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RESEARCH & ENGINEERING DIVISION

The Estimation of Asbestos in Liquids

1. Introduction

Since the original publication of the letter in Nature by Biles and Emerson¹ who reported observing asbestos fibres in some beers, TBA Industrial Products Ltd has undertaken a programme of research to develop suitable electron microscopic techniques to quantify the asbestos, if any, in various liquid media. Using such techniques, a number of wines and beers have been analysed. Since then electron microscopy has been used by a number of workers. Cunningham and Pontefract² have reported their results on a number of beers, wines and water samples. Recently Nicholson, Maggiore and Selikoff³ have discussed their findings of asbestos in a few parenteral drugs.

2. Limit of detection of electron microscopic techniques

In principle, a 1 μ long single fibril fragment can be identified by its electron diffraction pattern. However, a practicable lower limit of detection for asbestos in liquids was initially presumed to be about 100 fibrils per litre. Taking a typical fibril fragment to be about 1 μ long and 300 $\text{\AA}$ in diameter, it would have a mass of 2×10^{-15} g and 100 fibrils in a litre would be equivalent to 10^{-13} g/litre. If a 15 ml sample of this liquid was taken it should contain only 1 or 2 fibrils. An electron microscopic specimen would therefore distribute the 1 or 2 fibrils on the 1000 grid squares. An analysis of such a distribution/concentration would be statistically very unreliable, if indeed it was even practicable. In most such techniques a limited number of squares are examined, say five grid squares per specimen. If there was a probability of at least one fibril per square, then such a measurement may carry some weight. A concentration of 1 fibril per grid square from a 15 ml sample would be equivalent to about 60 000 fibrils per litre or 10^{-10} g/litre. This represents a realistic limit of detection.

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3. Background levels

It is obviously extremely important to ensure clean conditions during specimen preparation and examination, as contamination from the laboratory ware and the various analytical procedures could lead to very erroneous results. This is partly because in all electron microscopic techniques the ultimate sample examined is a minute fraction of the total sample and very large conversion factors are used to calculate the amount of asbestos actually present in the sample. Thus any contamination to the electron microscopic specimen also gets multiplied by the same large conversion factors. Finally, for a given concentration, the larger the volume of the samples the larger the actual asbestos content of the sample, thus reducing the importance of contamination in specimen preparation.

It is essential that background levels are measured on blanks at the same time as test specimens. In the work reported here, blank samples have generally ranged from 10^{-9} g/litre to 10^{-8} g/litre.

The details of the electron microscopic technique have been described by Rickards⁵ and in Appendix 1. Method A described in the appendix was used for the beer and wine samples and method B for the drug samples.

4. Samples

The following samples were analysed:-

- (a) Cans of one commercial British and one U.S. beer
- (b) Samples of test wines collected after differing amounts of wine had passed through the filters
- (c) Samples of initial water and final filtered beer collected from a brewery
- (d) A sample of streptomycin sulphate obtained from a leading international pharmaceutical manufacturer
- (e) A series of samples simulating the manufacture of human blood protein.

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5. Results

The chrysotile asbestos contents of these samples are shown in Tables I to V and are shown in a scalar diagram in Fig 1.

6. Discussion

As mentioned in Section 3 it has been our experience that the most reliable approach is to constantly carry out blank tests, simultaneously with test specimens, and ensure that the differences observed between the two are statistically significant. In this respect it is a little surprising that Cunningham and Pontefract did not observe any asbestos fibres in their blank runs. Contamination is particularly erratic when the specimen preparation involves exposure to air, e.g. evaporation under a heat lamp (Cunningham and Pontefract) or when the nitro-cellulose film is allowed to dry (Nicholson et al).

The significance of the contamination also increases in importance as the size of the sample decreases. TBA used 15 ml samples in initial studies but now prefer to use 500 ml to 1000 ml samples. Cunningham and Pontefract used samples of 400 ml but Nicholson et al used only 1 ml. This perhaps would explain why even the background level claimed by Nicholson et al is higher than the results obtained by other investigations (Fig 1).

One of the striking features in Fig 1 is that the results quoted by the three sets of investigators fall into three distinct bands with no overlap between them. This is all the more surprising considering the wide range of samples examined by Cunningham and Pontefract. The results suggest that very broadly each set of workers have been measuring essentially the background level inherent in their techniques and laboratories. Because of the very small samples used by Nicholson et al, one might expect the contamination to be more significant and this is indeed the case, even their background level being higher than all the results reported by others. TBA have devoted considerable effort to ensure the statistical significance of the observed data and hence very low background

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levels of 10^{-8} g/litre to 10^{-9} g/litre have been achieved. These are very close to the practicable limit of detection of 10^{-10} g/litre referred to earlier. Cunningham and Pontefract appear to have intermediate values in spite of their statement that their blank runs contained no asbestos at all, i.e. they claimed their background level as zero.

