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A STUDY OF FISCHER-344 RATS EXPOSED TO SILICA DUST FOR SIX MONTHS AT CONCENTRATIONS OF 0, 2, 10 or 20 mg/m^3

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LIST OF ABBREVIATIONS

Å	angstrom
AGT	average generation time
ANOVA	analysis of variance
atm	atmosphere
ATPD	ambient temperature pressure dry
BMDP	Biomedical Computer Programs, P-series
BrdUrd	5-bromodeoxyuridine
BTPS	body temperature pressure saturated
C_{DYN}	dynamic compliance ($cm^3/cm\ H_2O$)
CN	control group, 0 mg SiO_2/m^3
$DLCO_{rb}$	diffusing capacity of the lung for CO measured by a rebreathing technique ($cm^3/mmHg \cdot min^{-1}$)
EFR_x	expiratory flow rate at x% vital capacity (cm^3/sec)
ΔEFR_{25}	difference in the flow at 25% vital capacity above or below that flow estimated by a chord slope drawn from EFR_{50} to EFR_0 (cm^3/sec)
EKG	electrocardiogram
EPL	Experimental Pathology Laboratories, Inc. (Herndon, VA)
ERV	expiratory reserve volume (cm^3)
f	frequency of breathing (breaths/min)
FRC	functional residual capacity (cm^3)
FRC_b	functional residual capacity determined by Boyle's law (cm^3)
FRC_d	functional residual capacity determined by dilution (cm^3)
h	pressure which will theoretically distend the lung to one-half its volume at infinite pressure ($cm\ H_2O$)
HD	high dose group, 20 mg SiO_2/m^3
$\Delta HEFR_x$	difference in the flow at x% VC in the MEFV curves when helium/O_2 rather than air was the gas breathed

HR	heart rate (beats/min)
IC	inspiratory capacity (cm^3)
ID	intermediate dose group, 10 mg SiO_2/m^3
IRV	inspiratory reserve volume (cm^3)
LD	low dose group, 2 mg SiO_2/m^3
M_0	total area under the N_2 washout curve for 50 breaths $\sum_{j=1}^{50} X_j$, where X_j is the N_2 concentration in each breath
M_1	$\sum_{j=1}^{50} b_j \cdot X_j$, where b_j is the dilution number $(\frac{j \cdot V_T}{FRC_d})$
MANOVA	multivariate analysis of variance
mas	milliamp seconds
MEFV	maximum expiratory flow volume
MMAD	mass median aerodynamic diameter
p	probability
P	pressure (cm H_2O)
P_{ao}	airway pressure (cm H_2O)
P_e	esophageal pressure (cm H_2O)
P_L	transpulmonary pressure (cm H_2O)
P_{st}	static pressure (cm H_2O)
PBS	phosphate bufferd saline
PEF	peak expiratory flow (cm^3/sec)
PHA	phytohemagglutinin-p
QSC	quasi-static compliance ($cm^3/cm\ H_2O$)
QSC_{cs}	quasi-static compliance determined by chord slope ($cm^3/cm\ H_2O$)
R_L	pulmonary resistance ($cm\ H_2O/cm^3 \cdot sec^{-1}$)
R_{us}	upstream airway resistance ($cm\ H_2O/cm^3 \cdot sec^{-1}$)
RI	replicative index
RV	residual volume (cm^3)

s.e.	standard error of the mean
SPF	specific pathogen free
σ_g	geometric standard deviation
TLC	total lung capacity (cm^3)
TLC_d	total lung capacity determined by dilution (cm^3)
V	quasi-static volume (cm^3)
$\dot{V}$	airflow (cm^3/sec)
$\dot{V}_{30}$	airflow (cm^3/sec) at 30% of vital capacity
$\dot{V}_E$	minute volume (cm^3)
V_{max}	volume (% VC) at which maximum expiratory flow occurs
V_o	theoretical lung volume at infinite pressure (cm^3)
V_p	theoretical lung volume at a particular pressure (cm^3)
V_T	tidal volume (cm^3)
V_{TG}	volume of trapped gas (cm^3)
VC	vital capacity (cm^3)

ABSTRACT

The major objective of this study was to relate the results of a series of functional tests to the compositional and structural alterations in the rat lung induced by subchronic exposure to silica dust. Fischer-344 rats were exposed for 6 hours/day, 5 days/week for 6 months to either 0, 2, 10, or 20 mg SiO_2/m^3.

The general appearance of the exposed rats was not different from that of the controls. Interestingly, female rats exposed to silica dust, at all tested concentrations, gained more weight than the controls. The lung weight and the lung-to-body weight ratio was greater in the male rats exposed to the highest concentration of silica dust.

A series of respiratory physiology tests were performed on animals from each exposure group. Exposure to SiO_2 did not change the responsiveness of the animals to CO_2 induced hyperventilation. Silica exposure had no dose dependent effect on the partial pressure of arterial blood-gases or on the blood pH. None of the parameters of normal tidal breathing were affected by exposure. Exposed animals had normal EKG's. The lung volumes of the exposed rats were not different from those of the control animals. The quasi-static compliance, the diffusing capacity for CO, and the distribution of ventilation were not altered by silica exposure. The flow volume dynamics and the upstream airway resistance of Fischer rats were unaffected by the silica concentrations tested.

The amounts of protein, DNA, elastin and collagen, as well as the water content of the lungs from exposed animals were assessed. Significant dose dependent increases were observed in both collagen and elastin. However, frontal chest x-rays taken on rats from each exposure

did not exhibit any evidence of silica-induced lung disease.

Microscopic examination of the respiratory tissue of the silica exposed animals revealed accumulations of histiocytes near the end-airways. They were frequently accompanied by granulocytes and mononuclear cells. Type II cell hyperplasia, and in some cases focal fibrosis, were observed. Intralymphatic microgranulomas were commonly observed. The severity of these changes was related to the exposure concentration of silica dust.

Application of stepwise discriminant analysis to the individual functional and compositional variables measured in the lungs of each rat indicated which of these variables had the greatest power to discriminate among the exposure groups. Among the compositional variables, total lung weight, total collagen, and the amount of elastin and protein per unit dry weight were found to be most discriminating. None of the functional variables had significant discriminating power to distinguish the exposure groups.

INTRODUCTION

The work reported here is one part in a series of studies centered on a comprehensive comparison of morphologic and compositional parameters to the pulmonary function in rats exposed to toxic agents. Successful application of such functional tests to rodents would permit a more comprehensive appraisal of the pulmonary toxicity of inhaled chemicals as well as those administered by other routes but for which the lung is the target organ. To test the sensitivity of the functional measurements and to determine how structural and compositional changes are functionally manifested in the rodent, rats are being exposed to a variety of toxic agents. The compounds being used are ozone, acrolein, chlorine, silica dust (reported in part here), cadmium chloride aerosol, and tungsten carbide and cobalt.

Silica was selected as a test compound to produce a restrictive deep lung lesion and provide an opportunity to investigate the relationship between lung function, structure, and composition in animals with such a pulmonary affliction. The sequence of pathological changes in experimentally induced silicosis has been reviewed by Heppleston (1). In brief, the silica particles are ingested by macrophages leading to their death and the release of the silica particles. Macrophages accumulate in the areas of silica deposition and release a variety of chemotactic factors including fibrogenic factor which results in increased production of collagen leading to fibrosis.

The effect of inhaled crystalline silica on the human pulmonary system is apparently dependent upon the amount of dust inhaled, the percentage of free or uncombined silica in the dust particles, and the length of exposure (2,3). The pathology associated with silica

inhalation by humans manifests itself in a variety of ways, depending on exposure conditions and three forms of the disease have been described. These differ primarily in the duration of exposure before symptoms are manifested and in the rate with which the disease progresses, which may in part be dependent on the concentration of respirable silica in the inhaled air. The common form of silicosis has been recognized since antiquity as an occupational disease. It is generally associated with exposure to dust with a silica content of less than 30% and more than 20 years of exposure may be required before a chest radiogram is positive. There is very little respiratory impairment associated with the early stages of simple silicosis (2,3). Accelerated silicosis develops after shorter exposures to higher concentrations of silica dust. In accelerated silicosis, the time from first exposure to the development of silicotic nodules, which appear in chest radiograms, is shorter (5-15 years) than in simple silicosis. The disease develops much faster and often advances to a progressive massive fibrosis (2,3). The third form of the disease, acute silicosis, is often termed silicoproteinosis. In humans it develops after 1-3 years of exposure and progresses very quickly. There is rapid loss of pulmonary function and invariably it is fatal. The distinctive characteristic of this disease is the presence of a surfactant-like liquid in the alveoli. On a chest radiogram, few silicotic nodules are evident, and it is characteristic of diffuse massive fibrosis.

Because the fibrosis expected upon exposure of rats to silica is a progressive lesion requiring some time to develop, pulmonary endpoints were investigated at three time points using different subgroups of animals from each exposure chamber. Fischer-344 rats were exposed to

either filtered air, 2, 10, or 20 mg/m^3 silica dust for 6 hours/day, 5 days/week. Pulmonary function, lung composition, and histopathology were assessed in subgroups of animals after 3 months and 6 months of exposure and in an additional subgroup of rats exposed for 6 months and then maintained under specific pathogen free (SPF) conditions for an additional 6 months. This report will present only the findings in Fischer-344 rats exposed to 0, 2, 10, or 20 mg/m^3 silica for 6 months and assessed 6 days after their final exposure.

Fischer-344 rats were exposed to filtered air or silica dust for 6 months and then placed into a holding room for 6 days to avoid confoundment of the data by the acute effects of exposure, should any exist. To enable comparisons of function, composition, and structure in individual animals, each rat was first subjected to a series of pulmonary function tests, and immediately after testing the left lung was fixed for histologic examination and the right lung submitted for compositional analysis. To determine if any measured variables were significantly more sensitive to the induced changes, stepwise discriminant analysis was used. Subgroups of animals from each chamber were used solely for pathological examination. Cytogenetic effects of the agent under study were also investigated in separate subgroups from each chamber after 6 months of exposure.

Techniques have been developed to measure several parameters of pulmonary function in rodents and recent technological developments have increased the sensitivity of these determinations (4-8). Respiratory performance in these studies was based on ventilatory response to CO_2, arterial blood gas concentrations, and static and dynamic lung mechanics.

The most direct means of determining whether blood-gas exchange in the lung is adequate is to measure the blood pH and the concentrations of O_2 and CO_2. While systemic diseases and metabolic imbalances can offset these variables, data from their collective evaluation can generally be used to distinguish between respiratory and metabolic abnormalities. In cases of prolonged hypercapnea, often a complication of chronic lung disease, altered neural control of ventilation and related respiratory reflexes may become apparent. This condition can be detected as impaired responsiveness to inhaled CO_2, a condition currently believed to be the result of partially refractory CO_2 chemoreceptors in the aortic arch or the brainstem. Reduced ventilatory response (measured as a percent change in minute volume ($\dot{V}_E$)) appears to be directly related to the degree to which the receptors are refractory and to the CO_2 concentration of the blood (9).

Other measures of respiratory performance quantitate the actual mechanical status of resting and dynamic lungs. In general, alterations in normal breathing parameters (tidal volume (V_T), frequency of breathing (f), driving pressure, and inspiratory and expiratory airflow) are observed only in the presence of extensive lung disease. While changes in airway resistance or tissue elasticity during spontaneous normal breathing can be sensitive indicators of lung injury and may result in determination of ventilatory efficiency, diseases of the small airways or of the parenchymal interstitium can exist without overt impact on normal breathing patterns. Subtle changes in tissue elasticity can be detected by forcing the lungs to a fully inflated state (total lung capacity (TLC)) and controlling the deflation to minimal lung volume (residual volume (RV)). The resulting curve of the

expired volume versus the pressure induced by the elastic property of the lung tissue is known as the quasi-static compliance (QSC) curve. Divergent shifts in the typical sigmoidal shape of the deflationary curve may reflect degenerative alterations of the interstitium. These may include scarring or fibrosis in response to lung injury or progressive tissue destruction characteristic of emphysema. These changes in tissue elasticity may also result in altered resting lung volumes due to disturbances in the balance of the retractive forces of the lung and chest wall. Such disturbances can in turn affect the distribution of ventilation within the subcompartments of the lungs during tidal breathing. Thus, by examining the washout characteristics of residual lung nitrogen while pure oxygen is being breathed, the presence of poorly ventilated regions within the lungs can be detected. In extreme cases, these imbalances entirely alter the introduction of oxygen into the alveoli, resulting in reduced concentrations of oxygen in the arterial blood.

In the absence of severe regional ventilatory abnormalities, the ability of oxygen to diffuse across the blood-air membrane of the alveoli can be approximated by the diffusion of CO. Carbon monoxide has almost the same diffusion coefficient as oxygen (10) and because it binds almost irreversibly to hemoglobin, it functions well as an index of diffusion limitations across the alveolar surface. Reduction in the diffusion of CO indicates a thickening of the alveolar epithelial-endothelial barrier. Reduction in the alveolar surface area, as seen in degenerative emphysema, and mismatching of ventilation and perfusion can also reduce the diffusion index. This index, when considered in conjunction with other tests, can serve both as a diagnostic tool and an index of respiratory efficiency.

Small airway disease is characteristic of many degenerative processes in the lung. Because the small distal airways lack an extensive support structure, they are very sensitive to deformation or destruction of parenchymal tissue or changes in adjacent airways. Lesions in any structural component will affect not only the component directly, but the entire interdependent supportive framework of the small airways. This anatomical and functional interdependency is reflected in tests of small airway mechanics. The maximum expiratory flow volume (MEFV) maneuver stresses these airways in a manner which results in their dynamic collapse. Once a critical pressure drop along the airway is established, the fragile airway collapses and the maximum airflow is limited, regardless of the increased effort or imposed force. This phenomenon, known as effort independence, is reflected in the deflation portion of the MEFV curve. Whether or not these airways collapse prematurely, which is the case in some disease states, can often be detected upon inspection of the MEFV curve. By using helium, which is less dense but more viscous than air, the characteristic conversion of the forced airflow from turbulent to laminar can be further dissected. The lower density helium enhances all airflow which is turbulent in nature (at lung volumes at or near the total lung capacity) and as airflow becomes laminar at diminished lung volumes (where small airway constraints dominate the characteristics of airflow) the more viscous helium frequently results in reduced airflow. Comparison of the lung volumes at which air and helium airflows are converted from turbulent to laminar, and assessment of the degree to which helium enhances the airflow at specific lung volumes yields information relating to the site of airway obstruction or premature airway collapse.

Animal models have been developed to study various aspects of silicosis; however, they are limited in their ability to address the questions of structure versus function. The extensive functional data generated in this study should provide greater insight on how structural and compositional changes in the silicotic lung are presented functionally and on the physiological impact of these structural changes.

Animal Procedures and Exposures

The Fischer-344 rats used in this study were obtained from Charles River Laboratories, Inc. (Kingston, NY) in two shipments. The animals were received from the supplier at 5-6 weeks of age and held in our SPF facility for an additional 4-6 weeks before exposure.

Upon receipt, the animals were assigned to an exposure group as follows. Rats of the same age and sex were individually weighed and placed into holding bins, each bin holding animals within a 5 gram weight range. When all of the animals of a single age and sex had been weighed, the total number of animals weighed was reduced to the total number of animals needed for the experiment by removing equal numbers (±1) of animals from the bins holding the lowest and the highest weight groups. A random number table was used to assign each animal to a particular cage in a chamber (thereby determining its endpoint destination) and randomization of the numbers 1 through 4 resulted in the random assignment of animals to exposure groups. Animals from the lowest weight group were used first and randomly assigned to the appropriate positions in the four chambers before using animals from the next bin. This system resulted in groups of animals with the same mean weight in each exposure group. Each exposure chamber contained three subgroups of rats. One subgroup of animals was exposed for 3 months. After the exposure period, 24 animals from this subgroup in each exposure chamber were used for assessment of lung function, composition, and structure and an additional 8 animals were used for complete histopathology. A second subgroup at each exposure level was exposed for 6 months and assessed at the end of this exposure period. This subgroup also

included 24 animals for multiple pulmonary endpoint assessments and 8 rats for histopathology. In addition, each 6 month exposure subgroup included 8 male and 8 female rats for assessment of reproduction potential and 10 male rats for cytogenetic studies. A final subgroup in each chamber composed of 24 multiple pulmonary endpoint and 8 histopathology animals was exposed for 6 months and then maintained in conventional SPF animal quarters for 6 months prior to assessment of the specific endpoints.

All of the animals were neck tagged to provide permanent identification. The rats were individually housed in stainless steel, wire-mesh cages and provided a standard laboratory diet (Purina Chow) and water ad libitum. A 12-hour on/12-hour off light cycle was maintained in the animal room.

During this quarantine period, 10/285 and 10/310 rats from the first and second shipments, respectively, were sent to AnMed Laboratories, Inc. (New Hyde Park, NY) for health assessment. The rats sent for health assessment were selected from those animals on the high and low extremes of the weight range (see above). This service included: (1) determination of serum viral antibody status (Sendai Virus, Pneumonia Virus of mice, Reo Virus Type 3, Theiler's Virus, Kilham's Rat Virus, Rat Chronona Virus, and a zoonotic arenavirus which causes lymphocytic chorimeningitis); (2) culture of nasoturbinate washings for respiratory bacterial pathogens and mycoplasma; (3) culture of oropharyngeal swabs for Pseudomonas and Klebsiella; (4) preparation of fecal samples for detection of bacterial pathogens and parasites; (5) preparation of ileal wet mounts for protozoans; (6) inspection of the colon for helminths and of the bladder for Trichosomoides crossicauda;

and (7) scanning of the pelt for ectoparasites. Slides for histopathological examination were prepared from the lung, liver, kidney, ileum, spleen, and thymus. No murine pathogens of the helminth, viral, arthropod, protozoan, or mycoplasmal groups were isolated or otherwise detected. Klebsiella oxytoca was isolated from all of the animals submitted from the first shipment, but from none of the animals in the second lot. There is no evidence of this species being a pathogen of laboratory rats (11). Although this finding was undesirable, it was interpreted as not interfering with the use of these animals in the proposed protocol. The results of the pre-experimental health profiles of the animals submitted for evaluation have been provided in Appendix A.

Following the six month exposure period, sera from four animals, one from each exposure chamber, were submitted to AnMed Laboratories to assess the antibody status of these animals. All four animals had elevated antibody titers to pneumonia virus of mice (titers ranged from 160 to 320) (Appendix B). This virus produces silent infections in mice and can produce severe interstitial pneumonia after intranasal inoculation of mice. Although neutralizing antibodies have been detected in rats, clinical signs or lesions have not been reported (12).

Experimental and control animals were placed into the appropriate chambers the morning of their initial exposure. The animals were then continuously housed in the exposure chambers until the morning following their final exposure. Caging and light cycle in the chambers were identical to those in the holding rooms. The stainless steel cage units (each holding 8 rats, 2 rows of 4) were arranged in 3 tiers with 6 units per tier. Water was supplied to the animals ad libitum; however the food was removed during the daily 6 hour exposure period. Each animal

was weighed on the morning of its initial exposure and then biweekly, with approximately one-half of the rats in each chamber weighed each week according to the following schedule: control rats, Mondays; 2 mg/m^3 rats, Tuesdays; 10 mg/m^3 rats, Wednesdays; and 20 mg/m^3 rats on Thursdays. To facilitate the analysis of weight gain by the animals exposed to each concentration of silica dust, all of the rats designated for reproductive potential studies were weighed on the same day of the week (Monday) regardless of exposure concentration. These animals comprised the largest number of age matched animals entering the chambers on a single exposure day. Weight data from these rats are presented to provide an indication of the growth rate of the animals used for the assessment of all endpoints presented in this report.

The animals were briefly examined each day prior to exposure, when the food troughs were removed and clean catch pans were provided, and again following the exposure period when the food troughs were replaced. The animals were also inspected once daily on weekends. When the rats were weighed, they were examined more closely and provided a clean cage. The cage-packs were rotated through nine positions (3 tiers with 3 cage pack units/tier) by moving each pack one position after the biweekly weighing of the animals.

Rats were exposed to either filtered air, 2 mg/m^3, 10 mg/m^3, or 20 mg/m^3 silica dust for six hours/day, five days/week (holidays excluded). The rats exposed for 6 months received 126 ± 1 daily exposures. All of the animals were exposed for a minimum of two days the first and final weeks of exposure. In cases where the endpoint test procedures were time consuming, the starting dates were staggered while still adhering to the 127 exposure day regime and the minimum number of exposure days

per week. Following exposures, the rats were placed into an SPF animal room for 6 days before assessment of the selected endpoints.

Chambers

Exposures were carried out in stainless steel/Lucite chambers. Airflow through the 5 m^3 chambers was 1 m^3/min. Exhaust air from the 10 and 20 mg/m^3 silica chambers was passed through an electrostatic precipitator, a prefilter, and a HEPA filter before being discharged. Silica dust from the 2 mg/m^3 chamber was not electrostatically precipitated before the exhaust air passed through the filter beds. Continuous monitoring of the temperature in each chamber was under computer control and the average temperature during each 0.5 hr interval was recorded. The average temperature during the exposure of these animals was 22.5°C. The mean average daily temperatures ranged from 20.0 to 24.6°C and the minimum and maximum 0.5 hr averages recorded were 17.7 and 26.9°C, respectively.

Test Agent and Aerosol Generation

The crystalline quartz used in these studies was provided as a gift by Pennsylvania Glass Sand Corporation (Berkeley Spring, WV) as Min-U-Sil 5. A powder diffraction scan of this material employing a goniometer indicated that it was pure α quartz. The diffraction peaks observed at 1.540, 1.819, 2.280, 2.457, 3.36, and 4.28 Å (angstrom) were considered within experimental error of the published absorption peaks (13) of 1.541, 1.817, 2.282, 2.458, 3.34, and 4.26 Å, respectively.

Because the dust for these studies was generated using fluidizing bed generators which use brass beads as the bed matrix, the aerosolized material was sampled and analyzed by x-ray fluorescence for the presence of metals found in brass. This analysis qualitatively revealed the

presence of copper, tin, and trace amounts of lead. Atomic absorption spectroscopy indicated that copper and tin comprised approximately 1.1 and 0.1% of the dust by weight, respectively. No attempt was made to quantitate the lead contaminant with atomic absorption because of the extremely small amount indicated by the x-ray fluorescence technique. Although contamination of the silica with these elements was not desirable, the concentration of the metals was considered so low as to be innocuous.

The fluidizing bed units (described below) provided the required aerosol concentrations with satisfactory concentration control. The particle size of the generated dust generally increased slightly throughout the life of the bed (Figure 1). The mean mass median aerodynamic diameter (MMAD) and geometric standard deviation (σ_g) for the aerosols sampled from the three exposure chambers are provided in table 1. The mean MMAD of all of the cascade impactor analyses performed was 2.4 μm with a mean σ_g of 2.0.

The fluidized bed aerosol generators used in these studies are products of Thermo-Systems, Inc. (St. Paul, MN). A model 3400 was used to provide a chamber concentration of 2 mg/m^3, while the 10 and 20 mg/m^3 chambers were each equipped with a model 9310 generator. The automatic feed systems of the generators were not employed because the physical consistency of the silica powder was such that it tended to cake, rendering the feed mechanism ineffective. Instead, the silica powder was added directly to the bead beds after it was vigorously mixed by shaking with the 100 μm brass beads from the bed matrix. During the mixing process, the brass beads are coated with the silica particles. To disperse the particles, dry, filtered air is introduced through the

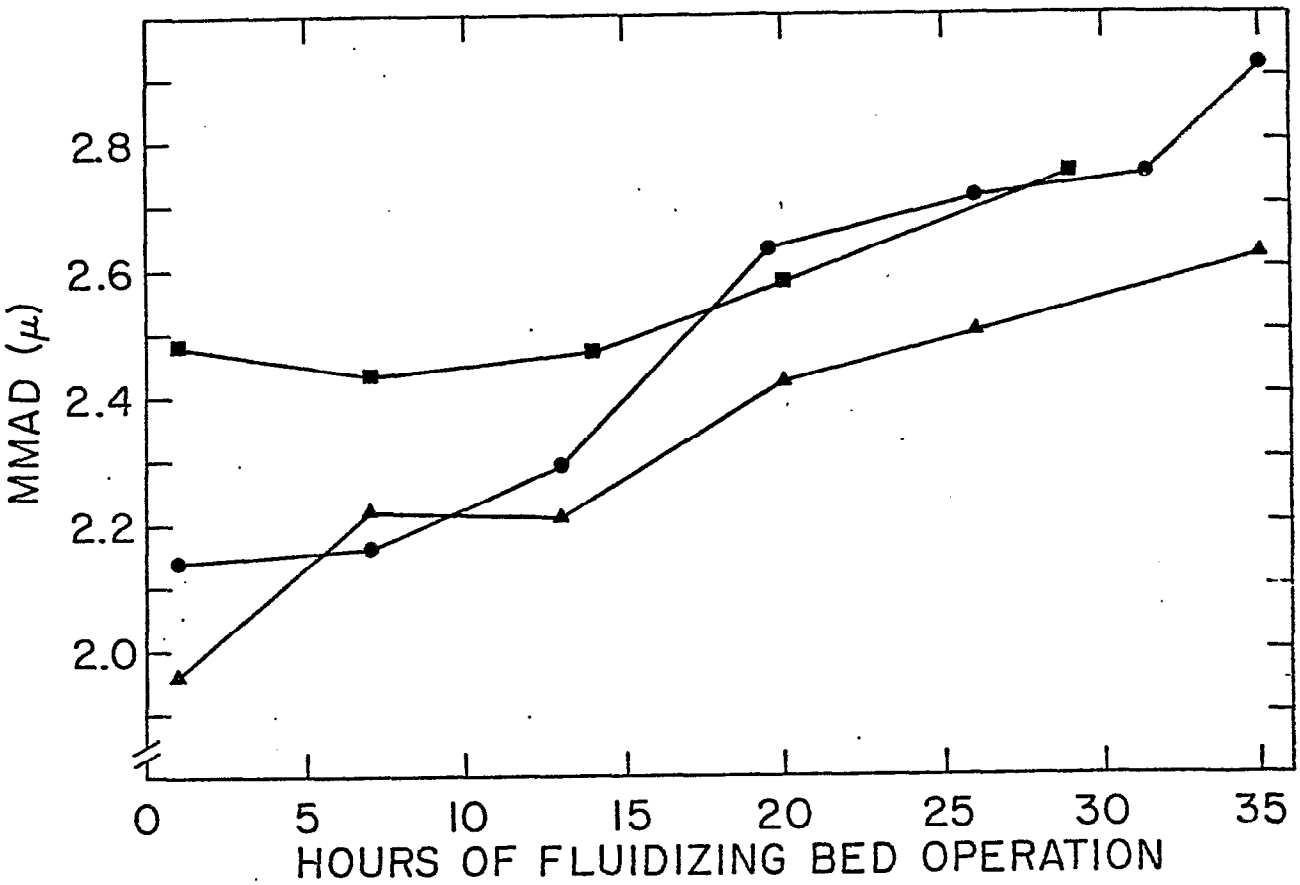

Figure 1. Change in silica particle size (MMAD) with increased operating time of the fluidizing bed generators associated with each exposure chamber; 2 mg/m^3 silica (●), 10 mg/m^3 silica (▲), and 20 mg/m^3 silica (■). MMADs were determined using an Anderson Cascade Impactor.

Table 1. Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (σ_g) of Silica Particles in the Animal Exposure Chambers

	Silica Concentration		
	2 mg/m^3	10 mg/m^3	20 mg/m^3
n	9	8	7
MMAD (μm)			
mean	2.43	2.32	2.46
s.e.	0.11	0.09	0.07
σ_g (μm)			
mean	2.02	2.05	1.96
s.e.	0.05	0.03	0.04

microporous stainless steel support screen at the bottom of the bed. The air strips the particles away from the beads and carries the resultant aerosol to the outlet of the generator. The delivery line between each generator and the air intake line of the exposure chamber was equipped with a 60 mCi Kr-85 neutralizing line source contained in a 2.4 mm O.D. nickel tube 30.5 cm long.

Monitoring of Silica Concentrations in the Exposure Chambers

The concentration of silica dust in each chamber was continuously monitored using a RAM-1 aerosol mass monitor (GCA Environmental Instruments, Bedford, MA) and the strip chart output from each unit was used to calculate the average daily concentration. During each exposure period, a gravimetric filter sample was collected and the chamber concentration during the collection period calculated by dividing the amount of material collected on the filter by the volume of chamber atmosphere sampled. The average daily concentration for each chamber was then determined by multiplying the average concentration recorded by the mass monitor by a correction factor derived by dividing the gravimetrically determined chamber concentration by the average mass monitor reading during the collection period.

The distribution of silica dust in the exposure chambers was assessed and the results are provided in Appendix C.

Respiratory Physiology

Respiratory performance, based on ventilatory response to CO_2, arterial blood gas concentrations, and static/dynamic lung mechanics, was evaluated in those animals designated for such assessment. For descriptive convenience, these three assessment procedures will be described in the order in which they were performed on each animal.

