LUNG FIBRE CONCENTRATION, DIMENSION, AND ASBESTOSIS SEVERITY: RELATIONSHIPS AMONG QUÉBEC CHRYSOTILE MINERS AND MILLERS

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I dedicate the fruits of my work to the memory of my father, my first teacher, whose lessons of life continue to deliver me faith, hope, and strength

ABSTRACT

The objectives of this study were: 1) to compare lung fibre concentration and dimensions found in two groups of former Québec chrysotile miners (Asbestos and Thetford-Mines) and, 2) to investigate the relationship between lung-retained mineral fibre concentration and dimension (length, diameter, and aspect ratio) to severity of interstitial lung fibrosis (asbestosis) within these groups.

Lung fibre content was determined for 86 former employees of chrysotile mines and mills in two Québec mining regions: Thetford-Mines and Asbestos (Jeffrey Mine). Six lung samples were selected for each subject from predetermined intrapulmonary sites. Tissue samples were digested in a solution of sodium hypochlorite and low-temperature ashed in a plasma asher. Fibres were assessed using transmission electron microscopy (TEM) and energy dispersive x-ray spectrometry (EDS). Aliquots of lung digest were analyzed using three separate fibre size categories: less than 5 micrometers (μm), 5 to 10 μm, and greater than 10 μm. Corresponding histological lung tissue sections were quantitatively graded for the interstitial lung fibrosis on the 12-point scale recommended by the College of American Pathologists. Asbestos bodies concentrations in lung digests were assessed separately using optical microscopy. Lung fibre concentration and dimension of asbestos fibres were compared between the two groups of workers using non-parametric statistical tests. Interstitial fibrosis as a function of fibre concentration and dimension was evaluated separately for the two occupational groups; each within three fibre length categories, by simple correlation and linear and multiple regression.

In summary, the concentrations of tremolite for short (<5μm) intermediate-length (5-10μm) and long (>10 μm) fibres were 4, 2, and 2 fold higher respectively among workers of Thetford-Mines than those from Asbestos. These differences were statistically significant between the two groups ($p < 0.05$ for all tremolite size categories). No consistent and biologically important difference was found among fibre dimensions in any of the size categories ($p > 0.1$ for all fibre types and size categories). These observations suggested that the much higher exposure in Thetford-Mines to asbestos fibres, tremolite

fibres being more apparent, is the most plausible factor which may explain the higher incidence of lung diseases among the former miners and millers of Thetford-Mines.

Concentration of short (<5 µm) tremolite fibres was the best predictor of asbestosis severity in both mining groups ($r=0.44$, $p<0.01$ and $r=0.39$ $p<0.01$ for Thetford-Mines and Asbestos, respectively). Chrysotile fibre concentration showed a lower correlation with asbestosis severity among subjects from Thetford-Mines ($r= 0.28$, $p<0.1$ and $r=0.39$, $p<0.01$ and for fibres <5 µm and fibres 5-10 µm respectively). Long (> 10 µm) amosite fibre concentrations showed a linear relationship with asbestosis severity in the group of miners and millers from Asbestos ($r=0.5$, $p<0.01$). Shorter commercial amphibole concentrations did not show this relationship and these fibres were largely absent from the lungs of miners from Thetford-Mines. Concentration of asbestos bodies from lung digests was significantly associated with fibrosis for subjects from Thetford-Mines only ($r=0.3$, $p=0.05$). Other variables (duration of employment, age, smoking, etc) had no significant predictive value for interstitial fibrosis in regression analyses for workers in either area, although age approached significance ($r =0.3$, $p=0.05$) in Asbestos miners and millers. Fibre dimension showed no linear relation with fibrosis severity, indicating no relationship between fibre dimension and severity of fibrosis for fibres in the three size ranges assessed. This observation is not in agreement with a previous study in which severity of asbestosis showed a linear, negative predictive trend with decreasing tremolite fibre length. However, in the present study, very long fibres (>20 µm) were not adequately assessed, and these deserve further investigation.

The high concentration of tremolite fibres in the lungs of subjects from Thetford-Mines, noted previously, explains the significant relation for both total fibres (measured by TEM/EDS) and asbestos bodies with fibrosis among this group, which was absent among the Asbestos group. In general, lung fibre concentartion measured by TEM/EDS, especially that of short (<5 µm) tremolite fibres, was a better predictor of interstitial fibrosis in the two groups than concentration of asbestos bodies, concentration of chrysotile fibres, or duration of exposure.

RÉSUMÉ

Les objectifs de la présente étude étaient doubles : 1) d'une part, comparer la concentration de fibres et leur dimension chez deux groupes de mineurs et d'ouvriers de l'industrie de la fibre d'amiante au Québec (Asbestos et Thetford-Mines) et 2) d'autre part, étudier la relation entre les caractéristiques des fibres minérales retenues dans le poumon et la gravité de la fibrose interstitielle de ces deux groupes de travailleurs.

La charge pulmonaire en fibres a été déterminée chez 86 employés des mines et moulins dans deux régions minières du Québec c'est à dire les mines de Thetford-Mines et la mine Jeffrey situé à Asbestos. Six échantillons de poumon ont été prélevés chez chaque patient à des sites déjà établis pour être digérés dans une solution d'hypochlorite de sodium et par la suite au four à plasma. Les fibres ont été caractérisées par microscopie électronique à transmission (MET) et spectre dispersif en énergies de rayons-X (EDS). Les fibres concentrées dans les aliquotes de tissu pulmonaire digéré ont été analyseés et ont été classées essentiellement dans trois catégories de taille: plus courtes que 5 µm, 5 à 10 µm et plus grandes que 10 µm. Les sections de tissu pulmonaire ont été quantifiées sur une échelle de douze points telle que recommandé par le Collège américain des pathologistes. Les corps asbestosiques ont été dénombrés séparément par microscopie optique. La concentration de fibres ainsi que leur dimension ont été comparées entre les deux groupes de travailleurs à l'aide de tests statistiques non paramétriques. La gravité de l'amiantose a été évaluée en fonction de la concentration de fibres et en fonction de leur dimension, séparément pour les deux groupes de travailleurs, et aussi pour les trois classes de fibres, par corrélation simple et régression linaire multiple.

En résumé, la concentration des fibres courtes (5 µm) de trémolite, la concentration des fibres intermédiaires (5-10 µm) et la concentration des fibres longues (10 µm et plus) 4, 2, et 2 fois plus élevées parmi les travailleurs de Thetford-Mines que chez les travailleurs d'Asbestos. Ces différences sont statistiquement significatives ($p < 0.05$ pour toutes les classes de fibres). On ne trouve pas de différence biologique importante en ce qui a trait aux dimensions des fibres qui ont été classées dans leurs trois classes de morphologie. Ces

observations permettent de suggérer une exposition plus intense à la fibre d'amiante chez les travailleurs de Thetford-Mines, en particulier une exposition intense à la fibre d'amiante trémolite. De plus la fibre de trémolite semble être le facteur le plus plausible pouvant expliquer l'incidence élevée des maladies pulmonaires chez les mineurs et les ouvriers de Thetford-Mines.

La concentration des fibres courtes de trémolite (5 µm) est le meilleur estimateur de la gravité de l'amiantose chez les deux groupes de mineurs ($r = 0.44$, $p < 0.01$ pour le groupe de Thetford-Mines et $r = 0.39$ $p < 0.01$ pour le groupe d'Asbestos). La corrélation entre la concentration de fibres d'amiante chrysotile et la gravité de l'amiantose est plus faible chez les sujets de Thetford-Mines ($r = 0.28$, $p < 0.1$ et $r = 0.39$, $p < 0.01$ pour les fibres < 5 µm et les fibres de 5 à10 µm, respectivement). La concentration des longues fibres (> 10 µm) d'amiante amosite montre une relation linéaire avec la gravité de l'amiantose chez le groupe de mineurs et d'ouvriers de la région d'Asbestos ($r = 0.05$, $p < 0.01$). Par contre les fibres courtes d'amosite ne montrent pas d'association avec la gravité de l'amiantose et ces fibres d'amosite sont pratiquement absentes chez les travailleurs de Thetford-Mines. La concentration de corps asbestosiques montre une relation statistiquement significative avec la fibrose pulmonaire chez les travailleurs de Thetford-Mines seulement ($r = 0.3$, $p = 0.05$). Les variables indépendantes telles la durée de l'emploi, l'âge, et le tabagisme, entre autres n'ont pas de valeurs prédictives pour la gravité de l'amiantose chez les travailleurs des deux régions minières bien que le facteur âge soit sur la frontière d'une association statistique ($r=0.3$, $p = 0.05$) chez les mineurs d'Asbestos. La dimension des fibres ne montre pas de relation linéaire avec la gravité de l'amiantose, ce qui indique qu'il ne semble pas y avoir d'association entre la dimension des fibres et la gravité de l'amiantose et ce dans les trois catégories de morphologie. Cette observation n'est pas en accord avec une étude antérieure dans laquelle la gravité de l'amiantose a montré une tendance de relation linéaire négative en fonction de la décroissance de la longueur de la fibre. Il faut préciser que nous n'avons pas testé les très longues fibres (20 µm) dans la présente étude et celles-ci devraient l'être éventuellement.

La concentration notable de fibres d'amiante trémolite dans les poumons des sujets de Thetford-Mines, déjà constatée dans le passé, explique la relation statistiquement significative entre la concentration totale de fibres (dénombrées par MET et EDS) ou l'association des corps asbestosiques et l'amiantose; on notera que la relation entre corps asbestosiques et fibrose est absente chez les travailleurs de la région d'Asbestos. En général, la concentration de fibres mesurées par MET / EDS, en particulier la concentration de fibres de trémolite < 5 µm est un meilleur estimateur de la gravité de l'amiantose, chez les deux groupes de travailleurs, que la concentration de corps asbestotique ou encore la concentration de fibres de chrysotile ou bien la durée de l'exposition.

CLAIM TO ORIGINALITY

Québec chrysotile miners and millers have been the subjects of many epidemiological and lung fibre content studies. There have been a large number of studies in which the lung fibre content of former miners and millers of Quebec chrysotile industry have been investigated. However, to the best of the author's knowledge, there has been no report in which the lung fibre burden of miners and millers of the two distinct mining regions were evaluated separately and compared for 1) fibre concentration and dimension, and 2) the relationship between fibre characteristic and severity of asbestosis. In addition, the use of three fibre length categories for the first time in this investigation provided a more reliable procedure to determine the possible effect of fibre dimension on the toxicity of asbestos fibres. Using this procedure, a wider range of fibre length was studied (see chapter 3).

This research makes the following original contributions:

1. This study investigated lung fibre content of two groups of former chrysotile miners and millers, one group from Asbestos (41 subjects) while the other comprised former miners and millers of Thetford-Mines (45 subjects). Since a consistent higher rates of various pulmonary diseases have been observed among the latter group, the first study hypothesis was considered the direct comparison of fibre concentration and dimension between a relatively a large number of subjects from two groups by statistical analysis. To cover a wide range of fibre length, three size categories (<5 µm, 5-10 µm and >10 µm) were selected, a procedure that was used for the first time to compare the lung fibre content of these populations.

2. The relationships of asbestosis severity and lung fibre characteristics were also investigated in this study. For the first time, this relationship was investigated for former miners and millers of Asbestos who had a history of exposure to commercial amphiboles (crocidolite and amosite). Moreover, comparison of the two groups of subjects, in the context of the presence of asbestosis and its severity and its association with various fibre characteristics and exposure variables, provided new knowledge that can be of importance

for epidemiologists, toxicologists, and hygienists, and the medico-legal and compensation systems.

3. Lung mineral fibres were analyzed in three steps for three size categories. This approach resulted in studying a much wider range of the fibre length and fibre length to diameter ratio (aspect ratio) and their relation with fibrosis severity. The use of this approach also allowed a better characterization of long fibres.

4. For the first time the possible relation between exposure variables (e.g. age, smoking, duration of exposure, etc.) and severity of histologically evaluated asbestosis were investigated for two distinct groups of Québec chrysotile miners and millers.

I, Ataollah Nayebzadeh, carried out the literature review, analysis of lung tissue samples by transmission electron microscopy and optical microscopy, sectioning of lung tissues for histological analysis, statistical analysis of data, and finally preparation of the thesis. The contribution of the people who kindly assisted me for above steps is acknowledged and appreciated in the next section.

Acknowledgement

First and foremost, I should thank God for providing me with the opportunity to complete my Ph.D. thesis and giving me strength and patience throughout all these years.

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ABBREVIATIONS AND SYMBOLS

AB(s)	asbestos bodies
ACGIH	American Conference of Governmental Industrial Hygienist (USA)
b_i	regression coefficient
D	diameter
DOE	duration of exposure
EDS	energy dispersive X-ray spectroscopy
EM	electron microscopy
f/g	fibre per gram (dry lung)
f/ml	fibre per milliliter (of air)
FYE	first year of exposure
GM	geometric mean
GMC	geometric mean concentration
GMD	geometric mean diameter
GML	geometric mean length
GM-GML	geometric mean of geometric mean length
GM-GMD	geometric mean of geometric mean diameter
GML/D	geometric mean of length to diameter ratio
GM-GML/D	geometric mean of geometric mean length to diameter ratio
GSD	geometric standard deviation
KS test	Kolmogorov-Smirnov test
keV	Kilo electron volt
Kv	Kilo-volt
L	length
LYE	last year of exposure

L/D	length to diameter ratio
Log_e	logarithm (natural)
m	meter
mg	milligram
min.	minute
mm	millimeter
mppcf	million particles per cubic foot
n	number of subjects
ND	not detected
NS	not statistically significant
OM	optical microscopy
OSHA	Occupational Safety and Health Administration (USA)
p	p-value
PCOM	phase contrast optical microscope
PEL	permissible exposure limit
r	correlation coefficient
R^2	coefficient of determination
SEM	scanning electron microscopy
SD	standard deviation
SMR	standard mortality ratio
TEM	transmission electron microscope
TLV	threshold limit value
TWA	time weighted average
Yr(s)	years
vs	versus
95% CI	95 percent confidence interval
μg	microgram
μm	micrometer
©	copyright

°	degree
°C	degree centigrade
<	less than
>	more than
%	percent
®	trade mark

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DEFINITIONS

Thetford-Mines: This includes several asbestos mines either open pit or underground, which are located in and around the town of Thetford-Mines in the Eastern Townships of the province of Québec (Canada). These mines and their mills form a large complex. Some of these mines are located within the town (central mines) and the rest outside the central area along the river in a 30 km area.

Thetford-Mines millers and miners(workers); Thetford-Mines subjects: In the thesis, all of these terms refers to the autopsy cases from Thetford-Mines (as defined above) whose lung tissues were studied for the mineralogical properties of fibres, and degree of fibrosis.

Asbestos : This includes a single chrysotile open pit mine, a chrysotile mill and also an asbestos factory, located within the Town of Asbestos in the Eastern Townships of Province of Québec (Canada). This mine is also referred to as the Jeffrey mine or as JM Asbestos in the literature.

Asbestos millers and miners (workers); Asbestos subjects: In this thesis, all of these terms refers to the miners, millers, and factory workers from Asbestos (as defined above), whose lung tissues were studied for mineralogical properties of fibres and degree of fibrosis.

Level of asbestosis: Various definitions have been used in the past by investigators to define the "degree of pulmonary interstitial fibrosis" due to exposure to different asbestos fibres. Therefore, this general term is used rather than "fibrosis severity" when discussing the previous literature, to avoid confusion.

Fibrosis severity: This refers to the progression of pulmonary fibrosis from respiratory bronchioles to alveolar ducts and further. In the present study, fibrosis severity was quantified from 0-4 by a pathologist and used in the calculation of fibrosis score. The method of grading will be discussed in detail in chapter 3.

Fibrosis extent: This refers to the proportion of pulmonary bronchioles involved histologically in the disease. In this study, fibrosis extent was quantified by a pathologist from 0-3 and used in the calculation of fibrosis score. The method of grading will be discussed in detail in chapter 3.

Fibrosis score: In the present study, this refers to overall degree of pulmonary fibrosis due to exposure to asbestos fibres. Fibrosis score is determined for each subject by multiplying the fibrosis severity score by the fibrosis extent score. This quantity ranges from 0-12 and is used as an overall measure of severity of asbestosis in statistical analysis.

Concentration of asbestos bodies: This refers to the number of mineral fibres coated with iron-containing proteins, which have the typical beaded dumbbell-shape. These gold to red or brown coated fibres are observed *in lung digestion aliquot* by phase contrast microscopy. The term *"ferruginous bodies"* is not used, because it has been shown that in these populations more than 95% of ferruginous bodies carry an asbestos fibre core. The unit used to express this variable is number of asbestos bodies per gram dry lung tissue.

Grade of asbestos bodies: In the present study, this refers to a semi-quantitative value (0-3) related to the method used for rating the abundance of asbestos bodies (as defined above) *in the histological sections* by using optical microscopy while examining the histological alteration of lung tissue by the pathologist. The observation of asbestos bodies in the histological specimen is classically used for the diagnosis of asbestosis.

Exposure variables: The term *"exposure variables"* in this thesis refers to; 1) demographic information for subjects such as age at start of exposure and age at death, 2) exposure related information such as duration of exposure and time since last exposure , and 3) smoking habit.

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL

1.1.1. Asbestos, its definition and mineralogy

Asbestos is a general term applied to certain fibrous forms, known as asbestifom varieties, of various silicate minerals. These are characterized in nature as long, thin, and separable fibrils possessing a crystalline structure, high tensile strength and flexibility.(1,2) A fibre is arbitrarily defined in the medical and environmental literature as a mineral particle whose length is at least three times greater than its diameter.(3) Mineralogists, however, prefer to use a greater aspect ratio (10:1 or greater) to classify a mineral particle as a fibre. The most commonly encountered asbestos mineral is chrysotile, which belongs to the serpentine group of silicates. The five other types of asbestos fibres, amosite $Fe_2Mg_5 Si_8O_{22}(OH)_2$, (fibrous cummingtonite or grunerite), crocidolite $Na_2(FeMg)_5 Si_8O_{22}(OH)_2$, (fibrous riebeckite), anthophyllite $Mg_7Si_8O_{22}(OH)_2$, tremolite and actinolite $Ca_2(MgFe)_5$

$Si_8O_{22}(OH)_2$ are members of the amphibole family, and each can occur either as asbestiform, or non-asbestiform varieties such as platy, acicular, and chunky.[4]

Chrysotile is one of three mineral structures (one of three polymorphs) of a fairly constant composition, $Mg_3Si_2O_5(OH)_4$.[5] Chrysotile asbestos occurs as very narrow tubular fibres that are often hollow, and have a tube-like cross-section. [5] Crocidolite and amosite both belong to the amphiboles, which are more brittle and less flexible than serpentine (chrysotile) fibres. These two asbestos fibres have usually been produced in Australia and South Africa.[2] Another commercial amphibole, anthophyllite, was produced only in Finland until 1974.[6] Tremolite exists primarily as a natural contaminant in the ores other exploited minerals, such as chrysotile, talc, and vermiculite.[6,7]

Asbestos fibres posses several invaluable physical properties such as fire resistance, poor conduction of heat and sound, ability to be woven into fabrics, and resistance to acids and alkalis (except chrysotile). Because of these properties asbestos fibres of different kinds were extensively used as a raw material in textile, fire proofing, insulation, floor tiling, and asbestos-cement industries. [2,4,6]

1.1.2. Health effects and pathogenicity of asbestos fibres

1.1.2.1. Asbestos-related diseases and fibre type

The respiratory diseases due to exposure to asbestos can be divided into two groups: malignant and non-malignant. Asbestosis, pleural plaques and diffuse pleural fibrosis are non-malignant lesions caused by exposure to asbestos.[3,8-9] Bronchogenic carcinoma of the lung and malignant mesothelioma of the pleura are malignant respiratory diseases related to asbestos exposure.[3,8,9] Other malignant diseases such as laryngeal cancer and gastrointestinal cancer have also been attributed to exposure to asbestos fibres. However, the causal relationship is not as strongly established as the one for asbestos exposure and respiratory disorders.[9,10]

Effects of asbestos type and the presence and severity of asbestos related diseases have been studied in several epidemiological studies for many years.[11-18] In general, all types of asbestos fibres can cause pulmonary fibrosis and lung cancer, and are considered potential carcinogens by the American Conference of Governmental Industrial Hygienists. [19] Tremolite has had limited commercial use; however, it contaminates chrysotile, talc, and vermiculite ore and it increases their toxicity disproportionately.[7-22]

Currently, the ACGIH recommends 0.1 f/ml as the maximum allowable concentration for all fibre type in the working environment for a 40-hour work week, and 8-hour exposure per day.[19] The permissible exposure limits for asbestos fibres in Québec are 1 f/ml for chrysotile and tremolite and 0.2 f/ml for amosite and crocidolite.[23]

Among various respiratory diseases related to exposure to asbestos, asbestosis is the main focus of a part of this work, and therefore will be discussed in more detail later.

1.1.2.2. The effect of fibre size

The belief that fibre length is important for the pathogenicity of asbestosis has a long history. [2] Several experimental studies on laboratory animals have suggested that longer fibres are associated with greater tumorigenic and fibrogenic activities.[24-29] Administration of short amosite and crocidolite fibres of different kinds by inhalation or injection to laboratory animals did not cause any fibrosis.[27-29] In all cases, animals given the same type of fibre but containing a substantial proportion more than 5 μm long developed the expected fibrosis, whereas the short fibres produced no more reaction than controls. Recently Miller and co-workers[30-31] reported the results of their work in which the tumorogenicity of several variables such as fibre concentration, dimension, and biopersistence were investigated through the inhalation or the injection of fibres in rats. The results suggested consistently that the number of fibres thinner that 1 μm and longer than 20 μm was the best predictor of risk of cancer. This effect, however, was absent for thicker (>1 μm) and shorter size categories of fibres (ie: >15 μm, >10 μm, > 8 μm and >5 μm). This evidence that the specific pathogenicity might be greater for long fibres is also suggested for mechanisms of fibrogenesis at the cellular level.[32] This may be partly related to the pathogenic and secretory function of pulmonary macrophages, the main cells responsible for the defense against foreign particles in the respiratory system.[33,34] With long fibres, the engulfment and digestion of fibres by macrophages is incomplete, resulting in damage to the membrane of macrophages and the leakage of tissue-damaging enzymes into the interstitial or alveolar spaces.[35] In vitro studies have also shown that long fibres have increased pathological activity.[36,37]

Despite the fact that the relationship between fibre size and the pathogenicity of asbestos fibres in animal models is well recognized, this relationship remains less certain for humans. Studies of the lung content of populations environmentally exposed to long, and usually much higher length to diameter ratio (aspect ratio) of tremolite asbestos indicate that this type of fibre is a potent mesothelial carcinogen. Yaziogliu and associates[38] reported on a population in Turkey and Langer and co-workers[39] from a population in Greece, with substantial incidence of mesothelioma and exposure to long fibres with an aspect ratio greater than 50. McDonald et al.[40] showed that long amphiboles (longer than 8 μm) accounted for an asbestos-related excess of mesothelioma in a case-control study of 78 cases. Case et al.[41] showed that the lungs of asbestosis cases contained a greater concentration of longer tremolite fibres (> 8 μm) than other workers without asbestosis (for fibres longer that 8 μm and aspect ratio 3:1, 0.96 f/μg for asbestosis and 0.14 f/μg without asbestosis $p<0.01$).

1.2. ASBESTOS PRODUCTION AND EXPOSURE IN QUÉBEC

1.2.1. History of Asbestos exploration

In the Eastern Township region of the province of Québec, deposits of chrysotile were noted in the 1847. However, the modern asbestos industry dates from the discovery of large deposits of chrysotile fibre in Québec in the 1870s.[6,15] Exploitation of chrysotile on a commercial scale began at the end of the 19th century in two regions; 1) the Thetford-

Mines (1877) and, 2) the town of Asbestos (1881). Then, the Québec mining region expanded around the towns of Thetford-Mines and Asbestos over the years. Mining at Thetford-Mines included 21 open pits or underground mines. A few of those were located near the original mine and from a center while the rest were located in a 30 kilometer area around the central mines. In and around Thetford-Mines, many companies started mining and several mills were developed. Thetford-Mines comprised 21 mining companies within a given distance. Six of the 21 companies were small and independent; the remaining 15 had been amalgamated into a large complex.(15,42) In Asbestos, one large open pit situated within the city itself was developed and named Jeffrey mine (43). The asbestos industry in the town of Asbestos also included a large mill for fibre processing and a factory for fabricating asbestos products (1924) using both local and imported fibres. The production of chrysotile in Québec peaked in 1977 when 1.6 million tones of chrysotile were produced.(6)

The mineralogy of the Québec asbestos mining region has been described previously.(44,45) Gibbs named more than 50 different minerals associated with asbestos mining in Québec. Recently the presence of several amphibole minerals such as tremolite, actinolite and traces of anthophyllite was confirmed in Asbestos.(46) Crocidolite and amosite, however, were not detected in the ores of the Québec chrysotile mining region.(43,46)

1.2.2. Occupational exposure to asbestos fibres in Québec

Chrysotile has been the main product of the Québec asbestos mining industry, and has been considered the most important fibre in the working environment. However, the work in an asbestos mine creates a dust cloud which contains various minerals, including tremolite, a non commercial amphibole. Gibbs(45) listed 20 likely mineral contaminants of the mill atmosphere and commercially milled fibre in the Québec asbestos mining and milling industry. Among them were brucite, magnetite, tremolite, actinolite, mica, olivine and chlorite.(45,47) However, Gibbs did not indicate the relative proportions of each mineral in the ore and airborne dust. (45,47)

Liddell provided a summary of past exposure levels for chrysotile workers of Québec.[48] The dust concentration was probably much higher than 100 f/ml before 1950 and during 1950s. It was reduced to 20 f/ml in 1960s and then to 5 f/ml in 1970s. Although these numbers are average concentrations levels for the Québec chrysotile mining industry regardless of locality of each mine, it has been indicated that the Thetford-Mines had been dustier mining region than Asbestos, and therefore, miners and millers employed in the Thetford-Mines were more heavily exposed.[15,49] It should also be noted that exposure levels in the Thetford-Mines or mills also varied substantially.[45] Although exposure differences between and within mines were indicated in previous reports, there has not been a quantitative estimation of these differences for each mine or mill in the literature. (6,15-16,45,47-48)

Exposure at Québec chrysotile mines and mills has been expressed as a cumulative index (duration x concentration).[15-16,47,50] Prior to 1964, all asbestos concentration measurements involved the use of a midget impinger, and all studies of exposure-response depended on these measurements. In the midget impinger method, all airborne particles were collected from air into water or isopropyl alcohol. Particles were then settled and counted at 100x magnification. The concentrations were reported as millions of particles per cubic foot (mppcf). The sensitivity of this method was adequate when the concentrations were high. However, it could not be used for personal monitoring and it was not specific to fibres.[51]

Based on midget impinger counts, cumulative dust exposure estimates (mppcf.year) were developed based on job classifications and the area in which tasks were performed. Attempt was made to estimate the concentration of fibre per milliliter of air (f/ml) from the use of available mppcf values, but no single conversion factor was achieved, and cumulative exposure values as mppcf.year continued to be used in epidemiological studies.[52]

1.2.3. Occupational lung diseases among miners and millers in Asbestos and Thetford-Mines

Cases of asbestosis were reported among Québec chrysotile miners and millers in the early years of this century.[53] Later, cases of lung cancer were reported by a pathologist from Thetford-Mines.[54] The issue of asbestos, asbestosis, and lung cancer among this population was addressed in 1958 by epidemiologists for the first time.[55,56] In 1966, a research program was established at McGill University to study the health effects of work in the Québec chrysotile industry.[15] Several epidemiological studies and lung-retained fibre studies were published since 1966. However, this section of the thesis focuses on the more recent publications, in which the mortality and morbidity of both mining regions were addressed.

In 1972, a study of radiographic changes among Québec miners was published.[50] The results of evaluating the chest radiographs of more than 11000 employees from both mining regions showed a higher prevalence of various radiological indices including small round opacities, small irregular opacities, pleural thickening, and pleural calcification among miners and millers from Thetford-Mines compared with those from Asbestos. In addition, the prevalence of lung opacities among workers from Thetford-Mines was more systematically related to the cumulative index of exposure. This trend persisted even after statistical adjustment for age and the level of exposure.

In 1972, the lung function of more than 1000 chrysotile miners and millers of Québec was studied by Becklake et al.[13,56] In nonsmokers and smokers, several function were tested such as inspiratory capacity (IC), vital capacity (VC), and forced vital capacity (FVC) were reduced. The observed changes were correlated with the cumulative index of dust exposure expressed as millions of particles per cubic feet year (mpcf.year). However, no distinction was made between different mining regions of Québec.

Gibbs [57] studied the prevalence and etiology of pleural calcification among Québec miners and millers. Among more than 13000 chest radiographs studied, 206 cases of pleural calcification were found. The prevalence of pleural calcification among persons employed at Thetford-Mines (198 cases, 2.5%) was 30 times higher than that among miners from Asbestos (6 cases, 0.08%). Gibbs [57] concluded that geographical location of each mine and, by implications, the mineralogical properties of the mines could be important factors affecting the exposure and disease of miners and millers. The presence of specific types of minerals or rocks in Thetford-Mines which were absent in Asbestos was suggested as a possible etiological factor responsible for the higher rate of calcification among miners of Thetford-Mines.

