NEDO project "Research and Development of Nanoparticle Characterization Methods" (P06041)

Risk Assessment of Manufactured Nanomaterials

-Carbon Nanotubes (CNTs)-

Interim Report issued on October 16, 2009

Executive Summary

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CONTENTS

On the Positioning of Interim Reports Released on October 16, 2009

One of the objectives of the project sponsored by New Energy and Industrial Technology Development Organization (NEDO), "Research and Development of Nanoparticle Characterization Methods", is to develop risk assessment of three different substance groups, TiO_2, C_{60}, and CNTs. The risks to be assessed are human health risks, with a primary focus on occupational risk management since the industries involving nanomaterials are still under development.

The scale of the industries handling nanomaterials at present is small, however, it is expected to be developed extensively in the future. The risk assessment of nanomaterials, therefore, is considerably different from those previously conducted by the National Institute of Advanced Industrial Science (AIST) on the substances with relatively long history of use and published in the Risk Assessment Series. The major difference is the emphasis on the framework to predict risks reflecting future changes of situations rather than presenting the fixed risk values based on the assessment of the available data. The changes of situations include the factors such as production volume, form of manufactured products, production methods, and methods of exposure management. These changes are technically defined as the changes of scenario.

Currently, with limited available data, it is not possible to develop hazard assessment and exposure assessment applicable to all the various scenarios. The only possible approach is to present a framework applicable to a number of substances and situations, with supplemental data generated by manufacturers. Such a framework is proposed in the interim reports.

Interim reports released on October 16, 2009 are the documentation of the current status in the process to develop final risk assessments. The purposes to release these interim reports include; firstly the conclusions obtained so far, though not final, are applicable to the management of occupational environment; and secondly, comments and advices are expected to be obtained on the released reports from many experts outside of the project, which would greatly contribute to improving the final outcomes of the risk assessment.

In these interim reports, the procedures to establish a provisional value of an acceptable exposure concentration in the occupational environment are presented. A method is proposed to establish an acceptable exposure concentration in those situations with a limited number of inhalation exposure studies. With TiO_2, a provisional value of an acceptable exposure concentration in the occupational environment is proposed. In the case of C_{60}, of which data with inhalation exposure studies is limited, only rough figures of acceptable exposure concentrations are estimated based on the comparison of particle burden in the lung between inhalation exposure and intratracheal instillation studies. In the final assessment, it is considered possible to propose standards of acceptable exposure concentrations with greater certainty by quantitative application of the data from intratracheal instillation studies. With CNTs, it has not been possible to

discuss standards of acceptable exposure concentrations in the interim report. The standards proposed in the interim reports are estimated primarily to prevent inflammation in the lung associated with inhalation exposure of particles. As described in "the principles and basic approaches to risk assessment of manufactured nanomaterials", no review of carcinogenicity studies has been conducted, however, some effort has been made to detect signs of carcinogenicity with various methods. Though it is premature to conclude, the provisional values presented in the interim reports are applicable at this time to risk management, of measures to prevent inflammatory responses in the lung in situations without possible chronic exposures.

With regard to risk management, measures easily taken by manufacturers are those for exposure control. With reference to these interim reports, risk reduction can be achieved through careful and wise control of exposures. It is sincerely hoped that these interim reports contribute to the risk management at manufacturing sites.

Critical reviews and comments on the interim reports are greatly appreciated for the successful completion of our project.

Regrettably, the results of toxicity studies conducted under NEDO Project have not been fully utilized in these interim reports, but should be incorporated into the final reports of risk assessment

October 16, 2009

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Chapter I: Preface

"The Nanomaterials Risk Assessment Document—Carbon Nanotubes (CNTs)" will be completed and released at the end of fiscal year 2010. This document is positioned as its interim report version (October 16, 2009).

This assessment has two main objectives. One objective is to assess human health risks of CNTs and present recommendations on risk management by using all currently available information. In this assessment, hazard assessment is made on the basis of available hazard information to propose the criteria for acceptable exposure concentrations in working environments. Another objective is to suggest a method for risk assessment of CNTs and other new materials. However, it is generally difficult to make risk assessment of new materials due to the non-existence of hazard- and exposure- information. In particular, the hazards and purpose of CNTs depend heavily on different materials due to its many variations. Therefore, this assessment is considered as a case study to examine the kind of information is obtained and the assessment method to be employed to enable more accurate risk assessments on the basis of limited data. The assessment document describes in detail all processes of a risk assessment, such as judgment grounds and handling of values, and enables CNT manufacturers and users to make their own risk assessment of their CNTs or other new materials using the same method. This assessment is intended to support independent risk assessments and controls used by CNT manufacturers and users.

This report is an outcome of the assessment of human health risks that may be caused by 2 types of materials, the single-walled carbon nanotubes (SWCNTs) and the multiwalled carbon nanotubes (MWCNTs). However, it does not cover ecological risks. This interim version (dated October 16, 2009) focuses only on CNT risks to workers in their work surroundings.

The structure of Chapter II and subsequent chapters are outlined below. Chapter II summarizes basic information about CNTs covered by this assessment. In Chapter III, we review the trends of regulation and control of CNTs in Japan, major developed countries, and international organizations. Chapter IV describes CNT hazard assessments. First, the results of existing studies and New Energy and Industrial Technology Development Organization (NEDO)'s studies on hazard information on SWCNTs and MWCNTs, as well as existing studies on hazard information on other nanoparticles and asbestos that are often compared with CNTs, are reviewed for comparison with those of studies on CNTs. Lastly, we estimate the acceptable exposure concentrations in working environments and propose their appropriate values on the basis of the above information. In Chapters V and VI, workers' exposure to CNTs is investigated. In Chapter V, we investigate workers' exposure to CNTs during the life cycle of a product (manufacturing, use, consumption, disposal/recycling, etc.) that contains this substance. In Chapter VII, we compare the acceptable exposures of SWCNTs and MWCNTs estimated in Chapter IV with the CNT exposures of CNT-handling workers'

estimated in Chapter V and discuss the risks associated with their working environments.

Chapter II: Basic Information

CNT is defined as fiber substances without any defect caused due to the graphite hexagonal-mesh plane (graphene sheet) in the form of a tube or 2-layer, 3-layer, or multi-layers with the nest accumulation (Endo and Takeuchi, 2007). Substances with a tube diameter of 100 nm or less are classified as CNTs. Those with a diameter greater than 100 nm are generally classified as carbon fiber (CF). However, there are CNTs with a minimum tube diameter of 0.41 nm. Tubes with a single-walled and multi-walled structures are called single-walled carbon nanotubes (SWCNTs) (Fig. II. 1A) and multiwalled carbon nanotubes (MWCNTs) (Fig. II. 1B), respectively. MWCNTs with double-walled structures are called double-walled carbon nanotubes (DWCNTs) and often differentiated from MWCNTs.

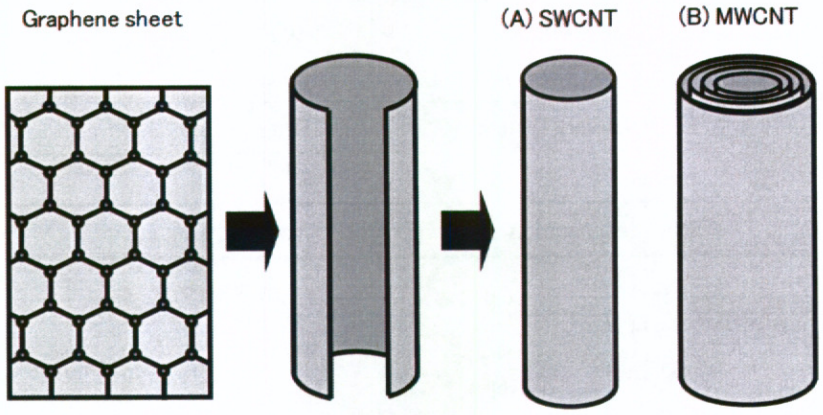

Fig. II. 1 Structures of SWCNT and MWCNT

CNTs have diameters of 100 nm or less, and they have extremely high structural integrity. Although these CNTs comprise only carbon atoms, they have metal or semiconducting characteristics, which depend on the chirality of the carbon atoms, and excellent mechanical properties. Currently, several applied studies on such excellent physical and chemical properties are being actively performed. The future applications of these properties to various fields and purposes are listed in Table II. 1.

Table II. 1 Expected CNT-applied fields and purposes

Field	Purpose	Remarks (parts to be used)
Electronics	Field-effect transistor	FET channel
	LSI wiring	Wiring materials
Energy	Fuel cell	Hydrogen storage and supported materials for electrodes
	Lithium-ion battery	Negative pole materials
	Electric double layer capacitor	Electrode
Electron emission	Field-emission display	Field-emission electron source (negative pole)
	X-ray tube	Field-emission electron so
Chemistry	Absorbent	Contaminant absorbent
	Sensor	
Nanotechnology	Scanning probing microscope	SPM probe
	Nano-manipulation	Nanotube pin set etc.
Composite material	Conductive resin	Filler
	Reinforced plastics	Filler
Medical care	Drug delivery system	Drug carrier
	Catheter	
	Bone regeneration	Artificial joint etc.

Chapter III: Domestic and International Trends of Regulation and Control

Chapter III describes the trends of regulation and control of CNTs in the US, UK, France, German, EU and Japan. In this executive summary, they are omitted. Refer to the main body of the assessment document (in Japanese) for more information.

Chapter IV: Hazard Assessment

1. Introduction

Potential human health hazards are caused by inhalation, dermal, and oral exposure to SWCNTs and MWCNTs. In this interim report (dated October 16, 2009), only the hazards caused by the inhalation of SWCNTs and MWCNTs are evaluated.

