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A COMPARISON OF LIGHT MICROSCOPY AND TRANSMISSION ELECTRON MICROSCOPY RESULTS IN THE EVALUATION OF THE OCCUPATIONAL EXPOSURE TO AIRBORNE CHRYSOTILE FIBRES

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Abstract— A comparison has been made between light microscope (LM) and transmission electron microscope (TEM) counts of airborne chrysotile fibres, collected on 30 membrane filters from different working processes. For TEM counts, fibres of length $l > 5$ μm were classified according to diameter d, namely $0.3\ \mu m < d < 3\ \mu m$ (microscopic fibres) and $d \leq 0.3$ μm (submicroscopic fibres); short fibres, $l \leq 5$ μm, were taken into account separately. A very good correlation has been found between LM and TEM counts, both for microscopic and total (microscopic + submicroscopic, $l > 5$ μm) fibres, for densities 40–500 fibres mm^{-2}. The median of the ratios between TEM and LM counts has been found to be 1.1 for microscopic fibres and 1.2 for total fibres. From TEM counts, submicroscopic fibres have been found to account for about 10% of total $l > 5$ μm fibres. The mean ratio between short fibres and those with $l > 5$ μm was in the range 0.8–3.6, depending on the working process. These results suggest the possibility of adopting factors, varying with the kind of working process, to be applied to LM counts in order to estimate the exposure to all the asbestos fibres considered important for a comprehensive evaluation of the risk.

INTRODUCTION

AT PRESENT, the membrane filter method (MFM), based on optical phase microscopy, is commonly used for measuring airborne asbestos dust levels in occupational environments. It has been used in most epidemiological studies relating asbestos dust exposure to lung disease.

The light microscope (LM) is considered a suitable instrument for routine control of airborne asbestos fibres, but, because of its low detection limit (approximately 0.2–0.4 μm), it cannot detect all fibres; consequently, the results obtained by this instrument are intended as an indication of exposure to fibres, rather than a concentration of all harmful fibres.

On the other hand, animal experiments have indicated the importance of fibres less than 0.5 μm in diameter (in particular, less than 0.25 μm) in the carcinogenic process (STANTON *et al.*, 1981; POTT, 1978). In addition, data on fibre recovery from human lung tissue have shown that most fibres are less than 0.5 μm in diameter (BIGNON *et al.*, 1978) and that the optically visible fibres are only a small fraction of the fibres measured by electron microscopy (POOLEY and CLARK, 1980). A different carcinogenic potential according to fibre size has been suggested to explain the incidence of mesothelioma in South African mines (TIMBRELL *et al.*, 1971).

The above findings indicate the inadequacy of LM in evaluating the true exposure

to all harmful airborne fibres: in order to make the optical data more representative of the true exposure, it is necessary to transform them, by appropriate conversion factors, into the true number of airborne fibres having pathogenic dimensions. To obtain such factors, the best available method nowadays appears to be electron microscopy, which is capable of resolving the finest asbestos fibres: traditionally it has been used for sizing submicron fibres, but its routine use is considered too expensive in terms of time and costs (WALTON, 1982).

Up to now, there have been few published studies on the direct comparison of the results obtained by light and electron microscopy (BECKETT and JARVIS, 1979, for amosite; HWANG and GIBBS, 1981, for crocidolite; CHATFIELD, 1979, for chrysotile). However, from the results of some electron microscope studies on airborne fibre dimensions, it is possible to calculate the percentage of fibres not detectable by LM and hence, indirectly, to estimate the relationship between the results obtained by the two instruments (GIBBS and HWANG, 1975, 1980; HWANG and GIBBS, 1981; RENDALL and SKIKNE, 1980; WINER and COSSETTE, 1979). A comprehensive analysis of these studies indicates the difficulty of establishing a generally valid relationship, since differences in size distribution of the airborne fibres can occur, owing both the mineralogical variety and to the stages of the production process (GIBBS and HWANG, 1975; HWANG, 1983).

These studies have obvious implications in the establishment of occupational hygiene standards for airborne asbestos fibre concentrations, compliance which is normally controlled by optical methods. Detailed and systematic studies involving different types of asbestos and different production processes are therefore required in order to correlate results from the two microscopical techniques.

