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Analysis of asbestos fibers and asbestos bodies in tissue samples from human lung

An international interlaboratory trial

by Bjørn Gylseth, PhD,¹ Andrew Churg, MD,² John MG Davis, MA, PhD,³ Neil Johnson, PhD,⁴ Arthur Morgan, DSc,⁵ Gunnar Mowe, MD,¹ Alan Rogers, MSc,⁶ Victor Roggli, MD⁷

GYLSETH B, CHURG A, DAVIS JMG, JOHNSON N, MORGAN A, MOWE G, ROGERS A, ROGGLI V. Analysis of asbestos fibers and asbestos bodies in tissue samples from human lung. *Scand J Work Environ Health* 11 (1985) 107–110. In order to compare methods of counting asbestos fibers in lung tissue, seven laboratories participated in an interlaboratory trial in which tissue samples from five human lungs were analyzed. In some laboratories, fiber concentrations were assessed with the light microscope and, in others, with either scanning or transmission electron microscopes. Within each laboratory the ranking of the results was similar, but there were marked differences in the absolute values obtained by the different laboratories. It is concluded that the laboratories participating in this trial appear to produce internally consistent results, but there is difficulty in directly comparing results from one laboratory to the next.

Key terms: biological samples, electron microscopy, optical microscopy, sample exchange.

Modern methods for the analysis of inorganic fibers, especially asbestos fibers, are based on the observation and counting of the fibers in different types of microscopes, including optical and electron microscopes. Differences in results often occur mostly due to the capabilities of the various analytical methods used. Furthermore, both systematic and random errors are often introduced during such analyses. Thus comparison of data from one study to another may be of limited value. One approach to assist in overcoming such problems is to standardize a method or introduce a reference method. Interlaboratory trials may prove to be a valuable tool for indentifying problem areas and have assisted in providing appropriate standard methods. Data from such interlaboratory trials for air and water samples have been reported in the literature (1, 2, 3) and work on international standards for the analysis of such samples is currently being given high priority.

Though a vast number of papers have appeared in the literature dealing with asbestos fibers in lung tissue samples, data from interlaboratory trials for such samples are scarce. One limited study, comprising two laboratories using almost similar analytical methods, has been published however (7, 8).

Experience from the analysis of water and air samples has shown that large variations in the results can occur, even when almost the same analytical procedures are employed. For tissue samples several other problems arise, such as an uneven distribution of fibers in the lungs, breakage of fibers during preparation, etc. Furthermore, the fixation, storage, and preparation of the tissue specimens prior to analysis may introduce certain errors. Detection of the fibers with light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) produce, in themselves, differences in resolution, and hence results differing by several orders of magnitude may be obtained.

The results from such analyses are often used in epidemiologic studies and in medicolegal cases, and hence it is important to have some knowledge as to the variability between the techniques used in various laboratories before direct comparisons are made of their results.

In order to stimulate an international discussion on the problems related to the standardization of a method for analyzing fibers in tissue samples, and to work out a background for a proposal for a reference method, a limited interlaboratory trial was initiated. The results of this study are presented in this report.

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Materials and methods

General information

In September 1982 an interlaboratory comparison was initiated in which samples of lung tissue from workers exposed to asbestos were distributed and analyzed. Participating laboratories were selected according to various criteria, including, for example, (i) personal communication and mutual interest, (ii) working actively in the field, (iii) laboratories using different methods, and (iv) geographic scattering. The study included three British, one American, one Canadian, one Australian, and one Norwegian laboratory.

Tissue samples

Lung tissue samples from six Norwegian workers with different occupations, all with definite or probable previous asbestos exposure, were included in the study. Samples, 0.3–1 cm³ in size, were cut from macroscopically homogeneous and undiseased lung

parenchyma, except for one sample (246/79), which comprised tissue from hilar lymph nodes. In order to keep the intersample concentration variation as low as possible, only a limited amount of tissue was taken from each lung; thus the number of participants was limited. The samples were preserved in formaldehyde and distributed as wet tissue in small airtight glass vials.

