ASSOCIATION OF TREMOLITE HABIT WITH BIOLOGICAL POTENTIAL: PRELIMINARY REPORT

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INTRODUCTION

Tremolite is an amphibole mineral which occurs naturally in three distinct morphological forms or mineral habits. It may occur as asbestos, splintery fibres or in massive crystalline deposits. Mineral habit is determined at the time of its crystallization and conversion to a different habit would require re-crystallization. The massive crystalline deposits, on crushing may yield elongated fibrous-looking particles referred to as acicular cleavage fragments (Langer *et at.,* 1979). Tremolite's ability to form these three habits imparts it with a range of physico-chemical properties.

The health effects from the occupational exposure to tremolite has been evaluated epidemiologically among vermiculite and talc workers (Reger & Morgan, 1990). Case reports have associated both pleural calcification and mesothelioma with environmental exposure to tremolite in Turkey, Cyprus and Greece. Experimental studies using various routes of administration in different animal species and *in vitro* assays have been used to

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compare tremolite specimens. These studies clearly indicate that tremolite possesses a range of biological activities, which should be expected considering the range of physico-chemical properties associated with the mineral's different habits of crystallization. The preliminary results of a comparison of several tremolite specimens which produced this range of biological activities are described in this paper.

EPIDEMIOLOGICAL AND CLINICAL STUDIES OF POPULATIONS INDUSTRIALLY EXPOSED TO TREMOLITE

The pulmonary hazards of fibrous tremolite exposure were evaluated among a group working in a vermiculite exfoliating plant. Twelve cases of benign pleural effusion occurred in the group over a 12 year period. The cause for this unusually high number of cases could not be established and a study was designed to determine the prevalence of pulmonary abnormalities in these workers. The vermiculite ore used in the plant was primarily obtained from Montana, which was reported to contain at least 0.006% - 0.41% fibrous tremolite (Banks, 1980; Lockey *et al.*, 1984). The study protocol involved a review of the industrial hygiene data, administration of a modified American Thoracic Society (ATS) respiratory questionnaire, physical examination and determination of single-breath diffusion capacity.

The study population consisted of 513 current employees with a history of exposure to vermiculite containing tremolite. The control group was made up of employees who were unexposed to dust. About 44% were current smokers and **20%** ex-smokers with no significant differences among the exposure groups. The medical component of the cross-sectional epidemiological study was correlated with exposure by job category, cumulative fibre exposure and time from first exposure to the vermiculite containing fibrous tremolite.

A statistically significant association was found between cumulative fibre exposure and symptoms, i.e., shortness of breath with wheezing, dyspnea on exertion and pleuritic chest pain. The radiographic survey involved 501 of the 513 individuals in the exposed group. Pleural and/or parenchymal changes were found in 4.4%. Eleven showed costophrenic angle blunting, and eleven showed pleural abnormalities. The mean cumulative exposure for an abnormal X-ray was 8.74 ± 11.71 (f/ml) x yr.

The health effects of exposure to vermiculite containing tremolite on miners and millers in Montana has been studied (McDonald *et al.*,(1986a,b; Amandus *et al.*, 1987a,b,c). The two studies were carried out separately, though in parallel, and each involved a cohort mortality study and a cross-sectional radiographic survey. Slightly different criteria were used to define each cohort and the McDonald cohort contained 406 men with 165 deaths and the Amandus cohort contained *575* men with 161 deaths.

Each research group used historical air samples to estimate an exposure index for each member of the cohort. The older measurements were all made with the midget impinger and a conversion was made from million particles per cubic foot (mppcf) to approximate the fibres per milliliter (f/ml). The exposures in the dry mill, before the installation of dust control equipment in 1964 were estimated by McDonald and co-workers (1986a) at $\approx$100 f/ml arid Amandus and co-workers (1987a) at $\approx$ 168 f/ml and 1965 to the close of the dry mill in 1974 $\approx$20 f/ml and $\approx$33 f/ml respectively. These were the highest exposures except for sweeping the floor in the dry mill which was $\approx$20% higher.

The McDonald cohort has an SMR for total mortality of 1.17 with 23 respiratory cancer cases (SMR = 2.45) and 4 mesotheliomas (3 pleural and 1 peritoneal). The SMR for total mortality of the Amandus cohort was 1.10 with 20 respiratory cancer cases (SMR = 2.23) and 2 mesotheliomas. The lung cancer SMR for more than 20 years since hire and all exposure levels for the McDonald and Amandus cohorts were 2.42 and 2.79 respectively. Both cohorts had an SMR of ≈ 2.5 for non-malignant respiratory disease.

The mortality experienced in Montana was compared to miners and millers of vermiculite in the Enoree region of South Carolina where the ore contains trace amounts of fibrous tremolite (McDonald *et al.*, 1988). This cohort was made up of 194 men. For men with > 15 years since onset of exposure, 51 deaths had occurred. The SMR for total mortality was 1.17, which was similar to Montana. All the air samples, except one, analyzed by phase contrast microscopy (using the NIOSH reference method) had mean concentrations < 0.01 f/ml (N = 58). The analysis of the same samples by analytical transmission electron microscopy were higher and the mean concentrations ranged from 0.01 - 0.32 fibres > 5 μm in length/ml. The average fibre diameter was 1.1 μm with an average length of 12.7 μm. Less than half the fibres sized had elemental compositions consistent with the tremolite-actinolite series. Of the 51 deaths, four were from respiratory cancer (SMR = 1.21), 3 of 4 lung cancers in lowest exposure group < 1(f/ml) x yr with no mesotheliomas or pneumoconiosis deaths.

ENVIRONMENTAL EXPOSURE IN TURKEY, CYPRUS AND GREECE

A series of tumours of the lung and pleura in southeast Turkey have been studied retrospectively in the Diyarbakir Chest Hospital (Yazicioglu *et al*, 1980). Between 1968 and 1976, 177 malignant lung cancers and 44 pleural tumours were admitted to the chest hospital. The geographic distribution of the tumours suggested an etiology due to asbestos exposure. The incidence of pleural neoplasms and pulmonary tumours were 11.4 times higher and 2.5 times higher respectively in areas where asbestos was extensively used compared to areas with no asbestos.

Of the 44 mesotheliomas found in the group of 221 malignant tumours, ten (6 females, 4 males) occurred among the 20-40 year old age group. The early age at which these tumours appeared, and the near equal distribution between the sexes, indicates the exposure was environmental and began in early childhood. Interestingly, many cases of benign pleural effusion were found as well. A similar observation was later reported (Lockey *et al.*, 1983) among the workers in a vermiculite exfoliation plant in the United States.

A radiographic cross-sectional survey of 7,000 individuals revealed that 6.6% (461) had pleural thickening and calcification, of the total survey group 1.5% (103) had interstitial pulmonary fibrosis. By 65 years of age, 50% of this group would have radiographic evidence of pleural abnormalities. The exposure continues from birth as long as the individual remains in this environment, which may explain why the changes occur so early and are very extensive. The clinical effects of these childhood exposures are found years later in individuals who moved away from these asbestos areas. This feature was noted by Constantopoulus and co-workers (1987b) in their observations pertaining to geographic distribution of plaques and tumours in Greece.