Allowing for the scatter of results it is, however, possible to draw the following conclusions:-

- (1) In the series of samples obtained from the brewery (Table III) there is no evidence that the use of asbestos filters introduced any additional asbestos fibres. The same conclusion can also be reached from Cunningham and Pontefract's results.
- (2) TBA examination, using 1000 ml samples of a drug, led to the lowest value recorded at TBA, a value 10 000 times less than that reported by Nicholson, Maggiore and Selikoff for a similar drug.
- (3) When the last sample in the test series (Table II) had an asbestos content of 10^{-4} g/litre, (comparable with levels reported by Nicholson et al for drugs) the electron microscope fields of view were so crowded that numerical counting was out of question. In fact an X-ray diffractometer was used to measure the amount of asbestos present by the technique described by Rickards⁴.

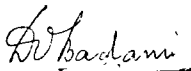
If therefore Nicholson et al's results were truly representative, then there should be sufficient asbestos in a litre of sample to give a strong X-ray diffraction pattern, since even 10^{-5} g can be detected by the TBA X-ray technique⁵.

Conversely if 10^{-8} g of asbestos were actually present in the ~1 ml samples used by Nicholson et al, it is equivalent to about 10^8 fibrils of the size that they claim are present in these. (Since their sample preparation involved actually grinding for 2 to 5 minutes before analysis, the authors could not in fact have had any information about the size distribution present in those actual samples). For their electron microscopic examination, Nicholson

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et al used half the filter and hence this should have still contained approximately 10^7 to 10^8 such fibrils (i.e. ten million to 100 million fibrils). The authors state that a known fraction of the air dried film was put in the electron microscope and two to four grid squares were examined. If, say, two squares contained 20 fibrils, then the film specimen on the grid would have about 20×200 fibrils, i.e. 4×10^3 fibrils. If these calculations are justified, the implication is that only about one tenth thousandth of the air dried film was examined in the electron microscope. There is no suggestion in the paper that even a duplicate film specimen was examined.

On the basis of the data presented by Nicholson et al, it is impossible to state, with any scientific credibility that there "is an indication of significant asbestos contamination of some parenteral drugs at this time". On the other hand, investigations carried out by TBA suggest asbestos levels of 10^{-9} g/litre. This corresponds, in terms of units used by Nicholson et al, to 10^{-12} grams of asbestos per gram of injection.


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References

1. Biles, B and Emerson, T. R. Nature 219,93 (1968)
2. Cunningham, H. M. and Pontefract, R. Nature 232,332 (1971)
3. Nicholson, W. J., Maggiore, C. J. and Selikoff, I. J. Science 177, 171 (1972)
4. Rickards, A. L. Analytical Chemistry, 44 1872 (1972)
5. Rickards, A. L. Analytical Chemistry, 45, 809,(1973)

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TABLE 1

Chrysotile Analyses of British and U.S. Beers

<u>Sample</u>	<u>Chrysotile Content (g/litre)</u>
U.S. Beer	5×10^{-9}
British Beer	8×10^{-8}
British Beer	3×10^{-8}
British Beer	4×10^{-8}
British Beer	8×10^{-8}

TABLE 2

Chrysotile Analyses of an experimental British Wine after passing through an asbestos cellulose sheet filter

<u>Sample</u>	<u>Chrysotile Content (g/litre)</u>
100 gal.	6×10^{-9}
9000 gal.	7×10^{-9}
10000 gal.	1×10^{-8}
17700 gal.	5×10^{-9}
20000 gal.	8×10^{-9}
23000 gal.	2×10^{-8}
25000 gal.	4×10^{-4} (measured by X-ray technique)

TABLE 3

Chrysotile Analyses of Water and Beer obtained at various stages through a commercial brewery

<u>Sample</u>	<u>Chrysotile Content (g/litre)</u>
Well head	2×10^{-9}
Well head	4×10^{-9}
Well head	3×10^{-8}
Water Tank Inlet	8×10^{-9}
Water Tank Inlet	2×10^{-8}
Water Tank	2×10^{-8}
Water Tank	4×10^{-9}
Fined Beer	3×10^{-8}
Rough Filtered Beer	4×10^{-9}
Rough Filtered Beer	4×10^{-8}
Fine Filtered Beer	6×10^{-8}
Fine Filtered Beer	1×10^{-9}

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TABLE 4

Chrysotile Analyses of Streptomycin Sulphate

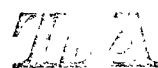
<u>Sample</u>	<u>Chrysotile Content</u>	
	<u>(g/litre)</u>	<u>(g/gram)</u>
Town Water	2×10^{-8}	2×10^{-11}
Post Asbestos Filter	3×10^{-8}	3×10^{-11}
Post Membrane Filter	2×10^{-9}	2×10^{-12}

