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Review of Published Studies on Gut Penetration by Ingested Asbestos Fibers

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During the 1970s, potential health risks associated with exposure to asbestos in drinking water became a national concern. One of the key questions that arose from debate over whether ingestion of mineral fibers could result in increased gastrointestinal cancer risk was whether fibers can penetrate the gastrointestinal mucosa and thus have some chance of residing in tissue. It is likely that such movement of a large number of fibers is a necessary precursor for carcinogenesis following ingestion of asbestos. Studies of the potential for fiber accumulation in tissues and body fluids following introduction of asbestos to the alimentary canal have provided seemingly contradictory observations. This review, which places particular emphasis on the impact of experimental and analytical limitations on the evidential strengths of each study, indicates the likelihood that a very small fraction of ingested microscopic asbestos fibers penetrates the gastrointestinal mucosa. A reliable estimate of the magnitude of long-term fiber retention in tissues as a consequence of chronic human ingestion of asbestos cannot be made at this time.

During the 1970s, potential health risks associated with exposure to mineral fibers in drinking water became a national concern. This concern developed primarily because of reports of some drinking water fiber concentrations in the range of 10^6–10^9 fibers/L (*1*) and published observations of increased risk of gastrointestinal and peritoneal cancer among asbestos workers (*2*). Inhalation of asbestos dust is accompanied by ingestion of many fibers cleared from the respiratory tract by mucociliary action. Thus a key question in the evaluation of cancer risks associated with the ingestion of asbestos involves the extent to which microscopic fibers, under normal alimentary canal conditions, can migrate through the gastrointestinal mucosa. Such movement of fibers could enable their residence in the bowel wall or, following hematogenous or lymphatic transport, the peritoneum and tissues at other sites. It is likely that such a movement of a large number of fibers is a necessary precursor for carcinogensis following ingestion of asbestos.

This paper will evaluate the published evidence for and against gut penetration and tissue accumulation of ingested mineral fibers. Particular attention will be paid to the influences of analytical techniques employed, fiber properties, and physiological factors on the capability of each investigation to provide useful information about fiber penetration. Most of the work on this question involves microscopic examination of tissues or body fluids for the presence of fibers as a consequence of a known ingestion exposure. Many of these exposures involve controlled laboratory tests with rodents, but a number of human environmental exposures have also been investigated.

Gut Penetration by Durable Particles Other Than Mineral or Synthetic Fibers

Studies involving ingestion of particles other than asbestos, mineral, or synthetic fibers provide some background information generally in support of the movement of a variety of durable particles through the gastrointestinal mucosa as at least an occasional event. Evidence for human intestinal uptake of particles as large as 75 μm is provided by the observation of starch granules in

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blood minutes after ingestion (3). Volkheimer reported that sleep, smoking, and caffeine increase the number of starch particles in the blood. Dyed cellulose particles were also identified in human blood and urine following ingestion of specially stained food (4). The cellulose fibers were found in urine several weeks after ingestion.

Various sizes of latex spheres have been studied. Latex particles of 0.22 μm diameter were reported to migrate from rat stomachs to lymphatics of the gastrointestinal mucosa and also to liver and kidney tissues (5). Mice that drank water suspensions of 2-μm diameter latex spheres for 2 months were found to have latex particles in macrophages in intestinal Peyer's patches (6). LeFevre et al. (7) later reported that mice that ingested 5.1-μm latex spheres had demonstrable accumulations of the particles in intestinal Peyer's patches, lungs, and mesenteric lymph nodes. The frequency of penetration was very small, and evidence for intestinal uptake of very large 15.8-μm latex spheres was not found. Although the difference in size may explain the absence of 15.8-μm sphere in tissues, a 50-fold lower number of large particles ingested appears to have created a corresponding loss of sensitivity for detection of the large particles by optical microscopy.

LeFevre et al. (7) did not find evidence for passage of large latex spheres from the intestinal lumen to the portal and peripheral blood as reported for larger starch granules by Volkheimer (3). The finding of some particles in mesenteric lymph nodes with a relative absence in the liver was offered as evidence for the restriction of transported particles to the lymphatic system. Most particles that penetrate the Peyer's patch epithelium are sequestered in macrophages and may be transported back into the intestinal lumen without further transport into the body (7).

