INTERIM REPORT

REGARDING THE BIOLOGICAL ACTIVITY

OF KAYLO DUST

to the

OWENS-ILLINOIS GLASS COMPANY

TOLEDO, OHIO

THE SARANAC LABORATORY

SARANAC LAKE, NEW YORK

OCTOBER 30, 1948

Submitted by:

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In cooperation with:

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INTRODUCTION

One year ago, on October 30, 1947, an interim report was submitted, covering the progress of certain animal experiments with Kaylo over a total period of 30 months. It was necessary at that time to recommend further experiments concerning certain phases of the problem. The present report deals with the final phases of the experiment then in progress and with the early observations made in the new series of experiments.

The original experiment has now been completed and the conclusions drawn are based upon the results of a full three years' exposure to an atmospheric suspension of Kaylo dust. Previous experience has shown that this is the required period over which exposures to a dust of doubtful biological activity must be continued before final conclusions are warranted. This experiment, in fact, constitutes an example of the importance of such prolonged study for, as is to be shown below, the findings at three years were strikingly different from those at two and one-half years and the tentative conclusion reached at that time must now be altered. Thus, at the time of the previous report, the evidence indicated that Kaylo acted as an inert dust in normal rats and guinea pigs; resulting only in the formation of clumps of dust cells, without fibrosis. However, it will be shown below that Kaylo is capable, on prolonged inhalation, of producing asbestosis in the lungs of guinea pigs, and that it should be handled industrially as a hazardous dust.

This report will be presented in four sections as follows:

A. Final observations, dust inhalation in normal guinea pigs.

B. Discussion.

C. Conclusions.

D. Progress report on new series of experiments.

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4. Final observations, dust inhalation in normal guinea pigs.

Reference to the Interim Report of October 30, 1947, will reveal that two groups of normal guinea pigs were placed in the dust rooms: one group in February 1945 and the second in August 1945. The animals were exposed to atmospheric suspensions of Kaylo dust for eight hours daily, five and one-half days a week throughout the experiment. The dust concentration, which varied somewhat from time to time, has averaged 116 million particles per cubic foot of air over the entire course of the experiment to date.

A brief review of the observations described on page 4 of the previous report will reveal that up to thirty months the reaction in guinea pigs was limited to the accumulation of clumps of macrophages, containing dust particles, in groups of adjacent air spaces. While occasional multinucleated giant cells were seen, there was no undue proliferation of tissue histiocytes and fibrosis was absent. A few unmistakable asbestosis bodies were identified. In general, the accumulation of cells of the types described above and their persistence as such over a full three year period, without the development of fibrosis, has been found to indicate that the agent responsible for them is not capable of producing fibrosis. Such agents are now considered to be "biologically inert," since the mere cellular accumulation has not been shown to affect pulmonary function or resistance to disease.

However, of nine animals sacrificed subsequent to thirty months in the present experiment, all have shown not only the above lesions but also have developed true fibrosis of a type characteristic of the response of guinea pigs to asbestos. This is a rather diffuse type of fibrosis which forms chiefly about the small air ducts, known as bronchioles, from which the air spaces, or alveoli, of the lung arise. The appended photomicrographs show the nature of the lesions being discussed. This condition is identical with asbestosis as can be seen by comparing

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Figure 2 with Figure 3.

The lesions in the tracheobronchial lymph nodes showed no change between thirty months and three years.

Chemical analysis of the lungs of guinea pigs that had inhaled Kaylo dust for periods up to 36 months yielded the data given in Table 1. It will be noted that as the period of exposure became longer the ash value gradually increased, indicating an accumulation of mineral matter in the lungs. There was a pronounced increase in the silica component up to about 30 months and then a slight decrease. The reason for this condition, which has been observed in experiments with other dusts, is not entirely clear. Since only two animals were analyzed for each exposure period, individual values were occasionally out of line but the general trend was clearly indicated.

B. Discussion.

The final observations in this experiment with Kaylo inhalation in normal guinea pigs, reported above and illustrated in the figures, prove that Kaylo is capable of producing the characteristic reaction, asbestosis. It is evident, therefore, that a considerable portion of the asbestos component in Kaylo remains unchanged during the manufacturing process or, if it is changed, that the altered product maintains its capacity to cause fibrosis. While the lesions up to 30 months showed no fibrosis, certain aspects of them were compatible with a preliminary stage in the development of asbestosis. These aspects were masked by the inert type of reaction to the materials other than asbestos in the dust. The characteristic early stage of the reaction to asbestos is the predilection of the fibers to localize in the lumen and wall of the small air ducts, known as terminal bronchioles. This is in contrast to particulate materials, which localize chiefly

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in the actual air spaces, or alveoli. In the present experiment the greatest portion of the inhaled dust, being particulate, lodged in the alveoli and masked the peribronchiolar deposition of some of the fibrous component. In retrospect, it is possible to discern that there is more evidence of dust cell activity in the region of the bronchioles than is characteristic of the reaction to an inert particulate dust. Little significance was attached to this at the time of the previous report because there was no evidence of the development of fibrosis.

The differences between the early reaction to Kaylo and that to asbestos may be interpreted as a modification of the lesions by the particulate components of Kaylo. "Modification" of silicosis is well recognized when lesions of that disease develop following inhalation of a mixed dust containing quartz together with materials of an inert nature, such as iron oxide.

It is of importance to note that while the peribronchiolar reactions observed incriminate Kaylo as capable of producing asbestosis, they exclude the possibility that it may cause silicosis, since, over a full three year period, there is in no animal any focus of hyaline nodular fibrosis characteristic of the reaction to quartz. Furthermore, it may be added that the animals show no evidence of the type of diffuse fibrosis which has been observed in this laboratory to follow inhalation of diatomaceous earth dust. In regard to hydrated mono-calcium silicate, a major constituent of Kaylo, it may be said that while there is no pointed evidence as to its effect on pulmonary tissue, there is much evidence to show that a variety of silicates, including some forms of calcium silicates, are biologically inert. It seems likely that the calcium silicate in Kaylo should conform with this previous experience.

Thus, the possibility expressed by Dexter Gardner in his early consideration of this problem, that Kaylo might produce two pneumoconioses, asbestosis and silicosis, with the resultant safety hazards, has been adequately studied, and concern over

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the latter condition can be dismissed. Further consideration must be given to asbestosis, however.

The laboratory has recently completed a large amount of work with asbestos, which has been reported to certain other supporting companies, but not as yet published. The following discussion of the problem is drawn largely from that work, and we would request that these comments be considered confidential. It is felt that the following information may be of aid to the Owens-Illinois health department in formulating a safety program, which certainly is necessary in view of the results of this Kayle experiment.

Asbestosis, both in man and animals, is a chronic, slowly developing peribronchiolar fibrosis, which in late stages extends from the original site of localization into the surrounding alveoli. The bronchiolar localization is apparently due to the fibrous nature of the mineral. Experimental studies indicate that the chief mode of action is by mechanical irritation of tissue by the fibers. The structure of these fibers, and the capability of repeated longitudinal division to molecular strands, is important in the mechanism of action, since the solid fibers of glass wool are not capable of producing the fibrosis. Chemical action is of little or no importance in causing reaction, since chrysotile rendered particulate by cutting and separation does not initiate fibrosis.

Certain investigations have indicated that a seemingly negligible proportion of fibrous asbestos is sufficient to produce the characteristic reaction. Thus, an inhalation experiment was carried out with ball-milled asbestos in which an attempt was made to eliminate all fibers from the material. It was found to be impossible to break up all of the fibers and about one per cent of the air suspended dust consisted of fibers. The characteristic peribronchiolar fibrosis developed in the exposed animals after forty months. Thus, it appears that very

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small numbers of fibers are capable of producing asbestosis, although the development of the lesions is delayed. The present experiment with Kaylo is also an example of this fact.

