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NIEHS ORAL ASBESTOS STUDIES

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Abstract

Epidemiologic data clearly associate inhalation of asbestos with an increased incidence of cancer. In addition to pulmonary and thoracic neoplasia, there are data which associate an increased incidence of gastrointestinal and peritoneal tumors. Controversy exists as to whether these latter types of neoplasia result from asbestos fibers that were ingested subsequent to clearance from the respiratory system. Exposure to ingested asbestos does occur in the general population through the presence of fibers in water and food.

The NIEHS oral asbestos studies in rats and hamsters represent a systematic attempt to assess the biological effects associated with primary ingestion of selected asbestos fibers. The objectives of the studies include: assessment of biological (carcinogenic) effects as a consequence of exposure to one of several types of asbestos; assess if an interaction may exist between a chemical carcinogen which is known to produce bowel cancer, and ingestion of asbestos. The specific experimental design of this series of ongoing studies will be presented.

Key Words: Asbestos; bowel cancer; cancer; epidemiology; fibers.

There is strong evidence that associates occupational exposure to chrysotile amosite, and crocidolite to a resulting high incidence of lung cancer. Exposure to these forms of asbestos has also been observed to result in an increased incidence of pleural and peritoneal mesothelioma and an excess risk of gastrointestinal cancer. Environmental exposure to asbestos through living in the neighborhood of asbestos factories or mines or through residing in households of asbestos workers also correlates with increased mesotheliomas [1].¹

It is plausible to speculate that the increased incidence of gastrointestinal cancer in occupationally exposed populations may be a consequence of asbestos fiber ingestion. Fiber ingestion in these circumstances may result through the swallowing of fibers cleared from the nasal or tracheobronchial tree. Direct ingestion of fibers deposited in the oral cavity also occurs.

Exposures of the general population to asbestos occurs through ingestion of materials and substances that contain fibers. For example, several million fibers per liter were found in Canadian tap water [2]; Great Lakes and St. Lawrence River water showed average concentrations of about 1.7 million asbestos fibers per liter [3]; water collected from the north shore of Lake Superior in the Silver Bay/Duluth region were found to have even higher fiber levels. A number of studies have reported the appearance of asbestos fibers in commercial beverages such as beer, vermouth, and soft drinks [2]. The fibers found in these products may be a result of the use of asbestos filters used in their preparation [2]. Food may contain asbestos through the use of asbestos filters or the use of talc, which has an asbestos impurity [4,5].

¹Figures in brackets indicate the literature references at the end of this paper.

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In response to a growing concern about the possible biological effects of ingested asbestos, a conference was held in 1973, co-sponsored by the National Institute of Environmental Health Sciences and the Environmental Protection Agency. The meeting confirmed that the preponderance of biological data concerning exposure to asbestos focused on the inhalation and not the ingestion route of exposure. A consensus of that international conference was that research was needed on health effects associated with asbestos ingestion. A Subcommittee of the DHEW Committee to Coordinate Toxicology and Related Programs (CCTRP) subsequently reviewed the existing data, recommended that additional research be undertaken, and prepared a draft research protocol that it felt would be responsive to the scientific needs. This protocol was widely distributed for comments both within and outside the Government. Based on the comments received, a final protocol was developed and submitted as part of its final report. In response to the Subcommittee's report, Congress appropriated specific funds directing the National Institute of Environmental Health Sciences to research the effects of oral asbestos ingestion. The NIEHS is conducting this research primarily through its research contracts program. The Environmental Protection Agency also contributed funds for these studies. The design of these studies is in concert with the recommendations of the CCTRP Subcommittee. The basic design of the studies provides for an evaluation of chrysotile, a serpentine asbestos; and amosite and crocidolite, fibers representative of amphibole asbestos; plus a non-fibrous tremolite, which does contain low levels of asbestiform fibers.

The studies call for asbestos to be fed continuously in the diet over the entire lifespan of the test animal. Each form of asbestos is contained at a one-percent level in a pelleted rodent diet of constant ingredient formulation (NIH Feed 31). The proposal to incorporate the asbestos within a pelleted diet form was approved only after studies indicated that the pelleting process did not alter the physical integrity of the fiber. The utilization of an asbestos diet in a pelleted form has obvious advantages: it minimizes fiber aerosols which would occur with greater ease in a non-pelleted form; it minimizes variations of asbestos concentration in the diet due to segregation of fibers that would occur during shipping, handling, and feeding. Incorporating asbestos into food rather than water eliminates settling and subsequent uneven distribution.

