INDUSTRIAL HYGIENE FOUNDATION OF AMERICA, INC.

MELLON INSTITUTE, 4400 FIFTH AVENUE

PITTSBURGH, PA. 15213

September 20, 1968

Proposal for

Johns-Manville Corporation

on

The Pathogenicity of Brake Lining Dust: Its relation to the development of "asbestos" bodies

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Broad statement of research objectives:

Since the discovery of ferruginous bodies in the lungs of adult city dwellers with a prevalence ranging from approximately 30 to nearly 50%,[1-4] commonly used products in which asbestos is a component have come under suspicion as the source of dust particles that, when inhaled, cause the development of these so-called asbestos bodies. This finding has been linked with the possible occurrence of pulmonary fibrosis, lung cancer, and mesothelioma in these people.[5]

Asbestos brake lining dust has been suggested as one of the most likely sources of the urban dust causing the development of "asbestos" bodies in the lungs; and, therefore, posing a serious threat to health.[1]

The proposed study is intended to determine the pathogenicity of brake lining dust and its ability to induce the production of "asbestos" bodies in the lungs.

1. Research Plan

a. Introduction and Specific Aims

There is now no reason for doubting the high prevalence of ferfuginous bodies in the lungs of adult city dwellers. We have recently found them in nearly 100% of about 30 unselected autopsied hospital cases.* However, we have also demonstrated that ferruginous bodies indistinguishable from asbestos bodies can be produced experimentally in the lungs of hamsters injected intratracheally with filamentous particles of respirable size composed of ceramic aluminum silicate, glass, or silicon carbide.[6] This successful demonstration establishes the non-specificity of ferruginous bodies. It follows that the categorization of a ferruginous body as an asbestos body may be erroneous unless the central filament has been identified as asbestos.

If one assumes that many of the ferruginous bodies found in the lungs of city dwellers are indeed asbestos bodies, then it is not unreasonable to assume further that the source of much of the fibrous dust that is responsible for the formation of these bodies is the brake linings of automobiles—as has been suggested by Thomson.[1] However, to imply that the inhalation of the brake lining dust carries with it the same disease-producing potential as the inhalation of asbestos dust, must be based on a further assumption: namely, that the asbestos fibers (embedded as they are in resin) possess the

*Unpublished data

same pathogenic potential as that of the asbestos fibers prior to their incorporation into the plastic of the brake band.

Since it cannot be denied that some, or even many, of the ferruginous bodies found in the lungs of city dwellers may be caused by the inhalation of brake lining dust, it would appear to be appropriate to investigate the pathogenicity of this dust as well as its ability to cause the development of ferruginous bodies. (A short-term, preliminary study has already demonstrated the development of ferruginous bodies in the lungs of hamsters injected with brake lining dust.) Inasmuch as the pathogenic potential of asbestos dust has two components, the fibrogenic and the cancerogenic, the ability of brake lining dust to produce pulmonary fibrosis as well as its ability to produce cancer are properly included in this investigation.

b. Method of Procedure

General: It will be advisable as a first step to introduce the brake lining dust into the lungs of the test animals by intratracheal injection. Inhalation studies may be needed but will require specially constructed chambers and are not covered herein.*

Three species of animals will be employed: guinea pigs, rats, and hamsters. Of these, only guinea pigs and hamsters have been found capable of producing ferruginous bodies. We have found no asbestos bodies in the lungs of rats that had inhaled asbestos dust or that had been injected with this dust intratracheally. All three species of animals are suitable for the study

* Inhalation studies will be the subject of a supplementary proposal later at an appropriate stage of program development.

of the fibrogenic potential. However, the hamster lung may react in an anomalous manner to the presence of the dust.[7, 8] It is proposed to study the sequential development of dust deposits and the tissue reaction to the dust by serial sampling of the animals injected intratracheally, but not those subjected to the inhalation technique. The reason for not sampling the latter animals is that it will be important to have as many animals as possible survive to the cancer—bearing age. The spontaneous deaths in this series will be utilized for the sequential study of the dust reaction in the lungs.

