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STROUP

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FINAL REPORT

COMPARISON OF AIRBORNE ASBESTOS LEVELS DETERMINED BY TRANSMISSION ELECTRON MICROSCOPY (TEM) USING DIRECT AND INDIRECT TRANSFER TECHNIQUES

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AUTHORS AND CONTRIBUTORS

This report was prepared by Jean Chesson of Chesson Consulting, Inc. under subcontract to Battelle. Jeff Hatfield of Battelle prepared an earlier draft of the Study 1 (EPA 1988) results. The R.J. Lee Group, Inc., Monroeville, PA performed the laboratory analysis of samples from Study 1 as part of the study reported in EPA (1988).

The EPA work assignment manager was Brad Schultz. Substantial contributions were also made by Cindy Stroup, Betsy Dutrow, and Joe Breen of the Exposure Evaluation Division in the EPA Office of Toxic Substances.

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EXECUTIVE SUMMARY

Background

Transmission electron microscopy (TEM) is the preferred analytical method for measuring asbestos concentrations in ambient atmospheres. The absence of a standard protocol for TEM analysis and the discovery and refinement of new techniques have resulted in a variety of procedures which may not necessarily provide comparable estimates of airborne asbestos concentration. An important difference between protocols is the use of direct and indirect transfer techniques. The direct transfer method was developed primarily to estimate structure concentration, whereas the indirect transfer method was developed primarily to estimate mass concentration. In a direct transfer the original filter is prepared for analysis with minimal disturbance of the particles upon it. In an indirect transfer, the particles are removed from the original filter and resuspended on a second filter prior to microscopic examination. Although the original spatial distribution of the particles is lost, indirect transfer is thought to provide greater control over analytical precision through improved distribution of materials over the surface of the filter.

Early TEM measurements of airborne asbestos used an indirect transfer method and expressed the results in terms of mass (ng/m^3). Fiber concentrations were not reported because it was thought that the indirect transfer technique might have broken up larger asbestos structures and artificially inflated the fiber count. The U.S. Environmental Protection Agency (EPA) has used the indirect transfer technique for many of its research programs, in part to overcome the problem of non-asbestos debris in some sampling situations, and in part because the type of filter most suited for direct transfer (polycarbonate) was thought to be more difficult to handle and transport in the field. However, improvements in the direct transfer technique applied to mixed cellulose filters have made direct transfer a feasible option.

Prior to carrying out a recent study of airborne asbestos levels in public buildings (USEPA 1988), EPA convened a meeting of microscopists and other asbestos measurement experts to determine the most appropriate analytical protocol. A direct transfer method using mixed cellulose ester filters was selected. A similar TEM protocol was later specified under the Asbestos Hazard Emergency Response Act (AHERA) to determine when an asbestos work site is sufficiently clean for the containment barriers to be removed.

To investigate the relationship between airborne asbestos levels measured by the two transfer techniques and possibly provide a basis for comparison with earlier studies based on indirect transfer, a subset of the samples collected in the 1988 EPA study were reanalyzed using an indirect transfer method. This document reports the results of the EPA analysis and extends the discussion to include data from six other studies.

Results and Conclusions

The investigation confirmed the generally held opinion that the direct and indirect transfer methods provide different estimates of airborne asbestos concentration. There is insufficient information, however, to determine the mechanisms responsible for the difference and thereby recommend one method over the other. The specific conclusions are listed below followed by recommendations for further research.

- **TEM analysis of air samples using indirect transfer methods tends to provide estimates of total airborne asbestos structure concentration that are higher than those obtained using direct transfer methods.** This conclusion is consistent with general opinion and implies that airborne asbestos levels estimated by one method are not directly comparable to those estimated by the other.

  Evidence. A review of available data (seven studies) revealed this relationship in every study despite variations in sampling, analytical, and counting protocols.

- **There is no single factor that can be applied to convert measurements made using an indirect transfer method to a value that is comparable with measurements made using a direct transfer method.** The quantitative relationship between estimates obtained by the two transfer methods is expected to depend on sampling and analytical protocols as well as the nature of the asbestos structures in the air.

  Evidence. In the studies considered here, measurements made by the indirect transfer method were 3.8 times to 1,700 times higher than measurements made by the direct transfer method. The highest value of 1,700 was estimated from a set of 45 samples collected in a school district. The lowest value of 3.8 was obtained in an interlaboratory study of 12 samples of amphibole.

- **Provided a single method is applied consistently, the choice of method is not as critical when measurements are to be used only for comparative purposes (for example, comparison of airborne asbestos levels inside and outside an abatement site). When measurements are to be interpreted in relation to a fixed standard, the choice of method is more important.**

  Evidence. Both methods appear to detect changes in airborne asbestos concentrations. Although the relationship between the two methods is not strong, higher concentrations determined from one method tend to correspond to higher levels obtained by the other. A statistically significant relationship of this type was found between measurements made by the two transfer methods in all seven studies. In a study designed to compare indoor and outdoor airborne asbestos levels, the same trend was revealed by both methods.

- **Based on data from the studies considered in this report, it seems unlikely that the larger airborne asbestos concentrations estimated by the indirect transfer method can be explained solely by breakdown of large asbestos structures into smaller components.** Alternative hypotheses involving interference by debris and association of unattached structures may also be important.

  Evidence. In the two studies for which data are readily available, the indirect transfer method counted more structures than the direct transfer method in all size categories. One would expect to count fewer large structures with the indirect transfer method if larger asbestos structures were being broken down into smaller ones.

Additional Research

The information needed to select the appropriate protocol for a given situation could be obtained with a relatively modest research program. A series of studies is suggested to:

- Further investigate structure size distributions for direct and indirect TEM preparations in order to distinguish among alternative hypotheses and thereby determine which method more accurately reflects biologically meaningful airborne asbestos concentrations; and

- Compare the spatial distribution of asbestos structures on samples prepared by direct and indirect transfer methods in order to characterize the precision of each method.

I. INTRODUCTION

Transmission electron microscopy (TEM) is the preferred analytical method for measuring asbestos concentrations in ambient atmospheres. A measured volume of air is drawn through a filter. Particles trapped on the filter are coated with a thin carbon film and the filter is dissolved by solvent leaving the carbon film supported on a fine metal mesh grid. The grid is examined with the transmission electron microscope. The absence of a standard protocol for TEM analysis and the discovery and refinement of new techniques have resulted in a variety of procedures which may not necessarily provide comparable estimates of airborne asbestos concentration. An important difference between protocols is the use of direct and indirect transfer techniques. The direct transfer method was developed primarily to estimate structure concentration whereas the indirect transfer method was developed primarily to estimate mass concentration. In a direct transfer the original filter is prepared for analysis with minimal disturbance of the particles that have been collected on its surface. In an indirect transfer, the particles are removed from the original filter and resuspended on a second filter prior to coating with the carbon film. Although the original spatial distribution is lost, indirect transfer is thought to provide greater control over analytical precision through improved distribution of materials over the surface of the filter (Cook and Marklund 1982, Chatfield 1985).

Early TEM measurements of airborne asbestos (e.g., USEPA 1975, 1979, 1980) used an indirect transfer method and the results were expressed as mass (ng/m^3). Fiber concentrations were not reported because it was thought that the indirect transfer technique might have broken up larger asbestos structures and artificially inflated the fiber count (Chatfield 1978). The U.S. Environmental Protection Agency (EPA) has used the indirect transfer technique for many of its research programs (e.g., USEPA 1983, 1985, 1986a, Tuckfield et al. 1988), in part to overcome the problem of non-asbestos debris in some sampling situations, and in part because the type of filter most suited for direct transfer (polycarbonate) was thought to be more difficult to handle and transport in the field. Comparability with earlier studies was also an issue.

Ortiz and Isom (1974) developed a direct transfer method for use with mixed cellulose ester filters, but data collected by Chatfield (1986) indicates that fiber loss is high. Burdett and Rood (1982) proposed a direct transfer method incorporating an etching step. Their method appears to give results comparable to direct transfer using a polycarbonate filter (Chatfield 1986), while benefitting from the easier handling and transport associated with a mixed cellulose ester filter. The NIOSH 7402 protocol (NIOSH 1985) also involves a direct transfer method with an etching step.

Prior to carrying out a study of airborne asbestos levels in public buildings (USEPA 1988), EPA convened a meeting of microscopists and other asbestos measurement experts to determine the most appropriate analytical protocol. A direct transfer method based on the Burdett and Rood protocol was selected. A similar TEM protocol (allowing either polycarbonate or mixed cellulose ester filters) was later specified in the Asbestos Hazard Emergency Response Act (AHERA, 40 CFR Part 763) to determine when an asbestos work site is sufficiently clean for the containment barriers to be removed. (The AHERA protocol differs from the study protocol by restricting fiber counting to asbestos fibers longer than 0.5 μm and with an aspect ratio of 5:1 or greater.)

To investigate the relationship between airborne asbestos levels measured by the two transfer techniques and possibly provide a basis for comparison with earlier studies based on indirect transfer, a subset of the samples collected in the 1988 EPA study was reanalyzed using an indirect transfer method. The purpose of this document is to report the results of the EPA investigation and extend the discussion to include data from other sources. Conclusions are presented in Section II. Section III describes the two transfer methods and discusses their advantages and disadvantages. Individual studies are described in Section IV and results analyzed in Section V. The discussion in Section VI suggests areas for future research.

II. CONCLUSIONS AND RECOMMENDATIONS

The results from the recent EPA study (USEPA 1988), together with a review of six other studies in the literature (Tuckfield et al 1988, Lee 1987 (two data sets), Burdett 1985a, Chatfield 1986, and Cook and Marklund 1982) lead to the following conclusions:

- **TEM analysis of air samples using indirect transfer methods tends to provide estimates of total airborne asbestos structure concentration that are higher than those obtained using direct transfer methods.** This conclusion is consistent with general opinion and implies that airborne asbestos levels estimated by one method are not directly comparable to those estimated by the other.

  Evidence. A review of available data (seven studies) revealed this relationship in every study despite variations in sampling, analytical, and counting protocols.

- **There is no single factor that can be applied to convert measurements made using an indirect transfer method to a value that is comparable with measurements made using a direct transfer method.** The quantitative relationship between estimates obtained by the two transfer methods is expected to depend on sampling and analytical protocols as well as the nature of the asbestos structures in the air.

  Evidence. In the studies considered here, measurements made by the indirect transfer method were 3.8 times to 1,700 times higher than measurements made by the direct transfer method. The highest value of 1,700 was estimated from a set of 45 samples collected in a school district. The lowest value of 3.8 was obtained in an interlaboratory study of 12 samples of amphibole.

- **Provided a single method is applied consistently, the choice of method is not as critical when measurements are to be used only for comparative purposes (for example, comparison of airborne asbestos levels inside and outside an abatement site). When measurements are to be interpreted in relation to a fixed standard, the choice of method is more important.**

  Evidence. Both methods appear to detect changes in airborne asbestos concentrations. Although the relationship between the two methods is not strong, higher concentrations determined from one method tend to correspond to higher levels obtained by the other. A statistically significant relationship was found between measurements made by the two transfer methods in all seven studies. In a study designed to compare indoor and outdoor airborne asbestos levels, the same trend was revealed by both methods.

- **Based on data from the studies considered in this report, it seems unlikely that the larger airborne asbestos concentrations estimated by the indirect transfer method can be explained solely by breakdown of large asbestos structures into smaller components.** Alternative hypotheses involving interference by debris and association of unattached structures may also be important.

  Evidence. In the two studies for which data are readily available, the indirect transfer method counted more structures than the direct transfer method in all size categories. One would expect to count fewer large structures with the indirect transfer method if larger asbestos structures were being broken down into smaller ones.

