

Induction of mesothelioma after intrapleural inoculation of F344 rats with silicon carbide whiskers or continuous ceramic filaments

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Abstract

Objective—To find whether continuous ceramic filaments (CCFs) and silicon carbide whiskers (SiCWs), which are used in many industries as reinforcing materials in advanced ceramic composites, are carcinogenic in the intrapleural inoculation assay.

Methods—Samples of SiCWs, CCF, International Union Against Cancer crocidolite, or saline were injected into the pleural cavities of female F344/N rats to find whether the samples of SiCW and CCF had the potential to induce mesotheliomas after the direct application of the materials to the surface of the pleural mesothelium.

Results—Rats injected with two of the three individual samples of SiCW or the crocidolite had significantly reduced life spans compared with the rats treated with saline, CCFs, or the third SiCW sample. Rats treated with either of the two SiCW samples or crocidolite developed mesotheliomas. By contrast, rats treated with saline or CCF did not. The two SiCW samples that induced shortened life spans also induced a higher rate of mesothelioma (87%–90%), than the crocidolite (57%) and the third SiCW sample (23%).

Conclusion—SiCWs but not CCFs could induce mesotheliomas after intrapleural injection in rats. The difference in biological activity between the SiCW samples could not be explained on the basis of their physical dimensions or biological activity toward cultured cells. Results from this study indicated that SiCWs should be handled with care as they might be carcinogenic if inhaled. However, there is controversy as to whether results of intrapleural injection assays are sufficient to determine a fibre's carcinogenic activity. The results also showed that a collection of fibrous materials such as SiCWs could have considerably different biological activities despite similar physical dimensions.

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Keywords: silicon carbide whiskers; mesothelioma; animal bioassay

Continuous ceramic filaments (CCFs) and silicon carbide whiskers (SiCWs) are used in the aerospace, automotive, and power generation

industries as reinforcing materials in advanced ceramic composites. The SiCWs can also occur as a byproduct of silicon carbide production for the abrasive industry.¹ The SiCWs are single crystal structures that can have a fine fibrous morphology similar to that of amphibole asbestos. The diameter of this fine SiCW is typically $\leq 1 \mu\text{m}$. There is concern that exposure to SiCWs may be associated with pulmonary fibrosis, lung cancer, and mesothelioma, biological effects known to occur after exposure to asbestos. In vitro studies in cultured cells have shown that SiCWs possess similar biological activity to asbestos.^{2,5} Alteration in normal cell division may be the mechanism that induces cellular damage by these whiskers.⁶ Lapin *et al*⁷ showed that in rats a three week exposure by inhalation of 500–7500 SiCW/cm³ results in pleural thickening and increased cellularity in the lungs. Vaughan *et al*⁸ showed that intratracheal instillation of SiCWs in rats results in granulomas, as does crocidolite asbestos 18 months after exposure. Intrapleural injection of SiCWs in rats has produced equivocal results. Pott *et al*⁹ showed that SiCWs produce a high incidence of abdominal mesotheliomas; Vaughan *et al*⁸ reported that intraperitoneal injection of SiCWs does not result in any mesotheliomas.

Continuous ceramic filaments are coarse fibres with diameters of 10–30 μm . In vitro studies in cultured cells have shown that CCFs do not show any adverse biological activity toward cultured cells.⁵ However, inhalation and intratracheal studies have not been reported for CCFs; such experiments would be impractical because of their size. Large diameter glass fibres similar to CCFs do not produce tumours after intrapleural implantation.¹⁰ Glass filaments do not produce a notable incidence of mesothelioma after intraperitoneal inoculation of high doses (up to 150 mg) of the material.⁹

The present study was conducted to find the in vivo potential of SiCWs and CCFs to induce mesothelioma. The in vivo assay chosen was intrapleural injections, which provide a useful screen to identify fibrous materials that do not produce a neoplastic response.¹¹ This approach does not produce false positive results as can happen when intraperitoneal injections are used.¹² The intrapleural assay was simpler to conduct than inhalation exposures. However, fibres that produce a positive response in the intrapleural injection assay should be further assessed by inhalation studies.¹¹ Inhalation bioassays have the advantage over injection assays in that they allow for the