Penetration of mouse intestine by 20- to 50-nm diameter carbon particles occurs almost exclusively through the epithelium covering Peyer's patches (8). India ink and ferritin microparticles are rapidly transported from the intestinal lumen by means of pinocytosis observed by electron microscopy of mouse Peyer's patch epithelial cells (9). Horseradish peroxidase uptake has been similarly described (10). Carbon particles from a 0.5% India ink suspension (20–25 nm diameter) were observed to pass almost exclusively through the posterior intestine of the Amazon molly, *Poecilia formosa* (11). Peyer's patches are apparently absent from the intestine of lower vertebrates. Intraperitoneal injection resulted in carbon particle accumulation in the heart, mesentery, and especially the head kidney of the fish.

A final observation of apparent passage of relatively large particles through the intestinal mucosa is provided by the presence of opal-phytoliths in digests of lymph nodes (12), and kidneys (13) of sheep that are thought to ingest up to 20 g of such particles with a daily intake of plant material.

As with many studies of ingested asbestos fibers, insufficient information is available concerning numbers of particles in nonasbestos exposures and particle detection limits achieved for the tissue preparation and microscope procedures employed to allow a comparison of the observations summarized above. In some regards, attempts to determine if ingested asbestos fibers penetrate the gastrointestinal mucosa provide a better test of durable particulate behavior in general. Fiber exposures can provide large numbers of particles available for penetration and the distinctive shapes and elemental compositions of mineral fibers allow better positive identification of the particles in the tissue milieu. On the other hand, the small size of most mineral fibers requires electron microscopic observation of tissues and consequent restrictions on the volume of tissue capable of being searched for fibers.

Experimental Factors to Consider in Evaluating Studies of Fiber Penetration of Tissues

Examination of tissues or body fluids with optical or electron microscopic techniques for identification of mineral fibers can be accomplished either by examination of thin tissue sections or bulk sample residues prepared by removal of carbonaceous and soluble material to concentrate inorganic particles. Thin-section examination provides information about the location of particle contaminants with respect to cells but is very insensitive for finding microscopic particles because of the very small volume of tissue present in a microscope field of view. A multitude of biological, analytical, mineralogical, and kinetic factors must be considered in order to evaluate the significance of a particular finding of the presence or absence of fibers in a sample.

Exposure

Important characteristics of the exposure dose include the size and number of fibers available for penetration of the gastrointestinal mucosa. Use of very large fibers or preparations in which fibers occur in large bundles or clumps for animal exposures may provide few particles capable of penetration. The ability to detect the passage of in-

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gested fibers to tissues or body fluids is increased by the ease with which the fibers can be identified microscopically. Durable, easily identified fibers, which are unlikely to be present through contamination of the samples or as a result of another exposure route (inhalation), are especially good tracers for ingested particle studies.

Asbestos and other mineral fibers possess sufficient durability to pass through the acid conditions of the stomach and penetrate the intestinal wall without extensive dissolution. Recent work demonstrates, however, that mineral fibers do differ considerably in their long-term resistance to dissolution while residing in the lungs or in acid solutions (*14*). Alteration of fiber sizes and shapes and splitting of some fibers to increase the number of fibers in tissue can occur during the dissolution process (*14*). Thus it is possible that the dissolution of less durable fibers following penetration of the gut may remove them prior to examination of tissues, whereas other types of fibers may undergo little change or be altered to create many thinner fibers. Phagocytosis, transport, and clearance of fibers are thought to be dependent on fiber size with smaller fibers more mobile. Thus, as these time-dependent processes take place, the number and sizes of fibers at any particular tissue site are likely to be changing. The time from last exposure, the number of particles, and the characteristics of the particles can therefore strongly influence the probability that detectable numbers of fibers will reside in tissue as a result of gut penetration.

