

Behavior of New Type of Rock Wool (HT Wool) in Lungs after Exposure by Nasal Inhalation in Rats

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Abstract

Objectives: Previous types of rock wool has been recently replaced with high-temperature wool (HT wool). HT wool is characterized by a chemical composition with a higher concentration of Al_2O_3 and a lower concentration of SiO_2 , lower biopersistence, and a higher melting point than previous types of rock wool. To evaluate the safety of HT wool, an asbestos substitute, we examined the biopersistence of HT wool in the lungs, based on changes in fiber count according to the length and fiber size (length and width), by performing a nose-only inhalation exposure study in rats.

Methods: Male Fischer 344 rats were exposed to fibers at the target exposure concentration of 30 mg/m³ continuously for 3 hours daily for 5 consecutive days. Rats were sacrificed shortly after exposure, and 1, 2, and 4 weeks after exposure, and their lung tissues were incinerated at a low temperature. Then, fiber counts and sizes in the lungs were analyzed using a phase contrast microscope.

Results: The fiber count in the lungs 4 weeks after exposure significantly decreased from the baseline value (shortly after exposure). The half-life of fibers calculated from the approximation curve was 34 days for all fibers and 11 days for fibers longer than 20 μm .

Conclusions: Both the length and width significantly decreased 4 weeks after exposure, probably because fibers were ingested by alveolar macrophages, discharged to outside of the body by mucociliary movement, or lysed by body fluid. In future studies, it is necessary to examine the long-term persistence of fibers in the lungs.

Key words: HT wool, inhalation, nose-only, clearance, biopersistence

Introduction

Asbestos excels in heat resistance, insulation performance, and durability. In Japan, it has been used as a material for building construction such as asbestos cement products and boards, a reinforcing material for synthetic resin such as vinyl flooring, board, and gear, a spray coating material for heat insulation or sound insulation, and a heat insulation material for boiler piping, furnaces, etc. At the same time, it has been reported to cause fibrosing lung disease, lung cancer, and malignant

mesothelioma in the pleura and peritoneum (1–3), and proved to have toxicity through many in vitro and in vivo experiments. Therefore, the use of asbestos has been banned or restricted all over the world (4–6). In Japan, the Enforcement Order of the Industrial Safety and Health Law, Industrial Safety and Health Regulations, and Ordinance on Prevention of Hazards due to Specified Chemical Substances were revised in 1995 to ban the manufacture, import, use, and sales of amosite, blue asbestos, and products containing either of them exceeding 1%. In addition, the use of chrysotile has partly been banned since October 2004. Under these circumstances, we are facing an urgent need to develop a safer fibrous substance as an asbestos substitute.

In the current market, man-made vitreous fibers (MMVF) of various kinds have been in use as asbestos substitutes. Rock wool (RW) is a kind of MMVF, which is extracted from molten soft rock such as slag (iron slag, copper slag, nickel slag, etc.) and natural stone (andesite, basalt, amphibolite etc.). Because

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RW excels in heat resistance, fire resistance, and sound absorption, it is mainly used as a fire- and heat-resistant material, heat insulation material, or sound absorption material (7). The International Agency for Research on Cancer (IARC) classifies the safety of RW as Group 3 (not classifiable in humans, and inadequate or limited data on animals with respect to its carcinogenicity) (8). Recently, a new type of RW, high-temperature wool (HT wool), has been developed. Its chemical composition has a high concentration of Al_2O_3 and a low concentration of SiO_2 , low biopersistence, and a high melting point (8–11). These characteristics of HT wool have enabled its use to be broadened to heat insulation and other purposes, and HT wool has extensively replaced previous types of RW. HT wool is also characterized by higher solubility at pH 4.5 and lower solubility at pH 7.4 than previous types of RW (8, 12). During an in vivo experiment performed to assess the safety of HT wool, no occurrence of pulmonary fibrosis, lung tumor, or intraabdominal tumor was observed in rats (8). However, the IARC rates the safety of HT wool as not classifiable (8), because data in humans are not available as it has only recently been applied for practical use.

The respiratory system is the most vulnerable to exposure to MMVF such as asbestos or RW. To evaluate the biological effects of MMVF such as asbestos and RW, many in vivo experiments have been performed, including short-term and long-term inhalation exposure studies, injection of MMVF into the pleura and peritoneum, and injection into the trachea. Reports by the IARC (8, 9) have proved that an inhalation exposure study, which simulates the condition of actual exposure of humans to a test substance, is the most suitable method to evaluate the effects on public health.

In the present study, to examine the persistence of HT wool in the lungs as an index of the effect of RW on the respiratory system, we conducted a short-term nose-only inhalation exposure study in rats using an original experimental system and HT wool. Then, we also studied the persistence of HT wool in the lungs by observing the behavior of fibers in the lungs in terms of both variations of fiber count according to the length and changes in size of the length and width.

Materials and Methods

This experiment was performed in accordance with the “Ethical Guidelines for Animal Experimentation” adopted by the Institutional Review Board of Kitasato University School of Medicine (Approval No. 2004022).

1) Materials

As a material, we used HT wool manufactured by R Co. Ltd. and provided by the Rock Wool Association of Japan (Tokyo). Fluorescent X-ray spectroscopy showed that the HT wool used in the study was chemically composed of 43% SiO_2 , 18% Al_2O_3 , 16% CaO , 10% MgO , 6% Fe_2O_3 , 2.1% TiO_2 , 2.1% Na_2O , 1.2% K_2O , and 0.6% S.