The important matter of progression of reaction after exposure to asbestos dust ceases has been extensively investigated. The results indicate that in contrast to silicosis, where progression sometimes for some time, there is no significant degree of progression in extent of the lesions. Rather, the size of the lesions decreases in asbestosis due to the tendency of the fibrous tissue to contract after the stimulus to its production is removed. The irritation of tissue by an asbestos fiber is apparently arrested within a few months after it enters the lung by the formation of the "asbestosis body" (a coating of the fiber by iron-containing material), with the result that the existing fibrous tissue contracts. Whether this contraction exerts a beneficial or deleterious effect on lung function is now under investigation.

Unfortunately, the studies with asbestos have not as yet completely clarified the problem of its effect on associated tuberculous infection. It appears that Kaylo may exert a slightly more marked adverse influence on the infection than does pure asbestos. The difference may well be due to the presence of the small amount of quartz in Kaylo or perhaps to other incidental factors. One purpose of the studies now under way is to clarify this problem.

C. Conclusions.

1. Kaylo, because of its content of an appreciable amount of fibrous chrysotile, is capable of producing asbestosis and should be handled as a hazardous industrial dust.

2. Kaylo does not produce silicosis in animals.

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3. Kaylo does not produce the diffuse cellular fibrosis of the lungs now recognized as resulting from inhalation of diatomaceous earth.

D. Progress Report on new series of experiments.

The three phases of the experiment, as outlined in the report of October 30, 1947 and in a letter dated March 3, 1948, are now under way. While little of significance has developed thus far, a review of the work is presented here. Progress and results to date of these experiments are given below under the title of each phase.

(1) The effect of Kaylo inhalation on tuberculous infection initiated at the onset of dust exposure.

Thirty experimental animals were infected with the attenuated strain (H_1) of tubercle bacillus used in this type of experiment and were placed in the dust room the next day. Thirty-three control animals were infected at the same time and kept in normal air. Of the experimental animals, six are now dead, two having been sacrificed at two months, two at four months and two having died.

One death occurred at five weeks, due to intercurrent pneumonia. The other was so recent that sections have not yet been prepared.

The animals killed at two months showed lesions comparable with those in the controls, both grossly and microscopically. The animals killed at four months have not been studied microscopically, but on gross examination the lesions appear similar in the two groups.

(2) The effect of Kaylo inhalation on regressing tuberculous lesions in guinea pigs.

In this phase of the experiment, twenty-five guinea pigs were infected, and groups of them were kept in normal air for two, four and six

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months before being placed in the dust room. At the present time the first two groups have been placed in the dust room; the third is to go in on December 15, 1948.

Only two animals of the first group have been sacrificed as yet, after two months in the dust. Microscopic observations have not been made, but in the gross, the lesions were comparable with those in control animals.

(3) The effect of accumulated Kayle in the lungs on a subsequent tuberculous infection.

Thirty normal guinea pigs were placed in the dust room on May 12, 1948, and on August 12, 1948 were infected with the attenuated tubercle bacilli. Five animals were sacrificed at this time for chemical analysis to establish the amount of dust inhaled. Control animals were infected on the same date. Animals were sacrificed recently, two months after infection. Grossly, the lesions in the experimental and control animals were comparable, but again microscopic observations have not been made.

The results to date in this experiment are of little significance, since the disease is a chronic one and any changes due to the inhalation of dust of any nature are known to be slow. However, it can be said that the experiment is progressing very satisfactorily, and that we anticipate none of the complications which interfered with the interpretation of the previous experiment in regard to the effect of Kayle on experimental tuberculosis.

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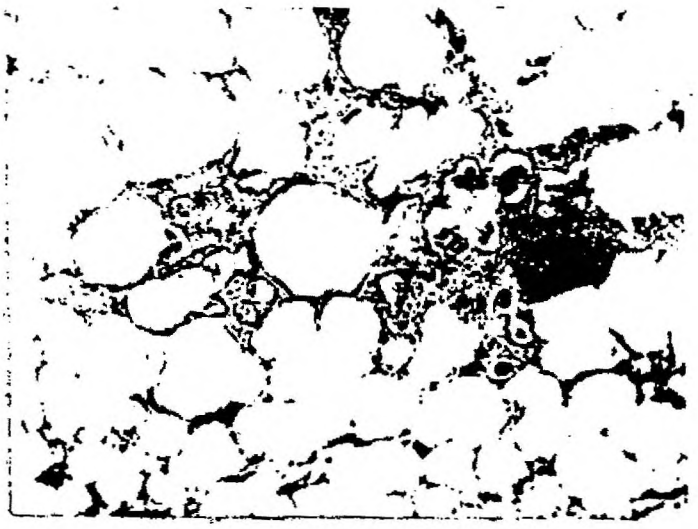

Fig. 1 - Kayle inhalation, 30 months. 200X

The photo shows a cross section of a bronchiole just above and slightly to the left of center. The other spaces are alveoli. Note the dark masses within some of the smaller spaces, especially to the right of the bronchiole. These are the macrophages and multinucleated giant cells, described in the text. Note the thinness of the wall of the bronchiole; it is virtually normal as is apparent on comparison with Figure 5. An asbestosis body is present in this field, and is revealed in Figure 4 at higher magnification.

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Fig. 2 - Kayle inhalation, 36 months. 200X

The bronchiole in this photomicrograph is just above and to the right of center. Note the thickness of the tissue between its lumen and that of the adjacent alveoli in comparison with the bronchiolar walls in Figures 1 and 3. Fine strands of fibrous tissue can be seen here, but they are more apparent, however less dense, in the area at the right of the field where fibrosis has extended to adjacent alveolar walls.

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Fig. 3 - Asbestos inhalation, 30 months. 200X

The bronchiole in this photomicrograph is just above and to the left of center. Note the thickening of its wall as in the Lagio animal at 36 months. Strands of fibrous tissue are apparent within the wall of this bronchiole, as well as in the adjacent alveolar walls.

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Fig. 4 - [illegible]

This higher magnification of part of the field shown in Figure 3 reveals a typical form of "asbestosis body," the curved dark elongated structure to the right of center.

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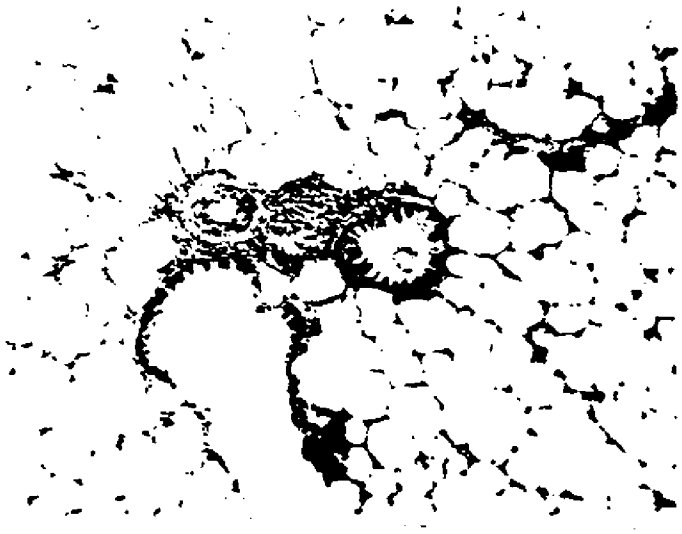

Fig. 5 - Normal guinea pig lung. 200X

This field shows two bronchioles, one in longitudinal, the other in cross section. The small associated blood vessel is seen above the former bronchiole. Compare the thickness of the wall of these bronchioles, with that of the bronchioles in Figures 1, 2, and 3. Note also the empty alveoli and thin alveolar walls.