The complete case information is given in table 1.

Methods

The specimens were either ashed or digested in the wet condition or after they had been dried to constant weight. In the former case, the dry:wet weight ratio was determined from pieces of adjacent tissue. The extraction procedures were based either on wet-digestion of the tissue with either sodium hypochlorite or potassium hydroxide solution or dry-ashing in a low-temperature plasma asher (LTA). Two laboratories used LM, four laboratories used SEM, and four laboratories used TEM. A fiber was

Table 1. Case information.

Case number	Occupation (when exposed)	Year of first exposure	Age at first exposure	Duration of exposure	Latency period since first exposure (years)	Age at death (years)	Cause of death and autopsy findings
123/80	Asbestos cement worker	1948	21	9 months	34	55	Drowning, pleural plaques
58/80	Chemical maintenance worker, carpenter	1929	27	40 years	51	78	Malignant pleural mesothelioma, pleural plaques
150/81	Plumber, chemical factory	1929	27	33 years	52	79	Myocardial infarction, pleural plaques
88/81	Insulation worker, shipyard	1938	17	2 years	43	60	Malignant pleural mesothelioma
127/80	Insulation worker, general insulation	1936	27	34 years	44	71	Bronchial cancer, interstitial fibrosis, pleural plaques
246/79	Asbestos cement worker	1945	21	33 years	34	54	Malignant pleural mesothelioma

Table 2. Methodological information.

Laboratory	Reference code	Method of preparation ^a	Method of analysis ^b	Magnification	Comment
A	0	NaOCl	LM	0.64 K	Fibers longer than 2 μ m counted
B	1	NaOCl	SEM	5 K	All fibers counted
C	2	KOH	SEM	2.8–7 K	All fibers counted
C	3	KOH	TEM	2.8–7 K	All fibers counted
D	4	LTA	SEM	4.5 K	All fibers counted
D	5	LTA	TEM	5 K	All fibers counted
E	6	NaOCl	TEM	10 K	All fibers longer than 0.5 μ m counted
F	7	KOH (LTA)	SEM	10 K	Fibers longer than 0.3 μ m counted
G	8	NaOCl	LM	0.45 K	Fibers longer than 5 μ m counted
G	9	NaOCl	TEM	8.3 K	Fibers longer than 2 μ m counted

^a NaOCl = sodium hypochlorite, KOH = potassium hydroxide, LTA = low-temperature plasma ashing, K = magnification $\times 1000$.

^b LM = light microscopy, SEM = scanning electron microscopy, TEM = transmission electron microscopy.

counted if its aspect ratio was greater than 3. Laboratory A counted all fibers longer than 2 μm , laboratories B, C and D counted all fibers irrespective of length, laboratory E counted all fibers longer than 0.5 μm , laboratory F counted all fibers longer than 0.3 μm , and laboratory G all fibers longer than 5 μm with LM and all fibers longer than 2 μm with electron microscopy. Two laboratories used both SEM and TEM, and one used LM and TEM. Asbestos bodies were analyzed by two laboratories using LM and by two using SEM. A brief description of the methods used by the different laboratories is given in table 2. Although the various laboratories use, in principle, reasonable similar methods, in practice they incorporate a large number of minor manipulations which are well known to alter the reported concentration of fibers in various ways.

