Chronic Effects of Dietary Exposure to Amosite and Chrysotile Asbestos in Syrian Golden Hamsters

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Bioassays of amosite, short-range (SR), intermediate-range (IR) or intermediate-range chrysotile asbestos in combination with the intestinal carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) were conducted with male and female Syrian golden hamsters. Amosite and both forms of chrysotile asbestos were administered at a concentration of 1% in pelleted diet for the entire lifetime of the hamsters starting with mothers of the test animals. Group sizes varied from 125-254. There was no adverse effect on body weight gain or survival by either type of asbestos or by IR chrysotile asbestos in combination with DMH.

A significant increase ($p < 0.05$) in adrenal cortical tumors was observed in male hamsters exposed to SR and IR chrysotile asbestos and in females treated with IR chrysotile asbestos when compared to the pooled control groups. However, statistical significance ($p < 0.05$) was lost when these dosed groups were compared with temporal control groups. Neither of the male or female amosite asbestos groups showed increased neoplasia in any tissue or organ compared to the control groups. The cocarcinogen studies using IR chrysotile asbestos and 1,2-dimethylhydrazine dihydrochloride were considered inadequate because there was no increase in intestinal neoplasia in the DMH group.

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 (J). 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-term animal toxicology studies to evaluate the ingestion of several minerals for carcinogenic effect. As a result the National Toxicology Program investigated the carcinogenic potential of the ingestion of various forms of asbestos in rats and hamsters. All of the studies were to encompass the lifetime of the animal, including exposure of the dams from which the test animals were derived.

This report represents the results of studies undertaken to determine the effects of the ingestion of amosite and chrysotile (short- and intermediate-range) asbestos in Syrian golden hamsters. The effects of intermediate-range chrysotile asbestos in conjunction with a known intestinal carcinogen, 1,2-dimethylhydrazine (DMH), were also studied.
Materials and Methods

Asbestos is a general term applied to certain natural mineral silicates when they appear in a fibrous form. Chrysotile is the fibrous member of the serpentine mineral group while amosite is an amphibole mineral. Two chrysotile test materials were selected for testing and are referred to as short-range (SR) and intermediate-range (IR) chrysotile. Intermediate-range chrysotile differs from short-range chrysotile in that the former contains fibers extending into relatively large sizes, both with respect to length and diameter.

The short-range chrysotile was purchased from the Union Carbide Corporation, Niagara Falls, New York, which referred to the material as COF-25. The chrysotile was mined from the New Idria serpentine mass located in the southwestern San Benito and western Fresno counties of California. Mineral and fiber characteristics of SR chrysotile are shown in Tables 1 and 2.

The intermediate-range chrysotile was purchased from the Johns Manville Company, which referred to the material as Plastobest-20. The chrysotile was obtained from the Jeffrey Mine, Asbestos, Quebec, Canada. Mineral and fiber characteristics of IR chrysotile are shown in Tables 1 and 2.

An amosite sample (S-33) from a mine in Penge, Transvaal, Republic of South Africa was purchased by the Bureau of Mines from the Atlas Asbestos Company, Montreal, Quebec, Canada. The sample was processed by a single pass through an air jet mill to improve the homogeneity of the amosite. Mineral and fiber characteristics of amosite are shown in Tables 3-5.

Table 1. Fiber characteristics of chrysotile asbestos.

| Fiber characteristics | Short-range | Intermediate-range |
|-----------------------|-------------|--------------------|
| Surface area, m²/g    | 59.0        | 27.9               |
| Density, g/cm³        | 2.577 ± 0.022 SD | 2.607 ± 0.016 SD   |
| Measurement, transmission electron microscopy | | |
| Fiber count/g         | 0.6081 × 10¹³ | 0.1291 × 10¹²      |
| Median length, µm     | 0.66        | 0.82               |
| Range of length, µm   | 0.088–51.1  | 0.104–783          |
| Median diameter, µm   | 0.059       | 0.089              |
| Range of diameter, µm | 0.019–1.57  | 0.019–11.5         |
| Median fiber aspect ratio (L/D) | 11.1698 | 8.435             |
| Frequency distribution by length (µm), optical microscopy | | |
| 10 percentile         | 1.3         | 1.4                |
| 20 percentile         | 1.7         | 1.9                |
| 30 percentile         | 2.2         | 3.0                |
| 40 percentile         | 2.6         | 5.4                |
| 50 percentile         | 3.1         | 14.0               |
| 60 percentile         | 3.8         | 29.0               |
| 70 percentile         | 4.5         | 48.0               |
| 80 percentile         | 5.8         | 76.0               |
| 90 percentile         | 7.8         | 130.0              |

Table 2. Chemical-instrumental analyses of chrysotile asbestos.

| Content, wt-% | Short-range | Intermediate-range |
|---------------|-------------|--------------------|
| Al₂O₃         | 0.66        | 1.47               |
| CaO           | 0.32        | 0.05               |
| Fe₂O₃         | 2.02        | 2.93               |
| MgO           | 40.62       | 40.62              |
| K₂O           | Not detected | 0.08              |
| SiO₂          | 39.77       | 39.90              |
| Na₂O          | 0.04        | 0.04               |
| TiO₂          | 0.03        | 0.04               |
| MnO           | 0.07        | 0.06               |
| Cr₂O₃         | 0.17        | 0.06               |
| NiO           | 0.17        | 0.06               |
| Co₂O₃         | 0.01        | Not detected       |
| CO₂          | 0.78        | 0.51               |
| H₂O          | 1.54        | 1.17               |
| H₂O⁺        | 12.69       | 12.81              |
| Benzene-extracted organics | 0.026 | 0.011             |

The homogeneity of the samples and the physical and chemical properties of the materials were extensively 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 Institute, Chicago, Illinois (Special Report and Addendum on project L6085, contract No1-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. The appropriate asbestos was incorporated to a level of 1% by weight into the test diet. All feed was pelleted with a Sprout-Waldron pelletor; the pellets were of oval configuration, % in. by % in. in size. Pelleted feed was packaged in 25-lb aliquots in standard paper feed-bags which were color coded to minimize the occurrence of feeding errors at the test laboratory. Each lot of blended feed was analyzed for asbestos concentration.

**Sources and Specifications of Test Animals**

Four groups (three chrysotile and one amosite) of disease-free, mated female outbred Syrian golden hamsters were obtained over a period of 20 weeks in 1977 from Charles River Lakeview Laboratories, New Field, NJ. The hamsters had been mated 6 days prior to shipping.

**Animal Maintenance**

Upon arrival, the mated female hamsters were weighed and sorted into weight ranges. They were then distributed randomly between control and treatment groups, which were housed in separate rooms. The first shipment of mated females was assigned to the short-range (SR) chrysotile study, the second to the intermediate-range (IR) chrysotile study, the third shipment to the IR chrysotile plus DMH study, the fourth group to the amosite study and their respective control groups. Each dam was placed in an individual cage with filter top in its respective room. Control or formulated diets were provided *ad libitum* on the floor of each cage. Water was provided *ad libitum* via bottles. The hamsters were not handled just before the litters were due to be born except when the cages were changed. Once the litters were born, they were left undisturbed until they were approximately 10 days of age. Then, the cages were changed weekly until the offspring

### Table 3. Fiber characteristics of amosite asbestos.

| Fiber characteristics |  |
|-----------------------|--|
| Surface area, m²/g     | 4.13 |
| Density, g/cm³         | 3.35 ± 0.026 SD |
| Measurements, transmission electron microscopy | |
| Fiber count/g          | 0.3466 x 10⁶ |
| 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 4. Chemical-instrumental asbestos of amosite asbestos.

| Al₂O₃   | 0.42 |
|---------|------|
| CaO     | 0.48 |
| Fe₂O₃   | 34.61 |
| FeO     | 2.24 |
| MgO     | 6.22 |
| K₂O     | 0.30 |
| SiO₂    | 50.36 |
| Na₂O    | 0.03 |
| MnO     | 2.66 |
| Cr₂O₃   | 0.03 |
| NiO     | 0.01 |
| CO₂     | 0.88 |
| H₂O     | 0.15 |

### Table 5. 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    | 83     | 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.-% | -      | 0.1    | 0.3    | 0.4    | 0.4    | 2.4      | 5.0      |
| Cumulative vol-% amosite | -     | 0.1    | 0.4    | 0.8    | 1.2    | 3.6      | 8.6      |
| Number of other particles | 11     | 8      | 8      | 1      | 0      | 1        | 1        |
| 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 |

*Calculated from particle number data, assuming rectangular cross section with third dimension equal to ½ measured width.
were 4 weeks of age, at which time they were weaned.

