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Chronic Effects of Dietary Exposure to Amosite Asbestos and Tremolite in F344 Rats

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Carcinogenesis bioassays of blocky (nonfibrous) tremolite and amosite asbestos alone or in combination with the intestinal carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) were conducted with male and female Fischer 344 rats. The minerals were administered at a concentration of 1% in pelleted diet for the entire lifetime of the rats starting with the dams of the test animals. One group of amosite rats also received chrysotile asbestos via gavage during lactation. Group sizes varied from 100 to 250 animals.

The offspring from mothers exposed to tremolite or amosite asbestos were smaller at weaning than those from untreated mothers and remained smaller throughout their life. The administration of dimethylhydrazine (DMH) did not affect body weight gain, either in amosite-exposed or nonexposed animals. Survival was comparable in the tremolite and control groups. The amosite-exposed rats showed enhanced survival compared to the untreated controls. DMH exposure reduced survival by approximately one year, although the amosite plus DMH groups survived slightly better than the DMH alone groups.

No toxicity or increase in neoplasia was observed in the tremolite-exposed rats compared to the controls. Significant increases ($p < 0.05$) in the rates of C-cell carcinomas of the thyroid and monocytic (mononuclear cell) leukemia in male rats were observed in amosite-exposed groups. However, the biological significance of the C-cell carcinomas in relation to amosite asbestos exposure is discounted because of a lack of significance when C-cell adenomas and carcinomas were combined and the positive effect was not observed in the amosite plus preweaning gavage group. The biological significance of an increased incidence of mononuclear cell leukemia is questionable, because of a lack of statistical significance in the amosite group when evaluated using life table analysis, lack of significance when compared to the tremolite control group, and the fact that no toxic or neoplastic lesions were observed in the target organs, i.e., gastrointestinal tract and mesothelium.

DMH caused a high rate of (62–74%) of intestinal neoplasia in amosite and nonamosite-exposed groups. Neither an enhanced carcinogenic nor protective effect was demonstrated by exposure to amosite asbestos.

Introduction

In November 1973 the National Institute of Environmental Health Sciences and the Environmental Protection Agency cosponsored a symposium on the possible biological effects of ingested asbestos. (*1*). This conference concluded that a paucity of definitive data existed concerning the effects of ingested asbestos and that specific research was needed.

A subcommittee of the DHEW Committee to Coordinate Toxicology and Related Programs was established to review existing data and to prepare a draft research protocol that would be responsive to the possible public health implication of ingested asbestos. This protocol was widely distributed for comment within and outside the government and a public meeting of the subcommittee was held on February 11, 1975. On the basis of the comments received, a revised final protocol was developed which called for the use of long-

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term animal toxicology studies to evaluate the ingestion of several minerals for carcinogenic effect. As a result, the National Toxicology Program has investigated the carcinogenic potential of the ingestion of chrysotile asbestos in hamsters and rats, amosite asbestos in hamsters and rats, crocidolite asbestos in rats and tremolite in rats. All of the studies were to encompass the lifetime of the animal, including exposure of the dams from which the test animals were derived.

Crystalline tremolite (not actually in asbestos fiber) was chosen for this study because up to 20 years ago it was a common contaminant of talc which was used in foods and pharmaceuticals. The grinding of tremolite in preparation for its intended use may result in the production of fibers which have the morphology of asbestos minerals. Stanton et al. (2), in reviewing intrapleural mineral deposition studies, speculated that the asbestos mineral hazard question may be directly related to fiber size in contrast to chemical composition. Therefore, the study of crystalline tremolite was deemed appropriate because of its past widespread exposure and the fact that it assumes fiber characteristics when ground in the processing of talc.

This report represents the results of those studies undertaken to determine the effects of tremolite or amosite asbestos in the diet fed to Fischer 344 rats. In addition, the study was designed to determine if the feeding of amosite asbestos modified the response of a known intestinal carcinogen, 1,2-dimethlhydrazine dihydrochloride (DMH). Reports on chrysotile and crocidolite asbestos will be reported later.

Table 1. Fiber characteristics of amosite asbestos.

Fiber characteristics	
Surface area, m^2/g	4.13
Density, g/cm^3	3.35 ± 0.026 SD
Measurements, transmission electron microscopy	
Fiber count/g	0.3466×10^{10}
Median length (μm)	4.37
Range of length, μm	0.85 – 995
Median diameter, μm	0.72
Range of diameter, μm	0.064 – 12.4
Median fiber aspect ratio (*l*/*d*)	6.4248

Table 2. Chemical-instrumental analysis of amosite asbestos.

	Content, wt-%
Al_2O_3	0.42
CaO	0.48
FeO	34.61
Fe_2O_3	2.24
MgO	6.22
K_2O	0.30
SiO_2	50.36
Na_2O	0.03
MnO	2.66
Cr_2O_3	0.03
NiO	0.01
CO_2	0.88
H_2O-	0.15
H_2O+	2.30
Benzene extracted organics	0.021

Materials and Methods

Test Materials

Asbestos is a general term applied to certain natural silicates when they appear in a fibrous

Table 3. Particle size distribution of amosite asbestos by particle number: SEM.

	Length interval, μm						
	0–1.99	2–3.99	4–5.99	6–7.99	8–9.99	10–19.99	20–39.99
Amosite mean width, μm	0.28	0.38	0.45	0.45	0.48	0.52	0.51
Amosite particles per interval	57	126	88	78	52	181	184
Total amosite particles, %	5.6	12.3	8.6	7.6	5.1	17.7	18.0
Cumulative % amosite	5.6	17.9	26.5	34.1	39.2	56.9	74.9
Amosite, vol-%[a]	–	0.1	0.3	0.4	0.4	2.4	5.0
Cumulative volume-% amosite	–	0.1	0.4	0.8	1.2	3.6	8.6
Number of other particles	11	8	1	0	1	1	0
Amosite particles per length interval by aspect ratio, %							
1:1–2.9:1	12	0	0	0	0	0	0
3:1–4.9:1	34	10	6	5	2	0	0
5:1–9.9:1	43	52	23	14	4	1	1
10:1–19.9:1	11	34	52	38	40	21	1
20:1–49.9:1	0	4	18	41	54	64	30
50:1–99.9:1	0	0	1	2	0	12	55
100:1–199:1	0	0	0	0	0	2	12
200:1–499:1	0	4	0	0	0	0	1
>500:1	0	0	0	0	0	0	0

[a]Calculated from particle number data, assuming rectangular cross section with third dimension equal to 1/2 measured width.

form. Amosite is a fibrous member of the amphibole mineral group, its chemical structure is $(Fe^{2+}Mg)_7Si_8O_{22}(OH)_2$. Mineral and fiber characteristics of amosite are shown in Tables 1–3.

The amosite sample identified as S-33 was purchased by the Bureau of Mines from the Atlas Asbestos Company, Montreal, Quebec, Canada. This material is from a mine in the area known as Penge, in the Transvaal, Republic of South Africa. Not a proper mineral name, amosite is a term used to describe the material from asbestos mines in South Africa. To develop homogeneity of the sample the amosite was processed by a single pass through an air jet mill.

Table 4. Fiber characteristics and chemical-instrumental analysis of tremolite.

