Br. J. exp. Path. (1981) **62,** 323

EFFECTS OF LONG TERM INHALATION OF ALUMINA FIBRES IN RATS

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Received for publication February 16, 1981

Summary.—Groups of rats were exposed by inhalation to atmospheres containing a refractory alumina fibre (Saffil Fibres, I.C.I.) either as manufactured or in a thermally aged form. Similar groups were exposed to UICC chrysotile A asbestos or clean air to serve as positive and negative controls respectively. Exposures continued for 86 weeks after which the animals were maintained to 85% mortality. Pulmonary reaction to both forms of alumina fibre was minimal; chrysotile asbestos provoked the expected progressive fibrosis. Pulmonary tumours (both benign and malignant) were confined to rats dosed with asbestos. The results support the predicted inert nature of these alumina fibres.

THE CARCINOGENIC POTENTIAL of asbestos in the work place and the general environment has been a topic of major concern for some time. The need to find a substitute for asbestos fibres in a variety of applications has been frequently stated. In addition the general question of the pathological effects of durable fibres in tissues continues to be examined experimentally in order to ensure the safety of such substitutes, and of man-made mineral fibres in particular. Saffil alumina fibre is a novel refractory material consisting of aluminium oxide containing about 4% silica, which is used mainly in high temperature (> 1200°) insulation. This fibre has been the subject of a series of toxicological investigations which has been outlined elsewhere (Pigott and Ishmael, 1981). This paper gives the detailed results of one of the investigations, an inhalation study.

Inhalation represents a potentially important route for human exposure to fibres and inhalation of asbestos may cause both lung fibrosis and neoplasia. Studies elsewhere have shown the rat to be an excellent model for experimental induction of lung cancer and fibrosis (Wagner *et al.*, 1974; Davis *et al.*, 1978) and Timbrell (1973) has demonstrated that the size of fibre which penetrates the rat lung resembles that in the human. In the study reported here rats were exposed by inhalation to Saffil fibres both as manufactured and after extensive thermal "ageing" (to simulate fibres which may be encountered during the removal of Saffil installations). A standard reference sample of UICC chrysotile A (Rhodesian) asbestos served as a positive control material. The main aim of the experiment was to establish any effects of the long-term inhalation of Saffil alumina fibres.

MATERIALS AND METHODS

Saffil fibres both as manufactured and after extensive thermal "ageing" were supplied by I.C.I. Ltd, Mond Division, The Heath, Runcorn, Cheshire. The "ageing" process results in a change in the microcrystalline structure of the fibres but has hardly any effects on the diameters found (Table I). Samples were preground before supply and characterized for length, diameter and chemical composition. Saffil fibres were supplied in 2 major batches with a third batch, derived from the second by ball milling, used during the last 8 weeks of exposure. Three batches of "aged" fibre were also supplied. During the last 6 weeks of exposure, samples prepared from Batch 3 by ball milling were used but were not additionally

TABLE I.—*Characteristics of materials tested*

	Saffil			"Aged" Saffil		
Batch	1	2	3	1	2	3
Treatment	As manufactured	As manufactured	Ball-milled for 24 h	> 1300° > 100 h	> 1200° > 100 h	> 1300° > 1000 h
Diameter distribution % (by number) less than						
5 μm	99	98	98	98	98	97
4 μm	89	87	87	86	86	86
3 μm	50	40	40	29	40	48
2 μm	11	6	6	1	2	6
1 μm	0	0	0	0·2	0	0
Median diameter (μm)	3·0	3·2	3·2	3·3	3·2	3·05
Length distribution						
Median length (μm)	35	62	10·5	57	53	56
% > 109 μm	11	26	0	14	22	8
% < 13·3 μm	0	0	66	7	9	5

characterized. The main properties of the batches of fibres used and the thermal treatments used to "age" the materials are shown in Table I. A UICC standard reference sample of chrysotile A (Rhodesian) asbestos (Timbrell, Gilson and Webster, 1968) was obtained from the Medical Research Council Pneumoconiosis Research Unit, Llandough Hospital, Penarth, Glamorgan. This sample has been extensively characterized (Rendall, 1972).

