Behavior of Rock Wool in Rat Lungs after Exposure by Nasal Inhalation

Yuichiro Kudo¹, Norihiko Kohyama², Toshihiko Sato¹, Yoshihito Konishi³ and Yoshiharu Aizawa¹

¹Department of Preventive Medicine and Public Health, Kitasato University School of Medicine, ²Faculty of Economy, Toyo University and ³Japan Association for Working Environment Measurement, Japan

Abstract: Behavior of Rock Wool in Rat Lungs after Exposure by Nasal Inhalation: Yuichiro Kudo, et al. Department of Preventive Medicine and Public Health, Kitasato University School of Medicine—To evaluate the safety of rock wool (RW) fibers, we examined the biopersistence of RW fibers in the lungs of rats, based on the changes of fiber number and fiber size in the length and width, in a nose-only inhalation exposure study. Twenty male Fischer 344 rats (6 to 10 wk old) were exposed to RW fibers at a fiber concentration of 70.6 (20.4) fiber/m³ and a dispersion density of 30.4 (6.6) mg/m³ [arithmetic mean (SD)] continuously for 3 h daily for 5 consecutive days. Five rats each were sacrificed shortly after exposure ended (baseline group) and at 1, 2, and 4 wk after exposure, and their lung tissues were ashed by a low temperature plasma-asher. The numbers and sizes of fibers in the ash samples were determined using a phase contrast microscope and a computed image analyzer. The fiber numbers in the lungs at 4 wk after exposure had significantly decreased from the baseline value, i.e. shortly after exposure (p<0.05). The half-lives of RW fibers calculated using the one-compartment model were 32 d for total fibers and 10 d for fibers longer than 20 µm in length. Fiber number was 53.6% of the baseline at 4 wk after exposure (baseline group=100%). Likewise, fiber sizes had significantly decreased at 4 wk after exposure (p<0.05), probably because fibers had been dissolved in body fluid, phagocytosed by alveolar macrophages or discharged from the body by mucociliary movement. In future studies, it will be necessary to examine the carcinogenicity of RW fibers through long-term inhalation studies. (J Occup Health 2006; 48: 437–445)

Key words: Rock wool, Inhalation, Nose-only, Clearance, Biopersistence, Carcinogenicity

Asbestos excels in heat resistance, insulation performance and durability, and thus has been used in building construction materials such as asbestos cement products and cement boards, reinforcing materials for synthetic resins such as vinyl flooring, spray coating materials for heat or sound insulation, and heat insulation materials for boiler pipes, furnaces, etc. However, asbestos has been reported to cause pulmonary fibrosis, lung cancer, and malignant mesothelioma of the pleura and peritoneum¹–³, and its toxicity has been proved through many in vitro and in vivo experiments. Therefore, the use of asbestos has been banned or restricted all over the world⁴–⁶. In Japan, the Enforcement Order of the Industrial Safety and Health Law, Ordinance on Industrial Safety and Health and Ordinance on Prevention of Hazards due to Specified Chemical Substances were revised in 1995 to ban the manufacture, import, use and sale of amosite and crocidolite, and products containing either of them at a level exceeding 1%. In addition, the manufacture, import, use and sale of chrysotile and products containing chrysotile at a level exceeding 1% have been banned since October 2004. 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 fibers (MMVF) have been used as asbestos substitutes. Rock wool (RW), a kind of MMVF, is made from molten soft slag such as iron slag, copper slag, nickel slag, etc. and natural stone, such as andesite, basalt, amphibolite etc. Because RW excels in heat resistance, fire resistance, and sound absorption, it is mainly used as a fire- and heat-resistant material, heat insulation material, and sound absorption material⁷. In a previous in vivo experiment using RW, pulmonary fibrosis was observed...
in rats, but the development of lung tumors was not reported\(^8\). Also, in a previous in vitro experiment, β-glucuronidase and lactic acid dehydrogenase were reported to be released from macrophages stimulated with RW\(^9\), although their levels were lower than those reported for chrysotile. Giant cell formation of cultured cells was also reported in an experiment with RW\(^10\). Based on these studies, 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 of carcinogenicity\(^11\).

