

The lake then remained deep for several thousand years and may even have overflowed. About 5,500 BP, changes in the hydrologic environment is reflected in an increase in carbonate production and the eventual coarsening of bottom sediments, culminating in the deposition of shallow facies at 3,100 BP. At 3,100 BP water level rose slightly and then fell again, maintaining this low position until about 2,500 BP. Trees found in positions of growth near and below present water level and dated at 1,890 BP (N-390) were submerged by rising waters at this time. The trees belong to the last period when water level was below that of today. The lake rose and remained deep until after European settlement. In the late nineteenth century it began to fall and in 1969 stood at its lowest level for the past 1,900 yr.

In nearby Lake Grotok, submerged trees have been dated at $1,865 \pm 85$ BP (GX-0149, E. D. Gill, personal communication). Synchronous oscillations in lakes 20 km apart provide strong evidence that the controlling factor was climate rather than local hydrologic variations. Furthermore, the major drying between 18,000 and 15,000 BP is in close agreement with evidence documented from lakes in different hydrologic settings in north Victoria and south-west New South Wales recorded by J. M. B.³ The rise at about 10,000 BP, reflecting an increase in precipitation or decrease in temperature, cannot yet be correlated accurately with events elsewhere in Australia, although Kershaw⁴ suggests a wet period in north Queensland at this time.

Effects of a Post-Glacial thermal maximum are weakly represented by a slow fall in water level and a corresponding rise in carbonate production from 5,500 to 3,100 BP. The magnitude and implied rates of these changes suggest conditions little different from those in the area today. The data therefore do not support the hypothesis of widespread Post-Glacial aridity so widely claimed in Australia⁵⁻⁷. Although temperatures from 5,500 to 3,100 BP may have been slightly higher than in the preceding interval, the degree of aridity would almost certainly not have been sufficient to cause the vegetation destruction and widespread landscape instability which are sometimes attributed to events of this time.

Conditions moderated at 3,100 BP when a slight rise of lake level occurred, but the major rise marking the beginning of the next relatively wet period did not occur until 1,900 BP.

Oscillations in Lake Keilambete can be matched in other lakes in a variety of settings in south-east Australia supporting the interpretations of the water level changes discussed here. The sequence of palaeoclimatic conditions probably has general application through south-east Australia and it may help provide a reliable basis for climatic correlations within Australia and between different continents in both hemispheres.

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Asbestos Fibres in Beverages and Drinking Water

The hazards of inhaling asbestos fibres¹⁻⁴, the high incidence of gastrointestinal cancer^{1,2,5-7} and abdominal neoplasms^{3,8,9} among asbestos workers and the fact that asbestos fibres may penetrate the mucosa of the stomach and the intestine of animals^{10,11} are known and so it is important to study the distribution of asbestos fibres in the environment. Asbestos fibres have already been found in beer¹², and the occurrence of fibres 0.5 nm in length and 0.01 nm in diameter suggests that such material may find its way through filtering systems into city drinking water supplies, and this we have been able to confirm.

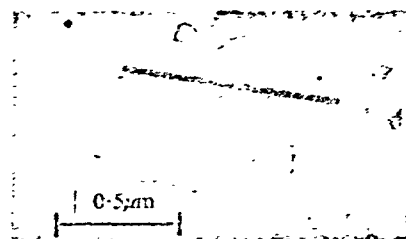


Fig. 1 Electron micrograph of asbestos fibre isolated from beer (enlarged 31,600 times).

The asbestos fibres in 400 ml. samples of beverages and water were concentrated by centrifuging in excess of 16,000g, drying at 100° C and ashing at 500° C¹². The ash was taken up in 1 ml. of distilled water and agitated in an ultrasonic vibrator to disperse the fibres and ash particles¹². Five μl. of this dispersion was evaporated with the aid of a heat lamp on 200 or 400 mesh carbon coated copper grids. Using a Siemens Elmiskop IA electron microscope at 80 kV and a magnification of 20,000, the number of fibres in each of five or ten grid holes was counted, and corrected for dilution. Precautions were taken to prevent contamination of samples from extraneous airborne fibres and no fibres were detected in distilled water

