Potential health risks from the use of fibrous mineral absorption granulates

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ABSTRACT Attapulgite (palygorskite) and sepiolite are fibrous clay minerals used commercially as components in a wide variety of products including oil and grease adsorbents, carriers for pharmaceuticals, cosmetics, and pesticides. They are also components of drilling muds and animal litter and they are used as paint thickeners. The current annual worldwide production of these minerals exceeds one million tons. Although fibrous in nature, the fibre length may vary greatly depending on the location of the geological deposits. American attapulgite is short (0.1–2.5 μm in length, median of 0.4 μm) but palygorskite from other parts of the world is much longer (30% longer than 5 μm). Several samples of these materials have been submitted to scanning transmission electron microscopy (STEM). This paper reports the results of microscopic evaluations and makes a comparison with the data from experimental carcinogenicity studies and it is concluded that fibre length is a most important carcinogenic property.

Attapulgite and sepiolite can occur in both long and short fibred varieties. Mesotheliomas were produced by intrapleural and intraperitoneal injections in animal experiments using the long attapulgite only.^{1,2} Extremely short fibrous varieties did not cause tumours after intraperitoneal or intrapleural injection (F Pott, unpublished data).

The analytical scanning transmission electron microscope (STEM) which can detect even very thin fibres was used to examine several samples of attapulgite and sepiolite. The materials were also compared with samples of erionite and crocidolite. In addition to determining fibre geometry the potential for contamination with asbestos fibres was also evaluated. Using the models of Stanton³ and Pott⁴ special attention was given to fibres longer than 5 μm and thinner than 1 μm . Although only a small carcinogenic potential is assumed in the case of shorter fibres with a length between 2 and 5 μm , it has been hypothesised that this potential may increase if a large number of these fibres are present.

Mineralogical description

Of the materials used in industry, the clays attapulgite (palygorskite) and sepiolite (meerschaum) play a major part as adsorption granulates. Unlike the plate form clays, such as bentonite (montmorillonite) and kaolin, attapulgite and sepiolite develop needle like crystals (fig 1). Chemically, they are magnesium silicates with varying proportions of Al and Fe. Apart from their length, their most significant property is their large effective surface; 130 to 310 m²/g for attapulgite and 150 to 250 m²/g for sepiolite.⁵ Depending on preliminary treatment, a distinction must be made between colloidal and calcined, thermally activated product types that have a different water solubility.⁶

Production and use

Bignon estimates the production of attapulgite and sepiolite in the western world to be at least 1.2 m tons a year.⁷ In the United States alone, about 900 000 tons a year of attapulgite are mined. The remainder is produced in Spain, France, and Turkey.

The main uses for attapulgite and sepiolite are listed in table 1. Adsorption granulates constitute the majority of attapulgite and sepiolite products. In the

Table 1 Uses of attapulgite and sepiolite^{6,7}

Thickener and thixotropic substances:
Paints, adhesives
Stabiliser in liquids:
Suspension fertiliser
Probe washing for oil drilling
Asbestos substitute:
Plaster filler, joint filler, underseal, sealants, brake linings
Sorption substances:
Old oil recycling, purification of hydrocarbons
Bases:
Pesticides
Catalysts
Liquid adsorption:
Oil, animal urine
Soil amelioration substances:
Horticulture in the United States

United States alone, where about 700 000 tons a year of attapulgite are used, more than two thirds is found in oil and grease adsorbents, as carriers for pesticides and as pet litter.

By comparison, in the Federal Republic of Germany the annual use of mineral adsorption granulates is estimated at more than one million tons. For animal litter alone, an annual production quantity of 145 000 tons a year mainly of sepiolite and attapulgite is reported (W Lohrer, personal communication 1984).

Biological effect

Because of its fibrous nature, samples of attapulgite were used in earlier animal experiments by the research teams of Stanton *et al*⁸ and Pott and Huth⁹ investigating the relation between carcinogenicity and fibre length. Although the intrapleural application of 40 mg short fibrous United States attapulgite per rat did not lead to an increased incidence of mesothelioma, mesotheliomas could be generated in 65% of rats after the intraperitoneal injection of 75 mg of a long fibrous palygorskite. Additional positive findings have been obtained by Wagner² with long fibrous Spanish attapulgite from Torrejon. Some cell tests^{7,9,10} found a strong haemolytic and cytotoxic effect in the case of short fibrous attapulgite specimens. Using Stanton's precisely measured original dust samples, Lipkin showed no cytotoxic effect for short fibrous United States attapulgite.^{11,12} Additional experiments carried out on organ cultures of the hamster trachea,¹³ hamster alveolar cells, and human tumour cells⁹ also showed no in vitro effect of short fibred attapulgite.

