

The pathogenicity of long versus short fibre samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection

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Summary. For many years it has been accepted that fibre dimensions are the most important factor in the development of asbestos related disease with long fibres being more dangerous than short for all types of asbestos. This information has been derived from *in vitro* experiments and injection or implantation experiments since the kilogramme quantities of specially prepared dusts that are necessary for long term inhalation have not been available. The present study has taken advantage of the availability of a sample of amosite produced so that most all fibres were less than 5 μ m in length. The effects of this dust were compared to dust prepared from raw amosite that contained a very high proportion of long fibres. Previous data on studies with UICC amosite, which was intermediate in length, were also available for comparison. At the end of 12 months of dust inhalation, significantly more short fibre amosite was present in the lung tissue compared to the long but while the long fibre dust caused the development of widespread pulmonary fibrosis, no fibrosis at all was found in animals treated with short fibre. One third of animals treated with long fibre dust developed pulmonary tumours or mesotheliomas but no pulmonary neoplasms were found in animals treated with short fibre dust. Following intraperitoneal injection, the long fibre amosite produced mesotheliomas in 95% of animals with a mean induction period of approximately 500 days. With short fibre dust, only a single mesothelioma developed after 837 days. In previous inhalation studies with UICC amosite, relatively little pulmonary fibrosis had developed and only two benign pulmonary tumours. This would suggest that to produce a significant carcinogenic response in rat lung tissue amosite fibres must be longer than those in the UICC preparation. Following the injection of UICC amosite, however, mesotheliomas developed in the same proportion of animals and with the same mean induction period as with long fibre dust. From this it would appear that while very short fibres exhibit little carcinogenicity to either lung or mesothelial tissues, mesotheliomas can be produced by dust preparations consisting of shorter fibres than are needed to produce tumours.

Keywords: amosite asbestos, fibre length, pulmonary fibrosis, inhalation, injection

The inhalation of asbestos dust may produce both pulmonary fibrosis and neoplasia in man and experimental animals and many experimental studies have been undertaken

in an attempt to determine the mechanisms by which asbestos fibres produce these pathogenic effects. A number of early studies suggested that the most important factor in fibrogenesis was the silica content of asbestos which stimulated collagen production by chemical action (Beger 1934, Kuhn 1941). As early as 1946, however, King *et al.* (1946) administered chrysotile fibres cut on a special microtome at lengths of 15 μm and 2.5 μm to rabbits by intratracheal injection. They reported a greater tissue reaction from those animals that had received the long fibre sample. Later, Vorwald *et al.* (1951) reported that animals which had inhaled chrysotile fibres in the 20–50 μm range had more pulmonary fibrosis than those breathing only dust with fibres below 3 μm in length. Scymczykiewicz & Wiecek (1960) obtained similar results when they administered fibrous and amorphous asbestos dust to guinea-pigs by intratracheal injection. They did not, however, give details of the asbestos type employed.

Hilscher *et al.* (1976) extended these studies using both the intratracheal and intraperitoneal injection of finely ground chrysotile and crocidolite. They found these fibres produced little or no fibrosis in either site. In contrast, longer fibres of the same asbestos types resulted in considerable fibrosis in both regions. Davis (1972) conducted a series of experiments using the intrapleural injection of a number of different mineral types including long and short fibre chrysotile. The short fibre samples were either synthetic chrysotile with a maximum crystal length of 1 μm or chrysotile fragmented by ultrasonic treatment until all fibres were below 1 μm in length. While the long fibre samples produced extensive fibrosis, the short fibre specimens produced almost no tissue reaction. Wright & Kuschner (1977) reported that following the intratracheal injection of long and short samples of both asbestos and glass fibre all the long fibre samples produced considerable fibrosis while the short samples did not.

The fact that fibre dimensions were impor-

tant in carcinogenesis as well as fibrogenesis was demonstrated by the work of Stanton *et al.* (1972, 1977, 1981). Stanton's group implanted numerous samples of carefully sized fibrous dust into pleural cavities of rats and reported that fibres > 8 μm in length and < 1.5 μm in diameter appeared most effective in producing mesotheliomas. These findings have been confirmed by Pott & Friedrichs (1972) and Pott *et al.* (1976) using intraperitoneal rather than intrapleural implantation of fibrous dusts.

Until recently it was difficult to examine the importance of fibre length on pulmonary pathology in long term inhalation studies because large amounts of specially prepared dusts are required for such work. In 1981, however, the Manville Corporation in the USA prepared several kilograms of short fibre amosite and supplied the Institute of Occupational Medicine in Edinburgh with sufficient of this material to undertake the present study.

Materials and methods

Amosite samples used for dust cloud generation.

Both the long and short amosite dusts used in these studies were prepared from the same batch of South African amosite. The short fibre amosite sample was prepared and characterized by the Manville Corporation in the USA by grinding in a ceramic ball milled system followed by sedimentation in water. The resulting material was so fine that only 37% of particles had an aspect ratio > 3:1 and were therefore regarded as fibres. The mean length of these fibres was 2.68 μm while the mean length of all particles was 1.42 μm . The crystallinity of both the fibrous and non-fibrous particles was checked by transmission electron microscopy (TEM). All particles examined showed a high degree of crystallinity as indicated by the presence of characteristic spot patterns seen in electron diffraction and well ordered periodic lattice fringes seen in high resolution images. No evidence of damage to the crystal lattice or loss of crystallinity induced during the pre-

paration process could be found. In an analysis of individual particles was made in the TEM by energy dispersive spectroscopy. The elemental composition of each particle was consistent with amosite samples from South Africa. The long fibre amosite sample prepared at this Institute from the same batch of amosite that was used to prepare short fibre material.

Dust cloud generation and monitoring.

The inhalation phase of this study was carried out in inhalation chambers of the type described previously by Beckett (1975). Animals were housed, fed and watered in the chamber throughout the exposure period. For the generation of the long fibre dust cloud, the modified Timbrell dust generator (Beckett 1975) proved satisfactory. Dust clouds generated from the short fibre material with this apparatus were found to contain aggregates. For this dust, the fluidized bed generator (Marple *et al.* 1975) model 3400 was used. In order to reduce the risk of dust aggregation, neither both dust clouds were exposed to a 204 source of β -particles to neutralize the electrostatic charge on the fibres before entry into the inhalation chambers (Liu & Pui 1974). The aliquots produced dust clouds consisted almost entirely of individual fibres and flocs.

The mass concentration of the dust clouds in the chambers was monitored daily by a gravimetric method throughout the 7 h of exposure using an open filter holder facing vertically upwards and a Casella MRE113A sampling instrument (Dunmore *et al.* 1977). The former sampler monitored the total concentration and the latter monitored the respirable dust concentration. A mean respirable dust airborne concentration of 10 mg/m^3 was achieved by adjusting the flow rates of the dust generators in response to each day's measurement.

