Performance of Asbestos Fibre Counting Laboratories by Transmission Electron Microscopy

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In France, the owners of buildings have been obliged since February 1996 to ascertain whether asbestos has been incorporated into surfacing materials, insulation products or false ceilings. In certain circumstances, there is also a requirement to measure the airborne asbestos fibre concentration. Three years (1996–1998) of asbestos fibre count reporting are evaluated for the proficiency testing scheme organized in France to evaluate the performance of laboratories using an indirect-transfer transmission electron microscopy procedure to measure the airborne asbestos fibre concentration. Each year eight filters are distributed to each participating laboratory. These filters are obtained by filtering a suspension containing chrysotile or amphibole fibres. In 1996, 36% of the laboratories were rated 1 (the best performers; i.e. those providing counts close to the reference value). Performance improved appreciably in the last round where 85% of the laboratories were rated 1.

Keywords: asbestos counting; proficiency testing; quality control; transmission electron microscopy

INTRODUCTION

In France, the owners of buildings, with the exception of houses which only contain one self-contained dwelling, have been obliged since February 1996 to ascertain whether asbestos has been incorporated into surfacing materials, insulation products or false ceilings. In certain circumstances, there is also a requirement to measure the airborne asbestos fibre concentration in order to determine compliance with technical limit values. The reference method to be used is the French standard X43-050 (AFNOR, 1996), which is related to the international standard ISO 13794 (ISO, 1997). A number of technical modifications concerning both the air sampling equipment and the analytical method have since been introduced, the most important being the description of a protocol to recover the dust after having ashed the filter without using ultrasonics. It has indeed been shown that when used to facilitate the recovery of dust, ultrasonics may significantly increase the fibre number concentrations (Kauffer et al., 1996). Briefly, in the ISO standard the filter to be ashed is placed in a beaker. In the French standard it is placed on an optical microscope slide inside a conical Pyrex tube. To ensure complete recovery of the dust after ashing, in the ISO standard distilled water and glacial acetic acid are added to the beaker which is then placed into an ultrasonic bath for a period of 5 min. In the French standard to re-suspend the residue the optical slide is first shaved with a blade in distilled water and the solution is then agitated manually.

As considerable differences between laboratories can be expected in asbestos fibre counting, it is particularly important to be able to evaluate the performance of laboratories carrying out these measurements. It is for this reason that many proficiency testing schemes are organized in the field of occupational or non-occupational hygiene for fibre counting. Most use a phase contrast optical microscope to count the fibres (Carton et al., 1981; Kauffer, 1989; Crawford et al., 1992; Brown et al., 1994; Schlecht and Shulman, 1995; Arroyo and Rojo, 1998), but some, especially in Germany and Italy, involve the use of a scanning electron microscope (Höfert et al., 1996a, b; Paoletti et al., 1996).
As part of the legislation on asbestos in buildings INRS was asked by the French Ministry in charge of health to organize a proficiency testing scheme. Every laboratory approved for measuring asbestos fibre number concentration inside buildings must take part in this scheme, and must evaluate eight filters each year by means of transmission electron microscopy. The first scheme was organized in 1996. The aim of the present paper is to describe the results obtained over the first three years, and include a discussion about the criteria used to classify the participating laboratories.

SAMPLE PREPARATION

Asbestos reference samples are prepared by filtering sonicated suspensions of asbestos fibres through cellulose ester membrane filters. Briefly the methodology can be described as follows:

1. As a starting point to obtain a fibre size distribution as representative as possible of that obtained by air sampling, asbestos fibres are first generated in an experimental dust chamber (Rihn et al., 1996). A cyclone is used to remove the coarse fraction of the fibres. The generated fibres are then collected on Nuclepore filters. As an alternative method, the coarse fraction may also be removed by separation in an Andreasen pipette.

2. This raw material obtained by air filtration on Nuclepore filters and/or sedimentation, is then diluted to a fixed volume of 250 ml (heptane + 1% propanol). The asbestos fibres are then dispersed throughout the liquid by ultrasonic agitation for 60 min.

3. An aliquot of this suspension depending on the expected density on the loaded filters that we wish to obtain is then diluted in a large volume of 5 l. (heptane + 1% propanol). Homogenization of the solution is obtained by ultrasonic agitation for 15 min.

4. This stock suspension is divided into five equal parts (1 l. each). Each part is again homogenized for 5 min then filtered on a 142 mm diameter mixed cellulose ester membrane filter (Gelman 0.45 μm pore size) using the Millipore® filtration system (ref. VT30142HW). (This filtration system is specially designed for hazardous waste filtration by pressure filtration, but, in this case, was used without the bottom plate to filter the liquid at normal atmospheric pressure.)