The respiratory system suffers the greatest effect in the inhalation of asbestos or MMVF such as RW. To evaluate the biological effects of MMVF many in vivo experiments have been performed, including short-term and long-term inhalation exposure studies, and intrathoracic, intraperitoneal and intratracheal injection studies. Reports by the IARC\(^11\) have demonstrated that inhalation exposure studies are the most suitable method of evaluating the effects of MMVF on human beings.

In the present study, we conducted a short-term, nose-only inhalation exposure study with rats to examine the persistence of RW fibers in the lungs as an index of the effect of RW on the respiratory system, using RW samples currently available on the market. We further monitored the behavior of fibers in the lungs from the viewpoint of changes both in fiber number and in fiber size by length and width, 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 No. 20040422).

Materials

As analyte material, we used a RW sample manufactured by NC Co. Ltd., Japan, provided by the Rock Wool Association, Japan. Fluorescence X-ray spectroscopy showed the RW sample was chemically composed of 39% SiO\(_2\), 33% CaO, 14% Al\(_2\)O\(_3\), 5% MgO, 1.8% Fe\(_2\)O\(_3\), and 0.6% S.

On manufacture, RW is produced in the form of lumps of fibers of different sizes (length and width). 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 by the method of Kohyama \textit{et al.}\(^\text{12}\). That is, a cylinder (6 cm in diameter: 28.3 cm\(^2\)) was filled with bulk RW and a pressure of 160 kg/cm\(^2\) (4.5 MPa) was applied twice using a manual press machine (Type BRM 32, Maekawa Testing Machine MFG Co.). RW fibers thus obtained were dispersed in an exposure chamber, and the fiber sizes were measured. Their geometric mean length (geometric standard deviation, GSD) and geometric mean width (GSD) were 15.49 µm (2.02) and 2.44 µm (1.59), respectively (Fig. 1). Then, to make it easier to generate RW in the nose-only inhalation exposure system, the pulverized RW fibers were mixed with glass beads (BZ-02, AS ONE Corp.) at a ratio of 1 (RW) to 39 (glass beads) in weight.

Nose-only inhalation exposure system

The materials prepared according to the procedure described above were treated as follows. Air was supplied from an air compressor to a material generator at a rate of 30 L/min, and the RW with glass beads were placed in the material storage tank of the material generator. The RW mixed with glass beads were fluidized by air from the air compressor, and separated from the glass beads. The resulting RW air-suspension was sent to a subchamber, diluted and homogenized to a specific 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 RW (10,000 cpm) in the exposure chamber, the concentration was monitored using a digital dust meter, and the amount of RW suspension to be generated was adjusted by applying feedback to the feeder. The rat holders were placed in the exposure chamber\(^11\).

Exposure study

Ten male Fischer 344 rats (6 to 10 weeks old) were used for each experiment, and the experiments were performed twice (20 rats in total). To acclimatize the rats to the environment of the laboratory, they were first housed in cages for one week with free access to water and food. The temperature was kept at 22°C and humidity
The experiment was conducted by exposing the rats to RW fibers continuously for 3 h a 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 exposure). To monitor the airborne fiber concentration in the nose-only exposure chamber, air sampling was performed using membrane filters (MF: pore diameter, 0.22 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. At specified times, 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 MF having an aspect ratio (length to width ratio) of 3 or higher were measured by phase contrast microscopy in accordance with the criteria in the Guidebook for Working Environment Measurement. To monitor the airborne fiber concentration 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 the end of exposure on the 5th day, 5 rats were sacrificed (“SA group”). Five rats each were also sacrificed one week (“1W group”), 2 wk (“2W group”), and 4 wk (“4W group”) after the end of the exposure period. The body weights of the rats were measured once a 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 exsanguination from the abdominal aorta and their lungs were resected. The resected lungs were stored in a weighing bottle at a temperature of −20°C. Subsequently, the lung tissues were thawed at room temperature, minced, and lyophilized to reduce their weight to a specific level. The weight after lyophilization was regarded as the dry weight of the lungs. The lyophilized lungs, dry weight of approx. 17 g, 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 the RW fibers, and the fibers were counted on an 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 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.). Fibers to be counted were those with an aspect ratio (length : width) of 3 or higher. Win Roof (image analysis software, Mitani Corp.) was used to obtain the number of fibers, and to categorize the length (L) into L≤5, 5<L≤20, and L>20. 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. Then, the fiber numbers obtained were converted to the fiber 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 SA group was 100%. Measurement of fiber sizes

