MONITORING AND EVALUATING MAN-MADE MINERAL FIBRES: WORK OF A WHO/EURO REFERENCE SCHEME

N. P. CRAWFORD
Institute of Occupational Medicine, Roxburgh Place, Edinburgh, EH8 9SU, U.K.

D. KELLO and J. O. JARVISALO
World Health Organization, Regional Office for Europe, 8 Scherfigsvej, DK-2100, Copenhagen, Denmark

Abstract—In the mid-1970s, a meeting held at WHO in Copenhagen launched a research programme on the health effects of man-made mineral fibres. As part of this programme, a WHO/EURO Reference Scheme was initiated in 1980 to improve comparability of data obtained from epidemiological studies and thus reduce the level of uncertainty in the risk assessment process. The scheme involved developing reference methods for evaluating airborne MMMF concentrations and size distributions by phase contrast optical microscopy (PCOM) and scanning electron microscopy (SEM). These methods were tested in a series of inter-laboratory counting trials. Interim progress in the scheme was published in 1985. This paper updates the work of the scheme and confirms that the previously published improvements in reproducibility of measurement have been maintained.

INTRODUCTION

The Regional Office for Europe of the World Health Organization (WHO) is concerned with the problems of industrialized society. The adverse health effects of exposure to chemicals, harmful dusts and fibres in all aspects of daily life rank high in the WHO priorities.

In the mid-1970s, a meeting held at WHO in Copenhagen launched a research programme on the health effects of man-made mineral fibres (MMMF). This programme was sponsored by the European MMMF industry through its Joint European Medical Research Board (JEMRB) and comprised: animal experiments conducted by the British Medical Research Council Pneumoconiosis Unit; an international epidemiological study co-ordinated by the International Agency for Research on Cancer (IARC); and environmental surveys performed by the Institute of Occupational Medicine (IOM), Edinburgh, U.K., to determine airborne fibre levels and fibre size distributions in several European MMMF factories.

For the epidemiological study, it was necessary to give special attention to the quality of measurements of airborne MMMF. Different measurement methods were used in different countries (ÖTERY et al., 1982). Furthermore, different laboratories did not always produce comparable results even when using the same method; this was particularly true for the subjective procedures of fibre counting and sizing. This is similar to experience with asbestos fibre counting which has resulted in the establishment of national and international quality assurance schemes such as RICE (CRAWFORD and COWIE, 1984), PAT (SCHLECHT and SHULMAN, 1986) and AFRICA (CRAWFORD et al., 1984). Consequently, a WHO consultation (WHO, 1981) held in Copenhagen in 1980 initiated a similar scheme, known as the WHO/EURO Reference Scheme. Its aims were to produce reference methods for sampling and evaluating
MMMF and to minimize inter-laboratory variation in the results obtained with these methods.

A technical committee of experts, the WHO/EURO Technical Committee for Monitoring and Evaluating MMMF, was established in 1981 to manage the scheme. This committee comprises representatives of the WHO Regional Office for Europe, JEMRB, IARC, the IOM and national laboratories in six countries (Czechoslovakia, West Germany, France, Poland, Sweden and the United Kingdom). The scheme has had three key operations:

(a) Development of a reference method for measuring airborne MMMF concentrations by phase contrast optical microscopy (PCOM).
(b) Development of a reference method for sizing airborne MMMF by scanning electron microscopy (SEM).
(c) Implementation of practical quality assurance schemes to minimize inter-laboratory differences in fibre evaluation by PCOM and SEM.

The technical committee co-ordinated the work through an appointed Central Reference Laboratory (the IOM) which has been responsible for organizing technical meetings and discussions, preparing and distributing samples for evaluation by participating laboratories, statistical treatment of the data and reporting the results.

This paper updates the progress reported in 1985 which the WHO/EURO Technical Committee has made in harmonizing fibre counts and size distributions obtained by the use of standard evaluation procedures and regular inter-laboratory sample exchanges (WHO/EURO TECHNICAL COMMITTEE, 1985a).