At weaning, the offspring were individually weighed and separated by sex. The test groups were randomly placed into groups of three males and three females and housed in polycarbonate cages for the remainder of the lifetime study. The dams were killed at this time. Twenty male and twenty female offspring were removed from the study for endo- and ectoparasite examination to confirm that the test groups were of a desired health status. Extra hamsters were not discarded at this time, in case animals had been missexed. Approximately 6 weeks after weaning, all missexed hamsters were killed along with their cage mates and were replaced with these alternates which had received maintenance identical to that received by the original hamsters. The remaining hamsters were killed. The experimental design insured that ingestion of asbestos spanned the entire phase of solid food consumption during the lifetime of the animal. Food consumption was not determined because of the hamster's habit of sequestering its feed in the bedding. Control hamsters were housed in separate rooms. The number of animals in the study is shown in Table 6.

Starting at 6 weeks of age, male and female hamsters in the intermediate-range chrysotile/1,2-dimethylhydrazine dihydrochloride (DMH) study (Table 1) were given oral doses of DMH (4 mg/kg) every other week for a total of 5 doses. The dose of DMH used in this study was based on the results of a pilot study carried out previously in the same facility. The latter was conducted in a manner similar to that reported in rats (2). The DMH (Aldrich Chemical Co., Milwaukee, WI) was used as received and was dissolved in 0.9% saline to a concentration of 1.5% (15 mg/mL). This stock solution was then diluted with saline to give the appropriate concentration for dosing. The solutions were made up within one hour prior to the dosing of the hamsters. All dosing was completed in less than 3 hr. The DMH was analyzed after each dosing.

During the test period, room temperature was maintained at 22 ± 2°C and the relative humidity ranged from 40% to 80%. To minimize contamination of room air with asbestos, each cage was totally enclosed. Incoming air was filtered to the cages through glass fiber filters while exit air was filtered through a fiberglass roughing filter followed by a bag housing filter. The cage atmosphere was negative relative to the room and the room was maintained at a slightly negative atmosphere in relation to corridor air. Air flow within the animal rooms was maintained with a minimum of 20 air changes/hr. Fluorescent lighting was provided 12 hr/day.

Clinical Examinations and Pathology

All hamsters were observed daily for signs of toxicity. Body weights of individual animals were recorded weekly for the duration of the study. All animals were allowed to die or were killed with pentobarbital sodium when moribund. A complete postmortem examination was performed on all animals not severely cannibalized or autolyzed. Thus, the number of animals from which particular organs or tissues were examined mi-

| Group                  | Sex | On test | Histopathologic evaluation | Missing | Cannibaled | Autolyzed | Missexed |
|------------------------|-----|---------|----------------------------|---------|------------|-----------|----------|
| SR chrysotile          | M   | 126     | 115                        | 0       | 3          | 6         | 2        |
| control                | F   | 126     | 114                        | 0       | 1          | 6         | 5        |
| IR chrysotile          | M   | 126     | 116                        | 0       | 0          | 8         | 2        |
| control                | F   | 126     | 119                        | 0       | 0          | 4         | 3        |
| DMH and IR chrysotile  | M   | 125     | 119                        | 0       | 0          | 3         | 3        |
| control                | F   | 128     | 120                        | 1       | 0          | 2         | 5        |
| Asbestos               | M   | 127     | 122                        | 0       | 0          | 4         | 1        |
| control                | F   | 126     | 119                        | 1       | 0          | 1         | 5        |
| SR chrysotile          | M   | 253     | 233                        | 0       | 1          | 10        | 9        |
| F                     | 252  | 228     | 1                          | 0       | 17         | 6         |
| IR chrysotile          | M   | 251     | 245                        | 0       | 0          | 3         | 3        |
| F                     | 252  | 244     | 1                          | 0       | 3          | 4         |          |
| DMH                   | M   | 127     | 127                        | 0       | 0          | 0         | 0        |
| F                     | 126  | 122     | 0                          | 0       | 0          | 0         | 0        |
| DMH and IR chrysotile  | M   | 176     | 173                        | 0       | 0          | 2         | 1        |
| F                     | 174  | 161     | 3                          | 0       | 6          | 4         |          |
| Asbestos               | M   | 252     | 248                        | 0       | 0          | 3         | 1        |
| F                     | 254  | 237     | 5                          | 0       | 5          | 7         |          |
croscopically varies and does not necessarily represent the number of animals that were placed on study in each group (Table 6).

Since the gastrointestinal tract was chosen as one of the target organs prior to the study, it was handled in a manner slightly different from the usual in standard rodent lifetime bioassays. Prior to placement in fixative, the entire esophagus was opened and pinned with the exterior surface adjacent to cardboard. The stomach and cecum were prepared similarly. Lengths of duodenum and ileum (2 cm) and two portions of jejunum were placed unopened in fixative. The remaining small intestine was opened and gently washed with saline and it was then carefully examined. Suspect lesions were processed separately and identified individually as to location. Likewise, the entire colon with anus was opened, examined, and pinned to cardboard 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 posterior end, with the mucosal surface inward.

All tissues were fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin. Tissues/organisms examined microscopically were: tissue masses, the above mentioned portions of gastrointestinal tract, mesenteric 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, gallbladder, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate/testes, ovaries/uterus, brain, pituitary gland, eyes and spinal cord. Selected sections were stained with Bennhold’s congo red to demonstrate amyloid.

**Data Recording and Statistical Methods**

The individual animal pathology data on this experiment were recorded in the 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 (3). Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Differences in survival were evaluated by Cox’s life table method (4).

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (enumerator) 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., lymphomas), the denominators consist of the numbers of animals necropsied.

For the statistical analysis of tumor incidence data, two different methods of adjusting for intercurrent mortality were employed. Each used the classical methods for combining contingency tables developed by Mantel and Haenszel (5).

The first method of analysis assumed that all tumors of a given type were fatal, i.e., they either directly or indirectly caused the death of the animal. 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 tumor of interest. 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 (4).

The second method of analysis assumed that all tumors of a given type were “incidental,” i.e., they were merely observed at autopsy in animals dying of an unrelated cause. According to this approach, the proportions of animals found to have tumors in treated and control groups were compared in each of five time intervals. For male hamsters these time intervals were 0–52 weeks, 53–78 weeks, 79–92 weeks, 93–103 weeks and beyond 103 weeks. For female hamsters whose median survival was considerably less than that of the males, the time intervals were 0–44 weeks, 45–52 weeks, 53–60 weeks, 61–68 weeks and beyond 68 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 (6).