Selection of an appropriate protocol in a given situation involves consideration of bias (systematic error) and precision (random error). The conclusions above, combined with opinions expressed by microscopists, indicate that the indirect and direct transfer methods differ with respect to bias and precision, but there is insufficient information to recommend one method over the other. The necessary information could be obtained with a relatively modest research program involving the analysis of existing data and experiments designed specifically for this purpose. It is recommended that studies be performed to:

- Further investigate structure size distributions for direct and indirect TEM preparations in order to distinguish among alternative hypotheses and thereby determine which method more accurately reflects biologically meaningful airborne asbestos concentrations; and

- Compare the spatial distribution of asbestos structures on samples prepared by direct and indirect transfer methods in order to characterize this component of precision.

These recommendations are discussed in more detail in Section VI.

III. DESCRIPTION OF ANALYTICAL METHODS

A variety of preparation techniques have been used in the analysis of airborne asbestos samples collected on membrane filters. Early work is described in USEPA 1978a and 1978b. The descriptions below summarize direct and indirect transfer methods as they are presently applied.

A. Direct Transfer

In a direct transfer the original filter is prepared for microscopic examination. The direct transfer technique retains the spatial distribution of particles on the filter and minimizes disturbance which might change their number and size.

1. Polycarbonate Filter

Polycarbonate filters are strong and smooth-surfaced with a sieve-like construction which makes them particularly suitable for direct transfer. After sample collection, a portion of the filter is carbon coated in a vacuum evaporator. Portions of the coated filter are placed on mesh metal grids and the filter material is dissolved in chloroform. The remaining carbon film is a replica of the original filter surface. Particles that were deposited on the surface of the filter are embedded in the carbon film.

2. Mixed Cellulose Ester Filter

Mixed cellulose ester filters are thicker, sponge-like, and have a more irregular surface than polycarbonate filters. For this reason, they are thought to retain fibers better during handling and transport. Due to their construction, however, they are likely to trap fibers below, as well as on, the filter surface. The irregular filter must be collapsed to form a continuous surface film suitable for carbon coating. Current protocols also include a plasma etching step to remove a thin layer of filter and further expose trapped particles before applying the carbon film. After carbon coating, the procedure is similar to that for polycarbonate filters with the exception that different chemicals are used to dissolve the filter material.

B. Indirect Transfer

The procedure for indirect transfer is the same irrespective of the filter type. A measured fraction of the filter is ashed in a low temperature plasma asher, the ash sonicated in liquid to redisperse the particles, diluted as needed, and filtered on to a second filter (usually a polycarbonate filter). The second filter is prepared for microscopic examination according to the direct transfer method described above.

A second type of indirect transfer, which does not involve ashing, can be used with polycarbonate filters. Particles are washed from the surface of the filter and redeposited on a second filter. Ultrasonic agitation may also be used to remove particulate matter from the filter surface.

C. Comparative Advantages and Disadvantages

The main advantage of the direct transfer method is that it is thought to most closely represent the number and size distribution of asbestos structures present in the sampled air. This claim is based on the lack of disruption of the sample, rather than conclusive data. Since health effects, although poorly understood, are thought to be related to fiber dimensions, a method that provides relevant size information is desirable. The reduced number of preparation steps reduces sample preparation time and hence cost. The direct transfer method is at a disadvantage in dusty atmospheres or with larger volume samples where debris may obscure asbestos structures. Also, any inhomogeneity in the spatial distribution of particles on the filter is retained and reduces the precision of estimated airborne asbestos concentrations.

Compared to direct transfer, the indirect transfer method is more likely to disrupt the number and size distribution of asbestos structures. Nevertheless, it may be necessary when the sample is obscured by a large amount of debris. Rinsing the cassette allows particles that may be adhering to the walls to be included in the sample. The redeposition phase also provides control of filter loading (particles may be concentrated to increase analytic precision or diluted to permit counting of otherwise overloaded samples) and the effect of uneven deposition of particles on the original filter is eliminated. Improper filtration can, however, produce an uneven distribution on the second filter.

Contamination of the sample by asbestos structures unrelated to the air being sampled is of concern with both transfer methods. (Serious problems have occurred with polycarbonate filters, USEPA 1986b.) With direct transfer, contamination of the filter surface is the main source of contamination. With indirect transfer, the additional preparation steps increase the opportunity for contamination from the filter (both surface and internal contamination) and other sources (Anderson et al, 1989).

IV. INDIVIDUAL STUDIES

A. Study 1 -- EPA/GSA Study of Commercial and Public Buildings

1. Study Design

A primary objective of the original study (USEPA 1988) was to determine if airborne asbestos levels are elevated in buildings that have asbestos-containing material (ACM). A total of 406 filters (387 air samples and 19 field blanks) were analyzed by TEM using a direct transfer technique. The samples were collected in 49 buildings (339 samples) and at 48 sites outside the buildings (48 samples).

As discussed above, previous EPA studies had used an indirect transfer technique. To investigate the relationship between measurements obtained using direct transfer with those obtained by indirect transfer, 30 of the original 406 filters were chosen for reanalysis using an indirect transfer technique. A better understanding of the relationship between the two transfer methods might allow comparisons between the results of different studies. The reanalysis also provided additional quality assurance. In this study, airborne asbestos levels measured using the direct transfer technique tended to be at the low end of the range commonly measured in indoor atmospheres. Reanalysis using indirect transfer confirmed that asbestos structures were present on the filters.

The thirty samples were chosen to represent a range of sample types and asbestos structure concentrations. Two filters were chosen at random from the 19 field blanks. Four filters were chosen at random from the 48 outdoor samples. The remaining 24 filters were chosen from the 339 indoor samples. Their selection was based on the direct TEM results. Of the 24, eight filters were chosen at random from the 282 samples which, with the direct transfer method, had no structures in ten grid openings. Eleven samples were chosen at random from the 52 samples which had either one or two structures in ten grid openings. All five of the filters with three or more structures in ten grid openings were chosen. (One structure in ten grid openings corresponds to 16.1 s/mm^2 of filter surface. Two structures in ten grid openings correspond to 32.3 s/mm^2 of filter surface; three structures in ten grid openings correspond to 48.4 s/mm^2 of filter surface.)

2. Sampling Protocol

Air samples were collected on 37-mm cellulose ester (Millipore®) filters with a pore size of 0.45 μm. Approximately 5,000 L of air were drawn through each filter at a rate of approximately 5 L/min for 16 hours. Each sample was collected over two consecutive weekdays during periods of normal building activity

3. Analytical Protocols

The direct transfer protocol used in the original study is described in Appendix B of USEPA (1988). Sample preparation involved collapsing a portion of the filter, plasma etching, and directly coating the filter with a thin layer of carbon by evaporative deposition under vacuum. The samples were cleared with acetone, leaving the particles attached to the carbon film. The results reported here are based on the examination of ten grid openings with a total area of 0.062 mm^2 at a magnification of approximately 20,000 X. (Additional grid openings were examined on selected filters to investigate the spatial distribution of asbestos structures. The results of those analyses are reported in USEPA (1988)).

The indirect transfer protocol used to analyze the 30 samples selected for this study was similar to that described in Appendix B-5 of Tuckfield et al. (1988). A known portion (approximately 1/4) of the original 37 mm filter was ashed, suspended in 100 ml of filtered water, sonicated, and a known aliquot (either 70 ml or 100 ml) was deposited on a 25 mm cellulose ester filter. Ten grid openings with a total area of 0.067 mm^2 were examined on each filter.

Counting procedures for grid examination are given in Appendix B of USEPA (1988) and were taken from Yamate et al. (1984). The type of asbestos (chrysotile or amphibole) and type of structure (fiber, bundle, cluster, or matrix) were recorded along with length and width measurements. (Non-asbestos fibers were also identified.) Total structure concentrations, as well as separate estimates for fibers and for bundles, clusters, and matrices (BCM), are available. Fibers were defined as asbestos structures with an aspect ratio of 3:1 or greater. No minimum length was designated.

4. Data Analysis

The asbestos structure concentration per cubic centimeter of air (s/cc) is calculated by estimating the number of structures deposited on the original filter from the number counted in ten grid openings and dividing by the volume of air sampled. The computation uses the effective area of the filter (385 mm^2 for a filter 25 mm in diameter and 855 mm^2 for a filter 37 mm in diameter). The estimated total number of structures on the original 37 mm filter (TS) is given by:

$$TS = n \times (855\ mm^2/0.062\ mm^2)$$

for the direct transfer method and by:

$$TS = n \times (385\ mm^2/0.067\ mm^2) \times (100\ ml/aliquot) \times (855\ mm^2/area\ ashed)$$

for the indirect transfer method. The symbol n is the number of asbestos structures counted in 10 grid openings, aliquot is the amount of material suspended on the 25 mm filter (either 70 ml or 100 ml) and area ashed is the area of the 37 mm filter ashed during the indirect preparation (expressed in mm^2). The estimated airborne asbestos concentration (s/cc) is obtained by dividing TS by the volume of air sampled (cc). The estimated concentration of asbestos structures on the original filter (s/mm^2) is obtained by dividing TS by 855.

The Wilcoxon Signed-Rank test was used to test whether the two transfer methods differed in their estimates of asbestos structures per square millimeter of filter. The test examines the median difference between two sets of measurements. The degree of association between the two transfer methods was measured by the Pearson and Spearman correlation coefficients. Both coefficients vary between +1 and -1 depending on the strength of the relationship and whether the relationship is positive or negative. The Pearson correlation coefficient is calculated from the actual airborne asbestos concentrations and detects linear relationships. The Spearman coefficient is a nonparametric measure of association based on ranks.

Summary statistics for the lengths and widths of asbestos structures were produced separately for the direct and indirect TEM measurements; the Wilcoxon Rank-Sum test was used to test for statistically significant differences.

5. Results

Asbestos structure concentrations for the 28 air samples are reported in Appendix B. The two field blanks, chosen at random from the 19 blanks analyzed with direct TEM in the original study, were found to have zero and three asbestos structures, respectively, in the ten grids counted for the indirect preparation. The three structures counted were all fibers. These counts correspond to structure concentrations of 0 and 96.2 s/mm^2 on the filter and 0 and 0.016 s/cc in the air when sampling 5000 liters of air. The average background contamination is 48.1 s/mm^2 or 0.008 s/cc. This is an order of magnitude less than all but three of the nonzero indirect TEM asbestos structure concentrations measured in the study.

The relationship between structures/cc measured by indirect TEM and fibers/cc measured by direct TEM is illustrated in Figure 1. (To maintain consistency with the other studies discussed below, structures per cubic centimeter are plotted against fibers per cubic centimeter because these are the units reported in the remaining studies.) The two field blanks are not included in this or any subsequent analyses.