The 1978 report attributed the malignant tumours, particularly those of the pleura, to be numerous outcrops of tremolite asbestos, which was used locally to make a whitewash or stucco for the walls, floors and roofs of the houses (Yazicioglu *et al.*, 1980). The whitewash contained fibrous tremolite and the non-fibrous minerals talc, chlorite and antigorite/lizardite. Although occasional chrysotile fibres were found in the environment, the investigators attributed the pleural reactions, pulmonary fibrosis, and the malignant tumours of the lung and pleural in the Cermik region to the tremolite asbestos.

Asbestos related diseases of the chest, including mesothelioma, have also been reported in the small Anatolian Village of Caparkayi in Turkey (Baris *et al.*, 1988a, b). Four cases of pleural mesothelioma were reported in a population of 425 over a three year period. All of the tumours occurred in women between 26 and 40 years of age. Again, the tumours occurred at a young age and in women indicating the non-occupational nature of the exposure.

In a radiographic cross-sectional survey of 167 individuals over 20 years old from the village, *63* abnormalities were found. Due to a migration of the younger people, the village population has a higher percentage of older individuals than would generally be expected (51% are over 20 years). Approximately 15% of the 167 individuals surveyed had calcified pleural plaques, interlobar fissure thickening and/or diffuse interstitial fibrosis.

Although no asbestos mine is **near** the village, a commonly used white stucco was described by Baris and co-workers (1988b) as "rich in tremolite asbestos including some very fine fibre." The report indicates that the high incidence of mesothelioma and some of the pleural and parenchymal abnormalities in the village are associated with exposure to tremolite fibres.

Located in the central mountains of the island of Cyprus is a large chrysotile mine which has been in commercial operation since 1904. Initially it was thought that the site would provide an opportunity to study human mesothelioma from exposure only to chrysotile. The first mesothelioma identified was found in a women who had never worked in the mine, although she lived in a village nearby. The lung tissue in this case contained asbestos bodies and amphibole asbestos. Fourteen cases in total were reported between the onset of the study in 1969 and March, 1986. No tremolite was found in the chrysotile specimen taken from the mine, although tremolite and chrysotile were found to be present in an environmental dust sample taken from the roof eaves of the houses (McConnochie *et al.*, 1987).

Of the 14 cases of mesothelioma identified, 7 were confirmed by a panel of pathologists. In one case the diagnosis was in doubt, and in another the individual may have had prior exposure to asbestos in South Africa. Eight cases were either chrysotile miners or the wives of miners, and two were residents of mining communities, leaving only two cases with environmental exposure. Examination of the lung tissue burden of both human and sheep (from within 5 miles of the mine) identified chrysotile and tremolite. The tremolite found was in a form that included long, thin fibres having a similar size distribution to crocidolite.

Further study has positively identified **13** cases of mesothelioma, 5 of which occurred in persons unconnected with the local asbestos mine. A stucco used in the region contained fine fibrils of chrysotile and long, thin tremolite fibres. The reports indicate that the distribution of tumours and the naturally occurring tremolite asbestos, particularly its use as stucco, suggests the mine is not the major source of disease (McConnochie *et al.*, 1989).

Six deaths occurring from malignant pleural mesothelioma have been reported among residents in the villages of Milea, Metsovo, Anilio and Votonosi in northwest

Greece (Constantopoulos *et al.*, 1987a). These six deaths occurred among seven mesotheliomas diagnosed in the region. Three mesotheliomas occurred in males and four in females. Bilateral pleural plaques, pleural thickening, restrictive lung function and mesotheliomas constitute the cluster of disease referred to as Metsovo lung (Constantopoulos *et al.*, 1985). Before 1940 virtually all of the inhabitants of the area painted their homes with a whitewash containing tremolite asbestos (Langer *et al.*, 1987). Exposure to the tremolite asbestos contained in the whitewash has been associated with Metsovo lung. A hypothesis for expecting such findings to be regional and occur in other parts of the world has been proposed (Constantopoulos *et al.*, 1987b).

EXPERIMENTAL ANIMAL STUDIES

The carcinogenic properties of four tremolite specimens were determined through single intrapleural injections into Syrian golden Hamsters (Smith, 1974; Smith *et al.*, 1979). The model was validated by determining the carcinogenicity of the four commercial asbestos varieties. The greatest proportion of tumours were produced with crocidolite, although, all the commercial asbestos specimens tested produced tumours, extensive pleural adhesions and densely fibrotic lesions. Two of the four tremolite specimens produce no tumours.

These results were compared to a specimen of tremolite talc which contained 50% fibrous tremolite, 35% talc (about 25% of which was itself fibrous or rolled), 10% antigorite and 5% chlorite. By light microscopy the fibres ranged from 2.5-16.5 μm in length with an average length of 5.7 μm and diameter of 1.6 μm. Intrapleural injection of a 25 mg dose of tremolite talc produced no tumours in 50 animals. An identical mass of three chrysotile specimens, one of which was tested both heated and unheated, produced $\approx 18 \pm 2$ tumours, and 1 mg injection of UICC crocidolite produced 4% tumours (Smith, 1974).

Three additional specimens, a non-asbestos tremolite (#275), a western tremolitic talc and a tremolite asbestos, were evaluated using the same bioassay (Smith *et al.*, 1979) The specimens were found to be 95%, 90% and 95% tremolite respectively. The non-asbestos tremolite produced no tumours at doses of 10 and 25mg while the western tremolitic talc and tremolite asbestos produced $\approx 2.5\%$ and 24% tumours respectively at 10 mg and 21% and 50% tumours at the 25 mg dose respectively.

Both of the tremolite specimens which produced no tumours were from the same geological locale, although, the tremolitic talc specimen evaluated in the earlier experiment contained a significant proportion of both platy and fibrous talc, antigorite and chlorite. Extensive pleural fibrosis was produced with tremolite asbestos, less with western tremolitic talc, and very slight pleural fibrosis with the two specimens that produced no tumours. In these experiments tumour production correlated with fibrosis (Kuschner, 1987).

Two tremolite asbestos specimens, both from the same lot, were evaluated by Stanton and co-workers (1981) using a pleural implantation model in rats. The fibre diameters of these specimens were distinctly smaller than the diameters of the specimen reported by Smith, 1974. A 40 mg dose of tremolite 1 and 2, containing 55.2 x 10^6 and 27.7 x 10^6 fibres $> 8.0\ \mu m$ in length and $< 0.25\ \mu m$ in diameter respectively, produced a tumour incidence of 22/28 and 21/28 respectively. For both of these specimens the model predicted a 100% probability of tumour induction.