Biological Factors

Since so little is known about mechanisms for passage through the gastrointestinal mucosa and subsequent movement of microscopic particles, physiological factors that may influence penetration are unknown. The time over which exposure takes place; the impact of massive fiber doses such as used in some animal ingestion studies; the nature of foods or fluids ingested with the fibers; the species of animal exposed and the relative permeability of its gastrointestinal mucosa; the role of mucosal tissue abnormalities; the age, sex, health, and other characteristics of individual exposed animals; dietary or environmental influences on the motility of the gut; and other factors may determine whether fibers are found when tissues from exposed animals are examined.

The selection of tissues or fluids for microscopic analysis is complicated by a number of considerations. The choice of any particular organ or site for analysis ultimately involves guesswork about where particles would travel after passing through the gastrointestinal mucosa, how long they would take to get there, how long they would reside there, and whether they would concentrate selectively in certain components of the organ. The sampling of portions of organs or body fluids is always coupled with the question of how representative the sample is of the whole. Sample selection must also consider the probability that the finding of a particular kind of fiber in the organ can be associated with a particular route of exposure such as ingestion rather than inhalation.

Analytical Factors

Electron microscopic methods for quantitative assessment of fiber concentrations in air and water have in recent years been successfully adapted to allow determinations of fiber concentrations in tissues and biological fluids. Analytical limitations present for air and water samples (*15*) are generally more severe for tissue analysis, particularly when trace concentrations must be measured (*16*). The primary concerns for tissue analysis are analytical sensitivity, accuracy, and prevention of sample contamination.

Sample characteristics, choice of sample preparation method, and choice of microscope type and conditions all determine the sensitivity of the analysis. Tissues from different organs vary greatly in their reaction to procedures for isolating inorganic particles from the organic matrix, so that the particles can be concentrated into smaller areas for microscope observation. Examination of thin sections frequently is incapable of detecting fiber concentrations likely to be present except in the case of lung tissue following high inhalation exposure. Electron microscopy is required to detect most asbestos fibers that generally have diameters less than 0.5 μm when present in tissues. Sensitivity is maximized through digestion and/or low-temperature ashing of large tissue volumes to produce concentrated residues that are examined by transmission electron microscopy (TEM). Scanning electron microscopy (SEM), although capable of sufficiently high magnification, produces images of sample surface features that are more difficult to search for individual asbestos fibers than TEM images.

Evaluation of reports of tissue analyses for fiber concentrations is impossible without knowledge of the detection limit achieved for each analysis. The detection limit is generally expressed as the concentration of fibers equal to the finding of one fiber in the portion of the sample examined.

Reported concentrations must exceed the detection limit by a factor of at least three or four before any degree of statistical significance is achieved.

The presence of the fibers in blank or control samples as a result of contamination obviously raises the number of fibers that must be observed in order to conclude that the fibers actually accumulated in the tissue. Conclusions made regarding the absence of fibers in tissue are weakened when presented without a demonstration of capability to count the fibers in positive control or spiked samples. When the percentage of recovery is found to be low, it should be used to estimate a more realistic detection limit for the analysis.

Some sample preparation techniques can produce large systematic losses of fibers. Other techniques can increase the number of fibers by fiber comminution. Chrysotile asbestos fiber concentrations are the most difficult to estimate because, in addition to difficulty in identification due to extremely thin diameters and the ease of introduction as contamination, these fibers are readily reduced to individual fibrils by surfactants, ultrasound, and other factors associated with sample preparation. Analytical precision is enhanced when sample preparations observed on the microscope contain a uniform distribution of particles. TEM grids produced by the carbon-coated Nuclepore filter method (*16*) are favored by many analysts for achieving uniform particle distributions in which the number of particles observed in replicate preparations by different laboratories is in reasonable agreement (*17*).

Evidence for and against Fiber Penetration

Several studies of tissues from subjects with occupational exposure to asbestos have resulted in reports of fibers in extrapulmonary tissues and fluids. Wyss (*18*) reported fibers observable by optical microscope in urine samples from asbestos workers. Langer (*19*) reported concentrations of asbestos bodies and uncoated fibers in the order lungs >> kidneys > pancreas > liver for asbestos workmen. Whether fibers arrived at these extrapulmonary sites via lung clearance and ingestion or via migration from the lungs and dissemination throughout the body cannot be determined.

