Asbestos Fibrils in Beverages. I. Gin

by

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The purpose of this study is to collect survey data on the presence of asbestos particles in commercially retailed beverages. This particular report is restricted to electron microscopic analyses of gin samples from four manufacturers.

MATERIALS AND METHODS

The general procedure of NICHOLSON, et al. (1972) was followed with modifications as described below. Samples of gin sold under four different labels were purchased as "miniatures" (0.1 pints) from retail outlets in Baltimore County, Maryland. The samples were prepared for analysis by filtering 10 ml of each sample through Millipore membrane filters (AA, pore size 0.8 μ, diameter 13 mm) in a Swinney hypodermic apparatus. The protocol for each sample was: filtration of 10 ml of deionized water through one filter, followed by filtration of 10 ml of sample through another filter, using the same syringe and Swinney adapter. All filtrations were done in a filtered-air hood with a laminar outflow pressure of 0.35 inches H_2O.

A total of 28 control and unknown samples were filtered: 14 control samples, 9 gin samples--3 from Company A, 1 from Company B, 3 from Company C, 2 from Company D--and 5 samples of water taken from the water supply of Company C.

Samples were prepared for electron microscopy by allowing the Millipore filters to dry on precleaned microscope slides in covered glass Petri dishes. The filters were subsequently ashed in the same vessels in an ashing oven at 500° C for 30 min. Slides bearing the ashed residue were covered with approximately 0.5% Formvar solution, which was allowed to dry. All operations after ashing were done in the filtered-air hood. When the Formvar film had dried, it was stripped from the glass slide by floating it onto the surface of Millipore-filtered water. The ashed residue adhered to the underside of the film, and was removed from the slide as the film was removed. 300-mesh copper grids (3.05 mm diameter) were deposited on the upper side of the film in areas overlying the ashed residue. The Formvar technique described here is

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routinely used in other EM applications where it is desirable to have a low contrast support film for the specimen to be examined.

The grids were examined in an RCA EMU 3-G electron microscope as follows. Samples of gins, supply water, and control water ("blanks") were assigned code numbers by one investigator. A second investigator was instructed to scan each of three grids from each coded sample at low magnification (2200 X) to ascertain any regions of particle or fiber concentration. Next, the region of greatest particle concentration was to be scanned at 9600 X over 4 contiguous grid squares (12,100 μ^2). Photographs were taken of any fiber observed in this area. If a grid appeared to be wholly negative, then 25 contiguous grid squares (each 55 x 55 μ) were scanned to confirm this appearance. The "blind" investigator recorded semi-quantitative findings for each sample during scanning as F, RF, VRF, N--fibers, rare fibers, very rare fibers, negative.

Each micrograph was scored for "asbestos fibrils" according to these predetermined criteria: (a) exposed to 30 minutes' ashing at 500° C (true of all fibrils); (b) 150-400 A diameter, and (c) where at least one fibril on the same grid displayed the internal "capillary" or microtubular morphology described by previous investigators for chrysotile asbestos (LANGER, et al., 1971; POOLEY, 1972). Each micrograph displaying material which did not meet all of the above criteria was recorded as "non-asbestos contamination."

As estimated maximum number of fibrils in each whole 10-ml sample was calculated by multiplying the number of fibrils observed in 4 grid squares by the factor (10.9 x 10^3). This factor is based on the assumption of a homogeneous distribution of fibrils throughout the sample, and it is derived from the areas of 4 grid squares, of the total grid, and of the total Millipore filter, as indicated in Table I.

TABLE I

Calculation of Factor for Estimated Total Number of Particles per 10 ml.

(A) open area of 4 grid squares, 300 mesh = 55 x 55 = 12,100 μ^2
(B) diameter of grid = 3.05 mm = 3,050 μ
(C) area of grid = pi x 1525 x 1525 = 7,302,462.5 μ^2
(D) (C)/(A) = 603.51
(E) area of grid = pi x 1.525 x 1.525 = 7.32 mm^2
(F) area of Millipore filter = pi x 6.5 x 6.5 = 132.67 mm^2
(G) (F)/(E) = 18.12
(H) (D) x (G) = 10,936 = conversion factor 10.9 x 10^3

RESULTS

The data obtained from each of the 28 samples tested, including blanks, is compiled in Table II. All gin samples from Companies A, B, and D were completely free of asbestos contamination. Asbestos was found in two of the 14 control samples; in both cases, the samples following these controls were negative. It should be noted that each odd-numbered sample is a control for the sample number immediately following it only. That is, the same syringe and Swinney adapter apparatus used for each even-numbered sample is first washed with distilled water; the filter through which this water is filtered is assigned the preceding odd number. Such "blanks," usually not included in published reports, are given here for the sake of completeness.

