CHAPTER 3

Hazard and Risk Assessment of Workplace Exposure to Engineered Nanoparticles: Methods, Issues, and Carbon Nanotube Case Study*

Eileen D. Kuempel[1] and Vincent Castranova[2]

[1]*Nanotechnology Research Center, National Institute for Occupational Safety and Health, Cincinnati, OH, USA*

[2]*Department of Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Morgantown, WV, USA*

Chapter Outline

* Disclaimer: The findings and conclusions in this chapter are those of the authors and do not necessarily represent the view of the National Institute for Occupational Safety and Health.

3.1 Introduction

Toxicology data from experimental studies in animals are frequently used in risk assessment when human dose-response data are not available. Collaborations among industrial hygienists, toxicologists, risk assessors, and other disciplines provide an opportunity to obtain scientific data and develop an improved basis for assessing the risk of exposure to nanomaterials. In this chapter, the components of the risk assessment process are described, with a focus on assessment of occupational risk of inhaled particles and potential adverse lung effects. A case study using rat subchronic inhalation data of carbon nanotubes (CNTs) and carbon nanofibers (CNFs) is presented, highlighting two studies that were the primary basis for the exposure limit recommended by the National Institute for Occupational Safety and Health (NIOSH) (NIOSH, 2013)—that is, the Ma-Hock et al. (2009) and Pauluhn (2010a) studies of multiwalled carbon nanotubes (MWCNTs). In addition, more recent studies of MWCNTs (Kasai et al., 2014) and of CNFs (DeLorme et al., 2012) are evaluated in conjunction with the case study. These examples illustrate the application of risk assessment methods to currently available toxicology data for estimating the risk of adverse lung effects from occupational exposure to engineered nanoparticles. Challenges in using such data in quantitative risk estimation are discussed, and research needs are suggested to reduce uncertainties in risk estimates.

The data used in various risk assessments of CNTs to date are based on rat studies of pulmonary inflammation and fibrosis (Pauluhn, 2010a; Aschberger et al., 2010, 2011; Kuempel, 2011; Nakanishi, 2011; NIOSH, 2013). Additional *in vivo* studies have reported cardiovascular responses, as well as genotoxicity and cancer, in rodents (Tables 3.1 and 3.2). A large number of *in vitro* studies have also been published, but these studies are not discussed here because they have not yet been used in risk assessment. Studies in humans are extremely limited at this time. One health surveillance study with only nine subjects reported no adverse health effects (Lee et al., 2014).

3.1.1 Risk Assessment Paradigm

Risk assessment is a process to systematically characterize the scientific evidence of potential adverse health effects from human exposures to hazardous agents (NRC, 1983).

Table 3.1 Hazard data examples: rodent studies of single-walled carbon nanotubes (SWCNTs)

Response	Dose & Duration[a]	Species & Exposure Route[b]	Reference
Pulmonary inflammation Granulomas	0.1 or 0.5 mg per mouse (7 & 90 d pe)	Mouse ($B6C3F_1$, male); IT	Lam et al. (2004)
Cell proliferation—lung epithelial cells	0.4 mg per rat (1 and 21 d pe)	Rat (F344, female); PA	Mangum et al. (2006)
Pulmonary fibrosis (early onset and persistent)	5, 10, 20, 40 µg per mouse (1, 3, 7, 28 & 56 d pe)	Mouse (C57BL/6, female); PA	Shvedova et al. (2005)
K-ras oncogene mutations in lung tissue; pulmonary fibrosis	5 mg/m^3 (5 h/d, 4 d); 1, 7, & 28 d pe	Mouse (C57BL/6), female; inhalation (whole body)	Shvedova et al. (2008)
Cardiovascular—oxidative stress and plaque formation	20 µg per mouse every 2 weeks for 10 weeks (7, 28 & 56 d pe)	Mouse (C57BL/6, male); PA	Li et al. (2007)
Pulmonary fibrosis; transforming growth factor beta (TGF-β), greater bioactivity than asbestos	40 µg per mouse (1, 7 & 28 d pe)	Mouse (C57BL/6, female); PA	Murray et al. (2012)
Pulmonary fibrosis, greater bioactivity than asbestos	40 µg per mouse (up to 1 yr pe)	Mouse (C57BL/6, female); PA	Shvedova et al. (2014)

[a]In addition to 0 dose (control); pe: post-exposure.
[b]IT: intratracheal instillation; PA: pharyngeal aspiration; IP: intraperitoneal injection.

various forms worldwide includes four main steps: (i) hazard assessment, (ii) dose-response assessment, (iii) exposure assessment, and (iv) risk characterization (NRC, 1983). Research studies in various fields, including toxicology, exposure measurement, and computational methods, are needed to provide data for risk assessment in order to inform risk management decision making. Risk communication and processes to obtain stakeholder input are integral components of the risk assessment process. In many cases, sufficient data are not available for a full risk characterization, and risk management decisions may need to be made on the basis of the limited data available. A higher level of precaution in controlling exposures is prudent when the extent of the hazard is not well known, as with many nanomaterials (Schulte and Salamanca-Buentello, 2007).

This classic risk assessment paradigm was recently re-evaluated by the National Research Council (NRC) in response to a charge from the U.S. Environmental Protection Agency (EPA) to recommend improvements to the risk assessment process as practiced (NRC, 2009). In its report, the NRC recommended retaining the four basic steps of the risk assessment process and recommended additional steps to improve the utility of risk assessment and the technical analyses supporting risk assessment. Among these, the NRC proposed adding an

Table 3.2 Hazard data examples: rodent studies of multiwalled carbon nanotubes (MWCNTs)

Response	Dose & Duration[a]	Species & Exposure Route[b]	Reference
Granulomatous inflammation Lipoproteinosis	0.1, 0.5, 2.5 mg/m^3 (6 h/d, 5 d/wk, for 13 wk)	Rat (Wistar, male); inhalation (head & nose)	Ma-Hock et al. (2009)
Pulmonary inflammation and fibrosis	0.1, 0.45, 1.68, 5.98 mg/m^3 (6 h/d, 5 d/wk, for 13 wk)	Rat (Wistar, male); inhalation (nose-only)	Pauluhn (2010a)
Pulmonary inflammation, fibrosis, mesothelial hyperplasia	0.2, 1, 5 mg/m^3 (6 h/d, 1 d, 5 d/wk, for 13 wk)	Rat (F344, male & female); inhalation (whole body)	Kasai et al. (2014)
Pulmonary inflammation, fibrosis, pleural migration	5 mg/m^3 (5 h/day, 5 d/wk, 12 d)	Mouse (C57BL/6, male); inhalation (whole body)	Mercer et al. (2013a,b)
Pulmonary inflammation and fibrosis	0.5, 2, 5 mg per rat (28 & 60 d pe)	Rat (Sprague-Dawley, Wistar, female); IT	Muller et al. (2005, 2008)
Bronchiolitis obliterans Peribronchial fibrosis	12.5 mg per guinea pig (3 month pe)	Guinea pig (three-color, male); IT	Grubek-Jaworska et al. (2006)
Granulomatous inflammation Pulmonary fibrosis	10, 20, 40, 80 ug per mouse (1, 7, 28, 56 d pe)	Mouse (C57BL/6, male); PA	Porter et al. (2010)
Cardiovascular (loss of coronary artery dilution)	26 mg/m^3, 5 h	Rat (Sprague-Dawley, male); inhalation (whole body)	Stapleton et al. (2012)
Inflammation in peritoneal cavity, associated with carbon nanotube length	50 µg (1, 7 d pe)	Mouse (C57B1/6, f)—all IP	Poland et al. (2008)
Mesothelioma	0.003–3 mg IP (25 to 52 wk pe)	Mouse (p53(+/−, m))	Takagi et al. (2008, 2012)
No mesothelioma (ground MWCNTs)	2, 20 mg (2 yr pe)	Rat (Wistar, m)	Muller et al. (2009)
Mesothelioma (rigid MWCNTs); no mesothelioma (tangled MWCNTs)	1 and/or 10 mg each of four types of MWCNTs (1 yr pe; up to 3 yr pe for tangled MWCNTs exposure group)	Rat (F344/Brown Norway F1 hybrid, m, f)	Nagai et al. (2011, 2013)
Mesothelioma	0.05 to 3.0 mg of one of four types of rigid MWCNTs (2 yr pe)	Rat (Wistar, m)	Rittinghausen et al. (2014)

[a]In addition to 0 dose (control); pe: post-exposure.
[b]IT: intratracheal instillation; PA: pharyngeal aspiration; IP: intraperitoneal injection.

initial step in problem formulation and scoping, as well as revisions to the risk management phase to evaluate both risk and nonrisk information (e.g., technical feasibility) in a systematic evaluation of potential options. Toward the goal of improving the utility of risk assessment, the revised NRC framework explicitly requires reporting of what options are available to

reduce the hazards or exposures that have been identified and how risk assessment can be used to evaluate the merits of the various options (NRC, 2009).

In the absence of epidemiology data on workers exposed to engineered nanoparticles, much of the current focus in risk assessment involves toxicology studies in animals to assess the hazard, determine dose-response and time course relationships, and identify modes of action. The design of toxicology research studies for use in risk assessment necessitates an interface between toxicology and risk assessment to develop adequate data for qualitative and quantitative analyses. Evaluating the key information needs in this process provides an opportunity to focus additional research efforts on generation of data necessary to reduce uncertainties in estimating the hazard and risk of exposure to nanoparticles. As with workers exposed to other chemicals or particles, nanotechnology workers are likely to have the highest exposures and greatest potential for adverse health effects associated with the production of nanoparticles and their use in commercial applications. The hazard and dose-response assessment steps are discussed further in the following sections, as these steps are used in the quantitative risk assessment. The exposure assessment step (which is beyond the scope of this chapter) is needed to characterize the risk in a given population.

