Respirable cellulose fibres were less toxic in vitro than the mineral fibres crocidolite and MMVF10. Short-term inhalation of cellulose caused an inflammatory lung response which resolved despite continuing exposure. Intraperitoneal injection of cellulose fibres induced sarcomas rather than mesotheliomas at the highest dose (10^9 WHO fibres), while the two middle doses (10^7 and 10^8 fibres) each produced a mesothelioma. Further work is required to examine the pulmonary effects of respirable cellulose fibres.

**Keywords:** cellulose; crocidolite; fibres; inhalation; inflammation; intraperitoneal injection; MMVF10; tumour

**INTRODUCTION**

Cellulose fibres are used to manufacture products such as textiles, paper and cardboard, to give structural strength to cement and other construction products and to insulate buildings. This manufacture and use can produce respirable airborne cellulose fibres.

Cellulose fibres have not been subjected to the same rigorous toxicity testing that has been applied to asbestos and man-made mineral fibres (Davis, 1993). Previously, there have been only limited numbers of short-term studies in animals using lung instillation and inhalation, all with relatively high doses. Those experiments produced some pathological changes, such as granuloma, alveolitis, epithelial hyperplasia and fibrosis (see references in Davis, 1993; Cullen et al., 2000).

We have studied cellulose fibres using a combination of in vitro, short-term inhalation and i.p. injection studies and made comparisons with mineral fibres.

**MATERIALS AND METHODS**

**Test materials**

The cellulose fibre was a thermo-mechanically processed wood pulp supplied by Laxa Bruks AB of Sweden. Respirable samples of crocidolite asbestos and the man-made mineral fibre MMVF10 were obtained from the Thermal Insulation Manufacturing Association (TIMA) fibre repository.

Fibre sizing

Nuclepore filters were loaded with known masses of fibres. Then respirable fibres were counted and sized by SEM and the data used to calculate the fibre number to mass ratio for use in determining dose in the in vitro and i.p. injection experiments.

Size distributions for crocidolite and cellulose have been published elsewhere (Cullen et al., 2000). In brief, the length distributions were similar for both fibres, but with more long fibres (>20 µm) in the cellulose and more short (<5 µm) fibres in the asbestos. However, classification according to diameter showed that cellulose fibres tended to be thicker than crocidolite fibres. These size differences made for large differences (65 times) in mass doses between the fibre types.

**In vitro experiments**

For the in vitro experiments we used two target cells: rat alveolar macrophages and the lung epithelial cell line A549. Subconfluent monolayers of cells were exposed for 18 h to the test fibres at concentrations of 6 × 10^5, 60 × 10^5 and 600 × 10^5 WHO fibres per culture well in a 96-well plate. In vitro toxicity was determined as release of the enzyme lactate dehydrogenase (LDH).

**Inhalation experiment**

In the inhalation experiment rats were exposed to an aerosol of cellulose fibres at 1000 WHO fibres/ml for 7 h a day, 5 days/week for periods of from 1 day up to ~3 weeks. WHO fibres (particles longer than 5 µm) were counted by optical microscopy.

After each exposure period rat lungs were lavaged and the various types of recovered cells were counted. There were six rats per time point. One
group of rats was allowed to recover for 28 days beyond the end of the exposure period.

**Lifetime i.p. injection study**

This test involved injecting fibres into the peritoneal cavity of rats and observing the rats over their lifetime for the development of tumours. This is a very severe test and, for man-made mineral fibres, a negative finding would exonerate a fibre from being labelled a carcinogen in Europe.

We used the same size-selected cellulose fibre prepared for the *in vitro* tests. Crocidolite obtained from the TIMA repository was used as a positive control and the saline used to suspend the fibres for injection was used as a negative control. Four doses of each fibre were used: $10^6$, $10^7$, $10^8$ and $10^9$ WHO fibres. All groups had 50 rats except for the two highest asbestos dose groups, which had 26 rats. The SEM counts of fibres gave the numbers of fibres longer than $5\mu m$ per unit mass. This resulted in masses for the highest dose of $10^9$ fibres of 1.78 mg for crocidolite and 116 mg for cellulose. Because of the mass size of the largest cellulose dose, all doses for each treatment group were divided into three injections spaced 7 days apart.

**RESULTS**

*In vitro results*

LDH concentrations are shown in Fig. 1 as percentage release compared with the maximum amount releasable using detergent to lyse untreated control cells. For macrophages, MMVF10 was the most toxic of the three fibres, whereas for A549 cells, crocidolite was generally more active than MMVF10. Both these mineral fibre types were more toxic than cellulose.