For the purpose of studying the ability of brake lining dust to produce lung cancer, only rats will be used because in a previous investigation, the intrapulmonary deposition of asbestos dust was associated with lung cancer only in rats. No tumors were found in guinea pigs or hamsters. On the other hand, to test the ability of the dust to produce mesotheliomas, hamsters will be used as well as rats. Here the dust will be injected into the pleural cavity.

The intratracheal injections will be accomplished under light ether anesthesia with the aid of an illuminated self-retaining speculum. This speculum allows the introduction between the vocal chords (under direct visual observation) of a blunted hollow needle attached to a syringe, and the subsequent expulsion of the dust suspension into the trachea.

The intrapleural injections will also be made under light ether anesthesia. The needle will be inserted in the right midaxillary line just below the axilla.

The dust will be suspended in distilled water so that the desired dosage will be contained in 1 ml water for rats and guinea pigs, and in 0.5 ml, for hamsters.

Animals surviving 30 months will be killed with ether. All animals regardless of the mode of death will be autopsied and the lungs distended with formalin under a pressure of 10 cm water. Selected lungs will be fixed with glutaraldehyde and osmic acid for electron microscopy. For optical microscopy, blocks of lung will be embedded in paraffin, sections will be cut at 6μ, and stained with hematoxylin and eosin.

Photographic records will be made on 35 mm film of pertinent lesions. The stromal reaction of the lung tissue will be followed by means of silver impregnation of the sections. Dust clearance and the topographic distribution of the dust (if not visible in the stained sections) will be studied by the microincineration technique.

Controls:

a. Laboratory controls, consisting of ten animals of each species picked randomly from the same shipment as that of the animals on test will be housed separately from the test animals. These will not be subject to any procedure but will receive food and water ad lib.

b. Intratracheal controls will consist of ten animals of each species injected intratracheally with water as often as the test animals.

c. Intrapleural controls will consist of 20 rats and 20 hamsters. Ten of each species will be injected intrapleurally with quartz dust and ten, with amorphous magnesium silicate (prepared by adding magnesium chloride to sodium silicate and adequately washing the resulting precipitate).

A summary of the experimental plan is given in tabular form on the following page.

Table 1

	Intratracheal Injections				Intrapleural Injections					Lab Controls
	Brake Lining Dust		Chrysotile[b]	H_2O	Brake Lining Dust		Chrysotile[e]	H_2O	Quartz[f]	
	D[a]	D/2			D[c]	D/2				
Rats	30	30	10	10	20	20	10	10	10	10
Guinea Pigs	20	20	10	10						10
Hamsters	20	20	10	10	20	20	10	10	10	10
	70	70	30	30	40	40	20	20	20	30

(a) The maximum intratracheal dosage (D) will be 28 mg for rats, 14 mg for hamsters, and 50 mg for guinea pigs. This may be administered in multiple injections. D/2 indicates half this dose.

(b) The dosage for rats will be 14 mg, 28 mg for guinea pigs, and 14 mg for hamsters.

(c) A single injection will be given. The maximal safe dosage to be determined. The animals will be allowed to live out their lives or killed at 30 months. Again, D/2 indicates half the maximum safe dose.

(e) Dosage to be the same as in (c) if tolerated.

(f) Dosage to be the same as in (c) if tolerated.

NOTE: The numbers of animals in Table 1 are the net numbers to provide statistical validity to the research program However, the program will be initiated by a larger number approximating double the number shown in the tabulation to allow for a 50% mortality rate during the program. The totals in the tabulation are 160 rats, 70 guinea pigs, and 140 hamsters, for a total of 370 animals; whereas the total number of animals to be used in the program will approximate 740. If additional animals are required, they will be included to assure statistical validity.

c. Significance of this Work

It has been established that urban dust contains filamentous particles of respirable size and that such filamentous particles are inhaled by city dwellers and deposited in their lungs. Some of these filamentous particles become coated with ferritin or a ferritin-like protein to become ferruginous bodies.

