MINERAL FIBRE IN THE LUNGS OF WORKERS FROM A BRITISH ASBESTOS TEXTILE PLANT

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INTRODUCTION

The material examined in this study consisted of random specimens of lung parenchyma which had originally been collected at autopsy for histopathological examination. The specimens of fixed tissue were embedded in paraffin wax blocks and represented samples obtained from individuals previously employed at a British asbestos textile plant who died between the years 1964 and 1975.

The asbestos textile plant where the various individuals had been employed had used chrysotile as its principal raw material, this being imported from Canada and Africa. Crocidolite was used between 1932 and 1969 and over this period represented approximately 5% of the total amount of asbestos processed.[1] No amosite was apparently used at any time for production purposes.

When these tissue samples were originally collected the assay of the specimens for their fibrous mineral content had not been considered. They represented a unique collection of biological material from individuals who for various lengths of time were employed in a plant which has been extensively studied for more than 30 years.

The plant has been the focus of several publications concerned with relating the effects of exposure to asbestos on health.[1-7] However, these studies have not been able to assess the effects of exposure to the various asbestos minerals processed. The British Occupational Hygiene Society used data from this factory in determining its hygiene standard[10] for chrysotile and the mesotheliomas occurring in this factory have been attributed to chrysotile exposure.[11] Information on the mortality of the workers at this plant has been shown to be very different from that of an American textile plant where crocidolite was not extensively used.[8,9] Mesothelioma, lung cancer and asbestosis have been causes of death of the workers in the British plant but not in the American operation where lung cancer and asbestosis cases have been more prevalent.

An examination of the limited working histories of the cases revealed that three had originally been employed in an asbestos fibre store some distance from the textile plant and for a further six, no information regarding period of employment was available. Results from these eight cases are therefore not reported here. The available information regarding the causes of death of the remaining 98 individuals showed that 20 were due to a mesothelioma, 24 to asbestosis and/or lung cancer and 54 were due to other causes.

The specimens have been examined to estimate fibre loadings, the relative concentrations of the various fibre types retained in the tissue and their physical characteristics. These results are reported here and compared with similar data obtained from 46 cases of exposure from an American asbestos plant.

A fibrosis category for the 98 British textile plant cases was also estimated from histopathological sections which had been previously cut from the paraffin wax blocks examined in this study. This information was used to compare fibrous grading with asbestos fibre lung burdens.

METHODS

In each case the tissue specimen was first extracted from its wax block in warm Xylene solution. When separated from the Xylene each specimen was immediately washed in ethanol to remove the wax-rich Xylene solution and dried at 80°C to evaporate the ethanol. The weight of the dry tissue specimen was then recorded. Tests with wet lung tissue specimens which had been prepared in wax and then subsequently extracted in the manner outlined have shown that the dry weight of the tissue recovered was on average 15% of the wet tissue weight. This value of dry tissue weight as a percentage of wet weight corresponds closely to figures obtained from wet lung tissue specimens dried to a constant weight at 80°C.[18]

The tissue specimens were placed in glass centrifuge tubes and digested with 5 mls of 5N KOH solution at a temperature of 80°C in a heated block. The KOH digests were then diluted with distilled water and centrifuged to the bottom of their respective tubes and the diluted KOH solution decanted. The residue was resuspended in distilled water and centrifuged again to remove residual KOH. The washed pellet was then dried in the centrifuge tube and the remaining organic material removed by oxidation at 300°C in an oxygen atmosphere. The ashed pellet was finally resuspended in distilled water whose pH had previously been adjusted to a value of 1.5 and filtered almost immediately onto 0.2 μm pore size polycarbonate (Nuclepore) filters to produce an even deposit. If the suspension was judged to be too concentrated aliquots of 50%–20% were taken. In this particular study this problem was only encountered in a few instances because of the small quantity of dried tissue available from each wax block which varied from 1.3 mg to 85.7 mg.