Assessment of CO_2 responsiveness under conditions free of anesthesia, restraint, or other invasive procedures which may have imparted artifacts, was achieved by whole-body barometric determination of V_T and f. A whole body plethysmograph was constructed from a 2.75 liter glass jar with a screw cover (Figure 2). The cover was provided with several ports for the introduction and exit of selected breathing atmospheres, insertion of a thermister probe, and communication with a differential pressure transducer (Setra Systems 239: $\pm$ 7.6 mm Hg, Natick, MA). A Gould Brush (Cleveland, OH) 2400 recorder was used to obtain permanent tracings of tidal breathing patterns. The plethysmograph was calibrated using a calibrated piston pump (1 cc displacement); phase related changes in the plethysmograph pressure up to 5 Hz were recorded for use in final analyses. A linear difference of 20% in V_T was noted between 1.0 and 5 Hz. All V_T data were corrected for this difference on the basis of f for the final determination of $\dot{V}_E$. The animal was allowed to acclimate to the system for 15 minutes while breathing air (20% O_2, 80% N_2) which was provided at 2 l/min. Representative tidal breathing data were collected for 15-25 seconds after closing the inlet airport, allowing about 10 seconds for atmospheric pressure equilibration, and closure of the outlet port. Next, a 10% CO_2, 20% O_2, 70% N_2 breathing gas mixture was passed through the plethysmograph (2 l/min) for 5 minutes. Previous testing had indicated that this duration and flow rate were sufficient to maximize the CO_2 response. After closure of the gas ports, the breathing patterns were monitored as described above. The temperature within the plethysmograph, the room temperature, and the barometric pressure were recorded during all experiments, although inclusion of these data into

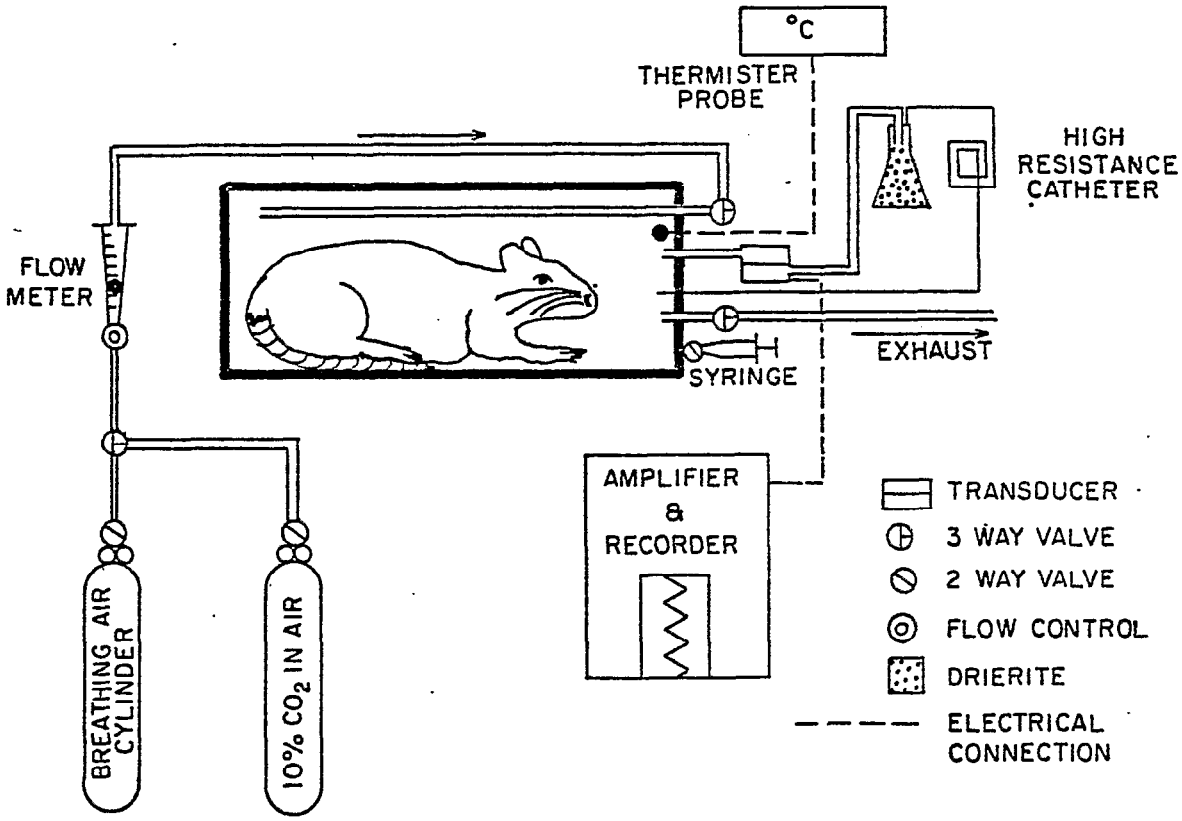

Figure 2. Schematic diagram of a modified Fenn-Brorbaugh plethysmograph.

calculations to determine V_T was found not to affect these volumes. Breathing frequencies were determined directly from chart recordings. Each V_T was also determined directly from chart recorder deflections and along with f was used to calculate estimated $\dot{V}_E$'s which could be used to determine the percent change in ventilation as follows:

$$\%\Delta V_E = \left[\frac{(V_T \text{ deflection}) \cdot f \text{ in } CO_2 - (V_T \text{ deflection}) \cdot f \text{ in air}}{(V_T \text{ deflection}) \cdot f \text{ in air}}\right] \times 100$$

Small differences in the actual V_T's due to pressure and temperature changes on a day-to-day basis did not affect the relative values of the V_T estimates made from strip chart deflection. Thus, chart deflection estimates of V_T were used to determine the percent change in "$\dot{V}_E$" with no apparent loss of accuracy in the overall determination of CO_2-enhanced ventilation.

Arterial blood gases were analyzed in approximately 10 of the 24 rats designated as multiple endpoint animals in each chamber. The time required for caudal artery cannulation and recovery from anesthesia (2-3% Ethrane, 30% O_2 in N_2) was too lengthy to permit blood gas measurements in all animals. Anesthesia appeared uniform through the entire surgical procedure, typically 10 to 15 minutes. Following cannulation, the animal was placed into a modified Bollman (14) restrainer and the rat's tail secured to the restrainer. After a minimum recovery period of 15 minutes, a 0.5 cm^3 blood sample was taken. This blood loss did not have any apparent effect as judged by comparison of the data obtained from bled animals and those that were not so treated. The caudal artery was ligated and the animal returned to its cage. Blood

gases (pO_2 and pCO_2) and pH were determined with an IL Model 113 pH/Blood Gas Analyzer (Instrumentation Laboratory, Lexington, MA). Generally, at least one hour elapsed before these rats were further evaluated.

A constant volume plethysmograph (2.2 liter) was used for the measurement of lung mechanics. This unit was maintained isothermal by an attached 16 liter insulated reservoir bottle filled with copper mesh (Figure 3).

Lung volume changes were measured as proportional pressure changes using a high frequency response differential pressure transducer (Setra System 239: $\pm$7.6 mm Hg) referenced to a 16 liter bottle filled with copper mesh. This transducer was embedded directly into the wall of the plethysmograph to minimize frequency damping. Intrathoracic pressure was measured with a second differential pressure transducer (Sanborn 268B: $\pm$ 40 mm Hg) via a water-filled esophageal catheter (PE-160) inserted to a depth of 10 cm from the upper incisor teeth. From the side of the 4 mm breathing port of the plethysmograph, a second water-filled catheter was connected to the reference side of the intrathoracic transducer. The electronic subtraction of the esophageal pressure (P_e) from airway pressure (P_{ao}) provided the transpulmonary pressure (P_L), the so-called driving pressure of the lungs. Prior to animal testing, the lengths of the esophageal and airway catheters were adjusted to ensure that a constant phase relationship existed between transpulmonary pressure and plethysmographic pressure. These pressures were in phase to a frequency of 6 Hz, confirmed using a piston pump (1 cm^3 displacement).

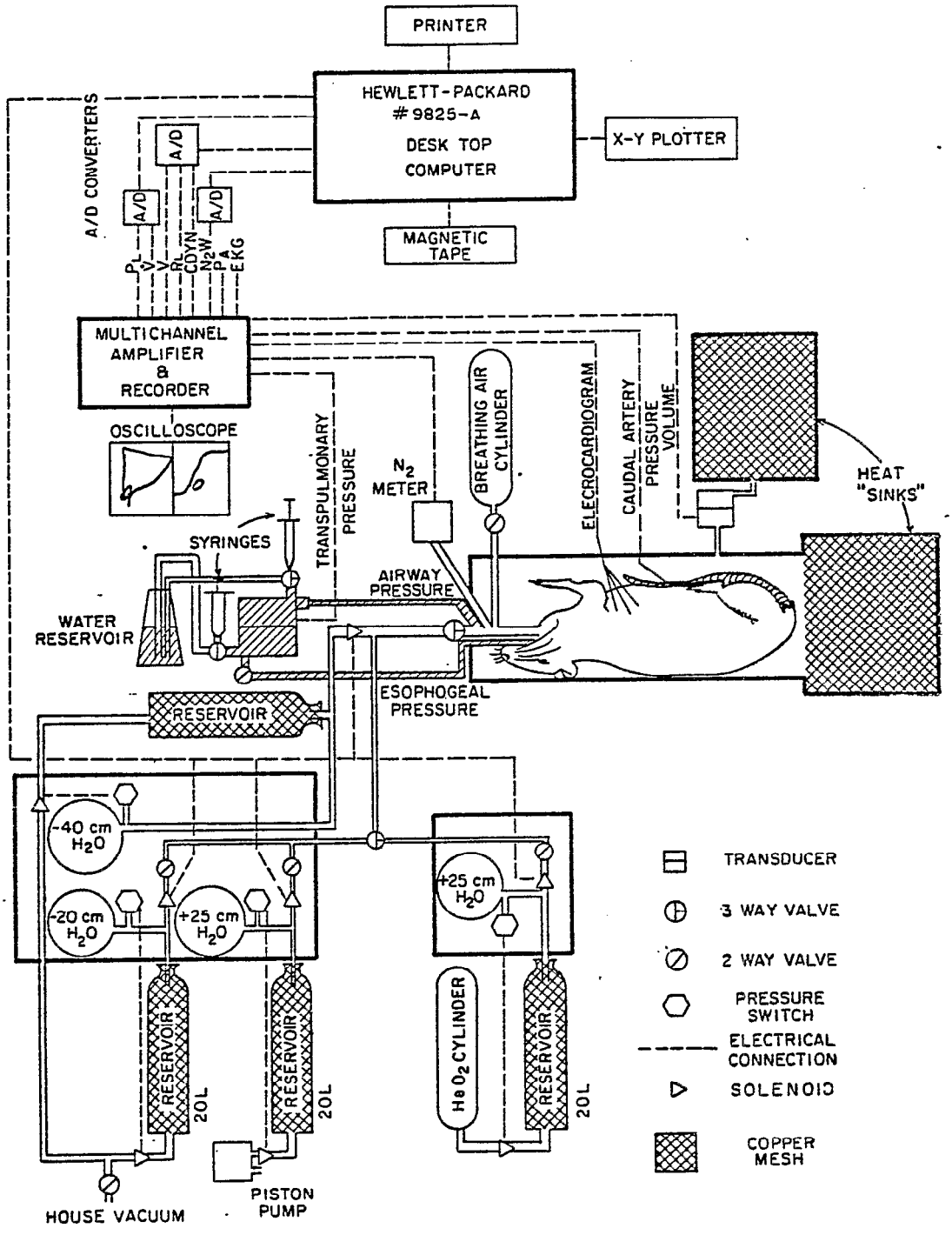

Figure 3: Schematic diagram of the plethysmograph and associated equipment used to access rodent pulmonary function.

Prior to the induction of specific breathing maneuvers, V_T, f, P_L, air flow ($\dot{V}$) as derived from V_T, pulmonary resistance (R_L), and dynamic compliance (C_{DYN}) were recorded. The V_T and P_L signals were conditioned by HP-8805C carrier preamplifiers. The R_L and C_{DYN} were calculated by an analog computer (HP-8816A Respiratory Analyzer, Waltham, MA) according to the method of Mead and Whittenberger (15). Airflow, as derived by the computer module, and C_{DYN} were conditioned through a HP-8802A medium gain preamplifier. Three-lead electrocardiograms (EKGs) were obtained from each animal just prior to its being placed into the plethysmograph. The lead (needle) configuration formed a triangle on the animal's chest. The indifferent electrode lead was attached at the base of the left front leg, the negative electrode was located at the base of the right front leg and the positive pole was positioned just below the animal's seventh rib. Heart rate and intervals of cardiac electrical activity, (P-R and QRS intervals) were measured from these tracings. Permanent records of all the waveforms were made using an eight-channel recorder (Gould, Brush 2800, Cleveland, OH).

Prior to any measurements, each animal was anesthetized with 75 mg/kg pentobarbital (Nembutal). Reliable anesthesia was achieved by injecting 67% of the total dose followed by the remaining 33% after the loss of righting reflex. This resulted in a relatively stable level of anesthesia for a period of approximately two hours, sufficient time for assessment and subsequent sacrifice.

A cannula, molded from teflon shrink tubing, was transorally inserted into the trachea of each rat, by-passing the effect of the nose on all of the measurements made on these otherwise obligate nasal

breathers. A shoulder was molded onto the tubing approximately 1 cm from the proximal tip to ensure an airtight seal with the glottis upon insertion of the tube. The rat was placed in the plethysmograph in a supine position. The dead space volume of the cannula, including all valving to the glottis insert, was manometrically measured. In all calculations, this volume was adjusted to BTPS (body temperature pressure saturated). The volumes of the tracheal cannulas used were between 1.55 and 1.90 cm^3. The "effective" dead space from the mouth opening to the distal end of the breathing port was 0.71 cm^3. To minimize the error introduced by this latter dead space on the parameters of spontaneous breathing, a bias flow of breathing air (approximately 400 cm^3/min) was introduced into the tracheal cannula through a side port to maintain fresh air in that space. The bias flow was suspended during all other measurements.

Before being assessed each rat was allowed to stabilize within the plethysmograph chamber for approximately 10 to 15 minutes. This period was determined by the stability of spontaneous breathing parameters, R_L and C_{DYN}. When these tracings had satisfactorily stabilized, their average values over a 0.5 minute period were recorded. Thereafter, a series of ventilatory maneuvers was performed on each animal to assess the following: apportionment of lung volume, QSC, multibreath N_2 washout, and characterization of the MEFV curve with air and helium. The TLC and RV were defined as those lung volumes corresponding to a transpulmonary pressure of +25 cm H_2O and -20 cm H_2O, respectively. Inflation and deflation of the lungs from the end of expiration (the end of a normal tidal breath) were achieved through the use of large volume, constant-pressure reservoirs controlled by solenoid valves.

Quasi-static volume (V)/P_L relationships were determined in a similar manner, but were measured at a specific inspiration rate (~3 cm^3/sec) to TLC followed by a slow deflation (~3 cm^3/sec) to RV. The resulting volume-pressure curves were recorded on tape with the HP-9825B-desk top computer and later plotted with an HP-9826A calculator plotter. Quasi-static compliance was estimated using the chord slope-(QSC_{cs}) between 0 and 10 cm H_2O P_L of the deflation limb of the V/P_L curve. This pressure range was selected because it is typical of the lower and upper limits, respectively, of tidal P_L. Exponential analysis of the V/P_L curve was performed to assess the theoretical elastic properties of the lung (16). Deflation lung volumes, corresponding to 5 cm H_2O pressure decrements from 25 cm H_2O to 0 cm H_2O, were fitted to the exponential: $V_p = V_o(1-\exp P/h)$, where V_o represents the extrapolated, theoretical lung volume at infinite pressure, P is the pressure (cm H_2O) at the particular lung volume (V_p), and h is the pressure (cm H_2O) which will distend the lung to one half V_o.

The functional residual capacity (FRC) was measured by neon dilution (FRC_d) as described by Takezawa et al. (17) and the Boyle's Law technique (FRC_b) (18). The "standard" gas used in the dilution measurements consisted of 0.532% Ne, 0.497% CO, and 22.01% O_2 in N_2. The volume injected was equal to the plethysmographically determined vital capacity (VC) adjusted to ATPD (ambient temperature pressure dry). From RV, a volume equal to the VC (ATPD) was injected from a syringe through a three-way valve. The lungs were then ventilated ten times in approximately ten seconds with this syringe using a stroke volume of 75% the VC. The constituent gases in the last VC-volume withdrawn were assayed with a gas chromatograph (Carle Basic GC 8700, Fullerton, CA). The

proportional Ne dilution and the VC (BTPS) were used to calculate the FRC_d after adjusting for the dead space of the equipment and subtracting the measured inspiratory capacity (IC). The FRC_b was determined by occluding the airway at end-expiration and comparing ΔP_{ao} to ΔV with each inspiratory effort. Calculation of $VP = V'P'$, corrected for dead space, yielded the FRC_b. These calculations were done on-line by the HP-9825 desk-top computer programmed for breath-by-breath calculation of the FRC_b. Both FRC_d and FRC_b represent estimates of the resting lung volume, including the trachea up to the naso-pharynx. The BTPS correction was based on the ambient barometric pressure and a body temperature of about 34°C, a body temperature previously recorded in similarly anesthetized rats.

Diffusing capacity for CO ($DLCO_{rb}$) was determined in conjunction with the rebreathing technique used to determine TLC by dilution as described above. The equilibrated concentrations of alveolar gas and the time from inspiration (gas injection) to the final expiration (expirate collection) were used in the Krogh (19) calculation.

Ventilatory homogeneity was evaluated by assessing multibreath N_2 washout. This was accomplished by sampling end-expiratory (alveolar) N_2 gas directly in the tracheal tube using a MedScience Nitrolyzer (St. Louis, MO) while the animal was breathing 100% O_2 which flowed by the tracheal tube opening at approximately 400 cm^3/min. A total of 50 breaths were sampled for each animal. The natural log of the end-expiratory N_2 concentration was plotted against the dilution value (V_T • breath/FRC_d) for each breath by the HP-9825B computer using data collected on-line during the maneuver. Moment analysis was then used to assess the degree of ventilatory inhomogeneity.

The MEFV curve, used to assess small airway mechanics, was an imposed expiratory maneuver. It was controlled directly by the HP-9825B computer which also collected all flow and volume data on-line. Three seconds after slow inflation to TLC, the tracheal port of the plethysmograph was exposed to a pressure sink of -40 cm H_2O by activating a wide bore solenoid valve (Skinner Valve - V53DB2VAC2, 1/4"-3/32" orifice, New Britain, CT). The tubing from the sink to the valve, as well as between the valve and tracheal port, was as large and rigid as practically possible. (With closed vials used to represent body mass, 10 cm^3 of air was injected into the closed plethysmograph; the time to peak flow for the system with the tracheal tube in place was 50 msec.) For each animal, peak expiratory flow (PEF), expiratory flow at 50, 25, and 10% VC (EFR_{50}, EFR_{25}, and EFR_{10}, respectively), and the percent expired VC at PEF were recorded. The ΔEFR_{25} was measured as the difference in flow at 25% VC above or below that flow estimated by a chord slope drawn from EFR_{50} to EFR_0. A positive ΔEFR_{25} is a measure of the degree of convexity (away from the volume axis) of the effort independent portion of the MEFV curve and conversely, a negative ΔEFR_{25} is a measure of curve concavity (toward the volume axis).

Using the MEFV and quasi-static compliance data, maximum-flow static recoil curves were derived for the determination of "upstream" airway resistance (R_{us}) during the MEFV maneuver. The R_{us} of each animal was calculated as the static pressure (P_{st}) divided by $\dot{V}$ at 30% of its lung volume ($\dot{V}_{30}$). The existence of airway obstruction and/or loss of tissue elasticity as the potential cause of the decreased flow could thereby be deduced.

To test density dependent changes in small airway mechanics, a He-MEFV curve was derived as described above, but with a 20% O_2, 80% He mixture for inflation. After bringing the animal to RV, it was inflated to TLC with the He:O_2 mixture and then rapidly exposed to the -40 cm H_2O pressure sink. Rebreathing the He:O_2 mixture or deriving multiple experimental curves did not significantly affect the enhanced R_{us} generally encountered in this maneuver. The difference in flow between the He and air MEFV curves at 50 and 25% VC ($\Delta HEFR_x = EFR_x(He) - EFR_x(air)$) were used as the index of altered density-viscosity transition in the small airways. When possible, isoflow points (i.e., as the % VC) where the He and air curves overlapped or crossed were noted.

Radiographic Techniques

Following assessment of pulmonary function, a single frontal radiograph was taken of each animal. The x-rays were taken with a Westinghouse, Newport 1958 portable x-ray system at 32 keV/20 milliamp seconds (mas) at a focal distance of 43 cm. To stop breathing motions the rat to be x-rayed was hyperventilated with 10 repeated intratracheal injections (air) of approximately 75% IC via the tracheal cannula to achieve apnea. The rat was then inflated to TLC with a volume equal to its IC and held at that volume for the x-ray. A 0.25 sec x-ray was taken with the animal in a supine position on a sheet of plexiglass suspended 43 cm above the Kodak Min-R cassette containing Kodak Min-R film (MR-1). The rat was then released from TLV and subsequently necropsied. The x-ray film was developed using a Payro-Automatic Processor and Eastman Kodak solutions. Each x-ray film was coded according to group of origin for blind evaluation. Evaluation included descriptive record for the individual rat x-ray films and an attempt to order the groups by exposure level.

Determination of Lung Composition

The right lung of each rat designated for multiple pulmonary endpoint assessment was weighed, homogenized in water using a Polytron Homogenizer (Brinkman Instruments), and the total volume brought to 10 ml with water. Suitable aliquots of the homogenate were then taken for determination of dry weight by freeze drying in tared tubes, and for chemical analyses.

Collagen content was determined and reported as total hydroxyproline in the sample. Hydroxyproline was determined by the method of Bergman and Loxley (20) after hydrolysis of the aliquot in 6 N HCl at 105°-110°C in an evacuated tube for 22 hr. Elastin was considered to be the insoluble protein remaining after treatment of an aliquot with 0.1 N NaOH at 98°C for 0.5 hr. It was determined by the method of Naum and Mogan (21) and compared with a sample of bovine ligamentum nuchae elastin (Sigma) as standard. Total protein was determined by the Hartree (22) modification of the Folin-Lowry method. The method of Burton (23) was used for DNA determinations after heating a sample in 5% perchloric acid at 90°C for 12 min (conditions found to give the maximum color).

Pathological Examination

The animals designated for pathological examination from each chamber were anesthetized with pentobarbital and then exsanguinated via the descending aorta. The thorax was opened and the heart and lungs were removed intact. The trachea was detached at the larynx and the thymus, heart, lymph nodes, epicardial fat, and esophagus were carefully removed from the respiratory tissue. The lungs were patted dry and weighed with the trachea still attached. The lungs were then infused with 2.5% glutaraldehyde in 0.1 M cacodylate buffer at a pressure of 25

cm water for 30 minutes. After the infusion period, the left lung of four randomly selected animals from each exposure group was submerged in this fixative for 3.5 hours, after which several tissue slices were removed for possible future electron microscopy studies. The tissue remaining from the left lung was then placed in 10% buffered formalin. The right lobes of these animals were placed into 10% buffered formalin immediately after the 30 minute infusion period. The following tissues were collected and stored in formalin: eyes, pituitary, thyroid, salivary glands, brain, cervical lymph node, larynx, trachea, thymus, peribronchial lymph node, heart, esophagus, stomach, small intestine, large intestine, cecum, liver, pancreas, kidney, adrenal glands, mesenteric lymph node, urinary bladder, gonads, seminal vesicle, epididymus, prostate, penis, sternum, diaphragm, rib junction, skeletal muscle, peripheral nerve, skin, spleen, and nasal cavity. All pathological examinations were done under contract by Experimental Pathology Laboratories, Inc. (EPL)(Herndon, VA). Microscopic examination was conducted on hematoxylin and eosin stained sections of lung, peribronchial lymph node, nasal turbinate, brain, kidney, liver, spleen, testes, and heart from eight animals from each exposure group.

The left lung of the animals in the multiple pulmonary endpoint groups was submitted for histopathologic examination. This provided pathology, respiratory physiology, and lung composition data on individual animals, and also served to determine whether the respiratory physiology testing battery itself induced pulmonary damage. These lung lobes were infused through the trachea with 2.5% glutaraldehyde in Sorenson's buffer for 30 minutes and then stored in 10% buffered formalin until embedded.

To provide data suitable for statistical evaluation, numerical values were generated from the lung histopathology sections by adding up the values which indicated the severity of the pulmonary lesions observed. The scored lesions included perivascular and peribronchiolar lymphoid proliferations, intralymphatic microgranulomas, hemorrhage, abnormal numbers of histiocytes, granulocytes, and mononuclear cells, fibrosis, and type II cell hyperplasia.

Cytogenetic Methods

On the day following the final exposure, rats designated for cytogenetic studies were briefly anesthesized with enflurane (Ohio Medical Products) and placed in modified Bollman restrainers (14). Tail veins were cannulated with hubless 23 gauge needles inserted into Clay Adams P.E. tubing attached to 1 ml syringes loaded with isotonic phosphate buffered saline (PBS, pH 7.3). After cannulation, the P.E. tubing was attached to similar tubing originating at a Watson-Marlow 10-Channel peristaltic pump with auto analyzer tubing (Gamma Enterprises). The pump delivered 5-bromodeoxyuridine (BrdUrd) (Cal Biochem) dissolved in PBS at a rate of 50 mg/kg body weight/hour for 24 hours (total volume ~45 ml). During this time, the animals were provided with food and water ad libitum. After 24 hours, the animals were intraveneously injected with colcemid (80 mg/kg body weight). One hour later the tubing was removed and one hour after that (total elapsed time, 26 hours) the animals were killed by continued exposure to enflurane.

Immediately after cessation of breathing, the chest cavity was opened and a sample (~3 ml) of blood was obtained by cardiac puncture using a heparinized 3 cc syringe. Whole blood (0.25 ml) was inoculated into 5 ml McCoy's 5A medium containing 10% fetal calf serum (Sigma), 2.5

µg/ml BrdUrd and 5 µg/ml phytohemagglutinin-P (PHA)(Burroughs Wellcome). Complete cultures were incubated at 37.5°C for 72 hours in darkness. Colcemid (0.1 mg/ml) (Gibco) was added to each culture four hours before termination. At termination the cultures were centrifuged and the pellet was resuspended in hypotonic KCl (0.075 M) for 15 min at room temperature. The cells were then fixed twice in methanol:glacial acetic acid (3:1) and stored at 0°C until slides were prepared (24).

Both femurs were removed and the bone marrow rinsed out using PBS. The resulting material was incubated in hypotonic KCl (0.075 M) for 20 min at 37°C, then fixed twice in methanol:glacial acetic acid (3:1) and finally stored at 0°C until slides were prepared (25).

The right epididymidis was removed and minced in PBS, and the large particles allowed to settle. The resulting supernatant containing sperm was spread onto clean microscope slides and fixed for 10 min in methanol:glacial acetic acid (3:1) (26,27).

All processed material (with the exception of sperm) was flame-dried onto microscope slides. After staining with Hoechst 33258 (0.5 mg/ml distilled H_2O) for 20 min, a coverslip was mounted onto the slide with phosphate:citric acid buffer (pH 7.0). The slide was then exposed to blacklight fluorescent tubes (~2.5 cm distance) for 25 min, the coverslip was removed and the slide stained with Giemsa (4% Harleco Giemsa and 4% methanol in distilled H_2O) for ~5.5 min (28). Sperm slides were stained with 0.22% eosin Y for 30 min.

One hundred randomly chosen metaphase cells in each sample were scored for the number of times they had replicated (one, two, or more replications), as distinguished by their BrdUrd staining patterns (29). Twenty-five second generation metaphase cells were scored for the number

of sister chromatid exchanges (SCEs) (25) and 50 first generation metaphase cells were scored for chromosomal aberrations (30). Finally, 500 sperm were examined from each animal to determine the frequency of morphologically abnormal specimens (26,27).

Reproductive Potential Methods

Six days after the final exposure eight male rats from each exposure group were individually housed with two unexposed females for seven days. Ten females from each exposure level were mated with unexposed males (1:1) that had previously been mated with unexposed females to assure that they were fertile. Females from these matings were sacrificed 19 days after the first mating, as determined by the presence of sperm in the vaginal smears. Upon sacrifice the numbers of viable embryos, late deaths, early deaths (reabsorptions), and corpora lutea were determined. Preimplantation losses (corpora lutea-(early deaths + late deaths + viable embryos)) were also evaluated.

Statistical Methods

Weight gain data were analyzed by one-way analysis of covariance with repeated measures, using an animal's weight on the first day of exposure as the covariate. If significant differences among exposure groups were indicated, each pair of adjusted group means was compared using an F-test. To adjust for the complication of multiple comparisons, the α-level (probability of falsely rejecting the null hypothesis) was divided by the number of comparisons made, according to the method of Bonferroni (31). Thus, a pair of adjusted means was considered to be significantly different if its associated p-value was less than 0.0083 (0.05/6), because six pair-wise comparisons were made. One-way analysis of variance (ANOVA) was used to compare the means of single variables

across exposure groups. The SCE data was subjected to a square root transformation prior to ANOVA to normalize the distribution and to provide greater homogeneity of variance. The data on percent abnormal sperm was analyzed after arcsin square root transformation. The replicative history cell cycle data was transformed to an average generation time (AGT) using the formula AGT = (BrdUrd exposure time/RI) where the replicative index (RI) = [(1 x frequency of 1st generation cells) + (2 x frequency of 2nd generation cells) + (3 x frequency of 3rd generation cells)]. When ANOVA indicated a significant difference among group means, Duncan's multiple range method of multiple comparisons (32) was used to investigate the source of the differences. In these cases, the exposure groups are reported in order of ascending mean values (control, CN; 2 mg SiO_2, LD; 10 mg SiO_2, ID, 20 mg SiO_2, HD); the means of those groups joined by a common underscore did not differ significantly.

In addition to ANOVA, quasi-static compliance data and flow-volume data were each analysed as sets of variables. These sets were compared among exposure groups by a multi-variate analysis of variance (MANOVA).

In each table and figure which reports the results of ANOVA and MANOVA, the p-value of the corresponding F-statistic is also reported. This value is the minimum level at which statistical significance would be indicated. Those p-values less than or equal to 0.05 were taken to indicate significant differences among the group means.

To investigate differences among exposure groups based on histopathologic and chromosome aberration data, values were non-parametrically ranked and were then analysed by the Kruskal-Wallis non-parametric test. When a significant difference was indicated among the groups, non-parametric multiple comparisons were performed according to the

method of Dunn (33) to identify the source of the differences.

For each of the above tests, the p-value reported is the minimum level at which the relevant test statistic would indicate statistical significance. Those p-values less than or equal to 0.05 were taken to indicate significant differences among group means for the corresponding variable(s).

Stepwise discriminant analysis was used to determine the degree to which the four exposure groups were distinct based upon physiology variables, lung composition variables, or all variables combined. This technique generated a set of linear functions of the variables under consideration which displayed the groups to be as distinct from one another as possible. The effectiveness of this distinction was measured by means of classification functions, which classified an animal into one of the four groups according to its values for each of the original variables; the classification thus obtained was compared with the true group classification of the animal to assess the percent of all animals correctly classified. For each animal, the classification functions were estimated using the data from all other animals. Thus, classification functions were estimated separately for each animal, and these estimates were independent of the data for that animal. This scheme, referred to as "jackknifed classification", reduced the bias in this analysis. The proportion of animals correctly classified has been reported using jackknifed classification.

Stepwise discrimant analysis operates in a stepwise manner to select those variables which make up a minimal set of variables which can distinguish among the groups. At each step, that variable (if one exists) which most improves the ability to discriminate among groups is

included, or that variable (if one exists) which adds no discriminating information to the information contained in the other included variables is deleted. (The F to enter and F to delete were 4.0 and 3.996, respectively.) This procedure continued until no single excluded variable could significantly improve the discrimination among the groups. The proportion of animals correctly classified (jackknifed) was then evaluated using this reduced set of variables. These variables are considered to be the "most important" in discriminating among the exposure groups. Although this interpretation is an accurate one, it could be misleading because the variables are only selected individually. Thus, if two or more variables each display little ability to distinguish the groups, they will not be selected by the stepwise discriminant algorithm even if those variables as a set are effective.