Table 1 Asbestos Fibres in Beverages and Water

Sample	Source *	No. of fibres × 10 ⁶ l. ⁻¹
Beer	Canadian 1	4.3
Beer	Canadian 2	6.6
Beer	USA 1	2.0
Beer	USA 2	1.1
Sherry	Canadian	4.1
Sherry	Spanish	2.0
Sherry	South African	2.6
Port	Canadian	2.1
Vermouth	French	1.8
Vermouth	Italian	11.7
Soft drink	Ginger ale	12.2
Soft drink	Tonic water I	1.7
Soft drink	Tonic water II	1.7
Soft drink	Orange	2.5
Tap water	Ottawa, Ottawa river (F)	2.0
Tap water	Toronto, Lake Ontario (F)	4.4
Tap water	Montreal, St Lawrence river (F)	2.4
Tap water	Quebec, Saguenay river (NF)	2.5
Tap water	Beauport, Quebec, St Lawrence river (5 km below Quebec City) (NF)	3.1
Tap water	Drummondville, Eastern Townships, Quebec, St Francois river (F)	2.9
Tap water	Asbestos, Eastern Townships, Quebec, Nicolet river (F)	5.9
Tap water	Therford Mines, Eastern Townships, Quebec, Lac à la Pêche (NF)	172.7
Melted snow	Ottawa, top 50 cm (2-3 weeks precipitation)	33.5
River water	Ottawa river, at Ottawa	9.5

* F, Filtration plant used, NF, no filtration plant used.

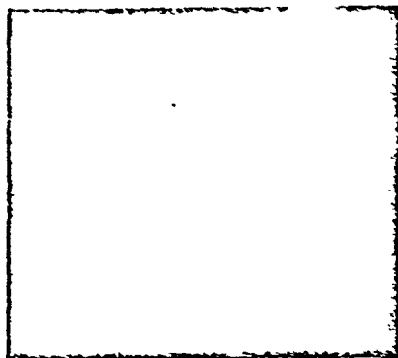


Fig. 2 X-ray diffraction pattern of asbestos fibre shown in Fig. 1.

blanks which were treated in the same manner as the liquid samples.

Twelve brands of Canadian beer, wine, sherry and port, six of American beer, six of European wine, vermouth and sherry and two of South African sherry were examined and all were found to contain asbestos fibres. Counts made on some of these brands are reported in Table 1. The fibres in Canadian beer (Fig. 1), sherry and water were identified as being chrysotile asbestos by comparing their electron diffraction pattern (Fig. 2) with that of pure chrysotile asbestos. Some of the European samples contained fibres of amphibole asbestos. Many asbestos fibres were hidden in clumps of debris such as mineral matter in water and diatomaceous earth which is used as a filtering aid in beer. As a result, the number of fibres reported in Table 1 must be considered a minimum estimate of those actually present. Most of the fibres found in Canadian beer and in filtered city water were less than 1 μm in length while most samples of wine, some American beer and unfiltered melted snow or river water also contained a considerable number of asbestos fibres ranging from 1 to 15 μm in length.

Tap water from three major Canadian cities, Ottawa, Toronto and Montreal, combined from 2.0 to 4.4 million fibres per litre. All of these cities have filtration systems and Ottawa tap water, drawn from the Ottawa river, had considerably fewer fibres than unfiltered river water. Many of Canada's major asbestos mines are located in the Eastern Townships of Quebec and filtered tap water from towns in this area did not differ greatly from that in the larger cities but unfiltered tap water in Thetford Mines, drawn from a small lake in the heart of an asbestos mining area, was exceptionally high in fibre content. The high level of asbestos fibres in melted snow in the Ottawa area gives some indication of one source of asbestos in surface waters.

The question as to the hazard of orally ingested asbestos has not yet been resolved. The amounts by weight of the asbestos found in these samples were exceedingly small—about 1 μg per 10 million fibres—obtained from counts on 1 mg samples of fibres of approximately the same size as those found in the beverages and water. Further work is required to determine the extent to which the fibres penetrate the walls of the digestive tract, the degree to which they may be transported to other organs of the body and the biological effects of their residence in various tissues. Studies of this nature are now in progress.

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BIOLOGICAL SCIENCES

Molecular Conformation of Deoxyuridine

THE structure determination of deoxyuridine was undertaken as part of a series of conformational studies of nucleic acid components. Such studies give information about the preferred conformation of nucleosides and nucleotides^{1,2}, and may clarify the conformational differences and changes that occur in nucleic acids. The conformational parameters of interest in deoxyuridine are the pucker of the sugar ring, the torsion angle of the sugar relative to the base about the glycosidic bond and the orientation of the C(5')-O(5') bond about the C(4')-C(5') bond.

