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CHRONIC INHALATION TOXICOLOGY OF FIBROUS GLASS IN RATS AND MONKEYS

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Abstract—A long term inhalation study was conducted with 500 F344 rats and 60 Cynomolgus monkeys in 5 treatments, in 4 of which they were exposed to aerosolized glass fibres of varying geometry and mass. Exposures of 5 or 15 mg/m^3 with long or short lengths, with and without binder were provided for 18 months to monkeys and for 21 months to rats which were subsequently held to 80% mortality. Biological responses evaluated include life table analysis, body weights, clinical signs, haematological testing, respiratory function, ophthalmological examinations, clinical biochemical analysis, and gross and microscopic pathological examinations. Both species demonstrated pulmonary macrophage aggregates and granulomata containing fibrous glass. Rats had grossly visible pleural plaques which were not seen in the monkeys. Fibrogenicity or carcinogenic responses were not seen except for a significantly increased incidence of mononuclear cell leukaemia in each fibre-exposed rat group.

INTRODUCTION

NIOSH (1974) sponsored an International Symposium to assist in the development of health effects criteria for occupational exposure to fibrous glass. In 1977 a criteria document was published which critically reviewed the research data and recommended a 5 mg/m^3 (total dust) standard. Two categories of glass fibres were studied with fibres exceeding 3.5 μm diameter considered as large fibres. These large fibres were found to be related to skin, eye, and upper respiratory tract irritation. A low incidence of pulmonary fibrosis and an excess of non-malignant respiratory tract disease was also found. With regard to small diameter fibres, animal studies have been able to demonstrate the potential for carcinogenic responses; however, the test methods have employed artificial routes of exposure which limit the direct extrapolation of these data to conditions of worker exposure. The criteria document specified that 'a need exists for studies of the effects of small diameter (< 3.5 μm dia.) on specific cohorts over long periods of time . . . Questions remain concerning the effect of fibrous glass larger than 3.5 μm diameter'. NIOSH (1977) considers occupational exposure to fibrous glass to present a greater hazard than exposure to inert or nuisance particulate.

Animal experimentation has well served the role of demonstrating the potential biological responses to fibrous glass. Implantation techniques with fibres of varying sizes have shown that biological activity relates to fibre geometry (STANTON and WRENCH, 1972; POTT, HUTH and FRIEDERICHS, 1976; WAGNER, BERRY and SKIDMORE, 1976; DAVIS, 1972). Long thin respirable fibres have been shown to produce tumours. Pulmonary fibrosis has also been demonstrated by WRIGHT and KUSCHNER (1975) and JOHNSON and WAGNER (1979). Fibres, including fibrous glass have also demonstrated the potential to produce genotoxic effects in *in vivo* systems (SINCOCK, 1977).

The object of this investigation was to conduct a long-term low level inhalation study with carefully characterised fibres for the purpose of: (1) assessing the adequacy of the OSHA standard and the NIOSH recommended standard relative to airborne particulate mass; (2) characterising the pulmonary responses from a physiological (obstructive or restrictive) and pathological (fibrotic or carcinogenic) approach.

EXPERIMENTAL METHODS

Animals

Sixty young male Cynomolgus monkeys and 500 Fischer 344 rats (received at 5 weeks of age) were randomly assigned to 5 experimental groups (4 exposed and 1 control for each species). The monkeys were obtained from Charles River Laboratories, Long Island, New York, and the rats from Charles River Laboratories, Wilmington, Massachusetts. All animals were quarantined and evaluated by experienced veterinarians in AALAC accredited facilities. The rats were randomly housed in individual cage compartments alternating male and female animals. The monkeys were kept in individual cages. All animals were fed Purina (rat or monkey) Chow *ad libitum* following daily exposures. The weights of all animals were recorded weekly for the first month and biweekly thereafter.

The animals were exposed for 7 hours per day, 5 days per week, for periods of 72 weeks/18 months (monkeys) and 86 weeks/21 months (rats), excluding holidays. The exposure chambers used were of the type described by HINNERS *et al.* (1968), featuring tangential inlets and constructed of stainless steel. They measured 1.83M square with a nominal volume of 5.43M and were maintained with a conditioned airflow of $1M^3$/min. The targeted exposure conditions were as follows: Treatment 1 geometry—>3.5 μm dia.; >20 μm/long, mass—15 mg/M^3, with binder. Treatment 2 geometry—<3.5 μm dia.; >10 μm long, mass—15 mg/M^3, with binder. Treatment 3 geometry—<3.5 μm dia.; >10 μm long, mass—5 mg/M^3, without binder. Treatment 4 geometry—<3-5 μm dia.; <10 μm long, mass—5 mg/M^3, without binder, and Treatment 5, filtered and conditioned air (control group).

