Biopersistence of Rock Wool in Lungs after Short-Term Inhalation in Rats

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To evaluate the safety of rock wool (RW), an asbestos substitute, we examined the biopersistence of RW fibers in rat lungs based on the changes of fiber number and fiber size (length and diameter) by a nose-only inhalation exposure study. Twenty-four male Fischer 344 rats were exposed to RW fibers at a concentration of 30 mg/m³ continuously for 3 h daily for 5 consecutive days. Six rats each were sacrificed shortly and at 1, 2, and 4 wk after exposure, and their lung tissues were ashed by a low-temperature plasma asher. Then the fiber numbers and fiber sizes in lungs were determined using a phase-contrast microscope and computed image analyzer. During the study period, the arithmetic mean (SD) values of fiber and weight concentrations were 78.5 (35.7) fibers/cm³, and 29.9 (28.3) mg/m³, respectively. The fiber number in lungs 4 wk after exposure significantly decreased from the baseline value (shortly after exposure) ($p < .05$). The half-life of fibers calculated from the approximate curve was 28 days for all fibers and 16 days for fibers with $L > 20 \mu m$, and the rate of decrease in fiber number was 46.3% at 4 wk after exposure (shortly-after group = 100%). Likewise, both length and diameter significantly decreased at 4 wk after exposure ($p < .05$), probably because fibers were phagostysed and digested by alveolar macrophages, discharged to outside of the body by mucociliary movement, or dissolved by body fluid. It will be necessary in the future to further confirm the safety of RW fibers by assessing the biopersistence of fibers in the lungs and their pathological effects in our ongoing study performed in accordance with the guidelines established in the “Methods for Determination of Hazardous Properties for Human Health of Man Made Mineral Fibers” (EC protocol).

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Asbestos excels in heat resistance, insulation performance, and durability, and thus has been used for building construction materials such as asbestos cement products and cement boards, reinforcing material for synthetic resin such as vinyl flooring and gear, spray coating material for heat or sound insulation, and heat insulation material for boiler pipes, furnaces, etc. However, it has been reported to cause pulmonary fibrosis, lung cancer, and malignant mesothelioma of the pleura and peritoneum (Doll, 1955; Ministry of the Environment, 1987; Morinaga et al., 1993), 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 (Koshi et al., 1972; Berry, 1981; Gormley et al., 1983). In Japan, the Enforcement Order of the Industrial Safety and Health Law was revised in October 2006 to ban the manufacture, import, transfer, offer, or use of asbestos and products containing asbestos at a level exceeding 0.1%, except for products such as sealing materials that cannot be substituted by other materials. Under these circumstances, the related industries are facing an urgent need to develop a safer fibrous substance as an asbestos substitute.

In the current market, various kinds of man-made vitreous fiber (MMVF) are used as asbestos substitutes. Rock wool (RW), a kind of MMVF, excels in heat resistance, fire resistance, and sound absorption, and is mainly used as fire- and heat-resistant material, heat insulation material, sound absorption material, etc. (Ministry of the Environment, 1987). At present, the International Agency for Research on Cancer (IARC) classifies RW as Group 3: limited evidence in experimental animals for the carcinogenicity, and inadequate evidence in humans for the carcinogenicity (IARC, 2002).

The biodurability (biopersistence or solubility) of MMVF in the lung regulates the safety of fibers. Long fibers are considered to have greater biological activity and pathogenicity than shorter...
ones. The biopersistence of long fibers (length > 20 µm) has been evaluated in many studies in rats, suggesting a correlation between biopersistence of long fibers and their pathogenicity (IARC, 2002). We developed an original nose-only inhalation exposure system and evaluated the biopersistence of Japanese- and European-made RW fibers in the rat lung. The results obtained suggested that the biopersistence of RW fibers was less than that of asbestos (Kudo et al., 2005, 2006). On the other hand, there are many kinds of RW fibers with different chemical compositions and sizes, showing different effects on the human respiratory system. In the present study, we conducted a short-term nose-only inhalation exposure study in rats to examine the biopersistence of RW fibers with different chemical compositions and sizes from the fibers used in our previous studies. We monitored the behavior of fibers in the lungs from the viewpoint of changes both in fiber number and size by length and diameter, to examine the biopersistence of RW fibers in the lungs.

