Determination of Mineral Fiber Concentrations in Fish Tissues

ALLAN R. BATTERMAN AND PHILIP M. COOK

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, MN 55804, USA

BATTERMAN, A. R., AND P. M. COOK. 1981. Determination of mineral fiber concentrations in fish tissues. Can. J. Fish. Aquat. Sci. 38: 952–959.

Submicroscopic inorganic particle concentrations in tissue have not been quantitatively determined in the past because of a lack of sample preparation techniques capable of achieving the sensitivity required. The determination of whether mineral fibres in water are accumulated in aquatic organisms requires transmission electron microscope examination of bulk tissue residues rather than thin sections. The sample preparation method used for this investigation involved removal of water and organic matter by freeze-drying and low temperature ashing. Lake trout with a lifetime exposure to Lake Superior water containing amphibole fibers contained similar amphibole fibers particularly in the kidney and with low concentrations in muscle tissue. Lake trout from two locations with widely different water fiber concentrations had corresponding differences in tissue fiber concentrations. Analysis of other fish raised under laboratory conditions suggests that ingestion is the primary route for fiber accumulation.

Key words: electron microscopy, fiber accumulation, asbestos, lake trout, brook trout, channel catfish, Arctic char

BATTERMAN, A. R., AND P. M. COOK. 1981. Determination of mineral fiber concentrations in fish tissues. Can. J. Fish. Aquat. Sci. 38: 952–959.

Le manque d'une technique de préparation des échantillons permettant d'atteindre la sensibilité requise est la raison pour laquelle les concentrations de particules inorganiques submicroscopiques dans les tissus n'ont pu être mesurées quantitativement dans le passé. Afin de déterminer si des fibres minérales présentes dans l'eau s'accumulent dans les organismes aquatiques, il faut examiner au microscope électronique des tissus en vrac plutôt que de minces coupes. Dans l'étude décrite ci-dessous, la préparation des échantillons comporte l'enlèvement de l'eau et de la matière organique par cryodessiccation et incinération à basse température. Des touladis ayant été exposés toute leur vie à l'eau du lac Supérieur contenant des fibres d'amphibole ont également de ces fibres, particulièrement dans le rein et, à de faibles concentrations, dans le tissu musculaire. Des touladis provenant de deux endroits dont la concentration des fibres dans l'eau était très différentes montrèrent des différences correspondantes de concentration des fibres dans les tissus. L'analyse d'autres poissons élevés dans des conditions de laboratoire donne à penser que l'ingestion est la principale voie d'accumulation des fibres.

Received December 4, 1980
Accepted April 24, 1981

Reçu le 4 décembre 1980
Accepté le 24 avril 1981

RECENT concern over the human carcinogenicity of asbestos and analogous mineral fibers when ingested (Lee 1974) has spurred interest in the development of methods for the quantitative determination of their presence in tissues. The discovery of mineral fibers in human urine has demonstrated that some fibers can pass through the human gastrointestinal tract following ingestion of water contaminated with amphibole[1] fibers (Cook and Olson 1979). The same type of fibers were

[1]Amphibole minerals are members of a class of hydrated silicates having a double-chain crystal structure. These minerals can crystallize during rock formation in an asbestiform habit (bundles of individual fibers). Amphibole minerals used commercially as asbestos (when minable as the asbestiform variety) are amosite (fibrous grunerite), crocidolite, anthophyllite, tremolite, and actinolite. Most amphibole particles in western Lake Superior water fall in the cummingtonite–grunerite series and appear to range from nonasbestiform to asbestiform in origin. A fiber in a water sample is defined as any particle with a length-to-width ratio equal to or exceeding 3:1. Some nonasbestiform amphibole mineral crystals, when crushed, form cleavage fragments that are microscopically very similar to, or identical with, small fibers, which result from the crushing of amphibole asbestos, in morphology, crystal structure, and chemistry. About 95% of the asbestos used in North America is chrysotile, a fibrous serpentine mineral with a distinct tubular microstructure.