To measure the sizes of fibers (length and width) in the air and in the lungs, fibers within the measurable range and with an aspect ratio of 3 or higher were measured using a phase contrast microscope at x 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 geometric standard deviation of the total fiber number and the distribution of the length and width of fibers were estimated. Moreover, to estimate the length and width distribution, a minimum of 200 fibers, which had entered the rats’ lungs, were counted for each rat in the two experiments. The geometric mean for a group of five rats was then calculated. One-way analysis of variance and multiple comparisons by Scheffe’s test were performed.

Results

Monitoring of fiber concentration in the exposure chamber

Table 1 presents the time course of fiber concentrations in the exposure chamber in each experiment. The mean values (SD) of the count obtained by the digital dust meter for the 1st and 2nd experiments (5 d each) were 9,359 (310) and 10,056 (956) counts/min. The mean fiber concentrations (SD) in the exposure chamber were 77.4 (310) and 10,056 (956) counts/min. The mean fiber concentrations (SD) in the exposure chamber were 77.4 (310) and 10,056 (956) counts/min. The mean concentration of fiber in the chamber was monitored 5 times a day (30, 60, 90, 120, and 150 min after the start of exposure).
Table 1. Fiber concentrations in the exposure chamber

<table>
<thead>
<tr>
<th></th>
<th>1st Experiment</th>
<th></th>
<th>2nd Experiment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Digital dust meter</td>
<td>Fiber concentration</td>
<td>Dispersion density</td>
<td>Digital dust meter</td>
</tr>
<tr>
<td></td>
<td>(counts/min)</td>
<td>(fibers/cm³)</td>
<td>(mg/m³)</td>
<td>(counts/min)</td>
</tr>
<tr>
<td>Day 1 (n=5)</td>
<td>9861 (274)</td>
<td>81.0 (19.5)</td>
<td>30.0 (6.2)</td>
<td>Day 1 (n=5)</td>
</tr>
<tr>
<td>Day 2 (n=5)</td>
<td>9237 (197)</td>
<td>72.8 (5.0)</td>
<td>27.0 (7.1)</td>
<td>Day 2 (n=5)</td>
</tr>
<tr>
<td>Day 3 (n=5)</td>
<td>9247 (97)</td>
<td>81.3 (4.9)</td>
<td>33.2 (7.8)</td>
<td>Day 3 (n=5)</td>
</tr>
<tr>
<td>Day 4 (n=5)</td>
<td>9313 (154)</td>
<td>65.0 (26.2)</td>
<td>29.2 (2.3)</td>
<td>Day 4 (n=5)</td>
</tr>
<tr>
<td>Day 5 (n=5)</td>
<td>9137 (81)</td>
<td>86.8 (12.0)</td>
<td>30.4 (4.3)</td>
<td>Day 5 (n=5)</td>
</tr>
<tr>
<td>Mean (n=25)</td>
<td>9359 (310)</td>
<td>77.4 (17.4)</td>
<td>30.0 (5.8)</td>
<td>Mean (n=25)</td>
</tr>
</tbody>
</table>

Arithmetic mean (SD).

Fig. 2a. Distribution of length of fibers generated inside the chamber.
Fig. 2b. Distribution of width of fibers generated inside the chamber.

