AN ANIMAL MODEL FOR INHALATION EXPOSURE TO TALC

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INTRODUCTION

In this paper the results of an inhalation experiment in rats using non-fibrous talc are given. In a previous communication (Wagner *et al.*, 1977) we gave the preliminary results of this study. We decided to use the Italian talc which is a major source of cosmetic talc used in Great Britain, and the effects of this material are compared with those of chrysotile asbestos and with non-treated controls.

MATERIALS AND METHODS

The main experimental material was Italian talc. The sample was obtained from a shipment of talc material imported from a mine in Northern Italy where talc has been produced for over 70 years. Talc from this particular mine was chosen for two reasons. Firstly, because it has been used in Great Britain for over 50 years, and secondly, because over 40% of the cosmetic grade talc used in Great Britain is obtained from this source.

This particular talc is referred to in the talc trade as Italian 00000 grade. It is imported in a ready milled form, with an upper particle size of 70μm and a mass median particle size of 25μm. The talc sample was found to contain 92% talc mineral by weight together with 3% chlorite and 1% carbonate minerals; quartz was also found in the powder at approximately the 0.5 - 1% level.

Extensive investigation of this particular grade of talc had been performed for a two-year period prior to the start of the animal experimentation, and samples which were over 30 years old had been examined as well as more recent imports. Virtually no change in the mineralogical composition of the material has been detected. No asbestos minerals of either the tremolite or chrysotile varieties have been detected in the many samples of this powder examined. A mineralogical study of the talc mine itself has shown that tremolite can be found in isolated sections of the mine but this could not be traced into the final product. This is probably due to the selective mining procedures adopted in the mine, where the talc is mined by hand.

For comparison, the sample of super fine chrysotile asbestos (SFA chrysotile) which we have previously shown to give a high mesothelioma rate after intrapleural inoculation (Wagner *et al.*, 1973) was included, and there were also controls exposed to neither material.

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The experimental animals were barrier protected caesarian derived rats of the Wistar strain bred at the Unit from a stock given to us by the Imperial Chemical Industries, Pharmaceutical Division, Alderley Edge, Cheshire.

In each group of animals there were equal numbers of males and females, and allocation to the treatment groups was at random. The rats were caged in fours except when in the inhalation chambers when they were caged in sixes. The inhalation chambers were in a separate room. The rats were fed on a proprietary brand of autoclaved cubes and water *ad libitum*. The animal house is supplied with filtered air. Except for the scheduled killings, each rat was allowed to live until it died or apeared to be distressed and a full necropsy examination was carried out.

INHALATION EXPERIMENT.

Rats were exposed in 1.4m³ chambers (Timbrell *et al.*, 1970) which hold 48 rats, caged in sixes. The dust clouds were generated for 7½ hrs. a day and 5 days per week. The respirable dust concentrations were measured daily using a Casella type 113A size selective gravimetric dust sampler, and variations were allowed for by adjusting the concentrations on the following days, so that the required dosage, calculated as the product of concentration and time, was achieved uniformly in a specific time. The SFA cloud was generated using the generator designed for the UICC standard reference samples of asbestos (Timbrell *et al.*, 1968) and in the cabinets about 80% by weight of the cloud was respirable. The talc cloud was generated using a Wright dust feed mechanism and about 40% was respirable. Exposure started in February 1973, with 48 rats exposed to talc and 48 to SFA chrysotile. After 6 months' exposure half of the rats were removed and transferred to ordinary cages, and were replaced by another 24 per dust. These rats were in turn removed and replaced after 3 months' exposure, and all exposure ceased after another 3 months. Thus, there were 96 rats exposed to each dust, 48 for 3 months, 24 for 6 months, and 24 for 12 months. There were also the same number of controls which were kept in ordinary cages in racks. At the start of each exposure period the majority of the rats were between 6 and 8 weeks old. The mean respirable dust concentration was 10.8 mg/m³ for each dust and the cumulative doses, i.e. the products of concentration and time, were approximately 4100, 8200, and 16400 mg/m³ hrs. for the 3 months, 6 months and 12 months exposures. Ten days after the end of each exposure period some rats were sacrificed, and there were also sacrifices one year later.

The amount of dust in the lungs was determined for the sacrificed rats using the method based on determination of the silica content (Morris *et al.*, 1967). For talc, the mean amounts of dust in the lungs were 2.8, 4.5 and 12.3 mg per rat at the end of exposures of 3, 6 and 12 months respectively. In contrast, the amount of SFA chrysotile was close to the detection limit of the method and was estimated as only 0.6 mg/rat after 12 months' exposure.

For the sacrificed rats an assessment was made of the severity of fibrosis in the lungs. Sections of both lungs were examined in random order, without knowledge of the dust or length of exposure. The sections were observed on a viewing screen of a Projectina microscope 4013 BK at a magnification of x85. The fibrosis in each lung was assessed on a seven point scale: – 1, nil; 2, minimal; 4, slight; 6, moderate; and 8, severe. Illustrative examples can be seen in Wagner *et al.* (1974).

The mean fibrosis scores of the rats sacrificed at the end of exposure, and one year later, are shown in Table 1.

TABLE 1. Inhalation Experiment - Mean Fibrosis at End of Exposure and One Year Later (Number of Rats)

Material	Time	Length of exposure 3 months	6 months	12 months
Italian talc	End of exposure	2.2 (8)	2.7 (6)	3.4 (6)
	1 year later	2.4 (8)	3.4 (4)	4.6 (4)
SFA chrysotile	End of exposure	2.8 (8)	3.0 (6)	3.2 (6)
	1 year later	2.2 (8)	3.2 (4)	4.2 (4)
Controls	End of exposure	1.8 (8)	1.9 (6)	1.3 (6)
	1 year later	1.6 (8)	1.5 (3)	1.9 (3)

The main features are that both Italian talc and SFA chrysotile produced fibrosis to a similar extent, and that there was some evidence of progression after exposure had discontinued in the longer exposed animals. the number of rats with lung tumors are shown in Table 2.

TABLE 2. Inhalation Experiment - Lung Tumors

Material	Exposure	Number at risk*	Number of lung tumors: Adenomas	Adenomatosis	Adenocarcinomas	Mesotheliomas
Italian Talc	3 months	39	0	0	0	0
	6 months	18	0	0	0	0
	12 months	24	2	0	0	0
SFA Chrysotile	3 months	40	0	0	0	1
	6 months	18	1	2	1	0
	12 months	22	3	3	2	0
Controls		71	1	0	0	0

*Number surviving at least 300 days from start of exposure.

One adenoma occurred in the control rats, there were two adenomata in the rats exposed to talc, and there were 13 lung tumors, including one mesothelioma and 3 adenocarcinomata in the SFA groups.

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