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Carcinogenic effect of mineral fibres in inhalation studies

By J. M. G. Davis*

Since the discovery by Lynch & Smith (1) and Wagner et al (2) that asbestos fibres cause both bronchial carcinomas and mesotheliomas in asbestos workers, many animal experimental studies have been undertaken in order to determine how these dusts exert their carcinogenic effects. Although Wagner in 1960 (3) showed that mesotheliomas could be produced in experimental animals by intrapleural injection, early attempts at producing tumours by inhalation techniques were unsuccessful, probably because they were not continued for a sufficiently long time. However, in 1967 Gross et al (4) exposed rats to very high levels of chrysotile dust and produced large numbers of tumours, mostly adenocarcinomas. Similar results were reported by Reeves et al in 1971 and 1974 (5 & 6) and Wagner et al in 1974 (7). Wagner's study used the UICC reference samples of chrysotile, crocidolite, amosite and anthophyllite was large enough for detailed comparisons to be made between the carcinogenic effects of the different dust types. It was found that Rhodesian chrysotile produced the most bronchial carcinomas and amosite the least. Canadian chrysotile, crocidolite and anthophyllite all showed similar levels of carcinogenicity. Only a few mesotheliomas were produced with Canadian chrysotile and crocidolite producing slightly more than amosite and anthophyllite while Rhodesian chrysotile, which produced the most carcinomas, produced none at all.

The surprising factor in these results was that crocidolite had been assumed from human studies to be the most carcinogenic type of asbestos, especially in relation to mesothelioma production, and this was not confirmed. The results from these inhalation studies did, however, agree with previous work by Wagner et al in 1973 (8) using injection techniques where crocidolite had again appeared to be no more carcinogenic than chrysotile.

From Wagner's inhalation studies some interesting data were produced regarding the effects of dust dose resulting from variations in the period of exposure. For these experiments exposure periods of one day, three months, six months, twelve months and twenty four months had been used. If the total number of lung tumours was considered, it was found that for all the asbestos types, there was a moderately good dose response effect. An occasional tumour was produced after only one day's dusting but from three months onwards tumour numbers increased with dose. However, a breakdown of the tumour types produced revealed an interesting finding. The maximum number of benign adenomas had been produced for all dust types by only three months of dusting and in some cases increasing the dose beyond this point greatly reduced the number of adenomas. For adenocarcinomas, however, the number of tumours increased up to twelve months of dusting but there was little difference between twelve and twenty four months. For squamous carcinomas on the other hand, by far the majority of these tumours developed from animals dusted for twenty four months. From this it is possible to conclude that while a moderate dose of asbestos is sufficient to produce adenomas, larger doses produce carcinomas with squamous carcinomas in particular being the response to excessive dust. At present there is no evidence as to whether all rat lung tumours start as benign adenomas which only progress if the carcinogenic stimulus is maintained or whether prolonged dusting causes tumours that are malignant from the start.

While Wagner had studied the effects of dust doses graded by time, Davis et al in 1978 (9) used constant exposure periods to explore the effects of mass versus fibre number for the UICC samples of crocidolite, amosite and Rhodesian chrysotile. At both equal mass, which at 10 mg/m^3 was the same as the dose used by Wagner, and at equal fibre number where the mass doses of chrysotile, crocidolite and amosite were respectively, 2 mg, 5 mg and 10 mg/m^3, it was found that chrysotile was the most carcinogenic dust. Both the chrysotile dust clouds had produced significantly more pulmonary adenomas and chrysotile

*J.M.G. Davis, M.A., Ph.D., Institute of Occupational Medicine, 8 Roxburgh Place, Edinburgh EH8 9SU/U.K.

was the only dust to produce carcinomas. (The numbers of tumours produced in this and subsequent inhalation studies by this group are listed in TABLE 1). As with Wagner's work, there was less mass dose effect for adenoma production, where the 10 mg and 2 mg chrysotile clouds had produced seven and six tumours respectively than for carcinomas where the equivalent figures were eight and two. While dust clouds of equal fibre number had been produced by counting all fibres > 5 um in length, it was found that the chrysotile cloud had the highest number of fibres over 20 um in length and it was suggested that this was probably the reason for the increased carcinogenicity of this material.

