Risk Assessment of the Carbon Nanotube Group

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This study assessed the health risks via inhalation and derived the occupational exposure limit (OEL) for the carbon nanotube (CNT) group rather than individual CNT material. We devised two methods: the integration of the intratracheal instillation (IT) data with the inhalation (IH) data, and the “biaxial approach.” A four-week IH test and IT test were performed in rats exposed to representative materials to obtain the no observed adverse effect level, based on which the OEL was derived. We used the biaxial approach to conduct a relative toxicity assessment of six types of CNTs. An OEL of 0.03 mg/m³ was selected as the criterion for the CNT group. We proposed that the OEL be limited to 15 years. We adopted adaptive management, in which the values are reviewed whenever new data are obtained. The toxicity level was found to be correlated with the Brunauer-Emmett-Teller (BET)-specific surface area (BET-SSA) of CNT, suggesting the BET-SSA to have potential for use in toxicity estimation. We used the published exposure data and measurement results of dustiness tests to compute the risk in relation to particle size at the workplace and showed that controlling micron-sized respirable particles was of utmost importance. Our genotoxicity studies indicated that CNT did not directly interact with genetic materials. They supported the concept that, even if CNT is genotoxic, it is secondary genotoxicity mediated via a pathway of genotoxic damage resulting from oxidative DNA attack by free radicals generated during CNT-elicited inflammation. Secondary genotoxicity appears to involve a threshold.

KEY WORDS: CNT; CNT toxicity; OEL; risk assessment

1. INTRODUCTION

Diverse applications of nanomaterials are advancing globally. Controlling the material size at the nanoscale markedly changes the properties of the materials chemically, physically, optically, electronically, and mechanically. Their application is thus expected in a wide range of fields, including medicine. However, there is widespread concern that the physiochemical changes they cause may result in unexpected adverse effects on human health. To promote the practical application of nanomaterials, we need first to respond to this concern. We must, therefore, assess the human health risk from exposure to nanomaterials.

There are numerous scenarios in which nanomaterials come into contact with humans and the environment. Although the current production...
output of nanomaterials remains small, the key challenge is to diminish health risks in the workplace, particularly those via the inhalation route. There have been many reports on toxicity of individual nanomaterials. Human health risk assessment has been promoted, and multiple occupational exposure limits (OELs) at the workplace have been proposed; however, these reports are based on toxicity data unique to individual nanomaterials. Many nanomaterials with the same chemical formula have different shapes and sizes of particles, and the properties and toxicity of these nanomaterials differ from each other. Therefore, there is not enough data on individual nanomaterials to be able to assess and manage the toxicity of entire nanomaterials. Toxicity assessment of nanomaterial groups is necessary to be able to formulate a risk management policy for nanomaterials. From this perspective, we established a national research project to assess and manage the health risks caused by the handling of nanomaterials such as titanium dioxide, carbon nanotubes (CNTs), and fullerenes. Due to space limitations, we focus on CNTs and describe the assessment of the CNT group, consisting of single-wall CNTs (SWCNTs), double-wall CNTs (DWCNTs), and multiwall CNTs (MWCNTs), which are available with constant properties and low impurities.

Hazard and risk assessments of a whole group would require a great deal of time and money; unfortunately, research facilities, funds, time, and toxicity data are all limited. To overcome this difficulty, we propose two methodologies that differ from those usually employed. One is the integration of intratracheal instillation (IT) data with inhalation (IH) data in toxicity testing of the rat lung exposed to CNTs by inhalation. The other is an extrapolation method for toxicity data, termed the “biaxial approach,” used to gain an understanding, via an overview, of the toxicity levels of various CNT materials in the group. An inhalation exposure test is essential to elucidate the adverse effects on the lungs of inhalation exposure: it is also regarded as the “gold standard.” However, test apparatus is large scale and costly, and is possessed by only a few research institutions. Aerosol inhalation testing requires especially sophisticated technology. It is impossible to test all known materials. We thus used the IH test for the essential parts and used the results of the IT test for those parts that can be estimated from the results of the IT test.

The biaxial approach is a framework for estimating the toxicity level of all the materials in a group. We assessed the toxicity of typical materials in the group in as much detail as possible and then multiplied the results by the relative value of the toxicity. This notion has long been advocated under the name of “bridging,” and many individuals and organizations are striving to put this method into practice. The key to this issue is how to obtain relative values for toxicity by employing an easier method and whether they can actually be used in practice.

In this study, we successfully completed our analysis by using biomarkers obtained in the IT test as a scale; details including the specific method are described in Section 2.5. Because the properties of nanomaterials are thought to depend on their sizes and shapes, it is obvious that a toxicity test must be performed with the samples accurately characterized, i.e., including information on their size and shape. However, in what way the different sizes and shapes of the materials affect their toxicity remains unknown. We have not been successful yet, to make the most of the advantage of the characterization results, in order to estimate the toxicity or classify the materials in terms of toxicity. In such a situation, it is not only practical to use the relative toxicity for estimating the toxicity, but also encouraging if we could relate the relative toxicity to the results of characterization. These results would also allow the estimation of to what degree size and shape contribute to toxicity.

Considering uncertainties inherent in these processes, we propose an OEL with a limited application period (a period-limited [PL]) on the assumption that it will be reviewed on the basis of long-term studies performed globally. This concept is related to adaptive management based on the notion that it is important to balance the promotion of technologies with the establishment of safety, since the purpose of this study is to assess the risks of an emerging technology. New materials represent numerous unknowns, and thus we consider it better to provide a rapid overview of the toxicity level of various known CNT materials, even though it would represent a somewhat rough estimation. Adaptive management is being applied to a widening range of decision-making processes, ranging from large-scale national projects for nanomaterial development, to a very specific but comprehensive strategy for monitoring chemicals in aquatic environments.

In this study, we describe the basic concept of the proposal and the results of key experiments. An exposure assessment is also performed using workplace concentration data, while taking into consideration the experimental results of dustiness tests. The
specific toxicity tests performed in the project have previously been reported in several papers.\(^{(4)}\)

2. HAZARD ASSESSMENT

It was technically difficult in Japan to perform a long-term IH test, so in order to collect overall information on all CNT materials of the group within the given project period, a subacute (four-week) inhalation test was applied only for the representative materials, and an IT test was performed to supplement information on long-term impact. No observed adverse effect levels (NOAELs) for rats were determined by integrating the results of the IH and IT tests. Genotoxicity tests were conducted because genotoxicity is a key area governing the risk assessment of chemical substances for human health, owing to the fact that classic genotoxic substances lead to carcinogenesis.\(^{(5)}\) The basics of the testing method are described below for a representative material of SWCNT (A). The other materials were studied in similar manner.