All materials are being fed to the F-344 strain of rat; whereas two forms of asbestos, chrysotile and amosite, are also to be tested in hamsters. Golden Syrian hamsters represent a second test species and are being fed a serpentine or amphibole form of asbestos. All studies encompass the lifespan of the animal, which is defined as the age at which the animal begins eating solid food until its death. To insure asbestos ingestion at a young age, these studies are initiated by feeding the asbestos diet to a nursing mother, which is removed once the pups are weaned. These latter animals that begin eating asbestos at two weeks of age constitute the test generation.

In the basic studies, the test group size is 500, composed of equal numbers of males and females. In each of the rat and hamster studies, there is a composite total of 1000 animals that receive diet which does not contain asbestos and serve as controls. The experimental group size allows one to detect a statistically significant increase in gastrointestinal tumors in the treated groups at a two percent increase above the control population.

In another rat experiment, two subsets of 200 animals each are to receive asbestos from the first to the 28th day of life by gastric intubation. The rat pups received 2.35 mg of an aqueous asbestos suspension daily. At weaning, the rats are placed on the appropriate asbestos diet for the remainder of their lifespan. One subset of 200 animals is to receive chrysotile while another subset is to receive amosite. The objective of these experiments is to see if a possibility exists that neonates may be a special risk population.

There is also scientific interest in determining if asbestos in the diet alters the expression of intestinal neoplasms induced by a known chemical carcinogen. Studies of this type are performed in rats that are fed either chrysotile or amosite. A similar study will be conducted in hamsters receiving the chrysotile diet. There are 350 animals in each of these three groups. The chemical carcinogen to be utilized was selected after a series of dose-ranging experiments of one-year's duration was performed in each species. In these dose-ranging studies, both dimethylhydrazine and methylazoxymethanol were evaluated. The

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results indicated that dimethylhydrazine was the chemical carcinogen of choice due to lower toxicity and greater specificity of intestinal tumor response. The dose selected is one that will produce approximately a 10 percent incidence of intestinal tumors. That dose for hamsters is 4 mg/kg, whereas for rats it was 7.5 mg/kg and 15 mg/kg in male and female rats, respectively. The dimethylhydrazine is administered by gavage once every fourteen days until five doses have been administered. The initial dose was administered at six weeks of age. Tables 1 and 2 summarize the design of the animal study. The animal testing phase of the experiments commenced in late 1975. Since the natural lifespan of the F-344 rat is 26-30 months and 18-23 months for the hamster, definitive interpretation of these studies is several years away.

Table 1. NIEHS oral asbestos study.

Golden Hamster

	<u>Chrysotile Intermediate</u>	<u>Chrysotile Short Range</u>	<u>Amosite</u>
Asbestos diet	500 ^a	500	500
Asbestos diet plus dimethylhydrazine ^a	350	ND	ND
Control diet ^b	500	250	250
Control diet plus dimethylhydrazine ^a	250	ND	ND

^a Number of animals (equal numbers of each sex).

^b Control allocations are descriptive only. Experimental response will be evaluated against total controls (1000). Subsets of control will reflect temporal differences in commencing phases of study which is expected to be aggregates of 250-350.

ND - Not done.

Studies conducted by Illinois Institute of Technology Research Institute, Chicago, Illinois.

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F-344 Rat

	Chrysotile Intermediate	Chrysotile Short Range	Amosite Intermediate	Crocidolite	Tremolite
Asbestos diet	500 ^a	500	500	500	500
Asbestos diet plus dimethylhydrazine ^a	350	ND	350	ND	ND
Prewaning asbestos gavage plus asbestos diet	200	ND	200	ND	ND
Control diet ^b	175	175	175	175	175
Control diet plus dimethylhydrazine ^a	250	ND	250	ND	ND

^a Number of animals (equal numbers of each sex).

^b Control allocations are descriptive only. Experimental response will be evaluated against total controls (1000). Subsets of control will reflect temporal differences in commencing phases of study which is expected to be aggregates of 250-350.

ND - Not done.

Studies conducted by Hazleton Research Laboratories, Vienna, Virginia.