In 1980, the result of 1891-1920 birth cohort of Québec chrysotile employees of both mining regions was published which included 4463 deaths among 10939 cohort members.[15] Among men, (1926-75) an excess mortality of 2% for the miners, millers and factory workers from Asbestos was reported. In Thetford-Mines the corresponding finding was 10%. A clear excess of death was observed among those who were exposed to the heaviest concentration of dust. The overall standard mortality ratio (SMR) for all causes of death was 1.07. Clear trends were found for SMRs to be higher with heavier exposure for total mortality, lung cancer, and pneumoconiosis. The follow-up of the cohort of Québec chrysotile miners and millers revealed further facts about the mortality and morbidity due to exposure to asbestos dust.[16] By this time, the total number of deaths in the cohort was 8000. The SMR for lung cancer had risen from 1.25 to 1.39 and death from mesothelioma had also increased approximately to one in 200 deaths. A higher risk of mesothelioma was observed among miners of Thetford-Mines. The standard mortality ratio for lung cancer was higher among miners at Thetford-Mines (1.89) than the SMR among miners of Asbestos (1.55) for cumulative exposures of more than 300 mppcf.year. Crude death rates for death due to pneumoconiosis and mesothelioma were also higher among miners and millers of Thetford-Mines in comparison with miners, millers, and factory workers from Asbestos.

In 1997, Liddell et al.[58], reported an average SMR of 1.07 for all causes of death for the same cohort of Québec miners. The SMR for deaths occurring between 1950 and 1992 was higher among miners and millers of Thetford-Mines (1.65) for cancer of trachea, bronchus and lung than that among miners of Asbestos (1.34). The SMRs for stomach cancer, larynx cancer and all causes of death were higher among miners and millers of Thetford-Mines. The higher rate of mesothelioma among miners and millers of Thetford-Mines was further reconfirmed in this study. A recent update of the birth cohort of Québec chrysotile miners and millers showed a higher rate of mesothelioma among miners from Thetford-Mines (33.7 per 100,000 subject year at Thetford-Mines vs 13.2 per 100,000 subject year at Asbestos).[59] McDonald A.D. et al.[59] also showed an increased risk associated with years of employment in a group of five central mines in Thetford-Mines but not in peripheral mines of the same region. She concluded that these observed differences within Thetford-Mines and between Thetford-Mines and Asbestos was related to mineral types and related to geological properties of each group of mines. However, this issue was not investigated.

1.2.4. Lung fibre content of Québec miners and millers

Pooley[60] examined the lung tissue of 20 asbestosis cases from the Québec mining industry. Tremolite fibre was found in 11 of the cases, often in large amount, and crocidolite and amosite fibres were found in two of the cases from Asbestos. He also reported that, in seven cases, non-chrysotile fibres were found to outnumber the chrysotile fibres. Pooley did not measure the size of the fibres and made no direct comparison between the two mining regions in regards to the concentration of fibres.

Rowland et al.[61] found a similar quantity of chrysotile and tremolite in the lung tissue of 47 cases. They suggested that chrysotile was probably removed from the lung of Québec miners while tremolite was retained. Later, it has been shown that chrysotile has a much shorter half time than tremolite in the pulmonary system.[41,49]

In 1983, Churg [62] compared the lung fibre content of two groups of miners of Québec chrysotile industry, with and without asbestosis. The lungs of asbestosis cases retained higher concentrations of chrysotile, tremolite and total fibres. However, the dimension of fibres were comparable between the two groups. Churg et al.[63] also investigated the lung fibre content of six mesothelioma cases from Thetford-Mines. They found that, on average, the lungs of these cases retained higher concentrations of chrysotile and tremolite than cases without mesothelioma.

Case and Sébastien [43] compared the lung fibre burden of cases with occupational and environmental exposure to asbestos fibres in Québec mining regions. The concentration of asbestos bodies among the residents of Québec mining regions was intermediate between an occupationally exposed group and a reference group. Also, he showed that the lungs of cases from Thetford-Mines retained higher proportions of tremolite (72%) than that among cases from Asbestos (39%). Commercial amphiboles were absent from lung of cases from Thetford- Mines. A large number of occupational cases (17/23) from Asbestos had crocidolite and/or amosite in their lungs. The lung amosite and crocidolite concentrations varied substantially among workers. Case and Sébastien [64] attributed the presence of crocidolite in the lungs of workers from Asbestos to the historical fact of asbestos fabrication and asbestos quality control testing using imported amphibole in Asbestos.

To examine the relationship between fibre type, concentration, and size with the presence of various lung diseases, Churg et al.[65] examined the lung tissue of 94 cases from Thetford-Mines. They found that mesothelioma and asbestosis were strongly associated with tremolite content, whereas pleural plaques and lung cancer showed no relationship to tremolite burden.

Dufresne et al.[66] investigated the lung fibre content of 12 mesothelioma cases from Asbestos. They reported the presence of crocidolite in seven cases. In another study, Dufresne et al. [67] found a tendency for the amphibole fibres to have a higher length to

diameter ratio among cases from Asbestos with lung cancer and asbestosis in comparison with cases showing asbestosis and no lung cancer from the same mining region.
The only study in which the lung fibre content of cases of two mining regions of Québec were directly compared for fibre type, size, and concentration was conducted by Dufresne et al.[68]. This study, was limited to 21 mesothelioma cases. The presence of crocidolite and amosite in the lungs of cases from Asbestos was confirmed. The results of fibre dimension and comparison showed that the length of tremolite fibres longer than 5 μm was higher among cases from Thetford-Mines.

Previous lung burden studies evaluated the relationship between asbestos-related diseases and lung-retained fibre. There have been limited attempts to directly compare the lung-retained fibre of cases from Thetford-Mines and Asbestos for fibre concentration and fibre dimension, in particular. Only in very recent studies was a clear stratification for fibre length used.[67,68] In the studies in which all fibres were counted with no classification for fibre length, most of the analytical effort was devoted to short fibres since 70% to 90% of fibres were shorter than 5 μm.[43,64]

1.3. LEVEL OF ASBESTOSIS AND LUNG FIBRE CONTENT.

1.3.1. Asbestosis

Asbestosis is a fibrotic interstitial lung disease which occurs after a long-term and heavy exposure to asbestos fibres. The pulmonary fibrosis that characterized asbestosis is the end stage of a long inflammatory process, which starts as alveolitis and develops at the point of maximum fibre deposition such as respiratory ducts bifurcations.[69] Under usual exposure conditions, the onset of symptoms and radiographically visible fibrosis rarely occurs earlier than 15-20 years from first exposure.[3,9,70] Asbestosis manifests itself radiologically as irregular linear opacities, primarily at the bases and the periphery of the lungs becoming visible in the mid and occasionally upper zones of the lung. Many patients with asbestosis are asymptomatic. In those with symptomatic diseases, the most frequent complaints are dyspnea and cough.[9] Asbestosis reduces the total lung capacity and the vital capacity of the lung as well as the diffusing capacity in advanced stages.[9] Asbestosis is a progressive disease, and it may continue to progress many years after exposure has ceased.[3,9] The histological hallmarks of asbestosis are the presence of an excess amount of collagen in the pulmonary interstitium together with asbestos bodies in the histological sections.[9,71]

1.3.2. Assessment of asbestosis

Asbestosis has a wide range in regard to both progress of fibrosis from the initial site (i.e.; severity) and the percentage of bronchioles afflicted by fibrosis (i.e. extent). [9,72] There is a divergence of opinions among pathologists for the number of asbestos bodies required for diagnosis of asbestosis.[73] To evaluate the degree of fibrosis, both **severity** and **extent** of asbestosis can semi-quantitatively or quantitatively be graded by histological examination of tissue. A number of protocols have been published for the histologic grading of asbestosis.[74,75] One of current grading schemes is that proposed by the College of American Pathologists and the National Institute for Occupational Safety and Health (NIOSH).[72]

According to this procedure the presence of asbestos bodies in the histological section is a condition for diagnosis of asbestosis. This condition , however, has been criticized by some investigators on the basis of the differential tendency for fibres to form asbestos bodies and the inadequate sensitivity of this approach to search for and grade asbestos bodies.[76] The grading scheme will be discussed in chapter 3.

1.3.3. Level of asbestosis, fibre type, and exposure parameters

The level of asbestosis associated with asbestos exposure varies depending on fibre type and the industrial setting or process where exposure occurs.[77] Specific mineral parameters such as fibre type, fibre size, and chemical composition of fibres can affect the level of asbestosis. The relationships between the level of asbestosis, mineralogical properties of fibres, and exposure parameters have been widely investigated, and are reviewed. The review is presented separately for Québec chrysotile workers and for workers in other asbestos industries around the world. A review of the literature on the association between the level of asbestosis and fibre dimension is also presented separately.

1.3.3.1. Level of asbestosis and lung fibre content outside Québec

The first studies on human asbestosis failed to demonstrate any clear correlation between the level of fibrosis and the lung asbestos content.(78,79) Ashcroft and Heppleston(80) in 1973, studied the relationship between the level of asbestosis and lung fibre content among 30 mesothelioma cases from various occupations. The result of that study, in which asbestosis was categorized into three levels, showed that in, "mild" and "moderate" asbestosis there was a progressive increase in concentration of asbestos fibres, both coated and uncoated, with increasing degree of fibrosis. However, in severe asbestosis no correlation existed between the fibre concentration and the form or extent of the pathological reaction.(80) The authors concluded that additional factors other than tissue burden must be involved in progression from "moderate" to "severe" asbestosis. Ashcroft and Heppleston(80) also showed that the diameter of fibres was not associated with the level of fibrosis. Whitwell et al.(81) found a progressive increase in median total coated and uncoated fibre count from patients with "mild" to "severe" fibrosis. Warnock et al.(82) graded the level of asbestosis based on macroscopic visual inspection and found no apparent correlation between the level of asbestosis and total fibre content assessed by TEM.

Wagner et al.(83) investigated the relationship between asbestosis grade and lung fibre content among 235 workers whose death was related to asbestos exposure. Asbestosis was graded by a four-level grading scheme, and fibre concentration was expressed as million of fibres per gram dry lung. They found an association between asbestosis grade and amphibole concentrations, but not chrysotile. Wagner et al.(84) also analyzed the lung tissue of 189 Naval dockyard workers, who were exposed to commercial amphiboles. They showed a correlation between grade of asbestosis and both the coded exposure and crocidolite concentration. Cases with "minimal" asbestosis contained significantly more amphibole, both crocidolite and amosite, on the average, than those with no evidence of asbestosis. Non-asbestos fibre concentrations were not associated with the grade of asbestosis. They also showed that the counts of the amphibole fibres crocidolite and amosite increase with the severity of asbestosis, whereas the fibre count after adjusting for amphibole presence, of chrysotile fibre and other non-asbestos fibres appeared to be unrelated to asbestosis grade . Wagner et al.(85) also compared the lungs of 36 former workers at an east London asbestos factory exposed to crocidolite, amosite and chrysotile who had died from asbestos related disease with 54 control patients, correlating the level of asbestosis with the type and amount of mineral fibre in the lung. Crocidolite, amosite, and total fibres content were strongly associated with asbestosis, lung carcinoma, and mesothelioma. The concentration of chrysotile and non-asbestos fibres were not associated with the grade of asbestosis. They did not investigate the relationship of fibre size and the level of asbestosis in their studies. No statistical parameters were provided in these studies..

Roggli et al.(86) studied the relationship between the lung fibrosis and the lung fibre content of 110 cases who were exposed to various type of asbestos fibres and had an asbestos-related disease. The level of asbestosis was quantitatively assessed by a grading method from 0 to 12. The best correlation was obtained for the histological grade of asbestosis versus the total fibre count obtained by scanning electron microscope (SEM) as well as uncoated fibre content ($r=0.57$, $p<0.05$) and ($r=0.56$, $p<0.05$), respectively. There was no significant correlation between the histological grade of asbestosis and the duration of exposure ($r=0.23$, $p>0.05$). Roggli et al.(86) also reported an independent correlation between the histological grade of asbestosis and the smoking history by pack-year ($r=0.23$, $p<0.05$). Fibre size and its relationship with the level of asbestosis was not studied by them. In another investigation, Roggli(87) evaluated the asbestosis grade and its relationship with lung mineral contents among 36 asbestos factory workers exposed to commercial amphiboles. He found a statistically significant relationship between both uncoated fibre content and total (coated and uncoated) fibre content and the histologic score ($r=0.46$, $p<0.01$ and $r=0.44$, $p=0.01$, respectively). There was no significant association between fibrosis score and patient age ($r=0.12$, p=NS), duration of exposure ($r=0.06$, p=NS), pack year smoking ($r=0.22$, p=NS) and asbestos body concentration ($r=0.26$, p=NS)

In 1987, Green et al.[88] evaluated the lung mineral content and the asbestosis grade (0-12) of 61 former employees of an asbestos packing materials plant, exposed to chrysotile. There was a significant correlation between the cumulative exposure and the level of asbestosis. A similar relationship was observed between the total lung fibre concentration and the fibrosis score. The total tremolite content showed a better correlation with the asbestosis grade than did the chrysotile content.

A histological examination of the lungs of 89 dead asbestos cement workers by Johanson et al.[89] showed a clear association between the number of ferruginous bodies and the level of asbestosis (0-4). No association between smoking history, duration of exposure and cumulative index of exposure and level of asbestosis was observed among cases.

Churg et al. [90] examined the lungs of workers with exposure to amosite. The level of asbestosis, graded from 0-4, was positively correlated with the lung amosite concentration ($r=0.62$, $p<0.01$).

Lung tissue from 76 deceased asbestos cement workers who were exposed to chrysotile asbestos and other commercial amphiboles was examined by Albin et al.[91] There was a weak correlation between fibre count or mass of amphiboles and the level of asbestosis. No correlation was found between either chrysotile or non-asbestos fibres and fibrosis ($\tau<0.1$, $p>0.2$). The concentration of tremolite displayed a better relation with the level of asbestosis($\tau=0.16$, $p=0.06$).

Green et al. [77] reported the analysis of lung tissues of 59 former textile workers principally exposed to chrysotile. Pulmonary fibrosis was graded from 0 to 12 according to the method of Craighead et al.[72] The level of asbestosis was correlated with cumulative exposure ($r=0.56$, $p<0.05$). There was a significant correlation between tremolite ($r=0.64$, $p<0.05$), chrysotile ($r=0.51$, $p<0.05$) and total fibre ($r=0.62$, $p<0.05$) concentrations. The concentration of non-asbestos fibres was not associated with the level of asbestosis ($r=0.0006$, $p>0.05$). The relationship between fibre size and the fibrosis score was not examined.

In conclusion, it appears that in most studies there was a correlation between the level of asbestosis in patients with asbestosis and lung tissue mineral fibre content. In most studies, amphiboles were better associated with level of asbestosis. Few studies, [77,91] addressed the relationship between asbestosis and lung fibre content among workers exposed principally to chrysotile.

1.3.3.2. Level of asbestosis and lung content among Québec chrysotile workers

The only investigation that specifically explored this relation among Québec chrysotile miners and millers was conducted by Churg et al.[92] In this study 21 specimens of autopsied lungs of chrysotile miners and millers of Thetford-Mines were analyzed and the correlation between the level of asbestosis with fibres concentration and other morphological characteristics of fibres were investigated. They found a positive correlation between concentration of tremolite fibres and the asbestosis score ($r=0.61$, $p<0.01$). The relationship between the concentration of chrysotile and asbestosis was less pronounced, but it was still significant ($r=0.44$, $p<0.05$).

1.3.4. Level of asbestosis and fibre dimensions

Churg et al.[92] investigated the relationship between the level of asbestosis and mean fibre length, mean fibre diameter, surface area, and total sample fibre length in his study on 21 former miners and millers of Québec. No correlation was found for chrysotile fibre size, surface area, or mass ($p>0.05$, all variables) and the level of asbestosis. Tremolite mean fibre length ($r=-0.7$, $p<0.001$), aspect ratio ($r=-0.6$ $p<0.003$), were negatively correlated with fibrosis grades. Churg et al. [90] suggested that the short fibres were actually more fibrogenic than were long fibres after observing the same trend among 20 former insulator workers exposed to amosite ($r=-0.26$ $p<0.01$ and $r=-0.28$ p=NS, for amosite fibre length and diameter respectively). However, the level of asbestosis was positively correlated with total fibre length ($r=0.37$ $p<0.001$), total fibre width ($r=0.33$, $p<0.003$), and total fibre aspect ratio ($p=0.4$, $p<0.001$). Their suggestion with regard to the effect of fibre length and the fibrogenicity of fibres was in contrast with the results of numerous in vitro [37,38] and animal [24-36] toxicological experiments studies and evidence from the McDonald et al.[40] study. A separate analysis of lung tissue in the same workers by Case[41] came to an opposite conclusion, the geometric mean concentration of fibres longer than 8 µm was higher among asbestosis cases than that among non-asbestosis cases ($p<0.01$). Later, two separate studies on 94 cases from Thetford-Mines and 144 former insulators by Churg et al.[65,93] showed that tremolite and chrysotile fibre dimensions did not discriminate between cases with and without asbestosis. However, the tremolite fibre length and aspect ratio were significantly higher among cases with pleural plaques than those without pleural plaques. The study did not investigate the correlation of fibre type and dimension with level of asbestosis as a continuous variable.

1.4. CAUTIONS IN INTERPRETATION OF THE RESULTS

The wide variety of preparation techniques and analytical methodologies that have been employed by various investigators makes it difficult to compare results from one laboratory to another. Inter-laboratory and intra-laboratory variations demonstrated some striking differences among and within laboratories, even when the same samples were analyzed. [94]. The analysis of lung fibres can be carried out by either SEM or TEM. The latter provides a higher concentration due to higher resolution. One important source of variation, complicating the comparison of the results, is the different cut-off for classification of fibres based on their length in previous studies.[65,67-68,93,95,96] As an example, Churg et al. [90, 92] analyzed all fibres with no classification for fibre size. Case and Sébastien [43] and Sébastien et al.[96] included fibres longer than 5µm and Dufresne et al. [67,68]used fibres longer and shorter than 5 µm.

Methodological variations mentioned above are also relevant factors when the relationship between the level of fibrosis and lung fibre content is compared. First, the type and dimension of asbestos fibre and level of exposure are different from one industry to another. Second, inter-laboratory variations related to both lung fibre analysis and asbestosis grading also complicate any comparison. Several examples of these variations were encountered in previous investigations. The inclusion of all fibre lengths in previous lung fibre studies might have affected the results according to one author.[41]

Level of exposure, type of fibre, and their mineralogical properties vary in different industries. This variation even exists between the two mining regions of Québec and even within Thetford-Mines.[47,59] Therefore, the interpretation of the results of the above-mentioned, often contradictory studies, requires caution. Undoubtedly, mineralogical composition of the ore body is the main determinant of the type of minerals that can be found in the airborne dust cloud during a mining operation. In addition to the type of minerals particles, the morphology including size parameters of minerals, in particular, fibres length might be different from one mining region to another. Previous investigations in Québec chrysotile mining regions through epidemiological studies and lung burden analysis further emphasized the possible role of mineral properties in relationship to prevalence and severity of lung diseases.[40,59,68,96]

In conclusion, lack of a consistent method of specimen preparation for both lung mineralogical analysis and histological examination of lung fibrosis as well as the lack of classification of fibres based on their length makes any comparison of the results of previously published studies difficult.

1.5. CONCLUDING REMARKS

The relationship between asbestosis and factors affecting its severity should be studied separately for each industry. Presently, there is a paucity of knowledge in regards to severity of asbestosis among Québec miners and millers, in particular for miner and millers at Asbestos, many with possible exposure to commercial amphiboles. Moreover, the associations between the level of asbestosis and smoking and other exposure parameters such as duration of exposure, years since last exposure, and cumulative exposure with pulmonary fibrosis remains incompletely explored. Finally, the effect of size of fibres, in particular fibre length, on the progression of pulmonary fibrosis deserves further investigation. Since contradictory results were observed in previous studies, any new investigation among miners and millers of Québec requires a well-defined strategy considering an appropriate laboratory procedure that would allow a better determination of fibre size categories and asbestosis grade.

The generalization of previous findings from other asbestos industries on the relation between asbestosis and lung fibre content in Québec miners and millers might not be scientifically or practically appropriate. This is due to 1) wide methodological and instrumental variations between previous studies, and 2) considerable difference between type, pattern, and level of exposure of Québec miners and millers to asbestos fibres and the workers of other industries.

Chapter 2

RESEARCH OBJECTIVES AND HYPOTHESES

2.1 OBJECTIVES OF THE STUDY

A better understanding is needed regarding: 1) the lung fiber concentration and dimension in miners and millers of the Thetford-Mines and the Asbestos chrysotile mining regions given the different incidence of pulmonary diseases between these two groups; and, 2) the relationship between fibre concentration and dimension with the severity of asbestosis and also the latter with exposure parameters among Québec chrysotile miners and millers. Such information on lung dust fibre content and its relationship with the disease may help in understanding the fibrogenic effects of fiber type and dimension in the progression of the disease. The relationship between the retained lung fibre data and severity of disease should show whether lung fiber concentration can be used as a surrogate for the cumulative index of exposure and as an indicator of the biologically effective dose in epidemiological studies.

2.1. Objectives of the study

2.1.1. To investigate the lung fiber content of two groups of Québec chrysotile miners and millers in regards to fiber type and concentration as well as fiber dimensions such as length, diameter and aspect ratio.

It has been shown that, despite exposure of miners and millers of Asbestos to crocidolite and amosite fibers, miners and millers from Thetford-Mines have had higher incidence of respiratory diseases.[12,15,16,50,56-59] While the literature on lung burden studies on these two populations included a small number of subjects, no direct quantitative comparison has been made between these two groups in regard to fiber type and, in particular, fiber dimension. [60-68] Apparently, there is a mineralogical difference between the two mining regions which may have resulted in exposure to different types of fibers and associated minerals with different morphology.[43,46,47,57,59] A comprehensive lung burden study accompanied by using adequate numbers of subjects from these two regions along with a precise classification for fiber dimension, can provide new insight into the possible differences between the two mining regions.

2.1.2. To evaluate the association between fiber type, concentration, and dimension (length, diameter and aspect ratio) and the severity of asbestosis among two groups of miners and millers of the province of Québec with two distinct exposure patterns.

Pathogenic effects of asbestos fibers and their role on the genesis of pulmonary fibrosis (asbestosis) have been studied by many investigators around the world. [71,77,80-92] However, this relationship has been inadequately addressed for populations of Québec miners and millers.[92] Presently, there is evidence based on experimental studies on laboratory animal [25-31] and in vitro investigations [36,37] that long fibres may have higher toxicity. The effect of long fibres was not investigated in previous human lung fibre burden studies. In general, relation between fiber size and the level of asbestosis requires further investigations.

The relationships between fiber properties and level of asbestosis among the two distinct populations of chrysotile miners and millers of Québec can reveal new facts on the fibrogenic effect of various asbestos fiber on the human respiratory system. For example, Thetford-Mines workers had a higher exposure to tremolite fibers and Asbestos workers had a history of exposure to crocidolite and amosite. The possible effects of fiber dimensions, in particular fiber length, in the progression of pulmonary fibrosis can also be investigated

2.1.3. To investigate the relationship between exposure and demographic parameters and the severity of asbestosis and their possible interacting or confounding effects with lung fibre burden variables.

In the assessment of the severity of asbestosis among Québec miners and millers, exposure parameters should be considered in addition to fiber properties. These exposure parameters such as duration of exposure, age at death and number of years since last exposure, as well as smoking will be studied in this survey. Possible interaction effects or confounding effects of these variables in asbestosis severity will be investigated.

2.2. RESEARCH HYPOTHESES

2.2.1. Former miners and millers from Thetford-Mines have been exposed to fibers in higher concentration. Fibres retained in the lungs of workers from Thetford-Mines have greater length and aspect ratio than those retained in the lungs of miners and millers of Asbestos .

2.2.2. There is a direct relationship between fiber concentration and severity of asbestosis. This relationship is different among miners and millers from Asbestos with their history of exposure to crocidolite and amosite. Crocidolite and amosite concentrations may have a positive correlation with the level of asbestosis among subjects from Asbestos.

2.2.3. There is a direct relationship between fibre length, diameter and aspect ratio and severity of asbestosis.

2.2.4. Concentration of fibers within a specific size category might be a better predictor of asbestosis severity than the others. Therefore, lung fiber concentration of a specific size category may be used as an indicator of exposure.

Note:

In this study, I have attempted to find determinates of interstitial fibrosis in asbestos miners and millers. The grading scheme for this fibrosis used was identical to one designed for grading of "asbestosis", which is a specific form of interstitial fibrosis always caused by asbestos exposure. By definition, true pathologic asbestosis requires identification, within fibrotic lung section, of typical asbestos bodies. My use of the term is however best regarded as nonspecific, even though most cases would be found to have true asbestosis on examination of sufficient tissue sections.

Chapter 3

SUBJECTS, METHODS, AND MATERIALS

3.1. SUBJECT SELECTION CRITERIA

A birth cohort (1891-1920) of approximately 11,000 workers employed in the chrysotile mining and milling industry in the province of Québec was constructed in 1966. By the end of 1993, 8000 deaths were reported among the members of the cohort.[15] Among 9780 miners and millers traced up to 1992, there had been 3850 deaths from all causes among cohort members from Thetford-Mines; the corresponding figure being 3770 death for the workers from Asbestos.[16] The average rate of autopsy among cohort members was 17% before 1975.[73] A similar rate is assumed to be valid for subjects after this date.[97] Based on the study objectives and available lung tissue samples, it was decided to include between 80 and 100 lung autopsy specimens (40 to 50 subjects from each group) of chrysotile miners and millers either in or outside the birth cohort.

To be included in the study a subject must meet each of the following criteria;

1) been employed at either Asbestos or Thetford-Mines (including mines, mills, and a small asbestos product factory at Asbestos), with a history of exposure to asbestos,

2) be deceased and autopsied,

3) have sufficient lung tissue for mineralogical analysis and histological examination, and

4) either medical or employment records are available to determine exposure variables and smoking habit.

Thetford-Mines Subjects: Approximately 200 autopsies were conducted during 1989-1991 at the Centre Hospitalier Régional de l' Amiante. Ninety-five cases out of 200 were former employees of Thetford-Mines for which all tissue samples were available. Cases with inappropriate lung tissue samples (e.g. dried specimen) and subjects for which occupational records were not available were excluded (approximately 25 subjects). Lung tissue samples and occupational histories were subsequently obtained for the remaining 70 subjects. Initially, a total number of 50 subjects out of 70 were included in the study. Later, five subjects were excluded due to the presence of large tumors in the tissue specimen. Eventually 45 subjects from Thetford-Mines were included in the study. (Table 3.1)

Asbestos Subjects: To include a similar number of subjects from the Asbestos (including both mine, mill, and factory workers), the same selection criteria were employed for subjects autopsied from 1983 to 1991 in the University Regional Hospital at Sherbrooke, Québec. The exact number of autopsies between 1983 and 1991 was not known. However, based on available information from other calendar years, the author estimates that the total number of autopsies during 1983-1991 could not be more than 100.[97] The lung tissue of many subjects was not available. From the approximately 45 subjects which had lung tissue available, 41 subjects had complete occupational records and were included in the study.

The total number of subjects from both regions included in this study was 86. Among the subjects from Thetford-Mines, 24 of the 45 were born prior to 1920, and were therefore, members of the Québec birth cohort. Among the subjects from Asbestos, 24 of the 41 were born within the years specified in the epidemiological birth cohort. (Table 3.1)

Table 3-1. The procedure for the selection of subjects.

	Asbestos	**Thetford-Mines**
Total number of cohort members [15]	10939	
The number of cohort members form each mining area [15]	5302	5637
Hospital	University Hospital Sherbrooke	Asbestos regional Hospital center
Number of deaths until the end of 1992 [58]	3331	4125
Rate of Autopsy*	17%	17%
Years of death of subjects studied	(1983-1992)	(1989-1991)
Number of autopsies (Miners and Millers)	<100**	95
Number of autopsies for which lung samples were available	41	95
Number of usable lung samples	41	70
Number of subjects initially included	41	50
Number of subjects excluded (see text)	0	5
Final number of subjects included	41	45
Number of subjects born 1891-1920	24	24
Total number of subjects included in this study	86	

* before 1975 [61,73]

** estimating based on available information on autopsy rate of different years and actual numbers of autopsies (n=28) of cohort from 1979 to 1984 [97]

3.2. LUNG TISSUE SPECIMENS

Forty-five formalin-fixed lung tissue specimens belonging to subjects from Thetford-Mines were sagittal slices of either right or left lungs. Therefore, adequate lung tissue was available for all subjects for both histological examination and mineralogical analysis. Forty-one formalin-fixed wet lung-tissue autopsy samples were available for the subjects from Asbestos. For 34 of these subjects, between 6 and 8 tissue blocks (6 to 8 cm^3 each) fixed in filtered formalin (10%) were available, adequate for both histological examination and mineralogical analysis. A small amount of "wet" formalin-fixed tissue samples was available for 7 of the subjects, adequate for mineralogical analysis only. Therefore, 7 paraffin-block sections were obtained for histological examination of these subjects (Courtesy of Dr. S. Massé, Centre Hospitalier Rouyn-Noranda, Québec). To determine whether or not tissues were appropriate for histological examination and mineralogical analysis, visual examinations were conducted. The selection of lung tissue specimens was supervised by Dr. B. W. Case, a pathologist and professor in the Department of Pathology, McGill University. All containers were marked by autopsy numbers and stored in a contaminant-free laboratory.

3.3. SECTIONING OF THE LUNG TISSUES

Since the degree of pulmonary fibrosis and the concentration of tissue mineral fibres may vary in different anatomical sections,[8,77] six sections from various areas of the lung parenchyma were selected for histological examination and two sections were used for fibre analysis. Due to this variation, it was necessary to select the sections in a consistent manner from all subjects. To avoid systematic error due to site-selection bias of sections which are not anatomically identical, an established sectioning method was used as is described in the following paragraphs.[72] The sectioning was performed in the autopsy room, Department of Pathology, Royal Victoria Hospital, under the supervision of Dr. B. W. Case.

