KEASBEY & MATTISON COMPANY

MANUFACTURERS OF ASBESTOS AND MAGNESIA PRODUCTS SINCE 1873

AMBLER, PENNSYLVANIA

EXECUTIVE OFFICES

March 11, 1940

ST/IUNAIF

K&M No. 1782

W. W. F. Shepherd, Esq.
Turner & Newall Ltd.
Spotland, Rochdale

Dear Mr. Shepherd:

I acknowledge receipt of your letter T&N No. 1862 dated February 13th, and have pleasure in handing you herewith copies of all three previous reports dated May 5, 1937, April 18, 1938, and December 9, 1938, issued by Dr. Leroy U. Gardner on his work on asbestosis for the industry in this country.

Yours sincerely,

KEASBEY & MATTISON COMPANY

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Encs.

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First Progress Report on Asbestosis Experiments

at the Saranac Laboratory. May 5, 1937.

To furnish a better understanding of the disease, asbestosis, and to provide standards as a basis for its diagnosis by x-ray films, a group of animal experiments has now been started.

It is expected that anatomical changes will be produced in the lungs of animals inhaling fibrous asbestos which will cast shadows on an x-ray film comparable to those seen in human beings. Since the animals can be killed as seems advisable it will be possible to compare the anatomical changes in their lungs with the shadows seen in the films.

To make certain whether the fibrosis in the lung is due to the chemical composition of asbestos or whether it is the result of a mild irritation in the walls of the air spaces resulting from the action of a fibrous foreign body (i.e.its physical structure) injection experiments are in progress. If no fibrosis results from accumulations of asbestos in other organs it may probably be assumed that chemical stimulation of the tissues is not responsible for the pulmonary fibrosis.

As a further check on the physical vs. the chemical hypothesis, the action of ground serpentine is being compared with that of chrysotile. Since they both have the same chemical composition the comparison should be instructive whatever the result.

To check the effect of mere fibrous structure, a search for other fibrous minerals was made. None other than those classified as asbestos could be discovered which had the same structural composition, However, because of our interest in the action of gypsum, a semple of satin spar (fibrous) and also one of soda tremolite were selected for comparative testing.

Finally, the action of various members of the asbestos group, amphibole, amesite, crocidolite and anthophyllite are all being compared with that of chrysotile.

It is too early to report more than the fact that the experiments have been started. For the inhalation of chrysotile, dust furnished by Mr. Fisher from a plant at Manville, N.J. is being employed. As it was received, the dust was not sufficiently fine for experiments of this type and we were forced to regrind it in a ball mill. Considerable time was spent in experimenting with a proper type of mill for the purpose. This difficulty was overcome and inhalation was begun on March 22.

In the dusting room we placed 80 guinea pigs, 20 rats, 8 rabbits and 3 cats. More of the latter will be procured as they become available. A dust concentration of approximately 175 million particles per cubic foot of air is now being maintained. This may later be changed. Over 90% of the particles are less than 5 microns in diameter. Since significant results cannot be expected to develop until exposureshave been continued for from 1 to 2 years there can be little to report before the expiration of that time.

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The various injection experiments are further advanced although it is too early to report any results. For this purpose all dusts have been analysed chemically and petrographically. They were then ground and fractionated by allutriation. Only particles 1 to 3 microns in diameter were used. Their composition was again checked by the same methods of analysis. The various tests are tabulated for your information.

Chrysotile (Thetford)

a. Intravenous Injections.

Have proved difficult. 7 rabbits have died, apparently from mechanical effects, without receiving significant quantities of the dust. Further attempts are in progress.

b. Intraperitoneal Injections - 5 guinea pigs, March 31, 1937

One killed after one month. No gross fibrosis.

Amphibole

a. Intravenous Injection. 4 rabbits still in progress. Have each received 11 doses totalling 0.55 grams. No fatalities.

b. Intraperitoneal Injection. 5 guinea pigs. Feb. 5, 1937

2 killed after 12 and 30 days respectively. Dust plaques without gross fibrosis.

Amesite

a. Intravenous Injection. 4 rabbits still in progress. Have each received 11 doses totalling 0.55 grams. No fatalities.

b. Intraperitoneal Injection. 5 guinea pigs on Feb. 5, 1937

2 died of infection.
1 killed after 1 month. Most of the dust absorbed; no fibrosis.

Crocidolite

a. Intravenous Injection. 4 rabbits have each received full dose of one gram in 20 injections. None killed.

b. Intraperitoneal Injection. 7 guinea pigs.

2 died of infection.
1 killed after 1 month. Pigmented dust plaques without fibrosis.

