

INDUSTRIAL HYGIENE FOUNDATION OF AMERICA, INC.
Mellon Institute, 4400 Fifth Avenue
Pittsburgh, Pa. 15213

June 1, 1966

Prospectus for An Investigation of:

- a. The Specificity of Asbestos Bodies and
- b. The Biologic Effects of Trace Substances
Associated with Chrysotile Asbestos.

INTRODUCTION

Thomson (1) found that about 30% of the people that had died in hospitals in Canetown (500 consecutive autopsies) had "asbestos bodies" in the lungs. The same author (2) found a similar prevalence of "asbestos bodies" among the hospital deaths in Miami, Florida. Cauna, Totten and Gross (3) examined the lungs of 100 non-selected hospital deaths for which autopsy permission had been obtained, and found that 40% of these had "asbestos bodies" in their lungs. Ian Webster (4) recently reported a 47% prevalence in Johannesburg.

There is, at present, a reasonable doubt that "asbestos bodies" are specific indicators of the inhalation of asbestos fibers. Such bodies have been found in coal miners and talc workers where they have been called "pseudo-asbestos bodies". Recently Davis (5) and Collet (6) have demonstrated the intracellular development of "asbestos bodies" secondary to amorphous material.

Asbestos occupies an anomalous position in its ability to produce lung damage, causing diffuse fibrosis and possibly also lung cancer. It is anomalous inasmuch as no other silicate has been shown to possess this ability. The fibrosis encountered in talcosis is limited to the tremolite variety, which is asbestos-like. On the other hand, the pneumoconiosis associated with the mining of mica (another silicate) has not been investigated experimentally, and the fibrogenic activity of the dusts coexisting with the mica has not been evaluated. Other silicates may evoke either minimal or relatively slight inflammatory changes, and are incapable of producing lung cancer.

Inasmuch as certain polycyclic hydrocarbons and trace metals known to be potent cancer-producing agents have been demonstrated in asbestos, particularly nickel, chromium and benzopyrene, the possibility exists that the trace metals and/or the polycyclic hydrocarbons may be solely responsible for the observed pathogenicity.

This possibility is supported by two findings:

1. The report of Braun and Truan (7) which indicated that the prevalence of lung cancer among Canadian asbestos miners was no greater than that of the general population. This was in contrast to the report of Doll (8) which indicated a greatly increased risk of lung cancer among British workers exposed to asbestos dust while fabricating the asbestos.

2. In a recent investigation in the laboratory of the Industrial Hygiene Foundation, lung cancers were found in rats exposed for 18 months to high concentrations of chrysotile dust. (9) Because rats exposed to chrysotile dust by other investigators failed to develop lung cancer, there is a good probability that the chrysotile dust that produced lung cancer was significantly different from that which did not have this effect. It is possible that this difference is

caused by a coating of nickel steel alloy applied to the asbestos dust particles during the continuous hammer milling process used for rendering the dust respirable. This method has not been used by previous investigators. Nickel has been found capable of producing lung cancer in trace amounts.

Although asbestos and glass are both silicates, the industrial health experience with workers of these two materials has been vastly different. No pulmonary disease has been reported in workers exposed to glass dust.

Nevertheless, the dust of filamentous glass has been labeled as highly dangerous in the lay press by association with asbestos. It is a fact that although the effect of flake glass dust on the lungs of animals has been reported, no similar study has been made of the dust of filamentous glass which does not have an associated resin or filler.

PROPOSAL

Phase 1.

It is proposed to examine the response of animals lungs to different inorganic fibrous materials of contrasting chemical composition, and to characterize the differences and similarities in the response.

Phase 2.

It is proposed to investigate the influence of trace minerals and polycyclic hydrocarbons (i.e., benzopyrene) associated with chrysotile ore or the subsequent processing on the long-term response of the lung to the dust.

