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

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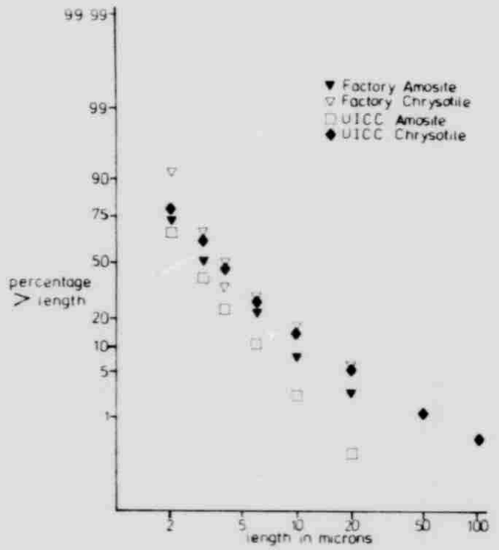
Following recommendations made at the New York Academy of Sciences' meeting on the Biological Effects of Asbestos in 1964, the UICC Working Group on Cancer and Asbestos organized the preparation of standard reference samples of the most important asbestos types, so that research workers in different countries could undertake experiments using identical material (Timbrell & Rendall, 1971/72). The physical and chemical characteristics of these minerals were described by Timbrell (1970), and these UICC materials have now been utilized in many animal experiments undertaken by a number of workers. However, the characteristics of dust clouds generated from the reference samples are in many ways different from those of factory dusts. For this reason, it was decided to undertake inhalation studies using rats to compare the effects of UICC amosite and chrysotile with those of amosite and chrysotile samples collected from the factory environment. These samples were taken from the bag filters of factories using only amosite or chrysotile so that there was no cross contamination with different asbestos types, although there was considerable contamination with materials other than asbestos.

The chrysotile sample was found to contain only 60% of chrysotile by weight, while the amosite sample was 90% amosite. The nonasbestos component of these dust samples was not characterized. Groups of 48 rats were exposed for a period of 12 months to dust clouds with a concentration of 10 mg/m^3 of air. After the dusting period, small groups of animals were killed for estimations of lung dust content and levels of pulmonary fibrosis, but the majority were allowed to live out their full lifespan in order to study the development of pulmonary neoplasms.

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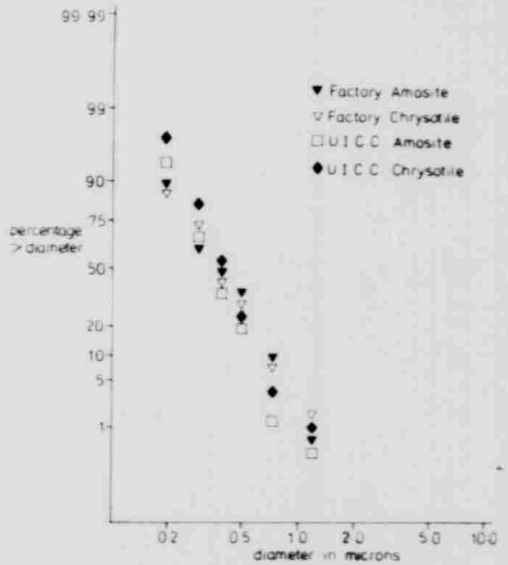
During the dusting period, a number of dust samples were collected
each chamber on membrane filters for measurement of fibre length
diameter distributions, which were undertaken using a Cambridge
ruments S600 scanning electron microscope. The results of these
urements (Figs 1 & 2) indicate that the UICC chrysotile cloud
ained a higher proportion of long fibres than did the factory
sotile, although the factory fibres tended to be thicker on average.
factory amosite cloud contained more long fibres than UICC amosite,
once again the factory fibres were thicker.

FIG. 1. LENGTH DISTRIBUTIONS OF FIBRES LONGER THAN 0.6 μm
(SCANNING ELECTRON MICROSCOPE MEASUREMENTS)



At the end of the 12-month dusting period, the mean weights of
stos in lungs of the different groups were as follows: UICC chry-
le, 1417 μg; factory chrysotile, 2003 μg; UICC amosite, 9169 μg;
factory amosite, 12,710 μg. This means that rats treated with the
ory samples, both of which contained impurities, had accumulated
asbestos than animals exposed to the pure UICC samples. The
sons for this may be different for the two asbestos types. In a
es of short-term experiments, Middleton et al. (1979) showed that
nals dusted in darkness, when they are most active, accumulated
e times more UICC chrysotile in their lungs than rats dusted in

FIG. 2. DIAMETER DISTRIBUTIONS OF FIBRES BROADER THAN 0.2 μm
(SCANNING ELECTRON MICROSCOPE MEASUREMENTS)



daylight. There was no similar effect with UICC amosite. They concluded that during the day the animals slept with their noses buried in each others' fur, and this acted as a filter for the long curly fibres of chrysotile but did not stop the shorter fibres of amosite. Since the factory chrysotile dust contained fewer long fibres, it may well be that the filtration effect was less and lung dust deposition greater compared with the UICC material. The higher lung burden of factory amosite is more difficult to explain but may be due to the different fibre dimensions of this material, affecting both deposition and retention.