In 1980 Davis et al (10) further explored the effects of asbestos dose on tumour production using the UICC reference samples of both amosite and Rhodesian chrysotile. In this study the same mass of each dust was administered for a year either at an even dose level over a five day week or as a peak dose all administered on one day during the week. Since there is a limit to the respirable mass that can be produced with a chrysotile cloud before dust flocculation begins to produce non-respirable particles, the chrysotile study had to be undertaken at a baseline dose of 2 mg/m^3 while the amosite baseline used was 10 mg/m^3 to give a better comparison with previous studies. Little or no difference in tumour production was seen between the peak and even dosing regimes but once again chrysotile appeared more carcinogenic than amosite considering the different mass doses. Further inhalation studie were reported by Davis et al in 1980 (11) in which samples of chrysotile and amosite dust collected from the factory environment were admin stered to rats. Unfortunately each sample was c taminated by non-asbestos material, especially the chrysotile, so that while the respirable mass of t dust clouds was the standard 10 mg/m^3 of air, the asbestos mass was only 6 mg and 9 mg for the chrys tile and amosite respectively. The factory chrys tile fibres were fewer in number but thicker than UICC chrysotile while the factory amosite fibres tended to be both longer and thicker than UICC amo site. With the chrysotile once again, the lower dose of factory dust (6 mg) had produced as many benign adenomas as previously found with a higher dose (10 mg) of UICC chrysotile while the number of carcinomas was reduced in the experiment using factory dust. The amosite studies confirmed the carcinogenicity of this material in rats since no tumours at all were produced by the factory amosit dust.

In injection studies Wagner et al (8) had found that a specially well separated 'superfine' sample of chrysotile was the most carcinogenic of a numbe or asbestos samples examined. In 1980 (12), the same authors reported inhalation studies in which the effects of this 'superfine' material had been compared with UICC Canadian chrysotile and a fine commercial grade of Canadian chrysotile which most closely compared to the 'superfine' specimen. In this study the UICC chrysotile sample produced the

Table 1. Tumour production during long-term inhalation studies in which rats were treated with a number of samples of asbestos and other mineral fibres. Davis et al. 1978-82

Dust type and mass dose	UICC Chrysotile A 10 mg/m^3	UICC Chrysotile A 2 mg/m^3	UICC Crocidolite 10 mg/m^3	UICC Crocidolite 5 mg/m^3	UICC Amosite 10 mg/m^3	UICC Chrysotile A peak dosing 2 mg/m^3 equivalent	UICC Amosite peak dosing 10 mg/m^3 equivalent	Factory Chrysotile 10 mg/m^3	Factory Amosite 10 mg/m^3	Wet Dispersed Chrysotile 4 mg/m^3	Wet Dispersed Chrysotile 4 mC/m^3 (reversed daylight)	Tremolite 10 mg/m^3	Brucite 10 mg/m^3	Ceramic fibre 10 mg/m^3
Fibre number/ ml of air > 5 µm	1950	390	860	830	550	-	-	200	700	110	120	1500	225	95
Adenomas	7	6	1	2	2	4	4	8	2	7	5	2	3	1
Total carcinomas	8	2	0	0	0	2	2	3	0	10	12	16	2	3
Adeno-carcinomas	6	1	0	0	0	1	2	2	0	5	5	8	1	1
Squamous carcinomas	2	1	0	0	0	1	0	1	0	5	7	8	1	2
Mesotheliomas	0	1	0	1	0	0	0	0	0	4	1	2	0	0

most adenomas and by far the most carcinomas, a finding which appeared to be related to a much higher number of fibres greater than 5 µm in length in this sample. These results suggest that whereas the number of long fibres may be very important in producing pulmonary tumours, they are less so in injection studies. However, since the dust samples in this study were sized only after collection from the dust clouds on nuclepore filters it is possible that the fibres in the material used for injection were different.

In 1981 Davis et al (13) reported the results of inhalation studies with chrysotile prepared by the wet dispersion process. This material produces very little dust when handled in industrial processes and the maximum respirable dust cloud that could be generated from it in experimental conditions was 4 mg/m^3 of air. This dust was administered to two groups of rats with one group dusted using a reversed daylight regime so that the animals were in their most active phase while inhaling dust. This procedure was adopted because it was believed it would lead to a higher level of pulmonary dust deposition. In practice this did not occur but it was found that in both groups of rats the 4 mg dose of wet dispersed chrysotile had produced as many pulmonary tumours as previously found with UICC chrysotile at a dose level of 10 mg/m^3 of air. Electron microscope studies of lung tissue from some of the animals treated with wet dispersed chrysotile indicated a possible reason for this high level of carcinogenicity. It was found that all the chrysotile fibres had separated into individual fibrils, many of which retained a length of more than 10 µm. Thus the number of fibre units within the tissue was far higher than in the dust cloud.