This paper reports a comparison between LM and transmission electron microscope (TEM) counts on airborne dust samples collected during three different working processes where chrysotile was used. The results are then analyzed and discussed in comparison with the other results previously published on the same subject.

MATERIALS AND METHODS

Samples were collected in a brake and clutch (BC) factory (8 samples) and at a railway workshop dealing with maintenance and repairs (22 samples); in the latter case, fibre exposure resulted from asbestos tapes and paperboards (ATP) handling, and from asbestos-cement (AC) board sanding. Fibre type was chrysotile, for all processes.

Air samples were collected by the MFM (AIA, 1979) on 25 mm and 37 mm filters. In order to detect a possible influence of fibre density on filters (observed fibres per mm^2) on the relationship between LM and TEM, samples were selected so as to cover a wide density range (10–1500 fibres mm^{-2} by LM). For each filter, a portion was examined by LM and another one by TEM, measurements being made on areas of the same order of magnitude. Counting criteria were those reported in AIA (1979) and it was verified that they were interpreted in the same way by the two operators who performed separately the LM and TEM analyses.

In order to eliminate coarse errors due to a possible uneven fibre distribution on the samples prepared for microscopic examination, for each sample we tested the goodness of fit of the Poisson distribution to that observed in LM and TEM counts. In ideal conditions fibre distribution is random and therefore describable by the Poisson law

(RAJHANS and BRAGG, 1975; LEINEWEBER, 1978; MILLETTE *et al.*, 1978). The Kolmogorov–Smirnov test was used to evaluate the goodness of fit. In addition, on the samples examined by LM, the relationship between the mean and the variance of the fibre number per microscopic field was studied by linear regression analysis (BATTISTI *et al.*, 1981).

Light microscopy: sample preparation and analysis

A filter portion, corresponding to one wedge-shaped quarter for 37 mm filters and to one-half for 25 mm filters, was cleared by acetone vapours and triacetin (AIA, 1979). Fibre counting was performed by a Leitz Ortolux phase-contrast microscope, with a magnification of 500×, and a practical detection limit *ca* 0.3 μm, checked by the test slides produced by the Asbestos Institute for Occupational and Environmental Safety and Health (AIA, 1979) and by the Health and Safety Executive/National Physical Laboratory, Mark I and Mark II versions (LE GUEN, in AIA, 1980, p. 83). Fibres were counted using a Walton–Beckett graticule (WALTON and BECKETT, 1977), following the rules and criteria now generally accepted and reported in AIA (1979); for each sample, the scanned area was between 0.16 and 0.79 mm^2, according to the fibre density on filter.

Transmission electron microscopy: sample preparation and analysis

Membrane filter preparation for TEM analysis was performed according to the 'collapsed membrane filter' method (NIOSH, 1977): a filter section (equivalent to that used for LM) was removed, placed on a microscope slide, sample side up, and fastened to the slide by a narrow transparent tape. The slide was exposed to acetone vapours to destroy the microporous structure of the filter (normally 2–3 min were sufficient to produce a smooth surface) and then coated with carbon (20–30 nm) by conventional techniques. Sections were cut from the coated filters and placed, sample side down, on 200-mesh electron microscope grids. The filter matrix was gently dissolved by acetone, leaving particles adhering to carbon film ready for TEM analysis.

Copper grids with opening of 85 × 85 μm were used; in the analysis of the filter the full grid opening was used as field of view for fibre counting.

A Siemens Elmiskop 102 transmission electron microscope was used at a magnification of $\times 10^4$. Fibre sizes were measured on the microscope screen with the help of known reference marks on it. The counting rules and criteria were the same as for LM (AIA, 1979); in particular, a fibre intersecting one side of the field of view was considered a half-fibre.

All the fibres belonging to one of the following size classes were considered for analysis: (1) $l > 5$ μm, 0.3 μm $< d < 3$ μm, $l/d > 3$ (microscopic fibres); (2) $l > 5$ μm, $d \leqslant 0.3$ μm, $l/d > 3$ (submicroscopic fibres); (3) $l \leqslant 5$ μm, $d < 3$ μm, $l/d > 3$ (short fibres). In the text, the whole of microscopic plus submicroscopic fibres will be reported as 'total' fibres ($l > 5$ μm).