**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 number 2004022).

**Materials**

As an analyte material, we used an RW sample manufactured by NT Co. Ltd. and provided by Rock Wool Association, Japan. Fluorescent x-ray spectroscopy showed that the RW sample used in the study was chemically composed of 40% SiO₂, 37% CaO, 14% Al₂O₃, 6% MgO, 0.9% S, 0.6% MnO, 0.6% TiO₂, and 0.3% Fe₂O₃.

Originally, RW is present in the form of lumps of fibers in different sizes (length and diameter). In general, animal experiments are conducted to evaluate the biological effects of MMVF in order to investigate the highest toxic level of fibers. Because the biological effect of fibers is known to vary depending on the size, the fiber size should be adjusted to obtain the maximum harmful effect. Therefore, we adjusted the size of RW fibers in accordance with the method by Kohyama et al. (1997). A cylinder (6 cm in diameter, 28.3 cm²) was filled with bulk RW, and pressure (160 kg/cm² = 4.5 MPa) was applied to it twice using a manual briquetting press machine (type BRM 32 of Maekawa Testing Machine MFG Co., Ltd.). The geometric mean length (geometric standard deviation, GSD) was 14.28 µm (2.25), and the geometric mean diameter (GSD) was 1.76 µm (2.08) (Figure 1). Then, to make it easier to generate RW fibers in the nose-only inhalation exposure study system, the pulverized RW fibers were mixed with glass beads (BZ-02, AS ONE Corp.) at a ratio of 1 (RW fibers) to 39 (glass beads) in weight.

**Nose-Only Inhalation Exposure System**

The materials prepared according to the procedure already mentioned were treated as follows: Air was supplied from an air compressor to a material generator 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 the materials (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 (Kudo et al., 2005; 2006).

**Exposure Study**

Twelve male Fischer 344 rats (6 to 10 wk old) were used for each experiment, and the experiments were performed twice (24 rats in total). To acclimatize the rats to the environment of the laboratory, they were first housed in cages for about 1 wk with free access to water and food. The temperature was kept at 22°C and humidity at 40% with fresh filtered air continuously being supplied.

The experiment was conducted by exposing the rats to the RW fibers continuously for 3 h/day for 5 consecutive days. The target airborne fiber concentrations was set to 30 mg/m³ in mass concentration and 50 ± 10 fibers/cm³ in fiber concentration. Each day during the experimental period, the rats fixed in the upper rat holders of the main chamber were replaced by the rats in the lower rat holders, rotating the positions among the upper and lower rat holders. During the exposure period, the fiber concentration in the chamber was monitored 5 times a day (30, 60, 90, 120, and 150 min after the start of the exposure experiment). To monitor the airborne fiber concentration in the chamber...
BIOPERSENCENCE OF ROCK WOOL IN RAT LUNGS

nose-only exposure chamber, air sampling was performed using membrane filters (“MF,” pore diameter 0.8 µm and diameter 25 mm, Millipore Corp.), T60A20 filters (“T60A20,” diameter 25 mm, Tokyo Dylec Corp.), and Nuclepore filters (“NF,” pore diameter 0.2 µm and diameter 25 mm, Nomura Micro Science Co.) set in a plastic holder. During a specified period of time, sample fibers were collected on MF for 1 min, on T60A20 for 10 min, and on NF for 5 min using an electric suction pump (GilAir-5: Gilian, USA) at a suction speed of 500 ml/min. The fiber concentration was confirmed by measuring the fiber number concentration (fibers/cm³) and mass concentration (mg/m³), and photomicroscopy was performed with a scanning electron microscope.