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Table 1

ANALYSES OF LUNGS OF GUINEA PIGS

EXPOSED TO DUST IN

INHALATION EXPERIMENT WITH TALC DUST

Animal Number	Duration of Exposure	Ash of Dry Tissue	Per Cent of Dry Tissue			Per Cent of Ash		
			SiO_2	CaO	MgO	SiO_2	CaO	MgO
	months	per cent	per cent	per cent	per cent	per cent	per cent	per cent
2	2 mos.	4.42	0.24	0.08	0.07	5.57	1.35	1.59
6		4.94	0.20	0.06	0.08	4.05	1.23	1.62
10	4 mos.	4.47	0.22	0.09	0.09	4.87	1.99	1.99
11		4.42	0.19	0.08	0.08	4.42	1.89	1.89
16	6 mos.	4.13	0.25	0.08	0.08	5.83	2.00	2.00
18		3.76	0.20	0.10	0.08	5.36	2.68	2.19
28	8 mos.	4.89	0.31	0.13	0.08	6.84	2.75	1.77
34		4.34	0.28	0.11	0.11	6.49	2.43	2.43
43	10 mos.	4.58	0.46	0.14	0.21	10.12	3.04	4.30
56		4.81	0.41	0.11	0.14	8.42	2.20	3.02
45	12 mos.	4.86	0.53	0.14	0.22	10.80	2.80	4.44
46		4.98	0.51	0.16	0.08	10.25	3.17	1.60
67	18 mos.	4.55	0.59	0.08	0.26	12.27	1.65	5.31
68		4.90	0.86	0.10	0.32	17.66	2.06	6.56
106	21 mos.	5.12	0.64	0.19	0.11	12.54	3.68	2.05
108		5.25	0.54	0.15	0.08	10.30	2.90	1.45
48	24 mos.	5.08	0.65	0.13	0.06	12.79	2.54	1.20
50		4.71	0.69	0.14	0.08	12.68	2.92	1.72
114	27 mos.	5.13	0.82	0.14	0.19	15.93	2.67	3.76
119		4.78	0.46	0.12	0.15	9.55	2.41	3.06
72	30 mos.	5.62	1.19	0.14	0.06	19.56	2.56	0.92
76		5.55	1.13	0.13	0.05	20.25	2.28	0.91
121	33 mos.	5.70	0.97	0.13	0.21	17.10	2.25	3.77
122		5.38	0.59	0.12	0.17	11.00	2.26	3.25
90	36 mos.	5.65	0.86	0.16	0.19	15.10	2.80	3.28
87		5.36	0.67	0.11	0.17	12.45	2.11	3.17
88		5.43	0.54	0.16	0.13	9.92	2.86	2.38

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INTRODUCTION

One year ago, on October 30, 1947, an interim report was submitted, covering the progress of certain animal experiments with Kaylo over a total period of 30 months. It was necessary at that time to recommend further experiments concerning certain phases of the problem. The present report deals with the final phases of the experiment then in progress and with the early observations made in the new series of experiments.

The original experiment has now been completed and the conclusions drawn are based upon the results of a full three years' exposure to an atmospheric suspension of Kaylo dust. Previous experience has shown that this is the required period over which exposures to a dust of doubtful biological activity must be continued before final conclusions are warranted. This experiment, in fact, constitutes an example of the importance of such prolonged study for, as is to be shown below, the findings at three years were strikingly different from those at two and one-half years and the tentative conclusion reached at that time must now be altered. Thus, at the time of the previous report, the evidence indicated that Kaylo acted as an inert dust in normal rats and guinea pigs; resulting only in the formation of clumps of dust cells, without fibrosis. However, it will be shown below that Kaylo is capable, on prolonged inhalation, of producing asbestosis in the lungs of guinea pigs, and that it should be handled industrially as a hazardous dust.

This report will be presented in four sections as follows:

A. Final observations, dust inhalation in normal guinea pigs.

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A. Final observations, dust inhalation in normal guinea pigs.

Reference to the Interim Report of October 30, 1947, will reveal that two groups of normal guinea pigs were placed in the dust rooms: one group in February 1945 and the second in August 1945. The animals were exposed to atmospheric suspensions of Kayle dust for eight hours daily, five and one-half days a week throughout the experiment. The dust concentration, which varied somewhat from time to time, has averaged 116 million particles per cubic foot of air over the entire course of the experiment to date.

A brief review of the observations described on page 4 of the previous report will reveal that up to thirty months the reaction in guinea pigs was limited to the accumulation of clumps of macrophages, containing dust particles, in groups of adjacent air spaces. While occasional multinucleated giant cells were seen, there was no undue proliferation of tissue histiocytes and fibrosis was absent. A few unmistakable asbestosis bodies were identified. In general, the accumulation of cells of the types described above and their persistence as such over a full three year period, without the development of fibrosis, has been found to indicate that the agent responsible for them is not capable of producing fibrosis. Such agents are now considered to be "biologically inert," since the mere cellular accumulation has not been shown to affect pulmonary function or resistance to disease.

However, of nine animals sacrificed subsequent to thirty months in the present experiment, all have shown not only the above lesions but also have developed true fibrosis of a type characteristic of the response of guinea pigs to asbestos. This is a rather diffuse type of fibrosis which forms chiefly about the small air ducts, known as bronchioles, from which the air spaces, or alveoli, of the lung arise. The appended photomicrographs show the nature of the lesions being discussed. This condition is identical with asbestosis as can be seen by comparing

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Figure 2 with Figure 3.

The lesions in the tracheobronchial lymph nodes showed no change between thirty months and three years.

Chemical analysis of the lungs of guinea pigs that had inhaled Raylo dust for periods up to 36 months yielded the data given in Table 1. It will be noted that as the period of exposure became longer the ash value gradually increased, indicating an accumulation of mineral matter in the lungs. There was a pronounced increase in the silica component up to about 30 months and then a slight decrease. The reason for this condition, which has been observed in experiments with other dusts, is not entirely clear. Since only two animals were analyzed for each exposure period, individual values were occasionally out of line but the general trend was clearly indicated.

B. Discussion.

The final observations in this experiment with Raylo inhalation in normal guinea pigs, reported above and illustrated in the figures, prove that Raylo is capable of producing the characteristic reaction, asbestosis. It is evident, therefore, that a considerable portion of the asbestos component in Raylo remains unchanged during the manufacturing process or, if it is changed, that the altered product maintains its capacity to cause fibrosis. While the lesions up to 30 months showed no fibrosis, certain aspects of them were compatible with a preliminary stage in the development of asbestosis. These aspects were masked by the inert type of reaction to the materials other than asbestos in the dust. The characteristic early stage of the reaction to asbestos is the predilection of the fibers to localize in the lumen and wall of the small air ducts, known as terminal bronchioles. This is in contrast to particulate materials, which localize chiefly

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in the actual air spaces, or alveoli. In the present experiment the greatest portion of the inhaled dust, being particulate, lodged in the alveoli and masked the peribronchiolar deposition of some of the fibrous component. In retrospect, it is possible to discern that there is more evidence of dust cell activity in the region of the bronchioles than is characteristic of the reaction to an inert particulate dust. Little significance was attached to this at the time of the previous report because there was no evidence of the development of fibrosis.

The differences between the early reaction to Kayle and that to asbestos may be interpreted as a modification of the lesions by the particulate components of Kayle. "Modification" of silicosis is well recognized when lesions of that disease develop following inhalation of a mixed dust containing quartz together with materials of an inert nature, such as iron oxide.

It is of importance to note that while the peribronchiolar reactions observed inexplicable Kayle as capable of producing asbestosis, they evaluate the possibility that it may cause silicosis, since, over a full three year period, there is in no animal any focus of hyaline nodular fibrosis characteristic of the reaction to quartz. Furthermore, it may be added that the animals show no evidence of the type of diffuse fibrosis which has been observed in this laboratory to follow inhalation of diatomaceous earth dust. In regard to hydrated mono-calcium silicate, a major constituent of Kayle, it may be said that while there is no pointed evidence as to its effect on pulmonary tissue, there is much evidence to show that a variety of silicates, including some forms of calcium silicates, are biologically inert. It seems likely that the calcium silicate in Kayle should conform with this previous experience.

Thus, the possibility expressed by Doctor Gardner in his early consideration of this problem, that Kayle might produce two pneumoconioses, asbestosis and silicosis, with the resultant safety hazards, has been adequately studied, and concern over

the latter condition can be dismissed. Further consideration must be given to asbestosis, however.