Printed in Canada (J6310)
Imprimé au Canada (J6310)

TABLE I Fish samples analyzed

Species	Location	Age (years)	Collection date (mo/yr)	No of fish	Estimated avg water fiber concn (fibers/L)		Food source
					Amphibole $\times 10^6$	Chrysotile $\times 10^6$	
Salvelinus namaycush	Split Rock, Minnesota	3–4	May 1978	2	100	< 1	Natural diet
S. namaycush	Huron Bay, Michigan	4–5	March 1980	1	1	Unknown	Natural diet
S. alpinus	Deception Bay, Hudson Bay, Quebec	7–12	Sept 1978	2	Unknown	0–500	Natural diet
S. fontinalis	ERL-D, Duluth, Minnesota	2–3	Feb 1980	1	50	< 1	Lab diet (Glencoe® Pellets)
Ictalurus lacustris	ERL-D, Duluth, Minnesota	5–6	May 1976	2	50	< 1	Lab diet (Glencoe® Pellets)

recently reported to be present in human liver, lung, and jejunum tissues as a consequence of ingesting Lake Superior water containing amphibole fibers (Carter and Taylor 1980). No studies of the accumulation of fibers by aquatic organisms have been reported with the exception of the optical microscope observation of particles in mussels (*Mytilus edulis*) exposed to asbestos tailings (Halsband 1974). Because mineral fibers contaminate some natural waters and fish are thus exposed to these fibers, we have investigated the possible accumulation of these fibers in fish tissue. Optical microscope examination of tissue preparations for mineral fibers is not practical because most of these particles have widths less than the resolving power of the optical microscope (Berkeley et al. 1965). Electron microscopy of standard histological preparations of ashed thin sections is not feasible because the approximately 70-nm-thick tissue sections would require astronomical numbers of fish in tissue to see one fiber in an entire section (Cook 1979). Since detection of concentrations of only a few hundred fibers or less per gram of tissue is required, a method is necessary which separates the fibers from larger pieces of tissue without altering them. This method must be able to eliminate all organic matter without changing the morphological, chemical, or physical characteristics of fibers.

To determine if freshwater fish used as human food can accumulate mineral fibers in their tissue, either by contact with the particles in contaminated waters or by ingestion of contaminated organisms of the food chain, several species of fish with different exposure histories were selected for analysis.

Methods

Exposure concentrations for fish collected from natural waters (Table 1) are approximations based on the assumption that the fish spent a significant portion of the year prior to capture in the collection area rather than distant areas with radically different fiber concentrations. All fish except the brook trout were frozen for storage and/or shipment prior to thawing and removal of tissue samples for analysis. Brook trout were collected from laboratory stock at the time of tissue preparation. Arctic char samples were obtained from frozen tail sections received after other portions of the fish were analyzed for toxic chemicals (I. Boulva and A. LeBeau, Department of Fisheries and Oceans, C.P. 15500, Quebec, Que. G1K 7Y7, 1979, personal communication).

The fish were dissected in a filtered air laminar flow hood in a clean room with a positive-pressure particle-free air supply. Dissecting instruments were cleaned with particle-free water. One set of instruments was used to remove the skin, another set to remove a block of tissue, and a third set to remove a subsection of tissue for actual analysis. The tissue sample was placed in a 25-mL Pyrex sample tube, weighed, frozen in liquid nitrogen, freeze-dried for ~ 5 h, and placed in a low temperature asher (LTA) at 150 W and 1.5 mL O_2/min for at least 16 h. The ash was suspended in ~ 15 mL of water acidified with 6 drops of 6 M HCl. Blank samples were prepared by processing an empty tube with the tissue sample tubes in each chamber of the LTA. The ash suspension was placed in an ultrasonic bath for 2 min, mixed on a vortex stirrer for 1 min, and filtered through a 25-mm 0.1-μm Nuclepore membrane filter. The filter was placed in a plastic Petri dish and dried for ~ 30 min at 50°C. One-half of the filter was carbon-coated in a vacuum evaporator. Small pieces (2 mm^2) were cut out of the carbon-coated filter and placed carbon side down on Formvar-film-coated electron microscope finder grids. The grids were placed on a piece of coarse metal screen on top of several layers of filter paper in a Petri dish. A small drop (10 μL) of 0.1-μm filtered chloroform was placed on each filter section and then the layers of filter paper were immediately saturated with filtered chloroform. The Petri dish was covered, and the chloroform allowed to dissolve all of the filter piece and Formvar film for ~ 12 h. The grid which resulted was covered with a thin carbon film in which sample particles were imbedded (Fig. 1). This technique prevents the loss of particles during transfer of the sample from the filter to the grid, preserves the distribution of particles originally in the sample, and leaves a replica of the filter (0.1-μm holes) which can be used to check on folds or breaks in the carbon film which would create areas of uneven particle distribution.