Originally, HT wool is present in the form of lumps of different sizes (length and width). In general, to evaluate the biological effects of MMVF, experiments on animals are conducted to determine the maximum harmful effect of the

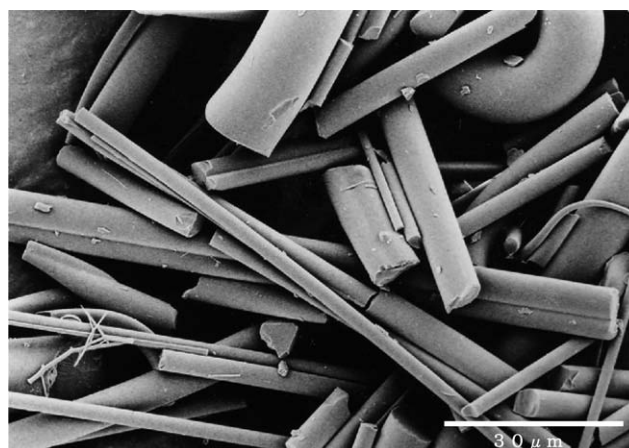


Fig. 1 Electron microscopic image of fibers before generation ($\times 1000$).

fiber. Because it is known that the biological effects of fibers vary depending on the size, it is necessary to make the fiber size as close to the value that will maximize harmful effects. Therefore, we adjusted the size of HT wool in accordance with the method suggested by Kohyama et al. (13). That is, a cylinder (6 cm in diameter, 28.3 cm^2) was filled with HT wool, and pressure ($160 \text{ kg/cm}^2 = 4.5 \text{ MPa}$) was applied to it twice using a manual briquetting press machine (Type BRM 32, Maekawa Testing Machine MFG Co., Ltd.). The geometric mean length (geometric standard deviation) was $26.2 \mu\text{m}$ (3.06), and the geometric mean width was $2.70 \mu\text{m}$ (2.97) (Fig. 1). Then, to make it easier to generate HT wool with the nose-only inhalation exposure study system, the pressurized material was mixed with glass beads (BZ-02, AS ONE Corporation) at a ratio of 1 (material) to 14.5 (glass beads).

2) Nose-only inhalation exposure system

The materials prepared according to the above procedure were treated as follows: Air was supplied from an air compressor to a material generator, as reported by Kudo et al. (14), at a rate of 30 L/min, and the materials were placed in the material storage tank of the material generator. The materials mixed with glass beads were fluidized by air from the air compressor, and separated from the glass beads. As a result, the materials were emitted into the air. The generated materials were sent to the subchamber, diluted and homogenized to a specified concentration, and transferred to the exposure chamber. The exhaust flow rate in the exposure chamber was set at 40 L/min. To maintain the concentration of HT wool fibers (10,000 cpm) in the exposure chamber, the concentration was monitored using a digital dust meter, and the amount of materials to be generated was adjusted by applying feedback to the feeder. The rat holders were placed in the exposure chamber.

3) Exposure study

A total of 24 male Fischer 344 rats (6 to 10 weeks old) were used. These rats were divided into four groups with six rats in each. (Unfortunately, one rat died on the 3rd day from the start of the study, and therefore, was excluded from the evaluation of the study results.) To accustom rats to the

environment of the laboratory, they were housed in cages for a week preliminarily. Water and food were given ad libitum. Fresh, filtered air was continuously supplied to the room, and the temperature was kept at 22°C and humidity at 40%.

The study was conducted by exposing the rats to HT wool fibers continuously for 3 hours a day for 5 consecutive days. With regard to the target exposure concentrations, the mass concentration was set at 30 mg/m³, and fiber concentration was set at 50±10 fibers/cm³. During the exposure study, the concentration in the chamber was monitored 5 times (30, 60, 90, 120, and 150 minutes after the start of the study). Membrane filters (“MF”, Millipore, 0.8 µm pore diameter, 25 mm diameter), T60A20 filters (“T60A20”, Tokyo Dylec Corp., 25 mm diameter), and Nuclepore filters (“NF”, Nomura Micro Science, 0.2 µm pore diameter, 25 mm diameter) were set in the plastic holder that had been arranged beforehand. During a specified period of time, sample fibers were collected on MF for 1 minute, T60A20 for 10 minutes, and NF for 5 minutes using an electric suction pump (GilAir-5, Gilian), and the exposure concentration was confirmed by measuring the fiber concentration (fiber/cm³) and mass concentration (mg/m³), and performing scanning electron microscopy (SEM).

To measure HT wool fibers in the nose-only exposure chamber, fibers collected on an MF that had an aspect ratio (length & width ratio) of 3 or higher (measured by phase contrast microscopy) were counted according to the criteria for fiber measurement (15). To measure the HT wool fiber mass concentration (mg/m³) in the nose-only exposure chamber (dust fibers emitted), fibers collected on T60A20 were measured using an electronic balance, and the mass concentration (mg/m³) was calculated based on the fiber count and the value before the collection.

On the 5th day of exposure, five rats were sacrificed shortly after the end of the exposure period (“shortly-after group”). In a similar way, six rats each were sacrificed one week (“1-week-after group”), two weeks (“2-weeks-after group”) and four weeks (“4-weeks-after group”) after the end of each exposure period. The body weights of the rats were measured once a week, and their appearances and condition were intermittently monitored for any change during and after the exposure study period.

4) Measurement of fibers in rat lungs

Under anesthesia with Nembutal, rats were sacrificed by bleeding from the abdominal aorta and their lungs were resected. The resected lungs were stored in a weighing bottle at -20°C. Subsequently, the lung tissues were thawed at room temperature, minced, and lyophilized to reduce their weight to a specified level. The weight after lyophilization was regarded as the weight of the dried lungs. The lyophilized lungs were incinerated in a low-temperature asher (Yanaco Corporation, Plasma Asher LTA-102) for 24 hours.

