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A Trial on the Quantitative Risk Assessment of Man-Made Mineral Fibers by the Rat Intraperitoneal Administration Assay using the JFM Standard Fibrous Samples

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Abstract: We tried to evaluate the carcinogenic risk of man-made mineral fiber based on the mesothelioma incidence in female F344 rats after intraperitoneal administration. Rats (female F344/Nslc, 5-week-old, n=330) were observed for 2 years after the intraperitoneal administration of 5 to 20 mg of 9 types of the JFM (Japan Fibrous Material Research Association) standard fiber samples (glass wool, rock wool, micro fiber glass, three types of refractory fiber, potassium titanate whisker, silicon carbide whisker, titanium oxide whisker), wollastonite (natural fiber) and UICC chrysotile B. All rats administered 10 mg of silicon carbide whisker had developed peritoneal mesothelioma within a year. The cumulative incidence of peritoneal mesothelioma at the end of the experiment was 85% for 10mg UICC chrysotile B, 77% for 10 mg of potassium titanate whisker, 70% for 5 mg of silicon carbide whisker, 20% for 5 mg of potassium titanate whisker, 20% for 20 mg of refractory fiber 2 and 10% for 20 mg of refractory fiber 1. Carcinogenicity was estimated 2.4 times for silicon carbide whisker and 0.23 for potassium titanate whisker in comparison with UICC chrysotile B. It has been well documented from several experimental studies that man-made fibers are safer than asbestos because of the different durability in the lung. Present results consistently suggest that man-made fibers with high durability have similar or higher risk as carcinogen than asbestos.

Key words: Man-made mineral fiber, Asbestos, Carcinogen, Risk assessment, Mesothelioma

Introduction

Natural and synthetic fibrous materials are widely used as insulator, friction material and other purpose, because of the unique physicochemical properties. Especially, synthetic fibers such as glass fiber and rock wool have been substituted to asbestos. However, progression of fine chemical industry led the production of new types of man-made mineral fibers. These fibers have very fine structure and some additional features such as colorless, superior friction profile at high temperature, conductivity of electricity and so on. It has been indicated that fine man-made mineral fibers could possibly induce similar biological effects to asbestos[1)]. Since it has been recognized that fibrous materials could not be determined by the *in vitro* test for screening the carcinogenicity, the animal assay may be the only method for the evaluation of their carcinogenic potency[2)].

JFM standard fibers including 9 different types of fine man-made mineral fibers are produced for the development of *in vitro* screening method on biological effects[3)]. For the accurate evaluation of the newly developed *in vitro* method, it is necessary to obtain their carcinogenic potency.

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Considering the difficulties on the cost of inhalation experiment and on the accuracy of intratracheal administration, we observed the carcinogenicity of JFM standard fibers by the intraperitoneal administration to rats. As known in a series of studies by Pott *et al.*, they have obtained enough evidences to conclude the dose response relationship on a number of fibrous materials by the intraperitoneal experiment using rats[4]. Therefore, we tried to estimate the carcinogenic risk on JFM samples in comparison with UICC chrysotile B by the intraperitoneal experiment.

Materials and Methods

Three hundred and thirty rats (F344/Jslc, female) were purchased from Japan Slc at 4 week-old. After a week of acclimatization, rats were divided into 24 groups and used for the experiment. JFM standard samples (Table 1) or UICC chrysotile B are administered intraperitoneally to the rats as suspended solution (1 mg/ml) in saline. Five milliliter was the highest volume of an administration to rat in a week. At first, all types of fibers were examined at 10 mg/rat of dose. Based on the tumor incidence at 10 mg/rat, doses for the second experiment were decided to gain (20 mg/rat) or reduce (5 mg/rat). Chemical composition, size distribution and other characteristics of JFM standard samples are described elsewhere[3].

Died, moribund or sacrificed rats were autopsied and the tissues were fixed in 10% neutral formalin. Histopathological examinations were performed under light microscopy on tissue slides stained by hematoxylin and eosin, PAS, Alcian blue (with or without hyaluronidase digestion) or Azan.