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Table 1 Physical characteristics of fibre samples

Sample	Length (μm) mean SEM	Diameter (μm) mean SEM	Fibre n/mg	Specific surface area (m^2/g)	Specific gravity
SiCW 1	4.5 (0.23)	0.42 (0.02)	7.6×10^6	3.0	3.4
SiCW 2	20.1 (1.01)	0.75 (0.02)	1.6×10^5	1.4	3.3
SiCW 3	6.6 (0.40)	0.32 (0.01)	1.1×10^7	3.6	3.2
Crocidolite	2.1 (0.31)	0.12 (0.01)	3.6×10^9	7.0	3.2
CCF (PRD-166)*	40-100	12	ND	1.5	4.3

*Values determined approximately by light microscopy.
ND = Not determined.

normal defence mechanism of the lung to operate as well as mechanisms of clearance and dissolution.

Materials and methods

The samples used in the present study were identical to those used in previous cell culture studies.⁵ Table 1 shows the characteristics of these samples. The distributions of lengths and diameters (table 2) were obtained from a bivariate analysis.⁵ Two hundred and twenty female F344/N rats, 6 to 8 weeks old, from the institute's barrier maintained colony were randomly allocated to the experimental groups (table 3). Each rat was injected intrapleurally with either saline (0.4 ml), or SiCW samples 1, 2, or 3 (20 mg); International Union Against Cancer crocidolite (20 mg); or PRD-166 (a CCF) suspended in saline (0.4 ml). The rats were killed by intraperitoneal injection of sodium pentobarbitone when moribund or when 20% of the longest surviving group of rats (injected with PRD-166) remained alive. All rats were necropsied and examined for gross lesions. The spleen, kid-

neys, liver, heart, lungs, trachea, and larynx were removed and fixed in 10% neutral buffered formalin (NBF). The lungs were inflated with NBF to 30 cm H₂O. The diaphragm and any identifiable lesion on the parietal chest wall and elsewhere in the thoracic cavity were also removed and immersed in 10% NBF. Fixed tissue was prepared for conventional paraffin embedding and examined by light microscopy with sections stained with haematoxylin and eosin. Only lung and thoracic lesions were routinely examined. The incidence of tumours was determined from the number of rats that developed pleural mesotheliomas. The animal survival data were analysed by the Kaplan-Meier method (SAS LIFE TEST Procedure; SAS Institute, Cary, NC) incorporating pairwise comparisons to find whether the survival of exposed rats was significantly different ($P \leq 0.05$) from that of rats treated with saline.

Results

The first rat died from respiratory distress at 166 days after inoculation with SiCW 2, and the first tumour was found 273 days after inoculation with SiCW 2. Rats inoculated with SiCW 1 or 2 had the shortest life spans (figure). Rats treated with crocidolite, the positive control, had intermediate life spans compared with the rats treated with the SiCW 1 or 2 and with rats treated with saline control (figure). Rats treated with PRD-166 had life spans similar to the rats treated with saline. Rats treated with SiCW 3 had life spans between the rats treated with saline and crocidolite. The life

Table 2 Length/diameter distribution (%) for SiCW samples

Diameter (μm):	0-0.9	0.1-0.19	0.2-0.29	0.3-0.39	0.4-0.59	0.6-0.79	0.8-0.99	> 1.0
SiCW 1	4.1	17.0	26.2	16.7	14.8	10.1	6.2	4.6
SiCW 2	—	1.7	8.1	15.2	18.5	18.1	13.2	25.4
SiCW 3	9.29	23.36	17.07	21.57	21.49	6.00	0.60	0.60
Length (μm):	0-1.9	2.0-3.9	4.0-5.9	6.0-7.9	8.0-9.9	10.0-14.9	> 15.0	
SiCW 1	25.2	32.2	17.9	7.9	8.0	5.5	3.0	
SiCW 2	—	—	7.6	8.1	9.5	24.2	46.9	
SiCW 3	21.26	30.24	12.21	8.70	8.69	10.18	8.70	

The fraction of rats surviving intrapleural injection varied with the different fibre inoculations. Saline and PRD-166 produced a similar response, which was different from that of crocidolite, the positive control. SiCWs 1 and 2 showed a similar response, also different from crocidolite.