In ribose and deoxyribose nucleosides, the most common type of pucker of the sugar is either C(2') endo or C(3') endo. In the deoxynucleosides the C(2') endo pucker is by far the most common. Since one of the nucleosides with a C(3') exo pucker is thymidine³, it was of particular interest to know whether deoxyuridine would also have this type of pucker. Even the same nucleoside in different environments can have a different type of pucker⁴, but these differences have only been observed in ribose nucleosides. There is therefore a need for further studies of deoxy nucleosides and nucleotides.

Crystals of deoxyuridine were obtained from aqueous solutions. The unit cell is monoclinic with dimensions $a=7.91$ Å, $b=6.71$ Å, $c=18.77$ Å, $\beta=96.6^\circ$ and the space group is $P2_1$. There are four molecules in the unit cell and therefore two molecules in the asymmetric unit. Intensity data were collected with a Hilger and Watts linear diffractometer using molybdenum $K\alpha$ radiation. The structure was solved by Patterson interpretation methods. The first step was the determination of the orientation of the plane of the pyrimidine bases in the unit cell using the $H(0,0)$ function of Tollin and Cochran⁵. The orientation of the pyrimidine base within the plane was determined by means of a rotation function⁶, and the positions of the molecules relative to the 2_1 axis were found using the $Q(X, Z_0)$ function of Tollin⁷. Full details of the structure determination will be given elsewhere. The R factor at the present state of refinement is 0.03.

The conformation of both molecules in the asymmetric unit is very similar. The pucker of both sugars is C(2') endo, or C(2') endo-C(3') exo with respect to the three-atom plane C(1')-O(1')-C(4'). Displacements of the C(2') atoms in molecules I and II relative to this plane are 0.553 and 0.333 Å respectively, and of atoms C(3') they are 0.111 and 0.210 Å respectively. Thus the sugar pucker in deoxyuridine is like that in the majority of deoxynucleosides and differs from that in thymidine.

The glycosidic torsion angle, $\phi_{\text{C}5'}$, defined by Donohue and Truett⁸ is -28° in molecule I and -24° in molecule II.

SYMPOSIUM ON INDUSTRIAL CHEMICALS AS FOOD CONTAMINANTS

Asbestos Fibers in Beverages, Drinking Water, and Tissues: Their Passage Through the Intestinal Wall and Movement Through the Body

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Methods were developed using an electron microscope to isolate and count asbestos fibers in liquids and tissues. Fibers were detected in beer, sherry, port, vermouth, soft drinks, and city drinking water at levels generally ranging from 1 to 11 million per liter. River water was higher in asbestos fibers than water passed through a city filtration system, and melted snow was considerably higher than river water. Asbestos fibers were also detected in parenteral solutions. Distribution studies on asbestos fibers administered to rats were performed with the electron microscope and were supplemented with studies using both tritiated and neutron-activated asbestos. Chrysotile fibers were injected into the stomachs of rats and 2-4 days later fibers were isolated from the blood, spleen, liver, kidney, omentum, muscle, lung, and brain. Highest levels of orally administered fibers were found in the omentum; intravenously injected fibers resulted in highest levels in the liver and the lung.

Chrysotile asbestos is a greyish, fibrous material composed of a specific crystalline form of magnesium silicate. The visible fibers are composed of millions of submicroscopic fibers (sometimes called fibrils) about 200Å in diameter arranged in bundles lying parallel to each other (Fig. 1). Upon extensive milling or abrasion the individual fibrils may be readily released from the bundles. Since asbestos has a crystalline structure, one of the best ways to identify it is by electron diffraction. The electron diffraction pattern of chrysotile asbestos is shown in Fig. 2.

It is well known that in the mining and milling of asbestos the inhalation of fibers can cause cancer of the lungs and pleura. There is also considerable evidence indicating a higher than normal incidence of gastrointestinal and peritoneal cancer in asbestos workers (1-5). Since asbestos is commonly used in the filtration of beverages,

we decided to see if we could detect the presence of fibers in some beverages which are often passed through filters containing asbestos. A preliminary report was published on this work earlier (6).