**Inhalation experiment**

The main finding from lavaging the lungs was an early inflammatory response after 1 day exposure, but subsequently the numbers and percentages of inflammatory granulocytes (mostly neutrophils) declined, despite continuing exposure to the cellulose aerosol (Fig. 2). Levels of granulocytes in the lavage from control animals were consistently low, typically <0.5% (for further details see Cullen et al., 2000).

Histopathology was carried out at the end of the inhalation exposure (day 18 from the start of exposure) and at the end of the 28 day recovery period. At the bifurcations of the terminal and respiratory bronchioles there were aggregations of macrophages and adjacent epithelial cells were rounded. There were some small solid lesions present formed by interstitialization of macrophages and fibroblasts that had the appearance of microgranulomas. There was no evidence of progression of these lesions during the 28 day recovery period and there were fewer macrophages at the bifurcations.

**Lifetime i.p. injection study**

Saline-treated animals tended to survive longest, although there was some overlap with the lower doses of cellulose and the lowest dose of crocidolite. Survival in the crocidolite groups was dose dependent, with animals receiving the higher doses dying sooner. For cellulose fibres there was less clear-cut evidence of a dose effect (0.055 significance level in a proportional hazards model), although there was a tendency for the high dose animals to die earlier.

At autopsy there were widespread adhesions and large granulomas in the peritoneal cavity of all animals receiving the highest dose ($10^9$) of cellulose fibres. Cellulose fibres could still be seen in the
lesions at the end of the experiment. With the $10^8$ dose of cellulose the granulomas were smaller and there were only slight adhesions. No granulomas or adhesions were seen in the lower dose cellulose groups nor in the saline or crocidolite groups.

The tumours found in the peritoneal cavity are summarized in Table 1. Note that for some animals it was not possible to obtain tissues for histology because of autolysis. The pathologists did not regard carcinomas as being treatment related.

In the two highest dose crocidolite groups most animals had mesothelioma, and there were fewer animals with this tumour type as the dose reduced. At the $10^9$ dose of cellulose there were no mesotheliomas, but nine rats had large tumours classified as sarcomas. Two animals had mesothelioma, one in each of the $10^7$ and $10^8$ dose cellulose groups, and an animal in the $10^6$ cellulose fibres group had an angiosarcoma. There were no mesotheliomas or sarcomas in the saline-treated group.

**DISCUSSION**

Cellulose fibres have a low intrinsic toxicity and this is confirmed by the in vitro experiments. However, the published short-term lung instillation studies showing pathological effects and the fact that cellulose fibres have been shown to be durable in rat lungs (Muhle et al., 1997) indicate the potential for these fibres to be harmful when inhaled by man.

The reason for the resolution of inflammation in our short-term inhalation study, in the face of continuing exposure to the cellulose fibre aerosol, is not clear and should not be taken to imply that inflammation would remain low during longer inhalation exposures. A temporary reduction in inflammatory cell numbers was reported with amosite asbestos inhalation (Cullen et al., 1997).

The i.p. injection test has been heavily criticized because of the unnatural route of administration of fibres (the peritoneal cavity rather than the lung) and the large doses of fibre injected. However, the assay has been used to test many fibre types and is part of the European fibre testing protocols.

The finding that cellulose at the highest dose ($10^9$ fibres) caused sarcomas was unusual in our experience with this test, as mineral fibres mostly produce mesotheliomas. The exact diagnosis of tumours produced following i.p. injection of fibres can be problematical due to the various histological patterns involved, and one point of view is that the sarcomas found in this study are an extreme in a continuum of mesothelioma types. The background incidence of mesothelioma is very low in this strain of rat (<1%). Thus, the incidence of mesotheliomas in the mid dose cellulose groups appears to be higher than that expected in control animals. However, the comparison relies on relatively few animals.

The implications for the ability of cellulose fibres to cause pulmonary carcinomas following inhalation remains unknown since long-term inhalation studies have not been undertaken. The present study re-emphasizes the need for such work.

**CONCLUSIONS**

In the in vivo tests cellulose fibres produced harmful effects, including tumours. The tumours in the peritoneal cavity included two rats with meso-
thelioma, but mainly comprised sarcomas (in nine 
rats), which are not normally seen with mineral fibres. 
Long-term inhalation studies with cellulose fibres are 
recommended.

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