Commercial grade glass fibres were used that are found in filtering and insulating products. The initial selection criteria were based on quantity production and fibre diameter in the needed size ranges. Based on the grinding and air classification testing, FG Insulation Fiberglass and FM Series Air Filter Media (Owens-Corning Fiberglass Corp. Newark, Ohio) were used for treatments 1 and 2 respectively and contained phenol-formaldehyde binder. Tempstran Code 100/475 glass fibre (Manville Corp., Denver, Colorado) was selected for treatments 3 and 4 where no binder was required.

Fibre Preparation

The fibre product selected generally met the diameter requirements; however, to meet the length requirements of the four different fractions, a series of procedures were employed. All materials required an initial grinding step and after evaluating numerous types of mills the Willy Bleuler Aperatebua ring mill grinder (Zollicon-Schweiz, Switzerland) was selected. Further processing was then necessary to remove under/oversized particles. Various techniques including centrifuging, wet filtration, settling, sonification and air classification were used to remove under/oversized fibre fractions.

Fibre Aerosol Generation

Different systems were used for different aerosols including some changes during the study; however, each system consisted of a metering device, a dispersing mechanism, and a delivery system. Bead chain feeders similar to that described by MARPLE *et al.* (1978) were eventually replaced by venturi aspiration, fluid atomisers because of extensive maintenance and improved efficiency for treatments 1 and 2. Similar improvement was seen by replacing the bead chain feeders used in treatments 3 and 4 with rotary platform feeders. The changes were required because of the projected production costs of this phase of the study. A demonstration study verified that the aerosolized fibre size distributions produced were still within the targeted exposure specifications.

Chamber aerosols were monitored at least twice daily by mass samples drawn at 10L/m and collected on 45mm Metricel DM450 filters in open-faced holders. Uniformity of the aerosol was demonstrated at various locations within the chamber with concurrent analysis of mass samples and photometry (Sinclair Phoenix, Phila. PA.). Mass distribution in each chamber was measured at least once a week with a cascade impactor. Size distribution was additionally measured from electrostatically precipitated samples by observation under 350–500 × magnification.

Ophthalmoscopic Examination

Pre-exposure and post-exposure eye examinations were performed on all monkeys in this study. The animals were restrained and the eyes dilated. Approximately 15 minutes later, the eyes were examined by a specially trained veterinarian using an ophthalmoscope for fundoscopic examination and a slit-lamp biomicroscope for examination of the iris, lens, cornea, and conjunctivae.

Pulmonary Function

Pulmonary mechanical properties (dynamic compliance and resistance) were determined by the method of FRANK *et al.* (1957) after direct recording of transpulmonary pressure, airflow, and integrated volume on an Electronics for Medicine (White Plains, New York) VR-6 oscillographic recorder. Dynamic lung volumes were obtained from recordings of flow and volume during forced breathing manoeuvres as described by MOORMAN *et al.* (1975). Nitrogen washout and closing volumes were measured by the method of BUIST *et al.* (1973). The volume of helium isoflow was assessed using the technique described by HUTCHEON *et al.* (1974). Pulmonary function evaluations were performed at three periods during the course of this study: once before the initiation of exposure, after 9 months, and after 18 months of exposure. All testing was done with the monkeys anaesthetised using a mixture of ketamine and xylazine as described by BANKNIEDER *et al.* (1978).

Gross and Microscopic Pathology

All animals which died or were killed (sodium pentobarbital, 50 mg/kg ip) underwent a complete necropsy. Following examination a standard set of 35 tissues was weighed and fixed in 10% formalin prior to being prepared for microscopic examination. Sections from each lobe of the lung as well as the other major organs or grossly observed masses were evaluated by Board certified pathologists.

Clinical Studies

Haematological indices including : haematocrit, haemoglobin, RBC, WBC, reticulocyte, platelet and differential counts in addition to serum chemistries: BUN, glucose, creatine, phosphorus, calcium, bilirubin, cholesterol, LDH, SGOT, and potassium were sampled following 12–16 hrs fast. The monkeys were evaluated at weeks 16, 32, 48, and 64 while the rats were only studied at termination.

