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

Risk Assessment of Manufactured Nanomaterials

-Titanium Dioxide (TiO_2)-

Interim Report issued on October 16, 2009

Executive Summary

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Isamu Ogura · Masashi Gamo · Junko Nakanishi

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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. Titanium Dioxide

Titanium dioxide (TiO_2) has the form of white powder at room temperature and has a chemical formula weight of 79.90. It has two types of representative crystal structure: rutile and anatase. It is widely used in the preparation of white pigment and cosmetics because of its good coloring and concealing properties. Brookite and TiO_2 (B) are other forms of TiO_2 polymorphs and reported to have better photocatalytic properties. Hydrothermal synthesis of these two forms has been reported, and further developments are expected.

1. Production and use

According to Kagaku Kogyo Nippo (the Chemical Daily) (2008), the amounts of TiO_2 produced and used in Japan in FY 2007 are as follows:

- Actual figures of TiO_2 shipped for domestic use is 160,716 tons (100). TiO_2 is used in paints (46), rubbers (1), chemical fibers (2), inks and pigments (22), plastics (11), papermaking (6), capacitors (1), and others (10). The three major uses (paints, inks and pigments, and plastics) account for approximately 80% of the total consumption. This value has hardly changed in comparison to that recorded 5 years earlier (FY 2003). The amounts used in papermaking and capacitors, which account for only a small proportion of the total, have decreased to almost 50% of the value recorded 5 years earlier.
- Production capacity of TiO_2 manufacturers in Japan: 309,000 tons (100) in total as of April 2008; Ishihara Sangyo Kaisha Ltd. (50), Tayca Corporation (19), Sakai Chemical Industry Co. Ltd. (19), Fuji Titanium Industry Co. Ltd. (6), and Titan Kogyo, Ltd. (5)
- Production capacity of TiO_2 manufacturers worldwide is 5,395,000 tons in total as of June 2008.

According to Kagaku Kogyo Nippo (the Chemical Daily) (2009), the domestic production in 2007 was 245,976 tons, which consisted of 39,071 tons (16%) of anatase and 206,905 tons (84%) of rutile. The production levels of rutile are much higher than those of anatase.

2. Occupational safety

In work environments in which workers may be exposed to TiO_2 dust, the Japan Society for Occupational Health recommends 1 mg/m^3 (respirable dust) and 4 mg/m^3 (total dust) as the occupational exposure limits (OELs) for Class 2 dusts. Table 1 shows the OELs and guidelines for TiO_2 dust in foreign countries.

Table 1. Occupational exposure limits and guidelines

Agency	TiO_2		PNOS/R	
	TWA (mg/m^3)	Comments	TWA (mg/m^3)	Comments
NIOSH	—	Potential human carcinogen	-	-
OSHA	15	Total	15	Total
			5	Respirable
ACGIH	10	Category A4 Not classifiable as a human carcinogen	10	Inhalable
			3	Respirable
MAK	1.5	Respirable	4	Inhalable
			1.5	Respirable

NIOSH (2005) Table 1-1

-TWA is calculated per shift.
-PNOS/R = Particles not otherwise specified or regulated.
-Total, Inhalable, and Respirable refer to the particle size fraction, as defined by the respective agencies.
-Because the definitions of terms, usage of guideline values, etc., vary across different agencies, please refer to the source material for details.

According to Baan (2007) - a report on the classification of carcinogenicity by a working group of the International Agency for Research on Cancer (IARC) - the carcinogenic hazards of carbon black, TiO_2, and talc, which are poorly soluble, low-toxicity particles have been reevaluated. In the reevaluation, TiO_2, which was classified as a Group 3 material (not classifiable as to its carcinogenicity to humans) in 1989, was changed to Group 2B (possibly carcinogenic to humans) on the basis of data from epidemiological studies and animal experiments.

Chapter II. Titanium Dioxide Nanomaterials

This chapter summarizes basic information on TiO_2 nanomaterials. There are several definitions of nanomaterials. The Ministry of Economy, Trade and Industry (METI) (2009) refers to the OECD and ISO definition and describes manufactured nanomaterials as: "materials in a solid state, which are manufactured from elements or other raw materials, and which are either nano-objects with at least one of three dimensions smaller than 100 nm, or nanostructured materials composed of the nano-objects (including aggregations of nano-objects)," We adhere to this definition in this report and consider TiO_2 nanomaterials to be TiO_2 particles with primary particle size ranging from approximately 1 to 100 nm and aggregates of the primary particles.

1. Volume of production and usage

The volumes of production and usage of TiO_2 nanomaterials in Japan have been reported as follows:

Production volume:	950 tons/year for domestic, 1,450 tons/year for foreign	METI (2009b)
	Approximately 2,500 tons (50% was exported)	MHLW (2009a)
Domestic usage:	1,250 tons/year in 2006	MHLW (2009a)

The domestic usage of 1,250 tons/year accounts for approximately 0.5% of the domestic demand for all TiO_2 materials, which is about 240,000 tons (MHLW, 2009a; the Chemical Daily, 2009). According to MHLW (2009a), the percentage-wise use of this material is as follows: cosmetics, 60%; toners, 33%; car paints, 5%; and others (as flame retardant, photocatalyst, etc.), 2%.

2. Physicochemical properties and uses of TiO_2 nanomaterials

In comparison to pigment-grade TiO_2, TiO_2 nanomaterials have the following two specific characteristics that stem from the fact that the primary particle size is in the nanometer range:

(1) Particle surface area per unit weight (specific surface area; e.g., m^2/g) is high.

(2) Band structure in the solid state changes to increase the absorption of ultraviolet rays and decrease the scattering of visible light.

Increased photocatalytic activity is a result of the first characteristic, and application of these materials in sunscreen formulations is possible due to the second characteristic.

Table 2 summarizes the sizes, crystal forms, and uses of TiO_2 nanomaterials. For practical applications, the surface of TiO_2 nanomaterials is modified to be well-dispersed in paints and well-mixed in resins (MHLW, 2009a (Reference 1, p. 6); Ishihara Sangyo, 2009).

- For cosmetics: surface treatment with silicone
- For toner: surface treatment with a silane coupler
- For car paints: surface treatment with alumina and zirconia

Table 2. Sizes and uses of TiO_2 nanomaterials

	Crystal form	Average primary particle size [nm]	Average secondary particle size [nm]	Specific surface area [m^2/g]	Uses
Nanometer size	Anatase	6 - 30	200 +	10 - 300	Photocatalyst and industrial catalyst support (solar cells)
	Rutile	10 - 50		20 - 150	Cosmetics, paints, additive for toners, filler for rubber, and antireflection coating
Pigment-grade	Rutile Anatase	200 - 400	550 +	5 - 15	Paints, ink, resins, paper, and cosmetics
Large particles	Rutile	700 - 1000	700 +	1 - 5	Paints for roads and outdoor walls, and cosmetics

partly modified from the data of the Japan Titanium Dioxide Industry Association (2008)

3. Importance of characterization

TiO_2 nanomaterials are used in widely varied forms. Therefore, risk assessment in such a situation requires a well-defined scenario (including the situation/conditions of usage). As a starting point, it is important to provide a definite description of the characteristics of TiO_2 nanomaterials.

Since the mid-1990s, Oberdörster *et al.* and Donaldson *et al.* have emphasized the importance of surface area as a parameter for describing the adverse effects of TiO_2, whereas Warheit *et al.* of DuPont have claimed that the surface chemistry is important.

Although variations in the photocatalytic properties and biological effects based on differences in the crystal structure have not been completely established, some studies reported that the anatase form shows higher activity than the rutile form. P25, a product of Degussa (now Evonik), consists of both anatase and rutile forms and shows high activity. Brookite, which is difficult to synthesize, is reported to have higher photocatalytic activity.

Nanoparticles are defined as particles with length smaller than 100 nm in three dimensions. However, even if primary particles, which are produced for a specific purpose, meet the abovementioned criterion, the apparent particle size often changes when the material is used because of particle aggregation. Since the photocatalytic functions and the degree of interaction with biological systems would depend on the degree of aggregation of the particles, it is important to know the details of the material's physical or chemical state.

Figure 1 shows transmission electron microscopy (TEM) images of primary particles and aggregates of TiO_2 nanomaterials. Nanoscale primary particles gather to form larger aggregates. Aggregates and agglomerates are two words used to describe the state in which particles gather (e.g. BSI, 2007):

-Aggregate(s): In aggregates, particles are bonded by strong chemical forces such as covalent bonding. The external surface area of aggregates is much smaller than the sum of the estimated surface area of all the particles that form the aggregate.

-Agglomerate(s): In agglomerates, weak bonding forces such as van der Waals interactions are responsible for holding together primary particles, aggregates, or their mixtures. Alternatively, these forms may be physically entangled. The external surface area of agglomerates is close to the sum of the surface area of the components.

Based on the information on aggregation/agglomeration of nanoparticles including that of TiO_2 nanomaterials, the following points have to be considered during risk assessment:

-Nanoscale primary particles tend to aggregate in air or water.

-The BET-measured specific surface area of primary particles is often retained in aggregates/agglomerates.

-There is little possibility that aggregates/agglomerates will disaggregate into the original primary particles in the human body.

-During hazard and exposure assessment, it is necessary to consider the size distribution of the nanomaterials in the site assessed.

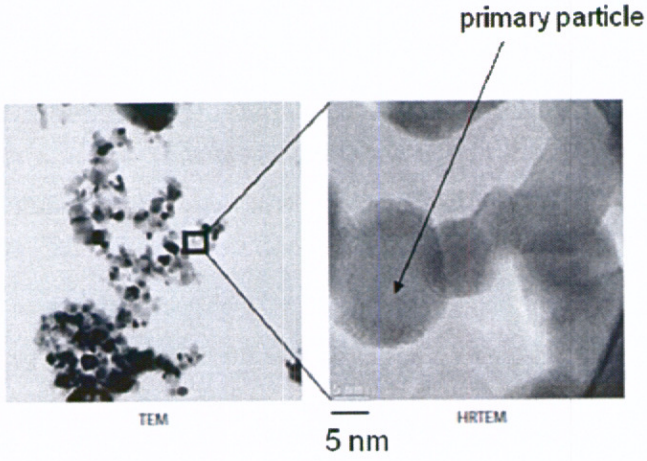


Figure 1. Primary particles of TiO_2 nanomaterials and their aggregates/agglomerates

Degussa Technical Information No. 1243, Degussa (now Evonik) (2005) (reprinted with the permission of Evonik)

Chapter III. Findings related to risk assessment of TiO_2 nanomaterials

In principle, risk assessment of nanomaterials can be carried out in the same manner as that of general chemical substances. The framework of risk assessment of chemical substances, which was initiated from a paradigm proposed by NRC (1983), has almost been established after much discussion over the last 30 years. This framework consists of the following 4 steps (Figure 2):

(1) Determination of the scenario and identification of the risk: This involves defining the situation and conditions to be assessed, choosing a target chemical substance, and qualitatively determining the toxicity and danger, and the possibility of exposure to the chemical.

(2) Exposure assessment: the degree of exposure in a given scenario is estimated.

(3) Hazard assessment: This involves determining the relationship between the dose (the amount of target chemical a person is exposed to) and adverse effects observed on the basis of epidemiological studies and/or animal experiments.

(4) Risk Characterization: the risk is estimated from the degree of exposure and adverse effect is estimated and compared with the criteria. The information needed to determine which course of action should be taken is summarized.

Determining the course of action (e.g., whether the risk is acceptable or not) on the basis of risk assessment is necessary for balancing the risk with the benefits. This is a risk management process. In this report, risk assessment is carried out by the following steps (1) to (4).