2. Hazard information

The information on the adverse effects of SWCNTs and MWCNTs is systematically arranged on the basis of the results of existing and NEDO studies. Due to the fact that there no human epidemiological studies on the adverse effects of SWCNTs or MWCNTs, the hazard information described below is based on the results of an *in vivo* test in laboratory animals and *in vitro* cultured cells. Hazard information on acute, chronic, genetic, and reproductive toxicities is required for the assessment of adverse effects of CNTs, but in this interim report, inhalation toxicities, which are considered to be the most important for assessment of hazard caused by inhalation exposure are first evaluated. Other toxicities will be evaluated in future reports.

2.1 Inhalation toxicity

For assessment of adverse effects of SWCNTs or MWCNTs inhalation, 3 types of *in vivo* tests—inhalation exposure, intratracheal instillation, and pharyngeal aspiration—are performed. Therefore, this section describes the test protocols and results of different test reports on these methods.

2.1.1 Inhalation exposure test

For SWCNT-based inhalation exposure tests, currently, there is only a short-term test case conducted by Shvedova et al. (2008a), which is presented in Table IV. 1. For this test, Shvedova et al. (2008a) used SWCNTs manufactured by using high pressure CO (HiPco) method. A female C57BL/6 mouse was made to inhale air containing SWCNTs at a concentration of 5.53 mg/m^3 for 4 days (5 h/day). Then, they performed a histopathological evaluation of the mouse's lung and measured the inflammatory biomarkers in a bronchoalveolar lavage fluid (BALF). It was reported that SWCNTs existed in air in aggregates and had a mass median aerodynamic diameter (MMAD) of approximately 4.2 μm. In this test, SWCNTs, which contains 17.7 wt% of iron as an impurity, is used as a sample. Consequently, a continuous inflammatory response for 28 days after the exposure was observed. However, it is difficult to determine whether the observed inflammatory response is caused by the inhalation of SWCNTs or iron impurities. NEDO planned 2 projects on inhalation exposure tests using SWCNTs (in which exposure to SWCNTs was assessed for

period of 1 and 3 months). NEDO is currently carrying out 1 of the projects.

Three short-term (several days) and 2 mid-term (1–3 months) inhalation tests using MWCNTs are presented in Table IV.

In the study reported by Mitchell et al. (2007), a male C57BL/6 mouse was made to air with MWCNT concentration of 0.3–5.31 mg/m^3 for 4 days (6 h /day). They performed a histopathological evaluation of the mouse's lung and measured inflammatory biomarkers in BALF. In a 14-day exposure test, the effect of MWCNTs on the immune system was observed. However, the biomarker level in the 1.0 mg/m^3 exposure group was greater than 5 mg/m^3. Therefore, there is no clear dose-dependent relationship.

In the study reported by Li, J.G. et al. (2007), a female Kunming mouse was made to inhale air with MWCNT concentration of 32.61 mg/m^3 for 5, 10, or 15 days. Immediately after the completion of exposure in all the mouse groups, the histopathological evaluation was performed on the mouse lungs in various groups. Hypertrophy of the alveolar walls was observed in all mouse groups at varying exposure periods.

In a study reported by Ma-Hock et al. (2009), female and male Wistar rats were continuously made to inhale air with MWCNT concentration of 2, 8, or 32 mg/m^3 for 5 days (6 h/day). They investigated the inflammatory biomarkers in BALF and measured the lung weights at 3 and 24 days after exposure completion. Increase in the numbers of inflammatory cells, particularly neutrophils and lymphoid cells, were observed at all exposure concentrations and in all observations. Furthermore, their lung weights were significantly increased in the 8 and 32 mg/m^3 MWCNT exposure groups. Inflammatory response, with the number of inflammatory cells in BALF as an indicator, was observed in the 2 mg/m^3 CNT exposure group on day 24 after exposure completion. This test shows an evident dose-inflammatory response relationship. On the basis of the results of this test, Ma-Hock et al. (2009) had female and male Wistar rats inhale air with MWCNT concentration of 0.1, 0.5, and 2.5 mg/m^3 for 13 weeks (6 h/day and 5 days/week) and investigated the effects of MWCNT on the bodies of the rats and performed histopathological evaluation on their lungs. These effects were not observed in all the MWCNT exposure groups, but significant multifocal granulomatous inflammation in the lungs and lung lymph, diffuse histiocytic and neutrophilic inflammation, and intra-alveolar lipoproteinosis in the interior alveoli were observed in all groups except for the 0.1 mg/m^3 exposure group. Minimal granulomatous inflammation in the lungs and lung lymph nodes was observed in the 0.1 mg/m^3 exposure group. Therefore, a "no observed effect concentration (NOEC)" was not established in this study. Consequently, the authors concluded that the 0.1 mg/m^3 concentration was the "lowest observed effect concentration (LOEC)".

In the NEDO project, a male Wistar rat was made to inhale air with a MWCNT concentration of 0.37 mg/m^3 for 4 weeks (6 h/day and 5 days/week). Subsequently, the rat's lung was histopathologically evaluated and inflammatory biomarkers in BALF were evaluated at 3, 7, 28, and 91 days after exposure. The important aspect of this test was that MWCNTs were dispersed in a liquid, then MWCNT-dispersed

solution was sprayed in air (wet method), and then the rat was exposed to air sufficiently diffused with MWCNTs (most of the MWCNTs were diffused in isolation). Currently, this is the only study in which MWCNTs were sprayed in air using the wet method to expose laboratory animals to CNT. The test results are outlined below. The lung wet weight of the rat significantly increased only at 3 days after the completion of the inhalation exposure in comparison to that in the control group; however, significant changes in other observation items, such as inflammatory biomarkers in BALF, were not observed. Consequently, we concluded that this exposure concentration (0.37 mg/m^3) had an minimal effect on rats.

Table IV. 1 Inhalation exposure studies using SWCNTs or MWCNTs (1/2)

Researcher	Sample information					Test conditions						Test results
	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Animal species	Exposure period	Observation points [day]	Observation item	Exposure concentration		
				Original	In air					[mg/m^3]	[m^2/m^3]	
Shvedova et al. (2008a) *SWCNTs	CNI	HiPco	508	0.8–1.2 × 100–1000	4200[a]	Female C57BL/6 mouse (8–10 weeks)	4 days (5 h/day)	1, 7, 28	Lung pathology, BALF	5.53	2.8[b]	Sustained inflammation
Mitchell et al. (2007) *MWCNTs	Shenzhen Nanotech Port	CVD	100	10–20 × 500–1500	700–1000[a]	Male C57BL/6 mouse (10 weeks)	7 days (6 h/day)	0?	Lung pathology, BALF	0.3	0.03[b]	No significant changes.
					1800[a]					1.0	0.10[b]	
										5.3	0.53[b]	
					700–1000[a]		14 days (6 h/day)	0?	Lung pathology, BALF	0.3	0.03[b]	Immune system was suppressed (no inflammation)
										1.0	0.10[b]	
					1800[a]					5.3	0.53[b]	
Li, J.G. et al. (2007) *MWCNTs	Shenzhen Nanotech Port	CVD	280	50 × 1000	-	Female Kunming mouse (11 weeks)	5 days	0	Lung pathology, BALF	32.61	9.13[b]	Hypertrophy of the alveolar wall was observed.
							10 days	0	Lung pathology, BALF	32.61	9.13[b]	
							15 days	0	Lung pathology, BALF	32.61	9.13[b]	

-: Not described/measured, a: MMAD, b: Calculated on the basis of the surface area

Table IV. 1 Inhalation exposure studies using SWCNTs or MWCNTs (2/2)

Researcher	Sample information					Test conditions						Test results
	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Animal species	Exposure period	Observation point [day]	Observation item	Exposure concentration		
				Original	In air					[mg/m^3]	[m^2/m^3]	
Ma-Hock et al. (2009) *MWCNTs	Nanocyl (NC7000)	-	250–300	5–15 × 100–1000	500–1300[a]	Female/male Wistar rats (8–9 weeks)	5 days (6 h/day)	3, 24	Lung weight, BALF	2.4	0.66[b]	Minimal inflammation
										8.4	2.31[b]	Significant increase in inflammatory biomarkers and lung weight
										29.8	8.20[b]	
					1300–2000[a]		13 weeks (6 h/day, 5 day/week)	0	Clinical observation, Lung pathology	0.1	0.03[b]	Minimal granulomatous inflammation
					900–1500[a]					0.5	0.14[b]	Significant inflammation
					700–800[a]					2.5	0.69[b]	
Tanaka & Morimoto (2009) (NEDO project) *MWCNTs	A company	CVD	77	30 × >1000	During meas.	Male Wistar rat	4 weeks (6 h/day, 5 day/week)	3, 28, 91	Lung pathology, BALF	0.37	0.03[b]	Minimal inflammation

-: Not described/measured, a: MMAD, b: Calculated on the basis of the surface area

2.1.2 Intratracheal instillation study

Intratracheal instillation and pharyngeal aspiration studies reported to date and available for this assessment are presented in Table IV. 2, Table IV. 3, and Table IV. 5. Due to the volume of study cases, only the way to read the tables and special notes will be described, while descriptions of individual studies will be omitted. The above tables describe the outline of sample data, test conditions, and results. The doses included in the test conditions are expressed in terms of CNT mass dose/body weight (mg/kg) and CNT surface area dose/body weight (m^2/kg). A column for test results is prepared to show when the animal was anatomized after a single instillation. A ● mark was indicated when inflammation was observed in a lung histopathological evaluation, and ▲ was indicated when a change in the number of inflammatory cells (neutrophil, etc.) was seen in the BALF test and not in the histopathological test. A △ mark was indicated when any change was seen in the inflammatory markers (cytokines, etc.) in BALF , and ○ was indicated when the above changes were not seen. For detailed descriptions, refer to the main body of the assessment document (in Japanese).