Fibre number concentrations and size distributions for the experiments

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process could be found. In addition, the analysis of individual particles was undertaken in the TEM by energy dispersive X-ray spectroscopy. The elemental composition of each particle was consistent with that of pure amosite samples from South Africa. The long fibre amosite sample was prepared at this Institute from the same source of amosite that was used to prepare the short fibre material.

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The mass concentration of the dust in the chambers was monitored daily by sampling throughout the 7 h of exposure using both an open filter holder facing vertically downwards and a Casella MRE113A dust sampling instrument (Dunmore *et al.* 1964). The former sampler monitored the 'total' dust concentration and the latter monitored the respirable dust concentration. The target mean respirable dust airborne concentration of 10 mg/m^3 was achieved by adjusting the generators in responses to each previous 7 days measurement.

Fibre number concentrations and fibre size distributions for the experimental dust

clouds were assessed from membrane filter snatch samples collected on 90 separate days. Fibres were counted from all these samples using phase contrast optical microscopy (PCOM) at a magnification of $\times 600$, and fibre sizing was undertaken by scanning electron microscopy (SEM) at a magnification of $\times 10\,000$. With PCOM only fibres with a length $> 5 \mu\text{m}$, a diameter $< 3 \mu\text{m}$ and an aspect ratio of greater than 3:1 were considered (ARC 1971, AIA 1979). With SEM examination all fibres longer than $0.2 \mu\text{m}$ were measured if their aspect ratio was greater than 3:1.

Animal inhalation studies. For the inhalation studies groups of 48 SPF male Wistar rats of the AF/Han strain were exposed to dust clouds of either long or short amosite for 7 h each day, five days a week for a total of 222 days during a period of 12 months. The animals were 10 weeks old at the start of dusting. Two batches of 36 and 25 undusted animals were maintained within the same unit as controls during the same overall time period.

Four animals from each experimental group were killed at the end of the 12 months dusting period and four more were killed 6 months later. The remaining animals were left for their full life span except that the study was terminated when the number of survivors in one group (the long fibre treatment group) had dropped to six. Estimations of early fibrotic lesions were limited to the small groups of animals from the first two killing dates. However, for the more advanced alveolar interstitial fibrosis occurring in the oldest animals, all those dying within 2 months of the final killing date were included. In practice this produced groups of 18 animals treated with long fibre amosite and 23 animals treated with short fibre amosite. Groups of 9 and 13 control animals of similar age were included in this estimation of interstitial fibrosis. Lungs from all animals in this study were examined histologically for the presence of neoplasms. Samples were also taken for histology from

other organs showing macroscopic abnormalities. Tables 3 and 4 include only animals surviving 18 months or more after the end of dusting since we have found that few neoplasms, and particularly pulmonary neoplasms, develop in the AF HAN rats before this time.

Tissue used for histological examination was fixed with Karnovsky's fixative and embedded in paraffin wax. Lungs were fixed by inflation at a standard pressure of 30 cm of fixative. Subsequently, the tracheas were ligated and the lungs excised and immersed in fixative. Sections were cut in the coronal plane at 1 mm intervals and were stained by either haematoxylin and eosin, Van Gieson's method for collagen or Gordon and Sweet's stain for reticulin.

Measurement of pulmonary fibrosis was undertaken by similar methods to those previously published by Davis *et al.* (1978) except that an electronic image analyser (Graphic Information Systems Limited, GDS1) was available for use in conjunction with the light microscope (Davis *et al.* 1985). Single lung sections were examined and the sections selected to contain the maximum area of lung parenchyma. As previously described, interstitial fibrosis was estimated using a $\times 2$ microscope objective lens and is expressed as a percentage of total lung tissue area. Peribronchiolar lesions are more numerous and smaller and so the lung tissue was scanned with an eyepiece graticule covering a tissue area of 2.92 mm² and divided into 100 squares. A $\times 4$ objective lens was used. Peribronchiolar lesions were recorded as a percentage of squares containing lesions of this type.

Lung dust estimations were performed on animals from the first two killing dates. Only the left lung was used so that the right lung was available for histological studies. (Studies in this laboratory have shown that the ratio of dust content between left and right lungs following experimental inhalation of fibrous dust such as asbestos in rats is approximately 0.6:1 and this correction factor was therefore used to estimate the

total pulmonary dust burden for each animal). Dust retained in the lungs was recovered by a low temperature plasma ashing process using a Nanotech PROO apparatus. Following tissue ashing the amosite residues were washed in 0.2 M HCl at room temperature before estimations of the amounts of retained fibre were made using the infra-red spectroscopy technique described by Bolton *et al.* (1983).

Animal injection studies. In addition to the inhalation studies the ability of the long and short amosite preparations to produce mesotheliomas was examined using the intraperitoneal injection assay. A dose of either 25 mg or 10 mg of dust suspended in 2 ml of Dulbecco's phosphate buffered saline was injected under ether anaesthesia into the peritoneal cavities of groups of 24 rats of the AF/Han strain. The dust was collected from the animals inhalation chambers by an elutriation process chosen to select the respirable fraction of the dust cloud (Bolton *et al.* 1982). The short fibre dust was dispersed in the saline by ultrasonic treatment. All animals were allowed to live out almost all of their dull life span and were killed when moribund.

Statistical techniques. Survival functions for each experimental group were estimated using the product-limit method (Kaplan & Meier 1958). The survival curves for the different series were tested for homogeneity using the Generalized Wilcoxon (Breslow) statistic in the statistical package BMDP (Dixon *et al.* 1983). In order to compare the overall mortality of the four groups of animals, the death of an animal as part of a planned kill was treated as control, all other deaths being treated as responses. Survival curves were also estimated for the response of developing a non-pulmonary tumour. All deaths of animals without non-pulmonary tumours were recorded as censoring events, whilst non-pulmonary tumours were treated as responses irrespective of whether the death was planned or unplanned.

Comparisons of levels of pulmonary and lung dust burdens were made using generalized linear models facilitated by the statistical package GENSTAT (Alvey *et al.* 1977). Differences between the means for pulmonary and non-pulmonary dust burdens were analysed using the Wilcoxon U-test (Siegal 1956).