The data show that concentrations measured by the indirect transfer method are greater than those measured by the direct transfer method. The result holds for total structure concentrations, fiber concentrations, and BCM (bundle, cluster,

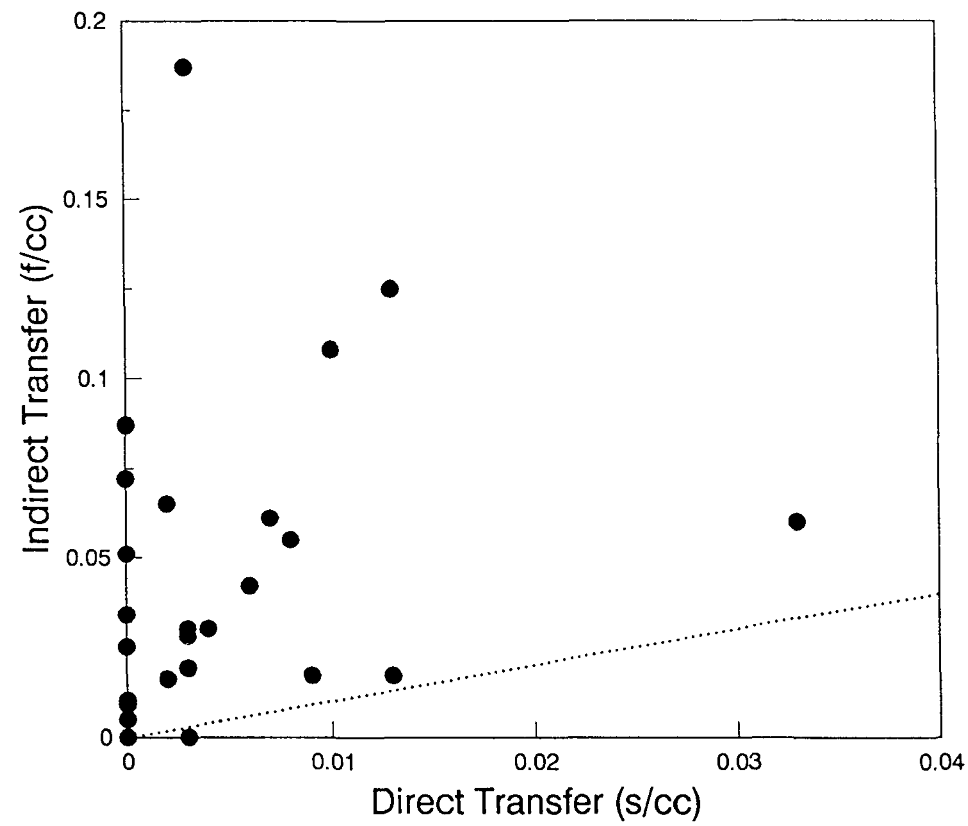

Figure 1. Airborne asbestos concentrations measured in Study 1 (EPA/GSA). If direct and indirect transfer techniques were equivalent, the points would fall on the dotted line.

and matrices) concentrations. The Wilcoxon Signed-Rank test yields p-values less than 0.0001 for all three comparisons.

The correlation coefficients are reported in Appendix C. None of the Pearson correlation coefficients are significantly different from zero at the 5 percent level. However a Spearman correlation coefficient of 0.37 suggests a weak correlation between direct structure concentration and indirect fiber concentration ($p < 0.05$). Spearman correlation coefficients for direct structure concentration versus indirect structure concentration (0.35), direct fiber concentration versus indirect fiber concentration (0.31), and direct BCM concentration versus indirect fiber concentration (0.34) are also significant at the 5 percent level. Recall that the Spearman correlation coefficient is sensitive to a broader range of relationships than the Pearson correlation coefficient.

The lengths and widths of chrysotile structures detected by the two transfer methods are summarized in Table 1. (Amphibole structures, which tend to be larger than chrysotile structures, were relatively rare in this study. On the 28 samples analyzed by both direct and indirect TEM, 11 amphibole structures were counted by indirect TEM and 8 amphibole structures by direct TEM.) Chrysotile fibers detected using the indirect transfer method tend to be shorter and thinner than those detected by the direct transfer method. The difference in fiber width is statistically significant ($p = 0.0003$, t-test), whereas the difference in fiber length is not ($p = 0.79$). No statistically significant size differences were found between chrysotile BCM detected by each method.

Chrysotile structure size distributions are shown in Table 2. Although the largest differences are seen with the smaller fibers, more fibers of all sizes were counted with the indirect transfer method (Table 1). Also, more BCM were counted with the indirect transfer method than with the direct transfer method and the average size of BCM counted with the indirect transfer method was no smaller than the average size of BCM counted with the direct transfer method. Therefore, it seems unlikely that the larger airborne asbestos concentrations estimated by indirect TEM can be explained solely by breakdown of large structures into smaller components.

Table 1. Summary Statistics for Length and Width of Chrysotile Asbestos Structures Measured with Direct and Indirect TEM

Fibers

	Direct		Indirect	
	Length	**Width**	**Length**	**Width**
Mean	0.94	0.08	0.88	0.06
Median	0.50	0.05	0.60	0.05
Standard Deviation	1.25	0.05	0.97	0.02
Standard Error	0.27	0.01	0.06	0.001
Sample Size	22	22	249	249

BCM

	Direct		Indirect	
	Length	**Width**	**Length**	**Width**
Mean	1.26	0.11	1.40	0.12
Median	0.80	0.05	1.00	0.10
Standard Deviation	0.81	0.11	2.01	0.13
Standard Error	0.27	0.04	0.15	0.01
Sample Size	9	9	173	173

Table 2. Size Distribution of Chrysotile Structures Measured in Study 1 (The Body of the Table Gives Number of Structures.)

a) Direct Transfer Method

Width Category	Length Category							
	1	2	3	4	5	6	7	Total
1	0	0	0	0	0	0	0	0
2	0	5	6	2	3	0	1	17
3	0	2	7	1	3	0	0	13
4	0	0	0	0	1	0	0	1
5	0	0	0	0	0	0	0	0
Total	0	7	13	3	7	0	1	31

b) Indirect Transfer Method

Width Category	Length Category							
	1	2	3	4	5	6	7	Total
1	1	8	9	2	11	0	0	31
2	0	53	86	26	63	4	6	238
3	0	6	56	18	52	3	4	139
4	0	0	1	1	6	3	2	13
5	0	0	0	0	0	0	1	1
Total	1	67	152	47	132	10	13	422

Key:

Structure Length (μm) Category		Structure Width (μm) Category	
1	$l < 0.25$	1	$w < 0.05$
2	$0.25 \le l < 0.5$	2	$0.05 \le w < 0.1$
3	$0.50 \le l < 0.75$	3	$0.10 \le w < 0.25$
4	$0.75 \le l < 1.0$	4	$0.25 \le w < 0.5$
5	$1.00 \le l < 3.0$	5	$0.50 \le w$
6	$3.00 \le l < 5.0$		
7	$5.0 \le l$		

B. Study 2 -- Phase III Abatement Study

1. Study Design

The main objective of the original study (Tuckfield et al. 1988) was to compare airborne asbestos levels in six schools before, during, and after removal of the asbestos-containing material. A secondary objective was to compare estimates of airborne asbestos concentrations made by TEM using direct and indirect transfer techniques.

Two side-by-side samples were collected at each sampling site. One sample was collected on a mixed cellulose ester filter (47 mm diameter, 0.45 μm pore size), and the other on a polycarbonate filter (37 mm diameter, 0.4 μm pore size). A total of 103 mixed cellulose ester filters were analyzed by TEM using an indirect transfer method. Based on the indirect TEM results, 25 polycarbonate filters were selected for analysis using a direct preparation technique. The filters were chosen to represent a range of airborne asbestos concentrations. Three of the 25 filters could not be successfully prepared for direct analysis due to heavy filter loadings. Thus, TEM analyses of 22 mixed cellulose ester/polycarbonate filter pairs were available for comparison.

2. Sampling Protocol

Approximately 10,000 L of air were sampled at a flow rate of 5 L/min. Samples were collected over 5 days during periods of normal activity. Each sampling pump was equipped with two orifices. The mixed cellulose ester filter was attached to one orifice, the polycarbonate filter to the other.

3. Analytical Protocols

The direct and indirect transfer protocols are given in Appendix B of the study report (Tuckfield et al. 1988). The mixed cellulose ester filters were ashed and sonicated prior to deposition on a polycarbonate filter. Subsequent steps for both protocols were similar. The polycarbonate filters were carbon coated and the filter material dissolved. Bundles, clusters, and matrices are recorded, but not included in the estimates of fiber concentration.

4. Results

The results of the 22 pairs of analyses are listed in Appendix B. Figure 2 shows that with the exception of one sample (Sample 85-324) estimates based on the direct transfer method are smaller than those based on the indirect transfer method. In several instances, fibers were detected on the mixed cellulose ester filter using the indirect transfer method, but not on the corresponding polycarbonate filter using the direct transfer method. Since different filter media were used, the observed differences could be due to the filter medium as well as the preparation method. It has been suggested that asbestos

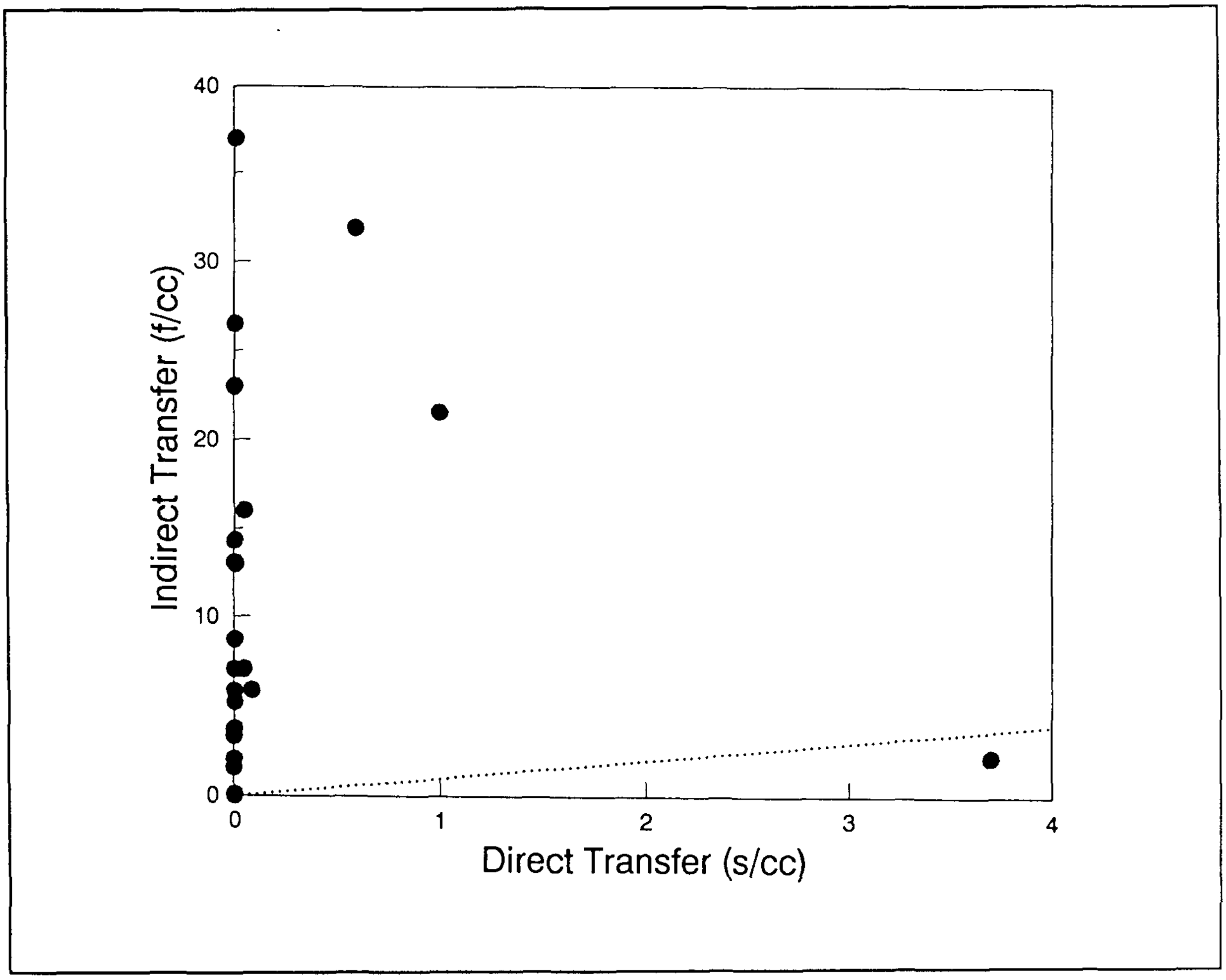

Figure 2. Airborne asbestos concentrations measured in Study 2 (Phase III). If direct and indirect transfer techniques were equivalent, the points would fall on the dotted line.

structures may have been lost from the filters during the on-off cycling of the pumps.