Albino rats of the Alderley Park (Wistar-derived) strain were supplied from the Animal Breeding Unit at ICI Ltd, Pharmaceuticals Division, Alderley Park, Cheshire. Standard laboratory diet and tap water were available *ad libitum* throughout the experiment.

The rats were exposed in 1·4m^3 inhalation chambers based on the design of Timbrell *et al.* (1970). Each chamber contained 8 cages and could house a total of 40 adult rats, though for a short period with young rats there was room for 50 (25 of each sex). Four chambers were used, one for each test fibre and one for an undosed control group. The chambers were housed within a Portakabin and filtered conditioned air was blown through the chambers continuously. A sensor in the common extract duct allowed feedback control of incoming air to maintain the chamber temperature at a nominal 25° ± 1° and the relative humidity nominally at 50% ± 10%. The construction of the chambers was such that the rats could be tended without the chambers being opened.

Dust clouds were generated using the dispensers described by Timbrell, Hyett and Skidmore (1968). After 2 months' exposure the dispensers were modified by the addition of a secondary jet in the lid of the bowl (Beckett, 1975). This modification resulted in a slight increase in the maximum concentration which could be generated and a much greater improvement in the stability of atmospheric levels achieved. Animals were exposed to fibres 5 days a week (except on bank holidays) for a nominal 6h period, though this was frequently extended by 2–3 h to increase cumulative exposure. Respirable dust concentrations were measured using size-selective gravimetric dust samplers (Casella Type 113A; Dunmore, Hamilton and Smith, 1964) and the collected samples were weighed at the end of each days' exposure. Variations in dosage rates were corrected by modification of the air flows on the following day. Occasional samples were taken with a portable dust sampler (Type L5-10, Rotheroe and Mitchell, Ltd) to determine total atmospheric concentration within the chambers.

Exposures were terminated after 86 weeks for the Saffil fibres and 77 weeks for asbestos. During week 88 of the experiment survivors were caged in groups of 2 or 3 in conventional caging in a separate room. Except for interim killings at 14 weeks and 27 weeks (2 of each sex per group) and 53 weeks (one of each sex per group) each rat was allowed to live until it died or appeared distressed, until 85% mortality (average of all groups in the experiment) was reached. At this point the remaining animals were killed.

Animals for interim killing, those in a distressed condition and survivors at termination were killed by overexposure to halothane BP (Fluothane, ICI Ltd) and subjected to immediate postmortem examination. Animals found dead were subjected to a similar postmortem examination as soon as practicable. The lungs were removed and inflated with formol saline and the nasal cavity was irrigated with this fixative. Samples of all grossly abnormal tissues and of the major organs were also taken for histopathology. The tissues were fixed in formol sublimate, embedded in paraffin wax and 5μm sections cut and stained with haematoxylin and eosin. After fixation, the lung lobes were separated and embedded whole in 3 blocks of paraffin wax. Each of these was trimmed down and a

median section from each lobe stained for histopathological examination. In addition further sections of lung were stained with van Gieson's stain for collagen and for reticulin by Gordon and Sweet's method. Additional stains were used as required.

Where asbestosis was diagnosed in a particular animal it was additionally assigned to one of 4 categories of severity, ranging from minimal to marked. These were defined as follows:

Minimal asbestosis (Category 1)—lesion confined to a few individual respiratory bronchioles with granular macrophages in these bronchioles. Epithelialization of a few associated alveoli may be present with aggregates of granular macrophages in alveolar spaces. Minimal fibrosis of alveolar ducts may be present.

Slight asbestosis (Category 2)—a focal lesion with more alveolar epithelialization than in Category 1. Aggregates of large mononuclear cells, probably Type II pneumonocytes, seen in some associated alveolar spaces. Minimal fibrosis.

Moderate asbestosis (Category 3)—a more extensive multifocal lesion involving the majority of respiratory bronchioles and also other areas within the lung parenchyma. Alveolar epithelialization is more marked than in the lesser grades and more granular/foamy macrophages are found in respiratory bronchioles and alveolar spaces. There is moderate fibrosis of alveolar walls.

Marked asbestosis (Category 4)—a diffuse lesion with extensive areas of alveolar epithelialization with or without squamous metaplasia. Numerous macrophages and Type II pneumonocytes in alveolar spaces with marked fibrosis of alveolar walls.

