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The effects of intrapleural injections of alumina and aluminosilicate (ceramic) fibres

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Summary. Groups of rats, 24 male and 24 female, approximately 8 weeks old, were dosed by a single intrapleural injection with a saline suspension of refractory alumina fibres (Saffil fibres ICI plc) either as manufactured or after extensive thermal ageing; or one of two aluminosilicate ('ceramic') fibres with different diameter distributions. Similar groups were dosed with a suspension of UICC chrysotile A asbestos or saline solution to serve as positive and negative controls respectively. Rats were maintained to 85% mortality and all decedents and terminal sacrifices were closely examined for the presence of mesothelioma. Malignant mesothelioma was diagnosed in ten rats, seven dosed with asbestos and three dosed with aluminosilicate fibre B. No mesothelioma was detected in any rat dosed with Saffil fibres or aluminosilicate fibre A or in negative controls. The results support the predicted inert nature of Saffil alumina fibres and provide further evidence for the importance of fibre dimension in the induction of mesothelioma. The implication of the results for inhalation exposures is discussed.

Keywords: carcinogenic, mineral, fibre, injection, mesothelioma

The need to substitute for asbestos in the work place has focused attention on the general question of the pathological effects of durable fibres in tissues in order to ensure the safety of substitutes of both synthetic and natural origin. A major concern arises from the association between inhalation of asbestos fibre and the occurrence of malignant mesothelioma which was first demonstrated by Wagner *et al.* (1960). These findings have since been confirmed and extended to the point where the link between exposure to asbestos (particularly 'blue' asbestos) and malignant mesothelioma of the pleura or peritoneum is well established.

Mesotheliomata have been induced in experimental animals by a variety of techniques, including intrapleural injections (Wagner & Berry 1969; Wagner *et al.* 1973) intraperitoneal injection (Pott *et al*, 1974, 1987, 1989) and implantation into the pleural cavity (Stanton & Wrench 1972; Stanton *et al.* 1977). Such investigations have shown that a variety of mineral fibres, of both natural and synthetic origin, can induce mesothelioma in these experimental regimes. These observations have led to the suggestion that the dimensions of a fibre rather than its chemical composition determine whether a material is carcinogenic

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under these conditions (Timbrell 1973; Stanton *et al.* 1977; Pott 1978; Pott *et al.* 1989). Consideration of the relevent dimensions indicated fibre diameter as the critical parameter. Saffil alumina fibre, a novel refractory material used mainly in high temperature (>1200°C) insulation, was designed with a specification of fibre diameter which was predicted as biologically inert. This fibre has been the subject of a series of toxicological investigations outlined elsewhere (Pigott & Ishmael 1982). Aluminosilicate fibres also find extensive use as insulators at temperatures in excess of 1000°C but have a wider spectrum of diameter than Saffil fibres. In the study reported here Saffil fibres both as manufactured and after extensive thermal 'ageing' (to simulate fibres which may be encountered during the removal of Saffil installations) and two samples of aluminosilicate fibres were assessed for potential to induce mesothelioma in rats after intrapleural injection. A standard reference sample of UICC* chrysotile A (Rhodesian) asbestos served as a positive control material.

Materials and methods

Saffil fibres, both as manufactured and after 'ageing' at temperatures above 1200°C for more than 1000 hours, were supplied by ICI Chemicals & Polymers Limited, The Heath, Runcorn, Cheshire, UK. The 'ageing' process results in a change in the microcrystalline structure of the fibre but has little effect on fibre diameter (Table 1). Samples were preground before supply, and characterized by optical microscopy for length and diameter. Chemical composition was also supplied. Aluminosilicate fibres were supplied as two samples of bulk fibre by the manufacturer. These samples were labelled A and B and differed in appearance; A was pale yellow/brown whereas B was pure white. This reflected the different starting materials; A was made from a calcined kaolin clay of natural origin and B from alumina and silica. Both fibres were heated to 600°C for 30 minutes to remove organic additives and surface coatings. This technique does not influence the chemical or physical properties of the fibres themselves. The resultant fibres were ground by hand in a porcelain mortar to reduce fibre length. The ground materials were passed through a 200 mesh (76 μm) sieve to remove 'shot' (large particulates found in fibres of this type) and very long fibres (which could cause syringe blockage on injection). A sample of the graded material was characterized for length and diameter by J.W. Skidmore, MRC Pneumoconiosis Research Unit, Llandough Hospital, Penarth, South Glamorgan, UK. The results are summarized in Table 1.

