Evaluation of the tumorigenic potential of vermiculite by intrapleural injection in rats

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Hunter, B., and Thomson, C. (1973). British Journal of Industrial Medicine, 30, 167-173. Evaluation of the tumorigenic potential of vermiculite by intrapleural injection in rats. Vermiculite, the geological name for a group of hydrated laminar minerals, has industrial application in areas previously restricted to the use of asbestos. In this assessment of its tumorigenicity, Rhodesian chrysotile asbestos or vermiculite was injected into the pleural cavity of rats. Mesotheliomata developed in 48% of the rats treated with asbestos. No tumours occurred which were associated with the vermiculite injections.

Epidemiological studies in man suggest that exposure to asbestos is associated with the development of mesotheliomata of the pleura (Wagner, Sleggs, and Marchand, 1960; Wagner, 1965; Gilson, 1966) and bronchial carcinomata (Doll, 1955). This association is supported by experimental studies in animals. Mesotheliomata have been induced by the subcutaneous injection of asbestos into the flanks of mice (Roe, Carter, Walters, and Harington, 1967) and following the intrapleural injection of asbestos into rats (Wagner, 1962; Wagner and Berry, 1969), hamsters (Smith, Miller, Elsasser, and Hubert, 1965), and fowls (Peacock and Peacock, 1965). Since the risk of developing mesothelioma of the pleura in man appears to be related to past exposure to asbestos dust, any alternative to the use of asbestos should be advocated.

Vermiculite is the geological name given to a group of hydrated laminar minerals which are aluminium-iron-magnesium silicates. Crude vermiculite consists of thin flat flakes containing microscopic droplets of water. On being subjected suddenly to high temperatures (700° to 1000°C) the flakes exfoliate to many times their original volume due to the formation of steam which forces the laminae apart. Exfoliated vermiculite takes the form of accordion-like granules containing numerous minute air spaces to which it owes its light weight, low thermal capacity, and high insulation value.

The industrial uses of vermiculite include thermal insulation, anti-condensation control, sound absorption, and fire protection. Vermiculite is used in the manufacture of asbestos-free boards and panels to comply with regulations which ban the on-site sawing of asbestos panels.

The potential of vermiculite as an alternative to asbestos necessitated testing for tumorigenic activity. This experiment was designed to see if mesotheliomata could be induced in rats by injecting vermiculite into the pleural cavity.

Materials and methods
The source of the vermiculite was the Palabora deposit mined by the Palabora Mining Company Limited, N.E. Transvaal, South Africa. The vermiculite was exfoliated using a Mantoc furnace situated at the premises of Mandoval Limited, Godalming, Surrey, England. After exfoliation the vermiculite was ground in a hammer mill to pass a screen with 240 meshes per inch, giving a nominal aperture of 63 microns.

Rhodesian chrysotile asbestos was obtained from the Medical Research Council Pneumoconiosis Research Unit, Penarth, Wales, as one of five samples contained in the Union Internationale contre le Cancer (UICC) standard reference asbestos samples.

Preparation of sample material
A 1-5 g sample of vermiculite was suspended in 10 ml physiological saline and exposed for six successive five-
minute periods to ultrasonic vibration; temperature was controlled by means of a cold water-bath. This treatment was necessary to reduce the majority of the vermiculite sample to a particle size of less than 10 microns. The resultant suspension was then made up to 12 ml (i.e., 25 mg in 0.2 ml).

The asbestos was made up to the required concentration in physiological saline and subjected to five minutes of ultrasonic vibration to aid wetting and to disperse aggregated fibres prior to injection. Since the vermiculite and asbestos samples had been subjected to ultrasonics, no further attempt was made to sterilize the materials in order to keep denaturation to a minimum. The resultant particle sizes of the vermiculite and asbestos are given in Table 1.