3.3.1. Subjects from Thetford-Mines

Using a laboratory scalpel, the following sections were cut from sagittal slices of lung specimen from each of the subjects from Thetford-Mines. Figure 3.1. demonstrates a schematic presentation of tissue sectioning .

(1) Upper lobe, mid parenchyma
(2) Upper lobe, peripheral subpleural
(3) Middle lobe, central
(4) Middle lobe, peri-hilar
(5) Lower lobe, mid-parenchyma
(6) Lower lobe, peripheral subpleural

All six sections were placed in plastic cassettes and numbered from 1 to 6. The cassettes were placed in filtered formalin (10%) and stored in the laboratory cabinets for subsequent histological examinations. Two sections adjacent to upper lobe, mid-parenchyma section (section 1) and lower lobe, peripheral subpleural section (section 6) were cut and placed in separate containers for mineralogical analysis.

3.3.2. Subjects from Asbestos

Whole lung slices were not available for subjects from Asbestos. For these subjects, having 6 to 8 wet tissue blocks, 6 sections for each subject were selected randomly for histological examination, placed in plastic cassettes and numbered from 1 to 6. Among those blocks, a sub pleural section and a parenchymal section were identifiable from their pathologic appearances and therefore selected and placed in separate containers for subsequent mineralogical examination. The selection of these two sections was performed according to the pathologist's instruction. A subpleural section has pleural tissue lining the exterior surface, and the parenchymal section is usually the largest available section among all sections with no pleural tissue on any surface. Figure 3.2. shows the methods of selection and sectioning of lung tissues for the subjects from Asbestos.

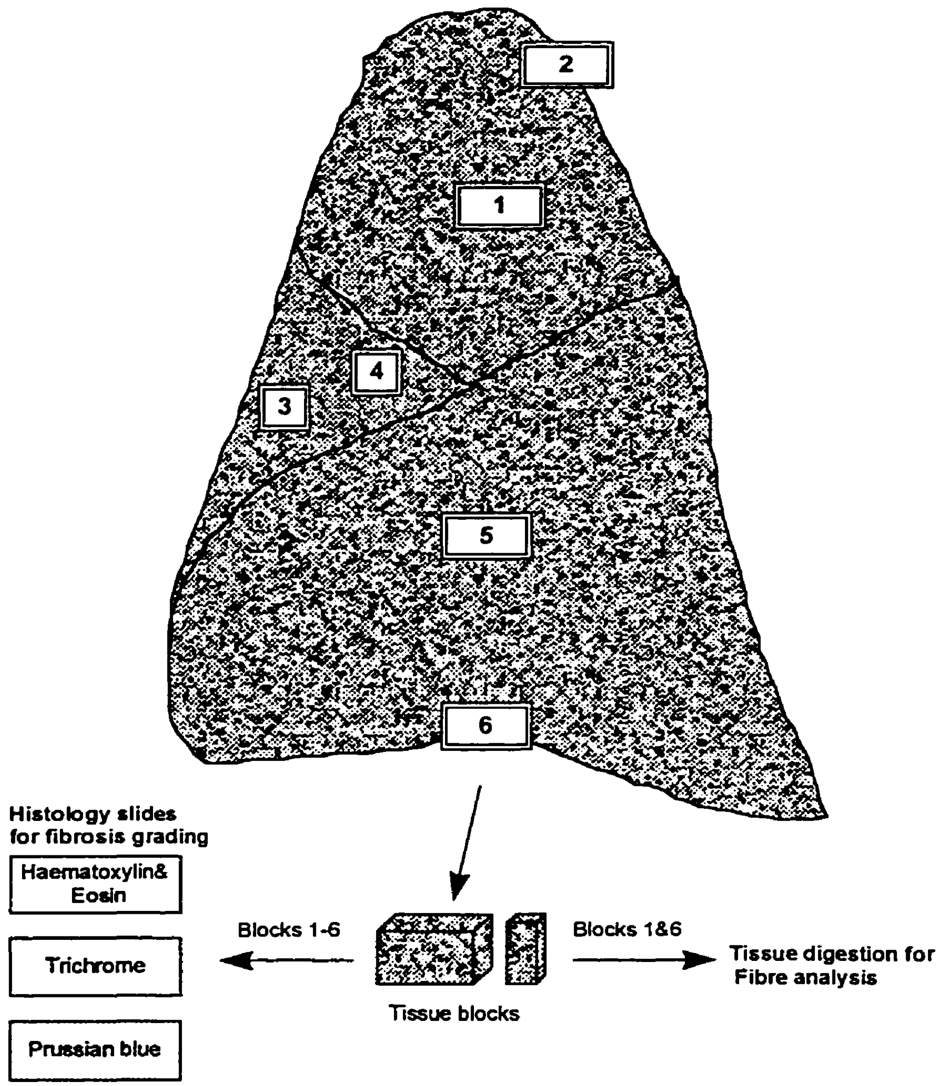


Figure 3.1. Schematic presentation of tissue sectioning from sagittal lung slices for cases from Thetford Mines; 1) upper lobe, mid parenchyma, 2) upper lobe, peripheral subpleural, 3) middle lobe, central 4) middle lobe, peri-hilar, 5) lower lobe, mid parenchyma, and 6) lower lobe, peripheral subpleural.

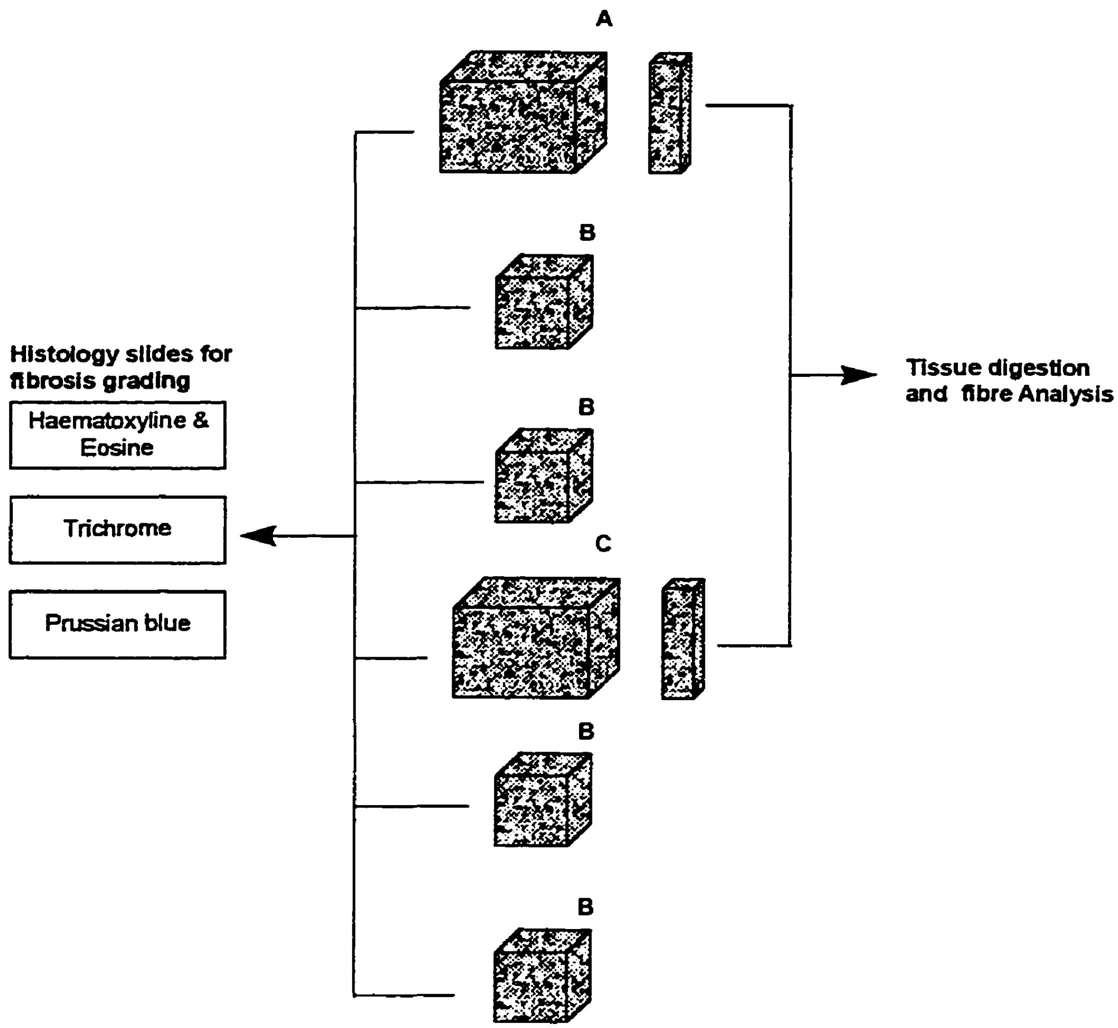


Figure 3.2. Schematic presentation of tissue sectioning from available tissue blocks of subjects from Asbestos. A) the largest block without attached pleural tissue (i.e. a parenchymal section), B) Four other smaller blocks, and C) a sub -pleural block lined by shiny pleural tissue.

3.4. HISTOLOGICAL EXAMINATION AND GRADING OF ASBESTOSIS

To investigate any association between mineralogical parameters and pulmonary fibrosis, the extent and severity of tissue fibrosis must be quantitatively evaluated.[77]. This was done through histological examination of lung tissue taken from the same tissue blocks as those used in mineralogical analysis.

3.4.1. Slide preparation and staining

Slides were prepared in the Histology Laboratory, Department of Pathology, McGill University. Tissue blocks were embedded in the paraffin using an embedding machine (LEICA Inc. Model. EG1160, Heidelberger, Germany) and then sectioned with an average thickness of 4 µm by the use of a microtome (Microm lab. Model HM330. Walldrof, Germany). Subsequently, the sections were dehydrated by rinsing in absolute ethanol four times (10 minutes each time) and four times in xylol. After rinsing the slide with tap water, staining was performed for five minutes. The slides were rinsed again in the tap water after staining and differentiated by dipping the slides into 1% HCL and water several times.

Three slides were prepared for each tissue block. Each slide had a different stain; either haematoxylin and eosin, trichrome, or Prussian blue. Thus, 18 slides were prepared for each subjects. The exception was the seven subjects from Asbestos for which only one paraffin block was available. For these subjects only three slides were prepared using of above stains. A total number of 481 tissue blocks were prepared. The total number of histology slides for microscopic examination of pulmonary fibrosis among 86 subjects from both mining regions was 1443.(Table 3.2)

Table 3.2. Number of tissue blocks and histology slides prepared for histological examination of pulmonary fibrosis for 86 subjects from Thetford-Mines and Asbestos.

	Asbestos n=41		**Thetford-Mines n=45**	
Type and number of specimens	Wet tissue	Paraffin block	Wet tissue	Paraffin block
	34	7	45	-
Number of tissue blocks made	204	7*	270	-
Number of slides	612	21	810	-
Total slides for each group	633		810	
Total number of slides for 86 subjects	1443			

n: Number of subjects
* one paraffin block was available for each case

3.4.2. Histological Grading of asbestosis

An evaluation of each slide was performed by pathologist Dr. J. Massé (Centre Hospitalier Rouyn-Noranda), using an optical microscope (Carson Group Inc. Olympus BX50. Ontario, Canada). Three different magnifications (100x, 250x, and 400x) were used for the examination of the slides. The grading scheme and the objectives of the study were discussed with the pathologist beforehand. The pathologist was blind to the geographical origin of the subjects and site of origin of the tissue blocks.

For each section, the following procedures were performed:

3.4.2.1. Grading for fibrosis severity

Identification and grading of fibrosis was performed using the haematoxylin and eosin stained slides. The grading scheme used was that of the College of American Pathologists and the National Institute of Occupational Safety and Health (NIOSH) originally designed to assess asbestosis. [72] This five point grading scale was used in this study for fibrosis severity and is presented in Table 3.3.

Table 3.3. Microscopic grading scheme for the severity of fibrosis.[72]	
GRADE	DESCRIPTION
0	no fibrosis is associated with bronchioles
1	fibrosis involves wall of at least one bronchiole with or without extension to the septa of adjacent alveoli
2	Fibrosis appears as in grade 1, plus involvement of alveolar ducts or two or more layers of adjacent alveoli; there is still a non fibrotic septum between adjacent bronchioles
3	Fibrosis appears as in grade 2, but with coalescence of fibrotic changes such that all alveoli between at least two adjacent bronchioles are thickened. Fibrotic septa; some alveoli may be obliterated completely
4	Fibrosis appears as in grade 3, but with formation of new spaces of a size larger that alveoli, ranging up to 1 cm; this lesion is called "honeycombing". Spaces may or may not be lined with epithelium.

3.4.2.2. Grading for the extent of fibrosis

The extent of fibrosis was assessed and graded by estimating the proportion of the respiratory bronchioles affected by the disease. The grades pertain to the number of bronchioles in the slide involving any degree of fibrosis as presented in Table 3.4. Haematoxylin and eosin (H&E) and trichrome stains were used for this step.

For each slide, the grade of severity and extent were multiplied. The resulting numbers for all slides belonging to a subject were averaged. This average value for each subject was used as fibrosis score to represent the existing level of interstitial fibrosis.

For the seven subjects from Asbestos, having only one paraffin block available, the severity and extent scores for that specific slide were multiplied. This value was used as the fibrosis score.

Table 3.4. Microscopic grading scheme for the extent of fibrosis. [72]

GRADE	DESCRIPTION
1	only occasional bronchioles are involved, most show no lesions
2	more than occasional involvement is seen, but less than half of all bronchioles are involved
3	More than half of all bronchioles are involved.

3.4.2.3. Histological grading of asbestos bodies

The grade of asbestos bodies was semi-quantitatively estimated by the use of slides stained with haematoxylin, eosin and Prussian blue. Asbestos bodies were graded on a scale of 0-3 using a method reported by Wagner et al.[83] Asbestos bodies were recognized by their clear straight central cores and beaded ferruginous coating. When asbestos bodies were few or absent on sections stained with haematoxylin and eosin, Prussian blue iron stains were used for their greater sensitivity. A semi-quantitative subjective rating of 0 (no bodies present), 1+ (one or occasional asbestos bodies), 2+ (moderate number of asbestos bodies), and 3+ (numerous easily detectable of asbestos bodies) was used. The individual scores for all slides from a subject were summed up and an average score for each subject was obtained.

3.5. LUNG TISSUE PREPARATION FOR FIBRE COUNTING

3.5.1. Tissue digestion and ashing

Upper lobe, central (section 1) and lower lobe, subpleural (section 6) specimens were used for the mineralogical part of the study using a digestion and low temperature ashing method (Figure 3.1). The method used for preparation of the lung tissue samples is demonstrated schematically in Figure 3.3. The tissue specimens were wet and formalin-fixed. Since the concentration of fibres in the lung tissue is expressed as number per milligrams dry lung tissue, 0.5 gram of wet tissue taken from both sections was used to calculate the dry/wet tissue weight ratio. The wet tissue sections were weighed with an electrobalance (Mettler Model AE 100. Zurich, Switzerland) and placed in a 70 °C oven overnight. Then, the tissue was weighed and the dry/wet ratio calculated. With a clean scalpel blade, tissue aliquots of approximately 50 mg from both sections were cut and digested in a 5.25% sodium hypochlorite solution (commercial bleach) in a 100-ml propylene centrifuge tube (Nalge Co. Model 3103-0050) at room temperature for 60 to 120 min. The volume of bleach was adjusted to a concentration of 1 mg of dry tissue per 1 ml of bleach. When the digestion was completed, the aliquot was then filtered through a 25-mm cellulose ester membrane filter (3 µm or 0.45 µm pore size) (Millipore, Massachusetts, USA). The volume of aliquot to be filtered, and the pore size of the membrane filter depended on the types of analysis, as indicated below.

For electron microscopic (EM) analysis of lung mineral dust, a 5-ml aliquot prepared as above was filtered through a membrane filter of 0.45 µm pore size. For optical microscopic analysis (OM) of ferruginous bodies, a volume of 25 ml was filtered through a filter of 3.0 µm pore size. After the filtration, each lung digest filter for EM analysis was then placed, particle-laden side face down, into a clean 30 ml glass bottle. The filters in the glass bottles were then ashed in a low temperature, oxygen plasma asher (LFE Corp. Model LTA 505. Massachusetts, USA) operated at 100 watts, with an O_2 flow rate of 10 cm^3 per minute for at least 3-5 hours.

After ashing, the ash in the glass bottles was resuspended with deionized and filtered water and gently sonicated for 5-10 minutes in an ultrasound bath (BRANSON, Model B12, Branson Cleaning Equipment Company, Connecticut, USA). For EM analysis, the suspension was

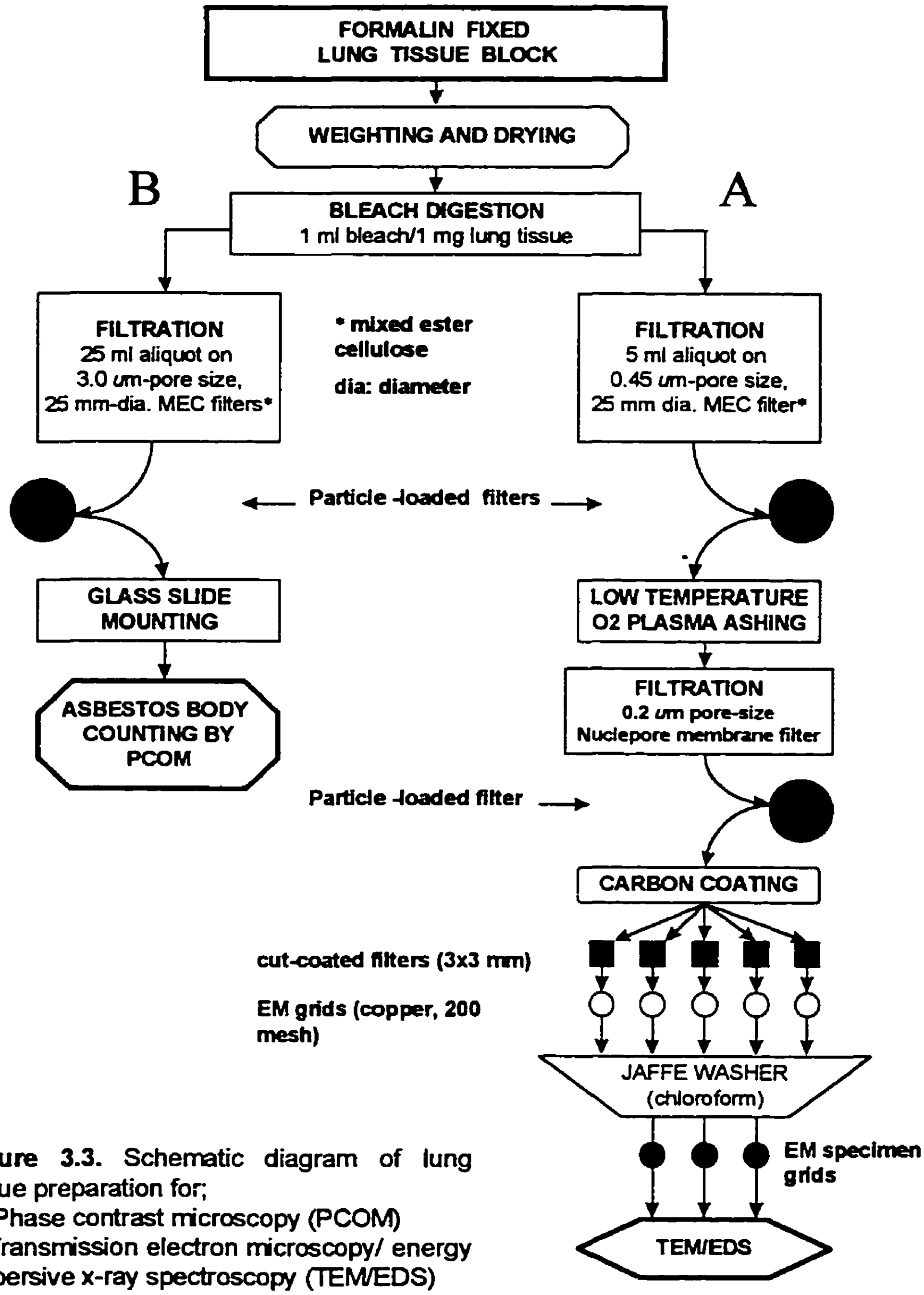


Figure 3.3. Schematic diagram of lung tissue preparation for;
A. Phase contrast microscopy (PCOM)
B.Transmission electron microscopy/ energy dispersive x-ray spectroscopy (TEM/EDS)

filtered through a 25-mm diameter and 0.2-μm pore size polycarbonate filter (Nuclepore, California, USA). The filters were then used to prepare the electron microscopic grids described in the next section.[98]

3.5.2. Electron microscopic (EM) specimen grid preparation

After carbon coating, the particle-laden polycarbonate filters from the above step were used to prepare the EM specimen grids by using the carbon replica technique (Figure 3.3). The polycarbonate membrane filters were first carbon coated in a vacuum evaporator (JEOL USA Inc. Model 4000 Massachusetts, USA). Five small squares of about 3x3 mm were then cut from the carbon-coated filter with a clean scalpel blade and each was placed on a 200-mesh copper EM grid (J.B. EM. Services Inc. Model Ve-5. Québec, Canada). The coated filters on EM grids were then cleared by chloroform in a Jaffe washer for 12-24 hours, leaving only the particles with carbon film on the grids. The specimen grids were then scanned with a transmission electron microscope (JEOL USA Inc. Model JEM-100CX II. Massachusetts, USA) to inspect the quality of the specimen grid. For each sample, four specimen grids with an even and discrete distribution of particles were chosen and kept in a grid box for further TEM analysis.[98]

3.5.3. Lung-retained fibre characterization

Two techniques of particle analysis were employed in the characterization of lung-retained fibre in this study. Transmission electron microscopy/energy dispersive spectroscopy (TEM/EDS) was used in the counting, the size measurement and the mineral characterization of each individual fibre. Phase contrast optical microscopy (PCOM) was utilized in the counting of asbestos bodies (AB).

3.5.4. Transmission Electron Microscopy/Energy Dispersive X-ray Spectroscopy

A JEOL 100CX electron microscope, in transmission mode, equipped with an X-ray energy dispersive spectrometer (EDS) (Princeton Gamma-Tech Inc. PGT Microanalysis

System, Model 4000T New Jersey, USA) was used in the counting, the size measurement, and the mineral characterization on the EM specimen grids.

An accelerating voltage of 80 kV with a magnification of 14,000 times was used for particle counting and measuring. At this magnification, an image of one millimeter on the fluorescent viewing screen of the EM is equivalent to 0.09 μm real dimension. The particle dimension was visually measured by comparing the particle size to the diameter of 10-mm and 50-mm concentric graticules at the middle of the fluorescent screen.

For analysis of the elemental composition, the TEM was operated at the same accelerating voltage and magnification as that used for particle sizing. The electron beam was condensed to achieve a beam diameter of about 1 mm focused on the particle image.

The display screen of the PGT System IV X-ray energy analyzer was set to display spectra in the range of about 0.95 keV and 7.8 keV. The system was set to collect the EDS spectrum of each particle for 30 seconds and subsequently stored them on eight-inch floppy magnetic diskettes. The dead time (the time required for the detector to recover sufficiently in order to accept the next pulse) of analysis was kept below 30% of clock time.

Identification of each element on the spectrum was based on KLM markers by the spectrum analyzer. A large energy peak, $K\alpha$, was determined first and then the secondary line, $K\beta$, with about 10% of the $K\alpha$ peak height.

Fibres were distinguished directly on the TEM screen by the operator based on the aspect ratio (length to diameter ratio) of 3:1. However, mineral type was determined by comparing the EDS spectrum of each single fibre with reference EDS spectrum after the end of analysis for each subject. The reference EDS spectrum of known standard mineral was supplied by the Registered Office of the Institute of Occupational Medicine Limited, Edinburgh, Scotland.[99] The EDS spectrum of four standard mineral fibres analyzed in the

Environmental Laboratory, Microscopy Unit at McGill University are shown in appendix (pages A-18 to A-21). Four different types of asbestos fibres; amosite, crocidolite, chrysotile, and tremolite were identified by comparing the collected spectra with the reference spectra. Other categories of fibre type, including other silicates, talc/anthophyllite and total fibres (sum of the fibre concentrations of various types), were also included.

The differentiation between tremolite and actinolite using EDS data can be difficult. However, this is not a serious problem, since there are no clear distinctions in biologic effect between the two minerals.[(8)] In contrast, the differentiation of talc and anthophyllite is an important issue, because these two minerals have very different biological activities.[(8)] Talc and anthophyllite can be differentiated using selected area electron diffraction, but this is a time consuming process that was not employed in this study since we expected a small number of talc and anthophyllite fibres to be detected in the lungs of this population.

3.5.5. Analysis of mineral fibres

The size of mineral fibres in the lung tissue is log-normally distributed. This fact implies that there is a much higher number of short fibres in the lung tissue than long fibres. Since the method of fibre counting used in any lung-burden study limits the total number of fibres counted (in our study 30 fibres), there is a risk of potential bias in estimating the mean length of fibres. Lack of any classification for the size of fibres will result in underestimation of concentration of long fibres and also inadequate characterization of fibre dimension. To avoid this potential bias in this study, the mineral fibres were divided into three size categories, according to their length, and analyzed separately. These categories were: 1) fibres shorter than 5 μm (short fibres); 2) fibres whose length ranged between 5 to 10 μm (intermediate-length fibres); and 3) fibres longer than 10 μm (long fibres). It should also be noted that this study uses the conventional definition of fibre, that is fibre defined in the medical and environmental literature as any particle with a minimum length-to-diameter ratio of at least 3:1.[(100)]

Fibres in all three size categories were counted and analyzed separately. Fibre size measurement and chemical analysis were performed concurrently one after the other on the same, *randomly* selected, fibres and grid openings. To reduce the limit of detection, at least 30 fibres or 30 grid openings (whichever was reached first) were observed, counted, and analyzed. Only fibres, both short and long, having length-to-diameter ratio (aspect ratio) of at least 3:1, were analyzed. The analytical protocol for fibres is shown in Figure 3.4.

Starting with the first specimen grid (out of four), all the fibres found in a grid opening were separately analyzed for the first size category (e.g. <5 μm). Upon the completion of the first grid opening, if the number of fibres reached 30, the fibre analysis was complete for that sample. On the other hand, if the number of analyzed fibres in the first opening was less than 30, fibre analysis proceeded as follows.

If the number of fibres was less than ten, fibre analysis proceeded to another grid opening on the same specimen grid until the number of fibres or the number of grid openings (whichever was reached first) reached 10 or more (a third of the total number of fibres or openings required). Then fibre analysis continued on the second and the third specimen grid with the same procedure as that for the first grid until at least 30 fibres or openings were analyzed. For samples with low fibre concentration (less than 10 fibres in 10 openings), the second specimen grid was used and the same protocol was continued until a cumulative number of 30 fibres or 30 openings was reached. For samples with high fibre concentrations, particularly for short fibres, one grid opening could contain 30 or more fibres, thus only one opening on the specimen grid was analyzed. After completion of counting, sizing, and analyzing of short fibres, the same procedure was followed for counting of intermediate-length fibres and then for long fibres on *randomly* selected grid openings. The concentration of fibres was expressed as number of fibres/mg dry lung tissue(f/mg). The limit of detection for fibre analysis for a sample of 15 mg of dry lung tissue was 70 f/mg.

3.6. OPTICAL MICROSCOPY FOR ASBESTOS BODY QUANTIFICATION

3.6.1. Slide preparation for optical microscopy

Millipore cellulose ester membrane filters with 25 mm diameter and 3.0 μm pore size as above were used for identification and numeration of asbestos bodies by the use of Phase Contrast Optical Microscopy (PCOM) (Figure 3.3). Each filter was placed on a 75 x 38 mm microscopic glass slide and cleared with 85 μl of DMF solution (a mixture of 35 ml dimethylfomamide, 20 ml of acetic acid, and 44 ml of water) and Eukitt (Kindler solution). The slides were placed in a oven for about 15 minutes at 65 °C.

3.6.2. Phase contrast optical microscopy for asbestos body counting

The asbestos bodies were counted using a Carl Zeiss phase contrast microscope at a magnification of 312× (Carl Zeiss Canada. Model G40-140. Ontario, Canada). The total number of asbestos bodies was calculated per each gram of dry lung tissue. In some subjects, the slide was overloaded with asbestos bodies, therefore, a lower amount of aliquot was used (15 or 10 ml) to prepare a new slide. Asbestos bodies were recognized by their clear straight cores and reddish to golden and beaded ferruginous coating as well as their dumbbell-like structure. Ferruginous fragments or bodies with other structures were not characterized as asbestos bodies.