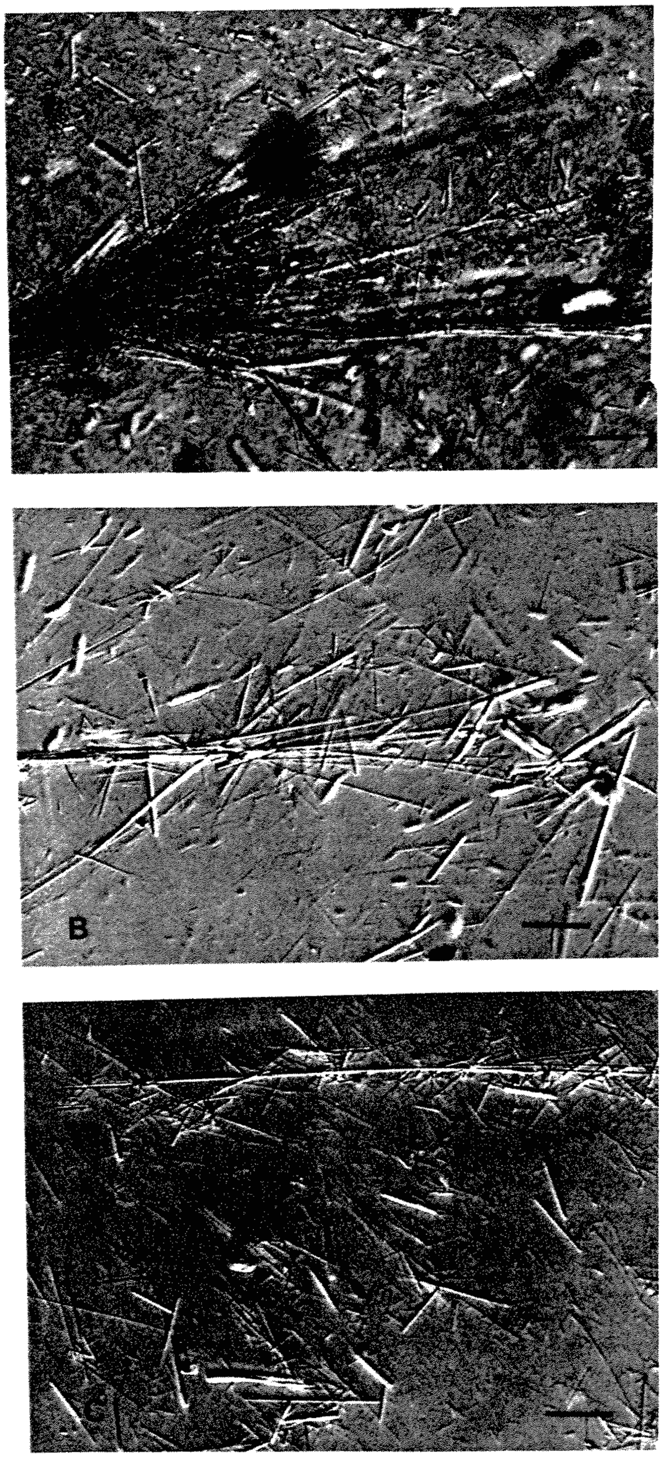
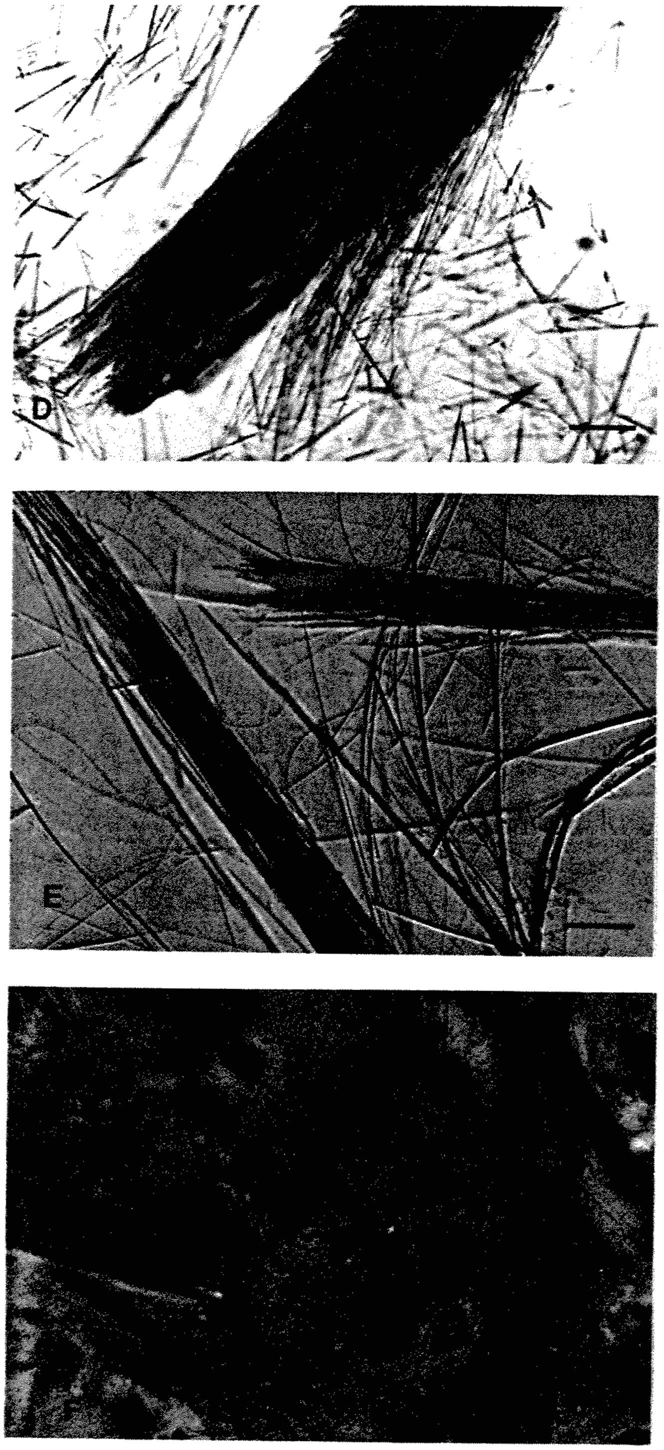


Figure 1 : Light photomicrographs of tremolite asbestos using Hoffman Interference Optics except (f), which was taken using a Zeiss bright field lens:
(a) Whitewash from **Metsovo, Greece;** (b) South **Korea;**
(c) Beneficiated from vermiculite **deposit** near **Libby,** Montana



(d) **Swansea**, Wales; (e) **Jarnestown,** California;
(f) **Inyo County.** California. (Bar represents 50 μm)

By intrapleural injection into rats Wagner (Wagner, 1982; Wagner *et al.*, 1982) evaluated three tremolite specimens while using either UICC crocidolite or Super-Fine Asbestos (SFA) chrysotile as positive controls. One tremolite specimen was beneficiated from a California tremolitic talc deposit by froth flotation. The starting material was $\approx$ 62% talc and $\approx$ 38% tremolite, and after froth flotation the material enriched to $\approx$ 95% tremolite (the remaining minerals were talc and calcium carbonate). The 20 mg dose injected intrapleurally, containing 1,020 x 10^6 fibres (3.3% of which were > 8 μm in length and < 1.5 μm in diameter) which produced no tumours in thirty-one rats. The positive control, 20 mg of SFA chrysotile, produced twenty mesotheliomas in thirty-two rats.