These results raised the interesting possibility that the high carcinogenicity of chrysotile in almost all published animal experiments might be due to some degree of fibril separation within the tissues, a phenomenon which may be more complete with wet dispersed chrysotile but not unique to this material. If this is so then the tissue dose of chrysotile fibre units would always be higher than indicated by examination of the dust cloud. If this occurs in rats it must also occur in humans and yet here the epidemiological evidence suggests that chrysotile may actually be less carcinogenic than the amphiboles. It may be that while chrysotile is potentially the most carcinogenic asbestos type, the fact that it tends to separate into individual fibrils in tissues also makes it the most easily removed from the lungs either by chemical dissolution or mechanical clearance. Chrysotile may survive long enough in rat lungs to produce tumours but be removed from human lungs during the very much longer period required for tumour induction in this long lived species.

In recent years there have been suggestions that some of the harmful effects of asbestos and especially chrysotile might be due not to the main commercially exploited mineral but to relatively small amounts of mineralogical impurities. Two such impurities frequently found are tremolite and brucite and studies using these materials have been undertaken in Edinburgh by Davis et al (14). Rats were treated with the standard 10 mg/m^3 dose and pulmonary tumours were produced in both studies. However, while the number of tumours in the Brucite study was low, tremolite produced more than previously reported from any similar study. This may indicate that chrysotile samples containing significant amounts of tremolite should be treated with caution.

Because of the accepted hazards of asbestos usage, industry has, where possible, substituted alternative fibrous materials especially man-made fibres and a number of animal inhalation studies have been undertaken in order to determine if these materials are completely safe. Early studies by Gross in 1976 (15) and Lee in 1969 (16) showed no carcinogenic effects in rats following prolonged inhalation of glass fibre. However, recently a number of reports were presented at the same scientific meeting where the results were more positive. Wagner (17), McConnell (18), Smith et al (19), Lee and Reinhardt (20) and Davis et al (21) between them examined the effects on experimental animals (mainly rats) of a number of glass fibre samples, slag wool, rock wool and ceramic fibre. Small numbers of pulmonary tumours were produced by most materials. The numbers were too low in most cases for detailed statistical comparisons but since spontaneous pulmonary tumours are extremely rare in rats the finding of even a few suggests the possibility of some hazard to workers if factory dust levels are uncontrolled. It was reported, however, that standard boron silicate glass fibres at least showed considerable evidence of solubility in tissue fluids and this phenomenon, as with chrysotile asbestos,may limit the hazard to humans where the tumour induction period is so much longer than in rats.

To summarise the results of animal inhalation studies with mineral fibres to date it may be said that while there has not been complete agreement a number of trends have emerged. Thus all the main

asbestos types show a dose response effect when different mass dose levels have been used. However, there appears to be a plateau effect above which further increases in dose do not increase tumour numbers. The plateau for benign pulmonary tumours appears to be lower than for carcinomas. In general dust clouds containing very large numbers of fibres greater than 5 µm in length appear to be the most carcinogenic but there are exceptions especially with chrysotile materials. With these there is evidence to suggest that fibril separation in the lungs produces a far higher number of fibres in the tissues than would be expected from the original dust clouds.

Where similar dusts have been used at the same dose in different laboratories, the results have sometimes been different. Thus Wagner et al (7) and Davis et al (9) both used UICC crocidolite, amosite and Rhodesian chrysotile at dose levels of 10 mg/m^3 of air for a one year exposure period. From all these dusts Wagner produced noticeably more tumours and indeed Davis et al failed to produce any malignant tumours with the UICC amphibole samples. Similar dust generators were used by both groups but data was not available for the fibre length distributions of all the dust clouds (T A B L E 2). It may be that subtle changes in dust generation procedures had resulted in dust clouds with a substantially different fibre length. It is also possible that the strain of rats used by Wagner was more susceptible to carcinogenesis than the one used in Edinburgh.

Where the same dust has been used more than once i similar studies in the same laboratory, agreement appears to be much closer. Thus Wagner et al (7 and (12) used UICC Canadian chrysotile at the sa dose level and produced closely comparable numbers of pulmonary tumours (T A B L E 3). Only the number of pleural mesotheliomas differed noticeabl in the two studies, with three occurring in the first and none in the second. In their studies with wet dispersed chrysotile, Davis et al (13) treated two groups of rats with the same dust dose. Although one group was dusted using a reversed daylight regime the lung dust burden was not significantly changed by this approach and the number of both benign and malignant pulmonary tumours was almost identical (T A B L E 3).