After incineration, distilled water that had been filtered with a Minisart (Sartorius) was added to the weighing bottle to suspend fibers, and the fibers were collected on a MF (pore diameter: 0.22 µm) using a suction filter and allowed to dry. The dried filter was put on a slide glass, and treated with acetone steam using Quick Fix until it became clear. At least

200 fibers were counted from each sample using a phase contrast microscope (Olympus, BX41). Fibers to be counted were those with a ratio of length to width (“aspect ratio”) of 3 or higher. Among the fibers counted, WHO fibers (fibers longer than 5 µm in length and shorter than 3 µm in width with an aspect ratio of 3 or higher) were also counted (8). WinRoof (image analysis software of Mitani Corporation, Tokyo) was used to obtain the number of fibers according to the length (L) (L≤5, 5<L≤20, and L>20). Then, the fiber count was converted into the count per weight of dried lung tissue.

The half-life of fibers in the rat lung was obtained from the exponential approximation curve assuming that the geometric mean fiber count in the lungs in the shortly-after group was 100% (9).

5) Measurement of fiber sizes (length and width)

To obtain the sizes of fibers (length and width) in the air and in the lungs, fibers within the measurable visual range and with an aspect ratio of 3 or higher were measured using a phase contrast microscope under ×400 magnification. At least 200 fibers were extracted from each lung for measurement. Fibers that were 0.36 µm or longer in size with an aspect ratio of 3 or higher were measured.

6) Statistical analysis

The geometric mean and standard deviation of the total fiber count, length and width were calculated. One-way analysis of variance and multiple comparisons by Scheffe’s test were performed.

Results

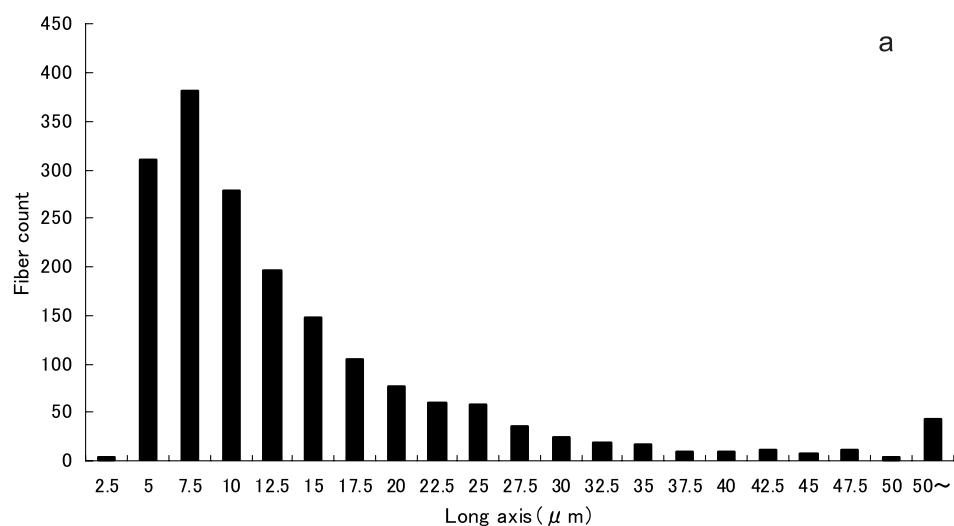
1) Monitoring of fiber concentration in exposure chamber

During the study period, the arithmetic mean (standard deviation) of the fiber concentration in the exposure chamber, which was measured 5 times a day, was approximately 31.2 (21.6) fibers/cm³, the arithmetic mean of the weight concentration was 11.0 (7.5) mg/m³, and the arithmetic mean of the value of the digital dust meter was 705×10 (262×10) counts/min. Figure 2 shows the frequency distribution (histogram) of the length and width of fibers, in which the geometric mean of the length was 10.04 µm (2.03) and that of the width was 1.71 µm (1.57).

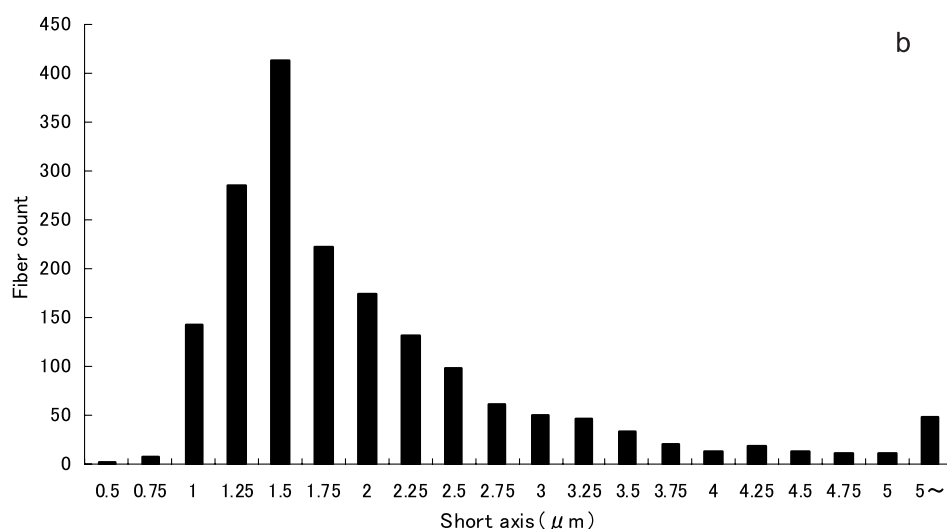
2) Changes in fiber count in all lungs

Table 1 and Figure 3 show the number of HT wool fibers accumulated in the lungs and the proportion on the assumption that the value shortly after exposure was 100%.