5. Eight 25 mm diameter filters are cut on an annulus of each 142 mm diameter filter so that for one density level and one type of asbestos fibre, forty 25 mm diameter filters are available for the scheme. In the first year (1996), two extra 25 mm diameter filters were cut in the central part of the 142 mm diameter filter to give an extra 10 filters.

6. As a quality check, three smaller filters (10 mm diameter in 1996, 20 mm diameter in 1997 and 1998) are cut on the remaining part (the central part) of each of the five 142 mm filters. By so doing, 15 control filters are available for each density level.

7. The entire procedure is repeated until eight different density levels are available.

CHECKING HOMOGENEITY

For each density level, the control filters (see step 6 above) are counted by optical phase contrast microscopy. A given density level is accepted if the following criteria are met:

- the ratio between the highest and lowest fibre density among the 15 control filters must be less than 2.5;
- the coefficient of variation of the 15 fibre densities must be lower than 25%;
- the fibre densities between each of the five 142 mm diameter filters must not be significantly different (p>0.05). This is verified by an analysis of variance on the logarithm of the density. Each observation is weighted by the number of fibres counted to stabilize the variance.1

At the end of the scheme, when all the results are available, another analysis of variance is performed to check that the differences in density between the five 142 mm diameter filters for the same density level are not significant. In this case, as each laboratory analyses only one 25 mm diameter filter for each density level, it is necessary to perform the analysis of variance using all the density levels to be able to take into account the differences between laboratories. Again, the logarithm of the density is used and each observation is weighted by the number of fibres counted.1

ORGANIZATION OF THE SCHEME

Each participating laboratory receives eight filters which differ in their asbestos fibre loading and/or type of asbestos. Until now, only one type of asbestos fibre has been used for each density level. No other fibres are included. Each laboratory is asked to send back the results within a month and a half, including the fibre number density, the number of fibres counted and the type of asbestos. The reference method is the X43-050 AFNOR standard.

Counting is stopped when:

1 Strictly speaking, as the area examined varies to a certain extent, this should also be taken into account in the weighting.
Performance of asbestos fibre counting laboratories

- at least 0.3 mm² of the 25 mm diameter filter has been analysed;
- at least 100 fibres have been counted.

For the schemes organized in 1996, 1997 and 1998 the fibre number densities per mm² were in the range 53–215, 256–2093 and 342–1214, respectively.

CATEGORIZATION OF LABORATORY PERFORMANCE

Due to a lack of information on other proficiency testing schemes for the counting of asbestos fibres by transmission electron microscopy, it was decided to adopt a categorization procedure similar to that used in the RICE and AFRICA schemes (Crawford et al., 1992).

For each scheme, the reference count was the mean (in 1996) or the median (in 1997 and 1998) of the counts provided by a reference group of laboratories. A standardized ratio, namely the ratio of a given density to the reference density, was computed for each laboratory and each density level. For classification purposes, three classes (each extending from 1/x to x) are defined as follows.

If six out of eight standardized ratios for one laboratory lie within a range extending from 0.43 to 2.30, the laboratory is classified in category 1. Failing that, if six out of eight standardized ratios lie within a range extending from 0.31 to 3.20, the laboratory is classified in category 2.

If more than two of the eight standardized ratios lie outside the outer limits or if the identification of the type of asbestos was wrong for one or several density levels, the laboratory is classified in category 3. If reference laboratories belong to this group they are removed from the reference group and the calculation has to be done again.

Laboratories belonging to category 1 are the reference laboratories for the next scheme. To initiate the system in 1996 the reference group was formed of six laboratories that had been involved in the field of asbestos counting by transmission electron microscopy for many years. It did not seem possible for us to include all the participants in the reference group as is the practice in the RICE scheme. This was mainly due to the fact that most of the laboratories taking part in the scheme in 1996 had only recent experience of asbestos counting by transmission electron microscopy. It is, however, encouraging to see that the number of reference laboratories has been increasing year after year. In 1996, six out of a total of 33 participants achieved reference classification, this figure rising slightly to eight out of 34 in 1997 then sharply to 17 out of 34 in 1998. It can therefore be expected that the number of laboratories included in the reference group will increase the longer the scheme goes on.