Randomly chosen grid openings with areas of ~ 9500 μm^2 were systematically scanned at a magnification of 10 000× or higher on a JEOL 100C electron microscope in the trans-

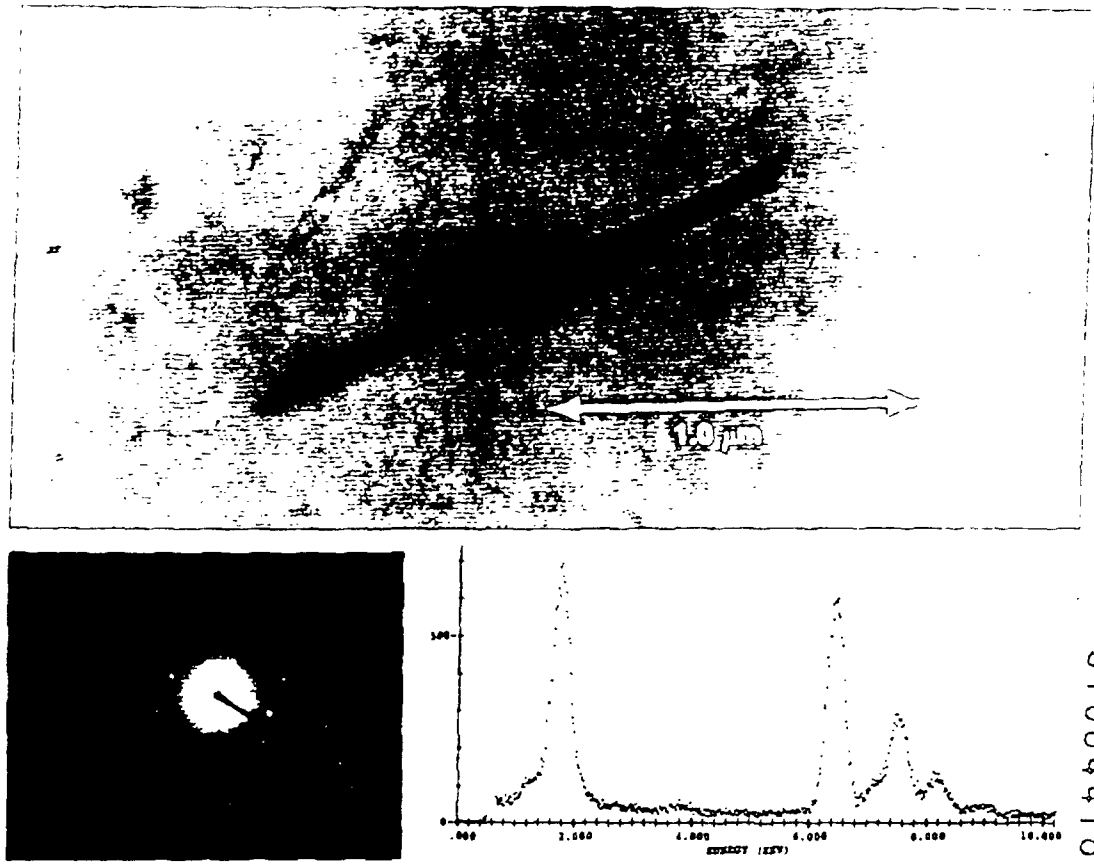

FIG 1. Electron micrograph of a fiber, 1.6 μm long, identified in a lake trout kidney preparation. The selected area electron diffraction pattern (*lower* left inset) established the mineral group as amphibole. The energy-dispersive X-ray spectrum (*lower* right inset) with major silicon and iron peaks established the mineral as grunerite.

mission mode. Grid openings with broken carbon film or other disruption of the sample were rejected. Each particle with an aspect (length to width) ratio ≥3 was identified by comparison of its morphology, selected area electron diffraction (SAED) pattern, and energy dispersive X-ray (EDX) spectrum with those obtained for standard mineral samples. Amphibole (Fig. 1) and chrysotile fibers produce characteristic SAED patterns and EDX spectra when oriented properly in the electron beam. Fiber size, orientation, or interfering particles cause difficulties with positive identification of fiber mineralogy. Fibers which possess amphibole or chrysotile morphology but SAED patterns and/or EDX spectra not clearly diagnostic for amphiboles, chrysotile, or some other mineral were classified as "ambiguous." Each sample was examined until 100 particles of interest were counted or at least 10 grid openings were scanned.

Fiber concentrations were calculated from the formula:

$$\text{No. of fibers/mg} = \frac{\text{no. of fibers} \times \text{filtration area } (\mu m^2)}{\text{area examined } (\mu m^2) \times \text{weight of sample (mg)}}$$

Fiber size distributions were analyzed and compared by computer program. Elemental ratios calculated from integrated EDX peak intensities were plotted on ternary diagrams to facilitate the comparison of mineral fiber elemental compositions for different samples.

