been reported (Maynard et al., 2004), there is evidence to suggest that it is possible for single SWCNT entities to enter the workplace air. Much current effort and development relating to SWCNT is geared towards producing such material. Certainly, based on the dimensions of SWCNT we would not expect them to be detected by PCOM; therefore, while their physical dimensions are consistent with fibers, they would not be counted as such by routine approaches. Due to the uniformity of their dimensions, a count of fibers greater than 200 nm would not provide a reliable index. In the Maynard study, much of the SWCNT were found as clumps of materials. Although such entities would clearly be seen by PCOM, they would not generally be expected to comply with the definition of a fiber and would not be counted as such.

At least some forms of CNT are expected to have a structure which would require them to be classified as fibers. However, it is clear that many of them would not be detected by current optical methods. For SEM (and to some extent TEM) methods that have been used, these are not used routinely and are not expected as part of current regulatory processes. Use of SEM or TEM methods would be necessary to adequately assess levels of CNT present in workplaces. However, as these methods do not currently benefit from the rigor and quality approaches used in PCOM methods, much work would need to be undertaken (e.g., to establish sample collection and preparation methods, graticule field selection, magnification limits, counting rules, and proficiency testing schemes) to develop appropriate approaches by which routine assessment using these methods would be possible.

CONCLUSION

CNT are an important class of new materials that have numerous properties which make them useful to technology and industry. The predicted increase in manufacture and use makes human exposure likely, and so CNT are beginning to come under toxicological scrutiny in order to assess the hazard they present. This review has sought to set out the toxicological paradigms that can be used to investigate the toxicity of CNT; building on the toxicological database on NP and fibers. We also describe the current workplace regulation regarding exposure to particles and fibers and make suggestions about potential problems associated with nanotube exposure...
measurement. We conclude that nanotubes could have features of both NP and conventional fibers. According to the current paradigm for fiber toxicology, based on mineral fibers and SVF, they would pose a problem if they were longer than about 20 μm, a length so far not evaluated in any of the toxicological studies. If CNT behave like NP, then the existing paradigm for LTLS surfaces such as graphite is that the surface area dose likely drives toxicity/inflammation. Such peer-reviewed literature as is currently available suggests that CNT may have toxic effects beyond those anticipated for their mass exposure. For example, they have more adverse effects than the same mass of NP carbon and quartz, a commonly used yardstick of harmful particles. They seem to have special ability to stimulate mesenchymal cell growth. However, it should be noted that there is, as yet, no definitive inhalation study available that would avoid the potential for artifactual effects due to large mats and aggregates forming during instillation exposure procedures. CNT may have their effects through oxidative stress and inflammation (Fig. 6), and this is borne out by some published toxicology studies. Additionally, the studies so far suggest that they may have an unexpected ability to cause granuloma formation and fibrogenesis. The NP toxicology paradigm also emphasizes the potential for NP to translocate from their portal of entry to other tissues, and this should remain a potential target in CNT research.

The increasing use of CNT in industry means that the safety of those who are working with nanotubes requires consideration of the workplace regulation in the light of the peculiar problems presented by monitoring such small materials and the uncertainty of the nature, mechanism, and exposure response for adverse effects. Until better information becomes available, CNT should be considered in the same way as other biopersistent fibers in workplace risk assessments implying similar control and assessment approaches. However, difficulties in applying PCOM methods means that assessment based on electron microscopy should be strongly considered. Clearly, more research is needed to gain an insight into the mechanism of these adverse effects and on the best ways to measure CNT in the air in order to protect those that are exposed to this new material.

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