

Comparison of Mounting Methods for the Evaluation of Fibers by Phase Contrast Microscopy

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The objectives of this study were to evaluate mounting methods for fiber examination of air sample filters by phase contrast microscopy (PCM) and to evaluate differences in fiber counts that might be due to fiber movement. Acetone/triacetin (AT) with various amounts of triacetin and acetone/Euparal (AE) where the mounting medium was placed between the cleared filter wedge and the coverslip were tested as a function of time. Field sample slides collected from a taconite iron-ore processing mill, a tremolitic talc-ore processing mill, and from around a crusher in a meta-basalt stone quarry were prepared with relocatable coverslips to revisit the same field areas on the slides. For each slide, three or four field areas were randomly selected and pictures were taken every 2 weeks to determine any sign of fiber movement over time. For 11 AT slides (named as AT-3.5) prepared with 3.5 μl of the mounting medium according to the NIOSH 7400 method, no fiber movements were detected over 59 weeks. On the other hand, AT slides prepared with larger quantities (10, 15, and 20 μl) of the mounting medium (named as AT-10) and AE slides prepared with ~ 10 μl mounting medium showed fiber movement from the eighth day at the earliest. Fiber movement began earlier for the slides mounted with excess triacetin than for those mounted with Euparal. The sample slide storage method, either vertically or horizontally, did not seem to accelerate fiber movement. Additionally, two other modified methods, dimethylformamide solution/Euparal (mDE) and dimethylformamide solution/triacetin (mDT), were also prepared where the mounting medium was placed between the cleared filter wedge and the glass slide. The findings of fiber movements were similar; when 3.5 μl of triacetin was used for the mDT slides, fiber movements were not detected, while fibers on slides prepared with 10 μl triacetin (mDT-10) moved around. No fiber movements were observed for the mDE slides at any time during 59 weeks. Once fiber movement started, fibers moved over distances measured from 4 μm and up to >1000 μm within 22 weeks. However, since then, no further fiber movements have been observed in any field sample slides. Additional sample slides, two Amosite and two chrysotile, were prepared from Union for International Cancer Control (UICC) samples using the AT method with 5 μl triacetin mounting medium. Fiber movements were also observed in these samples; chrysotile fibers began to migrate in 3 weeks, while Amosite fiber movement started after 3 months. Although fiber movement was observed for the AT-10, AE, and mDT-10 sample slides, fiber counts were not significantly different from AT-3.5 and mDE samples that exhibited no fiber movement. Although fiber counts would not be significantly changed by fiber movement, the type and amount of mounting medium for sample slide preparation remains critical for issues such as quality assurance and training of analysts by revisiting the same fibers.

Keywords: asbestos measurement; euparal; fiber counts; fiber movement; mounting method; phase contrast microscopy; relocatable coverslip; triacetin

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INTRODUCTION

The current standard method to estimate airborne fiber concentrations is to use a mixed cellulose ester membrane filter to collect fibers, to prepare sample slides using one of several mounting techniques, and then to examine the slides under phase contrast microscopy and count fibers meeting certain criteria (ISO 1993; NIOSH, 1994; HSE, 2006; WHO, 1997; OSHA, 1998; ASTM International, 2006). During the sample slide preparation, the dusty filter is collapsed and made transparent by exposing it to an organic solvent such as acetone (usually as a vapor). This procedure is important in providing a clear background of the filter so that counters are not confused with artifacts, which may, for example, be counted as fibers. The use of acetone vapor was first proposed by the Australian National Health and Medical Research Council (1976) and it has become a standard method worldwide. However, other solvents can be used in place of acetone. In the acetone vapor method, the cleared filter is often mounted with triacetin, but other media can be used. The most important requirement is that the refractive index (RI) of the mounting medium be sufficiently different from the fibers of concern, such as asbestos, that they are clearly visible. Permanence of the mounting medium is also another important factor to be considered in case samples should be revisited for the purpose of litigation or for performing a quality assurance check. Euparal, named by G. Gilson, Professor of Zoology at the University of Louvain, Belgium, is a substance based on the general formula of resin plus plasticizer and solvent (Shenton-Taylor and Ogden, 1986). The RI has been shown to be stable for ~40 years ranging from 1.480 and 1.485 (Ogden *et al.*, 1986) and it is well matched to the RI of a cleared membrane filter, providing a less granular background than other media (LeGuen and Galvin, 1981). LeGuen and Galvin (1981) investigated four different clearing and mounting techniques with mixed esters of cellulose membrane filters that sampled chrysotile asbestos generated in a chamber. Those four techniques were triacetin without clearance of the filter, acetone/triacetin (AT), acetone/Euparal (AE), and dimethylformamide solution/Euparal (DE). After considering several criteria such as simplicity of the sample procedure, background clarity, fiber contrast, permanence of filters, and RI of the cleared filter, they recommended the DE mounting method as the most suitable technique. Shenton-Taylor and Ogden (1986) tested the permanence of various mounting methods for membrane filters and reported that the filters prepared by

the DE method lasted longer than those prepared by the AT method; no change of background on the filters prepared by the DE method was observed for up to 5.5 years, whereas almost half of the examined fields of AT slides showed substantial deterioration after 2 years. Both previous studies (LeGuen and Galvin, 1981; Shenton-Taylor and Ogden, 1986) observed a slight movement of the fibers treated with the AT method due to the softening of the surface of the collapsed filter by adding triacetin and thus emphasized that the amount of mounting medium is critical; fibers were observed to move around if the amount of triacetin is excessive, whereas the coverslip might detach if the amount of triacetin is too little. On the other hand, the quantity of Euparal did not appear to be as critical.