Some of the respirable filamentous dust particles may be man-made, consisting of glass or rock wool; others may be derived from paper or wood ash,* as well as from other sources[9] in addition to asbestos products. In view of the density of automobile traffic in modern urban centers, it is theoretically possible that automobile brake lining dust may be found to be the most abundant source of filaments derived from asbestos in urban air.

Recent publications indicate that there is little relationship between asbestosis and the development of bronchogenic cancer in some individuals. It has, therefore, become important to determine whether or not the dust derived from automobile brake linings is pathogenic and, if so, to what degree. If our findings indicate that brake lining dust possesses a significant pathogenic potential, both industry and public health authorities are faced with problems. On the other hand, if as seems likely from a preliminary investigation, brake lining dust has no significant pathogenic potential from animal experiments, no problem exists.

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*Unpublished Data

2. Supporting Data

a. Previous work done in this or related fields—

Over the past twelve years, the lungs of workers from the asbestos industry have been examined in this laboratory. These had varying degrees of asbestosis. Most of these lungs were from workers who had worked in various plants of a leading asbestos fabricating company in this country, although two of the workers had mined chrysotile asbestos for another company in Canada.

In a comparative study of asbestosis caused by chrysotile dust in rats and hamsters, it was found that in rats this disease tends to be multi-focal and to heal with the formation of collagenous scars that contain imprisoned asbestos dust. No dust was found in the lung tissue between the focal scars. In hamsters, chrysotile asbestos resulted in a fatal, non-healing diffuse pneumonitis characterized by air spaces filled with macrophages, and the production of an argyrophilic stroma that did not convert to collagen. Asbestos dust was found diffusely distributed throughout the affected lung. [7]

The distribution of acid-insoluble mineral dust in human asbestotic lungs was investigated by Gross and Smith [10] using the micro-incineration technique. It was found that in asbestotic lungs there was a paucity of acid-insoluble mineral dust in general, that the distribution of this dust was highly irregular, that the amount of dust present was not proportional to the amount of fibrosis, and that there were regions of fibrosis that contained no demonstrable dust.

In a four-year study guinea pigs, hamsters, and rats were exposed to hammer-milled chrysotile dust. Out of a total of 91 rats exposed by inhalation (62 weeks) to chrysotile dust or injected intratracheally with the dust (14 to 21 mg.) and surviving 16 months or longer 28 or 31% developed primary lung cancers (including also one pleural mesothelioma). None of the guinea pigs or hamsters developed lung cancer. (Gross, et al.[11]).

An investigation of the prevalence of asbestos bodies in the lungs of autopsied hospital patients in Pittsburgh was conducted. It was found that these bodies were present in 43% of the lungs examined.[6]

Synthetic chrysotile was prepared by Dr. W. T. Granquist of Mellon Institute. The x-ray diffraction pattern of this synthetic material was that characteristic of chrysotile and the electron photomicrographs show tubular crystals that are characteristic of chrysotile. It was about 80% pure. The impurities consisted largely of unreacted magnesium chloride and sodium silicate as well as small amounts of amorphous silica. This material, when injected intratracheally into rats, evoked only a mild mobilization of macrophages. There was no alveolar wall thickening or stromal proliferation. Five months after the intratracheal injection, there was no evidence of its presence detectable in the lung sections by micro-incineration or by alteration of the alveolar morphology. This was in contrast to the appearance of the lung tissue following the intratracheal injection of the same dose (3.5 mg.) of hammer-milled natural chrysotile dust. Here, there was a severe inflammation involving the

respiratory bronchiole, adjoining alveolar ducts and all their evaginating alveoli. This resulted in collagenous scars.[12]