The mean of the total fiber count of all dried lungs tended to decrease during the period from shortly after exposure to 4 weeks after exposure. Although the rates of decrease in the number of fibers with a length of 5 or less (L≤5), or more than 5 and 20 or less (5<L≤20), and number of WHO fibers were low at a certain point, the number of fibers in the 4-weeks-after group was smaller than that in the shortly-after group (100%). At the same time, fibers with a length of greater than 20 (L>20) tended to decrease comparatively rapidly during the period from shortly after exposure to 4 weeks after exposure (Table 1



Total fiber count = 1,810



Total fiber count = 1,810

Fig. 2 a: Distribution of length of fibers generated. b: Distribution of width of fibers generated.**Table 1 Fiber counts in lungs and their proportions**

Sacrificed rat group	Total fiber count		L \leq 5 μm		5<L \leq 20 μm		L>20 μm		WHO fiber count	
	Geometric mean (GSD)	%	Geometric mean (GSD)	%	Geometric mean (GSD)	%	Geometric mean (GSD)	%	Geometric mean (GSD)	%
Shortly-after group	9.036 (1.22)	100.0	2.143 (1.17)	100.0	6.248 (1.23)	100.0	0.628 (1.43)	100.0	6.800 (1.23)	100.0
1-week-after group	8.133 (1.14)	90.0	2.018 (1.07)	94.2	5.637 (1.18)	90.2	0.458 (1.29)	72.9	5.996 (1.18)	88.2
2-weeks-after group	7.101 (1.32)	78.6	2.458 (1.33)	114.7	4.334 (1.32)	69.4	0.272 (1.69) ^a	43.3	4.582 (1.34)	67.4
4-weeks-after group	4.955 (1.14) ^{a,b,c}	54.8	1.594 (1.09) ^c	74.4	3.246 (1.18) ^{a,b}	52.0	0.094 (1.72) ^{a,b}	15.0	3.322 (1.19) ^{a,b}	48.9

a: Comparison with the shortly after group ($p<0.05$).b: Comparison with the 1-week-after group ($p<0.05$).c: Comparison with the 2-weeks-after group ($p<0.05$).Geometric mean: $\times 10^5/\text{lung}$.WHO fiber: length $\leq 5 \mu\text{m}$, width $< 3 \mu\text{m}$.

GSD: Geometric standard deviation.

%: Percentage when value of the shortly after group is assumed to be 100%.

n=6 (n=5 for the shortly after group).

L=Length of fiber (μm).

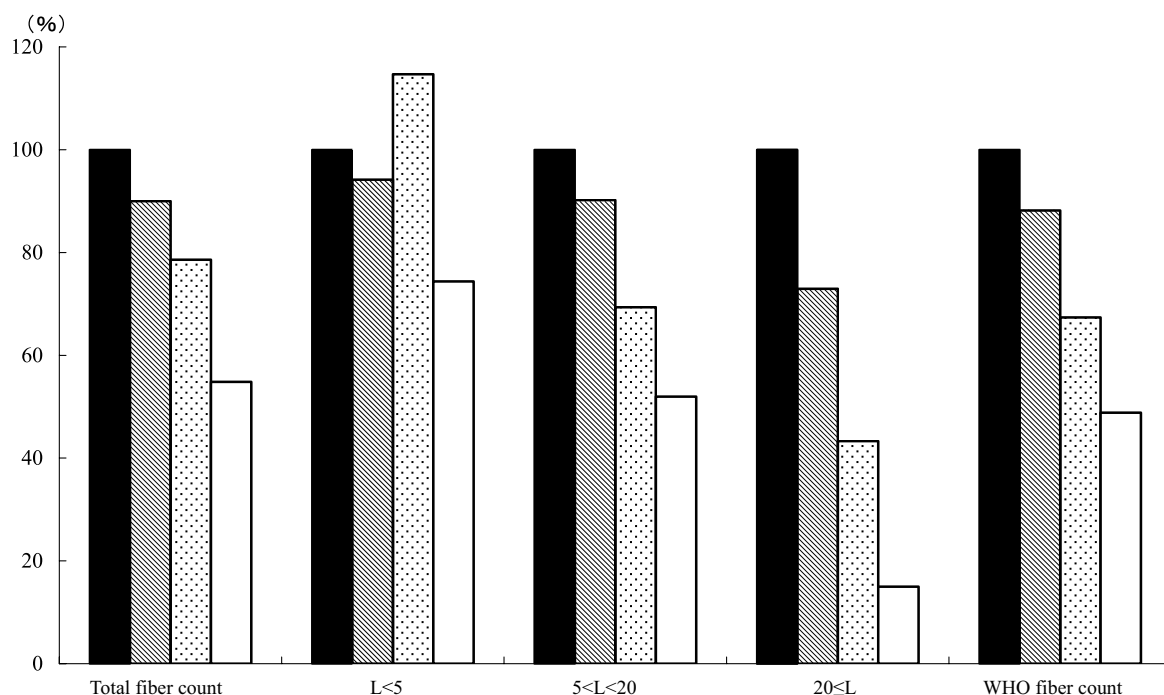


Fig. 3 Percentages of fibers in lungs. ■, Shortly-after group; ▨, 1-week-after group; ▤, 2-weeks-after group; □, 4-weeks-after group. Percentage when value of the shortly after group is assumed to be 100%. L=Length of fiber (μm).