Intratracheal instillation study with SWCNTs (Table IV. 2)

Warheit et al. (2004) conducted lung histopathological evaluation and BALF measurements at 24 h – 3 months after 1 and 5 mg/kg instillation of SWCNTs. Changes in the number of inflammatory cells and in other biomarkers were transient, and observed only 24 h after the instillation. However, multifocal granuloma and other changes were observed at later observation points in the lung histopathological evaluation. Considering the fact that a value of the measure of the inflammatory biomarker in BALF was recovered but a lesion in lung tissues was observed, the authors discussed that the mechanism for the development of SWCNTs pulmonary toxicity could be different from a toxicity paradigm of well known particles, such as crystal silica, asbestos, and silicon carbide whisker. In this study, approximately 15% of the subjects in the 5 mg/kg SWCNT-exposed rat group died within 24 h post-instillation. However, in this case, the injected SWCNT suspension (having very high viscosity) blocked the rats' upper respiratory tracts, thereby leading to death by asphyxiation. Consequently, the rats did not die from SWCNT toxicity.

In the study reported by Lam et al. (2004), death after lethargy, decreased activity, and body-weight losses (CarboLex exposure group), formation of granuloma, and significant interstitial inflammation (all in the SWCNT exposure group) were observed in rats at a dose of 0.5 mg/mouse (approximately 16.7 mg/kg). At a dose of 0.1 mg/mouse (approximately 3.3 mg/kg), significant clinical manifestations were not observed, but a light granuloma and other lesions were observed at 90 days after the instillation in the mouse groups exposed to the HiPco unpurified product and purified SWCNT.

The above studies have high historical values, but lack CNT characterization, including assessment of size distribution and impurity content. As mentioned above, artificial effects resulting from intratracheal instillation of aggregate CNTs are seen. Consequently, it is difficult to evaluate the adverse effects of

SWCNTs on an inhalation system from only these studies.

In most recent years, SWCNT intratracheal instillation studies have been conducted by Chou et al. (2008), Miyawaki et al. (2008), and Shvedova et al. (2008b).

In the study reported by Chou et al. (2008), foamy macrophages and multifocal granuloma were observed at 3 days and 14 days after the 0.5 mg/kg instillation. In the study reported by Miyawaki et al. (2008), significant pathological changes in tissues other than the lungs were not observed at 7 and 90 days after the 2.25 mg/rat (17.3 mg/kg) instillation, but the formation of foreign body granuloma was observed in 1 of 4 cases at 7 days after instillation and in 1 of 5 cases at 90 days after instillation. In the study by Shvedova et al. (2008b), the total number of cells in BALF, the number of inflammatory cells, such as neutrophils and macrophages, and values in inflammatory biomarkers such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, transforming growth factor (TGF)-β, and monocyte chemotactic protein (MCP)-1 were significantly increased. In addition, in the pathological test, formation of multifocal granuloma and collagen deposition in the lung tissues was confirmed.

For reference, NEDO is also conducting and analyzing the intratracheal instillation study using 2 types of SWCNTs.

Intratracheal instillation study with MWCNTs (Table IV. 4)

Muller et al. (2005) and Li et al. (2007) performed an intratracheal instillation study using MWCNTs. In addition to these 2 studies, NEDO's 2 studies (Tanaka and Morimoto, 2009; Naya et al., 2009) are now being implemented.

In the study reported by Muller et al. (2005), the increase in dose-dependent inflammatory biomarkers in BALF (LDH, proteins, neutrophils, and eosinophils) was observed. Furthermore, fibrotic responses, such as increase in hydroxyproline (OH-proline), and collagen depositions were observed at 60 days after the instillation of MWCNT. It was found that the inflammatory response in more shortly dispersed MWCNT samples was larger than in the MWCNT samples.

In the study reported by Li, J.G. et al. (2007), it was found that agglomerates of MWCNTs were deposited in the lining wall of bronchi at 16 days after the 0.05 mg (approximately 1.7 mg/kg) instillation, but the inflammation of the lung tissues was not observed. On the other hand, it was found that aggregated MWCNTs were deposited into the alveoli, and the net structure of the alveoli was severely destructed at 24 days after instillation. The result that toxicity observations were first observed approximately 1 month after instillation was seen only in this study.

NEDO is also conducting the intratracheal instillation study using 2 types of MWCNTs.

In the study reported by Tanaka & Morimoto (2009), a histopathological evaluation on lung tissues and assessment of inflammatory biomarkers in BALF were performed for an observation period of 3 days – 2 years after the CNT instillation of 0.2 mg/rat (approximately 0.67 mg/kg) or 1 mg/rat (3.3 mg/kg). The

results of the study confirmed the dose-dependent changes. In the 0.2 mg/rat exposure group, the increase in the lungs' wet weights was observed for 3 months after the instillation, while the significant increase in the number of total cells and neutrophils in BALF, and *HO-1* genes in the lung tissues were observed for 1 month after the instillation. For reference, the number of *HO-1* genes in BALF was also measured in this study. The number of BALF *HO-1* genes was not significantly increased in both the exposure groups and at all exposure periods. In addition, the histopathological evaluation on the lungs was performed to evaluate the inflammatory scores of the lungs by point counting method. The scores were significantly increased in the 1 mg/kg exposure group for 1 month after the instillation. The result of the histopathological evaluation on the lungs showed significant increase in inflammatory scores measured using point counting method at 1 month after the instillation[1].

In the study reported by Naya et al. (2009), histopathological changes and a significant increase in the BALF inflammatory biomarkers were observed in the 0.2 and 1 mg/kg exposure groups at only 3 days after instillation. However, these were minimal changes that were not found in 1 week or subsequent weeks after instillation. The 0.04 mg/kg exposure group was not affected at all the observation points.

[1] Due to the fact that not all test results have been obtained at this time, conclusions may be changed in the final assessment.

Table IV. 2 Intratracheal instillation studies using SWCNTs (1/2)

Researcher	Sample information					Test conditions					Test results				
	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation point [day]	Observation item	Dose		1 d	3 d	1–2 wk	1 mo	3 mo
				Original	In liquid				[mg/kg]	[m^2/kg]					
Warheit et al. (2004)	DuPont	Laser ablation	-	1.4 × > 1000	-	Male SD rat (8 weeks)	1, 7, 28, 91	Lung pathology, BALF	1	-	▲	-	●	●	▲
									5	-	▲	-	▲	▲	▲
Lam et al. (2004)	Rice Univ.	HiPco	-	-	-	Male B6C3F1 mouse (8 weeks)	7, 90	Lung pathology	3.3[a]	-	-	-	●	-	●
									16.7[a]	-	-	-	●	-	●
		HiPco (Purified product)	-	-	-				3.3[a]	-	-	-	●	-	●
									16.7[a]	-	-	-	●	-	●
	CarboLex	-	-	-	-				3.3[a]	-	-	-	○	-	○
									16.7[a]	-	-	-	●	-	●
Chou et al. (2008)	-	-	-	-	-	ICR mouse	3, 14	Lung pathology	0.5	-	-	●	●	-	-

-: Not described/measured, a: Calculated on the basis of the body weight described in the paper or on the basis of the typical body weight of the same species, b: Calculated on the basis of the surface area ●: Histopathological evaluation of the lungs revealed inflammation. ▲: The number of BALF inflammatory cells (e.g., neutrophils) changed. Results from histopathological evaluation of the lungs did not exist. Δ: BALF inflammatory biomarkers (e.g., cytokines) changed. ○: No change was observed.

Table IV. 2 Intratracheal instillation studies using SWCNTs (2/2)

Researcher	Sample information					Test conditions					Test results				
	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation point [day]	Observation item	Dose		1 d	3 d	1–2 wk	1 mo	3 mo
				Original	In liquid				[mg/kg]	[m^2/kg]					
Miyawaki et al. (2008)	CNI	HiPco	-	1 × > 1000	-	Male Wistar rat (5 weeks)	7, 90	Lung pathology	17.3[a]	-	-	-	●	-	●
Shvedova et al. (2008b)	CNI	HiPco	1040	1–4 × ?	-	C57BL/6 mouse (7–8 weeks)	1, 7, 28	BALF	1.3[a]	1.4[b]	▲	-	▲	▲	-
Naya et al., (2009) *NEDO's project	-	CVD						Ongoing							
Tanaka & Morimoto, (2009) *NEDO's project	A company	CVD						Ongoing							

-: Not described/measured, a: Calculated on the basis of the body weight described in the paper or on the basis of the typical body weight of the same species, b: Calculated on the basis of the surface area ●: Histopathological evaluation of the lungs revealed inflammation. ▲: The number of BALF inflammatory cells (e.g., neutrophils) changed. Results from histopathological evaluation of the lungs did not exist. △: BALF inflammatory biomarkers (e.g., cytokines) changed. ○: No change was observed.

Table IV. 3 Intratracheal instillation studies using MWCNTs (1/2)

Researcher	Sample information					Test conditions					Test results				
	Manufacturer	Manufacturing. method	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation point [day]	Observation item	Dose		1 d	3 d	1–2 wk	1 mo	3 mo
				Original	In liquid				[mg/kg]	[m^2/kg]					
Muller et al. (2005)	Original	CVD	378	9.7 × 5900	-	Female SD rat	3, 15, 60	Lung pathology, BALF	2.2[a]	0.15[b]	-	▲	○	○	-
									8.9[a]	0.59[b]	-	▲	▲	●	-
									22.2[a]	1.48[b]	-	▲	▲	▲	-
			307 (ground)	11.3 × 700 (ground)	- (ground)				2.2[a]	0.15[b]	-	▲	▲	△	-
									8.9[a]	0.59[b]	-	▲	▲	●	-
									22.2[a]	1.48[b]	-	▲	▲	▲	-
Li, J.G et al. (2007)	Shenzhen Nanotech Port	-	280	50	1000	Female Kunming mouse (11 weeks)	8, 16, 24	Lung pathology	1.7[a]	0.47[b]	-	-	○	●	-

-: Not described/measured, a: Calculated on the basis of the body weight described in the paper or on the basis of the typical body weight of the same species, b: Calculated on the basis of the surface area ●: Histopathological evaluation of the lungs revealed inflammation. ▲: The number of BALF inflammatory cells (e.g., neutrophils) changed. Results from histopathological evaluation of the lungs did not exist. △: BALF inflammatory biomarkers (e.g., cytokines) changed. ○: No change was observed.