Experimental

The asbestos fibers in 400 ml samples of beverages or water were concentrated by centrifuging in excess of 16,000 *g*, drying at 100°C, and ashing at 500°C. This temperature could not be exceeded because it distorts the electron diffraction pattern. The ash was dissolved in 1 ml of distilled water and lightly ultrasonicated to disperse the asbestos fibers evenly throughout the water. Five µl was placed on a carbon-coated electron microscope grid, the surface of which had previously been made hydrophilic by placing it in an ion flux to give it a positive charge. This allowed the asbestos-in-water suspension to spread and dry evenly over the surface of the grid. Five to 10 grid squares were scanned for each sample.

Figure 3 shows some asbestos fibers that were found in beer; note the appearance of the typical central canal. The more time that fibers remain in water the more of their magnesium is leached out and the central canal begins to disappear. The electron diffraction pattern also begins to fade.

We examined 12 samples of Canadian beer and wine, 6 of American beer, 6 sherrys and ports from other countries, and several local soft drinks. All were found to contain asbestos fibers. Those that we counted ranged from 1.1 to 12.2 million fibers per liter (mfl) (Table 1). Tap water in Ottawa contained 2.0 mfl and the Ottawa River from which it originated before filtration contained 9.5 mfl. Other large cities in Eastern Canada such as Toronto and Montreal, which also use surface water, had similar levels of fibers. Unfiltered water from Hull, across the river from Ottawa, also contained 9.5 mfl, the same as detected in the river water. Unfiltered St. Lawrence River water drawn at Beauport contained 8.1 mfl. It was surprising to find that two towns in the Eastern Townships (Drummondville and Asbestos), drawing

FIG. 1—An asbestos fiber approximately 4 μ m long showing how it is composed of a bundle of fibrils about 200Å in diameter.

FIG. 2—Electron diffraction pattern of a chrysotile asbestos fiber.

Cunningham
Pontepiacet fig 4

FIG. 3—Electron micrograph of asbestos fibers isolated from beer (enlarged 31,000 times).

water from rivers that we thought might contain large amounts of asbestos, were not exceptionally high. However, unfiltered tap water from Thetford Mines, which came from a small lake in the asbestos mining area, was quite high (172.5 mfl). Melted snow from outside our laboratories in Ottawa that had fallen during 2-3 weeks in March 1971 contained 33.5 mfl. This indicated that some of the asbestos was coming from the air; when we took a 24 hr air sample from outside our laboratory the day after a long rainy period, we found 545 fibers per liter of air. Most of the fibers found in Canadian beer and in filtered city water were less than 1 μ m long whereas most samples of wine, some American beer, and unfiltered melted snow or river water and air also contained a considerable number of asbestos fibers ranging from 1 to 15 μ m long.

To determine whether asbestos fibers consumed orally can pass through the wall of the intestine into the blood stream, we obtained a sample of very finely powdered chrysotile asbestos (7RF02, Johns-Manville Co., Asbestos, Quebec) and separated out fibers of about the same size found in our beverages (0.5-2 μ m long), using sedimentation in water (Fig. 4), as

follows: 2.5 g asbestos was shaken in 500 ml of water in a graduated cylinder and allowed to settle for 30 min; the top 350 ml, which contained about 10% of the asbestos, was then drawn off. This contained 1 mg or 9.4 billion fibers per ml.

In order to administer this orally to rats and still insure that they did not inhale any of the fibers we could not feed it or give it by intubation, for some of the fibers might dry on their lips and be inhaled later. We therefore anaesthetized the rats, opened the abdomen, and injected the asbestos directly into the stomach.

Two to 4 days later, uncontaminated blood was drawn from the orbital sinus by a capillary tube. The rats were then killed with an overdose of ether and were thoroughly vacuumed and washed with double distilled water and methanol to remove any fibers on their hair that might have come from their feces. Their abdomens were opened by covering their bodies with wet paper towels and then placing a new layer of wet towels in the incision after the skin had been penetrated. The major organs were removed and the brain was removed from the skull in the same manner. The tissues cannot be dried and ashed



FIG. 4—Electron micrograph of asbestos fibers separated by sedimentation in water for use in animal experiments. Width of field is 4 μ m.

directly, for when the preparation is later viewed with an electron microscope the asbestos fibers are completely obscured by the mineral matter. The tissues were therefore solubilized with Soluene (Packard Instrument Co.), and the asbestos fibers were separated by centrifuging, washed with methanol, and ashed at 500°C.