Another objective of this phase is to obtain definitive data on the long-term effect of dust derived from filamentous glass upon the lungs. Three varieties of such glass dust will be investigated:

1. with the phenol-formaldehyde-type of binder
2. with the binders used in textiles
3. without binder.

Phase 3.

A comparative study of the three main types of asbestos dust (chrysotile, amosite and crocidolite) is proposed to attempt to pinpoint the cause of biological activity - i.e., whether due to silicates *per se* or the associated trace metals or hydrocarbons. Positive findings would be a basis for identifying any possible health hazards and for suggesting specific engineering or other process controls to eliminate such hazards.

MATERIALS AND METHOD

Phase 1.

The following fibrous materials will be reduced to respirable particle size ($<3\mu$) and will be injected in amounts of 3.5 mg. to 30 mg. as aqueous suspensions:

1. chrysotile dust (Prepared by milling in Wright mill and with demonstrated carcinogenic potential)
2. Chrysotile dust (unmilled synthetic)*
3. Aluminum nitride
4. tricalcium phosphate (filamentous)
5. fibrous glass
 - a. with phenol-formaldehyde-type binder

5. b. with binder used for textiles
- c. without binder
6. magnesium hydroxide (brucite)
7. mineral wool
8. zinc oxide (filamentous)

*Synthetic chrysotile has been prepared at Mellon Institute by Dr. William Granquist. Electron photomicrographs show the characteristic tubular crystals.

These materials will be injected intratracheally into hamsters under ether anesthesia. Previous experience has demonstrated that asbestos bodies will form in response to chrysotile dust within five months. The animals will therefore be held for six months and then killed. Asbestos bodies will be sought in lung juice as well as in the lung tissues.

The lungs will be expanded with buffered formalin under a head of 10 cm. of water. Blocks removed from these lungs will be sectioned at 6 μ after paraffin impregnation. These sections will be stained by different methods and photographed sequentially to provide means for studying more completely the tissue reaction to these various materials. Search for the so-called asbestos bodies will be made using routinely stained sections, and sections stained for iron. In addition, unstained cleared sections will be examined under dark-field conditions for asbestos bodies. The latter glow when examined in this manner.

The difference in tissue reaction between the synthetic chrysotile dust and that which is known to be fibrogenic as well as carcinogenic will point to the contribution to pathogenicity made by one or more of the various trace substances which may be associated with chrysotile asbestos dust as a result of processing.

The development of "asbestos bodies", better termed Ferruginous bodies, to dusts other than asbestos would point to the non-specificity of these structures and to the desirability of collecting such "asbestos bodies" from human lungs in order to subject them to electron-probe analysis. Such analysis could give information in regard to the chemical make-up of the dust responsible for the development of the Ferruginous ("asbestos" body).

Phase 2.

In order to explore the etiologic role of the various components of chrysotile asbestos in the production of lung fibrosis and lung cancer, six series of rats will be injected intratracheally with the following:

1. chrysotile dust known to be carcinogenic (9)
2. synthetic chrysotile
3. synthetic chrysotile plus nickel
4. synthetic chrysotile plus chrome
5. synthetic chrysotile plus nickel, chrome and benzopyrene
6. nickel alone or with carbon as carrier
7. chrome alone or with carbon as carrier
8. benzopyrene with carbon as carrier

These rats will be held for two years and then killed. The lungs will be prepared for examination and sections cut in the same manner as in Phase 1.

Three other series of rats will be injected intratracheally with the following dusts:

1. filamentous glass with phenol-formaldehyde-type binder
2. filamentous glass with the binders used for textiles
3. filamentous glass without binder.

These animals will also be held for two years and then killed. The lungs will be prepared and studied as in the preceding groups, with special attention to asbestos bodies in lung tissues or juice.

The number of animals per series will be such that there will be no doubt regarding the statistical significance of the results.

Phase 3.

SUPPLEMENTARY PROPOSAL

(Note: The following supplement has been added to the original proposal of April 15, 1966).

