

# Use of Quantitative Analysis of Urine to Assess Exposure to Asbestos Fibers in Drinking Water In The Puget Sound Region

by Edwin S. Boatman,\* Tod Merrill,\* Angela O'Neill,\* Lincoln Polissar† and James R. Millette‡

An earlier epidemiologic and electron microscopy study of drinking water in the Everett area of Washington State indicated large numbers of naturally occurring chrysotile asbestos fibers in the water. The purpose of the present study was to determine whether significant numbers of asbestos fiber could be demonstrated in the urine of donors residing in that area for less than 3 yr and over 20 yr where the tapwater contained about  $200 \times 10^6$  fibers/L. A control group was obtained from Seattle where the tapwater asbestos fiber content was 100 times less. Urine samples, filtered control water, tapwater samples, and additional controls were processed for transmission electron microscopy by the use of the Nuclepore membrane filter-Jaffe wick procedure. Interference by mucos in the urine was reduced by treatment with hydrogen peroxide. Samples were taken over a period of 21 months. At no time during this period did the asbestos content of the urine samples consistently exceed that of the control waters. There was a significant difference ( $p < 0.05$ ) in the asbestos content of urine samples from subjects with  $< 3$  yr residence times versus  $> 20$  yr. Asbestos concentration in urine samples from Everett residents as a whole did not differ significantly from that in samples from Seattle residents. Variable degrees of chrysotile contamination of control water samples and of Nuclepore membrane filters presented a problem. At present, the data are inconclusive but would suggest no relationship between high concentrations of fibers in drinking water and the numbers estimated for voided urine.

## Introduction

There are now ample data to indicate that drinking waters taken from cities throughout the United States and Canada contain naturally occurring asbestos minerals (1-3). While chrysotile asbestos is the most prevalent form seen, amphibole asbestos is also found either alone or, more commonly, in admixture with the chrysotile variety. Millette et al. (4) found concentrations of chrysotile asbestos in drinking water ranging from greater than  $1 \times 10^6$  fibers/L to more than  $10 \times 10^6$  fibers/L in 186 out of 406 cities in the United States, and a study by Chatfield and Dil-

lon (5) indicated a similar range of concentrations in 71 locations across Canada. Other fluids, including wines (6) and beers (7), have also been shown to contain significant numbers of asbestiform fibers. From these and other observations, some obvious points of concern have arisen with respect to what happens to these fibers when imbibed. Do significant numbers penetrate the wall of the intestinal tract? Are they found in other organs of the body and body fluids? What is their effect, if any, of long-term exposure for 20 yr or more? Clearly, although it is easy enough to estimate the probable concentrations taken in during the consumption of water over time (without regard, of course, to the contributions from beverages and food), it is extremely difficult to determine the ratio of fibers retained or excreted. From the work of Cunningham et al. (8) on the accumulation of asbestos fibers in the feces of workers exposed to moderate to high industrial

\*Department of Environmental Health, University of Washington, Seattle, WA 98195.

†Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

‡Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268.

levels of chrysotile, it appears that around  $12\text{--}26 \times 10^6$  fibers/gm of feces (perhaps  $12\text{--}52 \times 10^8$  fibers in a 24-hr specimen) may be present. Control subjects yield about  $0.4 \times 10^6$  fibers/g of feces. About 85% of the fibers isolated (irrespective of group) were less than  $2.0 \mu\text{m}$  in length. Unfortunately, there are no data on the probable intake levels.

Although the results from animal experiments and human autopsy material have indicated the passage of ingested chrysotile asbestos fibers through the gastrointestinal wall and their presence in many organs of the body other than lungs, notably omentum, kidneys, urine and brain (9, 10), the long-term effects of ingestion of low to moderate numbers of fibers on body-organ function is unclear. Recent work by Hallenbeck et al. (11) indicates no evidence that asbestos fibers penetrate the gastrointestinal tract and migrate to various tissues. Contrary to these results, Sebastien et al. (12) have evidence that chrysotile and crocidolite asbestos fibers do pass across the wall of the gastrointestinal tract with the passage rate being higher for long fibers than for short ones.

Fibers other than asbestos may be found in the urine and in considerable numbers. In a single case, Bignon et al. (13) found attapulgite fibers at a concentration of  $300 \times 10^6$  fibers/L in the urine of a 60-yr-old woman treated with a drug containing attapulgite for 6 months at a dose of 9 g/day orally. The mean length for the fibers was  $0.93 \mu\text{m}$ .

Neoplastic changes in the lungs due to inhalation of asbestos are well documented, but evidence for a similar causation in other organs is less strong. Studies by Kanarek et al. (14) and Conforti (15) indicated a positive but low risk for tumors of the digestive tract in relation to imbibed asbestos for a population of the San Francisco Bay Area. Polissar et al. (16) studied cancer incidence and mortality with respect to asbestos in drinking water in the Puget Sound region (Washington State). Studying communities with long-term exposures of 20+ yr, the authors found few associations between imbibed asbestos and cancer and concluded that chance was the most likely explanation for the cancer incidents. From these results and those from previous studies, however, they proposed that the pancreas and small intestine were sites that should be included in follow-up studies.

Recently, Cook and Olson (17) published findings indicating the presence of significant numbers of amphibole asbestos in urine samples from residents of Duluth, Minnesota, who were drink-

ing unfiltered water derived from Lake Superior that was known to contain, on the average,  $30 \times 10^6$  fibers of amosite/L water. Subsequent epidemiologic studies in Duluth (18, 19) revealed no significant increase in the risks of various cancers in the exposed population.

Because of the epidemiologic and drinking water asbestos analysis data accumulated on the cohort groups in the Everett, Puget Sound region (16), it was decided to carry out a limited study on a small group of people from this area to ascertain whether or not significant numbers of chrysotile asbestos fibers could be demonstrated in the urine of people residing in that area for more than 20 yr and whose drinking water contained about  $200 \times 10^6$  fibers/L. These people would be compared to a control group where the level of chrysotile asbestos in the drinking water averaged  $2.0 \times 10^6$  fibers/L (i.e., the Seattle area, Tolt River supply).