The dissemination of fibers throughout the body has been demonstrated for fibers when introduced by injection into the blood (*20*) or subcutaneously (*21*). Occasional passage of fibers through membranes is suggested by the apparent cross-placental transfer of fibers to fetuses in pregnant rats injected through the femoral vein with chrysotile asbestos (*23*). SEM examination of tissues from mice injected intraperitoneally with amosite, crocidolite, or chrysotile asbestos demonstrated the penetration of the mesothelium by the fibers (*23*). Amphibole fibers have also been observed to migrate from the pleural cavity to the interior of the lungs and the kidneys (P. M. Cook, unpublished data).

More than 30 reports of examinations of tissues from animals and human subjects exposed to asbestos through ingestion (without inhalation exposure) were reviewed. Exclusion of multiple reports of the same observations reduced the number of reports to 19. Rather than discuss the experimental details and results reported for each study separately, Table 1 is provided as a summary and comparison of all 19 studies. Much of the information in Table 1 is presented to allow the reader some idea of how each study meets some of the criteria set forth in this review for evaluating studies of fiber penetration of tissues. Each reference should be consulted for a more detailed evaluation of its experimental design, data quality, and evidence for or against fiber penetration of the gastrointestinal mucosa. In many cases, incomplete information is available in these reports for defining analytical sensitivity, significance of sample contamination, fiber recovery efficiency of the sample preparation procedures, etc. The reduction of complex experimental design and results information to a few words that will fit in the table also requires that only the most generally correct characterizations can be present. For example, the microscope technique is listed as SEM where that technique was the primary method for determining fiber concentrations even though some tissue may have been qualitatively examined by TEM. Other relevant information, such as length of time from exposure to tissue collection, is not presented in the table.

Most of the headings in Table 1 are self-explanatory. The plus or minus symbols for "evidence for/against" indicate each author's conclusions with regard to whether the data reported supports fiber penetration. The fiber dose characterization ideally would provide the total number of fibers passing through the digestive tract. Many studies only report the mass of asbestos or percent of the diet. The duration of exposure varies, as shown in Table 1. In many cases, tissues were analyzed from animals at different time intervals following exposure. The variety of tissues analyzed in some cases is quite large. Positive or negative results for each type of tissue are indicated primarily on the basis of each author's

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Table 1. Summary of evidence for and against fiber penetration of the gastrointestinal mucosa.

Evidence for/against	Ref	Year	Species	No examined	Fiber type	Fiber dose Characterization	Exposure duration	Tissues analyzed	Tissue preparation method[b]	Microscope technique	Detection limit reported	Positive/negative control samples
+	(24)	1965	Rat	3	Chrysotile	No char., 6% diet	3 mos	Colon (+) Mesenteric nodes (-) Spleen (-)	Thin sections	TEM	No, qualitative	No/no
+	(20, 25)	1973	Rat	5 5 5	Chrysotile Chrysotile Control	9.6×10^8 fibers 9.4×10^8 fibers No fibers	Stomach injection	Blood (+) Omentum (+) Brain (+) Lung (-) Spleen (-) Heart (-)	Bulk, toluene, cent HTA, drop	TEM	No	No/contaminated
-	(26)	1974	Rat	10 5	Chrysotile Control	No char., 5% diet No fibers	21 mos	Intestine (-) Mesentery (-) Other organs (-)	Bulk, bleach, centr, wash, drop	TEM	Incomplete	No/contaminated
			Rat	10 10 5	Amosite tailings Control	No char., 400 mg No fibers	Single gavage	Intestine (-) Mesentery (-) Lung (?) Kidney (-)	Bulk, bleach, centr, wash, drop	TEM	No	No/yes
			Rat	10 10 10	Amosite tailings Control	No char., 10, 20% diet No fibers	6 days	Intestine (-) Mesentery (?) Lung (-) Kidney (-)	Bulk, bleach centr, wash drop	TEM	No	No/yes
	(27)	1976	Rat	2 2 2 2	Chrysotile Crocidolite Amosite Control	UICC 250 mg/week No fibers	1 yr + 1 mo free from exposure	GI tract (-)	Bulk, HTA, acid, filt	SEM	Incomplete, semiqualitative	No/yes
+	(28)	1977	Rat	10 10	Chrysotile Control	Fiber size only, 1% diet No fibers	6 weeks	Blood (?) Omentum (+) Lung (+) Kidney (+) Liver (+) Brain (+)	Bulk, LTA, acid, filt, LTA, drop	TEM	No	No/contaminated (less than 1973)
+	(29)	1977	Rat	5 2	Amosite Control	UICC, 0.1 mg Saline	1 hr on isolated jejunum segment	Epithelial surface penetration (+)	SEM preparation	SEM	Qualitative	No/yes
	(30)	1977	Human	282	Asbestos bodies	No char		Lung (+)	Bulk, LTA	OM	Incomplete	Yes/no
+	(31)	1979	Human	8 8	Amphibole Control	10^8 fibers/L in water $< 10^6$ fibers/L in water	Variable by subject	Urine (+)	Bulk, filt, LTA, filt, c-coat, wick	TEM	10 fibers/ml	Yes/yes
+	(32)	1979	Baboon neonate	1 1	Chrysotile Control	ca 3×10^{10} fibers in milk No fibers	9 day period	Kidney (+) Liver (-) Spleen (-)	Bulk, LTA, filt, LTA, filt, drop	TEM	80 fibers/mg	No/yes

