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INFORMAL REPORT

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

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FINAL REPORT

ON

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SUBMITTED TO

THE NATIONAL TOXICOLOGY PROGRAM

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November, 1984

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LIST OF ABBREVIATIONS

Å	angstrom
ANOVA	analysis of variance
ATPD	ambient temperature pressure dry
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
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 rather than air was the gas breathed
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
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)
ΔP_L	driving tidal pressure
P_{st}	static pressure (cm H_2O)
PEF	peak expiratory flow (cm^3/sec)
PVM	pneumonia virus of mice
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 $H_2O/cm^3 \cdot sec^{-1}$)
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)
VC	vital capacity (cm^3)

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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. To induce a fibrotic lesion, Fischer-344 rats were exposed to either 0, 2, 10, or 20 mg SiO_2/m^3 for 6 hours/day, 5 days/week for six months and then maintained in an animal room, equipped with a laminar flow unit, for six months prior to assessment of the end points.

A series of respiratory physiology tests were performed on animals from each exposure group. The results of these tests did not reveal any significant changes in those animals exposed to 2 or 10 mg SiO_2/m^3. However, almost every static and dynamic parameter measured in those animals that had been exposed to 20 mg SiO_2/m^3 was significantly affected, and the changes observed were consistent with a restrictive lung lesion. The minute volume of these animals was increased by 26% as a result of decreased tidal volume coupled with an increased breathing frequency. The driving tidal pressure was increased over that of the controls with a corresponding decrease in dynamic compliance. However, normalization of dynamic compliance to the functional residual capacity indicated a dependence on lung volume for this significant change. All subdivisions of lung volume were reduced in the 20 mg group with the most pronounced reductions in the functional residual capacity and residual volume. The overall lung compliance was reduced in the 20 mg group. The diffusing capacity for CO was reduced in part because of the loss in lung volume, in addition there was significant reduction in the homogeneity of the distribution of ventilation in these animals as measured by nitrogen washout. The 20 mg SiO_2/m^3 animals also

demonstrated significant alterations in airway function as demonstrated by reduced maximal flows at high lung volumes.

The amounts of protein, DNA, elastin, and hydroxyproline, as well as the water content of the lungs of exposed animals were assessed. The animals which had been exposed to 20 mg SiO_2/m^3 had significantly heavier lungs than those rats from the other exposure groups. However, the percent dry weight was similar among all of the groups. There was generally a dose dependent increase observed in the total amount of connective tissue, both elastin and collagen. However, when expressed in terms of dry weight the elastin concentration of the 2 and 10 mgSiO_2/m^3 groups was similar to that of the controls. When expressed in terms of amount per unit dry weight all of the tissue components were reduced relative to controls in the high dose group.

Microscopic examination of the respiratory tissues of the animals from the 10 and 20 mg SiO_2/m^3 groups revealed accumulations of histiocytes near the end-airways in most animals. Small birefringent crystals, presumably phagocytized silia particles, could occasionally be seen in these macrophages. Type II cell hyperplasia was evident in alveoli surrounding affected end airways. In the 20 mg SiO_2/m^3 group focal fibrosis with fibrotic aggregates and mononuclear cells forming "silicotic nodules" were common. In addition, alveolar proteinosis was observed in this group. Lymphoid proliferations around bronchioles and blood vessels often contained intralymphatic macrophages. Generalized reticuloendothelial cell hyperplasia was evident in the peribronchial lymph nodes.

Radiographic assessment of these animals demonstrated lungs of greater x-ray density in those animals which had been exposed to 20 mg

SiO_2/m^3. Whether this x-ray density was due to a fibrotic response or the material present in the alveoli of these animals is not known.

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 distinguish among the exposure groups. Among the compositional variables DNA, protein, and hydroxyproline all expressed as a ratio of dry weight and the total lung weight were found to be the most discriminating.

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 of 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 were exposed to a variety of toxic agents. The compounds which have been used are ozone, acrolein, chlorine, silica dust (reported in part here), cadmium chloride aerosol, and a combination of tungsten carbide and cobalt dusts.

Silica was selected as a test compound to produce a deep lung restrictive lesion and provide an opportunity to investigate the relationship of 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 macrophage fibrogenic factors resulting in the 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 duration 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 length of exposure before onset of symptons 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 as an occupational disease since antiquity. 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 or acute 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 is also acute and 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 they are rather diffuse.[2-3]

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 timepoints 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 then maintained for an additional 6 months before assessment of endpoints.

To enable comparisons of function, composition, and structure in individual animals, each rat was first subjected to a series of pulmonary function tests and upon sacrifice, immediately after testing, the left lung was fixed for histologic examination and the right lung submitted for compositional analysis. Stepwise discriminant analysis was then used to determine if any measured variables were significantly more sensitive to the induced changes. To evaluate the overall pathology induced by the test agent, subgroups of animals from each chamber were used solely for pathological examination.

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 concentrations of O_2 and CO_2 in the blood as well as the blood pH. While systemic diseases and metabolic imbalances can offset these variables, data from their collective evaluation can generally be used to distinguish between respiratory and metabolicly derived acid/base 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 (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 volume expired 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 characteristics 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, known as effort independence. Once a critical pressure drop along the airway is established, the fragile airways collapse and the maximum airflow is limited, regardless of the increased effort or imposed force. This portion of the MEFV maneuver is therefore effort independent. Whether or not these airways collapse prematurely, which is the case in some disease states, can 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 disected. 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 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 increased lung volume 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 vs. function. The extensive functional data generated in this study should provide greater insight on (1) how structural and compositional changes in the silicotic lung are presented functionally and, (2) on the physiological impact of these structural changes.

MATERIALS AND METHODS

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 three 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 eight animals were used for complete histopathology. A second subgroup at each exposure level was exposed for six months and assessed at the end of this exposure period. This subgroup also included 24 animals for multiple pulmonary endpoint assessments and eight rats for histopathology. In addition, each six month exposure subgroup included eight male and eight 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 eight histopathology animals, were exposed for six months and then maintained in conventional SPF animal quarters for six 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 the 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 includes: (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 Corononavirus, 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) examination of fecal samples for 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 viral, bacterial, or parasitic pathogens 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 (PVM) (titers ranged from 160 to 320) (Appendix B). Following the six month holding period sera from eight additional animals was sent to AnMed Laboratories for viral antibody assessment. Using the ELISA technique 6/8 of the animals were positive for PVM (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 three tiers with 6 units per tier. Water was supplied to the animals *ad libitum*; however the food was removed during the daily six 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. During the six month holding period following exposure to silica the animals were weighed the morning following their final exposure, then monthly, and on the morning of their designated endpoint assessment.

The animals were briefly examined each day prior to exposure, when the food troughs were removed and clean catch pans were provided, and again when the food troughs were replaced following the exposure period. 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 positions/tier) by moving each pack one position after 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. The rats exposed for six months were exposed for 127 weekdays with the exception of laboratory holidays (which are included in the 127 days). 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 six months before assessment of the selected endpoints. During this period the animals were individually housed in suspended wire mesh cages in a laminar flow SPF facility. A 12-hour on 12-hour off light cycle was maintained. A standard laboratory diet (Purina Chow) and water were provided ad libitum. The animals were inspected twice daily on weekdays and once daily on weekends. They were weighed monthly and provided a clean wire mech cage when weighed.

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 electrostatic precipitators, prefilters, and HEPA filters 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. The 0.5 hr temperature averages and the daily 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.

The dust ladened atmospheres for these studies were produced using fluidized bed aerosol generators, 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 microporus stainless steel support screen at the bottom of the bead. 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.

Because these fluidizing bed generators 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 spectroscopy 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 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 the cascade impactor analyses performed was 2.4 µm with a mean σ_g of 2.0.

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

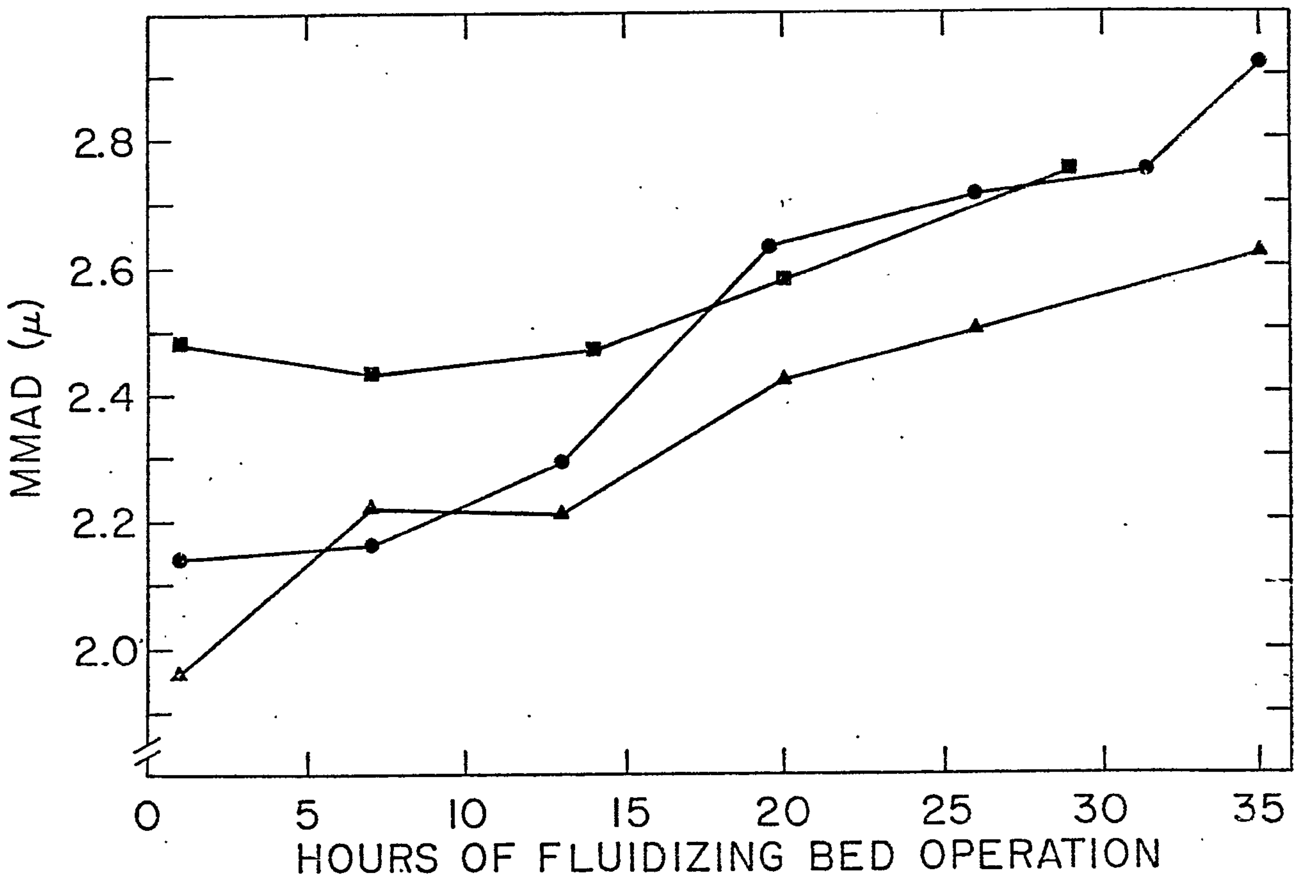

Figure 1. Change in silica particle size (MMAD) with increased operating time of the fluidizing bed generators associated with each exposure chamber; 2 mg/m^3 silica (●), 10 mg/m^3 silica (▲), and 20 mg/m^3 silica (■). MMADs were determined using an Anderson Cascade Impactor.

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

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 communcation with a differential pressure transducer probe, and communiation 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 cm^3 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 and 5 Hz. All V_T data were corrected for this difference on the basis of f for the final determination of V_E. A 15 minute period during which breathing air (20% O_2, 80% N_2) provided at 2 l/min was usually sufficient for the animal to aclimate to the system and permit the collection of representative tidal breathing data for 15-25 seconds. These data were collected after closing the inlet air port, allowing about ten 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 five minutes. Previous testing had indicated that this duration and flow rate were sufficient to maximize the CO_2 response. After closure of the

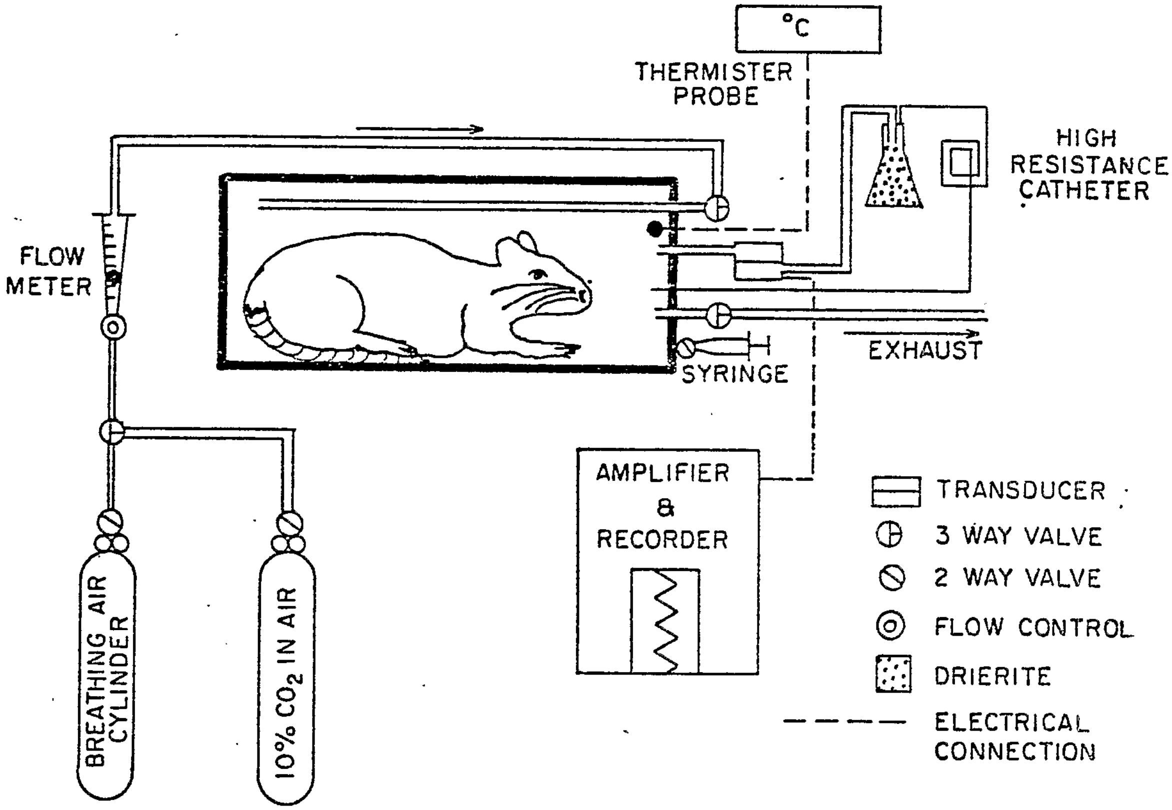

Figure 2. Schematic diagram of a modified Fenn-Brorbaugh plethysmograph.