2.1. Endpoint

Focusing on the effects of exposure through inhalation, we reviewed previous reports and found that pulmonary inflammation is critical for CNTs, similar to many poorly soluble low-toxicity (PSLT) particles, and that the development of fibrosis and tumors is triggered by persistent inflammation. In recent comprehensive reviews,\(^{(6–9)}\) their conclusion, that the main detrimental effects of CNT particles are pulmonary inflammation, granuloma, and fibrosis, is consistent with ours. We selected persistent pulmonary inflammation as the endpoint. This context indicates a threshold model in the dose-response relationship.

2.2. Materials

Table I shows the main physicochemical characteristics of the CNTs investigated in this study. SWCNT (A) and MWCNT (N) were selected as representative SWCNT and MWCNT materials owing to the presence of trace amounts of metals and the availability of these materials with consistent properties. SWCNT (A) was synthesized by water-assisted chemical vapor deposition (supergrowth CVD), with iron as the catalyst, at the National Institute of Advanced Industrial Science and Technology (AIST),\(^{(10)}\) and MWCNT (N) was synthesized using the conventional CVD method employed by Nikkiso Co., with an iron catalyst.

In this study, CNTs were dispersed as finely as possible to be able to evaluate their nanoscale-specific effects.\(^{(11)}\) SWCNT (A) was dispersed in phosphate buffered saline (PBS) containing Tween 80 as the dispersant and sonicated in an ultrasonic bath for four hours at 55 W and a frequency of 35 kHz. The temperature of the bath water was maintained at 0–10 °C during sonication, since flocculation of SWCNTs occurred at higher temperatures. Size distribution was measured by laser diffraction and observation under scanning electron microscope (SEM) and transmission electron microscope (TEM). The SWCNT suspension was prepared fresh daily before use for IT tests. The dispersants used were Triton X-100 for MWCNT (N) and Tween 80 for other CNTs.

For the IH test, the SWCNT (A) suspension was introduced into the whole-body inhalation system consisting of a pressurized nebulizer and a mist dryer connected to an exposure chamber (volume: 0.52 m\(^3\)). Throughout the exposure period, the size and number density of the SWCNTs in the chamber were analyzed in-line using a particle spectrometer consisting of a differential mobility analyzer and a condensation particle counter.\(^{(12)}\) Number-based geometric mean of length and width of primary particles, airborne particles in the exposure chamber, and dispersed particles in the suspension are described in Table I. Most airborne SWCNT (A) particles are tangled or rope-like, while MWCNT (N) particles are fibrous whose geometric mean of length and width are 0.063 \(\mu\)m and 1.1 \(\mu\)m, respectively. More detailed information including particle size distribution is shown in the Supporting Information.

2.3. Toxicity Study

For the IH test, Wistar rats were divided into three groups (10 rats/group): low exposure concentration of 0.03 ± 0.003 mg/m\(^3\), high exposure concentration of 0.13 ± 0.03 mg/m\(^3\), and negative control (NC, dispersant aerosol). Rats inhaled the aerosol six hours/day, five days/week, for four weeks, and were dissected at three days, one month, and three months after the last exposure. The data for CNT-exposed groups were compared with those for NC and the nickel oxide group.

For IT tests of SWCNT (A), Wistar rats were anesthetized and intratracheally instilled with SWCNT (A) suspension, crystalline silica particle...
Table I. CNT Materials Studied

| Material   | Producer          | Production | Impurity                  | Primary Particle Size (SD) | Airborne Particle Diameter × Length GM (SD) | Particle in Water Diameter × Length GM (SD) | BET Specific Surface Area (m²/g) |
|------------|-------------------|------------|---------------------------|----------------------------|---------------------------------------------|------------------------------------------|-------------------------------|
| SWCNT (A)  | AIST              | CVD        | Fe:0.015% N:0.01% (CVD)   | 2.8 nm (1.5)               | bundles 0.19 μm (1.6)–0.21 μm (1.7) × 0.66 μm (1.6)–0.69 μm (1.7) | 8.2 nm (1.7) × 0.23 μm (1.8)               | 1,064                         |
| SWCNT (C)  | CNI HiPco         |            |                           |                            |                                             |                                          |                               |
| DWCNT (T1) | Toray Industries, Inc. | CVD         |                           |                            |                                             |                                          |                               |
| DWCNT (T2) | CVD surface modified hydrophilic | CVD         |                           |                            |                                             |                                          |                               |
| MWCNT (N)  | Nikkiso Co.       | CVD        | Fe 0.0053%                | 44 nm (1.5) × >1 μm        | 63 nm (1.5) × 1.1 μm (2.7)                  | 48 nm (1.1) × 0.94 μm (2.3) long fiber 3.4 μm (2.2) | 69                            |
| MWCNT (M)  | Mitsui & Co.      | CVD        |                           | 70.1 nm (1.3)              |                                            |                                          | 23                            |
| MWCNT (S)  | Showa Denko K.K.  | CVD        |                           |                            | 0.218 μm (1.46) × 3.19 μm (2.01)            |                                          | 13                            |

Note: CVD, chemical vapor deposition; CNI, Carbon Nanotechnologies, Inc.; GM, number-based geometric mean, SD, standard deviation.

The genotoxicity of SWCNT (N) and MWCNT (N) was further evaluated in vivo with comet assays using the lung cells of rats exposed to SWCNT (N) or MWCNT (N) following IT. This is because the lungs are a major target organ of CNTs. The SWCNT (N) or MWCNT (N) was intratracheally instilled at a single dose of 0.2 mg/kg or 1.0 mg/kg or at a repeated dose of 0.04 mg/kg or 0.2 mg/kg once a week for five weeks to male SD rats. Rats were sacrificed three hours or 24 hours after the single instillation and were sacrificed three hours after the last instillation in the repeated-instillation groups.

All procedures and animal handling were carried out according to the guidelines described in the Japanese Guide for the Care and Use of Laboratory Animals, as approved by the Animal Care and Use Committee, University of Occupational and Environmental Health, and AIST, Japan.

