

TGG: JCL: MMG: AJO: RF
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TO: Distribution

FROM: T. G. Grumbles
DATE: February 22, 1989

Interoffice
Communication

SUBJ: INORGANIC FIBER TOXICITY TESTING RESULTS

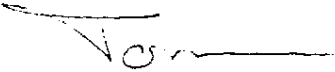
VISTA

Attached are three pieces of correspondence dealing with mineral fiber toxicity testing done by 3M. The test results were reported under Section 8(e) of TSCA.

The testing results confirm the difficulty of testing fibers with traditional toxicity testing protocols. The testing report also confirms the technical problems with inhalation studies when aerosols are necessary to effect exposures.

I don't believe the actual results add new information to the fiber toxicity database. They do further confirm the potential for lung damage and fibrosis as a result of exposure to high concentrations of fibers.

Anybody know what 3M is making?


T. G. Grumbles

dlj
.567

Attachment

cc: O. C. Kerfoot, Allen Nielsen-Ponca City, W. L. McClain

Dr. Gerald Vaughn
Professor of Zoology
University of Tennessee
Knoxville, TN 37916

Distribution: R. Poe, J. Fenton, W. R. Carradine-Ponca City
G. S. Hoenes, R. J. Tuttle

VVV 000000230

Frank A. Ubel, M.D.
Medical Director

2 pg.

COMPANY SANITIZED

8EHQ-0988-0752 S INIT.
88-880000060

3M

September 15, 1988

Document Processing Center (TS-790)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

SANITIZED

Attn: 8(e) Coordinator

Subject: TSCA 8(e) Notice of Substantial Risk

Dear Madam or Sir:

This notice is submitted pursuant to EPA's interpretation of Section 8(e) of the Toxic Substances Control Act. A preliminary verbal report of an animal toxicity test indicated that samples of inorganic fibers caused fibrosis following intratracheal insufflation in rats.

Sample Description

Inorganic fiber with diameter less than one micron and length ranging from less than 5 microns to greater than 100 microns.

Toxicity Study

Aqueous suspensions of sample were deposited in the lungs of albino rats by intratracheal insufflation. After six months, the lungs were fixed in 10% formalin, stained (hematoxylin and eosin) and examined by light microscope.

3M General Offices
Building 220-2E, 3M Center
St. Paul, Minnesota 55144-1000
612/733 5181

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Document Control Officer
Page 2
September 15, 1988

Results

Based on a verbal report, all samples caused pulmonary fibrosis to one degree or another.

Nature and Extent of Risk

This is a research and development material. About 1,000 kg have been manufactured in a closed process. During manufacture exposure may occur with transfer or shutdown and clean-up during which personnel use high efficiency filter respirators. No airborne fibers have been detected ($<.001$ fibers/cc). Approximately 200 potential customers have been sampled with quantities of less than 10 grams each and two customers have received kilogram quantities. The latter and all future recipients will be advised of these findings and that 3M has an exposure guideline of 0.2 fibers per cubic centimeter, time weighted average. They will also be advised to use high efficiency filter respirators and local exhaust ventilation.

A copy of the final toxicity study report will be submitted when available. If additional information is required, please contact:

Frank A. Ubel, M.D.
Director, Medical Department
3M Center, Bldg. 220-2E-02
St. Paul, MN 55144
612/733-5181

Sincerely,

Frank A. Ubel M.D.

bh (TS132.29)

Frank A. Ubel, M.D.
Medical Director

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18 pp

CONTAINS NO CBI

December 8, 1988

8EHQ-1288-0752 **3M** **FLWP** ...
IDCN 88-88000060
89-89000064

Document Processing Center (TS-790)
(Attn: Section 8[e] Coordinator)
Office of Toxic Substances
U. S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

63 DEC 22 PM 2:14
U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE

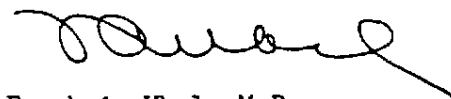
Re: EPA Document Control Number 8EHQ-0988-0752S

A confidential and a sanitized copy of the final report, "The Acute Toxicity of Fibrous Materials," is attached. The protocol, which has been non-confidential from its inception, is also attached. This submission was delayed because of the need to revise draft copies of the report.

3M has conducted no other toxicity studies and there are no plans to conduct such studies in the future.

Other information requested by the agency is being prepared and will be submitted when available.

The delay was discussed with EPA's Mr. David Williams.



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FAU/bh (TS140 2.26)

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SEP 22 PM 3:01
U.S. DEPARTMENT OF JUSTICE

THE ACUTE PULMONARY TOXICITY OF FIBROUS MATERIALS

SUBMITTED TO
F.D. GRIFFITH, PH.D.
3M
SAINT PAUL, MINNESOTA

BY
S.L. GAMBLE, M.S., INVESTIGATOR
J.W. CLAYTON, PH.D., STUDY DIRECTOR
COLLEGE OF PHARMACY
UNIVERSITY OF ARIZONA
TUCSON, ARIZONA
OCTOBER, 1968

VVV 000000234

S.L. Gamble
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Walter Clayton
J.W. CLAYTON, PH.D.

OCTOBER 3, 1988

VVV 000000235

INTRODUCTION

In this study animals were exposed to three inorganic fibers, asbestos, as a positive control, and saline as a negative control. Intratracheal instillation of particles was chosen as the method of exposure rather than inhalation exposure. There has been controversy over which method is the better choice. With the intratracheal instillation a suspension of particles in a carrier fluid is placed directly into the lumen of the trachea or airway. The carrier liquid is rapidly absorbed into the pulmonary circulation leaving the particles on the internal surface of the lung. In an inhalation exposure particles are deposited in the respiratory tract by inhalation of the aerosol by the test animals (1).