The filtered tissue extract was prepared for examination in a Philips EM 400T analytical electron microscope by the direct transference technique.[19] A layer of carbon was deposited onto the dust deposit on the filter and portions cut to the approximate size of gold specimen support grids. The carbon-coated filter portions were deposited carbon upon the grids and the filter material removed using a bath of chloroform.

The mineral fibres in the preparations were examined at a magnification of 20,000X, random areas being scanned to determine their concentration per unit area of the filter preparation. Each fibre when encountered was identified using an energy dispersive X-ray analysis sytem attached to the microscope. The quantity, length, diameter and identity of all fibres (i.e., particles observed in random areas of the grid with a 3:1 axial ratio) in the preparations were therefore recorded. The preparation, counting techniques and identification procedure adopted have been described in detail elsewhere.[12,13,14,15,16] The extent of fibrosis in the various cases was estimated from microscopic examination of histological sections and were graded on a scale of 0–4, 0 being normal, 1 minimal, 2 slight, 3 moderate and 4 severe. This grading has been described in more detail in a similar study of asbestos-related deaths in the United Kingdom in 1977.[16]

RESULTS

The average results for the concentrations of asbestos and other fibre types observed in the 98 cases examined are given in Table I from which it can be seen that all the asbestos mineral types were detected. The geometric mean values for the cases are presented together with arithmetic means because of the very wide range of fibre concentrations determined. Geometric mean values have also been employed as a means of presentation of other data in this paper. Chrysotile fibres were the most numerous of the asbestos particles observed but appreciable quantities of both crocidolite and tremolite were present together with minor concentrations of amosite and anthophyillite.

The combined size distributions of the major asbestos fibre types extracted from the samples are given in Table II. It can be seen from this table that the majority of the chrysotile fibres observed were less than 5 microns in length and finer than 0.25 microns in diameter. More crocidolite and tremolite fibres were longer than 5 microns when compared with chrysotile and a larger proportion of these fibres were greater than 0.25 microns in diameter with tremolite on average the larger of the fibre types. Some consideration must be given to the size of fibres detected in tissue specimens when the quantities of the various asbestos minerals are being assessed as the number of concentrations of the individual fibrous minerals do not equate directly to their mass concentrations because of size distribution differences. It is likely

Table I
Mean Concentrations of Number and Mass of Various Fibre Types
Determined in 98 Cases from a British Asbestos Textile Plant

Mineral Fibre Type	Fibre Concentrations 10^6 gram dry lung tissue			
	10^6 fibres/gram A.M.	10^6 fibres/gram G.M.	Range of concs.detected 10^6 fibres/gram	Fibre Mass ug/gram A.M.
Chrysotile	175.4	89.4	1.5 - 1389.6	6.0
Crocidolite	79.8	10.1	ND - 2056.9	8.4
Amosite	4.9	0.2	ND - 153.3	4.2
Tremolite	21.8	2.4	ND - 203.7	8.5
Anthophyllite	0.5	0.02	ND - 15.2	0.2
Mullite	31.8	11.3	ND - 246.8	-
Rutile	6.2	0.2	ND - 411.3	-
Iron	4.1	0.7	ND - 25.2	-
Others	3.8	-	ND - 29.7	-
Total	328.3	190.0	11.1 - 2508.4	-

ND - Not Detected
A.M. - Arithmetic Mean
G.M. - Geometric Mean

that the techniques used in the preparation of lung tissue specimens do enhance the number concentration of chrysotile fibres to a greater extent than amphibole fibres. This can be related directly to the fibrilar structure of chrysotile and is supported by the scarcity of fibre bundles observed in lung preparations.

In Table III the fibre concentration data collected has been presented on the basis of cause of death. This shows that on average larger concentrations of amphibole asbestos were detected in lung tissue where the cause of death was due to asbestosis and/or lung cancer than either mesothelioma or other causes of death. Crocidolite levels can be seen to be similar for mesothelioma cases, asbestosis and lung cancer cases, these levels being higher than the average for other causes of death. Table IV presents the average lung fibre burdens of chrysotile, amphibole and total asbestos on the basis of fibrosis grading together with average years of service for cases falling within each category. The chrysotile levels were found to increase in step with the fibrosis grading but the amphibole levels did not. It was also observed that the fibrosis grading did not increase directly with the average years of service. The average lung fibre burdens for the significant asbestos minerals are compared on the basis of years of service in Table V which show that the amphibole mineral fibres tend to accumulate with years of service but chrysotile levels are relatively static.