(7.4) mg/m³, respectively, with a mean value of 30.4 (6.6) mg/m³. Figure 2 shows the distributions of fibers’ lengths and widths dispersed inside the exposure chamber, in which the geometric mean (GSD) of the length was 15.49 µm (2.02) and that of the width was 2.44 µm (1.59).

Deposition rate of fibers in lungs

The total number of RW fibers inhaled by rats during
Table 2. Fiber numbers in lungs and their proportions

<table>
<thead>
<tr>
<th>Sacrificed rat group</th>
<th>Total fibers or equal to 5 μm (L≤5)</th>
<th>Fibers longer than 5 μm and shorter than or equal to 20 μm (5&lt;L≤20)</th>
<th>Fibers longer than 20 μm (L&gt;20)</th>
<th>WHO fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean (GSD)</td>
<td>%</td>
<td>Geometric mean (GSD)</td>
<td>%</td>
</tr>
<tr>
<td>SA group</td>
<td>9.43 (1.13)</td>
<td>100.0</td>
<td>2.12 (1.24)</td>
<td>100.0</td>
</tr>
<tr>
<td>1W group</td>
<td>7.42 (1.35)</td>
<td>78.7</td>
<td>2.04 (1.50)</td>
<td>96.3</td>
</tr>
<tr>
<td>2W group</td>
<td>7.68 (1.17)</td>
<td>81.5</td>
<td>2.12 (1.16)</td>
<td>100.3</td>
</tr>
<tr>
<td>4W group</td>
<td>5.05 (1.23)</td>
<td>53.6</td>
<td>1.59 (1.48)</td>
<td>74.9</td>
</tr>
</tbody>
</table>

a: Comparison with SA group (p<0.05), b: Comparison with 2W group (p<0.05), Geometric mean: × 10^5/lung, WHO fiber: Length > 5 μm and Width < 3 μm, GSD: Geometric standard deviation, %: Percentage assuming the value of the SA group to be 100%, n=5, L: Length of fiber (μm).

Fig. 3. Percentages of fibers in lungs.

The experimental period was calculated by the following equation:

\[
\text{Total number of RW fibers inhaled} = \text{Fiber concentration} \times \text{Respiratory volume} \times \text{Duration of exposure}
\]

The respiratory volume of the rats was estimated by the following equation:

\[
\text{Respiratory volume (mL/min)} = 2.1 \times (\text{Body weight [g]})^{0.75}
\]

Since the mean body weight of the rats was 131 (g), the respiratory volume was estimated as follows:

\[
2.1 \times (131 \text{ [g]})^{0.75} = 81.32 \text{ mL/min}
\]

Average RW fiber concentration in the exposure chamber was 70.6 fibers/cm³, as described earlier. Since the rats were exposed for 3 h daily for 5 consecutive days, the total number of RW fibers inhaled was estimated as follows:

\[
\text{Total number of RW fibers inhaled} = \text{70.6 fibers/cm}^3 \times 81.32 \text{ mL/min} \times 3 \times 60 \text{ min} \times 5 = 51.67 \times 10^5 \text{ fibers}
\]

Since the total fiber number inside the lungs corresponding to this number was found to be 7.09 × 10^5.
fibers shortly after exposure, the deposition rate of fibers in the lungs was estimated as follows:

\[
7.09 \times 10^5 / 51.67 \times 10^5 = 0.137
\]

Thus, the deposition rate in the lungs was 13.7%.

Changes in length and width of fibers in lungs

Table 2 and Fig. 3 show the numbers of RW fibers accumulated in the lungs and their proportions on the assumption that the value shortly after exposure was 100%. The mean of the total fiber number in dried lungs tended to decrease during the period from shortly after exposure to 4 weeks after exposure. Although the decrease rates of the number of fibers \( L \leq 5 \), \( 5 < L \leq 20 \), or in the number of WHO fibers were low at a certain point, the number of fibers in the 4W group was smaller than that in the SA group (100%). Also, the number of fibers \( L > 20 \) tended to decrease relatively quickly during the period from shortly after exposure to 4 wk after exposure. Multiple comparison by Scheffe’s test showed that the total fiber numbers, fibers \( 5 < L \leq 20 \), \( L > 20 \), and WHO fibers in the 4W group were significantly lower than those in the SA group (p<0.05).

