

# Accumulation of Ingested Asbestos Fibers in Rat Tissues Over Time

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With the use of the transmission electron microscope, asbestos fibers have been assessed in kidney cortex of four groups of rats previously exposed to intermediate-range feeding grade chrysotile asbestos. Newborn rats, from mothers gavaged with asbestos during pregnancy, were gavaged twice a week at the dose level of 50 mg/kg beginning at age day 7 until their natural death or sacrifice. The rats were divided into four groups by age: 0-200, 200-400, 400-600 and 600-800 days. Of the 20 rats comprising the four test groups, 17 were positive, average fiber recovery being  $5.34 \times 10^3$ /mg dry weight. Average fiber level in control tissues was  $0.23 \times 10^3$ /mg dry weight. Fiber recovery in tissues from control animals was shown to be significantly lower ( $p < 0.005$ ) than that from test tissues. Test groups showed highly significant differences ( $p < 0.005$ ) from each other in the fiber levels. Dose-response relationship was not significant ( $0.05 < p < 0.1$ ). The length distribution and the alterations in morphology of the recovered fibers are described. This study is consistent with the passage of chrysotile asbestos across the gastrointestinal wall.

## Introduction

A relationship between ingested asbestos and cancer incidence in humans was suggested (1-3) through epidemiological studies. Recovery of fibers in tissues and urine of humans exposed to asbestos has been reported (4-7). Short-term animal feeding studies (8-12) have shown that ingested fibers penetrate, migrate between, and are recoverable from gastrointestinal tract, lymph, distant tissues and urine.

The present study was undertaken to investigate whether penetration, migration and retention occur in tissues of animals chronically exposed to asbestos. Rats employed in a carcinogenicity study (National Cancer Institute Grant 21684, unpublished) were used. Kidney cortex was selected, since in our past study with baboons, the highest concentration of fibers was found in kidney cortex among ten organs under analysis (12). Selikoff (5) reported excessive kid-

ney cancer among insulation workers, although numerous asbestos fibers were recovered in liver. The relationship of fiber concentration in tissue with the time of exposure was tested in the present study by using rats gavaged for various time durations.

## Materials and Methods

Sprague-Dawley rats were gavaged with Environmental Health Sciences Sample No. 109C feeding grade intermediate-range chrysotile asbestos. The dosage and gavage schedule were as described earlier (13). Rats dying of natural causes between ages 0-800 days were grouped into four groups by age ranges at death: namely, 0-200 days, 200-400 days, 400-600 days and 600-800 days. Kidney tissues were collected from 20 randomly selected rats, five per age group. Four control rats, one in each age group, were randomly chosen to represent the level of asbestos in control tissues.

Tissue preparation for electron microscopic analysis was as described earlier (12). A Phillips 300 transmission electron microscope was used for chrysotile identification and for counting and sizing the fibers. The number of chrysotile fibers per grid hole followed a Poisson distribution (14); 50 grid holes were examined per sample, thus

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reducing the probability of a zero count to less than 5%. The detection limit of our analytical technique (13) was 88 fibers/mg dry weight if one fiber was found in 50 grid holes and if  $L = 1$ , where  $L$  was the weight of tissue (mg) per grid surface. Using the same preparative and counting techniques, blank preparations and tissue preservative were tested for the background level of fibers.

Characteristic morphology of chrysotile was revealed by some but not all fibers. Hence, fibers recovered in our tissues were classified as Class A, B, C, and D fibers according to definitions based on personal judgment of the morphological appearance of fibers (13). Blind-coded analysis was performed on samples from each test category on representative grids at the Health Effects Research Laboratory of the U.S. Environmental Protection Agency, Cincinnati, OH.

Among additional organs, lung and omentum of a test rat were analyzed.

## Results

When the method described was used, no problem was encountered in grid preparation. Transmission electron microscopic examination was confidently reliable due to minimal residue or debris on grids. The average fiber recovery of Class A + B + C fibers in test samples was 788 or  $5.34 \times 10^3$ /mg dry weight, whereas that in control samples was 10 or  $0.2 \times 10^3$ /mg dry weight. Fibers were at below detection level in blank grids (prepared to test preparative contamination) and grids prepared from tissue preservative.