Results

All rats administered 10 mg of silicon carbide whisker developed peritoneal mesothelioma within a year. In the group of rats administered 5 mg of silicon carbide whisker, incidence of mesothelioma was 70% at one year after the administration. As shown in Fig. 1, cumulative incidence of mesothelioma at two years of the experiment was 85% for 10mg UICC chrysotile B, 77% for 10 mg of potassium titanate whisker, 70% for 5 mg of silicon carbide whisker, 20% for 5 mg of potassium titanate whisker, 20% for 20 mg of refractory fiber

Table 1. JFM standard reference samples

Sample name	Short description of fiber and whisker
GW1	Glass wool (glass fiber sprayed by phenol resin)
RW1	Rock wool (sludge wool)
MG	Micro glass fiber
RF1	Refractory fiber (ceramic fiber, amorphous)
RF2	Refractory fiber (ceramic fiber, amorphous)
RF3	Refractory fiber (mullite fiber, chrystalline)
PT1	Potassium titanate whisker ($K_2Ti_8O_{17}$)
SC1	Silicon carbide whisker (SiC)
TO1	Titanium oxide (rutile) whisker (TiO_2)
WO1	Wollastonite (natural mineral fiber from China, $CaSiO_3$)

Chemical composition, size distribution and other data are reported by Kohyama *et al.* (1997).

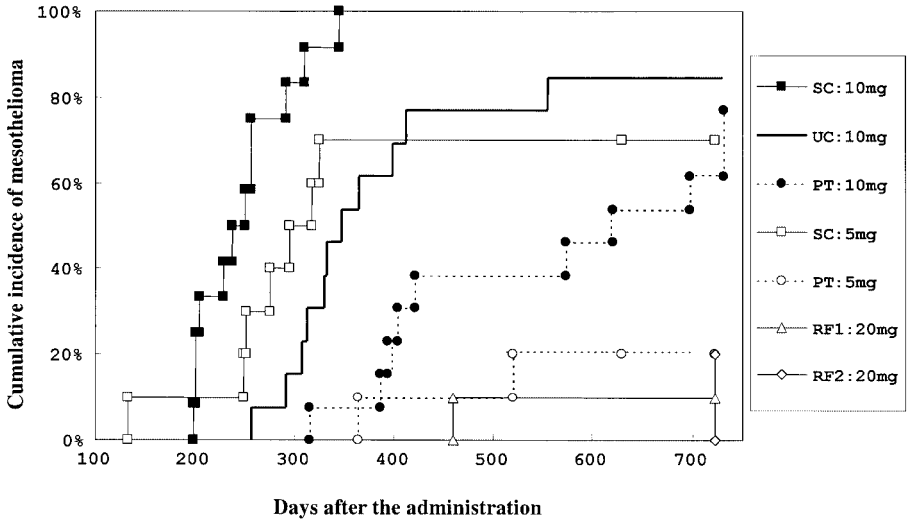

Fig. 1. Cumulative mesothelioma incidence in female F344 rats after the intraperitoneal administration of JFM standard fibers.

2 and 10% for 20 mg of refractory fiber 1.

The fastest development of peritoneal mesothelioma was identified in the rat administered 5mg of silicon carbide whisker at 133 days of the experiment. The rat was weakened with signs of severe anemia and ascites. In autopsy of the rat, hemorrhagic ascites full filled the abdominal cavity and numerous nodules ranged from 1 to 3 mm were spread on the abdominal wall and epithelium of the organs. (Figs. 2–5) These lesions were found in most (95%) rats developed peritoneal mesothelioma as the typical signs of the terminal stage.

Tumor cells spread the whole gamut of abdominal cavity, however, no metastases to other than the abdomen was found. Adhesive growth of the tumor between liver and diaphragm was common in the rats with mesothelioma and coagulation of deposited fibers at the same site was also common in the autopsied rats at the end of this experiment.

Microscopically, tumor cells showed a variety of characteristics including epithelial or sarcomatous structure and some of the extensive cases had the osseous formation in the tumor, as shown in Figs. 6–10.