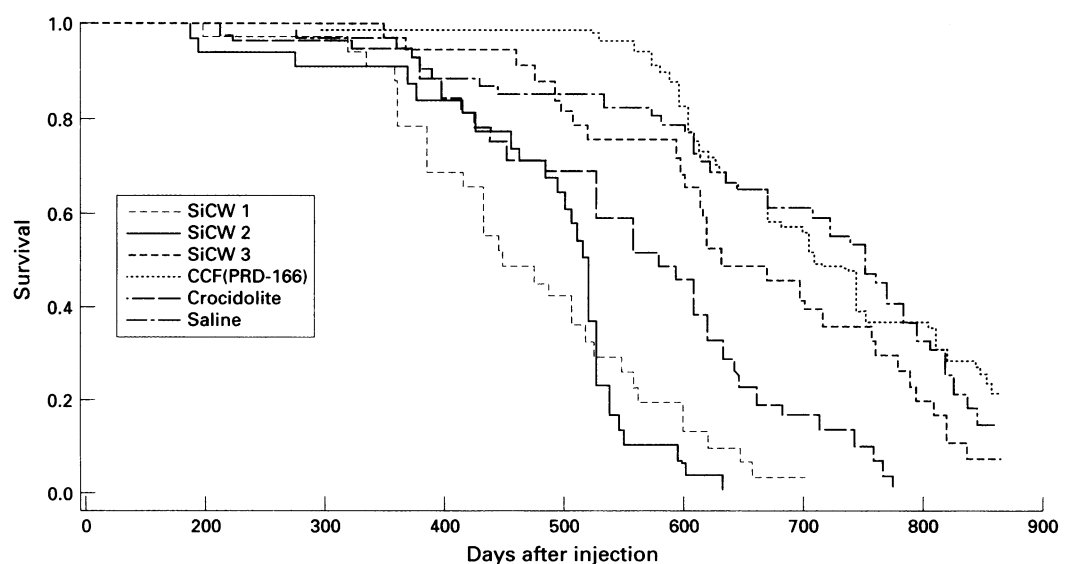


Table 3 Occurrence of pleural mesotheliomas by treatment group

Sample	Animals n	Animals with mesothelioma	Animals with mesothelioma (%)	Time of first tumour	Mean time to tumour (days (SEM))	Median survival time (days after injection (SEM))†
Saline	50	0	0	—	—	753 (25)
CCF (PRD-166)	50	0	0	—	—	708 (18)
SiCW 1	30	27	90	320	465 (25)	453 (21)*
SiCW 2	30	26	87	273	499 (15)	519 (20)*
SiCW 3	30	7	23	349	651 (30)	635 (26)
Crocidolite	30	17	57	416	608 (23)	548 (24)*

*P ≤ 0.05 v controls with pairwise comparisons with either generalised Savage or Wilcoxon test statistics corrected for multiple (five) comparisons.

†Determined with the SAS LIFE TEST procedure (SAS, Cary, NC).

spans of the rats treated with SiCW 1 or 2 and crocidolite were significantly shorter than those of the animals treated with saline. The life spans of the rats treated with PRD-166 or SiCW 3 were not significantly different from those of the rats treated with saline.

Histopathological examination of the thoracic contents from all rats showed that the three samples of SiCW varied in their ability to induce pleural mesotheliomas (table 3). The SiCWs 1 and 2 resulted in 90% and 87% respectively, of the treated rats developing pleural mesothelioma. Twenty seven per cent of the rats treated with SiCW 3 and 57% of those treated with the positive control (crocidolite) developed pleural mesotheliomas. No tumours were identified in the animals treated with saline or PRD-166 (table 3). The tumours identified, with one exception, were sarcomatous in appearance and, in all but one case, involved the visceral pleura. Fibres were found in sections from all treatment groups except those animals treated with saline or PRD-166.