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 calculations to determine V_T was found not to effect 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:

$$\% \dot{V}_E = \frac{(V_T \text{ deflection}) \cdot f \text{ in } CO_2 - (V_T \text{ deflection}) \cdot f \text{ in air}}{(V_T \text{ deflection}) \cdot f \text{ in air}} \times 100$$

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 deflections. Thus, chart deflection estimates of V_T were used to determine the percent change in "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. All rats so designated were not assessed for blood gases because of the time required for caudal artery cannulation and recovery from anesthesia (2-3% Ethrane, 30% O_2 in N_2). 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 its 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 bled. 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 in proportion to pressure changes using a high frequency response differential pressure transducer (Setra System 239: ± 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: ± 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 waterfilled 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

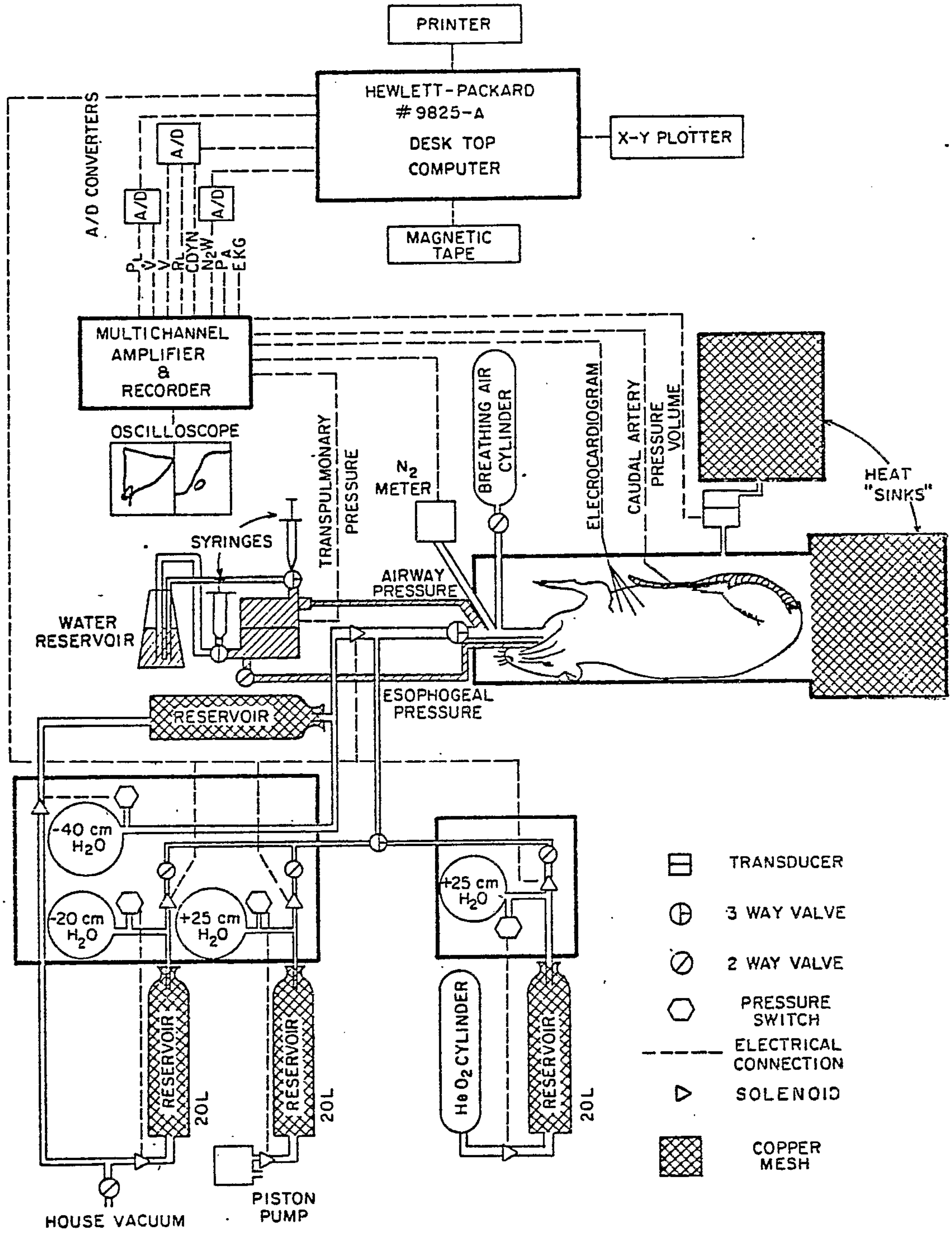

Figure 3: Schematic diagram of the plethysmograph and associated equipment used to access rodent pulmonary function.

were in phase to a frequency of 6 Hz, determined by using a piston pump of 1 cm^3 displacement.

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 to be assessed, by-passing the effect of the nose on all of the measurements made on these otherwise obligate nasal breathers. A shoulder had been 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 an 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: $V_p = V_o(1\text{-exp } P/h)$, 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 an 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 (TLC_d) 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 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 directly controlled 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 Britan, CN). 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 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, 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 at a focal distance of 43 cm. To stop breathing motions the rat to be x-rayed was hyperventilated with 10 repeated intratracheal injections, via the tracheal cannula, of approximately 75% IC 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 45 min.[21] It was dissolved with pancreatic elastase (Sigma, Type III) and determined by the method of Naum and Logan[22] and compared with a sample of bovine ligamentum nuchae elastin (Sigma) as standard. Total protein and elastin were determined by the Hartree modification[23] of the Folin-Lowry method. The method of Burton[24] was used for DNA determinations after heating a sample in 5% perchloric acid at 90°C for 12 minutes (conditions found to give 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 blotted 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 intesting, 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. (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 animal numbers were coded by treatment group and the slides were examined without knowledge of group. The diagnoses were entered into an HP-1000 computer and the incidence tables provided in the results section were printed after the the code was broken.

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. Microscopic examinations were made of hematoxylin and eosin stained sections of the left lung lobe, trachea and peribronchial lymph node from these animals. The examinations were conducted after the slides were coded as described above.

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 lymphoid proliferations, end airway cellular aggregations, alveolar histiocytosis, type II cell hyperplasia, intralympathic microgranulomas, fibrosis, alveolar proteinosis and abnormal numbers of granulocytes and monomuclear cells.

Statistical Methods

One-way analysis of variance (ANOVA) was used to compare the means of all single variables across exposure groups. When ANOVA indicated a significant difference among group means, Duncan's multiple range method of multiple comparison[25] was used to investigate the source of the differences. In these cases, the exposure groups are reported in order of ascending means (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 analyzed 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 report 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.

To investigate differences among exposure groups based on histopathologic data, the values were non-parametrically ranked and were then analyzed 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[26] 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).

In order to distinguish the four treatment groups on the basis of either functional, compositional, or all these variables combined, stepwise discriminant analysis was employed. In general, the more evident the distinction a particular variable makes among the groups, the more useful that variable is apt to be in deciding to which group an as yet unclassified animal belongs. The discriminant function, that linear correlation of the original variables, which yields the highest possible t-ratios for the differences among the groups is a logical choice for such applications. Stepwise discriminant analysis operates in a stepwise manner to select those variable which make up the minimal set of variables whi h can distinguish among the dosage groups. At each step, that variable (if one exists) which most improves the ability to discriminate among the groups is included, or that variable (if one exists) which adds no discriminating information is deleted. (In this study the selected 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. These variables are considered to be the "most important" in discriminating among the exposure groups. It should be noted however, that the variables are only selected individually, and thus, if two or more variables each display little ability to distinguish the groups, they will not be selected by the stepwise algorithm even if those variables as a set are effective.

The effectiveness of this discrimination was measured by means of classification functions, which categorized an animal into one of the four dosage groups according to its values for each of the reduced set of variables. For each animal, the classification functions were estimated using the data from all other animals. Thus, these functions were estimated separately for each animal, and the estimates were independent of the data for any particular animal. This scheme, referred to as "jack-knifed classification", reduces the bias in this type of analysis. The classification of animals was then compared with the actual grouping of the animals to assess the percent correctly classified.

Most statistics were computed using the Biomedical Computer Programs 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.

RESULTS

General Toxicology Parameters

Exposure Conditions. The mean daily concentrations of silica in the exposure chambers are 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 groups.

Animal Weights and Condition. Animals exposed to these three concentrations of silica did not show any outward signs of toxicity or discomfort. The mean weights of the animals on the day of their first exposure, the day following their final exposure, and the day of endpoint assessment are provided in Table 2. Because the endpoint assessment of these animals was time consuming, a limited number of animals were assessed each day. The rats were placed into the chambers on a staggered schedule. Therefore, the starting ages of the rats ranged from 10 to 12 weeks. At the beginning of exposures none of the groups differed in weight (Table 2). However, during the six month exposure period a significant weight differencedeveloped between the groups of rats exposed to 2 and 10 mg SiO_2/m^3. Although this difference persisted during the six month post-exposure period (Table 2) it was not considered exposure related.

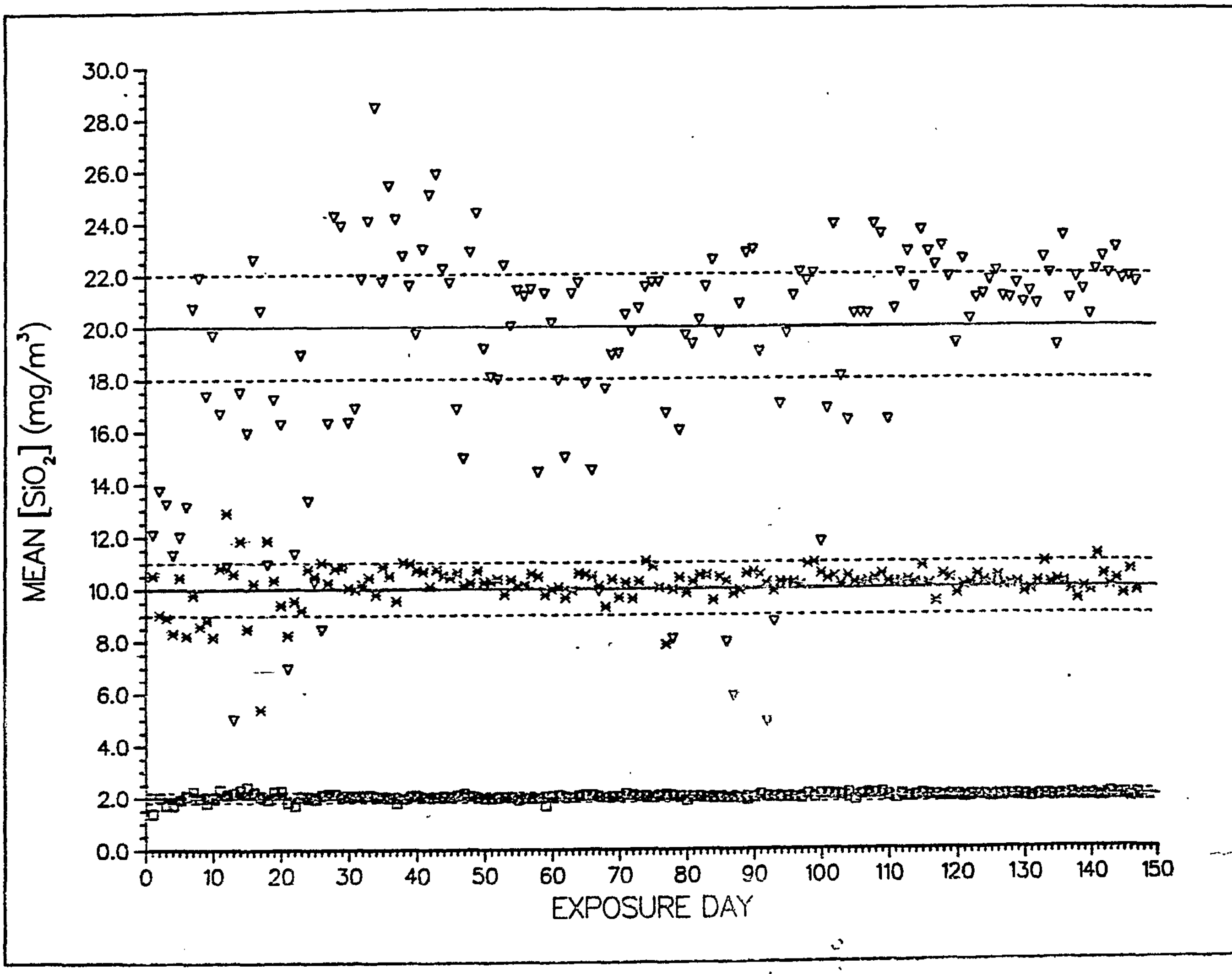

Figure 4: Daily mean silica-concentration in the animal exposure chambers: (□) 2.0 mg/m^3, (*) 10 mg/m^3, and (▽) 20 mg/m^3. The dashed lines indicate ± 10% of the target concentrations.

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

	Silica Concentration (mg/m^3)				p value
	0	2	10	20	
n	32	31	32	32	
Weight at 1st exposure (g)					
mean	212.1	204.4	212.5	213.6	0.3213
s.e.	4.8	4.3	4.0	4.1	
Weight at final exposure (g)					
mean	363.4	353.4	371.6	359.4[b]	0.0355[c]
s.e.	4.2	4.5	4.7	4.4	
multiple comparison[d]	LD HD CN ID				
Weight 6 months following exposure (g)					
mean	387.0	372.7	395.1	382.8	0.0094[c]
s.e.	4.6	4.9	5.3	3.6	
multiple comparison[d]	LD HD CN ID				

[a] Six hours/day, 5 days/week for 6 months, then maintained in animal rooms for 6 months.
[b] n=31.
[c] Statiscally significant at $\alpha = 0.05$ level using ANQUA.
[d] Pair wise comparison of means by the Duncan multiple range method.

Organ Weight and Organ-to-Body Weight Ratios. The weights of selected organs from those rats designated for pathological examination in each exposure group are provided in Table 3. Based on fresh organ weights the lungs from rats exposed to 20 mg SiO_2/m^3 were significantly heavier than those from the other exposure groups (Table 3). The lungs from this high dose group weighed 2.3 times as much as the lungs from control animals. This increased lung weight was maintained when lung-to-body weight ratios were considered (Table 4), while neither of the lower exposure groups demonstrated differences relative to controls. The volume displacement of the lungs from the 20 mg SiO_2/m^3 group were significantly increased to 125% of the volume of control lungs (Table 5).

The only other change observed in organ weights was a difference in fresh brain weight between the controls and the silica exposed animals (Table 3). However, this difference was of marginal statistical significane ($p = 0.0468$) and was not maintained when examined on a brain-to-body weight basis ($p = 0.3039$, Table 4). Therefore, the finding was considered an anomaly and not exposure-related.

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 D.

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 6). The range of the hyperventilatory response in the four groups tested was 84 to 107% the V_E recorded during exposure to

Table 3. Organ Weights of Control and Silica-Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	8	7	8	8	
LUNGS (g)					
mean	1.32	1.54	1.65	3.05	<0.0001[b]
s.e.	0.05	0.05	0.04	0.10	
multiple comparison[c]		CN LD ID HD			
HEART (g)					
mean	1.02	1.05	1.07	1.09	0.6836
s.e.	0.04	0.06	0.04	0.03	
SPLEEN (g)					
mean	0.79	0.85	0.86	0.80	0.7661
s.e.	0.07	0.08	0.05	0.04	
LIVER (g)					
mean	12.79	13.13	13.37	12.30	0.5465
s.e.	0.72	0.41	0.54	0.44	
KIDNEYS (g)					
mean	2.58	2.74	2.76	2.85	0.3006
s.e.	0.10	0.11	0.10	0.10	
ADRENAL GLANDS (g)					
mean	0.06	0.07	0.06[d]	0.06[d]	0.7411
s.e.	0.01	<0.01	<0.01	0.01	
TESTIS (g)					
mean	3.22	3.18	3.38	3.12	0.1098
s.e.	0.10	0.08	0.07	0.06	
BRAIN (g)					
mean	1.90	2.02	2.00	1.99	0.0468[b]
s.e.	0.03	0.05	0.03	0.02	
multiple comparison[c]		CN HD ID LD			
BODY WEIGHT (g)					
mean	379.8	389.2	411.8	383.8	0.1610
s.e.	9.1	16.1	9.9	7.3	

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] Statistically significant $\alpha = 0.05$ level using ANOVA.
[c] Pairwise comparison of means by the Duncan multiple range method.
[d] n = 7.