Fibers collected on the MF having an aspect ratio (length to diameter ratio) of 3 or higher were measured by phase-contrast microscopy in accordance with the criteria in the “Guidebook for Working Environment Measurement I” (Japan Association for Working Environment Measurement, 2000) as follows:

Criteria for Fiber Measurement
1. Single fiber: A fiber with an aspect ratio of more than 3 was counted as one fiber.
2. Curved single fiber: The whole length of a curved fiber was estimated through the straight line of the fiber, and measured along the curve.
3. Branched fiber: The whole branched fiber, including the branch, was counted as one fiber.
4. Tangled fibers: In cases where some fibers crossed one another, each crossing fiber was counted as one fiber. In cases where the correct number could not be counted because of tangles, the number was not counted.
5. Fiber with a particle: A fiber with a particle >3 µm in diameter was not counted.

Handling of RW Fiber Crossing the Border of the Counter Field
1. In cases where both ends of a fiber were within the counter field, the fiber was counted as one fiber.
2. In cases where only one end of a fiber was within the counter field, the fiber was counted as 1/2 fiber.

To measure the mass concentration (mg/m³) in the exposure chamber, the weight of fibers collected on T60A20 was measured using an electronic balance and compared with the weight before sampling.

Shortly after day 5 of exposure, 6 rats were sacrificed (“shortly-after group”). Six rats each were also sacrificed 1 wk (“1-wk-after group”), 2 wk (“2-wk-after group”), and 4 wk (“4-wk-after group”) after the end of the exposure period. The body weights of the rats were measured once per week, and their appearance and condition were intermittently monitored for any change during and after the exposure period.

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 the temperature of −20°C. Subsequently, the lung tissues were thawed at room temperature, minced, and lyophilized to reduce the weight to a specified level. The weight after lyophilization was regarded as the weight of dried lungs. The lyophilized lungs were ashed in a low-temperature plasma asher (Plasma Asher LTA-102, Yanaco Corp.) for 24 h.

After ashing, distilled water that had been filtered with Minisart (Sartorius K. K.) was added to the weighing bottle to suspend fibers, and the fibers were collected on an MF (pore diameter: 0.22 µm) using a suction filter and allowed to dry. The dried filter was put on a glass slide and exposed to acetone vapor using Quick Fix, making it transparent. At least 200 RW fibers were counted for each rat using a phase-contrast microscope (BX41 Olympus Corp.) in the same way as Hesterberg et al. (1996). Fibers to be counted were those with a ratio of length to diameter (aspect ratio) of 3 or larger. WinRoof (image analysis software, Mitani Corp.) was used to obtain the fiber number, distinguishing the length (L) in L < 5 µm, 5 < L ≤ 20 µm, and L > 20 µm. Among the fibers counted, WHO fibers (5 µm or longer in length and shorter than 3 µm in diameter with an aspect ratio of 3 or higher) were also counted (WHO, 1984). Then the fiber number obtained was converted to the number per weight of dried lung. The half-life of fibers in the rat lungs was calculated assuming that the geometric mean of the total fiber number/the total lung weight (fibers/mg) in the lungs of the shortly-after group was 100% (Hesterberg et al., 2001).

Measurement of Fiber Sizes (Length and Diameter)
To measure the sizes (length and diameter) of fibers 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 at ×400 magnification. At least 200 fibers of 0.36 µm or longer in length were counted for each rat.

Statistical Analysis
The geometric mean and standard deviation of the total fiber number, length, and diameter were calculated. One-way analysis of variance and multiple comparisons by Scheffé’s test were performed.

