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ANIMAL EXPERIMENTS WITH TALC

J. C. WAGNER,* G. BERRY,* T. J. COOKE,† R. J. HILL,*
F. D. POOLEY‡ and J. W. SKIDMORE*

Abstract—Italian talc has been tested on rats using three routes, intra-pleural inoculation, inhalation and ingestion. Groups exposed to superfine chrysotile asbestos and untreated controls were included for comparison. In all the experiments animals were allowed to live out their lives. The intra-pleural inoculation of talc produced no mesotheliomas in contrast to eighteen produced by the chrysotile asbestos. After ingestion, one leiomyosarcoma occurred with Italian talc and one with chrysotile asbestos. Whether these tumours are a consequence of the feeding is uncertain. The inhalation studies demonstrated that with equal dosage, talc can produce a similar amount of fibrosis as asbestos. However, the chrysotile exposed rats developed lung adenomas, adenomatosis and an adenocarcinoma, whereas the only lung tumour seen in animals exposed to talc was a small adenoma, which may have been an incidental finding.

INTRODUCTION

In these studies we have investigated the biological properties of a single type of talc, using the same methods as we have applied to various types of asbestos. Talc was introduced into rats by three routes—intra-pleural, inhalation and ingestion. We had facilities for testing a single talc dust, and decided to use the Italian talc which is a major source of cosmetic talc used in Great Britain. The literature on the biological effects of cosmetic and consumable (used in confectionery) talc is confusing and some of the results unjustifiably alarming. The more sensational findings have naturally attracted a lot of publicity. Therefore, we have been forced to devote a large amount of experimental resources in an attempt to put the possible hazards resulting from the inhalation of cosmetic and consumable talc into perspective. The inhalation study has not been completed and this is a preliminary communication, as far as this route is concerned.

MATERIALS AND METHODS

The main experimental material was Italian talc. The mineral sample used in this study 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. First, 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.

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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 70 μm and a mean particle size of 25 μm. 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 2-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 intra-pleural inoculation (WAGNER *et al.*, 1973) was included and there were also controls exposed to neither material.

The experimental animals were barrier-protected caesarian-derived rats of the Wistar strain bred at the Unit from a stock given to us by 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 was supplied with filtered air. Except for the scheduled killings, each rat was allowed to live until it died naturally or appeared to be distressed; full necropsy examinations were carried out.

INTRA-PLEURAL INOCULATION EXPERIMENT

The dose was 20 mg per rat made up as a suspension in physiological saline, with a concentration of 50 mg/ml. Injection was into the right pleural cavity using the method described by WAGNER and BERRY (1969). There were 48 rats injected with the talc, 48 with SFA chrysotile and 48 controls injected with saline. Injection was in January 1973 when the rats were between 8 and 14 weeks old, and the last animal died in September 1975.

The results are given in Table 1 which includes the mean survivals after injection and the numbers with a mesothelioma. As expected, mesotheliomas occurred in a proportion of the rats injected with SFA chrysotile. In fact, fewer occurred than was expected since in our previous experiments (WAGNER and BERRY, 1969; WAGNER *et al.*, 1973) this material produced mesotheliomas in 65% of the animals. However, in these previous experiments there was longer survival and after allowing for this the present mesothelioma rate was similar to that in our first experiment. The shorter survival in

TABLE 1. INTRAPLEURAL INOCULATION EXPERIMENT

Material injected	No. injected	Mean survival (days)	Number with mesothelioma
Italian talc	48	655	0
SFA chrysotile	48	598	18
Saline controls	48	691	0

the present experiment was probably due to the rats having lost their SPF status. Also, as expected, the mean survival of the SFA-injected rats was reduced in comparison with the controls. No mesotheliomas were observed in the talc-injected animals. However, injection site granulomas were common and a small pulmonary adenoma was found in one rat which died 25 months after injection. There was no other relevant pathology of the lungs in these animals. The mean survival was about a month less than the controls, but this difference could have been due to chance ($P > 0.25$).