Results

The individual values for both total fiber and asbestos bodies are presented in tables 3 and 4. While there was remarkable agreement in the measurements of coated fibers, there were considerable discrepancies in the results for uncoated fibers. The results obtained by the two laboratories using LM were fairly comparable, but those from laboratories using electron microscopy techniques showed a much larger variation, which was especially pronounced for laboratory F, using SEM. We are unable to account for such

discrepancies. One laboratory (C) obtained very high numbers for two samples both with SEM and TEM. Contamination may have been involved for those samples. For TEM the results in the lower concentration range were in fairly good agreement, but in the higher concentration range there was a great scatter. When the extreme values were excluded, a reasonable correlation (Pearson) was obtained between the laboratories for each sample ($r = 0.50-0.98$). For coated fibers the difference between the LM and SEM was much less pronounced, as would be expected as most bodies can be detected quantitatively by such techniques. The large scatter in the results makes a more-detailed statistical analysis inappropriate.

Total fiber counts only are presented in this communication; however, two laboratories (D and G) provided information on the identification of each type of asbestos fiber observed. These results have been reported elsewhere (5) and reveal certain consistencies but occasional large variations.

Conclusions

Two broad conclusions emerge from this study. The first is that there is overall agreement in the ranking of results from laboratories using a variety of different techniques and instruments. In general, all laboratories reported the "high" cases as high and the "low" cases as low. This trend can be seen if one

Table 3. Fiber concentrations in millions per gram of dried tissue.

Reference code ^a	Case number					
	123/80	58/80	150/81	88/81	127/80	246/79
0 (LM)	1.0	2.7	6.4	1.0	24.9	42.0
8 (LM)	0.4	0.5	3.7	0.9	16.2	30.7
1 (SEM)	0.7	1.7	4.8	3.1	12.8	28.3
2 (SEM)	14.7	— ^b	11.3	348 ^c	160	108 000 ^c
4 (SEM)	4.1	6.3	13.1	14.1	55	110
7 (SEM)	150	156	182	350	298	1 230
3 (TEM)	32.7	— ^b	20.3	655 ^c	245	239 000 ^c
5 (TEM)	11	10	50	33	177	633
6 (TEM)	32.6	9.1	22.5	6.2	41	63
9 (TEM)	4.7	4.9	11.4	9.3	89.2	640

^a See table 2 for an explanation of the abbreviations.

^b Fibers not detected due to large amounts of debris.

^c Contamination very likely.

Table 4. Concentration of asbestos bodies in millions per gram of dried tissue in lung tissue samples from four of the laboratories.

Reference code ^a	Case number					
	123/80	58/80	150/81	88/81	127/80	246/79
0 (LM)	<0.01	<0.01	0.28	0.14	6.61	0.86
1 (SEM)	—	0.01	0.03	0.17	1.67	1.45
4 (SEM)	0.04	0.02	0.27	0.15	2.6	0.3
8 (LM)	0.02	0.12	0.93	0.14	4.4	4.1

^a See table 2 for an explanation of the abbreviations.

reads the results from left to right across table 3, and it is reflected in the correlation calculation cited in the Results section. This observation suggests that most laboratories are reasonably consistent in the application of their own method and that estimates of fiber content in a given case or set of cases may reliably be compared to past data published by that laboratory.

The second conclusion is that there are marked variations in results from laboratory to laboratory. In part this variation is attributable to differences in the resolving power of the instruments used. TEM detects more fibers than SEM, which detects more fibers than LM. Another reason for the observed variation is the different counting criteria employed. However, it seems likely that additional factors are required to explain the high range of values observed. It is unclear whether these differences are intrinsic to the laboratory or the method (see the Introduction) or result from some other extraneous factors. For example, it is known that asbestos fiber concentrations vary 5- to 10-fold from one site to another within the lung (4, 6), and it is possible that the various samples of the same lung sent to the different laboratories actually contained quite different concentrations of asbestos. Due to the small samples provided in this study, replicate analysis for each specimen was not feasible. This problem can, however, be solved with the use of a common artificially produced sample and with attempts to standardize methods. Until the results of such a trial are available and if and until a standard method for counting asbestos fibers from lung is established, it appears unwise to attempt to draw conclusions by comparing

data from different laboratories. To assist in overcoming such problems, it is important that laboratories establish their own baseline population studies.

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