Separation of Asbestos Fibers by Length – Procedure for Obtaining Different-length Samples for Biological Experiments –†

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Abstract

A size-selective procedure has been developed in order to obtain samples of asbestos fibers of different lengths for use in biological experiments. Short chrysotile fibers less than 5 μm in length are widely suspected to be less carcinogenic than longer fibers. The bulk sample of U.I.C.C. standard asbestos (Chrysotile B) was aerosolized using a 2-component fluidized bed. It was then separated in a dry state by wire screens (200 mesh and 635 mesh) and a virtual impactor with a cut-off point of 2 μm. 0.1 g (sufficient weight for in-vitro tests) of a short-fiber fraction that passed through the wire screens and then onto the fine side of the virtual impactor, and 1.5 g of a medium fraction that passed through the 200 mesh screen but did not pass through the 635 mesh screen, were obtained from about 20 g of raw material. The length distribution of the short fiber fraction was 81% of fibers less than 5 μm long and 96% of fibers less than 10 μm long. X-ray diffraction analysis revealed that the crystallinity of the separated chrysotile did not change markedly. The present dry and mild separation process that was developed thus successfully avoids any artificial change in the physicochemical properties of asbestos fibers.

1. Introduction

The Carcinogenesis of asbestos has been widely recognized and that of other fibrous materials has been suspected. At present, the causes have been only partly identified and it is considered that a search for the causes will directly determine the direction of the search for substitutes for asbestos, which is attracting the attention of engineers and the industry. Asbestos is a natural mineral, pound with various mineral species, and is available in a wide range of compositions and sizes depending on the production locations. As a promotion of the studies of the biological effects of asbestos, the international conference on the biological effects of asbestos held in New York in 1964 adopted a resolution advocating the preparation of standard reference samples of asbestos dust. At the request of the conference participants, by 1966 five types of U.I.C.C. standard reference samples (International Union against Cancer Standard Reference Samples), namely Chrysotile A (product of Rhodesia), Chrysotile B (product of Canada), Crocidolite, Amosite, and Anthophylite, were produced. Each of the five U.I.C.C. standard reference asbestos samples were prepared in 500 kg packages to meet the requirements of research organizations throughout the world, to which they are available in packages of 500 g or 50 g. In this way, the U.I.C.C. standard reference asbestos samples became standard samples for the studies of its biological effects and have been popularly used in in vivo and in vitro experiments. From these studies of the biological effects, which represent one of the causes of carcinogenesis, a hypothesis that “fibers which are long and thin, and remain in lungs unaltered are carcinogenic whether they are asbestos or not” has been advanced by Stanton1,2 and Pott.3 Stanton emphasized that fibers of 0.25 μm or less in diame-
ter and 8 \mu m or more in length exhibit a particularly high carcinogenicity, and Pott also asserted that fibers of 0.5 - 2.5 \mu m in diameter and 3 - 20 \mu m in length are particularly carcinogenic. In order to carry out biological experiments to verify these hypotheses, such as they theory of Pott, samples of fibers of 3 \mu m or less in length and others of 20 \mu m or more in length are required in addition to the samples of 3 - 20 \mu m in length. This, in turn, requires a technique to separate the fibrous materials according to their length and diameter. Various methods have been tried to separate fibrous materials, but it has been difficult to obtain fine fibrous materials particularly of uniform length in a quantity (several grams) sufficient enough for biological experiments.

Stanton et al.\(^4\) obtained samples of fibers of various lengths and diameters by varying the grinding time of their grinding machines such as ball mills and separating the fibers by sedimentation in water. Spurny et al.\(^4\) made asbestos and glass fibers into fibrous aerosols by a vibrating type generator, and dispersed and separated them by sedimentation in water after a separation by elutoriator and cyclone. They reported that they were able to obtain several grams each of fine fibers containing 99\% of 3 \mu m long or smaller fibers and others consisting of 100\% of 10 \mu m long or smaller fibers. These methods are based on a separation by aerodynamic diameter of fibrous particles and are greatly dependent on the fiber diameter, and do not separate fibers directly according to their length.