Table IV. 3 Intratracheal instillation studies using MWCNTs (2/2)

Researcher	Sample information					Test conditions					Test results				
	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation point [day]	Observation item	Dose		1 d	3 d	1–2 wk	1 mo	3 mos
				Original	In liquid				[mg/kg]	[m^2/kg]					
Tanaka & Morimoto (2009) *NEDO project	A company	CVD	77	30 × > 1000	Under measurement	Male Wistar Rat	3, 7, 28, 91, 180, 365, 730	Lung pathology, BALF	0.67[a]	0.05[b]	-	●	○	○	○
									3.3[a]	0.26[b]	-	●	●	●	○
Naya et al. (2009) *NEDO project	B company	CVD	37	Under measurement	Under measurement	Male SD rat (8 weeks)	3,7, 28, 91, 180	Lung pathology, BALF	0.04	0.0015[b]	-	○	○	○	○
									0.2	0.007[b]	-	●	○	○	○
									1	0.037[b]	-	●	○	○	○

-: Not described/measured, a: Calculated on the basis of the body weight described in the paper or on the basis of the typical body weight of the same species, b: Calculated on the basis of the surface area ●: Histopathological evaluation of the lungs revealed inflammation. ▲: The number of BALF inflammatory cells (e.g., neutrophils) changed. Results from histopathological evaluation of the lungs did not exist. △: BALF inflammatory biomarkers (e.g., cytokines) changed. ○: No change was observed.

2.1.3 Pharyngeal aspiration test

Pharyngeal aspiration is an exposure method by which a mouse's lung is exposed to a test substance by aspiration. CNT-based pharyngeal aspiration tests reported to date are all for SWCNTs (Table IV. 5).

In the test reported by Shvedova et al. (2005), dose-dependent lung inflammatory responses and histopathological changes (formation of granulomas) were observed at doses of 10, 20, and 40 μg/mouse (approximately 0.5, 1, and 2 mg/kg, respectively). In the 20 and 40 μg/mouse exposure groups, formation of a small localized interstitial fibrotic lesion was confirmed at 21 days after exposure.

In the study by Mangum et al. (2006), significant changes were not observed at a dose of 2 mg/kg in the BALF test, but formation of a small localized interstitial fibrotic lesion was confirmed 21 days after exposure.

In the test reported by Li Z. et al. (2007), significant increase in *HO-1* genes was confirmed at doses of 10 and 40 μg/mouse (approximately 0.4 and 1.6 mg/kg, respectively) at 7 days after exposure, while damaged mtDNA in arteries was observed for 56 days after the exposure. It is difficult to evaluate the toxicological significance of SWCNTs merely on the basis of these results, but the authors argue about the systemic effects of SWCNTs and the necessity of further studies.

In the study reported by Shvedova et al. (2007), the short-term (1–7 days) increase of inflammatory cells in BALF after exposure and fibrotic responses, such as collagen deposition at 28 days after exposure were confirmed at the dose of 40 μg/mouse (approximately 1.9 mg/kg).

Mercer et al. (2008) exposed mouse groups to 2 types of SWCNTs, dispersed SWCNTs (average particle size, 0.69 μm) and non-dispersed SWCNTs (average particle size, 15.2 μm) at a dose of 10 μg/mouse (approximately 0.3 mg/kg) and compared the biological effects between the dispersed and non-dispersed SWCNTs exposed groups. In both SWCNT exposure groups, there was a transient increase in neutrophils and macrophages in BALF, but hypertrophy of the alveolar walls was confirmed only in the dispersed SWCNT exposure group. The authors discussed that the dispersed SWCNTs rapidly incorporated into the alveolar interstitium due to hard recognition of the dispersed SWCNTs by alveolar macrophages.

In the study reported by Shvedova et al. (2008a), dose-dependent lung inflammatory responses were observed at doses of 5, 10, or 20 μg/mouse (approximately 0.25, 0.5, and 1 mg/kg, respectively). The collagen concentration in the 10 μg/mouse exposure group continuously increased 28 days after the exposure. However, the SWCNT used in this test contains approximately 18 wt% of iron as an impurity.

Table IV. 4 Pharyngeal aspiration studies using SWCNTs (1/2)

Researcher	Sample information					Test condition					Test result				
	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation point [day]	Observation item	Dose		1 d	3 d	1–2 wk	1 mo	3 mo
				Original	In liquid				[mg/kg]	[m^2/kg]					
Shvedova et al. (2005)	CNI	HiPco	1040	1–4 × ?	-	Female C57BL/6 mouse (7–8 weeks)	1, 3, 7, 28, 60	Lung pathology, BALF	0.5[a]	0.5[b]	▲	▲	▲	▲	-
									1[a]	1.0[b]	▲	▲	▲	●	-
									2[a]	2.1[b]	▲	▲	●	●	-
Mangum et al. (2006)	Helix Material Solutions	CVD	300–600	< 2 × 500–40000	-	Female F344 rat (6 weeks)	1, 21	Lung pathology, BALF	2	0.90[b]	▲	-	●	-	-
Li Z et al. (2007)	CNI	HiPco	-	-	-	C57BL/6 mouse (2–3 months)	1, 7, 28, 56	Oxidative stress, DNA damage	0.4[a]	-	○	-	△	△	-
									1.6[a]	-	○	-	△	△	-

-: Not described/measured, a: Calculated on the basis of the body weight described in the paper or on the basis of the typical body weight of the same species, b: Calculated on the basis of the surface area ●: Histopathological evaluation of the lungs revealed inflammation. ▲: The number of BALF inflammatory cells (e.g., neutrophils) changed. Results from histopathological evaluation of the lungs did not exist. △: BALF inflammatory biomarkers (e.g., cytokines) changed. ○: No change was observed.

Table IV. 4 Pharyngeal aspiration studies using SWCNTs (2/2)

Researcher	Sample information					Test condition					Test result				
	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation point [day]	Observation item	Dose		1 d	3 d	1–2 wk	1 mo	3 mo
				Original	In liquid				[mg/kg]	[m^2/kg]					
Shvedova et al. (2007)	CNI	HiPco	1040	1–4 × ?	-	Female C57BL/6 mouse (7–8 weeks)	1, 7, 28	Lung pathology, BALF	1.9[a]	1.9[b]	●	-	▲	●	-
Mercer et al. (2008)	CNI	HiPco	-	-	690 (Area equivalent)	Male C57BL/6 mouse (25 weeks)	1, 7, 28	Lung pathology, BALF	0.3[a]	-	●	-	●	●	-
					1520 (Area equivalent)				0.3[a]	-	●	-	●	●	-
Shvedova et al. (2008a)	CNI	HiPco	508	0.8–1.2 × 100–1000	-	Female C57BL/6 mouse (8–10 weeks)	1, 7, 28	BALF	0.25[a]	0.13[b]	▲	-	-	-	-
									0.5[a]	0.25[b]	▲	-	▲	▲	-
									1[a]	0.51[b]	▲	-	▲	▲	-

-: Not described/measured, a: Calculated on the basis of the body weight described in the paper or on the basis of the typical body weight of the same species, b: Calculated on the basis of the surface area ●: Histopathological evaluation of the lungs revealed inflammation. ▲: The number of BALF inflammatory cells (e.g., neutrophils) changed. Results from histopathological evaluation of the lungs did not exist. △: BALF inflammatory biomarkers (e.g., cytokines) changed. ○: No change was observed.

2.2 Carcinogenicity

Currently, little information is available on the carcinogenicity of CNTs, but the information receives great attention due to the released paper of Takagi et al. (2008a).

Takagi et al. (2008a) administered Mitsui's MWCNTs (MWNT-7) into a p53+/- transgenic mouse's abdominal cavity at the dose of 3 mg/mouse (approximately 100 mg/kg). In the result, it was reported that 14 of 16 cases in the MWCNT administration group died of mesotheliomas up to 180 days after the injection. In this test, fullerene C_{60} and crocidolite of UICC grade were administered as negative and positive control substances in equal proportion, respectively. According to the report, mesothelioma was not observed in the fullerene administration group and solvent administration (control) group, but it was confirmed in 14 of 18 cases in the crocidolite administration group. In light of these results, which suggest that MWCNTs might have asbestos-like toxicity, the authors discussed that this potential toxicity is due to the similarity between the shapes of MWCNTs and asbestos. Immediately after the inclusion of this paper, Ichihara et al. (2008) and Donaldson et al. (2008) submitted Letters to the Editor indicating that the paper had critical defects. The details of their letters stated that the setting of the dose was exceedingly unrealistic, the number of fibers in MWCNTs was underestimated, light microscope photos were not presented as evidence of mesothelioma, these cases might die from intestinal strangulation, MWCNTs used in this test were insufficiently characterized, and the test system was not sufficiently verified. Subsequently, the authors argued against these indications (Takagi et al., 2008b; 2008c).

A conclusion in the test reported by Muller et al. (2009) was contrary to that in the above test reported by Takagi et al. (2009). Muller et al. (2009) administered MWCNTs into the abdominal cavity of a male Wistar rat at the dose of 2 or 20 mg/rat. The rat was observed for 2 years after administration, and it was reported that there was no difference between the incidence of mesothelioma in the MWCNT group and control group. For reference, UICC crocidolite was administered as a positive control substance at a dose of 2 mg/rat. The incidence of mesothelioma in the crocidolite administration group was significantly increased. Consequently, the authors estimated that short MWCNTs (average length, < 1 μm) used in this test would have no carcinogenicity.