Results

The results of this experiment are shown in Table 2. There were 5 rats in each group. Those in group 2 were given 9.4 billion fibers and killed 2 days later; those in group 3 received 94 billion fibers and were killed 4 days later. No asbestos fibers were detected in the blood of the control rats but 4.65 million per g (mfg) were found in the second group. This would be about 0.1% of the dose present in the blood at that time in rats weighing about 60 g. Four days after treatment the level of asbestos in the blood declined to 1.19 mfg, even though these rats received 10 times as much asbestos. The omentum surrounding the small intestine appeared to take up the largest

amounts of asbestos. The omentum consists partly of peritoneum and partly of adipose tissue. It is therefore interesting to note that in women asbestos workers, Keal (7) found a higher incidence of peritoneal cancer than of lung cancer. It is possible that the peritoneum may have a greater affinity for asbestos or else not be able to clear it as fast as other tissues. It is also possible that the omentum contained many small lymph nodes which could have filtered out the asbestos. The brain contained about 6 times more fibers than the controls, and the lung content was also appreciably higher. Unfortunately, counts could not be made on the livers or kidneys because of an excess of mineral matter isolated with the asbestos fibers.

The amount of asbestos in the tissues of the control rats was rather surprising and we still do not know its exact source. The rats could not have obtained this much asbestos from their drinking water. We do not know how much was in their feed or air but the air would appear to be

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Table 1. Asbestos fibers in beverages and water

Sample	Source ^a	No. of fibers/ L, × 10 ⁶
Beer	Canadian 1	4.3
Beer	Canadian 2	6.6
Beer	USA 1	2.0
Beer	USA 2	1.1
Sherry	Canadian	4.1
Sherry	Spanish	2.0
Sherry	South African	2.6
Port	Canadian	2.1
Vermouth	French	1.8
Vermouth	Italian	11.7
Soft drink	Ginger ale	12.2
Soft drink	Tonic water I	1.7
Soft drink	Tonic water II	1.7
Soft drink	Orange	2.5
Tap water	Ottawa, Ottawa River (F)	2.0
Tap water	Toronto, Lake Ontario (F)	4.4
Tap water	Montreal, St. Lawrence River (F)	2.4
Tap water	Hull, Quebec, Ottawa River (NF)	9.5
Tap water	Beauport, Quebec, St. Lawrence River (6 km below Quebec City) (NF)	8.1
Tap water	Drummondville, Eastern Townships, Quebec, St. Francois River (F)	2.9
Tap water	Asbestos, Eastern Townships, Quebec, Nicolet River (F)	5.9
Tap water	Thetford Mines, Eastern Townships, Quebec, Lac a la Truite (NF)	172.7
Melted snow	Ottawa, top 30 cm (2-3 weeks precipitation)	33.5
River water	Ottawa River, at Ottawa	9.5

^a F, Filtration plant used; NF, no filtration plant used.

one of the most likely sources. We have analyzed the tissues of 3 humans who died of natural causes and found somewhat similar levels of 0.114–0.378 mfg in brain, 0–0.253 in spleen, and 0.773–0.915 in peritoneum. Most of the fibers were 0.2–2.0 μ m long, but one fiber found in the peritoneum of a rat was 15 μ m long and one in the blood of another rat was 23.5 μ m long. The asbestos preparation with which the rats were dosed contained only a few fibers of this length, for most fibers over 20 μ m long were thicker than single fibrils and settled in less than 30 min when they were originally suspended in water.

This raises the question of how the fibers were absorbed—by piercing the gut like a needle or by engulfment by pinocytosis. It is most likely that a fiber 23 μ m long passed through by piercing but it is difficult to explain how the shorter fibers passed.

The ubiquitous presence of natural asbestos in the tissues of experimental animals and the te-

Table 2. Asbestos fibers found after intragastric injection (millions/g)^a

Tissue	Controls	Killed at 2 days (9.4 billion admin- istered)	Killed at 4 days (94 billion admin- istered)
Blood	0.00	4.65**	1.19
Spleen	2.33	4.11	3.45
Omentum	2.46	2.74	18.25
Heart	2.09	—	2.28
Brain	0.05	0.31	0.29*
Lungs	1.06	1.80	1.74

^a * = $P \leq 0.05$; ** = $P \leq 0.01$.

dious job of counting the fibers with an electron microscope limit the usefulness of the technique used in the aforementioned animal experiments. For this reason, tritiated asbestos was prepared by a simplified modification of a technique described by Turnock *et al.* (8) and neutron-activated asbestos by a technique similar to that of Holmes and Morgan (9).