The recording of fibres with a length $\leqslant 5$ μm can be useful in order to provide full information on the size distribution of fibres, particularly in view of future epidemiological studies.

Minimum diameter and minimum length of fibres detected by TEM were estimated to be, respectively 10^{-2} and 3×10^{-2} μm.

RESULTS

Statistical test of the random distribution of fibres on filters

For both TEM and LM counts performed on all the samples the null hypothesis (H_0 = fibres are randomly distributed on filter) is not rejected at an $\alpha = 0.20$ significance level. Therefore, the fibres can be considered as randomly distributed on filters. Table 1 reports an analysis of the relationship between the mean and the variance of the number of fibres detected in each LM graticule area; the results are reported as regression lines of the variance on the mean and as correlation coefficients (r).

TABLE 1. RELATIONSHIP BETWEEN MEAN ($\bar{x}$) AND VARIANCE OF THE NUMBER OF FIBRES PER LM GRATICULE AREA

$\bar{x}$ (range)	No. of filters	Regression line	r (P)
0.00–1.00	16	$y = 1.00x - 0.02$	0.98 ($P < 0.001$)
1.01–5.00	10	$y = 0.72x + 0.75$	0.85 ($0.01 < P < 0.001$)
0.00–5.00	26	$y = 0.95x + 0.07$	0.96 ($P < 0.001$)
0.00–12.00	30	$y = 1.50x - 0.57$	0.96 ($P < 0.001$)

In this analysis, samples have been classified according to fibre density, reported as mean number of fibres per graticule area; a very good agreement with the Poisson distribution has been obtained for the samples with a density up to 5 fibres per graticule area (about 650 fibres mm^{-2}), with a maximum agreement for the samples with density up to 1 fibre/graticule area.

As already reported (BATTISTI *et al.*, 1981), these results are in agreement with the particular suitability of the Poisson distribution to describe rare events.

Comparison between LM and TEM counts

All the LM and TEM counts performed on the 30 samples are reported in Table 2, in increasing order of fibre density determined by LM.

Comparing LM and TEM counts of microscopic fibres, it is possible to observe that, in four out of the five samples with the lower LM density (less than 20 fibres mm^{-2}), no fibre has been detected in the area examined by TEM. Moreover, the two samples with a very high LM density (more than 1000 fibres mm^{-2}) showed a particularly high ratio between LM and TEM counts.

As indeed samples with such densities are not generally accepted in the good practice of this kind of analysis, because of the poor reliability of the results, we considered separately the 7 above-mentioned samples in the overall analysis of the results.

Table 3 shows the results of the comparison between LM and TEM counts; TEM counts are reported both for microscopic fibres and for total fibres ($l > 5$ μm). Samples are classified according to working processes and also to fibre density ranges, where appropriate, in order to find out possible relationships between results and fibre density. The mean value of the ratios between the LM and TEM counts on each sample has been calculated for each working process and each density range. To evaluate the

TABLE 2. COMPARATIVE SUMMARY OF LM AND TEM COUNTS (fibres mm^{-2})

Filter	Process	LM counts	TEM counts		
			Microscopic fibres	Total fibres	Short fibres
1	ATP	9	ND	ND	83
2	AC	10	ND	1	9
3	AC	12	4	4	3
4	AC	13	ND	ND	ND
5	AC	16	ND	ND	ND
6	AC	42	54	66	226
7	ATP	45	7	7	78
8	ATP	48	33	33	43
9	ATP	50	40	40	137
10	ATP	58	83	119	291
11	AC	69	78	82	217
12	ATP	70	85	87	206
13	ATP	94	94	145	295
14	AC	113	184	189	548
15	AC	121	181	211	294
16	ATP	133	133	152	414
17	AC	167	119	149	1535
18	BC	179	218	325	402
19	ATP	225	552	552	421
20	AC	229	589	619	774
21	BC	291	346	412	301
22	BC	354	419	419	266
23	BC	406	415	467	353
24	BC	411	564	676	670
25	BC	439	768	858	914
26	BC	577	439	439	277
27	ATP	837	872	969	1239
28	BC	1000	2137	2137	129
29	AC	1218	609	740	845
30	AC	1446	346	526	955