The obvious advantage of the inhalation exposure is that it is a closer approximation to the 'real-life' situation of inhalation of intoxicants in the environment. However the disadvantages can be difficult to overcome. The generation of an aerosol of appropriate concentration and size can be technically difficult. The equipment for aerosol generation and animal exposure is complex and expensive. There is unequal dosing of the animals which makes statistics difficult. If animals are subjected to whole body exposure, toxicants can be absorbed through the skin and/or ingested. This introduces the factor of multiple routes of exposure.

Advantages to intratracheal instillation include the fact that the technique is relatively simple and inexpensive. The dose is administered directly into the respiratory tract and can be measured more accurately than in an inhalation

exposure. Intratracheal instillation also allows the administration of a large and therefore, effective dose of particulate in a short period of time versus a lengthy period of exposure to an aerosol. The major drawback of intratracheal instillation is the high probability that patterns of particle distribution in the lungs may be uneven and unlike those resulting from inhalation.

The objective of this study was to make a preliminary evaluation of the acute pulmonary toxicity of three samples of fibrous materials. Asbestos is used as a positive control as it is known to produce fibrosis after intratracheal instillation. The three test fibers and chrysotile asbestos are described in Table 1.

METHODS AND MATERIALS

Thirty five male Sprague Dawley (approx. 200 g) rats were divided into five groups: saline control, asbestos control, and the three inorganic fiber groups. Each rat was anesthetized with ether, and their trachea exposed by surgical methods. The materials in physiological saline were instilled intratracheally into the rat lungs at the bifurcation of the bronchi with a 1cc syringe and a 26g needle in a 0.5 ml dose. One dose per animal was given during the duration of the experiment.

The inorganic fibers, labeled T4098, T4099, and T4100 and the chrysotile asbestos control, labeled T4097 are described in Table 1. They were weighed on a Sartorius balance and mixed with saline in a closed sterile test tube on a vortex mixer in order to evenly suspend the fibers.

After administration of the various doses the rats were ear tagged and their wounds closed with clips. Each rat was observed for recovery. Notes were made on wound appearance and how well the rats recovered. The animals were housed in the University of Arizona Animal Care Facility. They were caged 2 to 3 animals per cage and fed water and food ad libitum. A 12 hour light cycle and 50% relative humidity were maintained for the experimental duration.

For six months post injection several markers of toxicity were recorded. These were changes in respiration, loss or gain of body weight (animals were weighed 3 times per week for three weeks, then once a week), appearance, and

general behavior throughout the observation period. At six months rats were euthanized with an intraperitoneal injection of pentobarbital. The lungs were dissected from the heart lung block, weighed, and fixed in 10% buffered formalin after inflation. After 24 hours fixation the lungs were processed for histological examination. Rats which succumbed during the six month period were, lacking significant post mortem autolysis, subjected to necropsy and lung histology. Lungs were examined for lesions and the presence or absence of fibrosis.

RESULTS

The rats were dosed in two phases at different times. Initial dosages of asbestos were chosen based on earlier experiments with asbestos (2). Arbitrarily 25 mg was chosen as the initial dosage for the three test fibers. T4098 could not be suspended in saline at that dosage so it was lowered to 12.5 mg, although two weeks later after extended mixing a 25 mg dose was obtained. Since the animals took that initial level of dose well the levels were raised to 18.75 mg (T4098) and 50 mg (T4099, T4100). After observing the first phase for two weeks it was decided to dose the rest of the rats available at higher doses as the rats recovered rapidly at the dosage levels given. Table 1 indicates the total number of rats and the doses given. Six rats died during dosing. Two died from ether overdose. Three others died while the fiber dose was being instilled. Upon dissection it was noted that the dose lodged in the trachea and prevented breathing. The last rat died when an internal hemorrhage occurred not involving the lungs. Some animals were blowing black bubbles from their noses after dosing but all seemed to recover rapidly with no after effects, and a negligible loss of dose.

The test fibers had different consistencies and therefore varied in their ability to be suspended in saline. T4100 broke into small pieces easily and mixed well such that instillation of the fibers was performed without plugging the needle. T4099 was more difficult to break apart but it mixed well in saline. T4098 absorbed fluid so that it was difficult to break up. Force was required to push the sample through the needle and it tended to remain in clumps. The bulk of the fibers was the limiting factor in determining dosage amounts.

Other than two episodes of apparent viral disease, the last occurring at sacrifice, all animals appeared healthy. The rooms the animals were housed in were free from Mycoplasma and Sendai virus but not from salivary gland virus. The animals only occasionally sneezed and there were no signs of labored breathing, no lethargy, no definite weight loss, and no ruffled coats. Two rats died during the six month observation period. Rat 4922 (75 mg T4100) died at nine days. It was the only rat that experienced labored breathing and was lethargic after dosing. The lungs were removed and examined. The second animal, Rat 4925 (75 mg T4099), died approximately 167 days after dosing. Although the body was autolyzed the lungs were removed for examination. Tables 2 and 3 show the body weights of Phase 1 and 2 in relation to days after dosing.