The laboratory has recently completed a large amount of work with asbestos, which has been reported to certain other supporting companies, but not as yet published. The following discussion of the problem is drawn largely from that work, and we would request that these comments be considered confidential. It is felt that the following information may be of aid to the Owens-Illinois health department in formulating a safety program, which certainly is necessary in view of the results of this Kyle experiment.

Asbestosis, both in man and animals, is a chronic, slowly developing peribronchiolar fibrosis, which in late stages extends from the original site of localization into the surrounding alveoli. The bronchiolar localization is apparently due to the fibrous nature of the mineral. Experimental studies indicate that the chief mode of action is by mechanical irritation of tissue by the fibers. The structure of these fibers, and the capability of repeated longitudinal division to molecular strands, is important in the mechanism of action, since the solid fibers of glass wool are not capable of producing the fibrosis. Chemical action is of little or no importance in causing reaction, since chrysotile rendered particulate by cutting and separation does not initiate fibrosis.

Certain investigations have indicated that a seemingly negligible proportion of fibrous asbestos is sufficient to produce the characteristic reaction. Thus, an inhalation experiment was carried out with ball-milled asbestos in which an attempt was made to eliminate all fibers from the material. It was found to be impossible to break up all of the fibers and about one per cent of the air suspended dust consisted of fibers. The characteristic peribronchiolar fibrosis developed in the exposed animals after forty months. Thus, it appears that very

small numbers of fibers are capable of producing asbestosis, although the development of the lesions is delayed. The present experiment with Kayle is also an example of this fact.

The important matter of progression of reaction after exposure to asbestos dust ceases has been extensively investigated. The results indicate that in contrast to silicosis, where progression continues for some time, there is no significant degree of progression in extent of the lesions. Rather, the size of the lesions decreases in asbestosis due to the tendency of the fibrous tissue to contract after the stimulus to its production is removed. The irritation of tissue by an asbestos fiber is apparently arrested within a few months after it enters the lung by the formation of the "asbestosis body" (a coating of the fiber by iron-containing material), with the result that the existing fibrous tissue contracts. Whether this contraction exerts a beneficial or deleterious effect on lung function is now under investigation.

Unfortunately, the studies with asbestos have not as yet completely clarified the problem of its effect on associated tuberculous infection. It appears that Kayle may exert a slightly more marked adverse influence on the infection than does pure asbestos. The difference may well be due to the presence of the small amount of quartz in Kayle or perhaps to other incidental factors. One purpose of the studies now under way is to clarify this problem.

C. Conclusions.

1. Kayle, because of its content of an appreciable amount of fibrous chrysotile, is capable of producing asbestosis and should be handled as a hazardous industrial dust.
2. Kayle does not produce silicosis in animals.

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3. Kaylo does not produce the diffuse cellular fibrosis of the lungs now recognized as resulting from inhalation of diatomaceous earth.

D. Progress Report on new series of experiments.

The three phases of the experiment, as outlined in the report of October 30, 1947 and in a letter dated March 3, 1948, are now under way. While little of significance has developed thus far, a review of the work is presented here. Progress and results to date of these experiments are given below under the title of each phase.

(1) The effect of Kaylo inhalation on tuberculous infection initiated at the onset of dust exposure.

Thirty experimental animals were infected with the attenuated strain (H_1) of tubercle bacillus used in this type of experiment and were placed in the dust room the next day. Thirty-three control animals were infected at the same time and kept in normal air. Of the experimental animals, six are now dead, two having been sacrificed at two months, two at four months and two having died.

One death occurred at five weeks; due to intercurrent pneumonia. The other was so recent that sections have not yet been prepared.

The animals killed at two months showed lesions comparable with those in the controls, both grossly and microscopically. The animals killed at four months have not been studied microscopically, but on gross examination the lesions appear similar in the two groups.

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In this phase of the experiment, twenty-five guinea pigs were infected, and groups of them were kept in normal air for two, four and six

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Only two animals of the first group have been sacrificed as yet, after two months in the dust. Microscopic observations have not been made, but in the gross, the lesions were comparable with those in control animals.

(3) The effect of accumulated Keyle in the lungs on a subsequent tuberculous infection.

Thirty normal guinea pigs were placed in the dust room on May 12, 1948, and on August 12, 1948 were infected with the attenuated tubercle bacilli. Five animals were sacrificed at this time for chemical analysis to establish the amount of dust inhaled. Control animals were infected on the same date. Animals were sacrificed recently, two months after infection. Grossly, the lesions in the experimental and control animals were comparable, but again microscopic observations have not been made.

The results to date in this experiment are of little significance, since the disease is a chronic one and any changes due to the inhalation of dust of any nature are known to be slow. However, it can be said that the experiment is progressing very satisfactorily, and that we anticipate some of the complications which interfered with the interpretation of the previous experiment in regard to the effect of Keyle on experimental tuberculosis.

Asbestos Litig.
Saranac Lake
1989 Depos.
Exhibit p.86

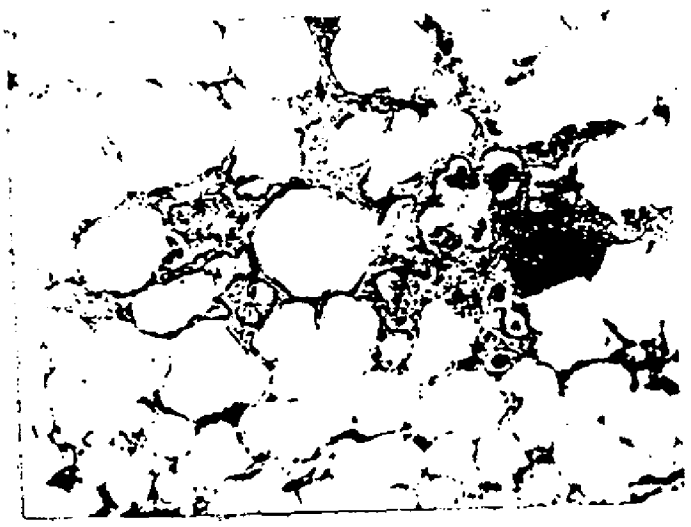

Fig. 1 - Kayle inhalation, 30 months. 200X

The photo shows a cross section of a bronchiole just above and slightly to the left of center. The other spaces are alveoli. Note the dark masses within some of the smaller spaces, especially to the right of the bronchiole. These are the macrophages and multinucleated giant cells, described in the text. Note the thickness of the wall of the bronchiole; it is virtually normal as is apparent on comparison with Figure 5. An asbestosis body is present in this field, and is revealed in Figure 4 at higher magnification.

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Asbestos Litig.
Saranac Lake
1989 Depos.
Exhibit p.87



Fig. 2 - Layla inhalation, 36 months. 200X

The bronchiole in this photomicrograph is just above and to the right of center. Note the thickness of the tissue between its lumen and that of the adjacent alveoli in comparison with the bronchiolar walls in Figures 1 and 5. Fine strands of fibrous tissue can be seen here, but they are more apparent, however less dense, in the area at the right of the field where fibrosis has extended to adjacent alveolar walls.

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Saranac Lake
1989 Depos.
Exhibit p.88



Fig. 3 - Asbestos inhalation, 30 months. 200X

The bronchiole in this photomicrograph is just above and to the left of center. Note the thickening of its wall as in the Eagle animal at 36 months. Strands of fibrous tissue are apparent within the wall of this bronchiole, as well as in the adjacent alveolar walls.

Asbestos Litig.
Saranac Lake
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Exhibit p.89



Fig. 4 – [illegible]

This higher magnification of part of the field shown in Figure 3 reveals a typical form of "asbestos body," the curved dark elongated structure to the right of center.