Table 1 shows the recommended amount of triacetin mounting medium by national and international standards. Sample collection with a 25-mm mixed cellulose ester membrane filter is recommended for all methods. The National Institute for Occupational Safety and Health (NIOSH) Method 7400 and Occupational Safety and Health Administration (OSHA) Method ID-160 suggest about 2.5–3.5 μl triacetin for ~25% of a collected filter area. The amount of triacetin recommended by the World Health Organization (WHO) and International Organization for Standardization (ISO) 8672 methods is similar to that in NIOSH 7400 and OSHA ID-160 but those standards do not clearly mention the proportion of filter area to be used. The American Society for Testing and Materials (ASTM) D7200 suggests about 1–2 drops with a 22-gage needle (approximately 4.5–9.0 μl) for a quarter of a 25-mm filter area and the recommended amount of the mounting medium is higher than the suggested amount for NIOSH 7400 and OSHA ID-160. The recommended amount in the UK Methods for the Determination of Hazardous Substances (HSG 248), ~30 μl for the quarter of a collected filter, is very high in comparison to the other methods.

Fiber counts from different mounting techniques have been compared by several researchers. Jankovic *et al.* (1986) observed no differences in fiber concentrations from sample slides prepared by a clearing solution (in this case, a mixture of dimethyl phthalate and diethyl oxalate), hot acetone vapor, and cool acetone vapor. However, different results were found by other researchers. Gonzalez-Fernandez and Martín (1986) reported that samples mounted with the mixture of dimethyl phthalate and diethyl oxalate produced about one-half the number of fibers per unit area of those examined after acetone clearance but without mounting medium or of those mounted with

Table 1. Recommended amount of triacetin by national and international standards

	Recommended amount of triacetin	Proportion of filter area
NIOSH 7400	3–3.5 μl	1/4 of a collected filter area
OSHA ID-160	2.5–3.5 μl	1/6–1/4 of a collected filter area
HSG 248	~120 μl	Entire filter (~30 μl for 1/4 of the filter)
WHO	~10 μl	Entire filter or a portion of it (~2.5 μl for 1/4 of the filter)
ISO 8672	1–3 drops (~4.5–13.5 μl) with a 22-gage needle	Total filter preferable (~1.1–4.4 μl for 1/4 of the filter)
ASTM D7200	1–2 drops (~4.5–9.0 μl) with a 22-gage needle	1/4 of a collected filter area

Note that all the standards cited in the table recommend using a 25-mm mixed cellulose membrane filter for air sampling.

the AT method. Walton *et al.* (1976) also observed different fiber counts among different mounting methods; clearance with acetone, but without mounting medium, and the AT method resulted in significantly higher counts than the triacetin and dimethyl phthalate and diethyl oxalate methods without clearance. The modified AT mounting procedure which added a heating procedure at the mounting stage did not result in statistically significant differences compared to the AT mounting method without the heating procedure (Furness and Reed, 1985). These studies focused on the comparison of fiber counts by different mounting methods immediately after the mount was prepared and studies have not compared fiber counts as a function of time when fiber movements have been observed in different mounting methods. During the period of those studies, tracking fiber movement over time would not have been easy due to the difficulty of visiting the same field areas. Although this could be done with precise stage movement controls and accurate slide placement, especially under computer-controlled alignment, not many optical microscopes had this facility, and it remains uncommon even today.

The current study includes two aims: (i) determination of fiber movement by tracking fibers over time from field samples and UICC reference filters and (ii) evaluation of any differences in fiber counts due to fiber movement. For the first aim, sample slides were prepared with relocatable coverslips to revisit the same field areas on any microscope. AT with various amounts of triacetin, AT-10 ($\geq 10 \mu\text{l}$ triacetin) and AT-3.5 ($\leq 3.5 \mu\text{l}$ triacetin), and AE were tested for fiber movements as a function of time. Additionally, two other modified methods, dimethylformamide solution/Euparal (mDE) and dimethylformamide solution/triacetin (mDT), were also tested where the mounting medium was placed between the cleared filter wedge and the glass slide (in the traditional method, the mounting medium is between the

filter wedge and the coverslip). For the study of fiber counts as a function of fiber movement, sample slides prepared were AT-10, AT-3.5, mDE, and dimethyl phthalate and diethyl oxalate (DP-DO).

METHODS

Aim 1—Investigation of fiber movement

Preparation of field samples. Field sample slides, one each collected from a taconite iron-ore processing mill, a tremolite talc-ore processing mill, and from around a crusher in a meta-basalt stone quarry, were prepared using different mounting methods mentioned above and described in detail below. The detailed information regarding field samples has been published elsewhere (Lee *et al.*, 2008; Lee *et al.*, 2010). Five to 11 sample slides were prepared for each mounting method with relocatable coverslips to revisit the same area. The relocatable coverslip, developed by one of the authors in this study, T.W.S. Pang, has two grids of circular viewing grid openings that are defined by a thin metal coating. See Fig. 1 for an example of a grid opening. Each grid contains 14 columns (from A to N) and 10 rows (from 1 to 10) and fibers are examined within the circular area, referenced to the intersection of a column and a row. Recording a position of a specific area of interest in a filter can also be done by using an England Finder Graticule™ (SPI Supplies/Structure Probe, Inc., West Chester, PA, USA). However, this graticule cannot be conveniently used because the Finder graticule and the sample slide cannot be used together, i.e. after finding and focusing an interest area of a sample slide, the sample slide should be removed and replaced with the England Finder to record the position.

AT mounting method: A filter wedge was placed on a dust-free glass slide with the dust side up and put under an acetone vaporizer (Quick Fix;

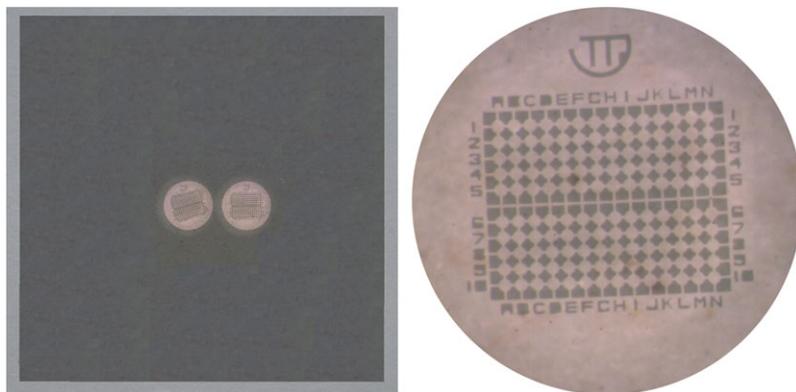


Fig. 1. Relocatable cover slip (left) and a grid of relocatable fields (right) (Diameter of a circular grid opening is nominally 100 μm).