RESULTS

Chamber Exposure

Table 1 presents the total integrated airborne mass for each treatment and species. This table also includes the percent mass in the targeted size range. The rats and monkeys in treatment 1 received 13.9 and 14.7 mg/M^3 respectively. Treatment 2 resulted in 14.9 (rats) and 15.6 mg/M^3 (monkeys). Treatment 3 resulted in 4.8 (rats) and 5.0 mg/M3 (monkeys). The fourth resulted in 4.8 mg/M^3 (rats and monkeys). While the average mass values presented appear close to the targeted values the tabulated data show standard deviations of approximately 4 mg/M^3 for the 15 mg/M^3 treatments and 1.5 mg/M^3 for the 5 mg/M^3 treatments. The percent mass in the targeted size range for each exposure group exceeded 90%, however, all treatments contained a majority of smaller than desired size particulate on a count basis. Figure 1 shows the size distributions for the four fibre exposed treatments.

TABLE 1. CHAMBER AEROSOLS

	Group Fibre Dimensions (dia., length)	Percent Mass in Required Size (%)	Total Mass (Measured) (mg/m^3)	Mass (Target) (mg/m^3)
1.	>3.5 μm. dia >20 μm. length with binder	97.1	13.9 (rats) 14.7 (monkeys)	15
2.	<3.5 μm. dia. >20 μm. length with binder	99.4	14.9 (rats) 15.6 (monkeys)	15
3.	<3.5 μm. dia. 9!V10 μm. length no binder	96.5	4.8 (rats) 5.0 (monkeys)	5
4.	<3.5 μm. dia. <10 μm. length no binder	91.1	4.8 (rats) 4.8 (monkeys)	5
5.	Filtered clean air	—	—	0

Pulmonary Function

Extensive evaluation of pulmonary mechanics, lung volumes, flow volume analysis, and distribution resulted in a few group differences. At the 9-month evaluation, a significant increase in dynamic compliance was demonstrated in both 15 mg/M^3

exposures and the 5 mg/M^3 >10 μm length treatments when compared with the controls. At 18 months, both 15 mg/M^3 groups demonstrated a significantly reduced expiratory reserve volume with a suggested trend toward increased inspiratory capacities. No indication of obstructive ventilatory impairment was demonstrated. Table 2 presents the pulmonary function results for the three testing periods.