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Asbestos Litig.
Saranac Lake
1989 Depos.
Exhibit p.90

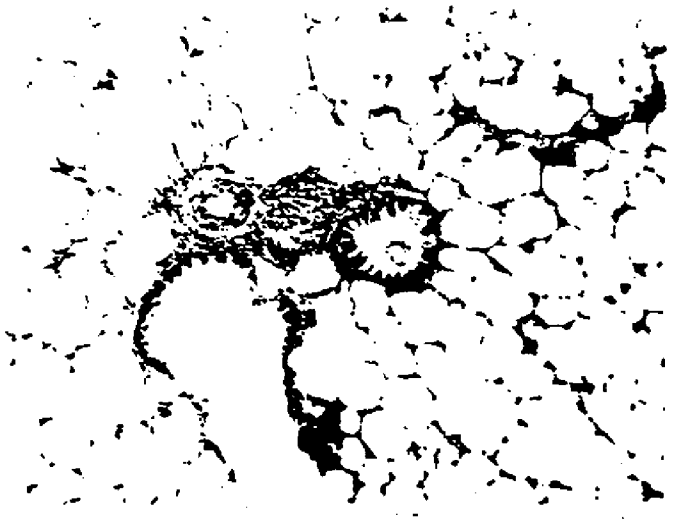

Fig. 5 - Normal guinea pig lung. 200X

This field shows two bronchioles, one in longitudinal, the other in cross section. The small associated blood vessel is seen above the former bronchiole. Compare the thickness of the wall of these bronchioles, with that of the bronchioles in Figures 1, 2, and 3. Note also the empty alveoli and thin alveolar walls.

Asbestos Litig.
Saranac Lake
1989 Depos.
Exhibit p.91

Table 1

ANALYSES OF LUNGS OF GUINEA PIGS

EXPOSED TO DUST IN

INHALATION EXPERIMENT WITH TALC DUST

Animal Number	Duration of Exposure	Ash of Dry Tissue	Per Cent of Dry Tissue			Per Cent of Ash		
			SiO_2	CaO	MgO	SiO_2	CaO	MgO
	months	per cent	per cent	per cent	per cent	per cent	per cent	per cent
2	2 mos.	4.42	0.24	0.08	0.07	5.57	1.35	1.59
6		4.94	0.20	0.06	0.08	4.05	1.23	1.62
10	4 mos.	4.47	0.22	0.09	0.09	4.87	1.99	1.99
11		4.42	0.19	0.08	0.08	4.42	1.89	1.89
15	6 mos.	4.13	0.25	0.08	0.08	5.83	2.00	2.00
18		3.76	0.20	0.10	0.08	5.36	2.68	2.19
29	8 mos.	4.89	0.31	0.13	0.08	6.84	2.76	1.77
34		4.34	0.28	0.11	0.11	6.49	2.45	2.45
45	10 mos.	4.58	0.46	0.14	0.21	10.12	3.04	4.30
56		4.21	0.41	0.11	0.14	8.42	2.20	3.02
45	12 mos.	4.86	0.53	0.14	0.22	10.89	2.90	4.65
46		4.99	0.51	0.18	0.08	10.25	3.17	1.60
67	18 mos.	4.85	0.59	0.09	0.26	12.27	1.85	5.51
68		4.98	0.86	0.10	0.32	17.65	2.08	6.56
106	21 mos.	5.12	0.64	0.13	0.11	12.54	3.68	2.08
108		5.23	0.54	0.16	0.08	10.30	2.90	1.45
48	24 mos.	5.08	0.68	0.13	0.08	12.70	2.54	1.20
50		4.71	0.60	0.14	0.08	12.52	2.92	1.72
114	27 mos.	5.13	0.82	0.14	0.19	15.93	2.67	3.76
119		4.78	0.46	0.12	0.15	9.55	2.41	3.06
72	30 mos.	5.62	1.18	0.14	0.05	19.56	2.46	0.92
75		5.53	1.12	0.13	0.05	20.25	2.33	0.91
121	35 mos.	5.70	0.97	0.13	0.21	17.10	2.25	3.77
122		5.38	0.59	0.12	0.17	11.00	2.25	3.25
90	36 mos.	5.65	0.86	0.16	0.19	15.10	2.80	3.28
87		5.36	0.67	0.11	0.17	12.45	2.11	3.17
88		5.45	0.54	0.16	0.13	9.92	2.86	2.38

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Saranac Lake
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Exhibit p.92

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INTERIM REPORT

REGARDING THE BIOLOGICAL ACTIVITY

OF KAYLO DUST

to the

OWENS-ILLINOIS GLASS COMPANY

TOLEDO, OHIO

by

THE SABANAC LABORATORY

SABANAC LAKE, NEW YORK

OCTOBER 30, 1948

Submitted by:

Arthur J. Vorwald, M. D., Director
The Edward L. Trudeau Foundation

In cooperation with:

Philip C. Pratt, M. D.
Thomas M. Durkan, M. D.
Anthony B. Delahant

cc: AB Delahant
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INTRODUCTION

One year ago, on October 30, 1947, an interim report was submitted, covering the progress of certain animal experiments with Kaylo over a total period of 30 months. It was necessary at that time to recommend further experiments concerning certain phases of the problem. The present report deals with the final phases of the experiment then in progress and with the early observations made in the new series of experiments.

The original experiment has now been completed and the conclusions drawn are based upon the results of a full three years' exposure to an atmospheric suspension of Kaylo dust. Previous experience has shown that this is the required period over which exposures to a dust of doubtful biological activity must be continued before final conclusions are warranted. This experiment, in fact, constitutes an example of the importance of such prolonged study for, as it is to be shown below, the findings at three years were strikingly different from those at two and one-half years and the tentative conclusion reached at that time must now be altered. Thus, at the time of the previous report, the evidence indicated that Kaylo acted as an inert dust in normal rats and guinea pigs; resulting only in the formation of clumps of dust cells, without fibrosis. However, it will be shown below that Kaylo is capable, on prolonged inhalation, of producing asbestosis in the lungs of guinea pigs, and that it should be handled industrially as a hazardous dust.

This report will be presented in four sections as follows:

A. Final observations, dust inhalation in normal guinea pigs.

B. Discussion.

C. Conclusions.

D. Progress report on new series of experiments.

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4. Final observations, dust inhalation in normal guinea pigs.

Reference to the Interim Report of October 30, 1947, will reveal that two groups of normal guinea pigs were placed in the dust room: one group in February 1945 and the second in August 1945. The animals were exposed to atmospheric suspensions of Keylo dust for eight hours daily, five and one-half days a week throughout the experiment. The dust concentration, which varied somewhat from time to time, has averaged 116 million particles per cubic foot of air over the entire course of the experiment to date.

A brief review of the observations described on page 4 of the previous report will reveal that up to thirty months the reaction in guinea pigs was limited to the accumulation of clumps of macrophages, containing dust particles, in groups of adjacent air spaces. While occasional multinucleated giant cells were seen, there was no undue proliferation of tissue histiocytes and fibrosis was absent. A few unmistakable asbestosis bodies were identified. In general, the accumulation of cells of the types described above and their persistence as such over a full three year period, without the development of fibrosis, has been found to indicate that the agent responsible for them is not capable of producing fibrosis. Such agents are now considered to be "biologically inert," since the mere cellular accumulation has not been shown to affect pulmonary function or resistance to disease.

However, of nine animals sacrificed subsequent to thirty months in the present experiment, all have shown not only the above lesions but also have developed true fibrosis of a type characteristic of the response of guinea pigs to asbestos. This is a rather diffuse type of fibrosis which forms chiefly about the small air ducts, known as bronchioles, from which the air spaces, or alveoli, of the lung arise. The appended photomicrographs show the nature of the lesions being discussed. This condition is identical with asbestosis as can be seen by comparing

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Figure 2 with Figure 3.

The lesions in the tracheobronchial lymph nodes showed no change between thirty months and three years.