The animals were sacrificed at 202 days (Phase 1) and 188 days (Phase 2) by intraperitoneal injection with sodium pentobarbital. The lungs and trachea were removed and the lungs inflated with 10% buffered formalin. Table 4 presents the lung weights for each animal.

After fixation in formalin the lungs were processed, sectioned and stained with hematoxylin-eosin and trichrome for examination by the pathologist. The results are presented in Table 5.

TABLE 1 DOSAGE GROUPS

<u>GROUP NO.</u>	<u>MATERIAL</u>	<u>NO. OF RATS</u>	<u>NO. OF RATS/DOSE(MG/RAT)</u>		
1	SALINE	3			
2	ASBESTOS	6	3(0.5)	3(7.5)	
3	T 4098	7	2(12.5)	2(18.25)	3(25)
4	T 4099	7	2(25)	2(50)	3(75)
5	T4100	6	2(25)	3(50)	1(75)

TABLE 2 PHASE I RAT BODY WEIGHTS

<u>DAYS</u>	<u>4901</u>	<u>4902</u>	<u>4903</u>	<u>4904</u>	<u>4905</u>	<u>4906</u>	<u>4907</u>	<u>4908</u>	<u>4909</u>	<u>4910</u>
<u>0</u>	223.0	232.6	228.3	229.0	221.2	225.4	223.2	232.7	230.5	227.8
<u>1</u>	205.0	206.6	226.0	232.6	219.1	229.5	225.8	231.5	230.7	205.3
<u>5</u>	219.1	182.0	251.1	256.4	251.9	260.0	261.7	255.3	261.1	227.0
<u>8</u>	225.3	184.4	247.1	263.9	260.4	269.0	263.5	256.6	272.1	241.8
<u>11</u>	251.0	204.8	274.1	288.8	276.8	290.0	288.0	276.5	290.0	258.8
<u>14</u>	260.0	224.5	278.3	288.0	287.0	298.0	289.0	282.0	300.5	264.0
<u>17</u>	270.5	235.0	287.0	311.0	300.0	298.6	305.5	290.6	306.5	273.7
<u>20</u>	275.4	243.8	289.4	318.0	309.7	307.5	318.4	298.5	316.2	274.0
<u>23</u>	283.6	257.2	296.5	320.0	320.0	308.4	323.5	307.9	323.0	291.0
<u>26</u>	294.0	277.1	310.0	326.6	329.0	307.0	326.0	305.0	328.5	299.5
<u>33</u>	311.5	300.1	330.7	342.2	339.5	323.3	343.4	320.0	345.8	322.8
<u>40</u>	332.6	317.6	353.7	359.7	360.0	344.0	364.6	339.5	360.3	342.7
<u>50</u>	350.0	333.7	364.3	363.2	371.5	345.3	375.0	350.0	377.0	344.2
<u>57</u>	360.2	337.8	373.0	376.0	377.3	355.5	380.0	357.4	391.5	350.0
<u>73</u>	384.2	360.0	396.4	397.5	404.4	380.0	401.0	375.4	413.0	382.0
<u>87</u>	388.5	360.0	404.0	402.8	416.0	395.5	409.6	375.0	420.5	386.8
<u>103</u>	387.0	370.0	408.0	400.0	413.0	401.5	415.0	375.0	428.0	390.0
<u>123</u>	416.0	386.0	430.0	427.5	433.5	412.0	436.5	400.0	456.0	407.5
<u>137</u>	428.0	396.0	444.0	441.5	445.0	420.0	453.0	410.0	466.0	420.0
<u>165</u>	426.0	403.0	450.0	414.5	426.8	405.5	440.0	396.5	457.5	422.5
<u>174</u>	425.0	404.5	453.0	422.0	445.0	408.0	433.0	398.5	452.0	414.0
<u>182</u>	425.0	408.5	460.0	438.0	461.0	425.0	444.0	403.0	465.0	428.0
<u>187</u>	428.5	408.0	457.0	435.0	451.0	417.0	448.5	400.0	465.0	427.0
<u>202</u>	433.0	418.5	447.0	428.5	455.0	417.5	462.0	411.0	472.5	424.5

TABLE 2 PHASE 1 RAT BODY WEIGHTS (CONT.)

<u>DAYS</u>	<u>4911</u>	<u>4912</u>	<u>4913</u>	<u>4914</u>	<u>4915</u>	<u>4916</u>	<u>4917</u>	<u>4918</u>
<u>0</u>	223.7	219.5	225.9	212.7	228.2	207.1	224.3	218.1
<u>1</u>	230.3	224.1	225.9	198.0	205.7	201.5	206.0	200.8
<u>5</u>	261.1	245.5	253.7	171.2	167.2	227.3	225.3	200.5
<u>8</u>	262.2	258.8	266.2	189.3	177.7	243.1	245.6	228.3
<u>11</u>	285.2	271.3	277.4	209.1	200.0	253.4	262.6	247.7
<u>14</u>	296.0	272.0	281.5	225.5	213.0	252.5	252.5	256.5
<u>17</u>	296.2	283.8	290.0	237.8	233.0	265.5	270.0	258.0
<u>20</u>	304.0	275.5	287.5	238.5	236.5	267.5	268.4	265.0
<u>23</u>	312.9	294.1	299.5	258.4	227.0	248.0	258.0	239.3
<u>26</u>	323.6	313.8	304.3	271.4	288.8	286.8	294.6	292.8
<u>33</u>	343.5	320.0	333.7	296.5	287.1	303.6	317.8	322.8
<u>40</u>	366.5	338.1	352.7	310.0	311.5	322.4	341.0	345.0
<u>50</u>	380.8	353.3	371.6	328.8	323.8	331.0	341.8	361.8
<u>57</u>	397.3	362.2	381.0	337.0	335.7	342.8	336.4	368.7
<u>73</u>	406.8	385.5	411.4	355.7	352.5	366.4	372.4	396.5
<u>87</u>	417.0	381.0	424.2	362.5	365.0	370.0	378.4	406.0
<u>103</u>	432.0	390.0	434.0	370.0	378.0	375.5	383.0	422.0
<u>123</u>	451.0	413.5	457.5	393.5	390.0	387.0	396.0	441.0
<u>137</u>	460.0	432.0	473.0	405.0	403.0	396.0	396.5	452.0
<u>165</u>	454.5	414.0	458.5	403.0	413.5	412.5	404.0	454.0
<u>174</u>	464.5	411.0	468.5	404.0	414.0	417.5	398.0	456.5
<u>182</u>	476.5	422.0	468.0	411.0	420.0	421.0	410.0	458.0
<u>187</u>	472.0	423.0	467.0	414.0	420.0	428.0	408.0	467.0
<u>202</u>	481.5	426.5	468.0	416.5	426.5	428.0	397.0	464.0