The tissue preparation technique described above was chosen after attempting several other techniques involving chemical digestion. The principle objective was to optimize the sensitivity of the analysis by increasing the amount of tissue processed per unit area of electron microscope grid examined. Digestion chemicals included Soluene®, KOH, H_2O_2, and a mixture of KOH–H_2O_2. Although these reagents all effectively digested muscle, kidney, and liver tissues, the digests when filtered through a Nuclepore filter (either 0.1-μm or 0.4-μm pore diameter) left too much residue to allow direct preparation of electron microscope grids. Therefore all chemical digestion residues on filters had to be low temperature ashed and refiltered prior to grid preparation. The resulting grids were suitable for electron microscope analysis and generally contained a slightly greater sample density than grids prepared by LTA without chemical digestion. The

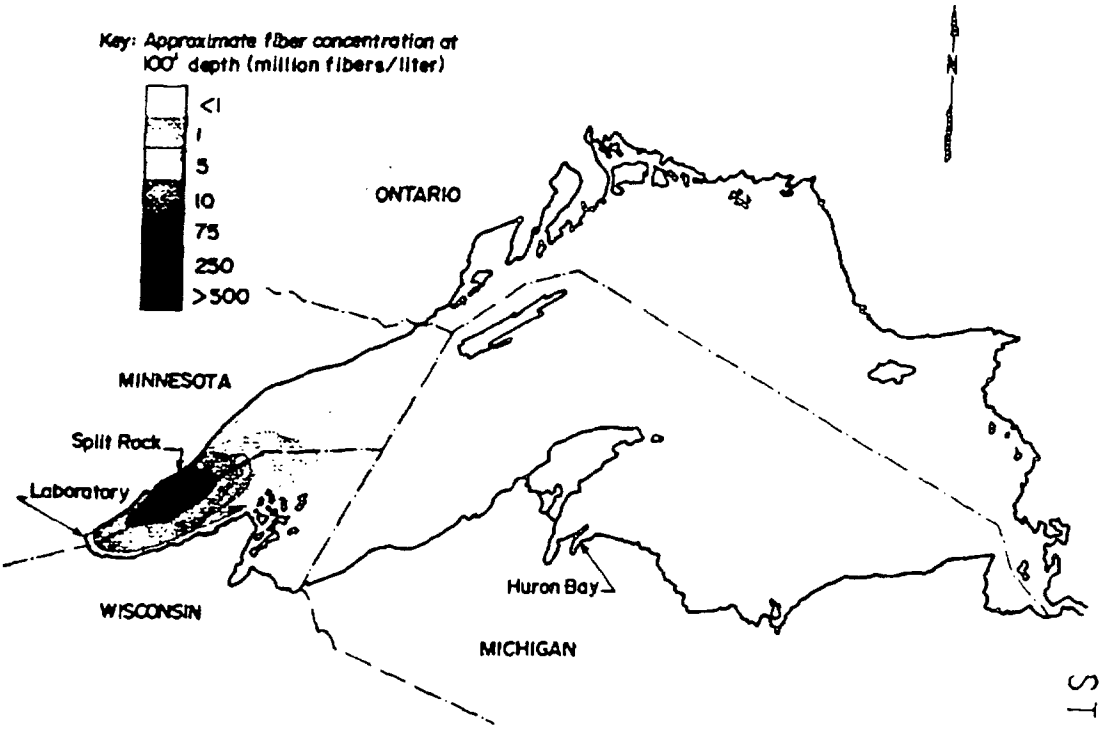

FIG 2. Estimated average amphibole fiber concentrations in subsurface Lake Superior waters at a depth of 30 m during the period of fish exposure influencing this study. The approximated distribution pattern is based on western Lake Superior water circulation, available measurements of fiber concentrations, and X-ray diffraction measurements of the amphibole mineral content of suspended solids and bottom sediments (Cook et al. 1976).

chemical digestion techniques were not chosen for this study, however, because membrane filters when ashed with the sample were found to add significant numbers of chrysotile fibers. Amphibole asbestos (crocidolite) fibers have also been found in one batch of membrane filters. Since the chemical digestion techniques also involved considerably more manipulation of the sample without greatly increasing analytical sensitivity, fish tissues were processed with the freeze-drying/LTA method. Average sample sizes processed were 1.0 g for muscle tissue and 0.3 g for kidney and liver tissue. Thus electron microscope identification of particles in a typical grid opening of 9500 μm^2 involved 0.00004 g of muscle tissue and required ~ 1 h to complete.

Fiber concentrations for Lake Superior waters were estimated from monitoring data accumulated for different locations primarily in western Lake Superior since 1974. Only measurements made with the carbon-coated Nuclepore filter Jaffe Wick method (Cook et al. 1976, Anderson and Long 1980) were used. Chrysotile fiber concentrations for the Deception Bay, Quebec area, were used as reported (Boulva and LeBeau personal communication).

