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Migration of Ingested Asbestos

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Tissue samples from one test and one control baboon were analyzed by transmission electron microscopy for the presence of chrysotile and crocidolite asbestos. The test animal had been gavaged with cumulative doses of 800 mg each of chrysotile and crocidolite asbestos. An earlier evaluation of these tissues led to the conclusion that ingested asbestos fibers do not penetrate the gastrointestinal tract of the baboon and migrate systemically. However, the present study involved more sensitive methodology, and penetration and migration were clearly demonstrated by the recovery of significant levels of asbestos from test stomach, heart, spleen, pancreas, and blood samples.

INTRODUCTION

Epidemiological studies have shown that asbestos workers have increased mortality due to pleural and peritoneal mesothelioma, and cancers of the lung, digestive system, and related organs (Selikoff, 1979). Exposure to asbestos is not restricted only to those who are occupationally exposed. Asbestos fibers have been found in air (U.S. EPA, 1979) and drinking water supplies (Millette *et al.*, 1980). Approximately 50% of an inhaled asbestos dose is cleared from the respiratory tract through mucociliary action and subsequently swallowed (Evans *et al.*, 1973). Thus, the gastrointestinal tract becomes the recipient of most inhaled and ingested asbestos. Increased rates of cancers in asbestos workers and others may be related to gut penetration and migration of asbestos. For example, statistical associations between asbestos fiber content in drinking water and several types of cancers (digestive system and related organs) have been shown for various populations within the United States and Canada (Conforti *et al.*, 1981; Kanarek *et al.*, 1980; Levy *et al.*, 1976; Mason *et al.*, 1974; Wigle, 1977).

We look to controlled animal studies for confirmation of human findings, especially with regard to dose. Several chronic animal ingestion studies have produced little evidence that ingested asbestos increases the incidence of gastrointestinal cancer (Bolton *et al.*, 1982; Cunningham *et al.*, 1977; Donham *et al.*, 1980; Gibel *et al.*, 1976; Hilding *et al.*, 1981; McConnell *et al.*, 1984a, b; Smith *et al.*, 1980; Ward *et al.*, 1980).

Research specifically into the area of gut mucosal penetration by asbestos fibers has produced conflicting results. The literature contains ingestion studies involving rats and baboons. Five rat studies (Cunningham *et al.*, 1977; Donham *et al.*, 1980; Patel-Mandlik and Millette, 1983; Sebastien *et al.*, 1980; Westlake *et al.*, 1965) and three baboon studies (Hallenbeck and Patel-Mandlik, 1979; Patel-Mandlik *et al.*, 1979; Patel-Mandlik and Millette, 1980) produced some evidence for asbestos gut penetration and migration. These studies were not conclusive

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because of various shortcomings: (a) the number of fibers observed on a grid opening basis was very small (Hallenbeck and Patel-Mandlik, 1979; Patel-Mandlik *et al.*, 1979; Patel-Mandlik and Millette, 1980, 1983); (b) a small number of control data were obtained (Sebastien *et al.*, 1980); (c) test and control data were not presented in a quantitative manner (Donham *et al.*, 1980; Westlake *et al.*, 1965); and (d) fiber count data were reported as fibers/g of tissue (Cunningham *et al.*, 1977). Unless fiber count data are reported in terms of fibers/grid opening, it is not possible to know whether the authors' conclusions were based on the observation of one or hundreds of fibers. Given the problem of background contamination, positive results are always more credible if they are based on the actual observation of many fibers.

Three rat studies (Bolton and Davis, 1976; Bolton *et al.*, 1982; Gross *et al.*, 1974) and one baboon study (Hallenbeck *et al.*, 1981) produced no evidence for asbestos penetration and migration. However, there was no discussion of asbestos recovery in these studies. Unless the methodology utilized in sample preparation has been evaluated as to fiber recovery, there is no way of knowing whether the sensitivity was sufficient to detect asbestos, especially if the levels may be very low.

Two human studies (Carter and Taylor, 1980; Cook and Olson, 1979) produced evidence for the penetration and migration of asbestos. Cook and Olson (1979) detected amphibole asbestos in the urine of Minnesota residents who ingested drinking water contaminated with 5×10^7 fibers/liter. In contrast to Cook and Olson's findings, Boatman *et al.* (1984) did not find significant levels of asbestos in the urine of humans exposed to 2×10^8 chrysotile fibers/liter. Cook and Olson and Boatman reported their data in fibers/liter instead of fibers/grid opening.

Carter and Taylor (1980) identified amphibole asbestos in postmortem tissues of persons exposed to a high oral intake of the mineral. However, they were not consistent in selecting equal masses of tissue, equal numbers of grid openings, and equal ash resuspension volumes for analysis.

Hallenbeck *et al.* (1981) analyzed tissue, blood, and urine samples from a baboon gavaged with 800 mg each of chrysotile and crocidolite asbestos by transmission electron microscopy. The results indicated that asbestos fibers did not penetrate the gastrointestinal tract of the baboon and migrate systemically. Since the test and control baboon tissues were frozen and stored at the end of the study, the purpose of the present research was to reevaluate the test and control baboon tissues using a different asbestos analytical methodology. This reevaluation had several advantages over the original study and other tissue studies in general:

1. Fiber recovery was defined.
2. Instead of the droplet transfer technique used in the original study, the Jaffe washer, considered the current state-of-the-art technique, was used to prepare electron microscope grids.
3. Five grams of tissue were used instead of 1 g (wet wt), greatly increasing the sensitivity of the technique. The minimum detection limit was reduced from 220 fibers/mg in the original study to an average of 24 fibers/mg dry tissue in the present study.