C. Study 3 -- Lee (1987)

1. Study Design

At the request of attorneys representing National Gypsum Company, 62 filters collected in EPA's 1983 study of airborne asbestos levels in schools (USEPA 1983) were analyzed by TEM using a direct transfer method (Lee 1987). The 62 filters were chosen because sufficient filter material remained for further analysis. In the original study samples were analyzed by TEM using an indirect transfer method. The objective of the study was to document exposure to airborne asbestos in schools. Forty eight asbestos-containing sites in 25 schools were sampled. An outdoor ambient sample, and an indoor control sample from an area without

ACM were also collected at each school. Of the 62 filters later analyzed by direct TEM, 46 were analyzed in the original study. (Most of the remaining filters are blanks that were collected but not analyzed.)

2. Sampling Protocol

Samples were collected on 47 mm diameter, mixed cellulose ester filters with a pore size of 0.45 μm. Approximately 10,000 L of air were sampled over 5 days at a rate of 5 L/min. Sampling took place during the hours of normal school activity.

3. Analytical Protocols

The indirect transfer protocol is given in Appendix E of USEPA (1983). A quarter of the filter was ashed, sonicated, and filtered onto a polycarbonate filter prior to carbon coating and microscopic examination. The protocol calls for recording of bundles, clusters, and matrices, but does not include them in the reported fiber concentration.

The direct transfer analysis was performed according to the protocol specified in the AHERA proposed rule (52 FR 15820, April 30, 1987). The filter was collapsed, etched, carbon coated and dissolved. Fibers are defined as structures longer than 0.5 μm with an aspect ratio of 5:1 or greater. Bundles, clusters, and matrices are included in the total structure count.

4. Results

Appendix B lists results for the 46 pairs of analyses. The relationship between the estimates obtained by direct and indirect transfer methods is illustrated in Figure 3. (Note that one extreme value is not included in Figure 3. Based on the size and type of particles on this filter, Lee (1987) suggested that this sample had been deliberately "spiked.") As expected, estimates based on the direct transfer method are lower than those based on the indirect transfer method. Although the 46 samples were selected according to availability and are not necessarily a representative sample of the experimental categories, the direct transfer results show a similar trend to that observed in the original study (Table 3). Measured airborne asbestos levels are lowest for outdoor samples and highest for indoor samples at sites with ACM.

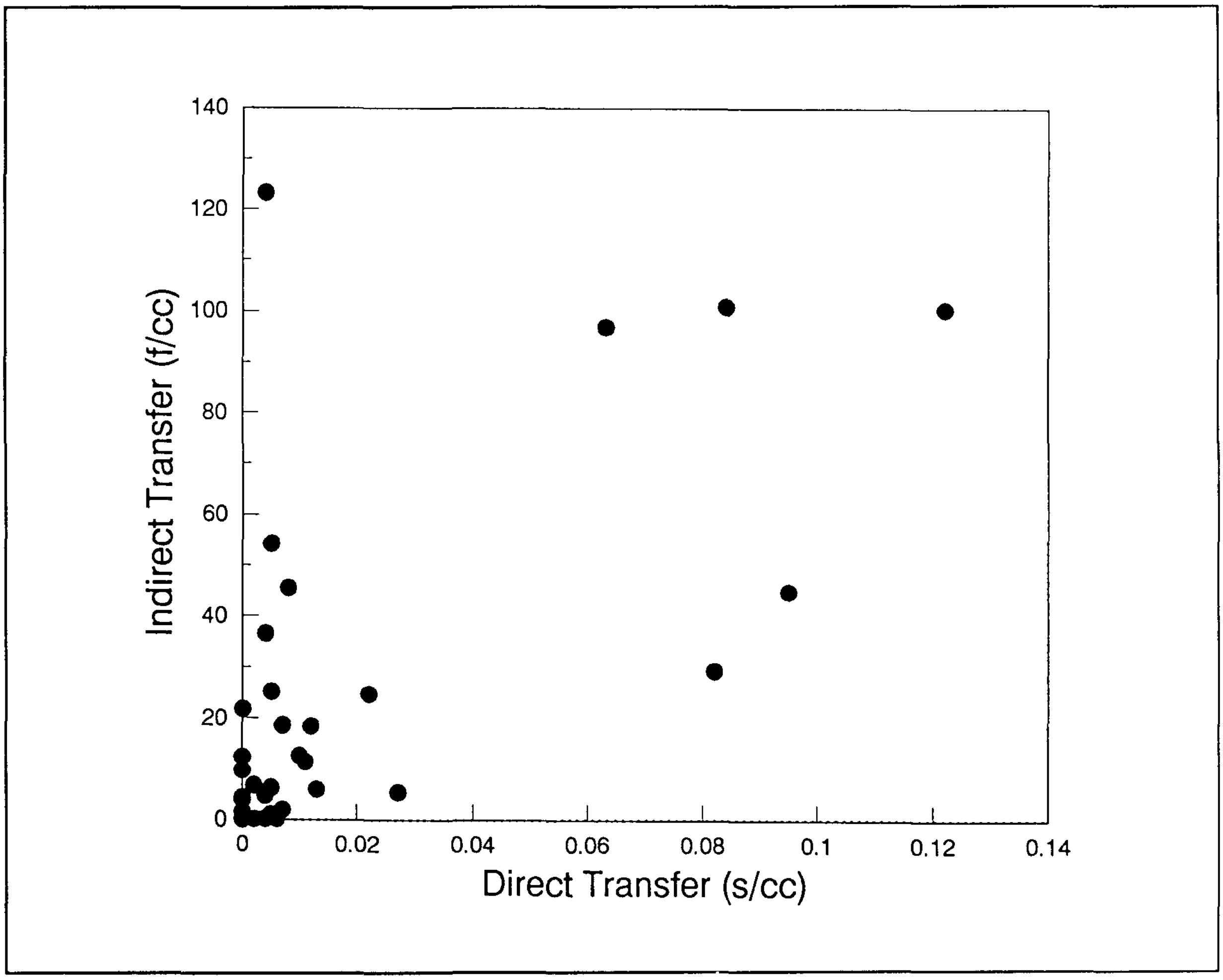

Figure 3. Airborne asbestos concentrations measured in Study 3 (Lee 1987). If direct and indirect transfer techniques were equivalent, the points would fall on the dotted line.

Table 3. Comparison of Mean Airborne Asbestos Levels Obtained by Direct and Indirect TEM Analysis of Study 3 Samples

Type of Site	**No. of Samples**	**Direct Transfer (s/cc)**	**Indirect Transfer (f/cc)**
Asbestos sites	27	0.021	29.49
Indoor Control	9	2560.400	4.80
Indoor Control excluding "spike"	8	.007	4.09
Outside Ambient	10	.001	0.28

D. Study 4 -- Burdett (1985a)

1. Study Design

Fifteen samples collected in EPA's 1983 study in schools (USEPA 1983) were reanalyzed by Burdett (1985a) as part of an inter-laboratory exchange. The basis for selection of the 15 samples is not stated, but they consisted of 6 samples from sites with ACM, 4 indoor controls, 4 outdoor samples, and 1 blank.

2. Sampling Protocol

The sampling protocol is described under Study 3 above.

3. Analytical Protocols

Burdett used the direct transfer method described in Burdett and Rood (1983). This protocol is similar to the AHERA protocol. A total structure count was obtained by summing the individual counts for fibers and bundles, clusters, and matrices. The indirect transfer method is described under Study 3 above.

4. Results

The fifteen pairs of results are listed in Appendix B and plotted in Figure 4. The relationship between the two sets of measurements is similar to that seen in Study 3. Table 4 shows that the increasing trend in measured airborne asbestos levels from outdoor ambient to indoor sites with ACM is apparent despite the small number of samples.

Table 4. Comparison of Mean Airborne Asbestos Levels Obtained by Direct and Indirect TEM Analysis of Study 4 Samples (Burdett 1985a)

Type of Site	**No. of Samples**	**Direct Transfer (s/cc)**	**Indirect Transfer (f/cc)**
Asbestos sites	6	0.059	27.48
Indoor Control	4	0.013	25.50
Indoor Control excluding "spike"	3	0.007	7.44
Outside Ambient	4	.001	0.76

E. Study 5 -- Toronto Subway

1. Study Design

Chatfield (1986) reports analyses of 8 samples collected in the Toronto Subway System. The samples were initially analyzed by TEM using a direct transfer method. Later they were reanalyzed

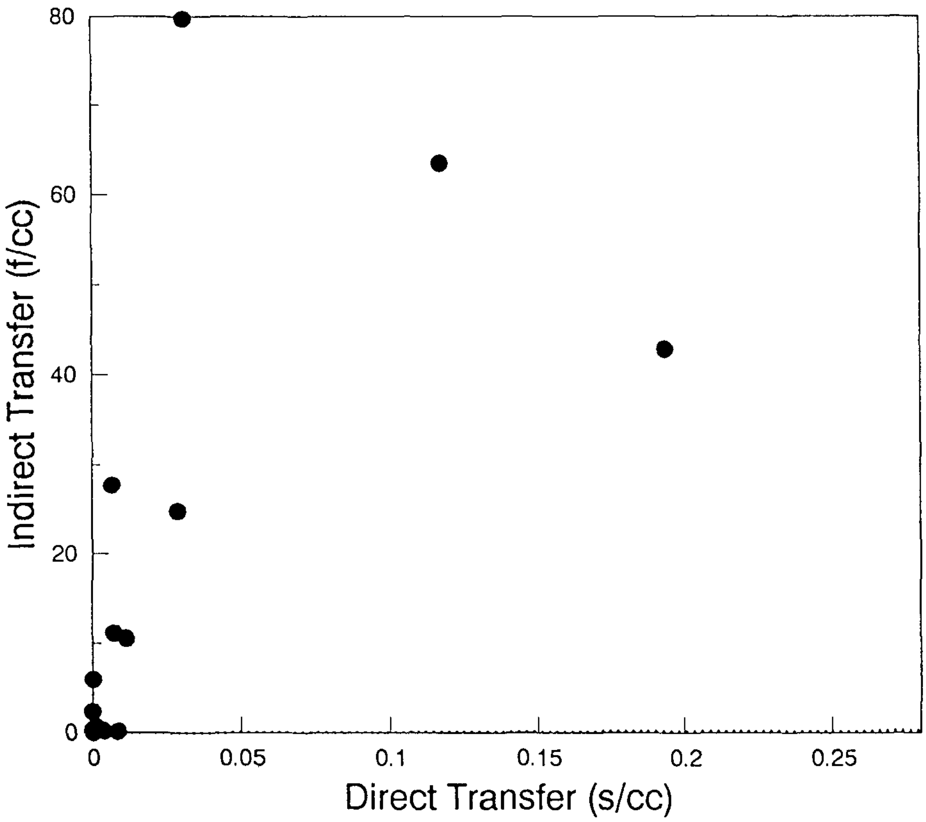

Figure 4. Airborne asbestos concentrations measured in Study 4 (Burdett 1985). If direct and indirect transfer techniques were equivalent, the points would fall on the dotted line.

using an indirect transfer method. The objective of the original study is not stated. Sampling and analysis details not given in Chatfield (1986) were obtained through personal communication with Dr. Chatfield.

2. Sampling Protocol

Samples were collected on 47mm, 0.4μm polycarbonate filters.

3. Analytical Protocols

The direct transfer analysis involved carbon coating and dissolution of the filter in chloroform following the Yamate protocol (Yamate et al 1984).

For the indirect transfer analysis, filters were washed in double-distilled water and the detached particulate ashed to remove organic material. After redispersion in water, the residual ash was prepared for analysis according to the direct transfer method. There was no ultrasonic treatment. Fiber concentrations are reported for both preparation methods. Bundles with a 3:1 aspect ratio or greater were counted as one fiber. To the extent possible, the individual components of a cluster were counted.