2.4. Results

2.4.1. IH Tests

In the IH tests of SWCNT (A), no persistent inflammation was noted at 0.03 mg/m³ or 0.13 mg/m³. The other results of the IH tests for biological effects are summarized in Table II. On the basis of these findings, it was concluded that four weeks of inhalation at 0.13 mg/m³ had no adverse effects. The
Table II. Biological Effects

| Findings | SWCNT(A) | MWCNT(N) | NiO |
|----------|----------|----------|-----|
|          | 0.13 mg/m³ | 0.37 mg/m³ | 0.12 mg/m³ |
| Lung weight | − | − | + |
| Cell analysis in BALF | − | − | + |
| Inflammation/ fibrosis-related gene HO-1 | − | − | + |
| Inflammation in lung | − | − | + |
| Fibrosis in lung | − | − | − |
| 8OH-dG in urea | − | − | − |
| Other organs | − | − | − |
| Final judgment | NO | NO | YES |

*Temporal increase at the beginning.
+ : Increase.
− : Negligible change.

In the two-year study, rats were instilled with MWCNT (N) at 0.67 mg/kg and 3.3 mg/kg. Acute inflammation started to decrease one month after instillation in the low-dose group (judged as “not influenced”), but significant inflammatory areas were noted up to 12 months in the high-dose group (judged as “influenced”). In the lung tissue, the outgrowth of bronchiolar and alveolar epithelial cells, an increase in fiber-phagocytizing macrophages in the alveoli, and infiltration of inflammatory cells were found. Only mild or transient fibrous lesions were noted during the observation period. No tumorigenesis or emphysematous changes were observed during the two-year observation period. No precancerous lesions, or fibrous changes, or persistent inflammation were noted at doses that correspond to the exposure level judged as noninfluential in our study.

2.4.3. Genotoxicity

No genotoxic effects of SWCNT (N), MWCNT (N), or MWCNT (M) were observed in bacterial reverse mutation assay or mammalian erythrocyte micronucleus tests.\(^{(13,14)}\) In \textit{in vitro} mammalian chromosomal aberration tests, SWCNT did not increase the number of structural or numerical chromosomal aberrations.\(^{(13)}\) MWCNT (N) and MWCNT (M) did not increase the number of structural chromosomal aberrations, regardless of metabolic activation, though the number of numerical aberrations was slightly increased by MWCNT (N) and distinctly increased by MWNT (M), in the absence of metabolic activation.\(^{(14)}\)

In comet assays, histopathological examinations of the lungs revealed that SWCNT (N) and MWCNT (N) caused inflammatory changes, including the infiltration of macrophages and neutrophils after a single instillation and repeated instillation at all doses. In lung cells, no changes in% Tail DNA were found in any group given SWCNT (N) or MWCNT (N). These findings indicate that SWCNT (N) and MWCNT (N) do not induce DNA damage in the lung cells of rats intratracheally instilled with SWCNT (N) or MWCNT (N), even at doses that elicited inflammatory responses, and suggest SWCNT (N)\(^{(15)}\) and MWCNT (N)\(^{(16)}\) to have no potential for genotoxicity \textit{in vivo}.

Details of the IH and IT toxicity tests and genotoxicity study and statistical analysis of the data have been reported previously.\(^{(17–20)}\)

Conclusion, after a similar IH test of MWCNT (N) had been performed, was that four weeks of inhalation at 0.37 mg/m³ had no adverse effects. No precancerous lesions, or fibrous changes, or persistent inflammation were noted at doses that correspond to the exposure level judged as noninfluential in our study.

2.4.2. IT Tests

IT exposure to SWCNT (A) up to 2 mg/kg did not produce mortality, changes in clinical signs, or changes in body weight during the observation period. Dose-dependent changes were observed in lung weight, BALF inflammatory cells, and biochemical parameters such as lactate dehydrogenase (LDH) value, protein content, interleukin (IL)-1β and IL-6 activity, and histopathology. In the 0.04 mg/kg SWCNT exposed group, almost no changes were observed during the observation period. In the 0.2 mg/kg SWCNT exposed group, reversible transient pulmonary inflammatory responses were observed after instillation. In the 1 mg/kg and 2 mg/kg SWCNT (A) exposed group, acute lung inflammation and subsequent granuloma, accompanied by increased lung weight, were observed. There were no fibrosis, atypical lesion, or tumor-related findings, even at the highest dose (2 mg/kg) of SWCNT exposed groups up to six months after instillation. In all groups, histopathological changes were observed only in the lungs and lung-associated lymph nodes but not in the other tissues examined (the liver, kidney, spleen, and cerebrum).
2.5. Data Analysis

2.5.1. Integration of IH and IT to Derive NOAEL

Considering these qualitative findings as well, we concluded that rats exposed to SWCNT (A) at 0.13 mg/m$^3$ and MWCNT (N) at 0.37 mg/m$^3$ in four-week IH tests, respectively, do not develop any adverse effects on the lungs, as described in Table II. We performed four-week IH tests with two doses of SWCNT (A)\(^{(17)}\) and MWCNT (N)\(^{(18)}\) due to the limited availability of research facilities and time when we started this study. We also performed several IT tests under various conditions for integration with IH test data. In doing so, we used the retained lung dose as a metric to combine the two kinds of test results, which was calculated assuming a deposition fraction of 0.1\(^{(21)}\) and no clearance for the IH test and instilled amount for the IT test. As shown in Fig. 1, the calculated retained dose immediately after four-week exposure to 0.13 mg/m$^3$ of SWCNT (A) in the IH test is equivalent to 0.06 mg/kg, while persistent inflammation started between 0.2 mg/kg and 1.0 mg/kg in the IT test. Integrating the four-week IH test results with IT test results allows us to confidently determine that the NOAEL for the IH test of SWCNT (A) is 0.13 mg/m$^3$. Here, if the 13-week IH test had been performed, the calculation would have shown the retained dose after 13 weeks of exposure to be 0.18 mg/kg, which is lower than 0.2 mg/kg, i.e., in the no-effect area observed in the IT test. This suggests that 0.13 mg/m$^3$ might be acceptable as the NOAEL for the 13-week IH test. Using a similar procedure, the NOAEL for the four-week IH test of MWCNT (N) was determined to be 0.37 mg/m$^3$.