INTRODUCTION

Because of the interest of a number of companies in biologic investigations similar to those planned for chrysotile asbestos, but using instead, amosite and crocidolite, an additional investigation is proposed to be undertaken concurrently with the one proposed April 15, 1966.

As in the proposal dealing with chrysotile asbestos, the purpose of the presently proposed concurrent investigation is to attempt to pinpoint the cause of the biologic activity of all three main types of asbestos dusts: whether this lies in the silicates per se, in the associated trace metals, or in the associated hydrocarbons.

Because neither amosite nor crocidolite is available as a pure synthetic material, the investigation proposed for these two types of asbestos is necessarily different from that proposed for chrysotile. Nevertheless, in order to make the investigation complete, it is desirable to include also chrysotile in the new investigation since different methods are involved.

Inasmuch as the biologic activity of asbestos seems to have two components: (a.) inflammatory--responsible for asbestosis, and (b.) neoplastic--responsible for lung cancer and mesothelioma, it is advisable to investigate both of them. This can readily be accomplished within the four year span contemplated for this investigation.

In order to determine whether the biologic activity of asbestos resides in the silicate, in the associated trace metals, or in the associated hydrocarbons, attempts will be made to remove the trace metals and the hydrocarbons from their respective silicates and to compare the biologic activities of such "purified" asbestos samples from which these associated materials have been removed.

PROPOSAL

As a prerequisite to any biologic testing (insofar as the lungs are concerned), it is necessary to reduce the asbestos to a respirable particle size. This will be done in the following manner: the amosite and crocidolite will be ball-milled and then fed into continuous hammer mills modified from a design by Holt and Young (10). The dusts emerging from the hammer mills will be collected in electro-

static precipitators. This method has been used successfully by us to reduce chrysotile asbestos to a particle size, 70% of which was respirable. A sufficient quantity of finely divided chrysotile dust is available for the purposes of this investigation.

All three types of asbestos dust will be deprived of the greater portion or of all of their hydrocarbon content in one of two ways:

1. by continuous extraction with various solvents
2. by heating beyond the decomposition point of the hydrocarbons.

The trace metals will be removed from the three types of asbestos dust by treatment with aqua regia followed by treatment with EDTA (ethylene diamine tetraacetic acid, a chelating agent).

One batch of each type of asbestos dust will be subjected to both procedures, i.e., removal of trace metals and removal of hydrocarbons.

Accordingly, each of the three types of asbestos dust will consist of five batches, each with a different composition:

1. the unaltered dust as collected in the precipitators
2. the dust minus the hydrocarbons (heat-treated). Here, it is anticipated that there may also have been some change in the chemical structure of the asbestos through loss of some molecules of water of hydration.
3. the dust minus the hydrocarbons (solvent extract) it is anticipated that the extraction may not be complete.
4. the dust minus trace metals
5. the dust minus trace metals then subjected to section to remove most of the hydrocarbons.

In order to investigate the inflammatory component of (15) batches of asbestos dust, these will be injected subcutaneously into rats and hamsters. The lungs of these animals will be examined to show definitive changes within one year after the introduction of the dusts.

In order to explore the lung cancer aspect of the neoplastic component of the biologic activity of asbestos dust, additionally rats will be injected with the same batches of dust as above and allowed to live out their lives. Most of the animals, however, will have died before the end of the third year.

The other (mesotheliomatous) aspect of the above neoplastic component will be investigated by injecting the 15 different batches of asbestos dust within the pleural cavity of rats and hamsters and allowing them to live out their lives. Most of these animals will also have died before the end of the third year.

For the investigation of the inflammatory component of asbestos dust, a large enough number of animals will be put on test to allow sampling at intervals throughout the year and still have an adequate number remaining to represent the 12 month old lesion.

A tabular summary of the protocol is attached.

