Precision of Analysis for Waterborne Chrysotile Asbestos by Transmission Electron Microscopy

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Abstract

Detailed examinations of water samples were carried out to determine the precision of analysis for chrysotile asbestos by transmission electron microscopy (TEM). Since the frequency distribution of counts fits a Poisson distribution, several statistical inferences were made, including (1) an estimate of precision, and (2) a model for determining the probability of observing chrysotile as a function of its concentration in water and magnitude of area scanned by TEM.

Introduction

Asbestos is a generic term for a number of fibrous silicate minerals such as chrysotile, amosite, grunerite, tremolite, crocidolite, anthophyllite, and actinolite. In 1970 chrysotile accounted for about 95% of the 750,000 short tons of asbestos used commercially in the U.S. (STATISTICAL ABSTRACT 1974; SPEIL, LEINEWEBER 1969). There are many reports which indicate that asbestos is an ubiquitous environmental contaminant. It has been found in ambient air (SELIKOF et al. 1972 ; HOLT, YOUNG 1973; HENRY et al. 1972), indoor air (HENRY et al. 1972), parenteral drugs (NICHOLSON et al. 1972), beverages (CUNNINGHAM, PONTEFRACT 1971; CUNNINGHAM, PONTEFRACT 1973; BILES, EMERSON 1968), food (MERLISS 1971), cosmetics (BLEJER, ARLON 1973), the vicinity of asbestos manufacturing operations (HARWOOD, BLASZAK 1974; HARWOOD et al. 1975; RICHARDS 1973) and water supplies (CUNNINGHAM, PONTEFRACT 1971; DURHAM, PANG 1975; COOK et al. 1974; KAY 1974; THE GREAT LAKES RESEARCH ADVISORY BOARD 1975; AMERICAN WATER WORKS ASSOCIATION 1974). Our knowledge of the health effects of asbestiform minerals is derived mainly from studies of occupationa exposure. There is reason to expect that a large fraction of inhaled asbestos is swallowed and thereby constitutes an ingestion exposure (EVANS et al. 1973). This ingestion exposure may be causally related to the excess of digestive system cancers in several occupational studies (SELIKOFF et al. 1973; SELIKOFF 1974; NEWHOUSE 1973).

Our interest in the health significance of environmental asbestos has led to our examination of electron microscopic (EM) methods for the detection of waterborne chrysotile. The concentration of asbestos in water is generally reported in terms of fibers

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or fibrils per liter. A fiber is a reducible unit of asbestos which is capable of further longitudinal splitting into fibrils. Henceforth, the term "unit" will be used to refer to either a fibril or fiber.

Published reports of the asbestos content of various media generally do not contain an estimate of precision (SELIKOFF et al. 1972; HOLT, YOUNG 1973; HENRY et al. 1972; NICHOLSON et al. 1972; CUNNINGHAM, PONTEFRACT 1971; CUNNINGHAM, PONTEFRACT 1973; BILES, EMERSON 1968; MERLISS 1971; BLEJER, ARLON 1973; HARWOOD, BLASZAK 1974; HARWOOD et al. 1975; RICHARDS 1973; DURHAM, PANG 1975; COOK et al. 1974; KAY 1974; THE GREAT LAKES RESEARCH ADVISORY BOARD 1975; AMERICAN WATER WORKS ASSOCIATION 1974). We suggest that it is very important to define the precision of EM methodology since only a very small percentage of a collected sample is actually examined. For example, only about 0.01% of a water sample is actually examined by EM. This paper describes the method used and results obtained in our effort to determine the precision of the analysis for waterborne chrysotile by transmission electron microscopy (TEM).

Experimental Procedure

The methods and statistics developed here apply to quantitation of chrysotile asbestos in drinking water. Each of two drinking water samples of groundwater origin were analyzed for their content of chrysotile asbestos using the following procedure.

1. A sample was mixed by multiple inversions.

2. 200 ml of water were filtered through a Millipore membrane filter of 0.45µm pore size and 47mm diameter within 24 hours of collection.

