IOEH 5763
Code 9
37
40
45
72
80

ARCHIVES OF ENVIRONMENTAL HEALTH, 1981; 36(Nov./Dec.): 298-303.

PLAINTIFF'S EXHIBIT
CAP-1381

INSTITUTE OF OCCUPATIONAL AND ENVIRONMENTAL HEALTH MONTREAL, CANADA

Biological Effects of Ingested Amosite Asbestos, Taconite Tailings, Diatomaceous Earth and Lake Superior Water in Rats

ANDERSON C. HILDING, M.D., Ph.D.*
Research Laboratory
St. Luke's Hospital
Duluth, Minnesota 55805

DAVID A. HILDING, M.D.
Department of Otolaryngology
Abraham Lincoln School of Medicine
University of Illinois at the Medical Center
Chicago, Illinois

DONALD M. LARSON, M.D.
ARTHUR C. AUFDERHEIDE, M.D.
Department of Pathology
St. Luke's Hospital
Duluth, Minnesota
and
Department of Pathology and Laboratory Medicine
University of Minnesota
School of Medicine
Duluth, Minnesota 55812

ABSTRACT. Because amphibole fibers were found in Lake Superior and in Duluth municipal water, this study was initiated to evaluate the carcinogenicity of ingested asbestos. Groups of 22-30 Sprague-Dawley rats were fed asbestos and related materials during their lifetime and were examined at autopsy after spontaneous death. Test materials were unfiltered Duluth city tap water, municipal water reservoir sediment suspension, taconite plant tailings, amosite asbestos (a "low-dose" group at 20 mg/rat · day and a high-dose group at 300 mg/rat · day) and diatomaceous earth; a control group drank fiber-free well water or filtered city tap water. Autopsy studies revealed no significant increase in the incidence of malignant tumors in any experimental group compared with that in the control group.

IN 1973 the discovery of amphibole fibers in Lake Superior, and in a taconite iron ore plant's tailings exhausted into the lake,[1-4] caused public alarm in residents of Duluth and other lakeshore communities drawing their municipal water supplies from the lake. When crushed or milled, many amphibole minerals produce microscopic-size fibers that are similar to one another in size and shape. The cummingtonite-grunerite mineral series (found in taconite iron ore) and amosite asbestos, suitable for commercial mining, are amphibole minerals. The term "amphibole fibers," as used in this study, refers to the total number of such fibers in the suspension without implication regarding their mineral source, whether it be cummingtonite-grunerite or amosite asbestos. Although the carcinogenicity of inhaled asbestos was well established,[5-7] the incident focused attention on how little was known of the biological effects, especially on gastrointestinal mucosa of ingested asbestos.[8,9] Workers inspiring considerable asbestos dust probably suffer some gastrointestinal exposure by swallowing mucus containing asbestos fibers cleared from the bronchial tree by ciliary action,[9-15] but there was no assurance that this was the equivalent to direct ingestion of amphibole-contaminated water. The presence of asbestos fibers in commercial beverages and other municipal water systems, possibly resulting from the use of asbestos filters in the first instance and asbestos-lined pipes in the second, caused concern about the carcinogenicity of ingested asbestos at the national level.[16] Accordingly, in 1973 we initiated an animal study to determine whether ingested min-

*Deceased

ASARCO ALV 0006487

eral fibers in various forms are carcinogenic to the rat gastrointestinal tract or to other rat tissues.

MATERIALS AND METHODS

One hundred ninety weanling Sprague-Dawley rats ate commercial rat chow as their basic nutrient. They were housed, five to a standard rat cage, until each rat died. A complete gross and microscopic thoracic and abdominal autopsy was performed on each animal. The gastrointestinal tract received special attention: the entire tract from esophagus to anus was removed, opened, washed, inspected, and transilluminated; tissue specimens were then removed from representative pre-selected sites in the tract and from all abnormal areas.

