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CAMBRIDGE

TO: E. S. Wood

DATE: January 6, 1977

FROM: Julie C. Yang

SUBJECT: Status on Animal Studies

CC: R. M. Vining
 H. A. Brown
 H. C. Duecker
 O. M. Favorito
 J. W. Wolter
 File: 71-001/-002



The death rate of hamsters when treated with various types of chrysotile and "tremolite - talc" had been reported in a paper presented at the Talc Symposium in Washington, D.C., May 8, 1973, copy of which is attached herewith. The tremolite - talc sample referred to in the text is the Johnson-Johnson talc, composite given in Table 3.

To compare his previous data (P.46, Table 5) with the Grace test, the up-to-date progress is as follows:

<u>Sample</u>	<u>183 days</u>	<u>245 days</u>
Control	0/61	0/61
100% Libby Tremolite	0/62	1/62 (mesothelioma)
50% Libby Tremolite-50% Libby Vermiculite	1/63 (malignant tumor)	1/63

I'll try to reach Dr. Smith for further comments and information on parallel tests made on crocidolite (if any) or other fibers shortly after he returns from vacation.

Julie C. Yang
 Julie C. Yang

JCY:mlr

attachment

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JOHNSON & JOHNSON TREMOLITE - TALC 600X

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EXPERIMENTAL STUDIES ON BIOLOGICAL EFFECTS
OF TREMOLITE TALC ON HAMSTERS

by

William E. Smith, M.D.¹

My name is William E. Smith. I am director of Health Research Institute at Fairleigh Dickinson University, Madison, N.J. I am a doctor of medicine. Over the past 25 years, I have studied more than 200 chemical materials for carcinogenicity in animals and have reported results of these tests in scientific journals. In the past 8 years my associates and I exposed animals to various mineral dusts, maintained them over their natural life spans, and studied them for pathologic changes by gross and microscopic examination of their tissues and organs.

The principal purpose of these latter studies has been to develop information on possible fibrogenic and/or carcinogenic properties of various preparations of mineral dusts. The principal support for this work was provided by Research Grant EC 00226 from the Bureau of Occupational Safety and Health, U.S. Public Health Service. Thus far, we have published five papers (listed in the bibliography section of this paper) describing some of our findings in animals exposed to various preparations of asbestos. Today, I would like to offer a brief summary of our experiments with asbestos and describe as yet unpublished data from studies we have made with a sample of talc containing a large amount of tremolite.

From our work, we identified the Golden Syrian hamster as a species that was capable of developing pulmonary fibrosis resembling asbestosis seen in man. Those experiments were done by repeated intratracheal injections of chrysotile or amosite asbestos.

The method used for those experiments, weekly intratracheal injections, is technically tedious and time consuming. We found that we could compare the relative fibrogenicity and carcinogenicity of mineral dusts more conveniently by depositing the dusts by a single injection into the pleural space of hamsters.

After single intrapleural injection of preparations of the chrysotile, amosite, anthophyllite, or crocidolite types of asbestos, we found that hamsters developed extensive pleural adhesions. These adhesions were composed mainly of round cells and multinucleated giant cells in the first few months after injection. Within 5 months, these adhesions became densely fibrotic. Adhesions induced by the chrysotile, amosite, anthophyllite, or crocidolite types of asbestos tended to extend over large areas of the surfaces of the lungs.

¹Director, Health Research Institute, Fairleigh Dickinson University, Madison, N.J.

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In contrast, we saw very little reaction when we gave hamsters intrapleural injections of a sample of tremolite talc, which I shall identify by our sample number, FD-14. After intrapleural injection of this tremolite talc, the particles were gathered into small depots on the lung surface. These depots contained round cells and multinucleated giant cells, but there was very little formation of fibrous tissue. The experiments thus show that fibrogenic activity of tremolite talc is very much less than the fibrogenic action of the four principal types of asbestos.

Also, in hamsters that had been given intrapleural injections of the four principal types of asbestos, we found intrathoracic tumors that we designate as mesotheliomas (table 1).

TABLE 1. - Number of mesotheliomas in groups
of 50 hamsters

Dose, mg	Chrysotile	Amosite	Anthophyllite	Crocidolite
25	9	-	-	-
10	4	3	3	10
1	0	0	-	2

From the data in table 1, it can be seen that the yield of mesotheliomas, in groups of 50 hamsters per sample, was related to dose. In response to a large dose (25 mg) of chrysotile, nine hamsters developed mesotheliomas. In response to a smaller dose (10 mg) of chrysotile, four hamsters developed mesotheliomas. In response to a still smaller dose (1 mg), there were no mesotheliomas. This experiment provides evidence to show that the carcinogenic action of chrysotile is related to dose, hence should be controllable by appropriate industrial hygiene measures to control dose.

At the 10 mg dose level, amosite and anthophyllite gave about the same yield of mesotheliomas as did chrysotile, but crocidolite induced more than double the number of mesotheliomas gotten with the other types of asbestos.

Also, crocidolite induced mesotheliomas in two hamsters treated with the lowest dose level (1 mg) whereas no mesotheliomas occurred in animals treated with that dose of chrysotile or amosite.

Table 2 shows results of tests for carcinogenicity of six preparations of chrysotile in groups of 50 hamsters each. Each of these preparations was tested at our highest dose level (25 mg per injection). The table gives data on the mean fiber length and mean fiber diameter of these samples. It can be seen that 8 to 10 mesotheliomas were induced by three of these samples: harsh chrysotile, soft chrysotile, and the same sample of soft chrysotile heat-treated to achieve some of the properties of harsh chrysotile. These three samples that proved carcinogenic had mean fiber length of 5.3 to 6.9 micrometers and mean fiber diameter of 0.2 to 0.8 micrometer.