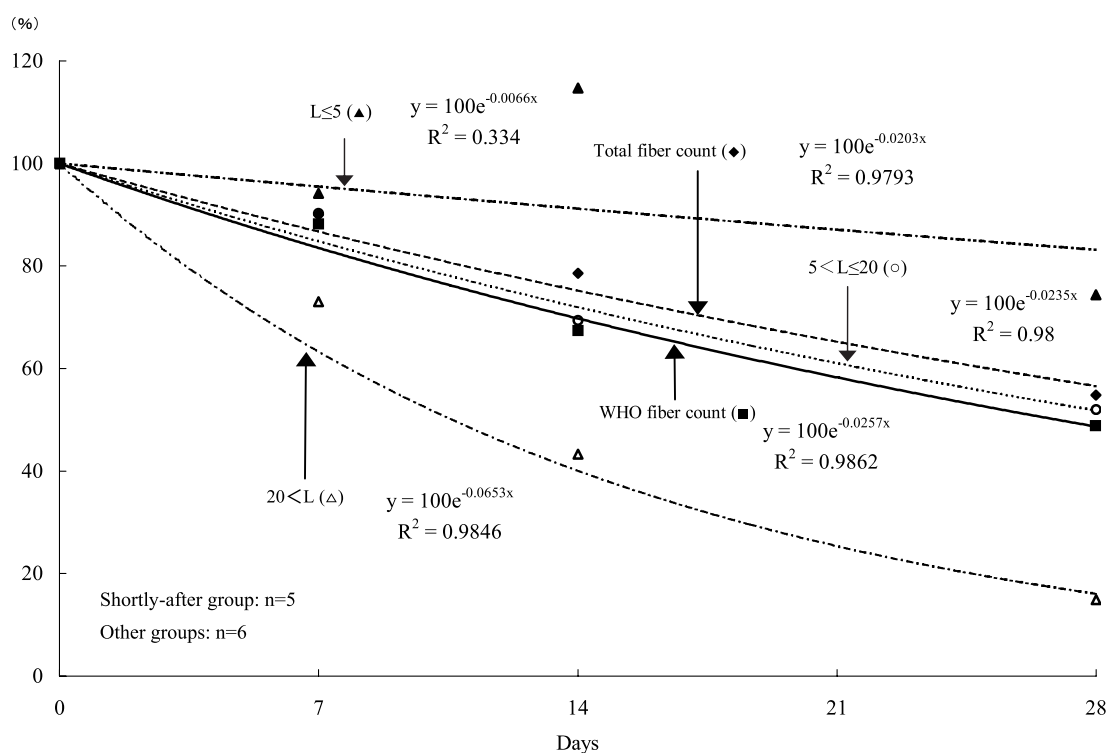


Fig. 4 Exponential approximation curve. (%): Calculated assuming that value of the shortly after group is 100%.

and Fig. 3). Multiple comparison by Scheffe's test showed that the total fiber count, 5<L≤20, L>20, and WHO fibers in the 4-weeks-after group significantly decreased compared to those in the shortly-after group (p<0.05) (Table 1 and Fig. 3).

3) Half-life of fibers

On the assumption that the total fiber count divided by

the weight of all lungs (fibers/mg) shortly after exposure was 100%, the half-life of fibers calculated from the approximation curve (Fig. 4) was 34 days for the total fiber count, 105 days for L≤5, 29 days for 5<L≤20, 11 days for L>20, and 27 days for WHO fibers. The half life of L>20 tended to be shorter than that of L≤20.