Fig 1 Sepiolite fibre, length 33 μ m and diameter of 0.1 μ m in sample from a Finnish deposit. Much thinner fibres of diameters down to 0.01 μ m are also visible.

Materials and methodology

TEST SAMPLES

We began our tests with four attapulgite samples from the United States (Georgia*), France (Mormoiron†), Spain (Lebrija), and France (de Cacerès).

These samples had been used in an injection experiment to investigate the potential to generate peritoneal mesotheliomas in rats; however, a carcinogenic effect could not be established for the first three of these materials but only for the last (F Pott, unpublished data). Sepiolite samples were also available from Spain (Uiacaluaro) and Finland. No commercial mining is done at the Finnish deposit (A Tossavainen and K Korhonen, 21st International Congress on Occupational Health, Dublin, September 1984).

For comparison, electron microscopic analyses were made with UICC crocidolite, a long fibrous Spanish attapulgite (Torrejon), and with erionite from Oregon. The latter two samples were provided by Dr Wagner who had shown an oncogenic potential in an intrapleural test.²

SAMPLE PREPARATION

Up to 5 mg of the substance was made into a suspension in 50 ml distilled water by a 30 minute ultrasonic treatment. After a dilution of 1:10, up to 20 ml

*Two samples of American attapulgite were available. One has been used in an injection experiment (F Pott, unpublished data) (Georgia 1), the other in an in vitro study¹⁰ (Georgia 2).

†This was actually a sample of a medicine used for gastric upsets, Gastropulgit.

Table 2 *Fibre concentrations and median values of the fibre dimensions for different types of attapulgite and sepiolite compared with erionite from Oregon and with UICC crocidolite. The number of fibres of any length and the fibre dimensions are determined at a magnification of 29 000 × under the STEM operation mode. The analysis of the fibres $L \geq 5 \mu\text{m}$ is made mainly at a magnification of 10 000 × in the TEM operation mode. The attapulgite from Torrejon and the erionite and the crocidolite were given ultrasonic treatment for a short time only.*

	Fibre concentration			Fibre dimensions			Carcinogenicity in rats
	No all L	All L	$L \geq 5 \mu\text{m}$	L	D	L/D	
		[F/mg] $\times 10^9$	[F/mg] $\times 10^6$	μm Median			
Attapulgite:							
Georgia 1	424	29.0	0.61*	0.8	0.04	20	— §
Georgia 2	200	72.0	0.41*	0.9	0.05	16	— §
Mormoiron	431	0.9	0.06*	0.7	0.07	11	— §
Lebrija	420	1.4	0.34*	0.5	0.07	7	— § ²
Torrejon	200	9.6	99.0*	1.9	0.04	46	+ † ²
Cacerès	201	8.1	240.0*	1.3	0.07	19	+ §
Sepiolite:							
Spain	100	20.0	180.0*	1.2	0.05	25	— [2]
Finland†	100	19.0	5500.0	2.9	0.05	64	?
Erionite:							
Oregon	200	0.33	64.0*	2.0	0.2	9	+ [2]
UICC crocidolite	120	2.7	130.0	1.2	0.16	7	+

*TEM operation mode at a magnification of 10 000 ×.

†Contains amphibole fibres (classified as anthophyllite).

§Pott, unpublished data.

of this suspension was filtered through Nuclepore-filters (diameter 25 mm, pore-size $0.2 \mu\text{m}$).

The filters for transmission electron microscopy were prepared by carbon coating and dissolving the filter material in a "Jaffewasher."

In the case of the attapulgite samples from Georgia, Mormoiron, and Lebrija three equal but independent preparations of each substance were analysed. Two preparations of the attapulgite from Torrejon, the erionite and the UICC crocidolite were analysed.

ANALYSIS BY STEM

Up to 431 fibres of any length per substance were counted under the STEM operation mode at a magnification of 29 000 × and their dimensions measured. Since the attapulgite and sepiolite samples are almost exclusively made up of extremely fine fibres of $< 5 \mu\text{m}$ length, the measured surface amounted to only about 1/1000 to 1/100 mm^2 . As a result, the sensitivity decreased to 10^6 to 10^8 fibres/mg per fibre found.