TABLE 3 PHASE 2 RAT BODY WEIGHTS

<u>DAYS</u>	<u>4919</u>	<u>4920</u>	<u>4921</u>	<u>4922</u>	<u>4923</u>	<u>4924</u>	<u>4925</u>	<u>4926</u>	<u>4627</u>	<u>4928</u>	<u>4929</u>
<u>0</u>	313.4	320.4	374.5	304.2	285.5	286.0	281.0	269.2	296.9	309.0	281.9
<u>1</u>	305.9	307.2	266.9	277.8	261.2	262.6	272.1	240.0	291.5	278.3	270.0
<u>3</u>	312.0	306.5	245.6	258.0	237.0	250.0	283.5	225.0	291.0	284.5	278.0
<u>6</u>	325.9	326.0	270.0	225.5	230.0	229.0	299.0	211.0	304.6	297.0	293.3
<u>9</u>	325.3	327.3	280.0	197.5	239.4	245.2	307.8	231.2	314.5	303.5	297.3
<u>12</u>	332.4	336.0	272.3	DEAD	272.5	252.4	326.1	247.5	323.4	317.4	302.4
<u>17</u>	345.4	351.0	297.7	----	308.0	292.2	332.0	265.7	332.5	340.0	312.3
<u>19</u>	348.4	362.6	304.5	----	314.6	304.9	338.2	281.0	337.0	350.0	313.2
<u>26</u>	363.1	381.8	321.0	----	347.5	335.5	363.4	311.0	366.5	372.6	337.1
<u>36</u>	360.0	374.4	318.5	----	358.5	347.9	367.0	326.0	372.0	377.0	330.0
<u>43</u>	373.5	380.0	327.0	----	361.4	353.8	371.0	326.2	381.1	385.0	353.8
<u>59</u>	410.9	417.1	356.9	----	394.5	384.4	410.0	357.4	417.7	422.5	381.5
<u>73</u>	422.0	430.0	352.5	----	405.8	392.0	418.8	372.0	430.0	440.0	384.0
<u>89</u>	416.0	432.0	352.0	----	413.0	393.0	409.0	376.5	438.0	446.5	376.5
<u>109</u>	426.0	441.0	373.0	----	436.0	413.0	427.0	399.0	457.0	461.0	404.0
<u>123</u>	443.0	460.0	384.5	----	454.0	433.0	450.0	411.0	471.0	480.0	415.5
<u>151</u>	439.1	460.0	375.0	----	440.0	428.5	448.0	407.5	476.0	475.5	408.0
<u>160</u>	435.0	454.0	374.0	----	434.0	433.0	452.0	407.0	462.0	478.0	409.0
<u>168</u>	444.5	464.0	382.5	----	438.0	431.0	DEAD	419.0	464.0	484.0	410.0
<u>173</u>	445.0	459.0	368.0	----	438.0	437.5	----	427.0	460.0	484.0	416.0
<u>188</u>	442.0	464.0	377.0	----	430.0	447.0	----	431.0	464.5	472.0	404.5

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TABLE 4 LUNG WEIGHTS AT SACRIFICE

<u>RAT</u>	<u>WT/GMS</u>	<u>LUNG/BODY</u>	<u>RAT</u>	<u>WT/GMS</u>	<u>LUNG/BODY</u>
4904-saline	5.99	1.40	4928	7.07	1.50
4905	7.68	1.69	4929	10.30	2.55
4906	7.70	1.84	4902-4099/25	7.71	1.84
4907-asb/0.5	6.70	1.45	4911	8.22	1.71
4908	6.37	1.55	4915-4099/50	9.17	2.15
4909	7.04	1.49	4916	7.60	1.78
4919-asb/7.5	6.00	1.36	4924-4099/75	6.00	1.34
4920	9.86	2.13	4925	5.34	1.18
4921	7.25	1.92	4926	6.37	1.48
4903-4098/12	7.64	1.71	4901-4100/25	6.56	1.52
4912	9.08	2.13	4910	10.66	2.51
4913-4098/18	7.39	1.58	4917-4100/50	9.09	2.29
4914	8.88	2.13	4918	12.33	2.66
4927-4098/25	8.48	1.83	4923	11.78	2.74