It would appear, therefore, that the rat at least provides a useful model for examining the carcinogenic potential of dusts administered by inhalation and that the same exposure conditions will produce consistent results when the same material is tested on more than one occasion. This model will obviously be useful in the future for screening new mineral fibres that come into industrial use, but i should also prove useful in studies of the tumour induction process itself. In this field, perhaps the most important area where new studies should be undertaken concerns the importance of fibre length. While Stanton et al (22 - 24) determined that following intrapleural implantation, the most car-

Table 2. Tumour production in long-term inhalation studies undertaken in two different laboratories. The UICC samples of Rhodesian Chrysotile, Amosite and Crocidolite were used at the same dose level on both occasions

	Wagner et al 1974			Davis et al 1978		
Dust type and mass dose	UICC Rhodesian Chrysotile 10 mg/m^3	UICC Amosite 10 mg/m^3	UICC Crocidolite 10 mg/m^3	UICC Rhodesian Chrysotile 10 mg/m^3	UICC Amosite 10 mg/m^3	UICC Crocidolite 10 mg/m^3
Fibre number/ml of air > 5 µm	not quoted	not quoted	not quoted	1950	550	860
Adenomas	2	5	5	7	2	1
Total carcinomas	13	1	9	8	0	0
Adenocarcinomas	7	1	3	6	0	0
Squamous carcinomas	6	0	6	2	0	0
Mesotheliomas	0	0	2	0	0	0

Table 3. Tumour production in long-term inhalation studies where the same dust type was tested on two occasions

	Wagner et al 1974	Wagner et al 1980	Davis et al 1980	
Dust type and mass dose	UICC Chrysotile B 10 mg/m^3	UICC Chrysotile B 10 mg/m^3	Wet dispersed Chrysotile 4 mg/m^3	Wet dispersed Chrysotile 4 mg/m^3 (reversed daylight)
Fibre number/ml of air > 5 um	not quoted	3750	111	117
Adenomas	1	0	7	5
Total carcinomas	7	5	10	12
Adenocarcinomas	6	2	5	5
Squamous carcinomas	1	3	5	7
Mesotheliomas	3	0	4	1

cinogenic fibres were those > 8 µm in length and < 1.5 µm in diameter, this finding has not really been confirmed in inhalation studies. In addition, while Stanton showed that long fibres were more carcinogenic than short, he was unable to prove that short ones were either completely safe or only relatively so. Delay in settling these points has resulted entirely from the difficulty in producing mineral fibre samples in uniformly short lengths, especially in the large quantities needed for inhalation studies. However, to some extent at least, these problems are being overcome and future studies should settle the points in question.

References

(1) LYNCH, K.M., and W.A. SMITH: Pulmonary asbestosis III. Carcinoma of lung in asbestos-silicosis. American Journal of Cancer 24 (1935), p. 56/64.

(2) WAGNER, C.J., C.A. SLEGGS., and P. MARCHAND: Diffuse pleural mesotheliomata and asbestos exposure in the North Western Cape Province. British Journal of Industrial Medicine 17 (1960), p. 260/271.

(3) WAGNER, J.C.: Experimental production of mesothelial tumours of the pleura by implantation of dusts in laboratory animals. Nature, London 196 (1960), p. 180/181.

(4) GROSS, P., R.T.P. DE TREVILLE., E.B. TOLKER., M. KASCHAK., and M.A. BABYAK: Experimental Asbestosis. The development of lung cancer in rats with pulmonary deposits of chrysotile asbestos dust. Archives of Environmental Health 15 (1967), p. 343/355.

(5) REEVES, A.L., H.E. PURO., R.G. SMITH., and A.J. VORVALD: Experimental asbestos carcinogenesis. Environmental Research 4 (1971), p. 496/511.

(6) REEVES, A.L., H.E. PURO., and R.G. SMITH: Inhalation Carcinogenesis from various forms of asbestos. Environmental Research 8 (1974), p. 178/202.

(7) WAGNER, J.C., G. BERRY., J.W. SKIDMORE., and V. TIMBRELL: The effects of the inhalation of asbestos in rats. British Journal of Cancer 29 (1974), p. 252/269.

(8) WAGNER, J.C., G. BERRY., and V. TIMBRELL: Mesotheliomata in rats after inoculation with asbestos and other minerals. British Journal of Cancer 28 (1973), p. 173-185.