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Anthophyllite

a. Intravenous Injection. 4 rabbits have each received full dose of 1 gram in 20 injections.

2 killed after 3 -1/2 to 6 months respectively. No evidence of fibrosis in the lungs, spleen, liver or bone marrow.

b. Intraperitoneal Injection. 5 guinea pigs.

3 killed after 1, 4 and 8 months respectively. Disappearing reaction with gross evidence of fibrosis.

Serpentine

a. Intravenous Injection. 4 rabbits have each received full dose of 1 gram in 20 injections. All alive and well.

b. Intraperitoneal Injection. 5 guinea pigs.

2 killed after 1 and 4 months respectively. Soft pigmented dust plaques without fibrosis.

Fibrous Gypsum - Satin Spar

a. Intravenous Injection. 4 rabbits have each received 11 of 20 injections, or a total of 0.55 grams. No fatalities.

b. Intraperitoneal Injections - not made.

Soda Tremolite

a. Intravenous Injection. 4 rabbits have each received total dose of 1 gram in 20 injections. All alive and well.

b. Intraperitoneal Injection. 5 guinea pigs.

1 killed after 1 month. Small pigmented dust plaques without fibrosis.

None of these early results is regarded as significant and no conclusions will be drawn until the observations have been continued for at least one year. It is not yet clear whether the difficulties with intravenous injection of chrysotile are merely a matter of technique or whether this substance is essentially toxic. We are attempting to discover the cause.

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Second Progress Report on Experimental Asbestosis

by the Saranac Laboratory, April 18, 1938

The inhalation experiment with asbestos dust has now progressed sufficiently to permit a more detailed discussion. The conditions defining this experiment are briefly summarized by the following data:

The Dust - is predominantly fibrous asbestos (chrysotile) with some serpentine, dolomite, chromite and magnetite. As received from the plant much of it was too coarse to be inhaled. It was therefore reground in a case-hardened steel ball mill until the maximum particle size was 3 microns and a great majority of the fibres were 1 micron or less in diameter. This reground material was too expensive to use in the ordinary 8x8x8 foot dusting chambers and it was so fine that it packed in the dusting hoppers. Therefore a limited amount of unground material was added to it to set up a dust cloud.

Dust Counts are made with a Greenburg-Smith impinger apparatus from time to time. The average concentrations maintained inside the animal cages daily, 6 days a week* are indicated by the following figures:

Particles more than 10 microns 645,000 per cubic foot of air (light field)

Particles less than 10 microns 86,100,000 per " " " " " "

471,300,000 " " " " " (dark field)

The last figure, obtained by counting the particles under dark field illumination, indicates that there were an excess of fines. In most dusts the ratio of dark to light field counts is 2 or 3 to 1; here it is over 5 to 1.

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*As the animals spent their entire lives in the dusting room they were subjected to a considerable but unmeasured concentration of dust during the remaining 24 hours of each day. Enough dust adhered to their bedding so that at night they were exposed to concentrations of the order of 2 million or less particles per cubic foot.

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Size Frequency Counts

Particles less than 2 microns	79.4%
Particles 2 to 5 microns	15.0
Particles 5 to 10 microns	3.4
Particles more than 10 microns	2.0

Again the great preponderance of small particles is indicated.

The first report made on May 5, 1937 pointed ou that the inhalation of high concentrations of very finely ground asbestos dust had not produced any significant changes in the lungs of guinea pigs, rabbits, white rats or cats during a period of 5 months.

On March 22, 1938 the dust exposures of these animals had been continued for a period of one year. Representatives of each species were killed and their lung tissues were examined microscopically for evidences of asbestosis. The lung tissues of the guinea pigs were also subjected to chemical analysis. The results have been somewhat surprising.

8 Guinea Pigs - failed to show enough evidence of reaction to the dust to be detected on gross examination of their lungs. Even in thin microscopic sections the changes were too slight to be visualized without magnification (see figure 1). When so observed it was found that only the very earliest stages of asbestosis had developed (figure 2). No fibrosis had appeared; the reaction was very largely one of the dust cells which had surrounded and walled off myriads inhaled fibres and particles of dust. Asbestosis bodies, which customarily form about asbestos fibres in the lungs of guinea pigs and human beings had developed but most of them were extremely small, as would be expected in dealing with such fine dust. A moderate number, presumably from the unground dust were much longer and often extracellular.