As stated above, it is difficult to reach a conclusion about the carcinogenicity of CNTs at this stage; carcinogenesis as an endpoint is not evaluated in this interim report (dated October 16, 2009). However, analysis of carcinogenicity is very important in evaluating the adverse effects of CNTs. Consequently, the analysis will be performed again in the final version of the report version.

3 Comparison between pulmonary responses to CNTs and other substances

This section describes the characteristics of adverse effects of CNTs by comparing the pulmonary responses exposed to CNTs and other substances, primarily in the intratracheal instillation study. Desirable

substances to be compared include low-solubility particles for the particle pathogenicity paradigm and asbestos for the fiber pathogenicity paradigm. Therefore, existing studies on pulmonary responses to low-solubility particle and asbestos exposure are reviewed to examine the similarities and differences in the responses of these substances and CNTs.

3.1 Response of titanium dioxide (TiO_2)

For information about the intratracheal instillation study on titanium dioxide particles, refer to Table IV. 6 in the main body of the assessment document (in Japanese) or Table 4 in the Executive Summary of assessment document of TiO_2 nanomaterial. The pulmonary inflammatory responses were transient at a high dose of 5 mg/kg. Recovery from the inflammatory responses was observed at 3 months after instillation in many studies (Rehn et al., 2003; Warheit et al., 2006, 2007a; Kobayashi et al., 2009).

3.2 Response of crystalline silica

For information about the intratracheal instillation study on crystalline silica, refer to Table IV. 7 in the main body of the assessment document (in Japanese) or Table 6 in the Executive Summary of assessment document of TiO_2 nanomaterial.

In the intratracheal instillation studies reported by Rehn et al. (2003), Warheit et al. (2006, 2007a), and NEDO's study performed by Kobayashi et al. (2009), crystalline silica was intratracheally instilled into rat lungs at doses of 1–5 mg/kg. Pulmonary inflammatory responses (inflammatory observation in the lung histopathological evaluation, increase of inflammatory cells, LDH, and protein in the BALF) that continued at least for 3 months after instillation were observed in all of the studies. Crystalline silica causes greater and sustained pulmonary inflammatory responses.

3.3 Response of nickel oxide (NiO)

For information about the intratracheal instillation study on nickel oxide, refer to Table IV. 8 in the main body of the assessment document (in Japanese) or Table 7 in the Executive Summary of assessment document of TiO_2 nanomaterial. In the study by Ogami et al. (2009), it was observed that pulmonary inflammation depended on the size of the nickel oxide particles at the dose of 2 mg/rat (approximately 6.7 mg/kg). In the nano-sized NiO-exposed group, sustained pulmonary inflammation for 6 months after instillation was observed. On the other hand, inflammation was transient in the micro-sized NiO exposed group.

In NEDO project, NiO was treated as a positive control. In the study reported by Nishi et al. (2009), the sustained pulmonary inflammation for 6 months after instillation was observed in the groups into which nano-sized NiO particles were intratracheally instilled at doses of 0.33 and 0.67 mg/kg.

3.4 Response of asbestos

Intratracheal instillation studies using asbestos (crocidolite, chrysotile, amosite, etc.) have been long conducted (since the 1980s). However, only short-term (1 month) effects after intratracheal instillation of asbestos were mainly examined. Some studies and cases are presented in Table IV. 5.

Lemaire et al. (1985) intratracheally instilled a chrysotile standard from Union Internationale Contre le Cancer (UICC) (containing a large amount of 5 μm chrysotile) and short chrysotile (containing a large amount of 0.5 μm chrysotile) extracted from the Quebec 4T30 chrysotile into male Wistar rats at a dose of 5 mg/rat (approximately 16.7–20 mg/kg). Lung tissues of the rats at 1, 7, 14, 21, and 60 days after asbestos instillation were histopathologically evaluated. Alveolitis and bronchiolitis obliterans were observed in the group exposed to short chrysotile and the group exposed to long chrysotile, respectively. This case illustrates that pathological observations depend on sample lengths.

Kamp et al. (1995) intratracheally instilled a UICC amosite standard (unknown diameter and length) into the male SD rat group at the dose of 5 mg/rat (approximately 16.7–20 mg/kg) and histopathologically examined the change in inflammatory biomarkers in BALF and the rats' lungs at 1, 7 and 4 weeks after instillation. In the result, a significant difference between the numbers of the BALF total cells was observed only 1 week after the injection, but the number of neutrophils, giant cells, and fibrosis scores significantly increased for 4 weeks after instillation. In addition, in the histopathological test on the lungs, the induction of neutrophils, giant cells, and macrophages was observed. This demonstrated the continuity in the fibrotic change.

Dörger et al. (2002) intratracheally instilled crocidolite (diameter, 0.3 ± 0.2 μm; length, 9.9 ± 7.8 μm) into 2 animal species, male CD rats and male SYR hamsters, at a dose of 5 mg/kg and examined the changes in inflammatory biomarkers in BALF at 1 and 7 days after instillation. In this study, a great difference in response between the 2 animal species was observed. For instance, significant increase in the protein concentrations in BALF was observed until 7 days post-instillation in rats, however, the change in inflammatory biomarkers in BALF was observed only 1 day post-instillation in hamsters.

Ogami et al. (2007) examined the long-term (6 months) effect of asbestos on rats after intratracheal instillation. They intratracheally instilled a UICC crocidolite (diameter, 0.2 μm and length, 1.3 μm – geometric averages) into male Wistar rats at a dose of 2 mg/rat (approximately 6.7 mg/kg) as a control substance for the test on silicon carbide whisker and potassium octatitanate whisker, and examined the histopathological change in the rats' lungs for 6 months after instillation. The degree of inflammation of the lung tissues, which is determined on the basis of an inflammatory score by the authors' point counting method, was significantly increased for 6 months after instillation. This inflammatory score in the crocidolite group is clearly smaller than that in the rat group into which Min-U-Sil-5 crystalline silica was instilled at the same dose.

There is 1 case, the intratracheal instillation study reported by Muller et al. (2005), in which the

pulmonary responses of CNTs were directly compared with those of asbestos in 1 test. A UICC chrysotile was intratracheally instilled into this group as a control substance for MWCNTs (diameter, 0.17 μm and length, 2.4 μm; geometric averages) into female SD rats at a dose of 2 mg/rat (approximately 8.9 mg/kg) and evaluated BALF inflammatory biomarkers at 3, 15, and 60 days after instillation and histopathologically examined the rats' lung tissues at 60 days after instillation. Significant increase in LDH, proteins, neutrophils, and eosinophils in the BALF and fibrotic responses, such as increase in hydroxyproline and intrapulmonary collagen deposition were observed. These responses were larger than responses in the 2 mg/kg MWCNT exposure group and equivalent to those in the 5 mg/rat (22.2 mg/kg) group.

Table IV. 5 Intratracheal instillation studies using asbestos (1/2)

Researcher	Sample information					Test conditions					Test results				
	Manufacturer (Supplier)	Sample name	Surface area [m^2/g]	Particle size [μm] Original	Particle size [μm] In liquid	Animal species	Observation point [day]	Observation item	Dose [mg/kg]	Dose [m^2/kg]	1 d	3 d	1–2 wk	1 mo	3 mo
Lemire et al. (1985)	National Research Institute for Occupational Diseases	UICC chrysotile B	26.8	> 2: 100% > 5: 42% > 10: 21%	-	Male Wistar rat	1, 7, 14, 21, 60	Lung pathology	16.7–20[b]	0.45–0.54[c]	○	-	●	●	-
	Original	short 4T30 chrysotile	38	> 0.5: 50% < 3: 98% < 8: 100	-				16.7–20[b]	0.63–0.76[c]	○	-	●	●	-
Kamp et al. (1995)	UICC	amosite	-	-	-	Male SD rat	7, 14, 28	BALF; lung pathology	16.7 – 20[b]	-	-	-	●	●	-

-: Not described/measured, a: Calculated on the basis of the body weight described in the paper or on the basis of the typical body weight of the same species, b: Calculated on the basis of the surface area ●: Histopathological evaluation of the lungs revealed inflammation. ▲: The number of BALF inflammatory cells (e.g., neutrophils) changed. Results from histopathological evaluation of the lungs did not exist. △: BALF inflammatory biomarkers (e.g., cytokines) changed. ○: No change was observed.

Table IV. 5 Intratracheal instillation studies using asbestos (2/2)

Researcher	Sample information					Test conditions					Test results				
	Manufacturer (Supplier)	Sample name	Surface area [m^2/g]	Particle size [μm]		Animal species	Observation point [day]	Observation item	Dose		1 d	3 d	1–2 wk	1 mo	3 mo
				Original	In liquid				[mg/kg]	[m^2/kg]					
Dörger et al. (2002)	-	crocidolite	-	0.3 ± 0.2 × 9.9 ± 7.8	-	Male CD rat	1, 7	BALF	5.0	-	-	▲	▲	-	-
						Male SYR hamster	1, 7	BALF	5.0			▲	○	-	-
Muller et al. (2005)	UICC	Rhodesian chrysotile A	-	0.17 × 2.4	-	Female SD rat	3, 15, 60	Lung pathology; BALF	8.9[b]	-	-	▲	▲	●	-
Ogami et al. (2007)	UICC	crocidolite	-	0.2 × 1.3	-	Male Wistar rat (9 weeks)	3, 7, 28, 91, 180	Lung pathology	6.7[b]	-	-	●	●	●	●

-: Not described/measured, a: Calculated on the basis of the body weight described in the paper or on the basis of the typical body weight of the same species, b: Calculated on the basis of the surface area ●: Histopathological evaluation of the lungs revealed inflammation. ▲: The number of BALF inflammatory cells (e.g., neutrophils) changed. Results from histopathological evaluation of the lungs did not exist. △: BALF inflammatory biomarkers (e.g., cytokines) changed. ○: No change was observed.