REFERENCE METHODS

The PCOM reference method is based on the membrane filter technique used to determine airborne respirable asbestos fibre concentrations (CEC, 1983). The sample is collected by drawing a measured quantity of air through a membrane filter by means of a battery-powered sampling pump. The filter is mounted on a glass slide and made optically transparent with acetone/triacetin. The number of respirable fibres in randomly selected areas of the filter is counted using PCOM at a magnification of about 500 x. Respirable fibres are those objects longer than 5 μm, narrower than 3 μm, and with a length/diameter ratio (aspect ratio) ≥ 3:1. In contrast to the asbestos fibre counting rules, fibres in contact with particles or other fibres are counted, provided they meet the above criteria; this avoids underestimation of the number of fibres present and minimizes ambiguities in the decisions microscopists have to make in these circumstances. The PCOM method also permits determination, if required, of the concentrations of non-respirable fibres, i.e. these fibres with the same length and aspect ratio characteristics as respirable fibres but with diameters ≥ 3 μm.

In the SEM reference method, samples are collected onto a polycarbonate filter (Nuclepore) or a PVC membrane filter (Gelman DM800) using the same sampling methodology as the PCOM method. After preparation, the samples are observed on an SEM at a magnification of 5000 x. Series of photomicrographs are recorded from randomly selected fields and fibre lengths and diameters are measured from optically enlarged images of these photomicrographs. Whilst the SEM method was primarily developed to determine fibre size, it also permits fibre number concentration to be evaluated if required.
Detailed specifications of both reference methods are given in a WHO Environmental Health Report (WHO, 1985b).

WORK PROGRAMME

The PCOM and SEM reference methods, once developed, were tested in a series of inter-laboratory sample exchanges. Special investigations of specific problems were also conducted where necessary. From 1981 to 1985 the work was confined to laboratories from France, West Germany, Sweden and the United Kingdom (2) and concentrated on fibre number and size measurements. In 1986, participation was extended to include laboratories from Italy, Norway, Denmark, Czechoslovakia, as well as an additional laboratory from France.

RESULTS

Phase contrast optical microscopy

Six slide exchanges were completed in the period 1981–1985. Twenty slides were evaluated in the first two exchanges. This number was reduced to eight in each of the subsequent exchanges. All samples had been collected in factories producing rock wool or glass wool and were generally in the density range 100–1250 $F/mm^2$.

Each of the five participating laboratories made one count on each sample using the PCOM reference method. In each exchange, the mean count was calculated for each sample and the ratio of each laboratory's count to this mean was obtained. For each laboratory, the arithmetic mean and standard deviation of these normalized results were calculated over all samples in the exchange. Two performance indices were derived for each laboratory:

(i) an inter-laboratory index, which was obtained by subtracting 1 from the mean of the normalized results and multiplying the result by 100; this gives the mean position of a laboratory's results, expressed as a percentage deviation from the mean of the group;

(ii) an intra-laboratory index, which is the coefficient of variation of the normalized results and gives a measure of the variability from sample to sample of the difference between a laboratory's count and the group mean. Ideally, both indices should approach zero.

The values of the indices obtained in each exchange are given in Fig. 1. The spread of each of the two indices was markedly reduced from the first through the third exchange, i.e. from about $\pm 40\%$ of the group mean to $\pm 20\%$ for the inter-laboratory index and from a range of 10–30% to 9–15% for the intra-laboratory indices.

Following the improvement, the scheme was extended to include more microscopists, i.e. from one per laboratory to up to three per laboratory. Consequently, the performance indices are higher in subsequent exchanges than in the third exchange. Nevertheless, the improvement in inter-laboratory reproducibility appears to have been maintained; maximum inter-laboratory systematic differences were 2.5-fold in the first exchange and 1.3–1.8-fold over the five subsequent exchanges.

