CONFIDENTIAL

Final Report

INVESTIGATION CONCERNING THE CAPACITY OF INHALED KAYLO DUST TO INJURE THE LUNG

to

Owens-Illinois Glass Company

Toledo, Ohio

by

The Saranac Laboratory

Saranac Lake, New York

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Submitted by:

Arthur J. Vorwald, M.D.
Director, The Saranac Laboratory
and The Trudeau Foundation

In cooperation with:

Thomas M. Durhan, M.D.
Philip C. Pratt, M.D.
Edward C.J. Urban, M.S.E.
Anthony B. Delahant
Donald A. Bailey

INVESTIGATION CONCERNING THE CAPACITY OF INHALED KAYLO DUST TO INJURE THE LUNG

I. INTRODUCTION

In 1943 the Owens-Illinois Glass Company had developed to the pilot plant stage a new industrial product, known as Kaylo, which could be used as a thermal insulating material and for other applications. According to information supplied by the company, this product was prepared by heating under steam pressure a suspension of calcium hydroxide, silica, and asbestos to form a hydrated calcium silicate. Studies at The Saranac Laboratory showed that although the principal component of Kaylo was the hydrated calcium silicate, some unchanged raw materials, including asbestos and free silica in the form of quartz or diatomaceous earth, were also present in the final product. Before Kaylo was used commercially on a large scale, it was important to determine whether or not any dust liberated from this product during manufacture, handling or fabrication would be likely to constitute a respiratory hazard to exposed individuals. Therefore, a comprehensive investigation was undertaken in which the effect of inhaled Kaylo dust on animals was studied. The investigation was started in February 1945 and was carried on for more than five years. It explored the nature of the pulmonary tissue reaction to inhaled Kaylo dust in normal animals and in animals harboring a tuberculous infection. In this final report of the study the principal findings given in previous interim reports will be reviewed briefly and the conclusions derived from the entire experimental program will be stated.

II. DUSTING MATERIAL USED

The material used for generating atmospheric dust in the experimental dust room was finely-divided Kaylo obtained from the dust collectors of the Berlin, New Jersey plant, in which Kaylo is manufactured. The material was received in separate shipments, as follows:

Lot #1 - Received in January 1946.
#2 - " " February 1947.
#3 - " " January 1949.

A. Composition: The results of chemical and petrographic analysis of a representative sample of the material as received at The Saranac Laboratory, are given in table 1. It will be noted that the only asbestos mineral revealed by the petrographic examination of this sample is chrysotile. Since Kaylo is a commercial product, it is possible that minor alterations in composition may occur from time to time in accordance with changes in the raw materials used. For example, the quartz value for lot #1 of Kaylo was less than 1 per cent but the quartz content of lots #2 and #3 was between 2 to 5 per cent. In the final shipment of Kaylo, received in January 1949 and first used in this experiment in September 1949, the asbestos component contained the mineral amosite as well as chrysotile. The fibers of amosite, in comparison with those of chrysotile, are less flexible and have a higher iron content. However, since experiments with animals have shown that both amosite and chrysotile are capable of causing asbestosis, it is unlikely that the substitution of the one mineral for the other in Kaylo would cause a significant change in the toxic properties of the final product.

D. X-Ray diffraction studies: The X-ray diffraction pattern of samples of two different shipments of Kaylo are shown in fig. 1. The prominent lines present are probably those of a hydrous calcium silicate, which is the principal component of Kaylo. Comparison of patterns a and b discloses that the quartz line is stronger in b than in a, indicating that the Kaylo received in January 1949 has a higher quartz content than has the earlier product, received in January 1945. Faint lines representing calcite and chrysotile are visible in both patterns. Diffraction lines produced by cristobalite are also present in pattern b.

C. Size-frequency studies: The size-distribution of Kaylo dust is shown by the graph in fig. 2. The lines of the graph represent the parent material in the dust machine and two samples of atmospheric dust collected in the dust room on different days. A magnification of 1200X was used in making the size-frequency counts. It will be noted that about 83 per cent of the particles in the parent material visible at that magnification are smaller than 5 microns in size; the corresponding values for the samples of atmospheric dust are 90 and 95 per cent. This difference in the two samples of atmospheric dust, which were derived from the same parent material, may have been due to the day-to-day variation in humidity inside the dust room.

III. REVIEW OF PREVIOUS REPORTS

During the course of the investigation with Kaylo dust five interim reports were issued.

The first report, dated May 13, 1946, revealed that in guinea pigs

exposed for 14 months to Kaylo dust the tissue reaction consisted only of microscopic focal collections of dust cells. The associated tuberculous infection had not been altered enough to be of significance.

In the second report, issued on October 30, 1947, it was noted that there was no evidence suggesting that Kaylo dust when inhaled into the lungs produces pulmonary fibrosis in rats exposed for 18 months or in guinea pigs exposed for 30 months. These findings seemed to show Kaylo dust to be a biologically inert material. In the experiment dealing with the effect of the dust on a tuberculous infection the results were inconclusive, indicating that the experiment should be repeated.

The third report, dated October 30, 1948, presented the final observations on the effect of inhaled Kaylo dust on normal uninfected guinea pigs. Contrary to previous tentative impressions, based upon an exposure of 30 months, which suggested Kaylo dust to be inert, the study of animals exposed for 36 months disclosed that the material is not inert but is capable, on prolonged inhalation, of producing asbestosis in guinea pigs. However, neither silicosis nor the diffuse fibrosis which is caused by inhaled diatomaceous earth developed in the exposed animals.

The fourth report, dated April 30, 1949, reviewed the results of the first 6 months of a new experiment in which the effect of inhaled Kaylo dust on a tuberculous infection in guinea pigs was studied. No definitely adverse effect of the dust on the infection during this 6-months period was noted.

The fifth report, dated January 1, 1950, supplemented the information given in the fourth report by extending the observations to infected animals

that had been exposed to Kaylo dust for periods up to 14 months. The findings at that time suggested that Kaylo dust had a slightly adverse influence on the course of a tuberculous infection in guinea pigs but that the effect was by no means as pronounced as that of quartz dust.

IV. INHALATION EXPERIMENT WITH KAYLO DUST

As a continuation of the previous interim reports and as a summary for this final report, a review of the entire program of study is reported.

In the inhalation experiment (Saranac Laboratory No. 1012, 1100, 1104) groups of guinea pigs, rats, and hamsters were placed in a cubical dust room, 8 feet in dimension, in which an atmospheric suspension of Kaylo dust was maintained. The room was operated for more than five years, from February 1945 to June 1950. About 450 animals were used in the experiment and some of them lived in the dust room for as long as 3 years.

A. Conditions of exposure

The atmospheric suspension of Kaylo dust was created by the action of a paddle that rotated inside a hopper, located in a corner of the dust room, which contained the finely-divided Kaylo product. The dust cloud generated in this manner floated out into the room where it was maintained for 8 hours on five days of the week and for 4 hours on Saturdays. At regular intervals during the experiment a few animals were killed and the tissues examined grossly and microscopically to determine the nature and the extent of the dust reaction. The tissue was also analyzed chemically to estimate the amount of Kaylo dust retained in the lungs of animals exposed for definite periods of time.

[illegible] counts of atmospheric samples collected in the dust room with the midget impinger were made regularly. The concentration of Kaylo dust to which the uninfected animals were exposed was generally within the range of 100 to 125 million particles per cubic foot of air and the overall average was 115 million. In the experiment with infected animals the concentration was higher, the average being 205 million.

B. Exposure of uninfested animals

Three species - guinea pig, rat, hamster - were employed in this phase of the investigation. A summary of the findings is given in table 2.