Results

Dust characterization

The respirable and total dust burdens were determined for both dusts in this study. The data are given in Table 1 as are the fibre size distributions for the long and short fibre amosite preparations. The short fibre amosite preparation contained very few fibres $> 5 \mu\text{m}$ in length, but the long fibre amosite dust contained a far higher proportion of fibres than any other amosite preparation previously examined in this laboratory (Bolton *et al.* 1978; 1982) with over 10% of fibres $> 10 \mu\text{m}$ in length and 3% of fibres $> 20 \mu\text{m}$ in length as seen by SEM. The size distributions of the two dusts were not expected since the same amosite was used in their preparation. The dusts were not identical. The characterization in general consisted of determining the number of fibres per ml of air. Figs. 2 and 3 illustrate the size distributions obtained by SEM. The number of fibres exceeding a given diameter are plotted against the

Table 1. Figures for dust mass and fibre content of the long and short fibre amosite dusts.

	Long fibre amosite
Total dust mass (mg/m ³ of air)	11.0
Respirable dust mass (mg/m ³ of air)	10.0
Fibres $> 5 \mu\text{m}$ in length (per ml of air)	2000
Fibres $> 10 \mu\text{m}$ in length (per ml of air)	111

pulmonary dust burden for each animal. Dust retained in the lungs was recovered by a low temperature plasma ashing using a Nanotech P100 apparatus. After ashing the amosite residues were dissolved in 0.2 M HCl at room temperature. Before estimations of the amounts of retained fibre were made using the infra-red spectroscopy technique described by Bolton (1983).

Injection studies. In addition to the inhalation studies the ability of the long and short fibre amosite preparations to produce mesotheliomas was examined using the peritoneal injection assay. A dose of 2.5 mg or 10 mg of dust suspended in 2 ml Dulbecco's phosphate buffered saline was injected under ether anaesthetic into the peritoneal cavities of groups of 24 rats of the Wistar strain. The dust was collected from the animals in inhalation chambers by an aspiration process chosen to select the representative fraction of the dust cloud (Bolton *et al.* 1982). The short fibre dust was dispersed in saline by ultrasonic treatment. All animals were allowed to live out almost all of their life span and were killed when required.

Statistics. Survival functions for the experimental groups were estimated by the product-limit method (Kaplan & Meier 1958). The survival curves for the series were tested for homogeneity by the Generalized Wilcoxon (Breslow *et al.* 1978). In order to compare the mortality of the four groups of animals the death of an animal as part of a planned or unplanned response was treated as a response. Survival functions were also estimated for the response to the induction of a non-pulmonary tumour. All animals without non-pulmonary tumours were recorded as censored events. Non-pulmonary tumours were treated as responses irrespective of whether the tumour was planned or unplanned.

Comparisons of levels of pulmonary fibrosis and lung dust burdens were made using the generalized linear models facilities in the statistical package GENSTAT (Alvey *et al.* 1977). Differences between the induction of pulmonary and non-pulmonary tumours were analysed using the Mann-Whitney *U*-test (Siegal 1956).

Results

Dust characterization

The respirable and total dust masses collected for both dusts in this study are given in Table 1 as are the fibre numbers $> 5 \mu\text{m}$ and $> 10 \mu\text{m}$ seen by PCOM.

The short fibre amosite preparation contained very few fibres $> 5 \mu\text{m}$ in length (Fig. 1) but the long fibre amosite dust cloud contained a far higher proportion of long fibres than any other amosite preparation previously examined in this laboratory (Davis *et al.* 1978; 1982) with over 11% of fibres $> 10 \mu\text{m}$ in length and 3% $> 25 \mu\text{m}$ in length as seen by SEM. The diameter distributions of the two dusts were close as would be expected since the same batch of amosite was used in their preparation but they were not identical. The long fibre preparation in general consisted of slightly thicker fibres. Figs. 2 and 3 illustrate the fibre size distributions obtained by SEM. The percentages of fibres exceeding a given length (diameter) are plotted against that length.

Table 1. Figures for dust mass and fibre number for the long and short fibre amosite dust clouds.

	Long fibre amosite	Short fibre amosite
Total dust mass (mg/m ³ of air)	11.9	11.6
Respirable dust mass (mg/m ³ of air)	10.0	10.0
Fibres $> 5 \mu\text{m}$ in length (per ml of air)	2060	70
Fibres $> 10 \mu\text{m}$ in length (per ml of air)	1110	12

When plotted on log-probability axes, the data are close to straight lines, indicating that the size distributions are approximately log-normal. Whilst there is a large difference in the length distributions of the two fibre samples (here indicated by the large separation between the two lines), the diameters of the short and of the long fibres are very similar. The slopes of the lines for short amosite are slightly steeper than those for the long-fibre amosite, indicating that the variances in the (logarithmically transformed) lengths and diameters for short amosite are slightly less than the corresponding variances for long amosite.

Pathological findings

There were no significant differences between the overall survival of animals treated with either long or short fibre amosite or the two groups of controls. Animals treated with the long fibre amosite preparation developed the same types of pathological change previously reported in similar inhalation studies from this Institute (Davis *et al.* 1978; 1982; 1985). At the end of the 12 month dusting period the main lesions present were deposits of granulation tissue around the terminal and respiratory bronchioles (Fig. 4). This granulation tissue consisted mainly of macrophages and fibroblasts but some foreign body giant cells were also present. At the end of the dusting period there was marked reticulin staining in the peribronchial deposits although relatively little collagen could be demonstrated by Van Gieson's stain. In lesions from older animals, however, collagen staining increased in intensity. In animals killed after the end of dusting, the overall area of peribronchiolar fibrosis per section was similar but the proportion of lung tissue occupied by these lesions was significantly less (Table 2; $P < 0.01$ variance ratio test). However, the number of lesions per lung section remained the same. The differences were partly due to the increase in size of the rat lungs during this period but also to the fact that there