Microscopic fibres: 0.3 μm $< d < 3$ μm; total fibres: microscopic and submicroscopic fibres ($l > 5$ μm); short fibres: $l \leqslant 5$ μm; ATP: asbestos tapes and paperboards; AC: asbestos–cement boards; BC: brake and clutch; ND: no fibre detected.

degree of association between the two different methods, the correlation coefficients between TEM and LM counts have been calculated.

Table 4 shows the percentages, obtained by TEM counts, of submicroscopic fibres with respect to total fibres ($l > 5$ μm) and of fibres up to 5 μm with respect to those more than 5 μm in length. Samples 1–5 have been excluded because no fibres, or very few, were detected in the scanned area. The percentage values turned out to be widely distributed.

DISCUSSION

TEM counts of microscopic fibres were expected to be roughly the same as LM counts. The results for the samples with densities in the range 40–1000 fibres mm^{-2} (Table 3) show a very good correlation between the two instruments ($r \cong 0.9$) and a median ratio between TEM and LM counts about or more than 1, with a maximum of 1.4 for AC samples. TEM counts are almost always higher: such a difference between

TABLE 3. CORRELATION COEFFICIENTS (r) AND RATIOS BETWEEN TEM AND LM COUNTS OF FIBRES LONGER THAN 5 μm

Process	LM density (fibres mm^{-2})	No. of filters	TEM (microscopic fibres)/LM				TEM (total fibres)/LM			
			r (P)	Ratio Median	Ratio Mean	s.d.	r (P)	Ratio Median	Ratio Mean	s.d.
AC	40– 250	6	0.85 ($0.01<P<0.05$)	1.4	1.5	0.6	0.86 ($0.01<P<0.05$)	1.6	1.6	0.6
ATP	40– 850	9	0.93 ($P<0.001$)	1.0	1.1	0.6	0.95 ($P<0.001$)	1.2	1.3	0.7
BC	150–1000	8	0.93 ($P<0.001$)	1.2	1.3	0.4	0.90 ($P<0.001$)	1.5	1.5	0.4
M	40– 250	15	0.89 ($P<0.001$)	1.2	1.3	0.6	0.92 ($P<0.001$)	1.5	1.4	0.7
M	251–1000	8	0.86 ($0.001<P<0.01$)	1.2	1.3	0.4	0.85 ($0.001<P<0.01$)	1.3	1.4	0.4
M	40–1000	23	0.90 ($P<0.001$)	1.1	1.3	0.6	0.91 ($P<0.001$)	1.2	1.4	0.6
M	8–1500	30	0.66 ($P<0.001$)	1.0	1.0	0.7	0.71 ($P<0.001$)	1.2	1.2	0.7

AC, ATP, BC, microscopic fibres, total fibres: refer to Table 2; M: mixed.

TABLE 4. TEM COUNTS PERCENTAGE OF SUBMICROSCOPIC FIBRES ($l>5$) AND RATIO BETWEEN $l\leqslant 5\mu$m AND $l>5$ μm FIBRES (FILTERS WITH LM DENSITY > 20 FIBRES mm^{-2})

Process	No. of filters	Submicroscopic fibres (%) ($l>5$ μm) Median	Mean	s.d.	$l\leqslant 5$ μm/$l>5$ μm Median	mean	s.d.
AC	6	9.5	10.8	7.6	2.7	3.6	3.4
ATP	9	2.3	10.0	13.8	2.4	3.0	3.1
BC	8	10.8	10.9	11.3	0.8	0.8	0.4
M	25	10.5	11.8	11.7	1.3	2.3	2.7

AC, ATP, BC, M: refer to Tables 2 and 3.