Table 4. 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	7	8	8	
LUNGS					
mean	3.47	4.00	4.01	7.96	<0.0001[b]
s.e.	0.12	0.20	0.08	0.33	
multiple comparison[c]		CN LD ID HD			
HEART					
mean	2.68	2.72	2.60	2.85	0.4575
s.e.	0.11	0.17	0.09	0.06	
SPLEEN					
mean	2.11	2.17	2.10	2.09	0.9817
s.e.	0.20	0.14	0.11	0.10	
LIVER					
mean	33.56	33.91	32.42	32.05	0.5682
s.e.	1.36	1.17	0.71	0.94	
KIDNEYS					
mean	6.77	7.08	6.70	7.43	0.1334
s.e.	0.18	0.32	0.18	0.27	
ADRENAL GLANDS					
mean	0.16	0.18	0.14[d]	0.16[d]	0.5408
s.e.	0.02	0.01	0.01	0.02	
BRAIN					
mean	5.01	5.23	4.87	5.21	0.3039
s.e.	0.14	0.23	0.12	0.12	
TESTIS					
mean	8.50	8.21	8.23	8.13	0.6137
s.e.	0.32	0.18	0.14	0.14	

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] Statistically significant $\alpha = 0.05$ level using ANOVA.
[c] Pairwise comparison of means by the Duncan multiple range method.
[d] $n = 7$.

Table 5. Displacement Volume of the Lungs from Control and Silica Exposed[a] Fischer-344 Rats.

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	7	7	8	8	
Displacement Volume (cm^3)					
mean	8.55	9.32	10.02	11.69	0.0058[b]
s.e.	0.51	0.80	0.44	0.58	
multiple comparison[c]	CN	LD	ID	HD	

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

Table 6. 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	100.8	100.9	106.6	83.6	0.2389
s.e.	8.2	7.9	10.4	6.1	
n	22	23	22	22	
pCO_2 (mmHg)					
mean	44.3	42.6	45.5	42.7	0.0536
s.e.	0.6	1.3	0.6	0.5	
n	11	12	11	11	
pO_2 (mmHg)					
mean	80.2	91.8	76.1	75.4	0.0370[b]
s.e.	2.8	5.1	3.7	5.3	
n	11	12	11	11	
multiple comparison[c]		HD	ID CN	LD	
blood pH					
mean	7.40	7.40	7.40	7.42	0.5611
s.e.	0.01	0.01	0.01	0.02	
n	11	12	11	11	

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

normal breathing air (CO_2<0.4%) with the 20 mg/m^3 animals being least responsive, although the difference was not significant.

Though statistically significant, exposure group differences among the arterial blood-gas partial pressures were marginal as well as inconsistent, and did not appear to be exposure related (Table 6). Blood pH values did not differ among the exposure groups.

Parameters of Spontaneous Breathing. Several measurements of normal tidal breathing were taken on each animal. As listed in Table 7, virtually all of the standard measures of breathing function were significantly affected by exposure to 20 mg/m^3, while no alterations were observed with the lower exposure concentrations. Tidal volume for the 20 mg/m^3 group decreased by 15%, but was more than offset by a 68% increase in breathing frequency; the product of these (V_E) was increased by 26%. Driving tidal pressure (ΔP_L) was increased by 15% with a corresponding fall of 32% in C_{DYN}. Pulmonary flow resistance, however, was not significantly affected by any level of silica-exposure. Normalization of R_L and C_{DYN} to FRC_d (Figure 5) did not reveal significant differences among the exposure groups, which could not be accounted for by the altered resting lung volume.

Electrocardiographic Data. Heart rate, as determined by EKG, was not significantly altered by silica-exposure (Table 8). Because of electrical noise in the processing of the EKG signal, only the P-R and QRS temporal patterns could be readily distinguished. All silica exposed groups exhibited EKGs which were similar to those of the control group.

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

Table 7. 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	22	23	24	23	
V_T (cm^3)					
mean	1.77	1.72	1.74	1.50	<0.0001[b]
s.e.	0.06	0.04	0.03	0.03	
multiple comparison[c]		HD LD ID CN			
ΔP_L (cm H_2O)					
mean	5.68	5.24	5.77	6.53	0.0002
s.e.	0.22	0.19	0.18	0.20	
multiple comparison[c]		LD CN ID HD			
f(breaths/min)					
mean	68	65	68	100	<0.0001[b]
s.e.	4	2	2	4	
		LD CN ID HD			
V_E (cm^3/min)					
mean	119.2	111.6	118.3	150.7	<0.0001[b]
s.e.	5.9	4.8	4.5	7.2	
multiple comparison[c]		LD ID CN HD			
R_L (cm H_2O/cm^3/sec)					
mean	0.50[d]	0.47[e]	0.44	0.47[f]	0.9595
s.e.	0.09	0.08	0.08	0.09	
C_{DYN} (cm^3/cm H_2O)					
mean	0.39[d]	0.34[e]	0.37	0.25[f]	<0.0001[b]
s.e.	0.02	0.02	0.02	0.02	
multiple comparison[c]		HD LD ID CN			

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] Statistically significant at $\alpha = 0.05$ level using ANOVA.
[c] Pairwise comparison of means by the Duncan multiple range method.
[d] n = 21.
[e] n = 22.
[f] n = 20.

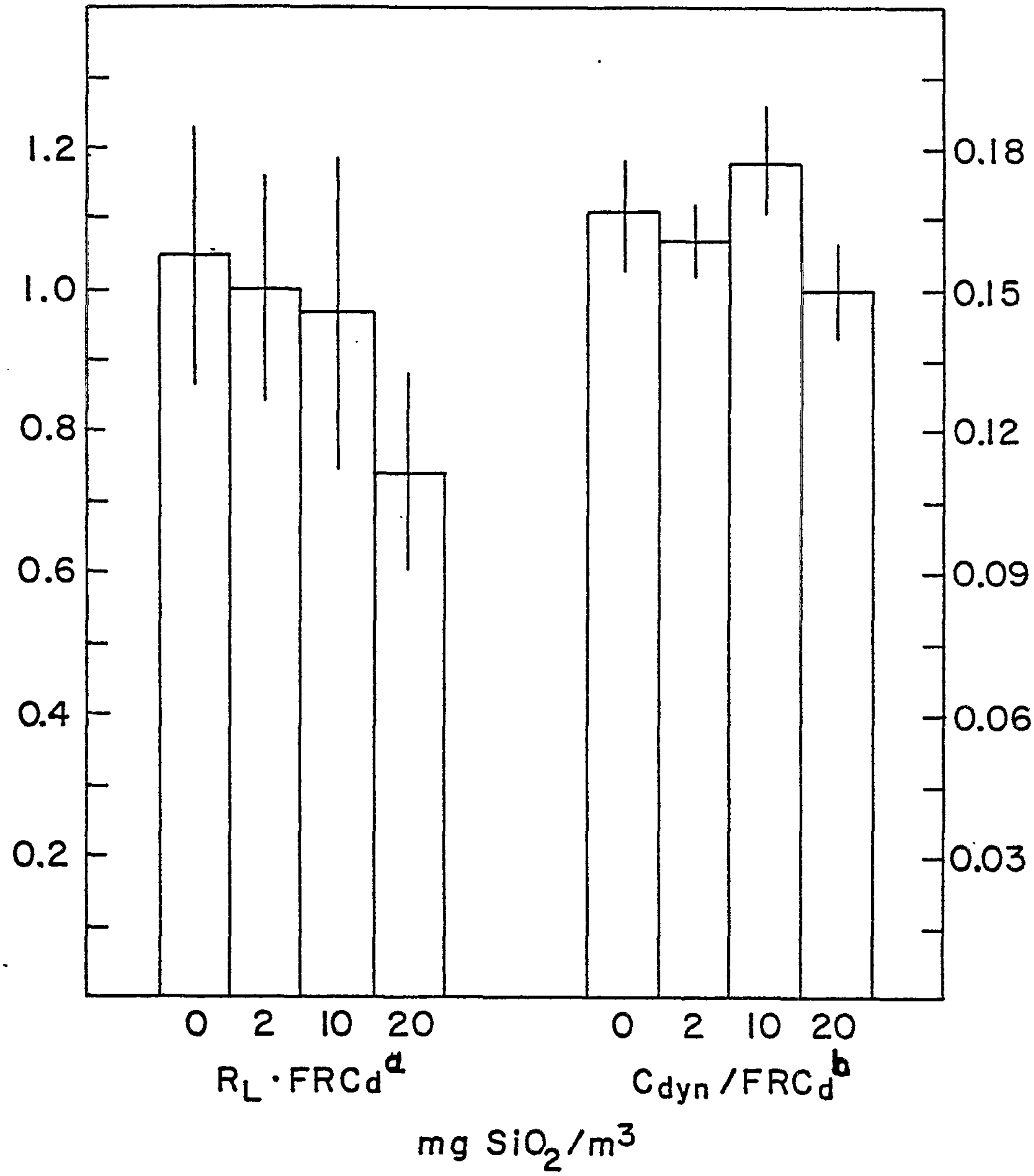

Figure 5: Pulmonary resistance (R_L) and dynamic compliance (C_{DYN}) normalized to the Functional Residual Capacity (FRC_d) of Fischer-344 rats exposed to SiO_2 for 6 months (6 hours/day, 5 days/week) then maintained in animal rooms for 6 months. The number of rats in the 0, 2, 10, and 20 mg/m^3 group was 21, 21, 23, 20, respectively.

a. p value of the F-statistic from one-way ANOVA = 0.5732.
b. p value of the F-statistic from one-way ANOVA = 0.1541.

Table 8. 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	19	21	21	16	
Heartbeats/min					
mean	312	340	313	303	0.0536
s.e.	8	10	10	11	
P-R (sec)					
mean	0.046[b]	0.046[c]	0.046	0.048[d]	0.1722
s.e.	0.001	0.001	0.001	0.001	
QRS (sec)					
mean	0.013[d]	0.012[c]	0.013	0.013[d]	0.5278
s.e.	0.001	0.001	0.001	0.001	

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] n = 18.
[c] n = 19.
[d] n = 15.

determination of lung volume 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 air volume in its estimate of FRC (Figure 6). No differences in trapped air volume were observed among the groups.

Figure 7 illustrates the impact of silica exposure on the subdivisions of lung volume. No significant changes relative to the control group were observed for either the 2 or 10 mg/m^3 exposure groups; however, those rats which had been exposed to 20 mg SiO_2/m^3 clearly exhibited restricted, i.e., reduced, lung volumes. On the average, approximately 1.6 cm^3 of total lung volume (~ 13%) was effectively "lost" in this group when compared to the mean control lung volume. Although all of the subdivisions of volume were significantly affected by this exposure regime, the apparent loss of volume was due in most part to disproportionate losses of the reserve volumes, FRC and RV (Figure 8).

Parenchymal Behavior and DL_{CO}. The QSC, reported as QSC_{cs} or h was not altered in the 2 and 10 mg/m^3 exposure groups when compared to controls (Table 9). At 20 mg/m^3, however, the QSC_{cs} was significantly depressed, though only slightly (7%). Multivariate analysis of the QSC curves expressed in terms of absolute lung volume indicated the significant impact of 20 mg/m^3 on overall lung compliance (Figure 9). The "h" value for this exposure group, which is a volume normalized estimate of lung compliance, did not differ from controls or the other exposure groups. Similarly, the volume (VC) adjusted compliance curves did not differ among any of the groups when evaluated by MANOVA (Figure 10).

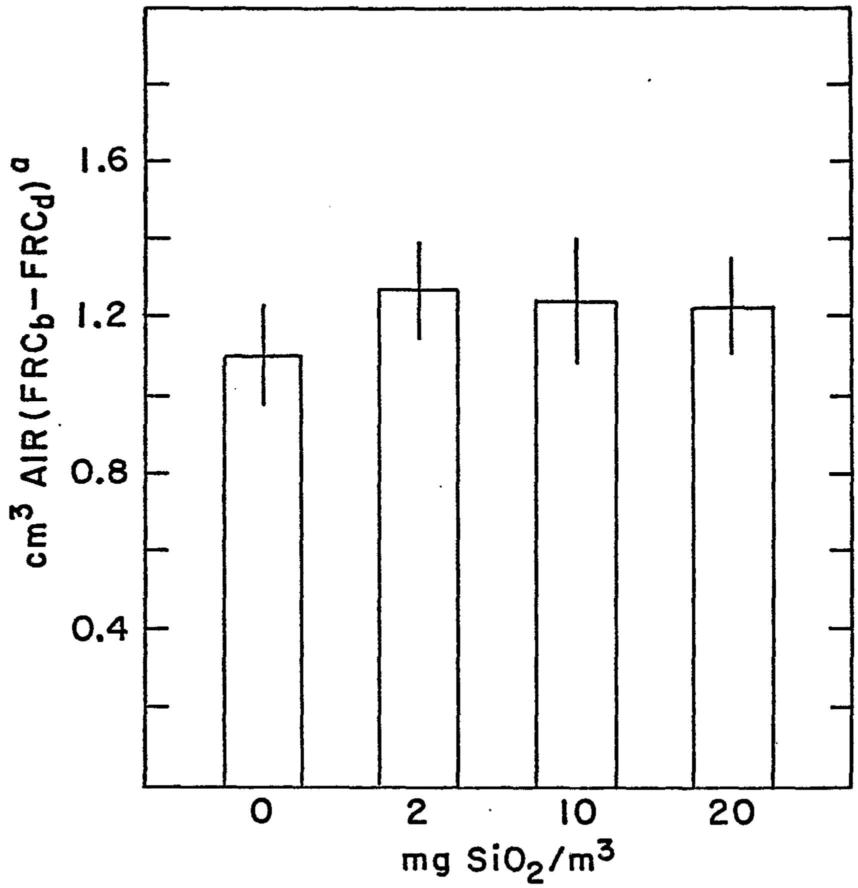

Figure 6: Trapped air in the lungs of Fischer-344 rats exposed to silica for 6 months (6 hours/day, 5 days/week) then maintained in animal rooms for 6 months. The data represent the means (± s.e.) of 22 control, 22 2 mg SiO_2/m^3, 23 10 mg SiO_2/m^3, and 23 20 mg SiO_2/m^3 rats.

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 = 0.8039.