A second tremolite specimen from Greenland, which contained 960 x 10^6 fibres (with no fibres > 8 μm in length and < 1.5 μm detected) in a 20 mg dose produced no tumours in 48 rats. A highly fibrous tremolite asbestos specimen from Korea contained 3,100 x 10^6 fibres (with 35% being > 8 μm in length and < 1.5 μm in diameter) in a 20 mg dose. This specimen produced 14 mesotheliomas in forty-seven rats. Due to a poor survival rate the positive control in this experiment, UICC crocidolite, produced only two mesotheliomas.

The tremolite asbestos from Korea has produced two mesotheliomas, two adenomas and sixteen carcinomas in thirty-nine animals using inhalation as the route of administration. The dust cloud contained $\approx$ 10 mg/m^3 with $\approx$ 1,600 fibres > 5 μm in length/ml. At the end of the exposure of 7 hrs/day for a total of 224 days in a 12 month period, the mean tremolite lung burden of four rats was 10.8 mg at the end of the exposure period. Additionally intraperitoneal injection of 25 mg of the tremolite asbestos from Korea produced tumors in twenty-seven out of twenty-nine rats (Davis *et al.*, 1985).

Recently, six tremolite specimens were studied by the intraperitoneal injection of a 10 mg dose in thirty rats (Davis, 1990; Davis *et al.* in press). Three were tremolite asbestos specimens from California, Swansea and Korea and contained 121 x 10^6, 8.0 x 10^6 and 48 x 10^6 fibres > 8 μm in length and < 0.25 μm in diameter per dose, which was 0.9%, 0.4% and 0.6% of the total fibres, and produced 100%, 97% and 89% tumours respectively. The mean survival time was less than 428 days for the animals in these three exposure groups. The splintery fibres from Italy contained 58 x 10^6 fibres per dose > 8 μm in length, and approximately 1.7% were < 0.25 μm in diameter and produced 67% tumours with a mean survival time of 755 days. Although the non-asbestos specimens from Carr Brae and Shinness contained no fibres > 8 μm in length and < 0.25 μm in diameter, the dose did contain 134 x 10^6 and 17 x 10^6 fibres per dose (> 8 pm) in length respectively, and produced 12% and 5.6% tumours respectively with too few tumours for a mean survival time to be calculated for the lifetime of the animals. Using the intraperitoneal model, the incidence of tumours - 10% with a 10 mg dose indicates it is unlikely that the non-asbestos tremolite from Shinness or Dornie would cause tumours by inhalation. (Ilgren & Wagner, 1991).

MATERIALS AND METHODS

Origin of the mineral specimens:

- Mixture of tremolite and richterite asbestos from **Libby**, Montana, **U.S.A.** obtained from W. Banks, Bureau of Mines, United States Department of the Interior. Epidemiology studies of the health effects of industrial exposure by Lockey *et al.*, 1983;

Lockey *et al.*, 1984; McDonald *et al.*, 1986a,b; Amandus *et af.*, 1987a,b,c.

- Tremolite asbestos from **Inyo** County, California obtained from G.J. Gill of Cypress Industrial Minerals, Denver, Colorado, U.S.A.
- Tremolite asbestos from **Metsovo,** Greece used as a whitewash and associated with pleural mesothelioma (Langer *et al.*, 1987).
- Mixture of tremolite asbestos and splintery fibres, Udaipur District, Rajastan State, **India.** obtained from Ward Scientific, Rochester, New York, U.S.A.
- Non-asbestos **respirable** tremolite from **Gouverneur,** New York, U.S. A. obtained from C.S. Thompson, R.T. Vanderbilt and Company, Norwalk, Connecticut, U.S.A. The same specimen (Sample No. 275) was used in experimental animal studies by Smith *et al.* 1979.
- Non-asbestos tremolite from **Gouverneur,** New York, U.S.A. used in NTP feeding studies, 1990 (Campbell *et al.*, 1979, Campbell *et al.*, 1986).
- Non-asbestos tremolite found in a vermiculite deposit in the Enoree region of **South Carolina** (McDonald *et al.*, 1988).

Respirable fractions of the following specimens were characterized mineralogically and used in experimental animal studies (Wagner *et al.*, 1982, Davis *et al.*, 1985, Davis, 1990, Addison and Davis, in press, Davis *et al.*, in press).

- Tremolite asbestos, **Jamestown**, California, U.S.A.
- Tremolite asbestos, **South Korea.**
- Tremolite asbestos, of unknown geological locale, obtained from **Swansea** Laboratory, Wales, United Kingdom.
- Splintery fibres, **Ala di Stura,** near Turin, Northern Italy.

UICC Reference Asbestos Specimens

- Crocidolite from the Cape Province, Republic of South Africa.
- Amosite from the Transvaal, Republic of South Africa.
- Anthophyllite from Paakila, Finland.
- Chrysotile A from Zimbabwe.
- Chrysotile B, a blend of chrysotile ores from eight Canadian mines.

The positive control for membranolysis was:

- Quartz, a commercially available silica flour referred to as Min-U-Sil 15 was obtained from the Pennsylvania Glass and Sand Company, Pittsburgh, PA.

The inhibitors of membranolytic activity, and their sources, are as follows:

- Poly (2-vinylpyridine-N-Oxide); (2-PVPNO) was obtained from Polyscience Inc, Washington, PA. The weight average molecular weight, of the 2-PVPNO polymer, as determined by light scattering, was 276,000.
- Pyridoxal 5-Phosphate, 1.5 H_2O was obtained from Sigma Chemical Company, St. Louis, MO.

Human Membranolytic Model

The ability of each of the mineral specimens to alter the permeability of a population of human erythrocytes was determined quantitatively. The HC_{50} is the concentration of particles (given in mg/ml) required to lyse 50% of the erythrocytes in a suspension containing 1.8×10^8 cells/ml (see Nolan *et al.*, 1981 for details).