Results

Minimum detectable particle concentrations are smallest where the amount of tissue prepared and the number of fields examined were largest. The poor precision for concentrations determined when only a few fibers are identified is reflected by the large range of the 95% confidence interval calculated on the basis of a Poisson distribution for counted particles (Hallenbeck et al. 1977). Blank samples did not contain detectable amphibole fibers. For the few blank samples containing chrysotile fibers the blank concentration is calculated on the basis of the weight of the corresponding tissue sample per grid opening. Thus, the comparision of fiber concentrations in tissue and blank samples is on the basis of fibers observed in each per unit area of the grid.

Fish with a lifetime exposure to Lake Superior water were studied primarily for the presence of amphibole fibers. The amphibole fiber distribution pattern in Lake Superior (Fig. 2) results from counterclockwise circulation of fine taconite tailings discharged 11 km northeast of Split Rock, Minnesota, during the period of 1956–80 (Cook et al. 1974). Lake trout, *Salvelinus namaycush*, captured at Split Rock are thought to have a much higher exposure to amphibole fibers than lake trout captured at Huron Bay, Michigan, far from the area of extreme amphibole fiber contamination. The Split Rock lake trout are likely to have occasionally migrated out of the area of high amphibole fiber concentrations, particularly by moving to the northeast along the north shore of Lake Superior.

Amphibole fibers provide a good test of fiber accumulation by fish because they are limited in distribution in natural

TABLE 2 Fiber concentrations in fish

Species	Location	Tissue	No of fish	No of preparations	x weight (g)	Total fields examined	Total No of fibers observed		Tissue concn [a] (fibers/milligram)		Blank concn [a] (fibers/mg)	
							Amphibole	Chrysotile	Amphibole	Chrysotile	Amphibole	Chrysotile
Salvelinus namaycush (Lake trout)	Split Rock	Muscle	2	6	1 228 (0 603–1 775)	85	5	108	1 0 (0 3–2 4)	27 0 (22 1–32 6)	<0 4 (0 0–1 5)	<0 4 (0 0–1 5)
		Kidney	2	3	0 388 (0 246–0 582)	31	202	0	1246 7 (1079 3–1427 9)	<6 2 (0 0–12 3)	<1 7 (0 0–6 2)	<1 7 (0 0–6 2)
		Liver	2	4	0 219 (0 139–0 407)	33	6	6	20 5 (7 52–44 7)	20 5 (7 52–44 7)	<3 0 (0 0–11 0)	<3 0 (0 0–11 0)
Salvelinus namaycush (Lake trout)	Hurst Bay	Muscle	1	2	1 335 (0 918–1 524)	29	2	1	1 4 (0 2–5 0)	0 7 (0 0–2 5)	<1 0 (0 0–3 7)	2 0 (0 2–7 1)
		Kidney	1	3	0 335 (0 169–0 541)	29	13	2	35 1 (18 7–60 1)	5 4 (0 7–19 5)	<3 9 (0 0–14 5)	7 8 (0 9–28 1)
		Liver	1	1	0 403	10	0	1	<6 5 (0 0–24 1)	6 5 (0 2–24 1)	<6 5 (0 0–12 1)	6 5 (0 2–24 1)
Ictalurus lacustris (Catfish)	Laboratory	Muscle	2	3	0 217 (0 078–0 494)	33	1	10	3 7 (0 1–23 2)	36 7 (17 67–67 5)	<5 5 (0 0–20 3)	11 0 (1 3–39 7)
		Kidney	1	1	0 041	6	0	0	<107 0 (0 0–394 7)	<107 0 (0 0–394 7)	<29 2 (0 0–107 6)	<58 4 (0 0–215 2)
Salvelinus fontinalis (Brook trout)	Laboratory	Muscle	1	1	0 805	6	0	2	<5 4 (0 0–20 1)	10 9 (1 3–39 3)	<3 3 (0 0–12 0)	<3 3 (0 0–12 0)
		Kidney	1	2	0 535 (0 065–0 1781)	11	0	5	<25 0 (0 0–92 1)	124 8 (40 4–290 8)	<27 5 (0 0–101 3)	<27 5 (0 0–101 3)
		Liver	1	2	0 364 (0 159–0 706)	16	0	0	<5 8 (0 0–21 5)	<5 8 (0 0–21 5)	<9 3 (0 0–34 3)	<9 3 (0 0–34 3)
Salvelinus alpinus (Arctic char)	Deception Bay	Muscle	4	6	3 380 (3 850–1 868)	64	0	20	<1 4 (0 0–4 9)	2 9 (1 7–4 4)	<4 3 (0 0–15 9)	8 7 (5 2–13 6)
		Kidney	3	3	0 142 (0 103–0 272)	22 5	1	28	8 3 (0 2–30 7)	230 5 (153 3–333 1)	<9 4 (0 0–34 6)	175 9 (105 9–280 0)

[a] Measured fiber concentrations or detection limits are reported as fibers per milligram of tissue (wet weight) with a 95% confidence interval for a Poisson distribution indicated within parentheses. Blank sample concentrations or detection limits are reported as calculated for the corresponding tissue sample weight per unit area of microscope examination.