Most statistics were computed using the Biomedical Computer Programs (BMDP) statistical package programs 7D, 8D, 2V, 4V, 3S, and 7M. Multiple comparisons were calculated by hand. All tests were conducted accepting the 0.05 level as significant.

General Toxicology Parameters

Exposure Conditions. The mean daily concentrations of silica in the exposure chambers have been provided in Figure 4. The mean daily concentration for subgroups of animals which entered their respective chambers on different days were 2.0 mg/m^3 for the 2.0 mg/m^3 chamber, 10.2 mg/m^3 for the 10 mg/m^3 chamber, and 19.3 mg/m^3 for the 20 mg/m^3 chamber. Because the exposure group averages were within 10% of the target concentration for any chamber, the exposed animals will subsequently be referred to as belonging to the 2, 10, or 20 mg/m^3 exposure group.

Animal Weights and Condition. Animals exposed to the three concentrations of silica tested did not show any outward signs of toxicity or discomfort. The weight data collected from the biweekly weighing of selected subgroups of exposed animals in each chamber (Figures 5 and 6) were analyzed using one-way analysis of covariance with repeated measures. The first weight was used as the covariate for each animal. The Bonferroni probability level of 0.0083 was used to protect the overall significance level of 0.05 for multiple comparisons among the groups. No significanct differences were found in the growth rates of the male animals during the exposure period. However, examination of the weight changes of the exposure groups between the final exposure and the endpoint assessment date, six days post-exposure, indicated that the high-dose animals gained significantly more weight than the control and 2 mg/m^3 rats ($p<0.05$ using Duncan's multiple range method). Interestingly, the female rats exposed to 10 and 20 mg/m^3 of silica dust appeared to grow faster than the control animals (Figure 6) (pairwise F-test

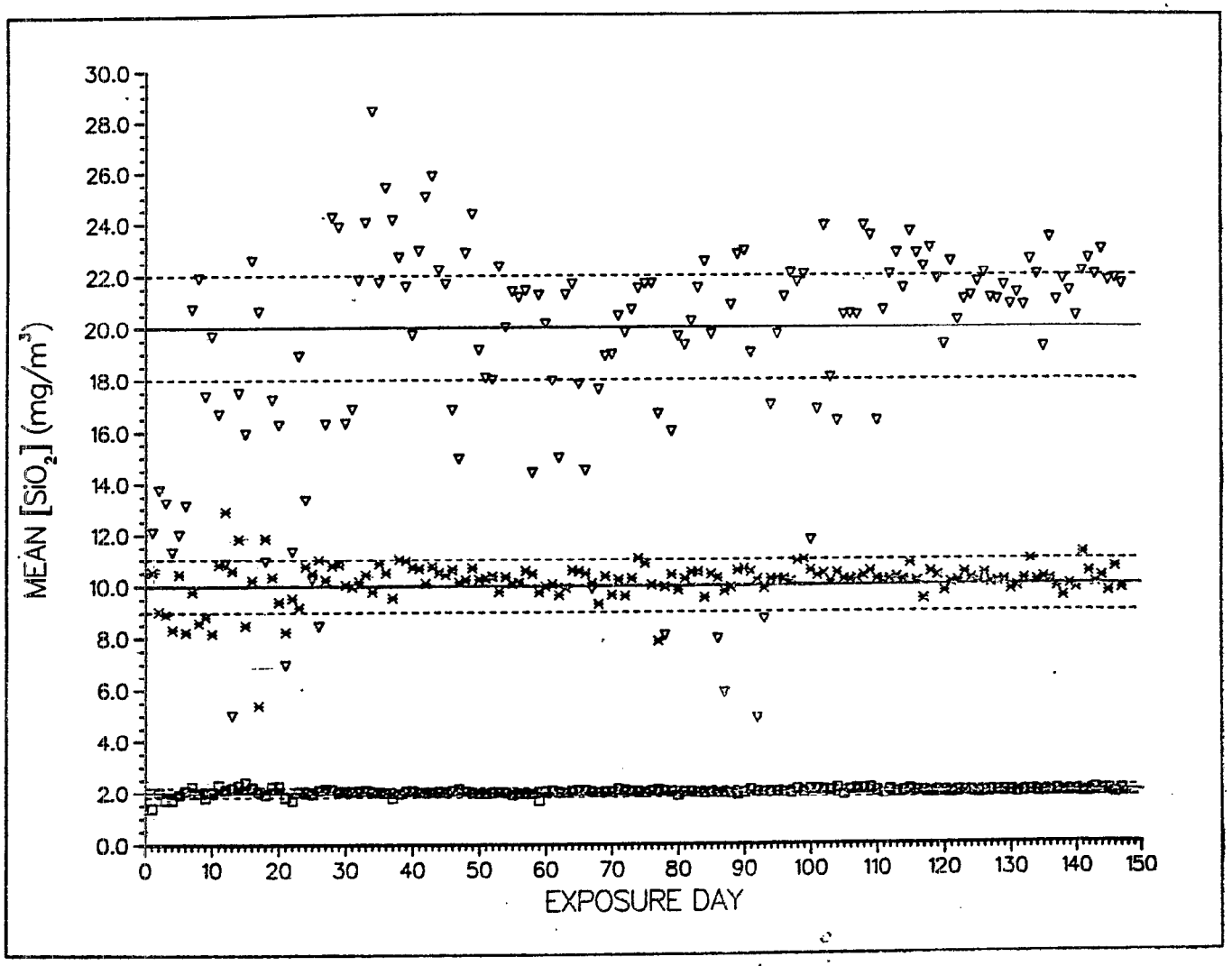

Figure 4: Daily mean silica-concentration in the animal exposure chambers: (□) 2.0 mg/m^3, (∗) 10 mg/m^3, and (▽) 20 mg/m^3. The dashed lines indicate $\pm$ 10% of the target concentrations.

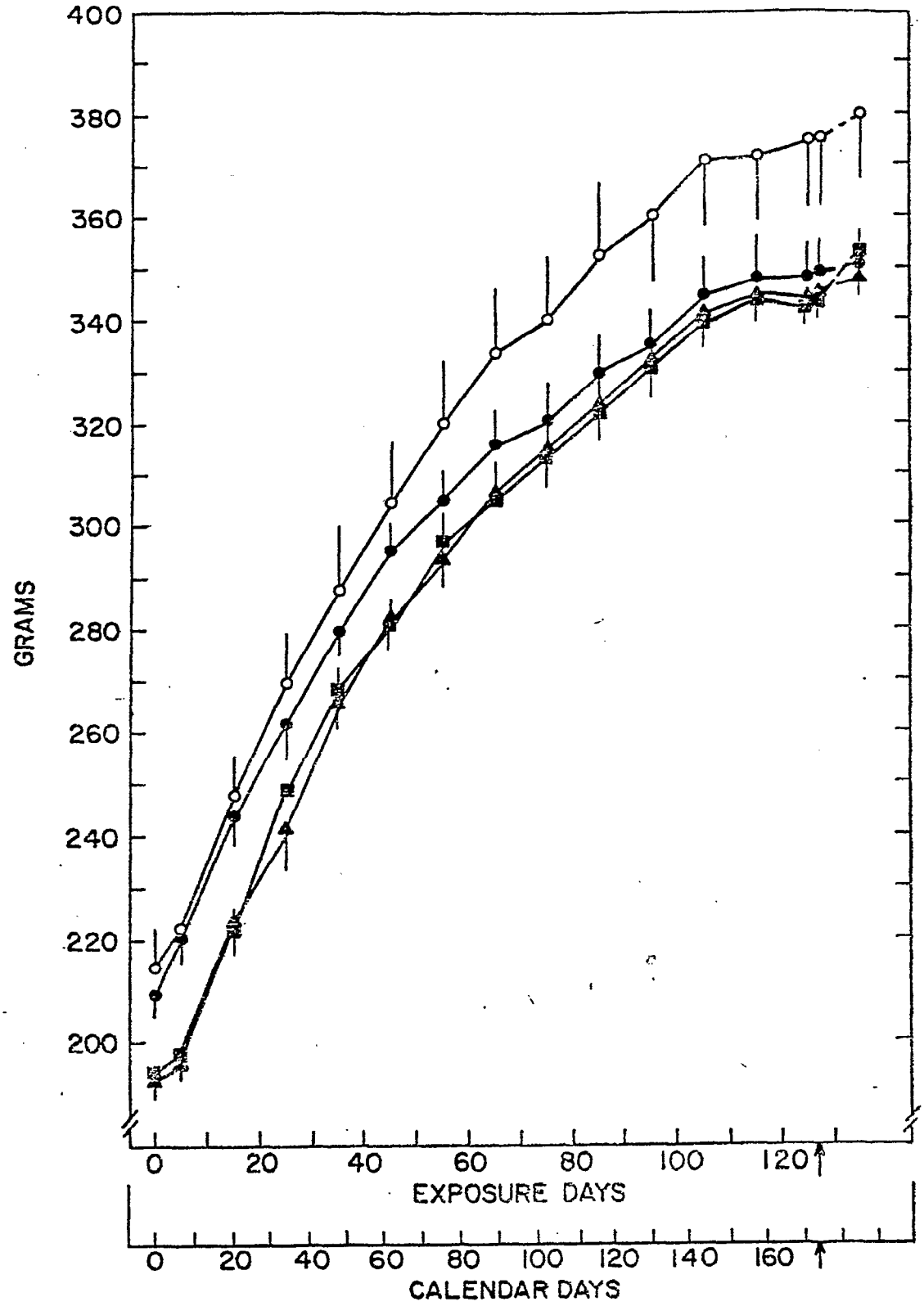

Figure 5: Weights of control and silica-exposed male Fischer-344 rats with increasing time of exposure. Exposures were for 6 hours/day, 5 days/week. The data are the means ± standard errors of 8 animals in each of the 0 mg SiO_2/m^3 (●), 2 mg SiO_2/m^3 (▲), 10 mg SiO_2/m^3 (o), and 20 mg SiO_2/m^3 (■) exposure groups. The dashed lines between the last two data points for each group indicate the weight gain during a six-day period after exposures were terminated (↑). (See text for details.) (A listing of the mean (± s.e.) of each subgroup weight at each time point is provided in Appendix D.)

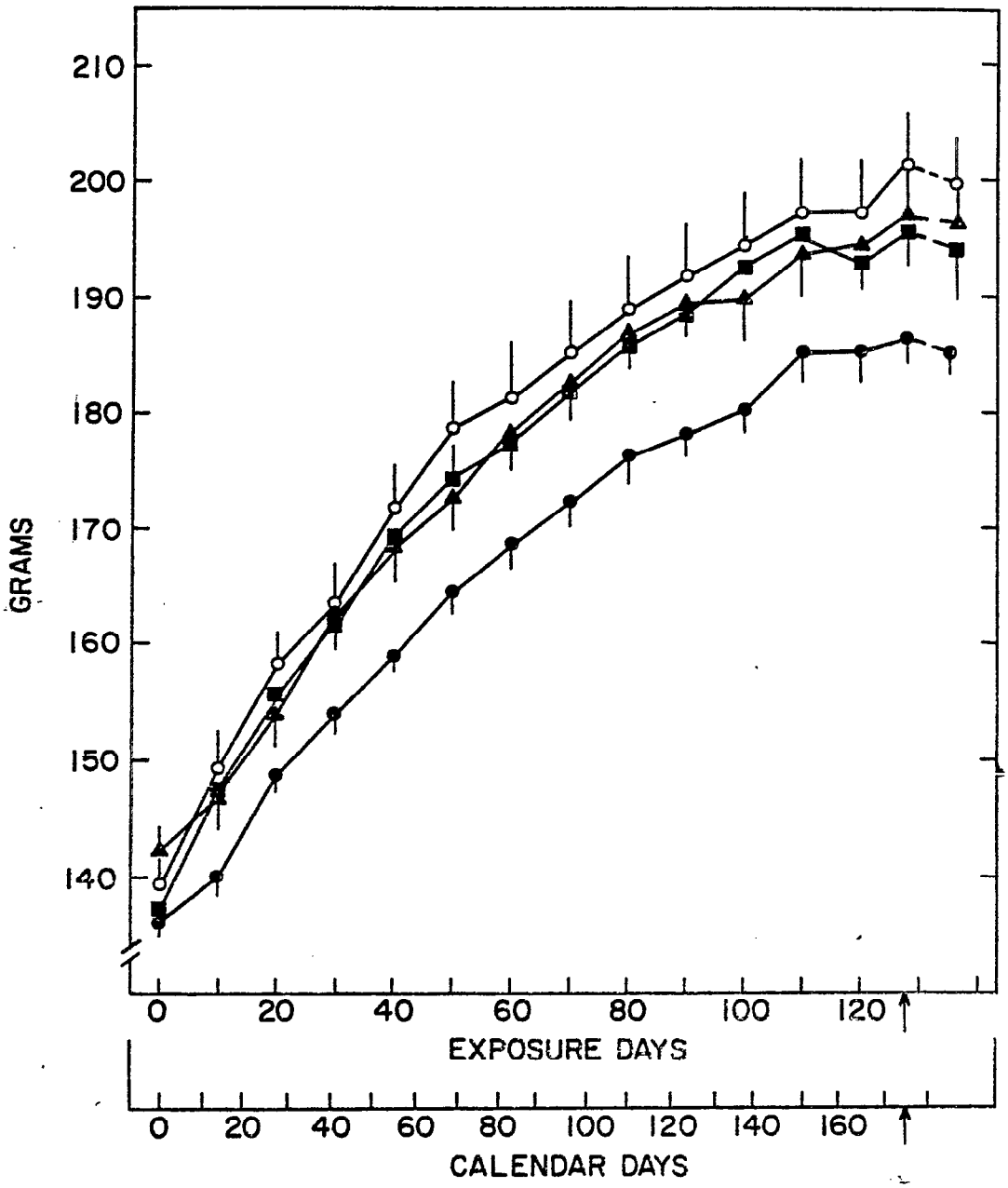

Figure 6: Weights of control and silica-exposed female Fischer-344 rats with increasing time of exposure. Exposures were for 6 hours/day, 5 days/week. The data are the means ± standard errors of 8 animals in each of the 0 mg SiO_2/m^3 (●), 2 mg SiO_2/m^3 (▲), 10 mg SiO_2/m^3 (o), and 20 mg SiO_2/m^3 (■) exposure groups. The dashed lines between the last two data points for each group indicate the weight gain during a six-day period after exposures were terminated (↑). (See text for details.) (A listing of the mean (± s.e.) of each subgroup weight at each time point is provided in Appendix D.)

p-values = 0.0033 and 0.0065, respectively). In the case of the females, there were no significant differences among the groups in post-exposure weight gain.

Organ Weight and Organ-to-Body Weight Ratios. The weights of selected organs from those rats designated for pathological examination from each exposure group have been provided in Table 2. The lungs from these animals exhibited a dose dependent increase in fresh weight (Table 2) which was also reflected in the lung-to-body weight ratio (Table 3). The fresh weights of the lungs exposed to 2, 10, and 20 mg SiO_2/m^3 increased to 108, 111, and 121%, respectively, of the control lung weights during the 6 month exposure period. The differences in lung weight and lung-to-body weight ratios, however, were not reflected in the data on the displacement volumes of the right lungs from these animals (Table 4). Although ANOVA indicated that slight differences ($p=0.0468$) existed among the fresh kidney weights of the silica exposed rats, these changes appeared random and were not considered exposure related. Also, liver weights of all of the exposure groups were slightly greater than those of control animals.

Respiratory Physiology

Each set of respiratory physiology variables will be presented in the order they were derived during the testing procedure. During assessment, an occasional datum for an animal could not be reliably determined, thereby resulting in a reduced sample size in the presented data. Individual pulmonary function data from all animals tested are provided in Appendix E.

Table 2. Organ Weights of Control and Silica-Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	8	8	8	8	
LUNGS (g)					
mean	1.19	1.28	1.32	1.44	0.0001[b]
s.e.	0.04	0.02	0.03	0.03	
multiple comparisons[c]		CN LD	ID HD		
HEART (g)					
mean	1.01	1.03	1.06	1.05	0.6418
s.e.	0.04	0.02	0.03	0.03	
SPLEEN (g)					
mean	0.70	0.85	0.86	0.90	0.1529
s.e.	0.09	0.05	0.04	0.05	
LIVER (g)					
mean	11.16	12.63	13.22	12.64	0.0312[b]
s.e.	0.37	0.66	0.52	0.27	
multiple comparisons[c]		CN LD	HD ID		
KIDNEYS (g)					
mean	2.32	2.52	2.55	2.51	0.0468[b]
s.e.	0.07	0.07	0.06	0.03	
multiple comparisons[c]		CN HD	LD ID		
ADRENAL GLANDS (g)					
mean	0.06	0.06	0.06	0.05	0.7371
s.e.	0.01	<0.01	<0.01	<0.01	
TESTIS (g)					
mean	3.12	3.30	3.25	3.24	0.0711
s.e.	0.08	0.01	0.04	0.04	
BRAIN (g)					
mean	1.92	2.00	1.94	1.95	0.1331
s.e.	0.03	0.03	0.03	0.01	
BODY WEIGHT (g)					
mean	359.6	378.8	382.6	386.5	0.1316
s.e.	13.0	5.1	8.6	3.2	

a. Six hours/day, 5 days/week, for 6 months.
b. Statistically significant at $\alpha = 0.05$ level, using ANOVA.
c. Pairwise comparison of means by the Duncan multiple range method.

Table 3. Organ-to-Body Weight Ratios (g/kg) of Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	8	8	8	8	
LUNGS					
mean	3.32	3.39	3.45	3.73	0.0061[b]
s.e.	0.08	0.06	0.10	0.09	
multiple comparisons[c]		CN LD	ID HD		
HEART					
mean	2.81	2.72	2.77	2.72	0.5807
s.e.	0.05	0.05	0.06	0.07	
SPLEEN					
mean	1.98	2.24	2.25	2.33	0.4960
s.e.	0.27	0.12	0.09	0.14	
LIVER					
mean	31.08	33.31	34.47	32.71	0.1056
s.e.	0.42	1.60	0.71	0.57	
KIDNEYS					
mean	6.45	6.64	6.67	6.49	0.2609
s.e.	0.07	0.13	0.09	0.08	
ADRENAL GLANDS					
mean	0.17	0.14	0.15	0.14	0.4204
s.e.	0.02	0.01	0.01	0.01	
BRAIN					
mean	5.37	5.29	5.07	5.04	0.1694
s.e.	0.20	0.05	0.12	0.04	
TESTIS					
mean	8.70	8.73	8.52	8.40	0.3370
s.e.	0.16	0.12	0.14	0.15	

a. Six hours/day, 5 days/week, for 6 months.
b. Statistically significant at $\alpha = 0.05$ level, using ANOVA.
c. Pairwise comparison of means by the Duncan multiple range method.

Table 4. Right Lung Displacement Volume of Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	8	8	8	8	
Displacement Volume (cm^3)					
mean	5.30	5.89	5.83	6.36	0.3584
s.e.	0.27	0.28	0.49	0.53	

a. Six hours/day, 5 days/week, for 6 months.

CO_2 Response and Blood-Gas Data. The CO_2 induced hyperventilation observed in silica-exposed rats was not different from that observed in control animals (Table 5). The range of the hyperventilatory response in the four groups tested was 78 to 107% the $\dot{V}_E$ recorded during exposure to normal breathing air (CO_2<0.4%) (Table 5).

Arterial blood-gas partial pressures and pH did not differ among the exposure groups. The differences in pO_2 among the groups were inconsistent and did not conform to a dose-response relationship, suggesting that this finding was not exposure related. Recovery from anesthesia, used to implant the arterial cannula, may not have been complete in some of the animals.

Parameters of Spontaneous Breathing. Several measurements of normal tidal breathing were taken on each animal. None of the variables measured differed significantly among the four exposure groups (Table 6). Normalization of R_L and C_{DYN} with FRC_d (Figure 7) did not result in significant differences among the exposure groups.

Electrocardiographic Data. Heart rate, as determined by EKG, was not significantly altered by silica exposure (Table 7). Because of electrical noise in the processing of the EKG signal, only the P-R and QRS temporal patterns could be readily distinguished. No silica exposed group exhibited EKGs which differed from the control group.

Table 5. CO_2-Induced Hyperventilation and Blood-Gas Data From Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
$\%\Delta \dot{V}_E$					
mean	94.7	96.7	78.0	107.0	0.2138
s.e.	9.3	8.2	6.4	12.8	
n	24	23	22	24	
pCO_2 (mmHg)					
mean	42.8	41.2	40.4	39.9	0.1489
s.e.	0.7	1.0	0.7	1.1	
n	9	12	14	10	
pO_2 (mmHg)					
mean	81.4	78.8	88.4	74.0	0.0164[b]
s.e.	2.9	2.3	4.3	1.3	
n	9	12	14	10	
multiple comparison[c]		HD LD	CN ID		
blood pH					
mean	7.41	7.41	7.40	7.42	0.4053
s.e.	0.01	0.01	0.01	0.01	
n	9	12	14	11	

a. Six hours/day, 5 days/week, for 6 months.
b. Statistically significant at $\alpha = 0.05$ level using ANOVA.
c. Pairwise comparison of means by the Duncan multiple range method.

Table 6. Parameters of Spontaneous Breathing of Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	23	24	23	22	
V_T (cm^3)					
mean	1.68	1.66	1.63	1.62	0.7935
s.e.	0.04	0.05	0.04	0.04	
ΔP_L (cm H_2O)					
mean	5.08	5.45	5.63	4.99	0.5064
s.e.	0.20	0.32	0.44	0.36	
f(breaths/min)					
mean	69	64	65	66	0.6072
s.e.	3	2	2	3	
$\dot{V}_E$ (cm^3/min)					
mean	114.9	106.0	106.6	107.2	0.5761
s.e.	5.9	5.1	4.8	4.5	
R_L (cm $H_2O/cm^3/sec$)					
mean	0.34	0.47	0.44	0.42	0.4650
s.e.	0.04	0.06	0.08	0.06	
C_{DYN} (cm^3/cm H_2O)					
mean	0.37	0.33	0.39	0.38	0.2131
s.e.	0.02	0.02	0.03	0.02	

a. Six hours/day, 5 days/week, for 6 months.

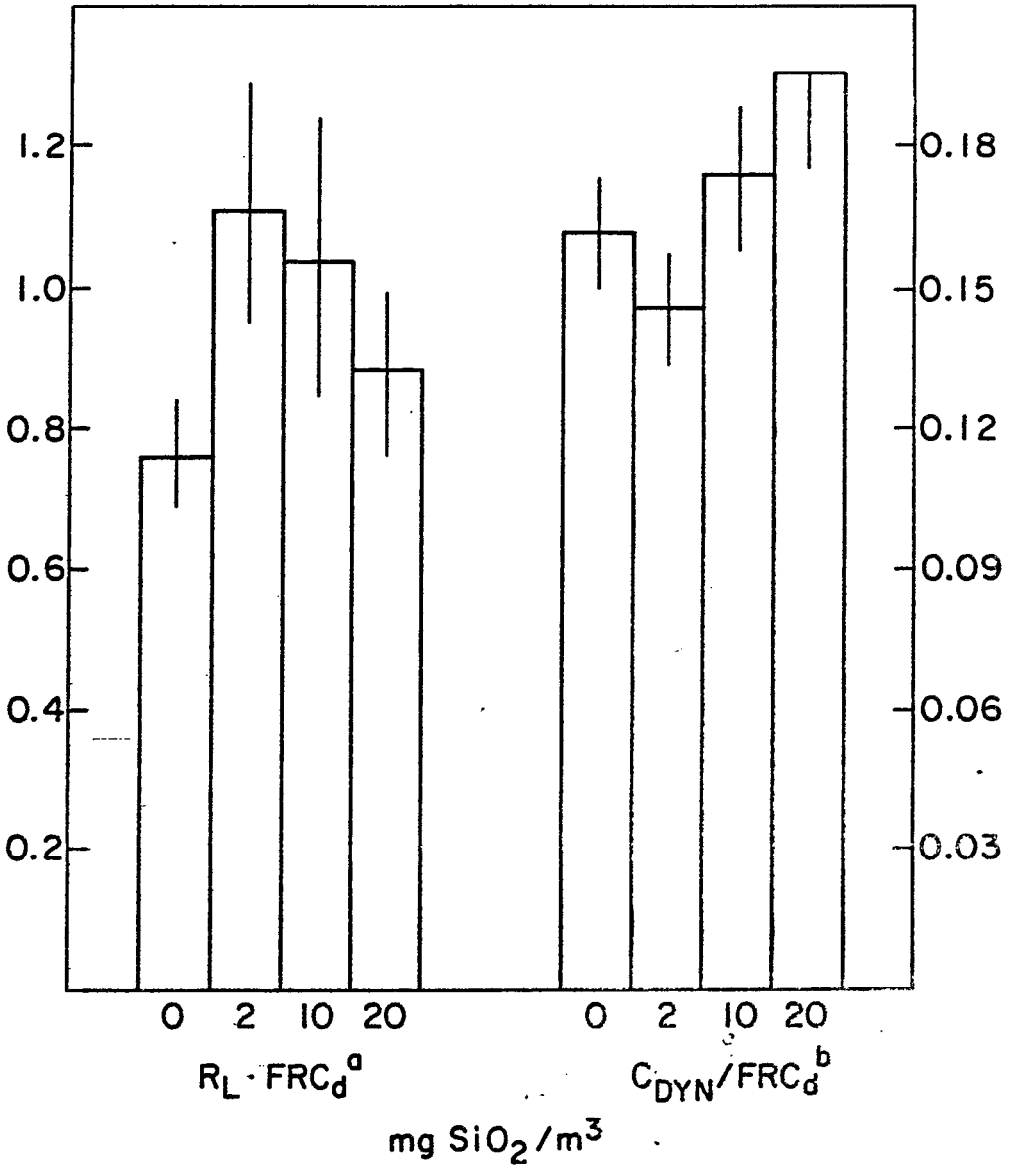

Figure 7: Pulmonary resistance (R_L) and dynamic compliance (C_{DYN}) normalized to the Functional Residual Capacity (FRC_d) of Fischer-344 rats exposed to SiO_2 for 6 months (6 hours/day, 5 days/week). The number of rats in the 0, 2, 10, and 20 mg/m^3 groups was 22, 23, 22, and 22, respectively.

a. p value of the F-statistic from one-way ANOVA = 0.3415.
b. p value of the F-statistic from one-way ANOVA = 0.1454.

Table 7. Analysis of Electrocardiogram Waveform Time Intervals of Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	20	21	19	18	
Heartbeats/min					
mean	356	370	337	355	0.1461
s.e.	8	9	10	13	
P-R (sec)					
mean	0.043[b]	0.045[b]	0.043[c]	0.045[c]	0.5806
s.e.	0.001	0.001	0.001	0.001	
QRS (sec)					
mean	0.011	0.011[b]	0.012[c]	0.012[c]	0.5014
s.e.	<0.001	<0.001	0.001	0.001	

a. Six hours/day, 5 days/week, for 6 months.
b. n=19.
c. n=18.

Lung Volumes. The apportionment of lung volume was determined using data from the QSC curve (VC, IC, and expiratory reserve volume (ERV)), the dilution derived TLC and FRC, and their arithmetically computed components, RV and IRV.

The neon dilution method was the primary technique used for the determination of lung volume (TLC_d) because it avoids confoundment of the data with the "trapped" air space volume. However, the concept of non-communicating air space was considered in the comparison of FRC_d to FRC_b. The latter measurement includes the "trapped" gas volume in its estimate of FRC (Figure 8). No differences among the groups were observed.

Figure 9 illustrates the impact of silica exposure on the divisions of lung volume. No statistically significant differences where found among the exposure groups for any lung volume subdivision. Normalization of lung volumes to TLC_d (Figure 10) did not disclose any effects of silica exposure on the divisions of lung volume.

Parenchymal Behavior and DLCO. The QSC, reported as QSC_{cs} or as h, was not significantly different among the control and silica-exposed groups (Table 8). Similarly, multivariate analysis of the QSC curves did not indicate any alteration in the slope of the curve when expressed as the actual volume (Figure 11) or as a fraction of the VC (Figure 12).

Diffusion capacity for CO was not different from the controls in those animals exposed to silica and normalization of the DLCO to TLC_d did not alter these findings (Table 8). (A comparison of the DLCO data from animals from which arterial blood was drawn for blood gas determinations to similarly treated, but unsampled animals, indicated that the loss of 0.5 to 1.0 cm^3 of blood did not significantly affect the estimation of DLCO.)

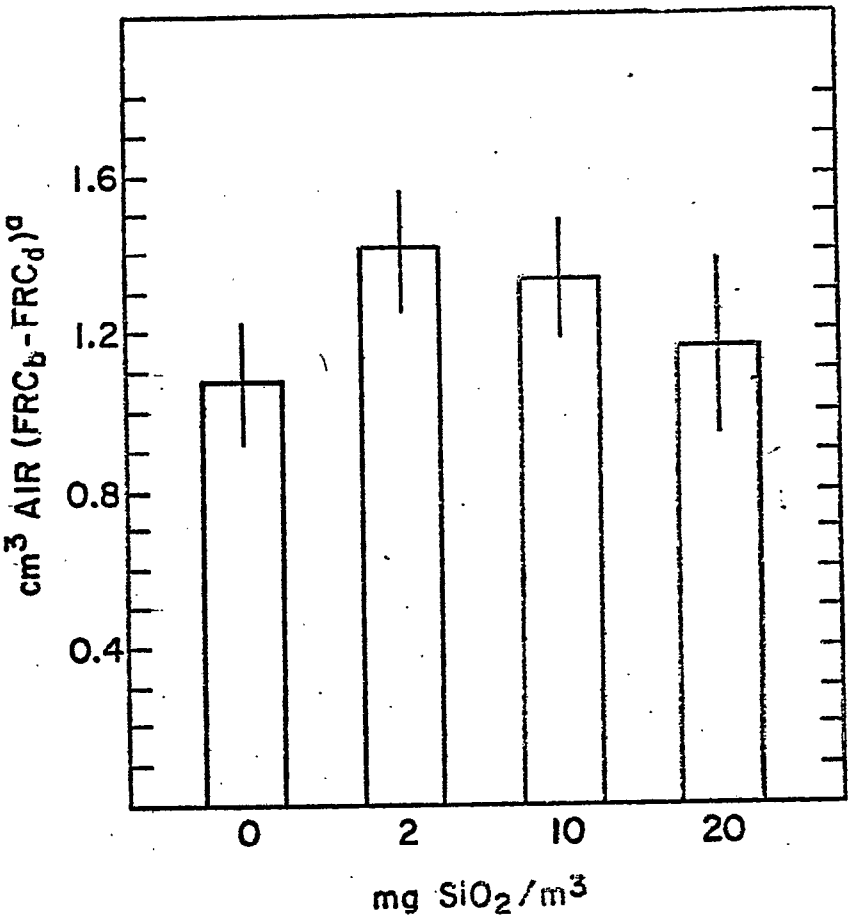

Figure 8: Trapped air in the lungs of Fischer-344 rats exposed to silica for 6 months (6 hours/day, 5 days/week). The data represent the means (±s.e.) of 23 control, 23 2 mg SiO_2/m^3, 21 10 mg SiO_2/m^3, and 22 20 mg SiO_2/m^3 rats.

