Experimental studies with palygorskite dusts

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ABSTRACT As the preliminary results of experimental studies on dust from the palygorskite group have led to some confusion a detailed description of the completed investigation is given for clarification. As in other experiments the biological effects have been shown to be associated with the physical characteristics of the fibres in these specimens. Samples of sepiolite and attapulgite from Spain and a single sample of palygorskite from the United Kingdom have been studied. Serious abnormalities were produced only by the palygorskite and one of the attapulgite dusts. The palygorskite is of no commercial interest and the attapulgite was from one small deposit and was used only in the preparation of drilling mud in the exploration of oil deposits.

The materials that we are concerned with are of the palygorskite group including palygorskite, sepiolite, and attapulgite.

Our interest in these materials was stimulated by several reports in the Swedish press. Apparently a young scientist had examined a white powder used as cat litter under the electron microscope and found it to consist of fine mineral fibres. This caused alarm as it was thought that the material was asbestos. A sample was sent to our colleague, Dr F D Pooley, who identified the material as sepiolite.

Shortly after this episode, we received a request from the major Spanish producers of this material (Tolsa) to undertake studies in animals to establish whether the use of this material could result in a possible hazard to man.

Tolsa was producing two forms of mineral from the palygorskite group in Spain, sepiolite and attapulgite. Mining was taking place in several areas, the sepiolite being produced from quarries near Madrid. Attapulgite was being mined on several sites, at Torreon el Rubio near Placencia in western Spain and at Lebrija near Jerez in the south. We decided to study two samples of sepiolite from Madrid, a sample of attapulgite from Torreon, and a further two samples from Lebrija. When we first visited Lebrija, attapulgite was being quarried and the first sample was taken from this deposit. A year later it was decided to remove the attapulgite layer and concentrate on the underlying fibrous marl. This marl is now being produced as the commercial fibre for cat litter. The second sample was from this mineral.

Previous studies had been undertaken on these materials by Pott who injected palygorskite intraperitoneally in rats¹ and Stanton who implanted attapulgite intrapleurally in rats.² The site of origin of Pott's material was not known and that used by Stanton originated in Attapulgis in Georgia, United States. In 1982 the preliminary results of our findings were presented at a meeting in Montreal.³ At that stage our inhalation studies were incomplete and in this paper we present the complete study.

The palygorskite group of clay minerals includes two important commercial minerals—namely, sepiolite and attapulgite, the latter being synonymous with palygorskite. Both minerals are fibrous in character with a crystalline structure akin to the amphibole mineral group. Palygorskite (or as it is referred to commercially, attapulgite) is produced in far larger quantities than sepiolite, with the United States being the most prominent producer. Deposits of the mineral occur world wide and the mineral is mined in Europe, Africa, and India.⁴ Sepiolite is produced commercially almost exclusively in Spain. Having similar structures, the two minerals may most readily be distinguishable by their chemistries. Sepiolite is considered to be one end member of the palygorskite group and is a magnesium silicate. Changes in composition by substitution of aluminium for magnesium produce other members of the series—namely, palygorskite. The quantity of magnesium and aluminium in palygorskite samples varies. The distinguishing chemical feature of the mineral, however, is an aluminium content of at least five times higher than sepiolite. Both materials have similar industrial uses as absorbent and filler materials and are marketed in granule and powder form. Both minerals occur as massive aggre-

gates of fine individual fibrous crystals that resemble chrysotile asbestos.

These aggregates only dissociate into individual fibres when dispersed in aqueous fluids. Substantial dispersion depends on the use of ultrasonics.