## Materials and Methods

### Selection of Specimens

In order to obtain urine samples in a precise and consistent fashion, male staff from the Everett Public Health Laboratories were recruited as donors. Personnel from the School of Public Health, University of Washington, were recruited as controls. All subjects were in good health, and none reported any history of kidney disease or occupational exposure to asbestos. Subjects also completed a questionnaire documenting their source of drinking water, personal consumption, and other beverages taken regularly. The ages, residence times, and other data of the donors are given in Table 1. Three standard prerinsed 1-qt plastic containers were given to each subject. One container contained 200 mL of  $0.1\text{-}\mu\text{m}$  Nuclepore-filtered distilled water (control), which was

Table 1. Asbestos analysis: donor urine.

Donor No.	Age	Years in residence (Everett)	Years at present address	Mucous threads <sup>a</sup>
101	55	24	6	Moderate
102	56	24	24	Moderate
103	62	30	13	Moderate
104	40	30	0.75	Moderate
107	28	1.5	1.25	Moderate
108	25	2.75	0.75	Scanty
109	44	1.5	1	Moderate
501 <sup>b</sup>	41	19 (Seattle)	19	Scanty
502 <sup>b</sup>	24	12 (Seattle)	2	Moderate
503 <sup>b</sup>	—	5 (Bellevue)	5	Moderate
504 <sup>b</sup>	25	3 (Bellevue)	3	Moderate

<sup>a</sup>Qualitative estimates by Nomarski interference optics.

<sup>b</sup>Control.

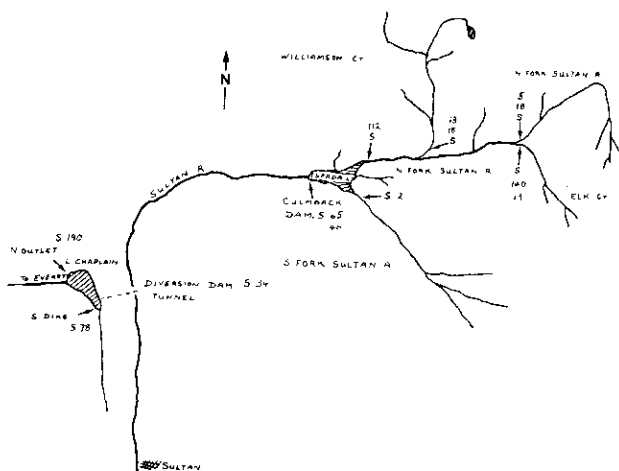


FIGURE 1. Sketch of Sultan River watershed showing sampling points and asbestos fiber concentrations. Not to scale.

simply opened and closed at the time of urination into the second and empty container. The third container was to be filled with tapwater from the place of residence. All urine samples were freshly voided, early-morning specimens that were delivered to the testing laboratory on the same day. Drinking water in the Everett area is supplied by the Sultan River system (Fig. 1).

The voided volume and specific gravity were recorded, an aliquot (10 mL) of each urine sample was centrifuged at 1,500 rpm for 10 min, and the deposit was mounted in a drop of methylene blue and examined by Nomarski interference optics for the presence of epithelial and other cell types, casts, and mucous strands. The presence of mucus was a consistent feature of the urine samples and, accordingly, presented a problem during the filtration procedure. After trials using various chemicals and enzymes (e.g., trypsin, urea, pronase, ozone, *N*-acetyl *L*-lysine, and hydrogen peroxide, it was found that the  $H_2O_2$  method of Cook and Olson (17) was the most effective in disaggregating the mucous strands. Consequently, the volume remaining with each urine sample was mixed with equal volumes of 30%  $H_2O_2$  and allowed to react at 40°C for 20 hr with intermittent shaking on a rotary shaker for the first hour. The maximum possible volume of urine (from 25 to 100, 50 mL being the general case) was filtered through 0.2- $\mu$ m filters with the minimum of negative pressure. Prefiltered control water samples (50 mL) were filtered through a 47-mm diameter, 0.2- $\mu$ m pore size Nuclepore filter, and tapwater samples through a 47-mm diameter, 0.1- $\mu$ m Nuclepore filter, each supported by a 2.0- $\mu$ m pore size Millipore backing filter. Although all tap

water samples were collected, not all were analyzed because of the consistently high levels of asbestos established. Low temperature ashing as in the Cook and Olson procedure was not used, and, after drying, an equatorial strip was cut from each Nuclepore filter, attached to a glass slide, and coated with carbon by rotation in a vacuum evaporator; then, small portions of the coated filters (2 mm<sup>2</sup>) were placed on 200-mesh copper-rhodium electron microscopy (EM) grids (Maxtaform, Fullham, NY). The grids were exposed to chloroform vapors in accordance with the modified Jaffe wick procedure of Chatfield et al. (20) and the filter matrix dissolved. After dissolution of the filter, the grid containing the particulates retained by the surface carbon film was observed by a JEOL 100S transmission electron microscope (TEM) operating at 100 kV at a magnification of 21,000 $\times$ . The tasks of preparation of the samples and the subsequent fiber analysis were distributed between two experienced observers.

## Controls

Additional controls, consisting of filtered  $H_2O_2$  solutions, urine samples, "spiked" with a predetermined number of chrysotile asbestos fibers, and urine and control water filtrations through Nuclepore membranes of different batch numbers of 0.1- or 0.2- $\mu$ m pore size, were also set up. Nuclepore membranes of different lot numbers were ashed to determine their endogenous asbestos fiber content.

## Sample Analysis

Twenty grid openings selected randomly from three or four grids of each sample were examined. Particulates suggestive of asbestos fibers were rated according to aspect ratio, morphology, and crystal structure (selected area electron diffraction, or SAED). In some instances, energy-dispersive X-ray analysis was also attempted. The concentrations of fibers in millions per liter were calculated by use of the standard formula of Anderson and Long (21).