4. Results

Results for the eight pairs of analyses are listed in Appendix B and illustrated in Figure 5. Reported concentrations are higher for the indirect preparation. Fiber length distributions are reported for two of the samples (Table 5). The increased number of fibers for the indirect method is due mainly to an increase in the number of short fibers. However, there is also a small increase in the number of longer fibers.

Table 5. Fiber Size Distributions of Two Samples from Study 5 (Chatfield 1986)

Fiber Length (μm)	**Sample SH43**		**Sample LA49**	
	Direct Transfer	**Indirect Transfer**	**Direct Transfer**	**Indirect Transfer**
0.50 - 0.73	4	70	2	4
0.73 - 1.08	1	56	1	1
1.08 - 1.58	1	14	0	1
1.58 - 2.32	0	9	0	0
2.32 - 3.41	2	3	0	0
3.41 - 5.00	0	2	0	0
5.00 - 7.34	0	2	0	0
7.34 - 10.77	0	0	0	0

F. Study 6 -- Lee (1987)

1. Study Design

As part of the investigation described under Study 3, 12 of the filters analyzed by direct TEM were reanalyzed by the same laboratory using an indirect transfer preparation technique. The filters were selected based on the results of the direct analysis. Unlike the larger set of 46 pairs of results described in Study 3, the set of 12 pairs does not include the effects of differences between laboratories.

2. Sampling Protocol

The sampling protocol is described under Study 3 above.

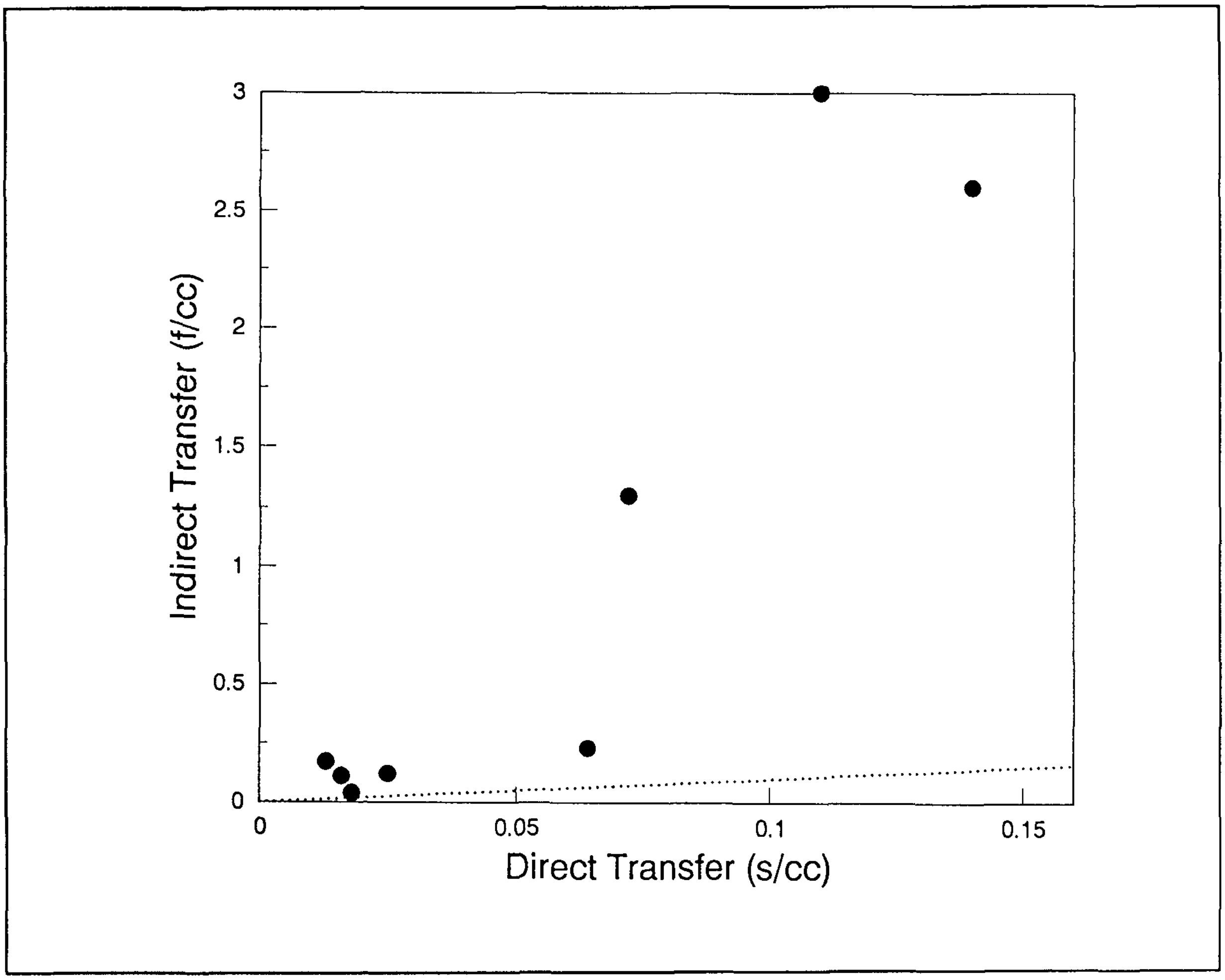

Figure 5. Airborne asbestos concentrations measured in Study 5 (Toronto Subway). If direct and indirect transfer techniques were equivalent, the points would fall on the dotted line.

3. Analytical Protocols

The direct transfer protocol is described under Study 3 above. Few details are given about the indirect protocol other than ashing lasted approximately 75 minutes and the objective was to duplicate the protocol used in Study 3 (Lee 1987).

4. Results

The 12 pairs of results are listed in Appendix B and plotted in Figure 6. The relationship between the two sets of measurements is similar to that seen in Studies 3 and 4. (All three studies use samples from the same EPA study, although not necessarily the same samples.)

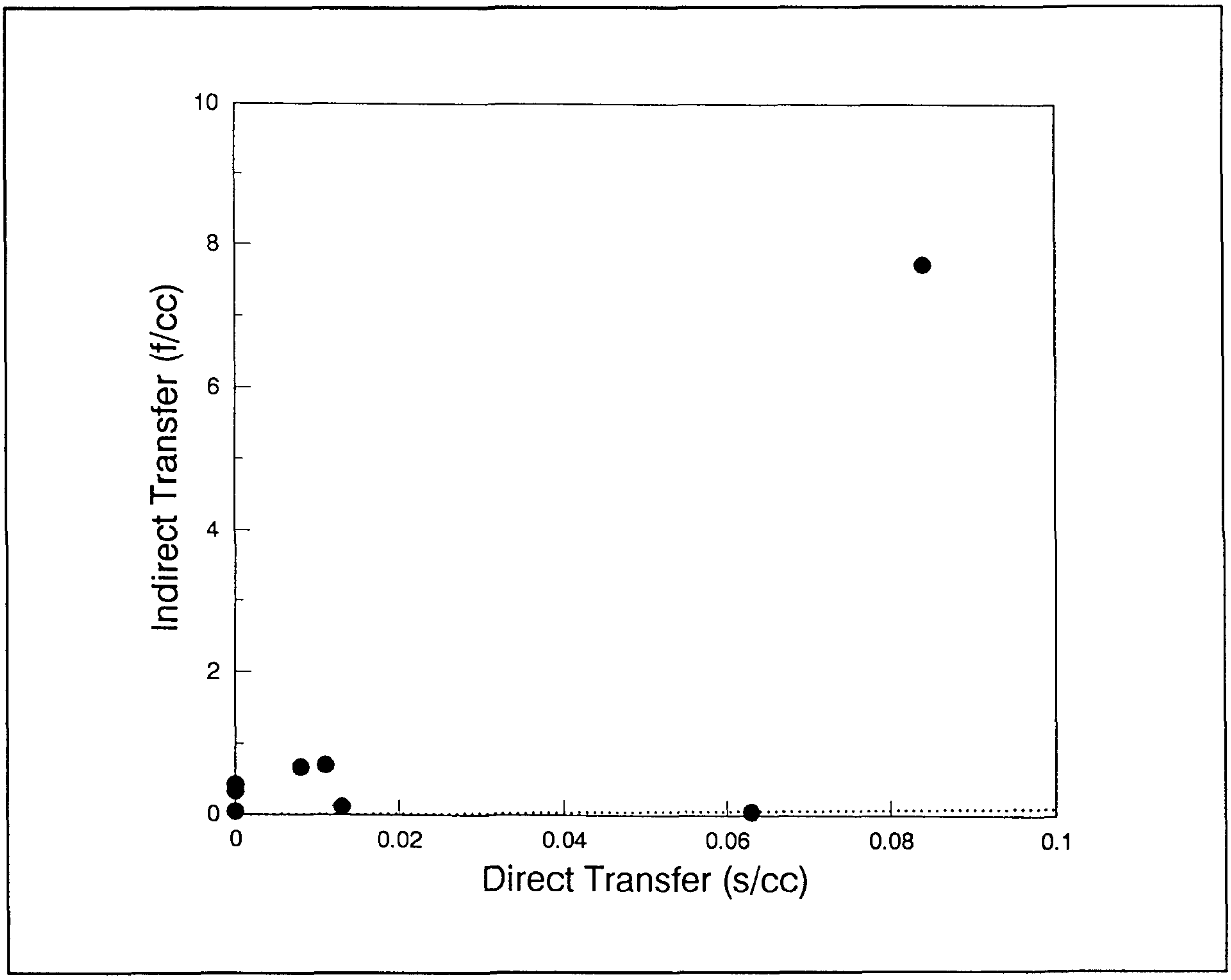

Figure 6. Airborne asbestos concentrations measured in Study 6 (Lee 1987). If direct and indirect transfer techniques were equivalent, the points would fall on the dotted line.

G. Study 7 -- Cook and Marklund (1982)

1. Study Design

Pieces of filter from twelve air samples collected by the Minnesota Department of Health in 1975 were sent to six laboratories as part of an interlaboratory study. Each laboratory analyzed all twelve samples using their standard analytical protocol. Unlike studies 1 through 6 in which chrysotile is the only, or predominant, type of asbestos, these samples contained amphibole.

2. Sampling Protocol

The samples were collected on 1.2 μm pore size mixed cellulose ester filters (Millipore®) over a period of approximately 55 hours so that visibly heavy sample loadings were achieved (approximately 5 cubic meters of air per square centimeter of filter).

3. Analytical Protocols

Each laboratory followed a different protocol. Three laboratories used some type of direct preparation and three used some type of indirect preparation. The protocols labelled "LTA/C-coat, Nuc-Jaffe" and "C-Coat, Direct/Jaffe" have been selected as being most similar to the direct and indirect transfer techniques currently in use. LTA refers to low temperature ashing, C-coat to carbon coating, and Jaffe to use of a Jaffe wick.

4. Results

The twelve pairs of results are listed in Appendix B and plotted in Figure 7. Cook and Marklund disagree with earlier claims by Peters and Doerfler (1978) that an increase in the number of fibers counted using an indirect transfer technique is caused by fracturing of larger fibers. Although the percentage of large fibers measured in with the indirect transfer technique was less than the percentage measured with the direct transfer technique, the number of fibers in every size category was greater using the indirect transfer technique.

H. Other Data

Additional data comparing measurements made by TEM using direct and indirect transfer methods are reported by USEPA (1978a), Steen et al. (1983), Sebastien et al. (1984), Burdett (1985b) and Chatfield (1986). These data are not included in the combined analysis in Section V below because they involve only a small number of samples, or differ in substantial ways from the six studies described previously.