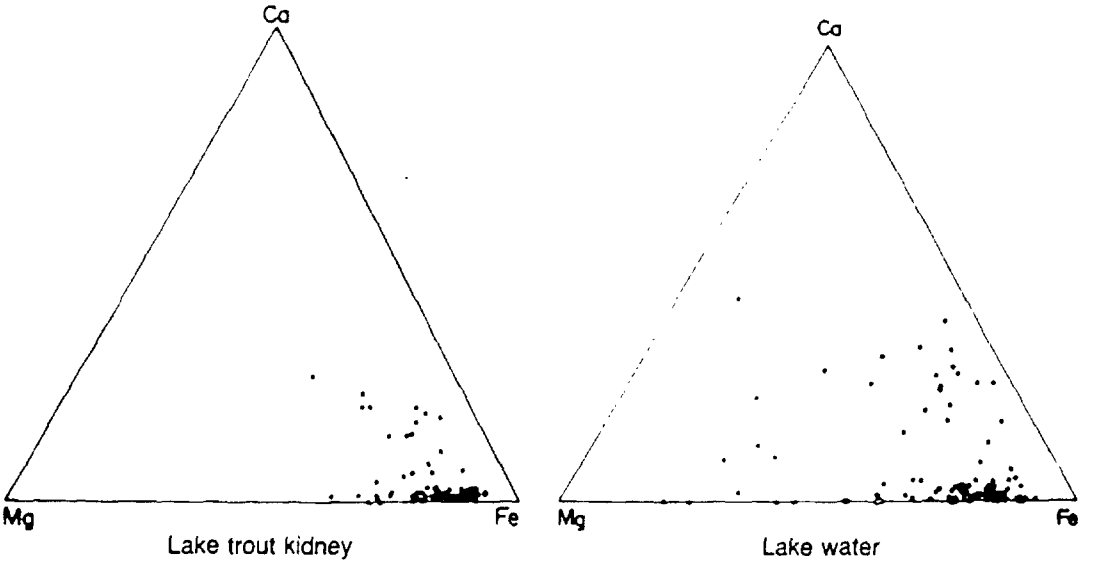

FIG 3 Iron–magnesium–calcium ternary diagrams for the major cation compositions of 100 amphibole fibers in both Lake Superior water and kidney tissue from lake trout exposed to the water. Plotted cation X-ray emission intensity ratio are calculated directly from integrated K_α peak intensities obtained after background subtraction from the energy-dispersive X-ray fluorescence spectra of individual amphibole fibers

waters and are not likely to present a contamination problem during analysis as are chrysotile fibers. No amphibole fibers were found in any of the blank samples analyzed. Large numbers of amphibole fibers were identified in kidney tissue of the lake trout, but only a few amphibole fibers were identified in lake trout muscle and liver tissues (Table 2). Although no difference in amphibole fiber concentration could be observed in lake trout muscle tissue for Split Rock versus Huron Bay fish, large differences existed for kidney and liver tissues. Only one amphibole fiber was found in all the tissue examined from catfish and brook trout raised in the laboratory, but only $\frac{1}{10}$ as much kidney tissue was examined for these fish when compared with the lake trout.

A check of the association between amphibole fibers in Lake Superior water and those in the lake trout tissue is provided by comparison of the elemental composition as determined by EDX analysis of individual amphibole fibers in the water and fish tissue. The unique amphibole mineral particle distribution found in Lake Superior water is also found in the fish. The major cation (iron–magnesium–calcium) compositions of 100 amphibole fibers observed both in Split Rock lake trout kidney tissue and in the lake water indicated similar amphibole mineral fiber mixtures of ~ 75% cummingtonite–grunerite [$(Fe,Mg)_7Si_8O_{22}(OH)_2$] and 20% actinolite [$Ca_2(Fe,Mg)_5Si_8O_{22}(OH)_2$] (see Fig. 3). The absence of aluminum in almost all amphibole particles identified precludes the presence of hornblende, a more common amphibole constituent of natural sediments and soils. The total of 15 amphibole fibers identified in Huron Bay lake trout samples fit the same amphibole mineral distribution pattern although too few fibers were identified to provide as strong an association.