REPORTS

Definite trends in regard to the inflammatory component are expected to be demonstrable in animals that will be killed prior to the 12 month period (as indicated under footnote (b) in the Tabular Summary). An interim report covering the findings in these 420 animals will, therefore, be issued at the end of the first year.

Because few, if any, tumors are expected to develop prior to 24 months, no interim report on the neoplastic component will be issued until the end of the third year. The final report covering the

entire investigation will be issued toward the end of the fourth year. This report will probably be in the form of several manuscripts suitable for publication.

SUPERVISION

Research will be performed under the direct supervision of Dr. Paul Gross, Director of the Foundation's Research Laboratory. The Foundation's studies on asbestos presently are being conducted under partial support of the U.S. Government through a National Institutes of Health grant and a contract with the U.S. Public Health Service, Division of Occupational Health (Dr. Lewis J. Cralley).

TABULAR SUMMARY OF PROTOCOL

Type of Asbestos	Batch (a)	Dust Injected Intratracheally			Dust Injected Intrapleurally	
		for Inflammatory Component		for Lung Cancer	for Mesothelioma	
		No. (b) Rats	No. (b) Hamsters	No. (c) Rats	No. (c) Rats	No. (c) Hamsters
Chrysotile	1	20	20	20	20	20
	2	20	20	20	20	20
	3	20	20	20	20	20
	4	20	20	20	20	20
	5	20	20	20	20	20
Amosite	1	20	20	20	20	20
	2	20	20	20	20	20
	3	20	20	20	20	20
	4	20	20	20	20	20
	5	20	20	20	20	20
Crocidolite	1	20	20	20	20	20
	2	20	20	20	20	20
	3	20	20	20	20	20
	4	20	20	20	20	20
	5	20	20	20	20	20
TOTALS		300	300	300	300	300

(a) The following batches are identified:

- No. 1 = dust as collected from electrostatic precipitators
- No. 2 = dust extracted with solvents
- No. 3 = dust heated to decompose hydrocarbons
- No. 4 = dust treated with aqua regia and EYTA
- No. 5 = dust treated as with No. 4 and No. 2

- (b) Two animals killed immediately after the dust injection
Four animals killed 4 days after the dust injection
Four animals killed 1 month after the dust injection
Four animals killed 6 months after the dust injection
Six animals killed 12 months after the dust injection
- (c) All animals allowed to live out their lives

REFERENCES

1. Thomson, J.G., et al. Asbestos as Modern Urban Hazard. S. Afr. Med. J. 37:77-81, 1963.
2. Thomson, J.G., et al. Asbestos as an Urban Air Contaminant. Arch. Pathol. 81:458-464, May 1966.
3. Cauna, Dzidra; Totten, Robert S.; and Gross, Paul. Asbestos Bodies in Human Lungs at Autopsy. JAMA 192:371-373, May 3, 1965.
4. Webster, Ian. Report of progress quoted in the Annual Report of the Pneumoconiosis Research Unit of the South African Council for Scientific and Industrial Research. 1965.
5. Davis, J.H.G. Electron-Microscope Studies of Asbestosis in Man and Animals. Ann. N.Y. Acad. Scie. 132:98-111, 1965.
6. Collet, A. Personal Communication, 1965.
7. Braun, Daniel C. and Truan, T. David. An Epidemiological Study of Lung Cancer in Asbestos Miners. Am Arch. Ind. Health 17: 634-653, June 1958.
8. Doll, R. Mortality from Lung Cancer in Asbestos Workers. Brit. J. Ind. Health 12:81, 1955.
9. Gross, P. and deTreville, R.T.P. Experimental Asbestosis: Studies on the Progressiveness of the Pulmonary Fibrosis Caused by Chrysotile Dust. To be published.
10. Holt, P.S. and Young, D.K. A Dust-Feed Mechanism Suitable for Fibrous Dust. Ann. Occ. Hyg. 2:245, 1960.