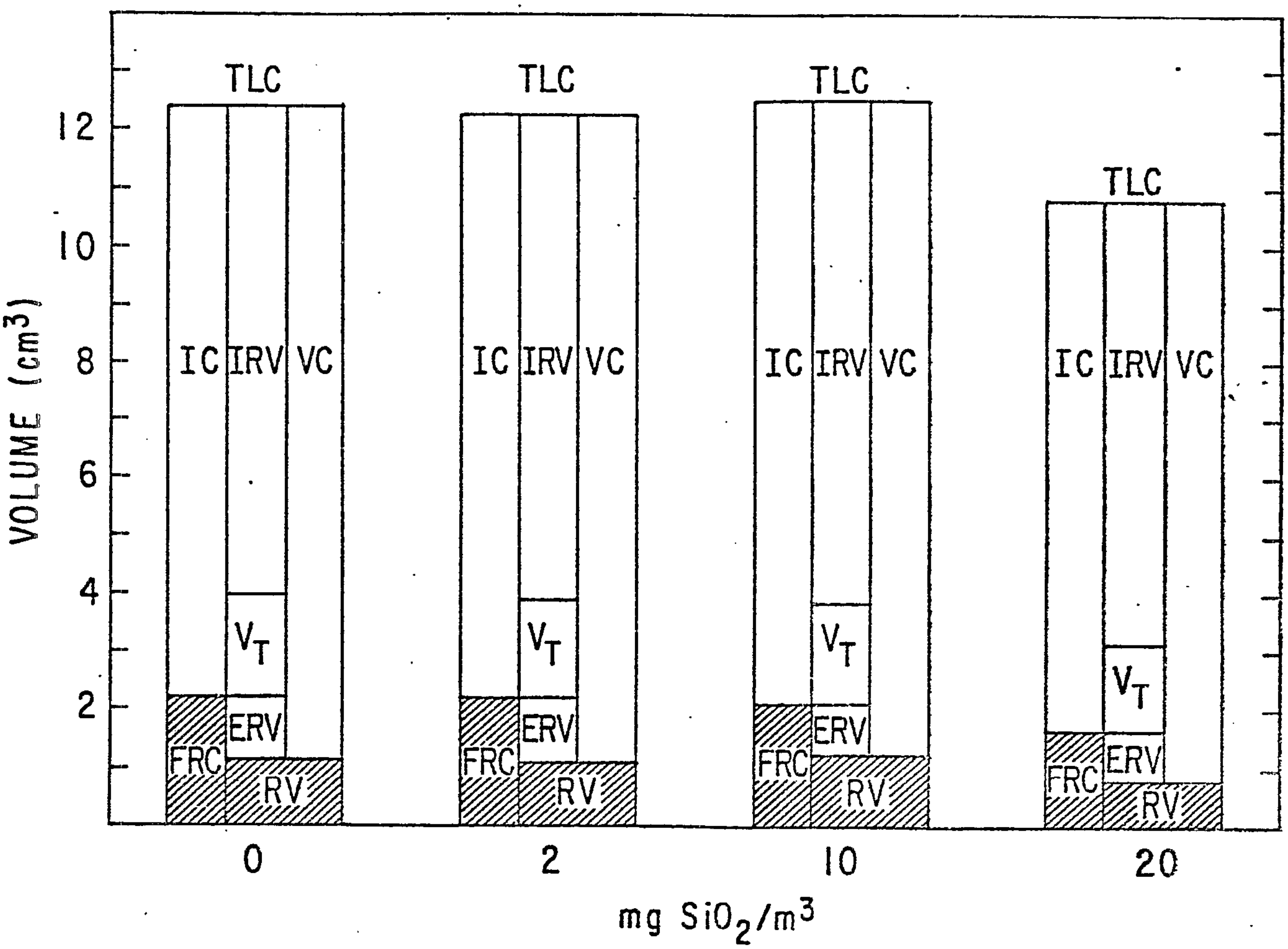

Figure 7: Divisions of lung volume in Fischer-344 rats exposed to filtered air or silica for 6 months (6 hours/day, 5 days/week) then maintained in animal rooms for 6 months. The data represents the means of at least 22 control, 22 2 mg SiO_2/m^3, 23 10 mg SiO_2/m^3, and 23 20 mg SiO_2/m^3 rats.

		p value
ERV:	Expiratory reserve volume	0.0049
	multiple comparison HD ID CN LD	
FRC:	Functional residual capacity	<0.0001
	multiple comparison HD ID LD CN	
IC:	Inspiratory capacity	<0.0001
	multiple comparison HD LD CN ID	
IRV:	Inspiratory reserve volume	<0.0001
	multiple comparison HD LD CN ID	
RV:	Residual volume	<0.0001
	multiple comparison HD LD CN ID	
VC:	Vital capacity	<0.0001
	multiple comparison HD LD CN ID	
V_T:	Tidal volume	<0.0001
	multiple comparison HD LD CN ID.	
TLC:	Total lung capacity	<0.0001
	multiple comparison HD LD CN ID	

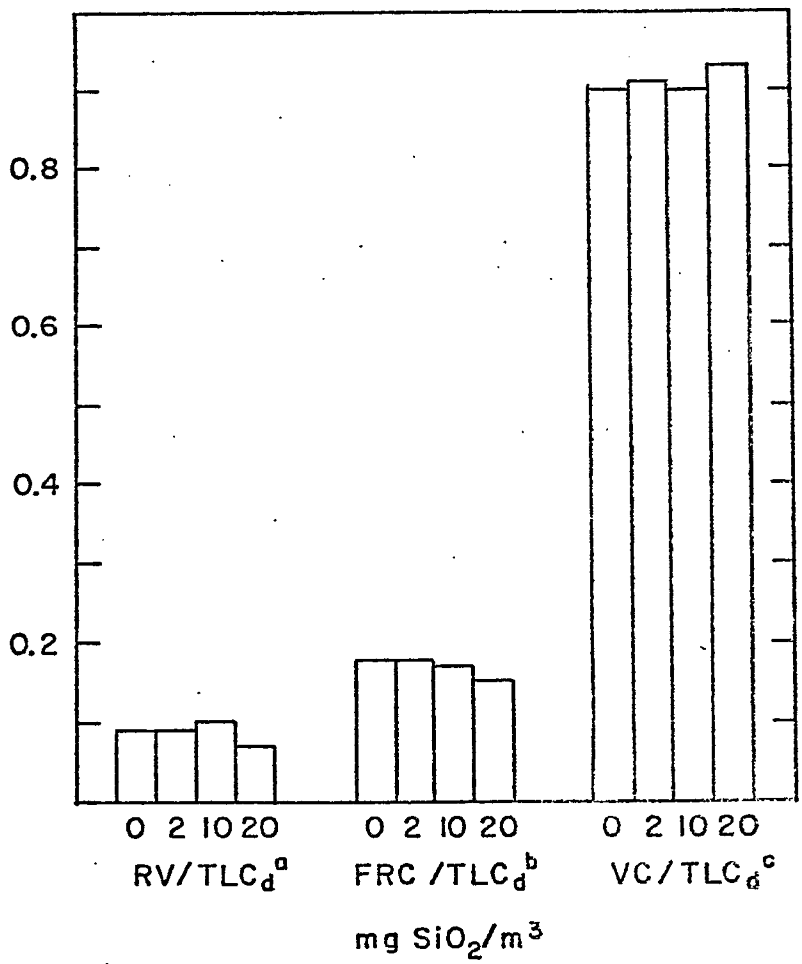

Figure 8: Normalized lung volume of control and silica exposed Fischer-344 rats (6 hours/day, 5 days/week) for 6 months then maintained in animal rooms for 6 months. The data represents the mean (± s.e.) of 22 control, 22 2 mg SiO_2/m^3, 23 10 mg SiO_2/m^3, and 23 20 mg SiO_2/m^3 rats.

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

					p value
[a] RV/TLC_d					0.0013
multiple comparison	HD	LD	CN	ID	
[b] FRC_d/TLC_d					0.0061
multiple comparison	HD	ID	LD	CN	
[c] VC/TLC_d					0.0013
multiple comparison	ID	CN	LD	HD	

Table 9. Physilogical 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	22	22	24	23	
QSC_{cs} ($cm^3/cm\ H_2O$)					
mean	0.94	0.93	0.97	0.87	<0.0001[b]
s.e.	0.01	0.01	0.01	0.02	
multiple comparison[c]		HD LD CN ID			
QSC_{cs}/FRC_d					
mean	0.426	0.447	0.481[d]	0.544	0.0022[b]
s.e.	0.018	0.025	0.026	0.021	
multiple comparison[c]		CN LD ID HD			
h ($cm\ H_2O$)					
mean	3.05	2.90	3.07	3.01	0.5270
s.e.	0.10	0.09	0.10	0.06	
$DLCO_{(rb)}$ ($cm^3/mmHg$)					
mean	0.180	0.173	0.183[d]	0.130	<0.0001[b]
s.e.	0.005	0.006	0.005	0.003	
multiple comparison[c]		HD LD CN ID			
$DLCO_{(rb)}/TLC$					
mean	0.014	0.014	0.015[d]	0.012	<0.0001[b]
s.e.	<0.001	<0.001	<0.001	<0.001	
multiple comparison[c]		HD CN LD ID			

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

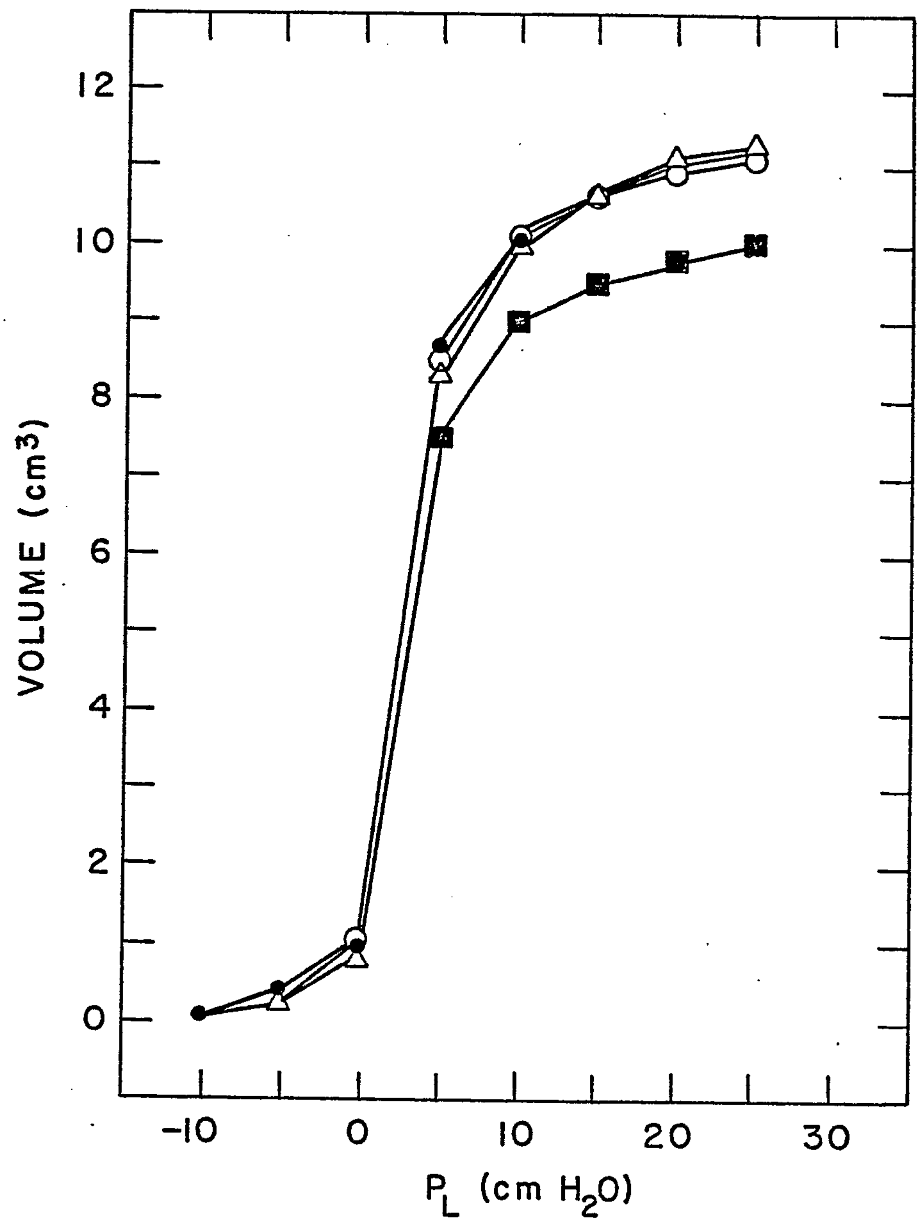

Figure 9: Quasi-static compliance curves of Fischer-344 rats exposed to silica for 6 months (6 hours/day, 5 days/week) then maintained in animal rooms for 6 months. The data represent the means (± s.e.) of 22 control (●), 22 2 mg SiO_2/m^3 (○), 24 10 mg SiO_2/m^3 (Δ), and 23 20 mg SiO_2/m^3 (■) rats. The p value of the F-statistic from one way MANOVA = <0.0001.

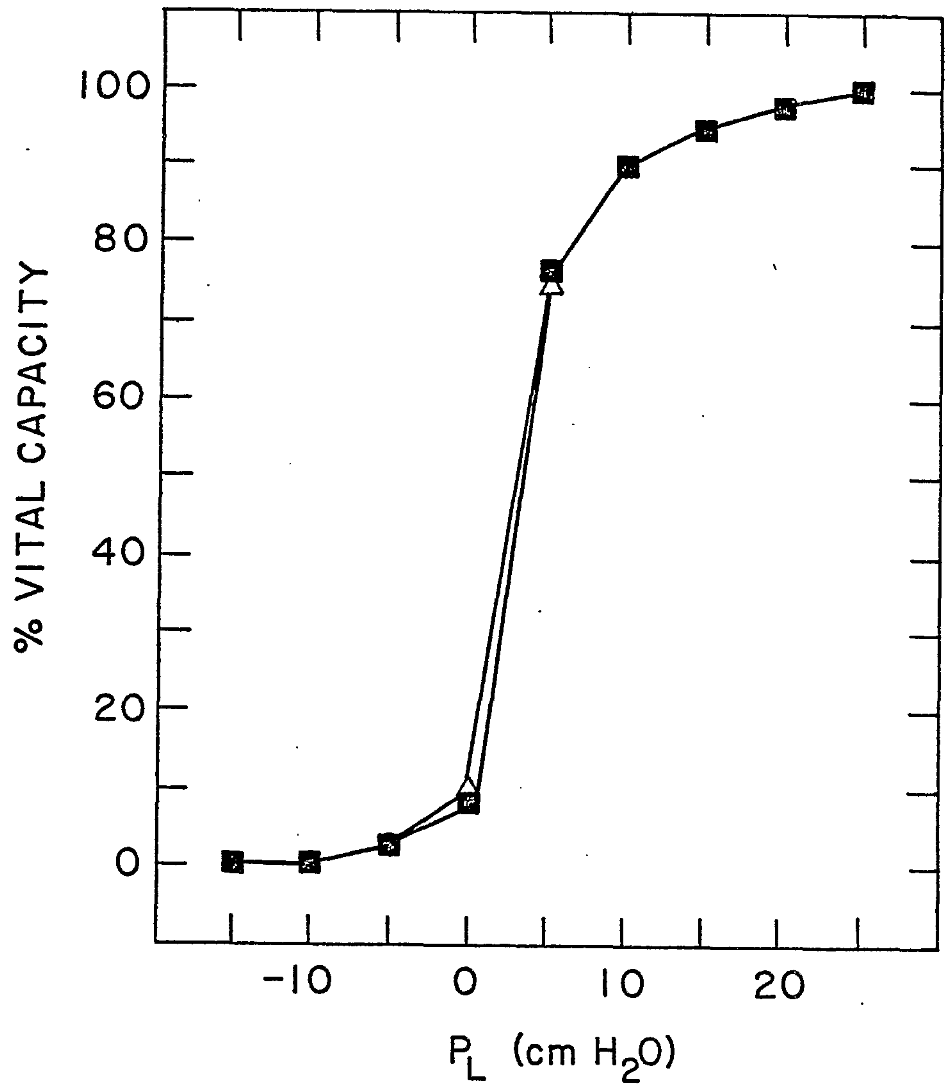

Figure 10: Quasi-static compliance as a function of vital capacity of Fischer-344 rats exposed to silica for 6 months (6 hours/day, 5 days/week) then maintained in animal rooms for 6 months. The means and s.e. bars of the 22 control (●), 22 2 mg SiO_2/m^3 (○), 24 10 mg SiO_2/m^3 (Δ), and 23 20 mg SiO_2/m^3 (■) rats often overlay each other and therefore may appear as a single curve. The p value of the F-statistic from one-way MANOVA = 0.1070.

Diffusion capacity for CO was significantly reduced only in the 20 mg/m^3 exposure group (28% relative to control (Table 9). Approximately 50% of this depression could be accounted for by the measured loss of lung volume, i.e., the reduced TLC (Table 9). (A comparison of the DL_{CO} 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 DL_{CO}.)