## Detectable Limits

The sensitivity of the asbestos analysis method is dependent upon the pore size of the filter used, the volume of urine or water filterable, the presence of interfering particulates (e.g., fragments of diatoms, residues of mucus, and other debris), and the number of grid openings searched (4). In our analyses, we obtained limits of detection ranging from 0.07 to 0.3  $\times 10^6$  fibers/L.

It is important to be aware that the areas of individual grid openings measured by light microscopy of 200-mesh microscope grids may vary considerably from batch to batch. In one sequence of measurements from different batches, we obtained readings ranging from 7995 to 9988  $\mu\text{m}^2$  for a single grid opening.

## Results

As shown in Table 1, it is seen that, of the seven donors from the Everett area, four had spent more than 24 yr in the area, and the remaining three, less than 3 yr. All were exposed, however, to high numbers of chrysotile asbestos fibers in their drinking water (Table 2). The numbers of fibers per liter calculated for urine, control water and drinking water are shown in Table 2, with summary statistics in Table 3. Although the concentrations of fibers in the urine varied throughout the 21-month sampling period, none was consistently and significantly higher than the concentrations of fibers estimated for the control waters.

There was, however, a significant difference ( $p = 0.05$ ) between the counts of fibers in the urine of donors with less than 3 yr residence time (Everett area) and the urine of donors with 24+ yr residence time. Note, however, that the fiber count in the urine of donor No. 102 was higher than that of all others of the Everett group; otherwise, the mean concentrations for long-term versus short-term exposure were similar. The concentration of fibers in the urine of Everett donors combined ( $0.97 \times 10^6$  fibers/L) was not significantly different from the concentration for Seattle/Bellevue control donors, even though the numbers of fibers in the drinking water of the latter were at least 100 times less. The results of various control samples processed with different batches of Nuclepore membrane filters are shown in Table 4. Overall, use of either of the two batches of 0.2- $\mu\text{m}$  Nuclepore membranes in the filtration step appears to yield about a fourfold increase in number of fibers present compared to filtration through a 0.1- $\mu\text{m}$  pore size membrane. It should be noted that a 2.0- $\mu\text{m}$  Nuclepore membrane is twice as

Table 2. Asbestos analysis: chrysotile fibers in urine, control water, tapwater.

Location	No.	Years in residence	Chrysotile fibers/L ( $\times 10^{-6}$ ) <sup>a</sup>		
			Urine <sup>b</sup>	Control water <sup>b</sup>	Tapwater <sup>b</sup>
Everett	101	24	0.85 $\pm$ 1.00	1.63 $\pm$ 1.34	230 $\pm$ 57
	102	24	2.70 $\pm$ 2.47	2.00 $\pm$ 1.94	320 $\pm$ 254
	103	30	0.68 $\pm$ 0.71	1.83 $\pm$ 1.07	295 $\pm$ 60
	104	30	0.70 $\pm$ 0.14	1.50 $\pm$ 1.11	297 $\pm$ 230
	107	1.5	0.70 $\pm$ 0.87	1.30 $\pm$ 0.89	235 $\pm$ 181
	108	2.8	0.57 $\pm$ 0.45	1.27 $\pm$ 0.72	259 $\pm$ 287
	109	1.5	0.27 $\pm$ 0.29	0.93 $\pm$ 0.55	383 $\pm$ 245
Seattle/Bellevue	501	19	2.03 $\pm$ 1.33	1.17 $\pm$ 0.15	1.17 $\pm$ 0.15
	502	12	0.33 $\pm$ 0.25	0.43 $\pm$ 0.06	2.70 $\pm$ 0.95
	503	5	0.43 $\pm$ 0.42	0.77 $\pm$ 0.15	3.07 $\pm$ 1.00
	504	3	0.50 $\pm$ 0.10	1.03 $\pm$ 0.25	2.50 $\pm$ 0.50

<sup>a</sup>Means of samples from November 1979 to August 1981,  $\pm 1$  SD.

<sup>b</sup>Nos. 101-104: sample ( $n$ ) for urine and control water = 4; for tapwater = 2. Nos. 107-109 and 501-504: sample ( $n$ ) for all procedures = 3.

Table 3. Asbestos analysis: chrysotile fibers in urine, control water, tapwater; statistical summary.

Group	Years in residence	Chrysotile fibers/L ( $\times 10^{-6}$ ) <sup>a</sup>		
		Urine	Control water	Tapwater
Everett				
Long term	27	1.23 $\pm$ 1.51	1.74 $\pm$ 1.28	285 $\pm$ 138
4		( $n = 16$ )	( $n = 16$ )	( $n = 8$ )
Short term	2	0.51 $\pm$ 0.55	1.17 $\pm$ 0.66	278 $\pm$ 214
3		( $n = 9$ )	( $n = 9$ )	( $n = 9$ )
Seattle/Bellevue	9	0.83 $\pm$ 0.95	0.85 $\pm$ 0.33	2.36 $\pm$ 0.98
4		( $n = 12$ )	( $n = 12$ )	( $n = 12$ )

<sup>a</sup> $\pm 1$  SD.  $t$ -test; Everett: urine: long term vs. short term. 1-tail test,  $p = 0.05$ ; control waters, NS (2-tail test); tapwaters, NS (1-tail test). Everett vs. Seattle/Bellevue: urine, NS (1-tail test); control waters and tapwaters,  $p = 0.01$  (2-tail test) and  $p < 0.001$  (1-tail test), respectively.

Table 4. Controls.