Environmental Monitoring Systems, Inc., Charleston, SC, USA) to clear the filter wedge. After waiting for 3–5 s, triacetin was dropped on the cleared filter wedge and a relocatable coverslip was lowered onto the glass slide. In order to determine whether the amount of triacetin is critical, as found by LeGuen and Galvin (1981), three slides, one slide, and two slides were mounted with two (10 μl), three (15 μl), and four (20 μl) drops of triacetin, respectively, using a needle size of B-D 21 G1 (Becton Dickinson and Company, Franklin Lakes, NJ, USA). These sample slides prepared with ≥ 10 μl triacetin were named as AT-10. In addition, 11 sample slides were prepared using 3.5 μl of the triacetin mounting medium according to the NIOSH 7400 method named as AT-3.5. The edge of the coverslip was sealed with a nail polish. Then, the prepared sample slides were left overnight at room temperature. Among 17 sample slides, 6 slides (4 slides prepared with 3.5 μl , 1 slide prepared with 10 μl , and 1 slide prepared with 20 μl triacetin) were kept vertically and the rest were kept horizontally in a storage cabinet. The slides kept vertically were always returned to the same vertical direction after each observation.

AE mounting method: The same method to clear the filter wedge was used as described in the AT mounting method, but after clearing the filter wedge, one drop of Euparal (~ 5 μl per drop) was placed on the cleared wedge and one drop on the underside of a relocatable coverslip and the coverslip was lowered onto the glass slide. Ogden *et al.*, (1986) recommended the natural form of Euparal resin to be used but a synthetic form of Euparal of unknown composition (BioQuip Products, Rancho Dominguez, CA, USA) was used in this study due to the difficulty of obtaining the natural form. The prepared sample slides were left overnight at room temperature and,

like the AT mounted sample slides, three slides were stored horizontally in the container and two slides were kept vertically.

mDE mounting method: A mixture of dimethylformamide (35% v), glacial acetic acid (15% v), and distilled water (50% v) was prepared and used to clear the filter. A relocatable coverslip was placed on a clean glass slide with the coated side of the coverslip facing up. A filter wedge was then placed on the relocatable coverslip by contacting the dusty surface of the filter with the coated side of the coverslip, i.e. the dust side down. This orientation is a modification from previously published Euparal methods where the Euparal is placed between filter wedge and the coverslip. The rationale for this change is to have the fibers and the grid markings as close as possible to the same plane to avoid the need for constant adjustment of the focus between them. Placing the clearing solution on the back of the filter wedge has the advantage of preventing washout of particles, which might occur with heavily loaded filters. Approximately, 20 μl of the clearing solution was dropped on the filter wedge and then the filter wedge was placed in a thermostat-controlled oven at a temperature ranging between 55 and 60°C for 30 min to evaporate the solution. One drop of Euparal resin (~ 5 μl per drop) was added on the glass slide and the relocatable coverslip was flipped over and gently lowered onto the glass slide. The mounted slide was then placed in the oven again for 60 min to polymerize the Euparal resin. This method does not require sealing the edge of the coverslip; but to maintain consistency with other sample slides, the edge of the coverslip was sealed with nail polish. The prepared sample slides were left overnight at room temperature and stored the same way as the AT

and AE mounted slides, i.e. four slides horizontally and two slides vertically in each container. In previous studies using the DE mounting method prepared in the same way as triacetin mounts (i.e. with the mounting medium between the filter wedge and coverslip), no fiber movements had been seen by LeGuen and Galvin (1981), while Shenton-Taylor and Ogden (1986) observed movement of a non-fibrous particle from one of nine grid openings but the movement was not the same kind of movement as that of chrysotile fibers in slides prepared by the AT method.

mDT mounting method: For this mounting method, clearing a filter wedge was done in the same way as for the mDE method. However, instead of Euparal, triacetin (10 μ l) was used to mount the filter wedge with a relocatable coverslip by placing it between the cleared filter wedge and the glass slide (mDT-10). The edge of the coverslip was sealed with nail polish and left overnight at room temperature. Three slides and two slides were then stored horizontally and vertically, respectively. In addition, four mDT sample slides were prepared in the same manner but using 3.5 μ l of the triacetin mounting medium (mDT-3.5) to determine any effect of different amounts of the mounting medium.

Examination of field samples. Table 2 shows a summary of the sample slides prepared using the different mounting methods. "Photographs were taken every 2 weeks using a NIKON 50i photomicroscope (NIKON Instruments Inc., Melville, NY, USA)." For the 11 AT-3.5 and 4 mDT-3.5 slides, three or four grid openings from each sample slide showing fiber(s) clearly were examined once every week over 59 weeks. For the other slides, three or four grid openings were selected and photographed every 2 weeks or until fiber movement was observed. The time periods for fiber examination were 94 weeks (658 days) for the AT-10 slides and 88 weeks (616 days) for the AE, mDE, and mDT-10 slides. In addition, 16 fibers from the field areas showing fiber movement were tracked to determine moving distances and the direction of movement as a function of time.

In order to determine whether there was any difference in the clarity of filter background, three sample slides per each mounting method were prepared: one media blank, one field blank, and one field sample collected from the taconite mill. Sample slides were prepared using both relocatable coverslips and clear coverslips. Two analysts at NIOSH examined the sample slides.

Preparation and examination of UICC samples. The field sample slides contained particles

meeting the dimensional criteria required for fiber counting, but these particles may not have been asbestos. Therefore, in addition to the field sample slides, several mixed cellulose ester membrane filters were prepared by filtration of aqueous suspensions of UICC reference Amosite and chrysotile asbestos per previously published method (Pang *et al.*, 1984; Pang *et al.*, 1988). From these filters, two Amosite slides and two chrysotile slides were prepared with vaporized acetone to clear the filter and \sim 5.0 μ l triacetin was used to mount the coverslips (AT method). For each slide, 10 grid openings with easily recognizable fibers were selected and photographed to record their original field positions. Then, the same openings were examined each week until fiber movement was observed and the fibers were tracked each month and photographed again in the fourth month.