INHALATION EXPERIMENT

Rats were exposed in 1.4 m^3 chambers which could hold up to 48 rats, caged in sixes. The dust clouds were generated for $7\frac{1}{2}$ h 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 U.I.C.C. 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 numbers 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/m^3 for each dust and the cumulative doses, i.e. the products of concentration and time, were approximately 4100, 8200 and 16 400 mg/m^3 h for the 3-month, 6-month and 12-month exposures. Ten days after the end of each exposure period some rats were sacrificed and there were also sacrifices 1 year later.

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 $\times 85$. 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 2. 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.

TABLE 2. INHALATION EXPERIMENT—MEAN FIBROSIS AT END OF EXPOSURE AND 1 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)

Most of the animals in the 6- and 12-month exposure groups had died by 6th June 1975, but over half of those in the 3-month groups were still living. Therefore, the 3-month groups will not be considered further in this paper.

The numbers of rats with lung tumours are shown in Table 3. None occurred in the control rats, there was one adenoma in the rats exposed to talc and there were seven

TABLE 3. INHALATION EXPERIMENT—LUNG TUMOURS

Material	Exposure	Number exposed	Sacrificed	Died	Number of lung tumours		
					Adenomas	Adenomatosis	Adenocarcinoma
Italian talc	6 months	24	10	12	0	0	0
	12 months	24	10	12	1	0	0
SFA chrysotile	6 months	24	10	8	0	1	0
	12 months	24	10	11	3	2	1
Controls		48	18	27	0	0	0

lung tumours, including one adenocarcinoma in the SFA groups. In addition, one rat in the SFA 1-year group had a widespread lymphosarcoma. However, as we showed previously (WAGNER *et al.*, 1974) tumours of this type (lymphomas and leukaemias) are an occasional finding in our rats independent of treatment.

INGESTION EXPERIMENT

Rats were fed the test materials with a dose of 100 mg per day per rat. The food mixture was prepared in batches sufficient for 5 days. The basic mixture consisted of equal amounts by weight of coarsely powdered Spillers small-animal diet and Horlicks malted milk. This mixture was chosen because the rats liked it and could be easily trained to eat it quickly and completely. 244 g of the basic mixtures was added to 16 g of the test material in a 20 × 30 cm polythene bag. The bag was sealed and the contents mixed by rubbing between the hands. The contents were then weighed into 5 equal parts and sealed in 5 × 13 cm polythene bags. On the day before a bag was required .5 cm^3 of deionized water were injected with a hypodermic syringe and the contents kneaded into a uniform stiff dough. This was rolled in the palms, still sealed in the bag, and then shaken out on a polythene rolling sheet. The cylinder of dough was then rolled out to the length of a dose-cutter on which it was then laid and divided into 32 portions by drawing a scalpel through the slots in the cutter. The doses were stored in the polythene bag until the next day. This improved the consistency, making them drier and firmer. The doses were administered by dividing the rectangular cages into four compartments. The mesh floor was covered with aluminium and the rats introduced. A daily record was kept for each rat of how much of the dose was consumed. In fact after the first 2 weeks, it was rare for the whole pellet not to be consumed and overall over 98% of the planned dose was consumed, the minimum consumption of one rat being 85%. There were 32 rats fed talc, 32 SFA chrysotile and 16 controls fed with the basic mixture only. Feeding started in February 1973, when the rats were between 21 and 26 weeks old, and was carried out on 101 days in the next 5 months. Except when the doses were being administered the rats had access to the normal diet.

At post-mortem the abdominal organs were examined and the entire alimentary canal removed and fixed *en bloc* in 10% neutral formalin, together with the liver, spleen, kidneys, heart, lungs and any suspected pathological lesion. Tissue for histological examination was taken from the liver, spleen, stomach, ileum, caecum, rectum, omentum, lesser omentum, mesentery and parietal peritoneum and any other site of pathology. Tissues were processed on a tissue processor, embedded in paraffin wax, sectioned at 5 μm bulk stained in haematoxylin and eosin and mounted in DPX. Care was taken during the post-mortem and while handling wet tissues not to introduce possible contamination particularly from glove powder. No steps were taken to eliminate possible contamination via chemical reagents such as the fixatives. Further tissue blocks will be taken for electron microscopical and electron probe identification of minerals.

Two animals from each treatment were sacrificed 3 months after the feeding had finished, and the last animal died in September 1975. The mean survivals from the start of feeding were 614 days for talc, 619 for SFA chrysotile and 641 days for the controls, ignoring the sacrificed animals.