Sample	Nuclepore membrane		Number of chrysotile fibers × 10 <sup>6</sup> /L
	Pore size	Batch number	
100 mL glass-distilled water (GWD)	0.1 μm	83B 9B27	0.14
100 mL GDW	0.1	93B 9A31	0.20
100 mL filtered H <sub>2</sub> O (0.1 μm) GDW	0.1	83B 9A21	0.14
100 mL filtered H <sub>2</sub> O (0.1 μm) GDW	0.2	83N 9A84 <sup>a</sup>	0.50
100 mL GDW + H <sub>2</sub> O <sub>2</sub>	0.1	83B 9A31	0.18
100 mL GDW + H <sub>2</sub> O <sub>2</sub>	0.2	83N 9A84	0.85
100 mL GDW + H <sub>2</sub> O <sub>2</sub>	0.2	83B 5F9 <sup>a</sup>	0.65
25 mL urine (A) + 25 mL asbestos H <sub>2</sub> O + 50 mL H <sub>2</sub> O <sub>2</sub>	0.2	83B 5F9	5.8
25 mL urine (B) + 25 mL asbestos H <sub>2</sub> O + 50 mL H <sub>2</sub> O <sub>2</sub>	0.2	83B 5F9	4.3
25 mL filtered H <sub>2</sub> O 25 mL asbestos H <sub>2</sub> O 50 mL H <sub>2</sub> O <sub>2</sub>	0.2	83B 5F9	8.9

<sup>a</sup>Batch 83N 9A84 gave 21.1 fibers/grid opening after ashing for 2.5 hr at radiofrequency, 50 W; and batch 83B 5F9 gave 6.4.

thick (i.e., 10 μm versus 5 μm thick) as a 0.1-μm pore size membrane. Prefiltration of the glass distilled water and H<sub>2</sub>O<sub>2</sub> in tests for asbestos contamination showed no contribution from these sources.

At the bottom of Table 4 are entries for urine samples with an added quantity of water containing a known amount of chrysotile asbestos fibers; these are compared with an asbestos control containing filtered distilled water in place of urine. It is seen that the mean concentrations of fibers calculated for the "spiked" urine ( $5.0 \times 10^6$ ) are 57% of the asbestos control concentration ( $8.9 \times 10^6$ ). Both of the spiked urine samples were shown to contain moderate amounts of residual material on the EM grids even after treatment with H<sub>2</sub>O<sub>2</sub>. It was further calculated that a loss (i.e., embedded in mucous residues) of one fiber/two grid openings would account for the 43% reduction in fiber counts for these urines.

The mean length of fibers found in the urine samples was 0.9 μm with a range of 0.5 to 1.2 μm. In the drinking water, fibers ranged in length from 0.3 to 5.0 μm with a mean of 0.8 μm. Electron diffraction showed 68% of these fibers to be positive for chrysotile. Among fibers counted as asbestos, 26% gave strong SAED patterns, 52% moderate patterns, and the remaining 22% were weak but discernible. About 32% of all fibers

examined failed to give a recognizable diffraction pattern and were not counted.

Light microscopy of centrifuged urine deposits nearly always showed residues of mucous threads (Fig. 2) which, following treatment with H<sub>2</sub>O<sub>2</sub>, were effectively disaggregated (Fig. 3). Scanning electron microscopy (SEM) of the deposit from 10 mL of an untreated urine sample filtered through a 0.2-μm Nuclepore membrane, dried and coated with gold-palladium, showed a homogeneous layer of mucus 1.0 μm thick covering the membrane surface (Fig. 4, arrow). The residue from an H<sub>2</sub>O<sub>2</sub>-treated and filtered urine sample processed for TEM and showing a single chrysotile asbestos fiber is seen in Figure 5. In this figure, note also the apparent blocking of pore apertures by residual material. (arrow).

## Discussion

Drinking water becomes contaminated by asbestos fibers as a result of geologic erosion, pollution (e.g., logging and the building of dams) and the internal erosion of asbestos-cement pipe. The consistently high concentrations of chrysotile fibers of about  $200 \times 10^6$  fibers/L found in the water supply of Everett, Washington, are unique for this area, but concentrations between 1 and  $100 \times 10^6$  fibers/L have been found in a number of

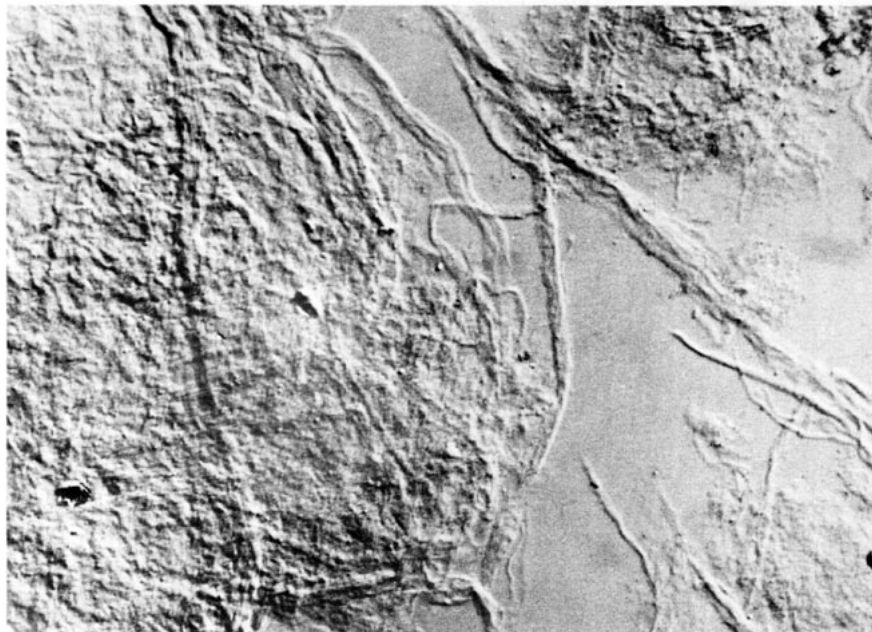


FIGURE 2. Light microscopy of urine sediment (10 mL) before treatment with 30% H<sub>2</sub>O<sub>2</sub>. Note abundance of mucous strands. Nomarski optics. × 200.