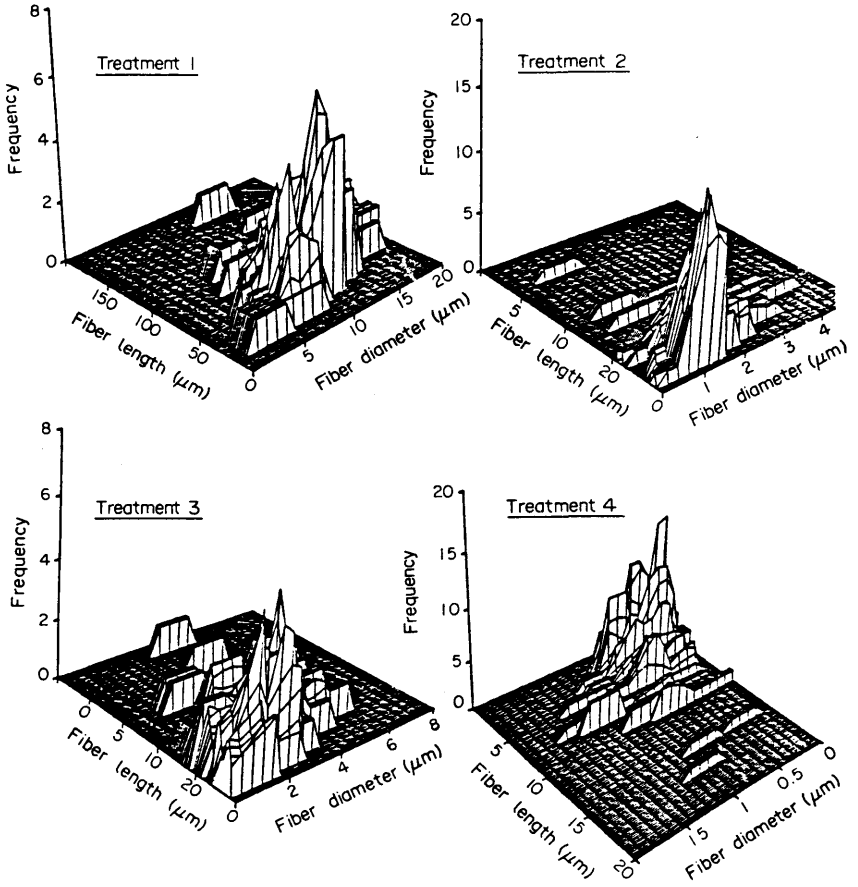

FIG. 1. Three-dimensional plots of frequency, length and diameter of the four aerosol treatments.

Pathological Evaluation

The only unequivocal effect induced by fibrous glass inhalation in monkeys was macrophage aggregates with phagocytised fibrous glass in the lungs and tracheobronchial lymph nodes. Pulmonary responses in the rat were characterized by macrophage aggregates and granulomas which contained fibrous glass fibres. Grossly visible plaque-like foci resulted from accumulations of granulomatous foci in pleural and subpleural locations. Lesions were limited to granulomatous foci. There was no fibrosis and there was no evidence in these animals that any further sequelae would result beyond that observed. There was no evidence of a fibrous glass induced fibrogenic response in either monkeys or rats. The most severe lesions in rats were in

TABLE 2. PULMONARY FUNCTION (Mean ± S.D.)

Pulmonary Parameter	Pre-exposure	9 Months	18 Months
Resistance (cm H20/1/sec)	5.1 ± 2.2	5.5 ± 2.0	6.0 ± 3.7
Compliance (ml/cm H20)	29.0 ± 12.1	34.0 ± 11.5*	23.6 ± 5.6
Inspir. Capacity (ml)	133 ± 33	144 ± 18	178 ± 45
Expir. Reserve Vel. (ml.)	167 ± 38	193 ± 45	199 ± 40*
Forced Vital Capacity (ml)	312 ± 74	338 ± 55	377 ± 68
Forced Expir. Flow (FVC/sec)			
at 50% FVC	3.3 ± 1.0	2.8 ± 0.5	2.6 ± 0.3
at 25% FVC	2.4 ± 1.0	1.2 ± 0.3	1.9 ± 0.5
Forced Expir. Vol. (%FEV/FVC)	79.6 ± 8.0	78.6 ± 6.4	78.1 ± 6.0*
Closing Volume (% of VC)	7.2 ± 3.6	10.4 ± 6.1	11.7 ± 3.7
Vol. He Isoflow (% of VC)	8.8 ± 4.7	10.5 ± 7.2	9.7 ± 3.0
Nitrogen Washout (% N_2/100ml)	0.7 ± 0.4	—	0.6 ± 0.3
Resistance (cm H20/1/sec)	5.0 ± 2.4	6.3 ± 4.4	8.3 ± 2.3
Compliance (ml/cm H20)	32.9 ± 13.2	33.3 ± 12.1*	24.8 ± 6.3
Inspir. Capacity (ml)	122 ± 23.5	152 ± 42.2	183 ± 38.1
Expir. Reserve Vel. (ml.)	175 ± 40.9	204 ± 33.3	196 ± 53.1*
Forced Vital Capacity (ml)	308.9 ± 60.5	357 ± 63.8	379.8 ± 83.3
Forced Expir. Flow (FVC/sec)			
at 50% FVC	2.97 ± 0.7	2.33 ± 0.6	2.46 ± 0.4
at 25% FVC	1.86 ± 0.8	1.45 ± 0.7	1.51 ± 0.5
Forced Expir. Vol. (%FEV/FVC)	78.7 ± 10.3	78.7 ± 7.7	75.0 ± 6.4*
Closing Volume (% of VC)	6.8 ± 3.6	7.0 ± 2.8	10.9 ± 4.9
Vol. He Isoflow (% of VC)	11.6 ± 9.1	9.6 ± 6.6	13.1 ± 5.9
Nitrogen Washout (% N_2/100ml)	0.7 ± 0.3	—	0.5 ± 0.4
Resistance (cm H20/1/sec)	4.9 ± 2.4	6.3 ± 2.9	7.4 ± 1.6
Compliance (ml/cm H20)	30.5 ± 11.5	33.4 ± 5.6*	23.0 ± 7.6
Inspir. Capacity (ml)	136 ± 33	150 ± 42	178 ± 47
Expir. Reserve Vel. (ml.)	161 ± 30	196 ± 36	212 ± 49*
Forced Vital Capacity (ml)	313 ± 53	348 ± 62	390 ± 76
Forced Expir. Flow (FVC/sec)			
at 50% FVC	3.4 ± 0.6	2.7 ± 0.6	2.5 ± 0.7
at 25% FVC	2.4 ± 0.9	1.4 ± 0.2	1.8 ± 0.7
Forced Expir. Vol. (%FEV/FVC)	80.4 ± 9.4	81.2 ± 4.4	77.7 ± 11.9*
Closing Volume (% of VC)	5.2 ± 1.8	7.6 ± 2.6	10.2 ± 2.8
Vol. He Isoflow (% of VC)	12.0 ± 13.1	11.0 ± 4.9	12.9 ± 4.2
Nitrogen Washout (% N_2/100ml)	0.9 ± 1.0	—	0.5 ± 0.3
Resistance (cm H20/1/sec)	4.3 ± 1.7	9.1 ± 7.0	9.0 ± 2.5
Compliance (ml/cm H20)	25.7 ± 9.5	27.4 ± 11.1*	22.2 ± 7.5
Inspir. Capacity (ml)	129 ± 31	161 ± 43	167 ± 47
Expir. Reserve Vel. (ml.)	169 ± 22	195 ± 26	212 ± 46*
Forced Vital Capacity (ml)	312 ± 49	356 ± 65	379 ± 80
Forced Expir. Flow (FVC/sec)			
at 50% FVC	3.1 ± 0.9	2.6 ± 0.7	2.4 ± 0.6
at 25% FVC	2.3 ± 1.2	1.3 ± 0.7	1.4 ± 0.6
Forced Expir. Vol. (%FEV/FVC)	78.2 ± 15.4	75.8 ± 8.3	70.2 ± 12.6*
Closing Volume (% of VC)	7.3 ± 4.2	9.5 ± 4.8	12.2 ± 5.8
Vol. He Isoflow (% of VC)	12.3 ± 6.2	13.4 ± 4.9	11.2 ± 6.0
Nitrogen Washout (% N_2/100ml)	0.9 ± 0.4	—	0.4 ± 0.2