Materials

DUSTS

The following dusts were used:

- (a) Lebrija attapulgitte from southern Spain.
- (b) Fibrous marl from Lebrija. There was only sufficient of this material for an intrapleural study.
- (c) Reported in this paper as Torrejon fibre. This material is another Spanish product and is now sold as drilling mud for exploration of the oil deposits in the North Sea and Persian Gulf. Preliminary studies showed this to be a long fibred fine type of attapulgitte. Our previous investigations suggested that this could possibly provide a hazard. Unfortunately, insufficient fibre was available for the inhalation studies.
- (d) Two samples of sepiolite from the Madrid deposit. One sample was a direct product of the initial milling of a crude sample that was used only for inoculation with and without being ultrasonically dispersed. The other sample was from a commercial product. We also performed inhalation investigations with this latter material.
- (e) Palygorskite (Leicester). This sample was from a disused quarry in the English Midlands.
- (f) Positive control UICC crocidolite from our standard sample prepared under a UICC contract.⁵
- (g) UICC chrysotile B prepared from Canadian samples.⁵
- (h) Negative control coating grade kaolin obtained from English China Clays Company and prepared in their mills in St Austell, Cornwall.

Methods

We received the materials used in the inhalation and intrapleural inoculation studies as rocks, granules, or fine dusts.

These were milled as required to a size suitable for producing a dust cloud in the inhalation chambers. Respirable fractions of these dusts were obtained aerodynamically for the inoculation studies.

ANALYSIS OF THE DUSTS

The fibrous forms of the respirable dusts necessary to calculate the number of fibres/ μg of dust were obtained by ultrasonic dispersion.

Weighed samples of each of the dusts were suspended in known volumes of distilled water, and aliquots of each of the samples were passed through a filter (Millipore 25 mm diameter, pore size $0.2\ \mu\text{m}$) in

a glass filter holder. The filter was then prepared for examination using the transmission electron microscope. The number of fibres/ μg of dust was calculated.⁶

CHARACTERISTICS OF DUSTS

Number of fibres/ μg of material

Lebrija attapulgitte—All fibres were found to be less than $2\ \mu\text{m}$ in length so no further analysis was required.

Fibrous marl—All fibres were found to be less than $1\ \mu\text{m}$ in length so no further analysis was required.

Torrejon attapulgitte

	%	No
Fibres/ μg respirable dust	100	15.77×10^6
No of fibres/ μg $\geq 6\ \mu\text{m}$ in length $< 0.5\ \mu\text{m}$ in diameter	0.54	0.085×10^6
No of fibres/ μg $\geq 6\ \mu\text{m}$ in length $< 0.2\ \mu\text{m}$ in diameter	0.054	0.085×10^6

Madrid sepiolite

	%	No
Fibres/ μg respirable dust	100	115.29×10^6
No of fibres/ μg $\geq 6\ \mu\text{m}$ in length $< 0.5\ \mu\text{m}$ in diameter	0	0
No of fibres/ μg $\geq 6\ \mu\text{m}$ in length $< 0.2\ \mu\text{m}$ in diameter	0	0

Palygorskite (Leicester)

	%	No
Fibres/ μg respirable dust	100	191.9×10^5
No of fibres/ μg $\geq 6\ \mu\text{m}$ in length $< 0.5\ \mu\text{m}$ in diameter	19.9	36.5×10^5
No of fibres/ μg $\geq 6\ \mu\text{m}$ in length $< 0.2\ \mu\text{m}$ in diameter	18.1	34.7×10^5

UICC crocidolite—The characteristics of the standard UICC samples have already been published.⁷

UICC chrysotile B—The characteristics of the standard UICC samples have already been published.⁷

Kaolin (coating grade)—As kaolin is not a fibrous dust, we have given percentages of particles in the different ranges.

Size range (μm)	No (%)
0.8-1.2	19.3
1.2-1.6	19.5
1.6-2.3	19.4
2.3-3.3	22.4
3.3-4.6	10.8
4.6-6.6	6.7
6.6-9.3	1.8
9.3-13.2	0.2

Dusts used for inoculation studies

The respirable fractions of the dusts to be used for inoculation were suspended in saline and mildly dispersed before injection.

Dusts used for inhalation studies

Owing to the importance of selective retention of fibrous dusts after inhalation, the size analysis of the inhaled material will be that obtained from the lung tissue after exposures. Therefore, the tables will be in the results section of this paper.