A UICC standard sample of chrysotile A (Rhodesian) asbestos (Timbrell *et al.* 1968) was obtained from the Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, South Glamorgan, UK. This sample has been extensively characterized (Rendall 1972).

Dispersions for dosing were prepared at 10% (w/v) in physiological saline by vigorous shaking. In the case of the UICC asbestos sample a 10% suspension takes the form of a thixotropic gel. To ensure uniformity this suspension was twice forced through a 25G × 1.5 cm hypodermic needle. All dosing suspensions were steam sterilized (120°C for 15 min) before use. Albino rats of the Alpk:AP (Wistar derived) strain were supplied from the breeding colony at ICI Pharmaceuticals, Alderley Park, Macclesfield, UK. Dose groups and the control group consisted of 24 rats of each sex and rats were randomly assigned to dose groups on receipt. Standard laboratory diet and tap water were available *ad libitum* throughout the experiment. Rats were dosed by means of a single intrapleural injection of 0.2 ml (20 mg suspended solids) of the appropriate suspension. The control group received saline only. Each animal was anaesthetized before dosing by intraperitoneal injection of pentobarbitone sodium solution (Nembutal, May & Baker Ltd, UK)

*Union Internationale Contre le Cancer.

Table 1. Dimensions of fibre samples tested. Figures are percentage of total fibres counted in the stated category

Length (μm)	Diameter (μm)						
	<1	1·0–1·5	1·5–2·0	2·0–3·0	3·0–4·0	4·0–6·0	Σ
Saffil fibres as manufactured							
<10	2.0	3.9	2.0	0	0	0	7.9
10–20	0.9	4.1	16.9	19.4	2.4	0	43.7
20–40	0.3	1.1	4.5	25.7	9.2	0	40.8
40–60	0.2	0.3	0.8	3.6	1.4	0.2	6.5
60–80	0	0	0	0.6	0.2	0.2	1.0
80–100	0	0	0	0	0	0	0
>100	0	0	0	0	0	0	0
Σ	3.4	9.4	24.2	49.3	13.2	0.4	99.9
Saffil fibres after thermal ageing							
<10	0.2	0	0	0	0	0	0.2
10–20	1.2	0.6	2.2	1.9	0.8	0	6.7
20–40	0.2	2.0	5.7	16.1	2.9	0	26.9
40–60	0.3	0.8	5.7	14.9	4.8	0	26.5
60–80	0.2	0.5	3.4	9.1	4.0	0.2	17.4
80–100	0	0.3	1.9	8.2	1.7	0	12.1
>100	0	0.3	2.3	4.3	2.9	0.3	10.1
Σ	2.1	4.5	21.2	54.5	17.1	0.5	99.9

Length (μm)	<0.5	0.5–1.0	1.0–1.5	1.5–2.0	2.0–3.0	3.0–5.0	5.0–7.0	7.0–10	Σ
Aluminosilicate fibre A									
0–10	8.7	7.9	1.6	—	1.6	—	—	—	19.8
10–20	1.6	6.3	3.9	0.8	5.5	4.7	0.8	—	23.6
20–40	—	5.5	3.9	6.3	6.3	11.0	1.6	—	34.6
40–60	—	—	2.4	1.6	1.6	7.1	4.7	1.6	19.0
60–80	—	—	—	—	—	—	—	1.6	1.6
80–100	—	—	—	—	0.8	—	—	—	0.8
>100	—	—	—	—	—	—	0.8	—	0.8
Σ	10.3	19.7	11.8	8.7	15.8	22.8	7.9	3.2	100.2
Aluminosilicate fibre B									
0–10	30.3	20.8	1.4	0.5	0.9	0.5	—	—	54.5
10–20	5.9	6.8	3.2	2.7	1.4	1.8	0.5	—	22.3
20–40	1.8	3.6	3.2	3.6	2.3	1..4	0.5	—	16.4
40–60	—	0.5	0.5	0.5	1.4	0.5	0.9	0.5	4.8
60–80	—	0.5	—	0.5	—	0.5	—	0.5	2.0
80–100	—	—	—	—	—	0.9	—	—	0.9
>100	—	—	—	—	—	—	—	—	—
Σ	38.0	32.2	8.3	7.8	6.0	5.6	1.9	1.0	100.8