The amphibole fiber counts on the samples were performed using electron microscopy. They were conducted by the staff at the Environmental Research Laboratory, Duluth, of the Environmental Protection Agency. This laboratory and this agency were not consulted by us for any other part of this study, therefore, they are not responsible for any conclusions or interpretations of our findings expressed herein.

The animals were divided into various groups, as follows.

Control Group. Twenty-eight rats drank fiber-free well water for 9 months after birth. For the remainder of their lives, they drank filtered Duluth municipal (Lake Superior) tap water that had an amphibole fiber count averaging one million fibers/L (mfl). Commercial rat chow was their only source of food.

Duluth municipal tap water (unfiltered). Thirty rats drank only unfiltered Duluth municipal tap water containing an average of 100 mfl, and ate only commercial rat chow.

Lake Superior water sediment. Twenty-two rats drank a suspension of Lake Superior sediment. The municipal water supply is drawn from Lake Superior and is stored in a four million gallon reservoir, which had not been cleaned for 14 yr prior to our sample procurement. A 2-L bucket was filled with sediment by dragging it along the bottom of the reservoir, the contents of which were diluted to 40 L with distilled water; this mixture was then shaken vigorously and allowed to settle overnight. The supernatant suspension, averaging 5,000 mfl, served as the sole drinking water source. These rats ate only commercial rat chow.

Taconite plant tailings. Some microscopic-size fibers of the *cummingtonite-grunerite mineral series* (found with taconite ore) are similar in size and shape to fibers found in Lake Superior, as well as to those of amosite asbestos. Therefore, 30 rats were exposed to taconite tailings. Thirty-liter samples of taconite tailings were obtained from the west launder of a taconite plant in Silver Bay, Minnesota. After 7 hr of settling, the top third of the sample was siphoned into glass containers; this decanted fraction of the sample contained an average of 100,000 mfl. For the initial 16 months of the study, the suspended tailings were mixed with cottage cheese (four parts tailings and one part cheese) and fed ad libitum to the 30 rats. Following its consumption (which proved to be prompt), commercial rat chow was offered. Beginning with the 17th month, and continuing for the remainder of the study, the tailings suspension (no longer mixed with cottage cheese) served as the sole source of drinking water.

Low-dose asbestos. Each of the 30 rats was offered a daily feeding of 20 mg asbestos mixed with cottage cheese (5 mg asbestos/g cheese). This asbestos-cheese mixture was offered to the rats in each cage in a single dish holding an amount sufficient to supply each animal with the designated dose. Following its consumption, commercial rat chow was available ad libitum. These rats drank only filtered Duluth municipal tap water. Chrysotile asbestos (Paperbestos No. 5, Johns Manville Co., Denver, CO) was used during the first 7 months. Beginning at 8 months, and continuing for the remainder of the study, the chrysotile was replaced by UICC Standard Reference Amosite Asbestos, which became available at this time from R.E.G. Rendall, Pneumoconiosis Research Unit, Johannesburg, South Africa; it was prepared as described by Timbrell.[17] The fibers in Lake Superior water and in taconite tailings resemble those of amosite asbestos. In Lake Superior water, the proportion of amphibole fibers exceeding 5 μm in length (approximately 6% of total amphibole fibers) is similar to that in amosite asbestos reference samples.

High-dose asbestos. This group of 20 rats was treated in a manner identical with that of the low-dose asbestos group, except that: (1) the amount of asbestos offered was different, and (2) this group did not receive chrysotile asbestos at anytime. Each rat ate a daily average of 300 mg of amosite asbestos mixed with cottage cheese to a concentration of 50 mg asbestos/g cottage cheese.

Diatomaceous earth. This group of 30 rats was also treated in a manner identical to that in the two asbestos groups except for the test material used. Each rat was fed a daily average of 20 mg diatomaceous earth (Johns Manville Co., Denver, CO) mixed with cottage cheese to a concentration of 5 mg diatomaceous earth/g cottage cheese. Diatomaceous earth was included among our test substances because it was a constituent of water filters commonly used in Duluth, Minnesota following the discovery of mineral fibers in Lake Superior.