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Table 1. Summary of evidence for and against fiber penetration of the gastrointestinal mucosa.

Evidence for/against	Ref.	Year	Species	No examined	Fiber type	Fiber dose Characerization	Exposure duration	Tissues analyzed	Tissue preparation method[b]	Microscope technique	Detection limit reported	Positive/negative control samples
+	(33)	1979	Baboon	1	Chrysotile	UICC, 120 kg Pregavage	4 days, gavage	Urine (+)	Bulk, filt, LTA, filt, LTA, drop	TEM	5×10^2 fibers/ml	No/yes
+	(34)	1980	Human	53[c]	Amphibole Chrysotile	10^8 fibers/L, in water None in water	Variable by subject	Liver (+) Lung (+) Jejunum (+)	Bulk, KOH, centr, HTA, drop	TEM	30 fibers/mg	Yes/yes
+	(35)	1980	Rat	5 5 15 8 2	Chrysotile Crocidolite Chrysotile Chrysotile Control	UICC, 10^{11} fibers UICC 10^{10} fibers NIEHS 10^{11}-10^{13} fibers NIEHS 10^{10}-10^{11} fibers No fibers	Single gavage Single gavage Diet 1-12 days Diet 1-12 days	Lymph Fluid (+) Lymph fluid (+) Lymph fluid (+) Lymph fluid (+) Lymph fluid (+)	Bulk, digestion, filt, c-coat, wick	TEM	Incomplete	No/yes
+	(36)	1980	Rat	10 10 6	Chrysotile Cellulose Control	UICC, 10% diet Cellulose fibers No fibers	2 yr	Colon (+) Colon (-) Colon (-)	Thin, 20 um, HTA	TEM	No	No/yes
+	(37)	1980	Baboon Newborn	1 1	Chrysotile Control	UICC, 3×10^{13} fibers No fibers	9 day period	Kidney cortex (+) Lymph nodes (+) Spleen (+) Colon (+) Esophagus (+) Kidney medula (+) Other sites (-)	Bulk, LTA, filt, LTA, drop		80 fibers/mg	No/yes
+	(38)	1981	Human	34	Chrysotile	None in drinking water	Unknown	Urine (+)[d]	Bulk, filt, LTA, filt, c-coat, wick	TEM	10 fibers/mL	No/yes
+	(39)	1981	Human	1	Attapulgite	5 g/day	Drug, 6 mos	Urine (+)	Bulk, undefined	TEM	No	No/yes
-	(40)	1981	Baboon	1 1	Chrysotile + Crocidolite Control	NIEHS, 800 mg NIEHS, 800 mg No fibers	Gavage, 8 times	Urine (-) Blood (-) Tissues (-)	Bulk, filt, LTA, acetone, drop Bulk, KOH, filt, LTA, acetone, drop	TEM	4×10^2 f/ml 4×10^4 f/ml 200 f/mg	Yes/yes

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Table 1 Summary of evidence for and against fiber penetration of the gastrointestinal mucosa.