3.1.2 Hazard Assessment

The hazard assessment seeks to identify the nature of any hazardous effects and the evidence regarding the biological mode of action. Many of the same adverse lung responses previously reported following inhalation of fibers or fine particles are being found with exposure to nanoparticles, although often at lower mass doses due to the increased total particle surface area (Oberdörster and Yu, 1990; Driscoll, 1996; Sager et al., 2008; Sager and Castranova, 2009) or volume (Bellmann et al., 1991; Oberdörster et al., 1992; Pauluhn, 2010a) per unit mass for nanoparticles compared with their fine-sized analogues. Recent results suggest that the surface area of nanomaterial agglomerates may be more predictive of biological response than the surface area of primary nanoparticles within the agglomerate (Murray et al., 2012; Sager et al., 2015). This suggests that the biologically effective surface area of the particle is that of the outer "envelope" that is in contact with the cell surface. The Sager et al. (2015) results also point to the importance of evaluating the size distribution of the nanoparticles to which humans, animals, or cells are exposed. Poorly soluble nanoparticles (e.g., metal oxides such as titanium dioxide [TiO_2] and aluminum oxide [Al_2O_2]) have been shown to cause greater inflammation response in rodent lungs compared with the same mass of larger-sized respirable particles of the same chemical composition (Bermudez et al., 2002, 2004; Oberdörster and Ferin, 1992; Sager et al., 2008) and in *in vitro* cell assays (Rushton et al., 2010). Common pathways for the pulmonary pathogenicity of inhaled particles of varying sizes and shapes include direct cytotoxicity (e.g., due to reactive surfaces), activation of oxidant release from phagocytes, and secretion of inflammatory cytokines and/or proliferative factors (Donaldson et al., 1996;

Castranova, 1998, 2000; Oberdörster et al., 2005). These pathogenic pathways have been linked to interstitial fibrosis in rodent models and to rat lung tumorigenesis associated with chronic exposures to various types and sizes of poorly soluble particles, apparently by a mode of action involving indirect (secondary) genotoxicity due to the earlier inflammatory and proliferative events (ILSI, 2000; Castranova, 2000; Schins and Knaapen, 2007; Baan, 2007).

Persistent granulomatous inflammation and interstitial fibrosis are also among the responses observed in rodents exposed to MWCNTs or single-wall carbon nanotubes (SWCNTs) by various routes of exposure (intratracheal instillation, pharyngeal aspiration, or inhalation) (Tables 3.1 and 3.2). On a mass-dose basis, SWCNTs appear to be more fibrogenic than MWCNTs due to the enhanced ability of SWCNTs to avoid uptake by alveolar macrophages and to enter the alveolar interstitium (Mercer et al., 2011). In addition, pulmonary exposure to SWCNTs has been associated with oxidative stress and enhanced plaque formation in the aorta, and intraperitoneal exposure to MWCNTs has been linked to mesothelioma in some studies (Tables 3.1 and 3.2). MWCNTs and SWCNTs have been shown in several studies to be more potent on a mass basis (i.e., a lower dose associated with a given adverse lung response, or a greater adverse response at a given dose) compared with ultrafine carbon black (Table 3.3) and other poorly soluble particles, including silica and asbestos (Elder et al., 2006; Lam et al., 2004; Muller et al., 2005; Murray et al., 2012; Shvedova et al., 2005, 2014). In contrast to the metal oxides, cellular responses to CNTs are not well predicted by the reactive oxygen species generation; rather, the nanostructured CNTs appear to act as a basement membrane substrate that enhances fibroblast proliferation and collagen production *in vitro*

Table 3.3 Adverse effect levels in rats after subchronic (13-week) inhalation exposure to carbon particles and carbon nanotubes

Study	Compound	Effect Level in Rats		Effect
		NOAEL (mg/m^3)	LOAEL (mg/m^3)	
Elder et al. (2006)	Ultrafine carbon black (Printex 90)	1	7	Pulmonary inflammation
Ma-Hock et al. (2009)	Multiwalled carbon nanotubes (BASF, Nanocyl)	n.d.	0.1	Granulomatous inflammation
		0.1	0.5	Alveolar proteinosis
Pauluhn (2010a)	Multi-walled carbon nanotubes (Baytubes) (Bayer)			Pulmonary inflammation
		0.1	0.45	Alveolar septal thickening
Kasai et al. (2014)	Multiwalled carbon nanotubes (Mitsui-7)	-	0.2	Pulmonary inflammation, Interstitial hyperplasia

NOAEL: No observed adverse effect level.
LOAEL: Lowest observed adverse effect level.

(Wang et al., 2010). *In vivo*, this would result in thickening of the alveolar septal air–blood barrier and a decrease in gas exchange between the lung and blood (Mercer et al., 2011).

Some types of MWCNTs and SWCNTs have also been shown to elicit similar biological effects as fibers in that the longer, thinner structures are more inflammogenic (Poland et al., 2008) and can penetrate from the lung subpleural tissue to the intrapleural space (Mercer et al., 2010). SWCNTs and MWCNTs have been shown to interfere with normal cell division in cell culture systems (Muller et al., 2008; Sargent et al., 2009) and *in vivo* (mice) (Sargent et al., 2014). MWCNTs can cause the two normal centrosomes to cluster, forming a single pole. The resulting mitotic spindles are monopolar rather than bipolar (Sargent et al., 2011). In addition, MWCNTs have been reported to form cross-bridges between multiple cell nuclei after pulmonary exposure (Muller et al., 2008). In contrast, SWCNTs appear to fragment centrosomes, causing multipolar mitotic spindle formation, abnormal chromosome division, and aneuploidy (Sargent et al., 2009). In comparison, chrysotile asbestos also interferes with the normal mitotic process but not by binding to centrosomes. Rather, asbestos fibers interact with mitotic spindles and interfere with cytokinesis by forming bridges to prevent normal separation of daughter nuclei (Asakura et al., 2010). MWCNTs have also been shown to translocate from the lungs to the mesothelial tissue lining the lung (Ryman-Rasmussen et al., 2009; Mercer et al., 2010, 2013b; Xu et al., 2012; Kasai et al., 2014), to lung-associated lymph nodes (as do other inhaled particles), and to other organs, including the liver and kidneys, with tissue damage observed in those organs (Reddy et al., 2010; Mercer et al., 2013b). Other nanoparticles (e.g., silver, iridium) have also been shown to translocate from the lungs via the systemic circulation to other organs and tissues (Takenaka et al., 2001; Semmler et al., 2004; Semmler-Behnke et al., 2007).

Compared with larger particles, nanoparticles have the unique ability to enter and interact with cells and cell organelles. Individual nanoparticles of TiO_2 have been observed inside cell organelles, including in the cell nucleus (Geiser et al., 2005) and in mitochondria, which can disrupt mitochondrial and cellular functions (Li et al., 2003). In addition, Mercer et al. (2010) have published electron micrographs showing individual MWCNTs within alveolar macrophages and epithelial cells. Spherical nanoparticles that are deposited in the nasal region have been shown to enter the brain via neuronal transport in the rat and cause inflammation in the olfactory bulb (Elder et al., 2006; Oberdörster et al., 2002, 2009).

The nature of the hazard and mode of action influence the extent to which information on larger particles of the same chemical composition or surface reactivity can be reliably extrapolated to nanoparticles. In the case of poorly soluble particles, a relationship between the particle surface area dose of nanoscale or larger particles and pulmonary inflammation or other adverse lung effects (including rat lung tumors in chronic studies) has been reported (Oberdörster and Yu, 1990; Driscoll, 1996; Sager et al., 2008; Sager and Castranova, 2009). Therefore, utilizing the available data for other particles and fibers may facilitate hazard

and risk characterization for classes of materials with common modes of action. However, additional data are needed to link the potential biological effects of the vast number of nanomaterials to given physicochemical properties (Rushton et al., 2010) in order to develop predictive hazard/risk grouping strategies.

3.1.3 Dose-Response Assessment

The basis for quantitative risk assessment is the data on dose-response relationship. Studies that provide epidemiologic data are generally preferred for risk assessment, since there is no uncertainty about extrapolation across species or about the species-relevance of the response endpoint. However, quantitative exposure data are often not available in epidemiologic studies, and in the case of nanoparticles no epidemiologic studies have been reported. Thus, experimental data in animals are used to examine dose-response relationships. Standard methods of risk assessment involve determination of either an adverse effect level (no observed or lowest observed) or a benchmark dose estimate. In either case, the animal dose must be extrapolated to humans, either by allometric scaling (i.e., based on body weight) or other data available on the factors that influence dose to the target tissue in each species (i.e., adsorption, distribution, metabolism, elimination). A potentially useful metric to scale human versus animal dose when evaluating pulmonary exposure–response is deposited dose per surface area of alveolar epithelium (Sections 3.2.3 and 3.3.1).

No observed or lowest observed adverse effect levels

A lowest observed adverse effect level (LOAEL) or no observed adverse effect level (NOAEL) approach has often been used as the point of departure (POD) in risk assessment of noncarcinogenic agents. The NOAEL is defined as the highest dose at which no adverse effects have been detected; and the LOAEL is the lowest dose at which adverse effects have been detected (EPA, 2012). A POD is the external exposure or internal dose to which uncertainty factors or low dose extrapolation methods are applied to derive an exposure limit that is considered acceptable (i.e., associated with no risk or low risk) in humans. Statistical evaluations are usually performed to determine an NOAEL, that is, the dose at which no statistically significant increase in adverse effects is observed. An important area of uncertainty in NOAEL estimation is that it is dependent on the limit of detection within a given study.

For noncancer endpoints, a common assumption in risk assessment is that low doses (e.g., where detoxification and clearance mechanisms are effective and any damage to cells is effectively repaired) would not be associated with any appreciable risk of adverse effects. The NOAEL is thus considered a threshold dose below which adverse effects would not be expected. The NOAEL (or LOAEL) is typically divided by "uncertainty factors" (otherwise known as "safety factors" or "adjustment factors") to account for uncertainty in the use of these estimates as PODs for risk assessment. Standard uncertainty factors typically include

the following four factors: (i) extrapolating the animal data to humans (both toxicokinetic and toxicodynamic factors), interindividual variability in the distribution of human responses (including most of the sensitive individuals in a population), uncertainty in estimating a chronic response from subchronic data, and/or the use of an LOAEL in the absences of an NOAEL. Factors of 10 for each have typically been used in the absence of other data (WHO, 2005; EPA, 2012). These uncertainty factors are intended to provide a sufficient margin of safety such that no "appreciable risk of deleterious effects in humans" (EPA, 2012) would be expected at exposures below the calculated exposure limits.

The assumption of a threshold dose for noncarcinogens may not be applicable in all cases (e.g., if exposure to a hazardous agent adds to a response associated with another environmental exposure or to background disease processes or incidence) and may not adequately account for interindividual variation in a population (NRC, 2009; White et al., 2009). Benchmark dose (BMD) estimates are generally preferred, if feasible, as a POD for either cancer or noncancer endpoints (NRC, 2009), as discussed in the next section.

Benchmark dose methods

A BMD estimate has several advantages over an NOAEL or LOAEL as a POD in risk assessment when sufficient dose-response data are available (Crump, 1984, 1995; NRC, 2009; EPA, 2012). A BMD is a risk-associated dose estimated by model curve fitting to the dose-response data. BMD estimates have been used in both cancer and noncancer risk assessments. Some examples of using BMD estimates in risk assessment of engineered nanomaterials include those using dose-response data in rodents for pulmonary responses to inhaled fine and nanoscale (ultrafine) particles (Kuempel et al., 2006; Dankovic et al., 2007; NIOSH, 2011) or to CNTs (Kuempel, 2011; NIOSH, 2013).