3.5 Summarized comparisons of biological responses

Pulmonary inflammatory responses to CNTs and other substances (inflammatory responses, primarily) in the intratracheal instillation studies mentioned above can be classified into type in terms of response change over time.

Pulmonary inflammatory responses were transient when they were exposed to a relatively high dose, 5 mg/kg of nano-, micron-, or submicron-TiO_2 particles. The inflammatory responses were significantly recovered 1–3 months after exposure. Although the responses varied slightly with different crystalline structures (Rehn et al, 2003; Warheit et al., 2007) and particle sizes (Kobayashi et al, 2009), it is believed that the biological effects of the TiO_2 particles are not exceedingly large.

In contrast, pulmonary inflammatory responses sustained for 3–6 months after exposure when they were exposed to crystalline silica and NiO particles at 1 mg/kg (Warheit et al, 2006; 2007) or lower doses (Nishi et al, 2009). Responses to these particles for a long-term (1 to 6 months) after exposure were greater than those at the initial stage (24 h–1 week) after exposure. Fibrotic responses such as collagen deposition in the lung tissues were also observed. These responses are greatly different from the responses to TiO_2 both quantitatively and qualitatively.

Biological responses to asbestos (crocidolite and chrysotile) are also believed to be similar to those to crystalline silica in terms of inflammatory sustainability. In the study reported by Ogami et al. (2007), the degree of inflammation in the lung tissues in the rat group exposed to crocidolite at 6.7 mg/kg had the tendency to increase for 3 days–6 months after exposure. Compared to the silica exposure rat group, the crocidolite exposure group's long-term inflammatory response (3–6 months) after exposure was not as large, but the short-time response (3 days–1 month) was larger. The long-term inflammatory response (3–6 months) to crocidolite after exposure is basically very similar to that to silica, nickel oxide, and asbestos.

The inflammatory response to CNTs is considered to lie in between inflammatory responses to low-toxicity particles TiO_2 and the particles or fibers such as silica, NiO, and asbestos. Namely, the sustainability of the responses greatly varied between doses. For instance, in the study of Muller et al. (2005) shown in Table IV. 3, a trend in recovery of inflammatory response could be observed at a dose of 2 mg/kg, but a trend for enhancement of the inflammatory response could be seen for 3 months after -exposure at a dose of 5 mg/kg. Furthermore, fibrotic responses such as collagen depositions in lungs were observed in some intratracheal instillation studies (Shvedova et al., 2008b; Mercer et al, 2008, etc.). Also in the intratracheal instillation studies using MWCNTs under the NEDO project (Tanaka & Morimoto, 2009; Naya et al., 2009), a relationship between inflammatory responses and doses was observed.

As mentioned above, a dose-response relationship of CNT exposure is clear. When adverse effects are limited to inflammatory responses, it is possible to control CNT risk by reducing exposure to a certain level or lower.

4. Estimation of acceptable exposure in working environment

4.1 Estimation of no-observed adverse effect level (NOAEL) for laboratory animals

4.1.1 Setting of assessment endpoints

Among the biological effects to SWCNT and MWCNT, "pulmonary inflammation," observed at minimum concentrations of SWCNT and MWCNT, is set as an assessment endpoint. It was reported that granuloma was observed in lung tissues despite the recovery of inflammatory biomarkers in BALF. Therefore, the presence or absence of pulmonary inflammation shall be determined mainly based on the results of histopathological evaluation on the lungs, and also based on the number of inflammatory cells and the results of measurement of other inflammatory biomarkers when the results of the histopathological tests are unavailable.

At this moment, NOAEL for SWCNTs cannot be determined from the results of existing inhalation exposures tests. We will determine the NOAEL for SWCNTs based on the future studies under the NEDO project.

Four inhalation exposures tests on MWCNTs have been conducted to date. In the inhalation exposure test under the NEDO project, a rat group was exposed to A company's MWCNTs with an air concentration of 0.37 mg/m3 for 4 weeks. Three days after exposure, a transient increase of the group's lung weight was confirmed, but inflammation of the lung tissues or a change of inflammatory biomarkers in BALF was not observed. Consequently, this MWCNT concentration shall be provisionally determined to be an air concentration equivalent to NOAEL for MWCNTs.

4.2 Calculation of deposition into lung

Based on the same method and parameters as in the TiO_2 risk assessment document, A company MWCNT's deposition into rats' lungs is calculated from an air concentration equivalent to NOAEL. In the result, a value of 6.0 μg/kg/day can be obtained. This value is divided by the product of estimated uncertainty factors (UF) of 2 (UF concerning TK difference: 1 × UF concerning TD difference: 1 × UF concerning extrapolation of exposure period: 2 × UF concerning individual difference: 1) to calculate an acceptable exposure in working environments. In the result, 3.0 μg/kg/day can be obtained. In this assessment, this value is considered as (A company's) acceptable MWCNT exposure to humans.

4.3 Estimation of acceptable exposure concentration

The above acceptable exposure is converted to an air concentration to estimate an acceptable exposure concentration of MWCNTs in working environments. (An acceptable exposure concentration of SWCNTs will not be estimated because the NOAEL cannot be determined.

First, an acceptable exposure is converted to an acceptable exposure concentration based on the same

calculation method as in the TiO_2 risk assessment document. In the result, the acceptable exposure concentration can be determined to be 0.21 mg/m^3. In this assessment, this value is proposed as an acceptable exposure concentration (time weighted average, TWA) in working environments exposed to (A company's) MWCNT on the assumption of working 8 hours a day and 5 days a week.

5. Relative comparison of CNT's hazard potential based on results of the intratracheal instillation studies

In this section, a relative comparison of CNT's hazard potential is made based on the results of intratracheal instillation studies to estimate allowable exposure concentrations of other CNTs whose results are generated only from the intratracheal instillation studies.

For a relative comparison of hazard potential, the number of neutrophils in BALF is used as an indicator. This value is the most commonly used as an indicator of pulmonary inflammatory responses and has been measured in many papers. Due to different methods for sampling BALF, however, an increasing rate of neutrophils compared with the negative control group is calculated instead of using an absolute value of the neutrophils in BALF. This increasing rate shall be used as an indicator for the relative comparison. In the existing intratracheal instillation studies using CNTs, as shown in Table IV. 2, values 24 h post-instillation were measured most frequently. However, it was judged that this time point was greatly affected by the artificial injection. Consequently, values 1 week after the instillation (1 month after injection in the absence of data) shall be used for the relative comparison.

First, an increasing rate of neutrophils in BALF 1 week and 1 month after intratracheal instillation (Table IV. 6) was calculated based on the results of intratracheal instillation tests and pharyngeal aspiration tests using SWCNTs in Tables IV. 2 and 4. Some samples have variations in values, but it has not been examined if these variations result from CNT properties, impurities, or diffusion methods in the intratracheal instillation. We will now increase the number of samples to sufficiently consider them and present the results of an allowable exposure concentration in working environment for each sample in the final report version.

Table IV. 6 Increasing rates of BALF neutrophils by intratracheal instillation of 1 mg/kg SWCNT

Researcher	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Increasing rate[a]	
				Original	In liquid	1 wk	1 mo
Warheit et al. (2004)	DuPont	Laser albation	-	1.4 × > 1000	-	1.2	0.7
Shvedova et al. (2008b)	CNI	HiPco	1040	1–4 × ?	-	4.7[b]	4.7[b]
Shvedova et al. (2005)	CNI	HiPco	1040	1–4 ×?	-	20	20
Shvedova et al. (2008a)	CNI	HiPco	508	0.8–1.2 × 100–1000	-	53[b]	-

-: No description measurements in papers

a: $\frac{\text{Number of neutrophils in BALF for each SWCNT exposure group}}{\text{Number of neutrophils in BALF for negative control group}}$

b: Estimated from test results at a dose near 1 mg/kg

Next, the increasing rate of BALF neutrophils at 1 week and 1 month post-instillation (Table IV. 7) was calculated based on the results of intratracheal instillation tests using MWCNTs in Table IV. 3. Only histopathological changes of the lungs exposed to MWCNTs were investigated in many of the studies. Only two studies under the NEDO project (Tanaka & Morimoto, 2009; Naya et al., 2009) enabled the comparison of increasing rates of BALF neutrophils. In the study of Tanaka & Morimoto (2009), the same sample as in the inhalation exposure test (Table IV. 1) was used. For this reason, the table also includes an increasing rate of neutrophils in the study of Naya et al. (2009) when an increasing rate of BALF neutrophils in the study of Tanaka & Morimoto (2009) is assumed to be 1.

The increasing rates of BALF neutrophils after intratracheal instillation at the dose of 1 mg/kg were small and had the same value in these two studies. For this reason, an acceptable exposure of B company's MWCNTs is estimated to be equivalent to that (0.21 mg/m^3) of A company's MWCNTs used in the inhalation exposure test.