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 significant impairment in washout efficiency in the 20 mg/m^3 exposure group (Table 10). The moment ratio, M_1/M_0, was increased 35% in this group indicating distal lung disease.

Flow-Volume Dynamics. Significant alteration in airway function was observed only in the 20 mg/m^3 SiO_2 exposure group after the flow data was expressed in terms of vital capacity (Table 11). The apparent flow augmentations were limited to maximal flows at high lung volumes (Figure 11). Exposure to silica at lower concentrations did not result in detectable airway effects, regardless of data presentation. Maximum expiratory flows, in terms of absolute volume units, i.e., cm^3/sec, were largely the same for all exposure groups (Table 12). The apparent inconsistency in these findings can be explained by the large decrement in VC to which the flows were adjusted. Analysis of the data by MANOVA confirmed the presence of significant flow dynamic alteration in the 20 mg/m^3 exposure groups relative to the controls and other treatment groups, only when data were expressed as volume adjusted flows (Figure 11).

Table 10. 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	19	21	22	18	
M_1/M_0					
mean	7.87	7.66	8.64	10.43	0.0006[b]
s.e.	0.38	0.37	0.53	0.59	
multiple comparison[c]		LD CN ID HD			

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] Statistically significant at α = 0.05 level using ANOVA.
[c] Pairwise comparison of means by the Duncan multiple range method.

Table 11. Points from the MEFV Curve Normalized to the Vital Capacity of Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	22	21	23	23	
V_{max}(% VC)					
mean	67.5[b]	72.5[b]	70.5	67.1	0.0172[c]
s.e.	1.8	1.2	1.2	1.2	
multiple comparison[d]		$\underline{\text{HD CN ID}}$ LD			
PEF (VC/sec)					
mean	9.7[e]	10.2	10.1	11.1	0.0001[c]
s.e.	0.1	0.2	0.2	0.3	
multiple comparison[d]		$\underline{\text{CN ID LD}}$ HD			
EFR_{50} (VC/sec)					
mean	8.3	8.2	8.3	9.4	0.0001[c]
s.e.	0.2	0.3	0.2	0.2	
multiple comparison[d]		$\underline{\text{LD CN ID}}$ HD			
EFR_{25} (VC/sec)					
mean	4.9	5.1	5.0	5.4	0.1800
s.e.	0.2	0.2	0.1	0.2	
EFR_{10} (VC/sec)					
mean	2.1	2.4	2.2	2.2	0.2417
s.e.	0.1	0.1	0.1	0.1	
ΔEFR_{25} (VC/sec)					
mean	0.7	1.0	0.9	0.7	0.3929
s.e.	0.2	0.1	0.1	0.1	

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] n=20.
[c] Statistically significant at α=0.05 level using ANOVA.
[d] Pairwise comparison of means by the Duncan multiple range method.
[e] n=21.

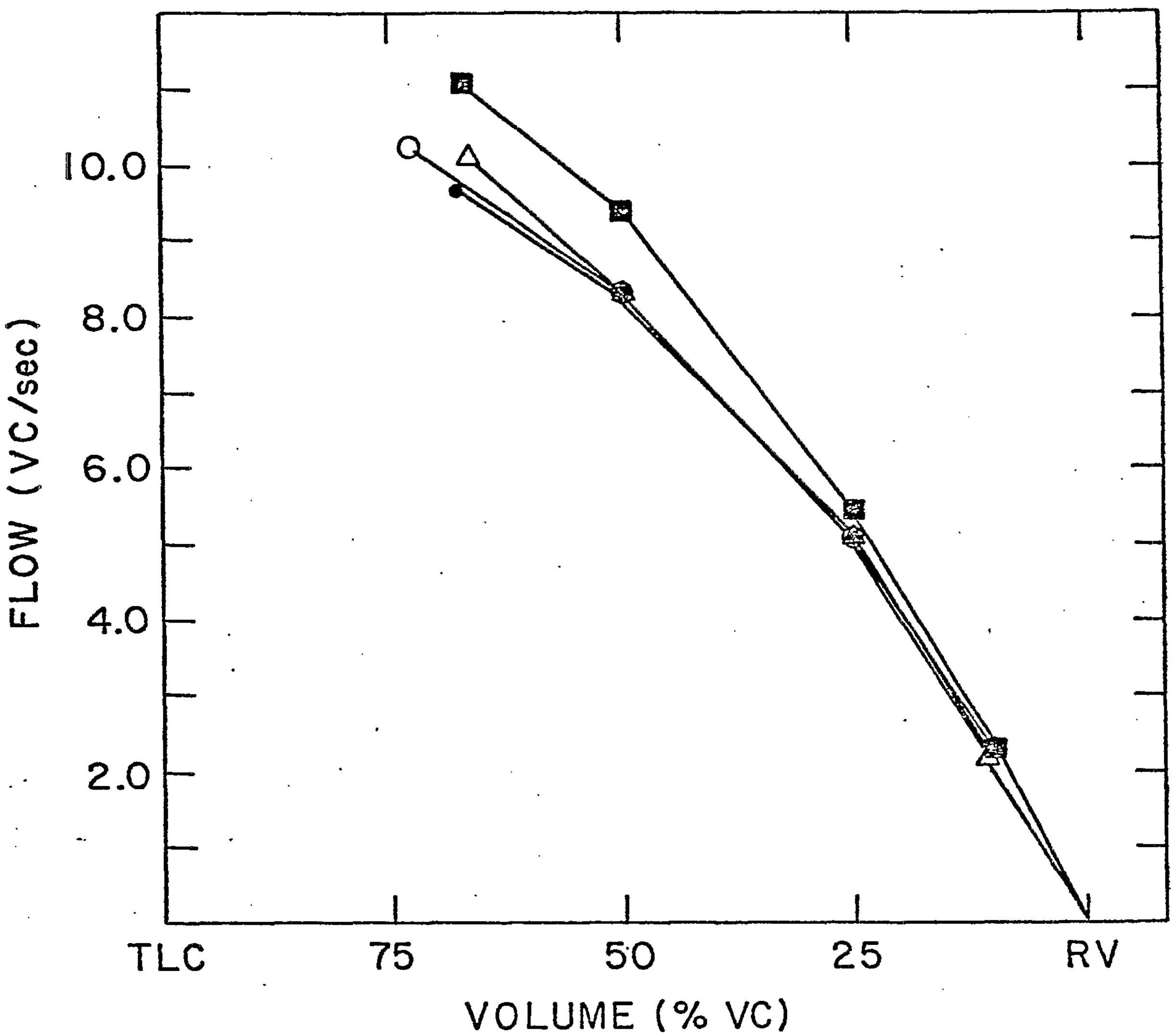

Figure 11: Maximum expiratory flow-volume curves of Fischer-344 rats exposed to silica for 6 months (6 hours/day, 5 days/week) then maintained in animal rooms for six months.

● Control, n = 22.
○ 2 mg SiO_2/m^3, n = 21.
△ 10 mg SiO_2/m^3, n = 23.
■ 20 mg SiO_2/m^3, n = 23.

The p value of the F-statistic from one-way MANOVA = 0.0287.

Table 12. Points from the MEFV Curve of Control and Silica Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	22	21	23	23	
PEF (cm^3/sec)					
mean	110.1[b]	113.3	113.5	110.7	0.7058
s.e.	2.2	2.3	2.6	2.9	
EFR_{50} (cm^3/sec)					
mean	92.9	91.5	93.6	93.9	0.8784
s.e.	2.0	2.9	2.1	2.0	
EFR_{25} (cm^3/sec)					
mean	54.3	56.4	56.6	54.0	0.6624
s.e.	2.2	1.9	1.5	1.8	
EFR_{10} (cm^3/sec)					
mean	23.8	27.0	24.8	21.6	0.0126[c]
s.e.	1.4	1.0	1.1	1.1	
multiple comparison[d]		HD CN ID LD			
ΔEFR_{25} (cm^3/sec)					
mean	7.8	10.6	9.8	7.1	0.1948
s.e.	1.7	1.2	1.0	1.3	

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for six months.
[b] n=21.
[c] Statistically significant at $\alpha = 0.05$ level using ANOVA.
[d] Pairwise comparison of means by the Duncan multiple range method.

The calculation of R_{us}, by relating MEFV and QSC at points of equal volume, did not reveal any silica-induced distortion of the effort independent flow during maximum expiration (Table 13). Changes in absolute flow rate, P_{st}, or R_{us} were not apparent. Augmentation of the MEFV curve with a low density $He{:}O_2$ mixture indicated the same type of apparent flow enhancement at high lung volumes as was revealed with air (Table 14).

Roentgenographic Findings

Evidence of silica-induced lung disease could be ascertained from the single frontal chest x-ray only in animals from the highest exposure group, 20 mg SiO_2/m^3. These radiograms, read without knowledge of group origin, generally exhibited a diffuse "haziness", best described as a ground-glass appearance with some peripheral striation. While not all of the rats in this exposure group presented this same impression, as a group they were clearly distinct from the other exposure and control groups. The control, 2 and 10 mg/m^3 did not exhibit noticeable abnormal radiographic densities and they could not be distinguished on the basis of treatment regime.

Lung Composition

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 E.

Lung Weight and Water Content. Although the 20 mg SiO_2/m^3 exposed animals did not differ in body weight from any other group these animals had significantly heavier lungs (Table 15). The fresh lung weight of these high dose animals was 189% of their control counterparts and the dry weight was double that of the controls. Although the lungs of the 20 mg SiO_2/m^3 animals increased markedly, the fresh to dry weight ratios did not change (Table 15).

Table 13. 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	21	21	21	23	
$\dot{V}_{30}$ (cm^3/sec)					
mean	62.5	65.3	65.7[b]	64.0	0.7151
s.e.	2.5	2.5	1.6	1.7	
P_{st} (cm H_2O)					
mean	20.5	19.0	22.7	22.4	0.1545
s.e.	1.2	1.2	1.4	1.3	
R_{us}					
mean	0.340	0.298	0.353	0.355	0.3074
s.e.	0.026	0.020	0.027	0.021	

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] n=23.

Table 14. 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	22	21	23	23	
$\Delta HEFR_{50}$ (cm^3/sec)					
mean	17.9	24.9	17.3	15.5	0.1095
s.e.	1.8	5.4	1.1	1.3	
$\Delta HEFR_{50}$ (VC/sec)					
mean	1.6	2.3	1.5	1.5	0.1832
s.e.	0.2	0.5	0.1	0.1	
$\Delta HEFR_{25}$ (cm^3/sec)					
mean	10.7	14.0	8.8	8.3	0.1989
s.e.	1.3	3.3	1.7	1.3	
$\Delta HEFR_{25}$ (VC/sec)					
mean	0.96	1.26	0.79	0.83	0.2978
s.e.	0.12	0.31	0.15	0.13	
Isoflow (% VC)					
mean	7.4[b]	8.9[c]	8.1[d]	7.9	0.9757
s.e.	1.5	2.9	2.2	1.8	

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] n=19.
[c] n=20.
[d] n=22.

Table 15. 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	24	23	23	23	
BODY WEIGHT (g)					
mean	389.3	370.4[b]	389.5[b]	382.5[b]	0.0229[c]
s.e.	5.3	4.1	5.8	4.2	
multiple comparison[d]		LD HD	CN ID		
LUNG WEIGHT (g)					
mean	1.40	1.48	1.48	2.64	<0.0001[c]
s.e.	0.02	0.02	0.02	0.08	
multiple comparison[d]		CN ID	LD HD		
LUNG-TOTAL DRY WEIGHT (mg)					
mean	301.6	319.6	324.9	599.3	<0.0001[c]
s.e.	7.3	4.6	7.0	18.6	
multiple comparison[d]		CN LD	ID HD		
LUNG-% DRY WEIGHT					
mean	21.57	21.54	22.09	22.74	0.1814
s.e.	0.47	0.23	0.52	0.46	

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

Lung Tissue Components. As would be expected with such substantial increases in lung dry weight, in the high dose animals, significant increases were also observed in all of the total amounts of the tissue components; protein, DNA, elastin and collagen (Table 16). In those animals exposed to 10 mg SiO_2/m^3 the amount of pulmonary DNA was also increased to 111% of control levels (Table 16). Total elastin was increased to 108, 109, and 140% of control elastin levels in the 2, 10, and 20 mg SiO_2/m^3 exposed animals, respectively (Table 16). Total lung collagen increased in a dose dependent manner in all of the exposure groups (Table 16). The amount of collagen in the lungs of rats exposed to 20 mg SiO_2/m^3 was 174% of the control lungs.

When the assayed tissue components were expressed in terms of dry weight, the 20 mg SiO_2/m^3 group consistently had the lowest concentration of each component (Table 17). This indicated that a tissue component, which was not analysed for, was dramatically increased by this exposure regime but not by exposure to either 2 or 10 mg SiO_2/m^3 (Table 17). The significant dose dependent increase observed in total hydroxyproline was also seen when expressed on the basis of dry weight with the exception of the 20 mg SiO_2/m^3 group (Table 17).

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

Table 16. Lung Composition of Control and Silica-Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	24	23	23	23	
TOTAL PROTEIN (mg)					
mean	189.5	200.5	202.6	294.3	<0.0001[b]
s.e.	4.6	3.0	4.6	10.1	
multiple comparison[c]		CN LD ID HD			
TOTAL DNA (mg)					
mean	6.2	6.7	6.9	9.6	<0.0001
s.e.	0.2	0.1	0.1	0.3	
multiple comparison[c]		CN LD ID HD			
TOTAL ELASTIN (mg)					
mean	7.7	8.3	8.4	10.8	<0.0001[b]
s.e.	0.2	0.1	0.2	0.2	
multiple comparison[c]		CN LD ID HD			
TOTAL HYDROXYPROLINE (mg)					
mean	2.88	3.19	3.47	5.06	<0.0001[b]
s.e.	0.07	0.06	0.09	0.14	
multiple comparison[c]		CN LD ID HD			

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] Statistically significant at $\alpha = 0.05$ level.
[c] Pairwise comparison of means by the Duncan multiple range method.

Table 17. Lung Composition Expressed as a Function of Dry Weight of Control and Silica-Exposed[a] Fischer-344 Rats

	Silica Concentration (mg/m^3)				
	0	2	10	20	p value
n	24	23	23	23	
PROTEIN (mg)/DRY WEIGHT (g)					
mean	631.9	627.3	623.3	491.0	<0.0001[b]
s.e.	4.7	2.6	3.3	7.0	
multiple comparison[c]		HD ID LD CN			
DNA (mg)/DRY WEIGHT (g)					
mean	20.8	20.9	21.3	16.0	<0.0001[b]
s.e.	0.1	0.1	0.2	0.2	
multiple comparison[c]		HD CN LD IN			
ELASTIN (mg)/DRY WEIGHT (g)					
mean	25.8	26.0	25.7	18.3	<0.0001[b]
s.e.	0.2	0.2	0.2	0.4	
multiple comparison[c]		HD ID CN LD			
HYDROXYPROLINE (mg)/DRY WEIGHT (g)					
mean	9.6	10.0	10.7	8.5	<0.0001[b]
s.e.	0.1	0.1	0.1	0.1	
multiple comparison[c]		HD CN LD ID			

[a] Six hours/day, 5 days/week, for 6 months, then maintained in animal rooms for 6 months.
[b] Statistically significant at $\alpha = 0.05$ level.
[c] Pairwise comparison of means by the Duncan multiple range method.

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.