diluted 1 in 10 with 10% aqueous ethyl alcohol. Supplementary anaesthesia was occasionally required and this was achieved with halothane BP vapour (Fluothane, ICI Pharmaceuticals). The anaesthetized animal was laid on its back and a 20G × 2.5 cm sterile hypodermic needle inserted through the skin at the level of the second nipple. The needle was then carefully inserted through the thoracic wall ensuring that the tip pointed dorsally and 0.2 ml of the dosing solution was injected into the pleural cavity. The needle was withdrawn and the animal allowed to recover. Dosing was arranged in replicates and took place over a period of 5 weeks. At the start of this period the rats were approximately 8 weeks old. Following injection the rats were caged in pairs in replicates in a room which contained animals from this experiment only. Each rat was allowed to live until it died or appeared distressed until 85% mortality (average of all groups) was reached. At this point the remaining animals were killed. Animals in a distressed condition and survivors at termination were killed by overexposure to halothane BP vapour and subjected to immediate post-mortem examination. Animals found dead were subjected to a similar post-mortem examination as soon as practicable. During the examination particular attention was paid to the thoracic cavity, the parietal and visceral pleura being closely examined. Care was taken to prevent contamination of abdominal tissues with thoracic material which may have contained test fibres. The lungs were removed and inflated with formol saline and samples of all macroscopically abnormal tissues plus adrenals, aorta, diaphragm, alimentary tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon) heart, kidney, liver, lungs, lymph node, mammary gland, ovary, prostate, seminal vesicle, spleen, testis (with epididymis), thymus, urinary bladder, uterus and cervix and voluntary muscle were taken for histopathology. The tissues were fixed in formol sublimate, embedded in paraffin wax and 5 μm sections cut and stained with haematoxylin and eosin. Additional stains were used when required.

Results

There were no specific clinical observations which could be related to treatment. Rats dosed with fibre tended to show a lower body weight gain compared to the control group in the period immediately following the injection but this was not statistically significant and did not persist. Overall mortality (Table 2) was broadly comparable between groups though there was a slight increase in the number of rats from the group dosed with

Table 2. Survival pattern for rats in the experiment. Termination was during weeks 122–123. Figures given represent numbers of surviving rats of either sex

	Number of survivors after				
	0 weeks	52 weeks	78 weeks	104 weeks	At termination
Control	48	48	44	19	7
UICC chrysotile asbestos	48	46	42	25	10
Saffil	48	48	47	29	17
'Aged' Saffil	48	48	45	31	9
Aluminosilicate fibre A	46*	46	42	23	10
Aluminosilicate fibre B	48	47	42	20	7

*Two rats were misdosed and excluded from the study.

Saffil surviving to termination. This was entirely attributable to the increased survival of females (11/24 *vs* 3/24 in controls) and is considered not to be biologically significant. Histological examination revealed changes related to the injected materials in rats from all dosed groups. There are summarized in Table 3.

There was evidence of intra-abdominal injection in a small proportion of animals in most groups. In some instances there was evidence for partial deposition of the administered dose in the peritoneum with the majority in the pleural space. Other changes were mainly age-related (not tabulated) and considered typical of the Alpk:AP rat and incidental to treatment.

Approximately one-third of the control (saline dosed) animals showed minimal chronic inflammatory cell infiltration and/or fibrosis in the pleural cavity, occasionally involving the pericardium. In one rat of each sex this was associated with some mesothelial proliferation. A small number of rats of either sex showed similar changes in the peritoneal cavity.