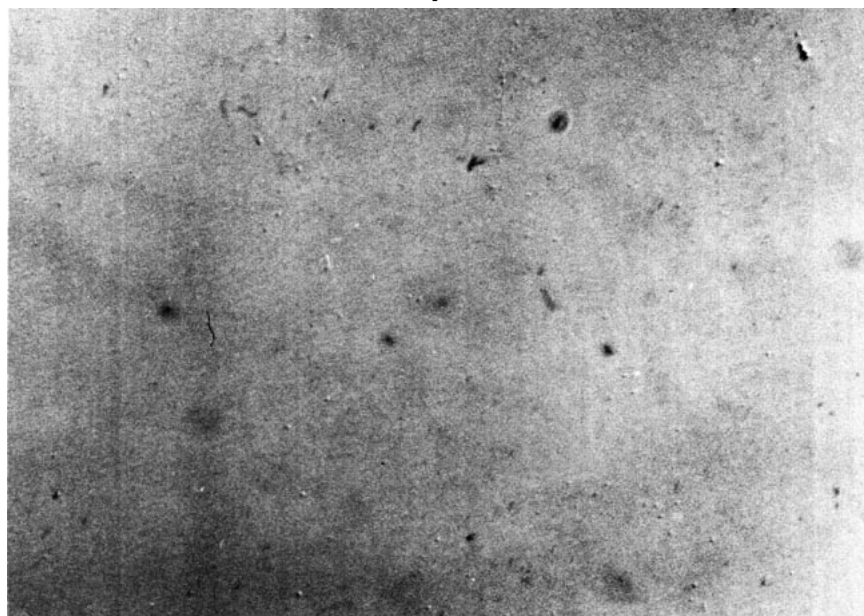


FIGURE 3. Light microscopy of urine sediment after treatment with 30% H<sub>2</sub>O<sub>2</sub>. Little residue is seen at this level of magnification. Nomarski optics. × 200.

water supplies in California (4). Most of this contamination on the West Coast is considered to be due to the erosion of asbestos-bearing rocks aided, at times, by marked fluctuations in the local meteorologic conditions. This combination results in relatively high concentrations of fibers in the water supply throughout the year and is

grounds enough for believing that unusually high numbers of fibers may be found in the urine of people drinking this water compared to appropriate controls. It has been variously estimated that approximately  $10^{-3}$  (17) to  $10^{-4}$  (22) of the ingested fibers find their way into the urine. In the first case, after 2 L water is imbibed, we would

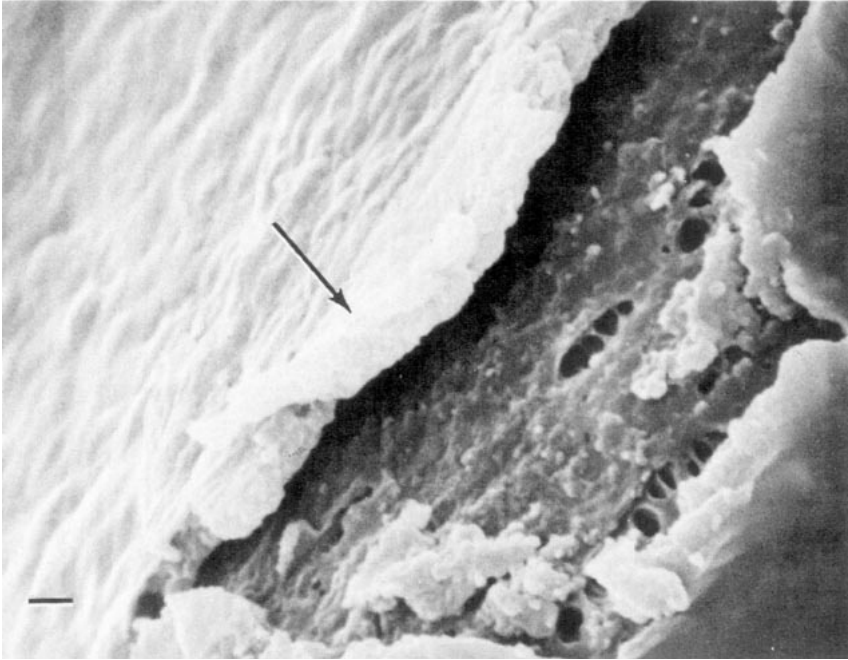


FIGURE 4. Scanning electron micrograph of a portion of the surface of a 0.2  $\mu\text{m}$  Nuclepore membrane following filtration of 10 mL of untreated urine. A part of the surface mucus has been disrupted exposing the underlying membrane. The thickness of the dried mucous layer (arrow) is about 1.0  $\mu\text{m}$ .  $\times 6050$ . Bar = 1.0  $\mu\text{m}$ .

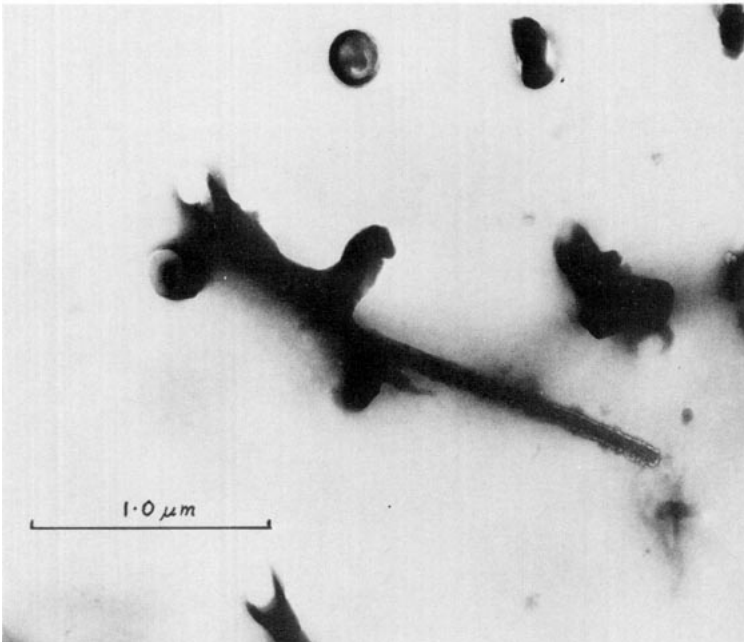


FIGURE 5. Transmission electron micrograph of a single chrysotile asbestos fiber (1.3  $\mu\text{m}$  long) isolated from urine. Carbon replica of a 0.2  $\mu\text{m}$  diameter pore plugged with debris is seen (arrow).  $\times 30,000$ . Bar = 1.0  $\mu\text{m}$ .

expect to find at least  $0.4 \times 10^6$  fibers/L in the urine in excess of control water without consideration of the real possibility that chrysotile fibers in urine may result from exposures other than water ingestion, i.e., food, beverages, and air via lung clearance of inhaled fibers. In the second case, only  $0.04 \times 10^6$  fibers/L would be assumed to be present, a figure well below our limit of detection.