(1) Guinea pigs. At the beginning of the experiment 100 uninfected guinea pigs were placed in the dust room and 6 months later 25 more were added to replace animals that had died in an epidemic of acute pneumonia. Since this pneumonia occurred at that time also in animals exposed to another dust in an adjacent room, the unfortunate epidemic was considered accidental. Epidemics of this kind, which occurred occasionally in experiments at that time, have since been controlled. Groups of guinea pigs, usually 2 to 4 in number, were killed at 2, 4, 8, 8, 12, 18, 21, 24, 27, 30, 33, and 36 months after the beginning of their exposure to Kaylo dust so that the course of the pulmonary reaction could be followed.

In guinea pigs exposed for periods up to 30 months there was only a mild tissue response to the inhaled Kaylo dust and the reaction was limited to the accumulation of clumps of dust-containing macrophages in groups of adjoining air spaces (fig. 3). The intervening alveolar walls were only slightly thickened by accumulated lymphocytes. Occasional multinucleated

were more visible but there was no extensive proliferation of [illegible] histiocytes or deposition of connective tissue. A few asbestosis bodies were seen (fig. 4). Microscopic study of the tracheobronchial lymph nodes, which became enlarged but remained soft, revealed clusters of inactive dust cells similar to those in the lungs but without asbestosis bodies. It is pertinent to point out here that animals exposed to asbestos dust for a similar 30-months period exhibit a severe tissue reaction with the production of fibrosis (fig. 5). For comparison, a lung section of a normal guinea pig is shown (fig. 6).

The 3 animals exposed to Keylo dust for more than 33 months (3 animals for 35 months and 6 animals for 38 months) showed not only the lesions like those of the 30-months animals but also exhibited a true fibrosis of a type characteristic of the response of guinea pigs to inhaled asbestos fibers. This reaction is a rather diffuse type of fibrosis which forms principally about the bronchioles, the small ducts from which the terminal air spaces, or alveoli, arise. A comparison of fig. 7 and fig. 8 reveals the similarity of the tissue reaction produced in the lungs by Keylo dust and by asbestos dust. The lesions in the tracheobronchial lymph nodes showed no change between 30 months and 38 months.

(2) Rats. This species was employed here as typical nodular fibrosis produced by inhaled quartz dust develops more rapidly in rats than in animals of other species. Forty rats were used and some of them were exposed to Keylo dust for as long as 38 months. Pneumonia was not a problem during the first year of the experiment but many rats exposed for 15 to 18 months had with terminal bronchiolitis and lobular

pneumonia. Since laboratory mice older than one year, even those not exposed to dust, are normally subject to this type of lesion, the high mortality rate is not considered significant. In order to follow the progression of the tissue reaction to the inhaled Keylo dust rats were killed in groups of 6 after 6, 9, 12, 15, and 18 months of exposure. It was revealed (Fig. 8) that throughout the experiment the reaction to the dust consisted of focal collections, in the alveoli, of macrophages which contained particles and spicules of dust. The only change observed as the exposure period became longer was an increase in the number of the alveolar foci. Similar dust-filled macrophages were seen in the tracheobronchial lymph nodes. In no animal was there any tissue reaction typical of silicosis. The peribronchiolar fibrosis of asbestosis was not discovered, though occasional asbestosis bodies were observed. In view of other experiments with pure long-fibre asbestos dust, typical peribronchiolar fibrosis would not be unlooked in 18 months exposure to Keylo dust.

(3) Hamsters. Twenty hamsters were placed in the dust room and after 6, 9, 12, and 18 months of exposure 5 animals were killed at each period for study; at the 3-months period 5 animals that did not receive dust were examined. Histologic examination revealed that the Keylo dust accumulated very slowly in the lungs. No fibrosis was seen except in one hamster, which had been exposed for 18 months. In that animal there was a trace of peribronchiolar fibrosis so slight that it could be definitely recognized only at high magnification. Asbestosis bodies in small numbers and all the usual alveoli were present.

C. Exposure of Tuberculous Animals

There are three types of experiment which are valuable for determining the effect of inhaled dust on the development of tuberculosis in experimental animals. In the simultaneous infection experiment normal animals are infected with attenuated tubercle bacilli by the inhalation method and then are immediately transferred to the dust room. Thus, the tuberculous disease and the reaction to the inhaled dust will develop simultaneously. In the sensitization experiment the animals, after being infected with the tubercle bacilli, are allowed to live in a clean atmosphere for several months before being exposed to dust. During this period the tuberculous disease generally regresses and some healing takes place. Following exposure of the animals to some dusts the lesions may continue to regress or, with other dusts, the tuberculous process may become active and spread. The predisposition experiment is one in which animals are exposed to dust for several months, are then infected, and are immediately returned to the dust room, where their exposure to dust is continued. In this way the effect of a previous dust exposure on the early course of a tuberculous infection can be studied.

In investigating the effect of inhaled Zeylo dust on the course of tuberculous infection a particular attenuated strain of human-type tubercle bacilli, known as the R_1 strain, was employed. This strain has a low degree of virulence for guinea pigs and, in the normal animal, produces a self limited disease which will progress for 2 to 3 months and subsequently heal. In an animal which has inhaled quartz dust or certain other toxic dusts, however, the disease usually progresses continuously and ultimately causes the death of the animal. Since experience has indicated that tubercle

? the H_1 strain have about the same degree of virulence for the pig as tubercle bacilli of the human strain have for man, it is to to obtain some correlation between the influence of various dusts H_1 tuberculous infection in guinea pigs and the effect of these dusts uberculous disease in industrial workers.

or infecting the animals with tubercle bacilli an inhalation technic ed. A glass tube was fitted over the animal's nose and into this a suspension containing a known number of bacilli of the H_1 strain prayed with a DeVilbiss atomizer. As the animal breathed, he inhaled tmospheric suspension of bacilli into the lungs, where they became lized.

(1) Simultaneous infection: guinea pigs infected at the time their exposure to Nayle dust was started. In the first experiment in animals were infected with tubercle bacilli at the time that their sure to Nayle dust was started (table 3), 25 guinea pigs were used but pidemic of pneumonia during the first 10 months reduced to 15 the number animals available for study. Two of these died at 14 months, apparently a progressive tuberculosis, and 2 others died from undetermined causes, not from tuberculosis. In the remaining 12 animals the course of the rculous disease was followed by killing 1 or 2 animals at 1, 5, 9, 12, , 18, and 24 months after infection. In 11 of the 12 animals the lesions re isolated, encapsulated, and regressing; in the remaining animal, which s killed at 18 months, the lesions were widespread throughout the lung t fibrosis had been produced, which indicated a tendency to heal. In an ttempt to develop a strain of guinea pig resistant to the infection that ad caused the pneumonia, some of the animals were used for breeding during

this experiment. The 3 animals that died from progressive tuberculosis (at 14 months) and the single animal, killed at 15 months, that exhibited widespread lesions were all used as breeders during the experiment but 4 other animals in the experiment, which were also employed for breeding, failed to show extensive disease. Owing to the element of uncertainty introduced by this factor of breeding, and also because of the high mortality from pneumonia, it was considered impossible to establish, from this experiment, definite conclusions about the effect of inhaled Keyle dust upon tuberculosis, and therefore the experiment was repeated.