A separate count under the transmission electron microscope operation mode (TEM) with a far greater sensitivity at a magnification of 10 000 × was made to search for fibres with a length of $\geq 5 \mu\text{m}$ and also for asbestos fibres. In doing this, 0.5 mm^2 or 50 fields of the transmission preparation, which had been transferred on a 400 mesh copper grid, were examined. Each fibre $L \geq 5 \mu\text{m}$ was analysed using energy dispersive x ray analysis and selected area electron diffraction.

Results

FIBRE CONCENTRATION MEASUREMENTS

Table 2 lists the calculated fibre concentrations based

on both the STEM (all lengths) and TEM (lengths $\geq 5 \mu\text{m}$) analyses and gives the median dimensions of fibres actually measured for all attapulgite and sepiolite specimens, as well as the Oregon erionite studied by Wagner and UICC crocidolite. The results of experimental carcinogenicity testing are also given. The American attapulgite, and the Spanish and Finnish sepiolite, showed the highest overall concentration of individual fibres when fibres of any length were examined (10^{10} to 10^{11} fibres/mg). For the attapulgite, erionite, and crocidolite samples which had proved to be carcinogenic in animal experiments, the concentrations of fibres of any lengths were significantly lower (10^8 to 10^{10} fibres/mg). The concentrations of long fibres (lengths $\geq 5 \mu\text{m}$) for these four carcinogenic fibre samples and for the two sepiolite samples amounted to 64 to 5500×10^6 fibres/mg. By contrast, as is seen in fig 2 and table 3 the non-carcinogenic attapulgite samples had few fibres longer than $5 \mu\text{m}$. Between three and eight fibres longer than $5 \mu\text{m}$ observed for these samples resulted in a fibre concentration calculated to be 0.06 to 0.6×10^6 fibres/mg longer than $5 \mu\text{m}$. Of these longer fibres, between 0 and 5 were found to be chrysotile, compared with one fibre of chrysotile observed on average in the filter and distilled water materials used in this study.

ANALYSIS OF FIBRE DIMENSIONS

The median values of the fibre dimensions measured

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at a magnification of $29\,000\times$ are listed in table 2. It is remarkable that the erionite and crocidolite fibres have a greater diameter than the attapulgite and sepiolite. On the other hand, the attapulgite from Torrejon and the erionite and sepiolite from Spain and Finland are distinctly longer than the three short fibrous attapulgite samples. Figure 3 gives a comparison of the three dimensional length and diameter distribution of four of the attapulgite samples and the four sepiolite, erionite, and crocidolite samples examined.

Discussion

EVALUATION OF THE METHOD

(1) Hand shaken suspensions of attapulgite and sepiolite samples contain both particle like fibre flocks, which are not countable as fibres, and thicker clusters of fibres. It was easier partially to disperse these flocks and clusters into thin fibres with a diameter of $0.01 \mu\text{m}$ for attapulgite than it was to do so with sepiolite using weak ultrasonic treatment.¹⁴ A complete dispersion with the same result was accomplished, this time using strong ultrasonic treatment. After an injection of hand shaken samples into rats and subsequent analysis of the lung ash, very thin fibres without any clusters were seen.¹⁴ Thus strong ultrasonic treatment is considered a suitable method of preparation. This does not apply to the attapulgite from Torrejon; as described above, it was treated

Fig 2 Fibres with $L \geq 5 \mu\text{m}$ in short fibrous attapulgite samples. Left: Attapulgite fibre, length $6.7 \mu\text{m}$, in sample from Georgia. Photograph taken in STEM operation mode, magnification $15\,000\times$. Right: Clusters of chrysotile fibres length $12 \mu\text{m}$ in attapulgite sample from Mormoiron. Photograph taken in STEM operation mode, magnification $6300\times$.

Table 3 Concentration of the attapulgite and chrysotile fibres with $L \geq 5 \mu\text{m}$ found in the attapulgite samples examined. All fibres were identified as attapulgite or chrysotile. (Upper limit of the 95% interval of reliability according to Poisson statistics in parentheses)

	No	Fibre concentration $L \geq 5 \mu\text{m}$ [F/mg] $\times 10^6$	
		Attapulgite	Chrysotile
Georgia 1	3	0.41 (< 1.50)	0.20 (< 1.10)
Georgia 2	5	0.41 (< 0.96)	0 (< 0.30)
Mormoiron	8	0.03 (< 0.07)	0.03 (< 0.07)
Lebrija	5	0 (< 0.25)	0.34 (< 0.79)
Torrejon*	102	99 (< 120.00)	No analysis made
Cacères	50	240 (< 320.00)	No analysis made

*Preparation with short term ultrasonic treatment.

ultrasonically for a short time and had completely dispersed into very fine fibres.