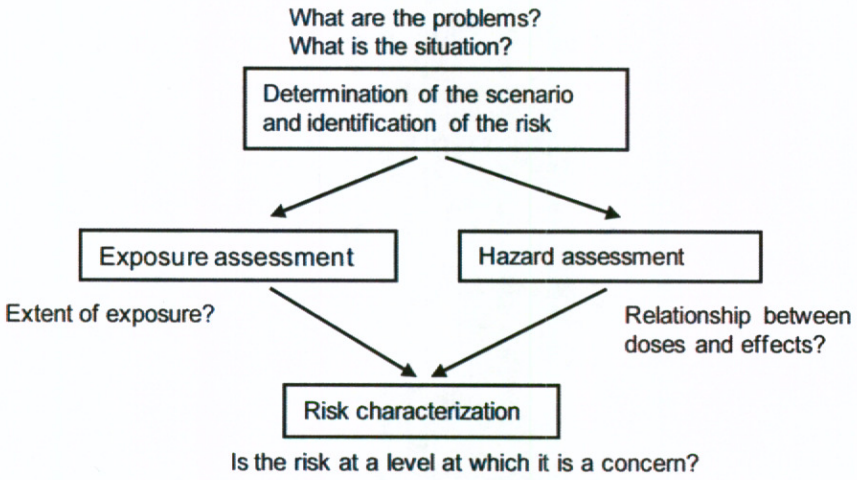


Figure 2. Framework of risk assessment of general chemical substances

During risk assessment, it is important to understand the differences between nanomaterials and general chemical substances.

-The degree of exposure and impact of general chemical substances is almost always assessed under conditions in which the substance exists as an individual molecule, as in the case of organic compounds. Nanomaterials, consist of primary particles that range in size from approximately 1 to 100 nm, and aggregates/agglomerates of the primary particles.

-Nanomaterials vary in shape, for example, they may be spherical, cylindrical, plate-like, tubular (single- or multi-walled), or dot-like.

-In practical usage, nanomaterials exist in various forms depending on their applications—they can be floating aerosols in the atmosphere, dispersed in liquids, mixed in polymer matrices, etc.

-Depending on the material, there is a possibility of dust explosion because these particles are fine.

Due to these differences, risk assessment of nanomaterials is very much complicated. The actual processes applicable to quantitative evaluation have to be discussed in detail. DuPont and an NGO (DuPont and Environmental Defense, 2007) have developed a framework of risk assessment, and NIOSH (2005) has proposed a recommended exposure limits (RELs). Both of these are useful in the discussions.

There are many unknowns with respect to nanomaterials risk assessment. For example, they are used in various forms, and methods for evaluating their toxicity have not been standardized. Discussions on risk management and regulations in Japan and other countries have focused on the following points:

-Voluntary control by private companies that produce or handle nanomaterials and the cooperation with the regulatory agencies

-Expectations of good working practices by companies that produce or handle nanomaterials

-Management based on risk assessment

In addition, some references are made to the control banding approach. This was originally developed in the United Kingdom for safety controls of general chemical substances in workplaces, particularly for small and medium enterprises without safety management expert.

Chapter IV. Hazard Assessment

In this interim report (October 16, 2009), we will address the issue of toxicity to human health upon inhalation exposure. Current knowledge indicates that the people who are most likely to be affected by TiO_2 nanomaterials (TiO_2 with primary particle size of 100 nm or less and aggregates/agglomerates of the primary particles) are workers in manufacturing workplaces, and exposure to nanomaterials by inhalation is considered to be the greatest problem.

The first section presents toxicity information on TiO_2 nanomaterials, which has been obtained from published studies and the results of NEDO projects. This includes outlines of tests, observed adverse effects, toxicokinetic examination, and inference of the toxicity mechanisms. (Note that toxicokinetic examination and toxicity mechanisms are not included in this interim report because these studies are still in progress.) The second section presents toxicity information on nanomaterials other than TiO_2. This information has been taken from published studies and is used to compare TiO_2 with other materials. In the final section, we discuss acceptable exposure and the acceptable exposure concentration in the working environment and also propose provisional values for these. Using the provisional values of acceptable exposures calculated in this chapter, the risk to human health is assessed in Chapter VI.

The provisional values of the acceptable exposure concentration are proposed with the intention that the atmospheric concentration of TiO_2 nanomaterials in the working environment will be controlled if the provisional values are not exceeded. However, it should be noted that the provisional values proposed here are no more than the values estimated on the basis of earlier tests on TiO_2 particles. In other words, examination of TiO_2 products that differ in size, shape, type, or manufacturing method may lead to different conclusions. This is a limitation in cases where the assessment is performed in situations where the events at a nanoscale level have not been theoretically estimated. It is also a universal caution when assessing a substance that is still in the developmental phase. At this point, assessment of all TiO_2 nanomaterials is impossible.

1. Summary of the toxicity information

This section summarizes information on the toxicity of TiO_2 nanomaterials from the results of published studies and NEDO projects.

Although information on acute toxicity, long-term toxicity, genotoxicity, and reproductive and developmental toxicity is required to evaluate the toxicity of TiO_2 nanomaterials, this interim report assesses only inhalation toxicity and long-term toxicity, which are the most important factors in assessing toxicity by inhalation exposure in the working environment. For inhalation toxicity, the test protocols and outlines of the

inhalation exposure tests and intratracheal instillation tests are summarized in this document. There is only one study on long-term toxicity (Heinrich *et al.*, 1995), in which TiO_2 particles with a primary particle diameter of 100 nm or less were used; therefore, studies in which pigment-grade TiO_2 particles (the primary particle diameter of which is in the order of microns or submicrons) were also examined.

1.1. Inhalation exposure test

In the inhalation exposure test, experimental animals in exposure chambers in a laboratory inhale nebulized test materials by inspiration. This method is regarded as the gold standard for assessing inhalation toxicity because the environment created in this method is very similar to the working environment in which humans are exposed to such materials (Morimoto and Tanaka, 2008). However, this test can only be performed in certain research institutes because it requires expensive large-scale equipment. Additionally, advanced techniques are required for aerosolizing the test substances because the concentration of the test substances is kept constant during the exposure period. Moreover, the method involves large sample loss because the samples aerosolized in the atmosphere cannot be recovered and a large sample amount is required in long-range tests. Therefore, there are only a few reports on inhalation exposure tests of nanomaterials, and at present, there are only 4 reports on inhalation exposure tests of TiO_2 nanomaterials (Table 3). Outlines of these 4 studies are given below.

Oberdörster *et al.* (1994) performed inhalation exposure studies using TiO_2 particles of two primary sizes. Male Fischer 344 (F344) rats were exposed to anatase-type TiO_2 particles with average primary size of 20 or 250 nm at a concentration of approximately 23 mg/m^3 for 12 weeks. The amount of test particles retained in the lung and the levels of biomarkers related to inflammation and cytotoxicity, including the number of inflammatory cells such as neutrophils and macrophages, lactate dehydrogenase (LDH) levels, and protein concentration in the bronchoalveolar lavage fluid (BALF) were measured immediately after exposure and at 6 months and 1 year after exposure. TiO_2 particles with a primary size of 20 nm took longer to clear from the lungs than particles with a primary size of 250 nm. Moreover, a significant increase in the number of total cells, neutrophils, and macrophages was observed in comparison to the controls. However, the increase in the number of inflammatory cells and biomarkers was only observed immediately after exposure. In other words, the effects were transient, and 6 months after exposure, no significant difference was observed in comparison with the controls. In the group exposed to TiO_2 particles with a primary size of 20 nm, "lung overload" was observed, in which the number of particles deposited in the alveoli exceeds the amount that can be cleared by the alveolar macrophages. This phenomenon is believed to cause a transient inflammatory response in the group exposed to TiO_2 particles with a primary size of 20 nm. On the other hand, there was no increase in the number of inflammatory cells and levels of biomarkers in the group exposed to TiO_2 particles with a primary particle diameter of 250 nm. The authors proposed that the difference among the groups could be explained on the basis of the difference in the surface area of the TiO_2

particles deposited in the lungs.

In a study performed by Bermudez *et al.* (2004), female F344 rats, female B6C3F1 mice, and female SYR hamsters were exposed to P25-TiO_2 particles (average primary size: 21 nm), which was also used by Heinrich *et al.* (1995). At 4, 13, 26, and 52 weeks after exposure, the TiO_2 particle burdens in the lung and lymph nodes and the lung responses were investigated. The results varied significantly depending on the animal species. More specifically, of the 3 species studied, rats showed the highest sensitivity, while hamsters exhibited the least sensitivity. In the case of rats and mice, while the groups exposed to 2 mg/m^3 or less of TiO_2 particles showed almost no effects, those exposed to 10 mg/m^3 showed a significant increase in the LDH level and protein concentration in the BALF and a significant decrease in the clearance of TiO_2 particles from lungs. In addition, lung overload, which has been described above, was observed in the rat and mice groups that inhaled TiO_2 particles at this concentration. On the other hand, unlike rats and mice, hamsters did not show a significant increase in the levels of inflammatory biomarkers and a decrease in TiO_2 clearance from lungs even when the animals were exposed to 10 mg/m^3 TiO_2 particles. The responses of the 3 species that were exposed to 10 mg/m^3 of P25-TiO_2 particles were approximately the same as those observed in a previous study in which the same 3 species were exposed for 13 weeks to 50 mg/m^3 of pigment-grade TiO_2 particles (the primary particle diameter of which is in the order of microns or submicrons) (Bermudez *et al.*, 2002). By comparing the results of the two studies, the authors concluded that a lower exposure concentration of TiO_2 particles with primary particle diameter in the order of nanometers was required to obtain the same effect as that of TiO_2 particles with primary particle diameter in the order of microns. Moreover, when the exposure concentration was expressed in terms of the particle surface area (m^2/m^3), not particle mass (mg/m^3), the exposure concentrations of the two tests was expected to be approximately the same.

In a study by Heinrich *et al.* (1995), female Wistar rats and female NMRI mice were exposed to P25 for 24 months or 13.5 months, respectively. P25 particles are TiO_2 particles that are manufactured by Evonik Degussa and consist of 80% anatase and 20% rutile. They have an average primary particle diameter of 10–40 nm. The average exposure concentration of TiO_2 in the exposure period was approximately 10 mg/m^3, and the cumulative particle exposure ($g/m^3 \times h$) calculated by multiplying the particle concentration with the exposure time was 88.1 for rats and 51.5 for mice. In rats, in comparison with the control groups, there was a significant increase in mortality, decrease in body weight, increase in lung weight, and decrease in clearance after 3 months or more of exposure, and the lung tumor significantly increased after exposure for 18 months or more. In mice, in comparison with the control groups, no significant difference was observed in the lung tumor, although there was a decrease in the body weight and an increase in the lung weight.

1.2. Intratracheal instillation test

In the intratracheal instillation test, test materials are dispersed in liquids and then directly instilled into the trachea of the experimental animal by using a syringe or other similar instrument. Subsequently, the biological effects on the lungs and other organs and the amount retained in the body at different observation time points are investigated. This method has been widely used as an alternative to the inhalation exposure test described above. It is easier than the inhalation exposure test in terms of technique and is cheaper and less laborious. Since this test method requires less sample than the inhalation test, intratracheal instillation tests have been performed as *in vivo* toxicity tests to study the inhalation toxicity of nanomaterials, which are often difficult to produce in large amounts. However, there are some specific problems with the intratracheal instillation test. In this test, test materials dispersed in liquids are administered to the lungs at once. This form of exposure is very different from that of inhalation in the actual working environment. Therefore, in general, it is difficult to directly estimate the acceptable exposure and acceptable exposure concentration from the results of intratracheal instillation tests. However, the Inhalation Specialty Section of the Society of Toxicology (SOT) recognizes the utility of the intratracheal instillation test in characterizing the potential toxicities of test materials to the lungs and for comparing the relative toxicities of different test materials to the lungs. This test has a certain utility when it is performed properly with an understanding of its limitations (Driscoll *et al.*, 2000). In our assessment, after estimating the acceptable exposure and acceptable exposure concentration from the results of inhalation exposure tests, we used the results obtained from intratracheal instillation tests to estimate the toxicity of other TiO_2 particles for which there are no results from the inhalation exposure test.

Table 4 summarizes the results of the intratracheal instillation tests using TiO_2 particles with a primary size of 100 nm or less.