Fiber characteristics	
Surface area, m^2/g	5.2 ± 0.5
Density, g/cm^3	2.91 ± 0.01 SD
Analyses, wt-%	
Al_2O_3	1.57
CaO	11.26
Fe_2O_3	0.27
MgO	26.71
K_2O	0.18
SiO_2	54.00
Na_2O	0.80
TiO_2	0.03
MnO	0.05
Li_2O	0.02
SnO	0.01
SrO	0.03
Bi_2O_3	0.01
CO_2	0.78
H_2O-	0.24
H_2O+	3.73
Benzene-extracted organics	0.003

The temolite sample used in this study was obtained from a single lense from the Governeur Talc Company, Governeur, NY. This 1200-lb lense was taken from the 500 ft level, American vein, No. 4 footwall stope, lower portion of the footwall bedding. The lense was crushed in a Denver Jaw Crusher and then to minus 14 mesh in a roll crusher. This material was then wheeler-milled at 204°C and bagged in 50-lb Kraft bags. The final particle size was nominal minus 325 mesh. To develop homogeneity of the sample, approximately 960 lb tremolite was blended in a 10 ft^3 V-type blender. Mineral and fiber characteristics of tremolite are shown in Tables 4 and 5.

After final blending the samples were weighed to 25 ± 0.5 lb and placed in fiberboard drums. These drums were shipped to a special warehouse at Research Triangle Park, NC. Each drum received a color marking unique to the mineral type. Homogeneity of the samples was verified by fluorescent X-ray spectrography for samples collected from six randomly selected drums. No significant differences were detected.

The homogeneity of the samples and the physical and chemical properties of the materials were characterized by the Bureau of Mines, U.S. Department of the Interior (Supt. of Documents No. I 28.23:8452) and by the Fine Particle Laboratories, Illinois Institute of Technology Research In-

Table 5. Particle size distribution for tremolite by particle number: SEM.

	Length interval, μm										
	0–0.99	1–1.99	2–2.99	3–3.99	4–4.99	5–5.99	6–6.99	7–7.99	8–8.99	9–9.99	>10
Mean width, μm	0.48	0.88	0.97	1.51	2.05	2.19	2.79	3.29	2.96	3.13	5.22
Number of particles per interval	59	291	194	106	53	40	31	19	9	13	58
% of all particles per interval	6.8	33.4	22.3	12.2	6.1	4.6	3.6	2.2	1.0	1.4	6.4
Cumulative % of all particles	6.8	40.2	62.5	74.7	80.8	85.4	89.0	91.2	92.2	93.6	100
Tremolite particles per interval	34	197	128	83	38	27	23	15	9	12	49
% of tremolite particles	5.5	32.0	20.8	13.5	6.2	4.4	3.7	2.4	1.5	2.0	8.0
Cumulative % tremolite	5.5	37.5	58.3	71.8	78.0	82.4	86.1	88.5	9.0	92.0	100
Talc-serpentine particles per interval	9	72	53	19	11	9	8	4	0	1	7
Other particles per interval	16	22	10	4	2	4	0	0	0	0	2
Tremolite particles per length interval, by aspect ratio, %[a,b]											
1:1–2.9:1	100	92	75	67	76	67	65	66	67	30	35
3:1–4.9:1	0	8	22	29	18	30	30	20	22	35	37
5:1–9.9:1	0	0	3	4	6	3	5	7	11	35	18
10:1–19:1	0	0	0	0	0	0	0	7	0	0	4
20:1–49:1	0	0	0	0	0	0	0	0	0	0	4
50:1–99:1	0	0	0	0	0	0	0	0	0	0	0
100:1–199:1	0	0	0	0	0	0	0	0	0	0	2

[a]Data for aspect ratio obtained from a second set of measurements.
[b]Total particles = 871, total tremolite = 615, total talc-serpentine = 193, and total other = 63.

stitute, Chicago, IL (Special Report and Addendum on project L6085, contract N01-ES-5-3157). Copies of these reports are available upon request from the National Toxicology Program.

Test Diets

The feed used was NIH-31 open formula rodent diet prepared by Zeigler Brothers, Inc., Gardner, PA. Tremolite or amosite asbestos was incorporated to a level of 1% by weight into the test diet. All feed was pelleted with a Sprout-Waldron pelleter; the pellets were of oval configuration, 3/8 in. by 3/4 in. in size. Pelleted feed was packaged in 25-lb aliquots in standard paper feedbags which were color coded to minimize the occurrence of feeding errors at the test laboratory.

Each lot of blended feed was analyzed for tremolite or amosite asbestos concentrations, pesticide contamination and nutrient content.

1% Chrysotile (Medium Range) Gavage.* The required amount of chrysotile (medium range), a gray powder with lumps, was weighed on a Mettler balance and placed in a beaker. Sterile water (for injection) was added to obtain the desired concentration and the suspension was then mixed in a magnetic stirrer for a short period of time. The suspension was administered by gavage, at a dose level of 0.47 mg/g of body weight, to the amosite and preweaning gavage (PWG) animals from birth to weaning (21 days).

Source and Specifications of Test Animals

Parental Generation (F_0). Weanling Fischer 344 (caesarean-derived) rats, which were barrier sustained and specific pathogen-free, were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA. These animals constituted the F_0 generation.

On arrival, animals were taken directly to the quarantine area and acclimated to laboratory conditions for approximately 2 weeks. At 24 hr after the animals arrived, eight animals of each sex were selected, sacrificed, and pathogen burden was determined for each animal. Pathogens examined for included ectoparasites, intestinal parasites, and bacteria. Serological tests were conducted for viruses. After approximately 2 months of quarantine the rats, both males and females, were randomized and divided into test groups by a computerized randomization process and placed on the appropriate designated diets.

*Animals were to receive 1% amosite, but were inadvertently gavaged with 1% chrysotile.

After at least 7 days exposure to the appropriate diets, the rats were placed in breeding cages (one male to two females). During the breeding period, the rats continued to be fed the designated diets; 20 days later (on the average), females were separated and housed individually in polycarbonate cages (Hazleton Systems, Aberdeen, MD). Males were removed from the breeding cages and re-housed two per cage.

Filial Generation (F_1). The F_0 females were allowed to deliver their F_1 litters naturally, and these were reduced to no more than eight pups (four/sex if possible) per litter. At birth, the litters from the F_0 dams within the control and treated groups were assigned randomly to the corresponding lifetime feeding phase groups such that birth dates were equally distributed. All pups assigned to the amosite and preweaning gavage (PWG) groups were exposed to the PWG phase of the study to assure exposure to asbestos from birth to weaning.

At 21 days after birth, the pups were weaned and given a temporary number, then selected, using a random number table, to be placed in their respective groups for the lifetime feeding study. Litters in which only one sex was present were excluded from those animals to be selected. The extra weanlings were discarded.

At 8 weeks of age, 1,2-dimethylhydrazine dihydrochloride (DMH) was administered by gavage to a control group and an amosite group every 14 days for a total of five doses. Males received 7.5 mg/kg, and females 15.0 mg/kg, based on a previous pilot study (3) which showed that these doses produced an approximate incidence of 15% intestinal neoplasia. Concentrations of DMH in the dosing solutions were determined within one hour prior to dosing and following dosing. The results of these determinations showed that the proper concentration of DMH was present in the dosing solution and had not deteriorated during dosing.

Animal Maintenance

The control and mineral exposed rats were placed in separate rooms with monitored temperature and humidity, and a controlled light cycle (12 hr light/12 hr dark). Temperature was maintained at 74 ± 4°F and humidity at 50% ± 10%. The rats were housed three per cage in polycarbonate cages covered with nonwoven polyester filter sheets and stored on Enviro-racks. Racks and filters were changed approximately once every 2 weeks. Cages and bedding were replaced twice per week. Control and treated diets and tap water

via automatic waterers were available *ad libitum*. Two water samples were collected and submitted for asbestos analysis. Stainless steel feed containers were changed once every 2 weeks.