Hydrogen Bonding Determinations

The ability of a mineral specimen's surface to bind 2-PVPNO was determined by adding various concentrations of the mineral to 20 ml of 50 μg/ml 2-PVPNO. The

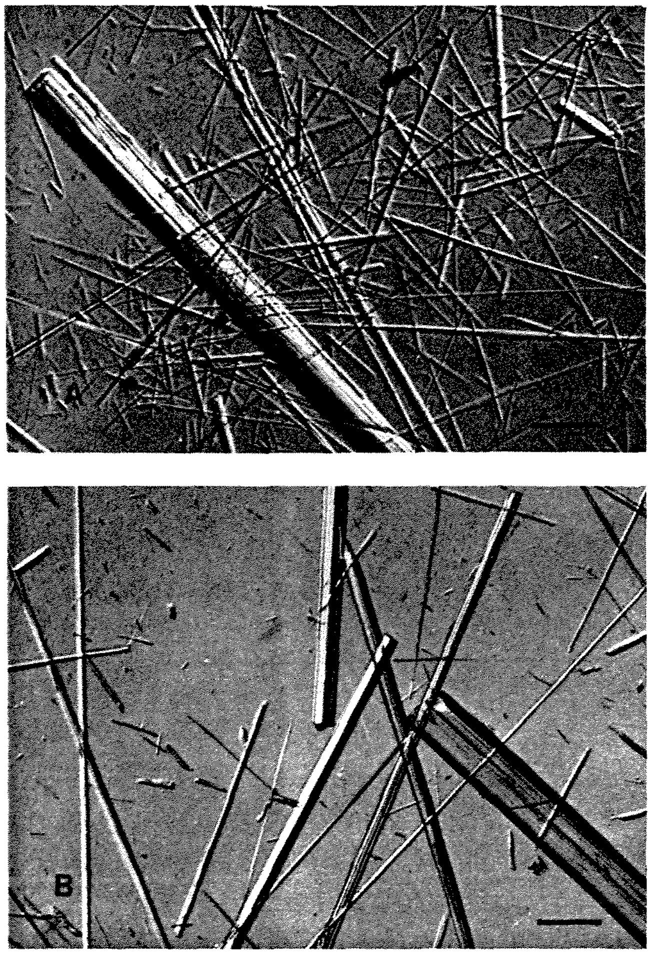


Figure 2. Light photomicrographs of splintery fibres using a) **Hoffman interference optics and b) a Zeiss hright field optics:**
a) Udaipur District, Rajastan State, India.
(Bar represents 50 pm)
b) Ala di Stura, near Turin, Italy. (Bar represents **100** μm)

suspension of mineral and polymer were sonicated using the same conditions as those for the membranolysis experiments and allowed to stand at room temperature for 60 minutes. The suspensions were vortexed occasionally to resuspend the mineral particulates. After an hour, the 2-PVPNO bound to the mineral surface was separated from the free 2-PVPNO by centrifugation at Fc $\approx$ 10,000 x g. The concentration of 2-PVPNO in the clear supernatant was determined by the absorbence of the polymer at 260nm. The amount of 2-PVPNO bound to a given amount of mineral was determined by this method.

Zeta Potential Measurements

The surface charge was approximated by determining the zeta potential using a commercially available instrument from Zeta-Meter, Inc., Long Island City, New York.

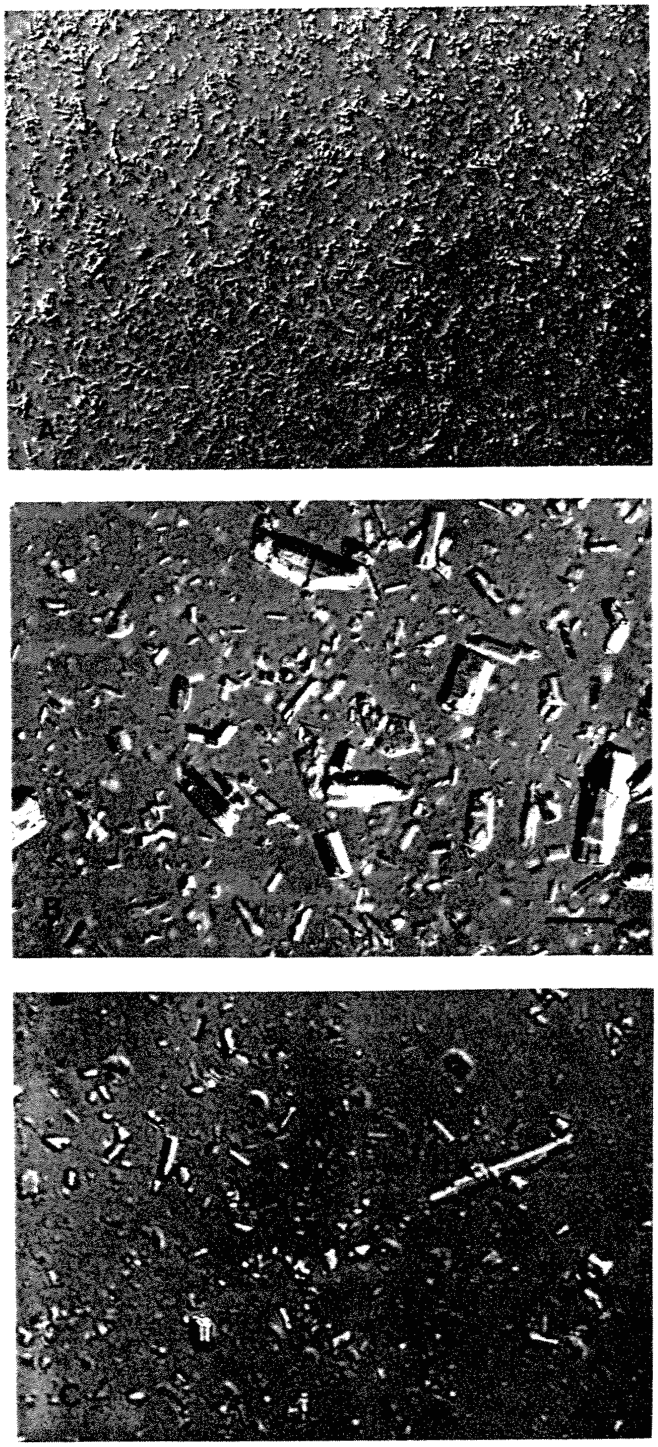


Figure 3. Light photomicrographs of non-asbestos tremolite specimens using Hoffman interference optics:
a) **Gouverneur, New York** used by Smith ***et al.***, 1979.
b) **Gouverneur, New York** used **in** NTP feed study, 1990.
c) Beneficiated from a vermiculite deposit, **Enoree region. South Carolina.** (Bar represents 50 μm)

TABLE 1: **Gross characteristics of minerals specimens**

Metsovo, Greece: Fibres visible with unaided eye. Fibre lengths up to 1 mm, fibre widths $<$ 0.5 mm. Fibres noted to protrude from matted clumps. Fibre color is gray-white.

Korea: Fibres visible with unaided eye. Fibre lengths up to 2 mm, fibre widths $<$ 1 mm. Few fibres visible. Much granular powder present associated with fine, matted clumps. Fibre color is light-tan.

Libby, Montana: Fibres visible with unaided eye. Fibre lengths up to 2 mm. fibre widths up to 0.5 mm. Specimen composed almost entirely of clumps of fibre. Fibre color is gray.