Selecting the inner limits (0.43–2.30) or the outer limits (0.31–3.20) was difficult because we were unable to confirm them with any other comparable data. It was obvious that the limits used in the RICE scheme (inner limit: 0.65–1.55, outer limit: 0.50–2.00) were unsuitable for two reasons. Firstly, in the RICE scheme, the reference density for a given density level is determined by a reference group of counters counting the same slide, whereas, in this case, the reference density is determined by reference laboratories counting different replicates. This, of course, introduces a higher level of variability. Secondly, in proficiency testing schemes such as the RICE scheme, to check the performance of fibre counting by phase contrast optical microscopy, the operator receives mounted filters and has nothing to prepare. On the contrary, in this scheme, each participant receives a filter and must prepare it in order to make the counting and identification of fibres by transmission electron microscopy possible. This involves several preparation steps (ashing of the filter, recovery of the dust and preparation of the grids) which may also increase the variability.

Based on the data of the first scheme, the number of laboratories, with six out of eight standardized ratios falling within the range 1/x–x for various values of x is shown in Fig. 1. This number changes quickly for x between 1.4 and 2.3, then more slowly between 2.3 and 3.2. No change is observed for x between 3.2 and 3.5. This confirmed the choice of the inner and outer limits. Moreover, as will be seen later, these
Table 1. Number of participating laboratories and number of laboratories in each of the three categories for the schemes organized in 1996, 1997 and 1998

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<tr>
<td>1</td>
<td>12</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>34</td>
<td>34</td>
</tr>
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</table>

RESULTS AND DISCUSSION

The number of participating laboratories and the number of laboratories in each of the three categories for the first three schemes are given in Table 1. Figures 2–4 illustrate the results obtained by the participating laboratories in 1996, 1997 and 1998. For each laboratory, the classification 1, 2 or 3 is given on the horizontal axis and the values of the standardized ratios (density/reference density) for the eight filters analysed by each laboratory are shown on the vertical axis. The laboratories are arranged on the horizontal axis in ascending order of the mean of the standardized ratios for the eight density levels. The inner and outer lines exhibit the inner and outer limits used in the categorization process of the laboratories. If the category number is followed by the letter r on the horizontal axis, this means that this laboratory belongs to the reference group. If a laboratory has not correctly identified the asbestos type present on a filter, the category number is followed by an asterisk (*). The scales of the vertical axes are logarithmic.

Both Table 1 and Figs 2–4 show a clear improvement with time of the results obtained by the participating laboratories. Looking at Fig. 2, it can be seen that in 1996 certain laboratories were clearly overestimating (right-hand side of the graph) or underestimating (left-hand side of the graph) the fibre densities. Such a dispersion of results could be expected for laboratories participating in the scheme for the first time. Moreover, many of them were new to the field of asbestos measurement by transmission electron microscopy. Several working meetings organized with the participating laboratories at the end of the first scheme made it possible to find explanations for the dispersion of the results. It became clear that overestimating the densities was, for the most part, due to misinterpretation of the X43-050 standard. In fact, some laboratories were using ultrasonics during the dust recovery process after ashing the filter, whereas the X43-050 standard recommends only manual shaking of the liquid suspension to make it homogeneous. Obviously this ultrasonic treatment can induce an increase in the fibre number densities, as already mentioned in the introduction. On the other hand, density underestimation was probably due to incorrect recovery of the dust. The action of a cell scraper on the optical slide on which the filter is ashed is vital to ensure complete recovery of the dust (Kohyama, 1989; Kauffer et al., 1996). The improvements in the results show that the discussions with the laboratories were useful and probably of some help.

Another way to appreciate the improvement in the results with time is to look at Tables 2 and 3 where the percentages of standardized ratios inside the 0.5–2.0 or 0.4–2.5 range are given for all the laboratories (Table 2) or for laboratories belonging to category 1 or 2 (Table 3). In 1998, 92% of all the standardized ratios were inside the limits 0.4–2.5 compared with 60% in 1996.
Fig. 3. Results obtained by the participating laboratories in 1997. The classification 1, 2 or 3 is given for each laboratory on the horizontal axis and the values of the standardized ratios (density/reference density) for the eight filters analysed by each laboratory are shown on the vertical axis (see text for more details).

Fig. 4. Results obtained by the participating laboratories in 1998. The classification 1, 2 or 3 is given for each laboratory on the horizontal axis and the values of the standardized ratios (density/reference density) for the eight filters analysed by each laboratory are shown on the vertical axis (see text for more details).