3. The filter was placed in a petri dish and allowed to dry in a vacuum dessicator.

4. A randomly selected 3.05mm punch from each filter was placed on a 200 mesh copper TEM grid of equal diameter. The grid substrate was carbon coated 0.25% Formvar.

5. Acetone was filtered through a 0.1µm Nucleopore membrane filter and used to charge the boiler of a condensation washer. The grid-punch combination was placed in the condensation washer for 2 hours with an acetone reflux rate of approximately 1 drop/second.

6. Ten grid squares were randomly selected and examined by TEM at 26,280x and 100kv. Chrysotile asbestos was confirmed by both morphology and selected area electron diffraction (SAED) pattern. In general, there are three types of information needed for the complete identification of asbestos: morphological information obtained by high magnification TEM, crystallographic information obtained by SAED patterns, and microchemical information obtained

by energy dispersive spectrometry. There is good agreement among investigators that the morphology and SAED pattern of chrysotile are sufficiently distinct to permit visual identification by TEM (CUNNINGHAM, PONTEFRACT, 1971; RICHARDS, 1973; KAY, 1974; SKIKNE et al., 1971; CHATFIELD, PULLAN, 1974; KAY, 1973)

7. Steps 4-6 were repeated for 9 more punches from the same membrane filter. Thus 10 punches were randomly selected from each membrane filter.

Results

For each of the two drinking water samples 10 grid squares from each of 10 grids were scanned for their content of chrysotile units. Hence the basic unit of observation is a single grid square. Table 1 presents the frequency distributions of counts. Selected descriptive statistics and estimation values are presented in Table 2.

TABLE 1

Frequency Distributions of Chrysotile Units by Grid Square

Count of Chrysotile Units/grid square	Number of Grid Squares Sample A	Sample B
0	95	93
1	4	7
2	1	0
TOTAL	100	100

TABLE 2

Selected Descriptive Statistics and Estimation Values

		Sample A	Sample B
(1)	Number of grid squares scanned, N	100	100
(2)	Standard deviation, s (units/grid square)	0.28	0.26
(3)	Variance, s^2	0.08	0.07
(4)	Variance test $x^2 = (N-1)\ s^2/\bar{x}$	127.4	93.1
	p-value	0.06	0.70
(5)	Point estimates and confidence interval		
	(a) Mean, $\bar{x}$ (units/grid square)	0.06	0.07
	(b) $8.41 \times 10^5\ \bar{x}$ (units/liter)	5.05×10^4	5.89×10^4
	95% confidence interval		
	Lower limit (units/liter)	1.85×10^4	2.36×10^4
	Upper limit (units/liter)	1.10×10^5	1.21×10^5

Discussion

The variance test was performed to test the Poisson goodness-of-fit for the distribution of chrysotile units per grid square (COCHRAN 1954). The results given in item 4 of Table 2 show a good fit for both water samples. Thus, we may state that the number of chrysotile units per grid square follows a Poisson distribution.

The number of chrysotile units is usually presented in terms of number per liter of water. The conversion from average units per grid square to units per liter is as follows.

Area of one grid square = 8.236×10^{-5} cm^2
Effective filter area = 13.847 cm^2
Volume of water filtered = 200 ml
Units/liter = (Average units/grid square)/(area of one grid square) x (total filter area) x (dilution factor) = $(8.41 \times 10^5)\bar{x}$

Since we recognize that the number of chrysotile units per grid square follows a Poisson distribution, confidence intervals can readily be obtained (PEARSON, HARTLEY 1958). The 95% confidence intervals are given in item 5 of Table 2. It is interesting to note that confidence intervals can be set up even if $\bar{x} = 0$. For example, the 95% confidence limits for counting 100 grid squares with $\bar{x} = 0$ are 0 and 3.10×10^4 units per liter.