Typical examples of Categories 2–4 are illustrated in Fig. 2(a)–(c).

RESULTS

Cumulative exposures to respirable dust are displayed in Figure 1. The mean respirable dust concentrations are shown in Table II. It may be seen that while the aged Saffil and Saffil groups were similar both quantitatively and qualitatively, the accumulated dose (in terms of respirable dust) was less than the group dosed with asbestos. In the case of asbestos, dosing was reduced between Weeks 35 and 50, partially due to failure of generation equipment and partially in a deliberate attempt to reduce dosage to a rate comparable with the other groups. Exposure

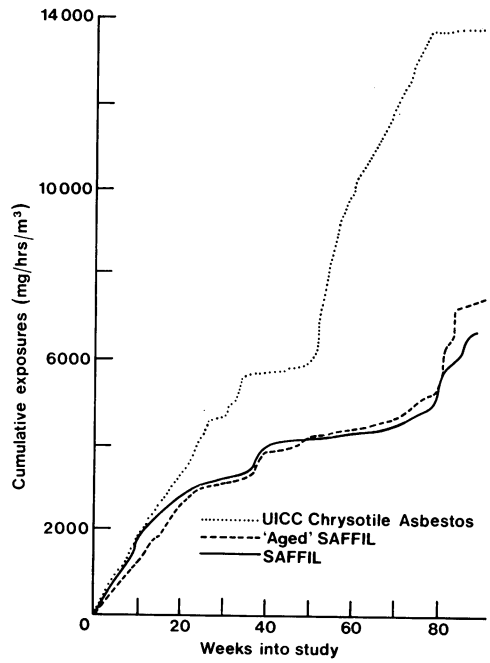

FIG. 1.—Cumulative exposures (respirable dust).

to asbestos was on alternate days only from Weeks 57 to 77 when it was discontinued in a further attempt to equalize dosage.

Total atmosphere samples were taken on a number of occasions from both the Saffil and aged Saffil chambers. These were taken over approximately 1 h periods during the exposure and, in conjunction with the integrated reading from the Casella dust samplers (taken over the whole exposure period), can be used to give an estimate of respirable fraction. In general these showed a low respirable fraction for total dust concentrations >10 mg/m^3 (Table II). Variation of generation conditions showed that higher respirable fractions could be achieved at lower air-flow rates, but this exercise produced low total atmospheric concentrations and a rapid build-up of fibre in the generator bowl which eventually resulted in equipment malfunction. The strategy adopted was therefore to use the higher flow rate, giving a reduced peak concentration which was sustainable over a longer

TABLE II.—*Characteristics of atmospheric dusts*

	Saffil	"Aged" Saffil	UICC chrysotile asbestos
Mean respirable dust concentration (mg/m³)*	2·18	2·45	4·57
Respitable fraction (total dust > 10 mg/m³)	2·5%	2·6%	ND
Respirable fraction (total dust < 5 mg/m³)	29%	37%	ND

ND = Not determined.
* Assuming total exposure of 35 h/week over 86 weeks' exposure.

period, resulting in increased cumulative exposure. The use of more extensively milled fibre during the last 6–8 weeks of exposure resulted in higher respirable dust levels but a proportion of this material was not "fibrous", *i.e.* the ratio of length to diameter did not exceed 3:1.

TABLE III.—*Survival patterns for the animals in the experiment. Termination was during Weeks* 128 *and* 129. *Figures given represent number of surviving rats of either sex*

	No. of survivors after				
Treatment	0 weeks	53 weeks*	80 weeks	106 weeks	At termination
Air control	50	35	26	16	5
UICC chrysotile asbestos	50	35	33	18	6
Saffil	50	30	29	18	6
"Aged" Saffil	50	38	38	17	5

* After interim kill: interim kills took place at 14 weeks (4 per group); 27 weeks (4); and 53 weeks (2).

The survival times for animals in the 4 chambers are shown in Table III. Numbers are for males and females combined. The variation in numbers at 53 weeks reflects several instances, mainly in male rats, of deaths by cannibalization after blood samples were taken for clinical chemistry. Blood sampling was discontinued as this loss rate was unacceptable. Survival times over the subsequent period were similar for all groups.