All animals receive a thorough pathologic evaluation at time of autopsy. In conformance with the NCI Carcinogen Bioassay protocol, some thirty tissues in addition to any gross lesions will be examined under light microscopy.

The rat studies are being performed through a contract with Hazleton Research Laboratories, Vienna, Virginia; whereas the hamster experiments are being performed by the Illinois Institute of Technology Research Institute, Chicago, Illinois.

As a biologist, I wish to emphatically state that the most difficult decision in the design of these studies was determining the types and specific forms of asbestos that were to be fed. The literature clearly indicated that some previous studies were flawed due to unwitting physical violence imposed upon the asbestos during its preparation. In some cases, there was concern about contamination by organic chemicals. In medical research circles, the issue still rages with respect to the size of fiber that may be associated with observed neoplastic response. It is necessary to relate size that produces optimal biological response to the distribution of fiber sizes to which there is general human population exposure. The common fiber found in municipal water supplies represents one of serpentine origin. From a numerical standpoint, the preponderance of these fibers is of the low micron and submicron lengths. To accommodate to these circumstances, it was decided that there would be two chrysotile asbestos materials used in the rat and hamster studies. These are referred to as the NIEHS short-range chrysotile and the NIEHS intermediate-range chrysotile.

NIEHS short-range chrysotile was mined from the New Idria deposits in California. This chrysotile is of very small fiber length and diameter. It is a single lot produced by Union Carbide and is referenced by them as COF-25. The NIEHS intermediate-range chrysotile originated from the Johns-Manville Jeffrey Mine in Canada. This material has general analogies to their Plastobest 20.

One method of comparing these two chrysotile samples is by comparing surface area determinations. Table 3 presents the results of such tests; the UICC chrysotile surface area values are listed for comparison. The UICC samples have been the asbestos source for the majority of biological studies over the past several years. As can be seen from the table, the NIEHS intermediate-range chrysotile compares quite favorably with the UICC Canadian chrysotile. The two-fold increase in surface area of the NIEHS short-range chrysotile compared to its intermediate-range counterpart reflects the much smaller fiber size found in this sample.

Table 3. Comparison of UICC and NIEHS chrysotile samples.

<u>Asbestos Identification</u>	<u>UICC Value (m^2/g)</u>	<u>IITRI Value (m^2/g)</u>
UICC Rodesian Chrysotile	21.3 ± 1.5	22.35
UICC Canadian Chrysotile	26.8 ± 0.7	27.7
NIEHS Intermediate Range Chrysotile		27.8 ± 2.7
NIEHS Short Range Chrysotile		59.0 ± 6.2

The amphibole samples, amosite and crocidolite, were prepared by the Ontario Research Foundation under the direction of the U.S. Bureau of Mines' College Park Laboratory. This asbestos, purchased commercially, has been processed by air jet milling to better standardize the range of fiber size contained in the material.

The tremolite sample was mined and milled to -325 mesh by the R. T. Vanderbilt Company, Balmat, New York. It was subsequently blended by the U.S. Bureau of Mines personnel to insure homogeneity of the sample.

All test materials are being extensively characterized as to chemical and fiber size characteristics. The characterization data include x-ray diffraction parameters, chemical composition, DTA, TGA, optical constants, density, and surface area. These studies are being performed by the U.S. Bureau of Mines. Exhaustive electron microscopic characterization of each material as to fiber length, fiber diameter, surface area, distribution of fiber size, and selective pore volume measurements are being performed by the Fine Particles Laboratory of the Illinois Institute of Technology Research Institute, Chicago, Illinois.

The characterization studies on tremolite and the short-range and intermediate-range chrysotile are nearly complete. The characterization of amosite and crocidolite are scheduled for completion by the end of the year.

Two recent ingestion studies that have been reported within the past year yielded variable results. In a British study, a group of 32 Wistar rats were fed 100 mg per day of UICC Canadian chrysotile prepared in milk powder on a five-day-a-week schedule for a total of 100 days of ingestion. There were 16 control animals which were fed only the malted milk. The animals were then allowed to live out their lifetime, which was a mean survival of 619 days for those animals on chrysotile versus 641 days for the controls. One gastric leiomyosarcoma was observed in the chrysotile group. No tumors of this type were found to occur in the controls [6].