The majority of rats dosed with asbestos showed nodular plaque formation consisting

Table 3. Non-neoplastic pathological findings: intercurrent deaths and terminal kill

	Males*						Females*					
Tissue/histopathological finding	1	2	3	4	5	6	1	2	3	4	5	6
Number examined	24	24	24	24	24	24	24	24	24	24	22	24
Pleura												
fibres in pleura	0	15	21	22	17	0	14	24	24	24	17	22
chronic pleurisy/focal fibrosis/plaques	6	20	22	23	24	22	11	19	24	24	20	24
mesothelial proliferation	1	1	4	5	16	13	1	1	6	2	15	14
Heart												
fibres in epicardium	0	0	2	6	2	2	0	0	3	6	2	0
chronic epicarditis/focal fibrosis/plaques	0	8	18	13	17	19	2	7	17	20	13	20
pericardial adhesions	0	0	4	4	6	5	1	2	1	1	3	3
mesothelial proliferation	0	1	7	3	3	6	0	5	4	1	2	9
Peritoneum												
fibres in peritoneum	0	2	4	4	2	4	0	5	5	0	4	1
chronic peritonitis/focal fibrosis/plaques	2	3	5	7	5	7	3	4	6	2	6	4
mesothelial proliferation	0	0	0	2	0	1	0	0	0	0	2	0
Thoracic lymph nodes												
fibres in histiocytes	0	0	12	13	0	0	0	0	17	15	0	0
Injection site												
intra-abdominal only	0	1	2	2	1	4	0	4	0	0	3	0
intra-thoracic and abdominal	0	1	2	1	1	1	0	1	5	0	1	1

* 1, Control (saline); 2, UICC chrysotile A asbestos; 3, Saffil; 4, 'aged' Saffil; 5, aluminosilicate fibre A; 6, aluminosilicate fibre B.

of a fine fibrous-encapsulated mass of amorphous calcifying material in the pleurae. Many of these plaques were on the diaphragmatic or parietal pleura. Associated with the plaques were further areas of chronic pleurisy/fibrosis with associated adhesion formation. Minimal chronic epicarditis/fibrosis with associated adhesions was seen in approximately one-third of animals. Similar changes to those in the pleura were seen in the peritoneum; mainly, but not exclusively, in those rats with clear evidence for intra-abdominal injection.

The reactions to the two forms of Saffil were very similar. In almost all animals there was a minimal focal chronic pleurisy/fibrosis with adhesion formation. Mesothelial proliferation was seen in some animals. Similar changes were seen in the peritoneal cavity of a few rats, mainly where the dosage had been intra-abdominal. The lesions were clearly less severe than those in asbestos-dosed animals with much less collagen present. Short Saffil fibres were seen in the medullary histiocytes of the thoracic lymph nodes in over half these animals. Minimal epicardial fibrosis/epicarditis was also common in animals from these groups. Pericardial adhesions with some Saffil fibres present, and mesothelial proliferation, were also seen in a number of rats.

The reaction to aluminosilicate fibres A and B was similar both qualitatively and quantitatively. Minimal to moderate focal chronic pleurisy/fibrosis, often with much associated mesothelial proliferation was seen in the majority of rats in these groups. In a small proportion of animals changes were also evident in the peritoneum but in these cases mesothelial proliferation was minimal. Overall the reaction was similar to, though somewhat more severe than, that seen in response to Saffil fibres.

The neoplastic findings are summarized in Table 4. A total of 406 tumours were observed, of which 313 were benign and 93 malignant. Malignant mesothelioma of the pleura or peritoneum was seen in ten rats, seven dosed with asbestos and three with aluminosilicate fibre B. These tumours occurred predominantly in male rats with the earliest seen 63 weeks after injection, though the majority were seen much later in the experiment. The histological appearance of the tumours varied though most were of either fibrous or mixed type (Table 5). Benign testicular mesothelioma (of the *tunica vaginalis*) was seen in one rat dosed with Saffil, two dosed with 'aged' Saffil and four dosed with aluminosilicate fibre A.

Other tumours observed were those commonly encountered in the Alpk:AP rat, pitui-

Table 4. Incidence of neoplasia

Group*	1	2	3	4	5	6
Number examined	48	48	48	48	46	48
Number with tumours	37	41	42	40	37	42
Number with multiple tumours	19	29	25	23	18	20
Number with malignant tumours	16	25	16	13	9	18
Total number of tumours	62	81	71	68	57	67
Total number of benign tumours	44	55	57	56	46	49
Excluding benign pituitary and mammary tumours	19	24	24	25	17	19
Total number of malignant tumours	17	26	16	14	10	19
Excluding malignant pituitary and mammary tumours	10	19	10	7	4	15
Malignant mesothelioma of the pleura or peritoneum	0	7	0	0	0	3

* 1, Control (saline); 2, UICC chrysotile A asbestos; 3, Saffil; 4, 'aged' Saffil; 5, aluminosilicate fibre A; 6, aluminosilicate fibre B.