FRC_b: Functional Residual Capacity by Boyle's Law.
FRC_d: Functional Residual Capacity by dilution.

a. p value of F-statistic from one-way ANOVA = 0.4652.

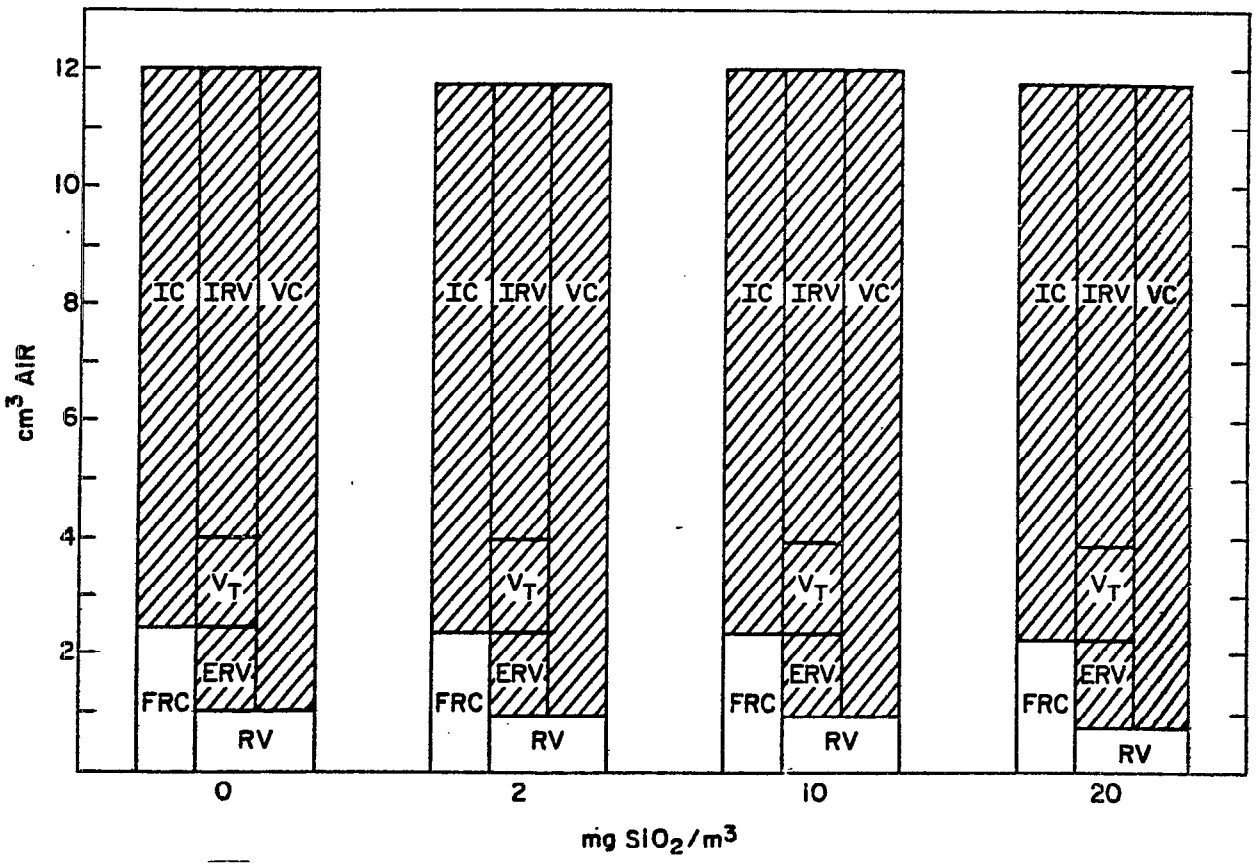

Figure 9: Divisions of lung volume in Fischer-344 rats exposed to filtered air or silica for 6 months (6 hours/day, 5 days/week). The data represent the means of at least 23 control, 23 2 mg SiO_2/m^3, 22 10 mg SiO_2/m^3, and 22 20 mg SiO_2/m^3 rats.

		p value
ERV:	Expiratory reserve volume	0.9906
FRC:	Functional residual capacity	0.8184
IC:	Inspiratory capacity	0.7544
IRV:	Inspiratory reserve volume	0.8151
RV:	Residual volume	0.5105
VC:	Vital capacity	0.7174
V_T:	Tidal volume	0.7935
TLC:	Total lung capacity	0.7342

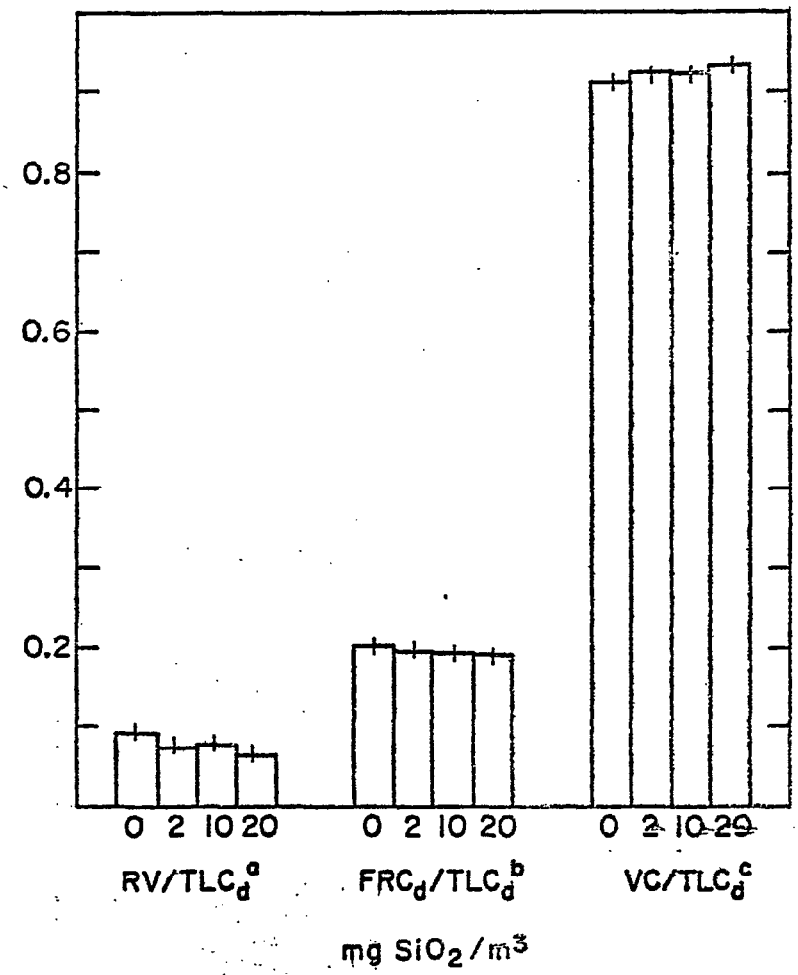

Figure 10: Normalized lung volume of control and silica exposed Fischer-344 rats (6 hours/day, 5 days/week for 6 months). The data represent the mean (±s.e.) of 23 control, 23 2 mg SiO_2/m^3, 22 10 mg SiO_2/m^3, and 23 20 mg SiO_2/m^3 rats.

FRC: Functional residual capacity
RV: Residual volume
TLC: Total lung capacity
VC: Vital capacity

a. p value of F-statistic from one-way ANOVA = 0.4155.
b. p value of F-statistic from one-way ANOVA = 0.8731.
c. p value of F-statistic from one-way ANOVA = 0.4155.

Table 8. Physiological Indices of Parenchymal Damage in Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	23	24	23	23	
QSC_{cs}(cm^3/cm H_2O)					
mean	0.81	0.79	0.82	0.81	0.7316
s.e.	0.02	0.01	0.02	0.02	
QSC_{cs}/FRC_d					
mean	0.355	0.361[b]	0.376[c]	0.404	0.5803
s.e.	0.023	0.021	0.024	0.037	
h(cm H_2O)					
mean	3.54	3.60	3.37	3.54	0.5195
s.e.	0.10	0.12	0.15	0.08	
$DLCO_{rb}$(cm^3/mmHg • min^{-1})					
mean	0.180	0.171[b]	0.168[c]	0.170	0.4791
s.e.	0.006	0.006	0.005	0.007	
$DLCO_{rb}/TLC$					
mean	0.015	0.015[b]	0.014[c]	0.014	0.4493
s.e.	<0.001	<0.001	<0.001	0.001	

a. Six hours/day, 5 days/week, for 6 months.
b. n=23.
c. n=22.

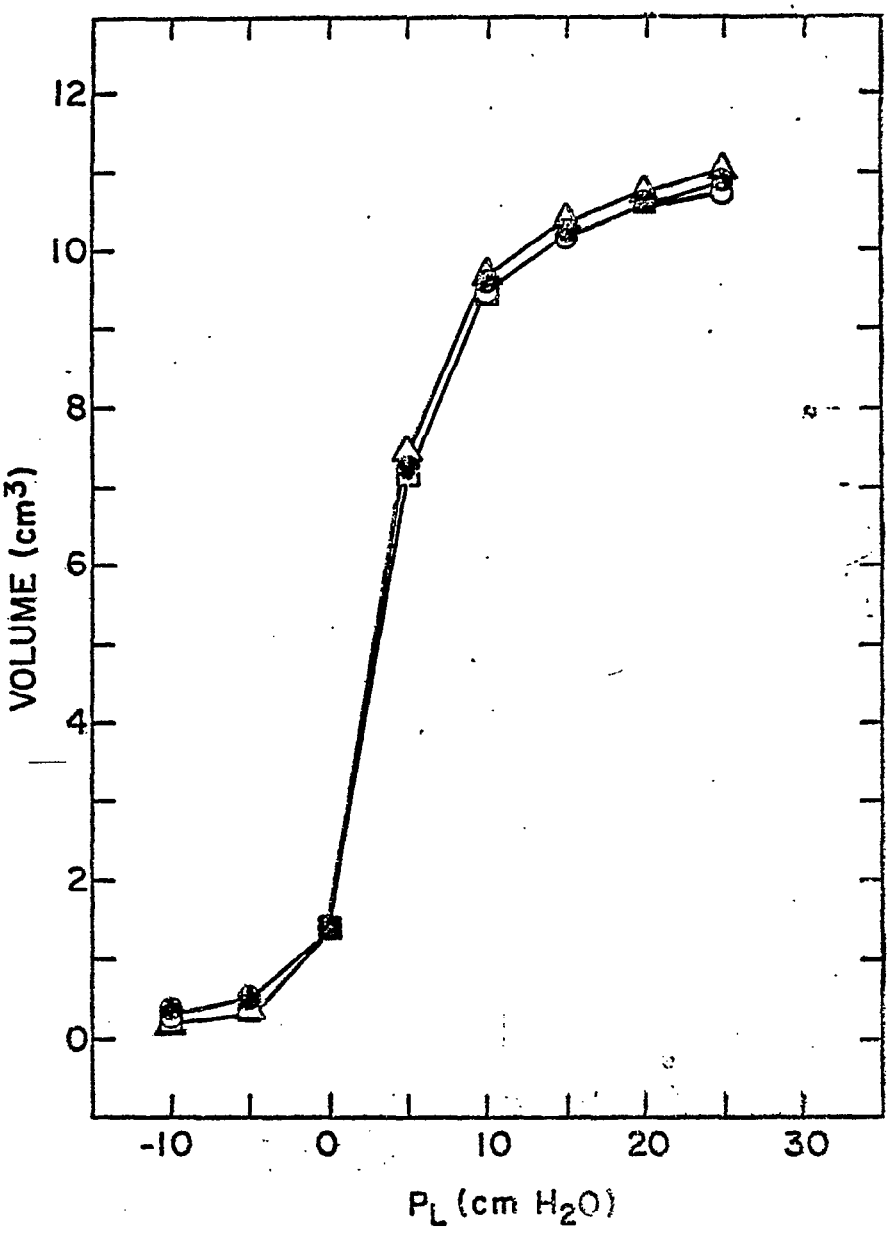

Figure 11: Quasi-static compliance curves of Fischer-344 rats exposed to silica for 6 months (6 hours/day, 5 days/week). The data represent the means (±s.e.) of 23 control (●), 24 2 mg SiO_2/mg (o), 23 10 mg SiO_2/m^3 (Δ), and 23 20 mg SiO_2/m^3 (■) rats. The p value of the F-statistic from one way MANOVA = 0.2282.

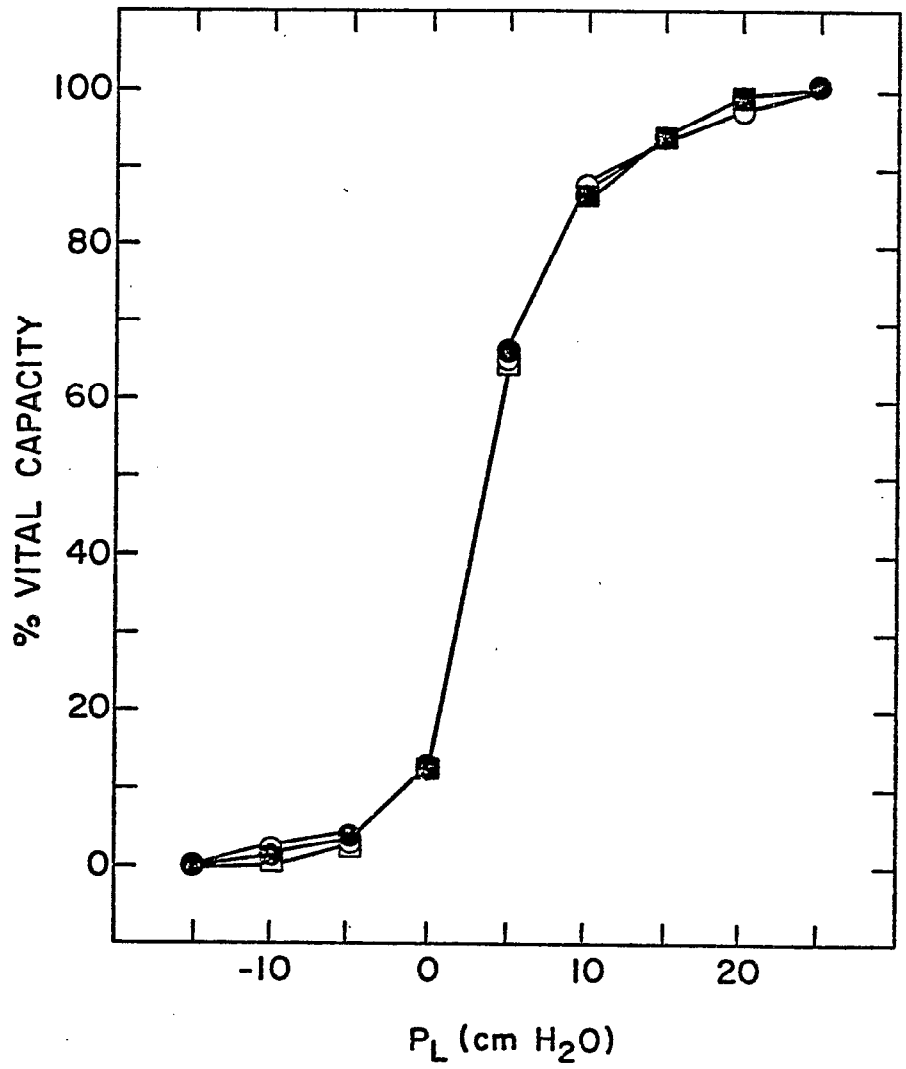

Figure 12: Quasi-static compliance as a function of vital capacity of Fischer-344 rats exposed to silica for 6 months (6 hours/day, 5 days/week). The means and s.e. bars of the 23 control (●), 24 2 mg SiO_2/m^3 (o), 23 10 mg SiO_2/m^3 (Δ), and 23 20 mg SiO_2/m^3 (□) rats often overlay each other and therefore may appear as a single curve. The p value of the F-statistic from one-way MANOVA = 0.1564.

Distribution of Ventilation. Moment analysis of the distribution of ventilation, estimated by the multi-breath N_2 washout for 50 tidal breaths of oxygen, found no impairment of washout efficiency in any of the exposure groups (Table 9).

Flow Volume Dynamics. No silica-induced alteration in airway function could be detected with the MEFV maneuver (Table 10). The convex shape (away from the volume axis) of the effort independent portion of the curves was similar at all exposure concentrations. Also, differences among the exposure groups were not apparent when the flow rate data were expressed in terms of VC/sec and analyzed by MANOVA (Figure 13).

The calculation of R_{us}, by relating the MEFV and QSC curves at points of equal volume did not indicate any statistically significant change in small airway function resulting from silica exposure (Table 11). Similarly, augmentation of the MEFV curve with a low density $He:O_2$ mixture did not indicate the presence of any abnormalities in the dynamics of the medium and small airways (Table 12).

Roentgenographic Findings

No evidence of silica-induced lung disease could be ascertained from the single frontal chest x-rays of the exposed rats. Each film was evaluated without knowledge as to its group of origin. The x-ray films of the silica exposed animals were all indistinguishable from those of the control animals.

Lung Composition Data

The right lung lobes from animals subjected to pulmonary function tests were assayed for protein, DNA, elastin, hydroxyproline (an index of collagen) and water content. The data from the individual animals in each exposure group have been provided in Appendix F.

Table 9. Moment Analysis of Multibreath N_2 Washout in Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	23	23	21	22	
M_1/M_0					
mean	6.70	7.46	7.34	8.19	0.3789
s.e.	0.56	0.64	0.55	0.65	

a. Six hours/day, 5 days/week, for 6 months.

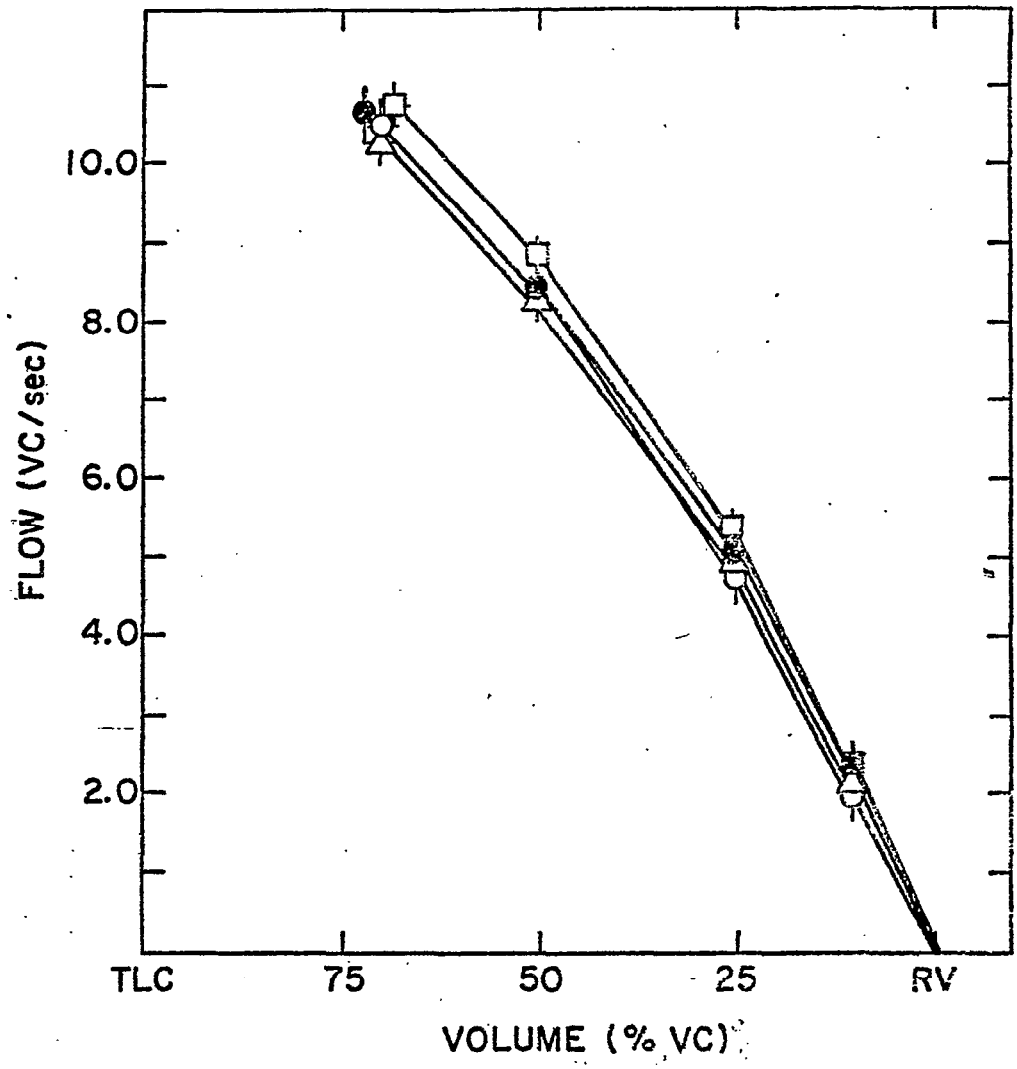

Figure 13: Maximum expiratory flow-volume curves of Fischer-344 rats exposed to silica for 6 months (6 hours/day, 5 days/week).

- ● Control, n=23.
- o 2 mg SiO_2/m^3, n=23.
- Δ 10 mg SiO_2/m^3, n=21.
- □ 20 mg SiO_2/m^3, n=23.

The p value of the F-statistic from one-way MANOVA = 0.4822.

Table 10. Normalized Points on the MEFV Curve of Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	23	23	21	23	
$\dot{V}_{max}$(% VC)					
mean	72.0	69.9	70.0	68.7[b]	0.5161
s.e.	1.4	1.4	1.7	1.5	
PEF (VC/sec)					
mean	10.7	10.4	10.3	10.8	0.5812
s.e.	0.3	0.3	0.2	0.2	
EFR_{50} (VC/sec)					
mean	8.5	8.6	8.3	8.8	0.6889
s.e.	0.3	0.3	0.2	0.2	
EFR_{25} (VC/sec)					
mean	5.1	4.8	4.8	5.4	0.3507
s.e.	0.3	0.3	0.2	0.2	
EFR_{10} (VC/sec)					
mean	2.4	1.9	2.1	2.5	0.1192
s.e.	0.2	0.2	0.2	0.2	
ΔEFR_{25} (VC/sec)					
mean	0.9	0.5	0.7	1.0	0.1975
s.e.	0.2	0.2	0.2	0.2	

a. Six hours/day, 5 days/week, for 6 months.
b. n=21.

Table 11. Analysis of Upstream Airway Resistance in Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	22	23	21	19	
$\dot{V}_{30}$ (cm^3/sec)					
mean	66.8	62.1	65.8	69.7[b]	0.3113
s.e.	3.2	3.0	2.8	2.4	
P_{st} (cm H_2O)					
mean	15.2	15.9	14.2	14.5	0.2089
s.e.	0.6	0.6	0.5	0.8	
R_{us}					
mean	0.244	0.272	0.224	0.214	0.0896
s.e.	0.021	0.019	0.012	0.012	

a. Six hours/day, 5 days/week, for 6 months.
b. n=21.

Table 12. Analysis of Density-Dependent (Helium) Maximal Flows for MEFV Curves for Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	23	23	21	23	
$\Delta HEFR_{50}$ (cm^3/sec)					
mean	26.2	25.0	22.9	25.8	0.7173
s.e.	2.0	2.2	1.8	2.5	
$\Delta HEFR_{50}$ (VC/sec)					
mean	2.4	2.3	2.1	2.4	0.6112
s.e.	0.2	0.2	0.2	0.2	
$\Delta HEFR_{25}$ (cm^3/sec)					
mean	14.4	13.8	11.5	14.0	0.6678
s.e.	1.8	1.7	1.8	1.6	
$\Delta HEFR_{25}$ (VC/sec)					
mean	1.3	1.3	1.0	1.3	0.5650
s.e.	0.2	0.2	0.2	0.1	
Isoflow (% VC)					
mean	7.2[b]	6.0[c]	6.5	7.6[b]	0.8175
s.e.	1.5	1.4	1.2	1.1	

a. Six hours/day, 5 days/week, for 6 months.
b. n=22.
c. n=21.

Lung Weight and Water Content. Exposure to the silica dust concentrations tested did not result in changes in total lung fresh weight. However, the total dry lung weight of the high-dose group was increased to 110% of controls (Table 13).

Lung Protein. The total amount of protein was increased in the rats exposed to 20 mg SiO_2/m^3 (Table 14). However, this difference from control protein content was offset by the increased dry weight of the lungs from this exposure group (Tables 13 and 15).

Lung DNA. THe total DNA content of the lungs of rats exposed to 20 mg SiO_2/m^3 was 115% the DNA content of control lungs. This increased DNA content was only 105% of control levels when expressed as a function of dry weight, approaching the $p = 0.05$ level of significance (Table 15).

Lung Elastin. The elastin content of the lung was a very sensitive indicator of the inhaled silica concentration with significant dose dependent increases at each exposure level. These changes were observed when this connective tissue component was expressed as the total amount (Table 14) or as a function of the lung dry weight (Table 15). Total elastin in the 2, 10, and 20 mg SiO_2/m^3 exposure groups was 107, 119, and 130%, respectively, of the amount in the lungs of control animals.

Lung Collagen. Dose dependent increases in total lung hydroxyproline, an index of collagen content, were similar to those observed in elastin. The amount of collagen relative to controls was 116, 128, and 136% in the 2, 10, and 20 mg SiO_2/m^3 exposure groups, respectively (Table 14). The amount of pulmonary collagen in each exposure group was significantly different from the amount in every other exposure group (Table 14). Similar dose dependent increases were observed when collagen was expressed on the basis of dry weight (Table 15). However, when expressed

Table 13. Body Weight and Lung Weight Data from Control and Silica-Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	22	23	22	24	
BODY WEIGHT (g)					
mean	357.6[b]	356.3[c]	358.3[c]	357.0	0.9955
s.e.	6.6	6.0	6.1	5.4	
LUNG WEIGHT (g)					
mean	1.32	1.28	1.32	1.36	0.6170
s.e.	0.04	0.03	0.05	0.03	
LUNG-TOTAL DRY WEIGHT (mg)					
mean	263.2	262.6	273.9	289.2	0.0179[d]
s.e.	6.4	5.4	7.3	7.4	
multiple comparison[e]		LD CN	ID HD		
LUNG-% DRY WEIGHT					
mean	20.10	20.59	20.94	21.36	0.0727
s.e.	0.34	0.35	0.42	0.27	

a. Six hours/day, 5 days/week, for 6 months.
b. n=23.
c. n=24.
d. Statistically significant at $\alpha = 0.05$ level using ANOVA.
e. Pairwise comparison of means by the Duncan multiple range method.

Table 14. Lung Composition of Control and Silica-Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	22	23	22	24	
TOTAL PROTEIN (mg)					
mean	166.2	165.5	173.2	181.9	0.0413[b]
s.e.	4.5	3.9	4.8	5.0	
multiple comparison[c]		LD CN	ID	HD	
TOTAL DNA (mg)					
mean	5.5	5.6	5.8	6.3	<0.0001[b]
s.e.	0.1	0.1	0.1	0.1	
multiple comparison[c]		CN LD	ID	HD	
TOTAL ELASTIN (mg)					
mean	6.9	7.4	8.2	9.0	<0.0001[b]
s.e.	0.1	0.1	0.2	0.2	
multiple comparison[c]		CN	LD ID	HD	
TOTAL HYDROXYPROLINE (mg)					
mean	2.5	2.9	3.2	3.4	<0.0001[b]
s.e.	0.1	<0.1	0.1	0.1	
multiple comparison[c]		CN	LD ID	HD	

a. Six hours/day, 5 days/week, for 6 months.
b. Statistically significant at $\alpha = 0.05$ level using ANOVA.
c. Pairwise comparison of means by the Duncan multiple range method.

Table 15. Lung Composition Expressed as a Function of Dry Lung Weight of Control and Silica-Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	22	23	22	24	
PROTEIN (mg)/DRY WEIGHT (g)					
mean	631.0	630.0	632.5	628.6	0.9611
s.e.	5.1	5.6	6.0	4.3	
DNA (mg)/DRY WEIGHT (g)					
mean	20.9	21.3	21.2	21.8	0.0518
s.e.	0.2	0.2	0.2	0.3	
multiple comparison[b]		CN ID LD HD			
ELASTIN (mg)/DRY WEIGHT (g)					
mean	26.5	28.2	30.1	31.3	<0.0001[c]
s.e.	0.4	0.2	0.4	0.4	
multiple comparison[b]		CN ID LD HD			
HYDROXYPROLINE (mg)/DRY WEIGHT (g)					
mean	9.4	10.9	11.6	11.8	<0.0001[c]
s.e.	0.2	0.2	0.3	0.2	
multiple comparison[b]		CN LD	ID HD		

a. Six hours/day, 5 days/week, for 6 months.
b. Pairwise comparison of means by the Duncan multiple range method.
c. Statistically significant at $\alpha = 0.05$ level using ANOVA.

as a function of dry weight, the collagen content of the lungs from the 10 and 20 mg SiO_2/m^3 exposure groups did not differ from each other.

Pathology

Selected tissues from two groups of animals were submitted to EPL for pathological examination. The first group consisted of eight male rats from each chamber which were designated for pathology. The second group was composed of animals from which respiratory physiology data had been collected and from which the right lung was submitted for lung composition analysis. These were studied to provide pathology data on the same animals used for pulmonary function and lung composition analysis. Submission of lung tissue from these animals also provided an opportunity to determine whether the pulmonary function test regime resulted in structural changes observable at the light microscopic level.