Oberdörster *et al.* (1992), Renwick *et al.* (2004), and Sager *et al.* (2008) compared TiO_2 particles of different sizes and demonstrated that smaller particles show larger effects when the doses (in terms of mass) are equal. In particular, Sager *et al.* (2008) reported that when doses are expressed on the basis of particle surface area, almost no difference is observed in the dose-response relationship of two types of TiO_2 particles. It should be noted that the conclusions drawn in these studies are based on the responses observed immediately after instillation (24 h), a time point at which there may be a substantial effect from unnatural instillation of liquids in the trachea. Moreover, the results were obtained by comparing TiO_2 particles that differ not only in particle size but also in crystal structure. However, in contrast to these observations, there are some reports in which little difference was observed in the toxicities of TiO_2 particles with primary size in the order of nanometers and those with primary size in the order of microns (or submicrons). Warheit *et al.* (2006, 2007a) proposed that the pulmonary toxicity of particles does not depend on the particle size and surface area and that surface properties are most important in determining pulmonary toxicity. Rehn *et al.* (2003) reported that groups exposed to two types of TiO_2 particles showed only slight signs of inflammation

and no difference based on surface treatment. The data, however, indicated that P25-TiO_2 particles showed a slightly higher reactivity than T805 TiO_2 particles, as shown in Table 4. For example, at a dose of 6 mg/rat, T805 caused significant changes for 3 days, while P25 resulted in changes for 21 days.

In a NEDO project, Kobayashi *et al.* (2009) performed intratracheal instillation tests on rats to compare the effects of TiO_2 particles among groups exposed to different types of TiO_2 particles (the primary sizes or agglomerations of which differed but manufacturer, manufacturing method, and crystal structure [anatase] of which were the same). In their tests, they performed histopathological evaluation of the lungs and measured the levels of inflammatory biomarkers in the BALF at each observation point after instillation. All the groups exposed to TiO_2 particles showed transient inflammatory responses, which recovered by 1 week or 1 month post-instillation. Regarding this recovery trend, there were almost no differences between the groups regardless of the primary particle size and agglomerations of instilled TiO_2 particles. However, with regard to the short-term (1 week) effects, some differences were observed among the TiO_2-exposed groups—smaller primary particles induced greater inflammation in short-term observations. On the other hand, no clear relationship was observed in the short-term effects of the TiO_2-exposed groups with different agglomerate sizes but the same primary particle size.

1.3. Long-term toxicity

To date, there is only 1 report on the long-term toxicity of nanosized TiO_2 primary particles (100 nm or smaller) (Heinrich *et al.*, 1995). The results of this are summarized in Table 5 together with those of studies in which pigment-grade TiO_2 particles were used (Lee *et al.*, 1985; Muhle *et al.*, 1991). High-doses of pigment-grade TiO_2 particles (Lee *et al.*, 1985) and nanosized TiO_2 primary particles (Heinrich *et al.*, 1995) led to an increase in the tumor incidence in rats.

2. Comparison of the biological responses to TiO_2 particles with those to other materials

Pulmonary responses arising from exposure to TiO_2 particles were compared with those caused by exposure to other materials, and this section discusses features of the toxicity of TiO_2 particles mainly on the basis of results from intratracheal instillation tests. The materials compared here are poorly soluble fine particles (other than TiO_2) such as crystalline silica and nickel oxide (NO). Tables 6 and 7 summarize the results of the intratracheal instillation tests with crystalline silica and NiO, respectively.

If the biological responses (mainly inflammatory responses) caused by other materials such as crystalline silica and NiO are summarized with a focus on the change in the response over time (that is, as persistence of inflammation), it would be easy to understand the variations arising from the different materials used.

Even when 5 mg/kg of TiO_2 particles, which is a relatively high dose for intratracheal instillation tests, was administered, the inflammatory responses recovered remarkably at 1–3 months post-exposure, although transient inflammatory responses were observed even at 24 h to 1 week post-exposure (Table 4). Although there were some differences based on the crystal structure (Rehn *et al.*, 2003; Warheit *et al.*, 2007) and particle size (Kobayashi *et al.*, 2009), it was estimated that the inflammatory responses arising from exposure to TiO_2 particles were not very large.

On the other hand, instillation of 1 mg/kg (Warheit *et al.*, 2006; 2007) or less (Nishi *et al.*, 2009) of poorly soluble, highly toxic particles such as crystalline silica and NiO caused remarkable pulmonary inflammatory responses that persisted for 3–6 months (Tables 6 and 7). The inflammatory responses arising from exposure to such particles were more pronounced in the long term (1–6 months) than in the short term (24 h to 1 week). Some tests recorded lung fibrotic responses, including collagen deposition in lung tissues, which differed both qualitatively and quantitatively from the responses caused by TiO_2.

It should be noted that the comparisons described here mainly focus on the inflammatory response, and biological effects other than inflammation, such as carcinogenicity, were not examined. Therefore, evaluation of biological effects other than inflammation may lead to different conclusions.

Table 3. Inhalation exposure tests using TiO_2 particles (1/2)

Study	Sample information						Test conditions						Test result
	Manufacturer	Product /sample name	Crystal structure	Surface area [m^2/g]	Particle size [nm]		Animal species	Exposure period	Observation time points	Observation items	Exposure conc.		
					Primary	In air					[mg/m^3]	[m^2/m^3]	
Oberdörster *et al.* (1994)	-	TiO_2-D	Anatase	77[b]	20	710[a]	Male F344 rat	12 weeks	4, 8, 12, 41, 64 weeks	BALF	23.5	1.8[c]	Inflammation recovered at 41 weeks.
	-	TiO_2-F	Anatase	6.2[b]	250	780[a]					22.3	0.14[c]	No significant change
Heinrich *et al.* (1995)	Evonik Degussa	P25	Anatase 80 /Rutile 20	48	10–40	800[a]	Female Wistar rat (7 weeks)	24 months (18 h/day, 5 days/week)	3, 6, 12, 18, 24 months	Lung pathology	10	0.48	Sustained inflammation
							Female NMRI mouse (7 weeks)	13.5 months (18 h/day, 5 days/week)	3, 6, 12, 18, 21 months	Lung pathology	10	0.48	No significant change

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

Table 3. Inhalation exposure tests using TiO_2 particles (2/2)

Study	Sample information						Test conditions						Test result
	Manufacturer	Product /sample name	Crystal structure	Surface area [m^2/g]	Particle size [nm]		Animal species	Exposure period	Observation time points	Observation items	Exposure conc.		
					Primary	In air					[mg/m^3]	[m^2/m^3]	
Bermudez *et al.* (2004)	Evonik Degussa	P25	Anatase 80 /Rutile 20	73[b]	21	1450[a]	Female B6C3F1 mouse (6 weeks)	13 weeks (6 h/day, 5 days/week)	Post-exposure 0, 4, 13, 26, 52 weeks	Lung pathology, BALF	0.5	0.04[c]	No significant change
											2	0.15[c]	
											10	0.73[c]	Sustained inflammation
						1440[a]	Female F344 rat (6 weeks)	13 weeks (6 h/day, 5 days/week)	Post-exposure 0, 4, 13, 26, 52 weeks	Lung pathology, BALF	0.5	0.04[c]	No significant change
											2	0.15[c]	
											10	0.73[c]	Sustained inflammation
						1290[a]	Female SYR hamster (6 weeks)	13 weeks (6 h/day, 5 days/week)	Post-exposure 0, 4, 13, 26, 49 weeks	Lung pathology, BALF	0.5	0.04[c]	No significant change
											2	0.15[c]	
											10	0.73[c]	
Grassian *et al.* (2007)	NanoAmor	-	Anatase	219	5	120[a]	Male C57BL/6 mouse (6 weeks)	1 day (4 h)	Post-exposure 0 day	Lung pathology, BALF	0.77	0.17[c]	No significant change
						123[a]					7.22	1.58[c]	Slight change
						128[a]		10 days (4 h/day)	Post-exposure 0, 1, 2, 3 weeks	Lung pathology, BALF	8.88	1.94[c]	Inflammation recovered at 3 wk

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

Table 4. Intratracheal instillation tests using TiO_2 particles (1/4)

Study	Sample information						Test conditions					Test result				
	Manufacturer	Product/ sample name	Crystal structure	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation time points [Day]	Observation items	Dosage		1 d	3 d	1 wk	1 mo	3 mo
					Primary	In liquid				[mg/kg]	[m^2/kg]					
Oberdorster *et al.* (1992)	-	TiO_2-D	Anatase	77[a]	20	-	Male F344 Rat	1	BALF	2.3[b]	0.18[c]	▲	-	-	-	-
	-	TiO_2-F	Anatase	6.2[a]	250	-				2.3[b]	0.014[c]	▲	-	-	-	-
Rehn *et al.* (2003)	Evonik Degussa	P25	-	77[a]	20	-	Female Wistar rat	3, 21, 90	BALF	0.8	0.06[c]	-	○	-	○	○
										1.5	0.12[c]	-	▲	-	○	○
										3.0	0.23[c]	-	▲	-	▲	○
										6.0	0.46[c]	-	▲	-	▲	▲
		T805	- (trimethoxyoctylsilane treated)	77[a]	20	-				0.8	0.06[c]	-	○	-	○	○
										1.5	0.12[c]	-	▲	-	○	○
										3.0	0.23[c]	-	▲	-	▲	▲
										6.0	0.46[c]	-	▲	-	▲	▲

-: Not described/measured, a: Calculated on the basis of the particle size, b: 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, c: 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. ○: No change was observed.

Table 4. Intratracheal instillation tests using TiO_2 particles (2/4)

Study	Sample information						Test conditions					Test result				
	Manufacturer	Product /sample name	Crystal structure	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation time points [Day]	Observation items	Dosage		1 d	3 d	1 wk	1 mo	3 mo
					Primary	In liquid				[mg/kg]	[m^2/kg]					
Renwick *et al.* (2004)	Evonik Degussa	-	-	49.8	29	-	Male Wistar rat	1	BALF	0.3[b]	0.015[c]	▲	-	-	-	-
										1.2[b]	0.059[c]	▲	-	-	-	-
	Tioxide	-	-	6.6	250	-				0.3[b]	0.002[c]	○	-	-	-	-
										1.2[b]	0.008[c]	○	-	-	-	-
Warheit *et al.* (2006)	DuPont	R100	Rutile	6	300	-	Male SD rat (8 weeks)	1, 7, 28, 91	Lung pathology BALF	1	0.006[c]	○	-	○	○	○
										5	0.030[c]	▲	-	○	○	○
		Nanorod	Anatase	26.5	20 × 233	-				1	0.027[c]	○	-	○	○	○
										5	0.13[c]	▲	-	▲	○	○
		Nanodot	Anatase	169.4	6	-				1	0.17[c]	○	-	○	○	○
										5	0.85[c]	▲	-	○	○	○

-: Not described/measured, a: Calculated on the basis of the particle size, b: 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, c: 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. ○: No change was observed.

Table 4. Intratracheal instillation tests using TiO_2 particles (3/4)

Study	Sample information						Test conditions					Test result				
	Manufacturer	Product /sample name	Crystal structure	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation time point [Day]	Observation items	Dosage		1 d	3 d	1 wk	1 mo	3 mo
					Primary	In liquid				[mg/kg]	[m^2/kg]					
Warheit *et al.* (2007a)	DuPont	R100	Rutile	5.8	300	2667	Male SD rat (8 weeks)	1, 7, 28, 91	Lung pathology BALF	1	0.006[c]	○	-	○	○	○
										5	0.029[c]	▲	-	○	○	○
		uf-1	Rutile	18.2	140	2144				1	0.018[c]	○	-	○	○	○
										5	0.091[c]	▲	-	○	○	○
		uf-2	Rutile	35.7	140	2891				1	0.036[c]	○	-	○	○	○
										5	0.18[c]	▲	-	▲	○	○
	Evonik Degussa	P25 (uf-3)	Anatase 80 /Rutile 20	53.0	25	2692				1	0.053[c]	▲	-	▲	▲	○
										5	0.27[c]	▲	-	▲	▲	●

-: Not described/measured, a: Calculated on the basis of the particle size, b: 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, c: 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. ○: No change was observed.