Chemical analysis of the lungs of guinea pigs that had inhaled Eyle dust for periods up to 36 months yielded the data given in Table 1. It will be noted that as the period of exposure became longer the ash value gradually increased, indicating an accumulation of mineral matter in the lungs. There was a pronounced increase in the silica component up to about 30 months and then a slight decrease. The reason for this condition, which has been observed in experiments with other dusts, is not entirely clear. Since only two animals were analyzed for each exposure period, individual values were occasionally out of line but the general trend was clearly indicated.

E. Discussion.

The final observations in this experiment with Eyle inhalation in normal guinea pigs, reported above and illustrated in the figures, prove that Eyle is capable of producing the characteristic reaction, asbestosis. It is evident, therefore, that a considerable portion of the asbestos component in Eyle remains unchanged during the manufacturing process or, if it is changed, that the altered product maintains its capacity to cause fibrosis. While the lesions up to 30 months showed no fibrosis, certain aspects of them were compatible with a preliminary stage in the development of asbestosis. These aspects were masked by the inert type of reaction to the materials other than asbestos in the dust. The characteristic early stage of the reaction to asbestos is the predilection of the fibers to localize in the lumen and wall of the small air ducts, known as terminal bronchioles. This is in contrast to particulate materials, which localize chiefly

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in the actual air spaces, or alveoli. In the present experiment the greatest portion of the inhaled dust, being particulate, lodged in the alveoli and masked the peribronchiolar deposition of some of the fibrous component. In retrospect, it is possible to discern that there is more evidence of dust cell activity in the region of the bronchioles than is characteristic of the reaction to an inert particulate dust. Little significance was attached to this at the time of the previous report because there was no evidence of the development of fibrosis.

The differences between the early reaction to kaylo and that to asbestos may be interpreted as a modification of the lesions by the particulate component of kaylo. "Modification" of silicosis is well recognized when lesions of that disease develop following inhalation of a mixed dust containing quartz together with materials of an inert nature, such as iron oxide.

It is of importance to note that while the peribronchiolar reactions observed intermediate kaylo as capable of producing asbestosis, they exclude the possibility that it may cause silicosis, since, over a full three year period, there is in no animal any focus of hyaline nodular fibrosis characteristic of the reaction to quartz. Furthermore, it may be added that the animals show no evidence of the type of diffuse fibrosis which has been observed in this laboratory to follow inhalation of diatomaceous earth dust. In regard to hydrated magnesium silicate, a major constituent of kaylo, it may be said that while there is no pointed evidence as to its effect on pulmonary tissue, there is much evidence to show that a variety of silicates, including some forms of calcium silicates, are biologically inert. It seems likely that the calcium silicate in kaylo should conform with this previous experience.

Thus, the possibility expressed by Doctor Gardner in his early consideration of this problem, that kaylo might produce two pneumoconioses, asbestosis and silicosis with the resultant safety hazards, has been adequately studied, and concern over

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the latter condition can be dismissed. Further consideration must be given to asbestosis, however.

The laboratory has recently completed a large amount of work with asbestos which has been reported to certain other supporting companies, but not as yet published. The following discussion of the problem is drawn largely from that work, and we would request that these comments be considered confidential. It is felt that the following information may be of aid to the Owens-Illinois health department in formulating a safety program, which certainly is necessary in view of the results of this Keylo experiment.

Asbestosis, both in man and animals, is a chronic, slowly developing peribronchiolar fibrosis, which in late stages extends from the original site of localization into the surrounding alveoli. The bronchiolar localization is apparently due to the fibrous nature of the mineral. Experimental studies indicate that the chief mode of action is by mechanical irritation of tissue by the fibers. The structure of these fibers, and the capability of repeated longitudinal division to molecular strands, is important in the mechanism of action, since the solid fibers of glass wool are not capable of producing the fibrosis. Chemical action is of little or no importance in causing reaction, since chrysotile rendered particulate by cutting and separation does not initiate fibrosis.

Certain investigations have indicated that a seemingly negligible proportion of fibrous asbestos is sufficient to produce the characteristic reaction. Thus, an inhalation experiment was carried out with ball-milled asbestos in which an attempt was made to eliminate all fibers from the material. It was found to be impossible to break up all of the fibers and about one per cent of the air suspended dust consisted of fibers. The characteristic peribronchiolar fibrosis developed in the exposed animals after forty months. Thus, it appears that very

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small numbers of fibers are capable of producing asbestosis, although the development of the lesions is delayed. The present experiment with Kaylo is also an example of this fact.

The important matter of progression of reaction after exposure to asbestos dust ceases has been extensively investigated. The results indicate that in contrast to silicosis, where progression continues for some time, there is no significant degree of progression in extent of the lesions. Rather, the size of the lesions decreases in asbestosis due to the tendency of the fibrous tissue to contract after the stimulus to its production is removed. The irritation of tissue by an asbestos fiber is apparently arrested within a few months after it enters the lung by the formation of the "asbestosis body" (a coating of the fiber by iron containing material), with the result that the existing fibrous tissue contracts. Whether this contraction exerts a beneficial or deleterious effect on lung function is now under investigation.

Unfortunately, the studies with asbestos have not as yet completely clarified the problem of its effect on associated tuberculous infection. It appears that Kaylo may exert a slightly more marked adverse influence on the infection than does pure asbestos. The difference may well be due to the presence of the small amount of quartz in Kaylo or perhaps to other incidental factors. One purpose of the studies now under way is to clarify this problem.

C. Conclusions.

1. Kaylo, because of its content of an appreciable amount of fibrous chrysotile, is capable of producing asbestosis and should be handled as a hazardous industrial dust.
2. Kaylo does not produce silicosis in animals.

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3. Kaylo does not produce the diffuse cellular fibrosis of the lungs now recognised as resulting from inhalation of diatomaceous earth.

D. Progress Report on new series of experiments.

The three phases of the experiment, as outlined in the report of October 31, 1947 and in a letter dated March 3, 1948, are now under way. While little of significance has developed thus far, a review of the work is presented here. Progress and results to date of these experiments are given below under the title of each phase.

(1) The effect of Kaylo inhalation on tuberculous infection initiated at the onset of dust exposure.

Thirty experimental animals were infected with the attenuated strain (R) of tubercle bacillus used in this type of experiment and were placed in the dust room the next day. Thirty-three control animals were infected at the same time and kept in normal air. Of the experimental animals, six are now dead, two having been sacrificed at two months, two at four months and two having died.

One death occurred at five weeks, due to intercurrent pneumonia. The other was so recent that sections have not yet been prepared.

The animals killed at two months showed lesions comparable with those in the controls, both grossly and microscopically. The animals killed at four months have not been studied microscopically, but on gross examination the lesions appear similar in the two groups.

(2) The effect of Kaylo inhalation on regressing tuberculous lesions in guinea pigs.

In this phase of the experiment, twenty-five guinea pigs were infected, and groups of them were kept in normal air for two, four and six

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months before being placed in the dust room. At the present time the first two groups have been placed in the dust room; the third is to go in on December 15, 1948.

Only two animals of the first group have been sacrificed as yet, after two months in the dust. Microscopic observations have not been made, but in the gross, the lesions were comparable with those in control animals.

(3) The effect of accumulated kaylo in the lungs on a subsequent tuberculous infection.

Thirty normal guinea pigs were placed in the dust room on May 17, 1948, and on August 17, 1948 were infected with the attenuated tubercle bacilli. Five animals were sacrificed at this time for chemical analysis to establish the amount of dust inhaled. Control animals were infected on the same date. Animals were sacrificed recently, two months after infection. Grossly, the lesions in the experimental and control animals were comparable, but again microscopic observations have not been made.

The results to date in this experiment are of little significance, since the disease is a chronic one and any changes due to the inhalation of dust of any nature are known to be slow. However, it can be said that the experiment is progressing very satisfactorily, and that we anticipate none of the complications which interfered with the interpretation of the previous experiment in regard to the effect of kaylo on experimental tuberculosis.