The incoming air in the animal rooms was filtered to remove particulate matter. Ten to fifteen changes of room air per hour were provided. Prior to initiation of the study, air samples were collected and analyzed for baseline asbestos determinations. Additional samplings were collected approximately every 6 months for analysis to assure personnel safety.

Other measures used for personnel protection included the wearing of fully protective disposable suits, gloves, boots and bouffant caps and the use of a dust/mist respirator mask. Personnel leaving the animal rooms were required to take showers. In addition, physical examinations, including pulmonary function tests and chest radiographs, were conducted at the initiation of the study, yearly thereafter, and at the end of the study.

Clinical Examinations and Pathology

Observations and Records. All animals were observed twice daily for moribund condition and mortality. Recorded weekly were individual body weights; signs of toxicity or pharmacologic effects; incidence, size and location of palpable tissue masses or nodules; and food consumption per cage.

Sacrifice and Gross Pathology. Animals were sacrificed when exhibiting any one of these conditions: palpable masses within the abdominal cavity (excluding retained testes); masses protruding from the rectum; rectal discharge of bright red fluid (an indication of the presence of a bleeding colonic or rectal neoplasm); large ulcerated masses in the area of the ears or on side of face (Zymbal gland tumors); large subcutaneous masses which have been ulcerated or infected; masses which interfere with breathing and eating or which severely hamper locomotion; huge tissue masses (>10 cm); central nervous system signs accompanied by weight loss (head tilt, circling incoordination, ataxia, paralysis); severe weight loss or emaciation; or comatose or very weak.

When the remaining animals of either the control and DMH or the corresponding amosite and DMH group of either sex was reduced to 10% of those starting the study, both groups within that sex were killed. When survival or untreated control or amosite or amosite and PWG group of either sex reached 10%, all remaining animals of these groups within that sex were killed. The tremolite-exposed groups were handled similarly. Animals were killed by exsanguination under sodium pentobarbital anesthesia (Nembutal, Abbott Laboratories, Inc., North Chicago, IL, or Diabutal, Diamond Laboratories Inc., Des Moines, IO). Final body weights were recorded and necropsies performed which included these additional procedures: blood smears taken from animals sacrificed *in extremis* or terminally sacrificed, touch preparations made from any enlarged spleen or lymphoid organ.

Since the gastrointestinal tract was considered as the target organ prior to the study, it was handled in a manner slightly different from that in standard rodent lifetime bioassays. Prior to placement in fixative, the entire esophagus was opened and examined. The stomach and cecum were opened and pinned with the exterior surface adjacent to paper; 2-cm lengths of duodenum and ileum and two portions of jejunum were placed unopened in fixative. The remaining small intestine was opened and washed gently with saline and the mucosal surface was then examined carefully using transillumination on a radiograph viewing box. Suspect lesions were processed separately and identified individually as to location. Likewise, the entire colon with anus was opened, examined, and placed on cardboard (serosal surface down) prior to fixation. The size and location of masses were recorded. Masses greater than 1 mm in diameter were removed as separate specimens for processing. After fixation and prior to embedding, the colon was "carpet-rolled" starting at the proximal end, with the mucosal surface inward.

All tissues were fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin. Tissues/organs examined microscopically were: tissue masses, the above-mentioned portions of gastrointestinal tract, mesenteric, celiac, iliocolonic, renal, iliac, mandibular, cervical, pancreatic and bronchial lymph nodes, mammary gland, salivary gland, thigh muscle, bone marrow (sternum), nasal cavity with turbinates, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, liver, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate, testes/epididymus, ovaries/uterus, brain, pituitary gland, eyes and spinal cord.

Data Recording and Statistical Methods

The individual animal pathology data on this experiment were recorded in the computerized

carcinogenesis bioassay data system. The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, and individual pathologic results.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (*4*). Animals were statistically censored as of the time that they died of other than natural causes or were missing; animals dying from natural causes were not statistically censored. Differences in survival were evaluated by Cox's (*5*) life table method.

The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., leukemia), the denominators consist of the numbers of animals necropsied.

For the statistical analyses of tumor incidence data, two methods of adjusting for intercurrent mortality were employed. Each used the classical methods of combining contingency tables developed by Mantel and Haenszel (*6*). The first method of analysis assumed that all tumors of a given type were fatal; i.e., they caused the death of the animal, either directly or indirectly. According to this approach, the proportions of tumor-bearing animals in the treated and control groups were compared at each point in time at which an animal died with a particular tumor. The denominators of these proportions were the total number of animals at risk in each group. These results were then combined by the Mantel-Haenszel methods to obtain an overall probability (p) value. This method of adjusting for intercurrent mortality is Cox's life table method (*5*).

The second method of analysis assumed that all tumors of a give type were "incidental"; i.e., they were merely observed at autopsy in animal dying of an unrelated cause. According to this approach, the proportions of male and female rats found to have tumors in treated and control groups were compared in each of five time intervals: 0-60 weeks, 61-86 weeks, 87-112 weeks, 113-126 weeks and beyond 126 weeks. The denominators of these proportions were the number of animals actually autopsied during the time interval. The individual time interval comparisons were then combined by the previously described methods to obtain a single overall result (*7*). For comparisons involving groups receiving DMH (which showed markedly reduced survival), somewhat shorter time intervals were utilized for the incidental tumor test: 0-52 weeks, 53-78 weeks, 79-92 weeks, 93-116 weeks (males), 93-102 weeks (females), beyond 116 weeks (males) and beyond 102 weeks (females).

In addition to these tests, one other set of statistical analyses was carried out for each primary tumor: the Fisher exact test based on the overall proportion of tumor-bearing animals (*8*). All reported p values are one-sided. Except where noted, the three alternative analyses gave similar results.

Results

Establishment of Test Groups

The experiment was designed to evaluate the effects of orally ingested tremolite or amosite asbestos during the entire life of the animal, starting from the time the rats were able to eat solid food. For this reason, the mated female rats had been on the test diets for approximately 12 weeks when the first litters were born. To minimize the chance that the mothers would reject or cannibalize their young, the litters were not handled during lactation, except for the group receiving asbestos via preweaning gavage.

Litter size and survivability of offspring were unaffected by the presence of amosite in the diets. The average number of live fetuses born to tremolite-exposed dams was 7.6 versus 7.8 for the control groups. The average number of live fetuses born to amosite-exposed dams was 8.5 versus 7.7 for the control groups. Significant mortality was induced in those pups which received the preweaning asbestos gavage (PWG). The average size of the litters in this group was 3.4 at weaning compared to 7.5 in the non-PWG amosite group. The average weight at birth of the tremolite-exposed pups was 4.7 g versus 4.8 g for the controls. The average weight at birth of the amosite-exposed pups was 4.7 g versus 4.8 g for the controls. The tremolite-exposed offspring were slightly smaller at weaning, 22.8 g versus 26.3 g (control). The amosite-exposed offspring were also slightly smaller at weaning, 23.2 g versus 27.4 g (control).

A summary of groups, number of animals and diets for the filial (F_1) animals is presented in Table 6.

Table 6. Summary of distribution and diets: a lifetime feeding study of tremolite or amosite asbestos in rats.

Test group	No. of animals		% of diet	DMH, mg/kg[a]	
	Male	Female		Male	Female
Control	118	118	0		
Tremolite	250	250	1		
Control	117	117	0	–	–
DMH	125	125	0	7.5	15.0
Amosite	250	250	1	–	–
Amosite + DMH	175	175	1	7.5	15.0
Amosite + PWG[b]	100	100	1	–	–

[a]Gavage with 1,2-dimethylhydrazine dihydrochloride.
[b]Animal was inadvertently gavaged during preweaning with intermediate (medium)-range chrysotile instead of amosite.