2.5.2. The Biaxial Approach

The NOAELs of SWCNT (A) and MWCNT (N) were estimated through the process described above, but these are values for representative materials selected from the group. To set NOAELs for other materials with different characteristics, we applied the “biaxial approach.” The concept is shown in Fig. 2. Material A is regarded as the representative material of a group. Materials B–E with different production methods, sizes, and surface characteristics are in the same group. When SWCNT (A) is selected as Material A and its NOAEL is estimated as described above, and for Materials A–E, levels of inflammation response, a–e, for example, are determined using IT tests, then NOAELs for B–E are estimated based on the ratio of b–e to a. Selection of the test system or endpoint to determine the hazardous strength, a–e, among the materials, is the question here. An indicator should be chosen whose quantitative endpoint can be measured simply and that has a strong correlation with inflammation. Ultimately, we selected the relative neutrophil count in BALF one month after instillation of 1 mg/kg CNT as an indicator: this is the ratio of increase rate BALF neutrophil count of CNT to that of SWCNT (A). Increase rate BALF neutrophil count is calculated as follows, where the PC is crystalline silica Min-U-Sil#5 and the NC is Tween 80 solution:

$$\text{Increase rate of BALF neutrophil count} = \frac{\text{Neutrophil count of CNT material} - \text{Neutrophil count of NC}}{\text{Neutrophil count of PC} - \text{Neutrophil count of NC}} \times 100.$$

The results are presented in Table III. These values indicate the level of CNT materials in terms of
Table III. Relative Hazard (IT: 1 mg/kg)

|          | Increase Rate /PC (%) | Ratio to SWCNT (A) (%) |
|----------|-----------------------|------------------------|
| SWCNT (A) | 1,064                | 269                    |
| SWCNT (C) | 1,000                | 120                    |
| DWCNT (T1) | 440                  | 58                     |
| DWCNT (T2) | 310                  | 39                     |
| MWCNT (M) | 37                   | 6                      |
| MWCNT (S) | 13                   | 5                      |

Increase Rate of BALF neutrophil count = \( \frac{(\text{Neutrophil count of CNT material} - \text{Neutrophil count of NC})}{(\text{Neutrophil count of PC} - \text{Neutrophil count of NC})} \times 100 \)

Fig. 3. Relationship between BET-specific surface area (m$^2$/g) and BALF neutrophil increase rate/PC (%) (See footnote of Table III).

2.5.3. Brunauer-Emmett-Teller-Specific Surface Area (BET-SSA)

We obtained interesting results in the process of investigating the data discussed above. Fig. 3 shows plots of the relative neutrophil count in BALF, representing the level of inflammatory response against BET-SSAs (m$^2$/g). The relationship between the two parameters was neither linear nor describable using a simple numerical formula, but continuity was noted in the data. It was demonstrated that the inflammatory response caused by instillation of unit mass of CNT correlated with the BET-SSA of the material, suggesting BET-SSA to be useful for predicting the pulmonary adverse effects of CNT.

2.5.4. Proposal of a Period Limited OEL

The NOAELs of SWCNT (A) and MWCNT (N) were estimated as described above. To be able to extrapolate rat data to humans, it is necessary to select metrics for the expression of doses. We examined the retained lung dose and lung deposition rate, normalized using the alveolar surface area, and concluded the lung deposition rate, i.e., the deposition per unit time per unit alveolar surface area, to be most appropriate. Although it is difficult to measure alveolar surface area, its relationship to body weight has been reported.$^{[22]}$ We therefore replaced alveolar surface area with body weight, and selected deposition per unit time per body weight for our metrics. The reason for selecting the lung deposition rate, not the retained lung dose, is considered in Section 5.

The OEL is derived from the subacute NOAEL$_R$ (four-week) for rats through the human equivalent concentration, HEC, corrected with normalizing factor for interspecies differences and exposure time:

\[
HEC = \text{NOAEL}_R \times \frac{t_R \times \text{day}_R}{t_{H} \times \text{day}_{H}} \times \frac{Q_R}{Q_H} \times \frac{DF_R}{DF_H} \times \frac{BW_H}{BW_R},
\]

where $t$ is time of exposure per day [hour/day], $\text{day}$ is days of exposure per week [days/week], $Q$ is respiratory minute volume [m$^3$/min], $DF$ is alveolar deposition fraction [-] where $DF_R = DF_H$ was assumed, $BW$ is body weight [kg], OEL = $\frac{HEC}{UF}$, and $UF$ is uncertainty factor.

For $UF$, we adopted 3 for interspecies difference because of its topical application, but not systemic inflammatory responses, and 2 for the difference in the exposure period (subacute to subchronic).

The OELs of SWCNT (A) and MWCNT (N) determined using this procedure were 0.03 mg/m$^3$ and 0.08 mg/m$^3$, respectively. Looking at Fig. 3, for dividing CNTs into two groups. In addition, when the BET-SSA was compared among the three types of MWCNT (Table VII), the value of MWCNT (N) was small (69 m$^2$/g). On the basis of the relationship shown in Fig. 3, the application of NOAEL of MWCNT (N) to all MWCNTs is not appropriate. We would therefore like to propose using the value of SWCNT (A), 0.03 mg/m$^3$, for all CNTs, including MWCNT and DWCNT. The BET-SSA of SWCNT toxicity. The relative neutrophil count is smaller than 1 for all materials, indicating the inflammatory activity per mass of SWCNT (A) to be the highest of the six CNT materials, and thus suggesting that safety can be guaranteed by regarding SWCNT (A) as representative of CNTs.
(A) exceeds 1,000 m²/g, which is close to the upper limit of external BET-SSA of CNT. This proposal is valid for managing risk safely. Here, the meaning of the determined OEL is more clearly defined. The toxicity test results used at this point are obtained from a subchronic equivalent animal study, extrapolated from the subacute test. Currently, numerous toxicity studies, including a two-year inhalation exposure test, are ongoing globally, with the results due in the near future. We therefore feel it appropriate to propose a time limit to our OEL, for example, of about 15 years, with a review of our results to be undertaken in about 10 years.

The period of 15 years was selected from two viewpoints. The first is based on a comparison of the human lifetime with that of the rat. OEL is derived based on the rat 13-week equivalent inhalation test, which corresponds approximately to 10–15 years of the human lifespan. The second viewpoint is that fixed criteria for working lifetime are not realistic for our fast-moving society, so that 10–15 years, one chapter of human life, is more appropriate to current conditions. However, when applying a period limited OEL, we must be careful to remain aware that lack of adverse effects during the working period of 15 years at the longest does not guarantee zero health effects after this period. To confirm this point, we performed observations for the IT test for two years. We were able to ascertain that no adverse effects were found throughout this two-year observation period. The period limited OEL is expressed as OEL (PL), with “PL” representing “period-limited.” Our proposal of period-limited criteria includes the potential for modifications to be made in response to changes in conditions, including input of new data.