TABLE 5. PATHOLOGY RESULTS

15

		LESIONS	FIBROSIS (+ OR -)
<u>SALINE</u>	<u>4904</u>	No significant lesions*	-
	<u>4905</u>	No lesions	-
	<u>4906</u>	No lesions	-
<u>ASBESTOS</u>	<u>4907</u>	Peribronchial granulomas with giant cells and epithelioid cells, pneumonia	+
<u>0.5 MG</u>	<u>4908</u>	No lesions	-
	<u>4909</u>	Peribronchial granulomas with giant cells and epithelioid cells, pneumonia, mild bronchiolitis obliterans	+
<u>7.5 MG</u>	<u>4919</u>	Mild peribronchiolar granulomas, mild pneumonia	+
	<u>4920</u>	Alveolar duct macrophages, mild peribronchiolar granulomas, edema and marked congestion	+
	<u>4921</u>	No significant lesions	+
<u>T4098</u>	<u>4903</u>	Alveolar duct macrophages, mild pneumonia	-
<u>12.5 MG</u>	<u>4912</u>	No significant lesions	-
<u>18.75 MG</u>	<u>4913</u>	Regular and brown pigmented macroph.	+
	<u>4914</u>	peribronchiolar nodules of brown macro. and/or with black fibers in association with fibrosis, bronch. obliterans	+
<u>25 MG</u>	<u>4927</u>	mild peribronchiolar nodules of brown macro. and/or black fibers, mild bronchiolitis obliterans	+
	<u>4928</u>	mild alveolar duct macrophages	+
	<u>4929</u>	very congested, brown pigment in macrophages around trachea	+
<u>T4099</u>	<u>4902</u>	Mild aggregates of alveolar duct macro., alveolar duct brown macro., Peribronchiolar granulomas of brown macro. and black fibers, mild bronch. obliterans, some distortion of small airways by fibrosis	+
<u>25 MG</u>	<u>4911</u>	Similar results (4902) seen in extra-pulmon. location, lung essentially normal	+
<u>50 MG</u>	<u>4915</u>	Peribronch. granulomas of brown macro. and black fibers with mod. bronch. obliterans	+
	<u>4916</u>	Alveolar duct foamy macrophages	+
<u>75 MG</u>	<u>4924</u>	Brown alveolar duct macro. peribr. granulomas with brown macro. and/or black	++

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		fibers, mild bronch. obliterations	
	<u>4925</u>	Animal died, too autolyzed, unable to evaluate	
	<u>4926</u>	Peribronchiolar granulomas of brown macroph. and/or black fibers, mild bronch. obliterations	++
<u>T4100</u>	<u>4901</u>	Mild alveolar duct macrophages	-
<u>25 MG</u>	<u>4910</u>	Alveolar duct macrophages, peribronchiolar nodules of black macro. or brown macro. with black fibers	+
<u>50 MG</u>	<u>4917</u>	Moderate peribronch. nodules of brown macro. or brown macro. with black fibers, moderate bronchiolitis obliterations	+
	<u>4918</u>	Mild alveolar duct macrophages peribronch. nodules of brown macro. or brown macro. with black fibers	+
	<u>4923</u>	Peribronch. nodules of brown macro. or brown macro. with black fibers	+
<u>75 MG</u>	<u>4922**</u>	Moderate to severe peribronchiolar granulomas consisting mostly of black fibers with bronchiolitis obliterations, an acute pneumonia with hemorrhage and edema	-

* Other than low level infection, no significant changes.
**Died nine days following intratracheal instillation.

DISCUSSION

The test mineral fibers made dosing difficult. Two, T4099 and T4100, made heavy suspensions in saline such that an uneven dose was given as the fibers settled quickly from the saline. T4098 absorbed the saline at the small quantities of saline involved producing clumps of fibers. Several animals were found to have clumps of fibers outside of the lungs, in or along the trachea.

For so many variables there were too few animals to obtain reproducible results. However, dosage levels were determined because of the physical properties of the fibers. Any higher than 25 mg per animal of T4098 would make it impossible to be suspended in saline and to get through the trachea. Of three animals attempted for T4100 at 75 mg per animal two died during dosing and one died nine days after dosing. Dosing at 50 mg was accepted by the animals. T4099 at 75 mg was tolerated by the animals and was the most easily suspended in saline, however the lesions and fibrosis were more severe.

The saline control animals were negative except for a low level infection probably due to salivary gland virus. The asbestos control animals produced fibrosis at both dosage levels.

The presence or absence of fibrosis was used as the infection evidence. All three fibers produced fibrosis at the highest levels. T4098 produced less severe fibrosis than asbestos. T4100 caused fibrosis more severe than asbestos but both were less severe than T4099.

REFERENCES

1. Brain, J.D., and Volberg, P.A. 1979. Deposition of Aerosol in the Respiratory Tract. Am. Rev. Resp. Dis. 120: 1325-1373.
2. Grimm, S., Wiersma, D.A., Clayton, J.W. 1986. Effects of Oral Difluoridone on Chrysotile Asbestos-Induced Pulmonary Fibrosis in Hamsters. Toxicologist 6:134.

UNT 189366

3M Medical Department
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I 8/25
8EHQ-0189-0752 FLWP
PDCN 88-880000060
89-890000067

CONTAINS NO CBI

EPA-OTS



3M

January 4, 1989

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Document Processing Center (TS-790)
(Attn: Section 8(e) Coordinator)
Office of Toxic Substances
U. S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

69 JAN -9 PM 2:15
OTS DOCUMENT CONTROL
OFFICE

Re: EPA Document Control Number 8EHQ-0988-07525

A copy of the protocol for "The Acute Toxicity of Fibrous Materials" is attached as requested by Judy Deranger on January 4, 1989.