The simultaneous infection experiment was conducted a second time (table 3) with 30 guinea pigs, instead of 25, and animals were killed in pairs for study at 2, 4, 6, 8, 12, 15, and 24 months after infection. Again the incidence of pneumonia was unusually high, 6 of the animals dying from that cause during the first 8 months of exposure and 2 more during the following 16 months. Of the remaining 22 guinea pigs 4 animals revealed, at 15, 20, and 24 months after infection, a spreading tuberculous disease, of minimal extent (fig. 2), which was confirmed by microscopic examination. Three other animals also had a spreading tuberculosis but in them the disease had extended to the spleen and other organs. These animals were considered exceptional and in the past have been classified as "sports". The term "sport" is given to a guinea pig in which the inhalation infection with the attenuated bacilli is not confined principally to the lungs and tracheobronchial lymph nodes but extends also to other organs and produces in them tuberculous changes of sufficient extent to be recognized in the gross. Since "sports" represent a departure from the normal pattern of tissue reaction to attenuated

tuberculi bacilli, whether the infection is combined with dust exposure or not, these animals must be included in assaying the effect of an inhaled dust upon a tuberculous infection. It is believed that "sports" are animals whose native resistance to the attenuated R_1 organism is unusually low. Support for this belief is given by experience with quartz dust, a definitely hazardous material, which reactivates an inhalation infection produced by attenuated R_1 bacilli but ordinarily is not associated with tuberculous extension to organs other than the lungs and tracheobronchial lymph nodes.

The control non-dusted group (table 3) of guinea pigs for this experiment was infected at the same time as the experimental group of animals subsequently dusted. The same culture of organisms was used for all animals. The tuberculous infection followed its usual course in the control group and resolved in all of the animals except one, a "sport", in which the disease spread.

Although a progressive tuberculous disease was noted in 4 guinea pigs of the second experiment, the degree of extension of the disease in them was slight. The remaining 23 animals, 8 of which died of intercurrent pneumonia, showed no spread and the infection followed the natural course of regression and healing as in the non-dusted control animals. It is believed, therefore, that the inhaled Kayle dust had no significantly adverse effect on the tuberculosis.

(2) Reactivation infection: guinea pigs, after being infected, lived in clean air for several months before being exposed to Kayle dust.

In the reactivation experiment (table 4) 20 guinea pigs were infected with the attenuated bacilli and allowed to live in a clean atmosphere. Animals

of this group were transferred to the dust room at 2 months (10 animals), 4 months (8 animals), and 6 months (4 animals) after infection. In order to follow the course of the disease reaction a pair of animals of the first sub-group (2 months in clean air) was killed after only 2 months of dust exposure and, in addition, animals from all groups were killed in pairs after 4, 6, and 12 months of exposure to the dust. Examination of the tissues of the dusted animals failed to reveal a significant reactivation of the tuberculous disease by the inhaled Doyle dust. Only one animal showed evidence of spreading pulmonary tuberculosis, minimal in extent (fig. 10). That animal was 1 of 3 that had lived in clean air for 2 months after infection and then had been exposed to the dust for 12 months.

In the control group (table 6) the tuberculous disease behaved in the normal manner and healed, with the exception that one animal (a "spare") developed a spreading disease and died from tuberculosis 5 months after infection.

(3) Preliminary infection: Guinea pigs were infected 2 months before dusty exposure to Doyle dust was started. An experiment of this kind is a severe test because a dust that is only very slightly toxic may produce tissue changes which, though minor in character, may be sufficient to alter profoundly the development of a fresh tuberculous infection. In this experiment (table 8), which was designed to study the effect of Doyle dust accumulated in the lung upon a newly-developing tuberculous disease, 80 guinea pigs were exposed to the dust for 6 months and then were infected with attenuated tubercle bacilli. The dust exposure was immediately resumed and carried on for another 21 months.

During the course of this experiment 5 animals were killed at the end of the preliminary 5 months dusting period. They showed only an occasional clump of dust phagocytes within the alveolar spaces. Of the dusted and infected animals, 7 died from intercurrent pneumonia, which eliminates them for purposes of analysis; 1 animal died at 14 months after infection from spreading tuberculosis; and the remaining 17 animals were killed in pairs at 2, 4, 6, 8, 10, 12, and 18 months and 3 were killed at 21 months after infection. Of the latter group of 17 animals, 1 showed many spreading tubercles, 10 revealed scattered spreading tubercles and 6 exhibited healed tubercles. These findings indicate that a tuberculous infection in guinea pigs that originates several months after a prolonged exposure of the animals to Kaylo dust was initiated may be unfavorably influenced by the inhaled dust. This influence, however, is minimal in degree.

The tuberculous infection in the control animals (table 5) in general regressed and healed. Of the control animals there was, as usual, 1 "spare animal", killed 10 months after infection, which revealed a few scattered isolated tubercles that were of the spreading type.

D. Analysis of tissue of exposed animals

Chemical analysis of the lungs of uninfected guinea pigs that had inhaled Kaylo dust for periods up to 36 months yielded the data reported in table 6. It will be noted that as the period of exposure became longer the ash values gradually increased, thus showing that mineral matter was accumulating 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 — [illegible]

entirely clear but it seems to be due part to dilution of the inhaled dust with the inorganic components mobilised as a result of the increased tissue reaction which developed, as already mentioned, in the guinea pigs between 30 and 36 months of exposure to Keyle dust. Since only 2 animals were analysed for each exposure period, individual values occasionally were out of line but the general trend was clearly indicated.

V. REVIEW OF EXPERIMENTAL FINDINGS

The findings derived from the various inhalation experiments will be reviewed briefly and some points of significance mentioned.

A. Findings for normal animals inhaling Keyle dust

The results of the inhalation experiment prove that Keyle dust, when inhaled into the lungs of guinea pigs for a prolonged period (30 to 36 months), is capable of producing the peribronchiolar fibrosis characteristic of the disease asbestosis. No fibrosis was formed by the inhaled dust in rats or in hamsters, with the exception that fibrosis was barely recognisable in a single hamster, exposed for 18 months. In animals exposed for 30 months or less the lesions showed no fibrosis but certain aspects of the lesions, although masked by the reaction to the inert components in Keyle dust, were compatible with an early stage in the development of asbestosis, a disease in which there is a predilection of the fibers to localise in the terminal bronchioles. This behavior is unlike that of particulate material, which is initially deposited chiefly in the alveoli rather than in the lumens of

[illegible]he bronchioles. Since the major porti[illegible] of Kaylo dust is particulate, a great deal of it lodged in the alveoli and the reaction overshadowed the developing peribronchiolar response to the asbestos fibers of Kaylo. It was noted that in the region of the bronchioles of animals which were exposed for less than 30 months there was evidence of more dust cell activity than is usual in the reaction to an inert dust. Little significance was attached to this observation at first, however, because there was no indication of fibrosis. The early reaction to Kaylo dust is somewhat different from that to asbestos dust, since the particulate components modify the type of lesion. [illegible] modification of the lesion is a well-recognized condition which follows the inhalation of a mixed dust containing quartz and certain inert material, such as iron oxide.

The rate of pneumonia among the animals exposed to Kaylo dust was remarkably high. Since the pneumonia rate has been [illegible] also in several inhalation experiments conducted with asbestos dust, it is possible that the [illegible]

[illegible] of producing asbestosis, the results showed that [illegible] silicosis in animals of the three species studied. In most of the animals, even those exposed for the full 3-year period, [illegible] characteristic of silicosis [illegible] animals indicated also that Kaylo dust did not produce the diffuse fibrosis which follows the inhalation of diatomaceous earth. In regard to the [illegible]

(ium silicate which is the major constituent of Kaylo it may be said that although there is no specific information about the effect of that compound on pulmonary tissue, there is much clinical and experimental evidence to suggest that it is biologically inert. Furthermore, in the inhalation experiment with Kaylo dust there was no deleterious effect noted which could be attributed to the calcium silicate component of the inhaled material. This observation is supported by our studies concerning similar calcium silicates in summ.