Table 2. Percentage of standardized ratios inside the range 0.5–2.0 or 0.4–2.5 for all the laboratories participating in the schemes

<table>
<thead>
<tr>
<th>Year</th>
<th>0.5–2.0</th>
<th>0.4–2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>48%</td>
<td>60%</td>
</tr>
<tr>
<td>1997</td>
<td>67%</td>
<td>78%</td>
</tr>
<tr>
<td>1998</td>
<td>86%</td>
<td>92%</td>
</tr>
</tbody>
</table>

Table 3. Percentage of standardized ratios inside the range 0.5–2.0 or 0.4–2.5 for laboratories belonging to category 1 or 2

<table>
<thead>
<tr>
<th>Year</th>
<th>0.5–2.0</th>
<th>0.4–2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>60%</td>
<td>78%</td>
</tr>
<tr>
<td>1997</td>
<td>81%</td>
<td>91%</td>
</tr>
<tr>
<td>1998</td>
<td>91%</td>
<td>96%</td>
</tr>
</tbody>
</table>
As the density ranges used in the first scheme were lower than those used in the two later schemes, we looked at whether this improvement was due to the use of samples with higher densities in the last two years. We also looked at the possibility of whether it could have been due to an improvement in the way the samples were prepared. To investigate these points, we studied the relationship between the logarithm of the variance and the logarithm of the mean density for all the density levels used during the three years the scheme has been running. Figure 5 gives the data obtained when checking the homogeneity of the filters: variance and mean are calculated from 15 control filters (see the Checking homogeneity section above). This shows a linear relation between the logarithm of the variance and the logarithm of the mean density. The fact that the variance is an increasing function of the mean is typical in fibre counting: for instance Brown et al. (1994) show that the variation in fibre density is approximately constant on the logarithmic scale. This means that the variance of densities is approximately proportional to the square of the mean density or that the logarithm of the variance is approximately a linear function of the logarithm of the mean density (see for instance Kendall and Stuart, 1969, p. 232). Furthermore as this relation does not depend on the year the scheme was organized it can be argued that the samples were prepared with the same care whatever the year and the fibre density level. Figure 6 shows the relation between the logarithm of the variance and the logarithm of the mean density from the results obtained by all the participants in all three schemes. It can be observed that the data are well described with regression lines: two for the schemes organized in 1996 and 1997, the other for that organized in 1998. The slopes of the lines are comparable while the intercepts are different, indicating that the decrease in the dispersion of the results of the laboratories occurred mainly between 1997 and 1998. As we have shown that the samples were prepared with the same care whatever the year the scheme was organized and the fibre density level, this would appear to indicate that the improvement stems from the laboratories and not from an improvement in the way the samples were prepared nor from the use of samples with higher densities.

Recently, the BCR (Bureau Communautaire de Référence) organized a trial to certify the concentration of asbestos fibres in dry lung tissues...
(Tussavainen et al., 1998). Six replicates of two different lung tissues were analysed by seven laboratories. For each sample, the anthophyllite and amosite/crocodolite concentrations, expressed in million fibres per g dry tissue, were measured. To allow a comparison with this proficiency testing scheme, it is possible to compute the standardized ratios (ratio of each individual result to the mean asbestos concentration measured by all the participants) for each of the two lung tissues and for each asbestos type. Table 4 gives the percentage of these standardized ratios within the 0.5–2.0 and 0.4–2.5 ranges along with the corresponding percentages obtained for the last proficiency testing scheme organized in 1998. In both cases all the participating laboratories are included and the proportions are similar. This would seem to indicate that the variability of the indirect method to determine the asbestos fibre number concentrations by transmission electron microscopy is similar whether applied to biological samples or to membrane filters.

The most critical choice that had to be made when the first proficiency testing scheme was launched in 1996 was to determine the inner and outer limits to categorize the laboratories. This is why, in the light of the three first schemes, it is interesting to try to estimate the probability of a laboratory being classified in category 2 or 3, assuming that its results are comparable with those of the reference group. To do this, the Shapiro–Wilk statistic was used for each of the three schemes to determine the best fit for the distribution of the standardized ratios of the reference group (normal, log-normal or normal after square root transformation) (Shapiro and Wilk, 1965). The probability $q$ of a standardized ratio being outside the inner or outer limits was then calculated from this theoretical distribution. Finally, the probability of success in the test (that is at least six out of eight standardized ratios inside the lower or outer limits) is given by (Ogden, 1984)

$$P = \frac{8!}{2!6!(1-q)^6} + \frac{8!}{1!7!}q(1-q) + \frac{8!}{8!}q^6(1-q)^8$$

where $q$ is the probability of obtaining a result outside the specified category limit and $1-q$ is the probability of obtaining a result inside the limits.