3.7. QUALITY CONTROL

Since the main objective of the study was to characterize the lung-retained fibre content and its association with the severity of fibrosis, it was of critical importance to avoid any conta-

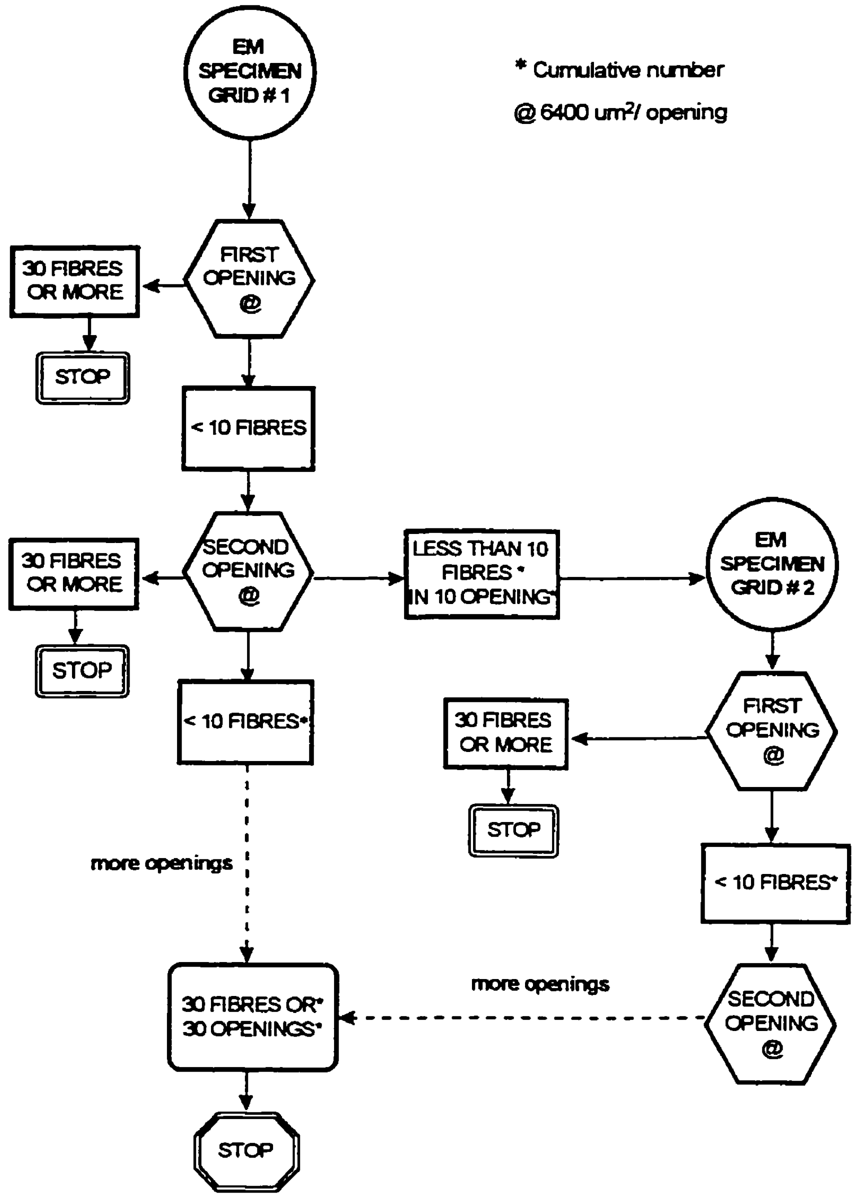


FIGURE 3.4. Schematic diagram of the method of fibre counting and analysis by transmission electron microscope /energy dispersive x-ray spectroscopy. Fibres with minimum length to diameter ratio of 3:1 were analyzed. For each sample, at least 30 fibres or 30 grid openings whichever was reached first, were counted and analyzed. The procedure was repeated three times for three size categories for each individual subject.

mination with dust and particles throughout the sample preparation. Therefore, several precautions were taken to avoid such contamination.

Contaminant-free containers were used for storage of all tissue samples. Disposable, dust-free, laboratory gloves and scalpels were used. Gloves and scalpels were discarded after the sectioning of each subject was complete. Laboratory films were used to cover the cutting board to avoid any cross-contamination. The film was discarded and replaced with a new one after each step. For each subject, a laboratory report was prepared containing the sites from which the samples were taken and the corresponding number. The tissue preparation was performed in a clean room (Department of Epidemiology, Biostatistics and Occupational Health, McGill University). Since this room is always under positive pressure and the air is filtered by using a HEPA filter, it is considered a particle-free environment. Laboratory containers, scalpels, and films that were used in the laboratory were discarded after the preparation of tissue from one specimen was complete. Deionized, distilled and filtered water was used in the clean room. All other chemicals such as formalin and sodium hypocholorite solution were also filtered through membrane filters (0.45 μm pore size) for particle removal before use.

3.8. OCCUPATIONAL HISTORY OF SUBJECTS AND EXPOSURE PARAMETERS

For all the subjects from both mining regions, the autopsy reports were available. Using this information, several parameters related to the history of exposure of each subject were extracted and entered into the databases. These parameters were sex, date of birth, date of death, age, year of start of exposure, age at start of exposure, year of end of exposure, age at the end of exposure, cause of death, duration of exposure, time since last exposure, and smoking (in pack-years).

3.9. CALCULATION OF FIBRE CONCENTRATION

The results of the lung-fibre analyses by TEM/EDS (number of fibres, fibre type and size) for each subject were stored in the databases. Then, the concentration of each mineral type was calculated by using a database program(dBase version V.)[99] and expressed as number of fibres per mg of dry lung tissue for each individual subject.

The number of fibres counted, over the area (30 fibres or more), the number of grid openings analyzed (6400 μm^2/opening), and the distribution of fibre types were computed for each subject. Fibres were classified into eight mineral categories: all fibres (i.e. total fibres and cleavage fragments with aspect ratio of 3:1 and more), chrysotile, tremolite, crocidolite, amosite, talc/anthophyllite, other silicates (including silica, mica, feldspars and clay minerals), and other minerals (calcite, apatite, metal fragments (e.g. iron. calcium, chromium, aluminum and titanium).

3.10. STATISTICAL ANALYSIS

3.10.1. Comparison of lung fibre concentration and dimension

The statistical analyses were performed using Minitab® statistical software (Minitab, release 10 Xtra).[101] Since the distribution of fibre concentration was log normal (see Chapter 4), the concentration of all fibres was logarithmically transformed by natural logarithm, Log_e. Non-detected values were given log transformed values of half the limit of detection (35 f/mg for asbestos fibres for 15 mg tissue filtered and 25 AB/g for asbestos bodies) of the analytical method. Geometric mean (GM) and geometric standard deviation (GSD), and 95% confidence interval (95% CI) were used to describe separately the lung fibre concentration data for subjects from Asbestos and Thetford-Mines.

For data analysis of fibre dimension, the length and diameter of each fibre type were also log transformed due to log-normal distribution of dimension parameters. The geometric mean diameter (GMD), the geometric mean length (GML) and geometric mean length to diameter ratio (GML/D) of each fibre type were computed for each subject. The geometric mean of each mineral type for each group was then calculated from these GMDs, GMLs, and GML/D of each subject (GM-GMD, GM-GML, and GM-GML/D). These geometric mean values were then shown in Tables.

The Mann-Whitney non-parametric test of significance was used to compare the geometric mean fibre concentrations (GMC) of fibres between subjects from Asbestos and Thetford-Mines at $\alpha=0.05$. The statistically significant p-values calculated by the statistical software as exact p-values were cited in the text as appropriate.[102] Those comparisons which were not significant at a p-value lower than 10% were indicated as $p>0.1$. The same statistical test was also used to compare the length, diameter and length/diameter ratio of fibres and also the concentration of asbestos bodies between the two groups.

To compare the occupational histories and the smoking habits of the two groups of miners and millers, the t statistical test was used, and the result was expressed as a probability value (p). The standard deviation and the range were also calculated for some parameters.

3.10.2. Association between fibrosis score and mineralogical parameters

The statistical analysis of data was performed separately for each group in four steps;

1. Statistical distributions of data, including dependent variable (i.e.: fibrosis score) and independent variables (i.e.: fibre concentrations and dimensions) were examined by using the Kolmogorov-Smirnov test (KS test) and normal plots.[103] Original values of fibrosis scores (0-12) were used as dependent variables in the regression models. Since fibre concentration and fibre dimension were log-normally distributed (KS test $p<0.05$ for all variables), logarithmic values (Log_e) of these variables were used as independent variables in the regression models.

2. To identify the determinants of fibrosis score, simple regression analysis was initially used. The association between each of the fibre parameters, such as fibre concentration and fibre dimension (length, diameter, and length/diameter ratio), for each size category and all fibre types with fibrosis scores were evaluated. The analysis was conducted separately for each study group. Regression coefficients and their respective standard deviations, t-ratios, and p-values as well as correlation coefficients and coefficient of determinations were used for interpretation of the results.[104] To evaluate the adequacy of the log-normal transformation, residual plots were used after performing each simple regression analysis.

All variables with their respecting statistical parameters showing their linear correlation with fibrosis score are reported in Chapter 4 (Results). Regression plots were used only for those variables which had a significant linear correlation with fibrosis score ($p<0.05$). In addition to regression analysis, stratification was used to examine the distribution of the geometric means and geometric standard deviations of fibre concentration and fibre dimension within three levels of fibrosis score. These levels were 0-4, 4-8 and 8-12.

3. To evaluate the possible interaction effects of exposure variables and fibre concentrations variables, multiple regression analysis was performed. Concentration variables, having a significant linear association with fibrosis score from a previous stage, and which also had potential biological importance, were examined for possible interaction effects. In each model, two main variables (e.g. concentration of short tremolite fibres and smoking) and one interaction variable (e.g. product of tremolite concentration x smoking) were included. The regression coefficient of the interaction term (b_1) and its significance level (p-value) were used for interpretation of the results.

4. Possible confounding effects of age, smoking, duration of exposure and time since last exposure were evaluated by multiple regression analysis. These variables were included in the regression models regardless of their regression coefficient and significance level obtained in the previous stage (e.g. simple regression). However, the inclusion of

($p<0.1$). To keep the ratio of number of subjects/number of variables to 10 at least, a maximum of four variables was included in each multiple regression model.[105] First, a simple model including a concentration variable was evaluated. Then, multiple models were constructed by adding exposure variables to the simple model one at a time. Several combinations of exposure variables and the first concentration variable were constructed. Results were interpreted by using the "changes of estimate method". In this approach the change of regression coefficient of the concentration variable from the simple model to multiple models were evaluated. The presence of a confounding effect was concluded when a change of more than 10% was observed on regression coefficient.

Since the type of fibres, level of exposure and disease incidence were different in two mining regions, no attempt was made to pool the two groups of subjects. The results are presented in various Tables in Chapter 4 or in the appendix.

Chapter 4

RESULTS

4.1. COMPARISON OF LUNG FIBRE CONCENTRATION DIMENSION, AND EXPOSURE VARIABLES

4.1.1. Occupational history of subjects

Information on work history and smoking habits extracted from autopsy reports and employment records are presented in Table 4.1. The average years of exposure were 36.8 and 37 years for Asbestos and Thetford-Mines miners and millers, respectively. The shortest and the longest duration of exposure were 14 and 50.5 years, and were experienced by two subjects from Thetford-Mines. Both Asbestos and Thetford-Mines subjects were comparable in both the calendar year of date of birth and first year of exposure (see Table 4.1). However, the calendar year of death was different in the two groups of workers (1988 vs. 1990 for Asbestos and Thetford-Mines workers, respectively

$p<0.01$). Another significant difference was the last year of exposure ($p<0.05$). The number of years since last exposure were also comparable, with an arithmetic mean of 11.3. However, the range for this parameter was wider among workers from Thetford-Mines (0 to 50 years) than for those from Asbestos (3 to 27 years). For all other parameters, most importantly "years of exposure" and "first year of exposure" there was no statistical difference ($p>0.1$ for both parameters). Cumulative indices of exposure for these subjects were not available. Ten and six workers were non-smokers from the Asbestos and Thetford-Mines, respectively. The averages of pack.year smoking was not different between the two groups ($p>0.1$).

4.1.2. Fibre counting, analyses, and statistical distribution

4.1.2.1. An overview of fibre concentrations

A total of 6982 single fibres were sized and analyzed for 86 subjects, 41 from Asbestos and 45 from Thetford-Mines (Table 4.2). The results are reported by classifying the mineral fibres into six groups. The category "other silicates" includes fibres and cleavage fragments of minerals having the elemental chemical composition of silica, clay, mica, feldspars with aspect ratio > 3:1. The category "other minerals" includes fibres and cleavage fragments of minerals having the elemental chemical composition of calcite and apatite, and metallic fragments such as iron, aluminum, titanium, chromium, and cobalt.

Seventy-two percent of fibres among subjects from Thetford-Mine were tremolite whereas 34.5% of fibres from Asbestos were tremolite (Table 4.2). A larger number of chrysotile fibres was identified in the lungs of Asbestos workers. For subjects from Asbestos a total of 230 fibres in the "other silicates" category were identified as talc/anthophyllite fibres. The number of talc/anthophyllite fibres in subjects from Thetford-Mines was 184. Much larger numbers of amosite and crocidolite fibres were detected in the lungs of Asbestos workers (0.28% Vs. 1.9% for amosite and 0.24% vs. 9% for crocidolite) than those in Thetford-Mines. The proportion of other silicates fibres was comparable in the two groups.

Table 4.1. Comparison of occupational histories and smoking habits of 86 former miners and millers from Thetford-Mines and Asbestos. Information was extracted from the employment records.

Variable	Asbestos n= 45		Thetford-Mines n=41		p*
	Mean (SD)	Range	Mean (SD)	Range	
Years of Exposure	36.8 (5.9)	*18-49*	37 (9.5)	*14-50.5*	>0.1
Year of Birth	1918	*1903-1939*	1919	*1908-1948*	>0.1
Year of Death	1988	*1983-1992*	1990	*1989-1991*	<0.01
Age at Death	69.8 (7.8)	*48-89*	70.1 (7.8)	*41-81*	>0.1
First Year of Exposure	1940	*1924-1966*	1942	*1923-1972*	0.1
Last Year of Exposure	1977	*1963-1984*	1979	*1959-1989*	0.03
Years Since Last Exposure	11.3 (4.2)	*3-27*	11.37 (9)	*0-50*	>0.1
Smoking**	41 (25)	*2-100*	40.5 (23)	*2.5-100*	>0.1

n= number of subjects
SD= standard deviation
*: t statistical test
**: pack-year

The total number of mineral fibres detected and analyzed was 3554 and 3428 for workers from Thetford-Mines and Asbestos , respectively.

The distribution of fibres based on their size category is presented in Table 4.3. The two groups of workers were similar in respect to the proportion of short (<5 μm), intermediate-length (5-10 μm) and long (>10 μm) fibres out of total fibres detected. Slightly more than 50% of fibres were short. Intermediate-length and long fibres constitute 31% and 17-18% of total fibres, respectively. The proportions of fibres from the three size categories were comparable in Asbestos and Thetford-Mines subjects.

Table 4.2. Number of mineral fibres observed, sized, and analyzed among 86 former miners and millers of Thetford-Mines and Asbestos by transmission electron microscopy/energy dispersive spectroscopy.

Thetford-Mines n= 45			Asbestos n= 41		
Fibre Type	**Number**	**Percentage of total**	**Fibre Type**	**Number**	**Percentage of total**
Tremolite	2568	72.2%	**Tremolite**	1180	34.5%
Chrysotile	223	6.2%	**Chrysotile**	546	15.9%
Amosite	10	0.28%	**Amosite**	67	1.9%
Crocidolite	7	0.24%	**Crocidolite**	311	9%
Other silicates	588	16.5%	**Other silicates**	525	15.3%
Other Minerals	158	4.4%	**Other Minerals**	799	23.3%
Total Fibres	3554	100%	**Total fibres**	3428	100%

n = number of subjects

Table 4.3. Distribution of detected fibres within three size categories and their percentage of total among 86 former miners and millers of Thetford-Mines and Asbestos by transmission electron microscopy/energy dispersive spectroscopy.				
Mining region	**Total Fibre**	**Fibre <5 μm**	**Fibre 5-10 μm**	**Fibre >10 μm**
Thetford-Mines n=45	3554	1798 (50.5%)	1106 (31%)	650 (18%)
Asbestos n=41	3428	1745 (51%)	1073 (31.5%)	610 (17.5%)

n= number of subjects

4.1.2.2. Statistical distribution of fibre concentration and dimension

Geometric mean concentrations of asbestos fibres of all types and sizes among both groups departed from a normal distribution (Kolmogorov-Smirnov $p<0.01$). Normal plots showed a higher frequency of lower geometric means for all fibre sizes and types, indicating a log-normal distribution. The distribution of geometric mean fibre size parameters (length, diameter, length to diameter ratio) also deviated from a normal distribution. However, the statistical test showed less significant values than those observed for fibre concentrations (KS test <0.05 for most parameters and 0.05 to 0.1 for some parameters). Due to the log-normal distribution of geometric mean concentrations geometric mean and geometric standard deviation of geometric means (GM-GMs and GSD-GMs) were calculated to demonstrate concentrations of the lung fibres for each group. An inspection of normal plots after $\log_e$ transformation showed that the concentration and dimension values were appropriately normalized by a $\log_e$ transformation, therefore, log-transformed concentration and dimension values were used in the subsequent regression and correlation analyses when the relationships of these parameters with the fibrosis score were investigated.

4.1.3. Comparison of fibre concentration

The geometric mean, geometric standard deviation concentration as well as the 95% confidence interval for geometric mean of fibre concentrations in both groups are shown in Tables 4.4, 4.5, and 4.6 for fibres <5 µm, 5-10 µm, and >10 µm, respectively.

For subjects from Thetford-Mines, the geometric mean concentrations (GMCs) of asbestos fibres were in the following decreasing order for fibres <5 µm: tremolite > talc/anthophyllite> chrysotile> crocidolite> amosite. This trend for fibres 5-10 µm, and >10 µm were tremolite> chrysotile> talc/anthophyllite> crocidolite> amosite, and: tremolite> chrysotile> amosite> crocidolite> talc/anthophyllite, respectively.

The GSD for the geometric mean concentrations of tremolite, chrysotile and talc/ anthophyllite were high, indicating a large variation in terms of fibre concentration among the subjects. The highest GSDs were found for short chrysotile fibres (13.3 and 8 for Thetford-Mines and Asbestos subjects, respectively). However, variations observed for the concentrations of amosite and crocidolite were lower. Overall, higher values of GSDs were found for short fibres than for long fibres.

For subjects from Asbestos, the GMCs of tremolite were still the highest for all size categories. In summary, the following decreasing trends were found for the GMCs of asbestos fibres among Asbestos miners and millers: for fibres >5 µm and fibres 5 to 10 µm; tremolite> chrysotile> talc/anthophyllite> crocidolite> amosite, and the trend for fibres > 10 µm was; tremolite> chrysotile> crocidolite> talc/anthophyllite> amosite.

Tremolite fibres were detected in the lung tissues of all subjects from Thetford-Mines and Asbestos. A notable difference was observed when the GM concentration of tremolite among subjects from Thetford-Mines was compared with that of Asbestos subjects. The concentration of short tremolite fibres in subjects from Asbestos was one fourth of that for subjects from Thetford-Mines and a statistical test (Mann-Whitney test) shows that this

Table 4.4. Geometric mean concentration (geometric standard deviation)*(95% confidence interval)* of fibres <5 μm /mg dry lung tissue. †

Fibre type	Thetford-Mines n=45	Asbestos n=41
Tremolite	28854***(4.48)*(9718-45297)*	6355***(8.15) (*3274-12320*)
Chrysotile	#334***(13.3) *(153-729)*	#2998***(8.73) *(1525-5943)*
Amosite	‡ 39* (2.16) *(31-49)*	†89* (5.36) *(52-150)*
Crocidolite	‡ 45*** (2.4) *(35-59)*	# 369*** (10.13) *(177-767)*
Talc/ Anthophyllite	1808* (6.3) *(1049-3171)*	1574* (7.5) *(834-2951)*
Other silicates	7021* (5.8) *(4113-11980)*	12594* (4.4) *(7879-20130)*
All fibres	43261* (4.8) *(26849-69703)*	39261*(3.4) *(26715-57756)*

n= number of subjects
† All fibres having length-to-diameter ratio greater than 3:1 and length <5 μm
‡ Fibres found among less than 50% of subjects
# Fibres found among more than 50% of subjects
Significance levels: *** p<0.01, ** 0.01<p<0.05, *0.05<p<0.1 (Mann-Whitney test)

Table 4.5. Geometric mean concentration (geometric standard deviation) *(95% confidence interval)* of fibres 5 -10 μm /mg dry lung tissue. †		
Fibre type	**Thetford-Mines** **n=45**	**Asbestos** **n=41**
Tremolite	5166** (5.6) *(3071-8690)*	2277**(4.1) *(1455-3568)*
Chrysotile	#199** (6.5) *(113-352)*	#525** (8.6) *(266-1037)*
Amosite	nd 35*	‡ 58* (3.1) *(41-84)*
Crocidolite	‡ 40***(1.65) *(34-46)*	#228*** (5.6) *(132-394)*
Talc/ Anthophyllite	#112** (4.8) *(70-180)*	#362** (8.5) *(220-597)*
Other silicates	399** (5.75) *(236-677)*	1037** (4.34) *(652-1635)*
All Fibres	6721 (5.6) *(3987-11327)*	6285 (3.9) *(4088-9662)*

n= number of subjects
† All fibres having length-to-diameter ratio greater than 3:1 and length 5-10 μm
‡ Fibres found among less than 50% of subjects
# Fibres found among more than 50% of subjects
Significance levels: *** $p<0.01$, ** $0.01<p<0.05$, *$0.05<p<0.1$ (Mann-Whitney test)
nd: Not detected in any subjects, half of the limit of detection was assigned

Table 4.6. Geometric mean concentration (geometric standard deviation) *(95% confidence interval)* of fibres >10 μm /mg dry lung tissue. †

Fibre type	Thetford-Mines n=45	Asbestos n=41
Tremolite	1124** (5.9) *(657-1919)*	#517** (3.7) *(334-801)*
Chrysotile	#122** (5.7) *(72-206)*	#206** (4.8) *(125-340)*
Amosite	‡42 ***(1.7) *(39-49)*	‡71 ***(2.6) *(52-97)*
Crocidolite	‡38***(1.4) *(34-42)*	#160 ***(4.4) *(100-255)*
Talc/ Anthophyllite	‡43.3 ***(3.5) *(52-111)*	#154 ***(3.6) *(102-230)*
Other silicates	#143 *(5.4) *(85-241)*	#171 *(3.4) *(116-252)*
All Fibres	1539* (6) *(926-2743)*	1576* (3.7) *(1045-2368)*

n= number of subjects
† All fibres having length-to-diameter ratio greater than 3:1 and length >10 μm
‡ Fibres found among less than 50% of subjects
# Fibres found among more than 50% of subjects
Significance levels: *** p<0.01, ** 0.01<p<0.05, *0.05<p<0.1 (Mann-Whitney test)

Table 4.7. Geometric mean concentration (geometric standard deviation) *(95% confidence interval)* of asbestos bodies per gram dry lung tissue counted by phase contrast microscopy.

	Thetford-Mines n=45	Asbestos n=41
Asbestos Bodies	27529 **(6) *(16058-47192)*	14016 **(6.5) *(7762-25336)*

n= number of subjects
Significance levels: *** p<0.01, ** 0.01<p<0.05, *0.05<p<0.1, (Mann-Whitney test)

difference was statistically significant ($p<0.01$). Concentrations of intermediate-length (5-10 μm) and long (>10 μm) tremolite fibres in workers from Thetford-Mines were twice as high as those for Asbestos workers (Tables 4.5 and 4.6) These differences were statistically significant ($p<0.05$, for both size categories). The maximum concentrations of tremolite found in subjects from Asbestos were 194,800, 13,400 and 5029 f/mg dry lung tissue for short, intermediate, and long fibres, respectively. Maximum concentrations in subjects from Thetford-Mines for corresponding size categories were 414,500, 210,000, and 81,600 f/mg.

Chrysotile was detected in 36/41 (88%) subjects from Asbestos. The lung tissues of 31/46 (67%) subjects from Thetford-Mines contained chrysotile fibres. Geometric mean concentrations of chrysotile (in three size categories) were higher in workers from Asbestos than those in Thetford-Mines workers. The difference in concentration of chrysotile fibres was statistically significant between these two groups ($p<0.01$, $p<0.05$, and $p<0.05$ for fibres <5 μm, 5-10 μm, and >10 μm, respectively)

Other commercial amphiboles, crocidolite and amosite, were detected in the lungs of Asbestos workers. Twenty out of forty-one subjects (51%) had detectable amosite in their lungs and 29/41 (71%) had crocidolite. Rare amosite fibres were detected in 5/45 (11%) and crocidolite in 6/45 (13%) Thetford-Mines subjects. The concentrations of these amphiboles in the Thetford-Mines group were just above the limit of detection of the analytical method.

The maximum concentrations of short amosite and crocidolite fibres in subjects from Asbestos were 4188 and 298,000 f/mg dry lung tissue, respectively. The concentration of long fibres (>10 μm) was 1043 and 10,000 f/mg for amosite and crocidolite, respectively. The GMC of the crocidolite was significantly higher for subjects from Asbestos ($p< 0.01$ for each size category). However, the GMC of amosite was statistically different only for fibres >10 μm ($p<0.01$).

Geometric mean concentrations of talc/anthophyllite were significantly higher for the Asbestos subjects for fibres 5-10 μm and for fibres >10 μm ($p<0.05$ and $p<0.01$, respectively).

In spite of the above-mentioned differences observed for the concentrations of asbestos fibres in the lung tissues of two groups of asbestos miners and millers of Québec, GMCs of total fibres were comparable, and statistically significant differences were not seen ($p> 0.10$ for all size categories).

The GMC of asbestos bodies was higher for subjects from Thetford-Mines. Statistical tests show that the observed difference approached statistical significance ($p<0.1$). The high geometric standard deviation for the GM asbestos bodies shows a significant variation and results in a wide 95% confidence interval around the GM for both groups (Table 4.7).

The GSD for the geometric mean concentrations of tremolite, chrysotile and talc/ anthophyllite were high, indicating a large variation in terms of fibre concentration among the subjects. The highest GSDs were for short chrysotile fibres (13.3 and 8 for Thetford-Mines and Asbestos subjects, respectively). However, variations observed for the concentrations of amosite and crocidolite were lower. Overall, higher values of GSDs were found for short fibres compared to long fibres.

4.1.4. Comparison of fibres dimension

The geometric mean lengths (GMLs) of tremolite fibre were 1.98, 5.98, and 12.78 μm for the subjects from Thetford-Mines for fibres < 5 μm, 5-10 μm, and >10 μm, respectively (Tables 4.8, 4.9, 4.10). For the subjects from Asbestos the GLMs for the same size categories of tremolite fibres were 2.00, 5.88, and 12.20 μm. The GMLs of short (<5 μm) tremolite the fibres were higher among the subjects from Asbestos than were those in the workers from Thetford-Mines, but this difference was not statistically significant ($p>0.1$). The opposite trend was observed for the length of tremolite fibres 5-10 μm; this difference being statistically significant ($p<0.05$). For tremolite fibres longer than 10 μm the lengths were slightly higher in the subjects from Thetford-Mines those that in the subjects from Asbestos. This difference was moderately significant ($0.05<p<0.1$). When the GMLs of chrysotile fibres were compared, no statistically significant differences were observed ($p>0.1$ for all size categories). Since the number of subjects with amosite and crocidolite was small for the workers from Thetford-Mines, no comparison for fibre dimension was made between the two groups. No significant differences in any size category were observed when the GMLs of "other silicates" were compared. No comparisons for any size-related parameters for total fibres and talc/anthophyllite fibres were conducted.

The geometric mean diameter (GMD) of tremolite fibres was higher in Thetford-Mines subjects for short fibres ($p<0.05$). However, for intermediate-length and long fibres, no difference in tremolite fibre GMD was observed ($p>0.10$). The GMD of intermediate-length chrysotile fibres was higher among Thetford-Mines subjects ($p<0.05$). When diameters of "other silicates" fibres were compared, no differences were observed.

The geometric mean length to diameter ratio (GM L/D) of fibres is also shown in Tables 4.8, 4.9 and 4.10 for the two groups. The GM L/D of asbestos fibres has naturally increasing trend from fibres <5 μm to fibres >10 μm and this trend was observed for chrysotile and tremolite in both groups. Chrysotile fibres had a larger GM L/D than any other fibrous mineral. For tremolite fibres, a significant difference between the two groups was observed only for short fibres ($p<0.01$). The GMs L/D of short fibres were 7.76 and 10 in the subjects from Thetford-Mines and Asbestos, respectively. For chrysotile fibres, GMs L/D were higher among the subjects from Asbestos only for short and long fibres, and the observed differences were moderately significant ($0.05<p<0.1$). Statistical comparisons for other silicates did not reveal any differences.

Table 4.8. Geometric mean (geometric standard deviation) of diameter (D), length (L) and length-to-diameter ratio (L/D) of fibres <5μm.