Pathology of the Lungs and Peribronchial Lymph Nodes. The pathology observed in the lungs and peribronchial lymph nodes from the animals designated for pathology and multiple endpoints was not different. Microscopic examination of these tissues revealed minimal to moderately severe accumulations of histiocytes near the end airways (alveolar ducts) in the lungs of most of the animals in the 10 and 20 mg SiO_2/m^3 group (Tables 18 and 19). These alveolar macrophages had foamy cytoplasm in which small (1 to 2 μ) birefringent crystals, presumably phagocytized silica particles, could occasionally be seen. The reaction seen around the end airway was often accompanied by an infiltration of mononuclear cells and granulocytes. Type II cell hyperplasia was evident in alveoli surrounding the affected end airways. In addition, in the 20 mg SiO_2/m^3 group focal fibrosis with fibrotic aggregates and mononuclear cells forming "silicotic nodules" were more common as was alveolar proteinosis. Lymphoid proliferations observed around bronchioles and blood vessels often contained intralymphatic microgranulomas composed of aggregates of eosinophilic macrophages. Birefringent crystals could be seen in some of these. Presumably these particles ascended the pulmonary lymphoid chains to the peribronchial lymph nodes where eosinophilic microgranulomas were abundant. A generalized reticuloendothelial cell hyperplasia was also evident in the peribronchial lymph nodes in addition to similar eosinophilic macrophages forming micrcogranulomas, sometimes with intracytoplasmic birifringent crystals. Sections of the trachea and nasal turbinates contained no significant changes.

Most of the lungs from animals in the 2 mg SiO_2/m^3 group contained a few pulmonary microgranulomas while larger numbers were observed in the peribronchial lymph nodes (Tables 18 and 19). The end-airway reaction was negative to slight.

In the control group no pulmonary microgranulomas or end-airway reactions were observed. One lung tumor was observed in the study and it was in a control animal (Table 19).

To graphically examine the severity of the scored lesions in animals from the multiple endpoint subgroups, the severity value for each lung lesion observed in the individual animals (from Table 17) was summed to provide a pathology score. The score for birefringent crystals was not included. The frequency of each score within the four exposure groups has been illustrated in Figure 12. Statistical assessment of these scores using the Kruskal-Wallis non-parametric test indicated a significant difference ($H = 28.31$, $p < 0.0001$) among the groups. Dunn's rank sum multiple comparison method indicated that the scores from the 10 and 20 mg SiO_2/m^3 groups were significantly higher than those of the control groups.

Pathology of Non-Respiratory Tissues. The changes observed in the peribronchial lymph nodes have been reported above. The changes observed in the brain, kidneys, liver, heart, spleen and testis (Table 18) were considered incidental or spontaneous lesions of the laboratory rodent and not related to silica exposure.

HISTOPATHOLOGY INCIDENCE TABLE

Table 18
Multiple Endpoint Animals

Control Group (L)

ANIMAL NUMBER	L17	L18	L19	L20	L21	L22	L23	L24	L41	L42	L43	L44
LUNG												
Broncho/Alveolar Carcinoma												
Lymphoid Proliferations	2	1	2	2	2	1	2	1	1	1	2	1
End Airways Cellular Aggregates	1			1								1
Alveolar Histiocytosis, Focal												
Type II Cell Hyperplasia												
Intralymphatic Microgranulomas												
Mononuclear Cells												
Fibrosis												
Granulocytes												
Birefringent Crystals												
Alveolar Proteinosis												
TRACHEA	X	N	X	X	X	X	X	X		X	X	X
Chronic Tracheitis									1			
PERIBRONCHIAL LYMPH NODE												
Lymphoid Hyperplasia	2	1		2	1	2						
Reticuloendothelial Cell Hyperplasia			2									2
Pigmentation				1	1		2	1	1	2	1	2
Congestion					2		2	1	2	2	1	2
Microgranulomas											3	
Birefringent Crystals												
NASAL TURBINATE	N	N	N	N	N	N	N	N	N	N	N	N
Submucosal Lymphoid Infiltrate												

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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 18
Multiple Endpoint Animals

Control Group (L)

ANIMAL NUMBER	L45	L46	L47	L48	L65	L66	L67	L68	L69	L70	L71	L72
LUNG												
Broncho/Alveolar Carcinoma												
Lymphoid Proliferations	1	2	1	2	2	2	1	1	2	1	1	2
End Airways Cellular												
Aggregates	1											
Alveolar Histiocytosis, Focal												
Type II Cell Hyperplasia												
Intralymphatic Microgranulomas												
Mononuclear Cells												
Fibrosis												
Granulocytes												
Birefringent Crystals												
Alveolar Proteinosis												
TRACHEA	N	X	N	N	X	X	X	X	X	X	X	X
Chronic Tracheitis												
PERIBRONCHIAL LYMPH NODE	X	X					X		X	X		
Lymphoid Hyperplasia				2	2							
Reticuloendothelial Cell												
Hyperplasia												
Pigmentation			2			2					1	2
Congestion			3			2		1			1	
Microgranulomas												
Birefringent Crystals												
NASAL TURBINATE	N	N	N	N	N	N	N	N	N	N	N	N
Submucosal Lymphoid Infiltrate												

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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

Table 18
Multiple Endpoint Animals

2 mg SiO_2/m^3 Group (A)

ANIMAL NUMBER	A17	A18	A19	A20	A21	A22	A23	A24	A41	A42	A44	A45
LUNG												
Broncho/Alveolar Carcinoma												
Lymphoid Proliferations	1	2	1	2	1	2	1	1	1	2	2	2
End Airways Cellular												
Aggregates	1	2	2	1	1	1	1	1	1	1	2	2
Alveolar Histiocytosis, Focal			1								2	2
Type II Cell Hyperplasia			1								1	1
Intralymphatic Microgranulomas		2	1	2		2				2	2	2
Mononuclear Cells											2	1
Fibrosis												
Granulocytes												
Birefringent Crystals												
Alveolar Proteinosis												
TRACHEA	N	N	N	N	N	X	N	X	X	N	N	X
Chronic Tracheitis												
PERIBRONCHIAL LYMPH NODE												
Lymphoid Hyperplasia	3	2	2	3	2	3	2	2	2	2	3	2
Reticuloendothelial Cell												
Hyperplasia	3				2	3				2	2	2
Pigmentation												
Congestion												
Microgranulomas	4	3	3	4	3	4	2	3	3	3	4	3
Birefringent Crystals	P											
NASAL TURBINATE	N	N	N	N	N	N	N	N	N	N	N	N
Submucosal Lymphoid Infiltrate												

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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 18
Multiple Endpoint Animals

2 mg SiO_2/m^3 Group (A)

ANIMAL NUMBER	A46	A47	A48	A65	A66	A67	A68	A69	A70	A71	A72	A92
LUNG												
Broncho/Alveolar Carcinoma												
Lymphoid Proliferations	1	2	1	2	2	2	1	1	2	2	2	2
End Airways Cellular												
Aggregates	1	1	1		1	1	1	1	2	1	1	
Alveolar Histiocytosis, Focal						1			1			
Type II Cell Hyperplasia					1	1			1			
Intralymphatic Microgranulomas		1		2	1	2			2	2	2	
Mononuclear Cells												
Fibrosis												
Granulocytes												
Birefringent Crystals												
Alveolar Proteinosis												
TRACHEA	X	X	X	X	X	X	X	X	X	N	X	X
Chronic Tracheitis												
PERIBRONCHIAL LYMPH NODE											I	N
Lymphoid Hyperplasia	2		2		3		2	3	3	2		
Reticuloendothelial Cell												
Hyperplasia			2		3		3	3	3			
Pigmentation		2				2						
Congestion		3				3						
Microgranulomas	3		3	4	3		4	4	4	3		
Birefringent Crystals												
NASAL TURBINATE	N	N	N	N	N	N	N	N	N	N	N	N
Submucosal Lymphoid Infiltrate												

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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 18
Multiple Endpoint Animals

10 mg SiO_2/m^3 Group (W)

ANIMAL NUMBER	W17	W18	W19	W20	W21	W22	W23	W24	W41	W42	W43	W44
LUNG												
Broncho/Alveolar Carcinoma												
Lymphoid Proliferations		3	1	2	3	2	4	3	3	3	3	3
End Airways Cellular Aggregates		2	2	3	2	3	3	1	2	3	2	1
Alveolar Histiocytosis, Focal	2	2	2	2	2	2	2	2	3	3	2	1
Type II Cell Hyperplasia	2	2	2	2	2	2	2	2	2	3	2	1
Intralymphatic Microgranulomas		4		3	3		3	3	3	4	4	3
Mononuclear Cells		2	2	2	2	2	2	2	2	2	2	1
Fibrosis		2	3	2	2	3	2	1	1	3	2	1
Granulocytes									2	2		
Birefringent Crystals											P	
Alveolar Proteinosis												
TRACHEA	X	X	N	X	X	X	N	N	N	X	X	X
Chronic Tracheitis												
PERIBRONCHIAL LYMPH NODE												
Lymphoid Hyperplasia	3		3	3	3	3	4	3	3			2
Reticuloendothelial Cell Hyperplasia		2			3				3	4	4	2
Pigmentation		2										
Congestion		2										
Microgranulomas	5	3	5	5		5	5	5	5	3	5	
Birefringent Crystals	P		P	P		P	P	P	P	P		
NASAL TURBINATE	N	N	N	N	N	N	N	N	N	N	N	N
Submucosal Lymphoid Infiltrate												

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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 18
Multiple Endpoint Animals

10 mg SiO_2/m^3 Group (W)

ANIMAL NUMBER	W45	W46	W47	W65	W66	W67	W68	W69	W70	W71	W72
LUNG											
Broncho/Alveolar Carcinoma											
Lymphoid Proliferations	4	3	3	3	3	3	2	2	2	3	1
End Airways Cellular Aggregates	3	2	2	2	3	3	2	2	2	2	2
Alveolar Histiocytosis, Focal	2	1	1	1	2	2	2	2		2	2
Type II Cell Hyperplasia	1	2	1	1	2	2	2	2		2	
Intralymphatic Microgranulomas	4	4	3	3	3	3	2	2	1	4	
Mononuclear Cells	2	1	2	1	2	2	1			2	
Fibrosis	3	1	2	2	2	3	2	2	1		2
Granulocytes	1				1		1				
Birefringent Crystals	P			P	P						
Alveolar Proteinosis											
TRACHEA	X	X	X	X	X	X	N	N	X	N	N
Chronic Tracheitis											
PERIBRONCHIAL LYMPH NODE											
Lymphoid Hyperplasia			3	3		2		3	3	3	3
Reticuloendothelial Cell Hyperplasia	2	1	4	3	2			4	4	4	4
Pigmentation	2				2	3					
Congestion	2	3				3					
Microgranulomas			5	5			5	4	4	4	4
Birefringent Crystals			P	P			P		P	P	P
NASAL TURBINATE	N	N	N	N	N	N	N	N	N	N	N
Submucosal Lymphoid Infiltrate											

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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 18
Multiple Endpoint Animals

20 mg SiO_2/m^3 Group (N)

ANIMAL NUMBER	N17	N18	N19	N20	N21	N22	N23	N24	N41	N42	N43	N44
LUNG												
Broncho/Alveolar Carcinoma												
Lymphoid Proliferations	2	2	2	3	4	4	3	3	2	3	3	3
End Airways Cellular												
Aggregates	2	2	2	3	2	2	2	2	2	2	2	3
Alveolar Histiocytosis, Focal	3	3	3	4	3	4	4	4	4	4	4	4
Type II Cell Hyperplasia	3	2	2	2	2	2	2	2	2	2	2	2
Intralymphatic Microgranulomas	2	3	3	4	5	5	4	4	3	4	4	4
Mononuclear Cells	2	2	2	2	2	2	2	2	2	2	2	2
Fibrosis	1	2	2	2	1	2	2	2	2	2	1	2
Granulocytes	2		1	1		1	1	1			1	
Birefringent Crystals												
Alveolar Proteinosis	4	4	2	4	3	4	3	5	5	4	5	3
TRACHEA	X	X	X	X	X	X	N	X	X	X	X	X
Chronic Tracheitis												
PERIBRONCHIAL LYMPH NODE				N								
Lymphoid Hyperplasia	2					4		3		3		3
Reticuloendothelial Cell												
Hyperplasia	2				3	5	2	3	4	4		2
Pigmentation		2	1								1	
Congestion		2	2								2	
Microgranulomas	2				3	4	2	3	3	4		
Birefringent Crystals						P			P			
NASAL TURBINATE	N	N	N	N	N	N	N	N	N	N	N	N
Submucosal Lymphoid Infiltrate												

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 18
Multiple Endpoint Animals

20 mg SiO_2/m^3 Group (N)

ANIMAL NUMBER	N445	N446	N447	N448	N465	N466	N467	N468	N469	N470	N471	N472
LUNG												
Broncho/Alveolar Carcinoma												
Lymphoid Proliferations	4	4	2	3	3	3	4	4	3	2	3	4
End Airways Cellular Aggregates	2	2		2	3	3	3	3	3	2	3	3
Alveolar Histiocytosis, Focal	3	4	3	4	4	4	4	3	2	3	3	3
Type II Cell Hyperplasia	2	2	2	2	2	2	3	3	2	2	2	3
Intralymphatic Microgranulomas	4	4	3	4	3	4	5	5	4		4	5
Mononuclear Cells	2	2		2	2	2	2	2	2	2	2	2
Fibrosis	2	2		2	3	3	3	3	2	2	2	3
Granulocytes	1	1			1	1	1	1	1		1	1
Birefringent Crystals												P
Alveolar Proteinosis	2	4	3	5	3	5	4	5	3	3	5	4
TRACHEA	X	X	N	N	X	X	N	X	X	X	X	X
Chronic Tracheitis												
PERIBRONCHIAL LYMPH NODE		I				I						
Lymphoid Hyperplasia	4		4		3		3	3	2			
Reticuloendothelial Cell Hyperplasia	5		5	4	5		3	4		2	3	
Pigmentation									2		1	2
Congestion									3	2	2	3
Microgranulomas	5		4	3	4		3	5	2			
Birefringent Crystals	P		P	P	P			P				
NASAL TURBINATE	N	N	N	N	N	N	N	N	N	N	N	N
Submucosal Lymphoid Infiltrate												

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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 19
Pathology Animals

ANIMAL NUMBER	Control Group (L)								2 mg SiO_2/m^3 Group (A)						
	L89	L90	L91	L92	L93	L94	L95	L96	A89	A90	A91	A93	A94	A95	A96
LUNG															
Broncho/Alveolar Carcinoma	P														
Lymphoid Proliferations	2	2	2	2	1	2	1	1	2	2	2	2	2	2	
End Airways Cellular															
Aggregates									1	1	1	1	1	1	
Alveolar Histiocytosis, Focal												2			2
Type II Cell Hyperplasia									1		1	2			2
Intralymphatic Microgranulomas										2	2	2	2	1	2
Mononuclear Cells												1			
Fibrosis															
Granulocytes															
Birefringent Crystals															
Alveolar Proteinosis															
TRACHEA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chronic Tracheitis															
PERIBRONCHIAL LYMPH NODE	N				N		X								I
Lymphoid Hyperplasia									3	3	3	3	3	3	
Reticuloendothelial Cell															
Hyperplasia										3		3			
Pigmentation		2	1	1		2									
Congestion		2		1		3									
Microgranulomas				1				1	3	4	4	4	4	3	
Birefringent Crystals															

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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 19
Pathology Animals