RESULTS

Detailed autopsies were performed on 187 of the 190 rats included in this study. Postmortem autolysis and occasional cannibalism precluded valid autopsy studies on three rats.

The frequency of cancer in the various groups is detailed in Table 1. No animal had more than one malignant tumor. Statistical evaluation of the results was provided by the Division of Biometry, School of Public Health of the University of Minnesota, Minneapolis. Analysis of the frequency of cancer in any of our test groups compared to that of our control group used the $2 \times k$ contingency table described by Armitage.[13] The departures from the null hypothesis of no difference were not statistically significant at the 5% level ($0.25 < P < .5$).

Breast fibroadenomas, encountered in every group, are common in aging Sprague-Dawley rats. None of these benign tumors showed evidence of transformation into a malignant tumor.

The morphology and location of the sarcomas in

ASARCO ALV 0006488

Table 1.—Frequency of Malignant and Benign Tumors after Exposure to Various Test Materials (in the Authors' Study)

Test Material	Dose	Mean Exposure Time (Days)	No. Rats Initial/Final	Malignant Tumors No.	Location/Type†	Benign Tumors No.	Location/Type‡
Control (filtered tap water)	1 mfl* ad lib	690	28/27	3	1 Lung(C), 1 Ovary(C) 1 Forestomach(C)	5	Breast(F)
Duluth city tap water, unfiltered	100 mfl ad lib	960	30/28	4	1 Salivary gland(C), 1 Skin-face(C), 1 Uterus(S) 1 Mediastinum(L)	9	Breast(F)
Lake Superior water sediment	5,000 mfl ad lib	840	22/22	3	1 Lung(C), 1 Skin-Ear(C) 1 Uterus(S)	7	5 Breast(F), 2 Pancreas (Adenoma)
Taconite tailings	100,000 mfl	870	30/30	3	1 Neck(S), 1 Chest Wall(S) 1 Mediastinum(L)	11	9 Breast(F), 1 Adrenal (Pheochromocytoma) 1 Pancreas (Adenoma)
Asbestos, low dose	20 mg/day	870	30/30	7	1 Breast(C), 2 Skin & Neck (Fibrous Histiocytoma), 1 Skin-Face(C), 1 Mediastinum(L) 1 Chest Wall (Mesothelioma)	15	Breast(F)
Asbestos, high dose	300 mg/day	750	20/20	1	Blood(Leukemia)	11	10 Breast(F), 1 Ileum (Leiomyoma)
Diatomaceous earth	20 mg/day	840	30/30	5	1 Salivary Gland(C), 2 Uterus(S) 1 Skin(C), 1 Peritoneum (Mesothelioma)	13	9 Breast(F), 1 Adrenal (Phoechromocytoma) 3 Pancreas (Adenoma)

NOTE: Cancer frequency is not significantly greater in any test group than in the control group; departures from null hypothesis of no difference are *not* statistically significant at the 5% level ($0.25 < P < .5$) by χ^2.
*mfl = Million amphibole fibers/L.
†(C) = Carcinoma, (S) = Sarcoma, (L) = Lymphoma.
‡(F) = Fibroadenoma.

rats suggested a myogenic origin. In every instance, these tumors behaved aggressively, infiltrating locally, but metastases were rare. In our rats, only two tumors (chest wall in an asbestos-exposed group and peritoneum in the group exposed to diatomaceous earth) demonstrated the histologic characteristics of a mesothelioma as described by Kannerstein et al.[19] The remaining malignant tumors had a conventional histologic structure.