These three active preparations of chrysotile were subjected to further milling to reduce the majority of particles to submicroscopic dimensions. The

table shows that this reduction in particle size eliminated the carcinogenic effect of these samples. It also greatly reduced their fibrogenic action.

TABLE 2. - Tests for carcinogenicity of six preparations of chrysotile in groups of 50 hamsters each

Chrysotile preparation	Mean fiber length		Mean fiber diameter		No. of mesotheliomas
	OM, micrometers	EM, micrometers	OM, micrometers	EM, micrometers	
Soft.....	32.5	6.9	2.2	0.18	8
Soft (heated).	-	-	-	-	10
Harsh.....	38.9	5.3	2.9	.2	9
Soft.....	-	.9	-	.03	0
Soft (heated).	-	-	-	-	0
Harsh.....	-	.4	-	.07	0

OM Optical microscope.

EM Electron microscope.

Now, we have tested a sample of tremolite talc for carcinogenicity in hamsters. Electron diffraction studies of this sample showed it to contain 50 percent fibrous tremolite and 35 percent talc (table 3). A majority of the talc particles were platy, but some were rolled or fibrous.

TABLE 3. - FD-14 (tremolite talc): Composition

	<u>Percent</u>
Selected area electron diffraction:	
Fibrous tremolite.....	50
Antigorite.....	10
Talc ¹	35
Chlorite.....	5

¹Of this about 75 percent is platy and 25 percent is rolled or fibrous.

In this sample, the fibrous particles had an average length of 5.7 micrometers and an average diameter of 1.6 micrometers (table 4). They are therefore in a size range that might be carcinogenic in our experiments with chrysotile. However, this sample of tremolite talc induced no mesotheliomas after intrapleural injection into hamsters.

TABLE 4. - FD-14 (tremolite talc): Size measurement¹

(Phase microscopy 10x ocular, 100x objective)

	Length, micrometers		Diameter, micrometers	
	Range	Average	Range	Average
Fibrous particles.....	2.5-16.5	5.7	1- 5	1.6
Platy particles.....	-	-	1-10	2.0

¹Ratio fibrous to platy particles: 3 to 1.

Table 5 gives detailed data on these tests which were conducted at our highest dose level (25 mg). In view of the long latent period before development of tumors, if we wish to compare relative carcinogenicity of different samples, validity of the comparisons depends on the number of animals surviving into the later periods of the tests. In table 5, the number of survivors in each group at each time period is shown as the denominator. The cumulative number of animals bearing mesotheliomas is shown as the numerator.

TABLE 5. - Yields of mesotheliomas in hamsters after intrapleural injection at 25-mg dose level^{1 2}

Sample	151 days	340 days	372 days	450 days	500 days	550 days	600 days	650 days	700 days
Soft chrysotile.....	0/44	0/33	1/30	1/20	4/11	4/9	7/3	8/1	(³)
Harsh chrysotile.....	1/41	4/26	4/21	7/16	7/13	7/9	8/5	9/2	(³)
Heat-treated soft chrysotile.....	0/37	1/35	1/32	1/27	4/17	5/15	8/4	10/1	(³)
Tremolite talc.....	0/52	0/35	0/34	0/29	0/27	0/23	0/20	0/15	0/7

¹Numerator: Cumulative number of hamsters with mesotheliomas.

²Denominator: Number of hamsters surviving on stated day.

³Survivors at 650 days in chrysotile groups were killed on that day. Pleural adhesions, but no tumors, were found in them.

We started with about 50 hamsters in each group. The first tumor was found at 151 days in an animal that had been treated with harsh chrysotile.

Now let us look at the situation as it was 500 days after start of tests. At that time, 4 mesotheliomas had occurred in the group treated with soft chrysotile and only 11 animals survived.

In the group treated with harsh chrysotile, 7 mesotheliomas had occurred and 13 hamsters were still living.

In the group given heat-treated soft chrysotile, there were 4 mesotheliomas and 17 survivors.

By comparison, no mesotheliomas had occurred in the group treated with tremolite talc, and there were 27 survivors.

The group on tremolite talc thus had many more animals at risk in the late stages of the tests, yet no mesotheliomas developed in them.

In the three groups on preparations of chrysotile, all of the animals had died or were killed to close out those groups by 650 days. At that time, the final yield of mesotheliomas in those groups was 8, 9, and 10, respectively.

By comparison, the group on tremolite talc had developed no mesotheliomas by 650 days, and there were still 15 animals living and at risk in that group at that time.

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Eight of these survivors died of various causes between 650 and 700 days. No mesotheliomas were found in them.

The remaining seven survivors on tremolite talc were killed for examination at 700 days. No mesotheliomas were found in them.

Throughout the tests, animals treated with the preparations of chrysotile in table 5 were found to have thick fibrous pleural adhesions extending over large areas of the lung surfaces, whereas animals treated with tremolite talc never showed more than small depots of the mineral particles with relatively little tissue reaction.

In summary, our experiments show profound differences between the biologic effects of tremolite talc, in respect to both fibrogenicity and carcinogenicity, as compared with the biologic effects of the four principal types of asbestos: chrysotile, amosite, anthophyllite, and crocidolite.

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