Mining region		Tremolite	Chrysotile	Amosite	Crocidolite	Other silicates
Thetford-Mines n=45	D	0.24** (1.23)	0.07 (1.7)	0.31 ‡ (1.5)	0.38 ‡ 1.9)	0.23 (1.56)
	L	1.98 (1.19)	2.39 (1.94)	1.84 ‡ (1.4)	2.29 ‡ (3.06)	1.62 (1.47)
	L/D	7.76*** (1.41)	32 * (2.76)	5.1 ‡ (1.62)	16.3 ‡ (2.18)	6.7 (2.09)
Asbestos n=41	D	0.21 ** (1.4)	0.05 (1.2)	0.13 ‡ (1.98)	0.09 # (1.56)	0.21 (1.37)
	L	2 (1.25)	1.9 (1.42)	1.5 ‡ (1.76)	1.84 # (1.38)	1.46 (1.22)
	L/D	10*** (1.27)	39 * (1.5)	11.7 ‡ (1.85)	20.3 # (1.77)	6.8 (1.27)

n= number of subjects
† All fibres having length-to-diameter ratio greater than 3:1 and length < 5 μm
‡ Fibres found among less than 50% of subjects
# Fibres found among more than 50% of subjects
Significance levels: *** p<0.01, ** 0.01<p<0.05, *0.05<p<0.1 (Mann-Whitney test)

Table 4.9. Geometric mean (geometric standard deviation) of diameter (D), length (L), and length-to-diameter ratio (L/D) of fibres 5-10 μm. †

Mining region		Tremolite	Chrysotile	Amosite	Crocidolite	Other silicates
Thetford-Mines n=45	D	0.39 (1.43)	0.075 **# (1.98)	nd	0.36 ‡ (1.4)	1.98 (1.55)
	L	5.98** (1.07)	5.96 # (1.16)	nd	8.8 ‡ (1.55)	6.15 (1.13)
	L/D	16.2 (1.36)	196 # (1.97)	nd	24.5 ‡ (1.67)	10.5 (1.91)
Asbestos n=41	D	0.32 (1.6)	0.049** # (1.23)	0.15 ‡ (2.5)	0.12 # (1.7)	0.53 (1.97)
	L	5.88** (1.1)	5.79 # (1.11)	6.03 ‡ (1.19)	6.19 # (1.1)	5.7 (1.1)
	L/D	17.9 (1.6)	117 # (1.3)	39.6 ‡ (2.33)	50 # (1.7)	10.6 (1.93)

n= number of subjects
† All fibres having length-to diameter-ratio greater than 3:1 and length < 5-10 μm
‡ Fibres found among less than 50% of subjects
# Fibres found among more than 50% of subjects
Significance levels: *** $p<0.01$, ** $0.01<p<0.05$, *$0.05<p<0.1$ (Mann-Whitney test)
nd: Not detected in any subjects.

Table 4.10. Geometric mean (geometric standard deviation) of diameter (D),length (L), and length-to-diameter ratio (L/D) of fibres >10 μm. †						
Mining region		**Tremolite**	**Chrysotile**	**Amosite**	**Crocidolite**	**Other silicates**
Thetford-Mines n=45	**D**	0.48 (1.9)	0.09 # (2.6)	0.35 ‡ (1.3)	0.28 ‡ (1.9)	0.67 # (2.32)
	L	12.78* (1.29)	14.3 # 1.8)	19.2 ‡ (1.7)	24.5 ‡ (1.64)	13.46 # (1.18)
	L/D	27.81* (1.5)	136.5*# (3.6)	54.2 ‡ (1.52)	144 ‡ (1.43)	20 # (2.32)
Asbestos n=41	**D**	0.45 (1.74)	0.053 # (1.29)	0.33 ‡ (1.67)	0.15 # (1.51)	0.73 # (3.03)
	L	12.2* (1.3)	14.15 # (1.96)	21.4 ‡ (1.57)	15.6 # (1.29)	12.64 # (1.23)
	L/D	27 (1.7)	266* # (1.38)	65 ‡ (2.05)	109 # (1.61)	17.3 # (3.17)

n= number of subjects
† All fibres having length-to-diameter ratio greater than 3:1 and length >10 μm
‡ Fibres found among less than 50% of subjects
# Fibres found among more than 50% of subjects
Significance levels: *** $p<0.01$, ** $0.01<p<0.05$, *$0.05<p<0.1$ (Mann-Whitney test)

Overall the geometric standard deviation (GSD) for tremolite and chrysotile lengths for all size categories ranged between 1.07 to 1.94. The GSD for the diameter of tremolite and chrysotile ranged between 1.23 to 2.6 for all size categories. Slightly higher GSD values were observed for GSD L/D of chrysotile and tremolite (1.41 to 3.6). In general, amosite and crocidolite had higher GSDs for the various size parameters in the three size categories than in the chrysotile and tremolite. No specific trend for GSDs was observed when the two groups of subjects were compared.

4.2. ASSOCIATION BETWEEN FIBROSIS SCORE AND FIBRE CHARACTERISTICS

4.2.1. Fibrosis scores among subjects from Asbestos and Thetford-Mines

Table 4.11 shows the results of the pathological examination of the lung tissue samples from 86 subjects from the two mining regions. The grading for seven subjects from Asbestos was conducted by the use of one tissue block for each case. The grades related to these blocks are included in the column "section 1". Among the subjects from Thetford-Mines one slide for eight subjects and two slides for two subjects were not gradable according to the pathologist due to the presence of excessive atelectasis, carcinoma, or hemorrhage. The lobe of origin of the lung tissue samples for the subjects from Asbestos was not known, therefore, the section numbers for this group were arbitrarily assigned and presented in Table 4.11. As expected, the grades for sections 1 to 6 (Table 4.11) for subjects from Thetford-Mines correspond to sections of known lobes as described in Chapter 3. The lower lobe, peripheral subpleural sections or sections 6, had the highest mean fibrosis score (4.7) followed by lower lobe, mid-parenchyma or sections 5 (4.2). The upper lobe, mid parenchyma sections had the lowest mean fibrosis score (3.2).

All subjects from both mining regions had a certain degree of pulmonary fibrosis. The fibrosis score ranged from 1 to 11 and 1 to 11.5 among the subjects from Thetford-Mines and Asbestos, respectively. The standard deviations were 2.7 and 3.26 for the subjects from Thetford-Mines and Asbestos , respectively. Grade of asbestos bodies ranged from 0 to 3 among subjects from both groups (Table 4.11).

The mean severity score was higher among the subjects from Asbestos (2.55 Vs. 2.11, p=0.01, t-statistics). The mean extent scores were not different between the two groups (1.8 for subjects from Asbestos and 1.7 for subjects from Thetford-Mines, p=0.37 t-statistics). The mean fibrosis score was higher for the subjects from Asbestos than for subjects from Thetford-Mines (5.36 vs. 4.25, p=0.06 t-statistics). Since the lobe of origin of tissue samples for subjects from Asbestos was not known, no attempt was made to compare the difference between the section (lobe) scores of the two groups.

4.2.1.1. Subjects with no asbestos bodies in their histological section

Asbestos bodies were not observed in the histological slides of ten subjects from Thetford-Mines (22%) and seven subjects from Asbestos (17%). However, these subjects were considered to be asbestosis cases, since: 1) they all had long-term exposure to asbestos, 2) the concentrations of asbestos bodies in the digestion aliquot for 13 subjects out of 17 (13/17) were more than 1500 per gram dry lung indicating a notable exposure to asbestos fibres (Table A-13, Appendix), 3) they all had a certain degree of pulmonary fibrosis, and had no other causes of fibrosis. Eleven of those subjects (11/17) were smokers; and 4) the concentrations of tremolite fibres and total fibres in all subjects (17) were more than one million fibres per gram dry lung tissue.

For four subjects (4/17) , the number of asbestos bodies in the digestion aliquot was less than 1500 per gram dry lung. However, the total fibre concentration among three of those subjects (3/4) was more than one million fibres per gram dry lung (Table A-13, Appendix).

Table 4.11. Statistical parameters of the grade of asbestosis among 86 subjects from Thetford-Mines and Asbestos.

Mining region	Statistical values	Average score for sections						Average score for subjects			
		Section 1	Section 2	Section 3	Section 4	Section 5	Section 6	Severity score	Extent score	Fibrosis score	Asbestos body grade
Thetford n=45	Mean	3.2	4.2	3.9	3.8	4.4	4.7	2.11	1.77	4.25	0.99
	Median	2	4	3	2	3	4	2	1.67	3.97	0.67
	SD	2.8	3.1	3.1	3.4	3.7	3.5	0.7	0.62	2.7	0.98
	Min	0	0	0	0	0	0	0.66	0.67	1	0
	Max	12	12	9	12	12	12	3.67	3	11	3
Asbestos* n=41**	Mean	5.31	5.8	5.29	5.5	5.3	5.1	2.55	1.83	5.36	1
	Median	6	6	4	4	4	4.8	2.8	1.82	5.8	1
	SD	3.5	3.8	4.33	4.7	4.2	3.35	0.85	0.69	3.26	0.82
	Min	1	1	0	0	0	1	1	0.83	1	0
	Max	12	12	12	12	12	12	3.8	3	11.5	3

* Sections arbitrarily numbered from 1 to 6.

** Column " section 1 " presents the results of 41 subjects from Asbestos. All other columns present the results of 34 subjects from Asbestos.

n: number of subjects

SD: Standard deviation

Max, Min: Maximum and minimum values observed

Therefore, the observed pulmonary fibrosis among these four subjects was also considered to be due to asbestos exposure. It should be noted that, for two subjects from Asbestos (2/4), the grade of asbestos bodies was evaluated based on examination of one histological section from an unknown lobe, thus, making the evaluation less reliable than the rest of the subjects for which six sections were used.

4.2.1.2. Statistical distribution of severity, extent, and fibrosis scores

Separate results of the test of normality for severity and extent did not indicate a significant deviation from normality for both groups (KS test $p>0.1$ for all variables and both groups). However, the same statistical test on the distribution of the fibrosis scores resulted in the rejection of the hypothesis of normality for workers from Thetford-Mines (KS test $p<0.05$). Visual examination of the probability plot indicated a log normal distribution for fibrosis scores among workers from Thetford-Mines. For Asbestos workers, however, the same statistical test showed no significant departure from normality (KS test $p>0.1$). A decision was made to use original values of the fibrosis scores for subsequent regression analysis and no transformation was conducted, since the fibrosis score was used as dependent variable in these analyses.

4.2.2. Association between fibre concentrations and fibrosis score

The results of simple regression and correlation analysis for the concentration of various fibre types within three size categories are summarized in Tables 4.12 and 4.13. Regression coefficients, their standard deviations and t-ratios, correlation coefficients, respective p-values, and coefficient of determinations are shown for each analysis. For each analysis the fibrosis score (0-12) was the dependent variable and the $\log_e$ fibre concentration was introduced as the independent or determinant variable.

Thetford-Mines subjects: For the subjects from Thetford-Mines (Table 4.12), tremolite (<5 μm and 5-10 μm), chrysotile (5-10 μm), and total fibres (<5 μm for all variables) showed a significant positive linear relationship with fibrosis scores ($p<0.05$). The regression coefficient ($b_1=0.74$, $p<0.01$) for short tremolite fibres (<5 μm) showed that the fibrosis score increases 0.74 unit when the fibre concentration increased one unit on a logarithmic scale. The regression coefficients for other fibre concentration variables were all smaller than that for tremolite (<5 μm). Small correlation coefficients and/or large standard deviations resulted in small t-ratios and nonsignificant p-values for many of the variables (See Table 4.12). The second largest regression coefficient was for short total fibres ($b_1= 0.73$, $p<0.05$) followed by that for intermediate-length chrysotile ($b_1= 0.6$, , $p<0.01$) and that for intermediate-length tremolite fibres ($b_1= 0.48$, $p<0.05$). Similarly, the highest linear correlation was found for short tremolite concentrations ($r=0.44$, $R^2=19.6$) and total fibre concentrations ($r=0.42$, $R^2=18.4$).

Chrysotile fibres (<5 μm) and talc/anthophyllite fibres (>10 μm) showed positive associations with the fibrosis scores, approaching statistical significance ($p=0.06$). The correlation coefficients for these two fibre types were $r=+0.28$ for both chrysotile (<5) and talc/anthophyllite fibres (>10 μm).

The concentrations of commercial amphiboles did not show any correlation with the fibrosis scores. The correlation coefficients were not statistically different from zero ($p>0.1$) for any size categories of crocidolite and amosite. Although the regression coefficients of some of these variables such as short amosite ($b_1=0.5$) and long crocidolite ($b_1=-0.8$) fibres were large, their corresponding p values were nonsignificant. Long chrysotile, tremolite, and total fibres did not show any linear relationship with the pulmonary fibrosis scores ($r<0.1$, $p>0.1$ for all variables). The lung-digest concentration of asbestos bodies also showed some positive linear relationship with fibrosis score ($r=0.4$, $p<0.05$). The histological grade of the asbestos bodies also had a significant correlation with the fibrosis score ($r=0.45$, $p<0.05$).

Since the fibrosis score was log-normally distributed among the subjects from Thetford-Mines, the regression and correlation analyses was also performed by using the $\log_e$ values of fibrosis score. The results did not show any difference with the regression and correlation coefficients reported in Table 4.12.

The results of the analysis of data by stratification of subjects based on their fibrosis score are summarized in Tables A-1 to A-3 (Appendix). The number of subjects in fibrosis levels (0-4), (4-8), and (8-12) were 25, 16, and 6, respectively. The geometric mean concentrations of tremolite (<5 μm), total fibres (<5 μm), and tremolite (5-10 μm) increased as fibrosis level increased. This trend was less obvious for other fibres such as chrysotile and talc/anthophyllite and no significant trend for the geometric means of amosite and crocidolite (all size categories) was observed. Figures 4.1 to 4.4 show the regression plots for the relationship of the $\log_e$ concentration of fibres, which showed a statistically significant linear relationship with fibrosis score, among the subjects from Thetford-Mines.

Asbestos: Table 4.13 shows the results of simple regression and correlation analyses for the subjects from Asbestos. The severity of asbestosis increased 1.67 units (on a 0-12 scale) for one unit increase on the long amosite concentration ($b_1=1.67$, $p<0.01$). The $\log_e$ concentration of long amosite fibres had a significant correlation with fibrosis score ($r=+0.5$, $R^2=24\%$). The second largest regression coefficient was found for short tremolite fibres ($b_1=0.6$, $p<0.01$, $R^2=15.8\%$). Intermediate-length crocidolite fibres, other silicates (<5 μm), and talc/anthophyllite fibres (>10 μm) showed a linear correlation with the fibrosis score approaching statistical significance ($r=+0.25$ to $r=+0.28$ $p=0.08$). Regression coefficients and correlation coefficients of other concentration variables including chrysotile and other silicates (all size categories) did not show any correlation with pulmonary fibrosis score ($r<0.25$ and $p>0.1$ for all fibre types/sizes). The regression coefficients for the number and grade of asbestos bodies were small and nonsignificant, indicating no statistical relationships with the fibrosis score ($b_1=0.21$ for lung digest concentration of AB, and $b_1=0.18$ for histological grade of AB, $p>0.1$ for both variables).

Table 4.12. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and fibre concentration (*Log_e fibre /mg of dry lung* or Log_e *f/g dry lung* for the number of asbestos bodies in digestion) and Log_e grade of asbestos bodies in histological slides among 45 subjects from Thetford- Mines.

Fibre type	Size Categories	b_1	SD	t ratio	r	p* value	R^2
Tremolite	< 5	0.74	0.22	3.24	0.44	0.002	19.6
	5-10	0.48	0.22	2.27	0.32	0.02	10.3
	>10	0.05	0.23	0.22	0.035	0.8	0.1
Chrysotile	< 5	0.3	0.15	1.96	0.28	0.06	8
	5-10	0.6	0.2	2.81	0.39	0.008	15.5
	>10	-0.01	0.23	-0.07	-0.01	0.9	0
Amosite	< 5	0.5	0.52	0.98	0.14	0.3	2.2
	5-10	ND	ND	ND	ND	ND	ND
	>10	0.1	0.74	0.14	0.02	0.88	0
Crocidolite	< 5	-0.39	0.46	-0.84	0.13	0.4	1.6
	5-10	0.7	0.81	0.9	0.13	0.37	1.8
	>10	-0.8	1.19	-0.71	-0.1	0.5	1.2
Other Silicates	< 5	0.23	0.2	1.15	0.21	0.13	4.5
	5-10	0.18	0.23	0.79	0.12	0.43	1.4
	>10	-0.1	0.23	-0.5	0.07	0.67	0.6
Talc Anthophyllite	< 5	-0.05	0.23	-0.25	-0.02	0.8	0.1
	5-10	0.4	0.33	1.2	-0.04	0.18	6.8
	>10	0.4	-0.05	-0.02	0.28	0.06	8.2
Total Fibres	< 5	0.73	0.23	3.2	0.42	0.03	18.4
	5-10	0.4	0.22	1.77	0.26	0.08	6.8
	>10	0.06	0.22	0.27	0.04	0.67	0.2
Conc. of AB	__	0.41	0.21	2.06	0.30	0.05	9

b_1 : Regression coefficient or the slope of the regression line
SD: Standard deviation for regression coefficient
r: Correlation coefficient
* : p-value based on non-zero regression and/or correlation coefficient
R^2: Coefficient of determination (%)
ND: No amosite fibre in this size category was detected among subjects

The results of the analysis of data by stratification of subject based on their fibrosis score are summarized in Tables A-7 to A-9 (Appendix). The number of subjects in fibrosis levels (0-4), (4-8), and (8-12) were 17, 14 and 10, respectively. An obvious increasing trend for the geometric mean of amosite fibres (>10 μm) from lower to higher fibrosis score levels was observed. Other fibre concentrations did not show a trend for the geometric mean concentrations for the three fibrosis score levels.

Figure 4-5 shows the regression plots for the relationship of the $\log_e$ concentration of fibres, which showed a statistically significant linear relationship with the fibrosis score, in the workers from Asbestos.

4.2.3. Association between fibre dimension and fibrosis score

Tables 4.14 to 4.19 show the relationships between the $\log_e$ fibre dimension parameters including fibre length, fibre diameter and aspect ratio with the fibrosis score. Tables 4.14, 4.15, 4.16 show these relationships for fibres <5 μm, 5-10 μm and >10 μm among the subjects from Thetford-Mines. No fibre dimension parameter of any kind showed a significant linear relationship with fibrosis score except for the diameter of long chrysotile fibres. Regression and correlation coefficients were small for these coefficients and failed to show that they were significantly different from zero. Generally, large standard deviations were observed for the regression coefficients. The probability values for regression and correlation coefficients were nonsignificant ($p>0.1$ for all variables). The coefficient of determinations (R^2) were also small, indicating small or no predictive ability of $\log_e$ fibre length , diameter and length to diameter ratio for fibrosis score.

Tables 4.17, 4.18, 4.19 show these relationships for fibres <5 μm, 5-10 μm and >10 μm among the subjects from Asbestos. No fibre dimension parameter of any kind showed a linear relationship with the fibrosis score. The regression and correlation coefficients were small and were not significantly different from zero ($p>0.2$ for all variables). The

Table 4.13. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and fibre concentration (*Log_e fibre /mg of dry lung* or Log_e *f/g dry lung* for the number of asbestos bodies in digestion) and Log_e grade of asbestos bodies in histological slides among 45 subjects from Asbestos.

Fibre type	Size Categories	b_1	SD	t ratio	r	p* value	R^2
Tremolite	< 5	0.6	0.22	2.71	0.39	0.01	15.8
	5-10	0.48	0.36	1.3	0.15	0.33	2.44
	>10	0.36	0.37	0.98	0.15	0.33	2.4
Chrysotile	< 5	0.14	0.24	0.57	0.09	0.57	0.8
	5-10	0.25	0.24	1.09	0.17	0.28	2.9
	>10	0.3	0.32	0.94	0.14	0.35	2.2
Amosite	< 5	0.33	0.3	1.08	0.17	0.28	2.9
	5-10	0.6	0.45	1.33	0.2	0.19	4.3
	>10	1.67	0.46	3.58	0.5	0.001	24
Crocidolite	< 5	0.28	0.22	1.31	0.2	0.19	4.2
	5-10	0.48	0.29	1.65	0.25	0.1	6.5
	>10	0.46	0.34	1.34	0.21	0.18	4.4
Other Silicates	< 5	0.61	0.33	1.83	0.28	0.08	7.9
	5-10	-0.02	0.35	0.07	-0.01	0.94	0
	>10	0.41	0.42	0.99	0.16	0.33	2.4
Talc Anthophyllite	< 5	0.05	0.26	0.2	0.032	0.84	0.1
	5-10	0.1	0.33	0.32	0.05	0.75	0.3
	>10	0.7	0.39	1.81	0.28	0.08	7.8
Total Fibres	< 5	0.59	0.41	1.41	0.22	0.16	4.9
	5-10	0.41	0.37	1.11	0.17	0.28	3
	>10	0.5	0.39	1.34	0.21	0.18	4.4
Conc. of AB	—	0.21	0.27	0.76	0.12	0.45	1.5

b_1 : Regression coefficient or the slope of the regression line
SD: Standard deviation for regression coefficient
r: Correlation coefficient
* : p-value based on non-zero regression and/or correlation coefficient
R^2: Coefficient of determination (%)

coefficients of determinations were also small, indicating no relationships between either $\log_e$ fibre length, diameter or and length-to-diameter ratio and fibrosis score.

The results of stratification by which the geometric mean length, diameter, and length to diameter ratio of different fibre types and sizes were compared for different levels of fibrosis score did not show any trend. The lack of trend was observed for both groups of subjects. The results of stratification are summarized in tables A-4 to A-6 for the subjects from Thetford-Mines and tables A-10 to A-12 for the subjects from Asbestos (Appendix).

Table 4.14. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and Log_e fibre length, diameter, and length-to-diameter ratio for fibres <5 μm among 45 subjects from Thetford-Mines. [#]

Fibre type	Dimension	b_1	SD	t ratio	r	p* value	R^2
Tremolite	L	-0.53	2.34	-0.23	-0.03	0.82	0.1
	D	-0.35	1.98	-0.18	-0.03	0.85	0.1
	L/D	-0.78	1.19	-0.66	-0.1	0.51	1
Chrysotile	L	0.22	0.9	0.24	0.05	0.81	0.3
	D	-1.64	1	-1.63	0.33	0.11	11.2
	L/D	0.31	0.58	0.53	0.11	0.6	1.3
Other Silicates	L	-1.24	1.13	-1.1	-0.18	0.28	3.1
	D	1.08	0.97	1.11	0.17	0.27	3.1
	L/D	-1.5	1.62	-0.93	-0.13	0.11	1.6

[#] No statistical analysis was conducted for amosite and crocidolite fibres, since these fibres were detected in the lung of only a few subjects (2 to 10 %) at very low numbers

b_1 : Regression coefficient or the slope of the regression line

SD: standard deviation for regression coefficient

r: Correlation coefficient

* : p-value based on non-zero regression and/or correlation coefficient

R^2: Coefficient of determination (%)

Table 4.15. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and Log_e fibre length, diameter, and length-to-diameter ratio for fibres 5-10 μm among 45 subjects from Thetford- Mines. [#]

Fibre type	Dimension	b_1	SD	t ratio	r	p* value	R^2
Tremolite	L	3.71	5.4	0.68	0.1	0.49	1.1
	D	0.24	1.16	0.21	0.03	0.83	0.1
	L/D	-1.99	1.32	-1.51	-0.22	0.14	5.2
Chrysotile	L	-1.24	4.8	-0.25	-0.06	0.80	0.4
	D	1.4	0.94	1.4	0.33	0.15	11.4
	L/D	-1.66	1.02	-1.01	-0.36	0.13	13.2
Other Silicates	L	0.37	3.9	0.09	0.01	0.92	0
	D	1.81	1.11	1.65	0.28	0.11	7.6
	L/D	-0.99	0.75	-1.29	-0.22	0.2	5

[#] No statistical analysis was conducted for amosite and crocidolite fibres, since these fibres were detected in the lung of only a few subjects (2 to 10 %) at very low numbers

b_1 : Regression coefficient or the slope of the regression line
SD: standard deviation for regression coefficient
r: Correlation coefficient
* : p-value based on non-zero regression and/or correlation coefficient
R^2: Coefficient of determination (%)

Table 4.16. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and Log_e fibre length, diameter, and length-to-diameter ratio for fibres >10 μm among 45 subjects from Thetford- Mines. #

Fibre type	Dimension	b_1	SD	t ratio	r	p* value	R^2
Tremolite	L	1.52	1.62	0.94	0.15	0.35	2.3
	D	0.04	0.63	0.06	0.01	0.95	0
	L/D	-0.74	0.99	-0.75	-0.11	0.46	1.4
Chrysotile	L	1.2	0.8	1.5	0.21	0.13	4.4
	D	-1.16	0.49	-2.35	-0.47	0.06	22.5
	L/D	0.59	0.39	1.52	0.33	0.14	10.7
Other Silicates	L	1.39	3.1	0.45	0.08	0.65	0.7
	D	-2.90	0.6	-0.48	-0.08	0.63	0.8
	L/D	0.34	0.6	0.57	0.1	0.57	1.1

No statistical analysis was conducted for amosite and crocidolite fibres, since these fibres were detected in the lung of only a few subjects (2 to 10 %) at very low numbers

b_1 : Regression coefficient or the slope of the regression line
SD: standard deviation for regression coefficient
r: Correlation coefficient
* : p-value based on non-zero regression and/or correlation coefficient
R^2: Coefficient of determination (%)

Table 4.17. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and Log_e fibre length, diameter, and length-to-diameter ratio for fibres <5 μm among 41 subjects from Asbestos.

Fibre type	Dimension	b_1	SD	t ratio	r	p* value	R^2
Tremolite	L	-0.1	2.44	-0.04	0.00	0.95	0
	D	-1.87	1.56	-1.2	-0.19	0.3	3.9
	L/D	3.93	2.14	1.83	-1.16	0.1	8.3
Chrysotile	L	2.36	1.57	1.5	0.25	0.14	6.2
	D	4.51	2.7	1.54	0.26	0.11	7.1
	L/D	0.74	1.4	0.53	0.09	0.6	0.8
Amosite	L	-1.06	2.87	-0.37	-0.15	0.72	2.2
	D	-0.2	2.42	-0.08	-0.03	0.93	0.1
	L/D	-0.64	2.55	-0.24	-0.09	0.81	1
Crocidolite	L	-3.3	2.17	-1.53	-0.3	0.14	9.6
	D	0.26	1.55	0.53	0.03	0.88	0.1
	L/D	-1.23	1.27	-0.97	-0.2	0.34	4.1
Other Silicates	L	-3.23	2.5	-1.29	-0.2	0.2	4.1
	D	-2.66	1.57	-1.59	-0.26	0.1	6.8
	L/D	2.39	2.1	1.14	0.18	0.26	3.2
Talc Anthophyllite	L	0.4	0.75	-0.55	0.03	0.59	0.9
	D	-0.43	2.38	0.8	0.00	0.82	0.2
	L/D	0.016	0.09	1.1	0.00	0.89	0.1

b_1 : Regression coefficient or the slope of the regression line
SD: standard deviation for regression coefficient
r: Correlation coefficient
* : p-value based on non-zero regression and/or correlation coefficient
R^2: Coefficient of determination (%)

Table 4.18. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and Log_e fibre length, diameter, and length-to-diameter ratio for fibres 5-10 μm among 41 subjects from Asbestos.

Fibre type	Dimension	b_1	SD	t ratio	r	p* value	R^2
Tremolite	L/D	-4.78	5.7	-0.85	-0.13	0.4	1.8
	L	-1.78	1.07	-1.55	-0.25	0.11	6.6
	D	1.64	1.1	1.51	0.23	0.14	5.5
Chrysotile	L/D	-1.51	5.35	-0.28	-0.05	0.78	0.3
	L	2.14	2.25	1.07	0.2	0.29	4.1
	D	-2.59	2.19	-1.18	-0.2	0.24	4.9
Amosite	L/D	-3.62	5.1	0.59	-0.2	0.27	4.2
	L	1.15	1.13	1.02	0.33	0.33	11
	D	-1.49	1.19	-1.26	-0.4	0.24	16.5
Crocidolite	L/D	-5.33	5.5	-0.83	-0.16	0.41	2.6
	L	0.11	1.26	0.01	0.00	0.93	0
	D	-0.3	1.25	-0.25	0.00	0.8	0.1
Other Silicates	L/D	3.07	5.28	0.49	0.03	0.62	0.7
	L	-1.14	0.78	-1.46	-0.24	0.15	5.9
	D	1.27	0.8	1.58	0.26	0.12	6.8
Talc Anthophyllite	L/D	-0.4	0.74	0.54	0.03	0.57	0.9
	L	-0.42	1.91	-0.22	0.11	0.82	0.2
	D	0.01	0.11	0.14	0.0	0.88	0.1

b_1 : Regression coefficient or the slope of the regression line
SD: standard deviation for regression coefficient
r: Correlation coefficient
* : p-value based on non-zero regression and/or correlation coefficient
R^2: Coefficient of determination (%)

Table 4.19. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and $\mathrm{Log_e}$ fibre length, diameter, and length-to-diameter ratio for fibres >10 μm among 41 subjects from Asbestos .

Fibre type	Dimension	b_1	SD	t ratio	r	p* value	R^2
Tremolite	L/D	0.17	1.05	0.08	0.01	0.9	0
	L	0.86	0.94	0.91	0.15	0.36	2.3
	D	-0.97	0.98	-0.99	-0.16	0.33	2.7
Chrysotile	L/D	1.83	3.14	0.58	0.1	0.56	1.2
	L	0.2	2.2	0.09	0.02	0.9	0
	D	0.43	1.76	0.25	0.05	0.8	0.2
Amosite	L/D	-0.17	1.53	-0.1	-0.02	0.92	0.1
	L	-0.63	1.44	-0.44	-0.1	0.66	1.2
	D	0.26	1.03	0.25	0.062	0.80	0.4
Crocidolite	L/D	-0.7	2.5	0.27	-0.01	0.29	0.3
	L	1.2	1.51	0.75	0.15	0.46	2.3
	D	-0.8	1.4	-0.56	-0.12	0.56	1.4
Other Silicates	L/D	-0.66	2.7	-0.24	0.00	0.81	0.2
	L	1.2	0.49	-1.3	0.15	0.46	2.3
	D	0.58	0.48	1.2	0.2	0.23	4.2
Talc Anthophyllite	L/D	0.21	0.16	1.29	0.24	0.2	5.8
	L	-1.28	0.95	-1.35	0.25	0.19	6.3
	D	0.017	0.2	0.92	0.17	0.36	3

b_1 : Regression coefficient or the slope of the regression line
SD: standard deviation for regression coefficient
r: Correlation coefficient
* : p-value based on non-zero regression and/or correlation coefficient
R^2: Coefficient of determination (%)

4.2.4. Association between exposure variables and fibrosis score

Tables 4.20 and 4.21 show the results of the simple regression and correlation analyses for each of the exposure variables as independent variables for both groups of subjects. For workers from Thetford-Mines, no exposure variables (e.g. duration of exposure, smoking, and age) showed statistically significant relationship with the fibrosis score. For the workers from Asbestos, only "age" showed some association with the fibrosis score ($r=+0.3$ and $0.1>p>0.05$). The regression and correlation coefficients of other variables such as duration of exposure , smoking, and years since last exposure, were small and non-significant ($p>0.1$).