Rats
One hundred and fifty 21-day-old female CFY rats, a hysterectomy-derived strain of Sprague-Dawley origin, were obtained from Carworth Europe, England. The rats were housed five to a cage in suspended cages fitted with wire-mesh floors. Animal room temperature and relative humidity were controlled at 21 ± 2°C and 50 ± 5% RH respectively, and lighting was controlled to give 12 hours of light (8 a.m. to 8 p.m. B.S.T.) per 24 hours. All rats had free access to tap water and to standard laboratory rat food (Spiller's Laboratory Small Animals Diet, autoclaved). The rats were distributed at random among three groups as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Injection treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>Control – physiological saline 0.2 ml/rat</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>Vermiculite – 25 mg in 0.2 ml physiological saline/rat</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>Chrysotile asbestos – 25 mg in 0.2 ml physiological saline/rat</td>
</tr>
</tbody>
</table>

Injections were given when the rats were 5 weeks old, each rat receiving a single intrapleural injection of the appropriate test material. The rats were anaesthetized with ether and the injection was made with a 1-inch sterile needle (American gauge 23G) into the right pleural cavity at the level of the second nipple.

The procedure was well tolerated and only two deaths occurred as a result of direct injection into the lung; these rats were replaced with appropriately treated spares. After injection all rats were maintained for 104 weeks or until death or culling for humane reasons. A full necropsy was performed on all animals except for a few which had been eaten by cage mates. Tissues for histological examination were preserved in 10% formalin. Microscopy was confined to the lungs of all rats and to all abnormal masses found within the thoracic cavity.

Results
Throughout the course of the study, growth rates showed no disturbance as a result of treatment with vermiculite or chrysotile asbestos (Fig. 1).

Table 2 gives details of survival for all groups. Rats injected with vermiculite had a survival rate equivalent or slightly superior to that of the saline controls, whereas the higher mortality rate among chrysotile asbestos treated rats was a direct result of treatment (see below). Macroscopic findings at necropsy are given in Tables 3 and 4, where all lesions have been recorded.

![FIG. 1. Group mean body weight.](http://oem.bmj.com/)

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PERCENTAGE PARTICLE SIZE OF SAMPLES OF ASBESTOS AND VERMICULITE</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Material</th>
<th>Size range (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;2</td>
</tr>
<tr>
<td>Rhodesian chrysotile asbestos (a)</td>
<td>71-53</td>
</tr>
<tr>
<td>(b)</td>
<td>78-7</td>
</tr>
<tr>
<td>Vermiculite (a)</td>
<td>37-23</td>
</tr>
</tbody>
</table>

(a) Estimations made by microscopy

(b) From UICC data sheet 1c (July 1968)
Evaluation of the tumorigenic potential of vermiculite by intrapleural injection in rats

One animal could be recorded several times, depending on the multiplicity of its lesions. This treatment of the data allows differentiation between treatment-related changes and the background pathology of the rat.

Among decedents treated with vermiculite or saline there was a high incidence of mammary gland and pituitary tumours. These tumours occur frequently in this strain of rat and in none of the groups was there evidence of an increase in the incidence, or of earlier onset. This pathology also occurred among rats injected with chrysotile.

### TABLE 2

**SURVIVAL PERIOD OF RATS**

<table>
<thead>
<tr>
<th>Treatment week</th>
<th>Number of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline control</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>26</td>
<td>50</td>
</tr>
<tr>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>80</td>
<td>41</td>
</tr>
<tr>
<td>104</td>
<td>21</td>
</tr>
</tbody>
</table>

One animal could be recorded several times, depending on the multiplicity of its lesions. This treatment of the data allows differentiation between treatment-related changes and the background pathology of the rat.