Abnormalities of the gut were found in only two rats. A rat fed talc had a leiomyosarcoma of the stomach. A tumour of this type may have occurred in a rat fed SFA chrysotile although the diagnosis is not certain (possibly it is a reticulum celled sarcoma). Other findings were an adrenal adenoma in a control rat, sarcomas of the

uterus in two rats fed talc, and in one fed SFA and a lymphosarcoma in a rat fed SFA. These latter findings are not considered as consequences of the feeding because of their location; also we have previously observed three sarcomas of the uterus out of 126 control rats (WAGNER *et al.*, 1974). The two leiomyosarcomas of the stomach could possibly be a consequence of the feeding although malignant tumours of the digestive organs and peritoneum do occur in our rats in the absence of treatment, and we found three in the group of controls referred to above, although none was a leiomyosarcoma.

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DISCUSSION

S. J. ROTHENBURG: Were the particle size distributions similar for the samples of talc and chrysotile?

J. C. MARTIN: In the inhalation experiments, how much dust was retained in the lungs at the end of exposure? Do you think that retention and clearance are the same for each dust tested; if not, can one really say that the fibrogenic potential is the same?

MR SKIDMORE: Whilst continuous sampling was used to determine respirable mass concentrations only occasional samples were obtained for microscopical examination. These indicated that the respirable fraction of the talc cloud contained approximately 1300 particles/ml larger than 1 μm. Between 1 and 2% of these were fibrous with lengths up to about 20 μm and diameters between 1 and 2 μm. The SFA cloud contained approximately 500 fibres/ml longer than 5 μm plus a substantial number of shorter fibres and non-fibrous particles of similar chemical composition.

Retention and clearance were certainly not the same for the two dusts. For talc, in those rats sacrificed at the end of exposure, there were 2.5, 4.7 and 12.2 mg talc per rat in the lungs after 3, 6 and 12 months' exposure. With the chrysotile much smaller amounts were found and even after 12 months, the mean was only 0.8 mg. One reason for using the inhalation route is to allow these differences to play their part; that is, the actual rather than the potential effect is determined.

E. K. CUNDY: You say that your mineral is free of fibrous particles of the tremolite type. Was any electron microscopy carried out on this sample to check the absence or otherwise of fibrous particles?

W. SMITHER: You said that you found no tremolite or asbestos in this talc. Have you found any fibres at all? This conference has learned that fibre morphology is the important factor. Would you care to characterize the fibres you found in talc? Would you say that they conform to the classical description of asbestos—that is a hydrated fibrous silicate? If so, must we change the classical description of asbestos or must we accept that there are asbestos fibres in cosmetic talc?

DR POOLEY: The examinations performed on the talc included X-ray diffraction analysis, differential thermal and thermal gravimetric analysis on the bulk material while the transmission electron microscope fitted with an energy-dispersive X-ray analyser was used to study single fibres in the samples in an effort to detect asbestos. The many fibrous particles analysed in the samples, i.e. those particles with a $> 3:1$ axial ratio, were found to be laths of talc or chlorite mineral.

Dr Smither poses a very interesting question concerning the definition of asbestos. If we look very closely at industrial silicate materials imported into Great Britain for use by the various manufacturing industries, we find that these materials contain numerous fibrous particles. One example is the mineral sepiolite which is a magnesium silicate which forms very fine fibre very similar to chrysotile but yet is not called asbestos. If you ask me whether or not the fibres found in talc look like commercial asbestos then I would say "no". The diameters of the fibres found in the talc samples were generally in excess of 1 μm whereas the majority of commercial asbestos minerals have fibre diameters which are below 1 μm.

W. SMITHER: Were there any ferruginous bodies in the pathological sections?

DR WAGNER: No.

J. R. LYNCH: Since most of what we know about the biological effect of asbestos indicates that the fibre shape may be the most important factor, it seems that we should regard all respirable mineral fibres, regardless of how they may be described industrially or mineralogically, as presenting a potential hazard similar to asbestos in the absence of evidence to the contrary.