3. Findings for tuberculous animals inhaling Kaylo dust

The inhalation experiments in which tuberculous guinea pigs were exposed to Kaylo dust indicate that when the animals were infected with attenuated tubercle bacilli at the time exposure to the dust was started, or were infected prior to that time, the inhaled Kaylo dust had little or no unfavorable effect of significance on the tuberculous infection. In most of the exposed animals the tuberculous disease healed in a normal manner and in the few animals that exhibited a spreading type of lesion the aggravation of the disease by the dust was minimal.

When the animals inhaled Kaylo dust for several months before being infected, the subsequent accumulation of the dusts in the lungs seemed to have a slightly unfavorable influence on the usual course of the tuberculous disease.

VI. SUMMARY

A. Kaylo dust, when inhaled for a prolonged period, is capable of producing

In the lungs of guinea pigs, but not of rats, the peribronchiolar fibrosis typical of asbestosis.

B. The inhalation of Keyle dust will not cause in guinea pigs, rats, or hamsters either siliconis or the diffuse fibrosis which sometimes follows the inhalation of diatomaceous earth.

C. The inhalation of Keyle dust does not significantly influence the usual course of tuberculosis in guinea pigs infected with attenuated tubercle bacilli, provided the infection is given to the animals before they are first exposed to the dust. In guinea pigs that have inhaled Keyle dust for several months before being infected, a continuation of the exposure appears to have a slightly unfavorable effect on the disease.

Table 1

ANALYSIS OF RAYLO

(Analysis of lot #2, received in January 1947)

Chemical analysis

SiO_2	43.30%
Fe_2O_3	1.62
Al_2O_3	1.97
TiO_2	0.12
MnO	0.09
CaO	24.96
MgO	7.10
K_2O	0.17
Na_2O	0.15
Loss below 105°C	4.52
" above 105°C	16.19
	99.74

Petrographic and X-ray diffraction analysis

The material consisted principally of clay-like masses which produced a definite X-ray diffraction pattern, presumably that of a hydrous calcium silicate. Quartz particles up to 30 microns in size were present, but they were not numerous and by weight probably made up less than 1 per cent of the sample. About 5 per cent of the material was calcite and approximately 15 to 20 per cent was chrysotile or serpentine, which was largely fibrous. The fibers and bundles present were usually several millimeters in length and were seldom shorter than 0.3 millimeter.

Table [illegible]

[illegible]

Exposure of Uninfected Animals

Nature of Experiment	Animals used	Maximum duration of exposure to [illegible] dust	Results
		months	
Uninfected animals exposed continuously to [illegible] dust until death.	123 G. pigs	33	Peribronchiolar fibrosis, like that of asbestosis, produced after 26 months of exposure. At 20 months of exposure reaction was limited to accumulation of clumps of dust-containing macrophages, without fibrosis. A few asbestosis bodies were seen. Tracheobronchial lymph nodes became enlarged but remained soft and without fibrosis.
	40 rats	18	Focal collections of dust-containing macrophages in the alveoli and in the tracheobronchial lymph nodes. Increase in number of focal collections was the only change observed as exposure period became longer. No silicosis or asbestosis.
	[illegible]	[illegible]	[illegible] No fibrosis changes in one animal which showed very slight fibrous reaction after 12 months of exposure. Asbestosis bodies in small numbers and at usual sites.

[illegible]

[illegible]

[illegible] of Infected Animals

([illegible] infection [illegible])

Nature of experiment	Animals used	Maximum duration of exposure to [illegible] dust	Results
		months	
Animals infected with tubercle bacilli* at beginning of exposure to [illegible] dust	35 g. pigs	21	[illegible]; [illegible] influence of [illegible] dust on a tuberculous infection not [illegible] established. [illegible] disease in 8 animals which had been used as breeders.
Controls to infection; no exposure to dust	35 g. pigs	0	Infection followed usual course; no spreading lesions. No pneumonia.
Animals infected with tubercle bacilli* at beginning of exposure to [illegible] dust. (Repetition of previous experiment)	30 g. pigs	24	Little or no significant adverse effect of inhaled [illegible] dust on a tuberculous infection was demonstrated. Localized spreads, all of slight degree, in 4 of 27 exposed animals [illegible] other 3 exposed animals [illegible] "[illegible]".
Controls to infection; no exposure to dust	30 g. pigs	0	Infection followed usual course; no spreading disease except in 1 "[illegible]".

*The guinea pigs were infected with tubercle bacilli of the low virulence R1 strain.

Table 4

RESULTS OF INHALATION EXPERIMENT WITH RAYLO DUST

Exposure of Infected Animals

(Reactivation phase)

Nature of experiment	Animals used	Maximum duration of exposure to Raylo dust	Results
		months	
Animals infected with tubercle bacilli* and then left in clean air for the following periods before being exposed to dust:			
2 months	10 guinea pigs	12	No definite evidence of reactivation of the tuberculous infection except in 1 animal, exposed to Raylo dust for 12 months, that had a slight spread of the disease.
4 months	6	12	No definite evidence of reactivation.
6 months	6	12	No definite evidence of reactivation.
Controls to infection; no exposure to dust	34	0	Infection followed its usual course and resolved in all animals except one, a "spore", in which the disease spread to other organs and caused the death of the animal 6 months after infection.

*The guinea pigs were infected with tubercle bacilli of the low-virulent Ky strain.

Table 5

SUMMARY OF INHALATION EXPERIMENT WITH KAYLO DUST

Exposure of Animals That Were Infected 3 Months After Their Exposure to Kaylo Dust Was Started

Nature of experiment	Animals used	Maximum duration of exposure to Kaylo dust	Results
Animals exposed to Kaylo dust for 3 months, then infected with tubercle bacilli*, and the exposure to dust continued	20 G. pigs	3 months (before infect.) plus 21 months (after infect.)	Spreading disease in 1 animal killed 2 months after infection and in 2 animals dying 14 months after infection.
Controls to infection; no exposure to dust	25 G. pigs	0	Infection followed the usual course and resulted, with the exception that 1 animal, killed 10 months after infection, showed restricted tuberculous [illegible]

*The guinea pigs were infected with tubercle bacilli of the low-virulent R_1 strain.

Table 5

ANALYSES OF LUNGS OF [illegible] GUINEA PIGS [illegible] TO DUST
IN INHALATION EXPERIMENT WITH KAYLO DUST

(Exposure of animals to dust was continuous until death)

Period of exposure to dust (months)		2	4	6	8	10	12	18	21	24	27	30	33	36
		%	%	%	%	%	%	%	%	%	%	%	%	%
Amount of ash (% of dried tissue)		4.42 4.94	4.27 4.42	4.15 5.74	4.50 4.34	4.50 4.61	4.66 4.09	4.35 4.90	6.12 5.25	5.02 4.71	5.15 4.73	5.62 5.53	5.70 6.33	5.63 5.56 5.23
Mineral components (% of dried tissue)	SiO_2	0.24 0.20	0.22 0.19	0.25 0.20	0.31 0.23	0.46 0.41	0.55 0.61	0.50 0.56	0.54 0.54	0.63 0.60	0.82 0.45	1.10 1.12	0.97 0.59	0.85 0.37 0.54
	CaO	0.06 0.06	0.08 0.08	0.08 0.10	0.15 0.11	0.14 0.11	[illegible] 0.16	[illegible] 0.10	0.19 0.16	0.13 0.14	0.11 0.12	0.14 0.13	0.13 0.12	0.15 0.12 0.15
	MgO	0.07 0.03	0.09 0.09	0.09 0.20	0.02 0.11	0.21 0.11	0.22 0.03	0.23 0.22	0.11 0.01	[illegible] 0.09	0.19 0.15	0.05 0.05	0.21 0.17	0.10 0.17 0.15
Mineral components (% of ash)	SiO_2	5.67 4.06	4.37 4.42	6.73 3.36	3.24 3.49	10.12 8.42	10.60 10.25	12.87 17.66	12.64 10.80	12.79 12.52	15.24 9.65	19.36 20.25	17.10 11.00	15.20 12.45 9.92
	CaO	1.45 1.25	1.39 1.35	2.05 2.65	2.45 2.45	[illegible] 2.20	[illegible] 3.17	1.28 2.65	[illegible] [illegible]	[illegible] [illegible]	[illegible] [illegible]	2.56 2.23	2.26 2.23	2.69 2.11 2.36
	MgO	1.59 [illegible]	1.39 [illegible]	[illegible] [illegible]	[illegible] [illegible]	4.50 [illegible]	4.66 [illegible]	5.31 [illegible]	[illegible] 1.45	1.20 1.72	5.73 [illegible]	0.92 0.91	5.77 5.28	3.29 3.27 [illegible]