MATERIALS AND METHODS

Preparation of Tissue Samples for Electron Microscopy

All tissue samples were rinsed with filtered (0.1 μm polycarbonate) deionized water before use. Five grams (wet wt) of tissue was obtained by cutting several small pieces from each tissue with a surgical blade. A new blade was used for each sample. A blank filter was also processed with the test and control samples for each tissue type analyzed. All tissue samples were prepared for analysis by transmission electron microscopy in the following manner:

1. Comparable wet weights (5 g) of test and control tissues were placed in preweighed beakers and dried in a vacuum drying oven at 90°C for 24 hr.
2. Beakers were removed from the oven, placed in a desiccator until cool, and then weighed on an analytical balance to determine the final dry weights of the tissues.
3. One hundred milliliters of filtered (0.2-μm fluoropore) 5% potassium hydroxide was added to each beaker. Beakers were covered with a glass petri dish and heated at 60–70°C for 24 hr on a combination stirrer/hot plate. The digestion process was facilitated with a magnetic stirring bar.
4. The tissue digest was cooled to approximately 30°C. (Digested tissue at 60–70°C damaged polycarbonate filters.) The digest was filtered through 47-mm-diameter 0.1-μm polycarbonate filters. The filters were covered during filtration and taken to dryness. Filtration units from Gelman Instrument Company were used along with 47-mm-diameter 0.4-μm polycarbonate filters as backing filters. Filtration time varied from 6 to 48 hr. Backing filters were discarded after use.
5. Filters were placed in covered petri dishes and kept inside a desiccator until dry. Once dry, the filters were placed inside 50-ml bottles and ashed in a low temperature plasma asher (13.56 MHz, 80 watts, 1 torr) for approximately 1½ hr. For a given tissue type, the test, the control, and blank were ashed together.
6. Ashes were suspended with approximately 30 ml of filtered water by ultrasonication in a water bath (55 KHz) for 15 min. Approximately 1 ml of filtered (0.2-μm fluoropore) 6 N HCl was also added to each suspension before ultrasonication to aid in the solubilization of the ash.
7. The suspended ashes were filtered through 0.1-μm polycarbonate filters with 5-μm cellulose ester filters as backing filters. The filters were covered during filtration and taken to dryness.
8. Steps 5, 6, and 7 were repeated to help reduce background debris and organic matter to a minimum.
9. The third and final polycarbonate filter with the sample deposit was dried in a desiccator, secured to the bottom of a petri dish with double stick tape, and then coated with a thin layer of carbon in a vacuum evaporator.
10. Random sections of the carbon-coated polycarbonate filters were removed with a surgical blade and transferred to 200-mesh electron microscope finder grids and subsequently placed in a Jaffe washer until dissolution of the polycarbonate filters was complete.
11. One grid opening from each of four grids was scanned at a magnification of 28,000× for test, control, and blank samples.

Recovery Study

A reference suspension of chrysotile (7×10^{-3} μg ml) was made from the same asbestos as administered to the test baboon (Hallenbeck *et al.*, 1981). Five milliliters of this suspension yielded about 25 fibers/grid opening. A "prespike" was prepared by adding 5 ml of reference suspension to 1 g (wet wt) of pig kidney in Step 1 above. The entire tissue preparation procedure then followed. A "postspike" was prepared by adding 5 ml of reference suspension to 1 g (wet wt) of pig kidney just before the second ultrasonication of the suspended ash. Chrysotile fiber counts from 10 grid openings per preparation were compared.

Fiber Classification and Counting Rules

Fibers were identified and classified as chrysotile or amphibole based on their morphology and electron diffraction patterns. A fiber was defined as any particle that had parallel sides and an aspect ratio ≥3:1. Tightly bound bundles of fibrils were counted as a single fiber and an estimate made of the number of fibrils in the bundle, the average width of the bundle, and the maximum length of the bundle. Clusters of fibrils were counted as a single fiber and an estimate made of the number of fibrils in the cluster.

STATISTICAL ANALYSIS OF DATA

Recovery Study

The Kruskal–Wallis one-way analysis of variance by ranks (Daniel, 1978) was used to test the null hypothesis that the suspension, prespike, and postspike fiber counts were equal ($\alpha = .05$). Chrysotile fiber counts were converted to fiber/cm^2 values to adjust for the individual variations in grid opening areas and were also adjusted for blank levels of chrysotile contamination before being analyzed by the Kruskal–Wallis test. Significant differences were found among the three groups. A multiple-comparison procedure (Daniel, 1978) was used to determine the source(s) of the significant differences.

Baboon Test, Control, and Blank Samples

(*i*) *Fiber count analyses.* All test, control, and blank fiber counts were converted to fiber/cm^2 values to adjust for the individual variations in grid opening areas. A Kruskal–Wallis one-way analysis of variance by ranks was performed on the test, control, and blank fiber/cm^2 values for each tissue to test the null hypothesis: test, control, and blank fiber/cm^2 values are equal ($\alpha = .05$). If statistically significant differences were found among test, control, and blank values, a multiple-comparison procedure was used to determine the source(s) of the significant differences.

Test and control fiber/cm^2 values were then adjusted for blank levels of asbestos contamination and concentrations in fibers/milligram tissue were calculated. A two-tailed Wilcoxon's rank sum test (Snedecor and Cochran, 1976) was performed on the test and control fiber/mg tissue values to test the null hypothesis: test and control fiber/mg values are equal ($\alpha = .05$). *P* values were obtained from Dixon and Massey (1969). Detection limits were also calculated for test and control tissues. These calculations were performed as follows.

Calculation of fibers/milligram tissue:

$$\left[\frac{\sum_{i=1}^{N} \text{No. fibers}^i}{\sum_{i=1}^{N} \text{Grid opening area (cm}^2)^i} - \frac{\sum_{i=1}^{N} \text{No. blank fibers}^i}{\sum_{i=1}^{N} \text{Grid opening area (cm}^2)^i}\right] \times \frac{A_1}{M} = \text{fibers/mg tissue}$$

N = number of grid openings examined for the sample
A_1 = 9.62 cm^2 = effective area of a filter
M = dry weight of tissue in milligrams

Calculation of detection limit in fibers/mg tissue:

$$\frac{F \times A_1}{A_2 \times M} = \text{fibers/mg tissue}$$

F = the observation of only one fiber divided by the total number of grid openings examined. For example, if one fiber was observed in four grid openings, F = 1/4 = 0.25
A_1 = 9.62 cm^2 = effective area of a filter
A_2 = average area of grid openings examined (cm^2)
M = dry weight of tissue in milligrams

The spleen samples were statistically analyzed in a different manner because a control spleen tissue sample was not available. Wilcoxon's rank sum test was used to compare test fiber/cm^2 values directly with blank fiber/cm^2 values.