When the distribution of the number of chrysotile units per grid square is understood, statistical tests can be carried out to test the hypothesis that two water samples have the same number of chrysotile units. After the statistical test was applied to the two water samples, there was not enough evidence to say that the two water samples contained different levels of chrysotile units per liter ($p>0.10$) (PEARSON, HARTLEY 1958).

Usually 10 to 20 grid squares are scanned in routine analyses. If the number of chrysotile units per grid square follows a Poisson distribution with the expected value, λ, we will be able to predict how many zero counts will be encountered. Given in Table 3 are the Poisson probabilities of the number of chrysotile units per grid square for four values of λ. The best estimate of λ is the sample mean $\bar{x}$. If we know the approximate value of λ for a given water sample, then the Poisson probability of finding a zero count in a single grid square, P_0, can be obtained as in Table 3. The probability that N grid squares will yield a zero count is simply P_0^N. Table 4 presents P_0^N for several values of λ and N. It is obvious that counting only 10 grid squares for $\lambda \leq 0.08$ would result in a zero count more than 45% of the time; the conventional counting of 20 grid squares would result in a zero count more than 20% of the time. For $0.06 < \lambda < 0.07$, 50 grid squares should be counted to reduce the probability of a zero count to less than 5%. In order to exclude the possibility that counts were made on background contamination, six blank grids were prepared by washing pure Millipore membrane filter punches onto grids. A total of 45 grid

squares were scanned and no chrysotile units were confirmed. If $0.06 \leq \lambda \leq 0.07$ there is only a 4-7% probability of observing no units in 45 grid squares. Thus it is unlikely that background contamination can account for the chrysotile units confirmed in the two groundwater samples.

TABLE 3

Poisson Probabilities as a Function of λ and Count/Grid Square

	λ			
Count of Chrysotile Units/Grid Square	0.05	0.06	0.07	0.08
0	0.951	0.942	0.932	0.923
1	0.048	0.057	0.065	0.074
$\geq$ 2	0.001	0.001	0.003	0.003

TABLE 4

Probability of a Zero Count as a Function of λ and Number of Grid Squares Scanned

	λ			
N	0.05	0.06	0.07	0.08
10	0.61	0.55	0.49	0.45
20	0.37	0.30	0.24	0.20
30	0.22	0.17	0.12	0.09
40	0.13	0.09	0.06	0.04
45	0.10	0.07	0.04	0.03
50	0.08	0.05	0.03	0.02

Increasing the number of grid squares to be scanned will inevitably increase cost and time. One can solve this problem by filtering large volumes of water. Another possible solution to the problem is based on the property that the sum of k Poisson variates with $\lambda_1, \lambda_2, \ldots \lambda_k$ is a Poisson variate with an expected value of $\lambda_1 + \lambda_2 + \ldots + \lambda_k$ (HALD 1952). For a given water sample, all λ's are of identical value. As an illustration of this point, the data obtained by scanning 100 grid squares was converted to units per grid data (see Table 5). The Poisson distribution of chrysotile units per grid square can be generalized to the Poisson distribution of chrysotile units per grid (CHAKRAVARTI, RAO 1959). It also follows that it is theoretically possible to stack several filter punches vertically on a single grid and still maintain a Poisson distribution of grid square and grid counts. This procedure may obviate the need to filter large volumes of water.

TABLE 5

Frequency Distributions and Means
of Chrysotile Units by Grid

Count of Chrysotile Units/Grid	Number of Grids	
	Sample A	Sample B
0	6	4
1	3	5
2	0	1
3	1	0
TOTAL	10	10
Mean (units/grid)	0.6	0.7

Conclusions

It is important to report the precision of an observed count of asbestos units. Equally important is an estimate of the probability of observing asbestos units as a function of concentration and area scanned. A model for specifying precision and probability has been described for chrysotile units in the 10^4 - 10^5 units per liter range. Further research is needed: (1) to determine whether the Poisson distribution also holds for higher concentrations of chrysotile, and (2) to verify that the distributions of grid square and grid counts follow a Poisson distribution for stacked punches.

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