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THE COMPARATIVE EFFECTS OF THREE CHRYSOTILES BY INJECTION AND INHALATION IN RATS

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In previous studies, we used chrysotile asbestos dusts which were specially prepared for experimental studies (Wagner et al., 1973, 1974). We decided, therefore, to compare their biological effects with those of the smallest particle-sized commercial chrysotile. One particular asbestos, the grade 7 Canadian fibre, is imported into the United States in large amounts, mainly as a filler for paints and plastic tiles (Wright, 1969). It is the final product from the Canadian asbestos mines.

MATERIALS AND METHODS

The three chrysotile samples and one talc sample chosen for the intrapleural and inhalation experiments were as follows:

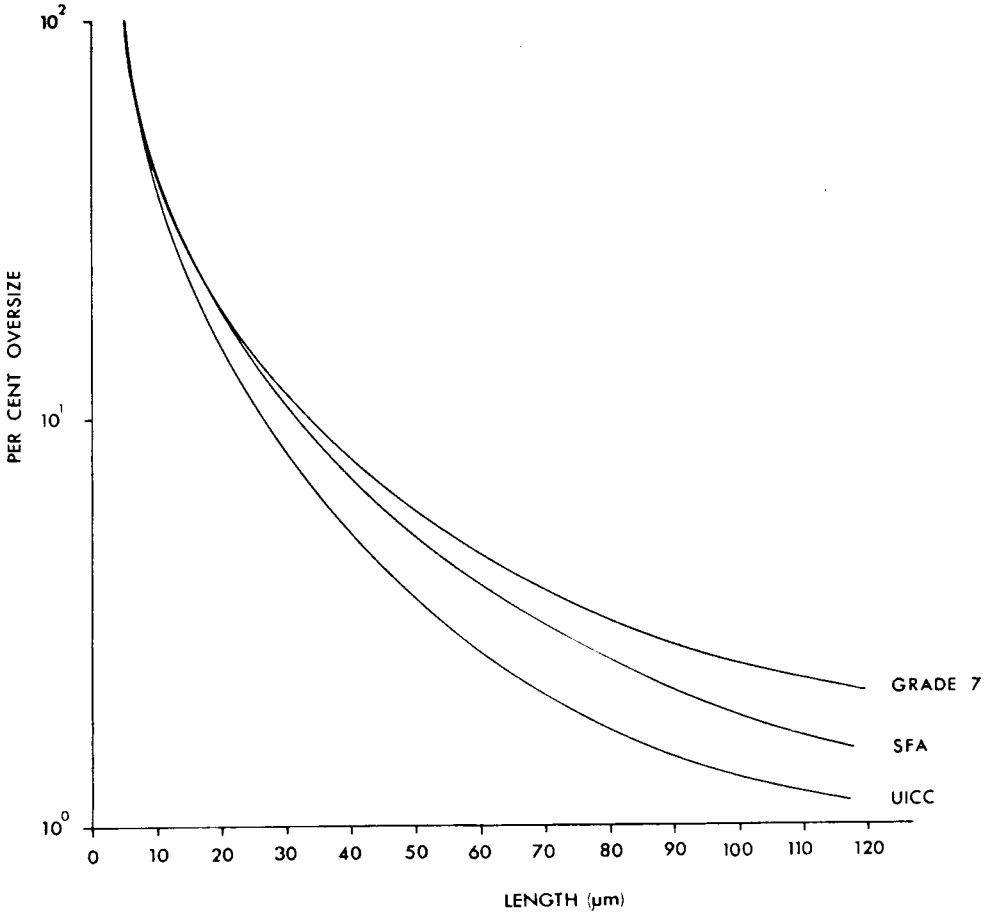
1. SFA chrysotile; a specially ground sample from the Normandie Mine in Canada
2. UICC Canadian chrysotile standard reference sample
3. Grade 7 chrysotile from the Bells Mine in Canada
4. Cosmetic grade talc 00000 code from Italy

The UICC Canadian chrysotile standard consists of a mixture of asbestos from eight Canadian mines (Timbrell et al., 1968) and has been prepared by milling to represent a 100% respirable material. Respirable fractions of the SFA, grade 7 and talc samples were prepared for the inoculation experiment. None of the three chrysotile samples was pure. The UICC sample was found by X-ray examination to contain a large proportion of the minerals brucite and pyroaurite, together with the silicates talc and chlorite. The SFA sample also contained pyroaurite and some talc and chlorite; while the Bells grade 7 sample contained brucite, chlorite, talc and also traces of tremolite. These impurities were present in the bulk material and at similar levels in the respirable airborne dust generated from each sample. The talc sample contained the mineral impurities chlorite and quartz (Wagner et al., 1977).