Respiratory Tissue. Histological changes observed in the animals designated for pathology and those seen in the left lung of the multiple endpoint rats were not different. The lungs from rats in the 20 mg SiO_2/m^3 group had the most severe exposure related lesions. These consisted primarily of inflammatory reactions near the end-airways. Histiocytes, which were prominent in these lesions, often contained several small (1-2 μ) birefringent crystals in their cytoplasm. In many cases scattered granulocytes and mononuclear cells were also observed. Type II cell hyperplasia, and in some animals focal fibrosis resembling the so-called silicotic nodules, were also present (Tables 16 and 17). Intralymphatic microgranulomas were common and often associated with perivascular and peribronchiolar lymphoid cuffs. In the 10 mg SiO_2/m^3 group, these changes were less severe, and in the low dose group, only

HISTOPATHOLOGY INCIDENCE TABLE

Table 16
Pathology Animals

	Control											2 mg SiO_2/m^3																	
ANIMAL NUMBER	G 8 1	G 8 2	G 8 3	G 8 4	G 8 5	G 8 6	G 8 7	G 8 8				E 8 1	E 8 2	E 8 3	E 8 4	E 8 5	E 8 6	E 8 7	E 8 8										
LUNG																													
Lymphoid Proliferations,																													
Perivascular and Peribronchiolar	1	1	1	1	1	1	2	1				1	2	2	2	2	2	1	1										
End Airways Crystals (Birefringent)													1	1															
Intralymphatic Microgranulomas														1		1													
Histiocytes	1	1	1	1	1	1	1	1				1	1	1	1	1	1	2	2										
Type II Cell Hyperplasia																			1										
Granulocytes																		2	1										
Mononuclear Cells																													
Fibrosis																													
Hemorrhage																													
TRACHEA	X	X	X	X	X	X	X	X				X	X	X		X	X	X	X										
Loss of Cilia																													
Tracheitis															1														
PERIBRONCHIAL LYMPH NODE	N	X	N	X	X	X	X	X				X		X															
Microgranulomas													2		2	2	1	3	1										
Lymphoid Hyperplasia													1			2													

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Key: P = Present
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N = No Section
2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
X = Not Remarkable
4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

Table 16
Pathology Animals

ANIMAL NUMBER	Control											2 mg SiO_2/m^3																	
	G81	G82	G83	G84	G85	G86	G87	G88				E81	E82	E83	E84	E85	E86	E87	E88										
HEART	X	X	X	X	X	X	X	X					X		X	X	X	X	X										
Chronic Myocarditis, Focal												2		2															

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N = No Section
2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
X = Not Remarkable
4 = Moderately Severe/High

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HISTOPATHOLOGY INCIDENCE TABLE

Table 16
Pathology Animals

	Control											2 mg SiO_2/m^3																	
ANIMAL NUMBER	G 8 1	G 8 2	G 8 3	G 8 4	G 8 5	G 8 6	G 8 7	G 8 8				E 8 1	E 8 2	E 8 3	E 8 4	E 8 5	E 8 6	E 8 7	E 8 8										
NASAL TURBINATE	X	N	X	X	X	X	X	X				X	X	N	X	X	X	X	X										
Submucosal Lymphoid Infiltrate																													
BRAIN	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X										
KIDNEYS	X	X	X	X	X	X	X	X				X	X	X	X	X		X	X										
Chronic Interstitial Nephritis																													
Tubular Casts																	2												
LIVER		X			X	X	X	X				X			X	X	X	X	X										
Chronic Pericholangitis	2		1	1									2	1															
Fatty Changes														1															
SPLEEN		X	X	X	X	X	X	X				X		X	X	X	X		X										
Hemosiderosis																		2											
Congestion	2												2					2											
TESTES	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X										

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HISTOPATHOLOGY INCIDENCE TABLE

Table 16
Pathology Animals

ANIMAL NUMBER	10 mg SiO_2/m^3											20 mg SiO_2/m^3																
	T 8 1	T 8 2	T 8 3	T 8 4	T 8 5	T 8 6	T 8 7	T 8 8				B 8 1	B 8 2	B 8 3	B 8 4	B 8 5	B 8 6	B 8 7	B 8 8									
LUNG																												
Lymphoid Proliferations,																												
Perivascular and Peribronchiolar	3	2	3	3	2	3	2	3				3	3	3	3	4	3	4	3									
End Airways Crystals (Birefringent)	2	2	3	3	2	3	2	3				2	2	2	2	1	2	2	2									
Intralymphatic Microgranulomas	3	2	3	2	2	3	2	3				4	4	4	4	4	3	4	4									
Histiocytes	3	3	3	3	2	3	2	3				2	3	2	3	2	3	3	3									
Type II Cell Hyperplasia	1	2	2	2	2	1	1	2				2	3	2	3	2	2	3	3									
Granulocytes		1	1	1	1	1	1	2				1	1	2	2	1	1	1	1									
Mononuclear Cells		2	1	1	1	1	1	1					3	1	2	1	3	3	2									
Fibrosis			1	2	1	2		2							2		2	2	1									
Hemorrhage																												
TRACHEA	X	X	X	X	X	X	X	X						X	X	X	X	X										
Loss of Cilia												3	1						1									
Tracheitis												3							2									
PERIBRONCHIAL LYMPH NODE																												
Microgranulomas	2	1	2	1	1	4	1	1				4	4	4	4	4	4	5	5									
Lymphoid Hyperplasia						3						3	3	3	3	3	3	4	4									

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X = Not Remarkable
4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

Table 16
Pathology Animals

	10 mg SiO_2/m^3											20 mg SiO_2/m^3																
ANIMAL NUMBER	T 8 1	T 8 2	T 8 3	T 8 4	T 8 5	T 8 6	T 8 7	T 8 8				B 8 1	B 8 2	B 8 3	B 8 4	B 8 5	B 8 6	B 8 7	B 8 8									
NASAL TURBINATE		X	X	X	N	X	X	N				X	X	X	X	X	X	X	N									
Submucosal Lymphoid Infiltrate	2																											
BRAIN	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X									
KIDNEYS	X		X	X	X	X		X				X	X	X	X		X											
Chronic Interstitial Nephritis																1		1	1									
Tubular Casts		1					1																					
LIVER	X	X	X	X		X	X	X				X	X	X	X	X	X	X	X									
Chronic Pericholangitis					1																							
Fatty Change																												
SPLEEN	X	X		X		X	X	X					X	X			X											
Hemosiderosis																												
Congestion			2		1							2			2	2		2	2									
TESTES	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X									

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Key: P = Present
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5 = Severe/High
N = No Section
2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
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4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

Table 16
Pathology Animals

ANIMAL NUMBER	10 mg SiO_2/m^3											20 mg SiO_2/m^3																					
	T 8 1	T 8 2	T 8 3	T 8 4	T 8 5	T 8 6	T 8 7	T 8 8				B 8 1	B 8 2	B 8 3	B 8 4	B 8 5	B 8 6	B 8 7	B 8 8														
HEART	X	X		X	X		X	X				X	X	X			X		X														
Chronic Myocarditis, Focal			2			2									2	1		1															

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HISTOPATHOLOGY INCIDENCE TABLE

Table 17
Multiple Endpoint Animals

	Control																												
ANIMAL NUMBER	G09	G11	G12	G13	G14	G15	G16	G33	G34	G35	G36	G37	G38	G39	G40	G57	G59	G60	G61	G62	G63	G64							
LUNG																													
Lymphoid Proliferations,																													
Perivascular and Peribronchiolar	1	2	1	2	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1							
End Airways Crystals (Birefringent)																													
Intralymphatic Microgranulomas																													
Histiocytes	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	2							
Type II Cell Hyperplasia																													
Granulocytes																													
Mononuclear Cells																													
Fibrosis																													
Hemorrhage																													
TRACHEA	X	X	X	N	X	X	X	X	X	X	X	X	X	X	X	X	X	N	X		X	N							
Loss of Cilia																													
Tracheitis																				1									

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HISTOPATHOLOGY INCIDENCE TABLE

Table 17
Multiple Endpoint Animals

2 mg SiO_2/m^3

ANIMAL NUMBER	E09	E10	E11	E12	E13	E14	E15	E16	E33	E34	E35	E36	E37	E38	E39	E40	E57	E58	E59	E60	E61	E62	E63	E64				
LUNG																												
Lymphoid Proliferations,																												
Perivascular and Peribronchiolar	1	2	2	2	2	1	2	2	1	2	2	2	2	2	1	3	2	1	3	1	1	2	1	1				
End Airways Crystals (Birefringent)																												
Intralymphatic Microgranulomas		1											2						2									
Histiocytes	2	1	1	1	2	1	2	2	1	1	2	1	2	2	2	2	2	2	2	1	2	2	2	2				
Type II Cell Hyperplasia																												
Granulocytes													1															
Mononuclear Cells																												
Fibrosis																												
Hemorrhage	3																	2										
TRACHEA	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Loss of Cilia																												
Tracheitis									1																			

EPL | Experimental Pathology Laboratories, Inc.

Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

Table 17
Multiple Endpoint Animals

10 mg SiO_2/m^3

ANIMAL NUMBER	T09	T11	T12	T13	T14	T15	T16	T33	T34	T35	T36	T37	T38	T39	T40	T57	T58	T59	T61	T62	T63	T64
LUNG																						
Lymphoid Proliferations																						
Perivascular and Peribronchiolar	1	2	2	2	2	3	1	3	1	1	3	3	2	2	2	2	3	2	3	2	2	2
End Airways Crystals (Birefringent)	2	1		2	1	2		2	1	1	1	1	2	1	1	1	2	1	1	1	1	1
Intralymphatic Microgranulomas				2	2	3		3			2	3	1	2	1	1	2	2	3	2	2	1
Histiocytes	2	2	1	2	2	2	2	2	2	2	1	1	2	2	2	2	3	2	2	2	2	2
Type II Cell Hyperplasia	1	1		1		1	1	1		1					2	1	2	1			1	
Granulocytes	1					1	1	1	1	1			1				1	1				
Mononuclear Cells	1	1		1		1	1	1	1								1	1				
Fibrosis																	1					
Hemorrhage																						
TRACHEA	X	X	X	X	X	X	X	N	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Loss of Cilia																						
Tracheitis																						

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Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

Table 17
Multiple Endpoint Animals

20 mg SiO_2/m^3

ANIMAL NUMBER	B06	B09	B10	B11	B12	B13	B14	B15	B16	B33	B34	B35	B37	B38	B39	B40	B57	B58	B59	B60	B61	B62	B63	B64				
LUNG																												
Lymphoid Proliferations,																												
Perivascular and Peribronchiolar	3	3	4	3	2	3	3	4	4	2	4	3	3	4	3	2	4	4	5	3	2	3	3	2				
End Airways Crystals (Birefringent)	2	1	1	2	1	1	1	2	2	2	2	1	2	2	2	2	1	2	2	2	1	2	1	1				
Intralymphatic Microgranulomas	3	4	4	3	2	3	4	4	4	3	4	3	4	4	2	3	4	4	5	4	2	2	3	2				
Histiocytes	2	2	2	3	2	2	2	3	2	2	3	2	3	2	2	3	2	2	3	2	2	2	2	2				
Type II Cell Hyperplasia	1	2	1	2	1	2	3	3	1	2	1	1	1	1	2	2	3	3	3	2	1	1		1				
Granulocytes	1			1		1	1	2				1	1	2	1	1	2	1	1	1	1		2	2				
Mononuclear Cells	1			1		1	3	3		2	1	1	1	1	1	1	3	2	2	1	1			2				
Fibrosis				1		1	3	3									3	2	3			1		1				
Hemorrhage		3																										
TRACHEA	X	X	X	X	X	N	X	X		X	X	X		X	X	X		X	X	X	X	X	X	X				
Loss of Cilia																												
Tracheitis									1				1				1											

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2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
X = Not Remarkable
4 = Moderately Severe/High

three rats had intralymphatic microgranulomas in their lungs (Tables 16 and 17). Lungs from the control group contained no specific alterations.

Each lung lesion observed in the individual animals (from Table 17) was scored for severity and the numbers were summed to provide a pathology score. The value for birefringent particles was not included in this pathology score. The frequency of each pathology score within the four exposure groups has been illustrated in Figure 14. The Kruskal-Wallis non-parametric test indicated that the mean scores of the four groups differed significantly ($H=75.15$, $p<0.0001$). Dunn's rank sum multiple comparison method indicated that scores from the 10 and 20 mg SiO_2/m^3 groups were significantly higher than those of the control group.

Non-respiratory Tissues. The peribronchial lymph nodes were examined only in those animals designated for pathology. In all of the animals from the 20 mg SiO_2/m^3 group, microgranulomas with associated lymphoid hyperplasia were observed (Table 16). All of the rats in the 10 mg SiO_2/m^3 group and six of the eight in the 2 mg SiO_2/m^3 group also had microgranulomas in the peribronchial lymph nodes (Table 16). Small transparent crystal-like particles could be seen but they were no longer birefringent. The peribronchial lymph nodes from the control animals were not remarkable.

No significant lesions were observed in any of the other organs examined.

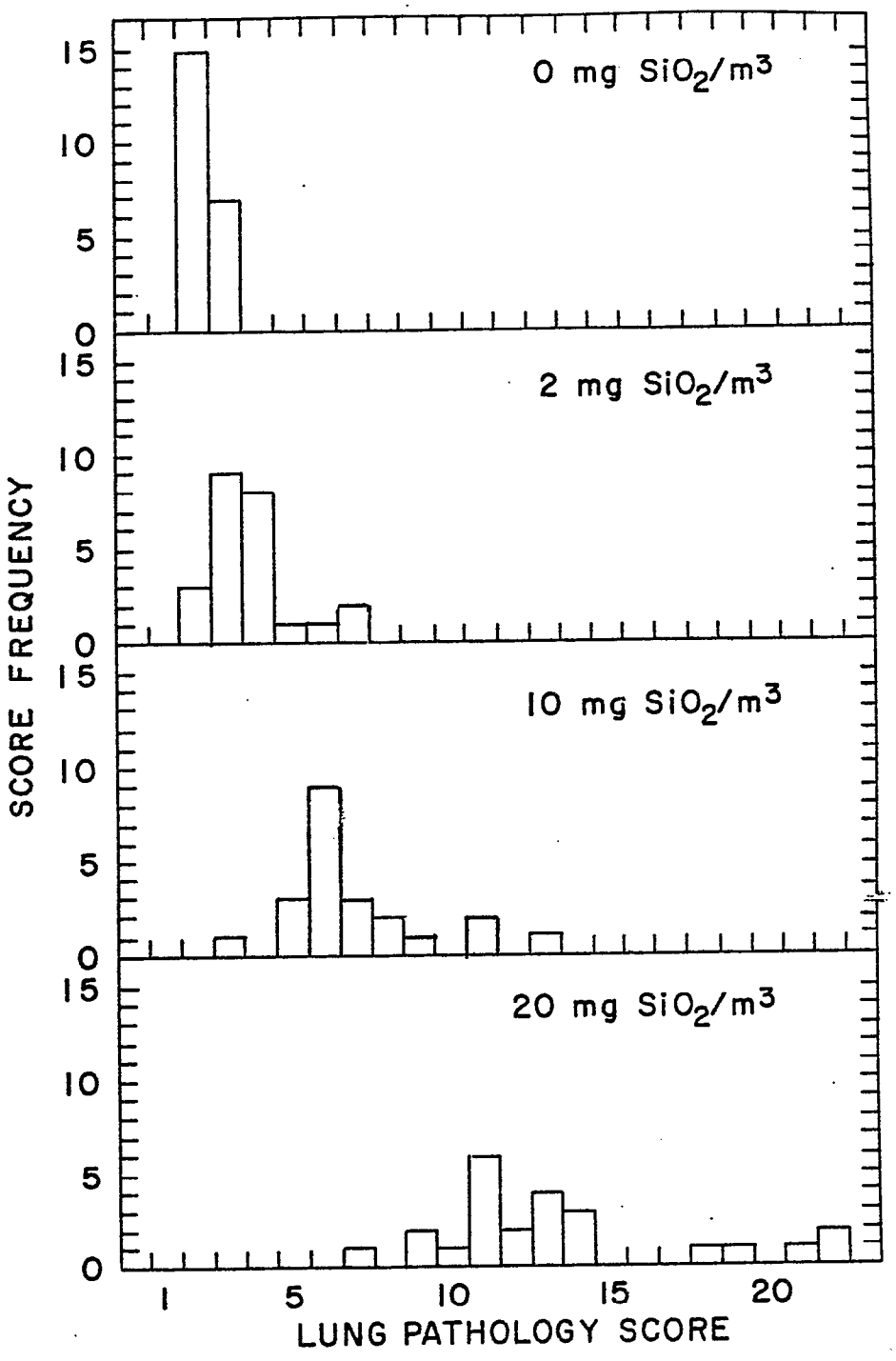

Figure 14: Frequency of lung pathology scores of Fischer-344 rats exposed to filtered air or silica dust for 6 months, 6 hours/day, 5 days/week (see text for details).

Cytogenetic Results

Bone Marrow. The results of the (SCE) and cellular proliferation studies in populations of bone marrow cells from control, 2, 10, and 20 mg SiO_2/m^3 silica-exposed rats have been provided in Table 18. The SCE data were normalized by square root transformation and compared using ANOVA. No significant differences were observed among any of the groups using AGT data and no significant differences were observed between the control and exposed animals in the relative proportions of first-, second-, and third-generation metaphase cells (cell proliferation kinetics, Table 18). Analysis of chromosomal aberrations was not possible because few first-generation metaphase cells were observed in the bone marrow of rats sacrificed after 26 hours of BrdUrd infusion.

Peripheral Blood Lymphocytes. The analysis of the SCE and chromosomal aberration data from peripheral blood lymphocytes of control rats and those exposed to silica was not possible. No metaphase cells were evident in cultures initiated from control or exposed animals, indicating that a defective lot of PHA or serum was used.

Sperm Morphology. Sperm samples from ten rats from each exposure group were examined for abnormal cells. Statistically significant differences were found to exist among the groups when the data were analyzed by either the Kruskal-Wallis non-parametric test or by one-way ANOVA after arcsin transformation of the square root of the frequency of abnormal sperm cells (Table 19). However, because the greatest portion of abnormal sperm were found in animals exposed to the lowest concentration of silica dust (2 mg SiO_2/m^3) combined with the absence of any dose response trend in the data, this positive result is considered an anomaly and not biologically relevant. The data from individual animals have been provided in Appendix G.

Table 18. Frequency of Sister Chromatid Exchanges (SCEs) and Average Generation Time (AGT) in Bone Marrow Cells of Fischer-344 Rats Exposed to Silica[a]

Silica Concentration (mg/m^3)	Animal Number	SCE/Cell[b] Raw Data	SCE/Cell[b] $\sqrt{x}$ Transformed Data	AGT (hr)[c]
0.0	1113	4.52(0.49)	2.05(0.12)	10.71
	1114	4.40(0.42)	2.00(0.13)	10.71
	1115	3.96(0.45)	1.91(0.11)	12.50
	1116	3.48(0.40)	1.74(0.14)	10.86
	1117	4.32(0.39)	2.00(0.12)	13.71
	$\bar{\bar{x}}$[d]	4.14(0.19)	1.94(0.06)	11.70(0.61)
2.0	1314	4.28(0.66)	1.89(0.18)	10.39
	1317	4.60(0.40)	2.07(0.12)	11.82
	1320	6.68(0.49)	2.55(0.09)	11.48
	1321	5.32(0.54)	2.23(0.12)	11.48
	1322	4.52(0.32)	2.09(0.08)	11.32
	$\bar{\bar{x}}$	5.08(0.44)	2.17(0.11)	11.30(0.24)
10.0	1513	3.80(0.37)	1.86(0.12)	11.37
	1514	6.32(0.68)	2.40(0.16)	10.57
	1516	6.00(1.49)	2.25(0.20)	11.48
	1519	5.76(0.50)	2.34(0.11)	11.37
	1520	4.92(0.50)	2.14(0.12)	11.65
	$\bar{\bar{x}}$	5.36(0.45)	2.20(0.10)	11.29(0.19)
20.0	1716	3.24(0.28)	1.76(0.08)	11.21
	1717	3.48(0.33)	1.76(0.13)	10.81
	1719	5.40(0.50)	2.27(0.10)	11.06
	1720	4.80(0.72)	2.07(0.14)	11.34
	$\bar{\bar{x}}$	4.23(0.52)	1.97(0.12)	11.16(0.15
p value[e]		0.1235	0.1718	0.7465

a. Six hours/day, 5 days/week, for 6 months.
b. Mean frequency of SCE/cell (±s.e.) among (n=25) cells from each animal.
c. AGT=BrdUrd exposure duration/(frequency I)+(2x frequency II)+(3x frequency III).
d. Mean of mean frequency of SCEs and AGTs (±s.e.) among n animals.
e. p value of F-statistic from one-way ANOVA.

Table 19. Percent Abnormal Sperm from Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0.0	2.0	10.0	20.0	p value
n	10	10	10	10	
Abnormal Sperm (%)					
mean	0.260	0.480	0.240	0.140	0.0165[b]
s.e.	0.163	0.068	0.058	0.085	
Arcsin $\sqrt{f}$					
mean	1.55	3.74	2.33	1.13	0.0280[c]
s.e.	0.84	0.45	0.52	0.61	

a. Six hours/day, five days/week, for 6 months.
b. p value of Kruskal-Wallis non-parametric test.
c. p value of F-statistic from one-way ANOVA.

Reproductive Potential Studies

Exposure to silica dust did not appear to affect the reproductive potential of male or female Fischer-344 rats. Although this finding is not surprising, the data are not considered reliable because of personnel changes implemented during the experiment (Appendix H).

Statistical Relationships Among Pulmonary Measurements

Discriminant Analysis. Stepwise discriminant analysis was used to identify those raw and normalized pulmonary function and lung composition variables which best distinguished among the four exposure groups. This technique selected and linearly combined a minimal set of variables which caused the exposure groups to appear as distinct as possible. The set was selected such that the addition of any other single variable to the set would not significantly improve the distinction among the groups. When completed, the effectiveness of the derived discriminating function was checked by means of classification functions, which classified the original animals studied into one of the four groups according to its values for each of the variables considered. The classification thus obtained was compared with the true group origin of the animal and used to assess the effectiveness of the classification functions.

The lung composition data used in these analyses were entered as total amount of each component in the lungs as well as the amount per unit dry weight (Table 20). Similarly, many of the pulmonary function variables were expressed as a function of another variable on which they were dependent (Table 20).

When stepwise discriminant analysis was applied to the lung composition data, four variables in this set had discriminating power. These were hydroxyproline, elastin/dry weight, total lung weight, and protein/

Table 20. Variables Used in Stepwise Discriminant Analysis of Pulmonary Function and Lung Composition Data

PULMONARY FUNCTION VARIABLES

Parameters of Spontaneous Breathing

- f
- ΔP_L
- $\dot{V}_E$
- V_T

Divisions of Lung Volume

- ERV
- FRC_b
- FRC_d
- FRC_d/TLC_d
- FRC_b-FRC_d
- $(FRC_b-FRC_d)/TLC_d$
- IC
- IRV
- RV
- RV/TLC_d
- TLC_d
- VC
- VC/TLC_d

Indices of Parenchymal Damage

- $DLCO_{rb}$
- $DLCO_{rb}/TLC_d$
- h
- P_{st}
- QSC_{cs}
- QSC_{cs}/FRC_d
- QSC volume at x cm H_2O pressure (x = -10, -5, 0, 5, 10, 15, 20, 25).
- QSC volume/VC at x cm H_2O pressure (x = -5, 0, 5, 10, 15, 20, 25).

Table 20, continued

Points on the MEFV Curve

EFR_x where x = 50, 25, or 10% of VC
EFR_x/VC
ΔEFR_{25}
$\Delta EFR_{25}/VC$
$\Delta HEFR_{25}$
$\Delta HEFR_{25}/VC$
$\Delta HEFR_{50}$
$\Delta HEFR_{50}/VC$
Isoflow
PEF
PEF/VC
R_{us}
V_{max}
$\dot{V}_{30}$

CO_2 Response

$\%\Delta\dot{V}_E$

LUNG COMPOSITION DATA

Lung Weight
Dry Weight
% Dry Weight
Hydroxyproline (total)
Hydroxyproline/Dry Weight
Protein (total)
Protein/Dry Weight
DNA (total)
DNA/Dry Weight
Elastin (total)
Elastin/Dry Weight

dry weight. The classification functions based on these variables were 70.3 percent successful in identifying the test animals as belonging to their appropriate exposure groups (Table 21). The classification functions performed best in identifying the control animals, misidentifying only 1 out of 22 as an animal exposed to 2 mg SiO_2/m^3 (Table 21).

When the pulmonary function data were assessed using stepwise discriminant analysis, none of the measured variables had significant discriminating power.

Table 21. Jackknifed Classification of Fischer-344 Rats Exposed to 0, 2, 10, or 20 mg SiO_2/m^3 by Classification Functions Derived from Stepwise Discriminant Analysis of Selected Variables

Lung Composition Data

	Number of Cases Classified into Group					
Group	0	2	10	20	Percent Correct	Discriminating Variables
0	21	1	0	0	95.5	Hydroxyproline
2	4	15	4	0	65.2	Elastin/Dry Weight
10	0	5	10	7	45.5	Lung Weight
20	0	0	6	18	75.0	Protein/Dry Weight
Total	25	21	20	25	70.3	

DISCUSSION

This study was conducted as part of a series of experiments to examine the relationships among pulmonary structure, composition, and function during the development of silicotic lesions in the lungs of rats. The experimental protocol provides for the assessment of pulmonary function, composition, and structure after rats have been exposed to silica dust for three months, six months, and for six months followed by a six month holding period prior to assessment. The studies reported here have dealt only with those animals exposed for six months and then assessed.

The finding that female Fischer-344 rats exposed to 10 and 20 mg SiO_2/m^3 grew faster than control rats was unexpected and cannot be readily explained. Similar results have not been reported for rats exposed to silica by either inhalation or instillation.

The lung weights and the lung-to-body weight ratios of the rats exposed to 20 mg SiO_2/m^3 were greater than that of the control animals when the subgroups (n=8) designated for pathology were considered (Tables 2 and 3). However, in the subgroups of animals (n=22 to 24) designated for multiple endpoint assessment, including lung composition analysis (Table 13), differences in fresh lung weight were not observed. However, the total dry weight of the larger subgroups of control lungs and lungs from animals exposed to 20 mg SiO_2/m^3 did differ significantly (Table 13). The mean liver weight of animals from the control group was less than that of any of the exposure groups and the kidney weight of the controls was also less than some of the exposure groups. These findings are not considered to be an effect of silica exposure because the organ-to-body weight ratios are not similarly

affected, the effects observed are not dose-dependent, and pathological changes were not observed in these tissues from exposed animals.

Rats exposed for six months to SiO_2 concentrations up to 20 mg/m^3 exhibited no statistically significant alteration in static and dynamic lung function. In fact, trends or patterns of impairment consistent with incipient interstitial fibrosis could not be discerned from the lung function data. The single statistically significant change observed in all of the data loosely classified as "functional", was the arterial pO_2. However, in the context of the overall functional characterization of the exposure groups, as well as the technical difficulties possibly attributable to recovery from post-cannulation anesthesia, the pO_2 reduction in the blood samples of the 20 mg/m^3 groups may likely be spurious.

Histopathological evaluation of the lung tissue demonstrated a dose-related pattern of silica-induced disease. Not only was the accumulation of birefringenet particles indicative of exposure group classification, clear indication of progressive lung tissue injury existed. The density of lymphoid-associated microgranulomata also appeared to be associated with increasing silica dose. Coexistent with the marked end-airway accumulations phagocytic cells and type II cell hyperplasia were interstitial fibrogenic activity and developed micronodules within the lung parenchyma which indicated the chronic nature of this lesion.

Lung tissue composition appeared to be the most sensitive indicator of exposure to silica dust. While no significant changes occurred in the fresh lung weights of the exposure groups, the total dry weight of lungs from the 20 mg SiO_2/m^3 group was greater than that of the control

and low dose groups. Protein was the only measured component which did not increase in a dose-dependent manner. Dose dependent increases in both elastin and collagen concentrations were observed as would be expected in this type of lesion. Similarly, the pattern of increase in DNA, indicative of cellular infiltration, may be attributable to the proposed macrophage mediation of silica induced lung disease. This dose-dependency was also observed in the pulmonary histopathology. However, the biological significance of these changes in terms of the impact on pulmonary function is unclear, considering the normal pulmonary function observed in these animals.

Whether the existent lesions observed histologically and chemically in the exposed animals were part of a progressive pulmonary disability remains speculative. However, the dose-dependent pattern of these lesions, in addition to the accumulation of silica particles within the lung and the lymphatics suggests that the disease may progress further and result in compromised pulmonary function.

The difference in the sensitivity of the lung compositional analysis and pulmonary function tests was evident when the measured variables were assessed using stepwise discriminant analysis. While four of the composition variables, hydroxyproline, elastin/dry weight, total lung weight, and protein/dry weight, had significant discriminating power, none of the pulmonary function variables did. Interestingly, what appeared to be considerable changes in lung composition, particularly increases in connective tissue, did not result in significantly impaired pulmonary function.

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APPENDIX A

PRE-EXPERIMENTAL HEALTH PROFILES OF THE SUBJECT ANIMALS

AnMed Laboratories, Inc.
Diagnostic Services and Consultation in Laboratory Animal Medicine.

Steven H. Weisbroth, D.V.M.,
President

1804 Plaza Avenue
New Hyde Park, N.Y. 11040
(516) 775-0033

SUMMARY PAGE

Client Organization: BNL--Dr. Kutzman
Date Necropsied: 12 January 1982

Group Designation: No identification
Date Completed: 10 February 1982

Species (N): rat (10)
Accession Nos.: 3577

Date Received: 11 January 1982

Services Performed: Test 120: Full battery diagnostic screen

INTRODUCTION

Ten (10) adolescent male rats were presented for pre-experimental health profiles. The report below describes the results and interpretation of screening examinations on this group of rats. Serum samples drawn from the animals at the time of necropsy were evaluated for antibodies to murine viruses.

FINDINGS AND INTERPRETATION

The results are summarized in Table 1 and the attached serologic report. It will be seen that the rats were in an excellent state of health. No murine pathogens of the helminth, viral, arthropod, bacterial, protozoan or mycoplasmal groups were isolated or otherwise detected.

Klebsiella oxytoca was isolated from 100 percent of the animals in the group. There is no evidence of this species as a pathogen of laboratory rats.

In summary, the group should be interpreted as free of common murine diseases and entirely suitable for any chronic study, including inhalation projects in barrier facilities.

AnMed Laboratories, Inc.