Clinical Signs

The incidence of clinical signs occurred at essentially comparable frequencies throughout the study groups except those that received DMH (see below). No distinct signs of compound effect were noted in any of the tremolite- or amosite-treated animals during the first 52 weeks of study. As the study proceeded, the incidence of clinical signs increased among all the groups. At intervals where there were a large number of moribund sacrificed animals in any one particular group, the clinical signs most frequently observed were supportive of the conditions for moribund sacrifice previously outlined in the Methods section. A comparison of clinical signs observed during the same selected intervals among all the groups revealed a larger number of palpable abdominal masses, tissue masses, and central nervous system signs, a well as red discharge and protruding masses from the rectum in the DMH and amosite and DMH groups. These findings were presumably due to the administration of DMH since they were not clinically observed with any frequency in any of the tremolite- or amosite-treated groups.

Body Weight and Food Consumption

Mean body weights were analyzed at selected intervals: birth, 3, 8, 11, 15, 24, 33, and 60 weeks for the males, and birth, 3, 8, 11, 16, 27, 48 and 60 weeks for the females by the method of Rao (*9*). The data revealed a 13% depressed mean body weight gain at weaning in both sexes of the tremolite groups and 15% in the amosite groups compared to the controls. The depressed weight gain in the tremolite- and amosite-exposed rats was more apparent at 8 weeks of age (tremolite:33% for males and 17% for females; amosite: 37% for males and 25% for females). Weight gain then paralleled the controls (except for DMH-exposed rats) for the remainder of the study but the mineral-exposed rats remained smaller throughout the study. Both male and female DMH-exposed groups gained less than their respective controls.

In the tremolite-exposed males and females, the average weekly food consumption was 97% that of the untreated controls. In the DMH, amosite, amosite and DMH, and amosite and PWG males, the mean weekly food consumption was 102%, 102%, 105%, and 107%, respectively, compared to the untreated control group and 98%, 101%, 105%, and 108% that of the untreated control for comparable groups of females.

Survival

Survival data of intervals prior to the final sacrifice of a group are summarized in Table 7. There were no significant differences in survival between the tremolite-exposed and control groups. Survival of males and females was approximately equal until 112 weeks, after which the females tended to live longer. When compared to the survival rates of the untreated control group, the amosite male survival at 118 weeks was higher, while amosite and PWG male survival was somewhat less. In female rats, the amosite group survival was better than the untreated controls, while the amosite and PWG group was about the same. The survival of both groups of DMH-exposed rats was considerably less than the untreated controls. The amosite plus DMH group was comparable to the DMH alone group.

Pathology

There were no apparent treatment related neoplasms in the digestive tract of the tremolite, amosite, or amosite PWG groups (Tables 8 and 9). Also, no specific type was increased, either at a particular location (e.g., cecum) or in the stomach, small or large intestine as a whole. In addition, the incidences of non-neoplastic diseases of the gastrointestinal tract such as enteritis, diverticulitis, ulceration or inflammation in general were comparable in the control and tremolite- or amosite-exposed rate (Tables 10 and 11).

There were no organs/tissues in the tremolite-exposed rats which showed an increased rate of neoplasia compared to the control groups. Organs which showed an increase in neoplasms in the amosite or amosite PWG groups compared to the control group were the thyroid and hematopoeitic system. The results are as follows.

Thyroid. Table 12 summarizes the incidence

Table 7. Survival of F344 rats in lifetime oral asbestos study at various time points.

Group	Age, weeks	Males No. alive/ total no.	Males Survival, %	Females No. alive/ total no.	Females Survival, %
Control	106	98/118	83	97/118	82
	120	70/118	59	71/118	60
	146	6/118	5	20/118	17
	148	–	–	14/118	12
Tremolite	106	206/250	82	207/247	84
	120	150/250	60	144/247	58
	146	36/250	14	33/246	13
	148	–	–	22/246	9
Control	106	95/117	81	92/117	79
	118	71/117	61	62/117	53
	142	7/117	6	20/116	17
	146	–	–	10/116	9
DMH	106	27/125	22	15/125	12
	118	16/125	13	–	–
	142	–	–	–	–
	146	–	–	–	–
1% Amosite	106	221/250	88	202/246	82
	118	117/250	71	162/246	66
	142	35/249	14	43/245	18
	146	–	–	28/245	11
1% Amosite + DMH	106	46/175	26	32/174	18
	118	26/175	15	–	–
	142	–	–	–	–
	146	–	–	–	–
1% Amosite + PWG	106	77/100	77	86/100	86
	118	52/100	52	56/100	56
	142	6/100	6	15/100	15
	146	–	–	9/100	9

of thyroid C-cell proliferative lesions. A significantly increased incidence of C-cell carcinoma was found in amosite-treated male rats ($p < 0.05$). This effect was not observed in amosite PWG male rats. Furthermore, the overall incidence of C-cell tumors (adenomas and/or carcinomas) was comparable between control and treated groups. C-cell hyperplasia was equivocally increased in amosite and amosite PWG female groups.

Hematopoietic System. A significantly increased incidence of mononuclear cell leukemia occurred in amosite ($p < 0.05$) and amosite PWG ($p < 0.01$) male rats (Table 13). However, neither group was significant when compared to the tremolite control group (39%). This increased incidence was not observed in amosite-exposed females.

Miscellaneous Neoplasms

Occasionally a somewhat higher or lower rate of commonly occurring neoplasms were observed in amosite treated groups. A statistically significant ($p < 0.05$) decrease in the rate of neoplasia was observed in the pancreas (Islet cell adenoma), adrenal medulla (pheochromacytoma), thyroid (follicular cell carcinoma) and preputial gland in at least one group of amosite-exposed rats compared to the controls. Similar observations were not observed in the tremolite-exposed groups.

Nonneoplastic Findings

A plethora of incidental lesions of aging Fischer 344 rats was found in all groups. Statistical analyses showed no obvious correlation between the incidence of specific lesion types and the type of treatment. Nonneoplastic lesions that were observed in more than 5% of the rats in any of the experimental groups are as follows: skin: epidermal inclusion cyst; lung: chronic inflammation (peribronchiolar and perivascular lymphoid cuffing); spleen: fibrosis, hemosiderosis, extramedullary hematopoeisis, lymphoid atrophy; lymph

Table 8. Number of tremolite-exposed F344 rats with primary epithelial neoplasms of the alimentary tract.

	Males[a]		Females[a]	
	Control	Tremolite	Control	Tremolite
Animals examined	118	250	118	250
Total alimentary	8(7)	12(5)	3(3)	7(3)
Oral/pharynx				
Papilloma	1(1)	1(0)	0(0)	1(0)
Carcinoma	3(3)	1(0)	2(2)	5(2)
Esophagus				
Total gastrointestinal	4(3)	9(3)	1(1)	1(0)
Total stomach	3(3)	2(1)	0(0)	0(0)
Nonglandular				
Papilloma	2(2)	1(0)		
Carcinoma	1(1)	1(0)		
Glandular				
Polyp				
Carcinoma				
Total small intestine	0(0)	3(1)	0(0)	0(0)
Polyp		1(0)	1(1)	
Adca in polyp[b]				
Carcinoma		2(1)		
Total large intestine	1(1)	4(2)	0(0)	0(0)
Cecum				
Polyp		1(0)		
Adca in polyp[b]				
Carcinoma				
Colon				
Polyp	1(1)	1(0)		
Adca in polyp[b]				
Carcinoma		2(1)		

[a]Values in parentheses are percentages.
[b]Adenocarcinoma arising in adenomatous polyp.