4.2.5. Evaluation of interaction and confounding effects

To evaluate the possible interaction and confounding effects of exposure variables, several multiple regression models were examined. For each group, two fibre concentration variables with the largest regression coefficients that were also statistically significant were selected. These fibre concentration variables for the subjects from Thetford-Mines were short tremolite and intermediate-size chrysotile fibre concentrations. For the subjects from Asbestos, these variables were short tremolite and long amosite fibre concentrations. The result of the regression models did not indicate the presence of any interaction effects between concentration and exposure variables in any of the study groups. The test of significance showed that the regression coefficients of interaction terms were not significantly different from zero ($p>0.1$ for all interaction terms).

The results of statistical analyses did not indicate any confounding effect of age, smoking, duration of exposure and time since last exposure on fibre concentrations in any study groups. The regression coefficients of the concentration variables changed less than 10% when various combinations of these variables and four exposure variables were examined. This indicated that the effect of short tremolite and chrysotile concentrations on fibrosis severity was not confounded by any of the exposure variables in any study groups.

Table 4.20. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and various exposure variables among subjects from Thetford-Mines.

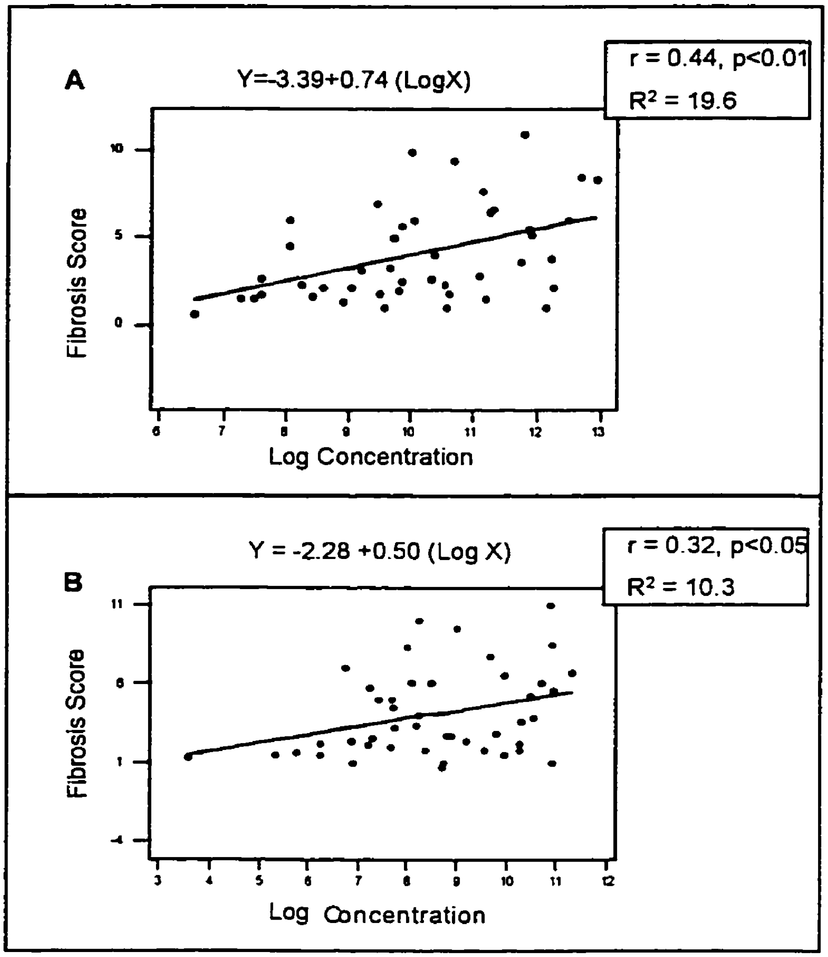
Variable	b_1	SD	t ratio	r	p* value	R^2
Duration of Exposure	-0.05	0.04	-1.2	-0.19	0.2	3.7
Age	-0.04	0.05	-0.84	-0.13	0.4	1.6
Smoking +	-0.01	0.01	-0.73	-0.11	0.46	1.2
Years Since Last Exposure	0.06	0.06	1.1	0.17	0.27	2.8
First Year of Exposure	0.03	0.046	0.7	0.1	0.43	1.1
Last Year of Exposure	-0.05	0.06	-0.83	-0.1	0.37	1.9

b_1 : Regression coefficient or the slope of the regression line
SD: Standard deviation for regression coefficient
r: Correlation coefficient
R^2: Coefficient of determination (%)
* : p-value based on non-zero regression and/or correlation coefficient

Table 4.21. Statistical parameters related to simple regression and correlation analysis between fibrosis score (0-12) and various exposure variables among subjects from Asbestos .

Variable	b_1	SD	t ratio	r	p* value	R^2
Duration of Exposure	0.13	0.23	0.09	1.5	0.14	5.5
Age	0.13	0.06	2.02	0.3	0.05	9.4
Smoking +	0.026	0.02	1.3	0.21	0.18	4.5
Years Since Last Exposure	0.1	0.12	0.93	0.14	0.35	2.2
First Year of Exposure	-0.04	0.06	-0.76	-0.12	0.45	1.5
Last Year of Exposure	-0.21	0.22	-0.94	-0.27	0.17	7.8

b_1 : Regression coefficient or the slope of the regression line
SD: Standard deviation for regression coefficient
r: Correlation coefficient
R^2: Coefficient of determination (%)
* : p-value based on non-zero regression and/or correlation coefficient



•**Figure 4.1**. Plot and regression equation showing relation between lung fibre content (log f/mg dry lung), and pulmonary fibrosis score (0-12) with significant level of $p<0.05$, for workers from Thetford-Mines; A) Tremolite fibres (<5 *um*), B)Tremolite fibres (5-10 *um*).

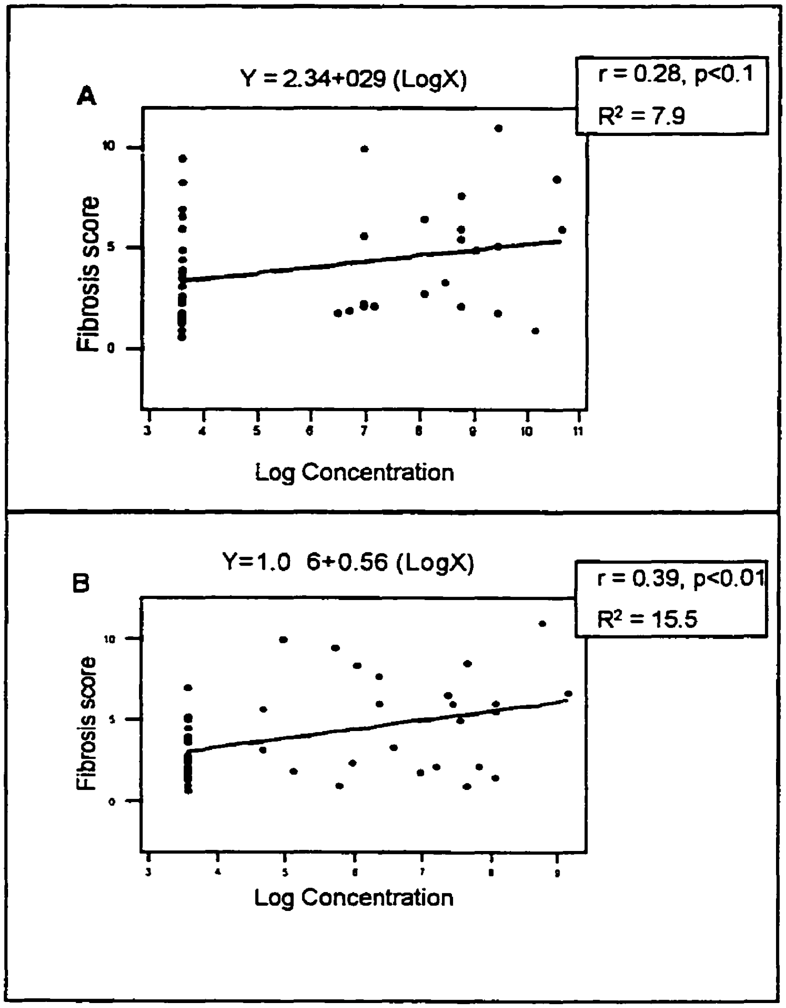


Figure 4.2. Plot and regression equation showing relation between lung fibre content (log f/mg dry lung), and pulmonary fibrosis score with (0-12) significant level of $p<0.05$, for workers from Thetford-Mines; A) Chrysotile fibres (<5 *um*), B) Chrysotile fibres (5-10 *um*).

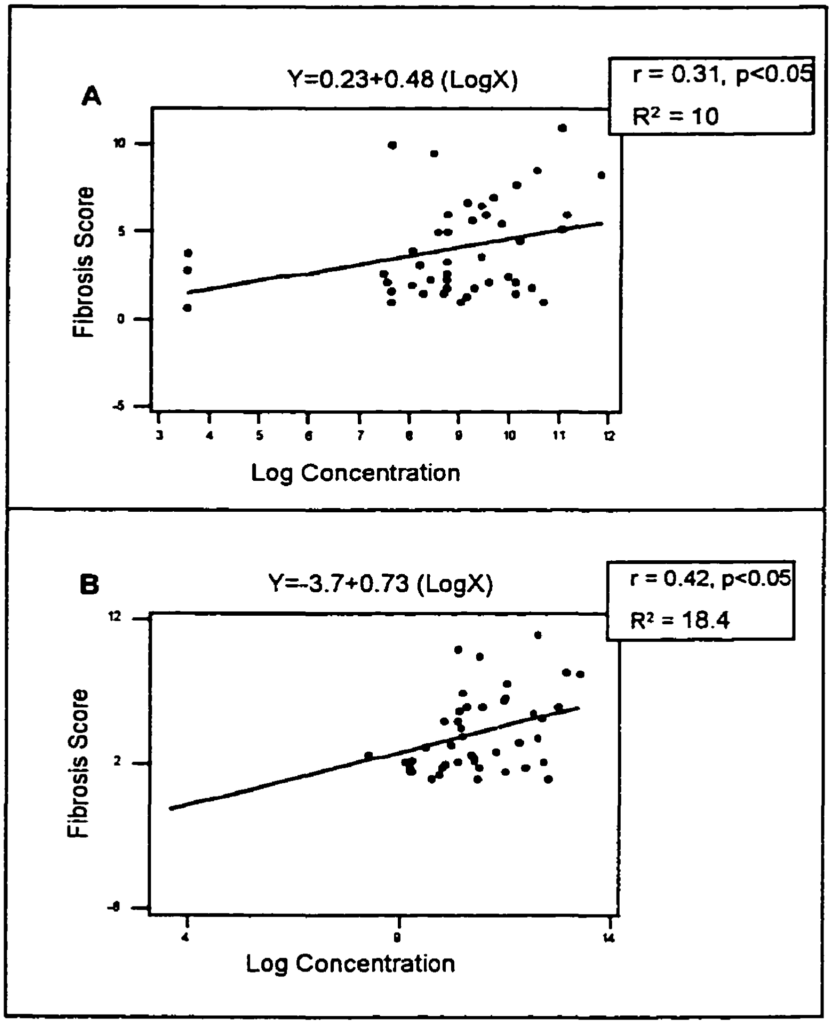


Figure 4.3. Plot and regression equation showing relation between lung fibre content (Log f/mg dry lung), and pulmonary fibrosis score (0-12) with significant level of $p<0.05$, for workers from Thetford-Mines; A) Other silicates (<5 *um*), B) All fibres (5-10 *um*).

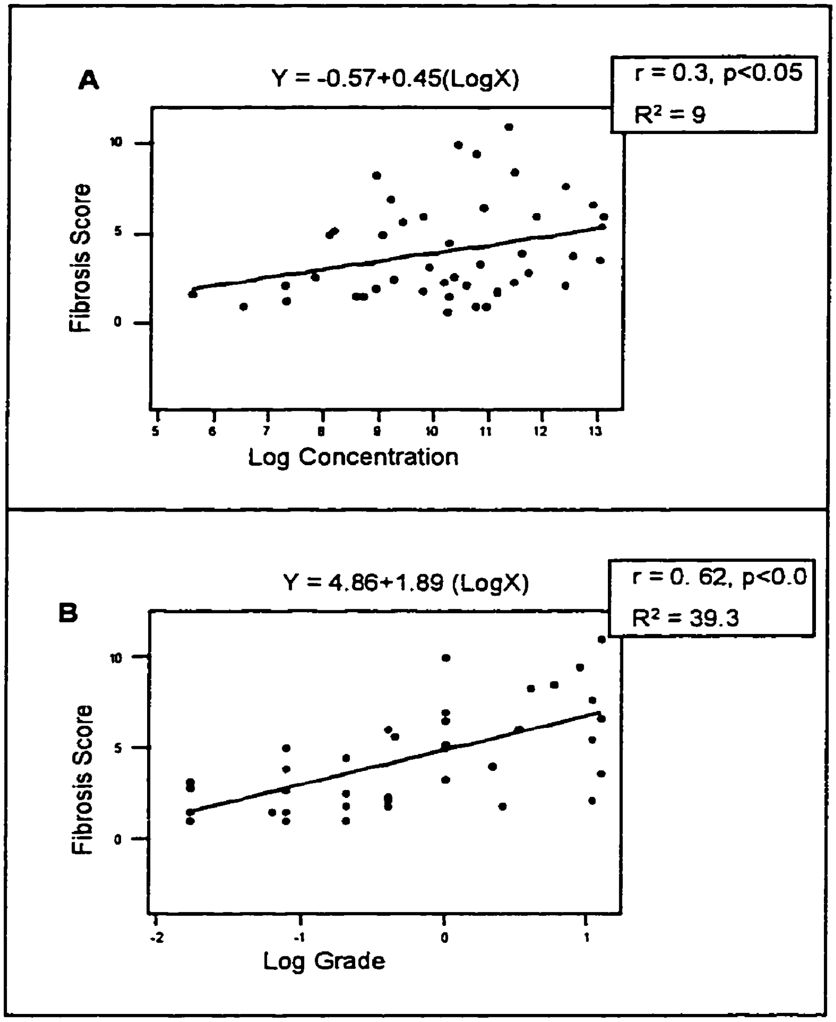


Figure 4.4. Plot and regression equation showing relation between asbestos bodies and pulmonary fibrosis score (0-12) with significant level of $p<0.05$, for workers from Thetford -Mines; A) ABs in digestiom (Log AB/g dry lung), and B) ABs in histoligical slides (Log grade).

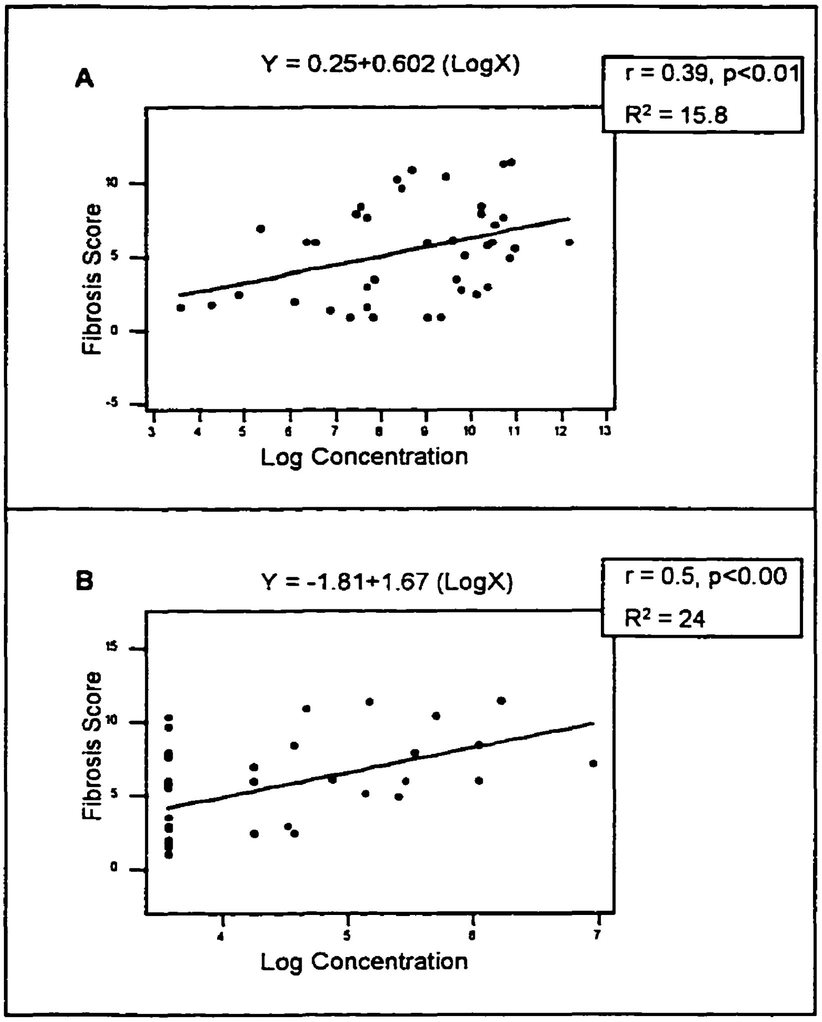


Figure 4.5. Plot and regression equation showing relation between lung fibre content (log f/mg dry lung), and pulmonary fibrosis score (0-12) with significant level of $p<0.05$, for workers from Asbestos ; A) Tremolite fibres (<5 *um*), B) Amosite fibres (>10 *um*).

Chapter 5

Discussion

5.1. COMPARISON OF FIBRE CONCENTRATION AND DIMENSION

The Québec chrysotile mining region has been, at least in the past two decades, a very convenient place in which to study the relative health effects of chrysotile and to some extent tremolite fibres. The area consists of two regions, separated by geography, geology, air fibre content, and disease incidence.[49] The incidence of asbestos-related diseases such as pleural calcification, radiographical abnormalities, and more strikingly, mesothelioma has always been higher in Thetford-Mines (among miners and millers) than in Asbestos.[49,59] A direct comparison of the lung fibre content of these two groups could reveal further differences in fibre concentrations and fibre dimension. Thus, it was important to select an adequate number of subjects with comparable exposure variables.

Two variables that could affect the retention of fibres in the lungs, the years of exposure and years since last exposure, were similar in the two groups of workers. Thus, it was assumed that any difference in the geometric mean lung fibre content of the two groups could not be attributed to these variables. In addition, since smoking habit did not differ between the two groups, the observed differences for fibre concentrations were unlikely to be related to increased deposition due to smoking.

The type, concentration, and dimension of fibres retained in lungs of miners and millers exposed to mineral fibres in these two regions have been addressed in several studies.[60-68]. Most of these focused on the relationship of asbestos-related diseases and lung fibre burden. In only one study was a direct comparison made between subjects from the two mining regions for both fibre concentration and dimension.[68] Lung fibre burden of 12 mesothelioma subjects from Asbestos and 12 mesothelioma cases from Thetford-Mines were compared. The geometric mean of tremolite fibres (>5 μm) were slightly higher among cases from Thetford-Mines.[68] Also, much higher concentrations of amosite and crocidolite were found in the lungs of cases from Asbestos.

The results of our study were comparable to those in the latter study of mesothelioma cases by Dufresne et al.[68] Although we had three categories of fibre size, the geometric mean concentration of short tremolite fibres (<5 μm) among 12 mesothelioma subjects from Asbestos studied by Dufresne et al. were higher than the ones among our subjects (8100 vs. 6350 f/mg dry lung). The geometric mean concentration of tremolite (5-10 μm) fibres concentration was comparable (2960 vs. 2990 f/mg dry lung) in our subjects and mesothelioma cases studied by Dufresne et al.

The presence of crocidolite and amosite in the lungs of subjects from Asbestos and the absence of these fibres in the lungs of the majority of subjects from Thetford-Mines have also been previously reported. [43,61,66-68,106] Our investigation reconfirmed the previous findings. While less than 15% of subjects from Thetford-Mines had rare amosite and crocidolite in their lungs at concentrations close to the detection limit of our analytical method, the lung tissue of 70% of subjects from Asbestos contained either crocidolite or amosite in much higher concentrations indicating a probable occupational exposure to these fibres.[43] The concentration of crocidolite was always higher than amosite within the Asbestos group, a fact that has been previously observed. [43,66,67]

For both study groups, we found a comparable size of short (<5 μm) mineral fibres of all types, except for tremolite. For short tremolite fibres, the GML was slightly higher for subjects from Asbestos (2 μm vs 1.98 μm). However, the GML of tremolite fibres 5-10 μm long in subjects from Thetford-Mines was significantly higher than that of subjects from Asbestos (5.98 μm vs 5.88 μm). The GM L/D of tremolite fibres >10 μm was also slightly higher for subjects from Thetford-Mines (27.8 vs 27). In general, the comparison of various fibre dimension parameters for the two groups did not show a consistent difference, which might have a biological significance.

As was mentioned by Gibbs,[45] non-commercial minerals occurring in the rocks mined are numerous, and many of them such as tremolite are likely to contaminate ambient air of the mines and mills. Although the percentage of tremolite in the ore body is small,[46] the high concentrations in the lung of miners and millers of both groups suggests a preferential accumulation in the respiratory system.[108]

In addition, the much higher concentration of chrysotile fibres in the lungs of subjects from Asbestos as compared to the subjects from the Thetford-Mines may not reflect a true difference of lung chrysotile content between these two groups. This is partly related to the limitation of our counting method. The counting method allowed for screening 30 microscopic fields or counting 30 fibres whichever was achieved first. Hence, due to the presence of a large number of tremolite fibres in the lung tissue of subjects from Thetford-Mines, the likelihood of detection of chrysotile fibres might have been reduced substantially.

The higher intensity of exposure to asbestos fibres is the most plausible, causative factor responsible for the higher rate of respiratory diseases among miners and millers from Thetford-Mines. If the concentration of tremolite dust is regarded as a surrogate for total asbestos fibre exposure level, it can be claimed that the former miners and millers of Thetford-Mines were exposed to two to four times as heavily as the miners and millers from Asbestos. The presence of the higher concentration of tremolite among subjects from Thetford-Mines may not indicate that this fibre was alone the main causative factor. Apparently, our subjects also had extremely high chrysotile fibre exposure. Yet, this exposure may not be reflected in the mineral analysis of lung content, because of methodological limitations as well as relatively faster clearance of chrysotile. Thus, we may not be able, based on the data obtained in the mineral content analysis, to elucidate the role of chrysotile versus tremolite in producing disease among the subjects from both regions. However, it can be suggested that although there are a few differences for fibre dimension, fibre concentration differs substantially between the two groups of workers and therefore can be regarded as a more significant factor that may be related to the higher rate of lung diseases in Thetford-Mines.

It should be noted that less than 5% of total fibres sized, counted and analyzed in this study were longer than 15 μm and around 2% were longer than 20 μm. Therefore, although three distinct size categories of fibres were characterized and compared for fibre dimensions and concentrations, the results of our study have a limited capability to address the possible effects of very long fibres (>20 μm).

Since chrysotile and its mineralogical properties, processes, and operations involved in both mining regions were similar, an other explanation seems probable. Gibbs[57] concluded that the cause might be related to some mineral closely associated with chrysotile such as mica or talc. Recently epidemiologists and toxicologists have suggested that tremolite might be responsible for observed differences.[42,49,59,109] The length of fibres has also been suggested by some as the morphological feature that may contribute to fibre toxicity, and there are both recent and older animal studies and in vitro evidence that to support this hypothesis.[26-27,30-31,110-113]

Currently, there is evidence that the carcinogenicity of chrysotile can be disproportionately affected by fibrous tremolite contamination.[22] It has been indicated that miners and millers at Thetford-Mines were more heavily exposed.[6,42,49] Our results provide further support for this opinion by quantitative comparison, but not for the effects of fibre size.

Recently the nature and distribution of amphibole minerals in Asbestos was investigated by a group of geologists from McGill University, Montreal, Canada.[46] The presence of several amphibole minerals such as tremolite, actinolite and a lower amount of anthophyllite was confirmed. In addition, it was shown that the bulk of the amphibole (tremolite) in this mining area occurs in serpentinite adjacent to or within felsic dykes, and that the chrysotile ores per se are amphibole-free. Although this survey explored the distribution of tremolite, there is still a need for a similar mineralogical investigation in Thetford-Mines ore bodies, followed by a comparison of the two mining regions.

5.2. MINERALOGICAL AND EXPOSURE CORRELATES OF ASBESTOSIS SEVERITY

The focus of the second part of this work was the severity of asbestosis and its relationship to fibre characteristics. Having a large number of lung tissue specimens from the two mining regions, which differ in disease incidence and exposure patterns, provided an opportunity to assess and compare the associations between fibre characteristics and the severity of asbestosis for the former workers of the two mining regions.

5.2.1. Fibre concentration

The results of this study showed both compatibility and contrast with previous investigations. In general, we found a stronger correlation between lung tremolite concentrations and fibrosis score than that for chrysotile with fibrosis score in both groups of subjects. The tremolite concentration showed a better correlation with the fibrosis score among subjects from Thetford-Mines than among subjects from Asbestos. Correlation of tremolite concentration with fibrosis was comparable with a previous study from the chrysotile textile industry.[77] Green et al. [77] reported a better correlation between tremolite concentration and fibrosis score ($r=0.64$, $p<0.0001$) than that for chrysotile concentration ($r=0.51$, $p<0.0001$), among textile workers. Churg et al.[92] also showed a better correlation of tremolite concentration with fibrosis score ($r=0.61$, $p<0.003$ for tremolite and $r=0.44$ $p<0.05$ for chrysotile) among formers miners and millers of Thetford-Mines. Albin et al.[91] found a non-significant correlation for chrysotile concentration with the severity of asbestosis among chrysotile cement workers (Kendall rank correlation $\tau<0.2$, $p>0.05$), and a significant correlation with tremolite (Kendall rank correlation $\tau=0.16$, $p<0.05$).

In the present study, the fibrosis score was correlated with chrysotile fibres (5-10 μm length) ($r=0.39$) for workers from Thetford-Mines. In general, chrysotile concentrations were less consistently related to the fibrosis score than were tremolite concentrations. Wagner and coworkers did not find any association between chrysotile and lung fibrosis among factory workers [85], naval dockyard workers [84], and 235 panel cases [83] (no statistical values were given).

The correlation coefficients for tremolite concentrations in our study were somewhat smaller than coefficients reported by Churg et al. [92] and Green et al.[77] The observed differences with the study of Green et al.[77] on textile workers were more likely related to different exposure patterns and exposure levels among chrysotile miners and millers and textile workers and, to some extent to methodological variations such as different fibre counting criteria. The smaller correlation coefficient among our subjects from Thetford-Mines ($r=0.44$) when compared with that obtained by Churg et al. [92]($r=0.61$ for tremolite concentration) is most likely related to the use of a larger number of sample, (for mineralogical analysis) per subject by Churg et al. (one sample each for 86 subjects in this study vs. four samples for 21 subjects by Churg et al.). When Churg analyzed four samples separately for each case (84 sites), the correlation coefficient was very similar for ours for Thetford-Mines subjects (0.44 vs. 0.45 for tremolite concentration). The use of a different grading scheme by Churg et al.,[92] in which fibrosis score was graded from 0 to 4, may have also contributed. In studies like this, the correlation coefficient would likely increase with a more extensive histological and mineralogical sampling of the lung for each subject when values are treated as separate since, in fact, intra-subject variation may be less than inter-subject variation.

Another important observation was that only concentrations of short and medium size tremolite fibres (Thetford-Mines) and short tremolite fibres (Asbestos) were correlated with the fibrosis score and this correlation was absent for long tremolite fibres among both groups ($p>0.1$ for both groups). Correlation coefficients of tremolite had a decreasing trend from short to long fibres in both groups of subjects, this trend was more apparent among Thetford-Mines workers. This finding shows that short tremolite fibres are better statistical predictors of the fibrosis score than long tremolite fibres. However, the present author cannot conclude that short fibre are more fibrogenic than long fibres based on the concentration data of this study. It should be emphasized that short fibres always outnumber long fibres regardless of the number of length categories used. Forty-six percent of tremolite fibres detected in the lungs of Thetford-Mines workers were shorter than 5 µm, 36% were 5-10 µm, and only 18% were longer than 10 µm. Short tremolite fibres were detected in the lungs of all Thetford-Mines subjects. However, medium size fibres were not found in the lung of two (4%) of subjects, and long fibres were not found in the lung of 5 (11%) subjects. This confirmed that short fibres are always better represented than long fibres in lung fibre burden studies due to the nature of the statistical distribution of airborne and inhaled fibres. The decreasing trend of correlation coefficients for tremolite is parallel to the decreasing concentration of tremolite fibres from short to long fibre categories. This trend, however, should not be taken to imply the presence of a decreasing fibrogenicity of tremolite from short to long fibres. It is also unknown to what extent short fibres are actually responsible of longer fibres which have fragmented during their residence in the lung.[41] Because of the aforementioned paucity of *concentration data* in the longer fibre categories, the author prefers to base his conclusion for the biological effects of *fibre dimension* based on the actual *fibre dimension data*. Thus, interpretation of concentration data is limited to the relationship between the fibre type and the severity of asbestosis. The issue of fibre length will be discussed later in this chapter by the use of dimension data, in which the direct correlation between fibre length, diameter and aspect ratio with the fibrosis score is addressed.