(*ii*) *Mass analyses*. The test, control, and blank data were also statistically analyzed using chrysotile and amphibole asbestos mass values.

The mass (μg) of each fiber was calculated as follows (Anderson and Long, 1980):

$$M = L \times W^2 \times D \times 10^{-6}$$

where

M = mass (μg)
L = length (μm)
W = width (μm)
D = density of fiber (g/cm^3) (chrysotile = 2.5 g/cm^3, amphibole = 3.25 g/cm^3)

Calculation of μg asbestos/mg tissue:

$$\left[\frac{\sum_{i=1}^{N} \mu\text{g asbestos}^i}{\sum_{i=1}^{N} \text{Grid opening area (cm}^2)^i} - \frac{\sum_{i=1}^{N} \mu\text{g blank asbestos}^i}{\sum_{i=1}^{N} \text{Grid opening area (cm}^2)^i}\right] \times \frac{A_1}{M} = \mu\text{g asbestos/mg tissue}$$

N = number of grid openings examined for the sample
A_1 = 9.62 cm^2 = effective area of a filter
M = dry weight of tissue in milligrams

TABLE 1
FIBER COUNT AND MASS DATA BY GRID OPENING FOR TEST SAMPLES

Baboon tissue	Chrysotile fiber count/ grid opening	Chrysotile (μg/grid opening × 10^{-7})	No. chrysotile clusters/grid opening (No. fibrils/ cluster)	Grid opening area (× 10^{-5} cm^2)	Amphibole fiber count/ grid opening	Amphibole (μg/grid opening × 10^{-7})	No. amphibole clusters/grid opening (No. fibrils/ cluster)
Stomach	99	>19.85[a]	2 (10) (TNTC)[b]	10.41	8	45.67	0 (0)
	110	>16.75[a]	1 (4)	10.41	4	1.45	0 (0)
	84	>15.19[a]	1 (TNTC)	11.05	1	0.88	0 (0)
	94	>12.17[a]	1 (7)	10.62	11	4.57	0 (0)
Heart	37	8.13	0 (0)	10.20	9	29.71	0 (0)
	40	13.19	0 (0)	10.00	4	2.90	0 (0)
	48	8.17	0 (0)	10.83	11	3.69	0 (0)
	48	11.16	0 (0)	10.60	11	14.59	0 (0)
Pancreas	24	2.40	0 (0)	11.25	1	0.09	0 (0)
	65	7.08	0 (0)	11.26	6	2.07	0 (0)
	24	2.51	0 (0)	10.41	1	0.21	0 (0)
	30	3.88	0 (0)	10.62	8	4.18	0 (0)
Blood[c]	44	7.88	0 (0)	11.26	22	23.03	0 (0)
	31	4.66	0 (0)	10.41	3	0.46	0 (0)
	33	4.13	0 (0)	11.47	2	0.54	0 (0)
	31	3.39	0 (0)	11.05	1	0.13	0 (0)
Blood[d]	12	1.66	0 (0)	11.04	9	4.66	0 (0)
	21	2.10	0 (0)	10.83	8	6.64	0 (0)
	7	1.12	0 (0)	11.25	5	>2.27[a]	1 (5)
	18	>1.38[a]	1 (5)	10.83	4	2.74	0 (0)
	12	2.12	0 (0)	10.82	7	14.39	0 (0)
	28	>2.65[a]	1 (TNTC)	10.83	5	1.77	0 (0)
	19	>2.09[a]	1 (TNTC)	11.04	10	4.49	0 (0)
	14	1.44	0 (0)	10.00	8	4.11	0 (0)

Kidney	14	1.13	0 (0)	10.62	2	0.27	0 (0)
	20	2.97	0 (0)	10.83	3	3.51	0 (0)
	21	3.82	0 (0)	10.41	2	0.33	0 (0)
	16	1.48	0 (0)	10.41	1	4.08	0 (0)
Lung	9	2.01	0 (0)	10.40	4	5.13	0 (0)
	11	1.35	0 (0)	10.00	2	0.18	0 (0)
	6	>0.89[a]	1 (4)	10.41	4	3.96	0 (0)
	7	0.47	0 (0)	11.25	2	1.77	0 (0)
Liver	12	>0.74[a]	1 (10)	11.04	2	0.51	0 (0)
	16	1.30	0 (0)	10.00	4	11.22	0 (0)
	20	2.30	0 (0)	10.61	2	1.22	0 (0)
	13	>0.89[a]	1 (10)	10.41	2	1.01	0 (0)
Spleen	93	8.04	0 (0)	10.41	5	1.52	0 (0)
	102	8.77	0 (0)	10.41	5	6.01	0 (0)
	103	12.12	0 (0)	9.80	9	10.95	0 (0)
	114	12.34	0 (0)	10.61	7	1.46	0 (0)

[a] The mass value calculated is an estimate less than the actual mass value due to the presence of a cluster(s).
[b] TNTC = too numerous to count.
[c] Blood taken at sacrifice.
[d] Blood taken at last gavage.