Table 4. Intratracheal instillation tests using TiO_2 particles (4/4)

Study	Sample information						Test conditions					Test result				
	Manufacturer	Product /sample name	Crystal structure	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation time point [Day]	Observation items	Dosage		1 d	3 d	1 wk	1 mo	3 mo
					Primary	In liquid				[mg/kg]	[m^2/kg]					
Sager *et al.* (2008)	Evonik Degussa	P25	Anatase 80 /Rutile 20	73[a]	21	204	Male F344 rat (10 weeks)	1, 7, 42	Lung pathology BALF	1.0[b]	0.076[c]	▲	-	▲	▲	-
										2.1[b]	0.15[c]	▲	-	▲	▲	-
										4.2[b]	0.31[c]	▲	-	▲	▲	-
	Sigma Aldrich	#224227	Rutile	1.5[a]	1000	-				21[b]	0.033[c]	▲	-	▲	▲	-
										43[b]	0.066[c]	▲	-	▲	▲	-
										86[b]	0.13[c]	▲	-	▲	▲	-
Kobayashi *et al.* (2009) * NEDO project	Ishihara Sangyo Kaisha, Ltd.	ST-01	Anatase	316	5	19	Male SD rat (8 weeks)	1, 3, 7, 28	Lung pathology BALF	5	1.58[c]	●	●	●	○	-
		ST-21	Anatase	66	23	28				5	0.33[c]	●	●	●	○	-
		ST-41	Anatase	10	154	176				5	0.05[c]	●	●	○	○	-
	Ishihara Sangyo Kaisha, Ltd.	ST-01	Anatase	316	5	18		1, 3, 7, 28, 91	Lung pathology BALF	5	1.58[c]	●	●	●	○	○
						65				5	1.58[c]	●	●	○	○	○
						300				5	1.58[c]	●	●	○	○	○

-: Not described/measured, a: Calculated on the basis of the particle size, b: 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, c: 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. ○: No change was observed.

Table 5. Long-term toxicity tests using TiO_2 particles

Study	Sample information						Test conditions			Observations
	Manufacturer	Product/ sample name	Crystal structure	Particle size [nm]		Surface area [m^2/g]	Animal species	Exposure period	Exposure concentration [mg/m^3]	
				Primary	In air					
Lee *et al.* (1985)	-	-	-	-	1500–1700	-	Male and female CD Rat	24 months (6 h/day, 5 days/week)	10, 50, 250	↑Bronchioloalveolar adenoma (male 12/77, female 3/74) (250 mg/m^3) ↑Squamous cell carcinoma (male 1/77, female 13/74) (250 mg/m^3)
Muhle *et al.* (1991)	Bayer AG	Bayertitan	-	-	1100	-	Male and female F-344 rats	24 months (6 h/day, 5 days/week)	5	Lung tumor rate was the same as that of controls
Heinrich *et al.* (1995)	Evonik Degussa	P25	Anatase 80 /Rutile 20	10–40	800	48	Female Wistar Rat	24 months (18 h/day, 5 days/week)	10.4	↑Mortality (90%) ↑Tumor (32/100) (benign squamous cell tumor, 20/100; squamous cell carcinoma, 3/100; adenoma, 4/100; adenocarcinoma, 13/100)
							Female NMRI mouse	13.5 months (18 h/day, 5 days/week)	10.4	No increase in tumor occurrence

-: Not described/measured in the paper.

Table 6. Summary of intratracheal instillation tests using crystalline silica particles

Study	Sample information					Test conditions					Test result				
	Manufacturer	Product/ sample name	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation time point [Day]	Observation items	Dosage		1 d	3 d	1 wk	1 mo	3 mo
				Primary	In liquid				[mg/kg]	[m^2/kg]					
Rehn *et al.* (2003)	DMT	DQ12	6.7[a]	900	-	Female Wistar Rat	3, 21, 90	BALF	3.0	0.020[c]	-	▲	-	▲	▲
Warheit *et al.* (2006)	U.S. Silica	Min-U-Sil 5	4	1500	-	Male SD rat (8 weeks)	1, 7, 28, 91	Lung pathology, BALF	1	0.004[c]	▲	-	▲	▲	▲
									5	0.020[c]	▲	-	▲	▲	●
Warheit *et al.* (2007a)	U.S. Silica	Min-U-Sil 5	5.2	200–2000	480	Male SD rat (8 weeks)	1, 7, 28, 91	Lung pathology, BALF	1	0.005[c]	▲	-	▲	▲	▲
									5	0.026[c]	▲	-	▲	▲	●
Kobayashi *et al.* (2009) * NEDO project	U.S. Silica	Min-U-Sil 5	5.0	1700	2000	Male SDrat (8 weeks)	1, 3, 7, 28, 91	Lung pathology, BALF	5	0.025[c]	●	●	●	●	●

-: Not described/measured, a: Calculated on the basis of the particle size, b: 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, c: 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 7. Summary of intratracheal instillation tests using NiO particles

Study	Sample information					Test conditions					Test result				
	Manufacturer	Product /sample name	Surface area [m^2/g]	Particle size [nm]		Animal species	Observation time point [Day]	Observation items	Dosage		1 d	3 d	1 wk	1 mo	3 mo
				Primary	In liquid				[mg/kg]	[m^2/kg]					
Ogami *et al.* (2009)	Nacalai Chemicals	NiO	0.328	2700	4800	Male Wistar rat (10 weeks)	1, 3, 7, 28, 91, 180	Lung pathology, BALF	6.7[b]	0.002[c]	-	○	○	○	○
	Vacuum Metallurgical	nNiOm	32.6	27	800				6.7[b]	0.217[c]	-	●	●	●	●
	Wako Chemicals	TiO_2	-	-	1500				6.7[b]	-	-	●	○	○	○
	U.S. Silica	Min-U-Sil 5	-	-	1600				6.7[b]	-	-	●	●	●	●
Nishi *et al.* (2009)	Nanostructured & Amorphous Materials	NiO	104.6	20	26	Male Wistar rat (9 weeks)	1, 3, 7, 28, 91, 180	Lung pathology, BALF	0.33	0.035[c]	-	●	▲	▲	●
									0.66	0.070[c]	-	●	●	●	●

-: Not described/measured, a: Calculated on the basis of the particle size, b: 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, c: 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. Acceptable exposures in the working environment

Since dust inhalation is considered to be the most important exposure route during the handling of TiO_2 nanomaterials in the working environment, this section assesses the acceptable exposures for inhalation exposure in the working environment.

3.1. Framework of assessment

The toxicity of TiO_2 particles differs greatly depending on the product. Moreover, even for the same product, the toxicities of different samples may vary greatly depending on the methods by which the samples have been prepared. In the case of TiO_2 particles, results of the inhalation exposure test, which is the most useful test for estimating the no observed adverse effect level (NOAEL), do not always exist because, as mentioned earlier, this test involves high costs and advanced techniques and requires large amounts of samples. For most types of TiO_2 particles, only the results of the intratracheal instillation test exist, and then available data are limited.

Under these circumstances, we adopt a "bi-axial approach" to estimate the acceptable exposures of TiO_2 nanomaterials. The concept of the bi-axial approach is shown in Figure 3, and it is described below. In this approach, the first step is to estimate the NOAEL for experimental animals on the basis of the results of the inhalation exposure tests for certain types of TiO_2 nanomaterials ("1" in Figure 3). The second step involves the estimation of the acceptable exposures of TiO_2 nanomaterials for humans by extrapolating the NOAEL for experimental animals to humans taking into consideration the uncertainty factor (UF), which will be described later (2). Next, information on the relative values of toxicity of TiO_2 particles is obtained by comparing the test results of TiO_2 particles for which there are results from the intratracheal instillation test but not from the inhalation exposure test (3). The final step is to combine the data on acceptable exposures of certain types of TiO_2 particles for humans (obtained in 3) and relative values of toxicity (obtained in 3) to calculate provisional values of the acceptable exposures of TiO_2 nanomaterials for which there are only results from the intratracheal instillation test (4).

After defining the assessment endpoints and dose metrics to be used for assessment, the NOAEL can be determined on the basis of available test results.

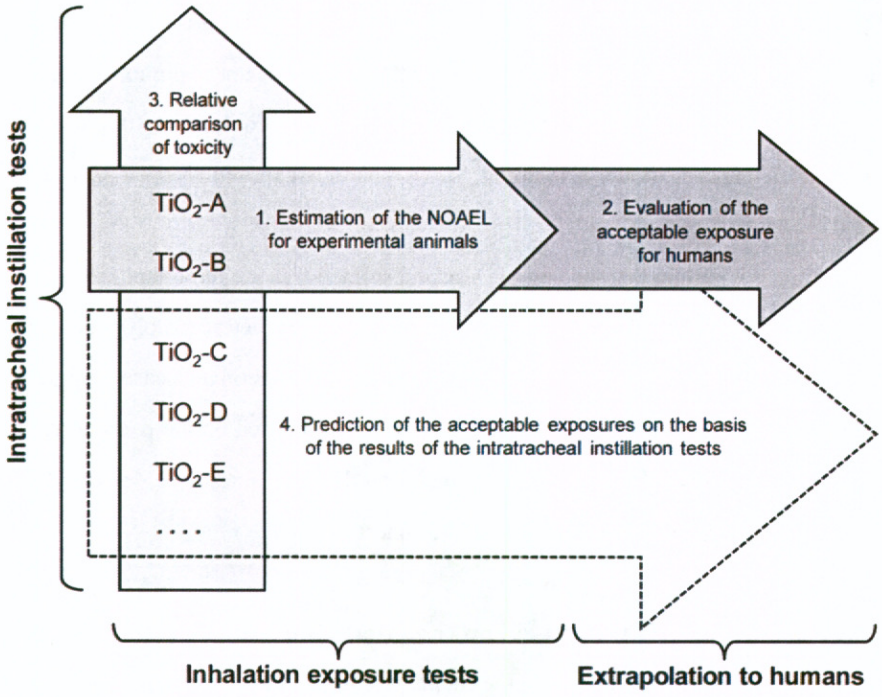


Figure 3. Concept of the bi-axial approach

Assessment endpoints

Among the effects on the lung due to exposure to TiO_2 particles, "lung inflammation" is taken as the endpoint in this report. This is an adverse effect that is observed at the lowest concentration. A review of earlier studies indicates a high correlation between the histopathological observations related to inflammation in the lungs and the number of inflammatory cells (including total cells, neutrophils, and macrophages) in the BALF or in the levels of inflammatory biomarkers (including LDH levels and protein concentration). When changes are observed in one of these factors, other factors also often change. Therefore, basically, the presence or absence of pulmonary inflammation is determined on the basis of the results from the histopathological evaluation of lungs in each test. However, in cases where only the number of inflammatory cells in the BALF or the levels of inflammatory biomarkers have been measured, the such results are also taken into consideration for assessment.

Dose metric for the assessment

As described above, although there are many studies on the relationship between particle size and biological effects, the conclusions drawn are not necessarily the same. Most studies have used particles that differ not only in size but also in crystal structure, impurities, or surface properties. Therefore, it is difficult to determine the effect strictly on the basis of size because all of these factors also contribute to the overall effect. A study in the NEDO project (Kobayashi *et al.*, 2009) showed differences in the effect depending on the

particle size, as described above. The effect, however, was only observed in short-term observations (within 1 week after intratracheal instillation), and the differences in the effect were not so pronounced even though the doses expressed on the basis of the particle surface area differed by approximately 30 fold at the maximum value (UF TiO_2 particle-exposed group: 1.58 m^2/kg, SF TiO_2 particle-exposed group: 0.33 m^2/kg, and F TiO_2 particle-exposed group: 0.05 m^2/kg).

Considering this, it is certain that the particle size and surface area are important properties that can be used as dose metrics in toxicity evaluation. However, since toxicity cannot be simply predicted only on the basis of these properties and some exceptions have been reported, evaluation on a case-by-case basis may be necessary. Therefore, this assessment provisionally describes doses based on the particle mass, which has been used in the original studies. In addition, doses based on the surface area are described in Tables 4-7. Detailed analysis on the issue of dose metrics should be undertaken in the future.

3.2. Determination of the NOAEL based on the inhalation exposure test

When "lung inflammation" is defined as a the endpoint of toxicity evaluation, the NOAEL can be taken as the maximum atmospheric TiO_2 particle concentration in which marked changes are not observed with respect to the inflammation of lung tissues or the number of inflammatory cells in the BALF during the observation period in the inhalation exposure test. Based on this definition, the NOAEL estimated from the last 4 inhalation exposure tests using TiO_2 particles was an atmospheric TiO_2 particle concentration of 2 mg/m^3 (shown in Table 3; exposure period of 13 weeks), as determined by Bermudez *et al.* (2004). P25-TiO_2 particles were used in this test (anatase 80%/rutile 20%, primary particle size: 21 nm, MMAD 1.4 μm).