The lungs of three of these guinea pigs were analysed chemically for their total mineral and silica content. The results obtained are indicated as follows:

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	Dry Tissue		Ash
No.	% Ash	% SiO_2	% SiO_2
8	5.02	0.51	10.23
11	4.58	0.46	10.08
12	5.16	0.54	10.54
Normal Controls	5.61	0.10	1.83
SiO_2 Controls	12.02	5.28	43.88

These figures indicate that the total weight of inorganic material in the lungs is not increased even though the sections reveal great numbers of fine particles within the dust cells. This could be explained on the basis of the very small size of the particles whose combined weight is negligible. That there are appreciable quantities of silica-containing foreign bodies is shown in the other columns; the dried lung tissue contains 4 or 5 times the normal amount of silica and in the ash the silica content is nearly 10 times the normal amount. When compared with the average figures for a group of animals exposed to pure quartz for one year it will be noted that the total amount of ash and the percentage of silica in the asbestos series is relatively low. The results of these chemical analyses seem to indicate that asbestos dust, even when very finely divided is not as readily inhaled as a particulate dust like quartz.

2 Rabbits - Autopsied after one year of exposure show slightly more cellular reaction in the lungs than the guinea pigs. The changes were visible on gross examination of thin microscopic sections but not on inspection of the lungs as they were removed from the body.

Microscopic examination of the sections disclosed considerable accumulations of phagocytic cells about the inhaled dust. The fibres were unchanged and no asbestosis bodies had developed. There was no evidence of fibrosis. A similar result was obtained in a previous experiment on rabbits.

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2 White Rats revealed changes comparable to those in the rabbits without the formation of asbestosis bodies. In two other animals killed on the 8th month the changes were similar in character but less intense. Five other rats have died of inter-current chronic pulmonary infections. They were discarded as their lungs showed little or no inhaled dust. The findings in this species confirm those obtained in a former experiment.

8 Cats died of infections within the first 3 or 4 months. Their lungs revealed little or no dust or reaction to it. One cat was killed after one year's exposure. Grossly there was no evidence of dust reaction. Microscopic sections showed numerous scattered phagocytes containing minute particles of asbestos. No long fibres or asbestosis bodies could be found.

The injection experiments with 1 to 3 micron particles of the various fibrous silicates and serpentines mentioned in the previous report have uniformly failed to produce scar tissue either in rabbits or in guinea pigs. Anthophyllite, crocidolite, soda tremolite and serpentine have now been observed for one year. Amosite and amphibole for 6 months. The difficulties attendant upon intravenous injection of chrysotile have not been overcome but the attempt has not been abandoned. All of these minerals are without effect upon the extrapulmonary tissues and none have produced asbestosis bodies.

Comments - At first sight the negative character of these inhalation results might seem discouraging for the original objectives are apparently not being attained. Instead of producing pulmonary asbestosis at a rapid rate the reaction is relatively insignificant. A whole year's exposure to a comparatively high concentration of exceedingly fine dust has not produced enough change to be visible without high magnification of microscopic sections.

The results are not only surprising but they challenge explanation. When they are compared with the findings of a previous inhalation experiment on guinea pigs they suggest that the original hypotheses accepted in planning

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the present experiment were erroneous. The very fine dust employed for this test has caused much less reaction than the coarser material used in the first one. This result was obtained in spite of the facts that the amount of fibrous material in the new dust was 3 or more times as great as that in the old, and that the atmospheric concentrations maintained were perhaps 10 or 15 times as great.

The factor of particle size is apparently the one responsible for the difference in results. Most of the asbestos fibres in the lungs of the new experiment animals are small enough to be entirely contained within the body of a dust cell. Some, originating from the unground material placed in the dust hopper to facilitate generation of dust are longer. The short intracellular fibres are treated by the lungs more or less like particulate dust. Instead of being concentrated about the terminal bronchioles as in the case with longer fibres, they are scattered widely throughout the air spaces. Few establish contact with the pulmonary framework as they are protected by the bodies of the dust cells which surround them. If their action be chemical, not enough are concentrated in any particular focus to expect appreciable reaction. What little effect there has been might readily be due to the limited number of long fibres. It is unfortunate that any unground fibre was added for otherwise it might have been possible to state with certainty that the short fragments caused no fibrosis.