ANIMAL NUMBER	Control Group (L)								2 mg SiO_2/m^3 Group (A)						
	L89	L90	L91	L92	L93	L94	L95	L96	A89	A90	A91	A93	A94	A95	A96
NASAL TURBINATE	X	X			X			X	X	X		X			
Submucosal Lymphoid Infiltrate			2	2		2	1				2		2	2	2
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
KIDNEY					X										
Chronic Nephritis	2	2	2	1		2	2	1	2	2	2	3	2	2	2
Tubular Casts	2	2	2			2	2	1			2	2	2	2	2
LIVER	X				X	X			X		X	X	X	X	X
Chronic Pericholangitis		2	2	1			2	1							
Necrotic Hepatitis															
Neoplastic Nodule										P					
HEART			X		X	X	X	X	X	X			X	X	
Chronic Myocarditis, Focal	1	2		2							2	2			2
Myocardial Degeneration															
SPLEEN		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hemosiderosis	2														
TESTIS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interstitial Cell Tumor															
MEDIASTINAL LYMPH NODE															
Lymphoid Hyperplasia	3														
Pigmentation	2														

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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

Table 19
Pathology Animals

ANIMAL NUMBER	10 mg SiO_2/m^3 Group (W)								20 mg SiO_2/m^3 Group (N)							
	W89	W90	W91	W92	W93	W94	W95	W96	N89	N90	N91	N92	N93	N94	N95	N96
LUNG																
Broncho/Alveolar Carcinoma																
Lymphoid Proliferations	3	4	2	3	2	2	2	2	3	4	4	4	5	4	4	3
End Airways Cellular Aggregates	3	3	2	2	2	2	2	2	3	3	3	2	2	3	3	2
Alveolar Histiocytosis, Focal	2	2	3	2	3	2	2	2	3	3	3	3	3	3	4	3
Type II Cell Hyperplasia	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	2
Intralymphatic Microgranulomas	4	4	2	2	3	3	3	3	4	5	5	5	5	5	4	4
Mononuclear Cells	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2
Fibrosis	3	3	2	3	3	3	2	2	3	2	3	3	2	3	3	2
Granulocytes	1	1	1	1	1				1		1	1		2	2	1
Birefringent Crystals		P		P		P				P		P	P	P	P	P
Alveolar Proteinosis									4	4	4	4	5	4	4	4
TRACHEA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chronic Tracheitis																
PERIBRONCHIAL LYMPH NODE													I			
Lymphoid Hyperplasia	4	3	4	4	4	4	3	4	2	4	4	3		3	3	3
Reticuloendothelial Cell Hyperplasia	4	4	4	4	5	5	5	4		3	4	5		5	5	5
Pigmentation																
Congestion									2							
Microgranulomas	4	4	5	5	4	5	3	4	2	5	5	5		5	5	5
Birefringent Crystals	P	P	P	P	P	P	P	P		P	P	P		P	P	P

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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 19
Pathology Animals

ANIMAL NUMBER	10 mg SiO_2/m^3 (Group (W)								20 mg SiO_2/m^3 Group (N)							
	W89	W90	W91	W92	W93	W94	W95	W96	W89	W90	W91	W92	W93	W94	W95	W96
NASAL TURBINATE		X		X	X		X	X			X			X		
Submucosal Lymphoid Infiltrate	2		2			2			3	2		2	2		2	2
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
KIDNEY									X							X
Chronic Nephritis	2	2	2	2	3	2	2	2		3	2	2	2	2	2	
Tubular Casts	2	2	2	2	2	2	2	2								
LIVER	X	X	X	X		X	X		X				X	X	X	
Chronic Pericholangitis					2			2		1	2					2
Necrotic Hepatitis												2				
Neoplastic Nodule																
HEART	X		X	X	X	X		X			X		X			X
Chronic Myocarditis, Focal		1					2		2	3		2		1	2	
Myocardial Degeneration										3						
SPLEEN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hemosiderosis																
TESTIS		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interstitial Cell Tumor	P															

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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

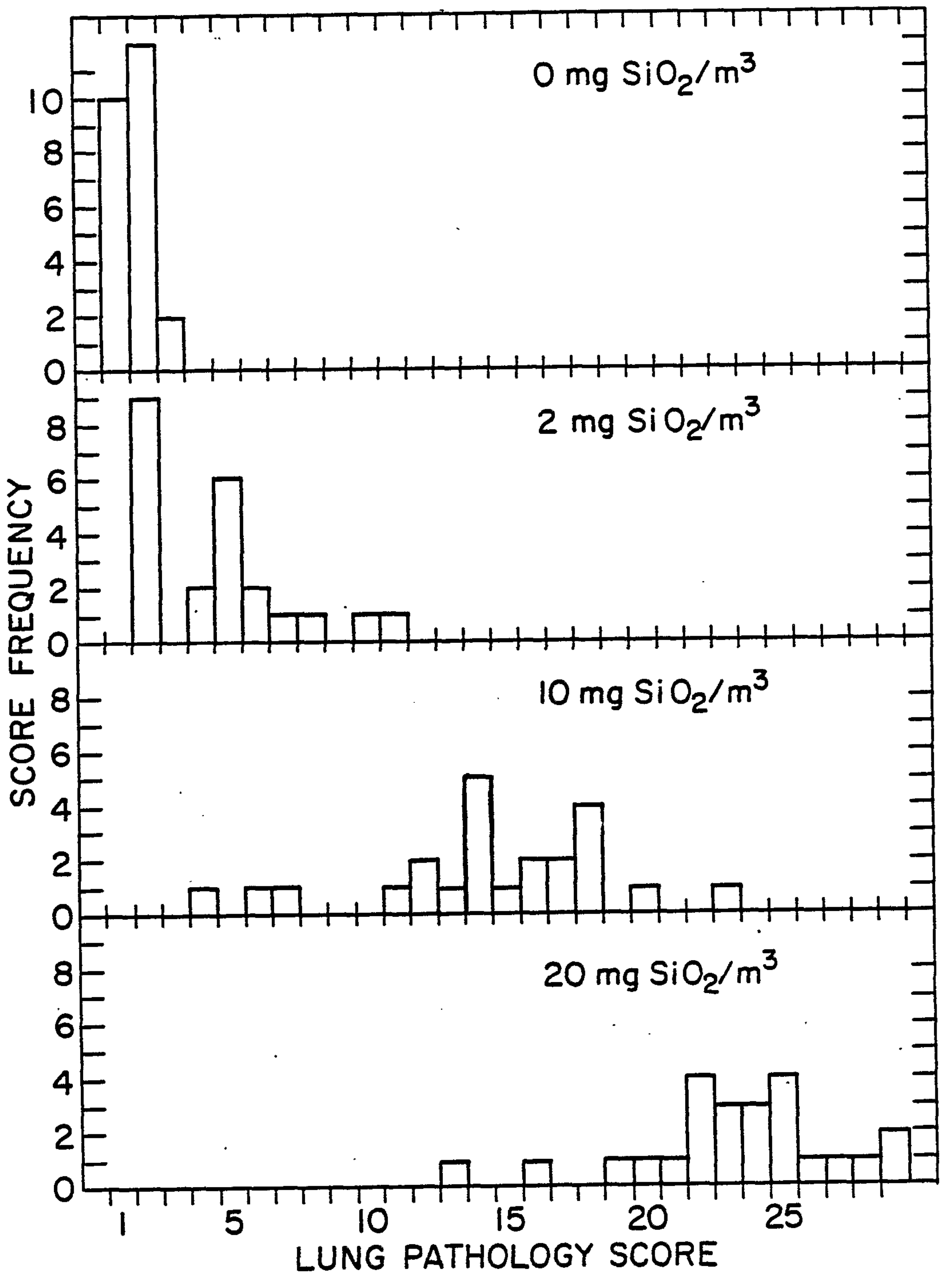

Figure 12: Frequency of lung pathology scores of Fischer-344 rats exposed to filtered air or silica dust for 6 months (6 hours/day, 5 days/week), and then maintained in animal rooms for 6 months (see text for details).

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 DNA/dry weight, protein/dry weight, hydroxyproline/dry weight, and total lung weight. The classification functions based on these variables were 73.1 percent successful in identifying the test animals as belonging to their appropriate exposure groups (Table 21). The classification functions performed best in identifying the 20 $mgSiO_2/m^3$ animals, being 100% correct.

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

PULMONARY FUNCTION VARIABLES

Parameters of Spontaneous Breathing

C_{dyn}
C_{dyn}/FRC_d
f
ΔP_L
R_L
$R_L \cdot FRC_d$
$\dot{V}_E$
V_T

Divisions of Lung Volume

ERV
FRC_b
FRC_d
FRC_d/TLC_b
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
M_1/M_0
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/VC (x = -10, -5, 0, 5, 10, 15, 20, 25).

Table 20, continued

Points on the MEFV Curve

EFR_x (x = 50, 25, or 10% VC)
EFR_x/VC (x = 50, 25, or 10% 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

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

Group	Number of Cases Classified into Group: 0	2	10	20	Percent Correct	Discriminating Variables
0	17	7	0	0	70.8	DNA/dry weight
2	7	12	4	0	52.2	Protein/dry weight
10	0	7	16	0	69.6	Hydroxyproline/dry weight
20	0	0	0	23	100.0	Lung weight
Total	24	26	20	23	73.1	

Pulmonary Function Data

Group	Number of Cases Classified into Group: 0	2	10	20	Percent Correct	Discriminating Variables
0	0	6	5	3	0.0	
2	2	9	6	0	52.9	DLCO
10	3	4	10	0	58.8	F
20	0	1	0	14	93.3	
Total	5	20	21	17	52.4	

Lung Composition and Pulmonary Function Data

Group	Number of Cases Classified into Group: 0	2	10	20	Percent Correct	Discriminating Variables
0	11	3	0	0	78.6	Protein/dry weight
2	4	9	4	0	52.9	DNA/dry weight
10	0	6	11	0	64.7	Hydroxyproline/dry weight
20	0	0	0	15	100.0	Lung weight
Total	15	18	15	15	73.0	

The pulmonary function variables with discriminating power in these studies were $DLCO_{rb}$ and f. When the animals were catagorized using the classification functions based on these variables only 52.4 percent of the animals were correctly classified (Table 21). Interestingly, none of the control animals were classified as controls while 93 percent of the 20 mg/m^3 group were correctly classified.

When the pulmonary function and lung composition data were combined only lung composition variables surfaced as having significant discriminating power. Overall, 73 percent of the animals were correctly classified. Again 100 percent of the 20 mg/m^3 group was correctly classified. The success rate varied slightly from that observed when only the composition variables were considered because all animals included in the analysis must have complete sets of data. If all of the variables to be considered were not available, the animal was deleted producing a slightly different result.

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 provided for the assessment of these endpoints after rats had been exposed to silica dust for three months, six months, and after exposure for six months followed by a six month holding period prior to assessment. The studies reported here address the data from those animals exposed for six months and then held for six months without further silica exposure before being assessed.

The mean lung weights of the animals exposed to 20 mg SiO_2/m^3 were greater than those of the controls and the lower exposure groups (Tables 3 and 4). Exposure to 2 and even 10 mg SiO_2/m^3 did not result in significantly increased fresh lung weights, lung-to-body weight ratios, or total lung dry weights. Exposure to 20 mg SiO_2/m^3 was necessary to affect these rather crude indicators of pulmonary toxicity.

The disparity between the mean weights of rats in the 2 mg/m^3 and 10 mg/m^3 groups was not considered to be exposure related. A dose response trend was not evident and the finding therefore considered spurious. Also a relevant explanation for the increased brain weights of all silica exposure groups is not available. Again there was not a dose-dependent trend and the disparity is eliminated when the data are considered on an organ-to-body weight basis.

There is a paucity of data available on the effects of inhaled silica dust on the constituents of the rat lung. In this study dose dependent increases were generally observed in total lung DNA, elastin, and hydroxyproline. However, when these lung components are expressed in terms of lung dry weight the responses were no longer dose-dependent. Instead the amount of protein, DNA, elastin, and hydroxyproline per unit dry weight decreased dramatically in the animals which had been exposed to 20 mg SiO_2/m^3. These data indicated that a tissue component which was not being assessed increased rather markedly as a result of this exposure regime (20 mg SiO_2/m^3, for 6 hours/day, 5 days/week, for six months, then maintained for six months without exposure prior to assessment). To determine what portion of the lungs was not being accounted for, the following assumptions were made, (1) that 5% of the total hydroxyproline measured by the assay employed comes from tissue components other than collagen(27) and that collagen is 13% hydroxyproline by weight.(28,29) Using these assumptions the total DNA, protein, elastin, and collagen accounted for 75% of the dry lung weight in the 0, 2, and 10 mg SiO_2/m^3 groups while only 59% of the dry weight of the 20 mg/m^3 group could be accounted for. The additional 16% of the dry weight in the high dose group was apparently the result of a unique increase of an unmeasured tissue component in this exposure group. It appears that increased amounts of lipid in these animals may have produced the observed results since increased pulmonary lipid has been noted in animals exposed to silica dust both by inhalation and instillation.(30-40) Although this is an inconsistent finding, it is usually the result of very high silica dosage by instillation or acute inhalation exposure. The increase in lipid content proposed here would indicate that exposure of SPF rats to 20 mg SiO_2/m^3 for six months with an equal amount of time for lesion development results in a disease state which more closely resembles acute silicosis in man rather than classical silicosis.

Morphologically, all exposure groups exhibited silica-induced lesions or deposited birefringent particles sufficient to identify the groups into their correct exposure categories when evaluated without knowledge of rat-group origin. Patchy thickening of the alveolar interstitium with concomitant development of fibrotic nodules was characteristic particularly in the highest dose group. The coexistence of lipoproteinosis and nodule formation conflicts with the early hypothesis of Heppleston[41] that these histopathologic phenomena are mutually exclusive. Some evidence of granulomata formation was apparent in the high-dose group most notably in lymphoid tissues and in association with significant accumulations of birefringent particles. Birefringent particles were also readily discernible within the tissue mass and the clustered histocytes.

The abrupt lung tissue response to silica which differentiated the 20 from the 10 mg/m^3 group was paralleled by significant decrement in the former group's overall physiologic competency. However, unlike the measures of tissue composition which revealed relatively minor, though statistically significant, alterations in the amounts of lung tissue and structural components at both 2 and 10 mg/m^3, no clear evidence of generalized dysfunction in lung mechanics or gas exchange was detected in animals exposed to less than the 20 mg SiO_2/m^3. Exposure to 20 mg SiO_2/m^3 resulted in a functional lesion which was largely restrictive in nature. Lung volumes were reduced, as was DL_{CO}, without remarkable airflow abnormality. Only the moment analysis of ventilation/distribution suggested a mild obstructive component of the disease which otherwise exhibited classical "fibrosis-like" dysfunction. Its possible that the presence of alveolar-filling lipo-proteinaceous material disrupted ventilation-homogenity which resulted in impaired N_2 displacement with tidal breathing of pure O_2.

Previous studies on the functional impact of silica on the rodent lung have utilized intratracheal instillation as the primary means of exposure.[40,42,43] In general, the results reported here agree with these earlier studies which relate to considerably higher lung tissue doses of silica than could have been obtained with the inhalation exposures utilized in this study. In addition, FRC and RV in these earlier reports generally increased[40,42,43], more typical of complications in end-stage disease in man and less characteristic of the pure silicotic disease state.[2,44] In the present study a consistent decrease in these volumes was observed after exposure to concentrations more comparable to those experienced by human workers.[44] In fact, the tissue composition alterations and the coexistence of the alveolar-fluid and nodular responses support the utility of the rat as a reasonable species for the study of the human disease. The relationship of the rat model of silicosis to human disease appears even stronger if applied to the more accelerated form of human silicosis, which has a significant component of lipo-proteinosis.[2,45]

While the functional, biochemical, and morphologic endpoints in the 20 mg/m^3 rats are fully compatible with one another and show significant correlation, at the lower exposure levels only the compositional and morphological analyses were effective in discerning and characterizing disease. At the lower concentrations innate compensation by the respiratory system appears to have been adequate to functionally mask the slowly progressive, diffuse fibrogenesis. Thus, no significant alteration in lung mechanics or interference with normal gas exchange was evident. Even at 20 mg/m^3, when much of the lung appeared to have accumulated lipo-proteinaceous material, the lung was able to supply the resting animal with sufficient gas exchange as indicated by normal blood-gas levels in the presence of significantly diminished diffusing capacity for CO.