Table 2 (Contd.)

Resistance (cm H20/1/sec)	5.2 ± 2.2	9.1 ± 7.2	7.9 ± 2.6
Compliance (ml/cm H20)	28.2 ± 10.6	24.1 ± 10.7*	20.2 ± 5.3
Inspir. Capacity (ml)	130 ± 20	132 ± 34	153 ± 44
Expir. Reserve Vel. (ml.)	163 ± 63	180 ± 69	252 ± 59*
Forced Vital Capacity (ml)	304 ± 46	315 ± 60	403 ± 68
Forced Expir. Flow (FVC/sec)			
at 50% FVC	2.8 ± 1.1	2.7 ± 0.8	2.5 ± 0.5
at 25% FVC	1.9 ± 1.2	1.4 ± 0.8	1.5 ± 0.6
Forced Expir. Vol. (%FEV/FVC)	74.2 ± 15.6	80.8 ± 8.6	71.5 ± 7.8*
Closing Volume (% of VC)	8.2 ± 6.3	9.1 ± 4.6	8.3 ± 3.8
Vol. He Isoflow (% of VC)	12.7 ± 11.8	12.8 ± 5.0	11.1 ± 4.0
Nitrogen Washout (% N_2/100ml)	0.7 ± 0.5	—	0.9 ± 0.7

*Significantly different from control values at the same time period ($p < 0.05$).

CONTROLS

TREATMENT 1

TREATMENT 2

TREATMENT 3

TREATMENT 4

Treatment 4 (1 μm dia., < 10 μm length, no binder) whereas the response in Treatment 1 (3.5 μm dia., > 20 μm length, with binder) was minimal.