Aim 2—Evaluation of differences in fiber counts due to fiber movement

Once fiber movement of AT-10, AE, and mDT-10 samples was observed from Aim 1 (see the Results section for the results), Aim 2 was performed to evaluate differences in fiber counts due to the instability of fibers. The AE and mDT-10 samples were not considered for this task because none of current standard methods recommended sample preparation using either method. All sample slides prepared with AT-3.5 method were examined within 2 weeks after the slide preparation. For the AT-10 and mDE mounting methods, samples were examined that had been prepared at least 6 months previously since from Task 1, all grid opening areas of AT-10 sample slides ($N = 6$ slides, 24 grid opening areas) showed fiber movement within 129 days (\sim 18.4 weeks) and further movement after 22 weeks was not significant. It might have been preferable to evaluate fiber counts due to an effect of fiber movement using the same slides tested for fiber movement (Aim 1) as a function of time. However, we were not able to do this because Aim 2 was planned after we had already observed fiber movement.

Prior to development of the current NIOSH 7400 method, NIOSH P&CAM 239 method (Asbestos fibers in air) was the standard method in the USA, with sample slide preparation using the dimethyl phthalate/diethyl oxalate (DP-DO) mounting method (NIOSH, 1977). The risk assessment for asbestos in the USA was largely based on results that had been obtained using this method and it would be useful if any future method modifications could be traceable to this method. Thus, sample slides using the DP-DO

Table 2. Summary of sample slides using different mounting methods

Techniques	Number of sample slides	Number of grid openings	Storage method	Amount of the mounting reagent (μl)	Tracking time period (weeks)
AT-10	6	24	Four horizontal; two vertical	10–20	94
AT-3.5	11	42	Seven horizontal; four vertical	3.5	59
AE	5	17	Three horizontal; two vertical	10	88
mDE ^a	6	24	Four horizontal; two vertical	10	88
mDT-10 ^a	5	20	Three horizontal; two vertical	10	88
mDT-3.5 ^a	4	23	Two horizontal; two vertical	3.5	59

^aUnlike the AT and AE methods, the mounting medium was placed between the cleared filter wedge and the glass slide.

method were additionally prepared according to the NIOSH P&CAM 239 method to determine differences of fiber counts compared to the other mounting methods. One reported drawback of this mounting method is the possibility of crystals growing on the prepared sample slide after a few days, which look similar to asbestos fibers. Thus, the DP-DO sample slides were examined within 2 days after the sample preparation.

The total number of sample slides for each mounting method was 17, 19, and 19 for the taconite mill, the talc mill, and the quarry, respectively. All sample slides were prepared with relocatable coverslips except for the AT-10 sample slides. One counter examined all sample slides according to NIOSH 7400 'A' counting rules, i.e. all fibers $>5 \mu\text{m}$ and with an aspect ratio $\geq 3:1$ regardless of fiber diameter. All sample slides were randomized carefully to avoid examining the slides in each set either on the same day or on the following day. A Leica DMRB microscope (Leica Microsystems Inc., Bannockburn, IL, USA) was used.

RESULTS

Aim 1—Investigation of fiber movement

AT and AE sample slides

Field samples: Only AT-3.5 sample slides ($n = 11$) did not show any fiber movement at all over 59 weeks, while the AT-10 and AE slides showed fiber movement from the eighth day at the earliest. Although the time of commencement of fiber movement was not the same for each method, more than half of

grid openings showed fiber movement within 2.5 months. By 129 days, all tested grid openings of the AT-10 samples showed fiber movement, while fiber movement of the AE samples was observed from $\sim 80\%$ of grid openings by 127 days. However, distortion of the filter background (i.e. cracking of the filter background) for the AT-3.5 samples was observed earlier than the AT-10 sample slides (Fig. 2). Almost 36% (4/11 sample slides) of AT-3.5 slides showed distortion of the filter background by the following week. Cover slips sealed with nail polish around the outside showed no evidence of slippage (on the macroscopic scale) during the period of fiber examination, whether the slides were stored horizontally and vertically. No consistent differences were observed between the samples from the different field locations in the start of first movement, suggesting the type of fiber (e.g. amphibole cleavage fragment or talc fiber) is not a factor in fiber movement.

UICC samples: Chrysotile fibers prepared with the AT mounting method started moving earlier than did Amosite fibers; chrysotile fibers began moving within 3 weeks, while Amosite fiber movement started after 3 months. Generally, for both chrysotile and Amosite fibers, fibers near the tip of the filter wedge moved at an earlier time than fibers near the center of the filter wedge. The direction of movement was more or less the same toward the tip of the wedge but not all fibers moved with the same speed. For example, a fiber (marked as A) in Fig. 3 stayed in the same location even after 20 weeks, while the other fibers in the vicinity of the fiber A all moved in one direction by almost the same distance. A long and thin Amosite fiber in Figure 4 has rotated ~ 90 degrees and chrysotile fibers in

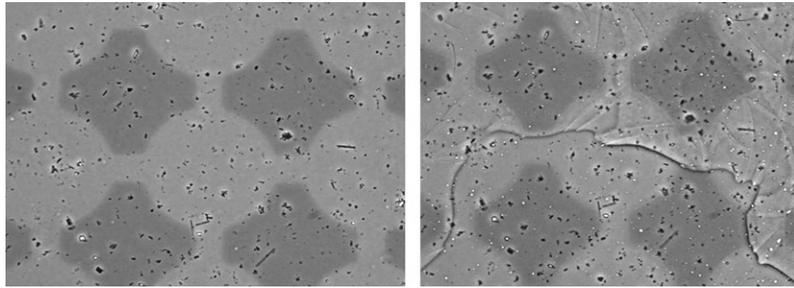


Fig. 2. Example of an AT-3.5 slide showing cracking of the filter background (left: original, right: after 10 weeks) (Diameter of grid opening is nominally 100 μm).