Table IV. 7 Increasing rates of BALF neutrophils by intratracheal instillation of 1 mg/kg MWCNT

Researcher	Manufacturer	Manufacturing method	Surface area [m^2/g]	Particle size [nm]		Increasing rate[a]		Ratio of increasing rate[b]	
				Original	In liquid	1 wk	1 mo	1 wk	1 mo
Tanaka & Morimoto (2009) *NEDO project	A company	CVD	77	30 × > 1000	Under measurement	1.5[c]	1.5[c]	1[c]	1[c]
Naya et al. (2009) *NEDO project	B company	CVD	37	Under measurement	Under measurement	1.1	1.9	0.8	1.2

a: $\frac{\text{Number of neutrophils in BALF for each MWCNT exposure group}}{\text{Number of neutrophils in BALF for negative control group}}$

b: $\frac{\text{Number of neutrophils in BALF for each MWCNT exposure group}}{\text{Increase rate of neutrophils in BALF for A company's MWCNT exposure group}}$

c: Estimated from test results at a dose near 1 mg/kg

Chapter V: Exposure Assessment of CNT Powder

In this chapter, we estimate CNT inhalation exposure of workers who directly handle CNT powder, assuming a certain emission and exposure scenario. To clarify the emission/exposure scenario assumed here, Table V. 1 shows the classification of exposure potential according to the material forms of nanomaterials, exposure control (measures against exposure), working scales, and exposure frequencies. Differences in the exposure potential due to material forms are as follows. When nanomaterials are fixed (e.g., when they are mixed in resins), the possibility of inhalation exposure to nanomaterials is regarded to be almost zero, except in special cases where the dust of nanomaterials or resins is scattered in the air due to abrasion or polishing. When nanomaterials are present in a liquid, inhalation exposure can occur only when the liquid itself is splashed (e.g., agitation, ultrasonication, processes involving foaming, and spraying). In contrast, there is a high possibility of inhalation exposure in cases where the dry powder of nanomaterials is handled.

If Class F (no exposure control) is changed to Class E (use of personal protective equipments) in Table V. 1, it is expected that the protective coefficient will be increased by a factor of 10 times or more, depending on types of protective masks to be used (Ministry of Health, Labour, and Welfare, 2008). Similarly, if Class F (no exposure control) is changed to Class D (use of a local ventilation equipment), the

protective coefficient can be technically increased by a factor of 10 to 100 times (or more), depending on the types and designs of the exhaust system (which also depends on cost). Class C (closed system/unattended operation/automatization) can be said to give null exposure except for opening of a closed system for sample collection, maintenance, and cleaning (In Table V. 1, opening of the closed system shall be included in Classes D to F). The closed system naturally requires high cost and involves impossible processes.

In this chapter, a certain emission and exposure scenario for direct handling of CNT dry powder is assumed to estimate the amount of CNT powder that a worker is exposed to by inhalation without exposure control. Due to the fact that most of the currently available data were obtained from research sites, the CNT handling scale can be said to be small. However, the exposure frequency is assumed to be high (8 h/day × 5 days/week). Therefore, estimated values are positioned in between FI and F2 in the classification of Table V. 1.

Table V. 1 Classification of exposure potential of nanomaterials based on material forms, exposure control, and working scale (or exposure frequency)

Class	Material form	Exposure control	Working scale[d] (or exposure frequency)	Exposure potential (low–high, 1–5)
A	Fixed state (e.g., mixed in resins)	–	–	1
B	Nanomaterials in liquids [a]	–	–	2
C	Dry nanomaterial powder	Closed system/unattended operation/automatization[b]	–	1
D1		Local ventilation equipment[c]	Small (low)	2
D2			Large (high)	3
E1		Only personal protective equipment[c]	Small (low)	3
E2			Large (high)	4
F1		No exposure control[c]	Small (low)	4
F2			Large (high)	5

a: Exposure can occur when the liquid itself is splashed (e.g., during agitation, ultrasonication, processes involving foaming, and spraying).
b: If an operation involves the opening of a closed system (sample collection, maintenance, cleaning, etc.), it will be regarded as Class D-F.
c: Class D-F operations in which workers directly handle the nanomaterial powder include the following: unpacking, weighing, subdividing, scooping, blending, charging into manufacturing/processing equipments, collection from manufacturing/processing equipments, transferring to other containers, packing/bagging, cleaning/maintenance, treatment of wastes, etc.
d: Examples of the working scale: laboratories (small); industrial production (large).

1. Emission scenario

(1) CNTs to be evaluated

Since CNTs have various fibrous diameters, lengths, structures, shapes, agglomeration states, and surface conditions, variations in emission characteristics resulting from these differences should be considered. However, it is difficult to evaluate CNTs based on these characteristics due to limited information currently available. In the interim report version (October 16, 2009), CNTs included in reports

from on-site investigations are considered.

(2) Processes and working scales to be evaluated

As mentioned above (Table V. 1), it is assumed that the possibility of inhalation exposure in a process for handling dry nanomaterial powder is high. In fact, processes in which emissions and exposures easily occur, as indicated in measurement reports on CNT manufacturing and use sites, were those for handling dry nanomaterial powder including collection, weighing, blending, transferring to containers, bagging, and maintenance.

Emissions and distributions of particle sizes may vary by process type and working scale (volume of CNTs handled, etc.). For this reason, assessments that consider types and scales are desirable. Due to limited data currently available, however, exposures in processes included in reports from on-site investigations are examined in the interim report (October 16, 2009). Variations in process types and work scales will now be considered.

(3) Concentration near CNT emission source

Data regarding environmental concentrations (in part, exposure concentration) near the CNT emission source that was obtained from on-site investigations is shown in Table V. 2.

Table V. 2 Environmental and exposure concentrations near CNT emission sources in on-site investigations

Source	Nanomaterials	Working scale	Process	Number concentration [number/cm^3]		Mass concentration [μg/m^3]
				Number of CNTs (electron microscope)	Number of particles (measuring instrument)	Total dust or inhalable dust
Maynard et al. (2004)	Laser ablation or HiPco SWCNTs (unpurified)	From laboratory to plant	Collection and cleaning	–	–	0.7–53 (CNT concentration on the basis of catalytic metal)
Han et al. (2008)	Thermal CVD method MWCNTs	Laboratory	Blending	172–193	–	210–430
			Weighing and spraying	2.0	–	37–190
Methner et al. (2007)	CNFs	Laboratory	Wet saw cutting of CNF composite	–	–	1,100 (Total carbon concentration)
			Weighing and mixing	–	–	64–93 (Total carbon concentration)
NEDO	AIST SG-SWCNTs	Laboratory	Removal of CNTs from substrates (interior GB*)	–	670 (< 0.5 μm) 81 (0.5–3 μm) 8.2 (> 3 μm) 750 (total)	–
			Transfer (interior GB*)	–	36 (< 0.3 μm) 9.1 (0.3–3 μm) 0.28 (> 3 μm) 45 (total)	–

*Interior GB: The CNT concentration on the inside of the glove box, not the worker's actual CNT exposure concentration.

2. Exposure scenario

(1) Exposure target

Workers who directly handle CNT powder shall be subjected to exposure assessment.

(2) Exposure metric

In evaluating CNT risks, it has not yet been determined which exposure metric is a proper adverse effects criterion. In this section, exposure concentration shall be expressed both as a number (number of agglomerates in the case of agglomerated CNTs) and as mass concentrations that have been reported. Exposure shall be expressed as the amount or CNTs deposited on the pulmonary alveoli per day and per body weight. ("Pulmonary inflammation" was set as an assessment endpoint in the hazard assessment discussed in Chapter IV. Based on this endpoint, standardization of exposure not per body weight but per lung weight and per pulmonary alveoli surface area is considered to be reasonable, but the exposure was provisionally calculated per body weight in the interim report (October 16, 2009).)

(3) Exposure control

Measures for exposure control including use of closing systems, local ventilation equipments, and personal protective equipments are generally taken in actual sites but, as mentioned above, cases in which these exposure controls are not made are assumed in this section (i.e., rate of particles removed by exposure control is zero).

(4) Exposure concentration

Exposure concentration is expressed as follows:

Exposure concentration = concentration of emitted particles near a source × environmental fate in the workplace × (1 - rate of particles removed by exposure control)

Environmental fate in the workplace include diluted concentration by diffusion of targeted particles, decreased concentration by particle deposition into floors and walls, changes in particle forms and sizes via inter-particle coagulation and between these particles and background particles, and physical and chemical particle changes.

In the working environment, particle emission and exposure often occur almost simultaneously. For this reason, all of the environmental fate except for diffusion are considered to make small contributions to alleviating the exposure.

Results of the on-site investigation in Table V. 2 are close to exposure concentrations. Consequently, the decrease in concentrations due to the environmental fate was ignored, while exposure control absence was assumed. In these result, exposure concentrations in the table were not changed.

(5) Alveolar deposition fraction

Pulmonary inflammation was set at an assessment endpoint in the hazard assessment discussed in Chapter IV. Based on this endpoint, the deposition of CNTs in pulmonary alveoli is considered. The alveolar deposition fraction of inhaled particles during light exercise was calculated with use of a model of Multiple Path Particle Dosimetry (MPPD2) (CIIT 2006; RIVM 2002). The parameters used for the calculation and the results of the calculation are shown in Table V. 3 and in Fig. V. 1, respectively. Particles with an aerodynamic diameter of approximately 20 nm had a high deposition rate, approximately 40%, while those with an aerodynamic diameter of more than 10 μm had a deposition rate of 0%.

In estimating exposure expressed as the amount deposited on the pulmonary alveoli based on the results listed in Table V. 2, the alveolar deposition fraction for each particle size was considered concerning exposure values obtained for different particle sizes (the difference between particle diameter from the intensity of scattered light and aerodynamic diameter was ignored for the calculation).

On the other hand, the alveolar deposition fraction was simply assumed to be 10% for values that are not used for different particle sizes (data on total or inhalable dust).

Table V. 3 Values used for calculating alveolar deposition fraction

Species	Human
Model	Yeh/Schum Symmetric
FRC (functional residual capacity)	3300 mL
URT (upper respiratory tract) dose	50 mL
Breathing frequency	20 min^{-1}
Tidal volume	1250 mL
Inspiratory fraction	0.5
Pause fraction	0
Breathing scenarios	Oronasal-Normal Augmenter

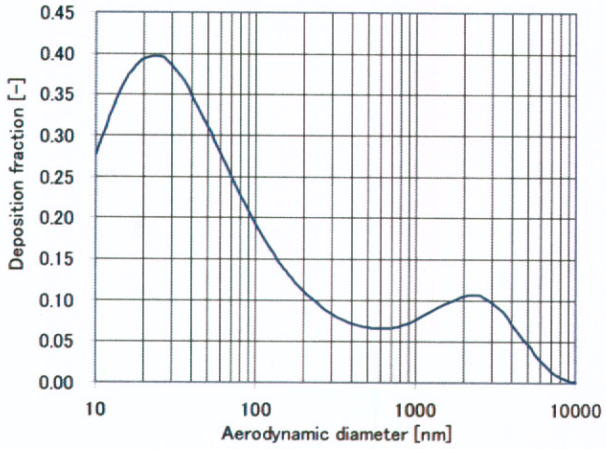

Fig. V. 1 Predicted alveolar deposition fraction related to particle size

Calculated based on the model of Multiple Path Particle Dosimetry (MPPD2) (CIIT 2006; RIVM 2002) during light exercise.