In USEPA 1978a, Samudra et al. describe the results of the analysis of a single sample by direct and indirect transfer by each of five analysts. The sample, which was part of a round-robin test, was collected on a 0.4 μm polycarbonate filter at 560 L/min for one hour at the Johns-Manville Plant in Waukegan, Illinois. Four of the five analysts obtained a higher estimate of airborne chrysotile fiber concentration with the indirect transfer method. The ratio of indirect to direct measurements ranged from 0.5 to 8.7 with an average of 4.9. The relative frequency of short fibers was greater with the indirect transfer method, but larger numbers of fibers of all lengths were counted with indirect transfer.

Steen et al. report airborne asbestos concentrations only for fibers longer than 5 μm. Their indirect measurements tend to be higher than their direct measurements, but compared to studies where all fiber sizes are reported, the magnitude of the difference is small.

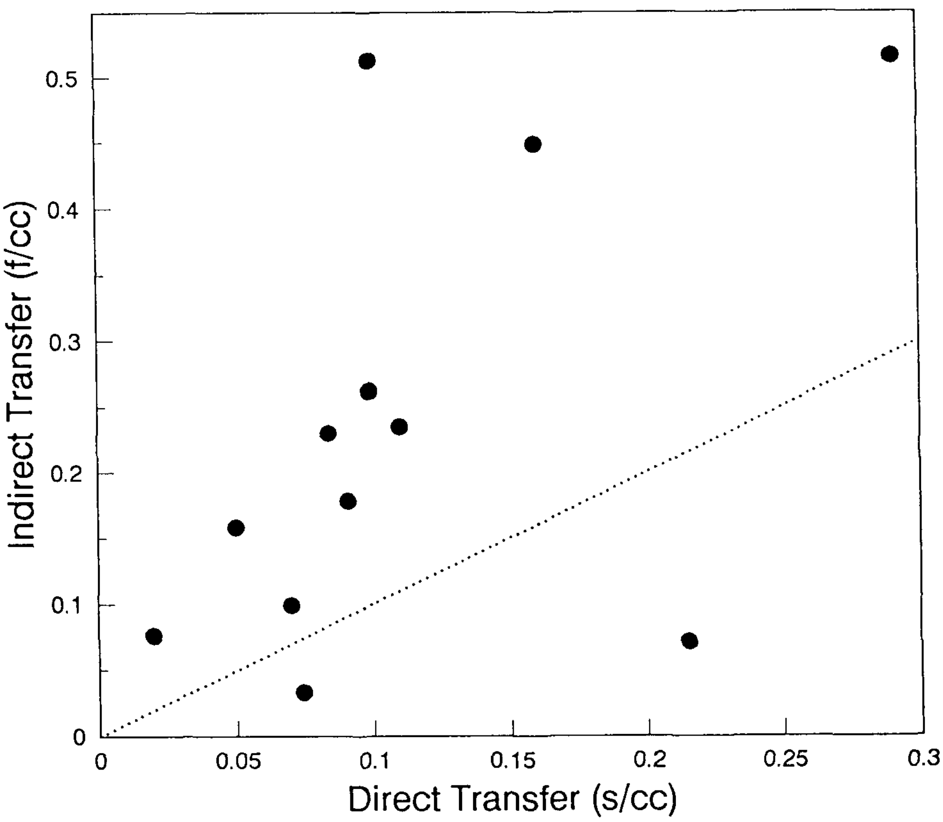

Figure 7. Airborne asbestos concentrations measured in Study 7 (Cook 1982). If direct and indirect transfer techniques were equivalent, the points would fall on the dotted line.

A study of ambient airborne asbestos concentrations in Quebec mining towns was preceded by a methodological study in order to determine how the air samples should be analyzed (Sebastien et al. 1984). Eighteen air samples were analyzed using an indirect transfer technique. Only four of the 18 samples had loadings sufficiently low to permit analysis by the direct transfer technique. The direct measurements were 0.006, 0.032, 0.002, and 0.007 s/cc. The corresponding indirect measurements were 0.084, 0.207, 0.016, and 0.244 s/cc. (Note that in Table VIII in Sebastien et al. the direct and indirect labels appear to have been reversed.) The authors conclude that more smaller fibers are counted by the indirect method and that fiber breakage during the ultrasound treatment is not sufficient to explain the increase.

As part of a study of fiber release from amosite insulation, Burdett (1985b) analyzed three samples by both direct and indirect transfer techniques. The concentrations obtained with direct transfer were 0.0005, 0.33, and 0.06 f/cc. The corresponding indirect measurements were 0.003, 0.43, and 0.25 f/cc. Slightly smaller fiber widths and lengths were measured with the indirect transfer method.

Chatfield (1986) compares fiber counts obtained using direct and indirect transfer methods on laboratory generated samples. The two transfer methods give comparable results for a sample generated with a single fibril aerosol. The indirect transfer method gives considerably higher counts for a sample generated with an aggregated chrysotile aerosol.

V. ANALYSIS OF COMBINED DATA

Table 6 summarizes the main features of the seven studies described in the previous section. The studies differ in details of the sampling and analytical protocols and in the type of asbestos structures included in the estimates of airborne asbestos concentration. Inter-laboratory differences such as quality of TEM specimen preparation, identification criteria, and analyst skill are also expected to contribute to differences in estimated concentrations. Therefore, it is not surprising that the relationship between direct and indirect measurements varies from study to study. In addition, variation from study to study is expected because of differences in the size distribution of asbestos structures in the sampled air.

A model of the form

$$Y_I = \alpha + \beta Y_D,$$

where Y_I, and Y_D are the indirect and direct measurements respectively, and α, and β are unknown parameters to be estimated, was fitted separately to each data set. Since Y_I and Y_D are both subject to measurement error, and no distinction is made between explanatory and response variables, standard linear regression techniques for estimating α and β are inappropriate. Instead, α and β are estimated by a nonlinear constrained maximum likelihood estimation technique (Britt and Lueke 1973). Y_I and Y_D are assumed to be normally distributed with variance σ^2.

An example of the use of this technique and additional references appear in Bishop et al (1981). (The model was also applied to $\ln(Y_I)$ and $\ln(Y_D)$ giving a model with measurement errors which increase with the mean. Since the pattern of results is essentially the same for both models, and interpretation of the log model is more difficult, only the results for the original model are presented.) If there were perfect agreement between the two TEM methods, α would be 0 and β would be 1. The strength of the relationship is indicated by the Pearson correlation coefficient. A correlation coefficient of 0 indicates no correlation. A correlation coefficient of 1 indicates maximum positive correlation. The Pearson correlation coefficient may be compared with the Spearman correlation coefficient which is based on ranks, and therefore is sensitive to other types of relationships in addition to linear relationships.

Table 6. Summary of Major Attributes of the Seven Studies

Study	Collection Medium			Lab		Protocol		Counting	
	Type	Diam.	Pore	Dir	Ind	Direct	Indirect	Direct	Indirect
1	MCE	37mm	0.45µm	A	A	Mod B&R	OTS	s/cc	f/cc
2[a]	PC	37mm	0.4 µm	B	B	Yamate	OTS	f/cc	f/cc
	MCE	47mm	0.45µm						
3	MCE	47mm	0.45µm	A	B	AHERA	OTS	s/cc	f/cc
4	MCE	47mm	0.45µm	C	B	B&R	OTS	s/cc	f/cc
5	PC	47mm	0.4 µm	D	D	Yamate	Wash	f/cc[b]	f/cc[b]
6	MCE	47mm	0.45 µm	A	A	AHERA	ETC	s/cc	f/cc
7	MCE	?	1.2 µm	E	F	C/J	LTA/C/J	?	?

[a]In this study the PC filter was analyzed by direct TEM and the MCE filter was analyzed by indirect TEM. In the remaining studies both methods were applied to the same filter.

Key:

Filter Type	MCE	mixed cellulose ester
	PC	polycarbonate
Laboratory	A	RJ Lee Group (formerly ETC)
	B	Battelle
	C	UK Health and Safety Executive
	D	Chatfield
	E,F	Unnamed
Protocol	Mod B&R	modified Burdett and Rood
	OTS	OTS method (USEPA 1983)
	Yamate	Yamate (1984)
	AHERA	AHERA proposed rule (52 FR 15820)
	B&R	Burdett and Rood (1983)
	Wash	Washing without ultrasonic treatment
	ETC	Ashed for 75 minutes, details not given
	C/J	Carbon coated, Jaffe wick
	LTA/C/J	Low-temp asher, carbon coated, Jaffe wick
Counting rule	s/cc	structures/cc (includes BCM)
	f/cc	fibers/cc (excludes BCM)
	f/cc[b]	Bundles with a 3:1 aspect ratio or greater were counted as one fiber. To the extent possible, individual components of a cluster were counted. (E. Chatfield, pers. comm.)

Estimates of α and β are given in Table 7 together with their 95% confidence intervals. For Study 2 the analysis was repeated with one extreme point (see Figure 2) excluded. Subsequent discussion refers to the analysis of the reduced data set. Confidence intervals for α all include 0. All seven studies have estimates of the proportionality parameter, β, which are greater than 1 (Figure 8), indicating that estimates based on indirect transfer measurements are larger than those based on direct transfer measurements. β is significantly greater than 1 ($p < 0.05$) in four of the seven studies.

The Spearman correlation coefficient indicates a statistically significant positive relationship between indirect and direct measurements for all studies except Study 6. A statistically significant linear relationship is indicated by the Pearson correlation coefficient in Studies 3 through 6. The apparent inconsistency between the two correlation coefficients for Study 6 is most likely due to one sample that had the largest concentration measured by both direct and indirect methods (see Figure 6). This sample would tend to increase the Pearson correlation coefficient, but have less effect on the Spearman correlation coefficient. A significant correlation is less likely to be obtained when there is a small number of samples in the data set.

The proportionality parameter, β, varies considerably between studies. The smallest value of 3.8 may reflect the type of asbestos involved--amphibole rather than predominantly chrysotile. All analyses in Studies 1 and 6 were done by Laboratory A. Therefore differences in the estimated values of β between these two studies cannot be attributed to laboratory differences. Protocols did differ slightly, however. Concentrations measured in Study 1 were low by both direct and indirect transfer methods. Over 80 percent of the original direct analyses were zero. Consequently, the range of concentrations in Study 1 may not be sufficient to gain a quantitative estimate of the relationship between the two transfer methods.

Studies 3, 4, and 6 involved samples from the same original study (USEPA 1983), although not necessarily the same samples. Assuming that the nature of the airborne asbestos material was similar across the three studies, differences between the estimates of β (1,670, 755, and 109) may reflect mainly differences between laboratories and protocols.