If these early results are substantiated on subsequent observation of the animals exposed for longer periods, it is believed that the chemical hypothesis of the action of asbestos will have to be abandoned. In its place it will be proposed that asbestos fibres act as mechanical irritants. Already there is considerable evidence to support such a hypothesis. The particulate serpentine which has the same chemical composition as the fibrous chrysotile has been proved to be incapable of producing a fibrosis in organs other than the lungs. When chrysotile is ground so finely that it approximates

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serpentine in physical state is loses its capacity to cause fibrosis. If its action were chemical in nature it should become more active as the particle size decreases for under such circumstances relatively more surface should be presented to the cells and fluids of the body. Quartz, whose action has been proved to be physico-chemical, behaves quite differently. The smaller the particles of free SiO_2, the more vigorous and rapid the reaction they promote. Their effects are not restricted to the lungs of certain species but can be elicited in any organ of any animal, bird or fish. In the case of asbestos, on the other hand, irritating properties are only manifested in the lungs, and then only in the lungs of certain species of animals. The smallest fibres, which should theoretically be most active are apparently inert while only the longer ones seem to cause fibrosis. It is inferred therefore that their action is mechanical and that the long stiff fibres are irritating only in the lung because this organ is in active motion during respiration. In sluggishly moving organs like the liver, spleen and subcutaneous tissue the factor of motility is minimized.

The most serious objection to discarding the chemical hypothesis at the present time is the imperfectly known relationship between the asbestosis body and the development of fibrosis. The asbestosis body must form as the result of chemical reaction. The question which remains unanswered is whether such chemical reaction is also responsible for the development of scar tissue. Some observers are of the opinion that the formation of the bodies is a protective mechanism by which the tissues attempt to coat the irritating asbestos fibre with a smooth non-irritating membrane. Our own experiments and those of others have demonstrated that asbestosis bodies recovered from human lungs by chemical digestion will not produce fibrosis in the subcutaneous tissues of guinea pigs. Their action must also be tested in the lungs before a final answer to this question can be given. The evidence now available suggests that the reaction responsible for the formation

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of the bodies is not the one producing fibrosis.

But on the other hand, it is known that asbestosis body formation occurs only in tissues which do develop fibrosis in response to local accumulations of asbestos fibres. Thus far only the lungs of guinea pigs, dogs and human beings have responded to the inhalation of asbestos dust by the formation of scar tissue and asbestos bodies. Injection of asbestos into other organs of guinea pigs produces only occasional bodies and little or no fibrosis. The asbestos corns of the skin in human beings do not contain the bodies. The lungs of species like the rabbit, white rat and cat have shown neither asbestosis bodies nor fibrosis. In their case it will have to be determined whether the lack of reaction is due to more effective upper respiratory protection which precludes the inhalation of effective quantities of asbestos fibres or whether it is due to chemical factors peculiar to these species which prevent reaction with asbestos. The possibilities are under investigation and if decisive results can be obtained the validity of one or the other hypothesis should be established.

Significance - while the inhalation experiments have failed to produce pulmonary fibrosis at the rapid rate anticipated they are nevertheless instructive. Verification of the proposed mechanical hypothesis and the establishment of a relationship between the length of fibre and the irritating properties of asbestos would explain the hitherto mysterious distribution of asbestosis in various departments of this industry. In the Thetford mills there seems to be little or no disease in spite of heavy atmospheric concentrations of dust. Here the fibrous elements of the dust are largely very short and hence perhaps not irritating. In the English plants it has been stated that the process of "mattress beating" is productive of the greatest amount of asbestosis. This process could readily break many fibres longitudinally, liberating a dust rich in fragments short enough to be inhaled but long enough to be irritating. In the American factories

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the amount of disease has not always paralleled the atmospheric concentration of dust. More cases have developed after exposuresto the "opening" machines than elsewhere. Since opening is a crushing operation it might produce the most effective length of fibre.

If these theoretical considerations can be substantiated, they should be of value in planning a program of prevention. Special precautions would then be instituted in departments where fibrous types of dust are generated. In processing mills dealing largely with the extremely fine asbestos powders employed for insulation only sufficient exhaust to conform to accepted standards of good housekeeping would be adequate. Until the hygienic significance of fibre length has been established all asbestos dust must still be considered hazardous.

Summary - An inhalation experiment upon guinea pigs, rabbits, white rats and cats using high concentrations of asbestos fibre found so finely that most of the particles are under 1 micron in maximum diameter has failed to produce evidence of asbestosis in one year's time. The amount and severity of the reaction is not nearly as great as that produced in a previous experiment by the inhalation of 1/10 to 1/15 this concentration of asbestos dust. The only other appreciable difference in the two experiments is the particle size of the dust. These observations together with those from collateral injection experiments suggest that the injury from asbestos may be mechanical in nature and produced only by fibres which exceed an unknown minimum length. If this ultimately proves to be the case it may explain the puzzling absence of asbestosis in certain very dusty parts of the industry. It may also direct attention to the points where dust collection is most important in preventing the development of asbestosis.