The term "benchmark dose" is defined as "...a statistical lower confidence limit for the dose corresponding to a specified small increase in level of [adverse] health effect over the background level" (Crump, 1984). In practice, the term "benchmark dose" is often used for the maximum likelihood estimate, whereas the BMD limit (BMDL) is the lower 95% confidence limit. The benchmark response (BMR) is the adverse response level associated with the BMD (BMDL). A BMR is typically in the low region of the dose-response data for example, a 10% response, which is near the statistical lower limit of detection in an animal bioassay. For dichotomous (yes/no) response data, a BMD can be defined as the dose associated with either an extra risk (relative to the background probability of having a normal response) or an excess risk (additional probability above background) (Crump, 2002). Excess risk is used in the example in this chapter because it provides an estimate of the exposure-attributable risk. The BMD is calculated as the dose, d, corresponding to the specified excess risk in the proportion of animals with a given adverse lung response (BMR):

$$BMR = P(d) - P(0)$$

where $P(d)$ is the probability of an adverse response at the BMD, and $P(0)$ is the probability of that adverse response in an unexposed population (Crump, 2002; EPA, 2006).

BMD methods and models are also available for continuous response data (Crump, 1995, 2002; EPA, 2010), although a detailed discussion is beyond the scope of the chapter. Briefly, BMD estimation using continuous data requires specifying a BMR level along a continuum of responses. Continuous response measures may be associated with normal biological structure or function, which can be perturbed in response to a toxicant and eventually result in a functional impairment. Toxicology studies can provide dose-response data for quantitative risk assessment based on continuous responses, as well as information on the biologically relevant level of response in animals and humans.

The BMD method is often preferred to obtain quantitative risk estimates for either cancer or noncancer endpoints (NRC, 2009). BMD estimates are also more useful in estimating the health benefits of reducing exposures, for example, in the context of developing recommended exposure limits, including for regulatory decision making (U.S. Supreme Court, 1980).

Comparison of BMD and NOAEL/LOAEL estimates

There are several advantages of BMD methods over the NOAEL/LOAEL approach: (i) The BMD curve fitting uses all of the data in the dose-response relationship, not just a single data point; (ii) whereas the NOAEL and LOAEL doses are dependent on the particular dose groups and spacing selected for the study (and tend to be higher in studies with fewer observations), the BMD method can provide dose estimates at a constant level of risk (e.g., 10%) for better comparison across studies; (iii) the BMD method takes appropriate statistical account of the sample size and provides estimates of the confidence limits on the BMD estimates; (iv) whereas an NOAEL or LOAEL approach assumes a threshold response regardless of the shape of the dose-response relationship, BMDLs are risk estimates derived from a statistical model fit to the dose-response data. A comparison of NOAELs and BMDs showed that the estimated risk associated with NOAELs were not negligible but ranged from 3% to 21% (Leisenring and Ryan, 1992). Finally, BMD methods provide a consistent framework for comparing the potency (severity of response at a given dose) of various substances and for extrapolating to doses associated with lower risks. As such, BMD methods may facilitate risk comparisons across an array of nanoscale and larger particles.

BMD methods require sufficient data to characterize the dose-response relationship. Dose-response relationships may show an increasing or decreasing trend, depending on the endpoint (e.g., an increase in an adverse effect or a decrease in a normal function associated with increasing dose). At least two dose groups in addition to the control group are generally needed for BMD modeling, although a reasonable BMD estimate may be obtained if the elevated response in the one exposed group is near the BMR (EPA, 2012). More dose

groups may be needed to adequately describe highly nonlinear relationships. If adequate dose-response data are not available for BMD estimation, an NOAEL or LOAEL may be used as the POD for low dose extrapolation or application of uncertainty factors (EPA, 2012). Toxicology study designs that take into consideration the BMD data requirements can greatly facilitate the study utility for quantitative risk assessment.

3.1.4 Interspecies and Temporal Extrapolation

As for most chemicals, data on the potential adverse health effects of nanomaterials on workers are limited. Thus, shorter-term (13-week) studies in rodents (e.g., on subchronic inhalation) often are used to estimate potential health hazards to workers. The LOAELs and NOAELs in studies of humans suffering particle exposures (presumably airborne) were reported to be generally lower than those in animals, suggesting that humans may be generally more sensitive (i.e., 53%, 21%, or 27%, respectively, of higher, similar, or lower sensitivity in humans than animals) (Kalberlah et al., 2002). Similar results were reported for exposures to gases.

Temporal evaluations in animals showed that the NOAELs and LOAELs following chronic exposures were often lower than those from shorter-term studies (Kalberlah et al., 2002). In an analysis of the U.S. National Toxicology Program of 46 subacute, subchronic, and chronic studies in rodents, Kalberlah et al. (2002) estimated that the effect concentrations (NOAELs or LOAELs) in subchronic (13-week) studies underestimated the chronic response by a factor of approximately 2.7 (geometric mean) (1.0–20, 10th and 90th percentiles). Most of those substances were reported to be respiratory irritants acting in the extrathoracic region (with a few acting in the tracheobronchial or pulmonary regions), On the basis of that analysis, the standard uncertainty factor of 10 to extrapolate from subchronic to chronic dose and response (EPA, 2012) would thus seem to be reasonable on average, although it may not be sufficiently protective in the case of some substances. For example, the limited data on substances acting in the tracheobronchial and pulmonary regions prevented a separate statistical evaluation of those substances (Kalberlah et al., 2002); such region-specific information would be useful in assessing the risk of adverse respiratory effects from exposure to airborne particles (including nanodiameter and microdiameter particles) that could deposit in these regions..

In the current example for respirable MWCNTs, the adverse lung responses are assumed to relate to the total estimated lung dose (deposited or retained), which are dose metrics that have been associated with fibrotic and other adverse lung effects from exposure to various other types of poorly soluble particles in animals and humans (e.g., Muhle et al., 1991; Kuempel et al., 2001a; Dankovic et al., 2007). In this case study example, instead of applying an exposure duration uncertainty factor, the total deposited or retained lung doses in rats (over the 13-week subchronic exposure), the total deposited or retained lung doses in rats (over the 13-week subchronic exposure) are converted to equivalent lung doses in workers assuming

exposures up to a 45-year working lifetime. In the absence of a validated lung model for CNT clearance in humans, the deposited or retained lung (alveolar) dose estimates provide bounds on the possible lung burdens in workers, that is, upper and lower, respectively, since some of the CNTs deposited maybe cleared by alveolar macrophages, although at a potentially lower rate than in the case of spherical particles (Pauluhn, 2010a; Mercer et al., 2013a; NIOSH, 2013). The pulmonary region is the focus of these case studies, based on the data available in the rodent studies; however, CNTs deposited in the tracheobronchial region could be a risk factor for diseases of the airways, including cancer (Schulte et al., 2012).

3.2 Case Study Example: Carbon Nanotubes

Three recent subchronic inhalation studies in rats of MWCNTs (Ma-Hock et al., 2009; Pauluhn, 2010a; Kasai et al., 2014) provide examples of dose-response data currently available for quantitative risk assessment of some engineered nanomaterials. These studies are relevant to occupational risk assessment, given that the target organ (lungs), exposure route (inhalation) and pattern (5 day/wk, 6 h/day), and lung responses in the rats were similar to those observed in humans with occupational exposures to other poorly soluble respirable particles (Attfield and Seixas, 1995; Kuempel et al., 2001a; Gardiner et al., 2001).

3.2.1 Data Description

The three MWCNT subchronic studies in rats had similar study designs, although the MWCNT material varied somewhat in their physicochemical properties. In Ma-Hock et al. (2009), the MWCNTs (produced by a vapor deposition technique) had a primary particle diameter of 5–15 nm and length of 1–10 μm; contained 9.6% Al_2O_3 and traces of iron and cobalt; and the specific surface area was 250–300 mg^2/g based on the Brunauer, Emmett, and Teller (BET) method (Brunauer et al., 1938). The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were approximately 1.2 and 2.7, respectively (median value reported). In Pauluhn (2010a), the MWCNTs (Baytubes, a proprietary product of Bayer MaterialScience, Leverkusen, Germany; production method not reported) had a primary particle diameter of ~10 nm and a median length of 200–300 nm; contained 0.5% Co; and the specific surface area (BET method) was 253 m^2/g (bulk). The MMAD and GSD were approximately 2.7 and 2.1, respectively (median value reported). In Kasai et al. (2014), the MWCNTs (produced by floating chemical vapor deposition) had a primary mean diameter of 90.7 nm and a mean length of 5.7 μm; the carbon content was >99.6% (with trace iron contaminant); and the specific surface area was 24–28 m^2/g. The MMAD and GSD range was 1.4–1.6 μm and 2.3–3.0, respectively. In a study of CNFs (vapor grown), by DeLorme et al. (2012), the diameter was 158 nm (range of 40–350 nm), length was 5.8 μm (range of 1–14 μm); content >99.5% carbon (with <0.003% iron); specific surface area (BET method): 13.8 m^2/g. Across the exposure groups, the MMAD was 1.9 to 3.3 μm

and the GSD was 2.0 to 3.1 (DeLorme et al., 2012). Estimated alveolar deposition fractions of MWCNTs or CNFs in rats were approximately 0.05 to 0.07 but may have been as low as 0.02 (NIOSH, 2013, Table A.2 and A.9; Section A.7.6). In each of these subchronic studies, rats were exposed by inhalation 6 h/day, 5 day/week, for 13 weeks. Lung responses were examined at the end of exposure in each study; postexposure follow-up was 3 months in the DeLorme et al. (2012) study (0 and 25 mg/m^3 groups) and up to 6 months for all groups in the Pauluhn (2010a) study.