One of five water supply samples was positive for asbestos. The three samples of gin from Company C had significantly more asbestos fibrils than the samples from any other source.

Examples of the fibrillar morphology observed are illustrated in Figs. 1-3. Fibrils were almost always individual or in very small groups; this is most likely because of the instability of macrofibers of chrysotile asbestos in solutions of less than pH 10.8 (NAGY and BATES, 1952). The dissolution of native chrysotile into unit fibrils was not discouraged by our technique, however, since the individual fibrils allow better resolution of their internal morphology, and thus allow for more positive identification of asbestos in electron micrographs.

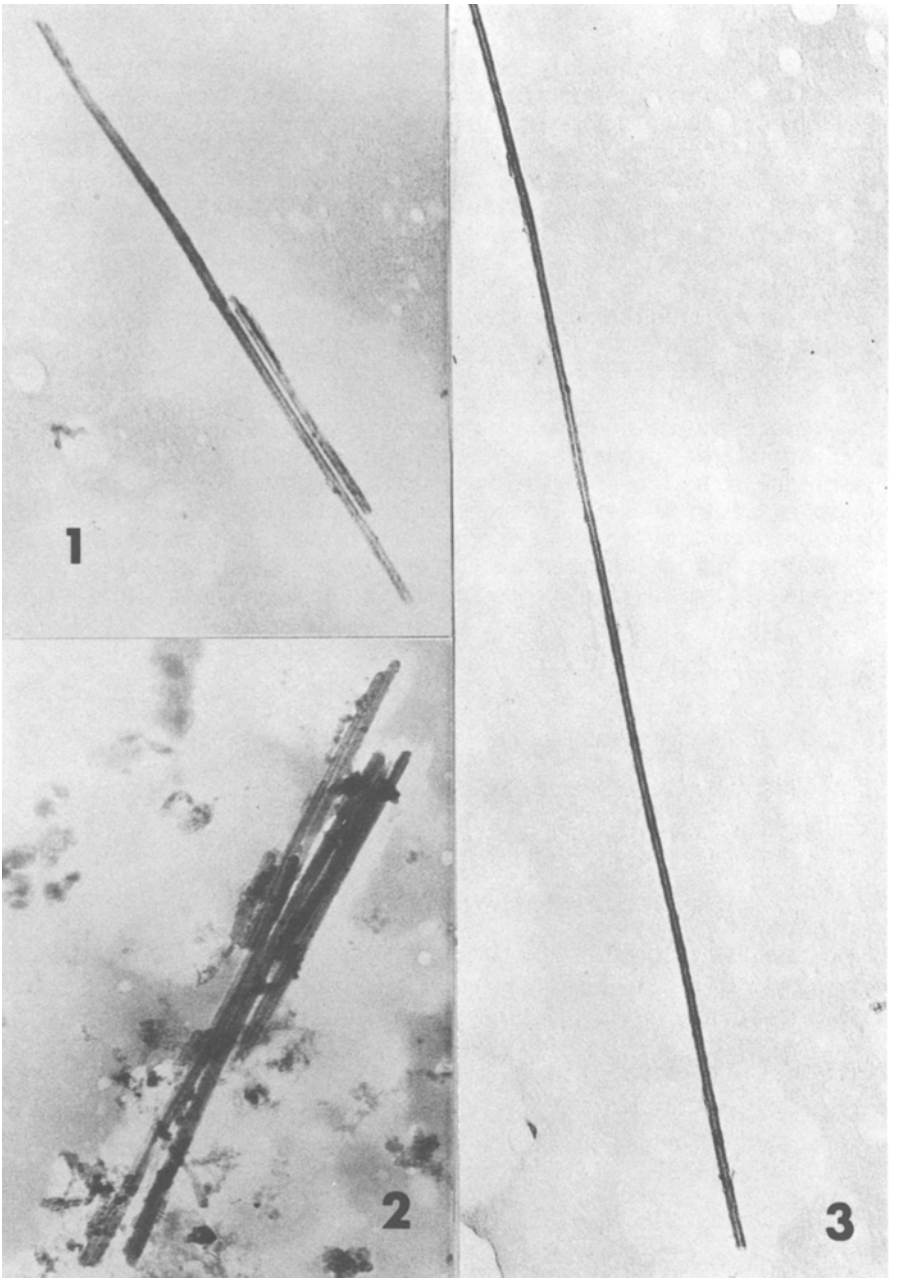
DISCUSSION

Increasing evidence of environmental asbestos contamination from other than airborne sources, or affecting organs other than the lung, is currently gaining attention as a possible hazard to health (BILES and EMERSON, 1968; CUNNINGHAM and PONTEFRACT, 1971; MERLISS, 1971; NICHOLSON, et al., 1972; SELIKOFF, et al., 1968; SELIKOFF, et al., 1972). Hence there is a need for independent investigators to find out where such contamination resides, and at the same time to develop a reliable survey method to monitor commercial substances. This method cannot be so exhaustive as to require the large amounts of time of a basic research project (e.g., 1969 to 1971, as reported by NICHOLSON, et al., 1972); but on the other hand, it must yield distinguishable results which are clearly indicative of asbestos contamination. In the practical sphere, an adequate survey method might be used by responsible government agencies to alert the responsible companies to undertake more thorough research into this aspect of their quality con-

TABLE II

Orig. No.	Code No.	Semi-quant.	FIBRILS, numbers of asbestos.	PRINTS, numbers, non-asb.	Sample	Projected fibrils (x 10^3) per 10 ml.
1	WS	WS			contr.	
2	24	N	0	0	gin A	
3	8	N	0	0	contr.	
4	9	RF	0	2	gin A	
5	1	RF	2	0	contr.	21.8