Half-life of fibers

Data obtained by plotting the number of fibers in the rat lungs against the measurement time points on a logarithm axis showed linear decreases (Fig. 4), therefore, the half-life was calculated using the one-compartment model. The half-lives based on this calculation were 32 d for the total fiber number, 86 d for \( L \leq 5 \), 31 d for \( 5 < L \leq 20 \), and 27 d for WHO fibers. The half-life of fibers \( L > 20 \) tended to be shorter than that of fibers \( L \leq 20 \).

Distribution and changes of fiber size

Table 3 lists the changes in the length and width of the fibers in the lungs in the SA, 1W, 2W and 4W groups expressed by the geometric mean (GSD). The average length and width decreased significantly in the 1W, 2W

Table 3. Changes in length and width of fibers in lungs

<table>
<thead>
<tr>
<th>Sacrificed rat group</th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean (GSD)</td>
<td>Geometric mean (GSD)</td>
</tr>
<tr>
<td>SA group</td>
<td>8.58 (1.94)</td>
<td>1.26 (1.43)</td>
</tr>
<tr>
<td>1W group</td>
<td>7.53 (1.87)a</td>
<td>1.18 (1.39)a</td>
</tr>
<tr>
<td>2W group</td>
<td>7.35 (1.80)a</td>
<td>1.17 (1.37)a</td>
</tr>
<tr>
<td>4W group</td>
<td>6.87 (1.75)a,b</td>
<td>1.14 (1.32)a</td>
</tr>
</tbody>
</table>

Geometric mean (GSD: Geometric standard deviation) (µm), a: Comparison with SA group (p<0.05), b: Comparison with 1W group (p<0.05), n=5.

Fig. 4. Clearance of RW fibers from rat lungs. (%): Calculated assuming that the value of the SA group is 100%.
and 4W groups as compared to the SA group ($p<0.05$).

**Discussion**

The fiber size and biopersistence of asbestos or MMVF have been shown in many previous epidemiological, physicochemical and animal studies to be important factors in their adverse health effects, especially carcinogenicity. With regard to inhaled fibers, which were 5 $\mu$m or longer in length and 3 $\mu$m or shorter in width, these previous studies reported that the thinner and longer the fiber is, the more carcinogenic it becomes. In addition, fibers that remain in the lung tissues for a long period of time without being dissolved or discharged from the body are considered to be more carcinogenic$^{15}$. It is said that fibers with a length of 20 $\mu$m or longer having a long half-life tend to cause fibrosis or cancer because of their low dissolution in the living body$^{11,15}$. Biopersistence is related to the quantity of fibers that remain inside the lungs, the so-called fiber retention quantity. Fiber retention quantity in the lungs is the number of fibers that have entered and been deposited in the lungs minus the number of fibers cleared by the lungs’ self-cleaning function. It indicates the amount of fibers present inside the lungs as a result of exposure. Fiber retention quantity in the lungs is based on the deposition-clearance balance: if the number of fibers deposited in the lungs is too large to be successfully cleared, or if the mechanism of clearance fails to work properly, fiber retention quantity in the lungs increases, thus inducing pulmonary fibrosis or cancer$^{15}$.