Evidence for/against	Ref	Year	Species	No examined	Fiber type	Fiber dose Characterization	Exposure duration	Tissues analyzed	Tissue preparation method[b]	Microscope technique	Detection limit reported	Positive/negative control samples
+	(41)	1981	Fish	2	Amphibole	ca 10^8 fibers/L	Lifetime, water and possibly diet	Kidney (++)[c] Liver (++) Muscle (++)	Bulk, LTA, filt, c-coat, wick	TEM	1-10 f/mg	No/yes
				1	Amphibole	ca 10^6 fibers/L						
				2	Chrysotile	Unknown						
-	(42)	1982	Rat	13	Chrysotile	UICC, 250 mg/week	~20 mos	9 sites (-)[d]	Bulk, LTA, acid, filt	SEM	Incomplete	No/no
				12	Amosite							
				13	Crocidolite							

[a]Positive or negative for fibers

[b]Preparation method steps: thin = removal of a thin tissue section for examination; bulk = processing a large volume of tissue to isolate and concentrate the inorganic particles; HTA = high temperature ashing; LTA = low temperature ashing; KOH, bleach, Soluene = chemicals used for digestion of tissue to remove organic matrix; acid = use of dilute acid to dissolve the nonsilicate portion of the ash; centr = centrifugation to isolate particles from suspension; filt = membrane filtration to remove particles from suspension usually prior to TEM grid preparation; c-coat, wick = preparation of a TEM grid by dissolving a piece of carbon-coated membrane filter by solvent wicking action to leave the particles embedded in the carbon film suspended on the grid; drop = preparation of TEM grid by evaporation of a small drop of a particle suspension on a carbon-coated TEM grid

[c]Samples were pooled as an exposure group (32 subjects exposed to amphibole fibers in drinking water) and a control group (no amphibole fibers in drinking water).

[d]Number of fibers in urine exceeded number in blank for each urine sample.

[e]Amphibole concentrations in replicate preparations of tissues were found to fit a dose-response relationship with concentrations greatest in kidney and very low in muscle tissue.

[f]Some fibers were found in tissues (no comparison to blank samples) but concluded to be insignificant on the basis of a lack of preferential retention in any specific tissue, particularly the mesenteric lymphatic tissues. The authors also indicated difficulty in identifying fine fibers (< 0.1 μm diameter) with the SEM

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conclusions. Question marks are entered where a reasonable uncertainty exists as to whether fibers identified in the tissue are from sample contamination or actually resided in the tissue.

The complexities of tissue sample preparation methods are difficult to condense into a short table entry. In Table 1 they are first categorized as "thin" (microtomed tissue section) or "bulk" (large tissue volumes processed to remove the organic matrix and concentrate inorganic particles). Thin-section analyses are invariably too insensitive to allow the observation of trace concentrations of fibers widely disseminated in tissues. For bulk preparations, a few key words have been added to provide some definition of each bulk preparation technique used. This provides some information concerning the quality of sample preparations, chances for contamination, and uniformity of sample distribution on TEM grids. These key words are provided in the order of the basic steps used for sample preparation. A general sequence involves a digestion and/or ashing (high or low temperature), a concentration of the residue suspended in water by centrifugation or filtration, and the preparation of TEM grids by direct transfer of sample from a filter or application of a drop of suspension to the surface of a carbon-coated grid.

The absence of a clear definition of detection limit, as indicated in Table 1 for many studies, makes the reports of negative tissue analyses difficult to evaluate. Since there can be no demonstration of zero fiber concentrations in tissues, the detection limit, often reported as a "less than" value for each sample, is essential for determining if any particular exposure has resulted in significant accumulations of fibers in the animal. Similarly, the absence of blank tissue control samples makes reports of positive tissue fiber concentrations difficult to accept because of the possibility of fiber contamination. Since it is possible during some sample preparation procedures to systematically lose large numbers of fibers or to fail to detect the fibers during microscope examination of the sample, positive tissue control samples (samples with a known trace concentration) are important for demonstrating that fibers, if present in tissues, can be detected quantitatively. Consequently, negative fiber presence studies in which this capability is not demonstrated have limited authority.