The highest concentration was found in samples from rats in age range 400-600 days. Tissues from rats in age range 0-200 days showed the lowest concentration (Table 1). For statistical comparisons, square-root transformations of data were used because the mean and the variance were proportional. Fiber recovery in test and con-

trol groups were statistically (15) for each of the four time intervals, and each showed significant difference at  $p < 0.005$ . The individual transformed data were combined to test an overall difference between test and control which was found to be significant at  $p < 0.005$ .

The dose-response effect was tested by comparing the fiber recovery in test samples from the four age groups. Group I animals (0-200 days) represented the lowest dosage of gavaged asbestos and group IV animals (600-800 days) represented the highest dosage, since, the older the animal, the higher the cumulative dosage it would have. The statistical evaluation of transformed data by weighted regression analysis (16) revealed that when Class A + B fiber counts were considered, no linear relationship was found at the 5% level of significance. However, when Class A + B + C fiber counts were considered, although there was no linear relationship between the age of animal and number of fibers, a quadratic relationship was found between the variables at  $p < 0.08$ .

Bundles and clusters of fibrils were recovered in all age ranges of rats to form 25.8% of total fibers. Fiber dimensions were measured; however, only length data were reported (13). Diameter was not meaningful, due to swelling or lack of sharp edges in some degraded fibers. The length varied from 0.08 to 5.71  $\mu\text{m}$ , where the median ranged from 0.23 to 0.46  $\mu\text{m}$  among the fibers in four groups of rats. The frequency of length distribution (Table 2) of Class A and B fibers combined was unimodal for rat groups I and III, bimodal for rat group IV and trimodal for rat group II. The mean values of four groups showed bimodal distribution. Fiber lengths and frequency distribution of fiber Classes A, B, C, and D recovered in representative samples from test rats in age groups II, III and IV have been described in tabular and nomogram forms (13).

One bundle of fibrils was recovered in omentum, and none in lung of one test rat.

Table 1. Fiber counts in the kidney cortex from rats gavaged with chrysotile asbestos.

Age group of rats <sup>a</sup>	Average kidney weight, mg	Chrysotile fibers/mg dry weight $\times 10^3$ in each class of fibers <sup>b,c</sup>		
		A + B	C	A + B + C
I	1230	1.24 $\pm$ 1.1 (BDL)	0.33 $\pm$ 0.3 (BDL)	1.57 $\pm$ 1.4 (BDL)
II	1722	2.66 $\pm$ 1.7 (BDL)	2.71 $\pm$ 2.0 (0.53)	5.37 $\pm$ 4.0 (0.56)
III	1870	8.00 $\pm$ 4.2 (BDL)	1.62 $\pm$ 0.6 (BDL)	9.63 $\pm$ 4.1 (BDL)
IV	2040	3.31 $\pm$ 1.2 (BDL)	1.47 $\pm$ 0.9 (0.34)	4.78 $\pm$ 2.0 (0.34)

<sup>a</sup>Each group consisted of five rats.

<sup>b</sup>Fiber classes are defined in the text.

<sup>c</sup>Fiber counts in kidney cortex of control rats are shown in parentheses; BDL denotes below detection level.

**Table 2. Frequency of length distribution of fibers recovered in kidney cortex of gavaged rats.**

Length range, $\mu\text{m}$	Fiber distribution, % <sup>a</sup>				Mean	Cumulative
	Group I rats	Group II rats	Group III rats	Group IV rats		
0-0.5	84.6	56.3	79.0	89.8	77.4	77.4
0.5-0.99	7.7	22.3	15.8	7.8	13.4	90.8
1.0-1.7	3.9	3.0	3.8	0.6	2.8	93.6
1.8-3.1	3.8	8.7	1.4	0	3.5	97.1
3.2-5.5	0	9.7	0	1.2	2.7	99.8
5.6-9.9	0	0	0	0.6	0.15	100.0

<sup>a</sup>Chrysotile fibers A + B sized by transmission electron microscopy.