The correlation of the fibrosis score with tremolite concentration was stronger than the correlation of chrysotile concentration for both groups of subjects. This finding shows that lung tremolite concentration is a better predictor of both past exposure and progress of disease than is chrysotile concentration. This observation should not be taken to imply that tremolite is more fibrogenic than chrysotile. It is known that the chrysotile miners and millers, including the study subjects, were exposed principally to chrysotile fibres, and tremolite constituted only a small fraction of the original airborne dust to which they were exposed.[21,41] Chrysotile asbestos fibres fragment into fibrils, and are cleared from the lungs more rapidly than amphibole fibres.[41] Thus a component of the lung fibrosis in these asbestos workers could be due to asbestos fibres that were subsequently cleared.[77] Moreover, the small diameter of chrysotile fibres makes the detection of these fibres more difficult than tremolite. These methodological limitations may cause an under-representation of chrysotile fibre exposure and possibly chrysotile fibre lung content in the final counting results. It would be more likely to observe a higher correlation between chrysotile concentration and fibrosis, if a more accurate estimate of chrysotile corrected for clearance could be made.

The concentrations of commercial amphiboles did not show any associations with the fibrosis score among subjects from Thetford-Mines. This association was also absent for crocidolite (all size categories) and short and medium size amosite fibres among subjects from Asbestos. Yet, the concentration of long amosite fibres (>10 μm) showed a significant positive correlation with the fibrosis score among subjects from Asbestos ($r=0.5$, $p<0.01$). This correlation was even stronger than the correlation found for tremolite and chrysotile. Lack of any correlation between commercial amphiboles and fibrosis among the subjects from Thetford-Mines was not surprising, since this population did not have any history of exposure to these fibres and only rarely were amosite and crocidolite fibres found in the lungs of a small proportion of subjects. The lack of association between the crocidolite concentration and the fibrosis score among subjects from Asbestos contrasted with the previous studies by Wagner et al. [85] in which subjects (factory workers) were exposed to both chrysotile and commercial amphiboles as well as by Churg et al.,[90] in which insulators were heavily exposed to amosite fibres. Wagner et al. [85] found increasing concentrations of crocidolite and amosite in cases of mild to severe asbestosis among 36 factory workers. Wagner et al.[84] also reported that the proportional counts of amosite and crocidolite increased with the level of fibrosis (from mild to severe) among naval dockyard workers, although no statistical values were given. Churg et al. [90] found a strong association between lung amosite concentration and the fibrosis score among 20 former insulation workers. Discrepancies between our results and the findings of Wagner et al.[85] and Churg et al.[90] may be due to different populations with different exposure patterns. Unlike our chrysotile workers, who had a minor exposure to amosite and crocidolite, subjects studied by Wagner et al. and Churg et al. were principally exposed to these fibres.[85,90] Cement workers studied by Albin et al. [91] were more similar to our subjects from Asbestos, since they were exposed mainly to chrysotile and small amounts of commercial amphiboles. However, Albin et al.[91] did not find a significant correlation between either crocidolite or amosite concentrations and severity of asbestosis (Kendall rank correlation (τ <0.15, p>0.05 for both fibres).

The same method of examination of data obtained from two populations, one exposed to chrysotile and tremolite and other exposed to amosite led Churg and co-workers[90,92] to conclude that amosite is more fibrogenic than chrysotile and tremolite. However, the author of this study did not attempt to make any fibre-to-fibre comparison in regards to fibrogenic potency, since the subjects of the present study (Asbestos only) had a short term and minor exposure to amosite. The geometric mean concentration of long amosite fibres was 71 fibre/mg dry lung almost at the detection limit of our analytical method. More importantly, the correlation coefficient applied to all 41 subjects from Asbestos, among which only 19 (41%) subjects had any long amosite fibres in their lungs. Although, the correlation coefficient and the slope of the regression line of long amosite fibres among the subjects from Asbestos was slightly higher than the those for tremolite fibres, tremolite concentrations, although non-significant for long tremolite, were relatively more consistently and systematically related to asbestosis for both groups. Also, a similar trend was observed for tremolite fibres in previous studies of chrysotile exposed workers.[77,88,91,92] Therefore, in this present population, interpretation should be limited to the determination of the best statistical predictor of pulmonary fibrosis at the time of death rather than identification of the most fibrogenic fibre which initiated its fibrogenic activity years before the death. The correlation between long amosite concentration and the fibrosis score was not significant when the data was reanalyzed for *19 subjects who had long amosite* fibres in their lungs ($r=0.32$, $p=0.11$).

In this study a positive correlation between the total number of asbestos fibres in the lung and the severity of pulmonary fibrosis was found among Thetford-Mines workers ($r=0.42$) but was absent among workers from Asbestos ($r=0.22$). Green et al.[77] and Roggli [87] found a correlation between total fibres and fibrosis score ($r=0.57$, $p<0.05$). Wagner et al.[85] showed that total fibre concentration among 36 factory workers was higher in those with a higher category of fibrosis. Whitwell et al.[81] and Ashcroft and Heppeleston[80] showed a progressive increase in the median total coated and uncoated fibre counts from patients with mild to severe fibrosis, respectively.

The presence of a correlation between total fibre concentration and the fibrosis score among Thetford-Mines workers, and lack of this correlation among Asbestos workers, is most likely affected by the different proportion of tremolite in the total fibres. While 72% of the total fibres (Table 4.2) were tremolite among subjects from Thetford-Mines, tremolite fibres constituted only 34% of the fibres detected in the lungs of workers from Asbestos (Table 4.2). This stronger representation of tremolite among Thetford-Mines workers is related to the higher proportion of tremolite in the lung. When the correlation between total fibre concentrations in three size categories and the tremolite concentrations of corresponding size categories are compared between the two groups, the correlation coefficients were higher among subjects from Thetford-Mines than those among subjects from Asbestos in all size categories (see Appendix, Table A-14)

No relationships were found either between the fibrosis score and non-asbestos fibres concentration or with short talc/anthophyllite fibres concentration. This observation was in agreement with previous findings for exposed populations other than chrysotile miners and millers. Green et al.[77] and Churg et al.[92] reported a non-significant relationship for non-asbestos fibres and asbestosis severity in chrysotile textile workers and Thetford-Mines miners, respectively. Wagner et al.[85] did not show any trend of non-asbestos concentrations with severity of asbestos in his study of factory workers. Long talc-anthophyllite fibres showed a weak correlation with fibrosis score for both groups.

A significant correlation between the fibrosis score and the concentration of asbestos bodies in tissue digests was found among the subjects from Thetford-Mines. This correlation was absent among the subjects from Asbestos. It was previously noted that workers at Thetford-Mines had a much higher lung tremolite content indicating higher exposure to tremolite as was indicated above. It is also known that tremolite fibres form asbestos bodies more readily than do chrysotile fibres. Thus, the stronger correlation between the concentration of asbestos bodies and the fibrosis score may also be related to the higher concentration of tremolite in the lungs of Thetford-Mines workers. When the correlation between tremolite concentrations and asbestos bodies concentrations are compared between the two groups, correlation coefficients were larger among Thetford-Mines workers than those among Asbestos workers (see Appendix, Table A-15).

A similar trend was found when the correlation between the grade of asbestos bodies and the fibrosis score was compared between the two groups. This correlation was significant among the subjects from Thetford-Mines ($p<0.05$) and non-significant among the subjects from Asbestos ($p>0.1$). The correlation coefficient of the grade of asbestos bodies was slightly larger than that for the concentration of asbestos bodies, in both groups. However, the grade of asbestos bodies, also classically recommended for the diagnosis of asbestosis, may be biased, since the pathologist in this study was aware of the presence of pulmonary fibrosis and its severity, and had been trained to look for asbestos bodies when there is fibrosis. The concentration of asbestos bodies, estimated in a different laboratory is less likely to be biased. Moreover, the concentration of the asbestos bodies is estimated by digesting of a considerable amount of tissue (25 mg) and expressing it as a continuous variable (AB/gr dry lung), while the grade of asbestos bodies is determined categorically on a thin section (4 μm) of the lung tissue.

Johanson et al.[89] reported a significant association between asbestos bodies concentration and fibrosis score among 89 cement workers ($p<0.01$ Kendall rank correlation). Roggli et al.[86] found a non-significant correlation between the asbestos bodies count and the fibrosis score ($r=0.26$, $p>0.1$) among 36 asbestosis cases. In general, the results of this work indicated that lung fibre concentrations, measured by TEM, provides a better predictor of lung fibrosis than does concentration of asbestos bodies.

It was not possible to determine a minimum concentration of fibres in the lung necessary to produce histologic asbestosis by using our data. Our subjects were chrysotile miners and millers with a long-term and heavy exposure to asbestos fibres. It has been strongly suggested that the minimum cumulative exposure necessary to invoke asbestosis is 25 f/ml.year.[115] The long duration of exposure (mean 36 years), and average level of fibre concentration during the years in which our subjects were employed (15 to 50 f/ml) led us to conclude that these subjects had a much higher cumulative exposure than 25 f/ml.year. The lungs of these workers usually contained very high concentrations of fibres. Thus, it does not provide a wide enough range of lung fibre concentrations for the determination of a threshold. In addition, no subject was found to have a zero fibrosis score, making the identification of a threshold level for fibre concentration unlikely.

5.2.2. Fibre dimension

The result of several regression and correlation analyses on three dimension parameters within three length categories for both groups did not show any association between the fibrosis score and the dimension-related variables. This finding was in disagreement with Churg's findings among 21 Thetford-Mines workers [92] and 20 shipyard workers.[90] Churg et al.[92] found a negative correlation between fibre length of tremolite and amosite fibres with the severity of asbestosis. He also found a similar relation between fibre aspect ratio and fibrosis score. He suggested that short fibres are actually more fibrogenic than long fibres. Later work by Churg et al. [93] using a similar fibre counting method but different statistical method on larger number of subjects did not appear to support his hypothesis.

None of the 21 cases studied by Churg and coworkers had a geometric mean fibre length of more than 5 µm. Geometric mean length and aspect ratio of tremolite fibres of his cases ranged between 2 and 4.5 µm and 12:1 to 22:1, respectively, indicating a coverage of a very narrow range of fibre length and aspect ratio.

Using the three length categories provided a more sensitive approach to characterizing fibre dimension. By counting and enumerating mineral fibres in three separate steps for fibres <5 µm ,5-10 µm, and >10 µm separately, long fibres were better represented. This method permitted the evaluation of the relationship between the fibre size and the fibrosis severity for fibres up to 20 µm. Moreover, a much wider range of fibre length and aspect ratio was evaluated. For instance, the geometric mean length of tremolite fibres detected in the lungs of the Thetford-Mines subjects was 1.25 µm (in category < 5 µm) to 18.5 µm (in category >10 µm). For the same group geometric mean aspect ratio tremolite fibres ranged from 6:1 (in category <5 µm) to 183:1 (in category >10 µm).

The results of *dimension data* did not support Churg's hypothesis that short fibres are more fibrogenic than long fibres, but neither did it support our hypothesis that there might be a positive correlation between the fibre length and the fibrosis score. However, our approach had a limited ability to evaluate the effects of very long fibres (eg: >20 µm). Only 5-6% of the total fibres detected for both groups had a length of more than 15 µm and 2-3% had a length of more than 20 µm. Therefore, the author does not generalize his finding for fibres beyond the studied ranges. It may also seem that the results of this study contradict the findings of previous animal studies in which the effect of fibre size was investigated. In general, animal experiments, using either inhalation or instillation methods of exposure to fibres, suggested a higher inflammatory reaction and fibrotic response of the lung tissue among animals exposed to long fibres than those exposed to short fibres for the same fibre type and concentration. (28,29,36,112-117)

While the results of this work evaluated the correlation between the fibre dimension and fibrosis for fibre length < 20 µm, the results of animal experiments suggest the possible effects of fibre length at ranges above our size categories (20-50 μm).[28-29,114-115] Recently Miller and co-workers[30,31] reported the results of their works in which the tumorogenicity of several variables such as fibre concentration, dimension, and biopersistence were investigated through inhalation or injection of fibres in rats. The results suggested consistently that the number of fibres thinner that 1 μm and longer than 20 μm was the best predictor of risk of cancer. This effect, however, was absent for thicker (>1 μm) and shorter size categories of fibres (ie: >15 μm, >10 μm, > 8 μm and >5 μm). The results of the present study did not indicate any correlation between fibre length and diameter and asbestosis severity for fibres length up to 15 μm. Yet, it might not be appropriate to rule out the possible association between the fibre size for very long fibres (>20 μm) on the asbestosis severity based on our observations. The effect of fibre length and diameter on asbestosis severity for very long fibres (>20 μm) on occupationally exposed populations deserves further investigations.

One may argue that very long fibres (eg >20 μm) are not able to penetrate into the alveolar region of the lungs because of their size. However, it has been suggested that the penetration of mineral fibres is managed mainly by their diameter and not by their length and that very long fibres can penetrate into the alveolar region of the lung as long as their diameter is less than 3 μm.[118] In an experimental study using human tracheobrochial casts, the deposition of fibres in the airways was shown to increase in the airways as the length increased.[119] In addition, pulmonary retention increases markedly in rat lungs with increasing fibre length above 10 μm.[120] Asbestos fibres as long as 50 to 100 μm have been detected in the lungs of Québec chrysotile miners and millers.[121]

It is noteworthy that a direct extrapolation of the results of animal experiments on humans might not be justified, since, these experiments have considerable methodological and biological limitations. The size classification of fibres in these studies, particularly in the earlier works, were inaccurate.[114,116,122] Unlike human exposure, the method of exposure in animal experiments is usually by injection [117] or instillation. [28] In studies in which inhalation exposures were used, animals were exposed to very high concentrations of fibres within a short period of time.[115,123] Physiological differences such as different lung size, histological anatomy, and life-span should also be considered. Human exposure to asbestos fibre in industry, in mining and milling in particular, is not comparable with the animal experiments. Unlike human exposure in the working environment, in animal experiments exposure time is usually between 6-18 months. Therefore, the interpretation of the results of animal experiments on human working populations requires caution. This caution should also be exercised when comparing the result of the present work with those obtained through experiments on laboratory animals.

Timbrell et al. (124) recommended that fibre surface area might be a better predictor of fibrosis than fibre length or fibre diameter. However, no attempt was made to include this parameter in the present study because fibre surface area can be calculated by the use of fibre diameter and fibre length. Therefore , the use of fibre surface area would have provided a variable with a high correlation with mean fibre length and diameter, with no significant independent contribution in explaining fibrosis severity. Churg et al.(92) found a correlation between geometric fibre surface area as calculated theoretically, and fibrosis score ($r= 0.5$) , which was still weaker than the correlation found for geometric mean fibre length ($r= 0.7$) for the same subjects.

It is unlikely that our finding were affected by inclusion of subjects without asbestos bodies in their histological sections. As mentioned in Chapter 4, those subjects had an established long term exposure to asbestos fibres. This was indicated by a high concentration of asbestos bodies in lung digest (more than 1500 AB per gram dry lung) for 10 subjects and having more than 1 million asbestos fibres per gram of dry lung tissue for all subjects.(125) In addition, some investigators have challenged the requirement for finding asbestos bodies in histological section because of different rate of body formation among chrysotile, tremolite, and other commercial amphiboles.(76) The controversy about the presence of asbestos bodies in histological sections is more related to the medico-legal and compensation aspects of asbestosis, and does not seem to be relevant to our research questions.

5.2.3. Exposure variables

In general, exposure variables did not show any important predictive ability for fibrosis severity in comparison with the fibre concentration. This lack of association was more apparent among subjects from Thetford-Mines. Among subjects from Asbestos, the association of age approached statistical significance. The results of multiple regression analysis did not support confounding effects of any exposure variables on the fibrosis score among subjects from Thetford-Mines. Models that included, age, time since last exposure, and smoking only slightly improved the model. The correlation between exposure variables and concentration variables (the ones selected for multiple regression) were small and non-significant. The duration of exposure did not show any predictive ability for either groups, indicating that this variable alone might not be an adequate surrogate for exposure in the studies of exposure-response of asbestosis subjects among the population of Québec miners and millers. Hypothetically, the exposure intensity, measured as airborne fibre concentration, might be more predictive of fibrosis. However, due to the unavailability of the cumulative index of exposure (mppcf.y or f/ml.y) or the exposure intensity (mppcf or f/ml) for the study subjects this relationship could not be examined, and no comparison between exposure intensity and lung fibre content could be assessed for their accuracy of predicting asbestosis severity.

Johanson et al.[89] found a correlation between age and fibrosis, but not between fibrosis and duration of exposure. Albin et al.[91] did not find any association for age and fibrosis. Green et al. [77] found an additive effect of age on the fibrosis score, but no correlation for peak exposure, time since last exposure, or latency. The correlation coefficients between age and concentration variables were small and non-significant, indicating no interaction effect between age and lung concentration. A lack of significant correlation was also observed for time since last exposure and concentration ($r=0.2$, $p>0.1$).

Smoking and asbestos exposure are both known to cause small airway disease[126]; and therefore, smoking can influence the pathological grade of asbestosis among workers exposed to asbestos fibres. Roggli et al. [86] found a significant correlation between pack-year smoking and asbestosis grade. Johanson et al. [89] and Albin et al.[91] found no significant correlation between cigarette smoking and the level of asbestosis. This possible influence of cigarette smoking on the histological grade of asbestosis is likely to be minor [77], yet, it was essential that it be investigated. Absence of a correlation between smoking and the fibrosis score in both study groups attests that the effect of smoking on the fibrosis among workers, who were exposed heavily to asbestos fibres for a long time, is minor if any, and thus, cannot be detected by the statistical approach used in this study for these subjects. Morphological studies comparing the lungs of asbestos workers who smoked and non-asbestos workers who smoked have shown that the contribution of cigarette smoking to pulmonary fibrosis is small or absent compared with the effect of asbestos exposure [77,126,128]. It is noteworthy that there is no conclusive evidence that smoking can affect the deposition and retention of asbestos fibres in the human lungs. In a study of Québec miners and millers, Churg et al.[65] found no relationship between pack-year of smoking and the parenchymal fibre concentration in the lung. The results of the present study did not show any correlation between pack-year smoking and lung fibre concentrations ($r<0.25$, $p>0.1$ for tremolite and chrysotile). Therefore, it is unlikely that the lung fibre concentrations in this study were influenced by cigarette smoking.

It is very likely that, among cases with heavy exposure to asbestos like our study subjects, the association of exposure variables cannot be observed because of the long term effect of asbestos fibres on the respiratory system. Moreover, our subjects were very similar both in regards to lung fibre concentrations and smoking habit, making the determination of the independent effects of smoking in fibrosis severity in this population unlikely. This explanation may also be valid when the interaction and confounding effects of exposure variables are addressed. However, since these effects were evaluated using multivariate statistical analysis on a relatively small sample size, the interpretation of the results requires caution. In general, lung fibre concentration measured by TEM had a stronger predictive ability than exposure variables studied in this research.

Human response to environmental contamination, in general, and to mineral particles in particular, can be influenced by a host factor. The observation that, even after long-term exposure to high dust levels, a substantial proportion of the workers, often the majority, exhibit no evidence of asbestosis [129] has led to a search for many host factors that might offer protection against the consequences of asbestos inhalation or that might enhance susceptibility. Physical dimensions of the lung and also immunological and genetic factors have been addressed in the literature.[56,69,130] However, there has been less research interest in what factors trigger the response in some individuals and not in others, nor is it known whether the response of the immunoregulatory mechanisms is genetically determined. In general, the evidences implicating the factors discussed above is inconclusive and does not suggest that this may be of a practical value for screening of workers. These issues, nevertheless, require further research.

5.3. LIMITATIONS

1) The initial step in conducting a lung fibre burden study is to select subjects from whom tissue specimens will be obtained. Ideally, the relationship between these subjects and the population for which the results of the study will be generalized should be addressed. However, individuals who are autopsied are not representative of persons who die in the population under study. This has been shown among chrysotile miners and millers of Québec, who have a five-fold increased necropsy rate for subjects with mesothelioma, lung cancer, and asbestosis.[131]

Selection bias in a study of this nature comes from selection of subjects for autopsy. Autopsy selection bias in lung fibre content studies among occupationally exposed groups is evident.[43,64] Due to current compensation standards, chrysotile miners and millers in Québec are more likely to have an autopsy if they have a potentially asbestos related disease and if they are of working age at the time of death.[43,64] Selected subjects of this study should, however, be representative of each mining region. In Québec, the rate of autopsy among the general population of workers is unknown.[73] The rate was 10% in 1986, but a previous calculation in the McDonald birth cohort showed a higher overall autopsy rate (17%) among workers up to 1975.[73]

The total number of autopsies among the subjects from Thetford-Mines was approximately 95 over three years(1989-1991) from which 45 subjects (47%) were selected for our study. This figure is not clear for the miners and millers from Asbestos. However, the author estimates that the total number of autopsies during 1983-1991 for this population could not have been more than 100. This means that 41% of cases autopsied were included in our study. It is clear that a marked selection for possible asbestos-related cancers can take place among those having been autopsied. However, for asbestosis, the question is less clear as this is rarely a cause of death among chrysotile miners and millers.[73] Although all of our subjects had asbestosis and their autopsy reports were available, the occurrence of selection bias remains very likely because they all had multiple causes of death such as lung cancer and mesothelioma. In general, necropsy selection bias, whether recognized or not, is always a factor in studies of lung asbestos fibre content, as necropsies may be performed because of work histories.[133] In the present study this inherent bias exists, thus, the investigators with knowledge of that should make the best use of available lung tissues.

2) Cumulative exposure index was not available for our subjects, precluding use of this variable in our analysis. Notably, Case et al.[106], compared lung fibre burden for 22 mesothelioma subjects from Asbestos and Thetford-Mines and while cumulative exposure did not differ between the two areas, the lung tremolite concentration was significantly higher among subjects from Thetford-Mines. Lung tremolite concentrations may provide a better measure of true cumulative exposure than environmental measurements.

3) To address the possible interaction and confounding effects of variables such as age and smoking, multiple regression analysis was used among possible methods. It is likely that the results of multivariate analyses were affected by two potential limitations 1) small sample size, and 2) correlation between the independent variables. Thus, the multivariate method used might not have adequate robustness to fully explore the interaction and confounding effects. This part of the statistical analysis might have provided a different result if a larger number of subjects were included. Therefore, the author cautions that interpretation of the results of multivariate analyses should be made carefully. These limitations, however, did not seem to affect the results of simple regression analyses and other statistical tests.

In this study, many statistical tests were performed and many p values obtained. On the one hand, the use of the statistical tests on a convenient sample (i.e. not chosen in a probabilistic fashion), such as the one used in this study, has its limitations.[133] On the other hand, because of multiple testing, p values probably should have been adjusted according to the number of the test before interpretation. Thus the author cautions about such limitations, although the author does not believe the general interpretation of results would be changed.

Chapter 6

Conclusion

The author would like to reiterate that this work cannot examine all the issues involved in the interpretation of lung-retained fibres and their association with severity of disease. In addition, since there are complex and inadequately-explored biological mechanisms in the genesis and progression of asbestos- related diseases, the presence of statistical measures of **correlation** and **association** used in this study should not be interpreted as indicators of **causation**.

The present study allowed the author to conclude the following;

1. The higher rate of respiratory diseases among miners and millers from Thetford-Mines is associated with higher inter-pulmonary fibre concentration, with tremolite being more apparent. If the concentration of tremolite dust is regarded as a surrogate for total asbestos fibre exposure level, it can be claimed that the former miners and millers of Thetford-Mines were exposed two to four times as heavily as miners and millers from Asbestos.

2. Differences between the two mining areas in lung-retained fibre dimension were minor, inconsistent, and therefore of dubious biological significance. Therefore, fibre dimension (within the study range) cannot explain the difference in mortality and morbidity observed between the two mining regions.

3. Concentration of fibres as measured by Transmission Electron Microscope/Energy Dispersive X-ray Spectroscopy, in particular short tremolite concentration, has better predictive ability and is a better biological indicator than either concentration of asbestos bodies estimated by optical microscope, or exposure variables such as duration of exposure for asbestosis severity evaluated histologically. It remains possible that detailed estimates of cumulative exposure based on industrial hygiene sampling methods would provide results better than lung fibre concentrations.

4. Although miners and millers of chrysotile mining regions in Québec were principally exposed to chrysotile asbestos, tremolite fibre concentration in lung tissue provides a better predictive ability for severity of pulmonary fibrosis. The latter may nevertheless have resulted from concomitant exposure to chrysotile fibres at least in part. However, the present author hesitates to make a conclusion on the potential fibrogenicity of chrysotile in comparison with tremolite, since chrysotile fibre may be cleared from the lung years before the time of death.

5. Among subjects from Asbestos, the concentration of long amosite fibres was the best predictor of asbestosis. Since only half of the subjects had long amosite fibres in their lungs and because in general tremolite concentration was more consistently associated with asbestosis severity, a conclusion for different fibrogenicity of amosite and tremolite (and the associated chrysotile exposure) cannot be drawn.

6. Correlation between histological grade and lung-digest concentration of asbestos bodies with asbestosis severity in Thetford-Mines subjects and the absence of these correlation in Asbestos workers are most likely affected by a higher exposure to tremolite in Thetford-Mines workers, resulting in a much higher tissue concentration of tremolite in the lungs of these workers.

Also, the presence of correlation between total fibre concentration and asbestosis severity in Thetford-Mines workers and lack of this correlation in Asbestos workers may also be affected by higher pulmonary concentration of tremolite in the lung of Thetford-Mines workers.

7. There is no association between fibre dimension (fibre length, diameter and aspect ratio) and asbestosis severity for asbestos fibres up to 20 μm in these populations. Our study was limited to concentration and dimensions of mineral fibres shorter than 20 μm, and mainly shorter than 10 μm. Future work may emphasize the possible contribution of very long fibres, which require separate assessment due to their rarity in fibre distribution.

8. Smoking, age, duration of exposure, and time since last exposure did not show interaction with tissue fibre concentration and did not show any significant modifying or confounding effects on the relation between tissue fibre concentration and severity of asbestosis.

9. This study suggests that the presence of asbestos bodies in histological sections as a criteria for the diagnosis of asbestosis might not be appropriate. Lung fibre concentration measured by TEM/EDS and asbestos bodies count in the lung-digest are better indicators of lung fibre content especially for chrysotile exposure. Future work, however, is necessary to study this relationship among other asbestos-exposed populations.