As mentioned earlier, many studies have been conducted to examine the effect of fibrous substances on the living body. In the present study, we observed the behavior of RW fibers in the lungs in a short-term, nose-only inhalation exposure experiment. Inhalation exposure experiments provide a condition that is closer to the actual exposure route in humans than other methods such as intratracheal or intrathoracic/intraperitoneal injections. When fibers are injected into the lungs, fibrous substances may be deposited unevenly, while in inhalation exposure experiments, deposition is characterized by uniform, non-localized distribution in the lungs$^{15}$. Inhalation studies involve chronic inhalation (exposure for more than a year), subchronic inhalation (exposure for about 3 months), and short-term inhalation (exposure for about 5 d). Short-term inhalation exposure studies are conducted to examine the biopersistence of substances in the lungs. They are often conducted as an acute inhalation toxicity study as a first step of a toxicity study. Chronic inhalation studies on pathologic effects such as carcinogenicity and fibrosis usually require a long period of time (a few years) and enormous cost. Recently, it has become clear that the biopersistence of MMVF correlates closely with the hazard to the lungs, and it has been reported that the greater the biopersistence is, the more likely carcinogenesis or fibrosis in the lungs$^{11}$. That is, biopersistence is an important factor for the biological effects of fibrous substances, and therefore, it has been adopted more often as an index of the hazard of MMVF in short-term inhalation exposure studies$^{15}$. 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$^{15}$. Inhalation exposure experiments are classified as systemic exposure and nose-only exposure. In comparison with a systemic exposure, a nose-only exposure requires smaller experimental apparatus and is less likely to be associated with individual differences in the deposition of inhaled substances in the lungs$^{17}$. Thus, it is more suitable for short-term exposure and observation of behavior in the lungs, with less adhesion of test substances to the skin.

The nose-only inhalation exposure system used in this experiment is an improved version of the traditional type in which a subchamber was set up just before the exposure chamber. There are two advantages in this approach. The first is that the subchamber can control the concentration of generated RW fibers, enabling a specific concentration to be supplied to the exposure chamber. The second is that the subchamber can select similarly-sized fibers and supply them to the main exposure chamber. Long and thick fibers that cannot be inhaled by rats precipitate in the subchamber, enabling the supply of only inhalable fibers to the exposure chamber. This method also set RW fibers to be generated consistently at a relatively high concentration for a specific period of time. Consequently, RW fibers were generated at almost the same concentration, because they were generated nearly at the target fiber concentration and dispersion density initially intended, although there were some daily fluctuations.

The fiber deposition rate in our study, shortly after the end of exposure for 3 h daily for 5 consecutive days, was 13.7%. In a previous study$^{18}$, 32 male Wistar rats (9 wk old, weighing 200–300 g) were exposed to RW fibers, for 6 h daily for 5 consecutive days for 3 months. In that study, the geometric means (GSD) of the length and width of RW fibers in the exposure chamber were 9.1 $\mu$m (2.3) and 0.83 $\mu$m (1.7), respectively, and the mean fiber concentration and dispersion density (SD) of RW fibers in the exposure chamber were 959 (249) fibers/cm$^3$ and 37.0 (10.9) mg/m$^3$, respectively, during the experimental period. The fiber deposition rate was 4.0–5.4% in rats sacrificed on the 7th d after 3 months of exposure. It is not possible to directly compare the previous and present studies because their experimental methods differed, but one reason for the difference in the deposition rates may be differences in the fiber concentration, fiber size, exposure period and body weight of rats.