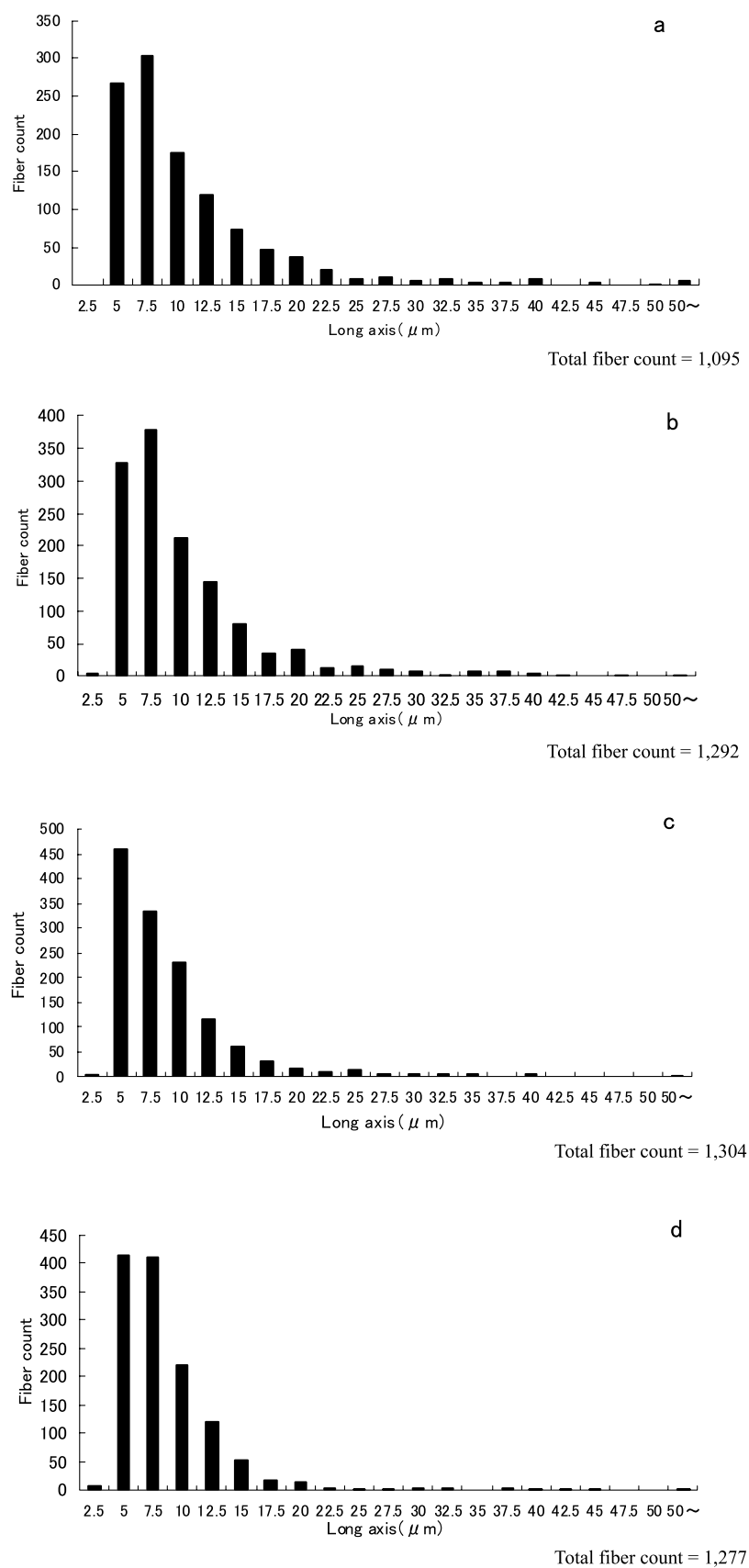


Fig. 5 Distribution of length of fibers in lungs. a: Shortly-after group. b: 1-week-after group. c: 2-weeks-after group. d: 4-weeks-after group. Horizontal axis indicates maximum value of each category.

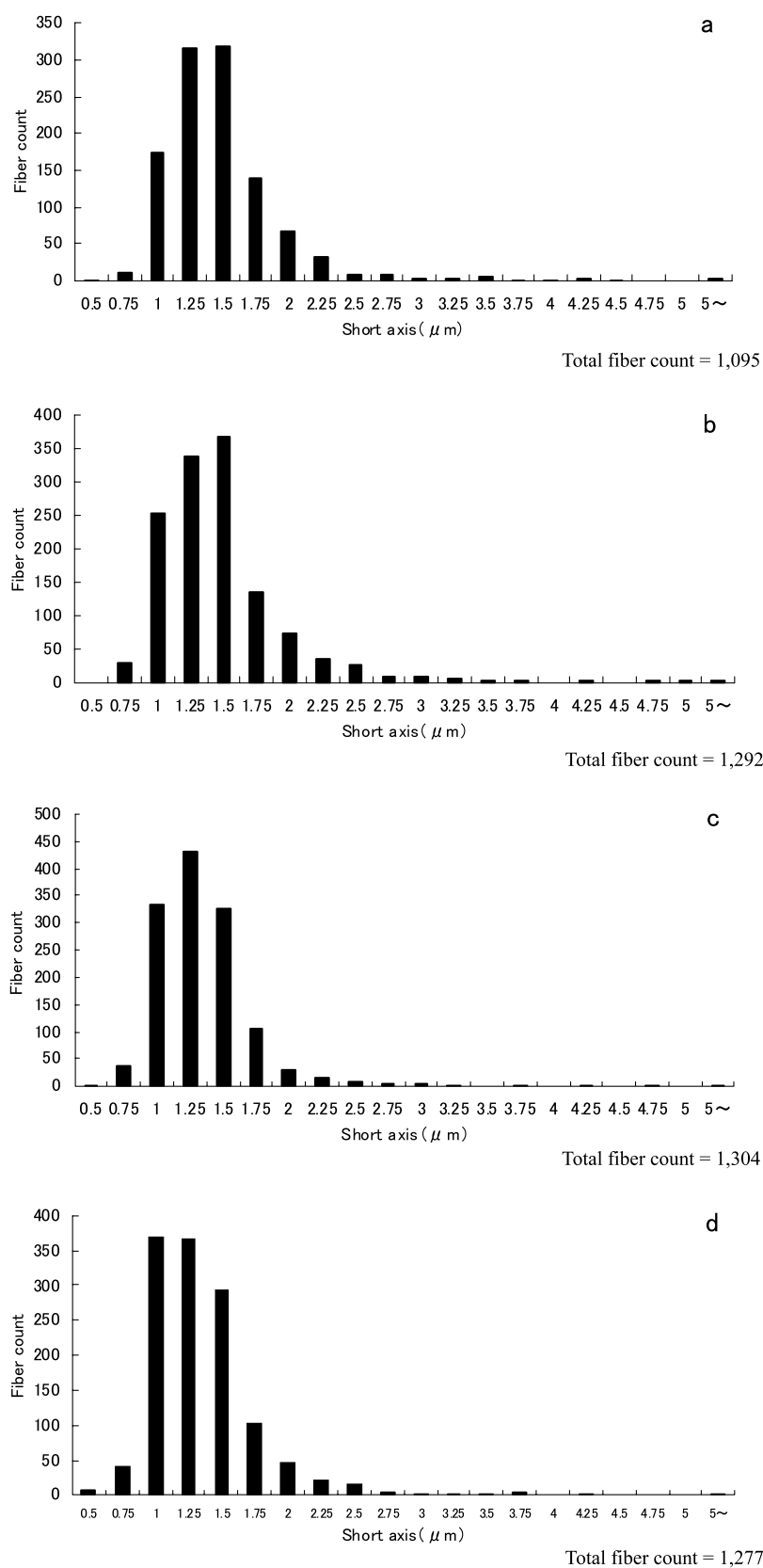


Fig. 6 Distribution of width of fibers in lungs. a: Shortly-after group. b: 1-week-after group. c: 2-weeks-after group. d: 4-weeks-after group. Horizontal axis indicates maximum value of each category.

4) Distribution of and changes in fiber size (length and width)

Figures 5 and 6 show the frequency distribution (histogram) of the length and width of fibers remaining in the lungs shortly after, 1, 2 and 4 weeks after exposure. Table 2 shows the time course of the changes in the geometric mean of the length and width of fibers in the lungs (geometric standard deviation), and microscopic images of fibers in the rat lungs are also presented in Figures 7 & 8.