The improved reproducibility was achieved by laboratories changing their counting level. Table 1 shows the geometric mean values of the ratio of counts made by each laboratory in exchange 2 to those made in exchange 1 for eight samples included in
both exchanges. Counts in the second exchange were elevated for all laboratories, the greatest mean increase (60%) being shown by laboratory D. Table 1 also summarizes the results obtained for four samples included in both the first and fifth exchanges. These indicated that mean count levels did not change too much in the 2–3 yr between exchanges 2 and 5 and that the reference level had remained reasonably constant during that time.

The IOM conducted the original factory environmental surveys for the epidemiological study during the period 1977–1980 and attempted to maintain a consistent level throughout. Five samples evaluated by the IOM during these surveys were included in exchange 2 of the reference scheme and another four survey samples were included in exchange 5. Comparison of the results indicated that the IOM counts in both exchanges were some 2–3 times higher than those made during the survey period. This was confirmed by CHERRIE et al. (1988) who evaluated additional survey and reference scheme samples when they conducted follow-up factory surveys in 1984. They
Monitoring and evaluating MMMF

Table 1. A comparison of counts obtained in PCOM exchange 1 and in subsequent exchanges

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Exchange 2</th>
<th>Exchange 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exchange 1</td>
<td>Exchange 1</td>
</tr>
<tr>
<td>A</td>
<td>1.19</td>
<td>0.71</td>
</tr>
<tr>
<td>B</td>
<td>1.21</td>
<td>1.48</td>
</tr>
<tr>
<td>C</td>
<td>1.16</td>
<td>1.18</td>
</tr>
<tr>
<td>D</td>
<td>1.60</td>
<td>1.65</td>
</tr>
<tr>
<td>E</td>
<td>1.15</td>
<td>0.89</td>
</tr>
</tbody>
</table>

also used this information to correct their factory measurements to approximate to the WHO/EURO reference level, so that they could compare factory concentrations in 1977–1980 and 1984.

Scanning electron microscopy

The SEM programme in the period 1982–1985 primarily comprised:
(a) formulation of a reference method;
(b) three slide exchanges to determine inter-laboratory variation and monitor any improvements made;
(c) visits to each laboratory by an experienced SEM operator to identify reasons for inter-laboratory differences in results;
(d) a workshop conducted immediately after the visits, where all SEM operators conducted a series of investigations at one laboratory. The work carried out during the visits and at the workshop was designed to permit aspects of an interim reference method to be more closely specified;
(e) an exchange of around 25 photomicrographs to assess the reliability of the reference method for evaluating fibres of length less than 1 μm.

The results of (a)-(d) have been published by the WHO/EURO Technical Committee (1985a) and it is appropriate to summarize these before describing the results of (e).

Details of an interim reference method were first agreed by discussion. Two exchanges of samples were then conducted. The number of samples was restricted to three because of the time-consuming nature of the evaluations: one was a glass wool factory sample, the other two were prepared in the laboratory from liquid suspensions of fine MMMF. Each laboratory evaluated each sample using the interim reference method. The evaluated fibre densities (\( f_{\text{SEM}} \text{ mm}^{-2} \)), geometric mean lengths and geometric mean diameters for one of the reference samples are given in Fig. 2, which is a reproduction of the Fig. 4 published in the WHO/EURO Technical Committee report (WHO, 1985a). Large inter-laboratory differences were apparent; geometric mean lengths ranged between 2.7 and 8.4 μm, with corresponding geometric mean diameters ranging between 0.14 and 0.51 μm.

Several investigations were carried out to determine the extent to which these differences were due to differences in interpretation of the interim reference method, variation in instrument performance and different subjective judgements made by SEM operators. Tests were carried out during the laboratory visits to assess SEM resolution, calibration of magnification and visibility of fine fibres (ca 0.05 μm dia.). It
became evident that differences in image quality existed between instruments and that detection of fine fibres on the SEM TV monitor was not always reliable compared with photomicrographs because of the lower signal-to-noise ratio found on the screen. In addition, the magnifications at which measurements were made ranged from 3000 to 50,000×. At the end of the visit, each laboratory re-assessed the sample illustrated in Fig. 2 from the first exchange, the results of which are also given in Fig. 2. The range of median lengths and diameters decreased markedly with an accompanied increase in evaluated fibre densities, indicating the greater detection of short thin fibres. Subjective errors associated in particular with short fibres were also identified in the workshop.