Arctic char, *Salvelinus alpinus*, were collected from two locations near the mouth of the Deception River at Deception Bay, an arm of Hudson Bay in Quebec, Canada. Chrysotile fiber concentrations as high as 670 million fibers per litre in the river mouth (Boulva and LeBeau personal communication) have been measured and are attributable to a chrysotile mining operation upstream and loading of asbestos onto ships at the bay. The anadromous nature of the Arctic char and the dependence of water fiber concentrations on asbestos mining and shipping operations make it very unlikely that the fish were exposed to average chrysotile fiber concentrations exceeding 10^6 fibers/L in the year preceding their capture. This uncertain exposure is reflected in the estimated average water exposure concentration (Table 1).

Muscle tissue samples from all four Arctic char analyzed contained chrysotile fibers but more chrysotile fibers were found in blank samples (Table 2). Higher chrysotile concentrations are reported for kidney tissue, but these concentrations are only slightly greater than corresponding blank sample concentrations. Only one amphibole fiber was found in all the Arctic char samples examined.

Diatom frustule fragments were particularly common in kidney and liver tissue of lake trout and kidney tissue of Arctic char (no liver analyzed). None was observed in fish raised in the laboratory. A conservative estimate of frustule fragments was made by counting each particle with a porous, diatom frustule-like appearance. All fragments analyzed by EDX gave spectra containing only a silicon K_α peak as expected for diatom frustules which are composed of amorphous silica. The concentrations in Split Rock and Huron Bay lake trout kidney tissue were 100 and 200 particles/mg, respectively. The identification criteria prevented counting many small fragments that were undoubtedly present in the samples.

Little comparison of amphibole fiber size distributions is possible since only lake trout kidney tissue contained enough fibers to allow a good measure of the incidence of different

sizes of particles Typical fiber size distributions in any media are characterized by large percentages of small particles The mean amphibole fiber length and width for Split Rock lake trout kidney tissues were 1.99 and 0.37 μm as compared to 1 50 and 0.28 μm for Huron Bay lake trout kidney tissue. Lake trout muscle tissue amphibole fibers (only seven identified) had a mean length of 1 06 μm and a mean width of 0.22 μm. The kidney preparations were often ......y with debris, so many short or thin fibers may not have been observed, and this affects both fiber concentrations and size distribution. Mean amphibole fiber lengths and widths typical for western Lake Superior water are 2.0 and 0 3 μm (Cook et al. 1976); storm resuspension of settled particles and proximity to the tailings discharge may raise these values Amphibole fibers transported to other parts of Lake Superior are expected to be primarily smaller particles

Discussion

The primary purpose for this investigation was to determine if freshwater fish can accumulate asbestos and analogous mineral fibers and thereby act as a vehicle for human ingestion. Although only seven amphibole fibers identical with those in Lake Superior water were found in Lake Superior lake trout muscle tissue, we conclude that trace concentrations of small fibers can be present in fillets from fish exposed to mineral fibers. The concentration in muscle tissue appears to be on the order of 10^{-2} the water exposure concentration (expressed as fibers per gram). Thus the human ingestion of fish from asbestos-contaminated waters is unlikely to be a concern as in the case of drinking water supplies.

The greater number of amphibole fibers in lake trout kidney tissue may be related to the large volume of blood flowing through that organ. The possibility that the urinary tract serves as an elimination route for these small particles as demonstrated for mammals (Cook and Olson 1979) has not been investigated. The 36-fold difference in amphibole fiber concentrations for Split Rock and Huron Bay lake trout kidney tissue parallels the great difference in water fiber concentrations at the two locations The tendency for lake trout found at Split Rock to migrate to areas with lower fiber concentrations and lake trout found at Huron Bay to migrate to areas with higher fiber concentrations may explain why the tissue fiber concentration difference is not even greater Another factor may be the drift of food organisms into the study areas from areas of higher or lower amphibole fiber water concentrations.

The absence of amphibole fibers in the catfish and brook trout raised in amphibole fiber-contaminated water on an artificial diet suggests that ingestion of food organisms containing the fibers is the primary route for fiber accumulation in the lake trout. If so, this study provides indirect evidence for the small volume of water normally ingested by freshwater fish. We intend to expose trout to a well-measured dose of asbestos in food and compare the amount accumulated in tissue to amounts found in fish exposed only through contaminated water The possible passage for microscopic mineral fibers through the gastrointestinal mucosa of fish and subsequent hematogenous or lymphatic transport to other tissues, particularly the kidney, is supported by reports of such a mechanism in mammals such as mice (LeFevre et al. 1978), rats (Sanders and Ashworth 1961), sheep (Nottie 1977), baboons (Patel-Mandlik et al. 1979), and man (Cook and Olson 1979). In a study of tissues of a neonate baboon that ingested asbestos, the highest fiber concentrations were found in the kidney (Patel-Mandlik 1980). A possible alternate pathway for fiber accumulation in fish, passage through gill membranes, seems to be of minimal significance since fibers were not found in fish exposed only to fibers in water The pattern of diatom frustule fragment occurrences in tissues also supports ingestion as the primary route for particle accumulation