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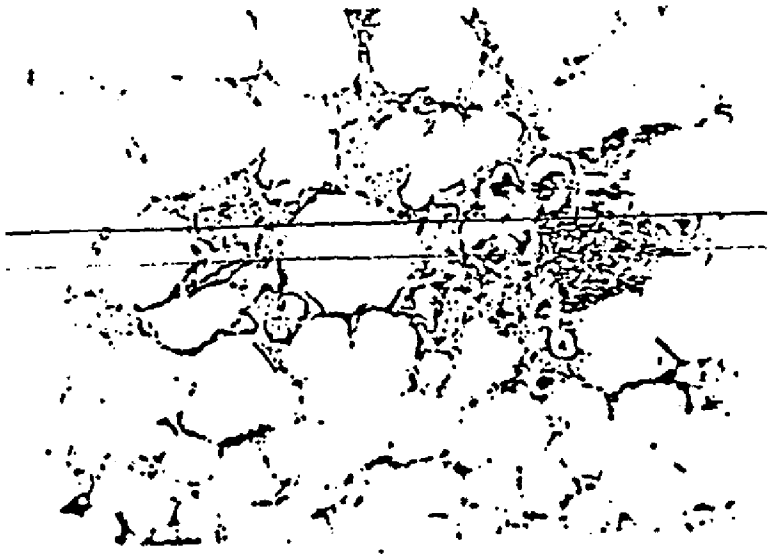

Fig. 1 - Xylo inhalation, 30 months. 200X

The photo shows a cross section of a bronchiole just above and slightly to the left of center. The other spaces are alveoli. Note the dark masses within some of the smaller spaces, especially to the right of the bronchiole. These are the macrophages and multinucleated giant cells, described in the text. Note the thinness of the wall of the bronchiole; it is virtually normal as is apparent on comparison with Figure 5. An asbestosis body is present in this field, and is revealed in Figure 4 at higher magnification.

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Fig. 2 - Mayle inhalation, 36 months. 200X

The bronchiole in this photomicrograph is just above and to the right of center. Note the thickness of the tissue between its lumen and that of the adjacent alveoli in comparison with the bronchiolar walls in Figures 1 and 5. Fine strands of fibrous tissue can be seen here, but they are more apparent, because less dense, in the area at the right of the field where fibrosis has extended to adjacent alveolar walls.

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Fig. 3 - Asbestos inhalation, 30 months. 200×

The bronchiole in this photomicrograph is just above and to the left of center. Note the thickening of its wall as in the acute animal at 36 months. Strands of fibrous tissue are apparent within the wall of this bronchiole, as well as in the adjacent alveolar walls.

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Fig. 4 - Kyle inhalation, 30 months. LXXI

This higher magnification of part of the field shown in Figure 1 reveals a typical form of "asbestosis body," the curved dark elongated structure to the right of center.

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Fig. 5 - Normal guinea pig lung. 200X

This field above two bronchioles, one in longitudinal, the other in cross section. The small associated blood vessel is seen above the former bronchiole. Compare the thickness of the wall of these bronchioles, with that of the bronchioles in figures 1, 2, and 3. Note also the empty alveoli and thin alveolar walls.

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Table 1

ANALYSES OF LUNGS OF GUINEA PIGS

EXPOSED TO DUST IN

INHALATION EXPERIMENTS WITH KATLO DUST

Animal Number	Duration of Exposure	Ash of Dry Tissue	Per Cent of Dry Tissue			Per Cent of Ash		
			SiO_2	CaO	MgO	SiO_2	CaO	MgO
	months	per cent	per cent	per cent	per cent	per cent	per cent	per cent
2	1 mos.	4.42	0.20	0.06	0.07	5.87	1.61	1.69
6		4.94	0.20	0.06	0.08	4.05	1.23	1.61
10	4 mos.	4.47	0.22	0.09	0.09	4.97	1.93	1.91
11		4.42	0.19	0.08	0.06	4.42	1.85	1.57
16	6 mos.	4.15	0.25	0.08	0.05	5.63	2.01	1.21
18		3.74	0.20	0.10	0.06	5.36	2.65	2.13
29	8 mos.	4.59	0.31	0.13	0.08	6.64	2.71	1.71
34		4.34	0.28	0.11	0.11	6.47	2.47	2.4
43	10 mos.	4.59	0.46	0.14	0.21	10.12	3.04	4.38
56		4.61	0.41	0.11	0.14	8.42	2.25	3.02
45	12 mos.	4.66	0.53	0.14	0.22	10.80	2.85	4.68
46		4.99	0.51	0.16	0.09	10.25	3.17	1.80
67	18 mos.	4.83	0.59	0.09	0.26	12.27	1.81	5.31
68		4.90	0.86	0.10	0.32	17.65	2.06	6.56
105	21 mos.	5.12	0.64	0.19	0.11	12.54	3.66	2.01
106		5.23	0.64	0.15	0.08	10.30	2.90	1.45
49	24 mos.	5.02	0.66	0.13	0.06	12.70	2.54	1.20
50		4.71	0.60	0.14	0.08	12.62	2.92	1.78
114	27 mos.	5.13	0.82	0.14	0.19	15.93	2.67	3.76
119		4.78	0.48	0.12	0.15	9.65	2.41	3.06
72	30 mos.	5.62	1.10	0.14	0.05	19.56	2.56	0.92
78		5.53	1.12	0.13	0.05	20.25	2.28	0.91
121	33 mos.	5.70	0.97	0.13	0.21	17.10	2.25	3.77
122		5.38	0.59	0.12	0.17	11.00	2.26	3.25
80	36 mos.	5.63	0.85	0.16	0.19	15.10	2.89	3.28
87		5.36	0.67	0.11	0.17	12.46	2.11	3.17
88		5.43	0.84	0.16	0.18	9.92	2.86	3.35

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INTERIM REPORT

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ON THE BIOLOGICAL ACTIVITY

OF KAYLO DUST

to the

OWENS-ILLINOIS GLASS COMPANY

TOLEDO, OHIO

by

THE SARANAC LABORATORY

SARANAC LAKE, NEW YORK

OCTOBER 30, 1948

Submitted by:

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INTRODUCTION

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The original experiment has now been completed and the conclusions drawn are based upon the results of a full three years' exposure to an atmospheric suspension of Kaylo dust. Previous experience has shown that this is the required period over which exposures to a dust of doubtful biological activity must be continued before final conclusions are warranted. This experiment, in fact, constitutes an example of the importance of such prolonged study for, as is to be shown below, the findings at three years were strikingly different from those at two and one-half years and the tentative conclusion reached at that time must now be altered. Thus, at the time of the previous report, the evidence indicated that Kaylo acted as an inert dust in normal rats and guinea pigs; resulting only in the formation of clumps of dust cells, without fibrosis. However, it will be shown below that Kaylo is capable, on prolonged inhalation, of producing asbestosis in the lungs of guinea pigs, and that it should be handled industrially as a hazardous dust.

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Final observations, dust inhalation in normal guinea pigs.

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A brief review of the observations described on page 4 of the previous report will reveal that up to thirty months the reaction in guinea pigs was limited to the accumulation of clumps of macrophages, containing dust particles, in groups of adjacent air spaces. While occasional multinucleated giant cells were seen, there was no undue proliferation of tissue histiocytes and fibrosis was absent. A few unmistakable asbestosis bodies were identified. In general, the accumulation of cells of the types described above and their persistence as such over a full three year period, without the development of fibrosis, has been considered to indicate that the agent responsible for them is not capable of producing fibrosis. Such agents are now considered to be "biologically inert, since the mere cellular accumulation has not been shown to effect pulmonary function or resistance to disease.

However, of nine animals sacrificed subsequent to thirty months in the present experiment, all have shown not only the above lesions but also have developed the fibrosis of a type characteristic of the response of guinea pigs to asbestos. This is a rather diffuse type of fibrosis which forms chiefly about the small air tubes, known as bronchioles, from which the air spaces, or alveoli, of the lung arise. The appended photomicrographs show the nature of the lesions being discussed. This condition is identical with asbestosis as can be seen by comparing

figure 2 with figure 3.

The lesions in the tracheobronchial lymph nodes showed no change between thirty months and three years.