The exposure concentrations in the Ma-Hock et al. (2009) study were 0, 0.1, 0.5, and 2.5 mg/m^3 (male and female Wistar rats); an LOAEL of 0.1 mg/m^3 was identified for granulomatous inflammation, in which 30% of rats had developed minimal or higher-grade inflammation based on histopathology. At 0.5 mg/m^3, 85% of the rats had developed lipoproteinosis. The exposure concentrations in the Pauluhn (2010a) study were 0, 0.1, 0.45, 1.62, and 5.98 mg/m^3 (male and female Wistar rats). The NOAEL was identified at 0.1 mg/m^3, and the LOAEL was 0.45 mg/m^3 for pulmonary inflammation, based on elevated polymorphonuclear leukocytes (PMNs) in bronchoalveolar lavage fluid (BALF), and on alveolar interstitial (septal) thickening, of which 90% of rats had developed minimal or higher-grade inflammation based on histopathology (Pauluhn, 2010a). The exposure concentrations in Kasai et al. (2014) were 0, 0.2, 1, and 5 mg/m^3 (male and female F344/DuCrlCrlj rats). The LOAEL for granulomatous lesions and changes in BALF was 0.2 mg/m^3. The LOAEL for interstitial fibrosis was 1 mg/m^3. In the DeLorme et al. (2012) study, the exposure concentrations were 0.54, 2.5, and 25 mg/m^3 (male and female Crl:CD Sprague Dawley rats). The NOAEL was 0.54 mg/m^3; and the LOAEL for minimal inflammation in the terminal bronchiole and alveolar duct areas was 2.5 mg/m^3.

3.2.2 Severity of Effects

Quantitative risk assessment involves estimation of the severity and likelihood of an adverse response associated with exposure to a hazardous agent (Piegorsch and Bailer, 2005; NRC, 2009). Although pulmonary fibrosis has not been studied in those working with CNTs, it has been associated with occupational exposure to various types of respirable particles and fibers, including carbon black (Gardiner et al., 2001), coal dust (Attfield and Seixas, 1995), silica (Park et al., 2002), and asbestos (Stayner et al., 2008). Chest radiography or computed tomography is used in medical examinations to identify the occurrence and severity of fibrosis. In animal studies, a more sensitive measure of pulmonary fibrosis is the amount of alveolar interstitial thickening. Since gas exchange occurs across the alveolar septal air–blood barrier, such thickening of the alveolar septum due to fibrosis can interfere with normal lung function.

The rat subchronic lung responses to inhaled MWCNT effects were relatively in the early stage (minimal or mild histopathology severity grades) for either pulmonary septal

thickening, including fibrosis (Pauluhn, 2010a; Kasai et al., 2014) or granulomatous inflammation (Ma-Hock et al., 2009; Kasai et al., 2014). In the Pauluhn (2010a) study, the alveolar septal thickening observed in response to CNT exposure persisted for at least 26 weeks after the end of the 13-week exposure (i.e., at week 39). Several toxicology studies in which mice were exposed to SWCNTs or MWCNTs via pharyngeal aspiration have also shown dose-dependent alveolar septal thickening, and this response persisted or progressed with longer postexposure time (Shvedova et al., 2005, 2008; Mercer et al., 2008; Porter et al., 2010). This progressive alveolar interstitial fibrotic response was verified in a 12-day inhalation study of mice with as long as a 336-day postexposure evaluation (Mercer et al., 2013a). Although limited information is available to evaluate whether the lung responses in animals exposed to CNTs are associated with functional impairment, changes in breathing pattern in SWCNT-exposed mice have been noted (Shvedova et al., 2005). In addition, alveolar septal thickening has been considered relevant to humans and indicates "fundamental structural remodeling" (e.g., in response to ozone exposure) (EPA, 1996; Stockstill et al., 1995). In the Ma-Hock et al. (2009) study, fibrosis was not evaluated, but a subsequent study of the rat lung tissue from the Ma-Hock et al. (2009) study reported no observed fibrosis (Treumann et al., 2013). The findings of granulomatous inflammation and lipoproteinosis observed in that study are also consistent with the development of pulmonary fibrosis in rodents and humans (e.g., from silica exposure) (Porter et al., 2004; Heppleston, 1975; Hoffmann et al., 1973). Therefore, these rat subchronic lung effects in response to CNT exposure may be considered to be in the range of early biological responses associated with altered structure and function (Schulte, 1989) (Figure 3.1).

A more detailed and quantitative scale of adverse effects has been developed for use in deriving inhalation reference concentrations (EPA, 1994). On the basis of that scale (from 0 to 10), these pulmonary changes observed in rats with subchronic exposure to MWCNTs may

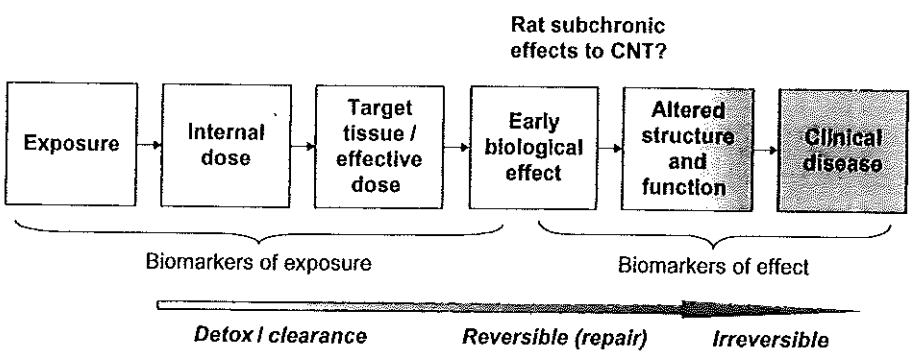

Figure 3.1
Biological continuum from dose to disease with consideration of the lung responses to CNT (carbon nanotubes) observed in the rat subchronic inhalation studies (Ma-Hock et al., 2009; Pauluhn, 2010a; Kasai et al., 2014). *Adapted from NRC (1987) and Schulte (1989).*

correspond somewhere in the range of levels 6–8, although the observed effects may not align exactly with one level:

- Level 6 (LOAEL): Degenerative or necrotic tissue changes with no apparent decrement in organ function
- Level 7 (LOAEL): Reversible slight changes in organ function
- Level 8 (LOAEL/FEL (defined below)): Pathologic changes with definite organ dysfunction that are unlikely to be fully reversible

These levels are consistent with the more qualitative evaluation depicted in Figure 3.1. Effect levels 6 and 7 are considered LOAELs, whereas level 8 is considered an LOAEL/FEL (EPA, 1994). An FEL is a "frank effect level," defined as an "exposure level that produces frankly apparent and unmistakable adverse effects, such as irreversible functional impairment or mortality, at a statistically and biologically significant increase in frequency or severity between an exposed population and its appropriate control" (EPA, 1994). Clearly, a goal in risk assessment is to estimate levels of exposure that are not likely to be associated with any material impairment of health or functional capacity, even if exposures occur over a person's full working lifetime (OSH Act, 1970).

3.2.3 Quantitative Risk Assessment Procedures

The risk assessment process based on animal data, focusing on rodent dose-response data of inhaled particles, is shown in Figure 3.2. An example of the steps in this process, as applied to rat subchronic inhalation studies of MWCNTs, is described in this section.

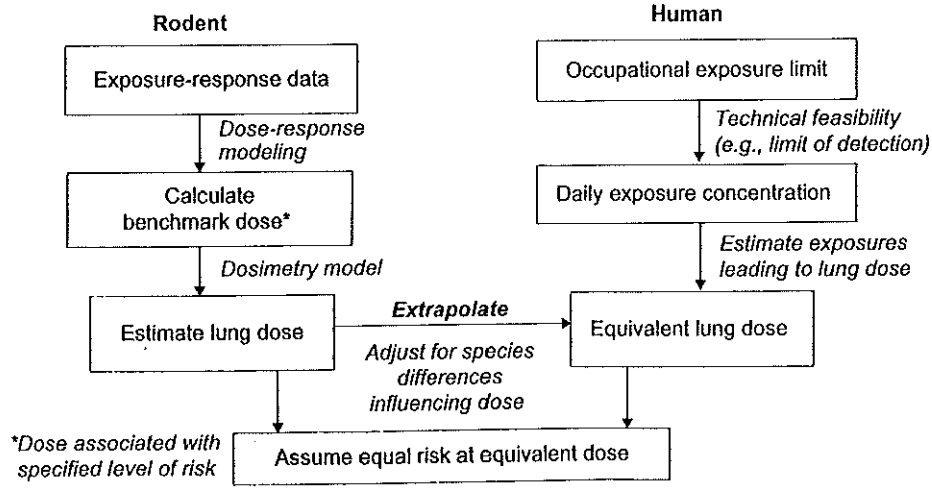

Figure 3.2

Risk assessment steps using animal data of airborne particles, e.g., carbon nanotubes, to develop occupational exposure limits. *Adapted from Oberdörster (1989) and Kuempel (2011).*

Step 1. Evaluation of the exposure (or dose) and response data

The exposure-response data from the three published subchronic inhalation studies of MWCNTs (Ma-Hock et al., 2009; Pauluhn, 2010a; Kasai et al., 2014) are evaluated for possible use in risk assessment because they provide data of relevance to workers (inhalation route of exposure, daily exposures), well-characterized materials (particle size data and chemical composition), and quantitative measures of dose and response. The rat lung responses to respirable MWCNTs are compared with those for CNFs (DeLorme et al., 2012).

The rat lung responses of granulomatous inflammation (Ma-Hock et al., 2009), pulmonary septal thickening (Pauluhn, 2010a), or both granulomatous inflammation and trichrome straining for collagen (Kasai et al., 2014) at minimal or higher severity (grade 1) based on histopathology are selected because they are sensitive, early-stage adverse lung responses to CNT exposure and are relevant to lung disease development in humans. When internal dose data are reported (e.g., lung tissue burden of CNTs), the dose-response data can be used in the estimate of BMD levels and extrapolated to humans based on the estimated equivalent dose in the lungs (e.g., NIOSH, 2013).

Step 2. Estimation of a point of departure

As described earlier (Section 3.1.3), a POD based on a BMDL is estimated by fitting statistical models (e.g., using the BMD software, BMDS (EPA, 2010, 2012)) to rat dose-response data, which, in this case, are the data from each study of CNTs in rats (Ma-Hock et al., 2009; Pauluhn, 2010a; Kasai et al., 2014). A subchronic inhalation study of CNFs in rats (DeLorme et al., 2012) is not included in these case study estimates because the comparable dose-response data for adverse interstitial responses of fibrosis or granulomatous inflammation by histopathologic evaluation were not reported. Other endpoints (e.g., percent PMNs or cell proliferation) might be used in other modeling comparisons; those endpoints (PMNs in male rats and cell proliferation in female rats) remained significantly elevated at the 25 mg/m^3 dose at 90 d after exposure (DeLorme et al., 2012). When estimated deposited lung doses were compared, the adverse lung responses to CNFs in rats (DeLorme et al., 2012) were similar to those observed in mice (Murray et al., 2012; NIOSH, 2013).