On face value, the data would suggest that significant numbers of chrysotile asbestos fibers do not pass into the urine, whether the concentration of fibers is low or 100 times higher. With respect to these urine samples, however, additional points need to be raised: (1) How many fibers were missed due to masking by mucous residues? (perhaps about 40%). (2) How many fibers were not counted due to deterioration of their morphology and negative selected area electron diffraction patterns? (about 30%). Such corrections would obviously not raise the counts significantly. (3) To obtain a convincing difference, the average numbers of fibers in the urine samples would have to be increased by a factor of 10 or more.

The reason for not finding a significant excess of asbestos fibers in urine samples from residents of Everett may be due to a number of additional factors. First, throughout this study the attainment of a high level of detection was thwarted by the inability to filter large volumes of urines due to the presence of mucous residues in spite of pretreatment with  $H_2O_2$ . Although a level of detection of less than  $0.2 \times 10^6$  fibers/L was obtained on urine where volumes of 50 mL or more could be filtered, the results were obscured by the generally higher background counts of the control water samples. Cook et al. (17) attained a similar detectable limit for amphibole fibers, and Hallenbeck et al. (10, 11) achieved a limit of  $0.44 \times 10^6$  fibers/L. Both groups of workers used techniques different from ours. Second, it may be that significant numbers of chrysotile fibers do not, in fact, gain access to the excretory mechanism of the kidneys and become voided with the urine.

Cook et al. (17) largely avoided the mucous residue problem by ashing the Nuclepore membrane following filtration of the urine and drying. It is likely, however, as the results suggest, that a significant increase in chrysotile contamination would occur. Although Cook and Olson were looking primarily for evidence of amphibole fibers, significant numbers of contaminating chrysotile fibers that sometimes exceeded  $1 \times 10^6$  fibers/L were found. For the past 2 yr, batches of "ashed" 47-mm diameter, 0.2- $\mu$ m pore size Nuclepore

membranes have consistently yielded between 6 and 21 fibers/grid opening. With this in mind, we chose to use the  $H_2O_2$  procedure to eliminate the residual mucus from human urine. On the whole,  $H_2O_2$  treatment appears satisfactory by light microscopy, but some mucous residues are somewhat refractory to treatment and, in addition, tend to rapidly plug the membrane pores (Fig. 5), thereby reducing filtration efficiency. A further disadvantage is that small asbestos fibers may become masked by refractory residues and escape being counted. The inability to see and to identify all fibers in a particular sample is a problem common to all asbestos analyses by TEM. Laboratory contamination by asbestos, particularly of the chrysotile variety, is also a significant problem of varying magnitude. Carter and Taylor (23) examined 300 grid openings on 65 "blank" grids and found 1.3 fibers/grid opening.

Comparison of chrysotile fiber counts of water filtered through 0.1- $\mu$ m pore size membranes and those after 0.2- $\mu$ m membrane filtration yielded a fourfold increase in counts for the latter (Table 4). As yet, we have not done enough of these comparisons to be able to ascertain the implication of these results.

Cook et al. (17) also compared amphibole fiber counts of "spiked" urine samples after filtration and ashing with similarly treated water/amphibole suspensions without added urine and found a 29% underestimate of amphibole numbers for the urines. We recorded a 43% reduction in fiber counts (Table 4) with urine "spiked" with chrysotile asbestos and processed using  $H_2O_2$  instead of ashing. Possibly, comparison of urine specimens between exposed and control persons is more appropriate than comparisons between urine and control water.

It is generally accepted that intralaboratory precision for asbestos counting in clean water using the interim method of Anderson and Long (21) is about 40-50%. This procedure also suggests that counts less than 5 fibers/20 grid openings should be considered not statistically significant. On this basis, the differences shown in Table 2 for urine largely represent counts in this range. From Tables 2 and 3, the number of fibers per liter from the Everett group of urine samples is not significantly different from the urine of the Seattle/Bellevue group. However, the control waters were significantly different due to the high counts of groups 101 to 104. In this case, it seems that there was a higher level of contamination in the early control water samples than later on. Furthermore, the fiber counts of the Everett urine samples were just significantly different ( $p$



< 0.1, two-sided *t*-test) from their control waters. This is most likely due to an unknown loss of fibers by masking by residues of debris. A 43% loss of fibers assumed, as judged by the "spiked" urine counts, is consistent with the difference between Everett urine samples and control water.

At this point, some functional and structural aspects of the kidneys should be reviewed. The output of urine in normal adult subjects varies from 600 to 2500 mL/day. The blood flow through the combined kidneys is 1200 mL/min. Each kidney has about  $1 \times 10^6$  nephrons, and each nephron consists of a glomerulus (filtration unit) connected to a long tubule consisting of four parts: (1)

proximal convoluted tubule, 60  $\mu\text{m}$  diameter (re-sorption); (2) Loop of Henle; (3) distal convoluted tubule, 20–50  $\mu\text{m}$  diameter; and (4) collecting tubule 100–200,  $\mu\text{m}$  diameter. From this last tubule follows the renal pelvises, ureter, and, finally, the bladder. It is the mucous coats of the ureters and bladder that give rise to mucus in the urine. Not to be forgotten is the generous system of lymphatics, which serves the kidneys, ureters, and bladder. A glomerular filtration unit (Fig. 6) consists of capillary endothelium punctuated by regularly spaced pores, 50–100  $\mu\text{m}$  in diameter; fused basement membranes (BM) of the overlying podocyte foot processes and underlying endothe-