TABLE 5

Chrysotile Analyses of Samples Simulating the Filtration Processes used in the Manufacture of Human Albumen

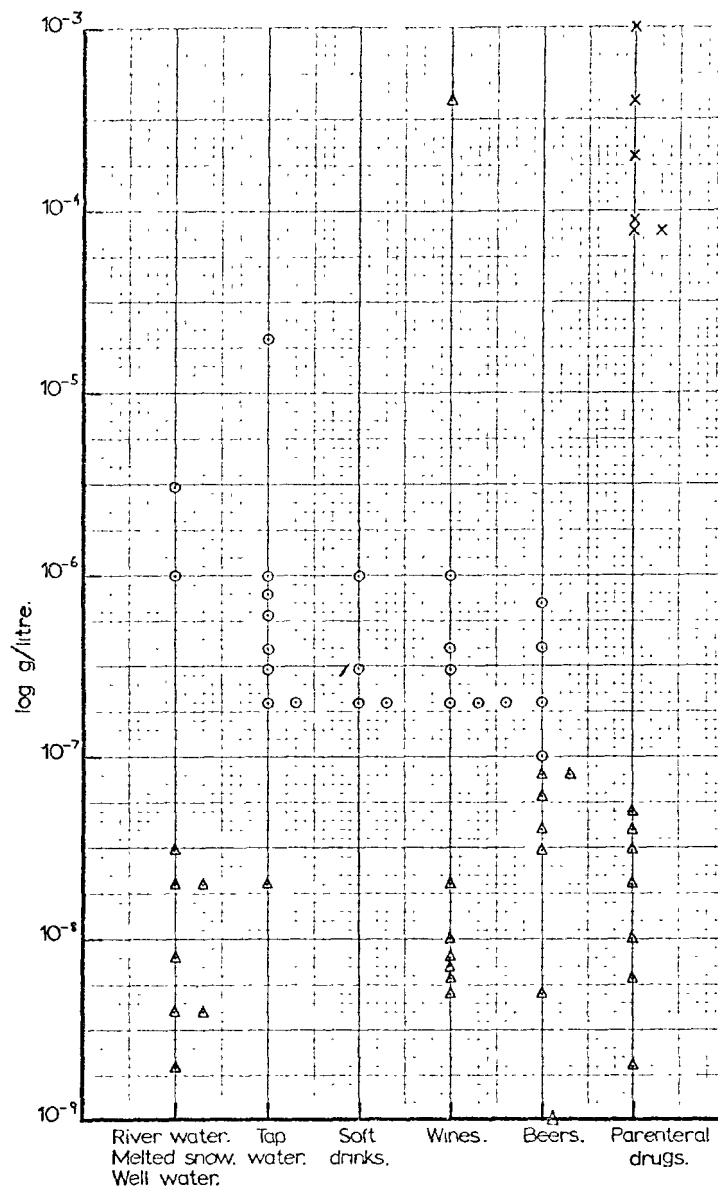
The samples were collected after passing through the filters in the sequence given in the Table.

<u>Sample</u>	<u>Chrysotile Content</u>	
	<u>(g/litre)</u>	<u>(g/gram)</u>
Post 15 x 40 cm EKS sheets		
1	$< 6 \times 10^{-9}$	$< 6 \times 10^{-12}$
2	3×10^{-7}	3×10^{-10}
3	3×10^{-8}	3×10^{-11}
Post "Lifeguard" pleated membrane		
1.	4×10^{-8}	4×10^{-11}
2.	2×10^{-8}	2×10^{-11}
3.	$< 6 \times 10^{-9}$	$< 6 \times 10^{-12}$
Post 20 Cox Filter Disks		
1.	6×10^{-8}	6×10^{-11}
2.	6×10^{-8}	6×10^{-11}
3.	3×10^{-8}	3×10^{-11}
Post 30" 0.22 μ m pore size Millipore pleated membrane		
1.	5×10^{-8}	5×10^{-11}
2.	$< 1 \times 10^{-8}$	$< 1 \times 10^{-11}$



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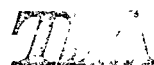
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o = Analyses published by Cunningham & Pontefract, Nature 232, 332 1971.

x = Analyses published by Nicholson et al Science, 177 171 1972.