In this example, the "dose" is the airborne exposure concentration, resulting in the estimation of a benchmark concentration (BMC) (maximum likelihood estimate) and a lower 95% confidence limit (BMCL) estimate. A challenge in using these data in risk assessment, as shown in the NIOSH (2013) risk assessment, is that the multistage model was the only one in the BMD model suite (EPA, 2010) that converged to a unique solution, or provided adequate fit to the data ($p > 0.1$ in a goodness of fit test) (EPA, 2012) in the Ma-Hock et al. (2009) and Pauluhn (2010a) studies. This is due to the steep dose-response relationship and the sparse data near the 10% BMR, which provided little information for the curve fitting and resulted in multiple solutions in several models. The dose-response data for granulomatous changes in Kasai et al. (2014, Table 2) revealed similar behavior, suggesting similar model-fitting issues.

Table 3.4 Benchmark dose estimates[a] and associated human working lifetime airborne concentrations—based on subchronic inhalation of MWCNTs in rats and estimated deposited lung dose[b] (NIOSH, 2013)

Rodent Study and Response[c]	Rat BMC (BMCL)[d] (mg/m^3)	Rat BMD (BMDL)[e] (μg/lung)	Human-equivalent BMD (BMDL) (mg/lung)	Human-equivalent BMC (BMCL): 8-h TWA & 45 work-years ($\mu g/m^3$)
Granulomatous inflammation (Ma-Hock et al., 2009)	0.060 (0.023)	21 (8.1)	5.4 (2.1)	0.51 (0.19)
Focal alveolar septal thickening (Pauluhn, 2010a)	0.10 (0.051)	28 (14)	7.2 (3.5)	0.77 (0.38)

[a]Benchmark response level: 10% excess (added) risk in exposed animal (EPA, 2010).
[b]Estimated deposited lung dose in rats and humans estimated using MPPD 2.0 model (CIIT and RIVM, 2006); aerodynamic particle sizes (MMAD, GSD): 2.74 (2.11).
[c]Responses are histopathology severity grade 1 or higher.
[d]BMC (BMCL)s; BMC: maximum likelihood estimate of the benchmark concentration; 95% LCL: 95% lower confidence limit of the BMC; dose-response data fit with multistage model (polynomial degree 2) (EPA, 2010). *P*-values for the rodent dose-response models: 0.99 for Ma-Hock et al. (2009) and 0.88 for Pauluhn (2010a) (deposited dose); 1.0 for Ma-Hock et al. (2009) and 0.93 for Pauluhn (2010a) (retained dose), respectively.
[e]BMD: estimated benchmark dose (maximum likelihood estimate): BMDL: estimated 95% lower confidence limit of the BMD.

The rat BMC (BMCL) estimates, as shown in Tables 3.4 and 3.5, Figures 3.3 and 3.4, are 0.060 (0.023) mg/m^3 for granulomatous inflammation (minimal or greater severity) in Ma-Hock et al. (2009) and 0.10 (0.051) mg/m^3 for focal septal thickening in Pauluhn (2010a). Similar BMC (BMCL) estimates were obtained based on the rat pulmonary response of granulomatous changes reported in Kasai et al. (2014, Table 2), i.e., 0.20 (0.056) mg/m^3 in the male rat data, or 0.31 (0.12) mg/m^3 in male and female (combined), also based on a multistage, polynomial degree 2, model. These BMC (BMCL) estimates are all based on the exposure (airborne concentration) and response (lung histopathology results) in each study.

Both Kasai et al. (2014) and Pauluhn (2010b) report BMC (BMCL) estimates. Kasai et al. (2014) reports a "benchmark exposure concentration" of 0.056 mg/m^3 MWCNT for granulomatous changes; although the specific BMDS model or data (male and/or female) are not mentioned, it is the same estimate as the BMCL estimated here based on the male rat data and the multistage (polynomial degree 2) model (gamma model provided identical estimates). Combining the male and female rat data, as done in this example, increases the sample size may increase the confidence (both statistical and biological) in the BMD estimates, assuming similar dose-response relationships in male and female rats. A more rigorous evaluation may be needed to verify that assumption. The pulmonary response of focal fibrosis in Kasai et al. (2014, Table 2) is considered inadequate for BMD modeling since the only responses in the exposed groups were 0 or 100%.

Table 3.5 Benchmark dose estimates[a] and associated human working lifetime airborne concentrations—based on subchronic inhalation of MWCNT in rats and estimated retained lung dose[b] (NIOSH, 2013)

Rodent Study and Response[c]	Rat BMC (BMCL)[d] (mg/m^3)	Rat BMD (BMDL)[e] (μg/lung)	Human-equivalent BMD (BMDL) (mg/lung)	Human-equivalent BMC (BMCL): 8-h TWA & 45 work-years ($\mu g/m^3$)
Granulomatous inflammation (Ma-Hock et al., 2009)	0.060 (0.023)	11 (3.8)	2.7 (0.97)	2.7 (1.0)
Focal alveolar septal thickening (Pauluhn, 2010a)	0.10 (0.051)	14 (6.5)	3.6 (1.7)	4.2 (1.9)

[a]Benchmark response level: 10% excess (added) risk in exposed animal (EPA, 2010).
[b]Retained lung doses in rats and humans estimated using MPPD 2.0 model (CIIT and RIVM, 2006); aerodynamic particle sizes (MMAD, GSD): 2.74 (2.11).
[c]Responses are histopathology severity grade 1 or higher.
[d]BMC (BMCL); BMC: maximum likelihood estimate of the benchmark concentration; 95% LCL: 95% lower confidence limit of the BMC; dose-response data fit with multistage model (polynomial degree 2) (EPA, 2010). P-values for the rodent dose-response models: 1.0 for Ma-Hock et al. (2009) and 0.93 for Pauluhn (2010a), respectively.
[e]BMD: estimated benchmark dose (maximum likelihood estimate): BMDL: estimated 95% lower confidence limit of the BMD.

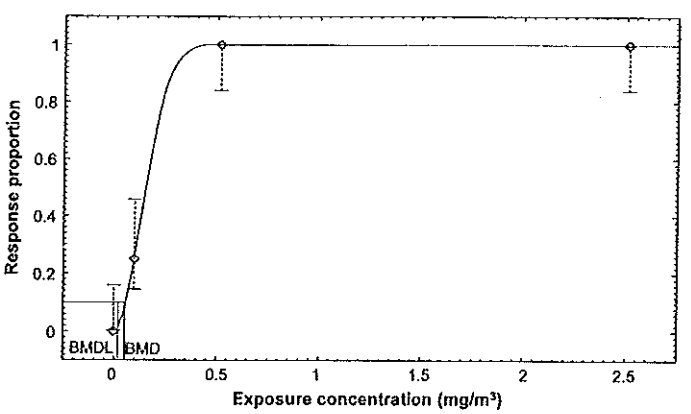

Figure 3.3
Benchmark dose estimation (Kuempel, 2011): Granulomatous inflammation (Ma-Hock et al., 2009). Multistage model, polynomial degree 2, $p = 0.99$. Rat subchronic BMC (BMCL), 10% excess risk: 0.06 (0.02) mg/m^3. (Note: BMD is a general term for a benchmark dose (maximum likelihood estimate) and BMDL is the 95% lower confidence limit estimate of the BMD. In this chapter, the term BMD is used to refer to the lung dose, while the term BMC (benchmark concentration) refers to a BMD based on an airborne exposure concentration).

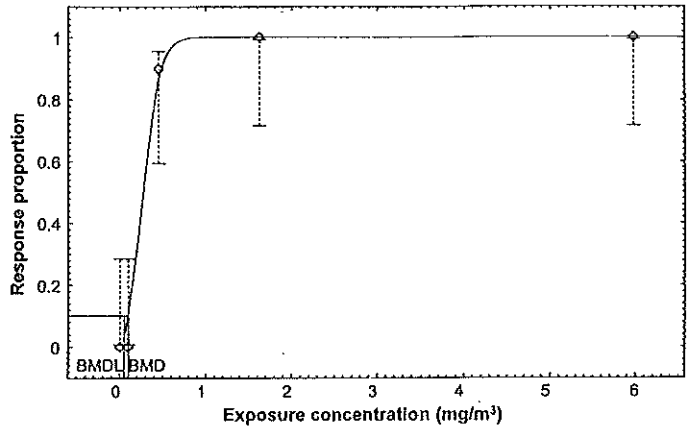

Figure 3.4

Benchmark dose estimation (Kuempel, 2011): Alveolar septal thickening (Pauluhn, 2010a). Multistage model, polynomial degree 2, $p = 0.88$. Rat subchronic BMD (BMDL), 10% excess risk: 0.1 (0.05) mg/m^3. (Note: BMD is a general term for a benchmark dose (maximum likelihood estimate) and BMDL is the 95% lower confidence limit estimate of the BMD. In this chapter, the term BMD is used to refer to the lung dose, while the term BMC (benchmark concentration) refers to a BMD based on an airborne exposure concentration).

Pauluhn (2010b) reports BMC (BMCL) estimates for some pulmonary inflammatory and fibrotic endpoints in BALF, i.e., 0.16, 0.78, and 0.2 mg/m^3 (BMCL) for PMN percent, PMN count, and collagen concentration, respectively (Figure 3 of Pauluhn, 2010b). PMN percent was reported to be significantly increased at the end of the 13-week exposure in rats in the 0.4 mg/m^3 and higher exposure groups (p-values < 0.01); the PMN percent was not significantly increased in rats in the 0.1 mg/m^3 dose group ($p > 0.05$) (Figures 8 and 9 in Pauluhn, 2010a). None of the histopathology responses were significantly increased in male rats (Table 3 in Pauluhn, 2010a); although no histopathology results are reported for female rats. The 0.1 mg/m^3 was regarded as the NOAEL in that study (Pauluhn, 2010a), and is the highest dose that did not have a statistically significant response in male rats. Pauluhn (2010b) uses 0.1 mg/m^3 as the POD for derivation of an OEL for MWCNT (Baytubes®) since that concentration is lower (more protective) than their BMCL estimates for the BALF endpoints. The BMCL estimate of 0.051 mg/m^3 (Tables 3.4 and 3.5) is based on the response of alveolar interstitial (septal) thickening (Table 3 in Pauluhn, 2010a); this BMCL estimate was also used as a POD in the NIOSH (2013) risk assessment. In addition to the POD selected, differences in other factors or assumptions—including those used in extrapolating the animal dose to humans or in accounting for uncertainty in the data—can contribute to differences in the OELs, such as that have been derived for CNTs (Table 3.6).