In addition to these tests, one other set of statistical analyses were carried for each primary tumor: the Fisher exact test based on the overall
proportion of tumor-bearing animals (7). 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 amosite or chrysotile asbestos during the entire life of the animal, starting from the time it was able to eat solid food. For this reason, the mated female hamsters had been on the test diets for approximately 2 weeks when the first litters were born. Of the females, 10–15% were not pregnant, aborted, or their litters died immediately after birth. Several more dams died after showing a prolapsed rectum in the week following birth. The incidences of infertility and neonatal deaths were unrelated to the test diet. To minimize the chance that the mothers would reject or cannibalize their young, the litters were not handled during lactation. Many of the pups which died during the nursing period were cannibalized by their mothers. In those pups in which a postmortem examination was possible, the stomachs were typically without food (milk), suggesting maternal rejection or inability to compete with litter mates. None of these observations were compound-related.

Approximately 2% of the offspring in all groups died between weaning and 14 weeks of age due to cage fighting or an enteritis of undetermined origin. Histologically, the disease was compatible with the acute form of proliferative ileitis (“wet tail”), a common disease of hamsters. Combinations of cage fighting and enteritis were also observed. These deaths were not compound-related, although cage fighting was more severe in the SR chrysotile and its temporal control group than in the other two portions of the study. Replacement hamsters were incorporated into the groups (in additional cages) to maintain group sizes until the animals were 12 weeks of age; from this time on, no additional hamsters were added to the experimental groups. The extra hamsters were killed.

**Body Weights and Clinical Signs**

Body weight gain was not adversely affected in any dose group, including the group given 1,2-dimethylhydrazine dihydrochloride (DMH). In fact, both types of asbestos appeared to increase body weight in most of the dosed groups.

No compound-related clinical signs were observed during the entire study. Occasional skin lesions and bite wounds were observed in both sexes, but were more apparent in males; these became less of a problem after the hamsters were 20 weeks of age.

**Survival**

Survival was not adversely affected by any of the test diets with the possible exception of DMH-treated female hamsters. Survival rates were actually higher in the amosite and SR and IR chrysotile groups relative to the temporal controls. The median life-span of females (control and treated) was shorter than that of corresponding groups of males (Table 7). The median survival of control

| Group                  | Sex | Median life span, weeks |
|------------------------|-----|-------------------------|
| IR chrysotile control  | M   | 83                      |
|                        | F   | 61                      |
| IR chrysotile          | M   | 86                      |
|                        | F   | 59                      |
| SR chrysotile control  | M   | 77                      |
|                        | F   | 57                      |
| SR chrysotile          | M   | 87                      |
|                        | F   | 63a                     |
| DMH and IR chrysotile  | M   | 82                      |
|                        | F   | 57                      |
| DMH                    | M   | 82                      |
|                        | F   | 54b                     |
| IR chrysotile and DMH  | M   | 90                      |
|                        | F   | 63a                     |
| Amosite control        | M   | 81                      |
|                        | F   | 55                      |
| Amosite                | M   | 84                      |
|                        | F   | 60                      |

*aSignificantly ($p < 0.05$) improved overall survival relative to controls (life table analysis).

*bSignificantly ($p < 0.05$) reduced overall survival relative to controls (life table analysis).
female groups was 55–61 weeks, compared to 77–83 weeks for control male hamsters (Table 7).

Pathology

The number of hamsters available for histopathologic examination is shown in Table 6. Most animals not examined pathologically were excluded because of autolysis or cannibalization. Review of the clinical records of hamsters lost to autolysis or cannibalization gave no indication that these animals had neoplasia.

A variety of neoplasms was observed in control (Tables 8 and 9) and asbestos-exposed hamsters (Tables 10–17). The proportion of control male or female hamsters bearing primary tumors was not statistically different among the four control groups. Thus, statistical comparisons were made with pooled controls as well as with temporal controls. Overall, the male hamsters had a slightly higher rate of neoplasia than the females.

Those organs with greater than 4% incidence of neoplasia in dosed or control groups were the adrenal gland, pancreas (islets of Langerhans), parathyroid, and reticuloendothelial system.

The only organ showing an increased rate of neoplasia in chrysotile-exposed hamsters compared to the controls was the adrenal cortex. In male hamsters, the incidence of cortical adenomas was significantly increased ($p < 0.01$) in the SR and IR chrysotile groups compared with the pooled controls but not in the DMH chrysotile or amosite groups. None of the chrysotile groups showed a significant ($p < 0.05$) increase in cortical adenomas relative to their temporal control groups. A similar increase in cortical adenomas was observed in the female IR chrysotile group compared with pooled controls, but this also ceased to be significant when compared with the temporal control group.

In only three other instances did specific tumor types show significant effects relative to pooled or temporal controls. The only statistically significant ($p < 0.05$) difference in tumor incidence observed in the amosite study was a decrease in islet-cell adenomas observed in female hamsters. Female hamsters administered SR chrysotile showed a significantly ($p < 0.05$) decreased incidence of islet-cell adenoma relative to pooled controls (Table 16). Male hamsters administered DMH showed a significantly ($p < 0.05$) increased incidence of leukemia or malignant lymphoma relative to pooled controls (Table 16).

The only group to show a significant ($p < 0.05$) increase in overall primary tumors was the male IR chrysotile group. This increase was due primarily to adrenal tumors. Male hamsters receiving SR chrysotile or DMH and IR chrysotile also

| Table 8. Incidence of primary tumors in male hamster control groups. |
|---------------------------------------------------------------|
| **Animals with primary tumors** | Short-range chrysotile control | Intermediate-range chrysotile control | DMH and intermediate-range chrysotile control | Amosite control |
|---------------------------------|-------------------------------|-------------------------------------|-----------------------------------------------|-----------------|
| Skin or subcutaneous tissue, all tumors | 21/115 (18%) | 26/116 (22%) | 27/119 (23%) | 21/122 (17%) |
| Lung or trachea, all tumors | 0/115 (0%) | 1/161 (1%) | 1/119 (1%) | 0/122 (0%) |
| Adrenal | | | | |
| Cortical adenoma | 7/115 (6%) | 7/115 (6%) | 3/117 (3%) | 8/119 (7%) |
| Cortical carcinoma | 3/115 (3%) | 3/115 (3%) | 4/117 (3%) | 3/119 (3%) |
| Pheochromocytoma | 2/115 (2%) | 5/115 (4%) | 3/117 (3%) | 3/119 (3%) |
| Other tumors | 0/115 (0%) | 3/115 (3%) | 2/117 (2%) | 1/119 (1%) |
| Pancreas | | | | |
| Islet-cell adenoma | 2/111 (2%) | 7/110 (6%) | 8/110 (7%) | 3/114 (3%) |
| Islet-cell carcinoma | 1/111 (1%) | 0/110 (0%) | 0/110 (0%) | 0/114 (0%) |
| Thyroid | | | | |
| C-cell adenoma | 3/109 (3%) | 3/106 (3%) | 0/107 (0%) | 1/106 (1%) |
| C-cell carcinoma | 1/109 (1%) | 1/106 (1%) | 0/107 (0%) | 1/106 (1%) |
| Other tumors | 0/109 (0%) | 0/106 (0%) | 1/107 (1%) | 0/106 (0%) |
| Parathyroid, adenoma | 0/72 (0%) | 1/71 (1%) | 1/64 (2%) | 0/64 (0%) |
| G.I. tract, all tumors | 2/115 (2%) | 1/116 (1%) | 2/119 (2%) | 1/112 (1%) |
| Pituitary, all tumors | 0/84 (0%) | 0/77 (0%) | 0/80 (0%) | 0/81 (0%) |
| Kidney, all tumors | 0/115 (0%) | 2/116 (2%) | 1/119 (1%) | 1/120 (1%) |
| Liver, all tumors | 0/115 (0%) | 0/116 (0%) | 0/119 (0%) | 0/120 (0%) |
| Leukemia or malignant lymphoma | 2/115 (2%) | 1/116 (1%) | 4/119 (3%) | 1/122 (1%) |
| Hemangioma or hemangiosarcoma | 0/115 (0%) | 0/116 (0%) | 3/119 (3%) | 2/122 (2%) |
| All other tumors | 1/115 (1%) | 0/116 (0%) | 3/119 (3%) | 1/122 (1%) |
showed an elevated incidence of primary tumors relative to pooled controls. However, when survival differences were taken into account by a life table analysis, these differences were not statistically significant. Female chrysotile groups showed little evidence of an increased incidence of primary tumors relative to temporal or pooled controls.