To answer the questions that have now been raised it is planned to continue the inhalation of the fine dust for another two years and to perform certain other injection experiments. To reproduce the pathological

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picture of generalized asbestosis another inhalation experiment with a dust composed of longer fibre will be started within the near future.

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Fig. 1



Fig. 3

Fig. 1
Guinea pig's lungs after 1 year's exposure to high concentration of exceedingly fine asbestos dust. Only reaction visible at this low magnification shown at points marked by arrows New Experiment

Fig. 3
Guinea pig's lung exposed for one year to 1/10 the atmospheric concentration of coarser asbestos dust Old Experiment



Fig. 2



Fig. 2
Higher magnification of an area marked in figure 1.

New Experiment

Fig. 4
Higher magnification of lung shown in figure 3.

Old Experiment

Progress Report on Asbestos Experiments

December 9, 1938

In reply to your letter of the 6th, I may say that any progress report of experiments on asbestos dust will necessarily be extremely brief.

In the original experiment, undertaken with the grant from the asbestos industry, animals have now been exposed to the very finely ground asbestos fiber for a period of 20 months. Concentrations of atmospheric dust ranging from 125 to 160 million particles per cubic foot of air have been maintained in the dusting room. Animals killed at intervals throughout this period have failed to exhibit any gross evidence of fibrosis. Minute asbestosis bodies have been observed since the second month but cellular reaction is still limited to widely scattered microscopic foci of early fibrous tissue. This is at variance with the experience in a previous experiment where concentrations of only 39.5 to 55.6 million particles per cu. ft. of air had already produced gross evidence of fibrosis in the lungs after 15 months exposure.

As previously reported, the assumption that grinding asbestos fiber to an extremely fine state of subdivision would accelerate the development of reaction in the tissues has not been justified. On the contrary such treatment seems to have had the opposite effect and almost destroyed their capacity to irritate the lungs. This has led to the hypothesis that the irritation from asbestos dust is not of chemical but is more probably of a mechanical nature.

Several collateral observations appear to support this view. In a series of intravenous injection experiments with four different varieties of asbestos, ground so that none of the particles exceeded 3u in diameter, one gram doses failed to produce fibrous changes in any organ of the body after one years time. Since free silica introduced in this manner in-

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variably does cause fibrosis of a characteristic nature and since such reaction is inversely proportional to the size of the injected particles, it seems logical to infer that the reaction to asbestos is of a different nature.

Chrysotile asbestos continues to give trouble, we have never been able to inject the full dose nor to keep an animal alive for more than 7 days thereafter. Either the physical or the chemical properties of this mineral render it unsuitable for injection into the veins. Three other forms, crocidolite, amosite and amphibole have produced no ill effects.

The reection to inhaled asbestos, unlike that to crystelline silica, is not a progressive one in experimental animals. In a previous experiment 8 months exposure followed by removal of the animals to a normal etmosphere for 2 or 3 years resulted in retrogression rather than progression of pulmonary changes. The asbestos fibers were present in the lungs in very appreciable numbers end they became coated with the material responsible for the formation of the asbestos body. They were still present at the end of the observation period but no fibrosis had appeared. Perhaps one might agree with Dr. Leary that the coating on the fibers is protective and that because of it they lose their capacity to irritate the tissues. These observations continue to suggest a mechanical form of irritation which is manifested only in the lungs because only this organ moves enough in normal function to be so irritated.

We are attempting to check these hypotheses by a new inhalation experiment with long-fibered crysotile. Another dust room has been assigned to asbestos and a suiteble apparatus has been perfected for breaking up this meteriel and generating a dust containing a high percentage of fibers. Concentrations of 120 million per cubic foot of air are being maintained in the dust room. A group of 100 guinea pigs, 20 rats, 20 mice and 6 cats have now been exposed a little over one month. The time of course is much too short

to anticipate results of any kind.

We are also making further checks by injecting short and long fibered asbestos directly into both lungs of a group of animals. To ascertain the influence of active motility on the part of the lung, we have induced an artificial pneumothorax on one side to put the organ at rest; the other lung continues to move normally with respiration. If our assumption is correct, we may expect that the long fiber material will produce fibrosis only on the side where active motion occurs; in the collapsed lung the long fiber should have little effect. The finely ground material should produce no reaction in either lung.

If, as anticipated, the long fiber inhalation experiment gives rise to pulmonary fibrosis we shall follow the development of the changes in x-ray films as originally proposed when the short fiber inhalation test was undertaken. However there is little point in undertaking this phase of the work until the monthly autopsy samples begin to reveal definite evidence of pulmonary change.

(signed) LEROY U. GARDNER

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