TABLE 2

FIBER COUNT AND MASS DATA BY GRID OPENING FOR CONTROL SAMPLES

Baboon tissue	Chrysotile fiber count/ grid opening	Chrysotile (μg/grid opening × 10^{-7})	No. chrysotile clusters/grid opening (No. fibrils/ cluster)	Grid opening area (× 10^{-5} cm^2)	Amphibole fiber count/ grid opening	Amphibole (μg/grid opening × 10^{-7})	No. amphibole clusters/grid opening (No. fibrils/ cluster)
Stomach	22	>2.12[a]	1 (6)	10.41	3	4.58	0 (0)
	20	3.94	0 (0)	11.48	3	0.82	0 (0)
	14	2.03	0 (0)	10.83	5	3.55	0 (0)
	16	1.87	0 (0)	10.41	5	2.16	0 (0)
Heart	11	7.23	0 (0)	10.41	0	0	0 (0)
	12	2.51	0 (0)	10.60	3	3.76	0 (0)
	6	0.80	0 (0)	10.00	4	2.58	0 (0)
	9	0.52	0 (0)	10.61	0	0	0 (0)
Pancreas	3	0.16	0 (0)	11.04	3	0.20	0 (0)
	9	1.22	0 (0)	10.83	7	2.61	0 (0)
	10	0.70	0 (0)	10.41	7	2.59	0 (0)
	7	0.33	0 (0)	10.00	2	0.27	0 (0)
Blood[b]	4	0.14	0 (0)	10.41	2	1.11	0 (0)
	2	0.09	0 (0)	11.70	3	0.46	0 (0)
	6	1.35	0 (0)	11.70	2	0.26	0 (0)
	11	0.80	0 (0)	10.41	0	0	0 (0)

Blood[c]	13	>1.71[a]	1 (15)	11.05	5	2.95	0 (0)
	11	>1.71[a]	1 (15)	11.04	4	>0.98[a]	1 (8)
	10	>0.96[a]	1 (10)	11.25	3	4.21	0 (0)
	8	1.39	0 (0)	11.26	0	0	0 (0)
	10	1.52	0 (0)	10.41	2	0.82	0 (0)
	10	1.22	0 (0)	10.40	2	1.21	0 (0)
	7	3.74	0 (0)	10.62	4	>1.63[a]	1 (6)
	8	1.39	0 (0)	10.41	3	2.23	0 (0)
Kidney	19	1.51	0 (0)	10.20	1	0.12	0 (0)
	12	0.83	0 (0)	10.00	0	0	0 (0)
	19	1.57	0 (0)	11.04	3	3.48	0 (0)
	23	2.47	0 (0)	10.31	2	0.91	0 (0)
Lung	5	0.41	0 (0)	11.04	1	2.66	0 (0)
	2	0.10	0 (0)	10.41	3	1.15	0 (0)
	1	0.04	0 (0)	10.62	2	0.89	0 (0)
	7	1.59	0 (0)	10.60	2	0.97	0 (0)
Liver	13	4.60	0 (0)	10.41	1	0.71	0 (0)
	14	>4.73[a]	1 (14)	9.79	0	0	0 (0)
	16	2.29	0 (0)	11.04	2	0.80	0 (0)
	16	2.50	0 (0)	11.04	6	5.40	0 (0)
Spleen[d]							

[a] The mass value calculated is an estimate less than the actual mass value due to the presence of a cluster(s).
[b] Blood taken at sacrifice.
[c] Blood taken at last gavage.
[d] Control tissue was not available.

TABLE 3
FIBER COUNT AND MASS DATA BY GRID OPENING FOR BLANK SAMPLES

Baboon tissue	Chrysotile fiber count/ grid opening	Chrysotile (μg/grid opening × 10^{-7})	No. chrysotile clusters/grid opening (No. fibrils/ cluster)	Grid opening area (× 10^{-5} cm^2)	Amphibole fiber count/ grid opening	Amphibole (μg/grid opening × 10^{-7})	No. amphibole clusters/grid opening (No. fibrils/ cluster)
Stomach	4	0.37	0 (0)	10.41	1	0.01	0 (0)
	2	0.09	0 (0)	10.83	2	0.24	0 (0)
	1	0.02	0 (0)	10.40	6	4.67	0 (0)
	0	0	0 (0)	10.41	4	3.75	0 (0)
Heart	3	0.37	0 (0)	10.60	0	0	0 (0)
	1	0.52	0 (0)	10.00	0	0	0 (0)
	2	0.04	0 (0)	10.20	1	0.85	0 (0)
	1	0.74	0 (0)	10.83	1	0.59	0 (0)
Pancreas	5	2.09	0 (0)	11.26	3	3.61	0 (0)
	7	0.95	0 (0)	11.04	5	0.70	0 (0)
	2	0.36	0 (0)	10.83	6	1.05	0 (0)
	1	0.14	0 (0)	11.25	2	0.44	0 (0)
Blood[a]	9	0.45	0 (0)	11.04	5	2.33	0 (0)
	2	0.90	0 (0)	11.47	0	0	0 (0)
	4	0.24	0 (0)	11.48	1	0.16	0 (0)
	3	0.21	0 (0)	11.47	2	4.97	0 (0)

Blood[b]	2	0.13	0 (0)	10.41	0	0	0 (0)
	4	0.27	0 (0)	10.83	0	0	0 (0)
	5	0.63	0 (0)	11.04	0	0	0 (0)
	1	0.04	0 (0)	11.25	0	0	0 (0)
	2	0.16	0 (0)	10.20	2	1.19	0 (0)
	1	0.51	0 (0)	10.41	1	0.06	0 (0)
	7	0.29	0 (0)	11.04	5	1.60	0 (0)
	2	0.17	0 (0)	11.48	3	1.24	0 (0)
Kidney	1	0.42	0 (0)	10.83	1	0.13	0 (0)
	6	0.59	0 (0)	10.62	0	0	0 (0)
	3	0.29	0 (0)	11.04	1	0.59	0 (0)
	5	>0.14[c]	1 (12)	10.83	1	0.08	0 (0)
Lung	4	0.38	0 (0)	10.83	3	0.71	0 (0)
	2	0.42	0 (0)	10.41	9	3.53	0 (0)
	2	0.11	0 (0)	10.41	2	>0.13[c]	1 (8)
	1	0.42	0 (0)	10.41	1	0.59	0 (0)
Liver	0	0	0 (0)	10.00	5	>1.48[c]	1 (8)
	1	0.04	0 (0)	10.41	8	5.38	0 (0)
	1	0.07	0 (0)	10.41	0	0	0 (0)
	0	0	0 (0)	11.47	0	0	0 (0)
Spleen	2	0.42	0 (0)	11.04	3	7.88	0 (0)
	11	1.19	0 (0)	11.04	3	0.76	0 (0)
	6	0.86	0 (0)	10.41	2	1.23	0 (0)
	12	3.46	0 (0)	11.04	1	2.03	0 (0)

[a] Blood taken at sacrifice.
[b] Blood taken at last gavage.
[c] The mass value calculated is an estimate less than the actual mass value due to the presence of a cluster(s).