## Discussion

Penetration of ingested asbestos fibers through gastrointestinal tract and recovery of migrated fibers in distant tissues have been reported in animal and human studies (6-13, 17-19). Contrary to this, other studies (20-22) indicate that ingested fibers do not penetrate or migrate. In the present study, recovery of chrysotile fibrils, bundles, and clusters in kidney cortex of gavaged rats clearly shows that migration occurs, and it supports our previous findings of chrysotile asbestos recovery in kidney cortex of a baboon (12) and humans (23). Since the clusters contained one or more bundles, 57 bundles were encountered within 39 clusters. Bundles formed 29.2%, and bundles and clusters formed 25.8%, of total recovery. Recovery of fibers in 17 out of 20 rats suggest that some animals may resist fiber penetration and/or migration. Biological variation was suggested by Sebastien et al. (17) in their studies with rats.

The presence of fibers in test samples was obviously not artifactual, since the results were based on up to 250 observations per age group of rats, and the background contamination from air, reagents and preparative procedures was minimal or below detection level. One gavage study (22) failed to demonstrate penetration and migration of ingested fibers due to contamination, resulting in fiber counts in the controls in the same range as the counts in test baboon blood and urine. Moreover, the detection limit of their methodology was low due to scanning of only 20 grid holes per sample and use of only one test animal.

By using square root transformed data on fibers of Class A + B or Class A + B + C a significant difference at  $p < 0.005$  was found between the fiber concentrations in test and control tissues. Controls were compared with each test group separately, as well as with the four test groups

combined. Fiber concentrations within the four age groups showed a statistically significant difference ( $p < 0.005$ ).

A further aim of this study was to determine whether there was a relationship between the amount of ingested chrysotile asbestos and concentration of fibers retained. There was a rise in fiber level in the tissues from group I to group III rats, followed by a drop in the tissues from group IV rats (Table 1). Rat group IV received cumulatively more fibers than rat groups III, II or I because the rats in this study were gavaged twice weekly at the dosage of 50 mg asbestos/kg body weight until death. No linear relationship was found. On accepting statistical significance for  $p$  values  $< 0.1$ , the quadratic model of regression analysis revealed a relation ( $p < 0.08$ ) between dosage and number of fibers of Class A + B + C retained in the kidney cortex. The quadratic relationship could signify time-related biological causes of low fiber recovery, such as degradation of fibers beyond TEM identity and elimination of fibers in feces and urine (6,18,19). No relationship was observed with Class A + B fiber counts.

The length of chrysotile asbestos Class A + B fibers recovered in test tissues showed a skewed distribution. Shorter fibers outnumbered the longer fibers. All the fibers were less than 9  $\mu\text{m}$  in length (Table 2). Most of the recovered fibers were in the length range of 0.31-0.5  $\mu\text{m}$ . It is not known whether shorter fibers selectively penetrate and disseminate or the fibers break into shorter lengths during the journey from oral entry into kidney cortex through the blood system. The health significance of such a large number of retained short-length fibers is not known. Hamilton et al. (24) reported that a sample containing chrysotile fibers shorter than 0.5  $\mu\text{m}$  was ineffective in coagulation of plasma. There was no particular pattern of fiber length in relation to the age group or rats (Table 2), except that fewer fibers recovered in group II tissues were in the length range of 0-0.5  $\mu\text{m}$  than in the other groups and showed trimodal distribution.

Morphological degradation was used as a criterion to form Classes A, B, C, and D of the recovered fibers. It was not possible within the scope of this study to use microprobe analysis for more precision. Degraded fibers have been reported previously (12,19) in gavage studies. It is suggested that there might be a degradative mechanism associated with the residence time of fibers *in vivo*. It is possible that during their passage and stay within the body system, fibers could be influenced by the body fluids, temperature, agitation and other factors.

Assuming that the degradative mechanisms affecting the morphology and diffraction property of the chrysotile preclude positive identification of all of the fibers present on the grid surface, the results expressed in fibers/milligram dry weight could be considered to be conservative measures of tissue fiber concentration. Mass concentration was not computed, since it is often very inaccurate because of the poor counting statistics associated with large fibers (bundles, clusters) that are fewer in number but represent most of the actual mass. Recovery of bundles and clusters suggests that, besides single fibrils, other forms could penetrate and migrate; that fibers accumulate in the same area of the tissue; or that the preparation procedure causes some fibrils to clump together.

Results from analysis of a test rat's lung and omentum were considered insufficient to derive information about fiber retention in these organs.

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