The pulmonary response to an insoluble fibrous dust was studied when a filamentous ceramic aluminum silicate of respirable size was injected intratracheally into rats. The tissue reaction was judged to resemble more that observed with limestone dust than that associated with feldspar, a silicate the pathogenicity of which is intermediate between quartz dust and limestone dust. Limestone dust is generally classified as "biologically inert".[13]

The specificity of asbestos bodies was investigated recently in this laboratory.[14] The development of bodies indistinguishable from asbestos bodies was demonstrated in the lungs of hamsters that had been injected intratracheally with filamentous dust of aluminum silicate, silicon carbide, and glass (all 1μ or less in diameter).[6] Because these bodies, regardless of the nature of the central fiber, are composed of ferritin(or a ferritin-like material), an iron-containing protein, they have been designated as ferruginous bodies. Although many of the ferruginous bodies that formed in response to non-asbestiform filaments were non-segmented clubbed rods, typically segmented forms were also seen. A method was developed in connection with this work for isolating and concentrating ferruginous bodies from human and animal lungs. It was shown that human asbestos bodies may also exist in the form of pale golden or yellow non-segmented straight rods with clubbed ends, similar to the ferruginous bodies recovered from the lungs of hamsters injected intratracheally with aluminum silicate, silicon carbide, and glass respectively.[6]

B. Personal Publications Pertinent to this Proposal—

- Gross, P., Grandquist, W. T., Spell, S., Leinweber, J., and deTreville, R. T. P.: The Pulmonary Response to Synthetic Chrysotile, to be published.
- Gross, P., Westrick, M. L., Schrenk, H. H., and McNerney, J. M.: The Effects of a Synthetic Ceramic Fiber Dust Upon the Lungs of Rats, A. M. A. Arch. Indus. Health 13: 161-166, 1956.
- Gross, P., and deTreville, R. T. P.: The Pulmonary Response to Fibrous Glass Dust, to be published.
- Gross, P., deTreville, R. T. P., Tolker, E. B., Kaschak, M. A., and Babyak, M. A.: Experimental Asbestosis: The Development of Lung Cancer in Rats with Pulmonary Deposits of Chrysotile Asbestos Dust, Arch. Environ. Health 15: 343-55, 1967.
- Gross, P. and Smith, K. W.: The Topographic Distribution of Mineral Dusts in Some Pneumoconiotic Lungs, Dis. Chest 35: 140-154, 1959.
- Gross, P. and deTreville, R. T. P.: Experimental Asbestosis: Studies on the Progressiveness of the Pulmonary Fibrosis Caused by Chrysotile Dust, to be published, Arch. Env. Health.
- Cauna, D., Totten, R. S., and Gross, P.: Asbestos Bodies in Human Lungs at Autopsy, J. A. M. A. 192: 371-373, 1965.
- Gross, P., Cralley, L. J., and deTreville, R. T. P.: Asbestos Bodies: Their Specificity, to be published, A. I. H. A. Journal.

- Gross, P., deTreville, R.T.P., Cralley, L.J., and Davis, J.M.G.: Pulmonary Ferruginous Bodies: 1. Their Development in Response to Filamentous Dusts Other Than Asbestos. 2. Method of Their Isolation and Concentrating Them, to be published.
- Gross, P.: The Pneumoconioses: Chapter 4, Part 1: Pathology of the Pneumoconioses, Edited by A.J. Lanza, Grune & Stratton, Inc., (1963).
- Gross, P., deVilliers, A.J., and deTreville, R.T.P.: Experimental Silicosis: The "Atypical" Reaction in the Syrian Hamster, Arch. Path., 84: 87-94, July, 1967.
- Cralley, L.J.: Keenan, R.G.; Lynch, J.R.: Exposure to Metals in the Manufacture of Asbestos Textile Products. Presented at Annual Meeting of American Industrial Hygiene Conference, Chicago, May 1967. JAIHA, to be published.