Discussion

These results showed that SiCWs could induce mesotheliomas when introduced into the pleural cavity, and that two SiCW samples were at least as carcinogenic as amphibole asbestos when given this way. The notable responses with two of the three SiCW samples indicated that SiCW should be handled with care and treated as a suspected carcinogen until the results of long term inhalation studies are available. The results of this study also showed that CCFs (PRD-166) did not induce mesotheliomas when injected into the pleural cavity of rats. This information combined with the negative *in vitro* data⁵ and the fact that the diameter of this material precludes inhalation showed that exposure to CCFs should not represent a significant health hazard. Shannon *et al*¹³ showed that the mortality of workers with glass filament is below that expected, and deaths due to lung cancer did not increase significantly. However, the extremely low airborne concentrations encountered in a continuous glass filament factory may preclude the ability of any workplace epidemiological study to investigate the potential risk of respiratory cancer from continuous glass filaments.¹⁴

The amount of published information providing details about the toxicological properties of SiCWs and CCFs is limited. The

results of the present intrapleural study support results from previous animal studies. Three studies have been reported involving SiCW implanted into the pleural cavity¹⁰ and injected into the peritoneal cavity.^{8,9} Pott *et al*⁹ gave an unequivocal positive result although many of the animals died of an infectious lung disease. Also, the intraperitoneal injection may be overly sensitive to fibres and particles such as silica.¹¹ By contrast, the study of Vaughan *et al*⁸ reported that intraperitoneal injection of SiCWs does not result in the development of mesotheliomas. However, Vaughan *et al*⁸ did not use a positive control such as crocidolite in their studies. Stanton *et al*¹⁰ reported a high incidence of pleural tumours in rats implanted with SiCWs. In a subchronic inhalation study in rats, pleural thickening and increased cellularity of the lung parenchyma have been found at 26 weeks after a 13 week exposure to a high concentration of SiCW (6 h/day, 5 days/week, 500–7500 fibres/cm³).⁷ The fibres in this study had an average diameter of 0.58 µm and average length of 4.7 µm. The relevance of the pleural thickening is not known; however, it has been reported to occur after exposure to erionite in rats¹⁵ and refractory ceramic fibres in hamsters.¹⁶ In studies with erionite and refractory ceramic fibres, high incidences of mesotheliomas have been reported.^{16,17}

Information on human exposures to SiCWs is also sparse. Exposure to SiCWs has been reported in workers exposed to silicon carbide in the abrasive industry as illustrated by the presence of SiCWs in lung tissue^{18,19} and in the working environment.^{20,21} Whether these fibres contribute to the pneumoconiosis associated with workers involved in silicon carbide production is unknown.²² A similar situation has been reported in the hard metal industry where the presence of tungsten oxide whiskers may contribute to the pneumoconiosis noted in some workers employed in the production of hard metal.²³

In the present study, the differences in the biological activity of the three SiCW samples could not be explained by differences in fibre morphology. The fibre length/diameter distributions were dissimilar. The SiCW 2 contained a disproportionate number of long, thin fibres, which are thought to be important in fibre carcinogenesis¹⁰ and this sample was highly carcinogenic. However, SiCW 1 had a similar carcinogenic potency as SiCW 2 but had a lower fraction of fibres ≥ 20 µm in length than SiCW 3, which was significantly less carcinogenic than either SiCW 1 or 2. The

difference in reactivity could not be explained on a fibre number basis as SiCW 3 (the least reactive SiCW) contained the highest number of fibres in the inoculum. Although fibre dimensions are a critical factor for carcinogenesis these results indicate that other aspects of a fibre must also be important. Surface chemistry may be an important factor in the development of tumours; however, the ability of SiCWs to induce hydroxyl radicals in vitro is limited compared with the natural crystalline minerals.²¹ In the case of SiCWs, surface chemistry may have a limited influence on their carcinogenic potency. The physical surface of the SiCW samples was different and it is unclear whether this might contribute to their divergent biological activity.

The in vitro activity of the three SiCW samples did not indicate the in vivo biological activity. In two cell lines (LEC and A549) SiCW 1 was the least toxic whereas SiCW 3 was the most toxic; however, SiCW 2 was the most toxic towards rat alveolar macrophages and rat tracheal epithelial cells.⁵ These results showed that physicochemical characterisation and in vitro cytotoxicity studies cannot necessarily predict accurate in vivo activity.

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