3.3. Estimation of the acceptable exposures for humans

The acceptable exposure is the highest amount of substance that has no adverse impact even if the target is routinely exposed to the substance. This assessment expresses the exposure as the amount deposited on the lungs per body weight per day (mg/kg/day), which is calculated by taking into consideration the atmospheric concentration, respiratory volume, and pulmonary deposition fraction. Acceptable exposures can be estimated by the following 2 steps. In the first step, the NOAEL, which is determined from animal tests (inhalation exposure tests), is converted into a value in the unit of the amount deposited on the lungs (mg/kg/day). In the next step, the acceptable exposure (the amount of deposited on the lungs) for humans is estimated taking into consideration the uncertainty arising from extrapolation from experimental animals to human. The following sections outline the evaluation methods in terms of the steps involved and also present the evaluation results.

Conversion into the amount deposited on the lungs

In the study by Bermudez *et al.* (2004), the NOAEL of P25-TiO_2 particles for experimental animals was determined to be 2 mg/m^3 for both rats and mice. To predict acceptable exposure for humans based on the NOAEL from inhalation exposure tests, the value was converted to the amount deposited on the lungs of experimental animals (rats and mice) per body weight per day (*DOSE*) using Equation (1).

$$DOSE = (C \times RMV \times T \times DF)/BW \quad (1),$$

where C is the atmospheric concentration of TiO_2 particles [mg/m^3] determined as the NOAEL by inhalation exposure tests, *RMV* is the respiratory minute volume [L/min], T is the exposure time (in min) per day [min/day], *DF* is the deposition fraction of TiO_2 particles on the lungs [-], and *BW* is the body weight of the experimental animal [kg]. This assessment assumes that $C = 2$ mg/m^3 and $T = 6 \times 5/7$ h/day $\times$ 60 min/h = 257 min/day on the basis of the test conditions described in the paper by Bermudez *et al.* (2004). *DF* is assumed to be 0.1 (10%) for both rats and mice on the basis of a study by Miller *et al.* (2000), and *RMV* is determined from Equation (2) obtained experimentally by Bide *et al.* (2000).

$$RMV = 0.499 \times BW^{0.809} \quad (2)$$

Bermudez *et al.* (2004) used 6-week-old female rats and mice but the body weight of these animals was not mentioned in their paper. Therefore, the calculation was performed using 177.3 g (Charles River Laboratories Japan, Inc., 2008a) and 21.5 g (Charles River Laboratories Japan, Inc., 2008b), which are the average body weights of approximately 18-week-old female F344 rats and female BALB/c mice, respectively. This is the same age as that of the animals at the end of the exposure period.

Substituting these parameters into Equation (1) yields the amount of P25-TiO_2 particles deposited on the lungs of rats and mice, that is, *DOSE*, as follows.

$$DOSE_{ra} = (C \times RMV_{ra} \times T \times DF)/BW$$
$$= \text{“}2 \times 0.499 \times (177.3 \times 10^{-3})^{0.809} \times 10^{-3} \times 257 \times 0.1\text{”}/177.3 \times 10^{-3}$$
$$= 0.036 \text{ mg/kg/day}$$

$$DOSE_{mo} = (C \times RMV_{mo} \times T \times DF)/BW$$
$$= \text{“}2 \times 0.499 \times (21.5 \times 10^{-3})^{0.809} \times 10^{-3} \times 257 \times 0.1\text{”}/21.5 \times 10^{-3}$$
$$= 0.053 \text{ mg/kg/day}$$

As shown above, the NOAEL, which is the amount deposited on the lungs per body weight per day, for rats was slightly lower than that for mice. In this assessment, to be on the safer side, the lower value obtained from the test with rats was used.

Uncertainty in extrapolation from rat to humans

The following uncertainty factors (UFs) are assumed in the estimation of acceptable exposures for humans.

Uncertainty resulting from differences in toxicokinetics (TK): Differences in the TK of experimental animals and humans are considered to depend only on the difference in the amount deposited on the lungs because "pulmonary inflammatory responses" caused by inhalation exposure, which is an endpoint of this assessment, are local effects, and effects on the same target in both experimental animals and humans are under assessment. Because this assessment involves a process in which the atmospheric concentration is converted into the amount deposited on the lungs per day, the difference between experimental animals and humans in terms of the amount deposited on the lungs has already been taken into consideration. Consequently, the UF arising from differences in TK was taken as 1 in this assessment.

Uncertainty resulting from differences in toxicodynamics (TD): Comparison of the results of past inhalation exposure tests using various experimental animals revealed that rats have a high sensitivity to particulate matter. This is believed to be due to the occurrence of "lung overload," a phenomenon specific to rats, and symptoms caused by lung overload will not be manifested in larger mammal such as dogs, monkeys, and humans (Borm *et al.*, 2006). Therefore, when extrapolating NOAELs obtained in tests using rats to humans, even if the UF resulting from differences in TD is defined as 1, the assessment will be on the safer side because in terms of pulmonary toxicity, rats are the most sensitive animal species.

Uncertainty resulting from the exposure period: This assessment aims at estimating the acceptable exposures and concentrations for exposure over a period of several years but not throughout the lifetime. However, use of an approximately 3-month exposure period in the test (Bermudez *et al.*, 2004) to evaluate the NOAEL of P25-TiO_2 particles is too short. Therefore, the UF resulting from the exposure period was taken as 2.

Uncertainty resulting from individual differences: This assessment is targeted at workers who are probably in good health and not sensitive. Therefore, the UF resulting from individual differences was taken as 1.

Summary of the estimation of the acceptable exposures for humans

In summary, for P25-TiO_2 particles (primary particle size: 21 nm, MMAD: 1.4 μm), dividing the amount deposited on the lungs (0.036 mg/kg/day) by the product of the UFs (2 (= 1 × 1 × 2 × 1)) yields an acceptable

exposure of 0.018 mg/kg/day.

The estimation procedure used and estimates of acceptable exposures in this assessment should be regarded as provisional ones in this interim report (2009.10.16) and may change in the final report (scheduled to be published in 2011) on the basis of new scientific data and improvement in the estimation procedures.

3.4. Relative comparison of the toxicities based on the results of intratracheal instillation tests

In the relative comparison of the toxicities of materials based on the results of intratracheal instillation tests, the increasing rate, which is defined as the ratio of the number of neutrophils in the BALF in the TiO_2 exposed group to that in the negative control group, was used as an index representing the extent of the effects. This was due to the amount of data and toxicological validity. The values of 1 week post-instillation (or 1 month post-instillation if no data were available) was used.

First, for P25-TiO_2 particles, for which the NOAEL was estimated from inhalation exposure tests, the increasing rate of the number of neutrophils in the BALF at 1 week and 1 month after intratracheal instillation were calculated. Table 8 shows the calculated increasing rate in 3 intratracheal instillation tests (Rehn *et al.*, 2003; Warheit *et al.*, 2007a; Sager *et al.*, 2008). Although there were differences (nearly double at the maximum) in the calculated increasing rate of the number of neutrophils in the BALF at 1 week and 1 month post-instillation in the 3 tests, an average values were used for relative comparison of the toxicity, as shown below.

Table 8. Comparison of the increase in the number of neutrophils in the BALF on the basis of 5 mg/kg intratracheal instillation of P25-TiO_2 particles

Study	Manufacturer	Product/sample name	Particle size [nm]		Increasing rate [a]	
			Primary	In liquid	1 wk	1 mo
Rehn *et al.* (2003)	Evonik Degussa	P25	20	-	-	20[b]
Warheit *et al.* (2007a)	Evonik Degussa	P25	21	2,692	18	13
Sager *et al.* (2008)	Evonik Degussa	P25	21	204	39[b]	25[b]
Average					28	19

-: Not described/measured in the paper.

a: $\frac{\text{The number of neutrophils in the BALF of the P25-TiO}_2\text{-exposed group}}{\text{The number of neutrophils in the BALF of the negative control group}}$

b: Estimated from test results in which a dose of about 5 mg/kg was used.

Next, for TiO_2 particles other than P25, the increasing rate of the number of nuetrophils in the BALF at 1 week and 1 month post-instillation were calculated and compared with the increasing rates observed for P25-TiO_2 particles (Table 9). As shown in Table 9, there was no other TiO_2 particle for which the increasing rate of the number of neutrophils in the BALF was larger than that of the P25-TiO_2 particles, and the ratios of these ranged from 0.03 to 0.3. This result indicates that the toxicity (assessed only on the basis of the pulmonary inflammatory responses) of TiO_2 particles other than P25 was 0.03–0.3 times that of P25-TiO_2 particles.

Based on the abovementioned bi-axial approach, this assessment assumes that there are corresponding differences between the acceptable exposures of P25-TiO_2 particles and those of other TiO_2 particles. The acceptable exposures of these TiO_2 particles could not be quantitatively calculated here because the relative comparison method for estimating toxicity is provisional. However, it can be hypothesized that the acceptable exposures of these TiO_2 particles, for which there are no results from inhalation exposure tests, will be larger than those of P25-TiO_2 particles, for which there are results from the inhalation exposure test.

Table 9. Comparison of the increase in the number of nuetrophils in BALF on the basis of 5 mg/kg intratracheal instillation of TiO_2 particles other than P25

Study	Manufacturer	Product/ sample name	Particle size [nm]		Increasing rate [a]		Ratio of the increasing rates [b]	
			Primary	In liquid	1 wk	1 mo	1 wk	1 mo
Rehn *et al.* (2003)	Evonik Degussa	T805	20	-	-[c]	4.2[c]	-[c]	0.2[c]
Warheit *et al.* (2006)	DuPont	R100	300	-	1.6	1.5	0.1	0.1
		Nanorod	20 × 233	-	2.5	1.5	0.1	0.1
		Nanodot	6	-	2.5	1.5	0.1	0.1
Warheit *et al.* (2007a)	DuPont	R100	300	2667	1.5	2.0	0.1	0.1
		uf-1	140	2144	2.5	1.5	0.1	0.1
		uf-2	140	2891	2.5	1.5	0.1	0.1
Sager *et al.* (2008)	Sigma Aldrich	#224227	1000	-	1.5[c]	0.7[c]	0.1[c]	0.04[c]
Kobayashi *et al.* (2009)	Ishihara Sangyo Kaisha, Ltd.	ST-01	5	19	7.6	-	0.3	-
		ST-21	23	28	8.7	-	0.3	-
		ST-41	154	176	1.4	-	0.05	-
		ST-01	5	18	8.4	1.5	0.3	0.08
				65	1.3	0.9	0.05	0.05
				300	1.7	0.6	0.06	0.03

-: Not described/measured in the paper.

a: $\frac{\text{The number of neutrophils in the BALF of the } TiO_2\text{-exposed group}}{\text{The number of neutrophils in the BALF of the negative control group}}$

b: $\frac{\text{The increasing rate of the number of neutrophils in the BALF of the } TiO_2\text{-exposed group}}{\text{The increasing rate of the number of neutrophils in the BALF of the P25-}TiO_2\text{-exposed group}}$

c: Estimated from test results in which a dose of about 5 mg/kg was used.

4. Provisional values of the acceptable exposure concentration in the working environment

On the basis of the acceptable exposures (the amount of deposited on the lung) determined above, provisional values of the acceptable exposure concentration in the working environment were estimated. Provisional values of the acceptable exposure concentration were calculated by converting the acceptable exposures (the amount of deposited on the lung) into the atmospheric concentration using Equation (3). The symbols in Equation (3) represent the same quantities as those in Equation (1).

Provisional value of the acceptable exposure concentration

$$= (\textit{acceptable exposure} \times BW)/(RMV \times T \times DF) \qquad (3)$$

For example, let us calculate the acceptable exposure concentration (time-weighted average: TWA) of P25-TiO_2 particles for 5 days/week, 8 h/day. By substituting *acceptable exposure* = 0.018 mg/kg/day, T = 8 [h/day] × 5/7 × 60 [min/h] = 343 min/day, BW = 60 kg, RMV = 25 L/min, and DF = 0.1 into Equation (3), we obtain the following value for the acceptable exposure concentration of P25:

$$\textit{Acceptable exposure concentration}_{P25} = (0.018 \times 60)/(25 \times 343 \times 0.1) = 1.2 \text{ mg/m}^3$$

Based on these calculations, 1.2 mg/m^3 is proposed as the provisional value of the acceptable exposure concentration of P25-TiO_2 particles (respirable dust, TWA).