EXPERIMENTAL ANIMALS

There were two types of exposure. These were intrapleural implantation and inhalation. Both these techniques have been developed by us, are well tried, and have been shown to produce results which are reproducible with asbestos and other dust.⁹

Intrapleural studies

SPF Fischer rats of F344 strain were randomised and earmarked for type of dust to which the animals were exposed. Forty rats, 20 of each sex, at about five weeks of age, were inoculated intrapleurally with a single injection of the allocated dust in saline, using a previously described method.¹⁰ Inoculation was undertaken under light ether anaesthesia. Animals were kept four to a cage behind rodent barriers, fed on standard irradiated diet, and given water as required. The animals were allowed to live out their natural life span but were killed if they appeared distressed. On death, a full necropsy and histological examination were undertaken routinely on both lungs, any pleural nodules, liver, and spleen. Furthermore, sections were taken of any relevant tissues. Mesotheliomas, if present, were recorded. When required, histochemical studies were undertaken to confirm the diagnosis.

Inhalation studies

Forty 6 week old SPF Fischer rats, 20 of each sex, were randomly allocated to each of the exposure chambers and identified by earmarking. They were exposed to a dust concentration of 10 mg/m^3 for six hours a day, five days a week (the dust having been milled to a suitable size from the original material). After three months, four animals, two of each sex, were removed from the dusting chamber, killed, and the lungs were removed to assess the severity of the

fibrosis. This was repeated after six months and after 12 months, after which dusting ceased and the remaining 28 animals were allowed to live out their normal life span and killed only if they showed signs of distress (apart from four rats killed after two years). When these animals died they were subjected to a full necropsy and the lungs, liver, spleen, kidneys, and any other relevant organs were examined histologically. Routine sagittal sections were taken through both lungs, which were inflated with formol saline. These sections were used to assess the severity of pulmonary fibrosis, which was done on a 1-8 scale that has been standardised and used in all experiments; lung tumours were noted.

The histological grading of the severity of the effects of exposure is recorded on an internationally accepted scale¹¹: grade 1 normal, grade 2 dust in macrophages, grade 3 early interstitial reaction, grade 4 first signs of fibrosis, grades 5, 6, and 7 increasing degrees of fibrosis, and grade 8 severe fibrosis.

CLASSIFICATION OF PROLIFERATIVE LESIONS AND TUMOURS

(1) Bronchoalveolar hyperplasia (BAH). Non-malignant proliferation of epithelia in response to dust exposure.

(2) Benign alveolar tumour (BAT).

(3) Malignant alveolar tumour (MAT).

(4) Adenocarcinoma.

(5) Squamous carcinoma.

(6) Adenosquamous carcinoma.

(7) Mesothelioma.

DUST EXTRACTION TECHNIQUE

Intrapleural studies

Granulomas were removed from the diaphragm or mediastinal tissue from the thoraces of rats inoculated with the dust under examination. These rats had survived for about two years after inoculation.

The granulomas were digested in a wet state in 40% KOH for about three hours in a boiling water bath. The resulting suspension was centrifuged at 4000 g and the supernatant discarded. The deposit was then resuspended in distilled water, centrifuged, and the supernatant again discarded. This was repeated three times.

The final washed deposit was then ashed at 300°C for four hours in the Dri-Block,* after which it was resuspended in 10 ml of distilled water. Aliquots of different volumes of the suspension were taken and passed through nuclepore filters. The dust laden filters were then prepared for examination under the transmission electron microscope (TEM).^{12 13}

*Dri-Block manufactured by Techne (Cambridge) Ltd, Duxford, Cambridge.

Table 1 Results: intrapleural inoculation

	Mesothelioma	Non-mesothelioma
Lebrija attapulgit	2*	38
Fibrous marl	0	32†
Torrejón attapulgit	14	26
Madrid sepiolite:		
Crude material not		
ultrasonicated	0	40
Crude sample ultrasonicated	1	39
Commercial sample	1	39
Palygorskite (Leicester)	30	2†
Crocidolite UICC	34	6
Chrysotile B	19	20
Kaolin	0	40
Saline control	1	39

*Includes one peritoneal mesothelioma.