Swansea, Wales: Fibres visible with unaided eye. Fibre lengths up to 1 cm, fibre widths **up** to 1 mm. Fibre visible associated with granular powder. Fibre occurs in matted clumps. Fibre color is gray-white.

Jamestown, California: Fibres visible with unaided eye. Fibre lengths up to 2.5 cm, fibre widths up to **3** mm. Specimen composed almost entirely of fibre. Fibres appear kinked, curled and splayed. Fibre color is predominately white.

Inyo County, California: Fibres visible with unaided eye. Fibre lengths up to 2 mm, fibre widths up to 0.5 mm. Fibres are present in matted clumps. Fibre color is white.

India: Fibres visible with unaided eye. Fibre lengths up to 1 cm, widths up to 1 mm. Fibres visible as individual bundles and as components of clumps. Fibre color is gray.

Ala di Stura, Italy: Fibres visible with unaided eye. Fibre lengths up to 1.5 cm, widths up to 2.0 mm. Specimen cornposed almost entirely of fibres. Fibres form straight, bladed crystals. Fibre color is light-gray.

Gouverneur, N.Y., Respirable: No fibres visible in specimen. Specimen consists of granular, white powder.

Gouverneur, N.Y., NTP: No fibres visible. Specimen consists of fine, white powder of small particle size.

Enoree Region, South Carolina: No fibres visible. Specimen consists of granular powder. The powder is grey in color.

Note: Some of these characteristics were better observed with the aid of a 10 power hand lens.

Each specimen was suspended at a concentration of 100 to 250 mg per liter in veronal buffer without saline **at** pH **7.4.** The particles were dispersed by ultrasound at 50 Watts of power for five minutes and allowed to cool back to room temperature. The electrophoretic mobility (EM) was then measured by determining the time (in seconds) required for 10 different particles to migrate 160 μm in an electric field. The field strength was varied from 10 to 20 V/cm to allow a tracking time of ≈ 3 seconds. Once the electrophoretic mobility (given in microns per sec/volts per cm) is determined, the zeta potential (ZP) *can* be calculated using the Helmholtz-Smoluchowski equation:

$$ZP = \frac{4\pi V_t}{D_t} \times EM$$

where:
EM = Electrophoretic Mobility at actual temperature; V_t = Viscosity of the suspending liquid at temperature, D_t = Dielectric constant of the suspending liquid at temperature;
ZP = Zeta Potential in electrostatic units.

TABLE 2. Characteristics of the Mineral Specimens by Polarized Light Microscopy

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Metsovo, Greece: Asbestos fibre bundles. Fibres with splayed ends, polyfilamentous. and parallel extinction. Asbestos constitutes about *50-75%* of specimen. Tremolite cleavage fragments are present, possibly constituting up to half of specimen. Other sificate minerals present. Figure la.

Korea: About 50% asbestos fibre (may be even less). Splintery fibres abundant ($\approx$25%). Cleavage fragments present as alteration products. Figure 1b.

Libby, Montana: About 90% asbestos. Polyfilamentous bundles of fibres. Parallel to near-parallel extinction, actinolite, other silicates fragments present. Figure Ic.

Swansea, Wales: About **75%-85%** asbestos fibre. polyfilamentous bundles, splayed ends, parallel extinction. Some cleavage fragments ($\approx$10%), and splintery fibres ($\approx$*5%)*. Figure 1d.

Jamestown, California: Over **90%** asbestos fibre (polyfilamentous, parallel extinction, splayed ends, etc.) Some cleavage fragments ($\approx$3%), splintery fiber ($\approx$2%), trace of chlorite (?). Figure le.

Inyo County, California: Asbestos fibres constitute -15-20% of specimen. Asbestos fibres exhibit polyfilamentous character, splayed ends, parallel extinction. Tremolite cleavage fragments present associated with carbonate minerals, talc, other silicates. Figure If.

India: Asbestos fibre constitutes $\approx$90% of specimen. Asbestos tibres exhibit polyfilamentous character. splayed ends, parallel extinction. Some cleavage fragments observed. non-fibrous silicates. some splintery fibres. Figure 2a.

Ala di Stura, Italy: About 90% laths (splintery fibre). no asbestos present, all laths have parallel extinction. laths display extreme length: width ratio. Figure 2b.

Respirable, Gouverneur, N.Y.: Non-fibrous fine powder. aggregates of particles, no asbestos present, small cleavage fragments observed in talc. some talc fibres visible. Figure 3a.

NTP, Gouverneur, N.Y.: Tremolite cleavage fragments. some talc, no asbestos present, some other silicates. Figure 3b.

Enoree Region, South Carolina: Tremolite cleavage fragments, some other silicates. no asbestos present, some elongate cleavage fragments noted (aspect ratio - 10:1). Figure 3c.

Mineralogical Characterization of the Specimens

Bulk powders were available for all the specimens examined (Table 1). An aliquot of each was characterized by polarized light microscopy (Table 2). Specimens found to contain polyfilamentous fibre bundles with splayed ends exhibiting parallel extinction, were classified as asbestos (Figure 1, see Langer *et al.* this volume). Invariably, these specimens contained fibres with high aspect ratios and very narrow diameters. The specimens in which the fibres exhibited the optical properties consistent with single crystals and oblique extinction in polarized light were classified as non-asbestos (Figure 2 and 3). These specimens invariably contained very few fibres with narrow diameters.

The specimen from India contained both polyfilamentous bundles with parallel extinction, which were classified as asbestos, and high aspect ratio fibres with oblique extinction which were classified as splintery fibres, which are not asbestos. The fibres were constituted of single crystals, of larger diameter than amphibole asbestos fibrils. The specimen from Ala di Stura contained predominantly splintery fibres (see Figure 2). The asbestos specimens contained variable amounts of cleavage fragments and/or splintery fibres and other impurities (Table 2). The four non-asbestos specimens contained no polyfilamentous asbestos fibre bundles.

Each specimen was examined by continuous scan X-ray diffraction and transmission electron microscopy using energy dispersive spectrometry. The complete results will be published elsewhere. Each specimen, with the exception of Enoree and Inyo County, produced a diffractogram in which the major crystalline mineral phase present was an amphibole.

Membranolytic Activity of the Mineral Specimens

The membranolytic activity of the tremolite asbestos specimens varied from 1.14 $\pm$ 0.19 mg/ml for Jamestown, California to 3.06 $\pm$ 1.06 for Metsovo, Greece, a factor of ~3-fold. The average HC_{50} for the six specimens is 1.94 $\pm$ 0.75 mg/ml. The HC_{50} of the specimen from India was approximately equal to Min-U-Sil 15 (Nolan *et al.*, 1981) while the specimen from Italy contained long, rigid fibres which could not be pipetted. The non-asbestos tremolite specimens from Gouverneur, New York ranged from an HC_{50} of 1.02 $\pm$ 0.22 mg/ml for the respirable tremolite to 5.21 $\pm$ 0.11 mg/ml for the NTP specimen. The UICC reference asbestos amphiboles varied from 1.76 $\pm$ 0.28 mg/ml for amosite to 4.05 $\pm$ 1.22 mg/ml for crocidolite a factor of 2.3 fold. The two UICC chrysotile specimens had any average HC_{50} of 0.07 mg/ml which was ~28-fold and ~45-fold more active than the average for the tremolite asbestos specimens or the average for the UICC asbestos amphiboles respectively (Table 3).