Size distributions and fibre counts are very dependent on the conventions adopted in determining the length of the fibres and on the interpretation of aggregates. The size distributions of these chrysotile clouds were obtained from several samples collected on Nucleopore filters (pore size, 0.2 μm), which were examined in the scanning electron microscope. The conventions adopted for counting and determining fibre length were those described by the Australian Department of Health (1976). Chrysotile fibres frequently are of non-uniform diameter, and in these evaluations the maximum diameter was recorded. Fibres shorter than 5 μm are considered to make a minimal contribution to lung damage, and the cumulative length and diameter distributions of the chrysotile fibres longer than 5 μm are shown in Figures 1 and 2. The grade 7 chrysotile fibres were longer than the SFA fibres, which were longer than those of the UICC chrysotile sample. The fibre diameters also differed: the UICC chrysotile fibres were narrower than those of the grade 7 and the SFA fibres. The numbers of fibres longer than 5 μm and the numbers of respirable particles per ml in the dust clouds are shown in Table 1. The UICC sample contained more fibres, because it was finer, and also contained fewer particles than the other two chrysotiles.

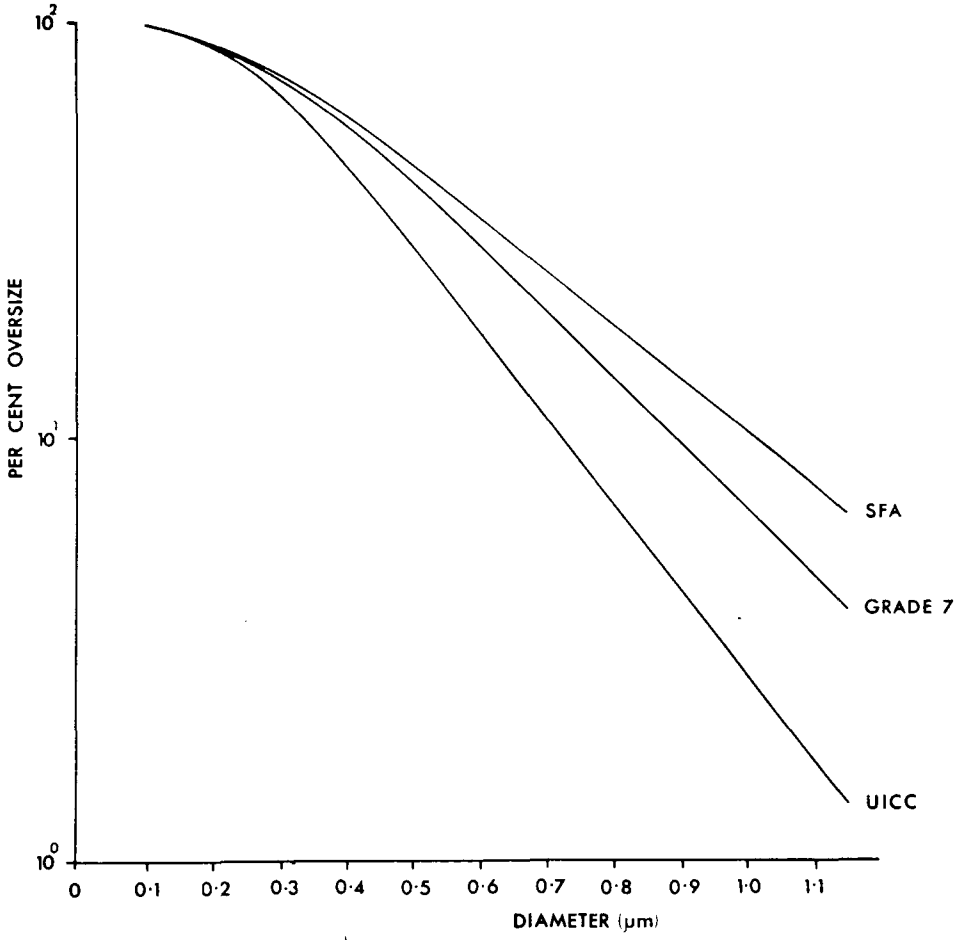
The experimental animals were barrier-protected, caesarian-derived rats of the Wistar strain. In the first experiment, 20 mg of experimental material, made up in physiological saline, were injected into the right pleural cavity. There were 48 rats, 24 of each sex, injected with each material and also 48 controls injected with saline. The rats were between 8 and 14 weeks old when injected and were allowed to live out their lives.

FIG. 1. LENGTH DISTRIBUTIONS OF FIBRES LONGER THAN 5 μm



In the second experiment, rats were exposed in inhalation chambers. There were 96 rats exposed to each dust; 48 for 3 months, 24 for 6 months and 24 for 12 months. The same number of controls were kept in ordinary cages. At the start of exposure, most of the rats were between 6 and 8 weeks old. The dust clouds were generated for seven-and-a-half hrs a day on five days a week. The mean respirable dust concentration was 10.8 mg/m^3, and the cumulative doses, i.e., the products of concentration and time, were 4100, 8200 and 16,400 mg/m^3 hrs for the 3-months, 6-months and 12-months exposures. Ten days after the end of each exposure a few rats were sacrificed; further sacrifices were made one year later for the assessment of fibrosis and dust in the lungs.