The total fiber number and fiber numbers counted by length tended to decrease with time from shortly after
exposure to the end of the 4th wk. In preceding studies, RW of all sizes decreased by 30–50% in the first 30 d after exposure\(^6\). Fibers that are inhaled and deposited in the lungs show different mechanisms for clearance depending on the site of deposition. Fibers deposited in the bronchioles are transferred to the pharynx by mucociliary movements and discharged from the body\(^7\). It is presumed that fibers deposited in the alveoli are cleared by either a) being dissolved by body fluid or phagocytosed and digested by alveolar macrophages (chemical clearance) or b) being transferred to the airway or lymphatic tissue by alveolar macrophages, and discharged from the body (physical clearance). Whether a fiber is phagocytosed or not depends on its length. Fibers with a length of 20 \(\mu\)m or shorter seem to be phagocytosed and digested by alveolar macrophages\(^11, 15\), while those with a length longer than 20 \(\mu\)m cannot be completely phagocytosed by alveolar macrophages. These fibers are presumed to be either a) dissolved by body fluid or b) folded transversely and crushed, shortening their length before being phagocytosed and digested by alveolar macrophages, or taken into pulmonary epithelial cells and transferred to lymphatic tissue, and thus discharged from the body\(^11, 15\). The fiber number is believed to be decreased by these mechanisms. Moreover, the rate of decrease in the number of fibers shorter than 20 \(\mu\)m slowed down in the 1W and 2W groups. A possible reason for this phenomenon is that fibers longer than 20 \(\mu\)m were dissolved by extracellular fluid and folded transversely with the fibers being crushed, thus increasing the number of shorter fibers (L< 20 \(\mu\)m) and, as a result, increasing the rate of accumulation in some indicators including the total fiber number\(^11\).

The half-life for long fibers with lengths of 20 \(\mu\)m or longer was especially short, 10 d. In a preceding study, the half-life was reported to be 111 d for WHO fibers and 53 d for fibers with a length of 20 \(\mu\)m or longer\(^9\). The half-life of fibers longer than 20 \(\mu\)m was shorter than those of fibers of the other sizes in the present study. We speculate that the number of fibers longer than 20 \(\mu\)m decreased rapidly, resulting in a short half-life, because they were folded transversely and became shorter. In contrast, the number of fibers 20 \(\mu\)m or shorter did not decrease rapidly and the half-life was accordingly longer. Because longer fibers were folded and became shorter, the number of fibers 20 \(\mu\)m or shorter increased, even though the shorter fibers were being continuously removed by phagocytosis by macrophages.

The distributions of fiber sizes (length and width) of the generated fibers were significantly different from those of the fibers in the lungs. It has been reported that fibers inhaled through the rat nose are mostly less than 80 \(\mu\)m in length and less than 1.5 \(\mu\)m in width\(^11\). Consequently, the difference in size distributions observed in this study may indicate size separation due to inhalation by rats. After fibers are inhaled into the lungs, both the length and width of fibers tend to decrease along with time in comparison with those shortly after exposure. In a preceding inhalation study on RW fibers manufactured in Denmark, the mean fiber length in the lung decreased from about 9 \(\mu\)m shortly after exposure to about 8 \(\mu\)m in the 4th wk, and the mean fiber width also decreased from about 0.7 \(\mu\)m shortly after exposure to about 0.6 \(\mu\)m\(^22\). In another inhalation study on RW fibers manufactured in Denmark, the mean fiber length in the lung decreased from about 11 \(\mu\)m shortly after exposure to about 10 \(\mu\)m in the 4th wk, and the mean fiber width also decreased from about 0.8 \(\mu\)m shortly after exposure to about 0.6 \(\mu\)m\(^19\). The length could have been reduced by the mechanism of fiber reduction postulated above, and the width could have been reduced by the action of body fluids.

In this study, we examined the behavior of RW fibers in rat lungs in order to determine their biopersistence following short-term, nose-only inhalation exposure. Our results suggest that RW fibers do not induce any overtly harmful health effects in a short-term inhalation exposure study. However, these results are clearly not applicable to the determination of safety of long-term inhalation. Consequently, it will be important to conduct long-term inhalation studies to further evaluate the carcinogenicity of RW fibers.

**Acknowledgments:** We would like to thank to Ms. Yumiko Sugiuira, Ms. Yoko Inoue, Ms. Yumi Komatsu, Ms. Michiyo Koyama, and Ms. Asuka Yamamoto of the Department of Preventive Medicine and Public Health, Kitasato University School of Medicine, and Mr. Shichiro Miyazawa and Ms. Noriko Nemoto of the Electron Microscopy Center, for their advice and encouragement.

**References**

7. Planning Division, Air Quality Bureau, Ministry of the