In a study reported in the East German literature, a statistically significant ($p < 0.01$) increased incidence of malignant tumors occurred in rats that received asbestos filter material in the diet [7]. The exact composition of the asbestos filter material was not given in this paper. In this study, 25 male and 25 female Wistar rats were given 50 mg/kg body weight per day of asbestos filter material which contained approximately 52 percent chrysotile asbestos. This asbestos containing filter material had been previously

powdered and added as a water suspension to the diet. In the group of animals which received the asbestos filter material, the average survival time was 441 days. Untreated controls had an average survival time of 702 days. Of the 42 treated rats available for pathologic evaluation, 12 malignant tumors were found. This is to be compared to seven tumors (two liver cell carcinomas and five mammary fibroadenomas) observed in 49 control animals. The tumor types observed in the animals fed the asbestos filter material included four kidney carcinomas, one lung carcinoma, three reticulum cell sarcomas, and four liver cell carcinomas. Two mammary fibroadenomas, as well as a lung adenoma, two cholangiomas, and two forestomach papillomas were also observed.

The NIEHS Oral Asbestos Studies should provide controlled data from large enough sample sizes to allow for initial formulation of basic principles as to the biological effects of exposure to ingested asbestos.

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Discussion

M. SCHNEIDERMAN: Dr. Moore completed his paper considerably earlier than the time allotted. Are there some questions concerning this particular elaborate set of experiments, and the experiment design? Are there some suggestions that people would have? When the results come in from these experiments, what kinds of doubts will exist in your mind? What sorts of things would you like to see answered that these are not going to answer? I hope that there are people here that have thought about these and might have some questions.

V. WOLKADOFF: Evidently Dr. Moore has brought up chrysotile, the short size range sample from Edra and the intermediate size sample from Jeffry, and an evaluation of the size by specific surface area. Amosite was size fractionated by air-jet milling, and tremolite, evidently by milling of some type, to minus 325 mesh. The chrysotile more or less has been characterized by specific surface area. Do you have information, within each of these categories, as to the crystallinity of the individual fibers, the four categories versus degradation of the crystallinity of individual fibers by the method of preparation, or is it too early to say? You mentioned data by x-ray diffraction, DTA, and optical microscopy. I also wanted to know if you are going to include the electron diffraction results in your studies.

J. MOORE: I think I mentioned the electron diffraction work as part of the study, and I'd rather let the Bureau of Mines personnel, who are here, or the IITRI personnel, answer your question with regard to the crystallinity. I'm sure it has been looked at, but I don't know if the data are in such a stage to make any comment about it.

WOLKADOFF: What about air-jet milling of amosite, do you have any data now?

MOORE: No, if I did, I would have presented it.

WOLKADOFF: The tremolite data also, you don't have anything then?

MOORE: No sir, it's not in complete form. As I mentioned in the paper, the characterization of the two chrysotile samples and the tremolite sample should be available within the next couple of months, and we would expect that the similar types of studies characterizing the amosite and the crocidolite will be done by the end of the year.

WOLKADOFF: Thank you very much.

W. CAMPBELL: All the data has been completed on tremolite, including optical microscopy, SEM, TEM, chemical data, and surface area. On chrysotile, the optical data is finished, the SEM data is about completed, and the TEM is about completed. So in answer to your question there are very extensive data available on the optical properties, the morphology, the crystallinity, the trace metals, and so forth. Surface area is just one of the many parameters being investigated.

MOORE: I may have misled you in my presentation by only showing the slide on surface area; I did that one because it did show a distinction between the two chrysotiles. I would point out that we do not wish to infer that there is a clear separation of fibers between these two materials. Certainly the intermediate range chrysotile sample does have fibers that are well into the size range of fibers that are found in the short range sample. The distinction between the two is the proportion of fibers that may exceed, with respect to length and diameter, those that were found only in the short range.

G. WRIGHT: You quite properly pointed out that the kind of occupational exposure which has led to what we know about tumor incidence is quite different from what's found in water supplies. In fact, the differences are very striking. On the other hand, in occupational exposure generally, and I say would say almost without exception, the percent of the total fibers that exceeds eight to ten and even five μm in length is of the order of less than five percent, and in many situations is only one or two percent. In the animal experiments that have been done by inhalation of asbestos, in general, the clouds created contained only one or two percent of what we call long fibers. For this reason, I think that to look at your samples in terms of percent, inferring that one, two, or three percent of long fibers present in a sample is acceptable when you're talking about short fiber samples is erroneous. We need to get around to the number of long fibers, not the percent. Now also I would like to ask if these experiments are designed to look at the occupational experience or at the water experience?