TEM and LM counts, in any case within the reproducibility of the MFM, can be attributed to the following causes: (1) operator's subjectivity in interpreting counting criteria (WALTON, 1982, p. 205); (2) uncertainty in determining LM detection limit (a non-negligible amount of fibres with a diameter near 0.3 μm has been found by TEM) (RENDALL and SKIKNE, 1980; WALTON, 1982, p. 234); (3) presence of a non-negligible amount of fibres with a non-uniform diameter, and in particular with $d < 0.3$ μm in the central portion and with $d > 0.3$ μm but $l < 5$ μm in the external portions: as the central portion is not detectable by LM and the external portions do not conform to the definition of fibre, such fibres are not counted by LM while they are by TEM. On the other hand, as the number of fibres crossing the sides of the TEM opening is negligible, we can exclude the possibility that the result is significantly affected by the fibres which can touch particulate matter out of the opening, in non-visible zones.

The lack of fibres detected by TEM in 4 out of the 5 samples with LM density less than 20 fibres mm^{-2} can be explained by a background on the samples prepared for the LM analysis due, e.g., to an ambient contamination or to the clearing procedure. This confirms (AIA, 1979) the importance of regarding as acceptable in the MFM the samples with a density of at least 20 fibres mm^{-2}, also of minimizing the 'blank' error.

The maximum of the agreement between the results obtained with the two instruments has been found for the ATP samples; such samples do not generally present non-fibrous particulate matter, which is known as disturbing in counting and as leading to ambiguity in interpreting counting criteria. On the other hand, the agreement is minimum for the AC samples, which show an opposite situation.

When TEM total fibres (submicroscopic + microscopic fibres, $l > 5$ μm) are considered, the median ratios between TEM and LM counts increase up to 25% as compared with the ratios found for microscopic fibres, previously discussed; the correlation coefficients are still very good ($r \cong 0.9$), and they are basically the same as those found for microscopic fibres.

In these conditions, and for the type of samples considered in this study, when considering the mean value for each process, TEM (including submicroscopic fibres in the counts) detects up to 60% more fibres than LM; the median increase for all the samples is 20%. As regards the kind of working process, results for total fibres ($l > 5$ μm) are similar to those previously reported for microscopic fibres: with increasing amounts of non-fibrous particulate matter, the difference between the results obtained with the two instruments increases. Finally, separate TEM counts of submicroscopic and microscopic fibres indicate that submicroscopic fibres are on average about 10% of total fibres ($l > 5$ μm), although with a wide distribution of values.

In Table 5 the results of this study are compared with those of other similar studies previously reported, concerning chrysotile fibres. Two studies (CHATFIELD, 1979, data reported also in WINER and COSSETTE, 1979; HWANG and WANG, 1983) have involved a comparison by applying the two techniques on the same filter: the three sets of data (the two above reported and ours) are considerably different; CHATFIELD (1979) reports, however, that TEM data can be over-estimated because of ultrasound used in preparing samples. Besides, LYNCH *et al.* (1970) have performed a similar comparison over a great number of samples: data are not reported but they conclude that the TEM counts of the longest fibres do not exceed the LM counts. In the other studies, fibre size distributions have been studied only by TEM: from such distributions it is possible to

TABLE 5. COMPARATIVE SUMMARY OF RESULTS FROM DIFFERENT STUDIES: RATIOS OF TEM COUNTS ($d < 3\,\mu m$; $l > 5\,\mu m$) TO LM COUNTS, AND FIBRE SIZE DISTRIBUTIONS AS MEASURED BY TEM (VALUES DERIVED FROM AUTHORS' DATA)

Process	No. of filters	TEM measurements: TEM/LM Mean ± s.d.	TEM measurements: Submicroscopic fibres (%) ($l > 5\,\mu m$) Mean	TEM measurements: $l \leqslant 5\,\mu m / l > 5\,\mu m$ ($d < 3\,\mu m$) Mean ± s.d.	Source and remarks
Asbestos–cement (boards sanding)	6	1.6 ± 0.6	10.8	3.6 ± 3.4	This study ODL: 0.3 μm
Insulation (tapes and paperboards)	9	1.3 ± 0.7	10.0	3.0 ± 3.1	
Brake and clutch	8	1.5 ± 0.4	10.9	0.8 ± 0.4	
Textile	NR			32.3	LYNCH *et al.* (1970)
Brake and clutch	NR			49.0	
Asbestos–cement (pipe)	NR			65.7	
Milling (drying)	2		38.8	5.1	GIBBS and HWANG (1975)
Milling (bagging)	2		43.7	13.1	ODL: 0.5 μm
Textile (carding)	2		42.5	26.4	Measurements by Scanning Electron Microscope