Since the toxicity (assessed only on the basis of pulmonary inflammatory responses) of other TiO_2 particles used in the tests reviewed in this assessment (such as the TiO_2 particles manufactured by DuPont, Ishihara Sangyo Kaisha, Ltd., etc.) was estimated to be lower than that of P25-TiO_2 particles by relative comparison on the basis of the results of intratracheal instillation tests, it is assumed that their acceptable exposure concentrations will be higher than that of P25-TiO_2 particles.

The estimation procedure used and estimates of the acceptable exposure concentration in this assessment should be regarded as provisional ones in this interim report (2009.10.16) and may change in the final report (scheduled to be published in 2011) on the basis of new scientific data and improvement in the estimation procedures.

5. (Appendix) Genotoxicity

Most of the positive responses in genotoxicity tests of TiO_2 have been reported in *in vitro* DNA damage assays and the *in vitro* micronucleus test. In some of the reports, different experimental conditions have been used, and these have not been described in detail. Therefore, it is difficult to comprehensively examine the relationship between the test results and experimental parameters such as the particle size and irradiation. However, comparison of different TiO_2 materials that show positive or negative responses within the same paper indicates that the smaller the particle size, the stronger the response under irradiation.

Two principle modes of genotoxicity can be considered for particles—primary and secondary. Primary genotoxicity is defined as the genetic damage elicited by particles in the absence of pulmonary inflammation, and secondary genotoxicity implies a pathway of genetic damage arising from oxidant DNA attack by reactive oxygen/nitrogen species generated during particle-elicited inflammation (Knaapen *et al.*, 2004; Schins and Knaapen, 2007). It was suggested that tumorigenesis induced by poorly soluble particles such as TiO_2 involves secondary genotoxicity (Knaapen *et al.*, 2004; Schins and Knaapen, 2007). Generation of hydroxyl radicals, which play a major role in genotoxic effects via oxidative DNA damage, was observed both intracellularly and extracellularly by electron spin resonance studies (Reeves *et al.*, 2008; Bhattacharya *et al.*, 2009). This suggests that the toxic effects of TiO_2 mainly arise from the hydroxyl radicals that are generated. It was also suggested that TiO_2 induces oxidative stress, the involvement of which is implicated in carcinogenesis, including carcinogen activation, DNA damage, and tumor promotion (Gurr *et al.*, 2005). Furthermore, it was reported that TiO_2 produces hydroxyl radicals, which are produced at higher levels by the anatase form than the rutile form, and that UV irradiation enhanced the production of hydroxyl radicals (Uchino *et al.*, 2002). These findings suggest that positive responses in the genotoxicity tests of TiO_2 via the mechanism of secondary genotoxicity.

However, in *in vivo* and *ex vivo* tests, while an increase in the *hprt* mutation frequency was observed in rat lungs exposed to TiO_2 by inhalation or intratracheal instillation, the formation of DNA adducts and 8-oxoGUA was not observed. To clarify the genotoxicity of TiO_2, it is necessary to perform *in vivo* genotoxicity tests corresponding to the *in vitro* tests in which positive responses are observed. Moreover, it is important to evaluate genotoxicity by a standard genotoxicity testing battery covering a wide range of mechanisms.

Chapter V. Exposure Assessment

Exposure to TiO_2 particles can be broadly divided into 2 categories: exposure at work in cases where the TiO_2 powder is directly handled (e.g., in a working environment in which TiO_2 particles are manufactured or used) and exposure through the lifecycle (production, use, consumption, disposal, and recycling) of products containing TiO_2 particles (e.g., cosmetics and paints). This chapter deals with TiO_2 inhalation exposure of workers in workplaces where the TiO_2 powder is directly handled.

We have reviewed studies on on-site investigations and laboratory dustiness tests with respect to release and exposure of TiO_2 particles to gather information on the potential release/exposure in each process, atmospheric concentrations, size, shape, and aggregation/agglomeration state of released TiO_2 particles. Subsequently, we estimate inhalation exposure of workers to TiO_2 nanomaterials (TiO_2 with primary particle size of 100 nm or less and aggregates/agglomerates of primary particles), assuming a certain emission and exposure scenario.

1. On-site investigations

The on-site investigation results from the NEDO project and published studies are presented in Table 10. This table summarizes the environmental concentrations and exposure concentrations measured in workplaces where TiO_2 nanomaterials and pigment-grade TiO_2 (primary particle size of approximately 200–300 nm) are manufactured and used. The findings from the data shown in Table 10 can be summarized as follows.

- In comparison with the background concentration of particles smaller than 100 nm (tens of thousands of particles/cm^3), the concentration of TiO_2 nanomaterials present in the form of unaggregated/unagglomerated primary particles and aggregated/agglomerated particles smaller than 100 nm was low and could not be detected.
- Observation of the released particles by electron microscopy showed many aggregated/agglomerated particles (several hundred nanometers to several micrometers in size).
- The particle number concentrations and mass concentrations of particles of several hundred nanometers in size or larger were found to be higher in workplaces than in control areas such as outdoors. This increase may be associated with the nature of work. The NEDO project results indicated that the increase in the particle number concentration was approximately 10–100 particles/cm^3 (Figure 4). The increase in the mass concentrations of respirable fraction was approximately 0.03–0.3 mg/m^3.
- The process by which TiO_2 nanomaterials are manufactured involves many steps that are common to

those of pigment-grade TiO_2 production. Therefore, information from pigment-grade TiO_2 production regarding the steps at which exposure may occur would be useful. Data from both TiO_2 nanomaterials and pigment-grade TiO_2 indicated that workers may be exposed to TiO_2 particles at steps in which they handle dry powder. In particular, it was considered that there is a high possibility of exposure in the bagging step. Other steps at which exposure can occur include milling, shoveling, cleaning, and maintenance.

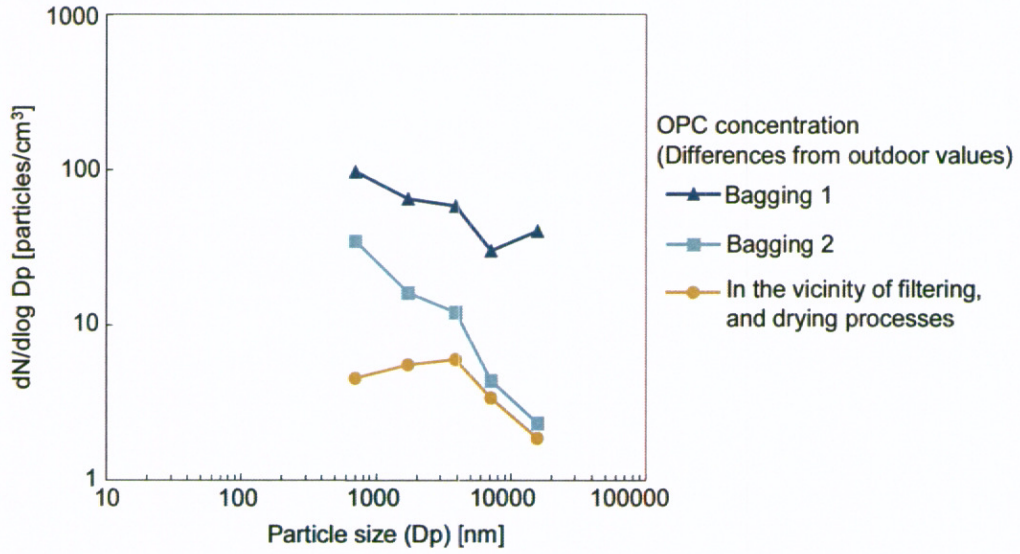


Figure 4. On-site investigations in a TiO_2 nanomaterial manufacturing facility: particle size distribution of the particle number concentration (NEDO project)

N is the particle number concentration, and Dp is the particle size obtained from an optical particle counter (OPC).

Table 10. Environmental concentration and exposure concentration in workplaces where TiO_2 particles are manufactured and used

Source	Nanomaterials, physical properties	Process	Particle number concentration [particles/cm^3]	Mass concentration [mg/m^3]		Measurement	Remarks
				Respirable dust or PM2.5	Inhalable dust or total dust		
TiO_2 nanomaterial							
Wake *et al.* (2001&2002)	Primary particle size 20–100 nm	Bagging	(4150–16615)[a]			CPC, APS	UK
		Dust prep	(4998–21167)[a]				
Ichihara *et al.* (2008 & 2009)	Primary particle size <100 nm, anatase	Bagging				CPC, impactor	Factory in Shanghai (materials are imported from Taiwan)
Berges *et al.* (2007)	Primary particle size 25–100 nm	Bin filling	(15000–156000)[a]	0.1–0.14	0.23	SMPS, NSAM, APS, impactor, filter, TEM	Europe
NEDO project	Primary particle size 15 nm, rutile, lipophilic surface treatment	Surface treatment, filtering		(0.067)[a]		CPC, OPC, impactor, filter, SEM	Japan
		Filtering, drying	(20000)[a]	(0.033–0.039)[a]			
		Bagging	(26000)[a]	0.31			
	Primary particle size 15 nm, rutile, no surface treatment	Bagging	(17000)[a]	0.12			
Pigment-grade TiO_2							
Fryzek *et al.* (2003)	Pigment-grade	Packing, micronizing, shoveling		2.7[b]		Filter	USA
		Maintenance		0.7[b]			
		Ore handling		0.6[b]			
		Dry and wet treatment		0.4[b]			
		Others		0.4[b]			
Boffetta *et al.* (2003)	Pigment-grade	Sulfate process: black end		0[c]		Filter	EU. Data on cumulative exposure per year. The inhalable dust concentrations were converted into the respirable dust concentrations using a conversion factor of 0.3.
		Sulfate process: white end		0.31–1.5– 4.2[c]			
		Chlorine process: black end		0–1.1–3.1[c]			
		Surface treatment, drying, packaging, blending		3.1–7.8–25[c]			
		Maintenance		0.25–1.3–3.3[c]			
		Acid plant		0[c]			
		Others		0–1.3–5.3[c]			

[a] Values in parentheses may largely be contributed by background particles

[b] Geometric mean values. Unknown whether it is the inhalable dust concentration or respirable dust concentration

[c] 25–50–75 percentile values

2. Laboratory dustiness testing

Dustiness testing is a test method that simulates the release of particles (Hamelmann and Schmidt, 2003: Liden, 2006). Dustiness means the propensity of a material to generate airborne dust during its handling. Dustiness has been used to assess general powder materials, not necessarily nanomaterials, mainly from the viewpoint of occupational hygiene. Various methods have been devised for dustiness testing (Hamelmann and Schmidt, 2003). Of these, the rotating drum method and continuous drop method are regarded as standard methods by the EU (European Committee for Standardization, 2006). These two methods simulate typical handling processes during which particles fall (such as bagging, filling, and weighing). Dustiness should be regarded as a relative value and depends on the test methods, apparatus, environmental conditions, and particle measurement methods.

The results of dustiness tests on the release of TiO_2 nanomaterials and pigment-grade TiO_2 from the NEDO project and published studies are summarized in Table 11. Figure 5 shows electron microscopy images of particles released from dustiness testing conducted in the NEDO project. Figure 6 is a graph of the particle concentration and particle size distribution observed in dustiness tests performed in the NEDO project and also reported in published studies. The findings from these can be summarized as follows.

Aggregation/agglomeration state of released particles

- In most tests, the emitted particles mainly consisted of aggregated/agglomerated particles of size in the order of submicrons to microns. Particles smaller than 100 nm or larger than 10 μm were minor.
- A few studies reported that the emission level of particles smaller than 100 nm was comparable to those of submicron- and micron-sized particles.
- Many submicron- and micron-sized aggregated/agglomerated particles were observed by electron microscopy. Particles smaller than 100 nm were also observed, although their proportion was not determined quantitatively.

Comparison with pigment-grade TiO_2

- Although based on only limited data, comparison of dustiness tests of TiO_2 nanomaterials and those of pigment-grade TiO_2 by the same method demonstrated that TiO_2 nanomaterials generate more emission of small particles (less than several hundred nanometers) than pigment-grade TiO_2. Regarding the emission of micron-sized particles, there were 2 cases—one in which TiO_2 nanomaterials generated more emission and the other in which TiO_2 nanomaterials generated as much emission as pigment-grade TiO_2.