RESULTS
Monitoring of the Fiber Concentration in the Exposure Chamber
Table 1 shows the fiber concentrations in the exposure chamber measured five times daily during the experiment period. This table also includes the results of preceding studies (Bernstein et al., 1996; Hesterberg et al., 1998). The arithmetic mean (standard deviation) of the total fiber number was 240.5 (62.9) fibers/cm³, while that of the number of fibers with L > 20 µm was 73.0
that the value shortly after exposure was 100%. The mean of the total fiber number of all dried lungs tended to decrease, as in the preceding studies, from shortly after exposure to 4 wk after exposure. Although the rates of decrease in the number of fibers with \( L \leq 5 \, \mu m \), \( 5 < L \leq 20 \, \mu m \), or in the number of WHO fibers were low at a certain point, the number of fibers in the 4-wk-after group was smaller than that in the shortly-after group (100%). At the same time, fibers with \( L > 20 \, \mu m \) tended to decrease relatively fast during the period from shortly after exposure to 4 wk after exposure (Table 3 and Figure 3). Multiple comparison by Scheffe’s test showed that the total fiber number, fibers \( 5 < L \leq 20 \, \mu m \), \( L > 20 \, \mu m \), and WHO fibers in the 4-wk-after group significantly decreased from those in the shortly-after group (\( p < .05 \)) (Table 3 and Figure 3).

Half-Life of Fibers

On the assumption that the total fiber number divided by the weight of all lungs (fiber/mg) shortly after exposure was 100%, the half-life of fibers calculated from the approximation curve (Figure 4) was 28 days for the total fiber number, 47 days for \( L \leq 5 \, \mu m \), 29 days for \( 5 < L \leq 20 \, \mu m \), 16 days for \( L > 20 \, \mu m \), and 27 days for WHO fibers. The half-life of fibers with \( L > 20 \, \mu m \) tended to be shorter than that of fibers with \( L \leq 20 \, \mu m \). The half-life of fibers with \( L > 20 \, \mu m \) in the preceding studies (Hesterberg et al., 1996, 1998) was 53 and 67 days, respectively.

Distribution of and Changes of Fiber Size (Length and Diameter)

Figures 5 and 6 show the frequency distribution (histogram) of the length and diameter of fibers retaining in lungs in the shortly-after and 1-, 2-, and 4-wk-after groups. Table 4 shows the change in geometric mean of the length and diameter of fibers in lungs (GSD). Table 4 also includes the results of the preceding study by Bernstein et al. in 1996.

With regard to the length, the frequency distribution changed with time as shown in Figure 5, and the number of fibers with \( L \leq 5 \, \mu m \) increased on and after wk 1 of exposure. As shown in Table 4, the mean length was 7.51 \( \mu m \) in the shortly-after group, but it decreased significantly with time (2- and 4-wk-after groups) to 6.35 \( \mu m \) in the 4-wk-after group (\( p < .05 \)). It also decreased significantly in the 4-wk-after group in comparison to the 2-wk-after group (\( p < .05 \)).

**FIG. 2.** (a) Distribution of length of fibers generated. (b) Distribution of diameter of fibers generated.
TABLE 2
Total fiber number for fiber sizes in exposure chamber (µm)

<table>
<thead>
<tr>
<th>Group</th>
<th>Length (µm)</th>
<th>Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.39 (0.98)</td>
<td>11.9 (2.4)</td>
</tr>
<tr>
<td></td>
<td>3.76 (1.21)</td>
<td>15.0 (2.3)</td>
</tr>
<tr>
<td>L ≤ 5</td>
<td></td>
<td>0.86 (1.72)</td>
</tr>
<tr>
<td></td>
<td>1.67 (1.59)</td>
<td>0.69 (0.61)</td>
</tr>
<tr>
<td>5 &lt; L ≤ 20</td>
<td>11.01 (1.45)</td>
<td>1.66 (1.54)</td>
</tr>
<tr>
<td>20 &lt; L</td>
<td>29.94 (1.36)</td>
<td>2.05 (1.51)</td>
</tr>
<tr>
<td>WHO fiber</td>
<td>14.51 (1.80)</td>
<td>1.63 (1.46)</td>
</tr>
</tbody>
</table>

Note. Geometric mean (geometric standard deviation).

aReported by Hesterberg et al. (1998).
bReported by Bernstein et al. (1996).

With regard to the diameter, the frequency distribution changed with time as shown in Figure 6. The number of fibers 1 µm or shorter increased significantly on and after wk 1 of exposure in comparison to the shortly-after group. As shown in Table 4, the mean diameter was 0.85 µm in the shortly-after group, but it decreased significantly with time (1-, 2-, and 4-wk-after groups) to 0.74 µm in the 4-wk-after group (p < .05).