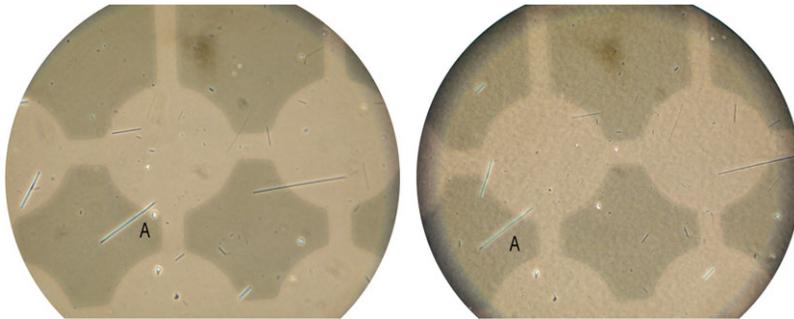


Fig. 3. Movement of Amosite fibers on a sample slide prepared by the AT mounting method (left: original, right: after 20 weeks) (Diameter of grid opening is nominally 100 μm).

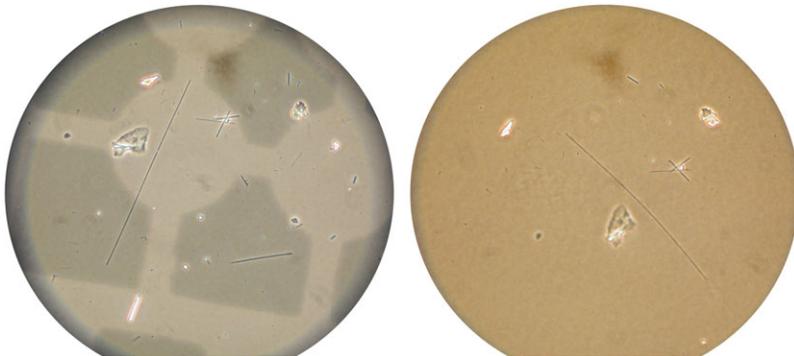


Fig. 4. Movement and rotation of Amosite fibers on a sample slide prepared by the AT mounting method (left: original, right: after 20 weeks) (Diameter of grid opening is nominally 100 μm).

Fig. 5 show that the distance between the two chrysotile fibers increased. Possible reasons for fiber movement in sample slides mounted with the AT method are discussed later.

mDT and mDE sample slides

Like the AT-3.5 sample slides, four mDT-3.5 sample slides did not show any fiber movement for 59

weeks, while 80% of grid openings for the mDT-10 slides showed fiber movements by 99 days. Figure 6 shows an example of fiber movement showing both change in the position and an increase of the distance between two fibers (marked as A and B) over time. For the mDE mounting method, no sample slides have shown fiber movement so far. There was no difference between slides stored horizontally and slides stored vertically.

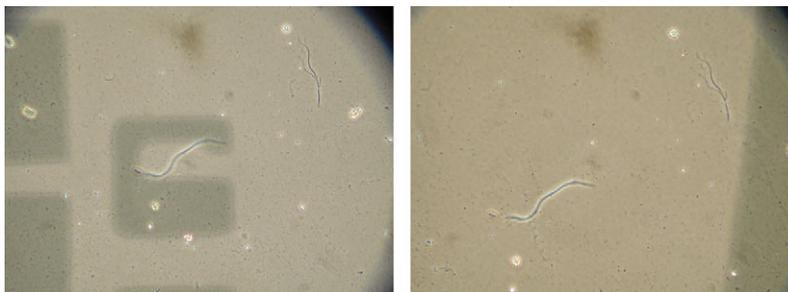


Fig. 5. Movement of chrysotile fibers on a sample slide prepared by the AT mounting method (left: original, right: after 3 weeks) (Diameter of grid opening is nominally 100 μm).

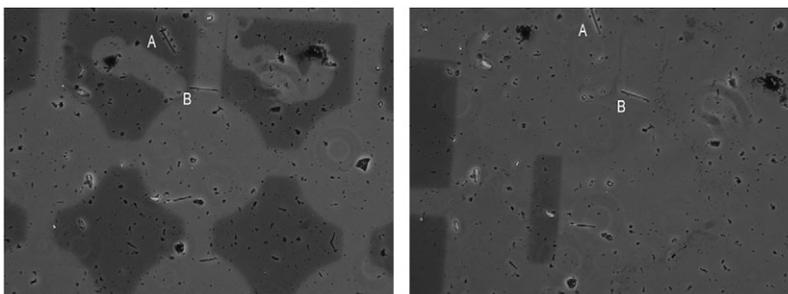


Fig. 6. Movement of fibers on a sample slide prepared by the mDT-10 method (left: original, right: after 21 weeks) (Diameter of grid opening is nominally 100 μm).

Clearness of filter background

Both analysts rated filter background as either good or excellent for all prepared sample slides and, unlike the previous study by LeGuen and Galvin (1981), they did not notice differences of the filter background among the four mounting methods and between the two types of coverslips (relocatable coverslip versus clear coverslip). The fiber counts of the laboratory media blank slides were either 0 or 1 and such low background counts would not affect the fiber counts of field samples.

Distance of fiber movement in field samples

Table 3 shows the distance of fiber movement over time for 16 fibers from 16 grid openings. The first measurement of the distance was obtained after ~ 22 weeks for the AT-10 slides and ~ 16 weeks for the AE and mDT-10 sample slides from the date of sample slide preparation. The distance traveled by the moving fibers for the vertical slides ranged from 4 to 80 μm , while the moving distance of fibers for the horizontal slides ranged from 8 to 1170 μm . Since the initial measurements of the moving distance, no further movement of the fibers was observed. The direction of fiber movement was not

consistent, for example, in slides AT-3 (3 fibers) and AE-4 (2 fibers), the direction of movement of all fibers was the same, while for slide AT-5, the two fibers moved in opposite directions and the two fibers moved in orthogonal directions for slide mDT-6.