Table 5. Histological type and time of diagnosis of mesothelioma

Age (weeks)	Site	Histological type
Rats receiving UICC Chrysotile A asbestos		
63	Pleural	Fibrous
97	Pleural	Fibrous
99	Pleural	Fibrous
100	Pleural	Mixed
116	Pleural	Mixed
120	Pleural	Epithelial
123*	Pleural	Fibrous
Rats receiving alluminosilicate fibre B		
91†	Peritoneal	Mixed
95	Pleural	Mixed
120	Peritoneal	Mixed

*Killed at termination.
†Female.

tary and mammary tumours predominating (Table 4).

Discussion

Incidence of mesothelioma

The major objective of this study was to investigate the incidence of mesothelioma in the test groups. In this context it is necessary to distinguish between the various histological forms of malignant mesothelioma of the pleura and peritoneum and benign mesothelioma of the testis. Mesothelioma of the *tunica vaginalis* is not uncommon in ageing Alpk:AP rats and is invariably confined to the testis and the epididymis. This was so for the seven cases diagnosed in this study overall and there was no evidence of intraperitoneal injection in any of these rats. None of the three affected groups showed an incidence above the historical control values and the occurrence of these tumours is regarded as incidental to treatment. Malignant mesothelioma of the pleura or peritoneum is rare as a spontaneous tumour in the Alpk:AP rat (< 1 in 10000) and the observed incidences in both the groups dosed with UICC chrysotile asbestos and aluminosilicate fibre B must be regarded as biologically significant. Malignant mesothelioma in rats dosed with asbestos was confined to males in this experiment. This is considered not to be a genuine sex-linked effect. In a similar previous experiment in this laboratory, 48 rats (24 of each sex) were dosed intrapleurally with 20 mg UICC chrysotile asbestos and six of the seven malignant mesotheliomata diagnosed occurred in female rats (unpublished observation). Thus, when both experiments are considered the incidence is similar for both sexes. This is consistent with experience in other laboratories (Wagner *et al.* 1973; Pott *et al.* 1974) where no sex-related differences in mesothelioma incidence were seen. Of the three malignant mesotheliomata diagnosed in rats dosed with aluminosilicate fibre B, two were of peritoneal origin but in both cases there was evidence for intraperitoneal rather than intrapleural injection. These must be regarded as significant; intraperitoneal injection has been used previously to assess the potential of fibres, including ceramic fibres, to induce mesothelioma (Pott *et al.* 1974, 1987, 1989; Davis *et al.* 1982).

It is evident from Table 3 that there is no general correlation between the observation of mesothelial proliferation in the test rats and the occurrence of mesothelioma. A more probable explanation of these relatively mild signs is that they reflect the irritant properties of the injected materials. Man-made fibres are generally mild to moderate skin irritants, a property which, at least for glass fibre, is related to fibre diameter, with coarse fibres being more irritant than fine (Heisel 1976). Pott *et al.* (1989) also observed that fibrosis is not a prerequisite for mesothelioma induction following injection of chrysotile asbestos.

Wagner *et al.* (1976) and Davis *et al.* (1982) reported a 10% incidence of mesothelioma after intrapleural inoculation of a respirable fraction of unspecified ceramic fibres. These materials were probably of similar composition to the aluminosilicate fibres tested here though the method of