TABLE 4
DRY WEIGHTS OF BABOON TISSUES

Tissue	Test (g)	Control (g)
Stomach[a]	0.9151	0.8288
Heart[a]	1.1200	1.3262
Pancreas[a]	1.7192	1.4030
Blood[b]	0.5983	0.6500
Blood[c]	0.2340	0.2420
Kidney[a,d]	1.1260	1.2694
Lung[a,e]	1.1138	1.2334
Liver[a]	1.2910	1.7315
Spleen[f]	0.7681	—

[a] Five grams wet weight of test and control tissues were analyzed.
[b] Blood withdrawn from the heart at sacrifice. Five milliliters was used for each asbestos analysis.
[c] Blood withdrawn during the last day of gavage. Approximately 2 ml was used for each asbestos analysis.
[d] Right kidney only.
[e] The lung tissue represented a combination of apical, cardiac, and diaphragmatic lobes.
[f] Only 2.5 g wet wt of test spleen was available. No control spleen was available.

Calculation of detection limit in μg asbestos/mg tissue:

$$\frac{F \times A_1}{A_2 \times M} = \mu g \text{ asbestos/mg tissue}$$

F = the mass of only one fiber divided by the total number of grid openings examined. For example, if one chrysotile fiber with a mass of 3.47×10^{-10} μg (mass of smallest fiber visible at 28,000× with a 3/1 aspect ratio) was observed in four grid openings, $F = 3.47 \times 10^{-10}$ μg/4 = 8.67×10^{-11} μg

TABLE 5
DETECTION LIMITS

Baboon tissue	Fibers/ mg tissue	μg Chrysotile/ mg tissue $\times 10^{-9}$	μg Amphibole/ mg tissue $\times 10^{-9}$
Stomach	26	9	10
Heart	19	6	8
Pancreas	14	5	6
Blood[a]	34	1	15
Blood[b]	46	16	20
Kidney	19	6	9
Lung	19	6	9
Liver	15	5	7
Spleen	30	10	10

[a] Blood taken at sacrifice.
[b] Blood taken at last gavage.

TABLE 6

STATISTICALLY SIGNIFICANT RELATIONSHIPS AMONG TEST, CONTROL, AND BLANK BABOON SAMPLES FOR CHRYSOTILE ASBESTOS

Tissue	Fibers/mg tissue[a]	Fibers/cm²[b]			μg/mg tissue[a]	μg/cm²[b]		
Stomach	T > C	T > C	C > B	T > B	T > C	T > C	C > B	T > B
Heart	T > C	T > C	C > B	T > B	T > C	T > C	C = B	T > B
Pancreas	T > C	T > C	C = B	T > B	T > C	T > C	C = B	T > B
Blood[c]	T > C	T > C	C = B	T > B	T > C	T > C	C = B	T > B
Blood[d]	T > C	T > C	C > B	T > B	T = C	T = C	C > B	T > B
Kidney	T = C	T = C	C > B	T > B	T = C[e]	NA[f]	NA	NA
Lung	T = C	T > C	C = B	T > B	T = C	T = C	C = B	T = B
Liver	T = C	T = C	C > B	T > B	NA	NA	NA	NA
Spleen[g]	—	—	—	T > B	—	—	—	T > B

[a] Test and control fiber counts/cm² or μg/cm² were adjusted for blank levels of chrysotile and then converted to concentration values of fibers/mg tissue or μg/mg tissue. A two-tailed Wilcoxon's rank-sum test of the null hypothesis (test concentrations are equal to control concentrations) was performed for fibers/mg tissue and μg/mg tissue for each tissue ($\alpha = 0.05$). Note that T = test, C = control, and B = blank.

[b] A Kruskal-Wallis one-way analysis of variance by ranks of the null hypothesis (test, control, and blank fiber levels are equal) was performed on fibers/cm² and μg/cm² of test, control, and blank samples ($\alpha = 0.05$). If there were significant differences between the three groups, a multiple-comparison procedure was used to find the source(s) of the differences.

[c] Blood taken at sacrifice.

[d] Blood taken at last gavage.

[e] Due to the occurrence of a cluster in the blank sample, test values were statistically compared directly to control values without adjustments for blank mass levels.

[f] NA = not applicable. Statistical mass analysis of data not possible due to a cluster(s) in one or more of blank, control, or test samples.

[g] Control spleen was not available. Wilcoxon's rank sum test was used to compare test fiber/cm² and μg/cm² concentrations directly with those of the blank sample ($\alpha = 0.05$).

A_1 = 9.62 cm² = effective area of a filter
A_2 = average area of grid openings examined (cm²)
M = dry weight of tissue in milligrams

RESULTS AND DISCUSSION

Recovery Study

The results of the Kruskal-Wallis test demonstrated that there were significant differences among suspension, prespike, and postspike counts in terms of fibers per square centimeter ($P < 0.005$). Differences were isolated via multiple comparison statistical tests which yielded the following results: (a) suspension and prespike counts were not significantly different (recovery = 95%), (b) suspension and postspike counts were significantly different (recovery >100%), (c) prespike and postspike counts were significantly different (recovery = 61%). Thus the true recovery probably lies in the range 61-100%.