6	15	N	0	0	gin A	
7	WS	WS			contr.	
8	5	N	0	0	gin B	
9	4	RF	0	1	contr.	
10	11	F	12	1	gin C	130.8
11	WS	WS			contr.	
12	25	F	12	3	gin C	130.8
13	13	RF	0	4	contr.	
14	23	F	22	0	gin C	239.8
15	14	N	0	0	contr.	
16	19	N	0	0	gin D	
17	2	N	0	0	contr.	
18	18	N	0	0	gin D	
19	16	VRF	0	1	contr.	
20	17	VRF	3	0	water C	32.7
21	22	N	0	0	contr.	
22	21	N	0	0	water C	
23	7	N	0	0	contr.	
24	10	N	0	0	water C	
25	20	RF	0	3	contr.	
26	3	N	0	0	water C	
27	6	RF	8	0	contr.	87.2
28	12	N	0	0	water C	

Original number indicates order of filtration and ashing; code number indicates order of analysis in EM. "WS" = "won't strip," i.e., Formvar film could not be stripped from slide after ashing. Other abbreviations given in text.



Figs. 1-3. Examples of fibrillar morphology as observed after ashing. Magnifications are 43,200 X; 48,000 X; and 57,600 X respectively.

trol procedures. The method described in this report, which is basically an application of those employed by previous investigators, adequately fulfills these two prerequisites. By using a blank control for each sample, unexplained contamination as found in #27 invalidates one sample only.

In the present instance, the type of asbestos observed is almost certainly chrysotile. The length distribution for chrysotile as determined for U.I.C.C. Standard Reference Asbestos Samples shows 83.8% of the fibrils observed to be less than 2 microns in length (RANDALL, 1972). In addition, the internal capillary substructure referred to earlier is considered to be very good evidence of the presence of chrysotile (NICHOLSON, et al., 1972).

Since the method of scanning is biased toward the finding of fibrils, the projected numbers of fibrils for each 10 ml sample represent a probable maximum. Thus Sample #14, for example, could be said "to contain not more than 23,980 fibrils per cubic centimeter." Therefore the method used in this investigation is recommended for determining whether asbestos has been added to liquids during the course of their manufacture.

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