Discussion

The use prohibition or restriction of asbestos stimulated the development of asbestos substitutes, since it has been

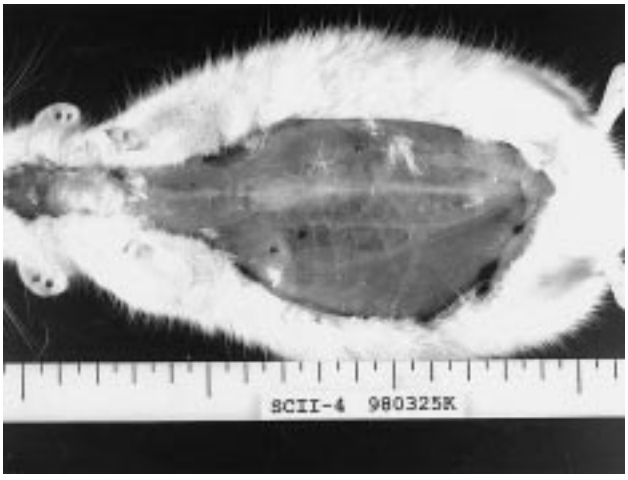

Fig. 2. Macroscopic appearance of rat bearing peritoneal mesothelioma (5 mg of silicon carbide whisker, 275 days).

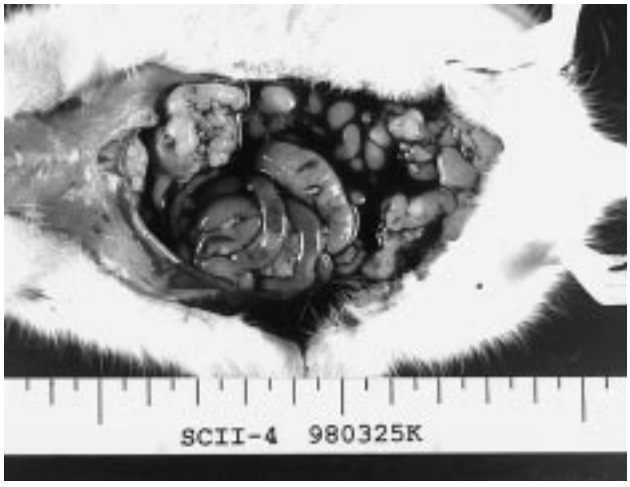

Fig. 3. Full filled with hemorrhagic ascites in abdominal cavity (5 mg of silicon carbide whisker, 275 days).

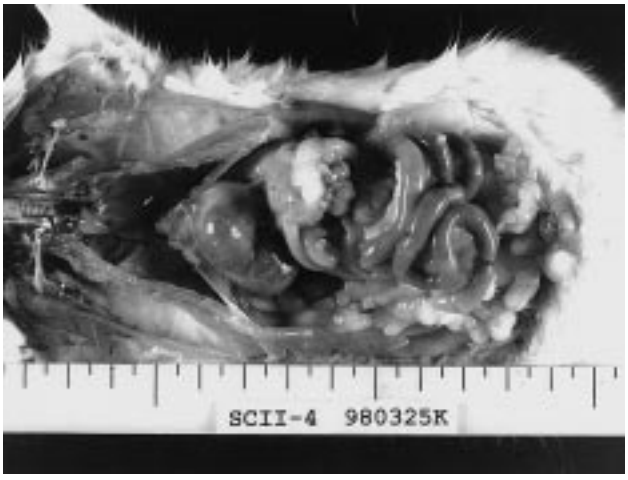

Fig. 4. Adhesion of liver and diaphragm and tumor nodules were observed (5 mg of silicon carbide whisker, 275 days).

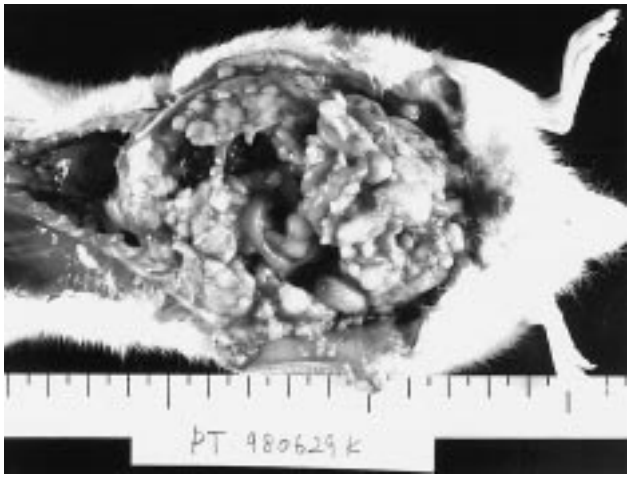

Fig. 5. Peritoneal mesothelioma extended abdominal cavity (10 mg of potassium titanate whisker).