Interim killings at 14, 27 and 53 weeks showed the presence of a low-grade pneumonitis, with foamy alveolar macrophage proliferation, perivascular lymphoplasmacellular cuffing and minimal alveolar epithelialization. This was attributed to respiratory infection and lesions were evident in both treated and control animals. Animals exposed to asbestos showed a minimal asbestosis at 14 weeks which progressed to slight asbestosis by Week 53.

Saffil fibres were seen in alveolar macrophages in both dosed groups and in the superficial mediastinal lymph node of one animal killed at 27 weeks. Reaction to these fibres was generally minimal but in one animal Saffil fibres were present in an area of alveolar epithelialization. Focal necrosis and regeneration of olfactory epithelium was seen in sections of nasal cavity in 2 Saffil-dosed animals and one from the asbestos group. Other lesions were typical of the Alderley Park rat colony and were not related to treatment.

Asbestosis became more marked as the study progressed (Table IV). All asbestos-exposed intercurrent-death and "terminal-kill" animals of both sexes showed some degree of asbestosis.

Saffil fibres, mainly fragmented, were seen in the lungs of most rats of both sexes exposed to Saffil in either form, both in cases of intercurrent death and of "terminal kill". Reaction was generally confined to the presence of groups of pigmented alveolar macrophages (Fig. 2d)

TABLE IV.—*Assessment of severity of asbestosis. Average scores for rats examined in the indicated time periods*

	Weeks				
	1–27	28–53	54–79	80–103	104–129
Male	1 (4)	2 (1)	2 (2)	3·8 (8)	3·3 (9)
Female	1 (6)	1·3 (3)	ND	2·7 (3)	3·8 (13)
Combined	1 (10)	1·5 (4)	2 (2)	3·5 (11)	3·6 (22)

The figures in brackets indicate the number of animals examined.
ND = No deaths during this period.
Scoring system based on the histological evaluation: 0 = no asbestosis; 1 = minimal asbestosis; 2 = slight asbestosis; 3 = moderate asbestosis; 4 = marked asbestosis.