### TABLE 3

**SUMMARY OF MACROSCOPIC FINDINGS: NUMBER OF RATS WITH SUSPECTED NON-NEOPLASTIC LESIONS**

<table>
<thead>
<tr>
<th>Site</th>
<th>Pathological findings</th>
<th>Saline control</th>
<th>Vermiculite 25 mg/rat</th>
<th>Asbestos 25 mg/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Fibrous adhesions</td>
<td>13</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Consolidation</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Congestion</td>
<td>9</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Subpleural haemorrhage</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Subpleural foci</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Abscess</td>
<td>1</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Collapse</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>Subcapsular motting</td>
<td>4</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Pallor</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Swollen lobes</td>
<td>8</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Subcapsular foci</td>
<td>5</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Kidney</td>
<td>Cortical pitting</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Subcapsular cyst</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Hydronephrosis</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Subcapsular motting</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lymph node</td>
<td>Congestion</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Enlargement</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Spleen</td>
<td>Enlargement</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Adrenal</td>
<td>Subcapsular foci</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>Abscess</td>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Ovary</td>
<td>Cyst</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Uterus</td>
<td>Hydrometra</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Skin</td>
<td>Ulceration</td>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Ear</td>
<td>Labyrinthitis</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Eye</td>
<td>Corneal opacity</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

| No. of rats examined | 28 | 21 | 25 |
| No. of rats not examined because of autolysis or cannibalism | 1 | 0 | 0 |

*D* = rats dying during study

*T* = rats killed at week 104
asbestos, although prior removal from the experiment, because of the debility caused by the subsequently confirmed mesotheliomata, tended to reduce the number of rats at risk of developing mammary or pituitary gland tumours.

Among the group treated with chrysotile asbestos, the majority of decedents showed macroscopic evidence of severe pulmonary damage, including abscess formation, extensive fibrous adhesions between the visceral and parietal pleura in 31 out of 40 rats, and suspected lung tumours in 21 out of 40 rats. Fibrous pleural adhesions were also noted in 13 out of 25 decedents in the group treated with vermiculite, but adhesions were not seen in the control rats.

Among rats killed at week 104, macroscopically visible lung masses were confined to the group treated with chrysotile asbestos. Fibrous adhesions, previously noted among decedents, were seen in 16 out of 25 rats injected with vermiculite and in 7 out of 8 injected with chrysotile asbestos. The distribution of other macroscopic lesions was unaffected by treatment.

**Histopathology**

Lung lesions characteristic of chronic respiratory disease of the laboratory rat were seen in rats from all groups. There was no significant difference between groups in their incidence or severity.

Chronic pleurisy was seen in a proportion of rats from all groups, but pleural adhesions and abscesses were confined to the rats injected with vermiculite or chrysotile asbestos. These changes were histologically characteristic of accidental infection of the pleura during the injection procedure: their greater severity in the rats given vermiculite or chrysotile asbestos is due to the exacerbating effect of the presence of free foreign material at the same time as an infection.

In the group of rats injected with chrysotile asbestos, 48% (i.e., 21 out of 40 decedents and 2 out of 8 killed at 104 weeks) bore tumours classified macroscopically as mesotheliomata (Table 5).

Representatives of the three types of mesothelioma described by Wagner and Berry (1969), consisting of tubopapillary (Fig. 2), spindle-celled, and mixed, were seen although the majority of tumours were of the mixed pattern.

Since there was only a small mortality before the occurrence of the first mesothelioma, in an asbestos-treated rat dying 399 days after injection, it is impossible to report the sequence of events in the pathogenesis of the tumours. There was evidence of phagocytosis of the asbestos by macrophages in the pleura of some early decedents, but few granulomata were seen in the asbestos group, and most of the animals exhibited either tumours or thickened fibrous pleura on death or at termination. Only in one case were granulomata seen in rats killed at week 104.

Examination of the first mortality in the group treated with vermiculite, 371 days after the injection, revealed numerous pigment-laden macrophages in the pleural membrane. By week 80, granulomata were seen in all rats (Figs. 3 and 4) but none displayed evidence of neoplastic reaction.