The severity of response in monkeys was similar for all exposed groups except for Treatment 1 in which the response was minimal. Treatment 1 did, however, have monkeys with mildly increased numbers of lymphoid nodules or aggregates in peribronchiolar and perivascular areas. The significance of the increase in the lymphoid aggregates is unknown but the most probable explanation would be a mild stimulation from an antigen such as the binder. The fibrous glass induced lesions were similarly distributed among all lobes of the lung in monkeys; in rats, the lesions were most prominent in posterior lobes in all but the treatment 4 group where there was more equal distribution throughout the lung. The only evidence of translocation of fibres occurred in macrophage transport to draining pulmonary lymph nodes in many animals (rats and monkeys) and to mesenteric lymph nodes in two rats.

The mononuclear cell leukaemia was statistically elevated when each fibre-exposed group was individually compared with the control group. Table 3 demonstrates the group differences. This study showed no other evidence of pulmonary or mesothelial carcinogenicity associated with inhaled fibrous glass. There were no significant treatment related changes observed for mortality/survival, ophthalmology, clinical observations, body weights, or haematology and clinical chemistry.

DISCUSSION

As a result of the technical difficulties and costs, few inhalation toxicity studies with fibreglass have been conducted. SCHEPERS *et al.* (1955), and GROSS *et al.* (1971) reported results of long-term inhalation studies in rodents. Other than dust-laden

TABLE 3. MONONUCLEAR CELL LEUKAEMIA (MCL) IN THE SPLEEN OF BOTH EARLY DEATH AND SCHEDULED SACRIFICE RATS

Group	MCL(Males) Total Examined	% MCL Males	MCL(Females) Total Examined	% MCL Females	MCL(Males & Females) Total Examined	% MCL Males & Females
1	17/50	34.0	20/50	40.0	37/100	37.0*
2	18/50	36.0	19/50	38.0	37/100	37.0*
3	20/50	40.0	15/49	30.6	35/99	35.4*
4	25/50	50.0	17/49	34.7	42/99	42.4**
5	10/50	20.0	11/49	22.4	21/99	21.2

* $p<0.05$ by Chi^2 test
** $p<0.01$

macrophages, no significant pathology resulted and the investigators concluded that fibrous glass exposures should be classified as 'biologically inert' dusts. More recent studies reported by HARDY (1979) also revealed no significant pulmonary function or histopathological response to short-term high level exposures. These inhalation studies suggest responses following exposures are similar to those exhibited by nuisance dusts.

Until recently, epidemiological investigations have not demonstrated excess mortality or morbidity from occupational exposures to fibrous glass. BAYLISS *et al.* (1976), however, showed a significant excess of non-malignant respiratory disease in workers exposed to special process small diameter fibres. In one of the largest industry-wide epidemiological studies, ENTERLINE *et al.* (1983) also found a significant excess in non-malignant respiratory disease deaths in exposed workers.

The grossly visible plaque-like foci that occurred in rats resulted from accumulations of granulomatous foci in pleural and subpleural locations. The decreasing severity of these grossly observed lesions among three of the test groups (i.e., Treatment $2>3>4$) was in direct contrast to the severity observed microscopically (i.e. Treatment $4>3>2$).

The pleural plaques may not have occurred in the monkeys because of different cellular responses or insufficient induction time. SELIKOFF (1965) has postulated that an interval of 20 years (humans) is necessary to develop plaques. PARKES (1982) concludes that there is no evidence that plaques lead to mesotheliomas or have an effect on life expectancy.

Increased incidence of mononuclear cell leukemia in the Fischer 344 rat could simply be due to the fact that aged Fischer 344 rats have an increased spontaneous incidence of this disease. However, only the exposed rats in this study demonstrated a significantly increased incidence of this leukemia. An alternate hypothesis could be that the presence of fibrous glass in the pulmonary and/or lymphoid tissues may have resulted in stimulation of a cell line with a high spontaneous incidence of neoplasia. SINCOCK (1977) has shown that fibrous glass can be both cytotoxic and genotoxic and that it is therefore plausible that fibrous glass may have a direct genotoxic effect on stem leukocytes in the pulmonary and/or lymphoid tissues resulting in an increased incidence or probability of leukaemia. ROBINSON *et al.* (1979) suggested that fibre exposed workers may have increasing lymphomas and leukemias.