TABLE 7
STATISTICALLY SIGNIFICANT RELATIONSHIPS AMONG TEST, CONTROL, AND BLANK BABOON SAMPLES FOR AMPHIBOLE ASBESTOS

Tissue	Fibers/mg tissue[a]	Fibers/cm²[b]			μg/mg tissue[a]	μg/cm²[b]		
Stomach	T = C	T = C	C = B	T = B	T = C	T = C	C = B	T = B
Heart	T > C	T > C	C = B	T > B	T = C	T > C	C = B	T > B
Pancreas	T = C	T = C	C = B	T = B	T = C	T = C	C = B	T = B
Blood[c]	T = C	T = C	C = B	T = B	T = C	T = C	C = B	T = B
Blood[d]	T > C	T > C	C = B	T > B	NA[e]	NA	NA	NA
Kidney	T = C	T = C	C = B	T = B	T = C	T = C	C = B	T = B
Lung	T = C	T = C	C = B	T = B	T = C[f]	NA	NA	NA
Liver	T = C	T = C	C = B	T = B	T = C[f]	NA	NA	NA
Spleen[g]	—	—	—	T > B	—	—	—	T = B

[a] Test and control fiber counts/cm² or μg/cm² were adjusted for blank levels of amphibole and then converted to concentration values of fibers/mg tissue or μg/mg tissue. A two-tailed Wilcoxon's rank-sum test of the null hypothesis (test concentrations are equal to control concentrations) was performed for fibers/mg tissue and μg/mg tissue for each tissue ($\alpha = 0.05$). Note that T = test, C = control, and B = blank.

[b] A Kruskal–Wallis one-way analysis of variance by ranks of the null hypothesis (test, control, and blank fiber levels are equal) was performed on fibers/cm² and μg/cm² of test, control, and blank samples ($\alpha = 0.05$). If there were significant differences between the three groups, a multiple-comparison procedure was used to find the source(s) of the differences.

[c] Blood taken at sacrifice.

[d] Blood taken at last gavage.

[e] NA = not applicable. Statistical mass analysis of data not possible due to a cluster(s) in one or more of blank, control, or test samples.

[f] Due to the occurrence of a cluster in the blank sample, test values were statistically compared directly to control values without adjustments for blank mass levels.

[g] Control spleen was not available. Wilcoxon's rank sum test was used to compare test fiber/cm² and μg/cm² concentrations directly with those of the blank sample ($\alpha = 0.05$).

Baboon Test, Control, and Blank Samples

Tables 1–3 contain the fiber count and mass data by grid opening for the test, control, and blank samples. Table 4 contains the dry weights of the baboon tissues analyzed. Table 5 contains detection limits.

One of the most significant aspects of this study was the consistency of the statistically significant relationships among test, control, and blank baboon samples as presented in Tables 6 and 7. The relationships presented in Tables 6 and 7 clearly indicate that the analytical results were not a product of the random occurrence of background asbestos. In all cases where statistical analyses could be performed, test sample concentrations were greater than or equal to control and blank sample concentrations; control sample concentrations were greater than or equal to blank sample concentrations. For a few tissues, control concentrations were greater than those for their respective blanks. This may have been attributable to environmental factors such as food, air, water, and intravenous fluids. In those tissues where test and control concentrations were equal, asbestos

TABLE 8
CHRYSOTILE ASBESTOS RECOVERED IN BABOON TISSUES

Baboon tissue	Concentration adjusted for blank fiber levels (fibers/mg tissue)		Concentration adjusted for blank mass levels (μg mg tissue $\times 10^{-3}$)	
	Test	Control	Test	Control
Stomach	9400[a,b]	1744[c]	15.7[a,b]	2.5[c]
Heart	3425[a,b]	540[e]	8.0[a,b]	1.6[d]
Pancreas	1649[a,b]	238[d]	1.6[a,b]	0[d]
Blood taken at sacrifice	4421[a,b]	184[d]	6.7[a,b]	0.2[d]
Blood taken at last gavage	5077[b,e]	2440[e]	5.9[b,f]	4.8[e]
Kidney	1139[b,f]	1069[e]	NA[g]	NA
Lung	493[b,f]	107[d]	0.7[f,h]	0.1[d]
Liver	1045[b,f]	749[e]	NA	NA
Spleen	11,623[i]	—	10.8[i]	—

[a] T > C (P = 0.028). Note that T = test, C = control, and B = blank.
[b] T > B (multiple-comparison procedure, P values unavailable).
[c] C > B (multiple-comparison procedure, P values unavailable).
[d] C = B (multiple-comparison procedure, P values unavailable).
[e] T > C (P = 0.020).
[f] T = C (α = 0.05).
[g] NA = not applicable. Calculation of mass concentration was not possible due to the occurrence of a cluster(s) in one or more of blank, control, or test samples.
[h] T = B (multiple-comparison procedure, P values unavailable).
[i] T > B (P = 0.028).

penetration and migration may have occurred without being detected because less than the whole organ was analyzed and the distribution of fibers in an organ may not be uniform.

Tables 8 and 9 contain the concentrations of asbestos recovered from the baboon tissues. The concentrations were adjusted for blank levels of asbestos. In a few cases, concentrations of zero resulted after adjustments for blank levels of asbestos were made.

Tables 8 and 9 should be referred to during the following discussion of individual tissues.

Stomach. Penetration of chrysotile asbestos into the stomach wall of the baboon was demonstrated by the significant level of chrysotile recovered from the test stomach sample. The test sample had chrysotile fiber and mass concentrations which were significantly greater than those of the control sample (P = 0.028). There was no indication that amphibole asbestos penetrated into the stomach wall of the baboon. Test amphibole fiber and mass concentrations were not significantly different from those of the control sample.