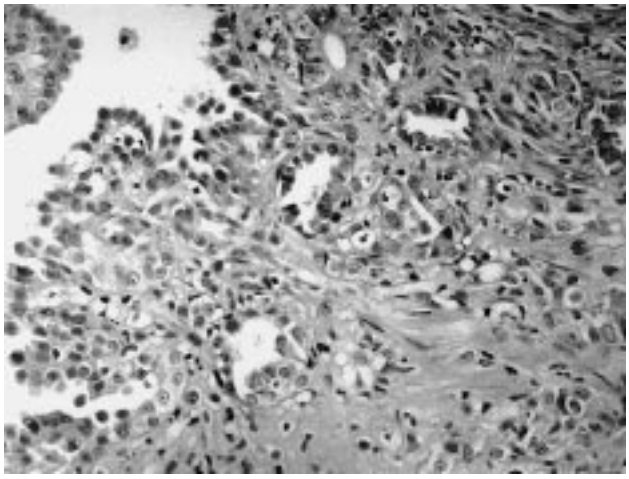

Fig. 6. Microscopic appearance of mesothelioma, showing papillary growth of tumor cell with round nuclei and less cytoplasm.
(H&E, × 100, 7% of mesothelioma rats, mean survival period was 373 days).

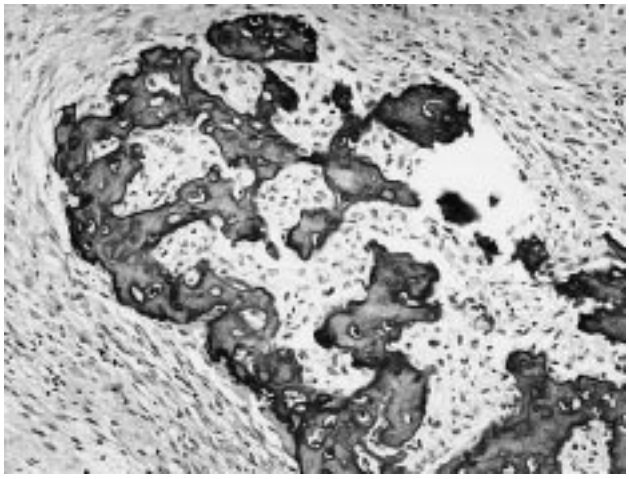

Fig. 7. Microscopic appearance of mesothelioma.
Osteogenesis appeared in tumor masses. Polygonal tumor cells occasionally showing mitotic figures. (H&E, × 100, 18% of mesothelioma rats, mean survival period was 336 days).

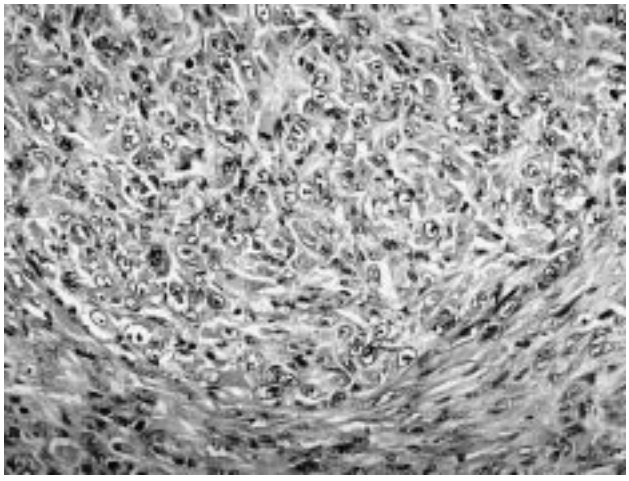

Fig. 8. Microscopic appearance of mesothelioma.
Atypical tumor cells were in close contact one another, having large vesicular nucleus with localized prominent nucleolus. (H&E, × 100, 18% of mesothelioma rats, mean survival period was 305 days).