Aim 2—Evaluation of differences in fiber counts due to fiber movement

The geometric means of fiber concentration ratios against AT-3.5 and mDE samples for the taconite mill, talc mill, quarry, and all-combined are summarized in Table 4. Figure 7 shows fiber concentration ratios of the individual sets of samples prepared using different mounting methods. Fiber concentrations of all AT-10 and AT-3.5 slides from the quarry were considerably lower than those of the corresponding mDE and DP-DO slides, unlike fiber concentrations of the taconite and talc mills. This might be because of the large number of samples with heavy loading of particles (fibers and other particulate matter) on the filter. With AT mounting methods, the filter wedge is cleared with an acetone vaporizer where the vaporized acetone is sprayed toward the filter wedge through a nozzle. If the velocity of the spray is high, it might washout dust

Table 3. Distance of fiber movement

Sample ID	Storage method	Moving distance ^a (μm)		
		Initial measurement ^b	After 32 weeks from the initial measurement	After 65 weeks from the initial measurement
AT-10 slides				
AT-2-C9	Horizontal	68	80	82
AT-3-C1	Horizontal	890	941	941
AT-3-C3	Horizontal	861	843	843
AT-3-I3	Horizontal	1170	1134	1134
AT-5-E3	Vertical	19	28	25
AT-5-L3	Vertical	80	129	120
AE slides				
AE-1-C1	Horizontal	8	11	12
AE-4-C1	Vertical	33	39	42
AE-4-F1	Vertical	31	41	48
AE-5-E1	Vertical	26	30	31
AE-6-E6	Horizontal	11	11	12
mDT-10 slides				
mDT-1-K1	Horizontal	732	700	700
mDT-3-C4	Vertical	4	5	5
mDT-5-D8	Vertical	4	4	4
mDT-6-A8	Horizontal	275	294	291
mDT-6-F2	Horizontal	208	200	200

^aThe moving distance is a rough estimation of a straight line from the original fiber location to the location on the date pictures were taken.

^bInitial measurement of the distance was obtained after ~ 22 weeks for the AT-10 slides and 16 weeks for the AE and mDT-10 slides from the date of sample slide preparation.

(including fibers) loaded on the filter and thus cause apparently lower fiber concentrations compared to other sample preparation methods. The lower fiber concentrations of AT-10 and AT-3.5 resulted in statistically significant differences when paired *t*-tests were performed for the quarry samples. For the taconite and talc mills, fiber concentrations of the AT-10 samples showing fibers' movement were not considerably different compared to those of AT-3.5 and mDE samples where no fiber movement was observed (ratio range: 0.72–1.22). Although the statistical test (paired *t*-test) showed significant differences from the comparison of AT-10 and AT-3.5 (*P*-value: 0.015) and the comparison of the AT-10 and DP-DO at the taconite mill (*P*-value: 0.003), all-combined data did not detect any differences (*P*-values >0.182). The comparisons of all-combined data from the taconite and talc mills (without quarry sample data) also did not detect any statistical differences; although the *P*-values are not listed in Table 4 for these comparisons, all *P*-values were >0.477 .

DISCUSSION

Aim 1—Investigation of fiber movement

Fiber movement. When sample slides were prepared with acetone to clear the filter wedge and $\sim 3.5 \mu\text{l}$ triacetin to mount the cleared filter wedge (11 AT-3.5 slides), fiber movement was not observed at all over 59 weeks (413 days). For field sample slides prepared with $\geq 10 \mu\text{l}$ mounting medium and for the UICC sample slides prepared with $5 \mu\text{l}$ mounting medium, experience with the AT mounting method appear to be similar to that in previous studies (LeGuen and Galvin, 1981; Shenton-Taylor and Ogden, 1986). Shenton-Taylor and Ogden (1986) observed small-scale movements of chrysotile fibers from the comparison of 2- and 6-week images. LeGuen and Galvin (1981) also reported movement of chrysotile fibers in the first 3 months after preparation. However, the amount of triacetin mounting medium used for the sample preparation was not reported in either study. In the present study, initial fiber movement was observed on Day 37 for the

Table 4. Summary of fiber concentration ratio and statistical analysis

Number of samples	Taconite	Talc	Quarry	All-combined
	17	19	19	55
FC ratio (geomean) ^a —against AT-3.5				
AT-10/AT-3.5	0.72	1.13	0.77	0.86
mDE/AT-3.5	0.89	1.06	1.95	1.24
DP-DO/AT-3.5	1.08	1.09	1.57	1.23
FC ratio (geomean) ^a —against mDE				
AT-10/mDE	0.81	1.07	0.4	0.7
AT-3.5/mDE	1.13	0.95	0.51	0.81
DP-DO/mDE	1.22	1.03	0.8	0.99
Statistical analysis (<i>P</i> -value) ^b				
mDE versus AT-10	0.106	0.590	<0.001	0.378
mDE versus AT-3.5	0.123	0.630	0.002	0.182
mDE versus DP-DO	0.060	0.655	0.041	0.485
AT-10 versus AT-3.5	0.015	0.311	0.294	0.557
AT-10 versus DP-DO	0.003	0.285	<0.001	0.856
AT-3.5 versus DP-DO	0.478	0.917	0.006	0.204

^aFiber concentration ratio (geometric mean).

^bPaired *t*-test was performed for each comparison.

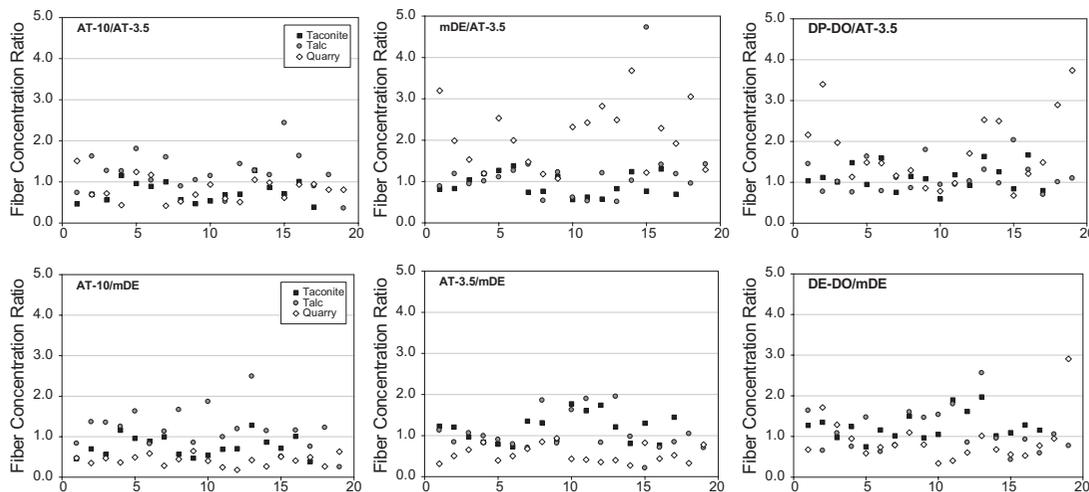


Fig. 7. Fiber concentration ratios at three sampling sites.