(6) Exposure frequency

It was assumed that workers would be exposed to CNT particles at a high exposure frequency, 8 h/day and 5 days/week (i.e., Exposure frequency = 8 h/24 h × 5 days/7 days = 0.24[-])

Assumptions in the set emission/exposure scenario are summarized in Table V. 4.

Table V. 4 Summary of the assumed emission/exposure scenario

Target CNTs, target processes, working scales, and generated concentration	Values reported in the on-site investigation. Most of them were obtained at the laboratory level.
Exposure control	No
Alveolar deposition fraction and breathing rate	Alveolar deposition fraction shall be assumed to be 10%, or calculated based on the model of MPPD2. The parameters for light exercise conditions were used.
Exposure frequency	High (8 h/day, 5 days/week)

3. Exposure calculation

The amount of exposure (the amount of CNTs deposited on the pulmonary alveoli per day per body weight) is expressed as the following equation:

Amount of exposure [number of particles/kg/day or μg/kg/day]

= exposure concentration [number of particles / m^3 or μg/m^3] × alveolar deposition fraction [-] × exposure frequency [-] × breathing rate during working [m^3/day] / body weight [kg]

The amount of exposure was calculated based on exposure concentrations (values in Table V. 2), alveolar deposition efficiencies (values in Fig. 1 or 0.1), an exposure frequency of 0.24, a breathing rate of

36 m^3/day (light exercise condition as shown in Table V. 3), and a body weight of 60 kg. The results are shown in Table V. 5.

Table V. 5 Estimated exposure based on the results of the on-site investigation (expressed as the amount deposited on the pulmonary alveoli per day and per kg of body weight)

Source	Nanomaterials	Working scale	Process	Number of particles [particles/kg/day]		Mass [μg/kg/day]
				Number of CNTs (electron microscope)	Number of particles (measuring instrument)	Total dust or inhalable dust
Maynard et al. (2004)	LASER ablation or HiPco SWCNTs (unpurified)	From laboratory to plant	Collection and cleaning	–	–	0.010–0.76 (CNT concentration on the basis of catalytic metal)
Han et al. (2008)	Thermal CVD method MWCNTs	Laboratory	Blending	2.5–2.8	–	3.0–6.2
			Weighing and spraying	0.03	–	0.53–2.7
Methner et al. (2007)	CNFs	Laboratory	Wet saw cutting of CNF composite	–	–	16 (Total caron concentration)
			Weighing and mixing	–	–	0.92–1.3 (Total carbon concentration)
NEDO	AIST SG-SWCNTs	Laboratory	Removal of CNTs from substrates (interior GB *)	–	18 (< 0.5 μm) 0.91 (0.5–3 μm) 0.060 (> 3 μm) 30 (total)	–
			Transfer (interior GB *)	–	1.0 (< 0.3 μm) 0.10 (0.3–3 μm) 0.0027 (> 3 μm) 1.6 (Total)	–

Exposure frequency of 8 h/day × 5 days/week and absence of exposure controls are assumed.
* Interior GB: Exposure is based on concentrations in the glove box but can be said to be excessive.

For more information on the results of the on-site investigation and the laboratory dustiness testing, refer to the main body of the risk assessment document written in Japanese.

Chapter VI: Exposure Assessment of CNT Applications

The possibility of exposure to CNT when the substance is actually applied to products was assessed for different phases in a CNT application's life cycle from use to disposal.

It is expected that the following four types of products are CNT applications as industrial products in large quantity. Consequently, use in an electric double-layer capacitor, a lithium-ion battery, a conductive plastic (not included in the interim report version), and a CNT-reinforced plastic (not included in the interim report version) was evaluated.

Since the electric double-layer capacitor is built into products, potential exposure to CNT during use of

the capacitor does not have to be considered except for special situations such as breakage resulting from an accident. Potential exposure during capacitor disposal is now being analyzed.

A lithium-ion battery is generally covered with a laminate case and package. Consequently, potential exposure to CNT during use of this battery is considered to hardly occur, except for special situations such as breakage or explosion resulting from an accident. Potential exposure during battery disposal was estimated below, assuming that CNT is used in all domestically-distributed lithium batteries.

Assuming that 10.2 tons of CNT that enters into a general waste treatment flow is all transferred to 9.7 million tons of waste (2006) excluding waste subject to direct final disposal and direct incineration, the concentration of CNT in the total quantity of waste becomes approximately 1.05 ppm. In a waste disposal site, dust is generated from the grinding and crushing of the waste by the shredder. Assuming that the composition of the dust generated in a general waste disposal site reflects the composition of the whole waste treated, a level of the CNT exposure was roughly estimated. Although most of information on dust concentration in working environments in the waste disposal site has not been released, according to a survey made as part of an investigation on a technique for treatment of asbestos-containing waste (Japan Environmental Sanitation Center, 2006), a dust concentration of 2.0–6.0 mg/m3 was observed at 4 measurement points within a crushing facility in an actual general waste treatment facility. Here, if a dust concentration is assumed to be 6.0 mg/m3 and the dust contains CNT at the concentration of CNT in a total quantity of the waste, 0.0064 μg/m3 can be obtained as an air concentration of CNT. Note that CNT is thought to form an aggregate with particles deriving from electrode materials or other battery waste.

Chapter VII: Risk Assessment

Chapter VII discusses human health risks caused by CNT inhalation exposure in working environments based on hazard and exposure assessment results (Chapters IV and V). In this interim report (October 16, 2009), however, qualitative risk assessment is limited due to the low perceived importance of quantitative risk assessment due to the acceptable exposure obtained from the hazard assessment and that estimated exposures from the exposure assessment are provisional values based on extremely limited data.

We will make a quantitative CNT risk assessment if more realistic hazard and exposure assessments can be done, and will refer to measures for risk control.

1. Risk assessment

A comparison of the acceptable exposure estimated in Chapter IV and workers' exposures estimated from CNT air concentrations in the working environment in Chapter V is shown in Table VII. 1. The

acceptable exposure in this table is the value for A company's or B company's MWCNTs. This substance is different from MWCNTs or CNFs that were used in the studies of Han et al. (2008) and Methner et al. (2007). For this reason, risk assessment by comparison of the acceptable exposure and workers' estimated exposures cannot be made. These values are shown in the same table for reference.

Comparison of the acceptable exposure and estimated exposures shows that the former value are close to the latter ones. However, estimated exposure values greatly vary with different work processes at individual sites, and the estimated exposure has a very wide range of values. Therefore, whether CNTs cause any risk cannot be determined based on the results of the acceptable exposure and estimated exposures. We think it necessary to assume more realistic emission and exposure scenarios and to grasp the difference between emission characteristics and adverse effects per CNT type in order to evaluate risks in a quantitative manner.

Table VII. 1 Comparison of the estimated exposure and acceptable exposure

CNT type	Researcher	Work process	Estimated exposure[a, b] [μg/kg/day]	Acceptable exposure[a] [μg/kg/day]
SWCNTs	Maynard et al. (2004)	Collection and cleaning	0.010–0.76	-
MWCNTs	Han et al. (2008)	Blending	3.0–6.2	3.0[d]
		Weighing and spraying	0.53–2.7	
CNFs	Methner et al. (2007)	Wet saw cutting of CNF composite	16[c]	
		Weighing and mixing	0.92–1.3[c]	

a: Both estimated and acceptable exposures represent the amount of CNTs deposited on the pulmonary alveoli per day and per kg of body weight.
b: Exposure frequency of 8 h/day × 5 days/week and absence of exposure control were assumed.
c: It is a total carbon and may includes carbon other than CNFs.
d: The value for A company's or B company's MWCNTs, which is different from those for CNTs determined in the on-site investigation.

2. Exposure control

This risk assessment was based on limited data, but as shown in Table VII. 1, workers' estimated exposures were close to the acceptable exposure. Based on this result, it is currently recommended that exposure should be at least reduced by a local ventilation equipment and so on when workers directly handle dry CNTs for many hours. In addition to this recommendation, we recommend that they wear protective masks.

According to the measurement reports on CNT production and use sites (refer to Chapter V), emissions and exposures occurred easily in the processes of handling dry CNT powder, including collection, weighing, blending, container-to-container transferring, bagging, and maintenance. It is necessary to note emissions and exposures in these processes.

Based on the measurement reports on the CNT production and use sites and the results of the laboratory

dustiness test (refer to Chapter V), CNTs are thought to be primarily emitted as agglomerates of sub-micron to micron size. Near the CNT emission source, an increase in dust concentration (mass concentration) was observed. The measurement of airborne dust concentration, as in the case of general dust, is often considered effective in on-site emission/exposure control (source determination, assessment of efficiency of exposure measures, etc.). In addition, general direct-reading aerosol monitors, such as a digital dust monitor and an optical particle counter covering particles of sub-micron to micron size, are also considered to be effective in daily CNT control.

One possible method to control even a trace amount of emission or to identify particles (separate from background particles) is to collect particles in the atmosphere or on the floors and walls with filters and observe these under an electron microscope or conduct a carbon analysis.

Due to the fact that this assessment is provisional and an interim report version (October 16, 2009), it should be noted that assessment methods and results in this temporary assessment document may be different from those in the final version.

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