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Summarized Findings of Screening Examinations: Table 1

Client Organization BNL--Dr. Kutzman — Date Necropsied 12 January 1982

Group Designation No identification — Date Completed 12 February 1982

Species (N) rat (10) — Serum Nos. 1-10

Date Received 11 January 1982 — Accession No. 3577

Examinations	Findings
1) Physical examination:	
A group of 10 male albino rate (mean wt-87.5g) was examined. They appeared in good health and no discharges from the nares, conjunctiva or anus were seen.	
2) Necropsy dissection:	10/10 NGL.
3) Fecal flotation:	10/10 No helminth ova or protozoan forms.
4) Fecal culture:	10/10 No Salmonella.
5) Direct cecum:	10/10 No helminths.
6) Intestinal wet mount:	10/10 No enteric protozoa.
7) Oropharyngeal culture:	10/10 No Pseudomonas, 3/3 (+) Klebsiella oxytoca.
8) Nasopharyngeal culture (PPLO):	10/10 No Mycoplasma.
9) Nasopharyngeal culture (BA):	10/10 Variably with Staphylococcus and K. oxytoca, No pathogens.
10) Nasopharyngeal culture (30% serum):	10/10 No Streptobacillus.
11) Middle ear:	10/10 No exudates.
12) Urinary bladder:	10/10 No helminths.
13) Blood film:	10/10 No hemoprotozoa.
14) Pelt:	10/10 No arthropods.
15) Liver (histopathology):	10/10 NML.
16) Lung (histopathology):	10/10 NML.
17) Kidney (histopathology):	10/10 NML.
18) Ileum (histopathology):	10/10 NML.
19) Other (list):	

ABBREVIATIONS, EXPLANATIONS

Abbreviations:

NGL = no gross lesion

NML = no microscopic lesion

NA = not applicable

TNP = Test not performed

(-) = indicated pathogen(s) not detected

(+) = indicated pathogen(s) detected

($\bar{x}$) = group mean or average

1) Physical examination involves clinical examination for exudates or abnormal discharges from body orifices, character of hair coat, posture, and attitudes of animals in diagnostic group.

2) Gross necropsy examination includes complete necropsy dissection of each animal in group with emphasis on observation of gross lesions.

3) Fecal flotation is performed using either pooled samples from shipping boxes or feces collected from the colon at necropsy. It is used to detect helminth ova and coccidia amenable to this procedure.

4) Fecal culture is oriented to screening for Salmonella and Citrobacter only, unless otherwise indicated.

5) Direct cecal examination under the microscope is used to supplement fecal flotation for helminth detection.

6) Intestinal wet mount examinations are performed by microscopy of small intestine contents for detection of intestinal protozoa, e.g. Hexamita, Giardia, etc.

7) Oropharyngeal culture is performed primarily to detect Pseudomonas and Klebsiella. Throat swabs are cultured in broth for 24 hours, then subcultured to differential media.

8) Nasopharyngeal culture (PPLO) is performed with nasoturbinate washings collected aseptically by pipette. When indicated, pulmonary culture is performed on selective media of pulmonary tissues collected aseptically from each animal at necropsy and ground in tissue mortars. Left side lobes are used. Mycoplasmas are determined on the basis of colonial, cultural and immunologic criteria.

9) Nasopharyngeal culture (BA) is performed by culture on blood agar (BA) of nasopharyngeal washings collected as in #8 above, for detection of bacterial pathogens.

10) Nasopharyngeal samples as collected in #8 above are cultured on 30% serum agar for detection of Streptobacillus moniliformis.

11) Middle ears are examined by puncture of tympanic membrane and aspiration of middle ear contents. Exudates, if any, are noted and cultured separately.

12) Urinary bladder mucosa of laboratory rats is examined under the dissection microscope for Trichosomoides crassicuada.

13) Giemsa-stained blood films are examined microscopically for hemoprotozoan forms, e.g. Hemobartonella.

14) Pelts are examined under direct low power microscopy for arthropod parasites. This procedure may be supplemented with Scotch tape examinations.

AnMed Laboratories, Inc.
Diagnostic Services and Consultation in Laboratory Animal Medicine.

1804 Plaza Avenue
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SEROLOGY REPORT

Client Organization: Brookhaven National Laboratory
Accession No.: 3577
Species: rat sera
Date Received: 11 January 1982
Group Designation: Dr. Kutzman
Date Completed: 10 February 1982

	AnMed Ident:	1	2	3	4	5	6	7	8	9	10
	Client Ident:										
____	MVM										
X	PVM	-	-	-	-	-	-	-	-	-	-
X	REO-3	-	-	-	-	-	-	-	-	-	-
____	MHV										
____	KV										
X	GD-7	-	-	-	-	-	-	-	-	-	-
____	RCV										
X	SEN	-	-	-	-	-	-	-	-	-	-
X	LCM	-	-	-	-	-	-	-	-	-	-
____	SV5										
____	MAV										
____	ECTR										
____	POLY										
X	KRV	-	-	-	-	-	-	-	-	-	-
____	THI										
X	SDAV	-	-	-	-	-	-	-	-	-	-
____	MYCO										
____	ECUN										
____	PMUL										
____	TREP										
____											

See Reverse For Abbreviations and Explanations

ALI Form 1008 H.

AnMed Laboratories, Inc.
Diagnostic Services and Consultation in Laboratory Animal Medicine.

1804 Plaza Avenue
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ABBREVIATIONS AND EXPLANATIONS

		Test Method
MVM	(Minute Virus of Mice). A parvovirus of rodents. ITD = 1:20	HI
PVM	(Pneumonia Virus of Mice). A paramyxovirus of rodents. ITD = 1:20	HI
REO-3	(Reovirus Type 3). A reovirus of rodents. ITD = 1:20	HI
MHV	(Mouse Hepatitis Virus). A coronavirus of mice. ITD = 1:10	CF
KV	(K Virus). A papovavirus of the mouse. ITD = 1:10	HI
GDVII	(Theiler's Virus, Murine Encephalomyelitis). A picornavirus of rodents. ITD = 1:20	HI
RCV	(Rat Coronavirus). A coronavirus of rats. ITD = 1:10	CF
SEN	(Sendai Virus). A paramyxovirus of rodents. ITD = 1:10	HI
LCM	(Lymphocytic choriomeningitis). A zoonotic arenavirus. ITD = 1:10	FA
SV5	(Simian Virus 5). A simian paramyxovirus infection of guinea pigs and hamsters. ITD = 1:20	HI
MAV	(Mouse Adenovirus). An adenovirus infection of mice. ITD = 1:10	CF
ECTR	(Ectromelia). A poxvirus of the mouse. ITD = 1:10	CF
POLY	(Polyoma). A papovavirus of mice. ITD = 1:40	HI
KRV	(Kilham's Rat Virus). A parvovirus of rats. ITD = 1:20	HI
THI	(Toolan's H-1). A parvovirus of rats. ITD = 1:20	HI
SDAV	(Sialodacryoadenitis Virus). A coronavirus of rats. ITD = 1:20	CF
EDIM	(Epizootic Diarrhea of Infant Mice). An unclassified mouse virus. ITD = 1:10	FA
LDV	(Riley's Lacticdehydrogenase Virus). A virus causing elevation of serum LDH. Presence of the virus is inferred from elevations of serum LDH.	
MYCO	(Mycoplasma pulmonis). A mycoplasma of rodents. ITD = 1:10	EL
ECUN	(Encephalitozoon cuniculi). A protozoan of rodents and rabbits. ITD = 1:25	IIR
PMUL	(Pasteurella multocida). A bacterial pathogen of rabbits. ITD = 1:20	FA
TREP	(Treponema cuniculi).A bacterial pathogen of rabbits. ITD = 1:10	RPR

HI ..... Hemagglutination Inhibition

CF ..... Complement Fixation

FA ..... Fluorescent Antibody

EL ..... Enzyme Linked Immunosorbent Assay

IIR ..... India Ink Immunoreaction

RPR ..... Rapid Plasma Reagin

ITD ..... Initial Test Dilution

NSA ..... Non-Specific Agglutination. *, **, ***, **** = Tested negative at dilutions 1:20, 40, 80, 160 respectively, but NSA at lower dilutions

AC ..... Anticomplementary factors in the serum. *, **, ***, **** = Tested negative at dilutions 1:20, 40, 80, 160 respectively, but AC at lower dilutions.

TC ..... Serum reacts with tissue control (medium used to propagate antigen).

AnMed Laboratories, Inc.
Diagnostic Services and Consultation in Laboratory Animal Medicine.

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SUMMARY PAGE

Client Organization:	BNL--Dr. Kutzman	Date Necropsied:	28 January 1982
Group Designation:	No identification	Date Completed:	16 February 1982
Species (N):	rat (10)	Accession Nos.:	3606
Date Received:	28 January 1982		
Services Performed:	Test 120: Full battery diagnostic screen		

INTRODUCTION

Ten (10) adolescent male and female rats were presented for pre-experimental health profiles. The report below describes the results and interpretation of screening examinations on this group of rats. Serum samples drawn from the animals at the time of necropsy were evaluated for antibodies to murine viruses.

FINDINGS AND INTERPRETATION

The results are summarized in Table 1 and the attached serologic report. It will be seen that the rats were in an excellent state of health. No murine pathogens in the helminth, viral, arthropod, bacterial, protozoan or mycoplasmal groups were detected or isolated.

In summary, the group should be interpreted as free of common murine diseases and entirely suitable for any chronic study, including inhalation projects in barrier facilities.

AnMed Laboratories, Inc.

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Summarized Findings of Screening Examinations: Table 1

Client Organization BNL--Dr. Kutzman — Date Necropsied 28 January 1982

Group Designation No identification — Date Completed 16 February 1982

Species (N) rat (10) — Serum Nos. 1-10

Date Received 28 January 1982 — Accession No. 3606

Examinations	Findings
1) Physical examination:	
A group of 8 male and 2 female albino rats (mean wt.=90 and 68g) was examined. They appeared in good health and no discharges from the nares, conjunctiva or anus were seen.	
2) Necropsy dissection:	10/10 NGL.
3) Fecal flotation:	10/10 No helminth ova or protozoan forms.
4) Fecal culture:	10/10 No Salmonella.
5) Direct cecum:	10/10 No helminths.
6) Intestinal wet mount:	10/10 No enteric protozoa.
7) Oropharyngeal culture:	10/10 No Pseudomonas or Klebsiella
8) Nasopharyngeal culture (PPLO):	10/10 No Mycoplasma.
9) Nasopharyngeal culture (BA):	10/10 Variably with Staphylococcus, E. coli; No pathogens.
10) Nasopharyngeal culture (30% serum):	10/10 No Streptobacillus.
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12) Urinary bladder:	10/10 No helminths.
13) Blood film:	10/10 No hemoprotozoa.
14) Pelt:	10/10 No arthropods.
15) Liver (histopathology):	10/10 NML.
16) Lung (histopathology):	10/10 NML.
17) Kidney (histopathology):	10/10 NML.
18) Ileum (histopathology):	10/10 NML.
19) Other (list):	Thymus: 10/10 NML. Spleen: 10/10 NML.

ABBREVIATIONS, EXPLANATIONS

Abbreviations:

NGL = no gross lesion

NML = no microscopic lesion

NA = not applicable

TNP = Test not performed

(-) = indicated pathogen(s) not detected

(+) = indicated pathogen(s) detected

($\bar{x}$) = group mean or average

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13) Giemsa-stained blood films are examined microscopically for hemoprotozoan forms, e.g. Hemobartonella.

14) Pelts are examined under direct low power microscopy for arthropod parasites. This procedure may be supplemented with Scotch tape examinations.

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SEROLOGY REPORT

Client Organization: Brookhaven National Laboratory
Accession No.: 3606
Species: rat sera (10)
Date Received: 28 January 1982
Group Designation: Dr. Kutzman
Date Completed: 16 February 1982

	AnMed Ident:	1	2	3	4	5	6	7	8	9	10
	Client Ident:										
	MVM										
X	PVM	-	-	-	-	-	-	-	-	-	-
X	REO-3	-	-	-	-	-	-	-	-	-	-
	MHV										
	KV										
X	GD-7	-	-	-	-	-	-	-	-	-	-
	RCV										
X	SEN	-	-	-	-	-	-	-	-	-	-
X	LCM	-	-	-	-	-	-	-	-	-	-
	SV5										
	MAV										
	ECTR										
	POLY										
X	KRV	-	-	-	-	-	-	-	-	-	-
	THI										
X	SDAV	-	-	-	-	-	-	-	-	-	-
	MYCO										
	ECUN										
	PMUL										
	TREP										

See Reverse For Abbreviations and Explanations

AnMed Laboratories, Inc.
Diagnostic Services and Consultation in Laboratory Animal Medicine.

1804 Plaza Avenue
New Hyde Park, N.Y. 11040
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ABBREVIATIONS AND EXPLANATIONS

		Test Method
MVM	(Minute Virus of Mice). A parvovirus of rodents. ITD = 1:20	HI
PVM	(Pneumonia Virus of Mice). A paramyxovirus of rodents. ITD = 1:20	HI
REO-3	(Reovirus Type 3). A reovirus of rodents. ITD = 1:20	HI
MHV	(Mouse Hepatitis Virus). A coronavirus of mice. ITD = 1:10	CF
KV	(K Virus). A papovavirus of the mouse. ITD = 1:10	HI
GDVII	(Theiler's Virus, Murine Encephalomyelitis). A picornavirus of rodents. ITD = 1:20	HI
RCV	(Rat Coronavirus). A coronavirus of rats. ITD = 1:10	CF
SEN	(Sendai Virus). A paramyxovirus of rodents. ITD = 1:10	HI
LCM	(Lymphocytic choriomeningitis). A zoonotic arenavirus. ITD = 1:10	FA
SV5	(Simian Virus 5). A simian paramyxovirus infection of guinea pigs and hamsters. ITD = 1:20	HI
MAV	(Mouse Adenovirus). An adenovirus infection of mice. ITD = 1:10	CF
ECTR	(Ectromelia). A poxvirus of the mouse. ITD = 1:10	CF
POLY	(Polyoma). A papovavirus of mice. ITD = 1:40	HI
KRV	(Kilham's Rat Virus). A parvovirus of rats. ITD = 1:20	HI
THI	(Toolan's H-1). A parvovirus of rats. ITD = 1:20	HI
SDAV	(Sialodacryoadenitis Virus). A coronavirus of rats. ITD = 1:20	CF
EDIM	(Epizootic Diarrhea of Infant Mice). An unclassified mouse virus. ITD = 1:10	FA
LDV	(Riley's Lacticdehydrogenase Virus). A virus causing elevation of serum LDH. Presence of the virus is inferred from elevations of serum LDH.	
MYCO	(Mycoplasma pulmonis). A mycoplasma of rodents. ITD = 1:10	EL
ECUN	(Encephalitozoon cuniculi). A protozoan of rodents and rabbits. ITD = 1:25	IIR
PMUL	(Pasteurella multocida). A bacterial pathogen of rabbits. ITD = 1:20	FA
TREP	(Treponema cuniculi).A bacterial pathogen of rabbits. ITD = 1:10	RPR

HI	Hemagglutination Inhibition	**EL**	Enzyme Linked Immunosorbent Assay
CF	Complement Fixation	**IIR**	India Ink Immunoreaction
FA	Fluorescent Antibody	**RPR**	Rapid Plasma Reagin

ITD ..... Initial Test Dilution

NSA ..... Non-Specific Agglutination. *, **, ***, **** = Tested negative at dilutions 1:20, 40, 80, 160 respectively, but NSA at lower dilutions

AC ..... Anticomplementary factors in the serum. *, **, ***, **** = Tested negative at dilutions 1:20, 40, 80, 160 respectively, but AC at lower dilutions.

TC ..... Serum reacts with tissue control (medium used to propagate antigen).

APPENDIX B

POST-EXPOSURE SEROLOGY PROFILE ON THE SUBJECT ANIMALS

The attached report stems from the sera of 4 animals from the 6 month SiO_2 assessment subgroups.

The sera were submitted to AnMed Laboratories to assess the viral antibody status of animals in the MIN-U-SIL study.

AnMed Laboratories, Inc.
Diagnostic Services and Consultation in Laboratory Animal Medicine

1804 Plaza Avenue
New Hyde Park, N.Y. 11040
(516) 775-0033

SEROLOGY REPORT

Client Organization: Brookhaven
Accession No.: 3955 c/pw
Species: Rat (4)
Date Received: 30 August 1982
Group Designation:
Date Completed: 17 September 1982

	AnMed Ident:	1	2	3	4
	Client Ident:	1086	1284	1484	1684
____	MVM				
X	PVM	320	320	320	160
X	REO-3	-	-	-	-
____	MHV				
____	KV				
X	GD-7	-	-	-	-
____	RCV				
X	SEN	-	-	-	-
X	LCM	-	-	-	-
____	SV5				
____	MAV				
____	ECTR				
____	POLY				
X	KRV	-	-	-	-
____	THI				
X	SDAV	-	-	-	-
____	MYCO				
____	ECUN				
____	PMUL				
____	TREP				
____					

See Reverse For Abbreviations and Explanations

ALI Form 1008

APPENDIX C

CHAMBER DISTRIBUTION OF SILICA DUST

To characterize the distribution of silica dust in the chambers employed in this study, two of the chambers were fitted with tubing to permit sampling of 27 stations throughout the chambers. The 27 stations sampled were located on 3 levels in the chamber with 9 sampling stations on each level. The 3 levels sampled corresponded to the first (top-most), the 3rd, and the 4th (bottom-most) tiers in the chamber. During the actual animal exposures, however, only the uppermost three tiers were utilized.

The values provided in Figures C-1 and C-2 are the decimal fraction (±s.e.) at each station of the average concentration throughout the chamber for a single distribution experiment.

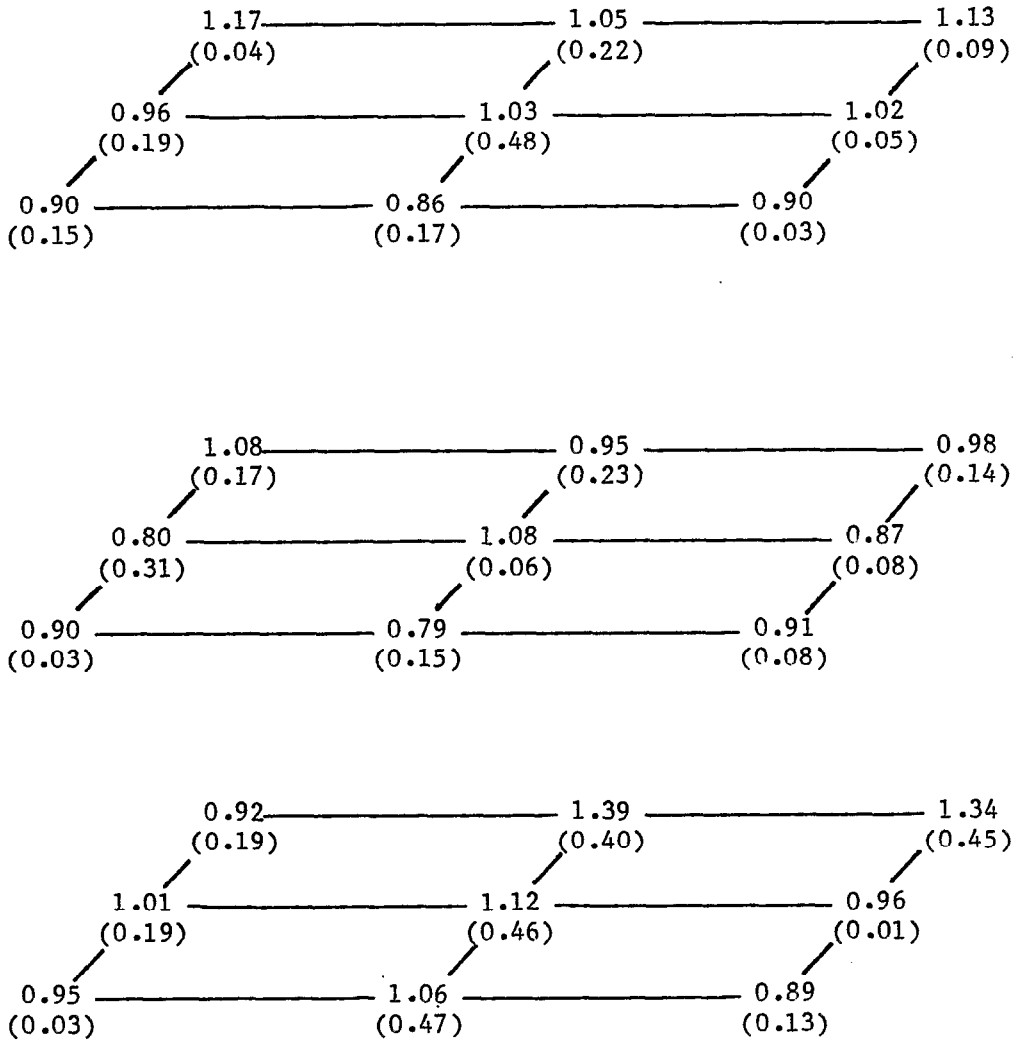

Figure C-1: Silica distribution in exposure chamber 5-C, the chamber used to expose animals to 10 mg SiO_2/m^3. Each value represents the mean (±s.e.)(n=3) decimal fraction, at a sampling station, of the average concentration throughout the chamber.

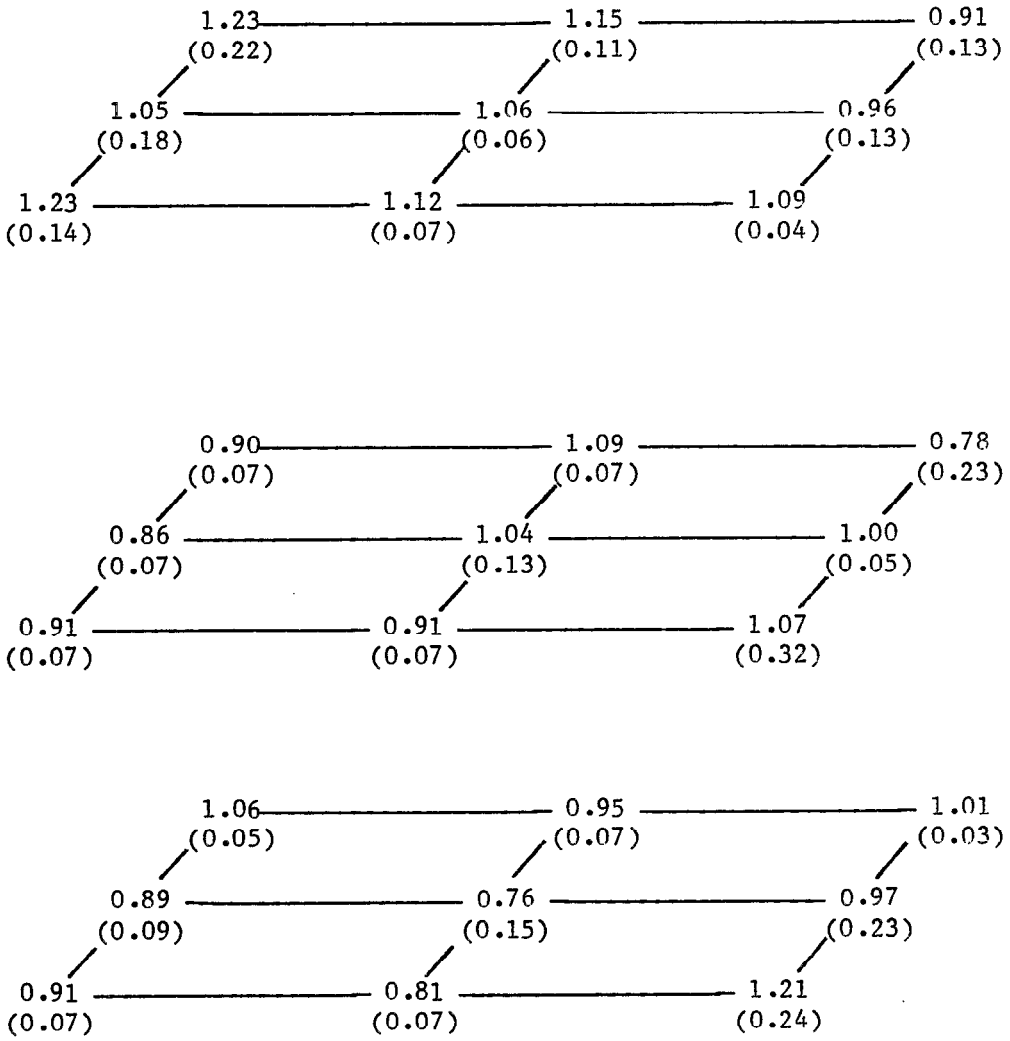

Figure C-2: Silica distribution in exposure chamber 5-D, the chamber used to expose animals to 20 mg SiO_2/m^3. Each value represents the mean (±s.e.)(n=3) decimal fraction, at a sampling station, of the average concentration throughout the chamber.

APPENDIX D

MEAN WEIGHTS OF SUBGROUPS OF F-344 RATS FROM EACH EXPOSURE CHAMBER (0, 2, 10, or 20 mg SiO_2/m^3)

Table D-1: Mean Weight of Subgroups (n=8) of Male Fischer-344 Rats Exposed to Silica Dust

Date Weighed	Control	2 mg SiO_2/m^3	10 mg SiO_2/m^3	20 mg SiO_2/m^3
2/22/82	209.6[a] (4.0)[b]	192.2 (3.5)	214.0 (8.3)	192.7 (5.2)
3/01/82	220.3 (4.1)	195.8 (2.9)	221.6 (8.2)	197.4 (5.6)
3/15/82	243.9 (4.5)	223.9 (2.6)	247.4 (10.1)	222.7 (5.7)
3/29/82	261.6 (6.2)	241.6 (7.5)	269.5 (12.1)	248.8 (6.5)
4/12/82	279.4 (5.3)	265.7 (3.8)	287.5 (12.3)	268.3 (5.6)
4/26/82	295.0 (5.8)	281.7 (4.1)	304.7 (11.8)	280.6 (5.3)
5/10/82	305.1 (6.0)	293.4 (5.1)	319.6 (11.9)	296.4 (6.2)
5/24/82	315.6 (6.9)	306.9 (6.1)	333.8 (12.1)	305.1 (6.0)
6/07/82	320.1 (7.4)	315.1 (6.6)	340.0 (11.9)	315.1 (6.3)
6/21/82	329.4 (7.2)	323.6 (6.8)	352.8 (12.8)	322.2 (6.7)
7/05/82	334.9 (7.7)	332.1 (8.1)	360.8 (12.8)	330.7 (6.1)

Table D-1, continued

Date Weighed	Control	2 mg SiO_2/m^3	10 mg SiO_2/m^3	20 mg SiO_2/m^3
7/19/82	344.8 (7.3)	341.2 (8.4)	370.4 (13.5)	340.9 (5.2)
8/02/82	347.7 (7.8)	345.4 (8.7)	371.2 (14.2)	344.3 (4.9)
8/16/82	348.0 (7.2)	344.5 (8.0)	374.6 (13.3)	342.5 (4.1)
8/18/82	349.6 (7.0)	345.2 (8.3)	375.0 (13.7)	343.3 (3.8)
8/24/82[c]	350.3 (7.3)	348.2 (8.3)	379.9 (12.3)	352.3 (4.0)

a. mean.
b. (+s.e.).
c. Six calendar days following termination of exposures.

Table D-2: Mean Weight of Subgroups (n=8) of Female Fischer-344 Rats Exposed to Silica Dust

Date Weighed	Control	2 mg SiO_2/m^3	10 mg SiO_2/m^3	20 mg SiO_2/m^3
3/01/82	136.1[a] (1.1)[b]	142.3 (1.8)	139.4 (2.9)	137.2 (2.3)
3/15/82	140.1 (1.7)	146.9 (2.3)	149.7 (3.0)	147.3 (2.2)
3/29/82	148.9 (1.3)	154.0 (2.3)	158.4 (2.9)	155.6 (2.2)
4/12/82	154.0 (1.4)	162.6 (2.6)	163.3 (3.4)	161.8 (1.7)
4/26/82	159.1 (1.3)	168.4 (3.2)	171.9 (3.6)	169.0 (1.9)
5/10/82	164.5 (1.5)	172.7 (3.0)	178.7 (4.3)	174.3 (1.8)
5/24/82	168.6 (2.2)	178.4 (3.4)	181.4 (4.9)	178.3 (2.3)
6/07/82	172.2 (2.0)	182.5 (3.4)	185.1 (4.6)	181.7 (2.5)
6/21/82	176.1 (1.8)	186.8 (3.7)	188.9 (4.4)	185.7 (1.9)
7/05/82	178.0 (1.9)	189.4 (3.6)	191.6 (4.5)	188.9 (1.6)
7/19/82	180.1 (1.8)	189.8 (3.6)	194.3 (4.7)	192.5 (2.1)

Table D-2, continued

Date Weighed	Control	2 mg SiO_2/m^3	10 mg SiO_2/m^3	20 mg SiO_2/m^3
8/02/82	185.3 (2.3)	193.7 (3.5)	197.3 (4.5)	195.2 (1.9)
8/16/82	185.0 (2.5)	194.3 (3.7)	197.0 (4.9)	192.8 (2.5)
8/25/82	186.4 (2.0)	196.9 (3.3)	201.4 (4.5)	195.6 (2.8)
8/31/82[c]	185.8 (2.0)	196.1 (3.2)	199.9 (4.1)	194.0 (4.3)

a. mean.
b. (±s.e.).
c. Six calendar days following termination of exposures.