comminution was different and organic additives were not burned out. The incidence of mesothelioma (6.25%) following injection of aluminosilicate fibre B is comparable to these previous experiments. The zero incidence seen with aluminosilicate fibre A is clearly different from the previous studies and may be attributable to the size differences between these fibres. There is considerable evidence (summarized by Pott 1978; Pott *et al.* 1989) to indicate that, for a range of fibrous inorganic materials of diverse chemical composition, both fibre diameter and fibre length can influence the incidence of mesothelioma after intrapleural or intraperitoneal injection. Stanton *et al.* (1977, 1981), using a technique for implantation of fibres in the pleural cavity, predicted that 'pleural sarcoma' was strongly associated with fibres <0.25 μm diameter, >8 μm length and less strongly with fibres up to 1.5 μm diameter, >4 μm length. Table 1 shows that for the fibres tested there is a similar correlation between the presence of 'fine long' fibres and mesothelioma though in this case fibres <0.5 μm diameter and >10 μm length is the category most probably associated with the observed incidence of mesothelioma. As both aluminosilicate samples A and B contained similar quantities of fibres in the range 1.0–1.5 μm diameter and there were a few Saffil fibres in this category, it seems unlikely that there is a strong association between fibres in this size range and mesothelioma. This suggests that the correlations proposed by Stanton *et al.* (1981) are too broad and the capability for mesothelioma induction is probably confined to submicron fibres. It should be noted that Pott *et al.* (1989) observed a considerably higher incidence of tumours after intraperitoneal injection of two samples of ceramic wool and of a basalt fibre than predicted from their content of sub-micron fibres. However, doses were higher than those used in previous experiments and the fibres concerned are likely to be durable in tissue. Thus it is possible that the absolute level of sub-micron fibres in the sample tested was sufficient to account for the enhanced tumour yield.

Relevance for risk assessment

The significance of results from injection studies for risk assessment of human inhalation exposures is debatable. Gross and Braun (1978) argue that extrapolation from such experiments is inappropriate and they give several examples for both natural and man-made fibres where the correlation is not observed. Wagner *et al.* (1982) in a series of experiments with man-made mineral fibres showed mesothelioma in response to intrapleural injection but not when the same material was administered by inhalation. Similarly Davis *et al.* (1982) showed mesothelioma (of low incidence) after intraperitoneal injection of ceramic fibre but not when the same fibre was administered by inhalation.

However, Hesterberg *et al.* (1991) have shown that a size-selected fraction from ceramic fibre can induce mesothelioma in hamsters when administered by inhalation. The fraction was selected to be highly respirable to the test animals (Bernstein *et al.* 1992), a process which also concentrated fibres predicted to be carcinogenic on the basis of their dimensions. This correlation may also extend to man. Baris *et al.* (1978) and Artvinli and Baris (1979) found a high incidence of mesothelioma in a population exposed to a fibrous zeolite (erionite) which was later shown to be carcinogenic to rats both by inhalation and by injection (Wagner *et al.* 1985).

These apparent inconsistencies are probably attributable to the effective dose received at the target tissue in the appropriate experiments. Intra-cavity injections deposit a high concentration of test material directly on to the target tissue. Inhalation exposures involve additional factors as fibres must first deposit in the alveolar region of the lung and subsequently penetrate lung tissue to reach the pleural space. The properties

required for a material to achieve this translation (and resist dissolution in transit) may be different from those required for tumour induction once the target is reached. Thus the injection techniques probably reflect an intrinsic property of the test material to induce mesothelioma but do not necessarily indicate an inhalation hazard.

Nevertheless, our results with the aluminosilicate fibres A and B may have relevance for human risk assessment. The results of Hesterberg *et al.* (1991) show a mesothelioma hazard from aluminosilicate fibres by inhalation after exposure of animals to high concentrations of fibres of the size predicted to be carcinogenic. The injection experiments used fibres which were representative of the bulk but which nevertheless would contain significant numbers with relevant dimensions. The fact that mesothelioma was seen in a low yield with one fibre and not at all with the other even when applied by a technique expected to maximize the yield of these tumours suggests that the risk associated with normal handling is low. Further work is needed to elucidate the dose–response relationship and to determine whether this reflects a low risk for individual 'active' fibres or the need for multiple fibres for tumour induction.

Saffil fibres both as manufactured and after extensive thermal ageing were not associated with mesothelioma induction in these experiments. It is concluded that Saffil fibres do not present a risk to man of mesothelioma. This is as predicted from consideration of the overall size range of the manufactured fibre and was an important design consideration (Pigott & Ishmael 1982). The inert nature of the material has been confirmed by an inhalation study (Pigott *et al.* 1981).

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