Δ = Analyses by T.B.A. of wines, beers & drugs



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APPENDIX 1

Electron Microscopic Estimation Technique

The principle of the electron microscopic analytical technique is that chrysotile asbestos can be uniquely identified in an electron microscope by its characteristic electron diffraction pattern and morphology. Quantitative estimates are obtained by counting the number of fibres visible on an electron micrograph. Samples are prepared for electron microscopic analysis by one of two methods as follows:-

Method 'A'

1. 15 ml samples are centrifuged and the residue of suspended solids collected at the base of the tube.
2. The residue is washed with filtered deionised water, re-centrifuged and suspended in hydrogen peroxide. A further cycle of washing and centrifuging is followed by suspension in 20% potassium hydroxide at 50°C overnight to digest some of the organic material.
3. After a final wash the residue is suspended in 0.1 to 0.5 ml of acetone and centrifuged directly onto an electron microscope grid at the bottom of the collecting tube.
4. Electron microscopic examination is carried out by first checking the suitability of the specimen followed by a general scan to establish, using selected area electron diffraction, that any fibres present on the samples are chrysotile. Provided that the distribution of the fibres on the grid is uniform, fine areas are chosen, randomly, by spinning the specimen stage controls.
5. Fibre counting is carried out by totalling the number of fibres present on an enlarged micrograph of the recorded field of view. A convenient way of expressing the numbers of fibres is to count in units of $340\text{\AA}$ diameter by $1\text{ }\mu\text{m}$ length. This unit has been termed "a micron fibril", and has a mass of $2 \times 10^{-15}\text{g}$.

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6. The amount of asbestos present in a sample is calculated from the total length of chrysotile observed on a grid square, knowing the fraction of the total sample represented by a grid square. The final results are expressed in grams.

Method 'B'

1. One litre of sample is filtered through a 47 mm, 0.05 um mean pore size, cellulose nitrate membrane filter using either a vacuum or pressure filtration vessel.
2. After washing and drying the sample is ashed at 450°C for two hours taking special precautions to prevent explosive ignition of the membrane⁵.
3. The ashed residue is suspended in 1 ml of filtered deionised water using an ultra-sonic bath. The ultra-sonic treatment is continued for a minimum of eight hours to ensure that complete homogenisation of the sample occurs. Also, any chrysotile is released from agglomerated particles, and reduced to micron fibrils.
4. A 10 ul drop of the suspension is dried onto a carbon coated electron microscope grid for examination.
5. The specimen is examined in the electron microscope, and if suitable, eight fields of view are recorded at 1000x mag. taking every third grid square across a diameter of the grid.
6. A second sample from the same sample suspension is also examined so that sixteen fields of view are recorded from each sample.
7. A blank sample is prepared by substituting filtered deionised water for the sample at stage 1 and proceeding through the process as though it were a real sample. Eight fields of view are recorded from the blank sample.

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8. The amount of chrysotile present in the original sample is obtained by measuring the total length of chrysotile fibrils present on a grid square and relating that figure to the original sample after subtracting the value obtained from the blank sample. The results are expressed in grams.
9. The statistical significance of the results over the blank is determined.

Method 'A' is a technique which can be applied to liquid samples which are difficult to filter. Method 'B' is the preferred method, and has been successfully applied to the analysis of samples of airborne solids collected from the urban atmosphere⁵.

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Asbestos in Liquids: Addendum

The limits quoted are the detection limits (2×10^{-9} g/l) for that particular series of samples. The sample volumes were 0.5 l. If the sample volumes had been 5 l, then the detection limit would have been 2×10^{-10} g/l. The volume of samples is obviously important in establishing the significance of the analytical results over the laboratory background. A further factor which determines the detection limit is the amount of total solids present in the samples. If the amounts are large, then the E.M. specimens have to be diluted so that any asbestos fibres can be identified. In this case, the total solids were low and the samples were made up to 1 ml after ashing, (samples of urban airborne solids are made up to 100 ml). If the solids contents had been high and we had been forced to dilute the samples to 10 ml then the detection limit would have been 2×10^{-8} g/l.

The centrifuging method, which was used to obtain earlier results, uses 15 ml samples, and deposits the solids directly on to an E.M. grid. However, the final sample analysed on the E.M. grid is not very much greater than that in the ashing method. For example:-

Centrifuging gives;

15 ml centrifuged on to 1 E.M. grid

Ashing gives;

1 litre filtered, ashed and transferred to 1 ml

One 10 μ l drop transferred to E.M. grid

10^3 μ l = 1 ml

10 μ l = 10^{-2} ml

∴ E.M. specimen represents $\frac{1}{100}$ th of a litre = 10 ml

Both methods are influenced by the presence of large amounts of total solids and as the ashing method is simpler to apply and is so reliable, it is the preferred method.

A final point in understanding the detection limits of these methods, is that as the contamination from the laboratory fluctuates then the detection limit of the analytical procedure will also vary. A determination of the background is of course carried out for each sample by processing a simultaneous blank sample.

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