The average results for the concentration of fibres detected in the lung tissue of 46 cases from an America textile plant are presented in Table VI. When compared with the British results in Table I, it can be seen that chrysotile and amphibole levels are lower. The marked reduction in the amount of amphibole fibre in the American cases is due mainly to the difference in the higher concentration of crocidolite observed in the tissues from the British cases. The tremolite levels in both groups of cases are similar. A greater range of all fibre concentrations were also detected in the British cases when compared with the American group.

The results from the British and American cases are compared further in Table VII where size distribution data has been used to calculate the average concentration of fibres longer than 5 microns for each asbestos type. In Table VIII this analysis has been extended further to compare the averages of fibres longer than 5 microns and finer than 0.25 microns. From both tables the most significant difference between the fibre concentrations is the greater proportion of long and thin amphibole fibres in the British cases. The major contributor to this difference being the significant amount of crocidolite occurring in the British cases.

DISCUSSION

In this study the asbestos mineral fibre detected in the lungs from 98 individuals previously employed in a British asbestos

Table II

Combined Size Distributions of Major Asbestos Fibre Types Observed in the Lungs of British Textile Plant Cases (%)

Length Ranges Microns	Chrysotile	Diameter Ranges Microns: Crocidolite			Tremolite/Actinolite		
	0-0.25	0-0.25	0.25-0.5	> 0.5	0-0.25	0.25-0.5	> 0.5
< 5	91.8	88.0	1.5	-	73.6	12.0	3.1
5-10	4.9	8.3	0.7	0.2	5.5	1.8	1.1
10-20	2.3	0.9	0.3	-	1:0	0.5	0.6
> 20	1.0	0.1	-	-	0.6	-	-

Table III

Geometric Mean Concentrations of Asbestos Fibres Detected in Lung Tissue and Expressed on the Basis of Cause of Death

Number of Cases	Cause of Death	Fibre Concentrations 10^6/gram of Dry Tissue: Chrysotile	Crocidolite	Amosite	Tremolite	Total Amphibole
20	Mesothelioma	64.5	13.0	0.4	2.0	24.8
24	Asbestosis &) Lung Cancer)	100.4	14.3	0.1	8.9	47.5
54	Other Causes	99.1	8.4	0.2	1.5	19.4

Table IV

Geometric Mean Asbestos Lung Fibre Burdens of Chrysotile, Amphibole and Total Asbestos Fibre Counts with Years of Service Compared on the Basis of Fibrosis Grading

Number of Cases	Years of Service	Fibrosis Grading	Mean Fibre Levels 10^6/gram of Dried Lung Tissue		
			Chrysotile	Total Amphibole	Total Asbestos
16	20.6	0	59.7	12.5	83.7
12	7.1	1	84.4	9.6	130.3
54	16.6	2	95.1	24.4	153.2
16	20	3	103.9	107.2	240.4

Table V

Geometric Mean Lung Fibre Burdens of the Significant Asbestos Minerals Compared on the Basis of Years of Service

Number of Cases	Years of Service Range	Mean Fibre Levels 10^6/gram of Dried Lung Tissue				
		Chrysotile	Crocidolite	Amosite	Tremolite	Total Amphibole
23	0-10	67.3	2.5	0.2	0.5	7.3
29	10-20	102.0	7.3	0.2	2.9	21.6
23	20.30	115.6	26.7	0.2	2.5	37.9
23	30-50	78.6	29.5	1.0	13.6	64.4

Table VI

Mean Concentrations of Number and Mass of Various Fibre Types Determined in 46 Cases from an American Asbestos Textile Plant