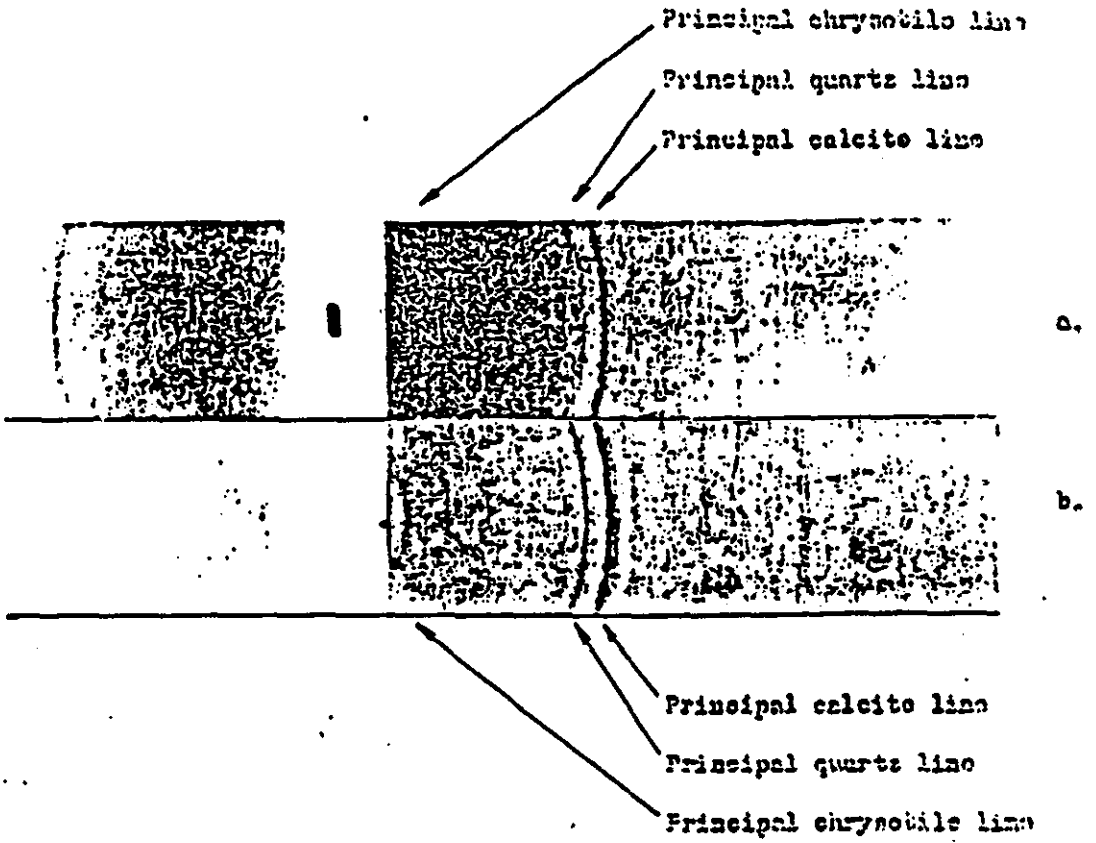

Fig. 1

X-RAY DIFFRACTION PATTERN OF KAYLO

a. Kaylo dust received in January 1945.

b. Kaylo dust received in January 1949.

Both patterns contain lines which represent a moderate amount of chrysotile and of calcite and a small amount of quartz. Faint lines representing what is probably the hydrous calcium silicate component of Kaylo are also present in both patterns. In addition, the original film of pattern b contains a very weak line corresponding to aragonite but the line is too faint to appear in the reproduced pattern.

The quartz content indicated by pattern a is less than 1 per cent and by pattern b, about 4 per cent.

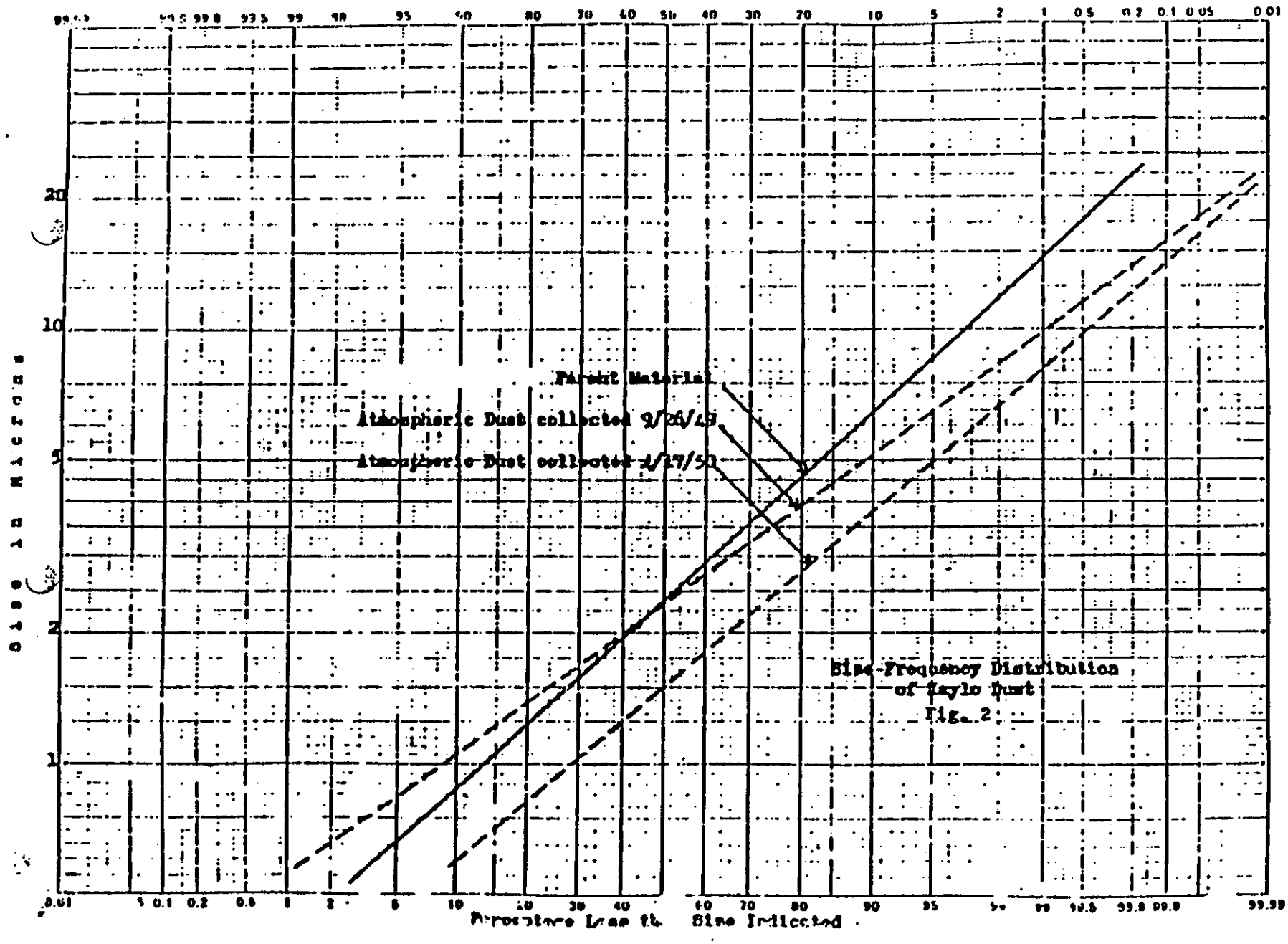
Size in Microns
Parent Material
Atmospheric Dust collected 9/26/49
Atmospheric Dust collected 4/27/50
Size-Frequency Distribution of Loyle Dust
Fig. 2
Percentage Less than Size Indicated