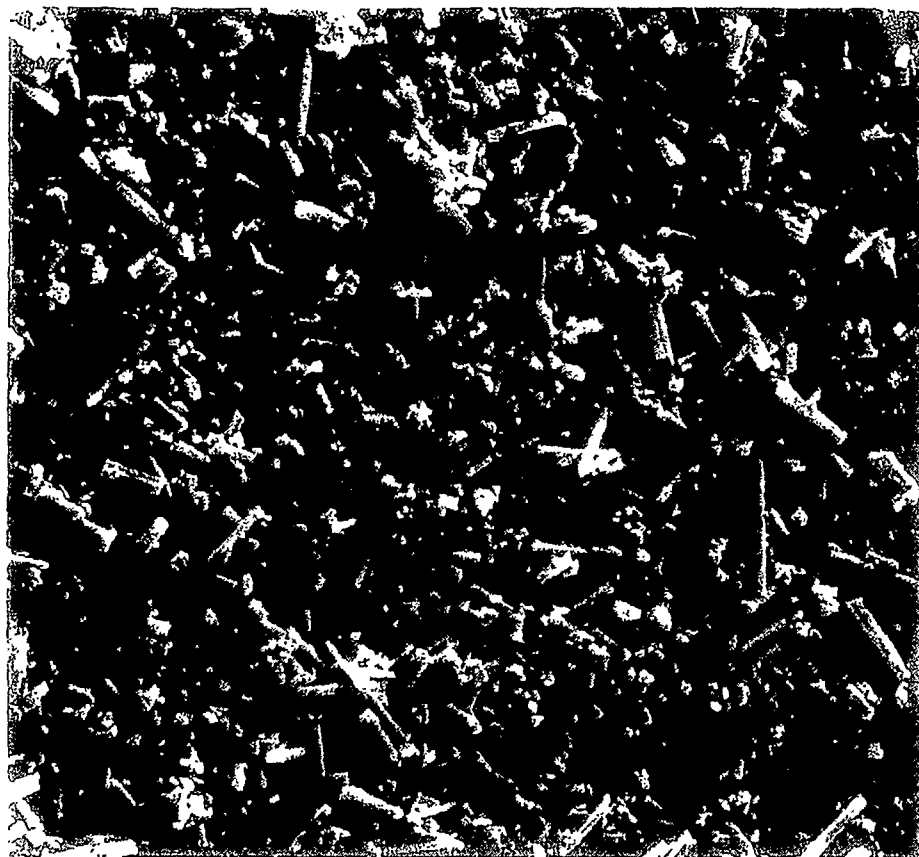


Fig. 1. Scanning electron microscope photograph of the bulk sample of short fibre amosite used in the inhalation and injection studies. $\times 6000$.

appeared to be some contraction of the loose granulation tissue originally formed. As these fibrotic lesions aged, the epithelial cells lining adjacent alveoli frequently became cuboidal in shape (Fig. 5). Animals treated with the short fibre amosite preparation showed no evidence of peribronchiolar fibrosis at any stage. At the end of the dusting period the lungs contained very large numbers of pulmonary macrophages packed with dust particles but these cells remained almost entirely free in the alveolar spaces. Sometimes large groups of these cells were aggregated in alveoli close to respiratory bronchioles (Fig. 6). There was no formation

of granulation tissue or thickening of alveolar septa at these points.

In general from about 18 months onwards, areas of lung tissue in animals exposed to long fibre amosite showed a progressive thickening of alveolar septa. In its earliest form this thickening was caused almost entirely by hyperplasia of type II pneumocytes but later there was considerable deposition of reticulin and eventually collagen in the septal walls (Fig. 7). Accumulations of dust were frequently visible among the fibrous tissue in the thickened septa. As shown in Table 2 these areas of alveolar interstitial fibrosis became more extensive as

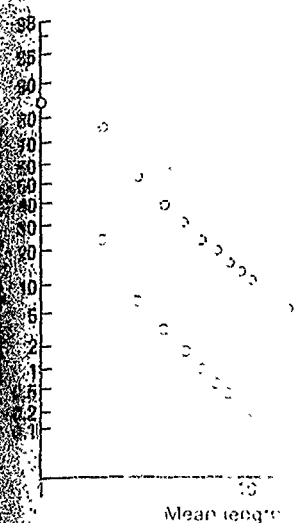


Fig. 2. Fibre length distributions for short fibre amosite dust. (Dusts: 1000 magnification 0.1 μ m; aspect ratio > 3 to 1. 2, short fibre amosite; 3, long fibre amosite).

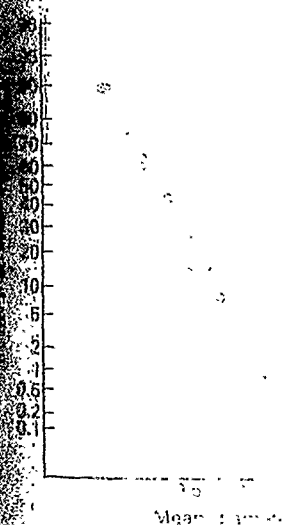


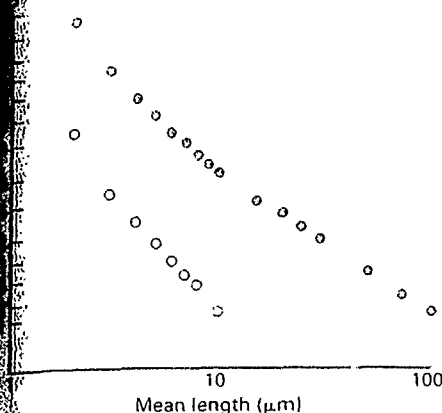
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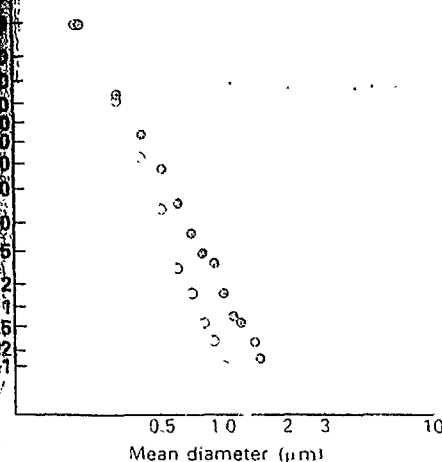
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Fibre length distributions of the long and short fibre amosite dust clouds. SEM sizing at 10 000 magnification, $>0.1 \mu\text{m}$ diameter $\times 3$ aspect ratio >3 to 1. $\circ$, short fibre amosite (700 fibres sized); $\bullet$, long fibre amosite (700 fibres sized).



Fibre diameter distributions of the long and short fibre amosite dust clouds. SEM sizing at 10 000 magnification, $>0.1 \mu\text{m}$ diameter $\times 3$ aspect ratio >3 to 1. $\circ$, short fibre amosite (700 fibres sized); $\bullet$, long fibre amosite (700 fibres sized).

the animals aged. In the oldest animals a mean of 11.0% of lung tissue was involved with interstitial fibrosis in the long fibre treatment group. The corresponding figure for animals treated with short fibre amosite was 0.15% which is comparable to figures seen in old control animals. In some areas the interstitial fibrotic element became progressively more marked with time but in some lesions hyperplasia of the epithelial cells lining the tissue spaces became more pronounced to produce a pattern of adenomatosis.

Three pulmonary adenomas, eight pulmonary carcinomas and two pleural mesotheliomas were found in the group of animals treated with long fibre amosite but no pulmonary tumours developed in the short fibre treatment group (Table 3). One adenoma and one carcinoma were found in control animals. The carcinoma was the first spontaneous malignant primary pulmonary tumour that we have ever found in our strain of rats was a small lesion (2 mm in diameter) which was not responsible for the death of the animal. One peritoneal mesothelioma was found in each of the group treated with amosite dust. However, both of these tumours were found in association with a testis and appear to have developed from the epithelial covering of the tunica vaginalis as reported from untreated populations of rats (Gould 1977). It is probable therefore that neither of these mesotheliomas was associated with amosite treatment.