Pertinent Literature References:

1. Thomson, J.G.; Kaschula, R.O.C.; and MacDonald, R.R.: Asbestos as a Modern Urban Hazard, S Afr Med J 37: 77-81, 1963.
2. Thomson, J.G. and Graves, W.M.: Asbestos as an Urban Air Contaminant, Arch Path 81: 458-464, 1966.
3. Cauna, D.; Totten, R.S.; and Gross, P.: Asbestos Bodies in Human Lungs at Autopsy, JAMA 192: 371-373, 1965.

4. Anjilvel, L. and Thuribeck, W.M.: The Incidence of Asbestos Bodies in the Lungs at Random Necropsies in Montreal, Can Med Assn J 94: 1179-1182, 1966.

5. Newhouse, M.L. and Thompson, H.: Mesothelioma of Pleura and Peritoneum Following Exposure to Asbestos in the London Area, Brit J Indus Med 22: 261, 1965.

6. Gross, P., deTreville, R.T.P., Cralley, L.J., and Davis, J.M.G.: Pulmonary Ferruginous Bodies: 1. Their Development in Response to Filamentous Dusts Other Than Asbestos. 2. Method of Their Isolation and Concentrating Them, to be published in AMA Arch Path

7. Gross, P. and deTreville, R.T.P.: Experimental Asbestosis: Studies on the Progressiveness of the Pulmonary Fibrosis Caused by Chrysotile Dust, Arch Environ Health, to be published.

8. Gross, P., deVilliers, A.J., and deTreville, R.T.P.: Experimental Silicosis: The "Atypical" Reaction in the Syrian Hamster, Arch Path , 84: 87-94, July, 1967.

9. Cralley, L.J.; Keenan, R.G.; Lynch, J.R.: Exposure to Metals in the Manufacture of Asbestos Textile Products. Presented at Annual Meeting of American Industrial Hygiene Conference, Chicago, May 1967. JAIHA, to be published.

10. Gross, P.: The Pneumoconioses: Chapter 4, Part 1: Pathology of the Pneumoconioses, Edited by A.J. Lanza, Grune & Stratton, Inc., (1963).

11. Gross, P., deTreville, R. T. P., Tolker, E. B., Kaschak, M. A., and Babyak, M. A.: Experimental Asbestosis: The Development of Lung Cancer in Rats with Pulmonary Deposits of Chrysotile Asbestos Dust. Arch. Environ. Health 15: 343-55, 1967.

12. Gross, P., Grandquist, W. T., Speil, S., Leinweber, J.: and deTreville, R. T. P.: The Pulmonary Response to Synthetic Chrysotile, to be published.

13. Gross, P., Westrick, M. L., Schrenk, H. H., and McNerney, J. M.: The Effects of a Synthetic Ceramic Fiber Dust Upon the Lungs of Rats, A.M.A. Arch Indus Health 13: 161-166, 1956.

14. Gross, P., Cralley, L. J., and deTreville, R. T. P.: Asbestos Bodies: Their Specificity, to be published, JAIHA

INDUSTRIAL HYGIENE FOUNDATION OF AMERICA, INC.

BUDGET ESTIMATE FOR THREE YEARS OF SUPPORT

(Detailed Budget for First 12-Month Period)

DIRECT COSTS		
Salaries:		$27,500
Paul Gross, M. D. — Principal Investigator	$10,000	
Research Technician	6,000	
Histopathology Technician	6,000	
Animal Caretaker	5,500	
Fringe Benefits (11%)		3,025
Supplies:		6,500
Animals, Feed, Bedding	4,500	
Histologic	1,000	
Photographic	1,000	
Travel:		500
Total Direct Costs		$37,525
INDIRECT COSTS		24,275
Equipment: (Charged at rate of $1,200/year)		1,200
Animal Racks and Cages	2,000	
Zeiss Microphotographic Apparatus	1,500	
Total First Year's Costs to Johns-Manville		$63,000
Estimated Total Three-Year Cost to Johns-Manville		$189,000

COPY FORM	INITIALS	DATE

BOX NUMBER	SEGMENT NO.	REQUESTING PARTY

NOTES 04-01172