The interim reference method was consequently modified to include a requirement that, particularly for poorer quality instruments, a photomicrograph be recorded for every field, even if no fibres are visible on the screen. Measurements would be made from the photomicrographs. The SEM magnification and the magnification of the final image for sizing were also specified more precisely (WHO, 1985b). A third sample exchange was then undertaken to check that the improved inter-laboratory variation observed at the end of the visits had been maintained and an exchange of about 25 photomicrographs was conducted to assess the reliability of the reference method to evaluate fibres of length less than 1 µm.

The results of the third exchange was shown in Fig. 3 [the Fig. 6 contained in the WHO/EURO Technical Committee report (WHO, 1985a)] and confirm the improved agreement obtained with the revised reference method. Table 2 lists the results of the photomicrograph exchange and illustrates that appreciable interlaboratory variation occurred in observing fibres <1 µm in length. The procedure defined in the reference method for reporting results now distinguishes between fibre lengths ≥1 µm or <1 µm.

**DISCUSSION**

PCOM and SEM reference methods have been established and their detailed specifications published in a WHO/EURO Technical Committee report (WHO, 1985b). Use of these methods has led to a reduction in inter-laboratory variation in both the PCOM and SEM quality assurance schemes.

The PCOM results are considered to have reached a plateau of optimum
Monitoring and evaluating MMMF

![Graph](image)

**FIG. 3. Results from exchange 3.**

**TABLE 2. RESULTS OF THE SEM MICROGRAPH EXCHANGE**

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>All fibres</th>
<th>Fibre density ($\mu_m^{-2}$)</th>
<th>$L &lt; 1\mu m$</th>
<th>$L \geq 1\mu m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5350</td>
<td>630</td>
<td>4700</td>
<td>2700</td>
</tr>
<tr>
<td>B</td>
<td>5450</td>
<td>1000</td>
<td>4450</td>
<td>2500</td>
</tr>
<tr>
<td>C</td>
<td>6300</td>
<td>2600</td>
<td>3700</td>
<td>2600</td>
</tr>
<tr>
<td>D</td>
<td>8200</td>
<td>3700</td>
<td>4500</td>
<td>2700</td>
</tr>
<tr>
<td>E</td>
<td>8950</td>
<td>3920</td>
<td>5000</td>
<td>2930</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Geometric mean fibre size ($\mu m$)</th>
<th>Length</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.5</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4.1</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.0</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.9</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>1.8</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

reproducibility, with maximum systematic laboratory counting differences being about 1.8 times. This is comparable to the smallest inter-laboratory variation achieved in analogous asbestos fibre counting schemes and compares with the value of 1.4 times previously reported by the WHO/EURO Technical Committee at a time when fewer exchanges had been completed with fewer microscopists taking part. The reference level has remained reasonably stable over a 2–3-yr period since the second exchange.

Airborne MMMF concentrations were measured by the IOM in a number of factories over the period 1977–1980 and again at surviving factories in 1984. Data obtained in the reference scheme, together with replicate counts made by the IOM on some samples collected in the early surveys, established that the IOM counts had increased by up to a factor of 3 at the second survey, relative to its counting level in the first survey period. Consequently, Cherrie et al. (1988) converted their epidemiological measurements to approximate to the reference level. Using this as a common basis, they were then able to compare airborne MMMF concentrations in the two surveys more accurately.
Epidemiological as well as experimental studies are approaching the stage where quantifying the risk of exposure to MMMF can be attempted. Bearing in mind that the WHO/EURO Reference Scheme was established to improve the comparability of data obtained from the epidemiological studies and thus reduce the level of uncertainty in the risk assessment process, it is regrettable that more laboratories do not participate in this programme. Participating laboratories significantly reduced the inter-laboratory variation of their data, suggesting that non-participating laboratories may not be generating comparable data.