Conclusions

It is difficult to conclude on the basis of the studies summarized in Table 1 that asbestos fibers do not cross the intestinal barrier. The possibility of such passage does not depend on the viability of Volkheimer's "persorption" mechanism (*3*). Most asbestos fibers typically are much smaller than starch granules and closer in length to the 2-μm (*6*) and 5.7-μm (*7*) diameter latex spheres observed by LeFevre et al. to penetrate intestinal Peyer's patches. The widths of many asbestos fibers are actually similar to the 0.02- to 0.05-μm diameters of carbon particles observed to penetrate the epithelium covering Peyer's patches (*8*).

Since it is not possible to design an experiment that demonstrates that no asbestos fibers are retained in tissues following ingestion, we must determine the presence or absence of fibers at a level of detection determined by the analytical considerations discussed earlier. Studies summarized in Table 1 that report the presence of fibers in tissue or fluids generally also report the lowest detection limits, and most offer evidence of negligible fiber contamination as a source of false-positive results. The studies that report negative results are fewer in number and either do not define the analytical detection limit, provide incomplete information, or report a less sensitive analysis than studies reporting positive results for similar experiments.

In addition to consideration of detection limits, which are a pure reflection of the amount of tissue examined, the efficiency of the microscopic technique for detecting individual fibers present in the sample preparation may indicate a further decrease in sensitivity. TEM examination of dispersed particulates is most efficient and allows electron diffraction and energy dispersive X-ray spectral identification of each fiber. One study (*42*) used SEM to detect fibers in bulk tissue preparations but observed: "However the working resolution of this instrument was such that it was difficult to be confident of identifying fine fibers (i.e., less than 0.1 μm in diameter) among residues."

Many of the studies summarized in Table 1 involve exposures to chrysotile asbestos. This is appropriate, since chrysotile is the most common fiber contaminant of water, food, and beverages. Chrysotile fibers, however, serve as poorer indicators of fiber penetration and transport to tissues because of their susceptibility to leaching and comminution and their common occurrence as a contaminant added during sample preparation. Amphibole fibers, including amosite and crocidolite asbestos, and attapulgite fibers were also studied and provide strong indications of fiber penetration. Crocidolite fibers were detected in

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lymph fluid of rats fed crocidolite in contrast to the finding of chrysotile fibers in the lymph fluid of other rats fed chrysotile (*35*). Attapulgite fibers were found in high concentration in the urine of a human subject known to have ingested a large amount of that mineral (*39*). Attapulgite is unlikely to be present as a result of a contamination introduced during or after sample collection. Finally, urine (*31*) and tissue (*34*) concentrations of amphibole fibers were shown to be well associated with exposure or absence of exposure to the same amphibole fibers in drinking water. These amphibole fibers existed as a mixture of cummingtonite-grunerite and actinolite with a unique range of elemental compositions that strengthened the association between the identity of fibers in the water ingested and the identity of fibers in the urine and tissue samples.

Perhaps more important than the weight of evidence in favor of the probability of some fiber penetration is the question of what fraction of ingested fibers may be involved. Very little information exists for providing an estimate of this fraction. Any estimate is subject to a number of assumptions and qualifications related to the nature of the data used. The study of fiber appearance in lymph fluid following ingestion or gavage of chrysotile or crocidolite in rats resulted in an estimate of a maximum daily passage of fibers to the lymph fluid of 10^{-4} to 10^{-7} times the number of fibers introduced to the stomach. (*35*). Amphibole fiber concentrations in human urine were observed to represent approximately 10^{-3} of the concentration of the same fibers in the drinking water consumed for up to 20 years prior to the sample collections (*31*). Both the lymph fluid and urine measurements provide a limited basis for an estimate because they do not account for all fibers that may move across the gastrointestinal mucosa. The kinetics of fiber transport and elimination are unknown and further complicate attempts to use the limited data for such estimates of fiber penetration. These estimates do, however, indicate the involvement of a very small fraction of ingested fibers in penetration and consequently low probability for significant tissue accumulations and increased risk of cancer.

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.

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