Table 2 Changes in length and width of fibers in lungs

Sacrificed rat group	Length (μm)	Width (μm)
	Geometric mean (GSD)	Geometric mean (GSD)
Shortly-after group	7.93 (1.79)	1.30 (1.33)
1-week-after group	7.48 (1.75) ^a	1.28 (1.36)
2-weeks-after group	6.66 (1.70) ^{a,b}	1.18 (1.30) ^{a,b}
4-weeks-after group	6.37 (1.62) ^{a,b}	1.16 (1.34) ^{a,b}

GSD: Geometric standard deviation.

a: Comparison with the shortly after group ($p < 0.05$).

b: Comparison with the 1-week-after group ($p < 0.05$).

n=6 (n=5 for the shortly after group).

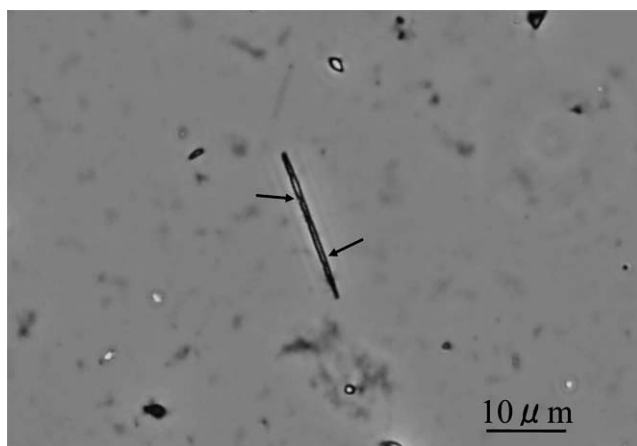


Fig. 7 Microscopic image of fibers in rat lung (4 weeks after exposure). The arrows indicate eroded parts of a fiber.

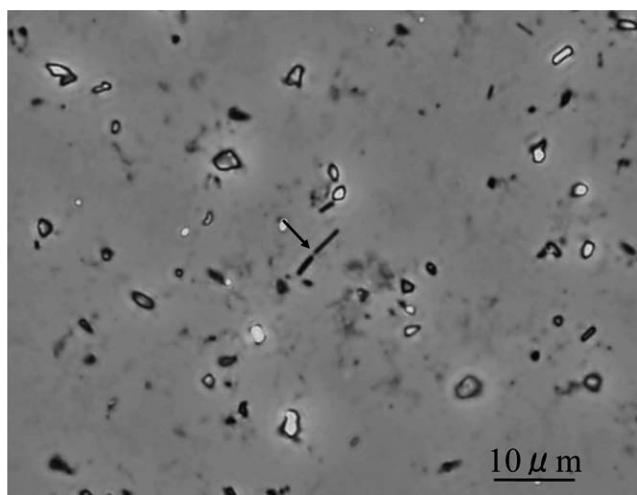


Fig. 8 Microscopic image of fibers in rat lung (4 weeks after exposure). The arrow indicates a part where a fiber is going to be folded transversely.

With regard to the length, the frequency distribution changed with time as shown in Figure 5, and the number of 10 μm or shorter fibers increased after the first week onwards. As shown in Table 2, the mean length was 7.93 μm shortly after exposure, but it decreased significantly with time (1, 2 and 4 weeks after exposure) to 6.37 μm in the 4th week after exposure ($p < 0.05$). It also decreased significantly in the 2- and 4-weeks-after groups in comparison to the 1-week-after group ($p < 0.05$) (Table 2).

With regard to the width, the frequency distribution changed with time as shown in Figure 6, and the number of 1.25 μm or shorter fibers increased with time (shortly after, 1, 2 and 4 weeks after exposure). The mean width was 1.30 μm shortly after exposure, but it decreased significantly with time (2 and 4 weeks after exposure) to 1.16 μm in the 4-weeks after group ($p < 0.05$). It also decreased significantly in the 2- and 4-weeks-after groups in comparison to the 1-week-after group ($p < 0.05$) (Table 2). As shown in Figures 7 & 8, fibers that were partly eroded or folded transversely were observed in the lungs.

Discussion

In many previous epidemiological, physicochemical studies and experiments on animals, the fiber sizes and biopersistence of asbestos or MMVF have been pointed out to be important factors in their harmful effects, especially carcinogenicity. With regard to inhalant fibers that are 5 μm or more in length and 3 μm or less in width, the thinner and longer the fiber is, the more carcinogenic it becomes. On the other hand, fibers that remain in lung tissues for a long period of time without being resolved or transferred are considered to be more carcinogenic (8). It is said that fibers that are 20 μm or longer in length and have a long half-life tend to cause fibrosis or cancers because of the low resolvability in the living body (8, 9).

As described above, various studies have evaluated the effects of fibrous substances on the living body. In the present study, we observed the behavior of HT wool in the lungs, using a short-term, nose-only inhalation exposure method. An inhalation exposure study provides conditions that are closer to the actual exposure route in humans than the conditions with an endotracheal administration method or injection method into the pleura or peritoneum. Inhalation studies are subdivided into chronic inhalation (exposure for more than a year), subchronic inhalation (exposure for about 3 months) or short-term inhalation (exposure for not more than 5 days). Short-term inhalation exposure studies are often conducted as the first phase of a toxicity study to examine the biopersistence of fibrous substances in the lungs. Short-term inhalation exposure studies have advantages over chronic inhalation studies in terms of the study period, number of animals required for experiments, and cost involved (9). It has been reported that the greater the biopersistence of MMVF, the more likely carcinogenicity or fibrosis in the lungs will be caused (9). Therefore, biopersistence has been adopted more often as an index of the hazard of MMVF in short-term inhalation exposure studies (9). Inhalation exposure studies are classified as systemic exposure studies and nose-only exposure studies. In comparison with systemic exposure studies, nose-only exposure studies require smaller

experimental apparatus and are less likely to be associated with individual differences in the deposition of inhaled substances in the lungs (16). Thus, they are more suitable for short-term exposure and observation of behavior in the lungs, with less adhesion of a test substance to the skin.