APPENDIX E

PULMONARY FUNCTION DATA FROM INDIVIDUAL FISCHER-344 RATS

Pulmonary Function Data from Individual Fischer-344 Rats
Abbreviations Used in Appendix E

Text Abbreviation	Definition	Appendix Abbreviation
	percent change in minute volume when breathing 10% CO_2, 20% O_2 instead of air	CO2RESP
C_{DYN}	dynamic compliance (cm^3/cm H_2O)	CDYN
$DLCO_{rb}$	diffusing capacity of the lung for CO measured by a rebreathing technique ($cm^3/mmHg^{-min}$)	DLCO
EFR_x	expiratory flow rate at x% vital capacity (cm^3/min)(where x=50, 25, or 10)	EFRx
f	frequency of breathing (breaths per min)	F
FRC_b	functional residual capacity (cm^3)	FRCB
$\Delta HEFR_x$	difference in the flow at x% VC in the MEFV curves when helium rather than air was the gas breathed (where x = 50 or 25)	DHEFRx
HR	heart rate (beats/min)	HR
IC	inspiratory capacity (cm^3)	IC
	isoflow points (as % VC) where the air and He MEFV curves overlap	ISOFLOW
	animal number	LABEL
M_1/M_0	$\sum_{j=1}^{50} b_j \cdot X_j \Big/ \sum_{j=1}^{50} X_j$	M1M0
	partial pressure of CO_2 (mmHg)	PCO2
ph	ph of arterial blood	PH
P_L	transpulmonary pressure (cm H_2O)	PL
	partial pressure of O_2 (mmHg)	PO2
P-R	EKG wave interval	PR

Text Abbreviation	Definition	Appendix Abbreviation
P_{st}	static pressure (cm H_2O)	PST
PEF	peak expiratory flow (cm^3/sec)	PEF
	EKG wave interval	QRS
QSC_{cs}	quasi-static compliance determined by chord slope (cm^3/cm H_2O)	QSCCS
R_L	pulmonary resistance (cm $H_2O/cm^3 \cdot sec^{-1}$)	RL
TLC_d	total lung capacity determined by dilution (cm^3)	TLCD
V_T	tidal volume (cm^3)	VT
$\dot{V}_{30}$	airflow (cm^3/sec) at 30% VC	V30
VC	vital capacity	VC
	lung volume (cm^3) at x cm H_2O pressure (N stands for -)	VOL(N)X
V_{max}	percent of VC at which peak expiratory flow occurs	VMAX

CONTROL GROUP

CASE NO.	LABEL	11 VT	12 PL	13 F	14 RL	15 CDYN	17 IC	18 VC	19 FRCB	20 TLCD	21 DLCO
1	1009	1.500	8.330	70	.390	.200	6.580	8.480	5.100	9.710	.144
2	1010	MISSING	MISSING	MISSING	.450	.120	MISSING	MISSING	MISSING	MISSING	MISSING
3	1011	1.540	4.520	65	.230	.430	10.230	11.730	3.150	12.880	.186
4	1012	1.800	5	60	.300	.350	8.480	10.060	3.460	10.880	.174
5	1013	1.440	5.800	90	.820	.200	8.470	9.810	3.430	10.540	.152
6	1014	2.010	3.500	85	.150	.500	9.690	11.660	3.540	12.960	.166
7	1015	1.390	4.050	116	.120	.540	9.020	11.230	3.630	11.920	.204
8	1016	1.650	4.500	51	.200	.360	8.580	10.040	2.720	11.100	.166
9	1033	1.980	5	71	.450	.420	9.440	10.950	3.790	11.810	.201
10	1034	1.670	5.330	57	.300	.300	10.330	11.230	3.180	12.390	.165
11	1035	1.500	5.930	64	.330	.330	9.860	11.110	3.210	12	.149
12	1036	1.850	5.330	90	.420	.230	10.340	11.740	3.430	12.550	.136
13	1037	1.910	6	83	.390	.380	9.620	10.650	2.470	11.840	.220
14	1038	2.110	5	61	.300	.440	10.810	11.790	5.050	13.210	.159
15	1039	1.700	4.500	49	.300	.420	9.840	11.190	3.720	13.270	.195
16	1040	1.550	5	74	.640	.450	10.620	11.710	4.060	12.930	.198
17	1057	1.570	4.830	62	.170	.500	9.820	12.310	3.310	13.610	.237
18	1058	1.930	5.510	68	.730	.350	7.710	8.680	3.270	8.680	.128
19	1059	1.670	5.630	59	.150	.340	9.920	11.400	3.900	12.260	.171
20	1060	1.810	4.750	55	.140	.370	10.990	11.850	2.680	13.070	.206
21	1061	1.440	3.790	59	.210	.480	10.530	11.530	3.190	12.570	.225
22	1062	1.480	5.170	74	.290	.400	9.340	10.690	3.630	11.660	.215
23	1063	1.520	4.500	53	.290	.330	10.100	11.400	3.470	12.380	.184
24	1064	1.540	4.830	64	MISSING	MISSING	9.870	10.830	3.210	12.060	.167

CONTROL GROUP

CASE NO.	LABEL	22 PST	23 V30	24 QSCCS	32 VMAX	26 PEF	27 EFR50	28 EFR25	29 EFR10	30 M1M0	33 HR
1	1009	MISSING	MISSING	.490	MISSING	123.700	98.300	55.400	35.600	4.100	MISSING
2	1010	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
3	1011	13.160	87.560	.840	67	145.200	115.600	79.100	35.300	5.700	MISSING
4	1012	17.680	89.740	.800	70	135.800	110	73.400	34.400	5.900	371
5	1013	12	78.330	.680	78	122.400	103.700	63.600	31.500	5.800	329
6	1014	14.840	49.390	.830	82	117.100	87.200	54.200	27.500	4.400	343
7	1015	18.100	73.060	.750	71	140.200	110.400	61.700	31.100	5.500	343
8	1016	12.950	59.590	.750	80	114.600	79.400	51.800	21.100	8.400	336
9	1033	20.840	77.300	.800	74	118.100	98.900	65.200	32.600	6.900	348
10	1034	12.210	70.310	.870	66	112.800	94.500	63.800	34.700	6.900	310
11	1035	20.210	42.480	.880	79	109.800	65.400	26.700	7	5.600	353
12	1036	14.530	59.670	.870	75	120.600	82.100	54.300	28.300	4.900	296
13	1037	14.110	80.550	.810	64	106.700	101.800	63.900	31.700	7.100	421
14	1038	16.630	67.030	.890	77	83.100	77.100	62	33.900	6.400	366
15	1039	16.530	83.240	.830	60	107.500	105	64.600	30.500	3.500	296
16	1040	13.260	79.430	.940	61	106.100	102.800	63.400	33.300	6.600	387
17	1057	14.110	77.430	.820	66	116.700	102.100	61.400	19.800	3.800	375
18	1058	15.890	43.620	.700	67	88.600	67.700	34.500	6.600	17	402
19	1059	12.210	61.250	.850	77	124.400	89.800	54.600	17.200	6.300	333
20	1060	16.530	53.770	.870	70	123.800	91.800	35.600	9.100	8.400	389
21	1061	12.630	53.460	.990	81	123.300	79.100	41.800	18.700	9.600	348
22	1062	19.890	38.190	.800	78	93.900	65.500	25.300	7.700	7.100	368
23	1063	8.740	65.070	.790	70	118.100	89.800	55.900	32.100	7	402
24	1064	16.530	78.470	.840	70	123	98.600	63.100	30.800	7.100	MISSING

CONTROL GROUP

CASE NO.	LABEL	34 PR	35 QRS	37 VOLN15	38 VOLN10	39 VOLN5	40 VOL0	41 VOL5	42 VOL10	43 VOL15	44 VOL20
1	1009	MISSING	MISSING	0	.0400	.0900	1.890	4.590	6.690	7.590	7.890
2	1010	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
3	1011	MISSING	MISSING	0	.0900	.0900	1.490	6.940	9.990	10.790	11.290
4	1012	MISSING	.0100	0	.0100	.0300	1.580	5.030	8.280	9.080	9.580
5	1013	.0429	.00750	0	.0400	.240	1.340	5.240	8.240	8.840	9.340
6	1014	.0423	.0108	0	1.770	1.820	1.970	8.170	10.270	10.970	11.370
7	1015	.0392	.0100	0	.710	.810	2.210	7.510	9.710	10.310	10.810
8	1016	.0344	.00920	0	.0600	.260	1.460	7.260	8.860	9.410	9.660
9	1033	.0436	.0141	0	.310	.360	1.610	6.960	9.410	10.210	10.710
10	1034	.0438	.0106	0	.0100	.0400	.890	6.790	9.490	10.490	10.890
11	1035	.0469	.0133	0	.650	.750	1.250	7.850	9.950	10.550	10.850
12	1036	.0325	.0125	0	.0900	.140	1.390	8.190	9.990	10.990	11.390
13	1037	.0418	.0100	0	.0300	.280	1.030	7.830	9.230	9.880	10.430
14	1038	.0540	.0150	0	.780	.880	.980	8.430	10.480	11.180	11.580
15	1039	.0500	.0115	0	.550	.800	1.350	8.050	9.950	10.500	10.950
16	1040	.0463	.0113	0	.0100	.0900	1.090	7.790	10.390	10.990	11.490
17	1057	.0400	.0110	0	1.890	2.010	2.490	8.690	10.990	11.640	11.890
18	1058	.0438	.0100	0	.260	.310	.960	6.510	7.760	8.310	8.560
19	1059	.0455	.00880	0	.0200	.130	1.480	7.480	9.880	10.630	11.080
20	1060	.0430	.0150	0	.560	.580	.860	7.810	10.260	11.060	11.460
21	1061	.0413	.00830	0	.190	.290	.990	7.340	10.790	11.340	11.590
22	1062	.0420	.0125	0	.640	.940	1.340	7.490	9.240	9.890	10.340
23	1063	.0440	.0120	0	0	.100	1.300	6.950	9.400	10.300	10.700
24	1064	MISSING	MISSING	0	.160	.210	.960	6.960	9.460	10.110	10.560

CONTROL GROUP

CASE NO.	LABEL	45 VOL25	47 DHEFR50	48 DHEFR25	49 ISOFLOW	50 PCO2	51 PO2	52 PH	53 CO2RESP
1	1009	8.390	29.300	20	MISSING	43.300	92.700	7.400	109
2	1010	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	57
3	1011	11.740	10.800	6.500	2.600	MISSING	MISSING	MISSING	22
4	1012	9.830	8.400	4.700	6.700	MISSING	MISSING	MISSING	107
5	1013	9.590	23	14.700	3.600	MISSING	MISSING	MISSING	67
6	1014	11.720	30.500	18	4.500	39.400	79.600	7.430	111
7	1015	10.960	21.500	9	10.900	43.400	70.900	7.410	176
8	1016	9.960	35.400	20	.800	MISSING	MISSING	MISSING	129
9	1033	11.010	32.100	19.900	8.400	43.600	90.400	7.430	121
10	1034	11.140	26.400	9.100	3	MISSING	MISSING	MISSING	35
11	1035	11	10	17.200	8.600	43	90.900	7.440	95
12	1036	11.890	44.300	18.700	.700	MISSING	MISSING	MISSING	49
13	1037	10.530	16.600	-3.100	28	MISSING	MISSING	MISSING	75
14	1038	11.730	45.300	26	10.400	47.200	77.900	7.380	92
15	1039	11.100	26.400	6.200	14.900	40.700	79.600	7.370	108
16	1040	11.710	34.400	27.300	.700	MISSING	MISSING	MISSING	124
17	1057	12.240	29.500	22.900	15.700	MISSING	MISSING	MISSING	49
18	1058	8.710	22.700	13.800	3.800	MISSING	MISSING	MISSING	77
19	1059	11.480	17.700	-5.700	17.300	41.700	67.900	7.400	114
20	1060	11.860	21.200	19.500	.700	MISSING	MISSING	MISSING	209
21	1061	11.590	27	10.100	10.900	43.200	82.600	7.420	128
22	1062	10.590	31.800	25	1.500	MISSING	MISSING	MISSING	134
23	1063	11.050	31	13	4.500	MISSING	MISSING	MISSING	41
24	1064	10.710	26.800	17.500	.400	MISSING	MISSING	MISSING	43

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	11 VT	12 PL	13 F	14 RL	15 CDYN	17 IC	18 VC	19 FRCB	20 TLCD	21 DLCO
25	1209	1.500	11.500	62	.360	.130	8.980	10.550	3.210	10.690	.0880
26	1210	1.050	4.500	66	.330	.400	8.930	10.040	3.920	10.280	.161
27	1211	1.400	6.500	64	.320	.300	8.410	10.100	3.590	11.040	.164
28	1212	1.710	5.500	54	.900	.220	8.590	10.200	3.050	10.880	.174
29	1213	1.930	4.730	46	1.360	.380	8.770	11.230	3.670	11.740	.145
30	1214	1.850	6.750	96	.420	.280	9.890	12.180	4.200	13.660	.194
31	1215	1.530	5.500	62	.350	.330	8.640	10.080	2.960	10.390	.175
32	1216	1.510	4	63	.470	.310	9.980	11.190	3.900	12.370	.178
33	1233	1.620	4.100	65	.760	.320	9.160	10.500	3.890	11.030	.149
34	1234	1.900	5.030	75	.360	.560	9.640	11.440	5.200	12.200	.217
35	1235	1.560	6	49	.760	.230	9.120	10.530	2.590	10.830	.160
36	1236	2	5	80	.800	.290	9.580	10.680	3.680	11.590	.183
37	1237	1.430	4.170	68	.150	.500	9.160	10.950	3.650	12.180	.167
38	1238	1.570	4	66	.240	.450	9.010	10.760	4.290	11.360	.177
39	1239	1.670	6.500	61	.830	.260	7.890	9.440	4.340	9.940	.157
40	1240	1.970	4	54	.200	.210	10.420	11.540	4.110	14.440	.187
41	1257	1.530	6.330	77	.600	.300	10.870	11.790	4.880	13.670	.226
42	1258	1.720	5.330	73	.300	.290	9.820	10.660	4.170	11.310	.176
43	1259	1.400	5.250	59	.210	.410	10.210	11.050	2.830	11.930	.185
44	1260	1.500	5	60	.150	.460	9.150	10.380	4.520	MISSING	MISSING
45	1261	1.810	6.670	67	.790	.350	9.590	10.290	3.190	11.410	.165
46	1262	1.700	5.080	58	.350	.300	10.020	11.540	3.700	12.310	.199
47	1263	2.060	4	50	.120	.250	9.210	10.740	3.460	12.240	.137
48	1264	1.860	5.250	60	.200	.290	10.020	11.440	4.140	12.690	.171

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	22 PST	23 V3Ø	24 QSCCS	32 VMAX	26 PEF	27 EFR5Ø	28 EFR25	29 EFR1Ø	3Ø M1MØ	33 HR
25	12Ø9	12.84Ø	8Ø.67Ø	.68Ø	74	123.9ØØ	1Ø4.9ØØ	69.5ØØ	3Ø.8ØØ	8.6ØØ	MISSING
26	121Ø	17.89Ø	56.11Ø	.76Ø	78	113.3ØØ	78	51.3ØØ	31	18.5ØØ	MISSING
27	1211	14.74Ø	67.79Ø	.68Ø	6Ø	123.3ØØ	117.8ØØ	51.7ØØ	23.9ØØ	5.1ØØ	4Ø7
28	1212	11.58Ø	48.Ø9Ø	.7ØØ	83	114.8ØØ	74.4ØØ	39.9ØØ	6.7ØØ	8.6ØØ	MISSING
29	1213	12.95Ø	43.87Ø	.74Ø	84	11Ø.8ØØ	71.5ØØ	29.7ØØ	11.9ØØ	6.2ØØ	31Ø
3Ø	1214	14.74Ø	51.35Ø	.8ØØ	7Ø	1ØØ.6ØØ	83.9ØØ	41.8ØØ	12.5ØØ	4	358
31	1215	17.47Ø	78.38Ø	.73Ø	61	128.8ØØ	115.3ØØ	67.9ØØ	33.5ØØ	8.7ØØ	3ØØ
32	1216	18.37Ø	77.6ØØ	.88Ø	7Ø	92.4ØØ	87.9ØØ	62.4ØØ	31.7ØØ	7	358
33	1233	21.26Ø	73.76Ø	.75Ø	74	116	93.1ØØ	64.7ØØ	37.1ØØ	8.2ØØ	338
34	1234	17.16Ø	65.69Ø	.83Ø	69	1Ø6.6ØØ	99.8ØØ	48.2ØØ	14.8ØØ	5.2ØØ	393
35	1235	17.47Ø	8Ø.23Ø	.78Ø	58	95.4ØØ	93.9ØØ	62.8ØØ	3Ø.8ØØ	8.8ØØ	339
36	1236	16.42Ø	78.34Ø	.81Ø	69	128.5ØØ	1Ø3.5ØØ	7Ø.1ØØ	29.8ØØ	7.1ØØ	41Ø
37	1237	13.Ø5Ø	74.88Ø	.8ØØ	67	113.8ØØ	1Ø7.2ØØ	61.5ØØ	15.9ØØ	5	338
38	1238	15.79Ø	66.41Ø	.83Ø	73	115.2ØØ	94.1ØØ	51.3ØØ	14.2ØØ	6.4ØØ	361
39	1239	18.32Ø	5Ø.64Ø	.66Ø	71	1Ø5.4ØØ	73	42.3ØØ	16.3ØØ	6.7ØØ	362
4Ø	124Ø	16.63Ø	42.63Ø	.88Ø	75	111.3ØØ	72.8ØØ	28.8ØØ	9.7ØØ	3.9ØØ	313
41	1257	19.47Ø	35.37Ø	.9ØØ	71	93.7ØØ	7Ø	27	1Ø.8ØØ	5.9ØØ	381
42	1258	14.95Ø	79.13Ø	.86Ø	59	1Ø4.9ØØ	1ØØ.7ØØ	65.9ØØ	31	1Ø.9ØØ	421
43	1259	16.95Ø	62.63Ø	.84Ø	76	126.8ØØ	83.4ØØ	47.3ØØ	16.3ØØ	9.5ØØ	358
44	126Ø	MISSING	MISSING	.81Ø	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	453
45	1261	17.79Ø	47.52Ø	.77Ø	69	134	119.8ØØ	7Ø.8ØØ	25.6ØØ	7.9ØØ	444
46	1262	16.84Ø	46.54Ø	.86Ø	67	114.4ØØ	9Ø.2ØØ	32.6ØØ	7.5ØØ	8.9ØØ	414
47	1263	13.89Ø	59.79Ø	.81Ø	64	93.3ØØ	86.5ØØ	48.3ØØ	13	5.2ØØ	387
48	1264	9.47Ø	61.2ØØ	.89Ø	65	112.8ØØ	98.6ØØ	49.9ØØ	18.7ØØ	5.2ØØ	329

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	34 PR	35 QRS	37 VOLN15	38 VOLN1Ø	39 VOLN5	4Ø VOLØ	41 VOL5	42 VOL1Ø	43 VOL15	44 VOL2Ø
25	12Ø9	MISSING	MISSING	Ø	.Ø7ØØ	.27Ø	1.57Ø	5.42Ø	8.27Ø	9.37Ø	1Ø.17Ø
26	121Ø	MISSING	MISSING	Ø	.61Ø	.61Ø	1.11Ø	6.36Ø	8.61Ø	9.36Ø	9.71Ø
27	1211	MISSING	MISSING	Ø	.19Ø	.24Ø	1.69Ø	5.69Ø	8.49Ø	9.19Ø	9.69Ø
28	1212	MISSING	MISSING	Ø	.11Ø	.26Ø	1.61Ø	6.16Ø	8.61Ø	9.41Ø	9.81Ø
29	1213	.Ø495	.Ø1ØØ	Ø	.5ØØ	.65Ø	2.45Ø	7.5ØØ	9.75Ø	1Ø.55Ø	1Ø.85Ø
3Ø	1214	.Ø48Ø	.Ø1Ø6	Ø	.78Ø	.98Ø	2.28Ø	7.38Ø	1Ø.Ø8Ø	11.13Ø	11.48Ø
31	1215	.Ø448	.Ø127	Ø	.Ø3ØØ	.Ø3ØØ	1.43Ø	5.78Ø	8.63Ø	9.38Ø	9.83Ø
32	1216	.Ø45Ø	.Ø15Ø	Ø	.1ØØ	.3ØØ	1.2ØØ	8	1Ø	1Ø.5ØØ	11
33	1233	.Ø463	.Ø1ØØ	Ø	.24Ø	.38Ø	1.34Ø	5.49Ø	8.74Ø	9.59Ø	9.94Ø
34	1234	.Ø444	.Ø1ØØ	Ø	.8ØØ	1.25Ø	1.8ØØ	7.9ØØ	1Ø	1Ø.65Ø	11.2ØØ
35	1235	.Ø496	.Ø1ØØ	Ø	1	1.15Ø	1.4ØØ	7.Ø5Ø	9.5ØØ	1Ø.25Ø	1Ø.6ØØ
36	1236	.Ø425	.ØØ88Ø	Ø	.Ø9ØØ	.34Ø	1.Ø9Ø	6.79Ø	9.19Ø	9.94Ø	1Ø.29Ø
37	1237	.Ø5ØØ	.Ø119	Ø	.Ø9ØØ	.24Ø	1.79Ø	8.Ø9Ø	9.79Ø	1Ø.34Ø	1Ø.79Ø
38	1238	.Ø413	.Ø1ØØ	Ø	.Ø5ØØ	.47Ø	1.75Ø	7.95Ø	9.55Ø	1Ø.15Ø	1Ø.75Ø
39	1239	.Ø45Ø	.Ø1Ø6	Ø	.25Ø	1.1ØØ	1.55Ø	6.45Ø	8.25Ø	8.9ØØ	9.15Ø
4Ø	124Ø	.Ø456	.ØØ75Ø	Ø	Ø	Ø	1.12Ø	9.97Ø	1Ø.72Ø	11.17Ø	11.32Ø
41	1257	MISSING	.Ø1ØØ	Ø	.22Ø	.77Ø	.92Ø	5.92Ø	9.72Ø	1Ø.82Ø	11.32Ø
42	1258	.Ø365	.Ø1Ø3	Ø	.Ø4ØØ	.59Ø	.84Ø	7.24Ø	9.34Ø	9.99Ø	1Ø.44Ø
43	1259	.Ø438	.Ø128	Ø	.24Ø	.59Ø	.84Ø	5.84Ø	9.24Ø	1Ø.14Ø	1Ø.64Ø
44	126Ø	.Ø425	.Ø123	Ø	.13Ø	.28Ø	1.23Ø	8.23Ø	9.33Ø	9.93Ø	1Ø.23Ø
45	1261	.Ø383	.Ø143	Ø	Ø	Ø	.69Ø	9.45Ø	9.9ØØ	1Ø	1Ø.3ØØ
46	1262	.Ø425	.Ø15Ø	Ø	.21Ø	.46Ø	1.51Ø	7.81Ø	1Ø.11Ø	1Ø.81Ø	11.11Ø
47	1263	.Ø523	MISSING	Ø	.13Ø	.48Ø	1.53Ø	8.Ø3Ø	9.63Ø	1Ø.23Ø	1Ø.53Ø
48	1264	.Ø425	.Ø135	Ø	.Ø2ØØ	.27Ø	1.42Ø	9.32Ø	1Ø.42Ø	11.Ø2Ø	11.42Ø

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	45 VOL25	47 DHEFR50	48 DHEFR25	49 ISOFLOW	50 PCO2	51 PO2	52 PH	53 CO2RESP
25	1209	10.570	46.800	23.300	1.400	42.100	90	7.410	MISSING
26	1210	10.110	13.700	8.900	6.700	MISSING	MISSING	MISSING	28
27	1211	9.940	8.800	13.700	0	MISSING	MISSING	MISSING	100
28	1212	10.110	16.700	13.600	MISSING	MISSING	MISSING	MISSING	123
29	1213	11.200	22.200	17	12.600	42.200	71	7.390	58
30	1214	11.780	19.900	8.300	5	41.700	77.600	7.450	113
31	1215	9.930	10.500	14.600	4.500	MISSING	MISSING	MISSING	144
32	1216	11.200	31.600	6.200	11.200	42.300	74	7.440	102
33	1233	10.340	23.700	-.200	22.200	MISSING	MISSING	MISSING	92
34	1234	11.300	36.600	35.300	4.500	MISSING	MISSING	MISSING	33
35	1235	10.900	27.300	6.900	19.800	MISSING	MISSING	MISSING	90
36	1236	10.590	31.600	15.400	1.900	39.600	84	7.420	81
37	1237	10.950	28.300	27.300	4.400	42.500	77.800	7.410	83
38	1238	10.760	34.200	17.800	3.900	39.300	69.600	7.400	132
39	1239	9.300	29.800	15.900	0	MISSING	MISSING	MISSING	153
40	1240	11.540	17.100	5	13.700	31.700	80.200	7.400	74
41	1257	11.670	28.700	13.200	3.700	MISSING	MISSING	MISSING	80
42	1258	10.660	24.700	19.400	.800	MISSING	MISSING	MISSING	48
43	1259	10.840	43.100	19.900	1.500	MISSING	MISSING	MISSING	75
44	1260	10.230	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	100
45	1261	10.300	29.400	15.400	2.900	42.600	90.300	7.400	108
46	1262	11.510	5.800	5.300	0	43.100	66.200	7.440	101
47	1263	10.780	20.500	5.900	4.700	45.400	78.200	7.390	99
48	1264	11.420	23.500	8.700	MISSING	41.900	86.200	7.390	206

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	11 VT	12 PL	13 F	14 RL	15 CDYN	17 IC	18 VC	19 FRCB	20 TLCD	21 DLCO
49	1409	1.770	13	75	1.030	.0900	8.840	9.850	4.320	10.410	.138
50	1410	1.320	8	63	.350	.190	8.470	10.240	2.970	10.530	.144
51	1411	1.750	4.250	62	.120	.410	9.220	11.600	3.740	12.310	.128
52	1412	1.700	4.100	69	.330	.360	8.880	10.890	4.410	12.390	.151
53	1413	1.720	5	54	1.360	.400	9.020	10.880	3.770	12.070	.144
54	1414	1.620	3.250	80	.130	.480	10.450	11.930	3.430	12.590	.183
55	1415	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
56	1416	1.500	6.750	50	1.360	.400	10.470	11.690	3.160	12.330	.199
57	1433	1.640	4.100	87	.480	.460	10.190	12	4.460	13.530	.211
58	1434	1.920	6	50	.300	.320	9.270	10.460	2.770	11.170	.163
59	1435	1.930	5.750	71	.410	.420	10.150	11.190	4.130	11.540	.186
60	1436	1.420	6	65	.240	.290	9.420	10.790	MISSING	12.660	.192
61	1437	1.300	4.500	62	.270	.490	8.880	9.800	3.500	10.680	.157
62	1438	1.650	4.750	66	.300	.410	9.590	10.500	2.940	11.070	.175
63	1439	1.790	9	66	.660	.350	10.800	12.100	3.290	13.150	.168
64	1440	1.500	3.920	54	.930	.280	9.310	10.750	4.430	11.850	.167
65	1457	1.590	5	62	.450	.700	11.160	12.190	3.920	13.270	.182
66	1458	1.550	6.830	55	.290	.260	9.340	11.490	3.820	11.930	.172
67	1459	1.370	4.500	68	.240	.370	10.450	11.510	3.500	12.230	.170
68	1460	1.770	4.750	69	.240	.510	10	11.390	6.080	MISSING	MISSING
69	1461	2.140	5.670	77	.0700	.410	10.540	11.640	2.810	12.360	.195
70	1462	1.630	4.080	65	.150	.470	9.440	10.730	3.580	12.890	.160
71	1463	1.440	5	69	.120	.500	8.720	10.500	4.510	11.280	.149
72	1464	1.400	5.330	62	.300	.360	9.200	10.190	2.970	11.240	.163

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	22 PST	23 V3Ø	24 QSCCS	32 VMAX	26 PEF	27 EFR5Ø	28 EFR25	29 EFR1Ø	3Ø M1MØ	33 HR
49	14Ø9	13.58Ø	46.98Ø	.67Ø	81	1Ø1.9ØØ	73.5ØØ	33.9ØØ	1Ø.8ØØ	12.5ØØ	MISSING
5Ø	141Ø	MISSING	MISSING	.64Ø	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
51	1411	11.68Ø	61.68Ø	.76Ø	7Ø	134	89.4ØØ	46.8ØØ	2Ø.9ØØ	3.5ØØ	MISSING
52	1412	11.58Ø	41.54Ø	.74Ø	76	121.7ØØ	72.9ØØ	26.4ØØ	6.4ØØ	4.6ØØ	315
53	1413	16.63Ø	45.1ØØ	.77Ø	8Ø	126	73.5ØØ	33.3ØØ	6	5.4ØØ	275
54	1414	14.32Ø	68.13Ø	.89Ø	76	137.8ØØ	96.5ØØ	56	19.2ØØ	7	344
55	1415	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
56	1416	12.74Ø	7Ø.38Ø	.87Ø	65	12Ø.7ØØ	1Ø4.5ØØ	56.7ØØ	33.1ØØ	9.3ØØ	297
57	1433	11.26Ø	72.24Ø	.86Ø	8Ø	114	93.3ØØ	64.1ØØ	21	4.9ØØ	34Ø
58	1434	17.26Ø	76.14Ø	.78Ø	66	97.6ØØ	94.7ØØ	62.4ØØ	3Ø.1ØØ	1Ø.6ØØ	327
59	1435	15.37Ø	7Ø.49Ø	.86Ø	56	1Ø1.6ØØ	97.5ØØ	54.6ØØ	17.2ØØ	11.8ØØ	398
6Ø	1436	15.86Ø	66.79Ø	.8ØØ	74	124.8ØØ	92.5ØØ	51.8ØØ	16.9ØØ	4.3ØØ	375
61	1437	16.42Ø	54.95Ø	.78Ø	78	94.1ØØ	7Ø.7ØØ	47	26.2ØØ	7.5ØØ	374
62	1438	14	85.39Ø	.84Ø	64	121.3ØØ	1Ø9.3ØØ	66.4ØØ	27.8ØØ	1Ø.4ØØ	278
63	1439	14.53Ø	66.15Ø	.96Ø	68	95.4ØØ	91.1ØØ	51.1ØØ	23.5ØØ	6.4ØØ	4ØØ
64	144Ø	14.32Ø	52.99Ø	.83Ø	71	11Ø.1ØØ	85.1ØØ	49.3ØØ	27.7ØØ	6.2ØØ	35Ø
65	1457	12.63Ø	77.54Ø	.96Ø	65	126.6ØØ	1Ø4.7ØØ	67.7ØØ	25.7ØØ	7.4ØØ	338
66	1458	16.63Ø	75.44Ø	.92Ø	67	1Ø6.1ØØ	1Ø2.8ØØ	59.2ØØ	34	5.6ØØ	286
67	1459	13.89Ø	73.Ø7Ø	.89Ø	68	119	99	56.5ØØ	24.8ØØ	8.1ØØ	353
68	146Ø	MISSING	MISSING	.94Ø	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	414
69	1461	13.2ØØ	71.85Ø	.84Ø	8Ø	129.3ØØ	99.2ØØ	62.3ØØ	29.3ØØ	8.4ØØ	29Ø
7Ø	1462	13.79Ø	49.22Ø	.82Ø	68	112.3ØØ	84.3ØØ	41.6ØØ	22	4.7ØØ	MISSING
71	1463	18.21Ø	83.2ØØ	.75Ø	66	1ØØ.1ØØ	96	71.6ØØ	38	7	329
72	1464	1Ø.74Ø	72.2ØØ	.75Ø	51	1Ø1.4ØØ	97	58.2ØØ	27.5ØØ	8.5ØØ	324