FIG. 2. DIAMETER DISTRIBUTIONS OF FIBRES LONGER THAN 5 μm



Fuller details of our experimental methods are given in our earlier papers (Wagner et al., 1973, 1974, 1977).

Table 1. Numbers of fibres and particles in dust clouds

Dust	Fibres/ml longer than 5 μm	Particles/ml (1-5 μm)
SFA chrysotile	430	669
Grade 7 chrysotile	1020	745
UICC chrysotile	3750	338
Italian talc	12	1300[a]

[a] Respirable particles > 1 μm

RESULTS

Intrapleural injection experiment

The mean lengths of survival and numbers of mesotheliomas are given in Table 2. Most mesotheliomas were obtained with SFA chrysotile, and the survival of that group was shortest. After allowing for the different survival times, the SFA was about twice as carcinogenic as the grade 7, which was three times as carcinogenic as the UICC sample. The carcinogenicity of the grade 7 was significantly ($P < 0.05$) different from that of each of the other two chrysotile samples.

Table 2. Results of intrapleural injection experiment in rats

Dust	Mean survival after injection (days)	Number of animals with mesotheliomas (out of 48)
SFA chrysotile	598	18
Grade 7 chrysotile	664	13
UICC Canadian chrysotile	683	5
Italian talc	655	0
Saline controls	691	0

Inhalation experiment

The mean weights of dust in the lungs of rats are shown in Table 3. Deposition and retention of dust in the lungs were low for all the chrysotiles, and the weights showed poor correlation with length of exposure. There was evidence of elimination of chrysotile during the year after exposure ended. In contrast, much more of the talc was retained in the lungs; the amount was related to the length of exposure, and there was no evidence of elimination.

Table 3. Weights of dust (mg) in lungs of rats at end of exposure by inhalation and one year later

Dust	Time	Length of exposure (no. of rats)		
		3 months	6 months	12 months
SFA chrysotile	End of exposure	0.4 (8)	0.3 (6)	0.6 (6)
	1 year later	0.3 (8)	0.3 (4)	< 0.1 (4)
Grade 7 chrysotile	End of exposure	1.4 (8)	1.5 (6)	2.7 (6)
	1 year later	< 0.1 (8)	0.3 (4)	0.3 (3)
UICC chrysotile	End of exposure	0.4 (8)	0.1 (6)	0.8 (6)
	1 year later	< 0.1 (8)	0.3 (4)	0.4 (2)
Italian talc	End of exposure	2.8 (8)	4.7 (6)	12.3 (6)
	1 year later	1.6 (8)	5.0 (4)	14.1 (4)

The mean fibrosis scores are shown in Table 4. There was progression of fibrosis during the first year after the end of exposure for 6 and 12 months for all dusts. However, this progression did not occur after 3 months' exposure, and in fact, on average, there was regression. The UICC chrysotile resulted in at least as much fibrosis as the other two chrysotiles and the talc on all six occasions ($P = 0.06$). The SFA and grade 7 chrysotiles and the talc gave very similar amounts of fibrosis.

Details of the lung tumours found at autopsy are given in Table 5. The UICC chrysotile produced 20 tumours, 10 of which were malignant; SFA produced 13, four of which were malignant, including the only mesothelioma in the experiment; the grade 7 produced nine tumours, only one of which was malignant. The tumour yield was significantly greater with UICC chrysotile than with grade 7 ($P < 0.05$, all tumours; $P < 0.01$, malignant tumours).

Table 4. Mean fibrosis scores[a] at end of exposure by inhalation and one year later

Dust	Time	Lengths of exposure		
		3 months	6 months	12 months
SFA chrysotile	End of exposure	2.8	3.0	3.2
	1 year later	2.2	3.2	4.2
Grade 7 chrysotile	End of exposure	3.1	2.9	3.3
	1 year later	2.1	3.2	4.3
UICC chrysotile	End of exposure	3.1	3.3	3.7
	1 year later	2.7	3.4	5.0
Italian talc	End of exposure	2.2	2.7	3.4
	1 year later	2.4	3.4	4.6
Controls	End of exposure	1.8	1.9	1.3
	1 year later	1.6	1.5	1.9

[a] 1: nil, 2: minimal, 4: slight, etc. (Wagner et al., 1974)