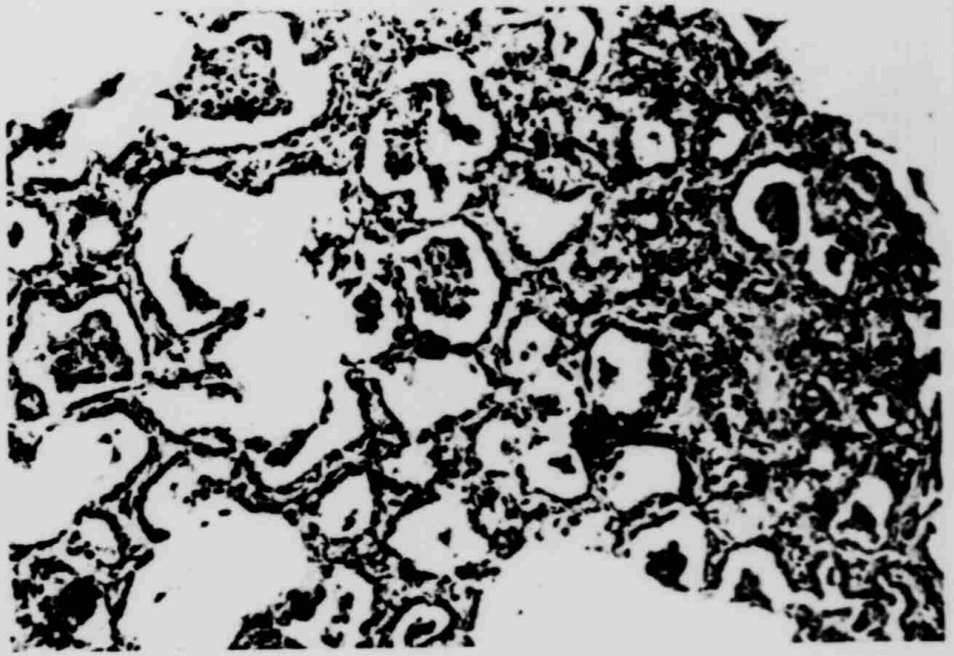
The levels of pulmonary interstitial fibrosis found in the four groups of animals at 12, 18 and 29 months after the start of dusting were recorded as a percentage of total lung tissue area, as reported by Davis et al. (1978). The results, illustrated in Table 1 together with the number of pulmonary tumours found, show that interstitial fibrosis is slow to develop and that only small amounts were present at the 12-month stage. In the older animals, however, it frequently became quite severe (Fig. 3). These results indicate that the sample of factory chrysotile produced very similar levels of lung pathology to UICC chrysotile. The levels of interstitial fibrosis and the

le 1. Levels of interstitial fibrosis and numbers of pulmonary ours produced by UICC amosite and chrysotile and by amosite and ysotile samples collected from the factory environment

osure	No. of animals	Level of interstitial fibrosis (% total lung area)	No. of pulmonary tumours	
			Benign	Malignant
C chrysotile				
2 months	4	0.48		
8 months	4	0.9		
9 months	6	9.15		
ifespan	43		7	8
tory rysotile				
2 months	4	0.0		
8 months	4	0.78		
9 months	6	7.7		
ifespan	42		8	3
C amosite				
2 months	4	0.87		
8 months	4	0.12		
9 months	6	2.6		
ifespan	40		2	0
tory amosite				
2 months	4	0.0		
8 months	4	0.75		
9 months	6	8.5		
ifespan	37		0	0

bers of benign tumours found in the lung tissue were extremely close, the only difference was the smaller number of bronchial carcinomas luced with the factory dust. However, with the number of animals olved, this difference was not statistically significant. With the amosite samples, it was found that the factory dust was more fibro- ic than the UICC sample, and levels of interstitial fibrosis in als treated with factory amosite were as high as with either of the tsotile samples. However, both amosite samples showed little carci- nicity, and the factory amosite produced neither benign nor malignant ours.

FIG. 3. AN AREA OF INTERSTITIAL FIBROSIS FROM THE LUNG OF A RAT 29 MONTHS AFTER THE START OF EXPOSURE TO A SAMPLE OF AMOSITE FROM THE FACTORY ENVIRONMENT (× 250)



These findings raise some interesting points regarding the importance of fibre length in the pathogenic effects of asbestos. Stanton & Wrench (1972) and Stanton et al. (1977) summarized theories on fibre length and pathogenicity and suggested that the most dangerous fibres were over 8 μm in length and less than 1.5 μm in diameter. Davis et al. (1978) reported experiments in which the different UICC asbestos samples had been administered to rats at different dose levels and confirmed that the chrysotile clouds, which alone contained significant numbers of fibres over 20 μm in length, were the most pathogenic. In this case, the results could be interpreted as indicating that carcinogenicity and fibrogenicity were associated with fibre length to the same degree. In the present study, however, the absence of most of the longest fibres from the factory chrysotile sample as compared with UICC chrysotile reduced the number of malignant tumours, but the number of benign adenomas and the levels of interstitial fibrosis in the two experiments were almost identical. With factory amosite, as compared with the UICC material, an increase in the number of relatively long