As with asbestos, there are subjective errors associated with the human operator in the perception of fibres in both the PCOM and SEM evaluations. These errors have been minimized by incorporating unambiguous counting rules in both reference methods and by participation in the reference scheme. Continued participation is most important; there is a tendency for laboratories to drift apart if inter-laboratory exchanges cease.

The WHO/EURO Technical Committee has had eight meetings since 1981, the last two of which have considered in detail its future activities. Until April 1986 the scheme was financed by the European manufacturing industry through JEMRB. In the current period, May 1986–April 1987, the scheme is being jointly financed at a reduced level by JEMRB and the participants. In addition, the participating laboratories now include not only the original reference group but also institutes from Denmark, Finland, France, Italy and Norway, representatives of which had attended the last Technical Committee meeting in Lyon in December 1985. Although development of other methods (e.g. automated image analysis and mass) was considered at their meeting, it was decided that future work should continue to concentrate on PCOM and SEM.

In the current period, the aims of the optical reference scheme are to maintain the improved inter-laboratory reproducibility previously achieved by the reference group and to ensure that new members also attain this performance. Because of the economic constraints, the frequency of slide exchanges has been limited to two during the course of the 1-yr period. Laboratories are divided into two groups, one of which comprises the reference laboratories. In each exchange, a batch of samples is sent to each group, the samples being in the density range 100–1000 $F$, mm$^{-2}$ selected from reference slides which have already been counted by the reference group. Each laboratory will evaluate the samples using the reference method or their own routine method, whichever they wish. Performance statistics will be calculated as before. In the longer term, it is hoped that the scheme can be continued in the same form. Additional samples would be introduced, including some low-density samples, once all participants had achieved an acceptable level of performance.

The reference group is continuing with its work in the SEM scheme, initially to resolve some remaining problems. Work which had previously been initiated is continuing to develop a visibility test specimen against which SEM operating performance can be judged. An investigation is continuing to determine the effect of gold coating on fibre diameters as a follow-on to preliminary work already undertaken. An acceptable method for discriminating between MMMF and other fibre types needs to be developed, especially as different regulations have been imposed for different types of fibre.

In conclusion, it is essential that researchers working with MMMF, whether they be engaged in human autopsy studies, animal experiments, epidemiology or industrial
hygiene measurements, should use reference procedures and participate in a scheme in
order to ensure quality and comparability of data. Such effort will not only decrease the
uncertainties inherent in the risk assessment but also increase the quality of assessing
environmental control, the prerequisite of any measure to reduce work-related risk
factors.

REFERENCES
mineral fibre concentrations in relation to epidemiology. In: Proceedings of the Sixth International
Symposium on Inhaled Particles, Cambridge, September 1985 (Edited by DODGSON, J. and McCALLUM,
R. I.). To be published.
COUNCIL OF EUROPEAN COMMUNITIES (1983) Council directive on the protection of workers from the risks
assurance scheme (RICE). Proc. Fifth Colloquium on Dust Measuring Technique and Strategy,
conditions at 13 European man-made mineral fibre plants. Institute of Occupational Medicine report
(IOM report no. TM/82/21). Also published (in 1984) by World Health Organization, Regional Office
for Europe: Biological Effects of Man-Made Mineral Fibres. Proceedings of a WHO/IARC conference in
association with JEMRB and TIMA, Copenhagen, 20–22 April 1982.
SCHLECHT, P. C. and SHULMAN, S. A. (1986) Performance of asbestos fiber counting laboratories in the
VICC/WHO consultation, Copenhagen. WHO Regional Office for Europe: EURO Reports and Studies
No. 48.
WHO/EURO TECHNICAL COMMITTEE FOR MONITORING AND EVALUATING MMMF (1985a) The
WHO/EURO TECHNICAL COMMITTEE FOR MONITORING AND EVALUATING AIRBORNE MMMF (1985b)
Reference methods for measuring airborne man-made mineral fibres (MMMF). WHO Regional Office
for Europe: Environmental Health Report 4.