On the other hand, Spurny et al.\(^4\) indicated that in order to separate fibers by length it is most effective to use interception effects, together with the use of a uniform microsieve and to proceed in such a way that the fibers are as closely perpendicular to the flow direction as possible. They have even carried out parts of the experiments to that effect. However, Spurny et al.\(^4\) concluded that their method involved a problem of reproducibility and needed improvements, suggesting that they could not obtain enough amount of fibers for the experiments with their methods.

A biological experiment was planned by separating fibers of 5 \mu m or shorter, which are supposed to be less harmful, and chrysotile of various fiber lengths from the U.I.C.C. standard reference Chrysotile B and comparing the toxicity of these fibers with that of the bulk sample. The research described in this paper was carried out as part of this experimental plan and was intended for the separation of fibers by length and obtaining the amount necessary for biological experiments. There were no specific requirements for the fiber diameter but fibers in splinter bundle form which are frequently encountered in aggregates of chrysotile fibers were eliminated.

In the plan of our biological experiment, about 1 gram of separated fiber sample is required for direct administration (in vivo test) to animals and about 0.1 gram for the cytotoxicity test (in vitro test). It is important for the samples not to be mixed with impurities during the separation process in order to obtain the same composition as that of the bulk sample, and to avoid the application of strong forces to destroy the whole or partial crystal structure. To satisfy these requirements, a fluidized bed was used to aerosolize the samples, stainless steel wire screens for sieves were used for the separation of the fibers by length, and a virtual impactor was used for the separation of fibers of small diameter. The whole method is based on dry type separation, which has the advantage of not changing the chemical composition, such as dissolution of the cations. Because a physical force was applied to the samples, the crystal structure was investigated by X-ray diffraction analysis.

2. The Experimental method for Separating Fibrous Materials by Length

It has been said that it becomes very difficult to obtain the fairly amount of new U.I.C.C. standard reference asbestos now 20 years after its first appearance. In this experiment, a limited amount of the standard sample (Chrysotile B) was separated by length and a method to separate fine fibers was investigated.

2.1 Classification apparatus and method

As shown in the flow diagram of Bold Figure 1, the continuous classification apparatus used in the investigation consists of a fiber feeder (1), fluidized bed (2), wire screen for separation (4), and a virtual impactor (3). The fiber feeder (1) is of the same type as that used in our previous report\(^6\) which chops fibers little by little in a grinder and transfers them pneumatically.

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feed rate was controlled by turning on and off the motor which pushed out the fibers towards the grinder.

The fluidized bed (2) is one of two types of fluidized beds I and II having different profiles. The fluidized bed I is of the same size as that used in our previous report and is made of glass 50 mm in diameter and 500 mm high, which was used for evaluating the separation efficiency. As fluidizing particles, 100 g of 80 - 120 mesh glass beads were used after being washed sufficiently with water to prevent the migration of impurities from the glass beads to the asbestos aerosol. One wire screen (4) with openings of 78 μm (200 mesh) or 19 μm (635 mesh) was inserted in the upper part of the fluidized bed for separation.

The fluidized bed II was prepared for a continuous separation of chrysotile fibers. The inside diameter of the fluidized bed was made as large as 70 mm to provide a larger filtering area for the wire screen. In addition, because the amount of the standard sample fiber used was small, the fluidized bed was designed to produce two types of separated samples simultaneously through the use of two kinds of wire screens, which was of 78 to 19 μm and was of less than 19 μm which was further separated by the virtual impactor.

Figure 2 schematically shows the fluidized bed II. The fluidized bed is made of glass 500 mm high and contains 200 g of 80 - 120 mesh glass beads. The wire screen (1) has openings of 78 μm and the downstream wire screen (2) has opening of 19 μm. Table 1 indicates the experimental conditions of the fluidized beds I and II.

![Diagram of classification apparatus of asbestos](image)

**Fig. 1** Schematic diagram of classification apparatus of asbestos
(1) Fiber feeder, (2) Fluidized bed I, (3) Virtual impactor, (4) Wire screen, (5) Vibrator, (6) and (6)’ Solenoid valve, (7) Controller, (8) Filter(fine), (9) Filter(coarse), (10) Compressor, (11) Vacuum pump, (12)Core air, (13)Sheath air, (14)Pressure gauge

![Detail of Fluidized bed II](image)