The nose-only inhalation exposure system used in the present study has two major advantages over traditional ones: the use of a mixture of test fiber particles and glass beads, and adoption of a subchamber. By mixing test fiber particles with glass beads, it has become possible to reduce the agglutination of the fiber particles and generate test fiber particles constantly. Adoption of a subchamber just before the exposure chamber has enabled test fiber particles to be supplied into the exposure chamber at a constant concentration, allowing test fiber particles to be generated constantly at a relatively high concentration for a specified period of time.

The total fiber count and the fiber counts classified according to length tended to decrease during the period from shortly after exposure to the 4th week. In particular, fibers with a length longer than 20 μm tended to decrease markedly. In a preceding study, WHO fibers decreased to about 30% and fibers with a length of 20 μm or longer decreased to about 7% 30 days after exposure (17). Fibers that are inhaled and precipitate in the lungs show different mechanisms for clearance depending on the site of precipitation. Fibers deposited in the bronchioles are transferred to the pharynx by mucociliary movement and are discharged from the respiratory system (8, 9). Of fibers deposited in the alveoli, those with a length shorter than 20 μm are considered to be ingested by alveolar macrophages and digested (8, 9). Those with a length longer than 20 μm cannot be completely phagocytosed by alveolar macrophages and, therefore, are dissolved by extracellular fluid, allowing the fibers to be folded transversely. They are subsequently ingested by alveolar macrophages (8, 9) or taken into pulmonary epithelial cells and transferred to lymphatic vessels (8, 9). The fiber count is believed to be decreased by these mechanisms. Moreover, the rate of decrease in the number of fibers with a length shorter than 20 μm slowed in the 1- and 2-weeks-after groups. A possible reason for this phenomenon is that fibers longer than 20 μm were dissolved by extracellular fluid and folded transversely with the fibers being crushed, thus increasing the number of shorter fibers (shorter than 20 μm) and, as a result, increasing the rate of accumulation in a number of indicators including the total fiber count (8, 9). As described earlier, HT wool is characterized by a chemical composition with a higher concentration of Al_2O_3 and a lower concentration of SiO_2 than previous types of rock wool, and is highly soluble at pH 4.5 (acidic condition) and poorly soluble at pH 7.4 (8, 12). It is reported that when fibers are inhaled into the lungs, they are ingested by alveolar macrophages and converted into phagolysosomes to be digested, and that the pH of phagolysosomes and the pH on the surface of the fibers in contact with alveolar macrophages are below 5 (acidic conditions) (10). Fibers longer than 20 μm , which could not completely be ingested by macrophages, are considered to come in contact with the surface of macrophages, dissolved and folded transversely under acidic conditions, and subsequently ingested by macrophages (10). These mechanisms may explain why the

number of fibers longer than 20 μm decreased and the half-life was shorter than that of the other fibers. Erosion and transverse folding were also observed in fibers in the lungs during the present study, suggesting that the above-mentioned mechanism also occurred in the course of the present study (Figs. 7 & 8).

The half-life was especially short (11 days) when the length of fibers was longer than 20 μm . In the preceding study, the half-life was 22 days for WHO fibers and 5 days for fibers with a length longer than 20 μm (17). With asbestos and other fibers that show high biopersistence, those fibers having lengths of 20 μm or greater are reported to have a longer half-life than shorter ones. The results of our experiment showed that fibers having a length of 20 μm or greater had a shorter half-life than those of shorter length. The reason for the decrease in fiber count observed during the present experiment may be attributable to phagocytosis by macrophages and dissolution by extracellular fluid (8, 9). Based on an inhalation exposure experiment conducted in the European Union countries, it is considered that fibers longer than 20 μm with a half-life of less than 10 days are unlikely to cause pulmonary fibrosis or carcinogenicity because of their low biopersistence (8, 9). Judging from the characteristics of the present HT wool fibers, our results suggest that HT wool fibers of longer than 20 μm are relatively safe, from the viewpoint that they have a shorter half-life.

The frequency distribution and mean length and width of generated fibers were significantly different from those of fibers in the lungs. It has been reported that many of the fibers that can be inhaled through the rat nose are less than 80 μm in length and less than 1.5 μm in width (18). That is, the difference may indicate the sizes of dust that can be inhaled by rats. After fibers are inhaled in the lungs, both the length and width tended to decrease with time in comparison to the length and width shortly after exposure. After fibers were inhaled into the lungs, the length was about 20% shorter and the width was about 11% smaller in the 4-weeks-after group compared with the shortly-after group. The length may have been shortened by phagocytosis by alveolar macrophages and dissolution by extracellular fluid. Fibers 20 μm or shorter are phagocytosed by alveolar macrophages, while those longer than 20 μm are not completely phagocytosed by macrophages but are dissolved by extracellular fluid, being folded transversely. This mechanism may explain the reduction in the fiber length (8, 9). The reason the percent decrease in fiber length remained at 20% may be that some fibers were not folded but remained as they were. The decrease in the width may have been caused by dissolution of fibers by extracellular fluid (8, 9).

Another report suggested that the reduction in fiber size was caused by a change in chemical composition (19). In a study in which changes in chemical composition of MMVF were observed over a period of a year, fiber sizes were assumed to decrease uniformly from the finding that there was no change in any of the constituents of RW (19). In a study on glass wool, oxides of alkali metals and alkaline-earth metals decreased, and the chemical constituents of fibers dissolved unevenly. It was also reported that fibers folded transversely were ingested by alveolar macrophages, causing the length and width to decrease (19).

In this short-term inhalation study using a nose-only inhalation exposure system, it was suggested that HT wool is a relatively safe substance, as reported in our preceding study. In future studies, it will be necessary to investigate the long-term persistence and pathological effects of fibers in the lungs to further ensure the safety of HT wool.

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