The numbers of tumours occurring in other tissue sites for the two groups of rats treated with amosite and the two control groups is illustrated in Table 4. Differences in the proportions of animals developing non-pulmonary tumours were not statistically significant when the time at risk was taken into account. The relatively high number of tumours in control group one appears to be related to the lack of planned kills in control groups which allows a higher proportion of animals to reach advanced age and increases their chance of developing a tumour.

One difference between the development

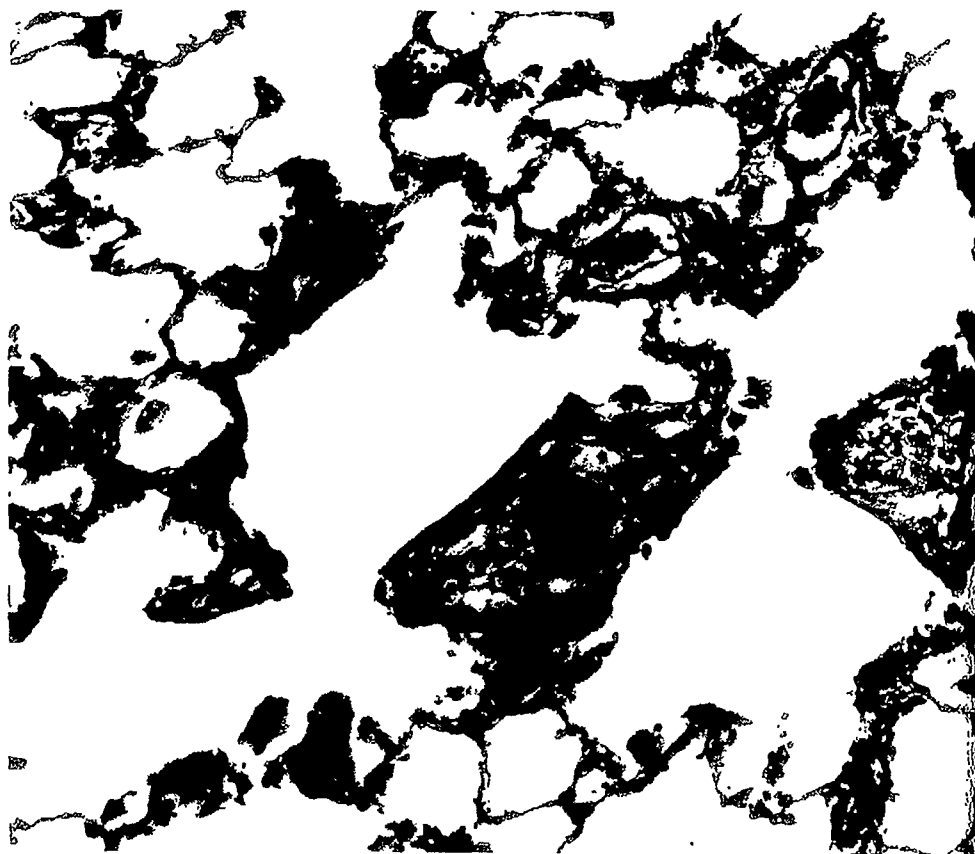


Fig. 4. Foci of loose granulation tissue in the walls of respiratory bronchioles and alveolar ducts from the lungs of a rat killed at the end of 12 months inhalation of long fibre amosite. $\times 450$.

pulmonary and non-pulmonary tumours was noticeable in this study. Non-pulmonary tumours began to be recorded after only 1 year of the study and in the animals treated with long fibre amosite the mean period of these tumours was 694 days from the start of dusting. In contrast, the mean induction period of pulmonary tumours was 891 days. This difference is statistically significant at the $P < 0.002$ level.

Lung dust burden

The retained dust burden of amosite in animals treated with either the long or short preparations is illustrated in Table 5. Less

dust was present in the lung parenchyma of animals treated with long fibre amosite than in those treated with short fibre at both the first and second killing dates. Analysis of variance shows that this difference is significant ($P < 0.01$). The figures suggest proportionately greater clearance of short fibres than long during the 6 months between the first and second killing dates. However, with the small group sizes, this difference was not significant.

Injection studies

The results from experiments in which doses of either 25 mg or 10 mg of the long and

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Table 2. Mean levels of pulmonary fibrosis produced by long and short fibre amosite dusts

	Long amosite			Short amosite			Control group 1		Control group 2	
Time after start of exposure (months)	12	18		12	18	27-29	27-29		27-29	
No. of rats examined	4	4		4	4	23	9		13	
Peribronchiolar fibrosis	15.6 (14.5-17.5)	11.2 (11.0-12.2)		0	0	—	0		0	
Interstitial fibrosis	0	0.45 (0-1.8)		0	0	0.15 (0-2.8)	0.5 (0-3.0)		0	

See text for units.
Figures in brackets are ranges.