**Fig. 2** Detail of Fluidized bed II.
(1) Wire screen(200 mesh), (2) Wire screen(635 mesh), (3) Vibrator, (4) Motor shaft, (5) Clean air inlet, (6) Aerosol outlet, (7) Fiber inlet from feeder, (8) Clean air inlet for fluidizing, (9) Glass beads, (10) Fiber outlet

| Table 1 Experimental conditions |
|--------------------------------|
| **Fluidized bed** | I | II |
| Bed diameter (mm) | 50 | 70 |
| Air flow into F.B. (l/min) | 10 | 17.0 |
| Air flow into feeder (l/min) | 6 | 7.5 |
| Superfacial velocity in F.B. (m/s) | 0.14 | 0.11 |
| Glass beads (g) | 100 | 200 |
| Asbestos feed rate (mg/min) | 1.8 | 1.4 |
| Switching cycle of feeder; 5s on/15s off 5s on/20s off Fluidizing and cleaning cycle; 110s/10s 110s/10s |

| **Virtual impactor** |
|---------------------|
| Nozzle diameter (mm) | 4 |
| Nozzle gap (mm) | 1 |
| Aerosol flow rate (l/min) | 26 |
| Core flow rate (l/min) | 29 |
| Sheath flow rate (l/min) | 9 |
| Fine flow rate (l/min) | 58.5 |
| Coarse flow rate (l/min) | 5.5 |
| 100% cut diameter (μm) | 2.0 |

The aerosol was then directed to the virtual impactor (Variable Impactor manufactured by Sankyo Dengyo; hereinafter called "V.I.") indicated by numeral (3) in Figure 1 and devel-
KONA fibers onto the fluidized particles. Using the vibrator (5) to shake off accumulated air supply side is closed and the valve (6') is opening to suck air into the bed and allow a temporary backflow of air in the fluidized bed. In addition, the wire screen frame is vibrated using the vibrator (5) to shake off accumulated fibers onto the fluidized particles.

In the fluidized bed I shown in Figure 1, the solenoid valve (6) on the clean air supply side is closed and the valve (6') is opened to suck air into the bed and allow a temporary backflow of air in the fluidized bed. In addition, the wire screen frame is vibrated using the vibrator (5) to shake off accumulated fibers onto the fluidized particles. In the fluidized bed II shown in Figure 2, fibers accumulated on the wire screens are shaken off onto the fluidized particles by allowing the air stream to flow in the reverse direction through the 200-mesh wire screen (1) as in the fluidized bed I while actuating the vibrator (3). Fibers on the 635-mesh wire screen (2) which is cylindrical and rotated by a motor are removed by the sucking force provided through the suction port (10) (Fiber outlet (10) in Figure 2) on the side as described later. The fibers on the wire screen (1) are guided to the filter holder from the suction port (10) by the air stream. The fibers caught at this point are used for biological experiments designated as a sample pass through the wire screen (1) but do not pass through the wire screen (2). The connections between the Solenoid valves and the V.I. and the other overall configuration are the same as those shown in Figure 1. Both fluidized beds I and II repeat the process of a 110-second generation and a 10-second shake-off. The cyclic operation and condition monitoring of the whole apparatus were carried out by a microcomputer, and the apparatus was operated nearly unattended.

Figure 3 shows the air-stream direction at the time of generation (fluidizing) and shake-off (cleaning) of the fluidized bed II. In the fluidizing process (a), chrysotile fibers dispersed in the fluidized bed ascend at low speed together with the air stream and pass through the 78 μm opening stainless steel wire screen (3) from the bottom, then pass through the cylindrical 19-μm opening wire screen (2) from the outside to the inside, and finally are sucked into the V.I. at a rate of 26 l/min. In this case, the fibers may be caught by the wire screens due primarily to the interception effects.

In the cleaning process (b), the air stream flow is reversed by the solenoid valve, and clean air is introduced into the bed through the clean air inlet (5) of Figure 2 and sucked from the fluidized bed bottom and through the fiber outlet (10) on the side of cylindrical wire screen. In this case, the frame holding the wire screen (1) is shaken several times by the vibrator. The fibers accumulated on the wire screen (1) drop onto the fluidizing particles. The fibers accumulated on the cylindrical wire screen (2) are caught on the filter in a filter holder connected to the fiber outlet (10). The wire screens are cleaned as described above and the fluidizing process is repeated.