Table 3.6 Occupational exposure limits (OELs) proposed for carbon nanotubes or nanofibers

Type of Carbon Nanotube or Nanofiber	Occupational Exposure Limit ($\mu g/m^3$)	Reference
MWCNT Baytubes	50	Pauluhn (2010b)
MWCNT (several types)	30	Nakanishi (2011)
MWCNT (based on Pauluhn, 2010b)	2	Aschberger et al. (2010, 2011)
MWCNT (based on Ma-Hock et al., 2009)	1	
CNT and CNF	1	NIOSH (2013)

CNF, Carbon nanofiber; CNT, carbon nanotube; MWCNT, multiwalled carbon nanotube.

In Ma-Hock et al. (2009), rats exposed to the lowest exposure concentration of 0.1 mg/m^3 developed granulomatous inflammation (1/10 in males and 4/10 in females) (Table 2 of Ma-Hock et al., 2009); these response proportions were not reported as being statistically significant (note: it would seem that a 4/10 response proportion in females, compared to 0/10 in controls, would be significant, especially given that the 0.5 mg/m^3 group with 3/10 responders was reported as significant, $p < 0.01$). However, this did not influence the BMD estimates of those data in this example (since all of these dose and response proportion data are included in the model).

In Kasai et al. (2014, Table 2), the granulomatous changes observed in histopathology examination were significant ($p < 0.01$) in male rats exposed at 1 mg/m^3 or higher concentration, while the female rat response was reported as significant only at the 5 mg/m^3 group (although the response proportion was 4/10 at 1 mg/m^3 compared to 0/10 in rats in either the 0.2 mg/m^3 exposure group or the control group). Focal fibrosis was significant ($p < 0.01$) in both male and female rats exposed to 1 mg/m^3, MWCNT but not in rats exposed to 0.2 mg/m^3 ($p > 0.05$). It is not reported whether the PMN responses shown in Figure 2 of Kasai et al. (2014) in female and male rats were significantly increased, although the other BALF parameters are generally significant ($p \leq 0.01$) (Kasai et al., 2014, Figure 2) (the authors only report that "no concentration-related changes were seen in male rats").

Step 3. Estimation of rat lung dose

For the two subchronic studies of CNT available at the time of NIOSH risk assessment (i.e., Ma-Hock et al., 2009 and Pauluhn, 2010a), the BMC (BMCL) estimates provide the basis for estimating an equivalent lung dose (deposited or retained) in rats. The amount of MWCNT deposited in the alveolar (or pulmonary) region of the rat lung at the end of the 13-week study (assuming no clearance) is calculated from data on the ventilation rate (which is related to body mass) (see Appendix), the exposure conditions, and the particle-size

specific deposition fraction in the pulmonary region. In the following example, the BMCL from the Ma-Hock study (Figure 3.3; Table 3.4) is used:

$$\begin{aligned}\text{Deposited Dose} = {} & \text{Airborne concentration} \times \text{duration} \\ & \times \text{ventilation rate} \times \text{deposition fraction} \\ & \text{e.g., } 0.023\ \text{mg/m}^3 \times (6\ \text{h/d} \times 5\ \text{d/wk} \times 13\ \text{wk}) \\ & \times 0.0126\ \text{m}^3\text{/hr} \times 0.072 \\ & = 0.0081\ \text{mg/rat lung}\end{aligned} \tag{3.1}$$

where the ventilation rate in the rat is calculated from: 0.21 L/min × 0.001 m^3/L × 60 min/h (Appendix). The ventilation rate is based on species and body weight (EPA, 1994, 2006), assuming 300 g average body weight for male and female rats (since Ma-Hock et al. (2009) did not report the body weights, the values from Pauluhn (2010a) of approximately the same age and rat species/strain were used). The deposition fraction is estimated based on the MMAD and GSD in the rat multiple-path particle dosimetry (MPPD) model (CIIT and RIVM, 2006; NIOSH, 2013, Table A.2).

Although the MPPD model has not yet been validated for CNT, using the measured aerodynamic dynamic diameter should provide a reasonable estimate of the deposition efficiency in the respiratory zone because aerodynamic diameter (which accounts for inertial behavior regardless of density and shape) accurately predicts the particle deposition efficiency in the respiratory tract regions (Hinds, 1999; Kulkarni et al., 2011). Deposited lung burden was used in this example as an estimate of the retained lung burden for CNT over the relatively short exposure period of the subchronic inhalation studies in rats because MWCNT clearance has been shown to be slower than predicted based on clearance data of other poorly-soluble particles (Pauluhn, 2010a,b). Additional comparisons of the MPPD-based model estimates from versions 2.0 and 2.1 as well as with Cobalt-tracer based measurements of retained MWCNT lung burdens reported in Pauluhn (2010b) are reported in NIOSH (2013, Sections A.6.1.1 and A.6.1.2).

Step 4. Estimation of human-equivalent lung dose

The rat lung dose is extrapolated to a human-equivalent dose, in this example, by adjusting for species-specific differences in the surface area of the pulmonary (or gas-exchange) region of the lungs. In making this extrapolation, it is assumed that rats and humans would have equal lung responses to an estimated equivalent dose per unit surface area of alveolar epithelial cells. The basis for this assumption is that the pulmonary region of the lungs (and specifically the alveolar epithelial cell surface) is the primary deposition target which results in interstitial fibrosis that has been observed in both rodents and humans exposed to various

types of airborne particles. Thus, the rat lung dose (0.0081 mg) is extrapolated to humans as follows:

$$\text{Human lung dose} = \text{Rat lung dose} \times \text{Human/rat alveolar surface area } (102\ m^2/0.4\ m^2) = 2.1\ \text{mg in human lungs} \quad (3.2)$$

where human and rat alveolar epithelial surface area are taken from morphometric analyses (Stone et al., 1992; Mercer et al., 1994).

Next is to estimate the workplace exposure scenario that would result in the human-equivalent lung dose. The estimated human 8-h time-weighted average (TWA) concentration over a 45-year working lifetime that would result in the human-equivalent lung dose in the pulmonary region of the lungs is calculated as:

$$\begin{aligned}&\text{Human-equiv. lung burden (mg)}/[\text{Air intake} \times \text{exposure duration} \times \text{deposition fraction}]\\ &= 2.1\ \text{mg}/[9.6(\text{m}^3/\text{d}) \times (5\ \text{d/wk} \times 50\ \text{wk/yr} \times 45\ \text{yr}) \times 0.099]\\ &= 0.00019\ \text{mg/m}^3 \end{aligned} \quad (3.3)$$

where the human-equivalent lung burden is from Eqn (3.2), the air intake is for the reference worker (ICRP, 1994) and the alveolar deposition fraction is based on the MMAD (GSD) as estimated in MPPD 2.0 (Yeh and Schum human deposition model) (CIIT and RIVM, 2006). As discussed above for the rat lung burden estimate, the aerodynamic diameter should provide a reasonable estimate of the deposited lung dose, while the retained lung dose estimates are more uncertain.

The benchmark dose and exposure concentration estimates shown in this example, based on deposited lung dose estimates (i.e., assuming no CNT clearance from the lungs), are shown in Table 3.4. In addition, Table 3.5 provides benchmark dose and exposure concentration estimates based on estimated retained lung dose in rats (at the end of 13 weeks) and equivalent retained dose estimates in humans (after 45-year working lifetime), assuming spherical particle deposition and clearance kinetics in MPPD 2.0 (CIIT and RIVM, 2006). The steps for deriving BMC (BMCL) estimates based on retained lung dose are similar to those described above for deposited lung dose, except the MPPD model-based estimates of retained dose (which account for time-dependent clearance of the deposited dose) are used instead of the estimated deposited dose in Eqns (3.1) and (3.3). The human-equivalent BMC (BMCL) estimates in Tables 3.4 and 3.5 indicate that working lifetime exposures to 0.2–2 $\mu g/m^3$ (as 8-h TWA concentrations, lower 95% confidence limits; based on deposited or retained lung dose estimates) would be associated with a 10% excess risk of early-stage adverse lung effects (pulmonary inflammation and fibrosis) in workers. These airborne mass concentration estimates are quite low relative to estimates for other poorly-soluble fine or ultrafine particles (e.g., Dankovic et al., 2007).

Step 5. Risk characterization

In order to perform risk characterization (step 4 of the risk assessment paradigm) (NRC, 1983, 2009), data are needed on the worker exposures. Because such data are limited (e.g., short-term or task-based area samples of airborne CNT concentration with few personal samples) (Bello et al., 2009; Lee et al., 2010; Johnson et al., 2010), it is not currently feasible to characterize the disease risk in workers producing or using CNT. However, these studies indicate the potential for workplace airborne concentrations of concern and strongly support the need for extra precaution in controlling exposures to CNT (Schulte and Salamanca-Buentello, 2007). NIOSH is currently evaluating exposure levels in ten industrial workplaces producing/using carbon nanotubes. Mean airborne exposures to MWCNT determined by elemental carbon (EC) in the inhalable fraction were approximately 10.6 $\mu g/m^3$ (arithmetic mean) and 4.21 $\mu g/m^3$ (geometric mean), while the respirable fractions were several-fold lower and typically near background EC levels (Dahm et al., 2012; Erdely et al., 2013). The NIOSH REL of 1 $\mu g/m^3$ was set at the limit of quantification (LOQ) for the analytical method (NIOSH Method 5040) (NIOSH, 2013).

3.2.4 Considerations in the Derivation of OELs

In addition to characterizing risk to workers given exposure, risk estimation is also used in developing occupational exposure limit (OELs), the final step in the risk assessment process (Figure 3.2). Details on the development of OELs are beyond the scope of this chapter. However, the specific basis for OELs should be well-documented since differences in the factors and assumptions used in the risk assessment (including those in the derivation of a POD, as discussed in this section) can contribute to differences in the derived OELs. For CNT and CNF, the proposed OELs vary by a factor of up to 50 (Table 3.6), although all of these proposed OELs are low airborne mass concentrations compared to other poorly soluble particles (e.g., OELs for carbon black or graphite are on the order of milligrams per cubic meter of air (NIOSH, 2007), compared to micrograms per cubic meter of air for CNTs) (Table 3.6).

Both hazard and risk-based factors and nonrisk factors (e.g., technological feasibility of measuring and controlling exposures) are typically considered in the development of an OEL. Such factors are also evaluated in conjunction with any exposure measurement data to characterize the risk in a given population and to assess the need for additional protective measures such as personal protective equipment and medical monitoring.

3.3 Discussion

Although the rat subchronic lung responses to MWCNT are early-stage (minimal or higher severity grade of granulomatous inflammation or alveolar septal thickening), a BMR is an

effect level (e.g., 10%) that is considered biologically and statistically significant. In risk assessment practice, a human-equivalent BMD (i.e., the dose associated with the BMR) would not be used directly to develop an OEL. Instead, the BMDL would typically be used as a POD to estimate doses associates with lower risk levels. Alternatively, a BMDL is treated like an NOAEL with the application of uncertainty factors (EPA, 2012).