†Only 32 rats inoculated.

The lengths and diameters of the fibres were measured.

Inhalation studies

Characterisation of the dust retained in the lungs was carried out using two methods: examination of ashed lung sections and examination of macerated lung tissue.

Characterisation was made on the mineral fibres extracted from formalin fixed lungs of rats exposed for 12 months in inhalation chambers to the appropriate dust cloud.

Examination of ashed lung sections—Serial paraffin sections were cut at 6 µm. One was stained by haematoxylin and eosin (H & E) and examined by light microscopy for areas containing dust. The adjacent unstained section was dewaxed and the areas previously identified as containing dust in the H & E sections were marked with a diamond, mounted on the microscope objective. The sections were then ashed in a muffle furnace at 450°C for three hours. The sections were then stripped off the slides using polyvinyl alcohol (PVA) and prepared for TEM.^{6 14}

Table 3 Characterisation of fibres recovered from lungs of animals in inhalation experiments

Palygorskite (Leicester)					
Diameter (µm)	% Frequency				
	0–	0.1–	0.2–	0.3–0.5	Total
Length (µm)					
0–1	17.5	5.7			23.2
1–2	16.0	6.3			22.3
2–3	11.2	8.3			19.5
3–4	3.8	3.3	0.2		7.3
4–6	7.3	7.2	0.8		15.3
6–8	3.0	4.8	0.3		8.1
8–10	0.7	2.0	0.2		2.9
10–25	0.5	0.5	0.2	0.2	1.4
Total	60.0	38.1	1.7	0.2	100.0

The areas marked by the diamond were selected for examination.

Examination of macerated lung tissue—Digestion was carried out in a similar manner to that used for the granulomas but using fixed lung tissue. The preparation of the extracted dust for examination under electron microscopy followed the same procedure.

Pathology results

INTRAPLEURAL STUDIES (TABLE 1)

In our experience after intrapleural inoculation of a strain of Fischer 344 rats, an incidence of mesotheliomas of more than 10% is regarded as a cause of concern. As may be seen from table 1, evidence of potential hazard is observed in the animals exposed to the crocidolite, palygorskite, chrysotile, and the attapulgit from Torrejón.

The findings from the crocidolite and chrysotile were expected from previous studies.⁹ The many tumours produced by exposure to palygorskite would be expected when the physical characteristics of the mineral specimen are considered (tables 2 and 3). This

Table 2 Characterisation of the respirable palygorskite

Inoculated after mild dispersion					Recovered from granuloma				
Diameter (µm)	% Frequency				Diameter (µm)	% Frequency			
	0–	0.1–	0.2–0.3	Total		0–	0.1–	0.2–	0.3–0.5
Length (µm)					Length (µm)				
0–1	0.9	2.6		3.5	0–1	12.0	2.7		14.7
1–2	11.6	9.5	0.4	21.5	1–2	15.7	3.7		19.4
2–3	10.0	8.7	1.3	20.0	2–3	11.7	8.0		19.7
3–4	6.1	9.1	0.9	16.1	3–4	5.3	2.7	1.0	9.3
4–6	9.9	9.1	0.9	19.9	4–6	12.7	6.3		19.0
6–8	4.3	4.8	0.9	10.0	6–8	4.3	3.3		7.9
8–10	1.3	1.3		2.6	8–10	2.3	1.7	0.3	4.3
10–25	4.7	1.7		6.4	10–25	1.6	4.0		5.6
Total	48.8	46.8	4.4	100.0	Total	65.6	32.4	1.3	99.9

being in the expected range for the particular animals used.

From the results in table 4, it may be seen that the only dust which should be regarded as a potential hazard was from the palygorskite sample.

ATTAPULGITE LEBRIJA AND FIBROUS MARL

Suspensions were made of the milled material and these were mildly dispersed. Aliquots of these suspensions were passed through a 0.2 µm pore size nucleopore filter and the dust laden filters were prepared for examination under the TEM. This method of preparation was also adopted for all subsequent preinoculation materials.