Neither the protocol nor the cover letter contains confidential information.

F. D. Griffith
F. D. Griffith, Ph.D.
Manager, Toxicology Services
(612-733-7635)

FDG/bh (TS143 2.20)

Enclosure

VVV 000000251

THE ACUTE PULMONARY TOXICITY
OF
FIBROUS MATERIALS

a Proposal

Submitted to
F.D. Griffith, Ph.D.
3M
Saint Paul, Minnesota

F.D. Griffith
9-7-87

J. Wesley Clayton
by
J. Wesley Clayton, Ph.D.
Department of Pharmacology and Toxicology
College of Pharmacy
University of Arizona

September 1987

Acute Pulmonary Toxicity of Fibrous Materials

Introduction

The objective of this proposal is to evaluate the acute pulmonary toxicity of three samples of fibrous materials which will be supplied by 3M. In this study, the test materials and an asbestos control will be suspended in physiological saline and injected intratracheally into the rats lungs at the bifurcation of the bronchi. Several markers of toxicity will be recorded at regular intervals throughout a post-injection period of six months, at which time, the surviving rats will be killed for necropsy and lung histology. Rats which succumb during the dosing or observation periods will, lacking significant post-mortem autolysis, be subjected to necropsy and histology of the lungs.

In the proposed study, the markers of toxicity that will be recorded are: changes in respiration, loss or gain in body weight, appearance, and general behavior throughout the observation period post-injection. The test procedure that is proposed is widely accepted in toxicology. A review which covers this area is Henderson (1984). A paper reporting specific results is Henderson, et al (1978, 1979). The results of the proposed study will be compared between and among control and test groups. Post-mortem autolysis potentially occurring from spontaneous deaths will be evaluated by Dr. Susan Wilson, DVM.

Test Materials

All samples will be sent to the Principal Investigator, J. Wesley Clayton, College of Pharmacy, the University of Arizona, Tucson, Arizona 85721. Although an adequate description of the test materials and the asbestos control has not yet been received, the list below shows the number of samples to be tested.

1. Saline control
2. Asbestos (positive control)
3. Fiber 1 chemical and physical properties will be supplied by 3M

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4. Fiber 2 chemical and physical properties will be supplied by 3M
5. Fiber 3 chemical and physical properties will be supplied by 3M

One dose of control or test materials will be intratracheally injected. The study will not result in a dose-response curve; results will be compared between and among the various groups of rats.

Work Plan

Sprague-Dawley, male rats identified with ear tags will be obtained from Hilltop Farms. The intratracheal injections will begin after a two-week quarantine period for the animals. During this time the animals will be housed in the Division of Animal Resources of the Arizona Health Sciences Center. Light cycle 12 on 12 hr - off and humidity at 50% RH will be controlled. Rats will be housed 4 per cage. Feed (Wayne Lab. Blocs) and water will be available ad libitum except immediately following dosing. Fasting will be 24 hours post-dosing. Cages will be changed twice weekly. The animals will be weighed regularly and examined by trained personnel. Dr. Wilson, DVM will advise as to the suitability of individual animals for the test. Rats will then be randomly assigned to three test and two control groups for each species. Each group will consist of ten male rats (175-200 grams). The intratracheal dosages could range from 0.5 mg per rat to 5 mg per rat. This dose range has been used in previous studies, Stoner et al 1985, Grimm et al 1986. The volume of the dose will be 0.5 ml of 0.15 M NaCl. To administer the dose of fibrous material, the animal will be anesthetized with methohexital. After anesthesia has been established a blunted, 19-gauge needle, attached to a syringe containing the fibers will be inserted into the trachea through the mouth and vocal folds. This procedure can be completed while the animal is fully anesthetized. Each rat will then be returned to its cage for observation. During the subsequent observation period of six months, the animals will be observed for survival, signs of toxicity (such as changed respiration) and body weights which will be measured three times weekly during the isolation period and the three weeks post-injection.

Thereafter, until termination of the study, the rats will be weighed weekly and at euthanasia, which will be done by intraperitoneal injection of pentobarbital. The inflated lungs will be dissected free from the heart lung bloc, weighed and fixed in 10% formalin instilled through the trachea, filling the lungs. The lung tissue will be stained with hematoxylin and eosin for microscopic examination. The data resulting from this study will be tabulated, graphs made of body weights plotted against time, and the pathologist's report. Animal remains will be incinerated.

The data from the proposed project will be recorded in bound laboratory notebooks along with SOPs. These items, along with correspondence and other applicable documents, tissues, blocks, slides and photomicrographs will be retained in the archives of the Quality Assurance Office, Room 252 in the College of Pharmacy, Dr. Clayton's laboratory. All reports will be prepared on a Xerox 860 Word Processor and stored on "write-protected" disks. All statistical computations (ANOVA, standard method, Grimm, et al., 1986) will be filed and accessible for review. The Quality Assurance Unit will audit and validate data entries and reports.

Facilities

The study proposed will be conducted in the laboratories located on the second floor of the College of Pharmacy at the Arizona Health Sciences Center, Tucson, Arizona 85721, which is the area occupied by the Department of Pharmacology and Toxicology. Animal work consisting of intratracheal injections will be carried out in the inhalation toxicology laboratory. Animals will be housed in the Division of Animal Resources connected by a limited access tunnel to the second floor of the College of Pharmacy.