TABLE 5. (continued)

Process	No. of filters	TEM measurements TEM/LM Mean ± s.d.	Submicroscopic fibres (%) ($l > 5$ μm) Mean	$l \leqslant 5$ μm/$l > 5$ μm ($d < 3$ μm) Mean ± s.d.	Source and remarks
Various	11	66.3 ± 63.9		39.2 ± 26.2	CHATFIELD (1979) WINER and COSSETTE (1979) ODL: NR
Mining	NR		38.2	76.5	GIBBS and HWANG (1980)
Milling (bagging)	NR		61.2	22.9	ODL: 0.3 μm
Mining	249*		76.9	1.3	RENDALL and SKIKNE (1980)
Insulation	91*		77.1	1.6	ODL: 0.4 μm
Brake and clutch	66*		61.1	0.8	Insulation: chrysotile and amosite
Mining	NR		67	82.3	HWANG and GIBBS, in WALTON
Milling (bagging)	NR		71	23.4	(1982, p. 147); ODL: 0.5 μm
Various	25	8.4	42.9	5.1	HWANG and WANG (1983) ODL: 0.3 μm(†)

ODL (Optical Detection Limit): limit assumed between submicroscopic and microscopic diameters; NR: not reported.

* No. of fibres sized (No. of filters not reported).

† Values are calculated as ratios between the mean counts of fibres for all the samples; they refer to TEM/Direct Method and LM/AIA Method.

calculate the proportions of submicroscopic fibres and of short fibres ($l \leqslant 5$ μm); in this case too, a remarkable disagreement among the various results is found. Possible causes of the discrepancies reported in Table 5 are: (1) different working processes, and also a different 'way of working' for the same process, on which the fibre size distribution can depend (GIBBS and HWANG, 1975; RENDALL and SKIKNE, 1980; HWANG, 1983); (2) the different diameter value adopted to differentiate submicroscopic from microscopic fibres; (3) the different mineralogical origin of the fibre. In particular, when considering only fibres with $l > 5$ μm, we found a lower percentage of submicroscopic fibres than any other author. This discrepancy too can be explained by the causes reported above. We think that a loss of fibres during the preparation for TEM is unlikely; in fact, by comparing with LM results, we verified that no loss of microscopic fibres longer than 5 μm occurred (as reported in Table 5) and there seems to be no reason to suppose a selective loss of thin long fibres ($d \leqslant 0.3$ μm, $l > 5$ μm), as could be possible for thin but very short fibres. Finally, it is clear that owing to both the discrepancies in reported results and the low number of studies undertaken, and especially of samples examined, it is not yet possible to derive generally valid conclusions about the percentage of submicroscopic fibres.

As regards the correlation between the two instruments when only the microscopic fibres are considered at TEM, HWANG and WANG (1983) report a correlation coefficient $r = 0.87$ (25 samples), which is in very good agreement with ours (see Table 3: $r = 0.90$; 23 samples with LM density of 40–1000 fibres mm^{-2}).

CONCLUSIONS

The results reported in this paper show a very good agreement between the two techniques considered and, therefore, suggest the possibility of deriving, for each working process, conversion factors which would allow a rough evaluation of the total number of airborne chrysotile fibres from LM results in occupational environments. In fact, LM might be a suitable instrument for routine control of asbestos dust, but it is unable to detect all the fibres which are considered to be harmful. The differences among the few results available up to now for this type of comparison indicate that the problem of correlating the two measurements is far from being solved. Consequently, it is not correct to determine the exposure to all the fibres considered as harmful, by using conversion factors derived from other surveys. It seems, therefore, clear that further systematic studies on a wider number of samples from different working processes are required to determine such factors.

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