sample slides prepared with 15 or 20 μ l triacetin and on Day 52 for the sample slides prepared with 10 μ l triacetin. The findings of this study reemphasize the importance of critical amount of the triacetin in sample slide preparation and support the amount recommended for the mounting medium in NIOSH 7400, OSHA ID-160, WHO, and ISO 8672; i.e. a maximum of 3.5 μ l triacetin might be appropriate to fix fibers on a collapsed filter wedge so that no fibers would move from their initial location. Sample slides prepared according to the ASTM D7200 and HSG

248 would most likely have an excessive amount of the mounting medium and thus, fibers may be expected to move. For the AE mounting method, the previous study by Shenton-Taylor and Ogden (1986) showed no fiber movement although filter deterioration was observed from 4 months. In the present study, however, the AE sample slides showed fiber movement from the eighth day. Two possible reasons can be suggested to explain the findings of the current study. For the AT mounting method, when too much triacetin is used as a mounting

medium, it may fill the layer between the collapsed filter and the coverslip and also soften the filter. Thus, fibers may move off the filter and migrate through the triacetin layer. For the AE mounting method, it is possible that condensed acetone still remained on the filter after exposing it to the vaporized acetone and thus, the Euparal solution cover might lead to a soft layer containing dissolved acetone remaining for some time, through which fibers might migrate.

The mDT-10 samples behaved similarly to the AT-10 and AE sample slides prepared with ≥ 10 μl mounting media. When the amount of the triacetin was reduced from 10 to 3.5 μl for the mDT method, movement of fibers was not observed. Also, no fiber movement was observed for the mDE slides over 88 weeks. It should be noted that the findings of these sample slides (mDT-10, mDT-3.5, and mDE slides) in the present study cannot be compared directly with the findings in previous studies because the mounting medium was placed at different locations (i.e. between the cleared filter wedge and the glass slide versus between the cleared filter wedge and the coverslip). However, the consistency of results between AT-3.5 and mDT-3.5 slides and between AT-10 and mDT-10 slides in this study argues that the placement of the mounting medium relative to the cleared filter and coverslip may make little difference.

The tremolitic talc-ore mill samples contain more flexible flatter talc fibers than the other two sampling sites, while the taconite ore mill and quarry samples contain mostly cleavage fragments, shorter and thicker than fibrous asbestos, while the UICC samples are chrysotile asbestos. Fiber movement did not seem to be influenced by these differences in fiber characteristics.

Distance of fiber movement. The distance fibers moved in the AT-10 and mDT-10 sample slides was greater than that for fibers on the sample slides prepared with the Euparal mounting medium (AE samples) shown in Table 3. Generally, once fiber movement started, the direction of fiber movement for individual fibers was the same over time but fibers on the same slide did not always migrate in the same direction. Unfortunately, an exact rate of movement could not be determined because the first measurement of the moving distance was made only after 22 weeks for the AT-10 slides and 16 weeks for the AE and mDT-10 slides. It is possible that when the amount of mounting medium ≥ 5 μl was used in the sample preparation, the mounting medium would overflow beyond the cleared filter wedge leading to fiber movement. Further fiber movement might not occur once there is no further flow of the mounting

medium toward the edge of the coverslip. Flow to the edge of the coverslip may have ceased before the first measurements of movement were made, which is why no further movement was observed for those fibers that had moved. The amount of fiber movement was not affected by horizontal or vertical storage. While it might be reasonable to assume slides kept vertically would show greater movement under the influence of gravity, fibers on mDT-3-C4 and mDT-5-D8 sample slides, kept vertically, migrated only 4 μm , whereas fibers on mDT-6-A8 and mDT-6-F2 sample slides, kept horizontally, migrated >200 μm (Table 3).

Group translation of fibers and particles was observed in some grid opening areas suggestive of movement of the coverslip. While linear movements <0.1 mm would not have been easily visible, rotations of more than a few degrees would have been obvious. Care was exercised to ensure slides were only handled by the edges and that the stage clips and microscope objective were not allowed to touch the coverslips.

Aim 2—Evaluation of differences in fiber counts due to fiber movement

The direction of fibers' movement on the same slide was not consistent and the magnitude of those movements also differed, so that it was not felt that fibers were leaving some areas to congregate in others. Overall, the comparison of fiber concentrations between samples showing fiber movement (AT-10 samples) and samples showing no fiber movement (mDE and AT-3.5 samples) resulted in no statistically significant differences (P -values >0.182). Unlike fiber concentrations at the taconite mill and the talc mill, the fiber concentrations of AT samples (AT-10 and AT-3.5) for the quarry-sampling site were significantly lower than the other mounting methods. As explained in the Results section, this significant difference might be caused from the sample slide preparation rather than from the fiber movement; excessive dust (including fibers and other particulate matter) on the filter may have been washed out during the clearance of the filter due to the velocity from the nozzle of the acetone vaporizer.

The fiber concentrations of DP-DO samples were also similar to those of AT-3.5 and mDE samples for all sampling sites (range of fiber concentrations: 0.8–1.22) except for the comparison of DP-DO versus AT-3.5 at the quarry (ratio: 1.57). These results support compatibility between the old and new NIOSH methods and the mDE method.