(2) No noteworthy deviations in the dimensions and concentrations of different preparations from any

one mineral sample were observed, according to the Kolmogoroff-Smirnov test on the 95% level, and to the 95% confidence interval of the Poisson distribution.

Attapulgite

Fig 3 Comparison of three dimensional length and diameter distribution of four attapulgite samples and four sepiolite, erionite, and crocidolite samples of different origin. Standardisation is made according to highest column which contains 19% to 60% of all fibres.

The length and diameter distributions of short and long fibred attapulgite, sepiolite, and erionite samples examined here may also be compared with the individual results of measurements by other authors.^{2 14 15} This comparison shows a close conformity, especially relative to the order of magnitude of the difference between the short and long fibred samples. Nevertheless, the median length and width dimensions reported by Zumwalde for Georgian attapulgite, 0.4 μm and 0.07 μm respectively, were somewhat shorter than those reported here.¹⁵

(3) The comparisons of the different samples 1 and 2 from deposits in Georgia may be regarded as an example of the reproducibility of the concentration measurements. Fibre concentrations of all lengths differ by 29 and 72×10^9 fibres/mg, by the factor 2.4. The findings $L \geq 5 \mu\text{m}$ based on a smaller number of fibres are virtually identical. This difference—observed despite comparable fibre lengths and diameters—is caused by differing proportions of non-fibrous contamination. According to the data in table 2, there is a close conformity between the attapulgite from Torrejon and a sample prepared by Hill and Griffith¹⁴ with intensive ultrasonic treatment.

In the sample of Hill and Griffith the concentration was 15.6×10^9 fibres/mg for all lengths and 85×10^6 fibres/mg $L \geq 5 \mu\text{m}$ by comparison with 9.6×10^9 and 99×10^6 fibres/mg in the data shown in table 2.

(4) From tables 2 and 3 it may be seen that the concentration of chrysotile and attapulgite fibres with a $L \geq 5 \mu\text{m}$ —in the case of short fibred attapulgite samples—is at least lower a factor of 100 than the long fibred attapulgite and UICC crocidolite. It exceeds the asbestos fibre concentrations $L \geq 5 \mu\text{m}$ found in talc samples by at least a factor of 10 when similar analyses were conducted.¹⁶ However, up to five chrysotile fibres with a $L \geq 5 \mu\text{m}$ observed per 50 counting fields has to be considered to lie within the range of the detection limit determined by blind samples. This is calculated from the 95% confidence interval of Poisson statistics. For two fibres found per 100 counting fields, 3.6 fibres per 50 counting fields are estimated. Therefore, the observance of three or five fibres longer than $5 \mu\text{m}$ for the attapulgite samples is not statistically significant.

BIOLOGICAL ASSESSMENT

Of the five attapulgite samples examined, four were definitely of the short fibrous type, whereas one sample (Torrejon) proved to be distinctly longer and was thus similar to the two sepiolite samples, the erionite and the crocidolite (see table 1). If one compares the distribution of the fibre dimensions with the carcinogenic effect of these dusts in most cases it will be found that the short fibrous dusts have not proved as

carcinogenic as the long fibrous ones. The Spanish sepiolite is one exception because, on the one hand it is a relatively long fibrous type and on the other it did not induce tumours.² The two explanations for this are (1) the sample of sepiolite used by Wagner's carcinogenicity test differed essentially from the sample which we examined, although the origin—so far as could be verified—was the same and (2) although the Spanish sepiolite has a larger proportion of longer fibres, so that carcinogenic effect is to be expected³ its firmness may be too low for the existing carcinogenic agent to take effect.

Obviously specimens classified as attapulgite from a mineralogical viewpoint may differ greatly with regard to their carcinogenic potential. At present, one cannot judge whether only fibre length, which proved to be greater in the carcinogenic samples,^{1 2} is decisive or whether a lower firmness was also important for the lack of a carcinogenic effect in the short fibrous samples.⁵

The surveys on carcinogenicity available to date were made using extremely sensitive tests—either the intrapleural test² or the intraperitoneal (F Pott unpublished data) in the rat. An important question: "To what extent are the samples examined representative of the entire deposit?" is difficult to answer.