Table 9. Incidence of primary tumors in female hamster control groups.

| Tumor Type                          | Short-range chryso tile control | Intermediate-range chryso tile control | DMH and intermediate-range chryso tile control | Amosite control |
|-------------------------------------|---------------------------------|----------------------------------------|-----------------------------------------------|-----------------|
| Animals with primary tumors         | 19/114 (17%)                    | 17/119 (14%)                           | 15/120 (12%)                                  | 15/119 (13%)    |
| Skin or subcutaneous tissue, all tumors | 0/114 (0%)                     | 1/119 (0%)                             | 1/120 (0%)                                    | 0/119 (0%)      |
| Lung or trachea, all tumors         | 0/114 (0%)                      | 0/119 (0%)                             | 0/119 (0%)                                    | 0/119 (0%)      |
| Adrenal                             |                                 |                                        |                                               |                 |
| Cortical adenoma                    | 4/112 (4%)                      | 6/118 (5%)                             | 3/120 (2%)                                    | 2/118 (2%)      |
| Cortical carcinoma                  | 0/112 (0%)                      | 0/118 (0%)                             | 0/120 (0%)                                    | 0/118 (0%)      |
| Pheochromocytoma                    | 0/112 (0%)                      | 0/118 (0%)                             | 0/120 (0%)                                    | 0/118 (0%)      |
| Other tumors                        | 0/112 (0%)                      | 0/118 (0%)                             | 0/120 (0%)                                    | 0/118 (0%)      |
| Pancreas                            |                                 |                                        |                                               |                 |
| Islet-cell adenoma                  | 2/109 (2%)                      | 5/116 (4%)                             | 5/116 (4%)                                    | 3/115 (3%)      |
| Islet-cell carcinoma                | 1/109 (1%)                      | 0/116 (0%)                             | 0/116 (0%)                                    | 0/115 (0%)      |
| Thyroid                             |                                 |                                        |                                               |                 |
| C-cell adenoma                      | 2/106 (2%)                      | 3/115 (3%)                             | 0/112 (0%)                                    | 1/106 (1%)      |
| C-cell carcinoma                    | 1/107 (0%)                      | 1/115 (0%)                             | 0/112 (0%)                                    | 1/106 (0%)      |
| Other tumors                        | 2/107 (2%)                      | 0/115 (0%)                             | 1/112 (0%)                                    | 0/106 (0%)      |
| Parathyroid, adenoma                | 3/68 (4%)                       | 1/77 (1%)                              | 1/74 (1%)                                     | 1/61 (1%)       |
| G.I. tract, all tumors              | 1/114 (1%)                      | 2/119 (2%)                             | 1/120 (1%)                                    | 1/119 (1%)      |
| Pituitary, all tumors               | 0/77 (0%)                       | 2/67 (3%)                              | 0/62 (0%)                                     | 0/79 (0%)       |
| Kidney, all tumors                  | 0/114 (0%)                      | 1/119 (1%)                             | 0/120 (0%)                                    | 0/119 (0%)      |
| Liver, all tumors                   | 0/114 (0%)                      | 0/119 (0%)                             | 0/119 (0%)                                    | 0/118 (0%)      |
| Leukemia or malignant lymphoma      | 2/114 (2%)                      | 1/119 (0%)                             | 3/120 (2%)                                    | 2/119 (2%)      |
| Hemangioma or hemangiosarcoma       | 0/114 (0%)                      | 0/119 (0%)                             | 1/120 (1%)                                    | 1/119 (1%)      |
| Uterus, all tumors                  | 3/113 (3%)                      | 1/119 (1%)                             | 2/120 (2%)                                    | 2/119 (2%)      |
| All other tumors                    | 3/114 (3%)                      | 0/119 (0%)                             | 1/120 (1%)                                    | 2/119 (2%)      |

Table 10. Incidence of primary tumors in male hamsters administered amosite asbestos.

| Tumor Type                          | Pooled controls (20%) | Amosite controls (17%) | Amosite-treated controls (3%) |
|-------------------------------------|-----------------------|------------------------|-------------------------------|
| Animals with primary tumors         | 95/472 (20%)          | 21/122 (17%)           | 57/248 (23%)                  |
| Skin or subcutaneous tissues, all tumors | 2/472 (<1%)          | 0/122 (0%)             | 0/248 (0%)                    |
| Lung and trachea, all tumors        | 0/470 (0%)            | 0/120 (0%)             | 0/248 (0%)                    |
| Adrenal                             |                       |                        |                               |
| Cortical adenoma                    | 25/466 (5%)           | 8/119 (7%)             | 13/246 (5%)                   |
| Cortical carcinoma                  | 13/466 (3%)           | 3/119 (3%)             | 7/246 (3%)                    |
| Pheochromocytoma                    | 13/466 (3%)           | 3/119 (3%)             | 4/246 (2%)                    |
| Other tumors                        | 6/466 (1%)            | 1/119 (1%)             | 2/246 (1%)                    |
| Pancreas                            |                       |                        |                               |
| Islet-cell adenoma                  | 20/455 (4%)           | 3/114 (3%)             | 11/234 (5%)                   |
| Islet-cell carcinoma                | 1/445 (<1%)           | 0/114 (0%)             | 0/234 (0%)                    |
| Thyroid                             |                       |                        |                               |
| C-cell adenoma                      | 7/428 (2%)            | 1/106 (1%)             | 7/221 (3%)                    |
| C-cell carcinoma                    | 1/428 (<1%)           | 0/106 (0%)             | 2/221 (1%)                    |
| Other tumors                        | 1/428 (<1%)           | 0/106 (0%)             | 2/221 (1%)                    |
| Parathyroid, adenoma                | 2/271 (1%)            | 0/64 (0%)              | 2/150 (1%)                    |
| G.I. tract, all tumors              | 6/472 (1%)            | 1/122 (1%)             | 6/248 (2%)                    |
| Pituitary, all tumors               | 0/322 (0%)            | 0/81 (0%)              | 0/182 (0%)                    |
| Kidney, all tumors                  | 4/470 (1%)            | 1/120 (1%)             | 2/248 (1%)                    |
| Liver, all tumors                   | 0/470 (0%)            | 0/120 (0%)             | 0/248 (0%)                    |
| Leukemia or malignant lymphoma      | 8/472 (2%)            | 1/122 (1%)             | 5/248 (2%)                    |
| Hemangioma or hemangiosarcoma       | 5/472 (1%)            | 2/122 (2%)             | 2/248 (1%)                    |
| All other tumors                    | 5/472 (1%)            | 1/122 (1%)             | 2/248 (1%)                    |
Table 11. Incidence of primary tumors in female hamsters administered amosite asbestos.