Table 2

SUMMARY OF INHALATION EXPERIMENTS WITH RATS AND

Exposure of Uninfected Animals

Nature of Experiment	Animals used	Maximum duration of exposure to Leyla dust	Results
Uninfected animals exposed continuously to Leyla dust until death.	125 M. pipe	months 30	Peribronchiolar fibrosis, like that of asbestosis, present after 30 months of exposure. At 30 months of exposure reaction was limited to accumulation of cells of dust-containing macrophages, without fibrosis. A few asbestosis bodies were seen. Tracheobronchial lymph nodes became enlarged, but remained soft and without fibrosis
	40 rats	18	Focal collections of dust containing macrophages in the alveoli and in the tracheobronchial lymph nodes. Increase in number of focal collections was the only change observed as exposure period became longer. No silicosis or asbestosis.
	20 hamsters	18	Dust accumulated very slowly. No fibrosis except in one animal which showed very slight fibrous reaction after 18 months of exposure. Asbestosis bodies in small numbers and at usual sites.

Table 2

RESULTS OF INHALATION EXPERIMENT WITH RABBITS

Exposure of uninfected animals

Nature of experiment	Animals used	Maximum duration of exposure to coal dust	Results
Uninfected animals exposed continuously to coal dust until death.	125 N. pigs	36 months	Peribronchiolar fibrosis, with foci of tuberculosis, produced after 36 months of exposure. At 33 months of exposure reaction was limited to accumulation of cells of dust-containing macrophages, without fibrosis. A few tubercular bodies were seen. Tracheobronchial lymph nodes became enlarged but remained soft and without fibrosis
	45 rats	18	Focal collections of dust containing macrophages in the alveoli and in the tracheobronchial lymph nodes. Increase in number of focal collections was the only change observed as exposure period became longer. No silicosis or tuberculosis.
	20 hamsters	18	Dust accumulated very slowly. No fibrosis except in one animal which showed very slight fibrous reaction after 18 months of exposure. Tubercular bodies in small number and at usual sites.

Table 1

ANALYSIS OF KAYLO

(Analysis of lot #2, received in January 1947)

Chemical analysis

SiO_2	63.60%
Fe_2O_3	1.69
Al_2O_3	1.97
TiO_2	0.12
MnO	0.09
CaO	24.94
MgO	7.10
Na_2O	0.17
K_2O	0.19
Loss below 105°C	4.52
" above 105°C	15.19
	99.78

Petrographic and X-ray diffraction analysis

The material consisted principally of clay-like masses which produced a definite X-ray diffraction pattern, presumably that of a hydrous calcium silicate. Quartz particles up to 30 microns in size were present, but they were not numerous and by weight probably made up less than 1 per cent of the sample. About 5 per cent of the material was calcite and approximately 15 to 20 per cent was chrysotile or serpentine, which was largely fibrous. The fibers and bundles present were usually several millimeters in length and were seldom shorter than 0.3 millimeter.

Table 2

SUMMARY OF INHALATION EXPERIMENTS WITH RATS AND

Exposure of Uninfected Animals

Nature of Experiment	Animals used	Maximum duration of exposure to Leyte dust	Results
Uninfected animals exposed continuously to Leyte dust until death.	325 S. pict	months 30	Peribronchiolar fibrosis, like that of asbestosis, produced after 20 months of exposure. At 30 months of exposure reaction was limited to accumulation of sheets of dust-containing macrophages, without fibrosis. A few asbestosis bodies were seen. Tracheobronchial lymph nodes became enlarged but remained soft and without fibrosis
	45 rats	18	Focal collections of dust containing macrophages in the alveoli and in the tracheobronchial lymph nodes. Increase in number of focal collections was the only change observed as exposure period became longer. No silicosis or asbestosis.
	20 hamsters	18	Dust accumulated very slowly. No fibrosis except in one animal which showed very slight fibrous reaction after 18 months of exposure. Asbestosis bodies in small number and at usual sites.

Table 3

EFFECT OF INHALATION EXPERIMENTS WITH KAYLO DUST

Exposure of Infected Animals

(Simultaneous infection plus)

Nature of experiment	Animals used	Maximum duration of exposure to Kaylo dust	Results
		months	
Animals infected with tubercle bacilli* at beginning of exposure to Kaylo dust	28 G. pigs	2d	Results inconclusive; adverse influence of Kaylo dust on a tuberculous infection not definitely established. Progressive disease in 3 animals that had been used as breeders.
Controls to infection; no exposure to dust	21 G. pigs	0	Infection followed usual course; no spreading disease. No pneumonia.
Animals infected with tubercle bacilli* at beginning of exposure to Kaylo dust. (Repetition of previous experiment)	32 G. pigs	2d	Little or no significantly adverse effect of inhaled Kaylo dust on a tuberculous infection was demonstrated. Confirmed spread, all of slight degree, in 4 of 27 exposed animals; the other 5 exposed animals were "sports".
Controls to infection; no exposure to dust	34 G. pigs	0	Infection followed usual course; no spreading disease except in 1 "sport".

*The guinea pigs were infected with tubercle bacilli of the low-virulent R1 strain.

Table 4

SUMMARY OF REACTIVATION EXPERIMENTS WITH RAYLO DUST

Exposure of Infected Animals

(Reactivation phase)

Nature of experiment	Animals used	Maximum duration of exposure to Raylo dust	Results
		months	
Animals infected with tubercle bacilli[a] and then left in clean air for the following periods before being exposed to dust:			
2 months	10 G. pigs	12	No definite evidence of reactivation of the tuberculous infection except in 1 animal, exposed to Raylo dust for 12 months, that had a slight spread of the disease.
4 months	8	12	No definite evidence of reactivation.
6 months	8	12	No definite evidence of reactivation.
Controls to infection; no exposure to dust	34	0	Infection followed its usual course and resolved in all animals except one, a "spread", in which the disease spread to other organs and caused the death of the animal 6 months after infection.

[a] The guinea pigs were infected with tubercle bacilli of the low-virulence R1 strain.