Chemical analysis of the lungs of guinea pigs that had inhaled Keylo dust for periods up to 36 months yielded the data given in Table 1. It will be noted that as the period of exposure became longer the ash value gradually increased, indicating an accumulation of mineral matter in the lungs. There was a pronounced increase in the silica component up to about 30 months and then a slight increase. The reason for this condition, which has been observed in experiments with other dusts, is not entirely clear. Since only two animals were analyzed for each exposure period, individual values were occasionally out of line but the general trend was clearly indicated.

4. Discussion.

The final observations in this experiment with Keylo inhalation in normal guinea pigs, reported above and illustrated in the figures, prove that Keylo is capable of producing the characteristic reaction, asbestosis. It is evident, therefore, that a considerable portion of the asbestos component in Keylo remains unchanged during the manufacturing process or, if it is changed, that the altered product maintains its capacity to cause fibrosis. While the lesions up to 30 months showed no fibrosis, certain aspects of them were compatible with a preliminary stage in the development of asbestosis. These aspects were masked by the wart type of reaction to the materials other than asbestos in the dust. The characteristic early stage of the reaction to asbestos is the predilection of the fibers to localize in the lumen and wall of the small air ducts, known as terminal bronchioles. This is in contrast to particulate materials, which localize chiefly

the actual air spaces, or alveoli. In the present experiment the greatest portion of the inhaled dust, being particulate, lodged in the alveoli and masked the peribronchiolar deposition of some of the fibrous component. In retrospect, it is possible to discern that there is more evidence of dust cell activity in the region of the bronchioles than is characteristic of the reaction to an inert particulate dust. Little significance was attached to this at the time of the previous report because there was no evidence of the development of fibrosis.

The differences between the early reaction to Kaylo and that to asbestos may be interpreted as a modification of the lesions by the particulate components of Kaylo. "Modification" of silicosis is well recognized when lesions of that disease develop following inhalation of a mixed dust containing quartz together with materials of an inert nature, such as iron oxide.

It is of importance to note that while the peribronchiolar reactions observed incriminate Kaylo as capable of producing asbestosis, they exclude the possibility that it may cause silicosis, since, over a full three year period, there is in no animal any focus of hyaline nodular fibrosis characteristic of the reaction to quartz. Furthermore, it may be added that the animals show no evidence of the type of diffuse fibrosis which has been observed in this laboratory to follow inhalation of diatomaceous earth dust. In regard to hydrated calcium-calcium silicate, a major constituent of Kaylo, it may be said that while there is no pointed evidence as to its effect on pulmonary tissue, there is much evidence to show that a variety of silicates, including some forms of calcium silicates, are biologically inert. It seems likely that the calcium silicate in Kaylo should conform with this previous experience.

Thus, the possibility expressed by Doctor Gardner in his early consideration of this problem, that Kaylo might produce two pneumoconioses, asbestosis and silicosis, and the resultant safety hazards, has been adequately studied, and concern over

the latter condition can be disclosed. Further consideration must be given to asbestosis, however.

The laboratory has recently completed a large amount of work with asbestos, which has been reported to certain other supporting companies, but not as yet published. The following discussion of the problem is drawn largely from that work, and we would request that these comments be considered confidential. It is felt that the following information may be of aid to the Owens-Illinois health department in formulating a safety program, which certainly is necessary in view of the results of this Raylo experiment.

Asbestosis both in man and animals, is a chronic, slowly developing peribronchiolar fibrosis, which in late stages extends from the original site of localization into the surrounding alveoli. The bronchiolar localization is apparently due to the fibrous nature of the mineral. Experimental studies indicate the chief mode of action is by mechanical irritation of tissue by the fibers. The structure of these fibers, and the capability of repeated longitudinal division to molecular strands, is important in the mechanism of action, since the solid fibers of glass wool are not capable of producing the fibrosis. Chemical action is of little or no importance in causing reaction, since chrysotile reduced particulate by cutting and separation does not initiate fibrosis.

Certain investigations have indicated that a seemingly negligible proportion of fibrous asbestos is sufficient to produce the characteristic reaction. Thus, an inhalation experiment was carried out with ball-milled asbestos in which an attempt was made to eliminate all fibers from the material. It was found to be impossible to break up all of the fibers and about one per cent of the air suspended dust consisted of fibers. The characteristic peribronchiolar fibrosis developed in the exposed animals after forty months. Thus, it appears that very

small numbers of fibers are capable of producing asbestosis, although the development of the lesions is delayed. The present experiment with Kaylo is also an example of this fact.

The important matter of progression of reaction after exposure to asbestos has ceased has been extensively investigated. The results indicate that in contrast to silicosis, where progression continues for some time, there is no significant degree of progression in extent of the lesions. Rather, the size of the lesions decreases in asbestosis due to the tendency of the fibrous tissue to contract after the stimulus to its production is removed. The irritation of tissue by an asbestos fiber is apparently arrested within a few months after it enters the lung by the formation of the "asbestosis body" (a coating of the fiber by iron-containing material), with the result that the existing fibrous tissue contracts. Whether this contraction exerts a beneficial or deleterious effect on lung function is now under investigation.

Unfortunately, the studies with asbestos have not as yet completely clarified the problem of its effect on associated tuberculous infection. It appears that Kaylo may exert a slightly more marked adverse influence on the infection than does pure asbestos. The difference may well be due to the presence of the small amount of quartz in Kaylo or perhaps to other incidental factors. One purpose of the studies now under way is to clarify this problem.

Conclusions.

Kaylo, because of its content of an appreciable amount of fibrous chrysotile, is capable of producing asbestosis and should be handled as a hazardous industrial dust.

Kaylo does not produce silicosis in animals.

3. Kaylo does not produce the diffuse cellular fibrosis of the lungs now recognized as resulting from inhalation of diatomaceous earth.

). Progress Report on new series of experiments.

The three phases of the experiment, as outlined in the report of October 30, 1947 and in a letter dated March 3, 1948, are now under way. While little of significance has developed thus far, a review of the work is presented here. Progress and results to date of these experiments are given below under the title of each phase.

(1) The effect of Kaylo inhalation on tuberculous infection initiated at the onset of dust exposure.

Thirty experimental animals were infected with the attentuated strain (R_1) of tubercle bacillus used in this type of experiment and were placed in the dust room the next day Thirty-three control animals were infected at the same time and kept in normal air. Of the experimental animals, six are now dead, two having been sacrificed at two months, two at four months and two having died.

One death occurred at five weeks, due to intercurrent pneumonia. The other was so recent that sections have not yet been prepared.

The animals killed at two months showed lesions comparable with those in the controls, both grossly and microscopically. The animals killed at four months have not been studied microscopically, but on gross examination the lesions appear similar in the two groups.

(2) The effect of Kaylo inhalation on regressing tuberculous lesions in guinea pigs.

In this phase of the experiment, twenty-five guinea pigs were infected, and groups of them were kept in normal air for two, four and six

months before being placed in the dust room. At the present time the first two groups have been placed in the dust room; the third is to go in on December 15, 1948.

Only two animals of the first group have been sacrificed as yet, after two months in the dust. Microscopic observations have not been made, but in the gross, the lesions were comparable with those in control animals.

(3) The effect of accumulated Kaylo in the lungs on a subsequent tuberculous infection.

Thirty normal guinea pigs were placed in the dust room on May 12, 1948, and on August 12, 1948 were infected with the attenuated tubercle bacilli. Five animals were sacrificed at this time for chemical analysis to establish the amount of dust inhaled. Control animals were infected on the same date. Animals were sacrificed recently, two months after infection. Grossly, the lesions in the experimental and control animals were comparable, but again microscopic observations have not been made

The results to date in this experiment are of little significance, since the is a chronic one and any changes due to the inhalation of dust of any nature to be slow. However, it can be said that the experiment is progressing satisfactorily, and that we anticipate none of the complications which interfered with the interpretation of the previous experiment in regard to the effect Kaylo on experimental tuberculosis.