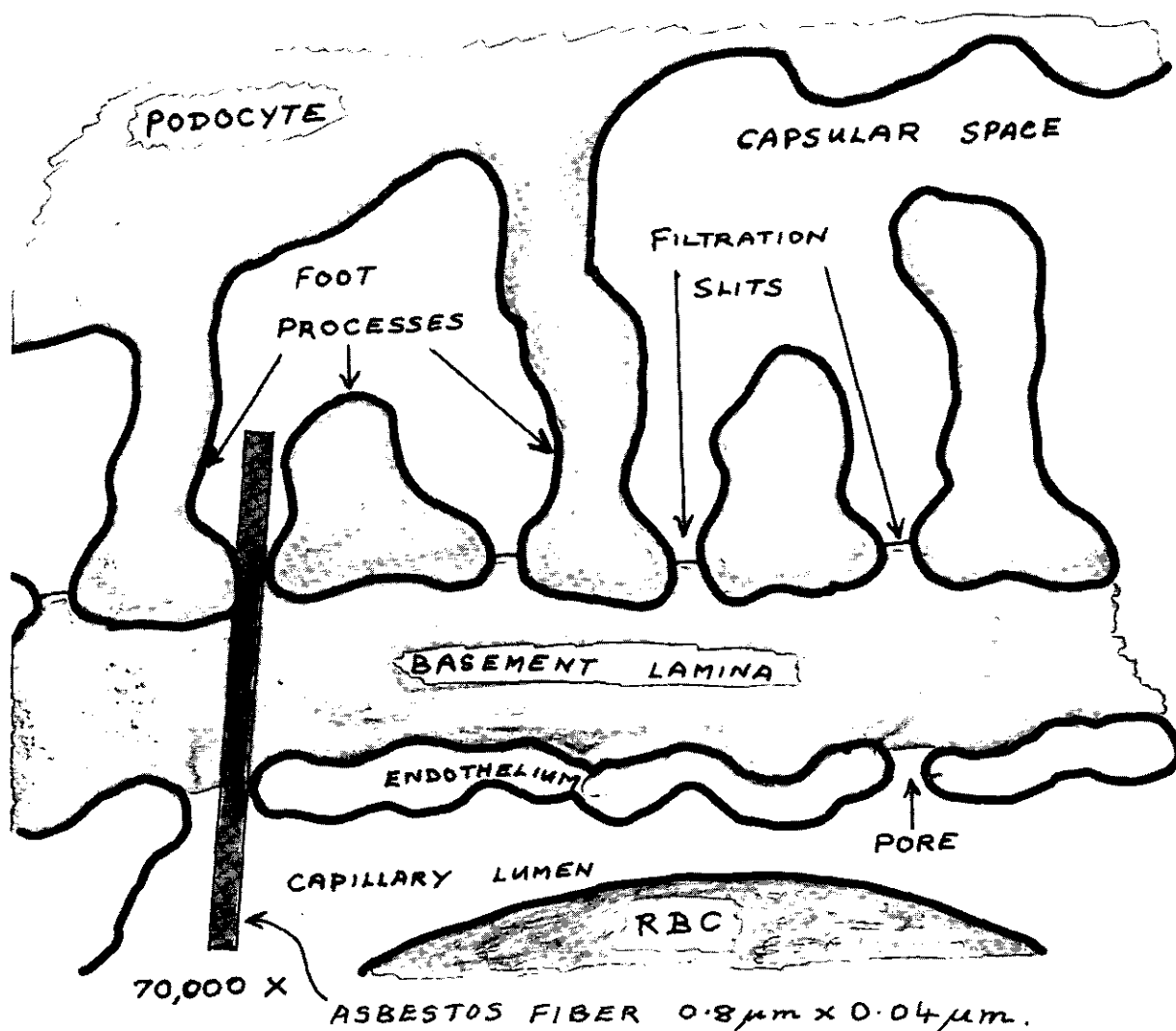


FIGURE 6. Sketch of a portion of the wall of a glomerular capillary and capsular space. Modified from Bloom and Fawcett (24). A hypothetical chrysotile asbestos fiber ( $0.8 \mu\text{m} \times 0.04 \mu\text{m}$ ) has been drawn in as a size reference.  $\times 70,000$ .

lial cell form a homogenous barrier, which is believed to be the principal filter to hold back large molecules in conjunction with filtration slits formed between any two adjacent foot processes.

Two potential sources of asbestos fiber contamination of renal tissue are therefore the blood plasma and the lymph, and two potential pathways for access into the urine are (1) entry through the endothelial pore–basement membrane–filtration slit network, and (2) into the bladder via the lymphatics. That particulate matter can be excreted along with the urine is suggested by the works of Cook and Olson (17) with waterborne amphiboles, Bignon et al. (13) with orally ingested attapulgite clay, and Hallenbeck et al. (10) with ingested chrysotile and crocidolite. At present, the question of how fibers find their way into the urine is unresolved. One might ask if fiber surface charge has anything to do with the process of entry or if it is simply a spearlike penetration by a relatively rigid rod-shaped object. An equally important aspect to consider is not the numbers of fibers retrieved from urine—thus safely and permanently removed—but how many fibers are sequestered in kidney tissue. The latter is an even more difficult problem to solve given the ubiquitous nature of contaminating chrysotile.

It is clear that there are substantial difficulties associated with the estimation of chrysotile asbestos fibers in human urine by the use of present techniques. The difficulties arise from the inherent chrysotile contamination of the 0.2- $\mu\text{m}$  Nucleopore filters, the presence of residual mucous material on the membrane, and, finally, sporadic environmental contamination of the sample during processing. At the present time, the results of the present study can only be regarded as inconclusive but would suggest no relationship between high numbers of chrysotile fibers in drinking water and the numbers estimated for voided urine. For whatever reason, no urine examined showed high numbers of chrysotile fibers during the 21 months of intermittent observation. The resolution of this problem will rest on future procedures to rid urine of obstructive mucous, on the acquisition of membrane filters free of endogenous chrysotile asbestos, and on obtaining a larger group of urine donors, which, to detect a significant difference between the mean test value and the mean control value, would require a considerable increase over the present numbers and consideration given to the frequency of sampling.