DISCUSSION
It is said that fibers with L > 20 µm, having a long half-life, tend to cause fibrosis or cancers because of their low dissolution in the living body (IARC, 2002).

TABLE 3
Fiber numbers in lungs and their proportions

<table>
<thead>
<tr>
<th>Sacrificed rat group</th>
<th>Total fibers</th>
<th>L ≤ 5 µm</th>
<th>5 &lt; L ≤ 20 µm</th>
<th>20 &lt; L µm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean (GSD)</td>
<td>%</td>
<td>Geometric mean (GSD)</td>
<td>%</td>
</tr>
<tr>
<td>Shortly-after group</td>
<td>13.25 (1.19)</td>
<td>119*</td>
<td>100.0</td>
<td>100*</td>
</tr>
<tr>
<td>1-wk-after group</td>
<td>11.63 (1.29)</td>
<td>105*</td>
<td>87.7</td>
<td>88*</td>
</tr>
<tr>
<td>2-wk-after group</td>
<td>10.77 (1.03)</td>
<td>74*</td>
<td>81.3</td>
<td>62.2*</td>
</tr>
<tr>
<td>4-wk-after group</td>
<td>6.14 (1.52)</td>
<td>54*</td>
<td>46.3</td>
<td>45.8*</td>
</tr>
</tbody>
</table>

5 < L ≤ 20 µm

<table>
<thead>
<tr>
<th>Sacrificed rat group</th>
<th>Geometric mean (GSD)</th>
<th>%</th>
<th>Geometric mean (GSD)</th>
<th>%</th>
<th>Geometric mean (GSD)</th>
<th>%</th>
<th>Geometric mean (GSD)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortly-after group</td>
<td>7.47 (1.19)</td>
<td>66 (10)*</td>
<td>100.0</td>
<td>100*</td>
<td>1.45 (1.15)</td>
<td>11 (1.5)*</td>
<td>100.0</td>
<td>100*</td>
</tr>
<tr>
<td>1-wk-after group</td>
<td>6.41 (1.31)</td>
<td>56 (13)*</td>
<td>85.8</td>
<td>104*</td>
<td>1.19 (1.37)</td>
<td>12 (4.4)*</td>
<td>81.5</td>
<td>107*</td>
</tr>
<tr>
<td>2-wk-after group</td>
<td>5.91 (1.12)</td>
<td>50 (7)*</td>
<td>79.2</td>
<td>75*</td>
<td>0.86 (1.19)</td>
<td>9 (2.4)*</td>
<td>59.4</td>
<td>82*</td>
</tr>
<tr>
<td>4-wk-after group</td>
<td>3.65 (1.26)</td>
<td>35 (6)*</td>
<td>48.8</td>
<td>54*</td>
<td>0.42 (1.65)</td>
<td>6 (1.1)*</td>
<td>29.0</td>
<td>49*</td>
</tr>
</tbody>
</table>

WHO fibers

<table>
<thead>
<tr>
<th>Sacrificed rat group</th>
<th>Geometric mean (GSD)</th>
<th>%</th>
<th>Geometric mean (GSD)</th>
<th>%</th>
<th>Geometric mean (GSD)</th>
<th>%</th>
<th>Geometric mean (GSD)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortly-after group</td>
<td>8.93 (1.19)</td>
<td>77 (10)*</td>
<td>100.0</td>
<td>100*</td>
<td>1.45 (1.15)</td>
<td>11 (1.5)*</td>
<td>100.0</td>
<td>100*</td>
</tr>
<tr>
<td>1-wk-after group</td>
<td>7.60 (1.31)</td>
<td>68 (16)*</td>
<td>85.2</td>
<td>88*</td>
<td>1.19 (1.37)</td>
<td>12 (4.4)*</td>
<td>81.5</td>
<td>107*</td>
</tr>
<tr>
<td>2-wk-after group</td>
<td>6.80 (1.10)</td>
<td>59 (9)*</td>
<td>76.2</td>
<td>76*</td>
<td>0.86 (1.19)</td>
<td>9 (2.4)*</td>
<td>59.4</td>
<td>82*</td>
</tr>
<tr>
<td>4-wk-after group</td>
<td>4.10 (1.27)</td>
<td>41 (6)*</td>
<td>45.9</td>
<td>53*</td>
<td>0.42 (1.65)</td>
<td>6 (1.1)*</td>
<td>29.0</td>
<td>49*</td>
</tr>
</tbody>
</table>