|                              | Pooled controls | Amosite controls | Amosite-treated |
|------------------------------|-----------------|------------------|-----------------|
| Animals with primary tumors  | 66/472 (14%)    | 15/119 (13%)     | 30/237 (13%)    |
| Skin or subcutaneous tissues, all tumors | 0/472 (0%) | 0/119 (0%)       | 2/237 (1%)      |
| Lung and trachea, all tumors | 0/471 (0%)     | 0/119 (0%)       | 0/234 (0%)      |
| Adrenal                      |                 |                  |                 |
| Cortical adenoma             | 15/468 (3%)     | 2/118 (2%)       | 6/234 (3%)      |
| Cortical carcinoma           | 0/468 (0%)      | 0/118 (0%)       | 0/234 (0%)      |
| Pheochromocytoma             | 0/468 (0%)      | 0/118 (0%)       | 2/234 (1%)      |
| Other tumors                 | 0/468 (0%)      | 0/118 (0%)       | 0/234 (0%)      |
| Pancreas                     |                 |                  |                 |
| Islet-cell adenoma           | 15/456 (3%)     | 3/115 (3%)       | 2/222 (1%)*     |
| Islet-cell carcinoma         | 0/222 (0%)      | 0/115 (0%)       | 1/456 (1%)      |
| Thyroid                      |                 |                  |                 |
| C-cell adenoma               | 6/440 (1%)      | 1/106 (1%)       | 4/215 (2%)      |
| C-cell carcinoma             | 1/440 (<1%)     | 0/106 (0%)       | 1/215 (<1%)     |
| Other tumors                 | 2/440 (1%)      | 0/106 (0%)       | 0/215 (0%)      |
| Parathyroid, adenoma         | 6/280 (2%)      | 1/61 (1%)        | 1/141 (1%)      |
| G.I. tract, all tumors       | 5/472 (1%)      | 1/119 (1%)       | 4/237 (2%)      |
| Pituitary, all tumors        | 2/285 (1%)      | 0/79 (0%)        | 0/149 (0%)      |
| Kidney, all tumors           | 1/472 (<1%)     | 1/119 (0%)       | 0/236 (0%)      |
| Liver, all tumors            | 0/472 (0%)      | 0/118 (0%)       | 0/234 (0%)      |
| Leukemia or malignant lymphoma | 7/472 (1%)   | 2/119 (2%)       | 3/2236 (1%)     |
| Hemangioma or hemangiosarcoma | 2/472 (<1%) | 1/119 (1%)       | 3/237 (1%)      |
| Uterus, all tumors           | 8/471 (2%)      | 2/119 (1%)       | 1/236 (<1%)     |
| All other tumors             | 6/472 (1%)      | 1/119 (1%)       | 2/237 (1%)      |

*p < 0.05 decrease relative to pooled controls (life table and incidental tests).

Table 12. Incidence of primary tumors in male hamsters administered short-range chrysotile asbestos.

|                              | Pooled controls | Short-range chrysotile controls | Short-range chrysotile |
|------------------------------|-----------------|--------------------------------|------------------------|
| Animals with primary tumors  | 95/472 (20%)    | 21/115 (18%)                   | 64/233 (27%)*         |
| Skin or subcutaneous tissue, all tumors | 2/472 (<1%) | 0/115 (0%)                     | 0/233 (0%)            |
| Lung or trachea, all tumors  | 0/470 (0%)      | 0/115 (0%)                     | 0/231 (0%)            |
| Adrenal                      |                 |                                |                        |
| Cortical adenoma             | 25/466 (5%)     | 7/115 (6%)                     | 26/229 (11%)*         |
| Cortical carcinoma           | 13/466 (3%)     | 3/115 (3%)                     | 8/229 (3%)            |
| Pheochromocytoma             | 13/466 (3%)     | 2/115 (2%)                     | 4/229 (2%)            |
| Other tumors                 | 6/466 (1%)      | 0/115 (0%)                     | 1/229 (<1%)           |
| Pancreas                     |                 |                                |                        |
| Islet-cell adenoma           | 20/445 (4%)     | 2/111 (2%)                     | 15/218 (7%)           |
| Islet-cell carcinoma         | 1/445 (<1%)     | 1/111 (1%)                     | 0/218 (0%)            |
| Thyroid                      |                 |                                |                        |
| C-cell adenoma               | 7/428 (2%)      | 3/109 (3%)                     | 3/207 (1%)            |
| C-cell carcinoma             | 3/428 (1%)      | 1/109 (1%)                     | 1/207 (<1%)           |
| Other tumors                 | 1/428 (<1%)     | 0/109 (0%)                     | 0/207 (0%)            |
| Parathyroid, adenoma         | 2/271 (1%)      | 0/72 (0%)                      | 3/132 (2%)            |
| G.I. tract, all tumors       | 6/472 (1%)      | 2/115 (2%)                     | 0/233 (0%)            |
| Pituitary, all tumors        | 0/322 (0%)      | 0/84 (0%)                      | 0/159 (0%)            |
| Kidney, all tumors           | 4/470 (1%)      | 0/115 (0%)                     | 3/232 (1%)            |
| Liver, all tumors            | 0/470 (0%)      | 0/115 (0%)                     | 0/232 (0%)            |
| Leukemia or malignant lymphoma | 8/472 (2%) | 2/115 (2%)                     | 3/233 (1%)            |
| Hemangioma or hemangiosarcoma | 5/472 (1%) | 0/115 (0%)                     | 4/233 (2%)            |
| All other tumors             | 6/472 (1%)      | 1/115 (1%)                     | 3/233 (1%)            |

*<p < 0.05 vs. pooled controls.

*<p = 0.152 (life table); p = 0.065 (incidental tumor test) and p = 0.019 (Fisher’s exact test) vs. pooled controls.
Table 13. Incidence of primary tumors in female hamsters administered short-range chrysotile asbestos.

|                                | Pooled controls | Short-range chrysotile controls | Short-range chrysotile controls |
|--------------------------------|-----------------|---------------------------------|---------------------------------|
| Animals with primary tumors    | 66/472 (14%)    | 19/114 (17%)                   | 28/228 (12%)                   |
| Skin or subcutaneous tissue, all tumors | 0/472 (0%)      | 0/114 (0%)                     | 3/228 (1%)                     |
| Lung or trachea, all tumors    | 0/471 (0%)      | 0/114 (0%)                     | 0/228 (0%)                     |
| Adrenal                        |                 |                                 |                                 |
| Cortical adenoma               | 15/468 (3%)     | 4/112 (4%)                     | 8/226 (4%)                     |
| Cortical carcinoma             | 0/468 (0%)      | 0/112 (0%)                     | 0/226 (0%)                     |
| Pheochromocytoma               | 0/468 (0%)      | 0/112 (0%)                     | 3/226 (1%)                     |
| Other tumors                   | 0/468 (0%)      | 0/112 (0%)                     | 1/226 (<1%)                    |
| Pancreas                       |                 |                                 |                                 |
| Inlet-cell adenoma             | 15/456 (3%)     | 2/109 (2%)                     | 2/217 (1%)<sup>a</sup>         |
| Inlet-cell carcinoma           | 1/456 (<1%)     | 1/109 (1%)                     | 0/217 (0%)                     |
| Thyroid                        |                 |                                 |                                 |
| C-cell adenoma                 | 6/440 (1%)      | 2/107 (2%)                     | 0/214 (0%)                     |
| C-cell carcinoma               | 1/440 (<1%)     | 1/107 (0%)                     | 0/214 (0%)                     |
| Other tumors                   | 2/440 (<1%)     | 2/107 (2%)                     | 0/214 (0%)                     |
| Parathyroid, adenoma           | 6/280 (2%)      | 3/68 (4%)                      | 3/139 (2%)                     |
| G.I. tract, all tumors         | 5/472 (1%)      | 1/114 (1%)                     | 1/228 (<1%)                    |
| Pituitary, all tumors          | 2/286 (1%)      | 0/77 (0%)                      | 0/132 (1%)                     |
| Kidney, all tumors             | 1/472 (<1%)     | 0/114 (0%)                     | 0/228 (0%)                     |
| Liver, all tumors              | 0/472 (0%)      | 0/114 (0%)                     | 0/228 (0%)                     |
| Leukemia or malignant lymphoma | 7/472 (1%)      | 2/114 (2%)                     | 2/228 (1%)                     |
| Hemangioma or hemangiosarcoma  | 2/472 (<1%)     | 0/114 (0%)                     | 1/228 (<1%)                    |
| Uterus, all tumors             | 8/471 (2%)      | 3/113 (3%)                     | 5/226 (2%)                     |
| All other tumors               | 6/472 (1%)      | 3/113 (3%)                     | 3/228 (1%)                     |

*<sup>p</sup> < 0.05 decrease relative to pooled controls (life table and incidental tumor test).