The authors thank Dr. Claris Hyatt, Health Officer, Snohomish Health District, and her staff for their splendid cooper-

ation during this study, and Roy Jones, Physical Scientist, EPA, Region X, Seattle, for his continued interest in this work from its conception. This Research was supported by Contract No. CI790578 from the U.S. Environmental Protection Agency.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## REFERENCES

1. Cunningham, H. M., and Pontefract, R. Asbestos fibers in beverages and drinking water. *Nature* 232:332–333 (1971).
2. Cook, P. M., Glass, G. E., and Tucker, J. H. Asbestiform amphibole minerals: detection and measurement of high concentrations in municipal water supplies. *Science* 185: 853–855 (1974).
3. Millette, J. R., Clark, P. J., and Pansing, M. F. Exposure to Asbestos from Drinking Water in the United States. Environmental Health Effects Research Report, Office of Research and Development, EPA-600/1-79-028, 1979.
4. Millette, J. R., Boone, R., and Rosenthal, M. Asbestos in Cistern Water. Environmental Health Effects Research Report, Office of Research and Development. EPA-657-093-7105, 1980.
5. Chatfield, E. J., and Dillon, M. J. A national survey for asbestos fibres in Canadian drinking water supplies. Environmental Health Directorate, Ottawa, 79-EHD-34, 1974.
6. Gaudichet, A., Bientz, M., Sebastien, P., Dufour, G., Bignon, J., Bonnaud, G., and Puisais, J. Asbestos fibers in wines: relation to filtration process. *J. Toxicol. Environ. Health* 4: 853–860 (1978).
7. Biles, B., and Emerson, T. R. Examination of fibres in beer. *Nature* 219: 93–94 (1968).
8. Cunningham, H. M., Pontefract, R. D., and O'Brien, R. C. Quantitative relationship of fecal asbestos to asbestos exposure. *J. Toxicol. Environ. Health* 1: 377–379 (1976).
9. Cunningham, H. M., Moodie, C. A., Lawrence, G. A., and Pontefract, R. D. Chronic effects of ingested asbestos in rats. *Arch. Environ. Contam. Toxicol.* 6: 507–513 (1977).
10. Hallenbeck, W. H., and Patel-Mandlik, K. J. Presence of fibers in the urine of a baboon gavaged with chrysotile asbestos. *Environ. Res.* 20: 335–340 (1979).
11. Hallenbeck, W. H., Markey, D. R., and Dolan, D. G. Analyses of tissue, blood and urine samples from a baboon gavaged with chrysotile and crocidolite asbestos. *Environ. Res.* 25: 349–360 (1981).
12. Sebastien, P., Masse, R., and Bignon, J. Recovery of ingested asbestos fibers from the gastrointestinal lymph in rats. *Environ. Res.* 22: 201–216 (1980).
13. Bignon, J., Sebastien, P., Gaudichet, A., and Jaurand, M. C. Biological effects of attapulgite. In: *Biological Effects of Mineral Fibres*, Vol. 1 (J. C. Wagner, Ed.) IARC, Lyon, France, 1980, pp. 163–181.
14. Kanarek, M. S., Conforti, P. M., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer incidence in the San Francisco Bay area. *Am. J. Epidemiol.* 112: 54–72 (1980).
15. Conforti, P. M., Kanarek, M. S., Jackson, L. A., Cooper, R. C., and Murchio, J. C. Asbestos in drinking water and cancer in the San Francisco Bay area: 1969–1974 incidence. *J. Chron. Dis.* 34: 211–224 (1981).
16. Polissar, L., Severson, R. K., Boatman, E. S., and Thomas

- D. B. Cancer incidence in relation to asbestos in drinking water in the Puget Sound Region. *Am. J. Epidemiol.* 116: 314-328 (1982).
17. Cook, P. M., and Olson, G. F. Ingested mineral fibers: elimination in human urine. *Science* 204: 195-198 (1979).
  18. Levy, B. S., Sigurdson, E., Mandel, J., Laudon, E., and Pearson, J. Investigating possible effects of asbestos in city water: surveillance of gastrointestinal cancer incidence in Duluth, Minnesota. *Am. J. Epidemiol.* 103: 362-368 (1976).
  19. Sigurdson, E. E., Levy, B. S., Mandel, J., McHugh, R., Michienzi, L. J., Jagger, H., and Pearson, J. Cancer morbidity investigations: lessons from the Duluth study of possible effects of asbestos in drinking water. *Environ. Res.* 25: 50-61 (1981).
  20. Chatfield, E. J., Glass, R. W., and Dillon, M. J. Preparation of Water Samples for Asbestos Fiber Counting by Electron Microscopy. Environmental Research Laboratory Report, Office of Research and Development, Cincinnati. EPA-600/4-78-011, 1978.
  21. Anderson, C. H., and Long, J. M. Interim Method for Determining Asbestos in Water. Environmental Research Laboratory Report, Office of Research and Development, Athens, GA, EPA-600/4-80-005, 1980.
  22. Millette, J., and Rosenthal, M. Fate of ingested particulates. In: *Proceedings, National Workshop on Substitutes for Asbestos*. EPA Report 560/3-80-001, Cincinnati, 1980, pp. 313-327.
  23. Carter, R. E., and Taylor, W. F. Identification of a Particular Amphibole Asbestos Fiber in Tissues of Persons Exposed to a High Oral Intake of the Mineral. Report, Environmental Health Advisory Panel, American Public Health Association, Washington, D.C., 1978.
  24. Bloom, W., and Fawcett, D. W. *Textbook of Histology*, 9th ed., Saunders, Philadelphia, 1968.