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Appendix

Table A-1. Geometric mean concentration (GM) and geometric standard deviation (GSD) of lung retained short fibers(<5 μm),by three levels of fibrosis score (0-12); fibers/ mg dry lung tissue for 45 subjects from Thetford-Mines				
Fiber type	**Fibrosis score**			
	0-4 **n=25**	**4-8** **n=15**	**8-12** **n=6**	**r**
Tremolite	14928 (5.09)	32080 (3.93)	140786 (3.79)	0.44***
Chrysotile	185 (9.8)	699 (16.5)	2039 (22.2)	0.28**
Amosite	35 (ND) (1)	50 (4)	35 (ND) (1)	0.14*
Crocidolite	48 (2.57)	44 (2.47)	35 (ND) (1)	0.13*
Other Silicates	3975 (7.08)	13877 (2.44)	18767 (6.21)	0.21*
Talc/ Anthophyllite	4117 (6.35)	2875 (4.97)	2853 (4.9)	-0.02**
Total Fibers	27065 (5.89)	66105 (2.37)	197008 (3.6)	0.42 ***

Limit of detection = 70 fibers /mg dry lung tissue for a 15 mg lung tissue
r = Correlation coefficient
n = Number of subjects
ND = Not detected in any subjects (half of the detection limit is assigned)
* $p>0.1$, ** $0.1>p>0.05$, *** $p<0.05$

Table A-2. Geometric mean concentration (GM) and geometric standard deviation (GSD) of lung retained fibers (5-10 μm), by three levels of fibrosis score (0-12); fibers/ mg dry lung tissue for 45 subjects from Thetford-Mines

Fiber type	Fibrosis score			
	0-4 n=25	**4-8 n=15**	**8-12 n=6**	**r**
Tremolite	3633 (6.32)	7376 (4.72)	13386 (4.69)	0.32***
Chrysotile	111 (5.22)	368 (8)	940 (5.37)	0.39***
Amosite	35 (ND) (1)	35 (ND) (1)	35 (ND) (1)	(ND)
Crocidolite	36 (1.27)	40 (1.72)	65 (3.45)	0.13*
Other Silicates	350 (5.74)	358 (6.24)	1118 (6.17)	0.12*
Talc/ Anthophyllite	113 (4.83)	96 (4.33)	235 (5.8)	-0.04*
Total Fibers	5223 (6.3)	8168 (5.17)	16155 (3.62)	0.26**

Limit of detection = 70 fibers /mg dry lung tissue for a 15 mg lung tissue
r = Correlation coefficient
n = Number of subjects
ND = Not detected in any subjects (half of the detection limit is assigned)
* $p>0.1$, ** $0.1>p>0.05$, *** $p<0.05$

Table A-3. Geometric mean concentration (GM) and geometric standard deviation (GSD) of lung retained long fibers (>10 μm), by three levels of fibrosis score (0-12); fibers/ mg dry lung tissue for 45 subjects from Thetford-Mines				
Fiber type	**Fibrosis score**			
	0-4 **n=25**	**4-8** **n=15**	**8-12** **n=6**	**r**
Tremolite	998 (6.88)	1475 (4.75)	829 (9.5)	0.035*
Chrysotile	107 (6.58)	202 (5.1)	65 (3.45)	-0.01*
Amosite	43 (1.72)	35 (ND) (1)	65 (3.24)	0.02*
Crocidolite	38 (1.34)	39 (1.61)	35 (ND) (1)	-0.1*
Other Silicates	156 (6.53)	107 (4.88)	327 (2.89)	0.07*
Talc/ Anthophyllite	78 (4.31)	162 (2.7)	193 (2.89)	0.28**
Total Fibers	1420 (7.59)	1964 (4.81)	1553 (4.8)	0.04*

Limit of detection = 70 fibers /mg dry lung tissue for a 15 mg lung tissue
r = Correlation coefficient
n = Number of subjects
ND = Not detected in any subjects (half of the detection limit was assigned)
* p>0.1, **0.1>p>0.05, ***p<0.05

Table A-4. Geometric mean (GM) and geometric standard deviation (GSD) for length, diameter, and length-to-diameter ratio of lung retained short fibers (< 5 μm) by three levels of fibrosis score (0-12) for 45 subjects from Thetford-Mines. #					
Fiber type	**Size Parameters**	**Fibrosis score**			
		0-4 n=25	**4-8 n=15**	**8-12 n=6**	**r**
Tremolite	L	1.99 (1.2)	2 (1.13)	1.98 (1.1)	-0.03*
	D	0.24 (1.24)	0.24 (1.2)	0.24 (1.22)	-0.03*
	L/D	7.46 (1.5)	7 (1.6)	8.32 (1.2)	-0.1*
Chrysotile	L	2.27 (2.15)	2.37 (1.78)	2.11 (1.63)	-0.05*
	D	0.05 (1.8)	0.06 (1.73)	0.06 (1.58)	0.33*
	L/D	24 (3.13)	35.88 (2.54)	33.18 (2.73)	0.11*
Other Silicates	L	1.72 (1.55)	1.48 (1.27)	1.5 (1.34)	-0.18*
	D	0.23 (1.66)	0.26 (1.45)	0.29 (1.47)	0.17*
	L/D	1.9 (1.39)	1.64 (1.1)	1.6 (1.05)	-0.13*

r = Correlation coefficient
n = Number of subjects
# No statistical analysis was conducted on amosite, crocidolite , and talc anthophyllite, since these fibres were detected in the lungs of only a few subjects at very low numbers (2 to 10% for amosite and crocidolite and 30 to 40% for talc anthophyllite)
* $p>0.1$, ** $0.1>p>0.05$, *** $p<0.05$

Table A-5. Geometric mean (GM) and geometric standard deviation (GSD) for length, diameter, and length to diameter ratio of lung retained fibers (5-10 μm) by three levels of fibrosis score (0-12) for 45 subjects from Thetford- Mines. #					
Fiber type	**Size Parameters**	**Fibrosis score**			
		0-4 **0=25**	**4-8** **0=15**	**8-12** **0=6**	**r**
Tremolite	L	5.99 (1.08)	6.07 (1.01)	5.83 (1.03)	0.1*
	D	0.41 (1.57)	0.39 (1.28)	0.39 (1.14)	0.03*
	L/D	16.25 (1.46)	15.5 (1.31)	14.6 (1.19)	-0.22*
Chrysotile	L	6.05 (1.12)	5.9 (1.09)	5.73 (1.28)	-0.06*
	D	0.08 (2.3)	0.08 (2.55)	0.12 (1.63)	0.33*
	L/D	73.18 (2.01)	97.3 (1.43)	28.61 (2.58)	-0.36*
Other Silicates	L	6.25 (1.37)	6.12 (1.1)	6.39 (1.14)	0.01*
	D	2.06 (1.5)	1.89 (1.38)	2.01 (1.75)	0.28*
	L/D	9.86 (1.74)	9.76 (1.6)	11.89 2.48)	-0.22*

r = Correlation coefficient
n = Number of subjects
# No statistical analysis was conducted on amosite, crocidolite , and talc anthophyllite, since these fibres were detected in the lungs of only a few subjects at very low numbers (2 to 10% for amosite and crocidolite and 30 to 40% for talc anthophyllite)
* p>0.1, **0.1>p>0.05, ***p<0.05

Table A-6. Geometric mean (GM) and geometric standard deviation (GSD) for length, diameter, and length to diameter ratio of lung retained fibers (>10 μm) by three levels of fibrosis score (0-12) for 45 subjects from Thetford-Mines. #					
Fiber type	**Size Parameters**	**Fibrosis score**			
		0-4 n=25	**4-8 n=15**	**8-12 n=6**	**r**
Tremolite	L	12.87 (1.38)	12.45 (1.12)	11.71 (1.52)	0.15*
	D	0.51 (2.25)	0.47 (1.230	0.61 (1.44)	0.01
	L/D	27.14 (1.63)	26.51 (1.27)	23.8 (1.25)	-0.11*
Chrysotile	L	13.01 (2.05)	17.79 (1.39)	26 (1.32)	0.21*
	D	0.12 (3.1)	0.06 (1.35)	0.09 (1.67)	-0.47**
	L/D	84 (4.45)	299 (1.67)	21.99 (1.51)	0.33*
Other Silicates	L	13.49 (1.16)	13.23 (1.23)	14.14 (1.2)	0.08*
	D	0.85 (1.850	0.41 (2.23)	1.08 (2.51)	-0.08*
	L/D	15.8 (1.91)	32.2 (2.58)	13 (2.88)	0.1*

r = Correlation coefficient
n = Number of subjects
# No statistical analysis was conducted on amosite, crocidolite , and talc anthophyllite, since these fibres were detected in the lungs of only a few subjects at very low numbers (2 to 10% for amosite and crocidolite and 30 to 40% for talc anthophyllite)
* $p>0.1$, ** $0.1>p>0.05$, *** $p<0.05$

Table A-7. Geometric mean concentration (GM) and geometric standard deviation (GSD) of lung retained short fibers(<5 μm),by three levels of fibrosis score (0-12); fibers/ mg dry lung tissue for 41 subjects from Asbestos				
Fiber type	**Fibrosis score**			
	0-4 n=17	**4-8 n=14**	**8-12 n=10**	**r**
Tremolite	1611 (9.62)	11070 (7.75)	9720 (3.75)	0.39***
Chrysotile	2307 (8.31)	5014 (10.95)	2277 (7.38)	0.09*
Amosite	63 (3.81)	90.2 (6.59)	153 (16.85)	0.17*
Crocidolite	203 (8.18)	510 (11.4)	653 (12.1)	0.2*
Other Silicates	6856 (6)	25285 (2.35)	13373 (3.5)	0.28**
Talc/ Anthophyllite	1366 (5.96)	2192 (12.72)	1262 (5.1)	0.03*
Total Fibers	25210 (3.88)	70403 (2.60)	36864 (2.87)	0.22*

Limit of detection = 70 fibers /mg dry lung tissue for a 15 mg lung tissue
r = Correlation coefficient
n = Number of subjects
ND = Not detected in any subjects (half of the detection limit is assigned)
* $p>0.1$, ** $0.1>p>0.05$, *** $p<0.05$

Table A-8. Geometric mean concentration (GM) and geometric standard deviation (GSD) of lung retained fibers(5-10 μm), by three levels of fibrosis score (0-12); fibers/ mg dry lung tissue for 41 subjects from Asbestos.				
Fiber type	**Fibrosis score**			
	0-4 **n=17**	**4-8** **n=14**	**8-12** **n=10**	**r**
Tremolite	1522 (3.73)	4372 (4.46)	1815 (3.57)	0.15*
Chrysotile	207 (5.71)	2277 (7.44)	329 (8.10)	0.17*
Amosite	39 (1.7)	90 (5.05)	62 (2.47)	0.2*
Crocidolite	136 (4.78)	223 (5.67)	562 (6)	0.25*
Other Silicates	793 (4.83)	1876 (3.72)	7.13 (3.85)	-0.01*
Talc/ Anthophyllite	270 (3.94)	682 (5.3)	246 (5.33)	0.05*
Total Fibers	3778 (3.72)	13279 (3.72)	52.39 (3.1)	0.17*

Limit of detection = 70 fibers /mg dry lung tissue for a 15 mg lung tissue
r = Correlation coefficient
n = Number of subjects
ND = Not detected in any subjects (half of the detection limit is assigned)
* $p>0.1$, ** $0.1>p>0.05$, *** $p<0.05$

Table A-9. Geometric mean concentration (GM) and geometric standard deviation (GSD) of lung retained long fibers(>10 μm), by three levels of fibrosis score (0-12); fibers/ mg dry lung tissue for 41 subjects from Asbestos.

Fiber type	Fibrosis score			
	0-4 n=17	4-8 n=14	8-12 n=10	r
Tremolite	349 (4.19)	875 (3.46)	483 (3.99)	0.15*
Chrysotile	111 (3.49)	561 (5.4)	146 (3.81)	0.14*
Amosite	40 (1.42)	94 (3.04)	128 (2.82)	0.5***
Crocidolite	108 (3.79)	165 (4.1)	295 (5.36)	0.21*
Other Silicates	130 (2.69)	205 (2.99)	211 (5.68)	0.16*
Talc/ Anthophyllite	97 (2.78)	204 (4.5)	2.24 (3.63)	0.28**
Total Fibers	932 (4.15)	3271 (2.75)	1392 (2.88)	0.21*

Limit of detection = 70 fibers /mg dry lung tissue for a 15 mg lung tissue
r = Correlation coefficient
n = Number of subjects
ND = Not detected in any subjects (half of the detection limit is assigned)
* $p>0.1$, ** $0.1>p>0.05$, *** $p<0.05$

Table A-10. Geometric mean (GM) and geometric standard deviation (GSD) for length, diameter, and length-to-diameter ratio of lung retained short fibers (< 5 μm) by three levels of fibrosis score (0-12) for 41 subjects from Asbestos.					
Fiber type	**Size Parameters**	**Fibrosis score**			
		0-4 n=17	**4-8 n=14**	**8-12 n=10**	**r**
Tremolite	L	2.05 (1.28)	2.07 (1.29)	1.99 (1.16)	0.00*
	D	0.23 (1.44)	0.19 (1.44)	0.21 (1.33)	-0.19*
	L/D	8.69 (1.2)	10.7 (1.3)	9.68 (1.28)	-1.16*
Chrysotile	L	1.7 (1.51)	2.1 (1.33)	2 (1.35)	0.25*
	D	0.05 (1.18)	0.05 (1.21)	0.05 (1.27)	0.26*
	L/D	32.6 (1.59)	41.7 (1.38)	38 (1.53)	0.09*
Crocidolite	L	1.99 (1.43)	1.78 (1.29)	1.72 (1.46)	-0.3*
	D	0.09 (1.48)	0.08 (1.48)	0.09 (1.83)	0.03*
	L/D	22.15 (1.72)	20.5 (1.37)	17.9 (2.33)	-0.2*
Amosite	L	1.63 (1.23)	1.25 (1)	1.55 (1.42)	-0.15*
	D	0.08 (2.17)	0.23 (1.23)	0.12 (1.89)	-0.03*
	L/D	20.9 (1.76)	5.37 (1.22)	12.94 (1.43)	-0.09*
Other Silicates	L	1.48 (1.25)	1.51 (1.23)	136 (1.8)	-0.2*
	D	0.23 (1.350	0.21 (1.31)	0.19 (1.49)	-0.26*
	L/D	6.32 (1.22)	7.24 (1.26)	7.04 (1.37)	0.18*

r = Correlation coefficient
n = Number of subjects
* p>0.1, **0.1>p>0.05, ***p<0.05

Table A-11. Geometric mean (GM) and geometric standard deviation (GSD) for length, diameter, and length-to-diameter ratio of lung retained fibers (5-10 μm) by three levels of fibrosis score (0-12) for 41 subjects from Asbestos.

Fiber type	Size Parameters	Fibrosis score 0-4 n=17	4-8 n=14	8-12 n=10	r
Tremolite	L	5.99 (1.12)	5.7 (1.07)	5.83 (1.06)	-0.13*
	D	0.41 (1.35)	0.27 (1.61)	0.28 (1.76)	-0.25*
	L/D	14.6 (1.32)	21.3 (1.62)	20.9 (1.78)	0.23*
Chrysotile	L	5.73 (1.16)	5.94 (1.07)	5.53 (1.09)	-0.05*
	D	0.05 (1.05)	0.05 (1.47)	0.05 (1.11)	0.2*
	L/D	125 (1.17)	111 (1.45)	114 (1.15)	-0.2*
Crocidolite	L	6.17 (1.11)	6.48 (1.22)	5.95 (1.07)	-0.16*
	D	0.14 (2.1)	0.12 (1.47)	0.12 (1.47)	0.00*
	L/D	44 (2.09)	61.7 (1.59)	46 (1.46)	0.01*
Amosite	L	5.88 (1.1)	6.31 (1.23)	5.86 (1.23)	-0.2*
	D	0.09 (2.66)	0.14 (2.17)	0.22 (2.70	0.33*
	L/D	65 (2.93)	45.6 (1.91)	26.8 (2.7)	-0.4*
Other Silicates	L	5.67 (1.06)	5.73 (1.1)	5.71 (1.11)	0.03*
	D	0.6 (2.1)	0.47 (1.91)	0.47 (1.89)	-0.24*
	L/D	8.98 (2.07)	12.1 (1.88)	12.21 (1.8)	0.26*

r = Correlation coefficient
n = Number of subjects
* p>0.1, **0.1>p>0.05, ***p<0.05

Table A-12. Geometric mean (GM) and geometric standard deviation (GSD) for length, diameter, and length-to-diameter ratio of lung retained fibers (>10 μm) by three levels of fibrosis score (0-12) for 41 subjects from Asbestos.					
Fiber type	**Size Parameters**	**Fibrosis score**			
		0-4 n=17	**4-8 n=14**	**8-12 n=10**	**r**
Tremolite	L	12.17 (1.230	11.73 (1.09)	13.11 (2.17)	0.01*
	D	0.41 (1.61)	0.46 (1.63)	0.51 (1.58)	0.15*
	L/D	29.6 (1.82)	25.6 (1.65)	25.43 (1.&0	-0.16*
Chrysotile	L	14 (1.17)	14 (1.2)	14.58 (1.25)	0.1*
	D	0.06 (1.43)	0.05 (1.18)	0.05 (1.29)	0.02*
	L/D	252 (1.53)	257 (1.25)	273 (1.34)	0.05*
Crocidolite	L	16.86 (1.23)	13.99 (1.41)	16.18 (1.8)	-0.01*
	D	0.14 (1.68)	0.15 (1.42)	0.14 (1.49)	0.15*
	L/D	119 (1.59)	93 (1.85)	11 (1.35)	-0.12*
Amosite	L	19.42 (1.16)	25 (1.14)	18.43 (1.5)	-0.02*
	D	0.34 (1.36)	0.35 (1.93)	0.29 (1.57)	-0.1*
	L/D	56 (1.18)	71.5 (2.51)	63 (2.06)	0.06*
Other Silicates	L	12.91 (1.25)	12.9 (1.25)	12.21 (1.19)	0.00*
	D	0.53 (5.1)	0.72 (1.93)	0.91 (2.82)	0.15*
	L/D	13.84 (2.84)	18.2 (2)	22.9 (5.8)	0.2*

r = Correlation coefficient
n = Number of subjects
* p>0.1, **0.1>p>0.05, ***p<0.05

Table A-13. Lung fibre concentrations (million f/gram dry lung), concentration of asbestos bodies (number /gram dry lung), fibrosis store and other information of 17 subjects for which no asbestos body was found in their histological sections								
Mining region	**Case number**	**Duration of exposure**	**Other causes of fibrosis**	**AB in digestion**	**Tremolite**	**Total fibres**	**Fibrosis score**	**Number of sections**
Thetford-Mines n=10	1	44	Smoking	7640	20.8	26.5	2	6
	2	42	Smoking	2670	5.9	13.1	2.33	6
	3	50	Smoking	1500	6.2	10.35	2.17	6
	4	44	None	2500	9.8	13.4	2.67	6
	5	29	Smoking	264	5	10.4	1.66	6
	6	38	None	28200	8.4	11.1	0.66	6
	7	18	Smoking	1520	7.4	20.2	1.33	6
	8	35.9	None	3530	190	290.8	5.2	6
	9	41	Smoking	9798	14.2	37	5	6
	10	15.5	Smoking	3199	19.8	37.9	5	6
Asbestos n=7	1	18	None	40	0.27	1.9	1.6	1
	2	33	None	66000	69.8	95.3	2.5	6
	3	45	None	4400	47.6	292.9	5.8	6
	4	30	Smoking	20240	19.3	25.3	8	6
	5	39	Smoking	10100	32.3	37.8	8	6
	6	40	Smoking	1220	9.1	12.1	1	6
	7	39	Smoking	199	3.4	12	3	1

Table A-14. Correlation between tremolite concentrations and total fibre concentrations among subjects from Asbestos (top) and subjects from Thetford-Mines (Bottom).

	Total <5 μm	**Total 5-10μm**	**Total >10μm**
Tremolite <5 μm	0.62***		
Tremolite 5-10 μm		0.82***	
Tremolite >10 μm			0.9***

	Total <5μm	**Total 5-10μm**	**Total >10μm**
Tremolite <5 μm	0.8***		
Tremolite 5-10 μm		0.93***	
Tremolite >10 μm			0.95***

Total: Total fibre concentration
***p<0.001

Table A-15. Correlation between tremolite concentrations and concentration of asbestos bodies among subjects from Asbestos (top) and subjects from Thetford-Mines (Bottom).

	AB	AB	AB
Tremolite <5 μm	0.3**		
Tremolite 5-10 μm		0.3**	
Tremolite >10 μm			0.43***

	AB	AB	AB
Tremolite <5 μm	0.52***		
Tremolite 5-10 μm		0.72***	
Tremolite >10 μm			0.73***

AB: Lung-digest asbestos body concentration
***p<0.001
** 0.05<p<0.1

Figure A-1. X-ray energy dispersive spectra and TEM image of RTI tremolite fibres.

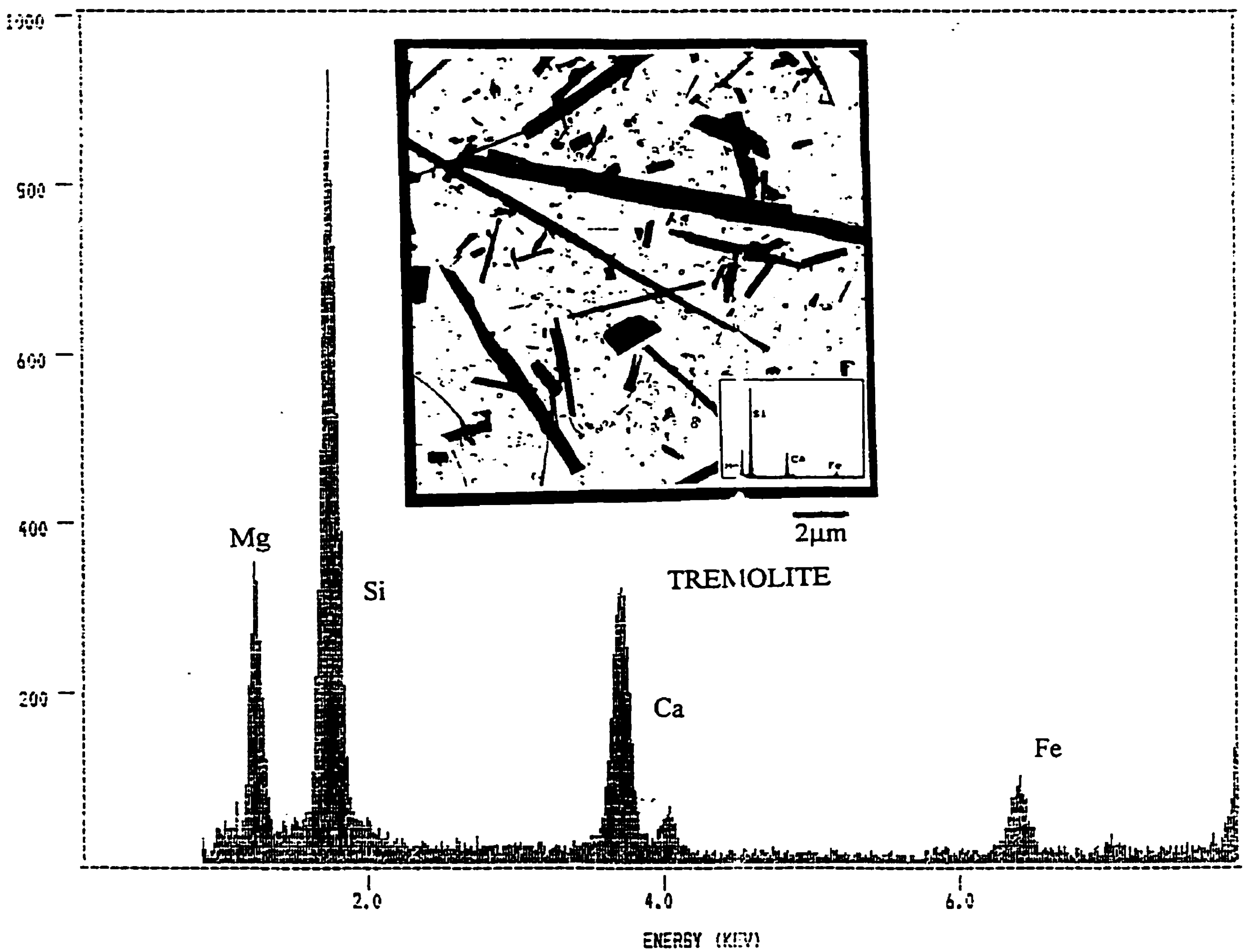

Figure A-2. X-ray energy dispersive spectra and TEM image of UICC chrysotile fibres.

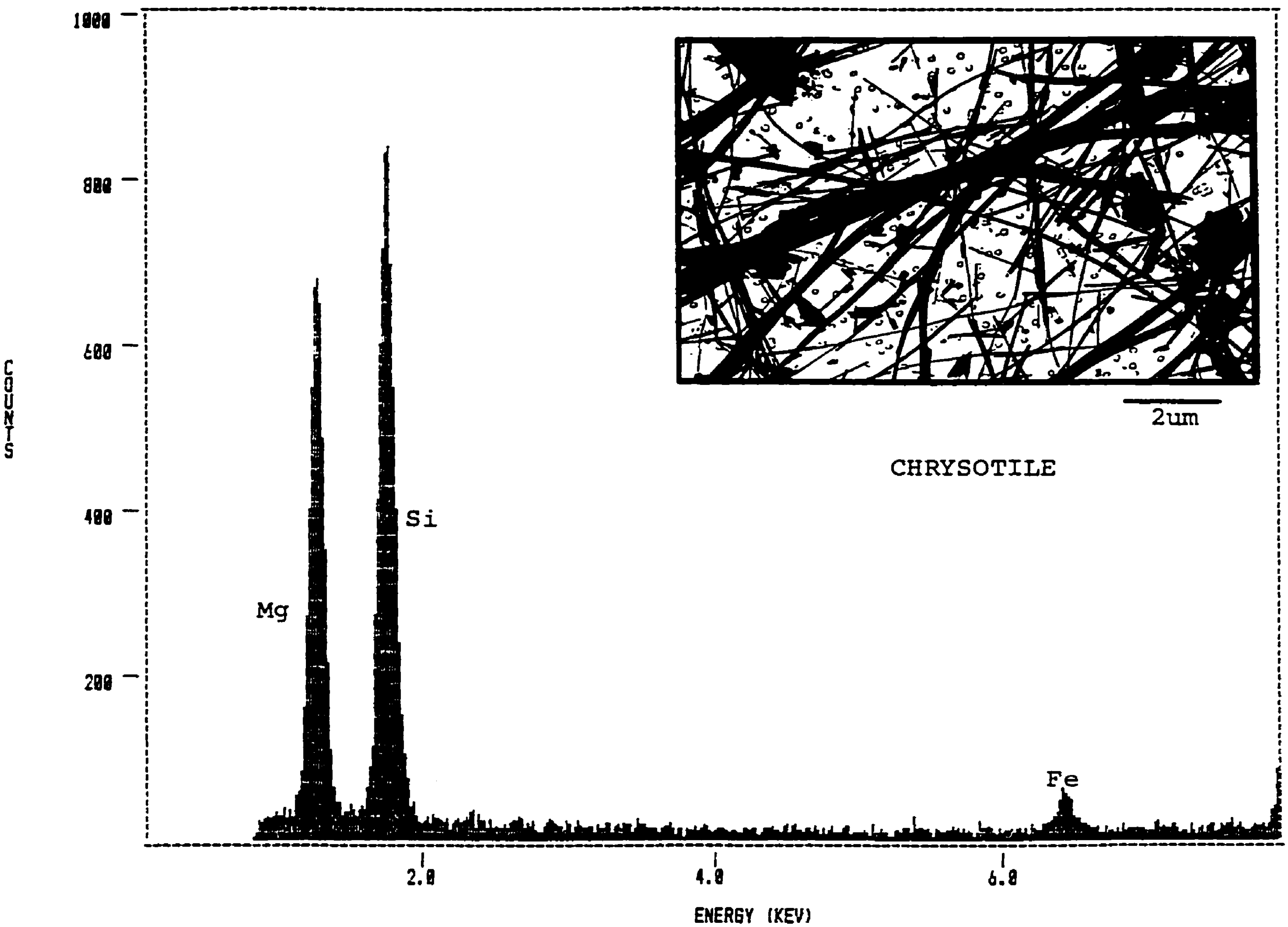

Figure A-3. X-ray energy dispersive spectra and TEM image of UICC amosite fibres.

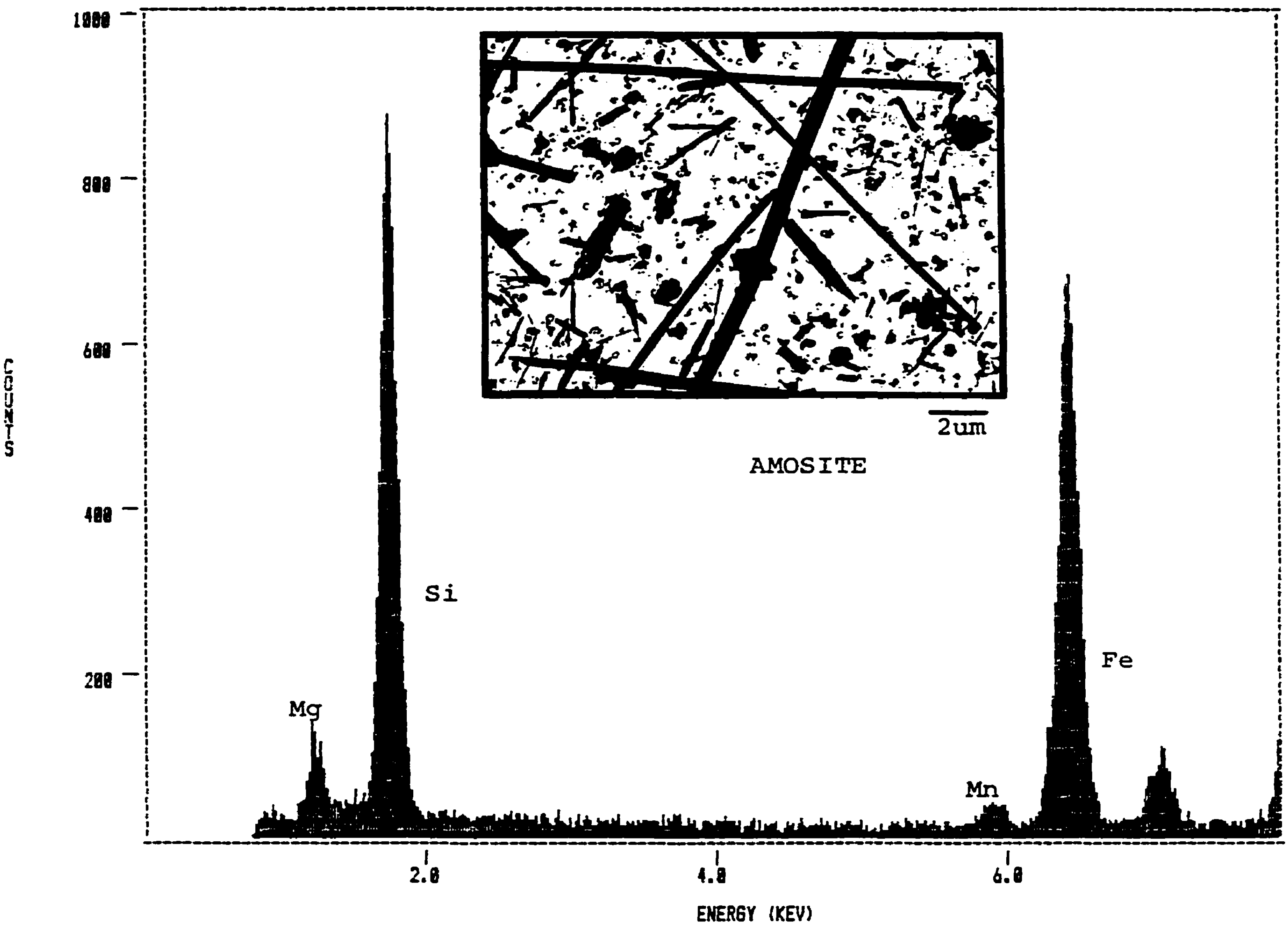

Figure A-4. X-ray energy dispersive spectra and TEM image of UICC crocidolite fibres.