Wagner et al. (1974) found that amosite produced fewer pulmonary
ırs in rats than other asbestos types after a 12-month inhalation
ıd; it may indeed be that amosite *per se* is a material of relati-
low carcinogenicity, although Selikoff et al. (1972) reported
ficant numbers of tumours in amosite-exposed workers. A more
y explanation of the findings reported in the present paper, how-
is that while fibrogenicity and carcinogenicity both depend on the
nce of relatively long fibres in dust clouds, different lengths are
ved in each process, and tumour production requires the longest
s. The effect of fibre diameter is still obscure. In the
nt study, both chrysotile samples had a higher proportion of fibres
1 μm in diameter than amosite, but chrysotile is much more likely
dergo longitudinal splitting within the tissues to produce very
fibres. The scanning electron microscope fibre sizing technique
for this work did not permit estimations of the proportions of
s below 0.2 μm in diameter because of resolution problems. It was
therefore, possible to study the importance of very thin fibres.
e animal inhalation studies within our Institute will include
mission electron microscope sizing of dusts from both dust clouds
ung tissue, which may resolve this problem.

SUMMARY

Inhalation studies were undertaken in rats to compare the patho-
effects of samples of UICC amosite and chrysotile with those of
:e and chrysotile samples collected from the factory environment.
length and diameter studies on the four dust samples showed that
ICC chrysotile cloud contained more long fibres than factory
›tile, although the factory fibres tended to be thicker. The
ry amosite cloud contained more long fibres than UICC amosite,
ain the factory fibres were thicker. The factory dusts contained
lerable amounts of impurities; in spite of this, the lung dust
ıt of asbestos at the end of 12 months' dusting was higher with
y dusts than with UICC material. The UICC chrysotile and fac-
hrysotile produced similar levels of interstitial fibrosis,
e factory dust produced fewer malignant lung tumours. The
y amosite produced much more interstitial fibrosis than UICC
e, but neither amosite cloud produced any malignant pulmonary
s. These results are discussed in relation to current theories on
portance of fibre dimensions in the pathogenesis of lung disease.

RESUME

Les auteurs ont entrepris des études par inhalation chez le rat afin de comparer les effets pathogènes d'échantillons d'amosite et de chrysotile de l'UICC à ceux d'échantillons d'amosite et de chrysotile recueillis en milieu industriel. Les études de la longueur et du diamètre des fibres dans les quatre échantillons ont montré que les fibres longues étaient plus nombreuses dans le nuage de poussières de chrysotile de l'UICC que dans le chrysotile d'usine, alors que les fibres d'usine étaient souvent plus épaisses. L'amosite d'usine contenait plus de fibres longues que l'amosite de l'UICC, mais les fibres d'usine étaient également plus épaisses. Les poussières d'usine contenaient des impuretés en quantité considérable; néanmoins, après 12 mois d'empoussièrement, la teneur des poussières pulmonaires en amiante était plus forte avec les poussières d'usine qu'avec les échantillons de l'UICC. Le chrysotile de l'UICC et le chrysotile d'usine occasionnaient des degrés comparables de fibrose interstitielle, mais les poussières d'usine provoquaient moins de tumeurs pulmonaires malignes. L'amosite d'usine produisait bien plus de fibrose interstitielle que l'amosite de l'UICC, mais aucun des deux nuages de poussières d'amosite ne provoquait de tumeurs pulmonaires malignes. Ces résultats sont examinés à la lumière des théories actuelles sur l'importance des dimensions des fibres dans la pathogénie des maladies pulmonaires.

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A STUDY BY ELECTRON MICROSCOPY OF THE EFFECTS OF CHRYSOTILE AND MAN-MADE MINERAL FIBRES ON RAT LUNGS

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INTRODUCTION

In undertaking the experimental programme of the European Insulation Manufacturers Association contract with the IARC, Institute of Occupational Medicine and Medical Research Council Pneumoconiosis Unit, it was decided that further information could be obtained by transmission electron microscopy. This paper reports preliminary results on the effects of inhaled man-made mineral fibres on the ultrastructure of the rat lung. A comparison is drawn with the changes resulting from inhalation of chrysotile.

MATERIALS AND METHODS

Twelve adult SPF Fischer rats were used: two were exposed to an atmosphere containing 10 mg/m^3 of each dust for seven hrs a day for five days each week for 50 weeks and then had a four-month post-inhalation period; two further animals were maintained under similar but dust-free conditions. The dusts used were UICC chrysotile B, glass fibre (Johns Manville Code 100), rock wool, resin-coated glass wool and uncoated glass wool. The physical dimensions of these dusts are reported elsewhere in this volume (Wagner et al.[1]). The animals were sacrificed by an overdose of anaesthetic (ether). The right lung was removed, cut into 2-mm

[1] See p. 363

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