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The WHO/EURO man-made mineral fiber reference scheme

by the WHO/EURO Technical Committee for Monitoring and Evaluating MMMF¹

WHO/EURO TECHNICAL COMMITTEE FOR MONITORING AND EVALUATING MMMF. The WHO/EURO man-made mineral fiber reference scheme. *Scand J Work Environ Health* 11 (1985) 123–129. At a consultation arranged by the World Health Organization's Regional Office for Europe in 1980 a reference method was specified for sampling and evaluating airborne dust samples collected in man-made mineral fiber (MMMF) workplaces. This method involves sampling onto a membrane filter and counting the fibers with the use of a phase contrast optical microscope (PCOM). The evaluation procedure has been tested by members of the WHO/EURO MMMF technical committee through a series of interlaboratory sample exchanges. The results from these trials have shown a consistent improvement in the reproducibility of MMMF counts, maximum systematic interlaboratory differences being reduced from 2.5 times to 1.4 times. This reduction has been achieved through harmonization of the subjective judgements made by microscopists. As this improved agreement was achieved, each laboratory's counting level changed; preliminary comparisons suggest that this occurrence would produce an approximately two- to threefold increase in the fiber concentrations reported during the epidemiology study of the Joint European Medical Research Board. Further work is currently being undertaken to quantify this change better. A reference method for sizing and counting airborne MMMF with a scanning electron microscope (SEM) has also been developed, and on the basis of preliminary experimental tests there has been some harmonization between the participating laboratories.

Concern has been expressed regarding the possible health effects associated with exposure to airborne man-made mineral fibers (MMMF). This concern led the European manufacturing industry to initiate an international collaborative retrospective epidemiology study (4). As part of this program environmental surveys were undertaken by the Edinburgh Institute of Occupational Medicine in several European MMMF factories to determine the airborne fiber levels and fiber size distribution (11). Wherever possible, parallel sampling exercises were carried out by the Institute and a prominent occupational hygiene laboratory in the country where the survey took place. During the course of these surveys it was noted that different methods of measuring airborne MMMF (both fiber number and mass) were in use in different countries. Furthermore, the different laboratories did not always produce comparable results when using the same method. This situation was particularly true for fiber counting and sizing, for which the subjective nature of the procedure can result in appreciable differences being obtained by different laboratories. This experience parallels that found in asbestos fiber evaluation. A World Health Organization (WHO) consultation took place in Copenhagen in April/May 1980 to consider means of ensuring the comparability of environmental measurements made during epidemiologic studies which were in progress.

Consequently a scheme was initiated to produce reference methods to sample and evaluate MMMF and to harmonize interlaboratory results obtained with these procedures. The aim of this program is to ensure that the results produced by different laboratories in epidemiologic studies are comparable. A more-detailed account of the background and aims of the scheme has been given in the report on the WHO consultation (13).

Organization of the scheme

The scheme, known as the WHO/EURO Man-Made Mineral Fiber Reference Scheme, is being partially funded by the European Manufacturing Industry through the Joint European Medical Research Board (JEMRB), the European Office of the World Health Organization (WHO/EURO), and the participating institutes. A technical committee of experts was formed to manage the scheme, and the Institute of Occupational Medicine (IOM), Edinburgh, the United Kingdom, was nominated as the central reference laboratory to organize the work. This committee, the WHO/EURO Technical Committee for Monitoring and Evaluating MMMF, comprises representatives of WHO, JEMRB, the Edinburgh Institute of Occupational Medicine, and the International Agency for Research on Cancer (IARC), as well as the following national institutions:

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Work to date

The experimental program, which began in 1981, was designed to minimize interlaboratory differences in fiber evaluation by phase contrast optical microscopy (PCOM) and by scanning electron microscopy (SEM). A PCOM reference method was agreed on as a consequence of the discussions at the WHO consultation (13), and an SEM reference method was developed during the experimental program. Several interlaboratory exchanges have been conducted to assess the performance with these methods, and special investigations of specific problems have been undertaken where necessary. The work has so far been confined to the French, German, Swedish, and British laboratories and has concentrated on fiber number and size measurements.