A health-based OEL is based on an exposure associated with a low risk of disease over a full working lifetime. However, the technologic feasibility of measuring or controlling exposures is also often considered in development of an OEL. For CNTs (as for other materials), there are limitations in the technical feasibility of the method to measure airborne mass concentrations. For example, the limit of quantification (LOQ) of NIOSH method 5040 for elemental carbon, including CNTs, is approximately 1 $\mu g/m^3$ as an 8-hour TWA concentration (NIOSH, 2013). Thus, the risk estimates at this LOQ are greater than 10% for early-stage adverse lung effects (see Section 3.2.3), which indicates the critical need to develop more sensitive measurement methods and to take additional precautionary measures (including engineering controls and use of personal protective equipment) when working with CNTs that may become airborne and inhaled.

3.3.1 Comparison with Other Methods

In addition to the benchmark dose method illustrated here, it is relevant to compare these estimates (Tables 3.4 and 3.5) with those based on other methods. For example, if an NOAEL or LOAEL of 0.1 mg/m^3 (Pauluhn, 2010a; Ma-Hock et al., 2009, respectively) is used as the starting point, the human-equivalent working lifetime 8-h TWA concentration would be <1 or 4 $\mu g/m^3$ based on the methods presented (using deposited or retained lung burden estimates), given that 0.1 mg/m^3 is also the BMC estimate based on the Pauluhn (2010a) study (Tables 3.4 and 3.5). As mentioned, these are human-equivalent concentrations corresponding to 10% excess risk of early-stage adverse lung effects; and no uncertainty factors have been applied to these estimates. The estimates for CNFs, starting with the NOAEL reported in the DeLorme et al. (2012) study, resulted in human-equivalent 45-year working lifetime concentration estimates of 1–4 $\mu g/m^3$ (8-h TWA), depending on the data and assumptions used to estimate the human-equivalent dose (NIOSH, 2013; see Section A.7).

A common method for extrapolating an NOAEL/LOAEL or BMD (BMCL) estimate from animals to humans (e.g., to derive a chronic inhalation reference concentration (RfC)) is the dosimetry adjustment factor (DAF) method for inhaled particles (EPA, 1994). In this method, the animal exposure concentration associated with an adverse effect (NOAEL, LOAEL, or BMC (BMCL)) is adjusted for differences in the animal versus human exposure pattern (hours per day and days per week), then multiplied by the DAF. The DAF for inhaled particles is a series of ratios used to adjust for the interspecies differences that influence the deposited particle dose in the respiratory tract, including the animal versus human ventilation

rate (V_E) (air volume inhaled per unit time); the animal versus human deposition fraction (DF) of particles in the relevant respiratory tract region(s); and a normalizing factor (NF) to adjust the deposited dose across species (e.g., the human versus rat surface area of the respiratory tract region(s) is typically used for insoluble particles, which deposit and clear along the surface of the respiratory tract) (EPA, 1994). Thus, a human-equivalent concentration would be calculated as:

$$\text{Effect Concentration (animal)} \times \left[V_E(\text{animal})/V_E(\text{human})\right] \times \left[\text{DF(animal)/DF(human)}\right] \times \left[\text{NF(human)/NF(animal)}\right]$$

As seen here, many of the same adjustments are made as in the case study example (Section 3.2). However, the DAF method is based on an average concentration (i.e., the response is assumed to be related to the chronic average exposure concentration rather than to the cumulative dose as in Section 3.2). Appropriate uncertainty factors would be applied to the human-equivalent concentration in deriving an exposure limit (e.g., RfC) (EPA, 1994).

In a recent risk assessment for MWCNTs, Pauluhn (2010b) started with the NOAEL of 0.1 mg/m^3 from a rat subchronic inhalation study (Pauluhn, 2010a) to estimate a human-equivalent concentration as the basis for an OEL, by applying a series of interspecies adjustment factors (AFs) to the rat NOAEL. The first AF was to adjust for rat versus human differences in the ventilation rate, which Pauluhn (2010b) expressed per kilogram of body weight: 0.14 (human)/0.29 (rat) = 0.5. These numbers were derived as follows: human reference worker breathing rate (8-h TWA) and weight: 9.6 m^3/70 kg (ICRP, 1994); and rat ventilation rate: 0.8 L/min/kg × 360 min (in 6-h rat exposure day) × 0.001 m^3/L. The second AF was to adjust for interspecies differences in the percentage of MWCNTs predicted to be deposited in the pulmonary region in each species, based on an MMAD of 3 μm (Pauluhn, 2010b): 11.8% (human)/5.7% (rat) = 2. The third AF was an NF to adjust the deposited lung dose in each species based on the total alveolar macrophage cell volume, assuming a rat-based volumetric overload mode of action (also expressed per kilogram of body weight), which resulted in an AF of 8.7×10^{10} (rat)/5.0×10^{11} (human) = 0.17 (Pauluhn, 2010b). The final AF was to normalize the retained lung dose based on an assumed constant factor of 10 times faster clearance in rats versus humans, based on first-order clearance kinetics. Combining these AFs, Pauluhn (2010b) derived an overall AF of: $0.5 \times 2 \times 0.17 \times 10 = \sim 2$. Dividing the rat NOAEL by this AF, a human-equivalent exposure concentration was calculated as: 0.1 mg/m^3/2 = 0.05 mg/m^3. No uncertainty factors were applied, and the human-equivalent concentration of 0.05 mg/m^3 was suggested as an OEL for MWCNTs (Pauluhn, 2010b). (Note: the ratios used by Pauluhn (2010b) are inverse to those used in the DAF method described above (EPA, 1994); whereas EPA would multiply a NOAEL (or other effect level) by the DAF, Pauluhn (2010b) divided the NOAEL by the AF. In other words, the EPA DAF = 1/AF (Pauluhn, 2010b)).

Extrapolation of an animal effect level to estimate a human-equivalent concentration is, of course, influenced by the various factors and assumptions used, and reasonable alternatives may exist based on the scientific literature. For example, in the Pauluhn (2010b) approach, the expression of the rat and human ventilation rates per body weight has a large effect on the first AF. Since ventilation rates are already derived from a nonlinear allometric relationship to body weight (EPA, 1994) (shown in the Appendix at the end of this chapter), typically these would not be adjusted again by body weight. If the whole animal or human ventilation rates are used instead, the first AF would be: 9.6 m^3 per human 8-h workday/0.085 m^3 per rat 6-h exposure day = 113 (versus 0.5 in Pauluhn (2010b)). The rat ventilation rate of 0.085 m^3 is calculated for a 0.35 kg body weight in a rat (based on equations given in the Appendix) and is similar to an estimate of 0.1 m^3 based on the values reported in Pauluhn (2010b), that is, 0.29 m^3/6-h per kg × 0.35 kg rat = 0.1 m^3/6-h. Thus, the ventilation rates are similar, but are expressed differently in the AF, resulting in a quantitatively different AF. For the second AF, no alternative assumptions would seem reasonable, since the pulmonary deposition percentages are based on the measured aerodynamic diameter of the particles; thus, the same human/rat pulmonary deposition AF of 2 is assumed here. For the third AF, an alternative assumption would be to adjust by the pulmonary surface area (Section 3.2.3; EPA, 1994) instead of using the alveolar macrophage cell volume to normalize the lung dose across species; this would result in an alternative AF of: 0.4 m^2 (rat)/102 m^2 (human) = 0.0039 (versus 0.17 in Pauluhn (2010b)). Regarding the fourth AF, additional issues are discussed below, but for simplicity in this example, the same rat/human AF of 10 is assumed. Thus, using these alternative assumptions, the total AF would be: 113 × 2 × 0.0039 × 10 = ~9. The alternative human-equivalent concentration would be: 0.1 mg/m^3/9 = 0.011 mg/m^3. This estimate is approximately five times lower than that of Pauluhn (2010b). However, this is not a large difference given the uncertainty in the various extrapolation methods. Actually, these estimates are reasonably consistent as low airborne mass concentrations relative to larger size (fine) respirable particles or to other ultrafine (nanoscale) particles (e.g., Dankovic et al., 2007).

In the BMD example in Section 3.2, instead of using an AF of ~10 based on a simple first-order (one compartment) clearance model (as in Pauluhn (2010b)), the interspecies lung dose extrapolation was based on an estimate of the actual lung dose (deposited or retained) for a given exposure scenario. The International Commission on Radiological Protection (ICRP, 1994) human respiratory tract model (which is used in MPPD (CIIT and RIVM, 2006)) includes three pulmonary clearance rate coefficients (three compartments) to estimate particle retention in the alveolar–interstitial region, including a fraction of the deposited dose that is cleared very slowly (approximately 10-year retention half-time). A simple one-compartment model assumed in Pauluhn (2010b) would underestimate the retained human lung burden (Kuempel and Tran, 2002). A higher-order long-term lung retention model that includes an interstitial-sequestration region (Kuempel et al., 2001b) has been shown to better fit several

human data sets, including those on coal miners experiencing high dust exposure (Kuempel, 2000; Tran and Buchanan, 2000) and workers in the nuclear industry exposed to lower nuclear doses (Gregoratto et al., 2010). Revisions to the ICRP model, including the alveolar–interstitial region based on the interstitial-sequestration model have been proposed (Bailey et al., 2008; Gregoratto et al., 2010). None of these models have been evaluated for CNTs, however, and the animal data have shown that MWCNT clearance is slower for a given mass dose than that of spherical poorly soluble particles (Pauluhn, 2010a,b; Muller et al., 2005). Thus, the BMD examples, based on either the deposited or the retained lung dose estimates (Tables 3.4 and 3.5), may represent the upper and lower bounds of the best estimate. That is, the deposited lung dose (assuming no CNT clearance) may overestimate the lung dose over time, whereas the retained lung dose (based on a poorly soluble spherical particle model) may underestimate the lung dose.

Despite these different approaches for dosimetric adjustment of a rodent adverse effect level (NOAEL or BMD) to estimate a human-equivalent dose, these various approaches all provide relatively low mass airborne exposure concentrations. By comparison, in a similar study design (13-week inhalation exposure) in rats exposed to ultrafine carbon black, the NOAEL was 1 mg/m^3 and the LOAEL for pulmonary inflammation and fibrosis was 7 mg/m^3 (Elder et al., 2005). Although dose spacing influences NOAEL and LOAEL values, these findings suggest that MWCNTs are approximately 10 times more potent in causing pulmonary inflammation and fibrosis compared with ultrafine carbon black.