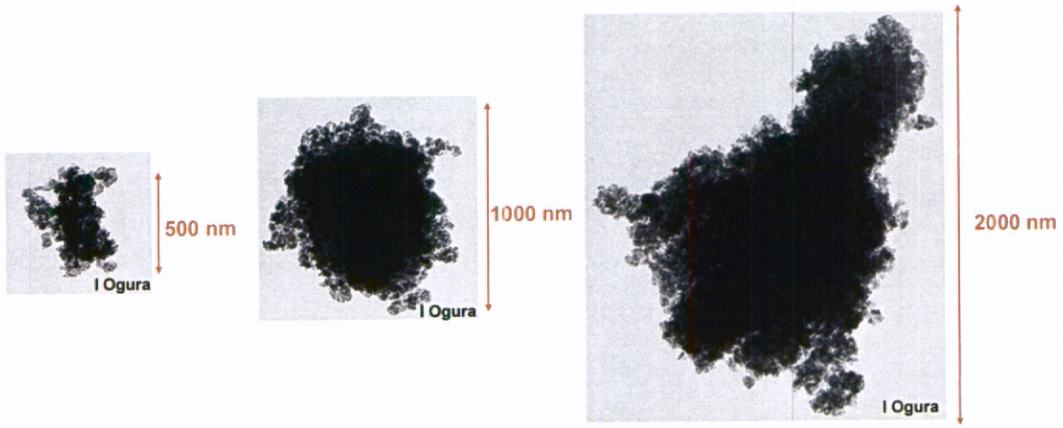
1

2

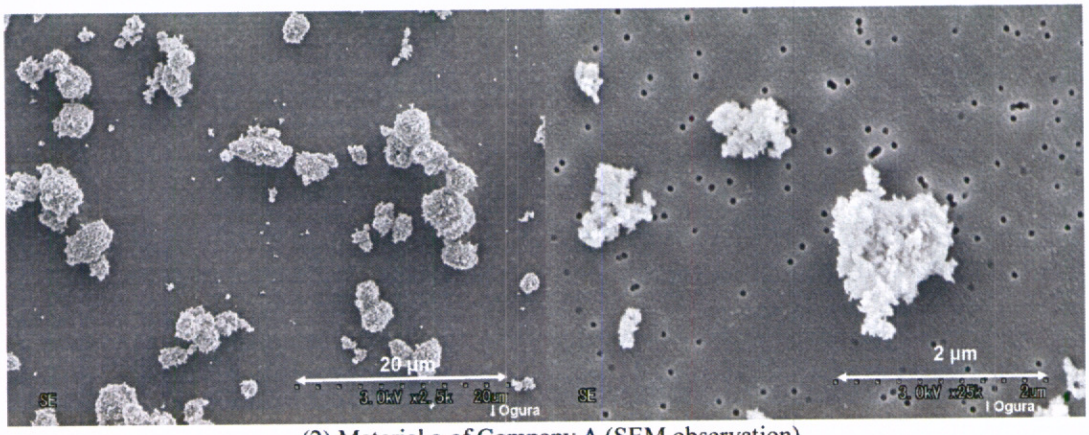
Table 11. Results of dustiness tests for TiO_2 particle emission

Source	Nanomaterials, physical properties	Type of dustiness tests	Particle number concentration [particles/cm^3]				Mass concentration [mg/m^3]			Measurement	Remarks
			<100 nm	100 nm –1 μm	1–10 μm	Total number	Respirable dust	Thoracic dust	Inhalable dust		
TiO_2 nanomaterial											
Tsai *et al.* (2009)	Evonik Degussa P25, primary particle size 21 nm, anatase/rutile	Rotating drum method	1.87	400[a]	86[a]	500[a]	1.0–1.8	69	804	SMPS, APS, impactor, SEM	
Schneider & Jensen (2008)	Kemira UV-TITAN M111, primary particle size 18.6 nm, rutile	Small-scale rotating drum method	48[a]	1600[a]	810[a]	2300[a]		910		FMPS, APS, filter	Mean concentration for 60 secs of continuous drop and subsequent 120 secs
Ibaseta & Biscans (2007 & 2008)	Millennium Inorganic Chemicals G5, primary particle size 5–12 nm, anatase	Drop method	1400[a]	1000[a]	2200[a]	4600[a]				ELPI, SEM	Dropped mass 49.5 g; height of fall 50 cm; height of sampling 50 cm; peak concentration at the instant of fall
Maynard (2002)	Evonik Degussa P25, primary particle size 20 nm, anatase/rutile	Vortex shaker method								SMPS, APS, TEM	Only particle size distribution was reported. Absolute values of the concentration were not reported.
NEDO project	Ishihara Sangyo Kaisha, Ltd. ST-01, primary particle size 7 nm, anatase	Vortex shaker method	0.64	250	150	410				SMPS, OPC, APS, CPC, TEM, SEM	
	Company A, primary particle size 15 nm, rutile, lipophilic surface treatment		0.12	220	800	1000					
	Company A, primary particle size 15 nm, rutile, no surface treatment		0.086	120	190	310					
Pigment-grade TiO_2											
Schneider & Jensen (2008)	Primary particle size 130–150 nm, anatase/rutile	Small-scale rotating drum method	-	13[a]	28[a]	41[a]		3.4		FMPS, APS, filter	
Bard *et al.* (2008); Mark *et al.* (2007)	Primary particle size 150 nm, anatase	Rotating drum method								SMPS, APS, CPC	

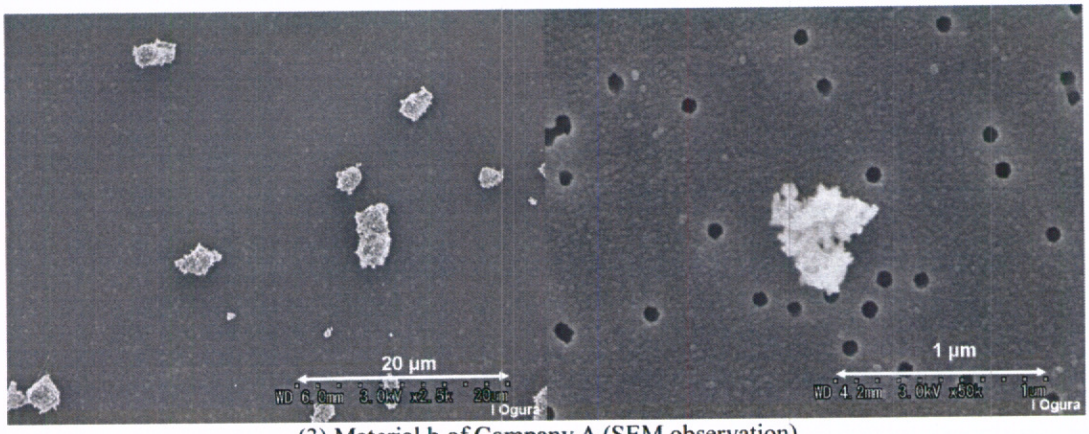
3 [a] Values were obtained from the figures.



(1) Ishihara Sangyo Kaisha, Ltd., ST-01 (TEM observation)



(2) Material a of Company A (SEM observation)



(3) Material b of Company A (SEM observation)

Figure 5. Electron microscopy images of particles released in dustiness tests carried out in the NEDO project

Primary particle sizes (described in the catalog) are as follows: (1) 7 nm, (2) 15 nm, and (3) 15 nm.
The primary particles formed aggregates/agglomerates ranging in size from several hundred nanometers to several microns.

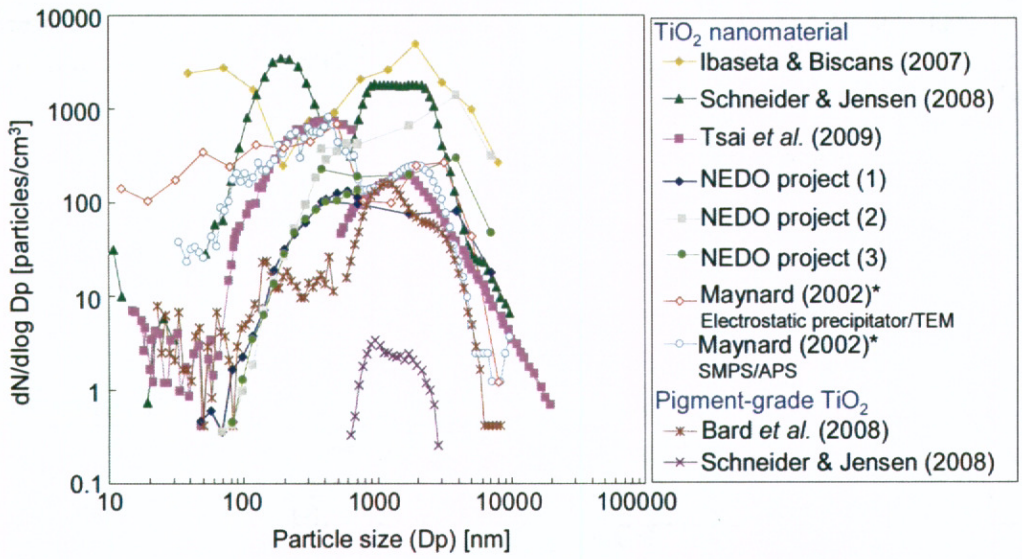


Figure 6. Particle size distribution of TiO_2 particles released by dustiness tests
* Concentrations reported by Maynard (2002) were expressed in an arbitrary unit.
N is the particle number concentration, and Dp is the particle size.

3. Estimation of the exposure of workers

In this section, an assumed emission/exposure scenario is presented, and the amount of TiO_2 particles to which a worker is exposed by inhalation is estimated in a case where the worker directly handles TiO_2 nanomaterial powder. The amount is expressed in terms of the amount deposited on the pulmonary alveoli per day per body weight.

To clarify the emission/exposure scenario assumed here, Table 12 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.

In this section, an emission/exposure scenario is presented assuming a case where a worker frequently handles dry TiO_2 powder on a large working scale without exposure control (Class F2 in Table 12). The amount of TiO_2 that a worker is exposed to by inhalation is estimated.

Table 12. 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 [1]–high [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).

Exposure concentration and the amount of exposure (the amount deposited on the alveoli per day per body weight) were estimated from the following equations:

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

Amount of exposure = exposure concentration × alveolar deposition fraction* × exposure frequency
× breathing rate at work / body weight

*For the deposition efficiencies, the values were calculated using the MPPD2 model (CIIT, 2006; RIVM, 2002) by particle size.

To estimate the values for the possible worst-case scenario, which would provide a baseline value for risk assessment to determine the level of exposure control, an emission/exposure scenario was assumed, which is described in Table 13.

Table 13. Summary of the assumed emission/exposure scenario

Target TiO_2 nanomaterials	Has relatively high emission properties among TiO_2 materials for which data are available.
Working scale, target process, generated concentration	A situation close to the possible worst-case scenario, in which a worker directly handles dry TiO_2 nanomaterials at an industrial scale.
Exposure control	No
Environmental fate	No decrease in the concentration and no changes in the particle size distribution due to environmental fate
Alveolar deposition fraction, breathing rate	Alveolar deposition efficiencies were calculated using the MPPD2 model. The parameters for light exercise conditions were used.
Exposure frequency	High (8 h/day, 5 days/week)

The particle size distribution for a certain concentration of emitted particles near a source is presumed to follow the trend shown in Figure 7 based on the assumption stated in Table 14. This assumption is based on the results from an on-site investigation in the NEDO project and dustiness tests of emission. The data on Bagging 1, which were obtained from an on-site investigation in the NEDO project, were measured under almost enclosed conditions. In addition, the TiO_2 nanomaterial handled in the case of Bagging 1 had a relatively high concentration of emitted particles in the dustiness tests in the NEDO project in comparison with the other types of TiO_2 nanomaterials (NEDO project (2) in Figure 6). Therefore, with respect to the concentration of emitted particles, the assumption made here would be a value close to that of the possible worst-case scenario.

Table 14. Assumption of the concentration of emitted particles near a source based on the particle size

Particle size [nm]	dN/dlog Dp [particles/cm^3]	Particle number concentration N [particles/cm^3]	Numerical basis
10–100	100	100	The results of dustiness testing indicated that the number concentration (dN/dlog Dp) of emitted particles smaller than 100 nm was as much as or less than that of emitted submicron- or micron-sized particles. The data for Bagging 1, obtained from an on-site investigation in the NEDO project, indicated that the dN/dlog Dp of particles of size 500–1000 nm was approximately 100 particles/cm^3. By combining these results, the dN/dlog Dp of particles of size 10–500 nm was assumed to be 100 particles/cm^3.
100–500	100	70	
500–1000	96	29	Based on the data for Bagging 1, obtained from an on-site investigation in the NEDO project
1000–3000	65	31	
3000–5000	59	13	
5000–10000	30	9.1	
Total		250	

N is the particle number concentration, and Dp represents the particle size.