Outline of the reference methods

The PCOM reference method (12) has been developed to measure the personal respirable and nonrespirable fiber concentration. It is based on the membrane filter technique used to determine the levels of asbestos fibers in air (1, 2, 6). The sample is collected by the drawing of a measured quantity of air through a membrane filter by means of a battery-powered sampling pump. After the filter is made optically transparent with the acetone/triacetin technique (10), the number of fibers on randomly selected areas are counted with the use of a phase contrast optical microscope, with a magnification of approximately $500\times$. "Fibers" are conventionally defined as objects with a length greater than $5\text{ }\mu\text{m}$ and an aspect ratio (length:diameter) greater than or equal to 3:1. Fibers of a diameter less than $3\text{ }\mu\text{m}$ are considered to be respirable, whereas those of a diameter greater than or equal to $3\text{ }\mu\text{m}$ are "nonrespirable." The counting rules adopted in this work for counting complex fiber/particle groups (13) differ appreciably from the rules commonly used for asbestos evaluations. In particular, fibers in contact with other particles or fibers are counted providing they meet the aforementioned criteria. This rule was adopted for the practical reason that, otherwise, few fibers would be counted. It has the added advantage of simplicity. This reference method is referred to in the current draft standard produced by the International Organization for Standardization (ISO) (9).

The SEM reference method (12) was primarily developed to assess the airborne fiber size distribution

in MMMF workplaces, with the estimation of fiber number concentration as a secondary aim. Samples are collected onto a polycarbonate filter (Nuclepore) or a polyvinyl chloride membrane filter (Gelman DM800) according to the same sampling methodology as for the PCOM reference method. After preparation, the samples are observed on a scanning electron microscope at a magnification of $5000\times$. A series of photomicrographs are then recorded from randomly selected fields, and the fiber length and diameters are measured from an optically enlarged image of these micrographs. Fibers are defined as all objects with an aspect ratio greater than or equal to 3:1, with no maximum or minimum length or diameter specified. The method should be used in conjunction with an SEM fiber visibility test specimen, developed by the WHO/EURO MMMF Technical Committee to ensure that fibers with a diameter greater than $0.05\text{ }\mu\text{m}$ are visible.

Results of the experimental program

Optical microscopy

The testing of the PCOM reference method has been undertaken by a series of slide exchanges. Four slide exchanges have been completed; in the first two exchanges 20 samples were evaluated, while in the third and fourth exchanges 8 samples were counted. All assessments were made on airborne dust samples collected in factories producing rock wool or glass wool. Five laboratories participated in these exchanges, each laboratory making one count on the samples with the reference method.

These data give an indication of systematic differences between laboratories and also each laboratory's internal consistency. The following two performance statistics have been used to describe these data:

1. An interlaboratory index, which gives a measure of the mean position of the laboratory's counts relative to the group.
2. An intralaboratory index, which measures the variability of this difference.

These statistics are calculated as follows. For each sample, the mean count is determined, and the ratio of each laboratory's count to this mean is obtained. For each laboratory the arithmetic mean and standard deviation of these normalized results are calculated over all samples in the exchange. The interlaboratory index is then obtained by the subtraction of 1 from the mean of the normalized results, and it is expressed as a percentage. The intralaboratory index is the coefficient of variation of the normalized results.

The values of the indices obtained in each exchange are given in figure 1. A marked reduction in the spread of each of the two indices occurred from the first through the third exchanges, ie, from

about $\pm 40\%$ of the group mean to $\pm 20\%$ in the case of the interlaboratory indices and from a range of 10–30% to 9–15% for the intralaboratory indices. Following the improvement it was decided to extend the scheme to include more microscopists, ie, from one per laboratory to up to three per laboratory. Furthermore, lower density samples were introduced to provide a more typical representation of fiber loadings encountered in factory situations. Consequently the performance indices are higher than those observed in the third exchange. However, for fiber densities in the density range generally accepted for optimum precision (ie, 100–1 250 fibers/mm²), the improvement in the interlaboratory reproducibility appears to have been maintained, as illustrated in table 1.