DISCUSSION

The most significant finding in our study was the total absence of gastrointestinal cancer in the animals exposed to the test materials. Any direct carcinogenic action of ingested asbestos fibers should have its primary impact on the gastrointestinal mucosa. Several long-term animal studies (most of them published since 1973) fail to identify with certainty any carcinogenic effect of ingested asbestos on the gastrointestinal tract (Table 2).[2,3,20–24] The one possible exception is the study by Ward et al.,[25] in which the authors concluded that "the experimental evidence suggested but did not prove that oral asbestos exposure in F344 rats may have increased the incidence of intestinal tumors occurring naturally." Intestinal tumors occurred in 32% of the rats receiving only intragastric amosite 3 times a week for 10 wk. This lifetime experiment lacked a control group, but according to the author, spontaneous intestinal tumors are rare in this animal strain. A large lifetime asbestos-feeding experiment is currently being conducted under the auspices of the National Institute of Environmental Health Sciences and the Environmental Protection Agency and directed by the recently established National Toxicology Program, using chrysotile, amosite and other forms of asbestos, F344 rats and hamsters. The final results of this study are expected to be published in the latter part of 1981.[16]

In humans, two epidemiologic surveys of populations drinking amphibole-contaminated water revealed no convincing increase in gastrointestinal cancer,[26–28] but several others showed an increased frequency of gastrointestinal carcinoma in working populations exposed to excessive amounts of airborne asbestos.[29–31] Inhaled asbestos is transported by the mucociliary mechanism of the respiratory tract, eventually reaching the pharynx, where a significant portion of the inhaled particulates is then swallowed.

If these inhaled and then swallowed fibers or the orally ingested asbestos fibers can penetrate the mucosa of the gastrointestinal tract, they might migrate to distant sites. Conceivably, the fibers could induce malignant transformation by direct action on the target tissues, or they might cause cancer indirectly by suppressing the immune defenses.[32–34]

Investigations addressing the questions of gut mucosal penetration by asbestos fibers[3,9,20,21,35–47] have produced conflicting results. Some experiments, employing intragastric injection of asbestos at laparotomy[36,42] demonstrated apparent gut penetration, but were later shown to be susceptible to unsuspected leakage of the injected material.[43] Gross et al.[9] fed asbestos to rats and found no evidence

ASARCO ALV 0006489

Table 2.—Summary of Long-Term (> 12 Months) Asbestos Ingestion Studies Previously Reported in Literature