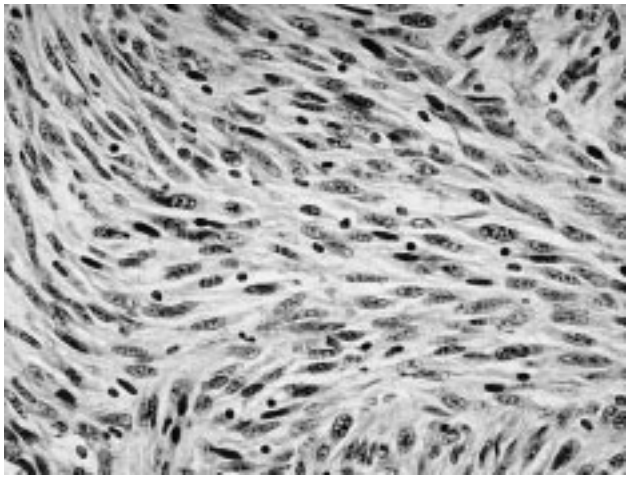

Fig. 9. Microscopic appearance of mesothelioma.
Large spindle cells form fibrosarcomatous appearance. (H&E, × 100, 14% of mesothelioma rats, mean survival period was 212 days).

used as a material with the excellent characteristics which originate from the unique shape and physicochemical properties. In addition to fiberglass used since the past of which the harmfulness of the asbestos was recognized, rock wool, slag wool and refractory fibers has been used. These fibers are amorphous and comparatively large diameter and length, because these are produced by the centrifugation method or the injection (spray) method. These materials are widely used as a building material, because the cost is enough as asbestos substitution.

In the meantime, the demand for application such as brake lining and plastics additives invented monocrystalline whiskers, which had supplemental properties such as color, homogeneity in dimension or electrical conduction property. Potassium titanate whisker, silicon carbide whisker, magnesium sulfate whisker and calcium sulfate whisker are used for these purposes. The carcinogenic mechanism of the asbestos has not come to the sufficient elucidation. There has been a limited positive result in the short term screening tests for carcinogenesis on asbestos[6, 7]. It is difficult to be

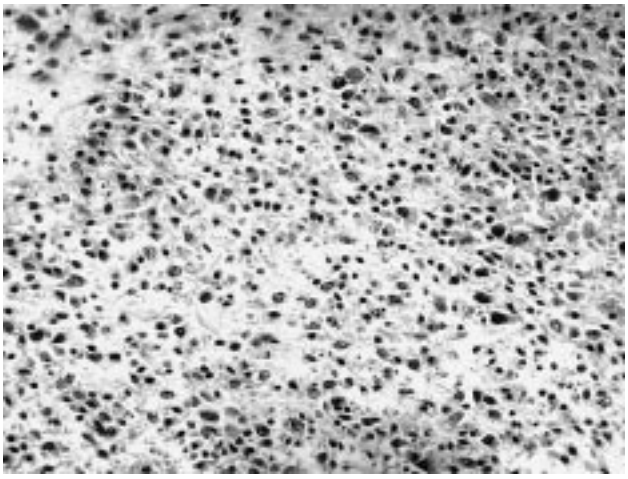

Fig. 10. Microscopic appearance of mesothelioma.
Rather small tumor cells appeared high anaplasticity and dysplasticity having chromatin-rich nuclei. (H&E, × 100, 4% of mesothelioma rats, mean survival period was 238 days).

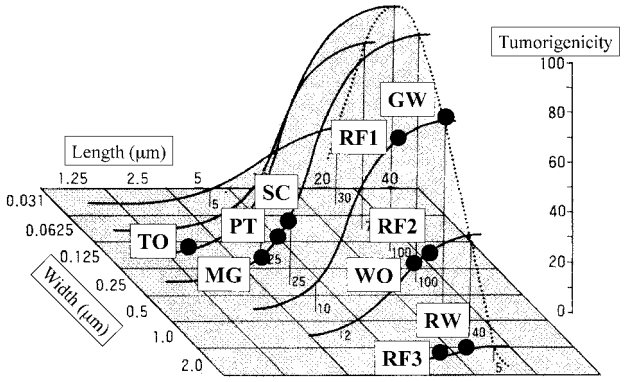

Fig. 11. Suspected tumorigenicity of JFM standard fibers on the hypothetical curve by Pott (Each spot indicates the geometric mean of length and width of JFM standard fibers).