Table 5. Lung tumours in rats after exposure by inhalation

Dust and length of exposure	No. of rats at risk[a]	Mean survival after start of exposure (days)	No. of rats with lung tumours				
			Adenoma	Adenomatosis	Adeno-carcinoma[b]	Squamous carcinoma[b]	Mesothelioma
SFA chrysotile							
3 months	40	680	0	0	0	0	1
6 months	18	772	1	2	1	0	0
12 months	22	670	3	3	2 (2)	0	0
Total	80	698	4	5	3 (2)	0	1
Grade 7 chrysotile							
3 months	39	671	0	1	0	0	0
6 months	18	721	5	0	0	0	0
12 months	24	598	1	1	0	1	0
Total	81	661	6	2	0	1	0
UICC chrysotile							
3 months	40	684	3	0	1 (1)	0	0
6 months	18	761	6	0	3 (1)	1 (1)	0
12 months	23	618	0	1	2	3 (3)	0
Total	81	682	9	1	6 (2)	4 (4)	0
Italian talc							
3 months	39	690	0	0	0	0	0
6 months	18	706	0	0	0	0	0
12 months	24	616	2	0	0	0	0
Total	81	672	2	0	0	0	0
Controls	71	678	1	0	0	0	0

[a] Rats that survived at least 300 days after start of exposure

[b] Numbers in brackets are those with metastases, defined as spread outside the lungs.

DISCUSSION

These experiments demonstrate the differences in biological effects which can occur with overtly similar chrysotile samples. In agreement with our previous work (Wagner et al., 1973, 1974), UICC chrysotile produced more lung tumours after inhalation than might be expected on the basis of the injection results. Problems of aggregation of fibres in the bulk samples could have resulted in the size distributions of the injected materials being quite different in solution from those measured for the clouds in the inhalation chambers, and this could be part of the explanation of the relative difference between the two experiments.

At the last symposium (Wagner & Berry, 1973), we said that injection and inhalation experiments supplement one another, and that injection experiments were 'the more readily interpretable' because they measured the biological activity unaffected by differential penetration of dust through the airways and alveoli. While the former statement remains true, we now have less confidence in the latter in view of our ignorance of the reasons why the two types of experiments give different relative hazards of different samples, both in the experiments described in this paper and in our previous experiments. There are clear dangers in extrapolating from injection experiments to man; a positive result in an injection experiment may be regarded as an indication that an inhalation experiment be carried out. This point is of particular relevance to present knowledge on the carcinogenicity of man-made glass fibres.

SUMMARY

Three samples of chrysotile, UICC Canadian chrysotile, a grade 7 Canadian chrysotile and a super fine sample (SFA) also from a Canadian mine, were compared in animal experiments using rats of the Wistar strain. All the materials contained impurities. The average length and diameter of the fibres contained in the UICC chrysotile cloud were less than for the other two chrysotiles, but the higher fibre count meant that the UICC cloud contained more fibres of all lengths.

In the first experiment, groups of 48 rats were injected intrapleurally with 20 mg of respirable dust. Mesotheliomas occurred with all samples; 18 with SFA, 13 with grade 7, and five with UICC chrysotile.

In the second experiment, rats were exposed to a respirable cloud of about 1 mg/m^3 for 35 hours a week. Groups of 48 rats were exposed for three months, 24 for six months and 24 for 12 months. Malignant lung tumours occurred with all the dusts; 10 with UICC chrysotile, 4 with SFA, and 1 with grade 7. However, only one of these tumours, obtained with SFA, was a mesothelioma.

RESUME

Lors d'expériences sur des rats de la souche Wistar, les auteurs ont comparé trois échantillons de chrysotile, de chrysotile canadien de l'UICC, de chrysotile canadien de classe 7 ainsi qu'un échantillon superfin (SFA) provenant également d'une mine canadienne. Toutes ces substances contenaient des impuretés. La longueur et le diamètre moyens des fibres contenues dans les poussières de chrysotile de l'UICC étaient inférieurs à ceux des deux autres chrysotiles, mais le plus grand nombre de fibres indiquait que le chrysotile de l'UICC contenait davantage de fibres de toutes les longueurs.

Dans la première expérience, des groupes de 48 rats ont reçu par voie intrapleurale 20 mg de poussières respirables. Des mésothéliomes sont apparus avec tous les échantillons; 18 pour le SFA, 13 pour le chrysotile de classe 7 et cinq pour le chrysotile de l'UICC.

Lors de la deuxième expérience, des rats ont été exposés à un nuage de poussières respirables d'environ 1 mg/m^3 pendant 35 heures par semaine. Des groupes de 48 rats ont été exposés pendant trois mois, 24 pendant six mois et 24 pendant 12 mois. Toutes les poussières ont provoqué des tumeurs malignes du poumon; 10 pour le chrysotile de l'UICC, 4 pour le SFA et une pour le chrysotile de classe 7. Mais une seule de ces tumeurs, induite par le SFA, était un mésothéliome.

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