Conclusions on the evaluation of differences in fiber counts due to fiber movement were based on

an assumption that the fibers were uniformly deposited on a filter, where fibers deposited in a random manner give the appearance of uniformity on a macroscopic scale (although this does not imply a constant number of fibers per microscopic field). Jones *et al.* (2001) observed relatively uniform deposition of particles across the filters collected from the cowled sampler positioned to the symmetrical vertical entry. In contrast, when the cowled sampler was positioned at angles between 0° and 180° to the flow direction of the surrounding air, non-uniform patterns of the filter deposit were observed due to the anisokinetic, anisoaxial sampling conditions (Baron *et al.*, 1994). The filter deposition patterns at a specific angle were similar regardless of the inlet flow rates. At 10 l min⁻¹ flow rate, the first vortices were observed at 60° and the vortices were more fully developed up to 165° causing non-uniform filter deposition patterns. At 180°, the uniform flow region was expanded but still causing a pair of vortices at the half of the filter area (note that there was no experimental study between 165° and 180°). In the current field study, the cowled sampler was positioned at a slight angle (typically, <60°) and thus a non-uniform pattern would not be expected. Additionally, no anisotropy was apparent on visual inspection of the filters. Although the fiber deposition pattern on the filter was not determined quantitatively in this study, examining a large number of sample sets and random selection of filter wedges for each mounting method should have ensured any possible bias that resulting from the non-uniform deposition would be minimal.

Clearness of filter background. Previous studies (LeGuen and Galvin, 1981; Shenton-Taylor and Ogdén, 1986) reported more fibers in the AT blank slides than those in the DE blank slides which was believed due to the more granular background with the AT method. Also, recent communication with several analysts indicated that fiber-like structures in the blanks of recent batches of mixed cellulose ester filters were observed when samples were prepared with the AT mounting method, while those structures were not found when the DE method was used. In the present study, however, when the mounting medium was placed between the cleared filter wedge and the glass slide for the mDE and mDT sample slides, two analysts at NIOSH did not notice any difference of the clearness of filter background compared to that of the AT and AE sample slides. The observations of the NIOSH analysts were based on only a few numbers of filters and so testing of an extended number of filters might be useful.

Ease of sample preparation

The procedures of the sample preparation by the AT mounting method required the least effort compared to the other mounting methods. In the early days of this method, a laboratory setup was required to clear a filter wedge with vaporized acetone (i.e. boiling acetone), which was not a suitable procedure for use in the field. The later invention of the acetone vaporizer made preparation of samples in the field much easier. The acetone vaporizer can be used in a field without requiring a fume hood because of the capability of adsorbing vaporized acetone into the charcoal bed under the exit port of the acetone vaporizer and also because acetone has a very high threshold concentration limit compared to other organic solvents. However, the finding of this study suggests that the use of an acetone vaporizer should be limited to filters that are not heavily loaded with dust. On the other hand, dimethylformamide solution involves handling of more toxic chemicals and requires a thermostat-controlled oven or hotplate. As a mounting medium, triacetin can be easily used without waiting, while Euparal resin requires additional heating time (at least 60 min for permanent storage) to be polymerized.

The optimal 3.5 µl quantity of triacetin was sometimes found to be too small to be dispersed widely enough to cover a filter wedge. Attempting to make the AT sample slides with 3.5 µl triacetin led to some mounting problems (i.e. the triacetin did not disperse sufficiently to cover the entire cleared filter wedge), while no mounting problems occurred when attempting to prepare AT samples with ≥10 µl. Sample preparation of the mDT slides using 3.5 µl triacetin was even more difficult than the AT slides prepared with 3.5 µl triacetin. Apparently, the clearance of the filter with dimethylformamide solution leads to a sticky surface over which the triacetin does not disperse well.

Generally, the sample slides mounted with triacetin included air bubbles more often than those mounted with Euparal mounting medium. Air bubbles can develop in the slides after preparation. Although air bubbles were not found in the mDE samples in the current study, air bubbles have been observed in other mDE slide preparations of field slides. However, the bubbles did not appear to grow in size even after 3 years, while the bubbles generated on the AT slides appeared to expand greatly over time until their presence led to problems with the filter background. Air bubbles were also trapped between the filter wedge and the Euparal for mDE method, but this can be easily fixed; one can reheat the slide to 60°C, remove the filter wedge

together with the coverslip, add more Euparal, and place it on a new glass slide.

CONCLUSIONS

Fiber movement has been studied using different mounting methods (AT-10, AT-3.5, AE, mDE, mDT-10, mDT-3.5 methods) with field samples collected from three sampling sites. In addition, two chrysotile and two Amosite samples from UICC material were prepared by the AT method. The tracking time period ranged from 59 to 94 weeks. For the field sample slides mounted with the AT-10, AE, and mDT-10 method, >80% of field areas showed fiber movement within 155 days (~22 weeks), while fiber movement was not detected at all for the mDE, AT-3.5, and mDT-3.5 slides. Once fiber movement started, fibers moved over distances measured from 4 up to >1000 μm . A possible explanation of fiber movement might be softening of the layer between the collapsed filter and the coverslip. The storage method, either vertically or horizontally, did not seem to cause a difference in the magnitude of fiber movement. The amount of movement as well as the direction of fibers' movement on the same slide was not always consistent. Similar results were observed in the sample slides prepared from filters containing UICC asbestos fibers; fiber movement of chrysotile fibers began in 3 weeks, while Amosite fiber movement started after 3 months. The findings of the current study confirm that the amount of the triacetin mounting medium to be used in sample preparation by the AT and mDT methods is critical to prevent fiber movement. The AT and mDT methods prepared with $\leq 3.5 \mu\text{l}$ triacetin for a quarter of a 25-mm filter area might be acceptable. The comparison of fiber counts between the samples showing fiber movement (AT-10 slides) and the samples showing no fiber movement (AT-3.5 and mDE slides) resulted in statistically non-significant differences. The fiber counts of DP-DO samples were also similar to those of AT-3.5 and mDE samples. In conclusion, although fiber counts would not be significantly changed by fiber movement, the type and amount of mounting medium for sample slide preparation remains critical for issues such as quality assurance and training of analysts by revisiting the same fibers.

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