nodes (various): lymphoid or reticulum cell hyperplasia, lymph-angiectasis, hemorrhage, pigmentation, chronic inflammation; heart: chronic inflammation; liver: degeneration, necrosis, fatty metamorphosis, toxic hepatitis (associated with leukemia), granuloma, angiectasis, pigmentation, focal cellular change; bile duct (extrahepatic): chronic inflammation, mucosal hyperplasia, cysts, fibrosis; pancreas (exocrine): atrophy, hyperplasia, ectopia; pancreas (endocrine): hyperplasia; kidney: chronic progressive nephropathy, cysts, pigmentation; pituitary gland: cysts, angiectasis, hyperplasia; adrenal (cortex): fatty metamorphosis, hyperplasia; adrenal (medulla): hyperplasia; thyroid: follicular cysts, C-cell hyperplasia; parathyroid: hyperplasia; testes: seminiferous degeneration, interstitial cell hyperplasia; prostate: abscess, chronic inflammation, glandular hyperplasia; seminal vesicles: cysts; ovary: follicular and parovarian cysts; uterus: hydrometra, endometrial cyst; mammary gland: cystic ducts, glandular hyperplasia, galactocele; mesentery: chronic inflammation; eye: cataract, hemorrhage, inflammation, retinal degeneration: zymbal gland: cystic ducts; bone: osteopetrosis, exostoses, marrow hyperplasia. Alimentary tract nonneoplastic lesions are noted in Tables 10 and 11.

1, 2-Dimethylhydrazine Dihydrochloride-Treated Groups

Two groups of male and female rats were exposed to 1, 2-dimethylhydrazine dihydrochloride (DMH) by gavage at levels of 7.5 mg/kg for males and 15.0 mg/kg for females, biweekly for a total of five doses. One group served as a positive carcinogen control and the other received amosite from weaning throughout life.

Exposure of rats to DMH or DMH with amosite was associated with a dramatically increased incidence of neoplasms of the intestinal tract, Zymbal's gland, and liver of male and female rats, and kidney in female rats. It is also noteworthy that survival in the DMH groups was shortened due to the presence of these neoplasms.

Table 9 summarizes the numbers of rats with primary epithelial neoplasms in the gastrointestinal tract by specific site and classification. Intestinal neoplasms, particularly the adenomatous polyps, were often multiple within a given animal.

Table 9. Number of amosite-exposed F344 rats with primary epithelial neoplasms of the gastrointestinal tract.

	Untreated control[a]		Amosite[a]		Amosite PWG[a]	
	M	F	M	F	M	F
Animals examined	117	117	249	250	100	100
Total gastrointestinal	4(4)	2(2)	7(3)	4(2)	3(3)	3(3)
Total stomach	1(1)	1(1)	2(1)	1(0)	0(0)	0(0)
Total small intestine	3(3)	0(0)	2(1)	3(1)	1(1)	1(1)
Duodenum						
Carcinoma			1(0)		1(1)	
Adca in polyp[b]						
Adenomatous polyp						
Jejunum						
Carcinoma	2(2)			1(0)		1(1)
Adca in polyp[b]			1(0)			
Adenomatous polyp				2(1)		
Ileum						
Carcinoma						
Adca in polyp[b]						
Adenomatous polyp	1(1)					
Total large intestine	0(0)	1(1)	3(1)	0(0)	2(2)	2(2)
Cecum						
Carcinoma						
Adca in polyp[b]						
Adenomatous polyp					1(1)	
Total colon	0(0)	1(1)	3(1)	0(0)	1(1)	2(2)
Ascending colon						
Carcinoma			1(0)			
Adca in polyp[b]						
Adenomatous polyp						
Transverse colon						
Carcinoma						1(1)
Adca in polyp[b]						
Adenomatous polyp						
Descending colon						
Carcinoma						
Adca in polyp[b]					1(1)	
Adenomatous polyp		1(1)	2(1)			1(1)

[a]Values in parentheses are percentages.
[b]Adenocarcinoma arising in adenomatous polyp.

The incidence of gastrointestinal neoplasia was dramatically increased with DMH treatment. However, the incidence appeared to be essentially comparable between groups receiving DMH alone and those receiving DMH with amosite. Furthermore, the number of animals with tumors either in the small intestine or in the large intestine was also essentially comparable between DMH alone and DMH with amosite. There was no difference in the time to tumor between the groups.

Evaluation of the incidence of the three categories of intestinal neoplasia (carcinoma, adenocarcinoma arising in an adenomatous polyp, and adenomatous polyp) by site (Table 14) reveals an increased incidence of duodenal carcinoma ($p < 0.05$) in the DMH with amosite-treated females, compared to female rats receiving DMH alone. In the jejunum, however, this incidence is reversed, with more carcinomas occurring in the female group receiving DMH alone.

In the large intestine the frequency of carcinoma arising in an adenomatous polyp and adenomatous polyps was greatest in the descending colon. In the cecum, the incidence of carcinoma was less in the DMH with amosite-treated group than those treated with DMH alone, in male rats. This effect was not observed in the female group. The appearance of carcinomas in the ascending colon was somewhat greater in DMH with amosite-treated males than in males receiving DMH alone. Adenocarcinoma arising in adenomatous polyp occurred more frequently in the transverse colon of male and female rats receiving DMH with amosite compared to rats receiving DMH alone.

Kidney Neoplasms

Almost without exception, the renal masses associated with DMH treatment were malignant

Table 10. Incidence of nonneoplastic lesions in the alimentary tract of F344 rats exposed to 1% tremolite in the diet.[a]

	Males[b]		Females[b]	
	Control	Tremolite	Control	Tremolite
Animals examined	118	250	118	250
Palate/tongue				
Inflammation	0(0)	0(0)	0(0)	4(2)
Necrosis	0(0)	0(0)	0(0)	1(0)
Hyperkeratosis	0(0)	1(0)	0(0)	2(1)
Acanthosis	1(1)	3(1)	1(1)	1(0)
Esophagus				
Inflammation	1(1)	0(0)	0(0)	1(0)
Necrosis	2(2)	1(0)	0(0)	0(0)
Hyperkeratosis	9(8)	18(7)	3(3)	4(2)
Acanthosis	1(1)	0(0)	0(0)	1(0)
Stomach-nonglandular				
Mineralization	13(11)	5(2)	4(3)	2(1)
Inflammation, chronic	19(16)	29(12)	25(21)	38(15)
Ulceration	10(8)	17(7)	9(8)	11(4)
Necrosis	20(17)	46(18)	17(4)	31(12)
Hyperplasia	3(3)	1(0)	0(0)	2(1)
Hyperkeratosis	18(15)	34(14)	15(13)	29(12)
Acanthosis	26(22)	54(22)	23(19)	45(18)
Stomach-glandular				
Hyperplasia	7(6)	1(0)	3(3)	0(0)
Small intestine				
Inflammation	0(0)	2(1)	0(0)	1(0)
Necrosis	2(2)	0(0)	1(1)	3(1)
Ulceration	0(0)	1(0)	0(0)	0(0)
Colon				
Parasitism	5(4)	32(13)	5(4)	3(1)
Inflammation	0(0)	5(2)	3(3)	0(0)
Necrosis	0(0)	3(1)	1(1)	1(0)
Hyperplasia	0(0)	1(0)	0(0)	1(0)
Cecum				
Parasitism	9(8)	2(1)	2(2)	1(0)
Inflammation	1(1)	2(1)	4(4)	1(0)
Necrosis	1(1)	4(2)	1(1)	3(1)
Hyperplasia	0(0)	0(0)	0(0)	1(0)
Rectum				
Necrosis	0(0)	1(0)	0(0)	0(0)
Anus (no lesions)				

[a]Incidence of nonneoplastic lesions that occur with a frequency of 1% or more in at least one group.
[b]Values in parentheses are percentages.

mesenchymal or mixed malignant tumors. Purely mesenchymal growths were classified according to their morphology (i. e., fibrosarcoma, undifferentiated sarcoma). Those having epithelial elements or epithelial-like elements were classified as mixed malignant tumors. In early stages, these neoplasms appeared as interstitial sclerosing growths near the inner cortex. Collagen formation was accompanied by proliferating, basophilic, primitive-appearing cells. Epithelial elements consisted of glands, ductlike structures or poorly differentiated solid tubules. The growths were often massive but rarely metastasized.