As improved reproducibility has been achieved, changes have occurred in the count obtained by each laboratory. The Edinburgh Institute of Occupational Medicine was responsible for conducting the environmental surveys in the JEMRB retrospective epidemiologic study between 1977 and 1980. Five samples counted by the Institute and laboratories B and C at this time were included in the second exchange to obtain an indication of long-term changes in level.

Institute counts in this exchange were around three times higher than those made four years earlier (Ratio of exchange 2 : 1978 counts made with phase contrast microscopy: IOM 2.8, laboratory B 4.5, laboratory C 1.1). Experience with reference schemes has shown that the mean count of a group rises towards that of the highest counter. This has been the case in our instance. Laboratory C has maintained a consistently high level, while the Institute, freed from the need to keep consistent counting standards for epidemiologic research purposes throughout a four-year survey period, has increased its count level. Laboratory B counts have also increased (by about 4.5 times) since 1978. More samples from the survey period are being introduced into the scheme to provide a more reliable estimate of changes in level.

Scanning electron microscopy

At the outset of the scheme there was no information about the differences which might be expected between laboratories using SEM to assess fiber size. Four laboratories had experience with SEM for determining MMMF size, and each had developed their own methodology. An interim reference method was agreed on by discussion, and it was then tested in two sample exchanges. The number of samples was restricted to three because of the time-consuming nature of these evaluations. Two were prepared from a liquid suspension of fine MMMF with no organic binder, and one sample was collected in a glass wool factory. The results of one of the samples from this exchange are illustrated in figure 2. The differences

Figure 1. Reference scheme results from four optical exchanges.

Table 1. Summary statistics for the optical exchanges.

	Exchange			
	1	2	3	4
Number of samples ^a	17	16	8	5
Geometric mean coefficient of variation (%)	30	22	16	19
Maximum interlaboratory difference	2.5	1.6	1.3	1.4

^a Within the density range 100–1 250 fibers/mm².

Figure 2. Results from one sample in exchange 1. (Inverted triangle = laboratory A, square = laboratory B, circle = laboratory C, diamond = laboratory D, and upright triangle = laboratory E)

between laboratories were large both in terms of fiber size and fiber number. For example, in the worst case, the geometric mean length estimates varied

Figure 3. Comparison of in-house (closed symbols) and reference scheme rules (open symbols) for one sample from exchange 1. (Inverted triangle = laboratory A, square = laboratory B, circle = laboratory C, diamond = laboratory D, and upright triangle = laboratory E)

Figure 4. Results from one sample evaluated on visits (open symbols) compared with exchange 1 results (closed symbols). (Inverted triangle = laboratory A, square = laboratory B, circle = laboratory C, diamond = laboratory D, and upright triangle = laboratory E)

from 2.7 to 8.4 μm , and the corresponding geometric mean diameters from 0.14 to 0.51 μm . In addition to evaluations by the reference method, those laboratories with their own in-house method reevaluated some of the samples using these methods. These data for a second sample from exchange 1 are shown in figure 3. Clearly these differences were smaller in magnitude than those between laboratories. It was concluded that the interlaboratory differences were due to variations in instrument performance and to subjective judgements by the operators.

To resolve these problems, a special experimental program was initiated. One SEM operator visited each laboratory to discuss the reference method and carry out a number of tests to assess SEM resolution, calibration of magnification, and visibility of very fine fibers (approximately 0.05 μm).

At the end of the visit each laboratory reassessed one of the samples from the first exchange. The results from these evaluations are shown in figure 4. There was a definite improvement in performance; for example the geometric mean fiber lengths ranged from 2.3 to 5.8 μm after the visit, compared with 2.7 to 8.4 μm before. Similarly the evaluated fiber density range changed from 4 100–7 700 fibers/ mm^2 to 7 900–10 600 fibers/ mm^2 . With this achieved im-

provement, evaluated fiber densities increased, while median length and diameter assessments decreased, the detection of more short thin fibers therefore being indicated. This occurrence is in fact due to closer specification of various problems in the subjective assessments made for low aspect-ratio fibers.