Heart. Penetration and migration of chrysotile asbestos were demonstrated by the significant level of chrysotile recovered from the test heart sample. The test

TABLE 9
AMPHIBOLE ASBESTOS RECOVERED IN BABOON TISSUES

Baboon tissue	Concentration adjusted for blank fiber levels (fibers/mg tissue)		Concentration adjusted for blank mass levels (μg mg tissue × 10^{-5})	
	Test	Control	Test	Control
Stomach	269[a,b]	72[c]	10.8[a,b]	0.6[c]
Heart	681[d,e]	87[f]	10.2[a,e]	0.8[f]
Pancreas	4[a,b]	61[c]	0.1[a,b]	0.02[c]
Blood taken at sacrifice	736[a,b]	0[c]	6.1[a,b]	0[c]
Blood taken at last gavage	2135[c,f]	553[c]	NA[g]	NA
Kidney	102[a,b]	57[c]	1.5[a,b]	0.7[c]
Lung	0[a,b]	0[c]	NA	NA
Liver	0[a,b]	0[c]	NA	NA
Spleen	531[h]	—	2.6[i]	—

[a] T = C (α = 0.05). Note that T = test, C = control, and B = blank.
[b] T = B (multiple-comparison procedure, P values unavailable).
[c] C = B (multiple-comparison procedure, P values unavailable).
[d] T > C (P = 0.028).
[e] T > B (multiple-comparison procedure, P values unavailable).
[f] T > C ($P < 0.001$).
[g] NA = not applicable. Calculation of mass concentration was not possible due to the occurrence of a cluster(s) in one or more of blank, control, or test samples.
[h] T > B (P = 0.028).
[i] T = B (α = 0.05).

TABLE 10
CHARACTERIZATION OF THE LENGTHS AND DIAMETERS OF CHRYSOTILE FIBERS[a] RECOVERED IN TEST STOMACH TISSUE

Length (μm)	Chrysotile diameter (μm)				
	0.018–0.036	0.037–0.071	0.072–0.125	>0.125	Total [%][b]
<0.2	0 (0)[c]	2 (0)	0 (0)	0 (0)	2 [<1]
0.2–0.5	1 (0)	61 (1)	21 (9)	0 (0)	83 [22]
0.51–1.0	1 (0)	114 (1)	47 (21)	7 (7)	169 [45]
1.1–2.0	0 (0)	70 (2)	27 (12)	3 (3)	100 [27]
2.1–5.0	0 (0)	9 (1)	6 (3)	0 (0)	15 [4]
>5.0	0 (0)	5 (3)	1 (1)	0 (0)	6 [2]
Total [%]	2 [0]	261 [70]	102 [27]	10 [3]	N[d] = 375

[a] Fibers = fibrils and bundles of fibrils. All entries were adjusted for blank chrysotile levels.
[b] The proportion of the total number of fibers in a specified length or diameter interval is shown in brackets.
[c] Numbers in parentheses refer to the number of bundles included in the cell total.
[d] N = Total number of fibers.

TABLE 11
CHARACTERIZATION OF THE LENGTHS AND DIAMETERS OF CHRYSOTILE FIBERS[a] RECOVERED IN TEST HEART TISSUE

Length (μm)	Chrysotile diameter (μm)				
	0.018–0.036	0.037–0.071	0.072–0.125	>0.125	Total [%][b]
<0.2	0 (0)[c]	0 (0)	0 (0)	0 (0)	0 [0]
0.2–0.5	2 (0)	23 (1)	8 (5)	0 (0)	33 [20]
0.51–1.0	0 (0)	37 (0)	39 (11)	3 (3)	79 [47]
1.1–2.0	0 (0)	14 (1)	26 (5)	0 (0)	40 [24]
2.1–5.0	0 (0)	4 (1)	6 (0)	1 (1)	11 [7]
>5.0	0 (0)	0 (0)	2 (0)	1 (1)	3 [2]
Total [%]	2 [1]	78 [47]	81 [49]	5 [3]	N^d = 166

[a] Fibers = fibrils and bundles of fibrils. All entries were adjusted for blank chrysotile levels.
[b] The proportion of the total number of fibers in a specified length or diameter interval is shown in brackets.
[c] Numbers in parentheses refer to the number of bundles included in the cell total.
[d] N = Total number of fibers.

sample had chrysotile fiber and mass concentrations which were statistically greater than those of the control sample (P = 0.028).

Penetration and migration of amphibole asbestos was indicated by the significant level of amphibole fiber recovered from the test heart sample. The test sample had an amphibole fiber concentration which was statistically greater than that of the control sample (P = 0.028). However, when the amphibole data were analyzed on a mass concentration basis, the test concentration was not statistically different from that of the control sample.

Pancreas and blood taken at sacrifice. Penetration and migration of chrysotile

TABLE 12
CHARACTERIZATION OF THE LENGTHS AND DIAMETERS OF CHRYSOTILE FIBERS[a] RECOVERED IN TEST PANCREAS TISSUE

Length (μm)	Chrysotile diameter (μm)				
	0.018–0.036	0.037–0.071	0.072–0.125	>0.125	Total [%][b]
<0.2	4 (0)[c]	0 (0)	0 (0)	0 (0)	4 [3]
0.2–0.5	3 (0)	35 (6)	5 (2)	0 (0)	43 [34]
0.51–1.0	0 (0)	48 (2)	14 (5)	0 (0)	62 [48]
1.1–2.0	0 (0)	9 (1)	6 (1)	0 (0)	15 [12]
2.1–5.0	0 (0)	2 (0)	2 (0)	0 (0)	4 [3]
>5.0	0 (0)	0 (0)	0 (0)	0 (0)	0 [0]
Total [%]	7 [6]	94 [73]	27 [21]	0 [0]	N^d = 128

[a] Fibers = fibrils and bundles of fibrils. All entries were adjusted for blank chrysotile levels.
[b] The proportion of the total number of fibers in a specified length or diameter interval is shown in brackets.
[c] Numbers in parentheses refer to the number of bundles included in the cell total.
[d] N = Total number of fibers.