(1) Animals, Necropsy, and Storage. The Division of Animal Resources is accredited by the American Association for Accreditation of Laboratory Animal Care. It is under the direction of Susan Wilson, D.V.M. This division has provided us with a double room that contains a high velocity hood with filtering systems. Histopathology services,

including interpretation by a veterinary pathologist, are available through the Division of Animal Resources. Prior to the conduct of animal studies at the University of Arizona, the proposal, written in lay language, must be approved by the Committee on Animal Care. This process has often required three weeks.

(2) Safety Procedures. The College of Pharmacy is a part of the University of Arizona Risk Management and Radioactive Safety programs. All hazardous materials are managed by this division, including receipt, inventory while on the premises, and disposal at approved sites maintained by the University of Arizona. In addition, this division provides classes for employees on safe handling of radioactive and toxic chemicals. They also monitor the laboratories to ensure that personnel are adequately protected.

(3) Good Laboratory Practice. The University of Arizona, Department of Pharmacology and Toxicology, has instituted procedures for compliance with GLP regulations. A departmental Quality Assurance Unit has been established to monitor toxicity studies conducted under grant or contract. DEC Rainbow computer with a printer will be used to store and retrieve data generated in this study in order to assure proper auditing and validation of laboratory data.

(4) Biostatistics. The University provides a resource center to aid in experimental design and statistical analysis of the data. In addition Dr. Thomas Moon of the Cancer Center and John Gaines of the Medical Center Biostatistics group are available for consultation.

The criteria used to evaluate the results and accomplishments of the proposed project will be the deviations of the test groups from the controls. The significance of the deviations will be based on a confidence level of $p < 0.05$ to assist in the judgment of effect.

(5) Personnel Responsibilities

All aspects of the project will be conducted in the laboratories of inhalation toxicology and biochemical toxicology located on the second floor of the College of Pharmacy and the adjacent Division of Animal Resources. The Principal Investigator, J. Wesley Clayton, will have the overall responsibility for the management of the project. This includes preparation of the proposal to the Committee on Animal Care; hiring of personnel, setting the Quality Assurance plan; developing Standard Operating Procedures with the Research Technician, preparing and assembling the requisite reports. The Principal Investigator will monitor the accounting and approve all orders for the purchase of equipment and supplies.

A Research Technician will see to it that SOP's are prepared and followed, check animals for suitability for the experiments, check solutions, prepare orders for reagents, maintain the stock of laboratory supplies, make data entries into the laboratory notebooks, and into the statistical program on the DEC Rainbow computer.

VVV 000000257

References

Henderson, R. F. (1984) Use of bronchoalveolar lavage to detect lung damage. Environ. Health Persp. 56, 115-129.

Henderson, R. F., Damon, E. G., and Henderson, T. R. (1978). Early damage indicators in the lung. I. Lactate dehydrogenase activity in the airways. Tox. Appl. Pharm. 44, 291-297.

Henderson, R. F., Rebar, A. H., and DeNicola, D. B. (1979). Early damage indicators in the lung. IV. Biochemical and cytologic response of the lung to lavage with metal salts. Tox. Appl. Pharm. 51, 129-135.

Stoner, J. S., J. W. Clayton, and S. D. Wilson 1985 Early Indicators of Pulmonary Change Induced by Combustion-Generated Particulates. Toxicologist 5:34.

Grimm, S., D. A. Wiersma, and J. W. Clayton 1986. Effects of Oral Difluoromethylornithine on Chrysotile Asbestos-Induced Pulmonary Fibrosis in Hamsters. Toxicologist 6:134.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

AUGUST FILE COPY

DATE: SEP 29 1988

Page 1 of 3

SUBJECT: Status Report* 8EHQ-0988-0752 S

Approved: *James F. Darr 9/30/88*FROM: David R. Williams, ^{DEW} Section 8(e) Coordinator
Chemical Screening Branch/ECADTO: James F. Darr, Section Head
Chemical Risk Identification Section/CSB/ECADNote

The 3M Company claimed the exact identity of the subject chemical substance to be TSCA Confidential Business Information (CBI); the Information Management Division (IMD/OTS) will be requesting 3M to substantiate this TSCA CBI claim. In the "sanitized" (i.e., non-confidential) version of its TSCA Section 8(e) submission, 3M stated that the subject chemical was an "inorganic fiber with [a] diameter [of] less than one micron and length ranging from less than 5 microns to greater than 100 microns."

Submission Description

3M reported that "aqueous suspensions of [inorganic fiber] sample were deposited in the lungs of albino rats by intratracheal insufflation" and "after six months the lungs were fixed in 10% formalin, stained (hematoxylin and eosin) and examined by light microscope." According to 3M, verbal reports received by the company indicate that "all samples caused pulmonary fibrosis to one degree or another." Finally, 3M stated that a copy of the final report from this study would be submitted to the Agency when that report becomes available.

Immediately upon receipt of this TSCA Section 8(e) submission, the Chemical Screening Branch provided copies of the submission to staff of the Chemical Control Division (CCD/OTS) for inclusion in the ongoing OTS review of available toxicological and exposure data on a number of man-made and naturally-occurring fibers.

Submission Evaluation

An EPA evaluation of the overall significance of the reported findings should be possible upon EPA's receipt of a full copy of the final report (including the actual experimental protocol, the results of gross/histopathological examinations, the results of statistical analyses, etc.) from the intratracheal insufflation study cited in the company's TSCA Section 8(e) submission.