### 3. EXPOSURE ASSESSMENT

The level of CNT inhalation exposure in the workplace by the particle size is estimated using information published in the open literature and the results of surveys in the workplace and dustiness tests performed as part of our project. Dermal exposure to CNTs can be avoided by wearing protective gloves. Animal studies have revealed that CNT possesses no sensitization effects and zero or very slight irritation effects. Accidental ingestion of materials adhering to the hands may occur, but exclusion of this information from the assessment should not be problematic.

#### 3.1. Field Surveys and Dustiness Test

There have been more than 10 reports of field surveys, including ours, of SWCNT. Exposure levels were measured in manufacturing plants in about half of the reported studies, and in laboratories in the others. However, there is limited information on the size distribution of released CNTs in these reports. To evaluate the release characteristics of CNT, we conducted dustiness tests of five types of SWCNT, one type of DWCNT, and six types of MWCNT, employing the vortex shaker method, and measured the relative release potential of CNT and the size distribution of released particles in the air. The release potential varied markedly among the materials, but the size distribution did not differ much. The average size distributions of SWCNT and MWCNT were determined and used for later analysis. As the OELs were defined as mass concentration, the measured number-based particle size distributions in the dustiness tests were converted to volume-based (i.e., approximately mass-based) particle size distributions by using equivalent spherical diameters obtained from aerosol measuring instruments.

#### 3.2. Estimation of Exposure Level by Particle Size

Four reports were selected for the following analysis, as shown in Table IV (Dₘ and Dₐ represent the mobility and aerodynamic diameters, respectively). We calculated the levels of respirable particles (<4 μm in aerodynamic diameter) using the total or inhalable dust levels reported, assuming half of the particles to be respirable. The exposure levels by particle size were calculated using the size distributions of particles obtained in the dustiness test.

### 4. RISK ASSESSMENT AND MANAGEMENT

#### 4.1. Risk Assessment

The OELs used here for SWCNT and MWCNT were 0.03 mg/m³ for SWCNT (A) and 0.08 mg/m³ for MWCNT (N). First, the OELs for MWCNT by particle size were determined taking account of the differences in pulmonary deposition fraction as a function of particle size. The geometric mean of mass median aerodynamic diameter for MWCNT (N) in the IH test was 1.25 μm (geometric standard deviation 2.4). The alveolar deposition fraction in humans for MWCNT with this size (assuming a log normal size distribution) was calculated to be
Table IV. Estimated Respirable Exposure and Hazard Quotient by Size

| Material (Reference) | Working Scale | Process                  | Total or Inhalable Dust ($\mu g/m^3$) | Particle Size ($\mu m$) | Estimated Respirable Exposure ($\mu g/m^3$) | OEL(PL) ($\mu g/m^3$) | Hazard Quotient |
|---------------------|--------------|--------------------------|---------------------------------------|-------------------------|--------------------------------------------|----------------------|----------------|
| SWCNT (unpurified)  | laboratory & plant collection, cleaning | 0.7–52.73 (based on catalytic metal) | $0.01 < D_m < 0.1$ | $0.000001 - 0.000080$ | 10 | $0.0000001 - 0.000080$ |
|                     | laser abrasion or HiPco (23)          | $0.1 < D_m, D_a < 1$ | 0.019–1.4 | 30 | 0.0063–0.047 |
|                     |                                          | $1 < D_a < 4$ | 0.33–25 | 30 | 0.011–0.83 |
|                     |                                          | Total |                            |                         |                            | 0.012–0.88 |
| MWCNT CVD approx. laboratory weighing, solution spraying | 332 | $0.01 < D_m < 0.1$ | 0.0062 | 31 | 0.00020 |
| 50 nm diameter, 1.5 $\mu m$ long (27) | blending (opening blender) | $D_m, D_a < 1$ | 29 | 90 | 0.32 |
|                     |                                          | $1 < D_a < 4$ | 137 | 71 | 1.9 |
|                     |                                          | Total |                            |                         |                            | 2.3 |
| MWCNT CVD (28)      | plant synthesis, weighing, bagging, dispersion | 113–193 | $0.01 < D_m < 0.1$ | 0.0021–0.0036 | 31 | 0.000069–0.00012 |
|                     |                                           | $0.1 < D_m, D_a < 1$ | 10–17 | 90 | 0.11–0.19 |
|                     |                                           | $1 < D_a < 4$ | 47–80 | 71 | 0.66–1.1 |
|                     |                                           | Total |                            |                         |                            | 0.77–1.3 |
| MWCNT CVD (29)      | laboratory bagging (manual) | 31–286 | $0.01 < D_m < 0.1$ | 0.00058–0.0054 | 31 | 0.000019–0.00017 |
|                     |                                           | $0.1 < D_m, D_a < 1$ | 2.7–25 | 90 | 0.030–0.28 |
|                     |                                           | $1 < D_a < 4$ | 13–118 | 71 | 0.18–1.7 |
|                     |                                           | Total |                            |                         |                            | 0.21–1.9 |
|                     |                                           | 63 (elemental carbon) | $0.01 < D_m < 0.1$ | 0.0012 | 31 | 0.000039 |
|                     |                                           | $0.1 < D_m, D_a < 1$ | 5.5 | 90 | 0.061 |
|                     |                                           | $1 < D_a < 4$ | 26 | 71 | 0.37 |
|                     |                                           | Total |                            |                         |                            | 0.43 |

Note: $D_m$, mobility diameter; $D_a$, aerodynamic diameter.
Table V. OEL by Particle Size ($\mu$g/m$^3$)

| Particle Size ($\mu$m) | SWCNT (A) | MWCNT (N) |
|------------------------|-----------|-----------|
| OEL (total)            | 30        | 80        |
| $0.01 < D_m < 0.1$     | 10        | 31        |
| $0.1 < D_m, D_a < 1$   | 30        | 91        |
| $1 < D_a < 4$          | 30        | 71        |

Note: $D_m$, mobility diameter; $D_a$, aerodynamic diameter.