Mineral Fibre Type	Fibre Concentrations 10^6/gram Dried Lung Tissue			
	10^6 fibres/ gram A.M.	10^6 fibres/ gram G.M.	Range of concs.detected 10^6 fibres/gram	Fibre Mass ug/gram A.M.
Chrysotile	58.1	29.3	1.6 - 319.7	1.9
Crocidolite	2.3	0.1	ND - 67.2	2.7
Amosite	1.8	0.1	ND - 16.8	3.8
Tremolite	15.8	2.2	ND - 95.8	17.1
Anthophyllite	0.2	0.03	ND - 2.7	0.01
Mullite	5.6	1.3	ND 43.6	-
Rutile	1.0	0.2	ND - 8.1	-
Iron	1.6	0.2	ND - 5.3	-
Others	0.3	0.02	-	-
Total	86.7	47.4	2.1 - 319.7	-

ND - Not Detected
A.M. - Arithmetic Mean
G.M. - Geometric Mean

textile plant who died between the years 1964–75 was found to consist of chrysotile in association with appreciable quantities of amphibole fibre. On average the most prominent amphibole mineral detected was crocidolite with a lower concentration of tremolite and only minor quantities of other amphibole fibre types. The quantity of asbestos was found to accumulate an average with years of service, this accumulation being more pronounced for the amphibole minerals than chrysotile. This provides further proof of the selective retention of amphibole fibre which has been demonstrated in many investigations of mixed fibre occupational exposures. There was no clear relationship between years of exposure and fibrosis grading indicating that exposures within a particular industrial operation have varied significantly for various individuals. An increase of amphibole asbestos mineral concentrations in tissue with fibrosis grading is more pronounced than corresponding chrysotile fibre levels. This would lend further support to the hypothesis that the major cause of asbestosis in Great Britain has been the result of the inhalation and retention of amphibole asbestos mineral.[16] The average levels of amphibole fibre in tissue were found to be larger in those cases where the cause of death was due to asbestosis and/or lung cancer when compared with either mesothelioma cases or other causes of death. Chrysotile levels did not, however, vary significantly with cause of death; these observations are similar to those reported elsewhere.[20]

Comparing fibre lung burdens of British textile plant workers with those of an American plant have revealed that there are some similiarities in the mineralogy of the asbestos dust retained. Tremolite levels are similar although chrysotile contents are on average higher in the British cases. The most significant difference between the two groups is the high level of crocidolite fibre in the British cases. If these mineralogical

Table VII

Comparison of the Geometric Mean Concentration of Asbestos Fiber Types and Proportion Greater Than 5 Microns in Length Detected in the Lungs of Cases from British and American Textile Plants

Fibre Type	Number Concentrations 10^6/gram of Dry Tissue			
	British Cases		American Cases	
	Total	> 5um in length	Total	> 5um in length
Chrysotile	89.4	7.5	29.3	3.2
Crocidolite	10.1	1.1	0.1	0.058
Amosite	0.2	0.05	0.1	0.049
Tremolite/Actinolite	2.4	0.3	2.2	0.34
Total Amphibole	24.7	2.8	3.5	0.6

Table VIII

Comparison of the Geometric Mean Concentration of Asbestos Fibre Types and Proportion Greater Than 5 Microns in Length and Less Than 0.25 Microns in Diameter Detected in the Lungs of Cases from British and American Textile Plants

Fibre Type	Number Concetnrations 10^6/gram of Dry Tissue			
	British Cases		American Cases	
	Total	> 5um in length < 0.25um diameter	Total	> 5um in length < 0.25um diameter
Chrysotile	89.4	7.5	29.3	3.2
Crocidolite	10.1	0.97	0.1	0.048
Amosite	0.2	0.02	0.1	0.023
Tremolite/Actinolite	2.4	0.2	2.2	0.1
Total Amphibole	24.7	2.1	3.5	0.2

differences are compared with the information on mortality of workers from both plants, it would appear that the mesothelioma cases occurring in the British factory can only be related to the more extensive use of crocidolite in their manufacturing operations. This conclusion could only have been obtained by a comparison of the mineralogy of the lung contents of workers from the two textile plants.

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