2.2 Wire screen for sieves

The wire screens of the sieves were used to separate the fibers. Compared to microsieves or nuclepore filters used by Spurny et al.,\(^{9}\) wire screens with smaller openings are difficult to manufacture but they are stronger than microsieves and easy to use when shaking off the fibers, with the further advantage of a higher porosity than that of nuclepore filters. The wire screen with openings of 19 μm (635 mesh) was the finest screen available. With respect to wire screens, an investigation was made regarding the use of filtering material for spherical particles and the screening of cylindrical particles.\(^{8}\) However, no investigation was made regarding the relationship between the wire screen openings and the penetration of fibrous aerosol per fiber length. In this investigation, the separated fibrous samples passing through the wire screen were confirmed by measuring the fiber sizes by scanning electron microscope (SEM).

2.3 Removal of fibers from wire screens

When filtering is continuously carried out by means of wire screens, the fibers gradually accumulate on the wire screens and lead to clogging, which requires the removal of such accumulated fibers to keep the wire screens in a regular condition. In the fluidized bed I shown in Figure 1, the solenoid valve (6) on the clean air supply side is closed and the valve (6') is opened to suck air into the bed and allow a temporary backflow of air in the fluidized bed. In addition, the wire screen frame is vibrated using the vibrator (5) to shake off accumulated fibers onto the fluidized particles.

In the fluidized bed II shown in Figure 2, fibers accumulated on the wire screens are...
2.4 X-ray diffraction analysis

The separated chrysotile fibers were analyzed for mineral species, checked for crystallinity, and inspected for the presence and composition of contaminants. The measurement conditions were as follows:
(1) Voltage and current of a copper X-ray tube; 35 kV and 20 mA, respectively.
(2) The slit system; 1°-1°-0.3 mm with a graphite monochromator.
(3) The radius of the goniometer; 185 mm, the step scanning width; 0.01°, and the scanning range; 2°-70°.

3. Results and Discussion

3.1 Experiment using fluidized bed I

The following results were obtained using the fluidized bed I through the evaluation experiments consisting of generation and separation. Figure 4 (a) shows a SEM photomicrograph of asbestos flowing out from the fluidized bed when no wire screens were used. Considerably long fibers or bundle-form fibers were seen and the long fibers were meandering, which are quite similar with the fibers contained in the bulk U.I.C.C. Chrysotile B. In addition, the distribution range of these fiber lengths was very wide, denying the accurate measurements by SEM.

Figure 4 (b) shows fibers which pass through 19 μm-opening wire screens and are caught on the coarse particle side of the V.I. Some fine fibers are included but fibers with large diameters and spherical particles are predominant.

Figure 4 (c) is a photomicrograph of the fine side of the V.I. Most of the fibers are shorter than 30 μm long. According to Burke and Esmen, when the aspect ratio (length/diameter) is 10, the fibers are separated with a fiber diameter of about 1/3 the cut-off point of 2 μm of V.I. A comparison between Figures 4 (b) and 4 (c) indicates that the V.I. separation greatly depends on fiber diameter. Figure 4 (d) represents a photomicrograph of fibers which pass through a wire screen of 78 μm-opening and caught on the fine side of the V.I. indicating the existence of long fibers exceeding 100 μm but there are no fibers nor fiber aggregates having both a big length and a large diameter.

Figure 5 shows the fiber length distributions measured on the fibers on Figure 4 (c) and (d). The fibers on Figure 4 (c) passing through the 19-μm-opening wire screen are nearly of the desired size, in which a fiber length of less than 5 μm accounts for 81% and that of less than 10 μm for 96%. The fiber lengths distribution of the U.I.C.C. Chrysotile B measured by Timbrell are also shown in this figure. These are the results obtained by optical microscope for bulk asbestos samples which were dispersed by alcohol and filtered, and the fibrous aerosol samples generated by the aerosol generator using a grinder were trapped on a filter. The fiber length distribution of the bulk samples is wide and slightly decrease after aerosolizing. This distribution is similar to that of the fibers.
Fig. 4  SEM micrographs of asbestos (U.I.C.C. Chrysotile B)
(a) Fibers flow out fluidized bed.
(b) Fibers penetrating 635 mesh screen and V.I. coarse side.
(c) Fibers penetrating 635 mesh screen and V.I. fine side.
(d) Fibers penetrating 200 mesh screen and V.I. fine side.