MOORE: I would hope that they would have relevance in both areas.

WRIGHT: What percent or what number of long fibers are still present in your so-called short sample?

MOORE: Well, at what length do you want to consider as a long fiber?

WRIGHT: Anything over 5 μm , because in water, you've said, it is under 5.

MOORE: I recall the raw data that are available on that; about 90 percent of the samples in the short range chrysotile would be below that.

WRIGHT: In other words, ten percent are still above 5 μm ?

MOORE: Right.

WRIGHT: Well, that's essentially what the occupational exposure is. I don't think you're looking at water related exposures.

W. BANK: I'll change the subject slightly. There have been some animal nutrition studies going on since 1965 in Japan, and more recently in the U.S., in which fibrous material, namely certain zeolites, have been fed to these animals. The results were that the animals gained weight faster, certain diseases seemed to disappear, and so on. It's recognized, however, that there is a possible long-range pathological effect that might be involved because of the fibrous materials. Have you heard or do you know of any such information?

MOORE: I'm not aware of that work coming out of Japan.

C. COOPER: I strongly support the observation made by George Wright that the 10 percent or even 5 percent of long fibers in your short fiber samples would leave serious doubts as to whether the results of these experiments would be applicable to water supplies. Another question that has bothered a number of people is whether or not consideration was given, in the design of this experiment, in the choice of samples, to actually including a sample of the material that has contributed a great deal to this whole controversy. That is, the amphiboles that are found in Lake Superior water, in the size distribution in which they were found. I can see the difficulties in doing this, but I wonder just what the course of reasoning was that led to this type of material not being included?

MOORE: We were advised, and I must say we also subscribe to the opinion, that with regard to injection studies we might, in an initial series of experiments, be better off by using materials that are known to have biological effects in test species. This is where we opted to go with the amosite, for example; it's probably the closest thing we have to being representative of a cummingtonite/grunerite which is the Lake Superior type of sample. The other problem that we had when we did discuss the possibility of using something from the water in that area, was the complete lack of agreement among people we talked to with regard to what actually should be the sample that would come out of that area. In addition there was the logistics of trying to get that sample; it was just that simple. I would also state that with regard to fiber size, maybe having too many fibers above 5 μ m to permit direct relevance or extrapolation to municipal water supply samples, as was pointed out by Dr. Wright and yourself, I assume you gentlemen would accept a negative.

A. SUNDARAM: You quoted Dr. Gibel's paper from East Germany. Do you believe that study was well conducted, showing a significant effect of asbestos by oral ingestion?

MOORE: All I can comment on is the information which was available in the reprint, which brings questions to mind which certainly aren't explained in the materials and methods. For example, how they prepared the material, actually what was the other 48 percent since they inferred that 52 percent of the material was chrysotile. I think this sample size data may have some problems as well.

SUNDARAM: I agree to that, but in addition there is a significant point worth noting. The paper never mentions the number of animals affected over the control. There should be a significant increase in the number of animals that had tumors, not a significant increase in the number of tumors, because it may be one animal that had twelve tumors or it may be 12 animals that had 12 tumors. So it is the number of animals that were affected that's more important than the number of tumors. This paper has been quoted many times even though it just appeared in 1976. So many people quote it, and I thought it's better to point out this significant question that we should not miss.

MOORE: I thank you for your point, because the paper does not indicate as to whether, for example, the 12 malignant tumors found in 42 treated animals came from 12 separate individuals, or whether it was one or more animals which may have had multiple tumors.

D. ALTON: I am really wondering whether a dosage of 100 milligrams per day per unit weight of rat for the lifetime of a rat is comparable to the ordinary ingestion of asbestos fibers by man. Is there any relationship between those two figures for rat and man.

MOORE: I don't remember quoting 100 milligrams per rat per day, but suffice it to say that the level of asbestos that's in a diet at the 1 percent level certainly is in the high range of exposure.

COSPER: I have a very crass and practical question to ask. I know it's a matter of public record what a study of this magnitude costs, what kind of investment it involves, but I think the group would be interested in knowing just how much a major study like this represents in cost.

MOORE: It's estimated that by the time the studies that I have outlined are completed, which will include the characterization of the materials as well, it will probably be somewhere around 3-4 million dollars.

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