In the fibrous marl the fibrous material present was found to be less than $1\text{ }\mu\text{m}$ in length (fig 2).

Subsequent examination of the filters prepared of the extracted dust from the granulomas in animals exposed to both these dusts showed the complete absence of the short fibre and the presence of granular material that on analysis was found to be mica (figs 3 and 4).

Examination of the dust extracted from the lung after inhalation again showed the complete absence of the short fibre and the presence of the granular material

Two results were obtained from the inhalation exposures, the degree of tissue reaction to the dust and the incidence of pulmonary tumours resulting from this treatment.

In a similar study on man made vitreous fibres we confirmed our opinion on the significant stages of fibrosis and the acceptable incidence of tumours.¹¹ The natural rate of pulmonary tumours in this particular strain of rats has been supported by a subsequent study.¹⁵ It was agreed that an interstitial reaction rating of four or more should be regarded as fibrosis. Bronchiolar alveolar hyperplasia was considered to be a reaction to an irritant and not a tumour. Benign alveolar tumour was a borderline between a severe reaction to irritation and early tumour. Malignant alveolar tumour was accepted as an early adenocarcinoma. In an exposed group of 40 rats two carcinomas and one mesothelioma were acceptable as

Just

[illegible]

Fig 1 *Milled Lebrija attapulgite mildly dispersed for inoculation.*

Fig 2 *Milled fibrous marl mildly dispersed for inoculation.*

Fig 3 *Lebrija attapulgite recovered from granulomas showing absence of fibre.*

Fig 4 *Fibrous marl recovered from granulomas showing absence of fibre.*

Fig 5 *Lebrija attapulgite recovered from lung after inhalation exposure.*

Fig 6 *Lebrija attapulgite in ashed lung section after inhalation.*

Fig 7 Milled Torrejon attapulgite mildly dispersed for inoculation.

(fig 5). The ashed sections showed a similar picture (fig 6).

ATTAPULGITE TORREJON

Inoculation

The preinoculation sample (fig 7) was found to be fibrous in nature. Table 5 shows the size characteristics of the original material after mild dispersion with fibres up to 8 μm in length with diameters less than 0.2 μm. Fibres in this category are in the range considered to have the potential to initiate malignant

change. The fibre extracted from the granulomas (fig 8) shows similar characteristics (table 5).

Inhalation

Not carried out because insufficient material was available.

MADRID SEPIOLITE

Inoculation

The preinoculation samples of both the crude and commercial products mildly dispersed showed similar

Table 5 Characterisation of the respirable Torrejon attapulgite

Inoculated after mild dispersion					Recovered from granuloma				
Diameter (μm)	% Frequency				Diameter (μm)	% Frequency			
	0–	0.1–	0.2–	Total		0–	0.1–	0.2–	Total
Length (μm)					Length (μm)				
0–1	40.5	1.0		41.5	0–1	40.5	0.2		40.7
1–2	22.5	4.0		26.5	1–2	21.8	5.8		27.6
2–3	10.5	10.0	0.5	21.0	2–3	11.0	9.2	0.5	20.7
3–4	3.0	2.5		5.5	3–4	5.5	2.0	0.7	8.2
4–6	1.5	1.5	0.5	3.5	4–6	1.3	1.0		2.3
6–8	1.0	1.0		2.0	6–8	0.5			0.5
Total	79.0	20.0	1.0	100.0	Total	80.6	18.2	1.2	100.0