8.4% using the multiple-path particle dosimetry 2 (MPPD2) model. On the other hand, the average alveolar deposition fractions in human for MWCNT in the particle size categories of $0.01 < D_m < 0.1$, $0.1 < D_a$ and $D_a < 1 \mu$m, and $1 < D_a < 4 \mu$m were determined to be 22%, 7.4%, and 9.5%, respectively, using the MPPD2 model considering the volume-based (i.e., approximately mass-based) particle size distributions of MWCNT in the dustiness tests. The OEL for the particles with a particle size of $0.01 < D_m < 0.1$ was therefore calculated to be $31 \mu$g/m$^3$ ($= 80 \times 0.084/0.22$). The OELs for MWCNT in the other size categories were determined similarly. They are shown in Table V. For SWCNT (A), there are no measured data for the aerodynamic diameter, but considering that the shape was a bundle-type and the diameter and length were about 0.2 $\mu$m and 0.7 $\mu$m in the IH test, respectively, the OELs for SWCNT by particle size were estimated as shown in Table V. Using the OELs by particle size derived here, a hazard quotient (HQ) was calculated for each particle size category. The results are shown in Column 8 of Table IV.

The exposure levels were not necessarily directly measured for individual workers, including those measured near the emission sources. The environment therefore corresponds to conditions with no exposure control, such as local exhaust ventilation. The raw values were not necessarily the average concentrations for eight hours, but were here regarded as the exposure level for eight hours a day to assess risk. It should be noted that the raw data were values of total or inhalable dust, and the detailed exposure levels by size of agglomerated particles were unclear. We chose to perform this analysis as a rough investigation of the contribution of agglomerated particle size to HQ. As shown in Table IV, the HQ of overall respirable particles exceeded 1 in some cases, but HQ was mostly due to particles with an aerodynamic diameter exceeding 1 $\mu$m in all these cases.

### 4.2. Risk Management

The HQ varies due to the diversity of the process and the material. In some cases, exposure may exceed the OEL if no exposure control measures are implemented. The results in Table IV indicate that the contribution of micron-sized respirable particles ($1 < D_a < 4 \mu$m) to HQ is predominant, whereas the contribution of particles smaller than 100 nm is very small. Inhalation exposure is likely to occur while handling dry CNT powder, such as during the processes of collection, weighing, mixing, transfer to containers, bagging, and cleaning. Inhalation exposure may also occur while handling liquids containing CNTs if liquid droplets become airborne (e.g., by sonication, stirring, foaming, or spraying). Exposure control measures for conventional dust (such as enclosure, local exhaust ventilation, and protective equipment) are known to be effective for CNT and many other nanomaterials as well. The particle collection efficiency of filters varies according to the particle size and, generally, the efficiency for submicron-size particles is the lowest. Fibrous substances are more efficiently collected by filters than spherical particles due to ease of interception. These points should be considered when choosing appropriate exposure control measures. Given that micron-sized respirable CNT agglomerates contribute substantially to the overall risk caused by CNTs, the exposure management (monitoring and exposure controls) of particles of this size should be the most efficient. Measurement of smaller CNTs such as isolated CNT fibers and agglomerates is generally problematic. However, the exposure management of larger CNTs may result in a simultaneous reduction in the concentration of smaller CNTs.

### 5. DISCUSSION

#### 5.1. The Biaxial Approach

As stated in Section 2.5.2, we chose the relative BALF neutrophil count in the IT test as an indicator to compare various CNTs for the inflammatory response they caused. We selected this indicator of inflammatory response because the IT test has an established reputation as a cost-effective alternative to the inhalation test, as long as certain caveats are clearly understood. Another reason is that polymorphonuclear (PMN) neutrophil and BALF cell information are used as inflammatory biomarkers.
Cell differentials, total cell counts, PMN, LDH, and protein in BALF are used to evaluate lung inflammatory response by exposure to SWCNT, MWCNT, or carbon fiber.\(^{(39,40)}\) In addition to neutrophil count, we measured BALF cell count, LDH, protein, and lymphocytes, and conducted histopathological examinations, all of which changed in parallel to neutrophil count. It has been argued that bolus exposure, such as IT and pharyngeal aspiration, caused greater pulmonary responses than IH exposure due to nonuniform deposition of particles.\(^{(9)}\) Inhalation exposure to SWCNT exhibited significantly greater inflammatory effects than bolus pharyngeal aspiration, but outcomes of inhalation exposure to respirable SWCNTs were very similar to those seen after pharyngeal aspiration leading to pulmonary toxicity.\(^{(40)}\) Castranova et al.\(^{(9)}\) suggested that when CNT of similar dispersion are applied, studies by bolus exposure can predict a pulmonary response that is consistent with short-term inhalation studies if dosed at an equal mass lung burden. Therefore, we employed the IT test as a simple approach in combination with the IH test. In the Tox21 Project, Lai proposed\(^{(41)}\) a nanomaterial evaluation method that compared the \textit{in vitro} and high-throughput screening (HTS) test results of various materials with the \textit{in vivo} and HTS test results of reference materials. This appears to be a method based on a similar concept to ours. Kuempel et al. of U.S. NIOSH also presented\(^{(42)}\) the idea of estimating the toxicity of unknown materials by combining \textit{in vivo} and \textit{in vitro} data and comparing them with the toxicity of benchmark materials: this strategy also has similarities to ours. They proposed the idea of bridging, referring to two earlier papers. The qualitative and conceptual concepts outlined are mainly based on the utilization of \textit{in vitro} data. We also investigated the use of \textit{in vitro} test results at the beginning of the project, but have yet to attain the expected results. Other reports discuss the need for simple and efficient methods of assessment\(^{(43–45)}\) but they are not yet practical. As explained in the REACH guidance,\(^{(46)}\) a range of \textit{in vitro} test data may be used in the future, which may simplify the risk assessment. The Toxic Substance Control Act (TSCA) requires a 13-week IH test for each CNT product before launch. This may be essential for temporary management, but, as it is expensive, takes time, and the results are obtained only under specific conditions, it may delay the development of novel substances.

In our strategy that employs the biaxial approach, a representative material that is chosen on the basis of its BET-SSA is examined using the IH test, while others are examined using the IT tests. The risk is managed while confirming the relative position of the material in terms of toxicity. This is an economical, safe, and realistic framework. The relative position of the biaxial approach among the various methodologies for toxicity assessment, ranging from theoretical calculations, quantitative structure-activity relationships (QSARs), \textit{in vitro} tests, \textit{in vivo} tests, to epidemiological analysis is depicted in Fig. 4. To the left, the methods, when established, would be simple to use and cost effective, but are yet to be developed and validated. To the right, the methods are more relevant to the human inhalation exposure and some are internationally standardized. However, animal tests are expensive. In the present state of science and technology for assessing the toxic effects of various nanomaterials used in occupational settings, we believe that our biaxial approach, which integrates IH and IT, strikes an optimal balance between cost and relevance.