considered that the component of the asbestos reacts with DNA by the carcinogenesis process, because of the insolubility of asbestos. In the beginning in 1980's, Stanton in the USA and Pott in Germany reported that there was the significant relation in tumorigenicity and dimensions of the fibers when a variety size of asbestos and man-made mineral fibers were administered to rats[4, 8]. Since the fibers are localized by the intrathoracic or intraperitoneal administration, the relationship between the tumorigenicity and the fiber dimension seems to be purely a result of the interaction between fiber and cell. Regardless of the fiber type, their hypothesis that the fiber of the specific size has the carcinogenicity has been supported as an important knowledge at present. However, the results of animal experiment on the newly produced man-made mineral fibers showed no or low tumor incidence on fibers which were classified as the carcinogenic fiber in the Stanton and Pott hypothesis[9, 10]. In addition, we observed a significant difference between acid leached crocidolite and original crocidolite on carcinogenic response in rats after intraperitoneal injection, in spite of their same dimension[11]. This result also supports the postulation that the oxygen free radicals may play an important role in the carcinogenic process induced by asbestos or other fibers[12–17]. We have compared tumorigenicity in experimental animals and oxidative DNA damage *in vitro* on 11 types of natural and man-made fibrous materials[18, 19]. A significant correlation was observed between tumorigenicity and 8-OH-dG, which is one of the products in DNA damaged by the reactive oxygen species. As an additional evidence, we observed that the tumorigenicity of chrysotile in the exposed rats had been diminished by the surface modification using the organic polymer. These results suggest that the chemical feature of fibrous dust is one of the important determinants for the carcinogenic potential[20].

Based on these backgrounds, the carcinogenicity of the JFM standard fibrous samples were examined and their carcinogenic risk was estimated from the results. According to the Pott's hypothesis, JFM standard sample ranges at the carcinogenic potential in this order, GW > refractory fiber 1 > refractory fiber 2 > WO > silicon carbide whisker > potassium titanate whisker1 > micro fiber glass > rock wool > refractory fiber 3 > titanium oxide whisker (Fig. 11).

It is proper when the fibers are compared in number of fibers. In this study, rats were administered 5 to 20 mg of the JFM standard samples. Yamato *et al.* counted the number of fibers using scanning electron microscopy as glass wool: $0.7 \times 10^3/\mu g$, rock wool: $1.7 \times 10^3/\mu g$, micro fiber glass: $65.4 \times 10^3/\mu g$, refractory fiber 1: $8.8 \times 10^3/\mu g$, refractory fiber 2: $8.7 \times 10^3/\mu g$, refractory fiber 3: $3.5 \times 10^3/\mu g$, potassium titanate whisker: $594 \times 10^3/\mu g$, silicon carbide whisker: $414 \times 10^3/\mu g$, titanium oxide whisker: $639 \times 10^3/\mu g$, wollastonite: $24.3 \times 103/\mu g$[21]. There were considerable differences between the number of fibers even in the equal weight. In comparison with the silicon carbide whisker and potassium titanate whisker, both fibers induced mesothelioma, the difference in number of fiber was small. However, approximately 50 times lower number of fiber than silicon carbide whisker was administered in the groups of refractory fiber 1 or refractory fiber 2. Because these refractory fibers contain long (over 100 μm) and thick (5 μm) fibers, the number of refractory fiber which may be

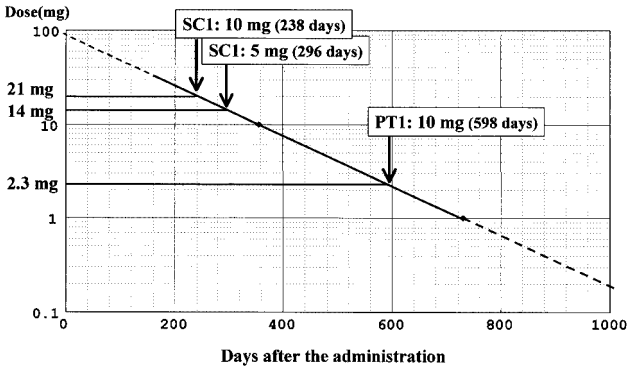

Fig. 12. Relative potency of JFM standard fibers to UICC chrysotile B in carcinogenicity test in F344 rats by intraperitoneal administration.