Note. Geometric mean: ×10⁵/lung. WHO fiber: 5 µm or longer in length and shorter than 3 µm in diameter. GSD: Geometric standard deviation. %: Percentage when value in the shortly-after group is assumed to be 100%. n = 6. L, length of fiber (µm). Asterisk, reported by Hesterberg et al. (1998): Percentage when value on day 1 (1 day after the end of exposure) is assumed to be 100%.

aComparison with the shortly-after group (p < .05).
bComparison with the 1-wk-after group (p < .05).
cComparison with the 2-wk-after group (p < .05).
FIG. 3. Percentages of fibers in lungs: ■ Shortly-after group; ⬇️ 1-wk-after group; ⬆️ 2-wk-after group; □ 4-wk-after group. Percentage when the value of the shortly-after group is assumed to be 100%. n = 6. L, length of fiber (µm).

FIG. 4. Clearance of RW fibers from rat lungs. (%): Calculated assuming that the value of the shortly-after group is 100%.
FIG. 5. Distribution of the length of fibers in lungs: (a) shortly-after group; (b) 1-wk-after group; (c) 2-wk-after group; (d) 4-wk-after group. The horizontal axis indicates the maximum value of each category.

The nose-only inhalation exposure study system used in this study had two major advantages over traditional ones: (1) mixture of the test fiber powder and glass beads and (2) adoption of a subchamber. Mixture of the test fiber powder and glass beads reduces the cohesive property of fibers, allowing fibers to be generated constantly. In addition, use of a subchamber makes it possible to send fibers generated to the exposure chamber at a constant concentration. This method allows the test fibers to be generated constantly at relatively high concentrations for a specified period of time. During the present study, animals were exposed to fibers for 3 h daily. This exposure time was selected in order to confirm the usefulness of this exposure system, which was newly developed for the present study, by first determining whether this system was able to generate fibers constantly for 3 h.
The present study produced fairly satisfactory results, judging from the fact that fibers were generated nearly at the target fiber concentration.

The materials mixed with glass beads were fluidized by air sent from the air compressor, being separated from the glass beads. As a result, only the materials were emitted into the air, and therefore rats did not inhale glass beads. Consequently, it was considered that the glass beads had no effect on the biopersistence of RW fibers in the lungs in the present study.

The total fiber number and fiber number by length tended to decrease during the period from shortly after exposure to wk 4. In preceding studies, fibers of all sizes decreased by 30 to 50% during 30 days after exposure (Hesterberg et al., 1996, 1998). Fibers that are inhaled and precipitate in the lungs show different mechanisms of clearance depending on the site of precipitation. Fibers deposited in the bronchioles are transferred to the pharynx by mucociliary movements and discharged from the body (Hesterberg et al., 2001; IARC, 2002). Of fibers deposited in the alveoli, those with a length shorter than 20 \( \mu m \) are phagocytosed by alveolar macrophages and digested (Hesterberg et al., 2001; IARC, 2002). Those with a length longer than 20 \( \mu m \) cannot be completely phagocytosed by alveolar macrophages. These are either (a) dissolved by body fluid, allowing the fibers to be broken and crushed to shorten in length, and subsequently phagocytosed by alveolar macrophages, or (b) taken into pulmonary epithelial cells and transferred to lymphatic vessels (Hesterberg et al., 2001; IARC, 2002). The fiber number is believed to be decreased by these mechanisms. Moreover, the rate of decrease in the number of fibers with a length shorter than 20 \( \mu m \) slowed in the 1- and 2-wk-after groups. A possible reason for this is that fibers longer than 20 \( \mu m \) were broken, increasing the number of shorter fibers (Hesterberg et al., 2001; IARC, 2002).