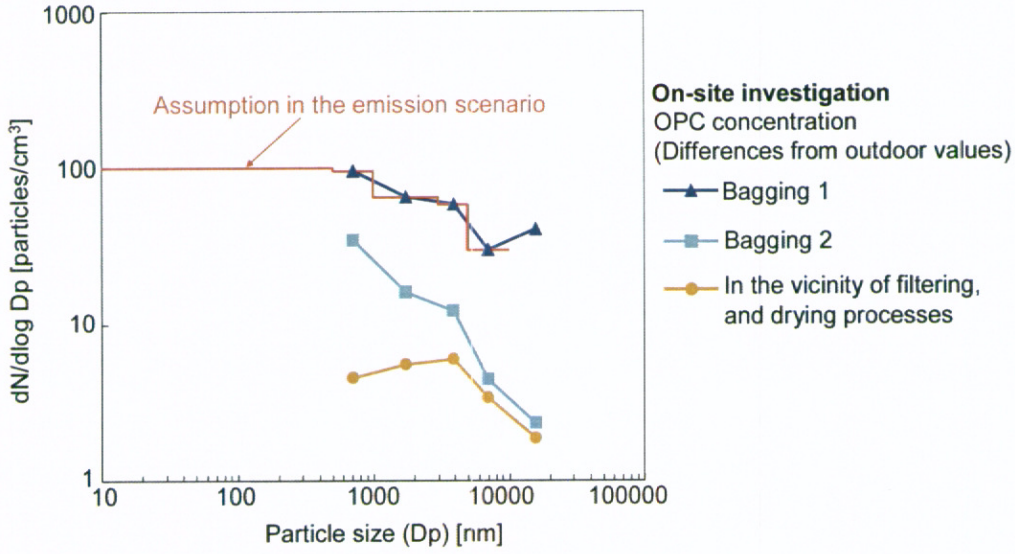


Figure 7. Assumption of the number concentration of emitted particles near a source based on the particle size

N is the particle number concentration, and Dp represents the particle size.

The estimated exposure concentration and the amount of exposure (the amount deposited on the alveoli) are shown in Tables 15 and 16, respectively. The number of particles, mass, and surface area are used as dose metrics in these tables, and the values classified on the basis of particle size, the total, and the values of respirable dust are listed.

Table 15. Results of calculation of the exposure concentration

Particle size	Particle number concentration [particles/cm^3]	Mass concentration [μg/m^3]	Surface area concentration [m^2/m^3]	Particle concentration [particles/cm^3] × deposition fraction	Mass concentration [μg/m^3] × deposition fraction	Surface area concentration [m^2/m^3] × deposition fraction
10–100 nm	100	0.030	0.0000030	15	0.0024	0.00000024
100 nm–1 μm	99	15	0.0015	7.7	1.5	0.00015
1–10 μm	53	5100	0.51	3.3	40	0.0040
Total	250	5100	0.51	26	41	0.0041
Respirable dust	220	400	0.040	—	—	—

Emission concentration in the worst-case scenario without exposure control is assumed.

Table 16. Results of calculation of the amount of exposure (the amount deposited on the alveoli per day per body weight)

Particle size	The number of particles [10^6 particles/kg/day]	Mass [μg/kg/day]	Surface area [10^{-4} m^2/kg/day]
10–100 nm	2.1	0.00034	0.00034
100 nm–1 μm	1.1	0.22	0.22
1–10 μm	0.47	5.7	5.7
Total	3.7	5.9	5.9

Exposure for 8 h/day × 5 days/week and emission concentration in the worst-case scenario without exposure control is assumed.

Chapter VI. Risk Assessment

This chapter discusses the risks faced by workers who directly handle dry TiO_2 powder at the manufacturing/processing site. There is a high possibility that these workers will be exposed to TiO_2 nanomaterials. The acceptable exposure (estimated from hazard assessment in Chapter IV) and the estimated amount of exposure in the worst-case scenario without exposure control (estimated in Chapter V) are used in this chapter to describe the risk level in such a scenario. The report concludes by proposing an acceptable exposure concentration in the working environment.

1. Risk assessment in the working environment

Hazard assessment, which was discussed in Chapter IV, involved inhalation exposure tests with P25-TiO_2. When lung inflammation is defined as the endpoint in the tests, the acceptable exposure (the amount deposited on the alveoli per day per body weight) was calculated to be 18 μg/kg/day (0.018 mg/kg/day) (refer to the next section for a converted value from the acceptable exposure (the amount deposited on the alveoli) to the acceptable exposure concentration (atmospheric concentration)). It should be noted that since relative comparison of the toxicity (the pulmonary inflammatory responses) on the basis of results of intratracheal instillation tests indicated that the toxicity of P25-TiO_2 was relatively high in TiO_2 nanomaterials when the dose is described based on the mass of particle, the acceptable exposure calculated here can be regarded as the lowest value for all TiO_2 nanomaterials.

Exposure assessment, which was discussed in Chapter V, involved the assumption of an operation in which a worker directly handles dry TiO_2 nanomaterials on an industrial scale. The amount of exposure (the amount deposited on the alveoli per day per body weight) in the possible worst-case scenario in which a worker is exposed to nanomaterials for 8 h/day for 5 days/week without exposure control was calculated to be 5.9 μg/kg/day. This estimated amount of exposure is lower than the acceptable exposure, and the ratio of the estimated amount of exposure to the acceptable exposure (referred to as the hazard quotient [HQ]) was calculated to be 0.3 (Table 17). An HQ value lower than 1 is desirable. If the amount of exposure was calculated per lung weight or alveolar surface area, not per body weight, then HQ was calculated to be 0.1 or 0.3, which does not differ much from the value calculated per body weight.

Thus, based on the fact that the estimated amount of exposure under the assumed worst-case scenario is lower than the acceptable exposure, which can be regarded as the lowest value for all TiO_2 nanomaterials, it can be stated that there is no concern of health risk to the worker. Although this result is based on a scenario in which there is no exposure control, it can be regarded as an approximation for actual cases. To obtain concrete information on individual cases, it will be necessary to conduct investigations such as monitoring

in the working environment. The amount of exposure can be reduced by using appropriate local ventilation equipment and protective masks.

Table 17. Comparison between the acceptable exposure and estimated amount to which a worker is exposed

Acceptable exposure[a]	18 μg/kg/day
Estimated amount to which the worker is exposed[a]	5.9 μg/kg/day
Hazard quotient (HQ)[b]	0.3 (= 5.9/18)

a: The amount deposited on the alveoli per day per body weight
b: Hazard quotient (HQ) = estimated amount to which the worker is exposed/acceptable exposure
An HQ value lower than 1 is desirable.

2. Proposed acceptable exposure concentration (provisional value)

In Chapter IV, we examined a provisional value of the acceptable exposure concentration of TiO_2 nanomaterials in the working environment. A proposed acceptable exposure concentration is given in Table 18. This value is calculated by converting the abovementioned acceptable exposure (the amount deposited on the alveoli per day per body weight) to an atmospheric concentration value using representative parameters (body weight, breathing rate, and alveolar deposition fraction). In this calculation, the assumption is that exposure occurs for 8 h/day, 5 days/week, over several years. The acceptable exposure concentration is obtained from the result of inhalation exposure tests using P25-TiO_2. In these tests, lung inflammation is defined as the endpoint. Regarding the other TiO_2 nanomaterials used in the tests reviewed in this report (such as the TiO_2 particles manufactured by DuPont, Ishihara Sangyo Kaisha, Ltd., etc.), since their toxicity (limited only to measurements of the pulmonary inflammatory responses) per mass of dose was estimated (from relative comparisons based on the results of intratracheal instillation tests) to be lower than that of P25 TiO_2 particles, the acceptable exposure concentrations of these are considered to be higher than that of P25 TiO_2.

Table 18. Proposed provisional value of acceptable exposure concentration

Acceptable exposure concentration	1.2 mg/m^3 (respirable dust, TWA)
Remarks	- Exposure for 8 h/day, 5 days/week over several years is assumed. - Lung inflammation is defined as the endpoint. The value was obtained on the basis of short-term inhalation exposure tests. - Because this value is based on the results obtained with P25-TiO_2 and the its toxicity per mass of dose is considered to be relatively high among TiO_2 nanomaterials, this acceptable exposure concentration can be said to be the lowest value for all TiO_2 nanomaterials

3. Problems and uncertainties in the estimated value

The estimation procedure used and the estimated values of the acceptable exposure and the acceptable exposure concentration in this assessment should be regarded as provisional ones in the interim report (2009.10.16) and may change in the final report (scheduled to be published in 2011) on the basis of new scientific data and improvements in the estimation procedures.

The main problems and uncertainties of the estimated values are summarized in Table 19.

Table 19. Main problems and uncertainties of the estimated values

- Issue of dose metric (The amount deposited on the alveoli per day per body weight based on the mass concentration was provisionally used in this report.)
- Influence of the surface area, primary particle size, and aggregation/agglomeration size
- Information on toxicity tests (particularly inhalation exposure tests) is limited
- Diversity due to differences in the types of TiO_2 nanomaterials
- Validity of the set values of UFs

4. Exposure control

Measurements in workplaces in which TiO_2 nanomaterials or pigment-grade TiO_2 are manufactured/used have demonstrated that potential steps in which TiO_2 particles can be emitted are those in which dry TiO_2 powder is handled, such as bagging, milling, shoveling, cleaning, and maintenance. Due caution should be exercised to prevent emission or exposure to the powder in these steps.

On-site measurement in workplaces in which TiO_2 nanomaterials are manufactured/used and results from dustiness tests (Chapter V) suggested that TiO_2 nanomaterials are mainly released in the form of submicron- to micron-sized aggregated/agglomerated particles. It was also observed that the dust concentration (mass concentration) increased in the vicinity of the emission source. For emission/exposure control in workplaces (identification of the emission source and operations that can emit dust, evaluation of efficiency of measures against exposure, etc.), similar to the case of general dust, measurement of the dust mass concentration in the atmosphere (respirable dust concentration is desirable to compare with the abovementioned acceptable exposure concentration) is considered to be effective in most cases. Use of general direct-reading aerosol monitors, such as a digital dust monitor or an optical particle counter (OPC) for submicron- to micron-sized particles, may also be effective methods for daily monitoring.

It must be noted that it is impossible to measure whether TiO_2 nanomaterials are released into the atmosphere in the form of primary particles or aggregated/agglomerated particles smaller than 100 nm by using the methods described above. Measurement of particles smaller than 100 nm in the atmosphere

requires more expensive equipment such as scanning mobility particle sizer (SMPSs), fast mobility particle sizers (FMPSs), and electrical low pressure impactors (ELPIs). However, as shown by on-site measurements in workplaces in which TiO_2 nanomaterials are manufactured/used and the results of dustiness tests (Chapter V), emission in the form of particles smaller than 100 nm is less than that of submicron- and micron-sized particles and generally tends to be buried in background particles. Therefore, it is difficult to detect the increase in the concentration of such extremely small particles. Emission of or exposure to particles smaller than 100 nm often coincides with the emission of or exposure to submicron- and micron-sized particles. Therefore, if the concentration of submicron- and micron-sized particles can be reduced, for example, by using local ventilation equipment, emission source enclosure or filters for particle removal, the concentration of particles smaller than 100 nm can also be reduced. That is, to perform exposure control targeted to submicron- and micron-sized particles is considered to be a practical, effective measure in the present circumstances.

It should be noted that in order to distinguish TiO_2 particles emitted in the workplace from background particles, it is necessary to compare the measurements before/after work (or the measurements at times when the work is not being performed) and during work, or to conduct simultaneous measurements in the workplace and in a control area (outdoors or at a place that is at a distance from the emission source).

Possible methods to control even a trace amount of emission or to identify particles (separate from background particles) are the observation under an electron microscope and the elementary analysis for titanium after collection of particles in the atmosphere or on the floors and walls with filters .

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