Table 15 summarizes the incidence of kidney tumors in control and DMH-treated groups. The high incidence of renal neoplasms was confined almost exclusively to treated female rats receiving either DMH alone or DMH with amosite ($p < 0.01$). The incidence rates for the two treated female groups was the same. Renal neoplasms occurred infrequently in male rats.

Zymbal Gland Neoplasms

Carcinoma was the most commonly observed neoplasm in Zymbal's gland. These neoplasms were composed of proliferating eosinophilic to basophilic squamous epithelial cells which formed thick fingers of tissue, masses of keratin and nests of sequestered cells. Some had sebaceous features with formation of sebum. Infiltration of adjacent tissues was not uncommon; however, metastases were rare. Table 16 summarizes the number of control and DMH-treated rats with Zymbal's gland neoplasms.

Table 11. Incidence of nonneoplastic lesions in the alimentary tract of F344 rats exposed to amosite asbestos.[a]

	Control[b]		Amosite[b]		Amosite PWG[b,c]	
	M	F	M	F	M	F
Tongue, number examined	117	117	249	250	100	100
Esophagus, number examined	115	117	249	246	100	100
Hyperkeratosis	12(10)	7(6)	4(2)	7(3)	12(12)	6(6)
Stomach, nonglandular, number examined	117	117	249	250	100	100
Mineralization	9(8)	3(3)	2(1)	2(1)	1(1)	0(0)
Inflammation, chronic	21(18)	21(18)	56(22)	60(24)	17(17)	18(18)
Ulceration	13(11)	4(3)	25(10)	30(12)	7(7)	10(10)
Necrosis	23(20)	13(11)	41(16)	37(15)	15(15)	11(11)
Hyperkeratosis	22(19)	24(21)	41(16)	56(22)	16(16)	17(17)
Acanthosis	31(26)	26(22)	62(25)	72(29)	21(21)	23(23)
Muscle degeneration	8(7)	2(2)	3(1)	3(1)	0(0)	0(0)
Stomach, glandular, number examined	117	117	249	250	100	100
Hyperplasia	6(5)	2(2)	0(0)	1(0)	0(0)	0(0)
Duodenum, number examined	117	117	249	249	100	100
Jejunum, number examined	117	117	249	249	100	100
Ileum, number examined	117	117	249	249	100	100
Colon, number examined	117	117	249	250	100	100
Parasitism	4(3)	2(2)	17(7)	6(2)	4(4)	8(8)
Cecum, number examined	117	117	249	250	100	100
Rectum, number examined	117	117	249	250	100	100
Anus, number examined	117	117	249	250	100	100

[a]Incidence of nonneoplastic lesions that occur with a frequency of 1% or more in at least one group.
[b]Values in parentheses are percentages.
[c]PWG = preweaning gavage.

Table 12. Number of F344 rats with thyroid C-cell proliferative lesions.

	Control[a]		Amosite[a]		Amosite PWG[a]	
	M	F	M	F	M	F
Animals examined	117	116	246	247	100	100
Total C-cell tumors	27(23)	24(21)	76(31)	65(26)	25(25)	29(29)
C-cell adenoma	16(14)	14(12)	26(11)	37(15)	11(11)	15(15)
C-cell carcinoma	11(9)	10(9)	50*(20)	29(12)	14(14)	14(14)
C-cell hyperplasia	21(18)	22(19)	58(24)	71(29)	23(23)	26(26)

[a]Values in parentheses are percentages.
*$p < 0.05$ vs. controls (incidental tumor and Fisher exact tests).

Table 13. Number of amosite-exposed F344 rats with mononuclear leukemia.

	Untreated control		Amosite		Amosite PWG	
	M	F	M	F	M	F
Animals examined	117	117	249	250	100	100
Mononuclear cell leukemia[a]	38(32)	40(34)	106*(42)	82(33)	49†(49)	34(34)

[a]Values in parentheses are percentages.
*$p < 0.05$ vs. controls (incidental tumor and Fisher's exact test).
†$p < 0.01$ vs. controls.

Approximately one quarter of all rats receiving DMH alone or DMH with amosite developed Zymbal's gland neoplasms ($p < 0.01$), while in control animals the occurrence was low (1–3%). The incidence appeared essentially comparable between the two DMH-treated groups.

Liver Neoplasms

The classification of hepatocellular proliferative lesions was based on the ILAR Monograph (*10*). Table 17 summarizes the number of control or DMH-treated rats with neoplastic nodules or hepatocellular carcinoma.

Table 14. Number of DMH-exposed F344 rats with primary epithelial neoplasms of the gastrointestinal tract.

	Untreated control[a]		DMH positive control[a]		DMH with amosite[a]	
	M	F	M	F	M	F
Animals examined	117	117	125	124	173	175
Total gastrointestinal	4(4)	2(2)	92(74)	77(62)	118(68)	114(65)
Total stomach	1(1)	1(1)	3(2)	4(3)	2(1)	1(1)
Total small intestine	3(3)	0(0)	18(14)	14(11)	19(11)	24(14)
Duodenum						
Carcinoma	0(0)	0(0)	11(9)	3(2)	13(8)	19(11)
Adca in polyp[b]						
Adenomatous polyp						
Jejunum						
Carcinoma	2(2)	0(0)	2(1)	11(9)	3(2)	2(1)
Adca in polyp[b]					1(1)	
Adenomatous polyp			1(1)			
Ileum						
Carcinoma			2(1)			
Adca in polyp[b]						1(1)
Adenomatous polyp	1(1)					
Total large intestine	0(0)	1(1)	81(65)	70(56)	110(64)	101(58)
Cecum						
Carcinoma			16(13)	7(6)	6(3)	6(3)
Adca in polyp[b]						
Adenomatous polyp					2(1)	
Total colon						
Ascending colon						
Carcinoma			10(8)	10(8)	20(12)	14(8)
Adca in polyp[b]			2(1)	4(3)	3(2)	7(4)
Adenomatous polyp			5(4)	7(6)	5(3)	8(5)
Transverse colon						
Carcinoma			1(1)		2(1)	2(1)
Adca in polyp[b]			8(6)	9(7)	21(12)	20(11)
Adenomatous polyp	8(6)	6(5)	22(13)	22(13)		
Descending colon						
Carcinoma				(1)	3(2)	2(1)
Adca in polyp[b]			20(16)	15(12)	25(14)	22(13)
Adenomatous polyp		1(1)	34(27)	27(22)	40(23)	31(18)
Colon (other)[c]						
Carcinoma				4(3)	2(1)	2(1)
Adca in polyp[b]						
Adenomatous polyp			1(1)	1(1)		1(1)

[a]Values in parentheses are percentages.
[b]Adenocarcinoma arising in adenomatous polyp.
[c]Colon (other) = site not identified.

Table 15. Number of DMH-exposed F344 rats with primary renal neoplasms.