Effect of Inhibitors of Membranolytic Activity and a Comparison of Surface Properties

The activity of all of the asbestos and non-asbestos tremolite specimens were inhibited by 2-PVPNO, a hydrogen bonding polymer. The dose response inhibition of non-asbestos tremolite, Gouvemeur, NY and tremolite asbestos, Korea were compared to quartz. The inhibition of lysis occurred in a similar concentration range to quartz. The ability of these three specimens to hydrogen bond 2-PVPNO correlated with their membranolytic activity (Figure 4).

The zeta potential of the two non-asbestos tremolite specimens from Gouvemeur, NY averaged –13.4 $\pm$ 4.1 mV, while six tremolite asbestos specimens were found to have an average zeta potential of –44.9 $\pm$ 10.4 mV (Table 3). The non-asbestos tremolite from Gouvemeur, NY is primarily prismatic with very few elongated or acicular cleavage fragments and the zeta potential is 70% less than for the asbestos analogues. The difference in zeta potential indicates the charge density on the surface of the non-asbestos tremolite maybe lower than on its asbestos analog. The anion transport inhibitor, pyridoxal 5-phosphate, which is known to block positively charged sites on the erythrocyte membrane was a more effective inhibitor for the non-asbestos tremolite than for tremolite asbestos or quartz (Figure 5). These differences may be due to the non-asbestos tremolite having a lower number of ionized sites on its surface.

DISCUSSION AND CONCLUSIONS

Eleven specimens thought to be in the tremolite-actinolite series were selected for study and compared to the UICC asbestos reference specimens and quartz. For all but two of the eleven specimens either epidemiological and/or experimental animal studies were available to indicate their biological potential. The habit of each tremolite specimen was

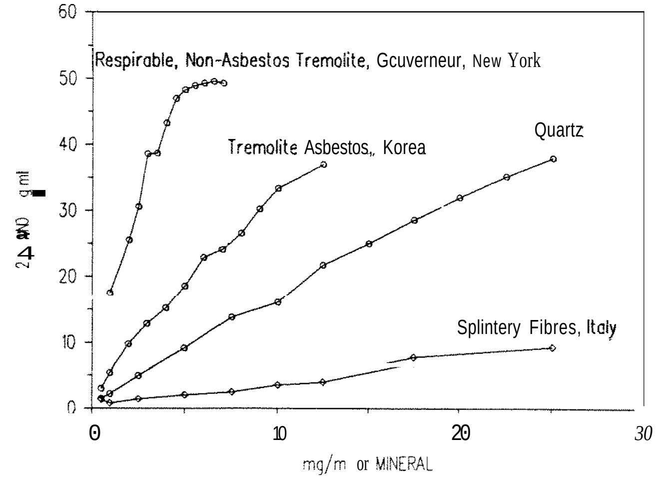

Figure 4. A comparison of the ability of three tremolite specimens having different habits *to* hind the hydrogen binding polymer, 2-PVPNO.

characterized as asbestos, splintery fibres or massive on the basis of their optical characteristics when examined by polarized light microscope (PLM). The splintery fibres and the massive form are non-asbestos, (for the criteria used see Langer *et al.*, this volume). The PLM observations were supported by the determination of each specimen's selected area electron diffraction characteristics, internal structure and morphology using transmission electron microscopy.

The specimens from Metsovo, Korea, Swansea, Jamestown, Inyo and India contain asbestos in the tremolite-actinolite series. Only about half of the asbestos fibres, analyzed by energy dispersive spectroscopy, from the vermiculite mine near Libby, Montana had an elemental composition consistent with that series. The other half of the

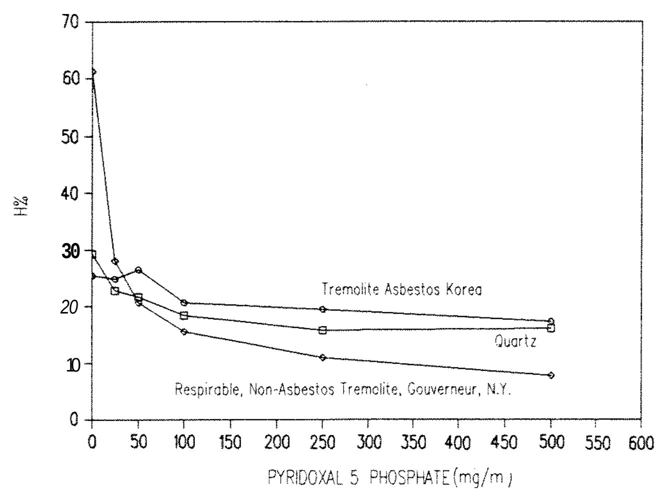

Figure 5. The effect of the anion transport inhibitor, pyridoxal 5-phosphate on the membranolytic activity of an asbestos and non-asbestos tremolite specimens compared *to* quartz.

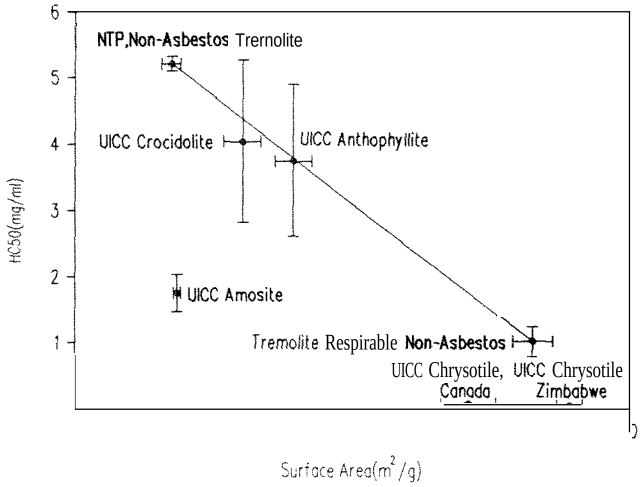

Figure 6. A comparison of the HC_{50}'s *vs* surface areas for two non-asbestos tremolite specimens and the UICC reference asbestos specimen. Values are given as mean ± standard deviation.

Figure 7. .. transmission electron photomicrograph of the specimen from Ala di Stura, Italy. The large fibre is > 3 μm in diameter with an elemental composition consistent with tremolite-actinolite series. The smaller fibre (~17.5 μm in length and ~0.˙5 μm in diameter) is a magnesium iron silicate which is consistent with the cummingtonite-grunerite series.

amphibole asbestos fibres contained either sodium or sodium and potassium (Nolan & Langer, unpublished data). The fibres from Libby found to be present in the tremolite-actinolite series were predominately actinolite asbestos (Moatamed *et al.*, 1986). The specimen from Rajastan, India contained a mixture of two fibers, tremolite asbestos and splintery fibres having oblique extinction.