Table 14. Incidence of primary tumors in male hamsters administered intermediate-range chrysotile asbestos.

|                                | Pooled controls | Intermediate-range chrysotile controls | Intermediate-range chrysotile controls |
|--------------------------------|-----------------|----------------------------------------|----------------------------------------|
| Animals with primary tumors    | 95/472 (20%)    | 26/116 (22%)                           | 78/245 (32%)<sup>a,b</sup>             |
| Skin or subcutaneous tissue, all tumors | 2/472 (<1%)      | 1/116 (1%)                            | 0/245 (0%)                             |
| Lung or trachea, all tumors    | 0/470 (0%)      | 0/116 (0%)                             | 1/244 (<1%)                            |
| Adrenal                        |                 |                                        |                                        |
| Cortical adenoma               | 25/466 (5%)     | 7/115 (6%)                             | 24/244 (10%)<sup>c</sup>               |
| Cortical carcinoma             | 13/465 (3%)     | 3/115 (3%)                             | 7/244 (3%)                             |
| Pheochromocytoma               | 13/466 (3%)     | 5/115 (4%)                             | 1/244 (1%)                             |
| Other tumors                   | 6/466 (1%)      | 3/115 (3%)                             | 1/244 (<1%)                            |
| Pancreas                       |                 |                                        |                                        |
| Inlet-cell adenoma             | 20/445 (4%)     | 7/110 (6%)                             | 15/226 (7%)                            |
| Inlet-cell carcinoma           | 1/445 (<1%)     | 0/110 (0%)                             | 1/226 (<1%)                            |
| Thyroid                        |                 |                                        |                                        |
| C-cell adenoma                 | 7/428 (2%)      | 3/106 (3%)                             | 5/216 (2%)                             |
| C-cell carcinoma               | 3/428 (1%)      | 1/106 (1%)                             | 4/216 (2%)                             |
| Other tumors                   | 1/428 (<1%)     | 0/106 (0%)                             | 1/216 (<1%)                            |
| Parathyroid, adenoma           | 2/271 (1%)      | 1/71 (1%)                              | 4/138 (3%)                             |
| G.I. tract, all tumors         | 6/472 (1%)      | 1/116 (1%)                             | 3/245 (1%)                             |
| Pituitary, all tumors          | 0/322 (0%)      | 0/77 (0%)                              | 0/182 (0%)                             |
| Kidney, all tumors             | 4/470 (1%)      | 2/116 (2%)                             | 1/245 (<1%)                            |
| Liver, all tumors              | 0/470 (0%)      | 0/116 (0%)                             | 0/244 (0%)                             |
| Leukemia or malignant lymphoma | 8/472 (2%)      | 1/116 (1%)                             | 10/245 (4%)                            |
| Hemangioma or hemangiosarcoma  | 5/472 (1%)      | 0/116 (0%)                             | 1/242 (<1%)                            |
| All other tumors               | 5/472 (1%)      | 0/116 (0%)                             | 2/245 (1%)                             |

*<sup>p</sup> < 0.01 vs. pooled controls.

<sup>a</sup> < 0.05 vs. intermediate-range chrysotile controls.

<sup>bp</sup> < 0.05 vs. pooled controls.
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Table 15. Incidence of primary tumors in female hamsters administered intermediate-range chrysotile asbestos.

|                          | Pooled controls | Intermediate-range chrysotile controls | Intermediate-range chrysotile controls |
|--------------------------|-----------------|---------------------------------------|---------------------------------------|
| Animals with primary tumors | 66/472 (14%)    | 17/119 (14%)                         | 39/244 (16%)                         |
| Skin or subcutaneous tissue, all tumors | 0/472 (0%) | 0/119 (0%)                           | 2/244 (1%)                           |
| Lung or trachea, all tumors | 0/471 (0%)    | 0/119 (0%)                            | 0/243 (0%)                           |
| Adrenal                  |                 |                                       |                                       |
| Cortical adenoma         | 15/468 (3%)     | 6/118 (5%)                           | 18/234 (8%)                          |
| Cortical carcinoma       | 0/468 (0%)      | 0/118 (0%)                           | 1/234 (<1%)                          |
| Pheochromocytoma         | 0/468 (0%)      | 0/118 (0%)                           | 1/234 (<1%)                          |
| Other tumors             | 0/468 (0%)      | 0/118 (0%)                           | 0/234 (0%)                           |
| Pancreas                 |                 |                                       |                                       |
| Islet-cell adenoma       | 15/456 (3%)     | 5/116 (6%)                           | 4/236 (2%)                           |
| Islet-cell carcinoma     | 1/456 (<1%)     | 0/116 (0%)                           | 0/236 (0%)                           |
| Thyroid                  |                 |                                       |                                       |
| C-cell adenoma           | 6/440 (1%)      | 3/115 (3%)                           | 2/223 (1%)                           |
| C-cell carcinoma         | 1/440 (<1%)     | 0/115 (0%)                           | 0/223 (0%)                           |
| Other tumors             | 2/440 (<1%)     | 0/115 (0%)                           | 1/223 (<1%)                          |
| Parathyroid, adenoma     | 6/280 (2%)      | 1/77 (1%)                            | 1/148 (1%)                           |
| G.I. tract, all tumors   | 5/472 (1%)      | 2/119 (2%)                           | 1/244 (<1%)                          |
| Pituitary, all tumors    | 2/285 (1%)      | 2/67 (3%)                            | 2/164 (1%)                           |
| Kidney, all tumors       | 1/472 (<1%)     | 1/119 (1%)                           | 0/243 (0%)                           |
| Liver, all tumors        | 0/472 (0%)      | 0/119 (0%)                           | 0/234 (0%)                           |
| Leukemia or malignant lymphoma | 7/472 (1%) | 0/119 (0%)                           | 2/244 (1%)                           |
| Hemangioma or hemangiosarcoma | 2/472 (<1%) | 0/119 (0%)                           | 1/244 (<1%)                           |
| Uterus, all tumors       | 8/471 (2%)      | 1/119 (1%)                           | 7/240 (3%)                           |
| All other tumors         | 6/472 (1%)      | 0/119 (0%)                           | 2/244 (1%)                           |

*<p < 0.05 vs. pooled controls.