Table 4. Comparison of the percentage of standardized ratios inside the range 0.5–2.0 or 0.4–2.5 between a trial organized to certify the concentration of asbestos fibres in dry lung tissues and the scheme organized in 1998 to evaluate the fibre densities on filters

<table>
<thead>
<tr>
<th>Range</th>
<th>Certification of asbestos fibres in lung tissues</th>
<th>Proficiency testing scheme on membrane filters</th>
</tr>
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<tbody>
<tr>
<td>Percentage of standardized ratios inside the range</td>
<td>0.5–2.0 85%</td>
<td>0.4–2.5 90%</td>
</tr>
</tbody>
</table>

In the process used to categorize the laboratory performance, it was explained in the Categorization section above that the reference laboratories for a given scheme were those classified in category 1 in the previous scheme. However, if the categorization procedure led to classifying a laboratory of the reference group in category 3, this laboratory was removed from the reference group and the calculation started again. It could be argued that this method, even if a means of rejecting outliers, will always maintain the probability of not being successful in the test at a low level. It is for this reason that the probability of failure was computed with two hypotheses:

H1—The reference group is as defined in the Categorization section above after rejection of laboratories considered as outliers.

H2—The reference group is defined without rejection of any laboratory except those for which a clear reason for their poor performance was found. This was the case during the second scheme for two laboratories (miscalculation of the results for one, very bad control of the recovery of the dust for the other).

Table 5 gives for the proficiency testing schemes organized in 1996, 1997, 1998 and for each of the theoretical distributions of the standardized ratios, the $p$-value of the Shapiro–Wilk test, the probability $q$ of obtaining a result outside the inner or outer limits, and the probability $P$ of success in the test for the two hypotheses H1 and H2. This table shows that for a laboratory whose results would be comparable with those of the reference group, the probability of being classified in category 1 or 2 varies between 89.0 and 99.8% or 98.8 and 100%, respectively, according to the year and the hypothesis. This means that the probability of a laboratory being classified in category 3 for no reason is very low (most often far below 1%). This is in agreement with other classifications based on the $z$ scores where a laboratory is classified as unsatisfactory when $|z|\geq3$, which corresponds to a probability of 0.3% for well behaved systems (Thompson and Wood, 1993).

As explained above the best fit for the distribution of the standardized ratios of the reference group among three possible theoretical distributions (normal, log-normal or normal after square root transformation) was used to calculate the probability
Table 5. The p-value of the Shapiro–Wilk test, the probability \( q \) of obtaining a result outside the inner or outer limits, and the probability \( P \) of success in the test for the two hypotheses \( H_1 \) and \( H_2 \) for the proficiency testing schemes organized in 1996, 1997 and 1998 and for each theoretical distribution of the standardized ratios

<table>
<thead>
<tr>
<th>Year</th>
<th>Theoretical distribution ((p))</th>
<th>(P^a), (q) category 1</th>
<th>(P^a), (q) category 2</th>
<th>Theoretical distribution ((p))</th>
<th>(P^a), (q) category 1</th>
<th>(P^a), (q) category 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>LN(^e) (0.412)</td>
<td>0.991 (0.060)</td>
<td>0.999 (0.030)</td>
<td>LN(^e) (0.077)</td>
<td>0.955 (0.107)</td>
<td>0.988 (0.065)</td>
</tr>
<tr>
<td>1997</td>
<td>LN(^d) (0.421)</td>
<td>0.998 (0.034)</td>
<td>1.000 (0.003)</td>
<td>N(^e)</td>
<td>0.977 (0.082)</td>
<td>0.998 (0.027)</td>
</tr>
<tr>
<td>1998</td>
<td>LN(^d) (0.421)</td>
<td>0.998 (0.034)</td>
<td>1.000 (0.003)</td>
<td>LN(^f) (0.056)</td>
<td>0.998 (0.027)</td>
<td>0.998 (0.027)</td>
</tr>
</tbody>
</table>

\(^a\)Except for two laboratories in 1997.
\(^b\)\(P\)=probability of success in the test.
\(^c\)\(q\)=probability of obtaining a result outside the specified category limit.
\(^d\)Log-normal distribution.
\(^e\)Normal distribution.
\(^f\)Normal distribution after square root transformation.

CONCLUSION

This study has used data from the first three years of the proficiency testing scheme organized in France to determine the performance of laboratories involved in the evaluation of asbestos fibre number density by transmission electron microscopy. The improvement in the performance of the laboratories over the three-year period demonstrates that reliable results can be obtained with indirect-transfer transmission electron microscopy provided that strict analytical procedures are respected. Although found in many schemes, this improvement was not obvious in this particular case where the analytical method is delicate to implement. Obviously a greater frequency in the operation of the scheme would have shortened the period of time needed for laboratories to produce consistently reliable data. The cost of sample preparation alone however makes this impossible.

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