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THE ACUTE PULMONARY TOXICITY OF FIBROUS MATERIALS

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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 anesthesized 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 pentobarbitol. 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

GROUP NO.	MATERIAL	NO. OF RATS	NO. OF RATS/DOSE(MG/RAT)		
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 1 RAT BODY WEIGHTS

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

DAYS	4911	4912	4913	4914	4915	4916	4917	4918
0	223.7	219.5	225.9	212.7	228.2	207.1	224.3	218.1
1	230.3	224.1	225.9	198.0	205.7	201.5	206.0	200.8
5	261.1	245.5	253.7	171.2	167.2	227.3	225.3	200.5
8	262.2	258.8	266.2	189.3	177.7	243.1	245.6	228.3
11	285.2	271.3	277.4	209.1	200.0	253.4	262.6	247.7
14	296.0	272.0	281.5	225.5	213.0	252.5	252.5	256.5
17	296.2	283.8	290.0	237.8	233.0	265.5	270.0	268.0
20	304.0	275.5	287.5	238.5	236.5	267.5	268.4	265.0
23	312.9	294.1	299.5	258.4	227.0	248.0	258.0	239.3
26	323.6	313.8	304.3	271.4	288.8	286.8	294.6	292.8
33	343.5	320.0	333.7	296.5	287.1	303.6	317.8	322.8
40	366.5	338.1	352.7	310.0	311.5	322.4	341.0	345.0
50	380.8	353.3	371.6	328.8	323.8	331.0	341.8	361.8
57	397.3	362.2	381.0	337.0	335.7	342.8	336.4	368.7
73	406.8	385.5	411.4	355.7	352.5	366.4	372.4	396.5
87	417.0	381.0	424.2	362.5	365.0	370.0	378.4	406.0
103	432.0	390.0	434.0	370.0	378.0	375.5	383.0	422.0
123	451.0	413.5	457.5	393.5	390.0	387.0	396.0	441.0
137	460.0	432.0	473.0	405.0	403.0	396.0	396.5	452.0
165	454.5	414.0	458.5	403.0	413.5	412.5	404.0	454.0
174	464.5	411.0	468.5	404.0	414.0	417.5	398.0	456.5
182	476.5	422.0	468.0	411.0	420.0	421.0	410.0	458.0
187	472.0	423.0	467.0	414.0	420.0	428.0	408.0	467.0
202	481.5	426.5	468.0	416.5	426.5	428.0	397.0	464.0

TABLE 3 PHASE 2 RAT BODY WEIGHTS

DAYS	4919	4920	4921	4922	4923	4924	4925	4926	4627	4928	4929
0	313.4	320.4	374.5	304.2	285.5	286.0	281.0	269.2	296.9	309.0	281.9
1	305.9	307.2	266.9	277.8	261.2	262.6	272.1	240.0	291.5	278.3	270.0
3	312.0	306.5	245.6	258.0	237.0	250.0	283.5	225.0	291.0	284.5	278.0
6	325.9	326.0	270.0	225.5	230.0	229.0	299.0	211.0	304.6	297.0	293.3
9	325.3	327.3	280.0	197.5	239.4	245.2	307.8	231.2	314.5	303.5	297.3
12	332.4	336.0	272.3	DEAD	272.5	252.4	326.1	247.5	323.4	317.4	302.4
17	345.4	351.0	297.7	----	308.0	292.2	332.0	265.7	332.5	340.0	312.3
19	348.4	362.6	304.5	----	314.6	304.9	338.2	281.0	337.0	350.0	313.2
26	363.1	381.8	321.0	----	347.5	335.5	363.4	311.0	366.5	372.6	337.1
36	360.0	374.4	318.5	----	358.5	347.9	367.0	326.0	372.0	377.0	330.0
43	373.5	380.0	327.0	----	361.4	353.8	371.0	326.2	381.1	385.0	353.8
59	410.9	417.1	356.9	----	394.5	384.4	410.0	357.4	417.7	422.5	381.5
73	422.0	430.0	352.5	----	405.8	392.0	418.8	372.0	430.0	440.0	384.0
89	416.0	432.0	352.0	----	413.0	393.0	409.0	376.5	438.0	446.5	376.5
109	426.0	441.0	373.0	----	436.0	413.0	427.0	399.0	457.0	461.0	404.0
123	443.0	460.0	384.5	----	454.0	433.0	450.0	411.0	471.0	480.0	415.5
151	439.1	460.0	375.0	----	440.0	428.5	448.0	407.5	476.0	475.5	408.0
160	435.0	454.0	374.0	----	434.0	433.0	452.0	407.0	462.0	478.0	409.0
168	444.5	464.0	382.5	----	438.0	431.0	DEAD	419.0	464.0	484.0	410.0
173	445.0	459.0	388.0	----	438.0	437.5	----	427.0	460.0	484.0	416.0
188	442.0	464.0	377.0	----	430.0	447.0	----	431.0	464.5	472.0	404.5

TABLE 4 LUNG WEIGHTS AT SACRIFICE

RAT	WT/GMS	LUNG/BODY	RAT	WT/GMS	LUNG/BODY
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

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

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

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

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