Hesterberg et al. (1998) assessed the persistence of fibers in the lungs by exposing animals to fibers for 6 h daily and counting at least 400 WHO fibers, and reported that the half-life was 67 days for fibers with \( L > 20 \mu m \) when the number of fibers counted on day 1 after exposure is assumed to be 100%. On the other hand, the half-life was 16 days for fibers with \( L > 20 \mu m \) in the present study, where animals were exposed to fibers for 3 h daily, and at least 200 fibers per rat were counted in accordance with the criteria for fiber measurement (Guidebook for Working Environment Measurement I) and with the conditions described in the preceding study (Hesterberg et al., 1996). However, the results of this study cannot be directly compared with those of the preceding studies because the concentration of fibers with \( L > 20 \mu m \), the method of calculating the half-life of RW, and the exposure time were different from those in the preceding studies. As for the measurement time point, it has been reported that 30% of inhaled fibers with \( L > 20 \mu m \) are cleared from the body within 24 h after inhalation (Bernstein et al., 1994). Therefore, the results obtained from the present study should be evaluated carefully.

On the other hand, the dissolution rate of RW fibers in the present study, calculated using Eastes’s formula (Eastes et al., 2000), was 2451 ng/cm\(^3\)/h, showing a much higher rate than that of RW fibers used in the study by Hesterberg et al. (1996) (25 ng/cm\(^3\)/h). Thus, RW fibers used in the present study were shown to be dissolved easier and cleared faster from rat lungs than RW fibers used in the study by Hesterberg et al. (1996).

After fibers are inhaled into the lungs, both the length and diameter tended to decrease with time in comparison to those measured shortly after exposure. Bernstein et al. reported in 1996 that there were no differences between the length or diameter measured shortly after exposure and those measured 4 wk after exposure, while both the mean length and diameter decreased in the present study. The reason for this seems to be that fibers with \( L \leq 20 \mu m \) were phagocytosed by alveolar macrophages and fibers with \( L > 20 \mu m \) were deposited in the trachea and discharged out of the body by mucociliary movement, as described earlier (Hesterberg et al., 2001).

We are now performing a 6-h inhalation exposure study on the basis of the number of fibers counted on day 1 after exposure, in order to compare the results with those of preceding studies in accordance with the guidelines established in the “Methods for Determination of the Hazardous Properties for Human Health of Man Made Mineral Fibers” (EC protocol) (Bernstein et al., 1999), which specifies that the concentration of fibers with \( L >

### TABLE 4

<table>
<thead>
<tr>
<th>Sacrificed rat group</th>
<th>Length</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean (GSD)</td>
<td>Geometric mean (GSD)</td>
</tr>
<tr>
<td>Shortly-after group</td>
<td>7.51 (2.05)</td>
<td>7.3 (2.0)*</td>
</tr>
<tr>
<td>1-wk-after group</td>
<td>7.16 (2.01)</td>
<td>—</td>
</tr>
<tr>
<td>2-wk-after group</td>
<td>6.80 (2.00)*</td>
<td>—</td>
</tr>
<tr>
<td>4-wk-after group</td>
<td>6.35 (1.93)*a,b</td>
<td>7.8 (2.0)*</td>
</tr>
</tbody>
</table>

*Note. GSD: Geometric standard deviation (\( \mu m \)). n = 6. Asterisk: Reported by Bernstein et al. (1996).

aComparison with the shortly-after group (\( p < .05 \)).

bComparison with the 1-wk-after group (\( p < .05 \)).
20 µm should be at least 100 fibers/cm³. It will be necessary in the future to further confirm the safety of RW fibers by assessing the biopersistence of fibers in the lungs and their pathological effects in our ongoing study performed in accordance with the EC protocol.

REFERENCES

Japan Environmental Sanitation Center. 1987. All about asbestos and zeolite, ed. Planning Division, Air Quality Bureau, Ministry of the Environment, Kawasaki.