Author	Test Material	Dose	Exposure Time (Days)	Animal Type†	No.	Malignant Tumors No.	Location/Type‡	Comments
Smith[36] (1965)	Amosite or chrysotile	1% diet ad lib	"Life"	H	45	3	1 Liver(C), 1 Mesentery(C) 1 Pancreas(L)	25 mg asbestos injected intraperitoneally x 1 into each animal
	Control	0	"Life"	H	15	0		
Gross[9] (1974)	Chrysotile	5% diet ad lib	630	R	10	0		
	Control	0	630	R	5	0		
	Chrysotile	10 mg/wk	to 538	R	31	2	Breast(C)	Asbestos fed initial 112 days.
	Crocidolite	5 mg/wk	to 616	R	33	0		
	Crocidolite	10 mg/wk	to 616	R	34	1	Node(L)	
	Control	0	to 644	R	24	5	3 Breast(C), 1 Thigh(S) 1 Node(L)	
Gibel[10] (1976)	Chrysotile	20 mg/day	441 ML*	R	42	12	1 Lung(C), 4 Kidney(C) 3 Node(L), 4 Liver(C)	20 mg filter was 53% chrysotile, 47% not clearly specified.
	Talcum	20 mg/day	649 ML	R	45	3	Liver(C)	
	Control	0	702 ML	R	43	2	Liver(C)	
Cunningham[37] (1977)	Chrysotile	1% diet ad lib	to 720	R	7	6	1 Brain(S), 1 Pituitary(C) 1 Node(L), 2 Kidney(C) 1 Peritoneum(S)	Asbestos feedings for 2 yr.
	Control	——	to 720	R	8	1	Peritoneum(S)	
	Chrysotile	1% diet ad lib	to 900	R	36	11	2 Thyroid(C), 1 Thyroid(S) 1 Chemodectoma Jugular Body, 1 Liver(C), 1 Ileum(S), 1 Colon(C) 1 Adrenal(C), 2 Node(L), 1 Bone(S)	
	Control	0	to 900	R	38	11	1 Thyroid(C), 1 Liver(C), 2 Adrenal(C), 1 Kidney(C), 1 Node(L), 5 Fat(S)	
Wagner[38] (1977)	Chrysotile	100 mg/day	619 ML	R	32	3	1 Stomach(S), 1 Uterus(S) 1 Node(L)	Asbestos feeding for 101 days of first 5 mo. of experiment.
	Talc	100 mg/day	614 ML	R	32	3	1 Stomach(S), 2 Uterus(S)	
	Control	0	641 ML	R	16	0		
Smith[5] (1979)	Amosite	0.5 mg/L ad lib	to 690	H	60	1	Lung(C)	130 mfl§
	Amosite	5 mg/L ad lib	to 690	H	60	3	2 Stomach(C), 1 Peritoneum Mesothelioma	1,300 mfl
	Amosite	50 mg/L ad lib	to 690	H	60	0		13,000 mfl
	Taconite tailings	0.5 mg/L ad lib	to 690	H	60	1	Uterus(S)	45 mfl
	Taconite tailings	5 mg/L ad lib	to 690	H	60	0		450 mfl
	Taconite tailings	50 mg/L ad lib	to 690	H	60	0		4,500 mfl
	Control	0	to 690	H	120		Node(L)	
Denham[39] (1980)	Chrysotile	10% diet	to 960	R	240	4	3 Colon(C), 1 Abdominal Mesothelioma	
	Control	10% diet	to 960	R	242	2	Colon(C)	
	Control	0	to 960	R	121	3	Colon(C)	
Ward[40] (1980)	Azoxymethane	7.4 mg/kg • wk x 10 wk	to 665	R	50	39	12 Ileum(C), 27 Colon(C)	Azoxymethane and saline administered subcutaneously; amosite administered intraperitoneally.
	Azoxymethane and Amosite	7.4 mg/kg • wk and 10 mg 3x/wk x 10 wk	to 665	R	50	44	15 Ileum(C), 29 Colon(C)	
	Saline and Amosite	1x/wk and 10 mg 3x/wk x 10 wk	to 665	R	50	17	1 Ileum(C), 16 Colon(C)	

*ML = Mean life.
†H = Hamster, R = Rat.
‡(C) = Carcinoma, (S) = Sarcoma, (L) = Lymphoma.
§mfl = Million amphibole fibers/L.

that fibers penetrated the mucosa or migrated. Cunningham et al.,[37] however, fed asbestos to rats and found numerous fibers in tissue suspensions of omentum, lungs, and kidney studied by electron microscopy. Storeygard and Brown[41] exposed the mucosa of a closed loop of bowel to amosite in suspension. In their subsequent study of the mucosa with a scanning electron microscope they reported fibers in the process of penetrating the mucosa.

Sebastien, Masse, and Bignon[42] administered asbestos (chrysotile or crocidolite) in gelatin capsules by gastric