(9) DAVIS, J.M.G., S.T. BECKETT., R.E. BOLTON., P. COLLINGS., and A.P. MIDDLETON: Mass and Number of Fibres in the Pathogenesis of Asbestos Related Lung Disease in Rats. British Journal of Cancer 37 (1978), p. 673/687.

(10) DAVIS, J.M.G., S.T. BECKETT., R.E. BOLTON., and K. DONALDSON: The effects of Intermittent High Asbestos Exposure (Peak Dose Levels) on the Lungs of Rats. British Journal of Experimental Pathology 61 (1980), p. 272/279.

(11) DAVIS, J.M.C., S.T. BECKETT., R.E. BOLTON., and K. DONALDSON: A comparison of the Pathological Effects in Rats of the UICC Reference Samples of Amosite and Chrysotile with those of Amosite and Chrysotile collected from the Factory Environment. In: Biological Effects of Mineral Fibres (Ed. J.C. Wagner) IARC Scientific Publication No.30 (1980), p. 285/292.

(12) WAGNER, J.C., G. BERRY., and J.W. SKIDMORE: The Comparative Effects of Three Chrysotiles by Injection and Inhalation in Rats. In: Biological Effects of Mineral Fibres (Ed. J.C. Wagner) IARC Scientific Publication No.30 (1980), p. 285/292.

(13) DAVIS, J.M.C., R.E.BOLTON., K. DONALDSON., A. WRIGHT., and A.D. JONES: Inhalation studies in rats using dust samples from chrysotile asbestos prepared by a wet dispersion process. Paper presented at XX International Conference on Occupational Medicine, Cairo. Conference report in press (1981).

(14) DAVIS, J.M.C., J. ADDISON., R.E. BOLTON., K. DONALDSON., A.D. JONES., and A. WRIGHT: The effects of long term inhalation of Tremolite and Brucite on the lungs of rats (1982). To be published.

(15) GROSS, P: The effects of fibrous glass dust on the lungs of animals. In: Occupational Exposure to Fibrous Glass. Proceedings of a Symposium Sponsored by NIOSH. (DHEW Publication No.NIOSH 76-151), (1976), p. 169/178.

(16) LEE, K.P., C.E. BAWAS., F.D. GRIFFITH., and R.S. WARITZ: Pulmonary response to glass fiber by inhalation exposure. Laboratory Investigation 40 (1979), p. 123/133.

(17) WAGNER, J.C: Effects of Inhalation and Intrapleural Inoculation of Man Made Mineral Fibres in Rats. Paper presented at WHO Conference on the Biological Effects of Man Made Mineral Fibres, Copenhagen (1982). Conference report in press.

(18) McCONNELL, E.E: Comparable Effects of Inhalation of Man Made Mineral Fibres in the USA and UK. Paper presented at WHO Conference on the Biological Effects of Man Made Mineral Fibres, Copenhagen (19 Conference report in press.

(19) SMITH, D.M., L.W. ORITZ., and R.F. ARCHULET Chronic Exposure of Laboratory Animals to Inhaled Small Diameter Glass Fibres. Paper presented at Conference on the Biological Effects of Man Made Mineral Fibres, Copenhagen (1982). Conference repor in press.

(20) LEE, K.P., and C.F. REINHARDT: Biological Studies on Other Man Made Mineral Fibres. Paper presented at WHO Conference on the Biological Effec of Man Made Mineral Fibres, Copenhagen (1982). Conference report in press.

(21) DAVIS, J.M.C., R.E. BOLTON., K. DONALDSON., A.D. JONES., and A. WRIGHT: The effects of inhalation of ceramic Aluminium Silicate Fibres in Rats. Paper presented at WHO Conference on the Biological Effects of Man Made Mineral Fibres, Copenhagen (198 Conference report in press.

(22) STANTON, M.F., and C. WRENCH: Mechanisms of mesothelioma induction with asbestos and fibrous glass. Journal of the National Cancer Institute 48 (1972), p. 797/821.

(23) STANTON, M.F., M. LAYARD., A. TEGERIS., E. MILLER., M. MAY., and E. KENT: Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension. Journal of the National Cancer Institute 58 (1977), p. 587/603.

(24) STANTON, M.F., and M. LAYARD: The Carcinogenicity of Fibrous Minerals. Definitions and Measurement Methods. (Ed. Gravatt CC et al) National Bureau of Standards Special Publication 50 (1978), p. 143/151.