Two analytical problems require further work. Techniques must be perfected which reduce chrysotile fiber contamination during tissue preparation to levels which do not interfere with the ability to detect chrysotile fibers in the tissue. The apparent association in this study of chrysotile fibers in blank samples with tissue samples having the highest concentrations of chrysotile may be due to cross contamination from the tissue tube to the blank tube in the low temperature ashing step. Amphibole fibers which are more massive than chrysotile fibers were not found in blank samples of tissues from unexposed fish. Also needed is the development of even more sensitive tissue digestion techniques to allow faster electron microscope examination of more tissue residue. Thus detection limits could be lowered and concentrations such as those reported for muscle tissue in this study could be determined with greater precision

Acknowledgments

The assistance of Vince Mattson and Anthony Carlson of this laboratory, and Jean Boulva, Fisheries and Oceans, Government of Canada, in obtaining the fish sample, is greatly apppreciated The laboratory assistance from Diane Huseby, Diana Longrie, and Mike Kline in preparing samples is also greatly appreciated Drawings were prepared by Barbara Halligan of this laboratory and the photographic work by Doug Lothenbach and Vicki Land of the University of Minnesota, Duluth

ANDERSON, C. H , AND J M LONG 1980 Interim method for determining asbestos in water EPA-600/4-80-005 U S Government Printing Office, Washington, D C 34 p

BERKLEY, C , J CHURG, I J SELIKOFF, AND W E SMITH 1965 The detection and location of mineral fibers in tissue Ann N Y Acad. Sci. 132 48–63

CARTER, R E , AND W F TAYLOR 1980. Identification of a particular amphibole asbestos fiber in tissues of persons exposed to a high oral intake of the mineral Environ Res 21· 85–93

COOK, P M. 1979. Preparation of extrapulmonary tissues and body fluids for quantitative transmission electron microscope analysis of asbestos and other mineral particle concentrations. Ann N.Y Acad Sci 330 717–724

COOK, P M , G E GLASS, AND J H TUCKER 1974 Asbestiform amphibole minerals detection and measurement of high concentrations in municipal water supplies Science 185. 853–855

COOK, P. M , AND G F OLSON 1979. Ingested mineral fibers elimination in human urine Science 204 195–198

COOK, P M , I B. RUBIN, C J. MAGGIORE, AND W J NICHOLSON 1976 X-ray diffraction and electron beam analysis of asbestiform minerals in Lake Superior waters, p 1–9, Vol 2, Session 34, Paper 1. Proceedings of an International Conference on Environmental Sensing and Assessment (Institute of Electrical and Electronics Engineers, New York, 1976)

HALLENBECK, W H., E. H. CHEN, K. PATEL-MANDLIK, AND A. H WOLFF 1977 Precision of analysis for waterborne

chrysotile asbestos by transmission electron microscopy. Bull. Environ. Contam. Toxicol. 17: 551–558.

HALSBAND, E. 1974. Der einfluss von asbestafallprodukten auf die miesmuschel. Wasser. Luft Betr. 18: 1–3.

LEE, D. H. K. 1974. Biological effects of ingested asbestos: report and commentary. Environ. Health Perspect. 9: 113–122.

LEFEVRE, M. E., J. W. VANDERHOFF, J. A. LAISSUE, AND D. D. JOEL. 1978. Accumulation of 2-μm latex particles in mouse Peye patches during chronic latex feeding. Experientia 34: 120–125.

NOTTLE, M. C. 1977. Plant particles in kidneys and mesenteric lymph nodes of sheep. Aust. Vet. J. 53: 405.

PATEL-MANDLIK, K. J. 1980. Distribution of orally administered chrysotile asbestos in newborn baboon body. EPA-600/1-80-022. U.S. Government Printing Office, Washington, D.C. 15 p.

PATEL-MANDLIK, K. J., W. H. HALLENBECK, AND J. R. MILLETTE. 1979. Asbestos fibers. I. A modified preparation of tissue samples for analysis by electron microscopy. 2. Presence of fibers in tissues of baboon fed chrysotile asbestos. J. Environ. Pathol. Toxicol. 2: 1385–1395.

SANDERS, E., AND C. T. ASHWORTH. 1961. A study of particulate intestinal absorption and hepatocellular uptake. Exp. Cell. Res. 22: 137–145.