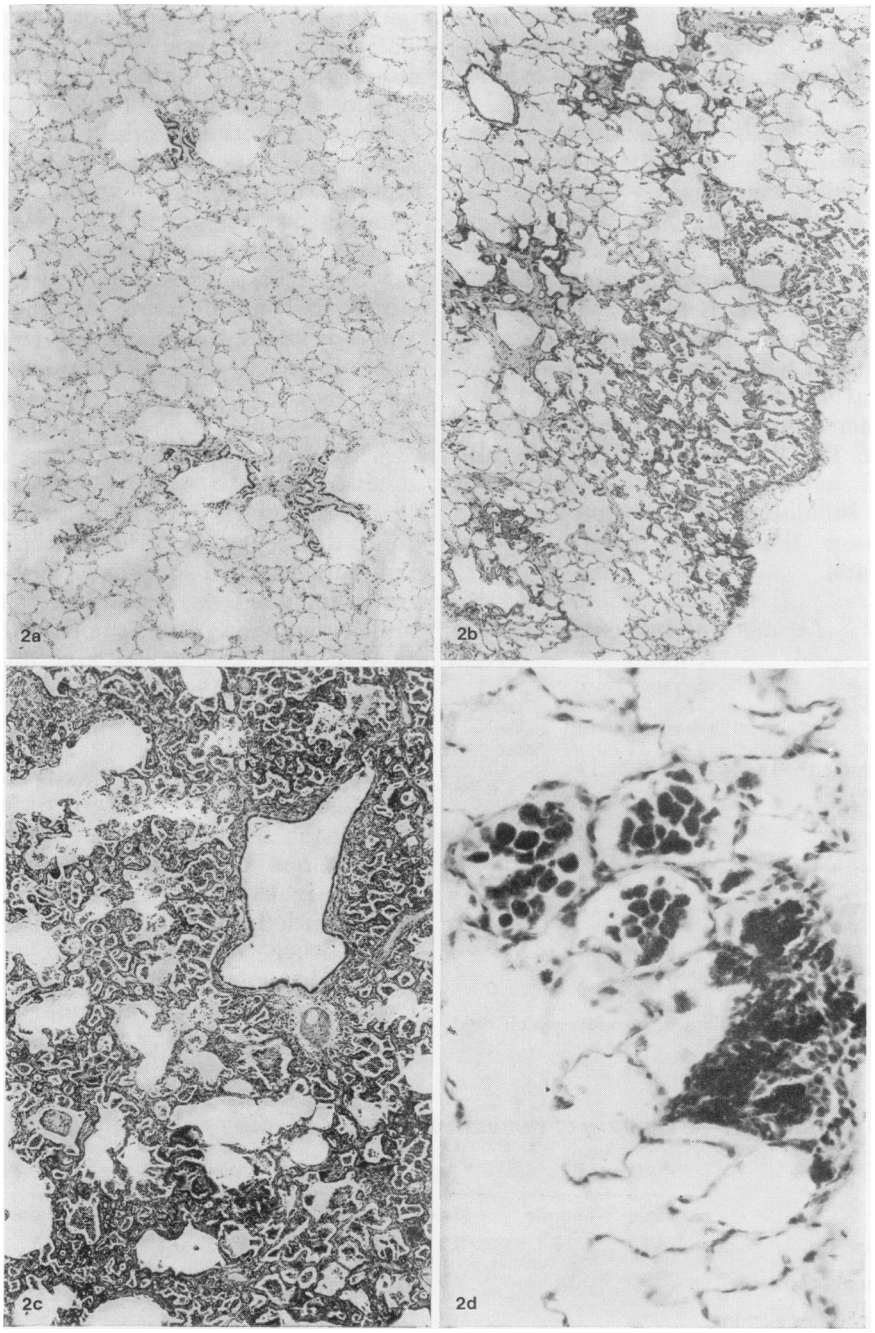

FIG. 2.—(a) Typical example of sections of rat lung showing slight asbestosis. H. & E. × 22. (b) Typical example of sections of rat lung showing moderate asbestosis. H. & E. × 22. (c) Typical example of sections of rat lung showing marked asbestosis. H. & E. × 22. (d) Clusters of pigmented alveolar macrophages in the lung of a rat dosed with Saffil fibres. Some fibre fragments may be seen in the macrophages. HVG stain. × 143.

in which fibre fragments could be seen when viewed under phase-contrast illumination. There was no fibrosis evident in the lungs of Saffil-treated animals. A minimal alveolar epithelialization was seen in control animals but the numbers showing this lesion were slightly higher in rats dosed with aged Saffil. This lesion was no longer evident after 106 weeks.

In a few animals small numbers of Saffil fibres were seen in nasal passages where there was evidence of slight irritation of the nasal mucosa with minimal focal necrosis. Degeneration of olfactory epithelium with replacement by respiratory epithelium was seen in all groups and is generally regarded as a spontaneous age-related change; inhalation of asbestos appeared to enhance this effect, particularly in female rats.

TABLE V.—*Incidence of pulmonary tumours*

	Control	UICC chryso-tile asbestos	Saffil	"Aged" Saffil
Males				
No. examined*	16	19	13	19
Adenoma	0	1	0	0
Squamous-cell carcinoma	0	1	0	0
Females				
No. examined*	18	19	19	19
Adenoma	0	4	0	0
Adeno-carcinoma	0	1	0	0
Squamous-cell carcinoma	0	2	0	0

* Excludes interim kills and animals which died before Week 28.

Pulmonary neoplasms both benign and malignant were seen only in asbestos-dosed rats with a higher incidence in females than in males (Table V). All were tumours of epithelial origin and appeared late in the experiment. The first benign tumour was seen at 109 weeks, malignant tumours at 128 and 129 weeks only. Pleural mesothelioma was not observed but one asbestos-dosed male rat showed mesothelial proliferation on the pleura. The only other tumour of the respiratory tract observed was an adenoma on the nasal septum of a female asbestos-dosed rat.

A total of 167 tumours were observed at sites other than the respiratory tract (Table VI) with no significant between-group differences in the overall incidence. Tumours confined to the asbestos-exposed group included one mesothelioma of the tunica vaginalis, a squamous-cell carcinoma of the oesophagus and an adeno-carcinoma of the colon. The first and last of these are not uncommon in control rats of this strain.