ASARCO ALV 0006490

25. Ward, J.M.; Frank, A.L.; Wenk, M.; Devor, D.; and Tarone, R.E. 1980. Ingested asbestos and intestinal carcinogenesis in F344 rats. *J Environ Pathol Toxicol* 3: 301–12.
26. Levy, B.S.; Sigurdson, E.; Mandel, J.; Laudon, E.; and Pearson, J. 1976. Investigating possible effects of asbestos in city water surveillance of gastrointestinal cancer incidence in Duluth, Minnesota. *Am J Epidemiol* 103: 362–68.
27. Masson, T.J.; McKay, F.W.; and Miller, R.W. 1974. Asbestos-like fibres in Duluth water supply. *JAMA* 228: 1019–20.
28. Sigurdson, E.E. 1977. Incidence of gastrointestinal cancer morbidity in residents of Duluth, 1969–74. *Proceedings of the "Colloquium on Asbestos and Human Carcinogenesis,"* pp. 77–82. Paris.
29. Britain's Advisory Committee on Asbestos. 1979. *Final Report*, Vol. I and II. London: Her Majesty's Stationery Office.
30. Kleinfeld, M. 1973. Biologic response to kind and amount of asbestos. *J Occup Med* 15: 296–300.
31. Selikoff, I.J. 1974. Epidemiology of gastrointestinal cancer. *Environ Health Perspect* 9: 299–306.
32. Hamilton, T.R.; Hamilton, E.W.; and Allison, S.B. 1978. Lymphocyte response to amosite asbestos: Mitogenic effect. *Clin Res* 22: 575.
33. Kagan, E.; Jacobson, R.J.; Young, K.Y.; Haidak, D.J.; and Nachnani, G.H. 1979. Asbestos-associated neoplasms of B-cell lineage. *Am J Med* 67: 325–30.
34. Kang, K.Y.; Sera, Y.; Okochi, T.; and Uamamura, Y. 1974. T-lymphocytes in asbestosis. *N Eng J Med* (letter to the editor) 291: 735–36.
35. Bolton, R.E., and Davis, J.M.G. 1976. The short-term effects of chronic asbestos ingestion in rats. *Ann Occup Hyg* 19: 121–28.
36. Cunningham, H.M., and Pontefract, R.E. 1973. 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. *J Assoc Off Anal Chem* 56: 976–81.
37. Cunningham, H.M.; Moodie, C.A.; Lawrence, G.A.; and Pontefract, R.D. 1977. Chronic effects of ingested asbestos in rats. *Arch Environ Contam Toxicol* 6: 507–13.
38. Davis, J.M.G. 1975. The use of animal experiments in the study of asbestos bioeffects. *Hefte Unfallheilkd* 126: 564–74.
39. Davis, J.M.G.; Bolton, R.E.; and Garrett, J. 1974. Penetration of cells by asbestos fibers. *Environ Health Perspect* 9: 255–60.
40. Hallenbeck, W.H., and Hesse, C.S. 1977. A review of the health effects of ingested asbestos. *Rev Environ Health* 2: 157–66.
41. Patel-Mandlik, K.J., and Hallenbeck, W.H. 1979. Asbestos fibers: I. A modified preparation of tissue samples for analysis by electron microscope. II. Presence of fibers in tissues of baboons fed chrysotile asbestos. *J Environ Pathol Toxicol* 2: 1385–95.
42. Pontefract, R.D., and Cunningham, H.M. 1973. Penetration of asbestos through the digestive tract of rats. *Nature* (Lond.), 243: 352–53.
43. Sebastien, P.; Masse, R.; and Bignon, J. 1980. Recovery of ingested asbestos fibres from the gastrointestinal lymph in rats. *Environ Res* 22: 201–16.
44. Storeygard, A.R., and Brown, A.R. Jr. 1977. Penetration of small intestinal mucosa by asbestos fibers. *Mayo Clin Proc* 52: 808–12.
45. Webster, I. 1974. The ingestion of asbestos fibers. *Environ Health Perspect* 9: 199–202.
46. Westlake, G.E.; Spjut, H.J.; and Smith, M.N. 1965. Penetration of colonic mucosa by asbestos particles: An electron microscopic study in rats fed asbestos dust. *Lab Invest* 14: 2029–33.
47. Westlake, G.E. 1974. Commentary: Asbestos fibers in the colonic wall. *Environ Health Perspect* 9: 227–28.
48. Gross, P., and Harley, R.A. Jr. 1973. The locus of pathogenicity of asbestos dust: A theory. *Arch Environ Health* 27: 240–42.
49. Cook, P.M., and Olson, G.F. 1979. Ingested mineral fibers: Elimination in human urine. *Science* 204: 195–98.
50. Wyss, V. 1953. Presenza di aghi di amianto nelle urine di operai esposti al rischio asbestosico. *Ross Med Indust* 22: 55–56.

ASARCO ALV 0006492