	Untreated control		DMH positive control		DMH with amosite	
	M	F	M	F	M	F
Animals examined	117	117	125	124	173	175
Total renal tumors[a]	0(0)	1(1)	3(2)	49(32)*	4(2)	56(32)*

[a]Values in parentheses are percentages.
*$p = < 0.01$.

A significantly increased incidence of neoplastic nodules and/or hepatocellular carcinomas occurred in groups receiving DMH alone and in groups receiving DMH plus amosite. Generally, females had a higher incidence ($p < 0.01$) than males ($p < 0.05$).

Miscellaneous Neoplasms

In several instances, DMH treatment with or without amosite led to statistically significant decreased incidences of certain spontaneous neoplasms, particularly of the endocrine system.

Table 16. Number of DMH-exposed F344 rats with Zymbal gland neoplasms.

	Untreated control		DMH positive control		DMH with amosite	
	M	F	M	F	M	F
Animals examined	117	117	125	124	173	175
Zymbal gland neoplasms[a]	1(1)	4(3)	33(26)*	34(27)*	55(32)*	39(22)*

[a]Values in parentheses are percentages.
*$p < 0.01$ vs. controls.

Table 17. Number of DMH-exposed F344 rats with hepatocellular neoplasms.

	Untreated control		DMH positive control		DMH with amosite	
	M	F	M	F	M	F
Animals examined	117	117	125	124	173	175
Neoplastic nodules[a]	9(8)	4(3)	18(14)*	29(23)*	27(15)*	32(18)*
Hepatocellular carcinoma[a]	1(1)	1(1)	9(7)*	10(8)*	7(4)†	8(5)‡

[a]Values in parentheses are percentages.
*$p < 0.05$ vs. controls.
†$p < 0.05$ vs. controls (incidental tumor and life table analysis).
‡$p < 0.05$ vs. controls (life table analysis only).

These included a reduced number of subcutaneous fibromas, pituitary adenomas in females, adrenal pheochromocytomas, pancreatic acinar cell adenomas and islet cell adenoma in males, mammary tumors, and interstitial cell tumors in male rats. However, many animals in these two groups died at an early age compared to the untreated controls.

Discussion

Tremolite (*11*) or amosite asbestos (*12*) was administered at a level of 1% in the diet to male and female F344 rats for their lifetime, including exposure of their dams to the test material. While the tremolite used in this study is considered crystalline or nonfibrous in its natural form, a small amount assumes a fibrous character during the crushing and milling process. However, the milling process used in the preparation of the tremolite for this study was identical to what is done in the commercial setting.

Starting at birth, one of three groups of neonate rats from amosite-exposed mothers were given chrysotile asbestos (instead of amosite) by gavage until weaning at which time they were given the 1% amosite diet. For all intents and purposes this group of rats should be regarded as being exposed to amosite asbestos for their lifetime. Two groups (control and amosite exposed) of weanling rats were exposed to five biweekly doses of 1,2-dimethylhydrazine dihydrochloride (DMH), a known intestinal carcinogen, to test the promotor or cocarcinogenic effects of DMH and amosite asbestos.

The clinicopathologic results in this study showed that the ingestion of tremolite or amosite asbestos did not adversely affect the fertility of the mothers or litter size of the F_1 bioassay animals. The average weight of the offspring at birth from mothers exposed to either mineral was comparable to the offspring of nonexposed mothers. However, the weight of the exposed offspring at weaning was slightly less than the control rats. The cause of the decreases in weight during lactation is not known. The differences in body weight gain became more apparant between weaning and 8 weeks of age. While the tremolite- and amosite asbestos-exposed rats paralleled the control animals in weight gain, they remained smaller throughout their life. The mean body weight of the male rats exposed to the chrysotile preweaning gavage (PWG) and subsequently to amosite asbestos was slightly higher than the amosite alone rats. This may be related to the mortality induced in the neonates by the PWG technique which would allow the remaining pups more milk during lactation. Exposure to DMH caused a small reduction in body weight gain in female but not in male rats.

No clinical signs were observed which could be attributed to the ingestion of either mineral. Starting at 9 months of age, the DMH-exposed rats showed signs attributable to DMH-related neoplasia, but no difference was noted between the DMH and DMH plus amosite groups.

The ingestion of either tremolite or amosite in the diet for the life of the rats did not adversely affect their survival. In fact, survival of female rats exposed to amosite or amosite plus chrysotile PWG was slightly better up to 112 weeks than the controls. Similarly, the survival of male rats exposed to amosite was slightly better than the untreated controls, although the amosite plus chrysotile PWG group showed slightly less survivability.

The most plausible explanation for the increased survival of the amosite exposed rats is their lower weight throughout the study. Yu et al. (*13*) have shown that rats of lower body weight caused by restricted caloric intake lived longer than rats that were allowed to eat an unlimited amount of food.

The survival of the rats (control and amosite) in this study compares favorably with other NTP bioassays (*14*). At 106 weeks of age (age at end of typical 2-yr bioassay) the percentage of male rats alive in this study was: untreated tremolite control, 83%; untreated amosite control, 81%; tremolite, 82%; amosite, 88%; and amosite plus PWG, 77%. The percentage of female rats alive at this time was: tremolite control, 82%; amosite control, 79%; tremolite, 84%; amosite, 82%; and amosite plus PWG, 86%. Haseman (*14*) in reviewing the 25 most recent NTP feeding studies found an average of 66% of control males and 73% of control females alive at 112 weeks of age.

The survival of control groups of males and females was similar at 106 weeks. In most 2-yr studies involving rats, more females generally survive to the end of the study than do males. However, the longer survival of female rats (control and tremolite or amosite exposed) was clearly demonstrated after 142 weeks.

The ingestion of either tremolite or amosite asbestos over the lifetime of these rats did not cause a biologically significant increase of neoplasms at any anatomic site when compared to the concurrent controls. The gastrointestinal tract was considered a potential target organ based on epidemiological studies in humans (*15*). The overall incidence of intestinal neoplasms in the control (male 4 and female 2%) and two amosite asbestos groups (male 3 and female 2%; male 4 and female 3%) was low, and there was no significant ($p < 0.05$) difference between the treated and control groups. Similar observations were noted in the tremolite groups and their respective controls. In addition, nonneoplastic lesions of the gastrointestinal tract were not increased. In summary, amosite asbestos did not cause any adverse affects in the gastrointestinal tract of either male or female F344 rats.

Rats exposed to DMH showed a high incidence (60–70%) of neoplasia of the gastrointestinal tract, primarily in the large intestine. This high rate of intestinal neoplasia was unexpected because a pilot study (*3*) using the same dosing regimen of DMH would have predicted an incidence of 15 ± 5% in this study. In a previous NTP bioassay, hamsters exposed to DMH and chrysotile asbestos also failed to develop the desired rate of intestinal tumors based on a similar pilot study (*28*). Apparently the neoplastic dose response to DMH is relatively steep and duplication of low rates of intestinal neoplasia are difficult to reproduce.

Because of the high background rate of DMH-induced neoplasia, it is not possible to determine with accuracy if amosite had a cocarcinogenic or additive effect in this study. Female rats exposed to DMH and amosite had a higher incidence (11% versus 2%) of neoplasia of the duodenum than the DMH controls. Conversely, they had a lower incidence (9% versus 1%) of neoplasms of the jejunum; thus the total number of animals with neoplasms of the small intestine was comparable. A similar situation was observed in the large intestine of male rats. The rats exposed to DMH alone had a higher incidence (13% versus 3%) of carcinoma of the cecum but a lower incidence (13% versus 26%) of neoplasms of the transverse colon.