Table 16. Incidence of primary tumors in male hamsters administered 1,2-dimethylhydrazine dihydrochloride (DMH) or intermediate-range chrysotile asbestos and DMH.

|                          | Pooled controls | DMH and intermediate-range chrysotile controls | DMH and intermediate-range chrysotile controls |
|--------------------------|-----------------|-----------------------------------------------|-----------------------------------------------|
| Animals with primary tumors | 95/472 (20%)   | 27/119 (23%)                                  | 29/127 (23%)                                  |
| Skin or subcutaneous tissue, all tumors | 2/472 (<1%) | 1/119 (1%)                                   | 0/127 (0%)                                   |
| Lung or trachea, all tumors | 0/470 (0%)    | 0/119 (0%)                                    | 0/127 (0%)                                    |
| Adrenal                  |                 |                                               |                                               |
| Cortical adenoma         | 25/466 (5%)     | 3/117 (3%)                                    | 3/127 (2%)                                    |
| Cortical carcinoma       | 13/466 (3%)     | 4/117 (4%)                                    | 2/127 (2%)                                    |
| Pheochromocytoma         | 13/466 (3%)     | 3/117 (3%)                                    | 4/127 (3%)                                    |
| Other tumors             | 6/466 (1%)      | 2/117 (2%)                                    | 0/127 (0%)                                    |
| Pancreas                 |                 |                                               |                                               |
| Islet-cell adenoma       | 20/445 (4%)     | 8/110 (7%)                                     | 6/114 (5%)                                    |
| Islet-cell carcinoma     | 1/445 (<1%)     | 0/110 (0%)                                    | 0/114 (0%)                                    |
| Thyroid                  |                 |                                               |                                               |
| C-cell adenoma           | 7/428 (2%)      | 0/107 (0%)                                    | 2/118 (2%)                                    |
| C-cell carcinoma         | 3/428 (1%)      | 0/107 (0%)                                    | 0/118 (0%)                                    |
| Other tumors             | 1/428 (<1%)     | 1/107 (1%)                                    | 0/118 (0%)                                    |
| Parathyroid, adenoma     | 2/271 (1%)      | 1/64 (2%)                                     | 0/81 (0%)                                     |
| G.I. tract, all tumors   | 6/472 (1%)      | 2/119 (2%)                                    | 3/127 (2%)                                    |
| Pituitary, all tumors    | 0/332 (0%)      | 0/80 (0%)                                     | 0/87 (1%)                                     |
| Kidney, all tumors       | 4/470 (1%)      | 1/119 (1%)                                    | 0/127 (0%)                                    |
| Liver, all tumors        | 0/470 (0%)      | 0/119 (0%)                                    | 0/127 (0%)                                    |
| Leukemia or malignant lymphoma | 8/472 (2%) | 4/119 (4%)                                    | 7/127 (6%)                                    |
| Hemangioma or hemangiosarcoma | 5/472 (1%) | 3/119 (3%)                                    | 2/127 (2%)                                    |
| All other tumors         | 5/472 (1%)      | 3/119 (3%)                                    | 1/127 (1%)                                    |

*<p = 0.257 (life table); <p = 0.038 (incidental tumor test); <p = 0.009 (Fisher's exact test) vs. pooled controls.

<sup>b</sup><p < 0.05 vs. pooled controls.
Table 17. Incidence of primary tumors in female hamsters administered 1,2-dimethylhydrazine dihydrochloride (DMH) or intermediate-range chrysotile asbestos and DMH.

| Animals with primary tumors | Pooled controls | DMH and intermediate-range chrysotile controls | DMH and intermediate-range chrysotile controls |
|----------------------------|-----------------|----------------------------------------------|----------------------------------------------|
| Skin or subcutaneous tissue, all tumors | 0/472 (0%) | 0/120 (0%) | 1/120 (1%) |
| Lung or trachea, all tumors | 0/471 (0%) | 0/119 (0%) | 0/122 (0%) |
| Adrenal | 15/468 (3%) | 3/120 (2%) | 2/120 (2%) |
| Pancreas | 15/456 (3%) | 5/116 (4%) | 2/119 (2%) |
| Thyroid | 1/456 (<1%) | 0/116 (0%) | 0/119 (0%) |
| Parathyroid, adenoma | 6/280 (2%) | 1/74 (1%) | 2/57 (4%) |
| Pituitary, all tumors | 5/472 (1%) | 1/120 (1%) | 2/122 (2%) |
| Kidney, all tumors | 2/285 (1%) | 0/82 (0%) | 0/59 (0%) |
| Liver, all tumors | 1/472 (<1%) | 0/120 (0%) | 0/122 (0%) |
| Leukemia or malignant lymphoma | 7/472 (1%) | 3/120 (2%) | 2/122 (2%) |
| Hemangioma or hemangiosarcoma | 2/472 (<1%) | 1/120 (1%) | 0/122 (0%) |
| Uterus, all tumors | 8/471 (2%) | 2/120 (2%) | 2/116 (2%) |
| All other tumors | 6/472 (1%) | 1/120 (1%) | 2/122 (2%) |

Table 18. Incidence of gastrointestinal tract tumors in hamsters administered amosite asbestos.

| Stomach (number examined) | M | F | M | F |
|---------------------------|---|---|---|---|
| Squamous cell papilloma   | 3 | 0 | 1 | 0 |
| Papilloma                 | 0 | 0 | 0 | 0 |
| Small intestine (number examined) | 207 | 207 | 180 | 180 | 180 | 180 |
| Adenoma                   | 1 | 0 | 0 | 0 |
| Adenocarcinoma            | 0 | 1 | 0 | 0 |
| Large intestine (number examined) | 207 | 207 | 180 | 180 | 180 | 180 |
| Squamous cell papilloma   | 0 | 0 | 0 | 0 |
| Rectum (number examined)  | 207 | 207 | 180 | 180 | 180 | 180 |
| Adenoma                   | 0 | 1 | 0 | 0 |
| Adenomatous polyp         | 0 | 0 | 0 | 0 |
| Fibroma                   | 0 | 1 | 0 | 0 |
| Squamous cell papilloma   | 0 | 0 | 0 | 0 |

Males and females administered DMH did not show a significant (p < 0.05) increase in intestinal neoplasia. Nor did the intermediate-range chrysotile produce a higher rate of intestinal neoplasia in DMH-dosed animals. A summary of all gastrointestinal tumors observed in this study is given in Tables 18 and 19. None of the treated groups showed an increased rate of neoplasia in the gastrointestinal tract which was the proposed target organ.

While this study was not designed to evaluate nonneoplastic disease, noteworthy lesions were observed. None appeared to be dosage related; rather, they were consistent with lesions that are normally found in aging hamsters. It was the pathologists' opinion that the most important le-
sion, responsible for many deaths, was generalized amyloidosis. The kidneys were particularly affected by diffuse accumulation of amyloid, which replaced glomeruli and infiltrated tubular interstitium to a point where the normal cortical architecture was obliterated. Other organs which showed significant accumulations of amyloid were adrenal gland, liver, spleen and the epithelium of the small intestine. Amyloid within the walls of blood vessels was observed in many tissues.

Many of the livers were cirrhotic, infiltrated with amyloid, and contained large cystic structures filled with a lightly staining proteinaceous fluid. These structures were interpreted as cystic bile ducts and are consistent with what others have termed “retention cysts.” At times, these cysts were so large and/or numerous that less than half of the livers remained.

Other nonneoplastic lesions that were observed in more than 5% of the hamsters in any of the experimental groups are as follows: (1) skin, chronic dermitis; (2) lung, interstitial pneumonitis; (3) spleen, lymphoid atrophy; (4) lymph node, hyperplasia; (5) heart, atrial thrombosis; (6) gallbladder, edema and calculi; (7) stomach (nonglandular), hyperkeratosis or acanthosis; (8) colon, intussusception, inflammation; (9) urinary bladder, chronic inflammation, hyperplasia; (10) adrenal gland, cortical and medullary hyperplasia; (11) thyroid gland, follicular atrophy; (12) pituitary gland, degeneration; (13) ovary, atrophy; (14) uterus, inflammation, endometrial hyperplasia; (15) vagina, acute inflammation, squamous metaplasia.