MR SKIDMORE: The talc sample we tested was mainly non-fibrous, i.e. less than 2% of the particles were fibres. Also these fibres were coarser than typical asbestos fibres. Our experiments have surely provided evidence that this talc has an actual and potential carcinogenic hazard at least an order of magnitude less than asbestos, but provide no evidence on the possible effects of non-asbestos *fibrous* material.

D. K. CRAIG: At Battelle, we have exposed groups of 100 hamsters to talcum powder in various exposure regimens, the maximum cumulative exposure being 6000 mg h/m^3, delivered over a year at a respirable concentration (as measured by the MRE horizontal elutriator) of 8 mg/m^3. This talcum powder came from Vermont. We observed no significant difference in the lung pathology of the control and the exposed animals, we saw no significant fibrosis in animals, we saw no ferruginous bodies in their lungs and we saw no fibres in any of the samples we took of the talcum powder aerosols.

G. W. WRIGHT: Since fibrous as well as non-fibrous respirable particles are reported in the air to which these animals were exposed, one cannot be certain which, or perhaps both, may be the fibrogenic agent. Which do the authors believe is the effective agent?

A similar situation has recently come to my attention in humans exposed to flux-calcined diatomaceous earth. The chest roentgenogram of persons thus exposed often shows a diffuse non-nodular pattern similar to asbestosis.

The dust examined by electron microscopy shows the presence of long thin fibres said to be quartz. This observation raises the possibility that it is these fibres that may account for the non-nodular component in these roentgenograms.

R. J. RICHARDS: Mineral particles do adsorb organic materials on to their surface; perhaps this effect should receive some consideration in relation to the biological potential of the "mineral".

A. MORGAN: Why did you select the SFA chrysotile for comparison? Electron micrographs of this material show that it is inhomogeneous and contains both very fine fibres and "chunks" of material which may consist of chrysotile compacted during milling. Tests such as protein adsorption and haemolysis show that it does not behave as a typical chrysotile. Would you hazard a guess as to whether it is the fibrous phase or the "chunks" which is responsible for the reported biological effects?

Dr WAGNER: The SFA chrysotile has produced a higher mesothelioma rate after intrapleural injection than any of the asbestos samples we have used, and as we were looking for gastrointestinal tumours after feeding for the first time we decided to include it for this reason. Actually in the injection and inhalation experiments other chrysotile samples, including one of the U.I.C.C. samples, were also used, but they are not reported in this paper which is primarily concerned with talc.

J. C. MCDONALD: Have you any information on the frequency of mesothelial tumours in animals exposed to talc containing tremolite?

G. W. GIBBS: Did you see pleural calcification in your animals?

Dr WAGNER: We have no experience of talc or tremolite in our previous experiments but we have an injection experiment in progress involving tremolite. I saw no pleural calcification in the talc experiments, in contrast to one of our previous experiments with samples of Canadian chrysotile.

M. KUSCHNER: Was the *pattern* of fibrosis in the lung produced by talc similar to that produced by asbestos? Secondly, did the *amount* of fibrosis in the pleura on intra-pleural instillation differ with each of these materials?

Dr WAGNER: In the lung the patterns were very similar but previously I have observed a slight difference between chrysotile and crocidolite. The pattern with chrysotile is similar to that described by Professor Heppleston with a focal alveolar lipoproteinosis around the respiratory bronchioles. The talc produced the same type of reaction. As far as the pleura is concerned the fibrosis was much more marked with the talc than the chrysotile.

V. TIMBRELL: Fibrous and flaky materials such as talc have in common the ability to produce large particles of small aerodynamic size, the aerodynamic size of a fibre being related to the diameter and that of a flake to its thickness. Thus thin flakes of talc can have diameters 25 μm or greater and yet be respirable. Like long fibres, such large flakes may not be cleared by macrophages, etc., and may thus remain in contact with the same cells indefinitely and produce fibrosis.

It is necessary to differentiate between fibrogenicity and carcinogenicity which may not be directly related. STANTON and WRENCH (*J. natn. Cancer Inst.*, 1972, **48**, 797) and ourselves have shown that the *carcinogenic* potential of fibrous materials is related to the particle shape and not that *fibrogenicity* is dependent on the particles being fibrous. It is thus understandable how inhaled talc particles may be fibrogenic without being carcinogenic.