TABLE 13
CHARACTERIZATION OF THE LENGTHS AND DIAMETERS OF CHRYSOTILE FIBERS[a] RECOVERED IN TEST BLOOD[b]

Length (μm)	Chrysotile diameter (μm)				
	0.018–0.036	0.037–0.071	0.072–0.125	>0.125	Total [%][c]
<0.2	3 (0)[d]	0 (0)	0 (0)	0 (0)	3 [2]
0.2–0.5	4 (0)	24 (5)	8 (6)	0 (0)	36 [30]
0.51–1.0	0 (0)	42 (2)	16 (12)	2 (2)	60 [50]
1.1–2.0	0 (0)	10 (0)	5 (5)	4 (2)	19 [16]
2.1–5.0	0 (0)	2 (0)	1 (0)	0 (0)	3 [2]
>5.0	0 (0)	0 (0)	0 (0)	0 (0)	0 [0]
Total [%]	7 [6]	78 [64]	30 [25]	6 [5]	N^e = 121

[a] Fibers = fibrils and bundles of fibrils. All entries were adjusted for blank chrysotile levels.
[b] Blood taken at sacrifice.
[c] The proportion of the total number of fibers in a specified length or diameter interval is shown in brackets.
[d] Numbers in parentheses refer to the number of bundles included in the cell total.
[e] N = Total number of fibers.

asbestos was demonstrated by the significant levels of chrysotile recovered from the test pancreas and sacrifice blood samples. The test samples had chrysotile fiber and mass concentrations which were statistically greater than those of their respective control samples ($P = 0.028$).

There was no indication of amphibole asbestos penetration and migration due to the insignificant levels of amphibole recovered from the test pancreas and blood samples. The test samples had amphibole fiber and mass concentrations which were not statistically different from controls.

Blood taken at last gavage. Penetration and migration of chrysotile asbestos was indicated by the significant level of chrysotile fiber recovered from the test blood sample. The test sample had a chrysotile fiber concentration which was statistically greater than that of the control sample ($P = 0.020$). The test sample mass concentration was not significantly different from that of its control.

Penetration and migration of amphibole asbestos was indicated by the significant level of amphibole fiber recovered from the test blood sample. The test sample had an amphibole fiber concentration which was statistically greater than that of the control sample ($P < 0.001$). A statistical analysis based on amphibole mass was not performed due to the occurrence of amphibole clusters in the test and control sample data.

Kidney, lung, and liver. Test sample chrysotile and amphibole fiber and mass concentrations were not significantly different from those of the control.

Spleen. Penetration and migration of chrysotile was indicated by the significant level of chrysotile recovered from the test spleen. The test sample had chrysotile fiber and mass concentrations which were significantly greater than those of its blank ($P = 0.028$).

Penetration and migration of amphibole was indicated by the significant level of amphibole fiber recovered from the test spleen. The test sample had an amphibole fiber concentration that was significantly greater than its blank (P = 0.028). However, when the amphibole data were analyzed on a mass concentration basis, the test concentration was not significantly different from that of its blank.

Penetration and migration of chrysotile asbestos was demonstrated by the significant fiber and mass levels of chrysotile recovered in the test stomach, heart, pancreas, sacrifice blood, and spleen samples. Characterizations of the lengths and diameters of the recovered chrysotile fibers are presented in Tables 10–14. Approximately 73% of the chrysotile fibers recovered from the test stomach, heart, pancreas, sacrifice blood, and spleen samples were 0.2–1.0 μm in length. Approximately 62% of the chrysotile fibers administered to the test baboon by gavage were 0.2–1.0 μm in length (Hallenbeck *et al.*, 1981).

CONCLUSIONS

1. The methodology used to prepare samples was sensitive and nondestructive to asbestos fibers.
2. Penetration and migration of chrysotile asbestos in the baboon due to gavage was demonstrated by both fiber count and mass data obtained from the test stomach, heart, pancreas, sacrifice blood, and spleen samples. Penetration and migration of chrysotile asbestos in the baboon due to gavage was indicated only by the fiber count data obtained from the test blood taken at last gavage.
3. Penetration and migration of amphibole asbestos in the baboon due to gavage was indicated only by fiber count data obtained from the test heart, blood taken at last gavage, and spleen samples. Amphibole penetration and migration

TABLE 14
CHARACTERIZATION OF THE LENGTHS AND DIAMETERS OF CHRYSOTILE FIBERS[a] RECOVERED IN TEST SPLEEN TISSUE

Length (μm)	Chrysotile diameter (μm)				
	0.018–0.036	0.037–0.071	0.072–0.125	>0.125	Total [%][b]
<0.2	15 (0)[c]	0 (0)	0 (0)	0 (0)	15 [4]
0.2–0.5	1 (0)	90 (4)	6 (5)	0 (0)	97 [25]
0.51–1.0	2 (0)	162 (2)	7 (7)	0 (0)	171 [44]
1.1–2.0	0 (0)	83 (0)	0 (0)	0 (0)	83 [22]
2.1–5.0	0 (0)	11 (0)	2 (1)	1 (1)	14 [4]
>5.0	0 (0)	4 (1)	0 (0)	0 (0)	4 [1]
Total [%]	18 [5]	350 [91]	15 [4]	1 [<1]	N^d = 384

[a] Fibers = fibrils and bundles of fibrils. All entries were adjusted for blank chrysotile levels.
[b] The proportion of the total number of fibers in a specified length or diameter interval is shown in brackets.
[c] Numbers in parentheses refer to the number of bundles included in the cell total.
[d] N = Total number of fibers.

may not have occurred to a great extent due to the larger size of amphibole asbestos fibers as compared to chrysotile asbestos fibers.

4. It appears that the length distribution of gavage fibers was not greatly changed in migrating through the body or during sample preparation.

5. Chrysotile concentrations in those test tissues (stomach, heart, pancreas, sacrifice blood, and spleen) where chrysotile penetration and migration due to gavage was demonstrated by both fiber count and mass data averaged 6100 fibers /mg tissue and 8.6×10^{-5} μg/mg tissue.

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