Only one intrathoracic tumour was found among

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**Table 4**

**Summary of Macroscopic Findings: Number of Rats with Suspected Neoplastic Lesions**

<table>
<thead>
<tr>
<th>Site of suspected neoplasm</th>
<th>Saline control</th>
<th>Vermiculite 25 mg/rat</th>
<th>Asbestos 25 mg/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>T</td>
<td>D</td>
</tr>
<tr>
<td>Lung</td>
<td>17</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Liver</td>
<td>8</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Spleen</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Lymph node</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pituitary</td>
<td>12</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>17</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Uterus</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Vagina</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Adrenal</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Harderian gland</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of rats examined</td>
<td>28</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>No. of rats not examined because of autolysis or cannibalism</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

D = rats dying during study
T = rats killed at week 104
TABLE 5
PULMONARY HISTOPATHOLOGY

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Number of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline control</td>
</tr>
<tr>
<td></td>
<td>D  T</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>0  0</td>
</tr>
<tr>
<td>Bronchial adenoma</td>
<td>0  0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0  1</td>
</tr>
<tr>
<td>Undifferentiated sarcoma</td>
<td>0  0</td>
</tr>
<tr>
<td>Granuloma</td>
<td>0  16</td>
</tr>
<tr>
<td><strong>No. of rats examined</strong></td>
<td>28  21</td>
</tr>
<tr>
<td><strong>No. of rats not examined</strong></td>
<td>1  0</td>
</tr>
</tbody>
</table>

*D* = rats dying during study  
*T* = rats killed at week 104

Rats injected with vermiculite, in a rat dying 672 days after injection. The tumour was situated in the mediastinum and consisted of several masses of fibrous stroma containing islands of basophilic cells with large pleomorphic nuclei and scant cytoplasm, with occasional formation of pseudo-acini. A tentative histological diagnosis of undifferentiated adenocarcinoma was made.

**FIG. 2.** Lung of rat dying 75 weeks after injection of chrysotile asbestos. A large mass of tubopapillary mesothelioma is seen on the visceral pleura, and compression and distortion of the lung parenchyma is evident. ×44.
FIG. 3. Lung of rat dying 84 weeks after injection of vermiculite. A granuloma is seen on the visceral pleura. ×44.

FIG. 4. Portion of granuloma on visceral pleura of rat dying 99 weeks after injection of vermiculite. Particles of vermiculite are visible within some of the macrophages. ×170.
Discussion

Wagner and Berry (1969), in a study of the induction of mesothelioma in SPF and conventional rats, obtained a 63.5% yield of mesothelioma equally distributed between the sexes in 96 SPF rats, following the intrapleural injection of 20 mg Canadian chrysotile asbestos. The first tumour was recorded in a rat dying 361 days after the injection. This incidence was higher than that of the rats obtained with crocidolite asbestos, and since earlier experimental work (Wagner, 1962) and epidemiological studies of mortality by district of residence (Oettlé, 1964) both showed that exposure to crocidolite carries with it a far greater risk of developing malignant disease, Wagner and Berry investigated the possibility that their sample of chrysotile asbestos was contaminated. However, they failed to discover any difference from other chrysotile samples (Wagner and Berry, 1969).

The results reported here show a 48% incidence of mesothelioma, with the first tumour found in a rat dying 399 days after the intrapleural injection of 25 mg of Rhodesian chrysotile asbestos. The lower incidence of mesothelioma and the slightly longer assumed latent period in our experiment may reflect an inherent difference between the Canadian and Rhodesian chrysotile asbestos. Further work is required to resolve this problem.

The carcinogenic properties of asbestos may be due to:
1. the chemical composition of the asbestos;
2. the chemical composition of organic materials associated with the asbestos, either naturally or following manufacturing processes;
3. the physical properties of the fibres, or a combination of these factors (Harington and Roe, 1964).

Asbestos injected into the pleural cavity undergoes a cyclic process of phagocytosis by macrophages which die and rupture, releasing the particles for re-phagocytosis. This process is accompanied by fibrosis. Eventually re-phagocytosis of the particles ceases, and they lie free within fibrous tissue (Davis, 1970; Allison, 1971).

Vermiculite shares many of its chemical components with various types of asbestos, but we have no knowledge of any natural or contaminating organic oils. The particles injected, unlike the fibrous particles of asbestos, were amorphous.

It is considered possible that the lack of carcinogenic potential of vermiculite is directly related to the ability of the animals to restrict the material within stable granulomata.

Although the concept of intrapleural injection is unrealistic when compared to the human situation, these findings demonstrate that the usage of vermiculite is unlikely to be associated with the degree of hazard imposed by asbestos.

References


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