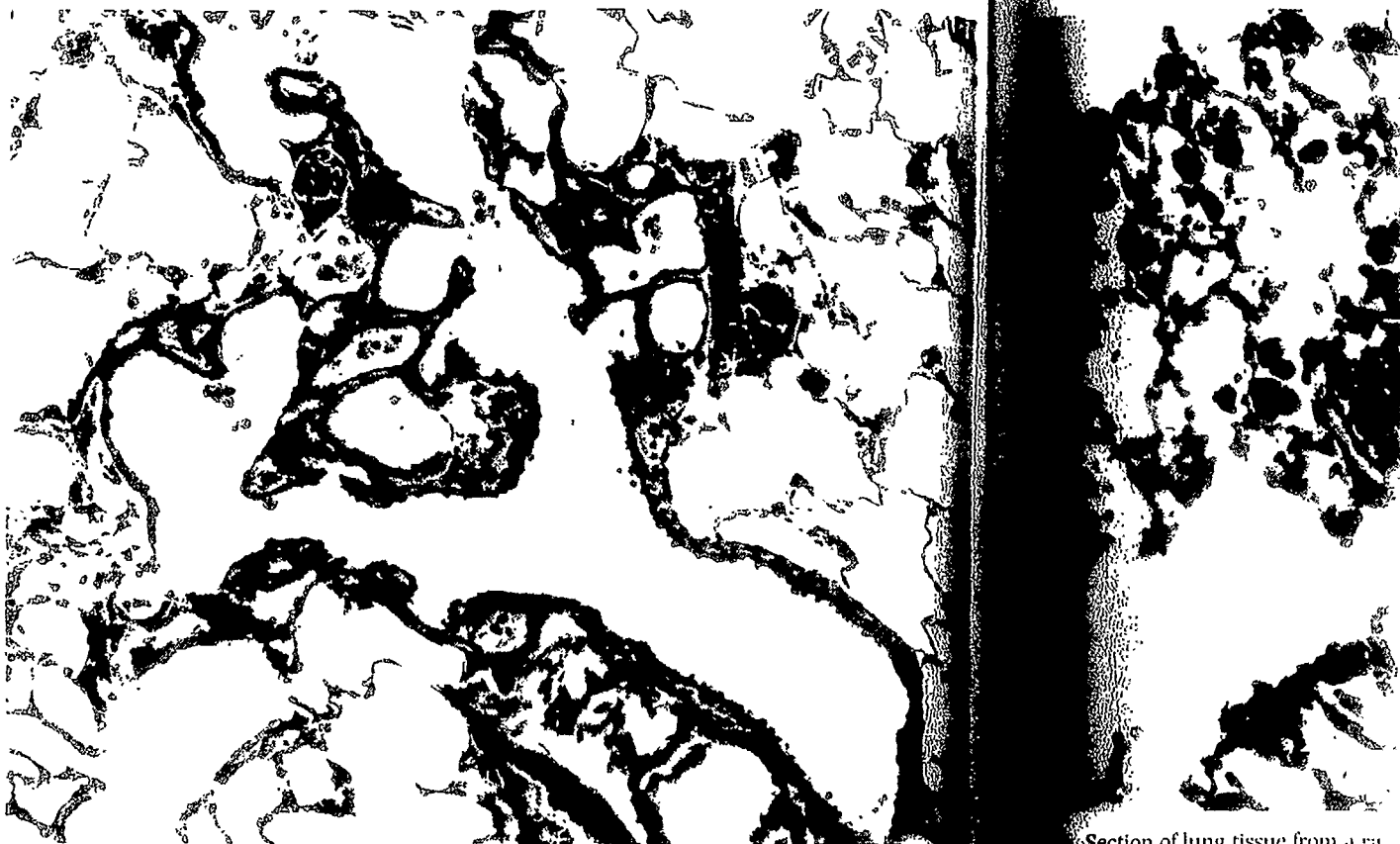


Fig. 5. Section of terminal and respiratory bronchioles from the lungs of a rat killed 6 months after the end of 12 months inhalation of long fibre amosite. Fibrotic foci are less marked than in Fig. 4, but now some alveoli are lined with cuboidal epithelium. $\times 350$.

Section of lung tissue from a rat showing pulmonary macrophages packed in the respiratory bronchioles. A normal. $\times 400$.

short amosite dusts were injected into the peritoneal cavity of rats are illustrated in Table 6. Data from previously published work with UICC amosite (Bolton *et al.* 1982) is included for comparison. The long fibre amosite produced mesotheliomas in almost all animals with a mean tumour induction period of 520–530 days. These figures are very similar to results obtained with UICC amosite. The short fibre amosite preparation produced only a single mesothelioma with an induction period of 837 days.

Discussion

The results from the long term inhalation

studies reported in this paper are in agreement with findings in many previous publications which showed the relatively low pathogenicity of short asbestos fibres. The short fibre amosite dust cloud used in the present study contained only 1% of fibres $> 5 \mu\text{m}$ in length and 0.1% $> 10 \mu\text{m}$ and was thus the shortest amosite preparation that has been available for long term inhalation experiments. After a 1 year period of dust inhalation at 10 mg m^{-3} this short fibre amosite failed to produce either pulmonary fibrosis or pulmonary tumours within the lifetime of the laboratory rat. The long fibre amosite sample with 30% of fibres $> 5 \mu\text{m}$ and 11% of fibres $> 10 \mu\text{m}$ was

other amosite preparation examined and produced pulmonary fibrosis in 30% of animals and large areas of bronchiolar fibrosis and alveolar fibrosis. The UICC standard sample of amosite which was used in our laboratory (Davis *et al.* 1982) had a fibre length distribution very similar to the present long and short fibre preparations. It was found to be highly fibrogenic compared to other amosite samples we have examined at the same concentration (10 mg m^{-3}). It produced only two benign pulmonary tumours in a group of 40 rats. The