The morphologic appearance of the neoplasms induced by DMH were comparable to those described previously in rats exposed to hydrazine compounds (*16*). In addition, the few intestinal neoplasms which occurred in the control and tremolite- or amosite (no DMH)-exposed rats were of the same morphologic types to those induced by DMH. The neoplasms observed in the kidney, liver and Zymbal's gland of DMH-exposed rats were consistent with those reported for these types of intestinal carcinogens (*17*).

A significantly ($p < 0.05$) increased incidence of C-cell carcinomas of the thyroid occurred in amosite-treated male rats. This effect was not observed in the amosite PWG male rats and the overall incidence of total benign and malignant C-cell tumors was comparable between control and treated groups. Therefore, this is not considered to be a treatment-related effect.

The incidence of mononuclear cell leukemia (synonyms—monocytic leukemia, Fisher rat leukemia) was elevated in amosite (42%) and amosite PWG (49%) male rats compared to the concurrent control group (32%). However, the rates were not significant when compared to the tremolite male control group (39%). This increased inci-

dence was not observed in treated female rats. Coleman et al. (*18*) reported an incidence of nearly 30% in male F344 rats within the age group of 24–40 months. In 2-yr-old F344 rats, Goodman et al. (*19*) reported 12% of males and nearly 10% of females had lymphoma/leukemia, a much lower incidence than in these studies. It is apparent from this study and above cited studies that the incidence of leukemia increases rapidly after 2 years of age. In view of considerable variation in the incidence of such disorders, the fact that the amosite-exposed male rats survived longer than their concurrent controls and lack of significance when compared to the tremolite control group, it is doubtful that the increase in the rate of leukemia is treatment related. More importantly, an increased incidence of neoplasia was not observed in target organs (GI tract and mesothelium). Even though it is known that certain types of asbestos are absorbed through the GI tract (*20,21*), it is difficult to envision how oral asbestos could cause an increase in leukemia without causing an increase of tumors in the proposed target tissues.

In summary, these effects represent only a modulation of neoplasms which occur in concurrent control groups and are known to occur in historical control rats of this strain. No uncommon or unique neoplasms were observed in any of the tremolite- or amosite-treated groups. In addition, the biological importance of the neoplasms in the absence of target organ neoplasia is questionable.

A large variety of nonneoplastic lesions, primarily lesions of aging, were observed in all groups. There was no obvious correlation between treatment and specific lesions. Therefore, tremolite or amosite at the level of 1% in the diet did not appear to cause any overt toxicity.

Studies on the effects of chronic ingestion of tremolite are not available. However, Stanton et al. (*2*) showed that the intrapleural inoculation of fibrous tremolite (two types) caused a high incidence of pleural sarcoma in Osborne-Mendel rats. In contrast, intrapleural studies of tremolite talc failed to show a carcinogenic response in hamsters (*22*). The tremolite used in the NTP study is a nonfibrous type and more closely resembles that used by Smith (*22*) than Stanton et al. (*2*).

Other studies involving the long-term ingestion of asbestos are few. Donham et al. (*23*) reported equivocal results in F344 rats which were fed a diet containing 10% chrysotile for their lifetime. While they did not observe a statistically significant ($p < 0.05$) increase in the number of tumors in exposed animals, the authors believed that there was a trend toward increased colon lesions in general, evidence of penetration of asbestos into the colonic mucosa, possible cytotoxicity to colonic tissues and suggested a possible relationship to peritoneal mesothelioma. Another equivocal study is that reported by Gibel et al. (*24*), who described an increase in malignant tumors in the lung, kidney, liver and reticuloendothelial system but no increase in intestinal neoplasia in Wistar rats fed asbestos filter material (20 mg/day) for a period of 8–14 months. Cunningham et al. (*25*) reported two studies in Wistar male rats using 1% chrysotile in the diet: one study of 24 months and one of 30 months. These authors concluded that trace amounts of ingested asbestos can penetrate the walls of the gastrointestinal tract, but evidence of carcinogenicity was inconclusive. Negative results were reported by Gross et al. (*26*), who fed rats a diet containing 5% chrysotile asbestos for a period of 21 months with no evidence of intestinal neoplasia.

Corollary studies to this investigation were conducted in Syrian golden hamsters (*27, 28*). The exposure regimen was similar in that male and female hamsters were exposed to 1% amosite asbestos (same source as the subject study) and short-range or intermediate-range chrysotile asbestos in their diet for their natural life-span. There was no adverse effect on body weight gain or survival, and no asbestos-related neoplasms were observed.

Another oral asbestos study in hamsters was reported by Smith et al. (*29*). They exposed groups of 30 male and female hamsters via drinking water for lifetime to amosite asbestos, mine tailings, beach rock or Lake Superior drinking water. They did not observe adverse effects on body weight or survival time in any of the groups. A peritoneal mesothelioma, one pulmonary carcinoma, and two early squamous cell carcinomas of the nonglandular stomach were found in the hamsters exposed to amosite but the incidence was not statistically significant ($p < 0.05$). They concluded that the study was essentially negative. A subsequent study in rats using similar materials also failed to elicit a carcinogenic response (*30*).

Except for the studies of Donham et al. (*23*), Smith et al. (*29*) and the NTP (*11, 12, 27, 28*), the other studies were conducted with relatively small numbers of animals. Also some were conducted for an insufficient period of time to adequately test the carcinogenic potential of ingested asbestos.

A long-term study of amosite asbestos designed to determine the promotor potential of asbestos

was reported by Ward et al. (*31*). They exposed 6-week-old male F344 rats three times per week for 10 weeks to 1 mg amosite asbestos in saline via gavage. Once per week during this same period, half of the rats received subcutaneous injections of 7.4 mg/kg azoxymethane (AOM), a known intestinal carcinogen in animals which produces effects similar to DMH. The rats were allowed to live out their lifespan or untl 94–95 weeks of age at which time they were killed. The authors reported an intestinal tumor incidence of 66.7% in AOM alone, 77.1% for amosite plus AOM and 32.6% for amosite alone. The authors concluded that while amosite did not significantly add to the incidence of AOM-induced intestinal neoplasia, amosite alone caused a relatively high rate of intestinal neoplasia. However, there was no untreated control group to compare to the treated groups. These results should also be viewed with some suspicion because the authors also reported a 14% incidence of Zymbal gland tumors in the rats exposed to amosite alone. The historical rate of Zymbal gland tumors in the Bioassay Program is 0.34%, indicating that this is a relatively rare tumor (*19*). However, AOM is known to induce Zymbal gland tumors with a sngle dose of 5.1 mg/kg in male F344 rats producing a 14% incidence of tumors in this organ (*17*); in this study 5.1 mg/kg AOM also caused a 24% incidence of intestinal neoplasia. A possible explanation for the incidence of Zymbal gland tumors in the amosite groups would be that they were inadvertently exposed to AOM. If this occurred, these rats would also be expected to show a high incidence of intestinal neoplasms.

Conclusions

Under the conditions of this lifetime bioassay, tremolite or amosite asbestos was not toxic, did not affect survival, and was not carcinogenic when ingested at a level of 1% in the diet by male and female Fischer 344 rats. While there were significant ($p > 0.05$) increases in the rate of C-cell carcinomas of the thyroid in male, and monocytic (mononuclear cell) leukemia in male rats exposed to amosite asbestos compared to untreated controls, their biological significance is questionable because of a lack of response in the concurrent amosite and preweaning gavage group or control group of the corollary study, nonaffect when all neoplasms of that organ are analyzed, lack of significance when examined using life table analysis or the absence of neoplasia in target organs. The cocarcinogenic studies using 1,2-dimethylhydrazine dihydrochloride (DMH) were considered flawed because of the high rate of intestinal carcinogenesis in both the DMH and amosite asbestos and DMH alone groups.

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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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