The Ala di Stura specimen contained predominately splintery fibres with oblique extinction. These non-asbestos fibres have diameters which vary as a function of length, *i.e.*, the long fibres tend to have larger diameters (Wylie, 1988). Also, the specimen contains a sub-population of iron magnesium silicate fibres with high aspect ratios and diameters <0.25 μm (Figure 7) (Nolan & Langer, unpublished data). This sub-population of fibres could have contributed to the high proportion of late occurring mesotheliomas after intraperitoneal injection in rats (Davis, 1990; Davis *et al.*, in press).

The two specimens from Gouverneur, New York and the Enoree region of South Carolina had optical characteristics consistent with non-asbestos tremolite (Figure 3). No iron was detected in the two specimens from Gouverneur, New York when examined by energy dispersive spectrometry. The tremolite specimen from the Enoree region contained barely detectable iron, indicating that the three specimens have elemental compositions consistent with tremolite.

The concentration of mineral required to lyse half the erythrocytes in suspension, the HC„ for the seven amphibole asbestos specimens ranged from 1.14 ± 0.19 mg/ml for Jamestown to 3.06 ± 1.06 mgiml for Metsovo with an average of 2.07 ± 0.76 mg/ml (Table 3). On average, the UICC reference amphibole asbestos specimens were less active, requiring 3.19 ± 1.25 mgiml to lyse half the erythrocytes. The two UICC chrysotile specimens were considerably more active than any specimen studied.

The range of HC_{50}'s for the two non-asbestos tremolite specimens from Gouverneur, New York were from 1.02 ± 0.22 mg/ml for the respirable tremolite used by Smith *et al.*, 1979 to 5.21 ± 0.11 mgiml for the specimen used in the NTP feeding studies. The respirable specimen was more active, and the NTP specimen less active, than any of the amphibole asbestos specimens studied. The two specimens differ in particle size distribution which is reflected in each having different surface areas per unit mass (Table 3). The surface area of the respirable non-asbestos tremolite is -5 fold greater than the NTP specimen and also -5 fold more membranolytic per unit mass.

A plot of the HC_{50}'s vs. surface area compares the two non-asbestos specimens to the UICC reference asbestos standards (Figure 6). The anthophyllite, crocidolite and the two non-asbestos tremolite specimens had similar HC_{50}'s per unit surface area. Although the amosite specimen had a surface area comparable with the NTP tremolite, the amosite was almost 3 fold more membranolytic. The UICC chrysotile specimens from Zimbabwe and Canada had HC_{50}'s of 0.06 ± 0.00 mg/ml and 0.08 ± 0.02 mg/ml and surface areas of 26.8 ± 0.7 m^2/g and 21.3 ± 1.5 m^2/g respectively. Although the surface area of the Zimbabwe specimen is comparable with the respirable non-asbestos tremolite, the chrysotile specimen is 17 fold more membranolytic. The membranolytic activity per unit surface area was greatest for chrysotile $>>$ amosite $>$ anthophyllite $\approx$ crocidolite $\approx$ non-asbestos tremolite were approximately the same. Although Smith and co-workers (1974) produced mesothetiomas, extensive pleural adhesions and dense fibrotic lesions by intrapleural injection of the UICC reference amphibole asbestos specimens, crocidolite and anthophyllite, comparable doses of the respirable non-asbestos tremolite produced no tumours and very slight fibrosis (Smith *et al.*, 1979).

The hydrogen bonding polymer, 2-PVPNO, inhibited the membranolytic activity of all the tremolite specimens studied. The dose-response inhibition of non-asbestos

TABLE 3. Comparison of the membranolytic activity, surface area, and zeta potential of tremolite asbestos, non-asbestos tremolite and amphibole asbestos reference samples.

Specimen	HC_{50} (mg/ml)	Surface Area Determined by N_2 Adsorption (m^2/g)	Zeta Potential (mv)
Tremolite-Actinolite Asbestos			
Metsovo, Greece	3.06 ± 1.06		−45.6
Korea	1.38 ± 0.47		−56.3
Libby, Montana	2.07 ± 1.72		−44.3 ± 2.3
Swansea, Wales	2.53 ± 0.05		−56.7
Jamestown, California	1.14 ± 0.19		−32.9
Inyo, California	1.48 ± 0.69		−33.6
India	2.81 ± 0.33		
Non-Asbestos Tremolite-Actinolite			
Respirable, Gouverneur, NY	1.02 ± 0.22	24.76 ± 1.09	−13.3 ± 5.8
NIEHS, Gouverneur, NY	5.21 ± 0.11(2)	5.20 ± 0.50	−13.5 ± 2.3
Ala di Stura, Italy	NP(1)		
UICC Reference Asbestos Specimens			
Crocidolite	4.05 ± 1.22	9.8 ± 1.0(3),	8.3 ± 1.0(4)
Amosite	1.76 ± 0.28	4.0 ± 0.1(3),	5.7 ± 0.3(4)
Anthophyllite	3.76 ± 1.14	11.8 ± 1.0(4)	
Chrysotile, Canada	0.08 ± 0.02	21.3 ± 1.5(4)	
Chrysotile, Zimbabwe	0.06 ± 0.00	26.8 ± 0.7(4)	

(1) NP- Not Pipettable
(2) Duplicate Determinations
(3) Campbell *et al.*, 1980.
(4) Timbrell, 1969.

tremolite from Gouverneur, New York and tremolite asbestos from Korea occur over a concentration range approximately the same as a standard quartz specimen. Although the two tremolite specimens have similar HC_{50}'s, the respirable non-asbestos tremolite bound significantly more polymer than the tremolite asbestos specimen (Figure 4).

At a physiological pH of 7.4, six asbestos specimens had an average zeta potential of −44.9 ± 10.4 mV while the two non-asbestos tremolites averaged −13.4 ± 0.1 mV. Surface charge heterogeneity has been previously reported between amphibole cleavage fragments and their asbestos analogues (Schiller *et al.*, 1980). The quartz surface charge can be reduced by blocking the ionized surface silanol groups and this reduced the mineral's membranolytic activity (Nolan *et al.*, 1981). One site on the erythrocyte membrane which would have the appropriate chemistry to bind an anion would be the receptor site for anion transport on the band 3 protein (Cabantchik *et al.*, 1978). The site specific anion transport inhibitor, pyridoxal 5-phosphate, binds to the erythrocyte membrane and inhibited the membranolytic activity of the non-asbestos tremolite, tremolite asbestos and quartz (Figure 5). Of the three minerals studied, the non-asbestos tremolite had the lowest zeta potential, i.e. surface charge, which may account for pyridoxal-5-phosphate being such an effective inhibitor of its membranolytic activity.

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