Carcinoma of the oral cavity was seen in 1 female dosed with aged Saffil and 1 rat of each sex dosed with asbestos. This tumour has been observed in undosed animals in this laboratory and these are not regarded as treatment-related.

The other tumours observed were those commonly encountered in the Alderley Park rat, pituitary and mammary tumours predominating.

TABLE VI.—*Summary of tumour incidence, excluding pulmonary tumours*

	Control		"Aged" Saffil		Asbestos		Saffil	
	Male	Female	Male	Female	Male	Female	Male	Female
No. examined*	19	19	19	20	19	20	13	19
No. of benign neoplasms	9	21	9	26	10	23	8	20
Excluding benign pituitary and mammary tumours	8	7	4	8	8	8	6	4
No. of malignant neoplasms	2	8	3	5	5	6	4	8
Excluding malignant mammary tumours	2	5	3	2	5	5	4	5

* Excluding interim kills.

DISCUSSION

An obvious feature of this experiment is the relatively low levels of respirable dust in the atmospheres. This represented the maximum achievable with both forms of Saffil fibres and the concentration of asbestos was reduced to ensure that dose rates were broadly similar. The low concentration achieved probably reflects an intrinsic property of Saffil fibres. Timbrell (1965) showed that the aerodynamic properties of glass fibres were almost independent of length. However, Burke and Esmen (1978) proposed a relationship between actual fibre diameter and aerodynamic diameter which included aspect ratio (fibre length/fibre diameter) in the equation and assigned values to constants of proportionality applicable to glass fibres on the basis of data from the literature. Assuming that these approximate to the values for Saffil fibres (which are essentially right cylinders and might be expected to be similar to glass fibres in their aerodynamic properties), and that the "respirable" limit coincides with an aerodynamic diameter of 7 μm, then the corresponding upper fibre diameter for "respirable" Saffil fibres can be calculated at 2·9 μm at an aspect ratio of 3 and 2·1 μm at an aspect ratio of 20. Not all fibres of "respirable" dimensions will penetrate either an elutriator or the respiratory tract, and the proportion deposited on the plates of an elutriator or in the upper respiratory tract increases sharply as the limit of the respirable range is approached (Timbrell and Skidmore, 1971). Also, Saffil fibres are made with an extremely narrow range of diameter (Table I). Thus even a small shift of aerodynamic equivalent diameter with aspect ratio will result in a significant reduction of the respirable fraction of Saffil fibres of high aspect ratio. Milling the fibre resulted in an increase in respirable fraction and an effective increase in dose rate for the last 6–8 weeks of exposure. This supports the proposition that aspect ratio is an important factor in the aerodynamic properties of these fibres as the effect of milling is to reduce fibre length. Fibre diameter is fixed at manufacture and is unaffected by mechanical attrition; "fibrous" cleavage seen when asbestos is milled (Assuncao and Corn, 1975) is not observed with Saffil fibres. The use of extensively milled fibre during the entire experiment would have resulted in a significant increase in respirable dust levels but the material then includes a proportion of non-fibrous particles. This would be concentrated in the respirable fraction and the evaluation of the material in fibrous form, the major objective of the experiment, could not be achieved by this means. In addition longer fibres may be particularly important in the aetiology of tumour induction (Pott, 1978) and fibrosis (Wright and Kuschner, 1977).

Separation of a "respirable" sample from the bulk was not attempted. While this could have been used to increase atmospheric levels of respirable dust, the cloud would not be representative of those encountered in the workplace where non-respirable dust predominates and exposure of the upper respiratory tract (in practice the major area for fibre deposition) would have been much reduced. Generation of higher levels of asbestos was not in general a problem and, even though exposure was restricted in the latter part of the experiment, a higher cumulative dose in terms of respirable dust was received by the rats in this group. The respirable fraction of the asbestos cloud was not measured but previous experimenters (Timbrell, 1970; Davis *et al.*, 1978) have reported between 70% and 80% respirable fraction in atmospheres generated from the same standard reference sample. This indicates that the total atmospheric dust was less, and probably very much less, than that encountered in the Saffil chambers where the respirable fraction was typically less than 10% (Table II).