### 5.2. Dose Metrics for Extrapolation to Humans

For extrapolation from rats to humans, we adopted the lung deposition rate per body weight (dose rate) (mg/kg/day) as the dose metric. This scale is generally used to evaluate the biological effects of chemicals on test animals and to convert them to human health effects. It may also be considered that retained lung dose should be employed because CNTs are bioresistant. However, the dose rate may be appropriate in such a case where tests are performed to identify the low dose range in which inflammation does not persist. Furthermore, to consider that the effects on rats and humans will be the same if the (normalized) retained lung dose is the same when extrapolating from rats to humans is to take a precarious position. Retained lung dose with extremely high PSPs (poorly soluble particles) were seen in a cohort study of mine workers, but no pronounced neutrophilic inflammation or associated cell growth was reported and no definite proof indicating an increase in cancer incidence has been shown.\(^{(47–49)}\) Therefore, using retained lung dose (normalized with alveolar surface area) as a metric would mean that the permitted amount of particles per alveolar surface area in humans is the same as in rats, which is far from what the findings accumulated thus far have shown.

We used the deposition rate of particles per alveolar surface area as the metric, after reconciling the exposure periods normalized to the lifespan of
animals (two years) and humans (70 years). Because it is not certain at this stage how the effects of the particles remaining in the lungs over the long term will differ among species, we have adopted an uncertainty factor of 3, the default for dynamics in interspecies extrapolation. Studies so far that have derived an OEL for MWCNTs are those by Pauluhn\textsuperscript{(50)} and Luizi\textsuperscript{(51)} based on the results of Ma-Hock \textit{et al.}\textsuperscript{(52)} and NIOSH\textsuperscript{(53)} reanalyzed their data. While NIOSH uses retained lung dose as the metric for interspecies extrapolation, Luizi\textsuperscript{(51)} used the concentration itself with an uncertainty factor. Pauluhn used retained lung dose normalized with the number of alveolar macrophages.

5.3. Relationship Between Properties of CNTs and NOAEL

It has been clarified that the biological effect was markedly influenced by the BET-SSA of CNT or by physical properties associated with the BET-SSA. According to the theoretical calculation of the BET-SSA of CNT by Peigney \textit{et al.}\textsuperscript{(54)} (Table VI), the BET-SSA of CNT decreases as the number of walls increases. In contrast, its dependence on the diameter of CNT is small. The calculated values in Table VI are consistent with those for the materials used in this study. The experimental BET-SSA of SWCNT (A) and MWCNT (N) are close to the values calculated for SWCNT and MWCNT. The number of walls was about 30, which was reasonable because the number for MWCNT (N) confirmed by a TEM was about 30.

From the test results of the three reports for the IH test of MWCNT, ours and those of Pauluhn\textsuperscript{(50)} and Ma-Hock \textit{et al.}\textsuperscript{(52)} the effect levels and BET-SSA are presented in Table VII. There are slight differences among these reports: the data were collected from the four-week IH test in our study, but from 13-week IH tests in the others. In Ma-Hock \textit{et al.}'s report, a very small effect was noted at the lowest observed effect concentration (LOEC). However, there were no major differences, so NOAEL may be similarly defined. It is interesting that the hazard levels corrected with the BET-SSA are similar, although the properties of the three materials are different, and our samples were dispersed while the other two materials were large.

\begin{table}
\centering
\begin{tabular}{lcccccccc}
\hline
Number of Walls & 1 nm & 3 nm & 5 nm & 10 nm & 20 nm & 30 nm & 40 nm \\
\hline
1 & 1.315 & 1.315 & 1.315 & 1.315 & 1.315 & 1.315 & 1.315 \\
2 & – & 742 & 706 & 681 & 669 & 665 & 663 \\
3 & – & 567 & 507 & 470 & 454 & 449 & 446 \\
4 & – & 498 & 413 & 366 & 346 & 340 & 337 \\
5 & – & – & 361 & 304 & 282 & 276 & 272 \\
10 & – & – & – & 190 & 155 & 146 & 142 \\
20 & – & – & – & – & 97 & 84 & 78 \\
30 & – & – & – & – & – & 65 & 58 \\
\hline
\end{tabular}
\caption{Specific Surface Area of CNT by Diameter and Number of Walls (m\textsuperscript{2}/g)}
\end{table}

\textit{Note:} Calculated with use of the equation given by Peigney \textit{et al.}\textsuperscript{(54)} All CNTs are assumed to be closed and only the external surface of each CNT is taken into account.
agglomerates. Thus we obtained findings similar to the relationship between the BET-SSA and IT test results discussed in reports on titanium dioxide (e.g., Borm et al.\textsuperscript{(55)}), which are very interesting and useful for the hazard-based classification of several CNT materials. We were able to obtain these findings because the objective of this study was to overview the hazard represented by various CNT materials, rather than to conduct a detailed investigation of the characteristics of individual CNT species.

### 5.4. The Shapes of Airborne CNTs

It is generally believed that the more finely the nanomaterials are dispersed, the more toxic they are. In our toxicity tests, therefore, we used particles that were as isolated and dispersed as possible to create a worst-case scenario. We prepared the test samples with the aim of performing toxicity tests using particles that retain the physical characteristics of nanoscale material, rather than that of large agglomerates, and performed IT and IH tests using samples at the nanoscale or close to it. When aerosol particles of MWCNT (N) in the exposure chamber were collected and observed with a SEM, particles existing as isolated fibers, bundles, and entangled agglomerates accounted for 72%, 18%, and 10%, respectively.

Although most airborne CNTs in the workplace are agglomerated, we used dispersed samples rather than agglomerated CNTs to be on the safe side. Our reason was twofold: (1) there are dispersed particles in the workplace, though with low probability; and (2) we cannot neglect the adverse health effects caused by exposure to dispersed particles from the viewpoint of risk assessment and risk management.

While the agglomerative state of CNT test samples in our study is different from that of airborne CNTs in a typical workplace, Table VII suggests that the toxicity level per unit mass of CNTs does not depend on the agglomerative state of the test samples. We therefore concluded that the difference in agglomerative states is not important, at least for the samples used in our tests.