It has recently been shown by WARD and REYNOLDS (1983) that the leukaemic cells comprising mononuclear cell leukaemia are the neoplastic counterpart of the normal rat large granular lymphocyte. It has further been shown by BOROWITZ *et al.*, (1981) that the heterogeneity of rat leukaemia is similar to that seen in human non-Hodgkin's lymphomas. STROMBERG (1985) has suggested the use of Fischer 344 rats as an animal model for this human disease. In view of the above, further studies are needed to more fully evaluate the potential risk of fibre exposures in relation to lymphocytic leukaemia.

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DISCUSSION

P. ELMES: Your use of the term 'pleural plaques' is misleading. In humans this describes lesions on the *parietal* pleura resulting from amphibole exposure. Your lesions appear to be in the surface of the lung itself. Is that correct?

W.J. MOORMAN: Yes, plaque-like lesions were seen on the surface of the lung in all fibre-exposed rats, but not in monkeys.

J.C. WAGNER: The slides showing the lungs give no indication of the underlying pathology. This macroscopic presentation could be misleading.

W.J. MOORMAN: Representative microscopic sections of the lungs of each group were presented. Multiple microgranulomatous lesions were seen in the fibre-exposed groups (rats and monkeys).

K. DONALDSON: Was there any evidence of fibre transport to spleen or other lymphoid tissue?

W.J. MOORMAN: Yes, the tracheobronchial lymph nodes were heavily laden with macrophages containing fibres.

H. MUHLE: Did you determine the number of retained fibres in lungs?

W.J. MOORMAN: Work is in progress.

R.O. MCCLELLAN: You noted that the OSHA and NIOSH standards were developed with consideration of not only pulmonary effects but also eye irritation. You described the pulmonary effects observed in both rats and monkeys, but you did not comment on effects on other organ symptoms. Specifically, did you observe any evidence of eye irritation? Were any special procedures used to examine the eye (cornea and conjunctiva)?

W.J. MOORMAN: Eye irritation was observed initially for the first two weeks. Pre- and post-exposure eye examinations were conducted on the monkeys using a direct ophthalmoscope for fundoscopic examination and an optical slit-lamp biomicroscope for examination of the iris, lens, conjunctivae and cornea; no effects were found.

R.O. MCCLELLAN: You described alterations in the pulmonary region of the respiratory tract. Would you comment on the procedures used to examine the nasal cavity, and any changes observed. On a related issue, did you examine the regional lymph nodes of the head and neck for the presence of fibres?

W.J. MOORMAN: Multiple cross sections of the nasal cavity were evaluated both grossly and microscopically. No significant effects were noted. The tracheobronchial lymph nodes showed intense numbers of fibre-laden macrophages.

R.O. MCCLELLAN: The 18-month observation period for the monkeys was relatively brief. Have you made any longer-term observations, or do you have any plans to make such observations? Two of your groups were exposed to fibres with phenol formaldehyde resin. What weight % of the mass was resin binder? Do you feel it contributed to the disease observed?

W.J. MOORMAN: No, the monkeys served for physiological evaluation of a larger (primate) species. The phenol-formaldehyde binder was approximately 12% in treatment no. 2. Treatment no. 1 contained phenol-formaldehyde binder at approximately 4.5%.

R.O. McCLELLAN: You described an increased incidence of mononuclear cell leukaemia in the rats. Were these incidental findings' observed in sacrificed rats, or was the incidence of leukaemia increased in animals that died and this considered to be the cause of death?

W.J. MOORMAN: The incidence of mononuclear cell leukaemia was significantly increased in all fibre-exposed rats. The diagnosis of leukaemia was made at termination and in early deaths.

J.M.G. DAVIS: What were the mean and maximum survival of rats in these studies? In studies with asbestos we have found few tumours in rats less than 24 months old, yet the final tumour total at the end of the study (33–36 months) may be 50%.

W.J. MOORMAN: The rats were fibre-exposed for 21 months and held to 80% mortality. The final sacrifice was at 27 months.

W.M. TER KUILE: Did you look at the kind of surface treatment of the glass fibres? For different purposes the surface is treated with phosphorus (or Na) to increase its chemical surface properties.

W.J. MOORMAN: Commercially available fibres were selected for use in this study. Size preparation methods included ring milling, flotation, sonification and air clarification.

J.L. ABRAHAM: Please do not publish this paper without including photo-micrographs of the lung lesions presented as plaques'. These could be adenomas from the gross photos presented. This is important to clarify.

W.J. MOORMAN: These lesions were not classical plaques as they did not contain connective tissue. The pathologist did not consider them to be adenomas.