A workshop was held immediately after this exercise, where the SEM operators completed another series of investigations at one laboratory. These exercises pinpointed a number of areas in the reference method which required closer specification. In particular the following points were incorporated in the reference method:

1. The SEM magnification and the magnification of the final image for sizing were specified more precisely. The original definition resulted in a range of final measurement magnifications from 3 000 $\times$ to 50 000 $\times$.
2. Differences in image quality were evident between instruments, as shown in figure 5 (—). As a solution a visibility test specimen was developed against which SEM operating performance could be judged. These specimens are currently being developed by the Institute of Occupational Medicine, Edinburgh.
3. For poorer quality instruments there is a need to record a micrograph for every field, even if no fibers are visible on the SEM screen. The detection of very fine fibers (diameter approximately 0.05 μm) on the SEM television monitor was not always reliable when compared to photomicrographs because of the lower signal-to-noise ratio found on the monitor.
4. Subjective errors were greater for short fibers. One laboratory consistently found more short fibers than the others. To reduce this problem, further work is in progress to assess the reliability of this method for fiber length of less than about 1 μm .

A third sample exchange was subsequently completed and the results, shown in figure 6, confirm the improved agreement obtained with the revised reference method.

Figure 6. Results of exchange 3. (Inverted triangle = laboratory A, square = laboratory B, circle = laboratory C, diamond = laboratory D, and upright triangle = laboratory E)

LAB A

4 μ m

LAB B

4 μ m

LAB C

4 μ m

LAB D

4 μ m

LAB E

4 μ m

Figure 5. Test area containing chrysotile asbestos viewed on five different scanning electron microscopes.

Discussion

A PCOM reference method for estimating airborne fiber concentrations in MMMF workplaces was formulated and tested through a series of interlaboratory slide exchanges. The results show an improvement in interlaboratory agreement for samples in the range of fiber densities recommended for optimal precision (ie, 100–1 250 fibers/mm²), maximum systematic interlaboratory differences being reduced from 2.5 times at the beginning of the MMMF exchanges to 1.4 times in the most recent exchange.

The five laboratories participating in the MMMF exchanges also took part in an international interlaboratory trial involving asbestos fiber counting (7). The maximum interlaboratory differences produced in this exercise was 3.5 times, when chrysotile samples were evaluated according to counting rules published by the Asbestos International Association (2). These rules have since been incorporated into a European directive (6). The better interlaboratory agreement for MMMF compared with asbestos probably reflects (i) the less ambiguous counting rules inherent in the MMMF reference method, (ii) the extent to which these laboratories have collaborated over the years, and (iii) the differences in fiber size distribution, MMMF being generally longer and thicker and hence more clearly visible to the microscopist.

This work has particular relevance to the JEMRB epidemiology study (4). During the period of this study the Edinburgh Institute of Occupational Medicine, which was responsible for making the factory environmental measurements, attempted to maintain a consistent counting level. In the course of the reference scheme, however, the Institute's level has increased, being now approximately two to three times higher than when the factory measurements were made. This occurrence represents an underestimation of exposure compared with the present reference level. Further work is currently being undertaken to quantify better the magnitude of the change in the Institute's counting performance.

An SEM reference method has been developed and has undergone preliminary tests. The extended time required for SEM size analysis has meant that less data are available to assess interlaboratory differences than for PCOM. From the limited number of samples exchanged, backed up by the experience of a workshop and laboratory visits, the indications are that there has been some harmonization between the laboratories. As with PCOM evaluations, there are subjective errors in the perception of fibers associated with the human operator. This has already been shown to be the case for asbestos (5). These errors can be minimized by the use of a reference method based on unambiguous counting rules and by continued participation in an interlaboratory quality control scheme. The latter is the most important;

there is a tendency for laboratories to drift apart once interlaboratory exchanges cease.

It is recognized that, with the increasing usage of MMMF, there are many situations developing where workers may be exposed to more than one variety of mineral fibers, either man-made or natural. In such circumstances it is important to be able to characterize the fiber types present. A reference method for fiber identification based on electron microscopy is therefore being developed.

To ensure the comparability of data, it is essential that researchers working with MMMF, whether they be involved in human autopsy studies, animal experiments, or epidemiology, should use reference procedures and should participate in a quality assurance scheme of this type.

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Appendix

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