Table 2. Estimated risk and regulatory number for JFM fibers based on their relative potency to UICC chrysotile B

	Risk level*[1] (WHO)	Risk level*[1] (EPA)	PEL*[2] (OSHA)	TLV*[3] (ACGIH)
Chrysotile	1.3×10^{-6}	4×10^{-6}	0.2	0.5
Tentative estimation using coefficient values from the rat study				
SC1	0.5×10^{-6}	1.7×10^{-6}	0.09	0.21
PT1	5.7×10^{-6}	17.4×10^{-6}	0.9	2.2

*[1]: Life time excess of one cancer death in 1,000,000 (fiber/ml). *[2]: Permissible exposure limit expressed as a time-weighted average (fiber/ml). *[3]: Threshold limit value expressed as a time-weighted average (fiber/ml).

responsible for the carcinogenicity seems to even less than the difference described above. It may lead the underestimation of carcinogenic potency on refractory fibers. Under the practical occupational exposure, harmful size of the fibers could be selected for inhalation because long and thick fibers would sediment easier than the short and fine fibers. Therefore, it may be difficult to conclude that the carcinogenic potency of refractory fiber is lower than that of silicon carbide whisker, chrysotile or potassium titanate whisker. As a future study, it is necessary to examine the carcinogenicity of these fibers in animals based on the dose of the number of respirable fiber.

According to the cumulative incidence of peritoneal mesothelioma in this study, carcinogenic risk for silicon carbide whisker and potassium titanate whisker was estimated as relative potential to UICC chrysotile B. Calculations are based on the assumption that the difference of number of fiber among silicon carbide whisker, potassium titanate whisker and UICC chrysotile B is negligible in comparison with the difference of carcinogenic potential. In fact, the difference was not greater than 10 times in measurement by Yamato *et al.* (Personal communication) As the second assumption, latency period (days) for 50% mesothelioma incidence is in inverse proportion to the logarithm of dose. Therefore, 10 mg of silicon carbide whisker was equivalent to 21 mg of UICC chrysotile B and 5 mg of silicon carbide whisker1 was estimated as 14 mg of chrysotile. On potassium titanate whisker, 10 mg of dose was equivalent to 2.3 mg of UICC chrysotile B (Fig. 12). Using these coefficient values, tentative calculation of the life time cancer risk was performed by referring the 1 in 1,000,000 cancer risk for chrysotile asbestos as 1.3×10^{-6} fiber/ml by WHO and 4×10^{-6} fiber/ml by U.S.EPA. Life time excess of one cancer death in 1,000,000 would be caused by exposure to silicon carbide whisker at concentration of 0.5×10^{-6} fiber/ml (WHO) or 1.7×10^{-6} fiber/ml (EPA) or by exposure to potassium titanate whisker at concentration of 5.7×10^{-6} (WHO) or $17x.4 \times 10^{-6}$ (EPA) (Table 2). Similarly, regulatory concentrations were calculated for these man-made mineral fibers. Permissible exposure limits (a time weighed average, OSHA) were estimated 0.09 for silicon carbide whisker1 and 0.21 for potassium titanate whisker1 by referring 0.2 fiber/ml for chrysotile. Threshold limit values (a time weighed average, ACGIH) were 0.9 fiber/ml for silicon carbide whisker1 and 2.2 fiber/ml for potassium titanate whisker1 by referring 0.5 fiber/ml for chrysotile. It is suggested that the aerodynamic feature, specific gravity and solubility must be considered for the evaluation of the biological effects of fibrous materials, because these factors affect deposition, transportation and dissolution of fibers after exposure[22]. The estimations on silicon carbide whisker1 and potassium titanate whisker 1 were speculated by the results from intraperitoneal experiment on JFM standard fibers. Therefore, it is possible to obtain a different dose response relationship on these fibers by another route of exposure or when the fiber having different size is used. These uncertainties should be cleared in the future, however, it is important for the risk assessment in industrial environment to recognize the similar or higher carcinogenic potential of man-made mineral fibers to asbestos.

Conclusions

1. Peritoneal mesotheliomas were induced in female F344 rats after intraperitoneal administration of 4 types of JFM standard fibers such as silicon carbide whisker, potassium titanate whisker, refractory fiber 1 or refractory fiber 2.
2. Carcinogenic potency was estimated 2.4 times for silicon

carbide whisker and 0.23 times for potassium titanate whisker of UICC chrysotile B.

3. Present results suggest that reasonable assessment can be established by the estimation of relative carcinogenic potency for man-made mineral fibers based on the data from the animal experiment.

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