- =====
- NOTE: This status report is the result of a preliminary evaluation of information submitted to EPA pursuant to Section 8(e), the substantial risk information reporting provision of the Toxic Substances Control Act (TSCA). The statements made in this report should not be regarded as expressing final EPA policy or intent with respect to the subject chemical(s). Any review of this status report should take into account the fact that the report may be based on incomplete information.

Current Production and Use

In view of 3M's TSCA CBI claim, no information about the initial TSCA Chemical Substance Inventory status of this inorganic fiber will appear in this status report.

In its TSCA Section 8(e) submission, 3M provided the following non-confidential information concerning the manufacture of and the potential for exposure to this inorganic fiber:

"This [inorganic fiber] is a research and development [(R&D)] material. About 1,000 kg have been manufactured in a closed process. During manufacture, exposure may occur with transfer or shutdown and clean-up during which personnel use high efficiency filter respirators. No airborne fibers have been detected (<.001 fibers/cc). Approximately 200 potential customers have been sampled with quantities of less than 10 grams each and two customers have received kilogram quantities. The latter and all future recipients will be advised of these [toxicological] findings and that 3M has an exposure guideline of 0.2 fibers per cubic centimeter [(cc)], time weighted average [(TWA)]. They will also be advised to use high efficiency filter respirators and local exhaust ventilation."

Comments/Recommendations

It should be noted that the Office to Toxic Substances (OTS) has received a number of TSCA Section 8(e) and "For Your Information" (FYI) submissions on a variety of naturally-occurring and man-made fibers.

- a) The Chemical Screening Branch will ask the 3M Company to ensure that the Agency receives a complete copy of the final report (including the actual experimental protocol, results of gross/histopathological examinations, results of any statistical analyses, etc.) from the cited intratracheal insufflation study.

In view of EPA's general interest in corporate actions taken on a voluntary basis in response to new chemical toxicity/exposure data, 3M will be asked to describe the nature and results, if available, of all studies (other than those reported already to EPA or those cited in the scientific literature) about which 3M is aware or that 3M has conducted, is conducting or plans to conduct that are designed to determine either the toxicity of or the exposure to these inorganic fibers.

- b) The Chemical Screening Branch will immediately provide copies of all reported information to the CCD/OTS for review and appropriate followup attention.

- c) The Chemical Screening Branch will send copies of this status report to NIOSH, OSHA, CPSC, FDA, NTP, OW/EPA, OSWER/EPA, OAR/EPA, ORD/EPA, CCD/OTS and RAB/ECAD/OTS; copies of this report will be sent also to the TSCA Assistance Office (TAO/OTS) for further distribution.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 30 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

CERTIFIED MAIL

Dr. F. A. Ubel
Medical Director
Building 220-2E-02
3M Company
St. Paul, MN 55144-1000

Dear Sir:

With regard to:

TSCA Section 8(e) submission on: Inorganic Fibers

Submitted by: 3M Company

Date submitted: September 15, 1988

EPA Document Control Number: 8EHQ-0988-0752 S

The Office of Toxic Substances (OTS) has completed a preliminary evaluation of the above referenced submission under Section 8(e), the "substantial risk" information reporting provision of the Toxic Substances Control Act (TSCA). The enclosed status report is the result of that preliminary OTS evaluation but does not necessarily represent EPA's conclusion on the subject inorganic fibers.

With regard to the above referenced TSCA Section 8(e) submission, please ensure that EPA receives a full copy of the final report (including the actual experimental protocol, results of gross and histopathologic examinations, results of statistical analyses, etc.) from the intratracheal insufflation study that was cited in the submission.

In view of EPA's general interest in corporate actions that are taken on a voluntary basis in response to chemical toxicity or exposure information, please describe the nature and results, if available, of all studies (other than those submitted already to the Agency or those published in the open scientific literature) about which 3M is aware or that the company has conducted, is conducting, or plans to conduct that are designed to determine the toxicity of or the exposure to these inorganic fibers.

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In responding to this request for information, or in otherwise communicating with EPA regarding this submission under Section 8(e), please refer to the EPA Document Control Number that has been assigned to the submission. As in the case of initial 8(e) submissions, all responses/correspondence will be placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA Section 8(e) policy statement ("Statement of Interpretation and Enforcement Policy; Notification of Substantial Risk" 43 FR 11110; March 16, 1978). Any confidentiality claims should be supported by submission of information as described in the enclosed item entitled "Support Information for Confidentiality Claims."

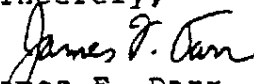
All available information requested by this letter should be transmitted to the EPA Document Processing Center at the address provided below within 20 working days of your receipt of this letter; any requested information or supplemental information that becomes available following your response to this EPA letter should be sent to EPA immediately upon your company's receipt of such information.

Document Processing Center (TS-790)
(Attn: Section 8(e) Coordinator)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, D.C. 20460

Should you have any questions or comments prior to responding to the Agency's request for additional information, please contact Mr. David R. Williams of the Chemical Screening Branch/ECAD at (202)-382-3468.

The Environmental Protection Agency looks forward to continued cooperation with 3M in its ongoing efforts to evaluate and minimize the potential risks posed by chemical substances to health or the environment.

Sincerely,


James F. Darr, Section Head
Chemical Risk Identification
Section/CSB/ECAD (TS-778)

Enclosures

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