The update of MPPD from version 2.0 to 2.1 (ARA, 2009) included revised rat deposition efficiency prediction equations (Raabe et al., 1988), which have resulted in increased predicted respirable particle deposition fractions in the head/extrathoracic region and, consequently, lower predicted deposition fractions in the rat pulmonary region (Owen Price, ARA, personal communication). For the MWCNT airborne particle sizes, this results in approximately half the estimated deposited dose of MWCNTs in the rat pulmonary region (thus, the rat pulmonary deposition fraction reported by Pauluhn (2010b) would also be about half) (NIOSH, 2013). The lower estimated dose associated with the same response proportion in the rat would result in lower rat BMD (BMDL) and human-equivalent BMC (BMCL) estimates (by a factor of approximately 2) than those shown in Tables 3.4 and 3.5. As additional data become available (e.g., in animal studies) to help evaluate current lung dosimetry models for CNT, the uncertainties in CNT dose and risk estimation may be reduced.

3.3.2 Research Needs

Toxicologic studies in animals and *in vitro* cell systems provide essential data for assessment of the hazards and risks associated with nanoparticles. Additional research needs for nanoparticle risk assessment (which may also apply to risk assessment of other substances)

include: (i) determination of responses not only in the organ of initial exposure but also in distal organs; (ii) identification of the nature of the hazards, including the severity of the effect and mechanism of action in animals and relevance to humans; (iii) determination of a biologically effective dose metric that is associated with these adverse effects; and (iv) generation of quantitative dose-response data in animal studies that are relevant to estimation of equivalent dose and disease in humans. In addition, toxicologic studies can provide more specialized data that are needed to develop mechanistically based risk models, including (v) kinetic data on the dose to the target tissue over time, to measure internal dose and develop dosimetry models; and (vi) time course of the dose and response, to develop biologically based models linking early biological responses and later disease outcomes. For fibrous particles such as CNTs and CNFs, additional dose metrics (e.g., fiber or tube count) may be needed to investigate the dose-response relationships and disease risks in workers (Schulte et al., 2012). Data on workplace exposures to nanomaterials are critically needed in order to characterize the risks and to take appropriate risk management measures to protect workers' health. Improvements in the sensitivity and specificity of measurement and analytical methods are needed for nanomaterials, including CNTs (NIOSH, 2013), in order to detect and quantify low mass concentrations. These low airborne mass concentrations are of concern based on the hazard data from the animal studies and the risk estimates derived from those data (e.g., case study example in this chapter).

3.3.3 Future Directions

Nanotechnology is capable of synthesizing nanoparticles of various sizes, shapes, dissolution rates, surface charge, hydrophobicity, surface functionalization, surface reactivity, chemistry, and so on. Given the vast array of nanoparticles that are being developed, it will be necessary to develop strategies to more efficiently and effectively assess the hazards and risks of nanoparticles to which workers may be exposed. The development of *in vitro* assays that can predict *in vivo* responses would facilitate initial hazard evaluation tests and screening to identify less hazardous nanomaterials (Rotroff et al., 2010). These assays require validation, although some promising studies are emerging. For example, the *in vitro* and *in vivo* dose-response relationships for inflammation-related responses have been shown to correlate well when dose is expressed as particle surface area and the reactivity of the surface is taken into account (Donaldson et al., 2008). More recently, *in vitro* cell assays of oxidative stress were shown to correlate well with *in vivo* acute lung responses in rats based on the particle surface area dose of several spherical metal and metal oxide nanoparticles (Rushton et al., 2010). Zhang et al. (2012) also reported good correlation between *in vitro* and *in vivo* assays of oxidative stress and acute pulmonary responses. Development of a models of the relationships between bioactivity and physicochemical properties (i.e., quantitative structure activity relationships, QSAR) (e.g., Liu et al., 2011, 2013; Gernand and Casman, 2014) may also facilitate comparative potency and hazard ranking strategies.

Future advancements in risk assessment methods may include models to predict disease response based on early biological responses, such as cell signaling and gene expression data (Thomas et al., 2007, 2009). There is also a need to confirm to what degree bolus exposures (intratracheal instillation or pharyngeal aspiration) of biopersistent nanoparticles such as MWCNTs provide similar responses to an equivalent dose by inhalation. Preliminary data suggest that pharyngeal aspiration of a well-dispersed suspension of SWCNTs results in a level of pulmonary inflammation and fibrosis, which is similar to that seen after a 4-day inhalation resulting in the same lung burden of SWCNTs (Shvedova et al., 2008; Mercer et al., 2008). In addition, a one-day (6-h) inhalation exposure in rats showed a similar dose-response relationship for pulmonary septal thickening and fibrosis at 90 days after exposure (Ellinger-Ziegelbauer and Pauluhn, 2009) as the 13-week inhalation study (Pauluhn, 2010a) based on estimated deposited lung dose. Therefore, it appears that shorter-term exposure studies may provide data for comparison with the subchronic studies and expand the database to evaluate the hazard of various types of CNTs.

Chronic exposure studies are needed to evaluate the potential adverse effects that exhibit a long latency, such as lung cancer or mesothelioma. A key area of uncertainty is the carcinogenic potential of the various types of CNTs (Grosse et al., 2014). Recent intraperitoneal studies in rats have reported mesothelioma at similar doses of MWCNTs (as mass or fiber number) to the intraperitoneal doses of asbestos that have been associated with mesothelioma (Takagi et al., 2008, 2012; Nagai et al., 2011, 2013; Rittinghausen et al., 2014). Studies on carcinogenicity of CNTs by inhalation are limited, although a recent study reported that MWCNTs were cancer promotors in mice exposed by inhalation (5 mg/m^3, 5 h/day, 5 day/wk) for 3 weeks (starting 1 week after intraperitoneal injection of the tumor initiator methylcholanthrene) and examined 17 months after exposure for lung tumor formation (Sargent et al., 2014). Cancer bioassay data are still limited or lacking for SWCNTs and for many other types of CNTs or CNFs. More rapid assays are needed for cancer screening (e.g., Wang et al. (2014) biotransformation assay).

Currently, some short-term studies of CNTs have included positive controls, for example, crystalline silica, asbestos, ultrafine carbon black (Lam et al., 2004; Muller et al., 2005; Shvedova et al., 2005, 2014; Murray et al., 2012) for which chronic study data are available in animals and in epidemiologic studies. These data provide a linkage between short-term effects in animals and chronic effects of relevance to humans. Such linkages provide opportunities for comparative potency analyses of these well-studied particles (also known as reference or benchmark particles) and engineered nanoparticles, especially if information is available to indicate the same mode of action (Kuempel et al., 2012a).

In the absence of complete information on the hazards and risks associated with exposure to nanomaterials, a higher level of precaution is needed to control exposures in the workplace (Schulte and Salamanca-Buentello, 2007). Animal studies indicate that inhaled

nanoparticles, including CNTs, may be more hazardous on an equal-mass basis than larger particles of the same chemical composition. Primary prevention through effective control of airborne exposure during production, use, or disposal of nanomaterials is essential to protect workers from developing occupational respiratory diseases (Kuempel et al., 2012b; Schulte et al., 2012).

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Appendix: Pulmonary Ventilation Rate Calculations

Species-specific average ventilation rates can be calculated based on the following allometric scaling equation:

$$\ln(V_E) = b_0 + b_1 \ln(BW) \tag{A.1}$$

where V_E is the minute ventilation (L/min); BW is body weight (kg); and $b_0 + b_1$ are the species-specific parameters; for the rat, $b_0 + b_1$ are -0.578 and 0.821, respectively (in Table 4.6 of EPA (1994)).

Minute ventilation (V_E) (L/min) is itself the product of the tidal volume (V_T) (L) and the breathing frequency (f) (min^{-1}) (EPA, 1994):

$$V_E = V_T \times f \tag{A.2}$$

Rat Ventilation Rate

The default value for minute ventilation in the multiple-path particle dosimetry (MPPD) 2.0 rat model (CIIT and RIVM, 2006) is 0.21 L/min, based on the default values of 2.1 mL (V_T) and 102 min^{-1} (f):

$$0.21\ (L/min) = 2.1\ (ml) \times 102\ (min^{-1}) \times (1/1000)\ (L/ml) \tag{A.3}$$

This minute ventilation corresponds to a rat weighing 300 g, based on Eqn (A.1):

$$0.21\ (L/min) = \text{Exp}\ [-0.578 + 0.821 \times \ln\ (0.3)] \tag{A.4}$$

Minute ventilation values for the rats in the subchronic inhalation studies (Ma-Hock et al., 2009; Pauluhn, 2010a) were also calculated on the basis of body weight. Pauluhn (2010a) reported male and female rat body weights of 369 and 245 g, respectively, in the control (unexposed) group at 13 weeks. Since the alveolar septal thickening response data were reported for 10 male rats per dose group, the male rat body weight (and calculated minute ventilation) was used to estimate deposited and retained lung dose in the Pauluhn (2010a) study. Ma-Hock et al. (2009) did not report the rat body weight, although the rat strain (Wistar) and study duration (13 weeks) were the same as in Pauluhn (2010a). Since the granulomatous inflammation response data in Ma-Hock et al. (2009) were combined for the 10 male and 10 female rats in each dose group (because response proportions were statistically consistent), an average rat body weight in male and female rats of 300 g was assumed, based on the 300 g body weight used in the default minute ventilation in MPPD 2.0 (CIIT and RIVM, 2006) and the male and female average body weight of 307 g reported in Pauluhn (2010a).

Thus, based on Eqn (A.1), a minute ventilation of 0 0.21 L/min is calculated for female and male rats in Ma-Hock et al. (2009) (same as MPPD 2.0 default), and 0.25 L/min for male rats

in Pauluhn (2010a). Assuming the same breathing frequency (102 min^{-1}), a tidal volume of 2.45 mL is calculated (Eqn (A.3)) and used instead of the default value in MPPD 2.0 (CIIT and RIVM, 2006) in estimating the rat lung dose in the Pauluhn (2010a) data.

Human Ventilation Rate

In the human MPPD 2.0 model (CIIT and RIVM, 2006), the default pulmonary ventilation rate is 7.5 L/min, based on default values of 12 min^{-1} breathing frequency and 625 mL tidal volume. The "reference worker" ventilation rate is 20 L/min (ICRP, 1994) or 9.6 m^3/8-hr (given 0.001 m^3/L, and 480 min/8-h). In these estimates, 17.5 min^{-1} breathing frequency and 1143 mL tidal volume were used in MPPD 2.0 to correspond to a 20 L/min reference worker ventilation rate.

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