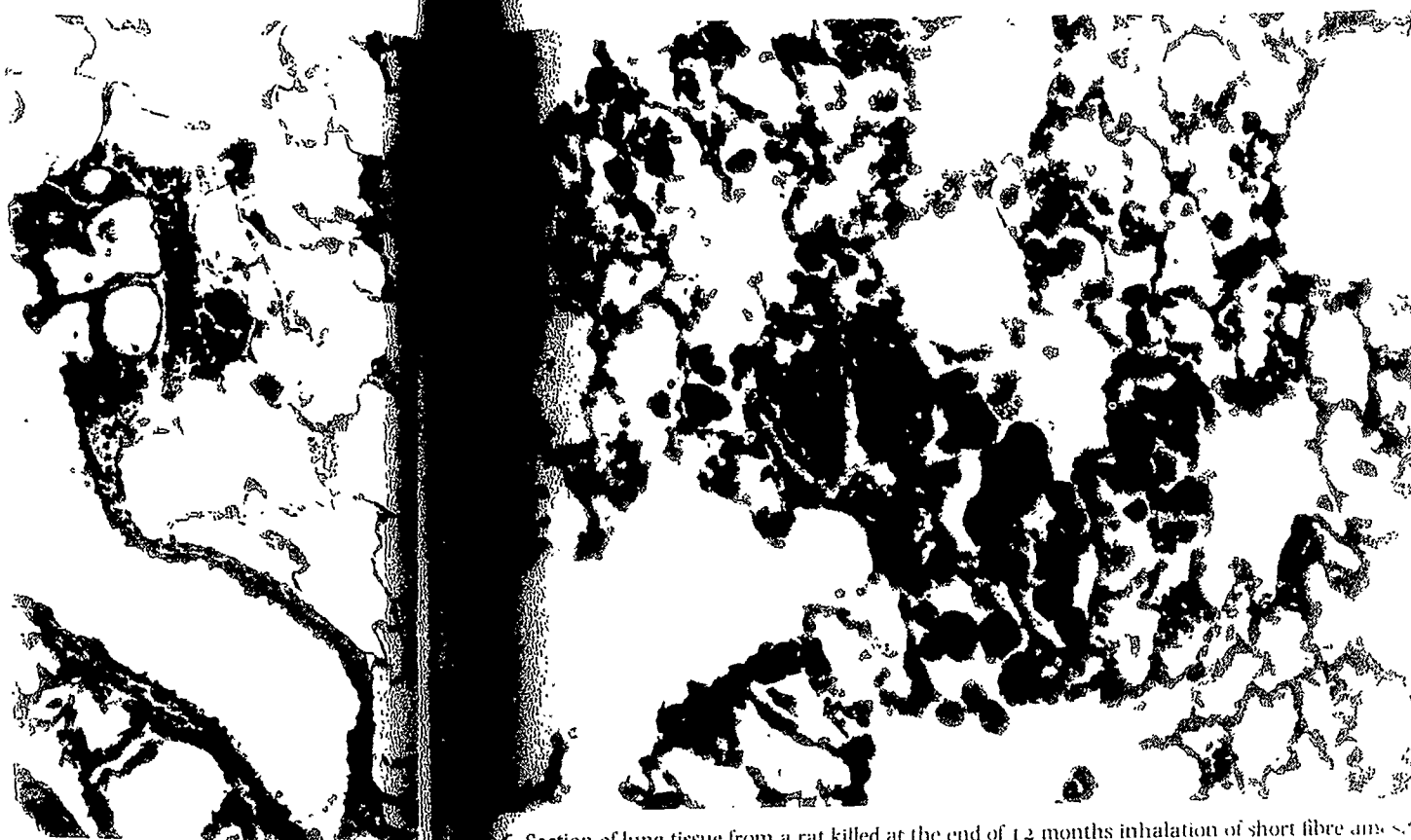


Fig. 5. Section of lung tissue from a rat killed 6 months after the end of inhalation of short fibre amosite. The dust is marked than in Fig. 4, but now some

Fig. 6. Section of lung tissue from a rat killed at the end of 12 months inhalation of short fibre amosite. Many pulmonary macrophages packed with dust are present and these are particularly aggregated around the respiratory bronchioles. Apart from the presence of these cells, however, the lung structure appears normal. $\times 400$.

As reported in this paper are in agreement with findings in many previous publications which showed the relatively low pathogenicity of short asbestos fibres. The fibre amosite dust cloud used in the present study contained only 1% of fibres $> 5 \mu\text{m}$ length and 0.1% $> 10 \mu\text{m}$ and was the shortest amosite preparation that has been available for long term inhalation studies. After a 1 year period of dust inhalation at 10 mg m^{-3} this short fibre preparation failed to produce either pulmonary fibrosis or pulmonary tumours within the lifetime of the laboratory rat. The long fibre amosite sample with 30% of fibres $> 10 \mu\text{m}$ and 10% of fibres $> 10 \mu\text{m}$ was long enough

to be considered as a long fibre preparation. In any other amosite preparation we have examined and produced pulmonary tumours in over 30% of animals and large amounts of peribronchiolar fibrosis and alveolar interstitial fibrosis. The UICC standard reference sample of amosite which was tested previously in our laboratory (Davis *et al.* 1978) had a fibre length distribution roughly midway between the present long and short fibre preparations. It was found to be only moderately fibrogenic compared to other asbestos dusts we have examined at the same airborne concentration (10 mg m^{-3}) and produced only two benign pulmonary adenomas in a group of 20 rats. Thus it would

appear that to produce a significant carcinogenic response in rat lung tissue in studies of this size, amosite fibres must be longer than those in the UICC preparation. These findings, however, contrast with those from experimental injection studies. In these studies a long fibre amosite preparation produced more than 90% of mesotheliomas in a group of rats while the short dust produced only a single tumour. Results from injection studies with UICC amosite (Bolton *et al.* 1982) are in this case almost identical to those from the long fibre amosite dust. From this it would appear that while very short fibres are not suitable for carcinogenicity studies, longer fibres

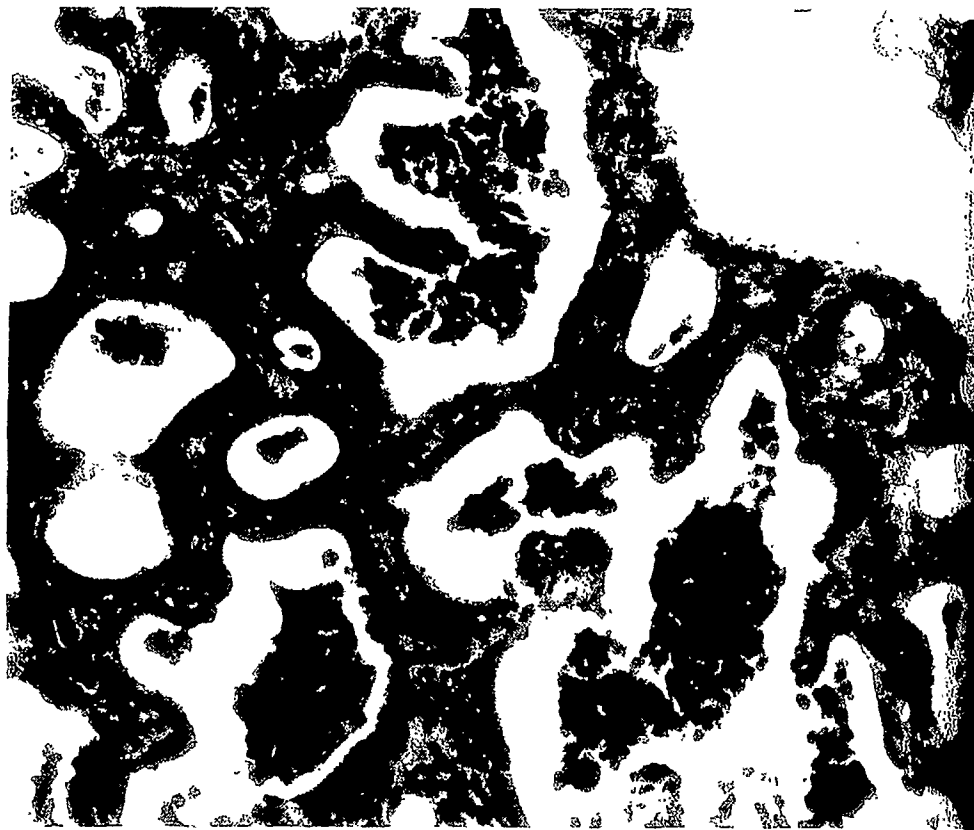


Fig. 7. Section of lung tissue from a rat killed 20 months after the end of twelve months inhalation of long fibre amosite. The normal lung architecture has been replaced by a lesion similar to human 'honeycombing' where the lung spaces no longer correspond to the original alveoli. The honeycomb spaces are lined by cuboidal epithelium and the thickened septa between them are fibrous, but also contain many cells. $\times 400$.

Table 3. Numbers of pulmonary tumours and mesotheliomas found in rats treated with three different preparations of amosite asbestos and in two control groups of these animals

Tumour type	Long amosite	Short amosite	UICC amosite*	Control group 1	Control group 2
No of rats	40	42	43	36	25
Adenoma	3	0	2	1	0
Adeno-carcinoma	3	0	0	0	0
Squamous-carcinoma	4	0	0	0	0
Undifferentiated-carcinoma	1	0	0	0	0
Pleural mesothelioma	2	0	0	0	0
Peritoneal mesothelioma	1	0	0	0	0

* Davis et al. 1978.