Furthermore, it is important to note that the negative result from the carcinogenicity test with attapulgite (sample Georgia 1) (F Pott, unpublished data) cannot be predicted from the strong cytotoxicity and the haemolytic activity that was found *in vitro* in the case of an attapulgite sample of a similar type (sample Georgia 2).¹⁰ The attapulgite samples "Georgia 1" and "Georgia 2" are virtually identical in the distributions of fibre dimensions (table 2) and in their infrared spectra. The different reactions in carcinogenicity test, by comparison with an *in vitro* test, show that positive findings from short term tests may be misinterpreted with respect to carcinogenic potential. Carcinogenicity tests with fibrous dusts on laboratory animals cannot, as yet, be replaced by *in vitro* tests. Based on their findings, Wagner *et al* have warned against drawing conclusions from *in vitro* tests without taking *in vivo* mechanisms also into consideration.¹⁷

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References

- 1 Pott F, Huth F, Friedrichs KH. Tumorigenic effect of fibrous dusts in experimental animals. *Environ Health Perspect* 1974; **9**:313-5.
- 2 Wagner JC. Health hazards of substitutes. In: *Proceedings of the world symposium on asbestos, Montreal, 24-27 May 1982*. Montreal: Canadian Asbestos Information Centre, 1982:244-66.
- 3 Stanton MF, Layard M. The carcinogenicity of fibrous minerals. In: *Workshop on asbestos: definitions and measurement methods*. 1978:143-51. (National Bureau of Standards spec publ 506.)
- 4 Pott F. Some aspects on the dosimetry of the carcinogenic potency of asbestos and other fibrous dust. *Staub-Reinhaltung der Luft* 1978; **38**:486-90.
- 5 Förster H, Burchard W-G. Kleintierstreu mineralogisch betrachtet. *Fortschritte der Mineralogie* 1984; **62**:57-8.
- 6 Nobel D. *Attapulgit-Eigenschaften und Anwendung*. Bremen: Sonderdruck der Chemie Mineralien KG, 1981.
- 7 Bignon J, Sebastien P, Gaudichet A, Jaurand MC. Biological effects of attapulgit. In: Wagner JC, ed. *Biological effects of mineral fibres*. Vol 2. Lyon: International Agency for Research on Cancer. 1980:163-82. (IARC sci publ No 30.)
- 8 Stanton MF, Layard M, Tegevis A, et al. Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. *J Natl Canc Inst* 1981; **67**:956-75.
- 9 Chamberlain M, Davies R, Brown RC, Griffiths M. In vitro tests for the pathogenicity of mineral dusts. *Ann Occup Hyg* 1982; **26**:583-92.
- 10 Harvey G, Page M, Dumas L. Binding of environmental carcinogens to asbestos and mineral fibres. *Br J Ind Med* 1984; **41**:396-400.
- 11 Lipkin LE. Cellular effects of asbestos and other fibers: correlations with in vivo induction of pleural sarcoma. *Environ Health Perspect* 1980; **34**:91-102.
- 12 Lipkin LE. Failure of attapulgit to produce tumours: prediction of this result by in vitro cytotoxicity test. In: Beck EG, Bignon J, eds. *In vitro effects of mineral dusts*. Berlin: Springer-Verlag, 1985:539-42.
- 13 Woodworth CD, Mossman BT, Craighead JE. Induction of squamous metaplasia in organ cultures of hamster trachea by naturally occurring and synthetic fibers. *Cancer Res* 1983; **43**:4906-12.
- 14 Griffiths DM, Hill RJ. The effects of dispersion of fibrous clays in vitro and in vivo. *Ann Occup Hyg* 1983; **27**:405-14.
- 15 Zumwalde R. *Industrial hygiene study of the Engelhard Minerals & Chemicals Corporation*. Cincinnati: National Institute for Occupational Safety and Health, 1977.
- 16 Rödelberger K, Lojewski H-G, Brückel B, Weitowitz H-J. Zum Fasergehalt von Pudern auf Talkumgrundlage. *Staub-Reinhaltung der Luft* 1984; **44**:62-6.
- 17 Wagner JC, Griffiths DM, Hill RJ. The effect of fibre size on the in vivo activity of UICC-crocidolite. *Br J Cancer* 1984; **49**:453-8.

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- 3 Weinstein L, Swartz MN. Pathogenic properties of invading micro-organisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. Philadelphia: W B Saunders, 1974:457-72.