The treatment-related effects seen in this study were in the respiratory system. All intercurrent deaths and terminal-kill asbestos-treated animals showed some degree of lung damage which increased

with time, even after exposure had ceased. This is entirely in agreement with previous studies with UICC chrysotile A (Rhodesian) asbestos in rats (Wagner *et al.*, 1974; Davis *et al.*, 1978). The methods used to assess the degree of asbestosis were different in both these previous studies but the results were broadly similar. Asbestosis was minimal over the first 6–12 months of exposure and then progressed over the next 12 months. A similar pattern emerges from the present study (Table IV) when a simple scoring system is applied to the subjective diagnosis. While this is by no means a definitive system it indicates that in terms of asbestosis this experiment is comparable to those previously reported.

This response may be contrasted with the very different effects elicited in response to Saffil fibres. There was an apparent slight increase in alveolar epithelialization in the group dosed with "aged" Saffil which was more prevalent in females than males. In the majority of cases the lesion was minimal and was not observed after 106 weeks.

The lesion also appeared in some control animals and 1 female from the group exposed to Saffil and may be at least partly attributable to intercurrent infection. Perivascular lymphocytic cuffing was also seen in these groups. This may also be attributed to intercurrent infection and was again seen most frequently in the group exposed to "aged" Saffil. The response to Saffil fibres was mainly confined to the appearance of small foci of pigmented macrophages and may be regarded as typical of a nuisance dust. There was no evidence of progression of the lung lesion beyond that described, but the appearance of fibres in the mediastinal lymph nodes in a significant proportion of animals from both groups dosed with Saffil indicated that fibres may also be transported *via* macrophages into the lymphatic system.

Saffil fibres are readily identifiable in tissue in the light microscope, especially when phase-contrast or dark-field illumination is used. The majority of fibres observed were very short compared to those in the atmosphere and many non-fibrous particles were present. This may partially reflect the size-selective properties of rat lungs (Timbrell and Skidmore, 1971) but the possibility of fragmentation within the tissues as described by Botham (1976) for glass fibres cannot be ruled out.

In the upper respiratory tract and nasal passages there were no clear-cut distinctions between the groups. The incidence of rhinitis/sinusitis with or without epithelial hyperplasia was slightly elevated in the group exposed to "aged" Saffil. This is probably a further indication of the presence of intercurrent infection though it is possible that the high proportion of non-respirable fibres present was a contributory factor. Neither this group nor the Saffil-dosed group showed a significant response to the treatment though the deposition of fibre in the upper respiratory tract must have been considerable with such a high proportion of non-respirable material present.

Pulmonary tumours were confined to the asbestos-dosed animals. The incidence is broadly comparable with previous studies though adenoma was observed somewhat less frequently than by Wagner *et al.* (1974) for this standard reference sample of chrysotile. However, the excess observed by these workers was largely attributable to one of the 2 experiments described where lung adenoma also occurred in untreated animals. The incidence reported here is comparable with that described by Davis *et al.* (1978) and may be typical of this sample of chrysotile asbestos. None of the malignant lung tumours observed had metastasized to other organs but 3 female animals had multiple tumours occurring in different lobes of the lung. These were all thought to be primary tumours rather than metastases and were counted as single tumours except in one instance where they were of a different histological type.

Mesothelioma of the pleura was not seen in this study and has not been reported

in the rat after inhalation of this reference sample of Rhodesian chrysotile asbestos. Mesothelioma was not expected in either of the Saffil-dosed groups, as induction of this tumour is believed to depend on the presence of fine fibres (Pott, 1978; Stanton *et al.*, 1977; Wagner, Berry and Skidmore, 1976) probably in the sub-micron range. Such fibres are not present in Saffil as manufactured and the ageing process does not affect the diameter distribution. The incidence and types of other tumours, in both the treated groups and controls, was typical of the strain of rat used.

It may be concluded that inhalation of Saffil fibres, either as manufactured or after thermal "ageing", is not associated with an increase in pulmonary or other tumours. The pulmonary reaction to Saffil fibres observed in this study is also consistent with their classification as biologically inert materials.

We gratefully acknowledge the skilled technical assistance of many members of the Central Toxicology Laboratory during the course of this experiment, in particular, Mrs K. Starr, Mr R. Walker, Miss A. Whitehead, Mrs K. Pearson, Miss E. Williamson and Mrs J. Riley and thank Mrs J. J. Burton for secretarial work.

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