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	34 PR	35 QRS	37 VOLN15	38 VOLN1Ø	39 VOLN5	4Ø VOLØ	41 VOL5	42 VOL1Ø	43 VOL15	44 VOL2Ø
49	14Ø9	MISSING	MISSING	Ø	.Ø1ØØ	.16Ø	1.Ø1Ø	4.51Ø	7.61Ø	8.51Ø	9.21Ø
5Ø	141Ø	MISSING	MISSING	Ø	.86Ø	.91Ø	1.76Ø	4.81Ø	8.26Ø	9.26Ø	9.76Ø
51	1411	MISSING	MISSING	Ø	.Ø8ØØ	.Ø8ØØ	2.38Ø	7.63Ø	9.98Ø	1Ø.78Ø	11.18Ø
52	1412	.Ø425	.Ø1ØØ	Ø	.Ø1ØØ	.Ø1ØØ	2.Ø1Ø	6.91Ø	9.41Ø	1Ø.11Ø	1Ø.61Ø
53	1413	.Ø472	.ØØ75Ø	Ø	.Ø6ØØ	.21Ø	1.86Ø	6.86Ø	9.36Ø	1Ø.11Ø	1Ø.46Ø
54	1414	.Ø425	.Ø113	Ø	.37Ø	.42Ø	1.47Ø	8.27Ø	1Ø.37Ø	11.Ø7Ø	11.67Ø
55	1415	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
56	1416	.Ø588	.Ø1ØØ	Ø	.Ø2ØØ	.12Ø	1.22Ø	6.47Ø	9.72Ø	1Ø.67Ø	11.22Ø
57	1433	.Ø364	.Ø1ØØ	Ø	.Ø1ØØ	.16Ø	1.81Ø	8.11Ø	1Ø.51Ø	11.26Ø	11.61Ø
58	1434	.Ø5ØØ	.Ø125	Ø	.Ø9ØØ	.14Ø	1.19Ø	6.34Ø	8.89Ø	9.74Ø	9.99Ø
59	1435	.Ø325	.Ø125	Ø	.Ø4ØØ	.Ø9ØØ	1.Ø4Ø	7.54Ø	9.84Ø	1Ø.49Ø	1Ø.84Ø
6Ø	1436	.Ø413	.Ø125	Ø	.17Ø	.37Ø	1.37Ø	6.77Ø	9.27Ø	1Ø.Ø7Ø	1Ø.37Ø
61	1437	.Ø388	.ØØ75Ø	Ø	.12Ø	.22Ø	.92Ø	7.Ø7Ø	8.72Ø	9.32Ø	9.52Ø
62	1438	.Ø483	.Ø135	Ø	.11Ø	.21Ø	.91Ø	7.11Ø	9.31Ø	9.76Ø	1Ø.31Ø
63	1439	.Ø381	.Ø138	Ø	.1ØØ	.4ØØ	1.3ØØ	8.7ØØ	1Ø.9ØØ	11.5ØØ	11.9ØØ
64	144Ø	MISSING	MISSING	Ø	.34Ø	.54Ø	1.44Ø	7.54Ø	9.64Ø	1Ø.14Ø	1Ø.64Ø
65	1457	.Ø45Ø	.Ø113	Ø	Ø	.72Ø	1.Ø3Ø	11.33Ø	11.83Ø	11.98Ø	12.Ø3Ø
66	1458	.Ø5ØØ	.Ø125	Ø	.15Ø	.25Ø	2.15Ø	7.Ø5Ø	9.85Ø	1Ø.7ØØ	11.15Ø
67	1459	.Ø425	.Ø1ØØ	Ø	.Ø7ØØ	.37Ø	1.Ø7Ø	7.47Ø	1Ø.Ø7Ø	1Ø.82Ø	11.27Ø
68	146Ø	.Ø4ØØ	.Ø125	Ø	Ø	.4ØØ	1.39Ø	1Ø.19Ø	1Ø.79Ø	11.14Ø	11.19Ø
69	1461	.Ø425	.Ø135	Ø	.9ØØ	1	1.1ØØ	6.85Ø	1Ø.1ØØ	1Ø.85Ø	11.3ØØ
7Ø	1462	MISSING	MISSING	Ø	.Ø9ØØ	.34Ø	1.29Ø	7.24Ø	9.49Ø	1Ø.14Ø	1Ø.49Ø
71	1463	.Ø363	.Ø138	Ø	.18Ø	.33Ø	1.78Ø	7.63Ø	9.28Ø	9.88Ø	1Ø.18Ø
72	1464	.Ø45Ø	.Ø15Ø	Ø	Ø	Ø	.98Ø	9.13Ø	9.68Ø	9.83Ø	9.98Ø

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	45 VOL25	47 DHEFR50	48 DHEFR25	49 ISOFLOW	50 PCO2	51 PO2	52 PH	53 CO2RESP
49	1409	9.850	24.100	17.400	1.700	MISSING	MISSING	MISSING	62
50	1410	10.240	MISSING	MISSING	MISSING	42.400	82	7.400	MISSING
51	1411	11.380	33.200	24.600	0	43.300	72.100	7.400	127
52	1412	10.760	19.600	4.800	14.100	41	72.600	7.400	81
53	1413	10.860	22.800	-5.100	14.400	39.900	76.700	7.430	58
54	1414	11.970	32.200	4.700	3.500	42	82.700	7.430	59
55	1415	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	127
56	1416	11.470	31.100	20.300	5.300	MISSING	MISSING	MISSING	74
57	1433	12.060	22.900	16.700	7.800	35.500	110.600	7.360	16
58	1434	10.440	21	11.100	8.200	MISSING	MISSING	MISSING	56
59	1435	11.040	30.200	14.800	20.200	40.700	105.300	7.440	115
60	1436	10.620	14.400	.200	6.900	MISSING	MISSING	MISSING	110
61	1437	9.670	21.300	10.800	8.500	MISSING	MISSING	MISSING	66
62	1438	10.410	12.900	13.800	1.500	MISSING	MISSING	MISSING	33
63	1439	12.050	21.600	10.600	1.300	35.400	122	7.400	80
64	1440	10.690	2.100	-5.200	8.200	38	104	7.340	57
65	1457	12.030	26.900	16.200	3.700	39.700	94.500	7.360	83
66	1458	11.400	19.500	21.500	.800	MISSING	MISSING	MISSING	110
67	1459	11.570	39.800	24.400	1.400	41.300	76.400	7.400	105
68	1460	11.390	MISSING	MISSING	MISSING	40.500	81.200	7.398	92
69	1461	11.600	23.300	13.300	5.200	MISSING	MISSING	MISSING	55
70	1462	10.790	14.500	10.800	12.800	41.700	82.200	7.430	98
71	1463	10.530	25.500	6.500	1.200	43.700	75.700	7.440	MISSING
72	1464	10.230	21.500	9.600	9.800	MISSING	MISSING	MISSING	52

20 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	11 VT	12 PL	13 F	14 RL	15 CDYN	17 IC	18 VC	19 FRCB	20 TLCD	21 DLCO
73	1609	1.430	10	56	.360	.150	8.730	10.780	2.990	11.630	.136
74	1610	1.880	5	53	.0800	.480	8.180	10.110	3.620	10.110	.119
75	1611	1.540	3.130	50	.150	.530	8.170	10.390	2.970	11.210	.0990
76	1612	1.350	3.270	75	.760	.430	8.550	10.260	4.020	10.380	.138
77	1613	1.770	5.500	63	1.140	.200	8.940	10.680	2.770	10.800	.181
78	1614	MISSING	MISSING	MISSING	MISSING	MISSING	8.350	10.660	4.010	12.150	.0970
79	1615	1.430	5	100	.390	.280	9.190	11.140	3.310	12.180	.193
80	1616	1.680	4.750	73	.850	.260	8.910	10.600	3.500	11.240	.186
81	1633	1.710	4.200	58	.330	.370	9.910	11.110	3.440	12.140	.189
82	1634	1.850	6	74	.420	.400	9.320	10.250	3.060	11.170	.155
83	1635	1.890	5.500	84	.410	.350	9.350	10.940	MISSING	11.780	.193
84	1636	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
85	1637	1.470	4.830	71	.230	.420	10.980	12.200	4.390	12.910	.192
86	1638	1.600	4.670	64	.380	.550	9.670	11.150	3.140	12.100	.171
87	1639	1.430	4.100	73	.590	.470	9.720	11.230	3.040	12.380	.182
88	1640	1.840	3.770	64	.580	.460	11.370	12.020	2.710	13.050	.196
89	1657	1.780	5.330	58	.970	.330	11.390	12	4.560	12.560	.209
90	1658	1.660	4.250	62	.120	.390	10.360	11.550	3.560	12.380	.187
91	1659	1.840	8.830	55	.410	.220	10.590	11.450	3.770	14.010	.208
92	1660	1.580	3.880	72	.200	.500	10.140	10.860	2.770	11.940	.178
93	1661	1.470	2.120	62	.380	.470	9.110	9.830	4.100	10.090	.142
94	1662	1.540	5.250	69	.200	.430	9.160	10.510	3.340	11.440	.199
95	1663	1.480	5	51	.120	.270	8.280	10.380	2.210	11.030	.170
96	1664	1.470	5.370	64	.180	.430	9.990	11.090	3.700	11.480	.197

20 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	22 PST	23 V3Ø	24 QSCCS	32 VMAX	26 PEF	27 EFR5Ø	28 EFR25	29 EFR1Ø	3Ø M1MØ	33 HR
73	16Ø9	MISSING	MISSING	.71Ø	MISSING	128.3ØØ	97.9ØØ	64.6ØØ	36.4ØØ	8.4ØØ	MISSING
74	161Ø	12.42Ø	64.21Ø	.67Ø	73	128.5ØØ	98.7ØØ	54.6ØØ	19.2ØØ	8.2ØØ	MISSING
75	1611	12.42Ø	51.32Ø	.68Ø	82	1Ø4.6ØØ	7Ø.9ØØ	46.9ØØ	28.6ØØ	4	MISSING
76	1612	16.21Ø	58.57Ø	.72Ø	82	12Ø.2ØØ	78.5ØØ	45.7ØØ	16.6ØØ	13.9ØØ	32Ø
77	1613	12.95Ø	67.69Ø	.73Ø	76	116.6ØØ	9Ø	58.1ØØ	25.3ØØ	6.6ØØ	387
78	1614	MISSING	MISSING	.73Ø	MISSING	1Ø7.6ØØ	7Ø	42.7ØØ	31.6ØØ	MISSING	MISSING
79	1615	2Ø.42Ø	79.64Ø	.76Ø	71	138.4ØØ	1Ø7.4ØØ	68.1ØØ	32	5.6ØØ	398
8Ø	1616	13.79Ø	75.67Ø	.75Ø	59	124.9ØØ	1Ø4.1ØØ	65.4ØØ	26.9ØØ	6.1ØØ	MISSING
81	1633	17.26Ø	78.Ø7Ø	.83Ø	65	126.5ØØ	1Ø8.2ØØ	67.8ØØ	3Ø	6.2ØØ	3Ø6
82	1634	12.95Ø	71.11Ø	.81Ø	66	116.5ØØ	94.9ØØ	6Ø.9ØØ	27.4ØØ	7.9ØØ	328
83	1635	12.95Ø	76.16Ø	.76Ø	71	129.2ØØ	93.5ØØ	66.7ØØ	35.6ØØ	6.3ØØ	358
84	1636	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
85	1637	MISSING	83.93Ø	.96Ø	69	124.8ØØ	111.4ØØ	66.5ØØ	23.6ØØ	8.6ØØ	2Ø4
86	1638	MISSING	7Ø	.84Ø	71	122.8ØØ	93.2ØØ	62.5ØØ	24.5ØØ	6.5ØØ	3ØØ
87	1639	14.32Ø	77.38Ø	.86Ø	64	113.5ØØ	112	64.3ØØ	26	6.1ØØ	4ØØ
88	164Ø	17.68Ø	63.12Ø	.98Ø	65	12Ø.3ØØ	1Ø3.6ØØ	48.3ØØ	19.6ØØ	9.4ØØ	381
89	1657	16.95Ø	83.43Ø	1.Ø1Ø	56	113.2ØØ	11Ø.4ØØ	66.6ØØ	28.2ØØ	16.2ØØ	36Ø
9Ø	1658	16.21Ø	69.18Ø	.88Ø	64	1Ø7.7ØØ	1Ø1	55	21.9ØØ	7.8ØØ	457
91	1659	13.47Ø	4Ø.59Ø	.89Ø	76	118	69.9ØØ	26.7ØØ	5.8ØØ	5.6ØØ	326
92	166Ø	18.32Ø	81.27Ø	.87Ø	74	128.2ØØ	1Ø2.1ØØ	72	37.8ØØ	8.9ØØ	364
93	1661	12.63Ø	61.83Ø	.83Ø	66	91.1ØØ	88.8ØØ	51.2ØØ	31.6ØØ	13.5ØØ	39Ø
94	1662	13.47Ø	69.74Ø	.77Ø	69	129	1Ø1.6ØØ	53	28.9ØØ	7.6ØØ	348
95	1663	5.Ø5Ø	61.24Ø	.66Ø	57	1Ø6	1Ø2.6ØØ	64.8ØØ	3Ø.3ØØ	5.8ØØ	335
96	1664	16.74Ø	8Ø.36Ø	.87Ø	67	91.7ØØ	88.7ØØ	74.3ØØ	37.2ØØ	1Ø.9ØØ	421

20 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	34 PR	35 QRS	37 VOLN15	38 VOLN1Ø	39 VOLN5	4Ø VOLØ	41 VOL5	42 VOL1Ø	43 VOL15	44 VOL2Ø
73	16Ø9	MISSING	MISSING	Ø	.Ø5ØØ	.15Ø	2.Ø5Ø	6.6ØØ	9.15Ø	9.85Ø	1Ø.45Ø
74	161Ø	MISSING	MISSING	Ø	.Ø3ØØ	.18Ø	1.93Ø	6.13Ø	8.53Ø	9.28Ø	9.73Ø
75	1611	MISSING	MISSING	Ø	.Ø2ØØ	.27Ø	2.22Ø	6.47Ø	8.82Ø	9.57Ø	9.82Ø
76	1612	.Ø45Ø	.Ø1ØØ	Ø	.22Ø	.22Ø	1.72Ø	6.22Ø	8.82Ø	9.52Ø	9.92Ø
77	1613	.Ø45Ø	.Ø113	Ø	.Ø3ØØ	.13Ø	1.73Ø	5.58Ø	8.83Ø	9.68Ø	1Ø.33Ø
78	1614	MISSING	MISSING	Ø	.11Ø	.31Ø	2.31Ø	7.51Ø	9.61Ø	1Ø.11Ø	1Ø.51Ø
79	1615	.Ø367	.Ø132	Ø	.15Ø	.15Ø	1.95Ø	6.55Ø	9.65Ø	1Ø.35Ø	1Ø.75Ø
8Ø	1616	MISSING	MISSING	Ø	.Ø9ØØ	.19Ø	1.69Ø	6.14Ø	8.99Ø	9.64Ø	1Ø.Ø9Ø
81	1633	.Ø5Ø3	.Ø168	Ø	.11Ø	.11Ø	1.21Ø	6.76Ø	9.51Ø	1Ø.36Ø	1Ø.81Ø
82	1634	.Ø55Ø	.Ø125	Ø	.13Ø	.23Ø	.93Ø	6.83Ø	9.Ø3Ø	9.63Ø	9.93Ø
83	1635	.Ø399	.ØØ85Ø	Ø	.Ø9ØØ	.29Ø	1.59Ø	6.74Ø	9.29Ø	1Ø.14Ø	1Ø.39Ø
84	1636	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
85	1637	.Ø48Ø	.Ø15Ø	Ø	.22Ø	.47Ø	1.22Ø	8.72Ø	1Ø.82Ø	12.17Ø	12.22Ø
86	1638	.Ø475	.Ø138	Ø	.Ø8ØØ	.18Ø	1.48Ø	8.33Ø	9.88Ø	1Ø.63Ø	1Ø.88Ø
87	1639	.Ø425	.Ø1ØØ	Ø	.11Ø	.16Ø	1.57Ø	8.Ø6Ø	1Ø.Ø1Ø	1Ø.66Ø	1Ø.91Ø
88	164Ø	.Ø425	.Ø125	Ø	.25Ø	.45Ø	.65Ø	9.1ØØ	1Ø.93Ø	11.45Ø	11.85Ø
89	1657	.Ø438	.Ø15Ø	Ø	.11Ø	.21Ø	.61Ø	7.56Ø	1Ø.61Ø	11.26Ø	11.81Ø
9Ø	1658	.Ø388	.ØØ75Ø	Ø	.Ø9ØØ	.44Ø	1.19Ø	7.44Ø	9.89Ø	1Ø.79Ø	11.19Ø
91	1659	.Ø425	.Ø125	Ø	.16Ø	.56Ø	.86Ø	6.91Ø	9.86Ø	1Ø.76Ø	11.26Ø
92	166Ø	.Ø425	.Ø125	Ø	.12Ø	.42Ø	.72Ø	8.Ø2Ø	9.72Ø	1Ø.32Ø	1Ø.72Ø
93	1661	.Ø45Ø	.Ø1ØØ	Ø	.11Ø	.41Ø	.71Ø	7.26Ø	8.81Ø	9.26Ø	9.71Ø
94	1662	.Ø425	.Ø113	Ø	.16Ø	.41Ø	1.36Ø	6.96Ø	9.Ø6Ø	9.76Ø	1Ø.16Ø
95	1663	.Ø53Ø	.Ø13Ø	Ø	.Ø1ØØ	.Ø9ØØ	2.Ø9Ø	6.29Ø	8.69Ø	9.29Ø	9.89Ø
96	1664	.Ø44Ø	.Ø14Ø	Ø	.2ØØ	.3ØØ	1.1ØØ	7.65Ø	9.8ØØ	1Ø.4ØØ	1Ø.7ØØ

20 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	45 VOL25	47 DHEFR50	48 DHEFR25	49 ISOFLOW	50 PCO2	51 PO2	52 PH	53 CO2RESP
73	1609	10.800	52	34.400	.400	MISSING	MISSING	MISSING	131
74	1610	9.930	7.300	6	2.500	MISSING	MISSING	MISSING	110
75	1611	10.470	26.100	19.200	8.100	MISSING	MISSING	MISSING	87
76	1612	10.220	32.300	15.600	4.500	40.300	72.300	7.390	32
77	1613	10.730	41.100	23.500	9.300	MISSING	MISSING	MISSING	152
78	1614	10.660	22.600	15.900	MISSING	42	70.400	7.430	40
79	1615	11.200	27.700	17.100	9.200	43.500	71.300	7.490	113
80	1616	10.440	34.600	11	7.600	41.100	71.600	7.400	240
81	1633	11.210	45	22.400	7.300	40.400	69.600	7.460	118
82	1634	10.180	33.600	19	2	39.100	73.800	7.420	8
83	1635	10.840	35.700	8.800	11.100	MISSING	MISSING	MISSING	15
84	1636	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	156
85	1637	12.220	25	23.500	3.400	41	75.800	7.430	138
86	1638	11.230	25.300	7.400	10	30.800	82.300	7.440	53
87	1639	11.260	6.100	7.300	13.800	MISSING	MISSING	7.380	41
88	1640	12.020	15.100	7.600	1.800	41.600	73.900	7.370	164
89	1657	12	29.400	18.100	13.500	MISSING	MISSING	MISSING	87
90	1658	11.550	21.200	10.600	1.100	MISSING	MISSING	MISSING	67
91	1659	11.450	28.500	4.600	13.400	MISSING	MISSING	MISSING	116
92	1660	10.720	23.800	14	13.900	39.100	78.500	7.430	32
93	1661	9.710	17.900	7.900	4.200	MISSING	MISSING	MISSING	132
94	1662	10.360	9.400	4.400	20.700	MISSING	MISSING	MISSING	207
95	1663	10.340	26.300	19	4	MISSING	MISSING	MISSING	200
96	1664	11.100	6.300	4.300	5.600	MISSING	MISSING	MISSING	128

APPENDIX F

LUNG COMPOSITION DATA FROM INDIVIDUAL FISCHER-344 RATS

Lung Composition Data from Individual Fischer-344 Rats

Appendix Heading	Definition
DNA	total lung DNA (mg)
DRYWT	total dry weight of the lungs (mg)
ELASTIN	total lung elastin (mg)
LABEL	animal number
OHPR	total lung hydroxyproline (mg)
PROTEIN	total lung protein (mg)

CONTROL GROUP

CASE NO.	LABEL	54 DRYWT	6 OHPR	7 PROTEIN	8 DNA	9 ELASTIN
1	1009	321.640	2.580	193.600	6.540	6.870
2	1010	MISSING	MISSING	MISSING	MISSING	MISSING
3	1011	259.440	2.340	172.500	5.400	6.980
4	1012	214.587	1.770	132.800	4.820	5.790
5	1013	241.155	2.460	146	5.230	6.770
6	1014	255.041	2.740	154.200	5.800	6.980
7	1015	260.662	2.590	158	5.580	7.220
8	1016	MISSING	MISSING	MISSING	MISSING	MISSING
9	1033	223.260	2.300	136.900	5.140	6.110
10	1034	285.740	2.460	173.100	5.780	7.660
11	1035	279.576	2.590	184	6.040	7.610
12	1036	331.254	2.290	221.600	6.030	6.980
13	1037	269.206	2.640	174.600	5.470	7.050
14	1038	291.448	2.700	179.400	5.610	7.640
15	1039	250.714	2.270	164.700	5.140	6.680
16	1040	218.484	2.030	133.200	4.850	6.060
17	1057	288.024	2.680	183.600	5.670	7.470
18	1058	236.432	2.290	142.400	4.790	6.420
19	1059	252.126	2.440	166.900	5.360	6.890
20	1060	290.195	2.670	178.500	5.880	7.760
21	1061	263.074	2.610	172.300	5.340	7.270
22	1062	250.444	2.590	161.200	5.330	6.840
23	1063	265.472	2.650	171	5.440	7.180
24	1064	243.236	2.500	156.400	5.170	6.420

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	54 DRYWT	6 OHPR	7 PROTEIN	8 DNA	9 ELASTIN
25	1209	289.560	2.590	208	5.550	7.410
26	1210	261	3.110	161.700	5.560	7.750
27	1211	243.136	2.570	155.700	5.300	7.260
28	1212	250.222	2.820	161.700	5.470	7.050
29	1213	258.423	3.130	163.200	5.870	7.180
30	1214	264.244	3.180	172.800	5.660	7.450
31	1215	243.089	2.600	151.800	5.420	6.860
32	1216	333.747	3.240	212.200	7.090	9.130
33	1233	259.969	2.640	159	5.820	7.090
34	1234	250.131	2.750	159.900	5.330	6.870
35	1235	256.184	2.630	166.800	5.210	7.190
36	1236	251.030	2.850	161	5.310	7.050
37	1237	249.781	2.890	151.100	5.530	7.160
38	1238	267.862	3.090	169.400	5.950	7.730
39	1239	220.168	2.520	133.100	4.790	6.510
40	1240	270.336	2.720	169.400	5.510	7.720
41	1257	325.714	3.020	196.900	6.660	8.500
42	1258	279.480	3.040	162.800	5.620	7.930
43	1259	261.239	2.700	159.700	5.400	7.280
44	1260	MISSING	MISSING	MISSING	MISSING	MISSING
45	1261	260.604	2.830	167.800	5.510	7.110
46	1262	272.272	3.010	169.300	5.740	7.690
47	1263	239.037	2.740	143.900	5.020	7.030
48	1264	231.855	2.910	148.700	5.220	6.940

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	54 DRYWT	6 OHPR	7 PROTEIN	8 DNA	9 ELASTIN
49	1409	328.406	2.970	230.900	6.510	8.460
50	1410	MISSING	MISSING	MISSING	MISSING	MISSING
51	1411	253.920	3.240	154.700	5.900	8.090
52	1412	236.572	2.810	145.900	5.220	7.380
53	1413	231.162	2.640	155.500	5.440	6.650
54	1414	284.284	3.370	183.300	6.020	7.940
55	1415	292.556	3.040	169.800	6.130	8.510
56	1416	305.532	3.040	197.700	6.260	8.990
57	1433	308.441	3.050	186.900	6.620	9.420
58	1434	268.732	3.030	174	5.590	8.560
59	1435	333.355	3.820	201.700	6.860	10.550
60	1436	275.058	3.300	178	5.500	8.770
61	1437	221.597	3.050	143.500	4.750	6.580
62	1438	273.273	3.330	174.200	5.360	8.250
63	1439	241.274	3.120	157.100	5.180	7.770
64	1440	244.080	3.270	148.900	5.320	8.080
65	1457	295.320	3.160	182.600	6.010	8.860
66	1458	338.528	3.980	210.900	7.150	9.820
67	1459	260.166	2.810	162.300	5.400	7.590
68	1460	MISSING	MISSING	MISSING	MISSING	MISSING
69	1461	251.648	2.840	167.800	5.400	7.230
70	1462	270.230	3.600	173.400	5.840	8.080
71	1463	234.549	2.810	144.400	5.090	7.210
72	1464	277.636	3.230	166.600	5.530	8.260

20 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	54 DRYWT	6 OHPR	7 PROTEIN	8 DNA	9 ELASTIN
73	1609	382.096	3.550	248.100	7.520	9.780
74	1610	228.212	2.740	142.100	5.370	7.540
75	1611	237.104	3.040	148.300	5.930	8.230
76	1612	229.503	3.070	138.300	5.050	7.850
77	1613	330.484	3.420	210.900	6.560	9.770
78	1614	264.792	3.180	170.700	6.230	8.330
79	1615	313.536	3.450	184.900	7.040	9.710
80	1616	270.974	3.460	162.700	6.100	9.130
81	1633	297.976	3.640	193.900	6.180	9.790
82	1634	275.604	3.240	176.900	5.940	8.520
83	1635	266.630	3.100	172.100	5.960	8.480
84	1636	271.677	3.460	167	6.120	9.650
85	1637	288.934	3.420	176.600	6.200	8.750
86	1638	293.703	3.260	182	6.460	9.230
87	1639	259.773	3.090	160.100	5.520	8.330
88	1640	330.681	4.320	200.400	6.830	9.940
89	1657	310.590	3.490	201.400	6.290	9.010
90	1658	309.495	3.670	192.400	6.750	8.940
91	1659	333.600	3.840	212.200	7.310	9.830
92	1660	310.224	3.390	193.200	6.500	9.670
93	1661	257.070	3.180	166.300	5.220	7.680
94	1662	276.963	3.200	183.200	5.970	8.290
95	1663	288.345	3.290	193	6.480	9.200
96	1664	312.228	3.740	187.800	6.850	10.400

APPENDIX G

ABNORMAL SPERM DATA FROM INDIVIDUAL FISCHER-344 RATS

Table G-1: Percent Abnormal Sperm from Fischer 344 Rats Exposed to 0, 2, 10, or 20 mg SiO_2/m^3 6 hours/day, 5 days/week for 6 months.

Silica Concentration (mg/m^3)	Animal Number	% Abnormal Sperm	arcsin p
0	1113	0.00	0.00
	1114	0.00	0.00
	1115	0.00	0.00
	1116	1.60	7.26
	1117	0.00	0.00
	1118	0.00	0.00
	1119	0.40	3.63
	1120	0.60	4.44
	1121	0.00	0.00
	1122	0.00	0.00
2	1313	0.60	4.44
	1314	0.60	4.44
	1315	0.40	3.63
	1316	0.60	4.44
	1317	0.40	3.63
	1318	0.80	5.13
	1319	0.40	3.63
	1320	0.60	4.44
	1321	0.40	3.63
	1322	0.00	0.00
10	1513	0.20	2.56
	1514	0.00	0.00
	1515	0.00	0.00
	1516	0.40	3.63
	1517	0.40	3.63
	1518	0.00	0.00
	1519	0.20	2.56
	1520	0.40	3.63
	1521	0.40	3.63
	1522	0.40	3.63
20	1713	0.00	0.00
	1714	0.00	0.00
	1715	0.80	5.13
	1716	0.20	2.56
	1717	0.00	0.00
	1718	0.40	3.63
	1719	0.00	0.00
	1720	0.00	0.00
	1721	0.00	0.00
	1722	0.00	0.00

APPENDIX H

SUMMARY OF REPRODUCTIVE POTENTIAL DATA ON FISCHER-344 RATS EXPOSED TO SILICA DUST

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: November 1. 1983

TO: R. S. Kutzman

FROM: A. L. Carsten

SUBJECT: Silica dust animals

Attached is a summary data sheet on the animals used in the silica dust experiment for the NTP. Although it may appear that there are slight differences between some of the groups, not necessarily in the expected direction, I have no confidence in stating whether there is or is not an effect. As you know, there was a change in technical support during the course of the experiment. Normally, this would not present a problem because of the rather routine nature of the determinations. However, the replacement technician became ill during this period and although he continued to work, I feel that he was unable to reliably score the results of this study. On this basis I feel that the data is not firm enough to make a statement.

ALC: jaw

Table H-1: Dominant lethal test data from control and silica exposed male Fischer-344 rats. Each male was caged with two unexposed female rats beginning six days after removal from the exposure chamber.

	Silica Concentration (mg/m^3)			
	0	2	10	20
Males Tested	8	8	8	8
Females Bred	16	16	16	16
Number Pregnant	15	12	14	16
% Pregnant	93.8	75.0	87.5	100.0
Number Scored	12	11	11	15
Corpora Lutea				
mean	11.4	11.3	11.6	10.4
s.e.	0.7	2.3	2.3	3.2
Viable Embryos				
mean	8.8	10.1	10.3	9.2
s.e.	3.0	1.5	1.2	1.5
Early Deaths				
mean	0.2	0.0	0.2	0.2
s.e.	0.4	0.0	0.6	0.4
Late Deaths				
mean	0.0	0.1	0.0	0.0
s.e.	0.0	0.3	0.0	0.0
Preimplantation Loses				
mean	2.4	1.1	1.1	4.4
s.e.	3.4	0.8	2.0	11.6

Table H-2: Dominant lethal test data from control and silica exposed female Fischer-344 rats. Each female was caged with an unexposed male rat beginning six days after removal from the exposure chamber.

	Silica Concentration (mg/m^3)			
	0	2	10	20
Females Bred	8	8	8	8
Number Pregnant	5	4	4	7
% Pregnant	62.5	50.0	50.0	87.5
Number Scored	5	3	4	7
Corpora Lutea				
mean	12.2	13.7	12.2	12.6
s.e.	0.8	1.2	0.5	1.6
Viable Embryos				
mean	5.0	3.3	6.0	6.0
s.e.	2.0	1.5	1.2	2.9
Early Deaths				
mean	2.8	3.7	3.0	3.7
s.e.	1.6	1.2	1.6	2.8
Late Deaths				
mean	0.0	0.0	0.0	0.0
s.e.	0.0	0.0	0.0	0.0
Preimplantation Loses				
mean	4.4	6.7	3.2	2.9
s.e.	3.0	2.9	1.0	1.6