To produce tritiated asbestos, 200 mg of asbestos and 0.1 ml of tritiated water (5.77 mCi/ml) were sealed in a stainless steel bullet with an internal volume of 0.4 ml and heated in a muffle furnace at 375°C for 1 week. Upon removal from the bullet the asbestos was dried for 3 hr at 138°C.

For neutron activation, 1 g of asbestos was irradiated at 10^{14} neutrons/cm²/sec for 3 weeks and then allowed to stand for several weeks to permit decay of the short-lived isotopes. Combined activity of all isotopes was measured with a gamma counter (Packard Instrument Co.) but best results were obtained with a multichannel analyzer with an attached computer by which the entire gamma ray spectrum was scanned and the individual peaks were identified and calculated. With the latter instrument, it was possible to distinguish between isotopes in tissues which had leached out of asbestos and intact asbestos fibers.

Both tritiated and neutron-activated asbestos preparations were used to determine the tissue distribution of asbestos given orally and injected intravenously into rats. However, when given orally, much of the radioactive components of both preparations were leached out in the gastrointestinal tract, possibly because of the acid in the stomach. These soluble isotopes were then absorbed and became incorporated into the tissues to produce such high background levels that it was very difficult to determine the activity of the

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Table 3. Distribution of intravenously injected ³H-asbestos in rats

Tissue	Per cent of dose/g		
	After 6 min	After 1 hr	After 24 hr
Blood	0.198	0.017	0.008
Brain	0.077	0.057	0.040
Omentum	0.023	0.028	0.012
Liver	1.140	1.132	1.637
Heart	0.170	0.111	0.207
Kidney	0.209	0.201	1.358
Lung	7.991	4.224	3.868
Spleen	1.393	1.630	2.105

intact asbestos fibers even when the tissues were solubilized and subjected to extensive centrifuging and washing steps. The present experiments indicate that small amounts of asbestos fibers pass through the walls of the intestine and concentrate in the peritoneum, liver, lungs, and other organs, but the levels detected are so small that further work is required to be sure of the exact amount.

It has been known for several years that asbestos fibers may penetrate the mucosa of the stomach and intestine of animals (10, 11) but previously no experiments had shown that orally ingested fibers may pass completely across the walls of the gastrointestinal tract and enter the blood.

Intravenously administered asbestos fibers are rapidly removed from the blood and most of them are deposited in the tissues within 6 min after injection (Table 3). At this time, highest levels are found in the lungs, liver, and spleen, but during the next 24 hr the levels in the lung tend to fall and the levels in the liver, spleen, and muscle increase slightly. The omentum had the lowest of all tissues checked. When neutron-activated asbestos was injected intravenously into rats, recovery of the labeled components in the various organs and carcass 1 hr later was close to 100% and approximately 70% of the activity could be recovered in the asbestos fiber fraction when the tissues were solubilized and the fibers were centrifuged (Table 4).

We have recently injected neutron-activated asbestos intravenously into rats on the 15th day of pregnancy and found considerable activity in the whole fetus and fetal liver. Up to 25% of the activity of the fetal liver could be isolated in the asbestos fraction by solubilization and centrifugation; it gave a spectral pattern similar to that of neutron-activated asbestos standards. This is additional support for the hypothesis that the

Table 4. Distribution of intravenously injected neutron-activated asbestos in rats

Tissue	Per cent of dose/g		Per cent of dose/tissue	
	Centrifuged	Total	Centrifuged	Total
Blood	0.12	0.17	1.13	1.69
Brain	0.11	0.13	0.18	0.22
Omentum	0.10	0.12	0.16	0.21
Liver	3.91	6.02	43.89	67.78
Heart	0.34	0.31	0.31	0.28
Kidney	0.41	0.62	0.82	1.23
Lung	8.06	11.34	12.29	18.14
Spleen	3.72	6.90	2.20	3.99
Skin	—	0.02	—	1.09
Carcass	—	0.04	—	6.61
Total				101.24

fibers cross other membranes such as the intestinal wall. We do, however, plan to do further confirmatory work on this.

In conclusion, we have presented techniques for isolating asbestos fibers from various beverages, water, and tissues. We have also simplified techniques for producing and counting radioisotopic tracers. Results obtained by these techniques indicate that asbestos fibers are now ubiquitous in our environment and can enter the body through numerous routes and distribute themselves throughout the various tissues.

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