### 5.5. Mechanisms

The key mechanism for toxicity of nanomaterials is the induction of oxidative stress via the formation of free radicals, reactive oxygen species (ROS)/reactive nitrogen species (RNS).\textsuperscript{(56–58)} A wide range of nanomaterials, including CNTs, has been shown to generate ROS, both \textit{in vitro} and \textit{in vivo}.\textsuperscript{(57,59,60)} Excessive generation of ROS or RNS may lead to an imbalance between oxidant and antioxidant mechanisms that manifests in the condition of oxidative stress.\textsuperscript{(56,57,59)} Oxidative stress may play a role in the induction or enhancement of inflammation.\textsuperscript{(56,59)} Inflammation and resulting fibrosis have been regarded as a significant risk factor in pulmonary carcinogenicity.\textsuperscript{(40,61)} In this context, pulmonary inflammation appears to be a useful indicator for evaluation of pulmonary toxicity induced by nanomaterials.

Genotoxicity may be produced by direct interaction of nanoparticles with genetic materials or by indirect damage from nanoparticle-induced ROS.\textsuperscript{(62)} Primary genotoxicity can be defined as genetic damage elicited by particles in the absence of inflammation, and secondary genotoxicity can be defined as genotoxic damage resulting from ROS/RNS that are generated during particle-elicited inflammation mediated by activated phagocytes.\textsuperscript{(62,63)} Greim and Ziegler-Skylakakis\textsuperscript{(64)} mentioned that inflammation leads to secondary genotoxicity, and that prevention of inflammation will prevent both genotoxicity and tumor induction. They also noted that inflammation exhibits a nonzero threshold. Both positive and negative results from genotoxicity studies of CNT have recently been reported.\textsuperscript{(62,65,66)} These inconsistencies might be due to differences in test conditions, such as cell types, exposure time, concentrations, the specific endpoint measured, the dispersal of the materials, the presence of trace amounts of

### Table VII. NOAELs or LOEC Reported for MWCNT

| Report          | This Report           | Pauluhn\textsuperscript{(50)} | Ma-Hock et al.\textsuperscript{(52)} |
|-----------------|-----------------------|-------------------------------|----------------------------------|
| Material        | MWCNT (N)             | Baytube                       | Nanocyl NC 7000                  |
| Diameter of primary particles (nm) | 44                     | 10 (as produced)              | 5–15                             |
| Airborne particles | diameter 63 (nm)      | aggregates (as administered/ as exposed) | aggregates                       |
| BET-SSA (m$^2$/g) | 69                    | 253–259                       | 250–300                          |
| NOAEL (mg/m$^3$) | 0.37 (four week)      | 0.1 (13 week)                 | 0.1 (13 week LOEC)               |
Risk Assessment of CNT

metals, and the physicochemical characteristics of the CNT tested. In our studies,\(^{13-15,67}\) no evidence for genotoxicity of SWCNT and MWCNT was detected except for numerical chromosomal aberrations induced by MWCNT.\(^{13}\) Numerical chromosomal aberrations are also reported to be induced by SWCNT\(^{60,68,69}\) and MWCNT.\(^{60,70,71}\) Bolt et al.\(^{72}\) noted that oxidative stress has been addressed as an important mechanism of indirect genotoxicity and ROS-mediated processes of carcinogenesis have a practical threshold and genotoxicity only at the chromosome level has a perfect statistical threshold.

No reports are available on carcinogenicity of CNTs in humans.\(^{66}\) Although the carcinogenicity of CNTs is a major concern, it has not been much studied using experimental animals. SWCNT did not show any evidence of lung tumor one year after aspiration in mice\(^{73}\) or 754 days after IT in rats.\(^{74}\) MWCNT produced mesotheliomas in p53 heteroknockout mice after intraperitoneal injection\(^{75}\) and in Fischer 344 rats after intrascrotal injection.\(^{76}\) Thin MWCNTs with high crystallinity were inflammogenic and mesotheliomagenic in rats after intraperitoneal injection.\(^{77}\) Most recently, pharyngeally aspirated SWCNTs have been suggested to act as tumor promoters in the murine metastasis/dissemination model\(^{78}\) and inhalation of MWCNTs produced lung tumors in mice using a two-stage initiation-promotion protocol.\(^{79}\) However, no data are available on long-term carcinogenicity studies of CNTs arriving in the human body by familiar routes. Further studies using appropriate routes of exposure are required to elucidate the carcinogenicity of CNTs and provide information that can be used in risk assessments.

6. CONCLUSION

We performed a toxicity assessment of the CNT group rather than of individual CNT materials. An OEL of 0.03 mg/m\(^3\), the value of the most toxic material in the group, was adopted for SWCNTs, DWCNTs, and MWCNTs as a common criterion.

The OEL was derived based on integration of IT and IH tests. Considering uncertainties in the data processing, we proposed limiting the period of application to 15 years on the assumption that the values will be reviewed whenever new data are obtained. We abbreviated this to OEL (PL). This can be regarded as a type of adaptive management, which is adopted to balance the development of technology with ensuring safety in developing eras of innovative technology.

We had to conduct toxicity assessments of numerous materials to obtain the above results. Given our limited research resources and time, we proposed and utilized two methodologies to achieve this objective. One was the integration of the IT data and the IH data. Because inhalation testing of particles is expensive and requires sophisticated technology, we deemphasized the IH test and supplemented it with the IT test. Without this methodology, we would not have been able to complete the risk assessment of the CNT group. The IT test should be substituted for the IH test in the toxicity assessment of various materials. The use of this method helps to advance the currently underdeveloped risk assessment and management of particulate chemicals.

The second is the biaxial approach. We used the IT test for this approach as well. This method allowed us to estimate the toxicity level of all the materials in this group. We selected an OEL of 0.03 mg/m\(^3\) as the criterion for the CNT group by virtue of the analysis using the biaxial approach. The selection of this value was shown to lean toward the safe side.

The risk assessment by particle size was performed on the basis of the published exposure data at workplaces handling CNTs.

Our study indicates that monitoring may efficiently control exposure and adopting an effective facility design that limits micron-sized respirable particles (1 < Da < 4 \(\mu\)m), since agglomerate particles of this size markedly contribute to HQ.

Our genotoxicity studies indicated that CNTs did not directly interact with genetic materials; they also support the concept that, even if CNTs are genotoxic, it is of the secondary type, mediated via a pathway of genotoxic damage resulting from oxidative DNA attack by ROS/RNS generated during CNT-elicited inflammation. Secondary genotoxicity is considered to involve a threshold.

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