Table 7. Parameter Estimates for Model $Y_I = \alpha + \beta Y_D$, where Y_I and Y_D are Measurements Obtained Using the Indirect and Direct Transfer Methods Respectively (95% Confidence Intervals in Parentheses)

Study	Sample Size	α	β	Pearson Correlation	Spearman Correlation
1	28	-0.07 (-0.23, 0.10)	24 (-11, 59)	0.26	0.37^{*}
2	22	11 (6, 16)	0.09 (-3.0, 3.1)	-0.07	0.41^{*}
2†	21	2.6 (-11.6, 16.9)	110 (-4, 220)	0.41	0.55^{**}
3	45	-4.3 (-17.8, 9.3)	1,700 (1,000, 2,300)	0.65^{***}	0.67^{***}
4	15	-2.6 (-25.4, 20.3)	760 (220, 1,300)	0.61^{**}	0.72^{**}
5	8	-0.6 (-1.3, 0.05)	28 (18, 37)	0.92^{***}	0.81^{**}
6	8	-1.2 (-3.8, 1.4)	110 (28, 190)	0.74^{*}	0.32
7	12	-0.20 (-0.61, 0.22)	3.8 (0.4, 7.2)	0.52	0.57^{*}

† Sample 85-324 excluded

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

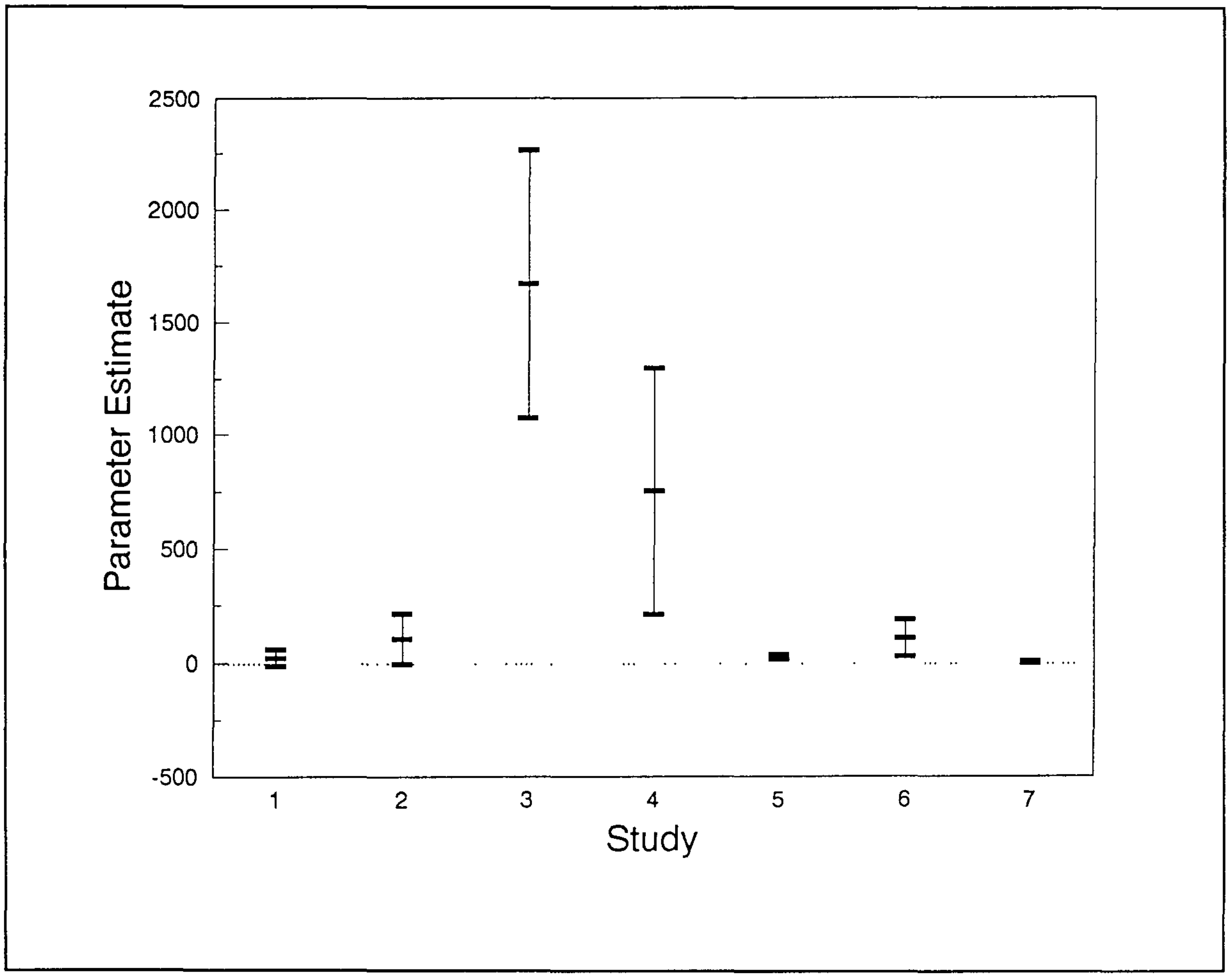

Figure 8. Estimated values of the proportionality parameter, β, for Studies 1 through 7. Vertical lines indicate the 95 percent confidence interval.

VI. DISCUSSION

An analytic method should be sufficiently accurate for its intended purpose. Accuracy has two components: bias and precision. Bias refers to a systematic deviation of the measured value from the true value of the quantity being measured. In this case the objective is to characterize exposure in a biologically meaningful way, that is, in terms of the number and type of structures that are inhaled. Precision refers to the uncertainty associated with repeated measurements of the same quantity. The direct transfer method is often characterized as being less biased than the indirect transfer method, whereas the indirect transfer method is considered more precise by some researchers. Neither of these claims is supported by extensive data. Bias and precision are discussed in turn below, together with suggestions for further research that could assist in selecting the appropriate analytical method for a given situation.

A. Bias

Bias must be considered within the context of the application. If measurements are to be used in a comparative manner (e.g., comparing airborne asbestos levels inside and outside a building), a bias that applies equally to both sets of measurements may not affect the comparison. If, however, the objective is to measure exposure in order to assess risk, a bias may have a significant impact on the interpretation of the data. Although the details are controversial, it is thought that the dimension of asbestos structures is important in determining the incidence of disease. Special attention should be devoted to minimizing bias with respect to asbestos structures that contribute most to disease incidence. (Note that the contribution is determined not only by relative potency of asbestos structures of different sizes, but also by their relative abundances.) An ideal measurement method would mimic the effect of respiration, etc. on complex structures (BCM) so that those that readily disintegrate would be represented by their individual components, while those that are firmly linked would be counted and sized as single structures.

The studies considered in this paper all support the generally accepted belief that airborne asbestos concentrations estimated by an indirect transfer method are larger than those estimated by a direct transfer method. Breakdown of larger structures during the ashing, sonication, and resuspension steps is assumed to be the main explanation for the difference. Fiber size information from Studies 1 and 5, however, does not provide strong support for this hypothesis. Although more small fibers are counted using an indirect transfer method, there is not a corresponding decrease in the number of large fibers and BCM, nor in the size of the BCM.

Chatfield (1986) provides two additional hypotheses for the larger structure counts obtained with an indirect transfer method. First, with the direct transfer method, structures may be hidden by organic debris. (This hypothesis was also suggested by Sebastien et al, 1984.) The effect is likely to be greatest for small structures, but applies to structures of all sizes. During indirect transfer the debris is removed by ashing, thereby improving visibility and increasing the structure count. Second, with the direct transfer method, small structures loosely associated with larger structures (for example, touching but not bonded) are counted as a single structure. During indirect transfer, these structures are disassociated from the larger structures and are counted as individual structures.

All three mechanisms may play a role to a varying degree under different circumstances. Note that predictions depend on the size distribution of asbestos structures in the sampled air. When only small fibers are present, the breakdown hypothesis would predict little difference between direct and indirect preparations whereas the debris hypothesis would predict higher measurements with the indirect preparation. When the majority of structures are complex, the breakdown hypothesis would predict higher measurements with the indirect preparation whereas the association hypothesis would predict little difference.

Given that measurements by indirect TEM are generally higher than those by direct TEM, it is important to determine whether indirect measurements incorporate a positive bias (because, for example, the additional preparation artificially inflates the number of fibers) or the direct measurements incorporate a negative bias (because, for example, fibers are covered by debris). Fiber size data should be available for Studies 2, 3, and 4, and could be analyzed to distinguish between competing hypotheses. The number of structures counted, particularly those in the larger size categories, could limit the investigation. A designed experiment in which samples were prepared according to carefully specified protocols would provide more conclusive information. Experimental factors include preparation method, filter loading (low to high), and prevalence of complex structures.

B. Precision

Other considerations being equal, the method with the highest precision is preferable. For TEM analysis of airborne asbestos, the spatial distribution of asbestos structures on the surface of a filter is important in determining precision. Only a tiny fraction of the original filter area is examined with the electron microscope. It is assumed that the area is representative of the entire filter surface in order to estimate the concentration of asbestos in the sampled air. (Other aspects of the protocol including counting rules, filter loading, and area of filter examined also affect precision. These are not discussed further here because they can be varied independently of the transfer method. The effect of procedures such as ashing and resuspension that are uniquely associated with indirect transfer method would be included in any overall study of precision.)

Chatfield (1984, 1986) has argued that the spatial distribution of asbestos structures on the filter is closer to random (i.e., follows a Poisson distribution) when an indirect transfer method is used. If structure counts per grid opening are available for Studies 1, 2, 3 and 4, Chatfield's claim can be tested. Efforts are underway to obtain these data. The question may also be addressed experimentally by preparing samples by both techniques and examining the filter in greater detail than is done during routine analysis. A relatively simple statistical design and analysis would be sufficient to detect marked differences and could provide a definite recommendation. A more sophisticated experiment is needed to explore heterogeneity on various spatial scales in order to determine the advisability of preparing more than one portion of the filter or analyzing multiple grids.

Since breakup of structures (resulting in a positive bias) and uneven spatial distribution of structures on the filter (resulting in decreased precision) are claimed to be the major disadvantages of the indirect and direct transfer methods respectively, further research to support or reject these claims would be a valuable and relatively low cost contribution to the continuing discussion over the choice of analytical protocol.

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Appendix A: Definition of Asbestos Structure Types

Fiber: Structure with an aspect ratio (length to width) of 3:1 or greater with substantially parallel sides.

Bundle: Structure composed of fibers in a parallel arrangement with each fiber closer than one fiber diameter.

Cluster: Structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group or groups of fibers closely spaced and randomly oriented.

Matrix: Fiber or fibers with one end free and the other end embedded or hidden by a particulate. Combinations such as a matrix and cluster, matrix and a bundle, or bundle and a cluster are categorized by the dominant fiber quality-cluster, bundle, and matrix, respectively.

Appendix B: Data Listings

Table B-1. Results from Study 1 (EPA 1988)

Sample ID	Air Volume (liters)	Direct TEM Structures(s) s/cc	Direct TEM Structures(s) s/mm^2	Direct TEM Fibers(f) f/cc	Direct TEM Fibers(f) f/mm^2	Direct TEM BCM(b)* b/cc	Direct TEM BCM(b)* b/mm^2	Indirect TEM Structures(s) s/cc	Indirect TEM Structures(s) s/mm^2	Indirect TEM Fibers(f) f/cc	Indirect TEM Fibers(f) f/mm^2	Indirect TEM BCM(b)* b/cc	Indirect TEM BCM(b)* b/mm^2
7159	4575.1	0.000	0.0	0.000	0.0	0.000	0.0	0.038	201.6	0.009	50.4	0.028	151.2
7206	4138.2	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
7275	4380.2	0.000	0.0	0.000	0.0	0.000	0.0	0.037	191.6	0.025	127.7	0.012	63 9
7518	4973.9	0.000	0.0	0.000	0.0	0.000	0.0	0.101	588.4	0.051	294.2	0.051	294 2
7542	5383.9	0.000	0.0	0.000	0.0	0.000	0.0	0.071	449.7	0.034	214.2	0.037	235.6
7622	4742.8	0.000	0.0	0.000	0.0	0.000	0.0	0.076	419.0	0.072	396.9	0.004	22.1
7958	5818.8	0.000	0.0	0.000	0.0	0.000	0.0	0.010	63.9	0.000	0.0	0.010	63.9
7962	6524.5	0.000	0.0	0.000	0.0	0.000	0.0	0.005	35.2	0.005	35.2	0.000	0.0
7964	6031.6	0.000	0.0	0.000	0.0	0.000	0.0	0.014	95.4	0.010	71.5	0.003	23.8
8006	5232.2	0.000	0.0	0.000	0.0	0.000	0.0	0.156	952.7	0.087	532.4	0.069	420.3
8110	5331.3	0.000	0.0	0 000	0.0	0.000	0.0	0.007	44.7	0.000	0.0	0.007	44.7
6796	4218.6	0.003	16.1	0.000	0.0	0.003	16.1	0.038	186.2	0.036	149 0	0.008	37.2
6823	5262.1	0.003	16.1	0.000	0.0	0.003	16.1	0.000	0.0	0.000	0.0	0.000	0 0
6853	3548.1	0.004	16.1	0.000	0.0	0.004	16.1	0.068	283.6	0.030	126.1	0.038	157.6
6855	4451.3	0.003	16.1	0.003	16.1	0.000	0.0	0.039	203.1	0.028	147.7	0.011	55.4
7539	3219.9	0.009	32.3	0.009	32.3	0.000	0.0	0.028	183.6	0.017	82.1	0.011	41.4
7564	3737.0	0.007	32.3	0.007	32.3	0.000	0.0	0.144	627.7	0.061	266.3	0.083	361.4
7637	4313.0	0.003	16.1	0.000	0.0	0.003	16.1	0.250	1258.7	0.187	944.0	0.062	314.7
7859	4906.3	0.003	16.1	0.003	16.1	0.000	0.0	0.034	197.3	0.019	109.6	0.015	87.7
7874	5762.1	0.002	16.1	0.002	16.1	0.000	0.0	0.144	971.2	0.065	439.4	0.079	531.9
7905	4924.5	0.006	32.3	0.006	32.3	0.000	0.0	0.100	575.6	0.042	239.8	0.058	335.8
8098	4798.3	0.003	16.1	0.000	0.0	0.003	16.1	0.000	0.0	0.000	0.0	0.000	0.0
8216	5722.6	0.002	16.1	0.002	16.1	0.000	0.0	0.022	147.2	0.016	105.1	0.006	42.0
6780	4539.9	0.033	177.4	0.030	161.3	0.003	16.1	0.082	435.4	0.060	319.3	0.022	116.1
6868	4324.9	0.010	48.4	0.006	32.3	0.003	16.1	0.163	823.2	0.108	548.8	0.054	274.4
7212	3269.1	0.013	48.4	0.008	32.3	0.004	16.1	0.147	561.3	0.125	477.1	0.022	84.2
7258	4348.5	0.013	64.5	0.013	64.5	0.000	0.0	0.029	148.7	0.017	85.0	0.013	63.7
7588	5024.0	0.008	48.4	0.000	0.0	0.008	48.4	0.092	539.3	0.055	323.6	0.037	215.7