4. Numbers of tumours occurring

	Long amosite
Number of rats examined	8
System	
Pleural/peritoneal	—
Orogenital	—
Endocrine	3
Musculo, skeletal and	
Connective-tissue	1
Endothelial	—
Vascular	—
Total	4

Benign: M. Malignant.

5. Estimates of the lung dust content with long and short fibre amosite asbestos.

	Long amosite	Short amosite
After end dusting		
Dust	3570 μ g (1590)	564
Fibres	3080 μ g (370)	147

Figures quoted are the means of rats in each case.

Figures in brackets are standard deviations.

6. Mesothelioma production: numbers of animals developing mesothelioma at the doses of amosite dust

	Long amosite	Short amosite
Number of animals developing mesothelioma	2	0
Mean tumour induction period in days	180	—

Table 4. Numbers of tumours occurring at sites other than lung

	Long amosite		Short amosite		Control group 1		Control group 2	
	40		42		36		25	
Number of rats examined	B	M	B	M	B	M	B	M
Respiratory System								
Pleural/peritoneal	—	—	—	2	1	3	1	—
Ovarian	—	1	1	1	—	2	2	—
Endocrine	3	—	—	—	6	2	3	—
Musculo, skeletal and integumentary	1	4	1	7	—	5	2	3
Vascular	—	5	—	3	—	3	—	1
Total	4	10	2	13	7	15	8	4

B, Benign; M, Malignant.

Table 5. Estimates of the lung dust content of rats treated with long and short fibre samples of amosite asbestos.

Time after end of dusting	Long amosite	Short amosite
0	3570 µg (1590)	5640 µg (370)
6 months	3080 µg (370)	4470 µg (580)

The figures quoted are the means of groups of four rats in each case.

Figures in brackets are standard deviations.

Mesothelial tissues at the doses examined. Mesotheliomas can be produced by dust preparations consisting of shorter fibres than

are needed to produce pulmonary tumours. Taken together these findings may explain both the comparative rarity of mesotheliomas in both rats and humans following dust inhalation and also their characteristically long latent period between first exposure and tumour development. Fibres >25 µm in length are unlikely to be transported through lung tissues to reach the pleural or peritoneal mesothelium. If this length were necessary to transform mesothelial cells then mesotheliomas might not occur at all even in heavily exposed individuals. Medium length fibres (8–15 µm) probably represent a size that is difficult but not impossible to move. After a long period of time enough of these may reach mesothelial tissues to produce

At the end of twelve months inhalation of long fibres replaced by a lesion similar to human asbestosis. The honeycomb structure between them are fibrosed and also

found in rats treated with three different samples of these animals

UICC amosite*	Control group 1	Control group 2
43	36	25
2	1	0
0	0	0
0	1	0
0	0	0
0	0	0
0	0	0

Table 6. Mesothelioma production in rats following the intraperitoneal injection of three samples of amosite dust

	Long amosite		Short amosite		UICC amosite
	25 mg	10 mg	25 mg	10 mg	25 mg
Number of animals developing mesotheliomas	20 (95%)	20 (88%)	1 (4%)	0	10 (91%)
Mean tumour induction period in days	520	535	837	N.A.	505

mesotheliomas in a few individuals. Short fibres (shorter than 5 μm) are relatively easily moved (Morgan *et al.* 1978) but do not appear to be very carcinogenic.

While almost all workers, who have reported experimental studies on fibrous dusts, agree that 'short fibre' preparations are at least relatively harmless, a series of papers by Kolev (1976; 1982; 1984) has reported that crocidolite or anthophyllite ground until all dust particles were isometric could produce mesotheliomas in rats and the isometric crocidolite sample apparently produced more mesotheliomas than a fibrous control sample of crocidolite. These results are difficult to evaluate since detailed size distributions of the injected dust samples were not given but one possible explanation comes from the photographs and histological descriptions of some of the tumours produced. These are described as a giant cell type and a sarcoid type. This type of histological pattern was found by Wagner and Wagner (1972) and Wagner (1976) following the intrapleural injection of isometric quartz particles and the tumours were classified as thymomas. Tumours of similar giant cell and sarcoid type (classified as malignant histiocytomas) were also found in the pleural cavities and lungs of rats treated ceramic fibre dust (Davis *et al.* 1984). Although this dust did contain some long fibres the majority of dust particles were isometric. From these results it seems possible that rats respond to a number of isometric dusts with a formation of tumours that may look superficially like mesotheliomas but are in fact of reticulo-endothelial origin.

From a very large amount of published experimental work there is now a general consensus that fibre dimensions are the major factor in disease production and recently Pott & Ziem (1983) suggested that because of this attempts to produce less dangerous asbestos materials by modifying the fibre surfaces would not prove successful. Bignon & Jaurand (1983), however, reported that acid treatment of chrysotile caused a reduction in fibrogenicity and carcinogeni-

city and suggested that while fibre dimensions were important the surface chemistry of fibres does play a part in disease production. As the authors point out, however, the findings of Bignon's group need not be taken as proof that the chemical composition of fibres is a factor in carcinogenicity. It is likely that changes in fibre chemistry could also effect fibre length and durability and that these factors may influence the experimental results.

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Enzyme activities and heart muscle and skeletal muscle

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Summary. A prominent feature of arterial disease is the presence of intimal smooth muscle cells. In this study the effect of asbestos on the heart muscle was investigated. The mean serum creatinine 165 $\mu\text{mol/L}$ and the mean serum uric acid 0.45 mmol/L of adenine nucleotides were measured. Examination disclosed no changes in the activities of hexokinase, an important glycolytic enzyme in heart and aorta, whereas the hexokinase activity remained unchanged in skeletal muscle. Fat metabolism was measured by the activity of acyl-CoA-dehydrogenase. Acyl-CoA-dehydrogenase activities were markedly increased in the heart muscle. The levels were found in the heart muscle to be similar to the pool of adenosine phosphates in the heart muscle. The animal model described offers a useful tool for studying the effect of asbestos on the heart muscle.

Keywords: adenine nucleotides, aorta, heart muscle.

The increased incidence of cardiovascular disease has been reported in patients with asbestosis (Ejner et al. 1974). In experimental animals, arterial lesions, characterized by intimal necroses and calcification (Ejner et al. 1979; Ejner et al. 1979a) as well as accumulation of glycosaminoglycans (Ejner et al. 1979a) and collagen (Ejner et al. 1979c), have been described. A similar disease has also been seen in patients (Ejner et al. 1979d; Ejner et al. 1979). A myocardial disease, char

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