Fig 8 *Torrejon attapulgite recovered from granulomas.*

Fig 9 *Milled sample of Madrid sepiolite mildly dispersed for inoculation.*

Table 6 Characterisation of the respirable Madrid sepiolite

Inoculated after mild dispersion					Recovered from granuloma				
Diameter (μm)	% Frequency				Diameter (μm)	% Frequency			
	0–	0.1–	0.2–	Total		0–	0.1–	0.2–	Total
Length (μm)					Length (μm)				
0–1	20.0	2.4		22.4	0–1	25.5	0.8		26.3
1–2	28.0	6.5		34.5	1–2	31.1	2.0		33.1
2–3	16.7	6.2		22.9	2–3	19.8	3.4		23.2
3–4	8.3	1.1	0.3	9.7	3–4	5.3	1.5	0.3	7.1
4–6	5.9	2.4	0.3	8.6	4–6	8.2	1.6	0.5	10.3
6–8	1.6	0.3		1.9	6–8				
Total	80.5	18.9	0.6	100.0	Total	89.9	9.3	0.8	100.0

characteristics (fig 9). These again were found to have fibres up to 6 μm in length with diameters less than 0.3 μm (table 6). The undispersed sample inoculated did not contain as many fibres as the other two samples. The measurements made on these fibres showed them to be less than 2 μm in length.

Figure 10 shows the fibre extracted from the granulomas arising from the rats inoculated with the mildly dispersed sepiolites (table 6).

Inhalation

The material examined from both macerated lung tissue and lung sections showed the presence of fibre

agglomerates as well as discrete fibres (figs 11 and 12). Measurements made on discrete fibres were found to be up to 6 μm in length with diameters up to 0.2 μm (table 7).

PALYGORSKITE (LEICESTER)

Inoculation

Examination of both preinoculation (fig 13) and post-inoculation (fig 14) material shows little apparent difference. Table 2 shows the size characteristics of the original material mildly dispersed and also the recovered dust. Fibres were up to 25 μm in length with diameters less than 0.2 μm and therefore are well

Fig 10 Madrid sepiolite recovered from granulomas.

Fig 11 Commercial sample of Madrid sepiolite recovered from lung after inhalation exposure.

Fig 12 Commercial Madrid sepiolite in ashed lung section after inhalation.

Experimental studies with palygorskite dusts

Table 7 Characterisation of fibres recovered from lungs of animals in inhalation experiments

Madrid sepiolite (commercial)				
	% Frequency			
Diameter (μm)	0–	0.1–	0.2–	Total
Length (μm)				
0–1	59.8	0.8		60.6
1–2	24.6	2.2		26.8
2–3	8.4	0.8		9.2
3–4	2.2			2.2
4–6	0.4	0.8		1.2
Total	95.4	4.6		100.0

within the range considered to be potentially tumour-genic. This feature of fibre size was more pronounced than in the Torrejon attapulgite.

Inhalation

Figures 15 and 16 show material from the macerated lung and ashed sections respectively. These again show the presence of long fibre as seen in the inoculation extract (table 3).

Discussion

These experiments were designed to establish if there

was a potential risk to those handling the various absorbent clays. Exposure to dust from two of these samples is inadvisable. These are the palygorskite from Leicester and the attapulgite from Torrejon el Rubio. Fortunately, the palygorskite which produced the more severe condition after both implantation and inhalation is not exploited for commercial use. It was from a small deposit in a disused quarry and was sent to us as a curiosity. The attapulgite from Torrejon el Rubio was from a single sample. The actual production from this quarry is a minor operation. The material was being used in the "drilling mud" used in the Persian Gulf and the North Sea. It was considered prudent to advise the Health and Safety Executive and the petrochemical industry of these findings before publication of the complete study.

The lesions produced after the inoculation of the sepiolites were one mesothelioma from both the commercial product and the ultrasonically dispersed crude sepiolite sample.

Neither the undispersed crude sepiolite sample nor the fibrous marl produced tumours.

Two mesotheliomas, one pleural and one peritoneal, were found in the animals inoculated with the attapulgite from Lebrija.

The findings in these experiments are specific for the actual samples examined. It must be emphasised that representative samples from all deposits of

Fig 13 Milled palygorskite (Leicester) mildly dispersed for inoculation.

Fig 14 *Palygorskite (Leicester) recovered from granulomas.*

Fig 15 *Palygorskite (Leicester) recovered from lung after inhalation exposure.*

Fig 16 *Palygorskite (Leicester) in ashed lung section after inhalation.*

fibrous clays should be studied for physical characterisation and biological effects before the deposits are exploited for commercial use.

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