*Bundles, clusters, and matrices

Table B-2. Data Listing of Results from Studies 1 Through 5 (See Text for an Explanation of Units of Concentration.)

	Direct Transfer		Indirect Transfer	
Study	**Sample ID**	**s/cc**	**Sample ID**	**f/cc**
1	7159	0.0000	7159	0.009
1	7206	0.0000	7206	0.000
1	7275	0.0000	7275	0.025
1	7518	0.0000	7518	0.051
1	7542	0.0000	7542	0.034
1	7622	0.0000	7622	0.072
1	7958	0.0000	7958	0.000
1	7962	0.0000	7962	0.005
1	7964	0.0000	7964	0.010
1	8006	0.0000	8006	0.087
1	8110	0.0000	8110	0.000
1	6796	0.0030	6796	0.030
1	6823	0.0030	6823	0.000
1	6853	0.0040	6853	0.030
1	6855	0.0030	6855	0.028
1	7539	0.0090	7539	0.017
1	7584	0.0070	7584	0.061
1	7637	0.0030	7637	0.187
1	7859	0.0030	7859	0.019
1	7874	0.0020	7874	0.065
1	7905	0.0060	7905	0.042
1	8098	0.0030	8098	0.000
1	8216	0.0020	8216	0.016
1	6780	0.0330	6780	0.060
1	6868	0.0100	6868	0.108
1	7212	0.0130	7212	0.125
1	7258	0.0130	7258	0.017
1	7588	0.0080	7588	0.055
2	CD13	0.0010	CD14	3.740
2	CD21	0.0000	CD22	5.850
2	CD10	0.0040	CD9	36.950
2	FB4	0.9980	FB3	21.600
2	J23	0.0000	J24A	14.350
2	J55	0.0020	J56	8.730
2	J58	0.0072	J57	13.000
2	J60	0.0000	J59	3.350
2	J62	0.0010	J61	2.060
2	85-451	0.0010	KE22	13.100

Table B-2. (continued) Data Listing of Results from Studies 1 Through 5

	Direct Transfer		Indirect Transfer	
Study	**Sample ID**	**s/cc**	**Sample ID**	**f/cc**
2	85-484	0.0010	KE23	7.075
2	85-450	0.5930	KE24	31.900
2	85-445	0.0000	KE36	1.595
2	85-435	0.0000	KE37	5.240
2	85-389	0.0000	X005-3-3	0.096
2	85-308	0.0841	081-10M	5.890
2	85-324	3.7000	081-11	2.200
2	85-319	0.0000	081-12	26.500
2	85-307	0.0000	081-4-3	0.010
2	85-82	0.0460	102-11	7.060
2	85-87	0.0460	102-13	16.000
2	85-361	0.0020	102-4-3	23.000
3	73	0.0040	1	123.173
3	72	0.0630	4	96.908
3	150	0.0000	19	21.729
3	153	0.0820	20	29.487
3	52	0.0100	23	12.548
3	53	0.0000	24	0.228
3	49	0.0000	26	0.019
3	59	0.0000	30	12.282
3	60	0.0050	31	25.163
3	61	0.0000	32	0.170
3	66	0.0000	33	1.479
3	68	0.0080	35	45.464
3	118	0.0000	41	0.365
3	117	0.0000	42	0.007
3	80	0.0040	43	0.179
3	76	0.0840	45	100.916
3	137	0.0000	51	0.063
3	138	0.0000	52	0.007
3	140	0.0000	53	0.000
3	96	0.0050	57	6.294
3	101	0.0270	58	5.487
3	111	0.0070	59	1.913
3	135	0.0060	61	0.135
3	128	0.0110	62	11.340
3	134	0.0020	63	6.787

Table B-2. (continued) Data Listing of Results from Studies 1 Through 5

	Direct Transfer		Indirect Transfer	
Study	**Sample ID**	**s/cc**	**Sample ID**	**f/cc**
3	133	0.0070	64	18.527
3	125	0.0000	65	9.669
3	126	0.0000	66	0.092
3	63	0.0040	75	0.161
3	55	0.0950	78	44.918
3	70	0.0000	81	3.872
3	71	0.0040	82	4.724
3	88	0.0050	85	54.176
3	85	0.1220	89	100.358
3	81	0.0020	94	0.100
3	92	0.0120	96	18.319
3	91	0.0000	97	0.146
3	162	0.0040	103	36.529
3	43	0.0000	112	4.347
3	45	0.0000	114	1.564
3	46	0.0000	115	0.217
3	40	0.0130	117	5.953
3	93	23043.8800	119	10.548
3	106	0.0220	122	24.763
3	157	0.0050	124	1.018
3	145	0.0000	126	0.000
4	1	0.0066	9	27.700
4	2	0.0310	27	79.700
4	3	0.0000	36	0.200
4	4	0.0071	74	11.130
4	5	0.0000	84	2.319
4	6	0.0012	86	0.637
4	7	0.0000	87	0.000
4	8	0.0000	88	0.314
4	9	0.0084	93	0.113
4	10	0.1935	95	42.843
4	11	0.1171	105	63.520
4	12	0.0036	115	0.216
4	13	0.0000	117	5.953
4	14	0.0111	119	10.548
4	15	0.0287	122	24.762

Table B-2. (continued) Data Listing of Results from Studies 1 Through 5

Study	Direct Transfer Sample ID	Direct Transfer s/cc	Indirect Transfer Sample ID	Indirect Transfer f/cc
5	LA39	0.1400	LA39	2.600
5	LA47	0.0640	LA47	0.230
5	SH43	0.0720	SH43	1.300
5	SH51	0.1100	SH51	3.000
5	LA23	0.0160	LA23	0.110
5	LA41	0.0130	LA41	0.170
5	LA49	0.0180	LA49	0.039
5	QP45	0.0250	QP45	0.120
6	72	0.0630	72	0.047
6	76	0.0840	76	7.723
6	128	0.0110	128	0.702
6	118	0.0000	118	0.323
6	66	0.0000	66	0.421
6	68	0.0080	68	0.667
6	40	0.0130	40	0.120
6	45	0.0000	45	0.037
7	7144A	0.0990	7144A	0.262
7	7144B	0.1100	7144B	0.235
7	7144C	0.0910	7144C	0.178
7	9040	0.1000	9040	0.513
7	9041	0.1600	9041	0.448
7	9042	0.2910	9042	0.516
7	9061	0.0740	9061	0.033
7	9062	0.2150	9062	0.071
7	9063	0.0200	9063	0.076
7	4221	0.0500	4221	0.158
7	4222	0.0700	4222	0.099
7	4223	0.0840	4223	0.230

Appendix C: Correlation Coefficients for Study 1 (USEPA 1988)

Table C-1. Measures of Association Between Direct and Indirect TEM Measurements Obtained in Study 1 (The Asymptotic Standard Error is Given in Parentheses.)

Comparison	Pearson Correlation	Spearman Correlation
Indirect s/cc vs. direct s/cc	0.21 (0.11)	0.35 (0.16)
Indirect f/cc vs. direct s/cc	0.26 (0.12)	0.39 (0.16)
Indirect f/cc vs. direct f/cc	0.16 (0.13)	0.31 (0.17)
Indirect f/cc vs. direct b/cc	0.37 (0.12)	0.34 (0.17)
Indirect b/cc vs. direct s/cc	0.08 (0.11)	0.31 (0.16)
Indirect b/cc vs. direct f/cc	0.06 (0.11)	0.29 (0.16)
Indirect b/cc vs. direct b/cc	0.07 (0.14)	0.11 (0.18)
Indirect s/mm^2 vs. direct s/mm^2	0.15 (0.10)	0.29 (0.16)
Indirect f/mm^2 vs. direct s/mm^2	0.20 (0.10)	0.34 (0.17)
Indirect f/mm^2 vs. direct f/mm^2	0.12 (0.12)	0.28 (0.17)

Table C-1. (continued) Measures of Association Between Direct and Indirect TEM Measurements Obtained in Study 1 (The Asymptotic Standard Error is Given in Parentheses.)

Comparison	Pearson Correlation	Spearman Correlation
Indirect f/mm^2 vs. direct b/mm^2	0.28 (0.11)	0.27 (0.19)
Indirect b/mm^2 vs. direct s/mm^2	0.04 (0.10)	0.23 (0.16)
Indirect b/mm^2 vs. direct f/mm^2	0.04 (0.10)	0.26 (0.17)
Indirect b/mm^2 vs. direct b/mm^2	0.02 (0.14)	0.00 (0.19)

s = Structure Concentration
f = Fiber Concentration
b = BCM Concentration
cc = Cubic Centimeter of air
mm^2 = Square Millimeter of Filter

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16. Abstract (Limit: 200 words)

A subset of air samples from a 1988 EPA study were reanalyzed for asbestos by TEM using an indirect transfer technique. The samples were originally analyzed using a direct transfer technique. This document presents the results of the reanalysis and extends the discussion to include data from six other studies. The development of the two techniques and their respective advantages and disadvantages are described.

The data support the general opinion that TEM analysis of air samples using indirect transfer methods tends to provide estimates of total airborne asbestos structure concentration that are higher than those obtained using direct transfer methods. There is no single factor that can be used to convert measurements made by one method to a value that is comparable with measurements made by the other because the quantitative relationship is expected to depend on details of the sampling and analytical protocols and the nature of the asbestos in the air. The ratio of indirect measurements to direct measurements ranges from 3.8 to 1,700 for the studies considered.

Additional research is needed to determine which transfer technique more accurately reflects biologically meaningful airborne asbestos concentrations. Breakdown of larger structures into smaller ones during indirect preparation does not appear to be sufficient to explain the difference in measured concentrations. Alternative hypotheses involving interference by debris and association of unattached structures may also be important.

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