Table 5

SUMMARY OF INHALATION EXPERIMENT WITH KAYLO DUST

Exposure of Animals That Were Infected 3 Months After Their Exposure to Kaylo Dust Had Started

Nature of experiment	Animals used	Maximum duration of exposure to Kaylo dust	Results
Animals exposed to Kaylo dust for 3 months, then infected with tubercle bacilli*, and then exposure to dust continued	22 G. pigs	3 months (before infect.) plus 21 (after infect.)	Spreading disease in 1 animal killed 2 months after infection and in 2 animals dying 14 months after infection.
Controls to infection: no exposure to dust	25 G. pigs	0	Infection followed the usual course and resulted, with the exception that 1 animal, killed 10 months after infection, showed scarcely isolated spreading tubercles.

*The guinea pigs were infected with tubercle bacilli of the low-virulent S_1 strain.

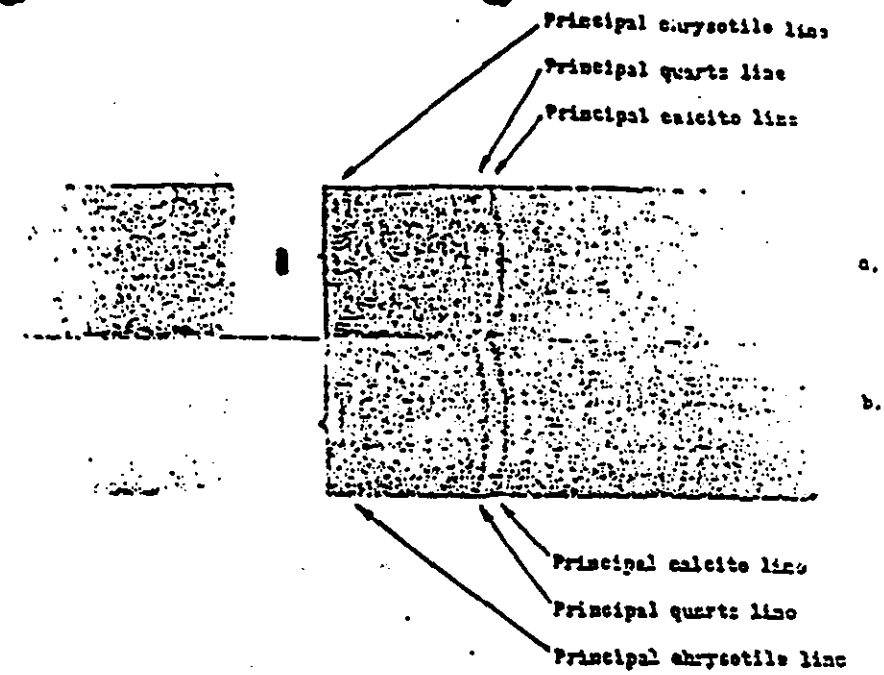

Fig. 1

X-RAY DIFFRACTION PATTERNS OF KAYLO

a. Kaylo dust received in January 1945.

b. Kaylo dust received in January 1949.

Both patterns contain lines which represent a moderate amount of chrysotile and of calcite and a small amount of quartz. Faint lines representing what is probably the hydrous calcium silicate component of Kaylo are also present in both patterns. In addition, the original film of pattern b contains a very weak line corresponding to amosite but the line is too faint to appear in the reproduced pattern.

The quartz content indicated by pattern a is less than 1 per cent and by pattern b, about 4 per cent.

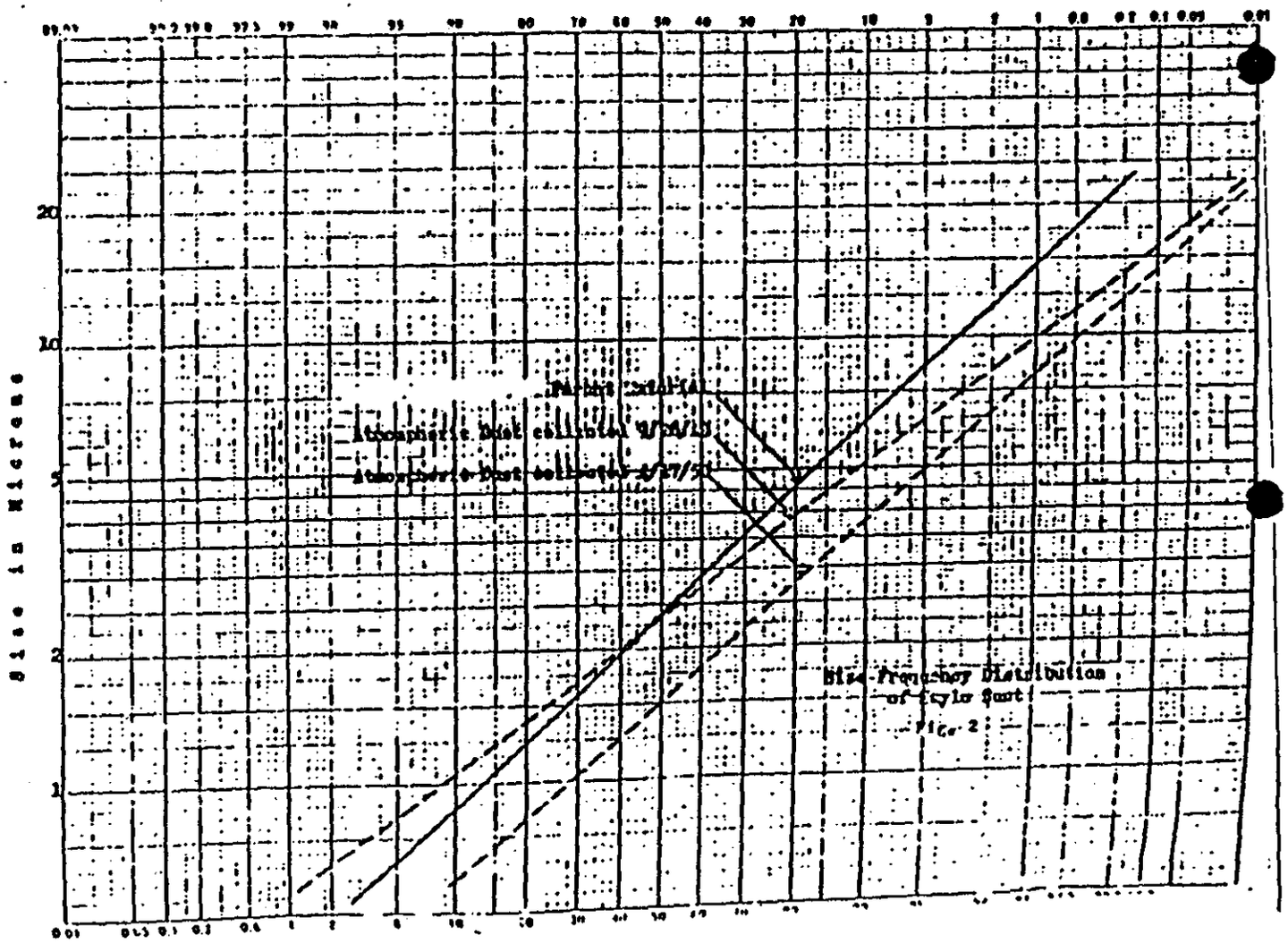
Size in Microns
Atmospheric Dust collected 9/24/51
Atmospheric Dust collected 9/27/51
Size Frequency Distribution of Cyclo Dust
Fig. 2

Table 3

ANALYSES OF LUNGS OF GUINEA PIGS EXPOSED TO DUST IN INHALATION EXPERIMENT WITH RAZED DUST

(Exposure of animals to dust was continuous until death)