### Discussion

The clinicopathologic results in this study showed that the chronic ingestion of 1% amosite (8) or chrysotile (SR and IR) (9) asbestos in the diet did not have any adverse effect on body weight gain and survival seemed to be enhanced. An explanation for these observations is not apparent.

The major organ which showed a statistically significant ($p < 0.05$) increased rate of neoplasia was the adrenal cortex in male and female hamsters exposed to IR chrysotile asbestos and males exposed to SR chrysotile asbestos when compared with pooled controls. However, statistical significance was lost when these groups were compared to their temporal controls. It is difficult to imagine how oral asbestos, even though it is known to be absorbed through the gastrointestinal tract (10), could cause an increased tumor rate in the adrenal cortex without causing similar increases in tumors in other abdominal organs and tissues, i.e., gastrointestinal tract and peritoneum. For these reasons, the biologic importance of adrenal tumors in this study is doubtful. The overall increase in total primary tumors in male IR chryso-

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**Table 19. Incidence of gastrointestinal tract tumors in hamsters administered chrysotile asbestos.**

| Organ                     | Pooled  | Short-range | Intermediate-  | DMH       | DMH and Intermediate- |
|---------------------------|---------|-------------|---------------|-----------|-----------------------|
|                           | controls| chrysotile  | chrysotile    | DMH       | chrysotile            |
|                            | M       | F           | M       | F       | M     | F       | M    | F     | M    | F    |
| Stomach (number examined) | (464)   | (468)       | (222)  | (224)  | (244) | (242)  | (127) | (118) | (170) | (160) |
| Squamous cell papilloma    | 3       | 0           | 0       | 0       | 1     | 0       | 0     | 0     | 2     | 0     |
| Carcinoma *in situ*       | 0       | 0           | 0       | 0       | 1     | 1       | 0     | 0     | 0     | 0     |
| Papillary adenoma         | 0       | 0           | 0       | 0       | 1     | 1       | 0     | 0     | 0     | 0     |
| Small intestine (number examined) | (467) | (469)       | (226)  | (227)  | (244) | (244)  | (127) | (120) | (170) | (159) |
| Adenoma                   | 1       | 0           | 0       | 0       | 0     | 0       | 0     | 0     | 0     | 0     |
| Adenomatous carcinoma     | 1       | 0           | 0       | 0       | 0     | 0       | 0     | 0     | 0     | 0     |
| Large intestine (number examined) | (464) | (468)       | (222)  | (226)  | (241) | (243)  | (126) | (118) | (170) | (159) |
| Papilloma                  | 0       | 0           | 0       | 0       | 0     | 0       | 0     | 0     | 1     | 0     |
| Adenoma                   | 0       | 1           | 0       | 0       | 0     | 1       | 0     | 0     | 0     | 0     |
| Papillary adenoma         | 0       | 0           | 0       | 0       | 1     | 0       | 0     | 0     | 0     | 0     |
| Adenocarcinoma            | 0       | 1           | 0       | 1       | 0     | 0       | 0     | 0     | 0     | 0     |
| Lipoma                    | 0       | 1           | 0       | 0       | 0     | 0       | 0     | 0     | 0     | 0     |
| Adenomatous polyyp         | 0       | 0           | 0       | 0       | 0     | 0       | 1     | 0     | 0     | 0     |
| Rectum (number examined)  | (472)   | (472)       | (233)  | (228)  | (245) | (244)  | (127) | (122) | (173) | (161) |
| Adenoma                   | 1       | 1           | 1       | 0       | 0     | 0       | 0     | 0     | 0     | 0     |
| Papillary adenoma         | 0       | 0           | 0       | 0       | 0     | 0       | 1     | 0     | 0     | 0     |
| Fibrosarcoma              | 0       | 0           | 0       | 0       | 0     | 1       | 0     | 0     | 0     | 0     |
| Squamous cell carcinoma   | 0       | 0           | 0       | 0       | 0     | 1       | 0     | 0     | 0     | 0     |
| Fibroma                   | 0       | 1           | 0       | 0       | 0     | 0       | 0     | 0     | 0     | 0     |
tile hamsters can be explained primarily on the basis of an increased incidence of adrenal tumors in this group. The enhanced survival of animals in the chrysotile groups also contributed to the elevated incidence of primary tumors observed in these groups compared with controls.

The only other instance of an increased rate of neoplasia was statistically significant (p < 0.05) increase in leukemia or malignant lymphoma in male hamsters exposed to DMH when compared to pooled controls. Again, statistical significance was lost when this group was compared to its temporal control group. This finding also loses importance because it was not observed in the DMH plus IR chrysotile group.

Other such studies involving the long-term ingestion of asbestos are few. Donham et al. (11) 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 and possible cytotoxicity to colonic tissues and they suggested a possible relationship to peritoneal mesothelioma. Another equivocal study is that reported by Gibel et al. (12), 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. (13) reported two studies in male Wistar rats administered 1% chrysotile in the diet, one study of 24 months and one of 30 months. No increase in intestinal tumors was found compared to the control rats. Negative results were reported by Gross et al. (14), who fed rats a diet containing 5% chrysotile asbestos for a period of 21 months with no evidence of intestinal neoplasia.

The only oral asbestos study in hamsters was reported by Smith et al. (15). They exposed groups of 30 male and female hamsters via drinking water for lifetime to amosite asbestos, mine tailings, beach rock, and 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). Their other studies were considered negative. They concluded that the study was essentially negative.

Except for those by Donham et al. (11) and Smith et al. (15), these studies were conducted with relatively small numbers of animals. Also, some were conducted for periods of time insufficient for adequately testing the carcinogenic potential of ingested asbestos.

The results of the combination study (IR chrysotile plus DMH) also did not yield a significant increase in tumors above the background level observed in the DMH group alone or in the untreated control group. The DMH failed to yield a background level of intestinal tumors high enough to provide a valid test of the cocarcinogenic potential of chrysotile asbestos. For this reason, a cocarcinogenic potential of oral asbestos should be considered untested. However, the DMH plus chrysotile provides an additional IR chrysotile group for comparative purposes.

It is not clear why the DMH-dosed group of hamsters failed to show an increased incidence of intestinal neoplasia. The pilot study suggested that this dose of DMH should have caused an incidence of approximately 15%. DMH solutions rapidly decompose if they are at room temperature or if they are not properly buffered.

The only long-term study designed to determine the cocarcinogenic potential of asbestos was reported by Ward et al. (16). They administered 1 mg amosite asbestos in saline by gavage to 6-week-old F344 rats three times per week for 10 weeks. 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. All surviving rats were killed at 94–95 weeks of age. They reported an intestinal tumor incidence of 66.7% for 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 with which to compare the treated groups. These results should as well 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 an uncommon spontaneous tumor. However, AOM is known to induce Zymbal gland tumors with a single 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. An appropriate explanation for the high incidence of Zymbal
gland tumors in the amosite group would be that those animals were inadvertently exposed to AOM. If this occurred, animals would also be expected to show a high incidence of intestinal neoplasia.

Conclusion

Under the conditions of this bioassay, amosite asbestos and short-range chrysotile and intermediate-range chrysotile asbestos were not carcinogenic when ingested by male and female Syrian golden hamsters. While there were significant increases in the rates of adrenal cortical adenomas in male and female hamsters exposed to intermediate-range chrysotile asbestos compared with pooled control groups, these incidence rates were not significantly different when compared with the temporal control groups. Additionally, the biological importance of adrenal tumors in the absence of target organ neoplasia is questionable. The cocarcinogen studies using IR chrysotile asbestos and 1,2-dimethylhydrazine dihydrochloride were considered inadequate because there was no increase in intestinal neoplasia in the DMH group.

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