![Images of SEM micrographs of asbestos](image)

Fig. 5  Fiber length distributions of U.I.C.C. Chrysotile B
*: length distribution measured by Timbrell (5)

In order to estimate the separation efficiency, using the fluidized bed I, a 3-hour aerosol generation was carried out and the amount of asbestos caught at the lower reaches of the wire screen was measured. The generation efficiency was determined from the ratio of the amount of collected asbestos and the amount fed (0.331 g) for 3 hours estimated from the feed speed, the results of which are shown in Table 2. Because of the shortage of asbestos sample, results when no wire screens were used have not been obtained but considering the results for glass fibers reported earlier, a generation efficiency passing through the 78 μm opening wire screen of Figure 4 (d), which was measured in this investigation. Comparing the distribution of bulk samples, that of the fibers passing through the 78 μm opening wire screen, and that of the fibers passing through the 19 μm opening wire screen, we came to the conclusion that it is possible to separate fibers by length using this method.
of about 10% may be assumed, indicating that the amount of fibers passing through the wire screens is extremely small. When the V.I. is used for separation, the amount of asbestos trapped on the fine side would become further lower.

Table 2 Generation efficiency of U.I.C.C.

| No. | Screen (mesh) | Generated fiber (mg/3h) | E (%) |
|-----|---------------|-------------------------|-------|
| 1   | 200           | 6.25                    | 1.9   |
| 2   | 200           | 6.50                    | 2.0   |
| 3   | 200           | 5.68                    | 1.7   |
| 4   | 635           | 1.31                    | 0.40  |
| 5   | 635           | 1.70                    | 0.51  |

Fiber mass fed for 3 hours : 0.331g

3.2 Experiment using fluidized bed II

In the continuous operation using the fluidized bed II, about 0.1 g of fibers which passed through a 635 mesh wire screen and were caught on the fine side of the V.I. and 1.5 g of fibers which passed through a 200 mesh wire screen but did not pass through the 635 mesh were obtained using about 20 g of U.I.C.C Chrysotile B over a total period of 350 hours.

Figure 6 shows the X-ray diffraction patterns of separated fibers smaller than 635 mesh and from 200 mesh to 635 mesh, fibers in the fluidized bed, and bulk samples for Chrysotile B obtained in this investigation. Chrysotile B contains impurities of plate-form brucite and pyroaurite. The brucite and pyroaurite were collected in large quantities in the samples which passed through 200 mesh screen and remarkably decreased in the samples which passed through the 635 mesh screen, which was much less than that in the bulk sample. According to Figure 4 (b), this may be attributed mainly to the separation process using the V.I.

In order to compare the crystallinity of each sample, the peak widths of the (002) and (004) diffraction lines observed in the vicinity of 12°, 2θ and 24° 2θ were measured. For all samples, the decrease in crystallinity was scarcely observed as compared to that of the bulk sample as shown Figure 7. In particular, the fact that the crystallinity of the fibers remaining in the fluidized bed for a long time is not degraded indicates that the fluidized bed does not give any serious effect on the crystallinity unlike the grinder such as a ball mill.
4. Conclusion

A classification apparatus experimentally built for this investigation was operated nearly unattended, except when filling the fiber feeder with asbestos, for a total of about 350 hours and 0.1 g of asbestos fibers which passed through the 19 μm (635 mesh) opening screen and were caught on the fine side of the virtual impactor with a cut-off point of 2 μm and 1.5 g of asbestos which passed through the 78 μm (200 mesh) opening screen but were unable to pass through the 635 mesh screen were obtained from about 20 g of U.I.C.C. standard reference asbestos (Chrysotile B). The former consisted of short fibers was not enough amount for use in injection or inhalation experiments on animals but was offered for cytotoxicity tests (in vitro tests) and interesting results have been obtained.\(^\text{10}\)

Fibers obtained by this method are, in principle, not subjected to any alteration of their chemical composition since separation is carried out in a dry state, and the X-ray diffraction results have not indicated any obvious degradation in crystallinity, suggesting that the process is an superior separation method for fibrous samples to be offered for biological experiments. In addition, the fibers can be prepared as fibrous aerosols containing fine fibers, which can be directly used in inhalation tests.

In order to separate fibers using wire screens, a further quantitative investigation must be carried out in future regarding the fiber size and separation efficiency.

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