Period of exposure to dust (months)		2	4	6	8	10	12	18	21	24	27	30	33	36
		%	%	%	%	%	%	%	%	%	%	%	%	%
Amount of ash (% of dried tissue)		4.42 4.04	4.47 4.42	4.15 3.74	4.59 4.34	4.50 4.01	4.06 4.09	4.05 4.90	5.12 5.23	5.03 4.71	5.13 4.70	5.02 5.53	5.70 5.39	5.63 5.36 5.45
Mineral components (% of dried tissue)	SiO_2	0.24 0.30	0.22 0.19	0.25 0.20	0.31 0.28	0.26 0.41	0.63 0.51	0.69 0.06	0.54 0.54	0.66 0.60	0.02 0.46	1.10 1.12	0.97 0.69	0.68 0.67 0.54
	CaO	0.09 0.06	0.09 0.09	0.05 0.10	0.13 0.11	0.14 0.11	0.14 0.18	0.09 0.10	0.19 0.16	0.15 0.14	0.14 0.12	0.14 0.13	0.13 0.12	0.18 0.11 0.16
	H_2O	0.07 0.05	0.09 0.09	0.08 0.08	0.09 0.11	0.21 0.14	0.22 0.08	0.29 0.32	0.11 0.08	0.06 0.00	0.19 0.18	0.05 0.05	0.21 0.17	0.19 0.17 0.13
Mineral components (% of ash)	SiO_2	5.67 4.03	4.87 4.42	6.65 5.58	6.84 6.49	10.12 8.42	10.60 10.25	12.27 17.05	12.64 10.30	12.70 12.62	16.93 8.65	19.56 20.26	17.10 11.00	16.10 12.45 9.92
	CaO	1.36 1.25	1.29 1.05	2.00 2.00	2.75 2.45	3.04 2.20	2.80 3.17	1.85 2.08	3.58 2.90	2.64 2.92	2.57 2.41	2.06 2.23	2.26 2.28	2.09 2.11 2.06
	H_2O	1.59 1.02	1.99 1.69	2.00 2.19	1.77 2.05	4.39 3.02	4.06 1.80	5.81 6.54	2.03 1.46	1.20 1.72	3.76 3.00	0.92 0.91	3.77 3.26	3.28 3.17 2.53

Fig. 3

Fig. 4

Fig. 5

Fig. 3. Lung tissue of a guinea pig exposed for 10 months to Reylo [illegible] by inhalation and then killed. A cross section of a bronchiole is seen just above and slightly to the left of center of the figure. The other spaces are alveoli. Note the dark masses within some of the smaller spaces, especially to the right of the bronchiole. These masses are macrophages and multinucleated giant cells. Note the thinness of the wall of the bronchiole; it is virtually normal, as is apparent on comparison with the tissue section of a normal guinea pig (Fig. 6). An asbestosis body is present in this field and is revealed in Fig. 4 at higher magnification. (200 X)

Fig. 4. High magnification of the tissue section shown in Fig. 3. This higher magnification of part of the field of Fig. 3 reveals a typical form of asbestosis body, the dark curved elongated structure to the right of center. (1200 X)

Fig. 5. Lung tissue of a guinea pig exposed for 10 months to Keyl's Blend asbestos dust by inhalation and then killed. The bronchiole in this photomicrograph is just above and to the left of center. Note the thickening of its walls. Deposits of fibrous tissue are apparent within the lumen of this bronchiole as well as in the [illegible]

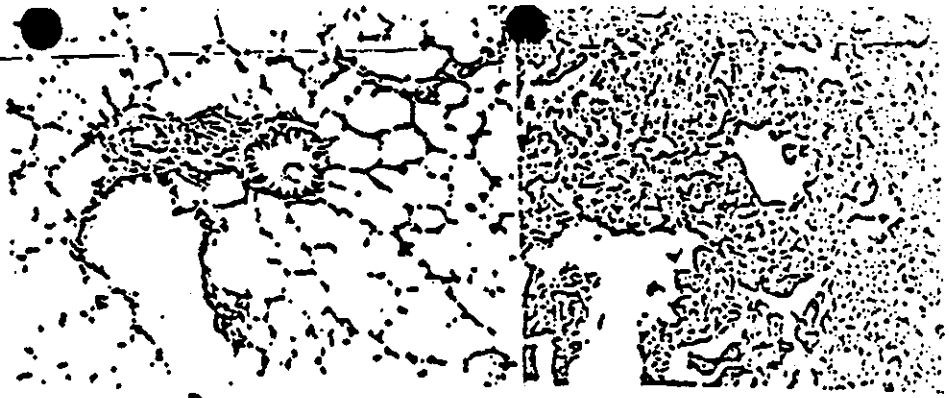

Fig. 6

Fig. 7

Fig. 8

Fig. 6. Lung tissue of a normal guinea pig. This field shows two bronchioles one in cross section at the right of center and the other in longitudinal section. The small associated blood vessel is visible above the bronchiole in longitudinal section. Compare the thickness of the wall of these bronchioles with the thickness of wall of the bronchioles in figs. 5, 6, and 7. Note also the empty alveoli and the thin alveolar walls in the normal animal. (200 X)

Fig. 7. Lung tissue of a guinea pig exposed for 30 months to Keyle dust by inhalation and then killed. In this photomicrograph a bronchiole is just above and to the right of center. Note the thickness of the tissue between the lumen of the bronchiole and the lumen of the adjacent alveoli in comparison with the thin bronchiolar walls in figs. 5 and 6. Note also that the greatly thickened bronchiolar walls of the animal exposed to asbestos dust for 30 months (fig. 5) are similar to those of this animal, exposed to Keyle dust for 35 months. Fine strands of fibrous tissue are visible near the bronchiole in this photomicrograph but they are more apparent, because they are less dense, in the area at the right of the field where fibrosis has extended to adjacent alveolar walls. (200 X)

Fig. 8. Lung tissue of a rat exposed for 18 months to Keyle dust by inhalation and then killed. The photomicrograph reveals simple accumulation of cells in alveoli. The cytoplasm of the cells is packed with particles of dust. Note that the alveolar septa, even those of spaces containing numerous dust cells, are thin and delicate. (200 X)

Fig. 9

Fig. 10

Fig. 11

Fig. 9. Lung tissue of a guinea pig infected with attenuated tubercle bacilli and immediately exposed to Keyle dust by inhalation for 24 months, and then killed. The figure is a slightly magnified photograph of the routine histologic preparation of the lungs, including both lungs from apex to base. The cluster of dark foci of irregular outline in the lower center (median lobe) and at the right (left caudal lobe) are foci of active spreading tuberculosis. The widely-distributed minute dark foci are merely lymphoid cell clusters and are unrelated to the infection. The extent of the tuberculous activity is seen to be exceedingly small. (1.5 X)

Fig. 10. Lung tissue of a guinea pig infected with attenuated tubercle bacilli and left in clean air for 2 months, then exposed for 12 months to Keyle dust by inhalation, and killed. The dark zone, irregular in outline, in the left upper quadrant (right cephalic lobe) is an area of active spreading tuberculosis. A smaller but similar focus is visible at the lower margin of the left cephalic lobe. Again, as in fig. 9, the extent of the tuberculous process is very small. (1.5 X)

Fig. 11. Lung tissue of a guinea pig exposed to Keyle dust by inhalation for 3 months, then infected with attenuated tubercle bacilli and exposed to the dust for an additional 16 months, and killed. The numerous poorly-defined dark zones irregular in outline, are all sites of active, spreading tuberculous disease. The tuberculous involvement exhibited in this photograph is the most extensive noted in any animal killed during the experiment. The involvement is relatively slight in comparison with the progression which occurs in animals exposed to quartz dust under similar conditions; in fact, all of the animals exposed to quartz dust would have died within 12 months after infection because of almost complete loss of functioning pulmonary tissue. (1.6 X)