Though generally consistent with infiltrative disease, the hazy pattern and occasional striations in the x-rays of the 20 mg/m^3 rats were largely non-specific. Whether this increased x-ray density was due to increased connective tissue or the material present in the alveoli could not be discerned.

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 compositional variables, DNA/dry weight, protein/dry weight, hydroxyproline/dry weight and total lung weight, had significant discriminating power, only two of the 63 functional variables entered had significant discriminating power, DL_{CO} and F. In addition the compositional variables correctly classified 73% of the animals by exposure group while 52% were correctly classified using only the functional variables.

Overall, the functional characterization of the animals, after exposure to 20 mg SiO_2/m^3, suggests that the lesion was almost exclusively parenchynal, with restricted lung volumes, reduced DL_{CO}, and minimally affected airways. Interestingly, what appeared to be considerable changes in lung composition after exposure to 2 and 10 mg SiO_2/m^3, particularly increased connective tissue, did not result in 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.

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

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):	

See Reverse Side for Explanation of Examinations and Abbreviations ALI Form 1001

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
New Hyde Park, N.Y. 11040
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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

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

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

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

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:	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.

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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.
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):	Thymus: 10/10 NML. Spleen: 10/10 NML.

See Reverse Side for Explanation of Examinations and Abbreviations ALI Form 1001

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.

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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

ALI Form 1008

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APPENDIX B

POST-EXPOSURE SEROLOGY PROFILE OF SUBJECT ANIMALS

The serology report bearing accession number 3955 presents the findings from four animals sacrificed six days after their final exposure. The sera were submitted to AnMed Laboratories (New Hyde Park, NY) for analysis.

The serology report bearing accession number 4334 presents the findings from eight animals sacrificed six months following their final exposure.

The microbiology report presents the finding after culturing swabs taken from the trachea of rats sacrificed six months following exposure to Min-U-Sil.5. These procedures were conducted at Brookhaven. The swabs were cultured on blood agar plates, inarbuted at 36^{o}C in a 5% CO_2 atmosphere, or on MacConky agar plates, in air at 35^{o}C. The plates were examined for growth after 18 to 24 hours of incubation.

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B-5 1804 Plaza Avenue
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SERAOLOGY REPORT

Client Organization: Brookhaven
Accession No.: 3955

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

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SEROLOGY REPORT

Client Organization Brookhaven

Accession No. 4334 SPW

Species Rat (8)

Date Received 16 February 1983

Group Designation

Date Completed 14 March 1983

	AnMed Ident:	1	2	3	4	5	6	7	8								
	Client Ident:	1289	1291	1493	1089	1091	1490	1689	1690								
	MVM																
ELISA	PVM	+	+	+	+	+	–	+	–								
HI	REO-3	–	–	–	–	–	–	–	–								
	MHV																
	KV																
HI	GD-7	–	–	–	–	–	–	–	–								
	RCV																
HI	SEN	–	–	–	–	–	–	–	–								
CF	LCM	–	–	–	–	–	–	–	–								
	SV5																
	MAV																
	ECTR																
	POLY																
HI	KRV	–	–	–	–	–	–	–	–								
HI	THI	–	–	–	–	–	–	–	–								
CF	SDAV	–	–	–	–	–	–	–	–								
	MYCO																
	ECUN																
	PMUL																
	TREP																

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MICROBIOLOGY REPORT

Culture No.(Rat No.)	Date	Source	Organisms Cultured
1089C	2-8-83	Trachea	Viridans streptocci Streptoccus group D Staphylococcus sp. Staphylococcus aureus
1091C	2-8-83	Trachea	None
1094C	2-8-83	Trachea	Streptococcus group D Citrobacter freundii
1095C	2-8-83	Trachea	Viridens streptococci
1289	2-9-83	Trachea	None
1290	2-9-83	Trachea	Staphylococcus aureas Viridans steptococci
1291	2-9-83	Trachea	Viridans streptococci
1489	2-9-83	Trachea	Viridans streptococci Staphylococcus sp.
1490	2-9-83	Trachea	Viridans streptococci Streptococcus group D Staphylococcus aureas
1491	2-9-83	Trachea	Viridans streptococci
1690	2-9-83	Trachea	Viridans streptococci Streptococcus group D Staphylococcus aureas
1491	2-9-83	Trachea	Pseudommas aeruginosa
1689	2-9-83	Trachea	Viridans streptococci
1690	2-9-83	Trachea	Viridans streptococci Staphylococcus sp.
1293	2-10-83	Trachea	Viridans streptoccoci Staphylococcus aureus
1492	2-10-83	Trachea	Staphylococcus sp.
1293	2-10-83	Trachea	Viridan streptococci Staphyloccus aureus
1492	2-10-83	Trachea	Staphylococcus sp.
1691	2-10-83	Trachea	Viridans streptococci
1693	2-10-83	Trachea	Staphylococcus aureus

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.

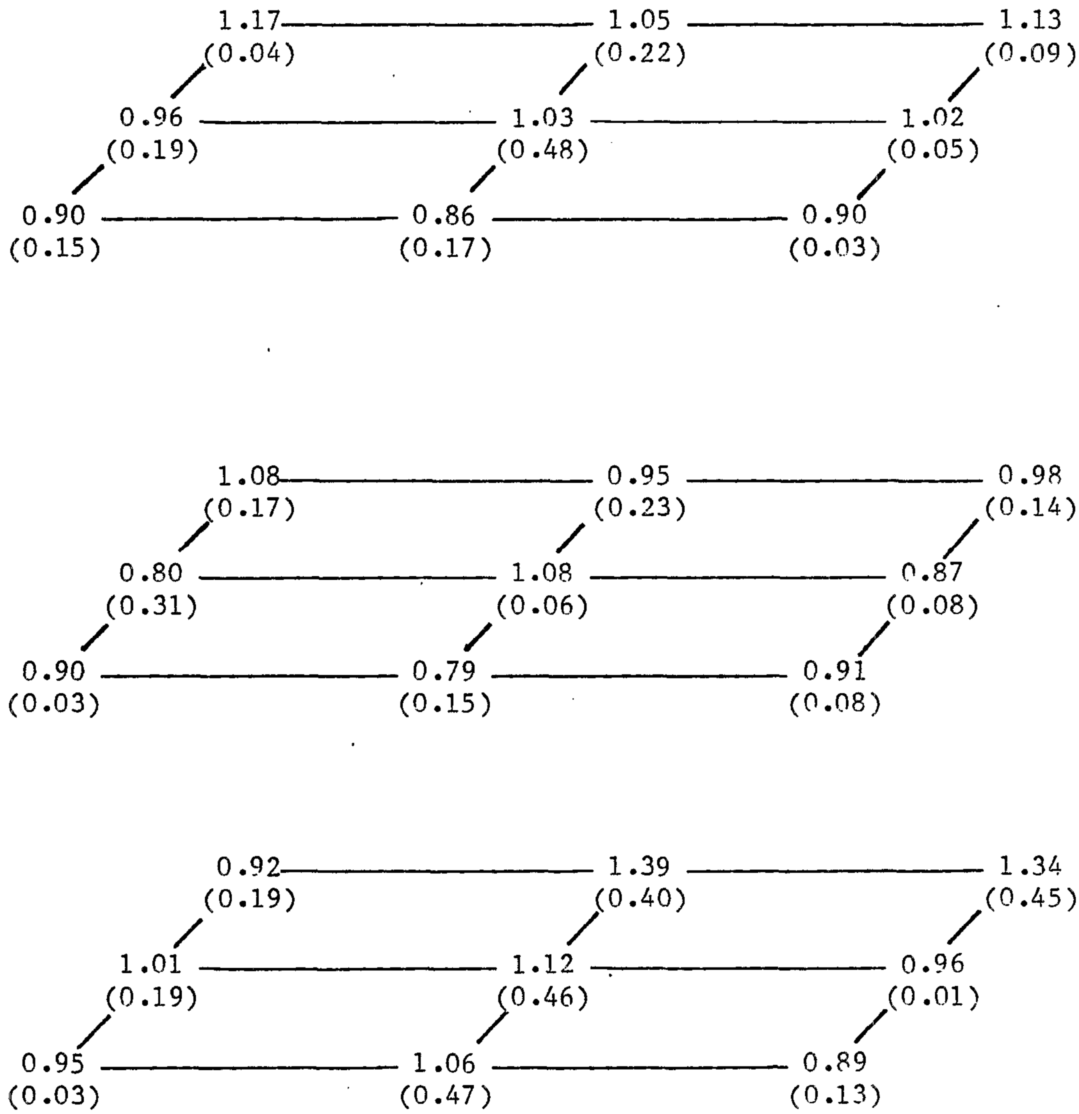

Figure C-1: Silica distribution in exposure chamber 5-C, the chamber used to expose animals to 10 mg SiO_2/m^3. Each value represents the mean (±s.e.)(n=3) decimal fraction, at a sampling station, of the average concentration throughout the chamber.

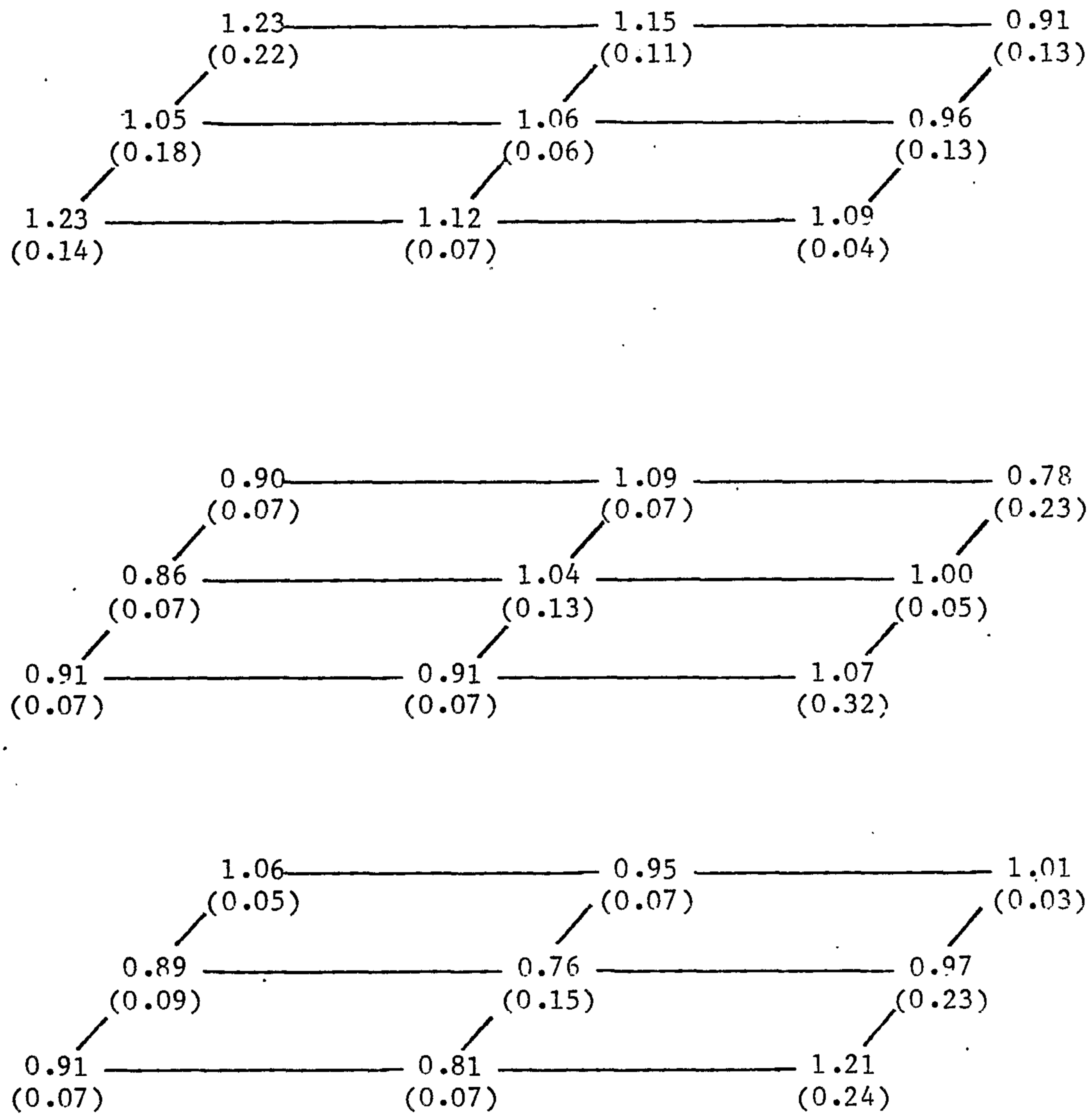

Figure C-2: Silica distribution in exposure chamber 5-D, the chamber used to expose animals to 20 mg SiO_2/m^3. Each value represents the mean (±s.e.)(n=3) decimal fraction, at a sampling station, of the average concentration throughout the chamber.

APPENDIX D

PULMONARY FUNCTION DATA FROM INDIVIDUAL FISCHER-344 RATS

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

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)	DHERFx
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/M_0	$\sum_{j=1}^{50} b_j \cdot X_j \Big/ \sum_{j=1}^{50} X_j$	M1MO
	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 sec^{-1})	RL
TLC_d	total lung capacity determined by dilution (cm^3)	TLCD
V_T	tidal volume (cm^3)	VT
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 -)	VOLNX
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	1017	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
2	1018	1.730	6.500	57	.730	.370	10.180	11.190	3.340	12.590	.177
3	1019	1.610	6	53	1.810	.230	10.850	11.540	3.050	12.470	.194
4	1020	2	6.500	63	.270	.370	11.040	11.280	3.360	12.460	.173
5	1021	1.790	6.500	53	.700	.340	11.160	12.230	3.770	13.480	.196
6	1022	1.680	5.750	67	1.040	.240	10.030	11.180	3.250	12.220	.159
7	1023	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
8	1024	1.440	4	71	.240	.450	9.720	11.190	2.880	12.220	.163
9	1041	1.500	4.380	58	.110	.420	8.830	10.200	3.440	11.360	.139
10	1042	1.380	4.580	92	.320	.330	9.760	11.160	3.620	12.480	.154
11	1043	1.470	4.250	53	.170	.410	9.750	11.100	3.290	12.110	.196
12	1044	1.380	4.250	108	.730	.310	9.370	10.950	2.640	12.100	.181
13	1045	1.920	6.830	66	MISSING	MISSING	11.060	11.960	2.790	13.490	.226
14	1046	1.660	5	69	.600	.430	10.410	11.300	3.160	12.410	.206
15	1047	2.300	6.170	73	1.140	.400	10.560	11.630	2.720	12.740	.185
16	1048	1.980	5.500	53	.300	.430	9.620	10.610	4.680	11.760	.168
17	1065	2.300	5.170	59	.690	.370	10.750	12.210	2.720	13.350	.167
18	1066	1.830	5	51	.150	.400	11.340	12.600	3.360	13.370	.174
19	1067	1.590	6.330	107	.190	.390	8.980	9.890	3.510	11.360	.153
20	1068	1.820	6	70	.100	.480	10.540	11.550	3.460	12.900	.196
21	1069	1.830	5.670	57	.170	.440	9.570	10.810	3.590	12.030	.165
22	1070	1.670	5.670	72	.270	.370	9.520	10.250	3.890	11.290	.183
23	1071	2	7	59	.490	.550	10.580	11.930	3.550	13	.190
24	1072	2.150	8	83	.320	.390	10.150	11.240	3.700	12.310	.214

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	1017	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	303
2	1018	16.200	54.550	.870	67.300	109	93.500	47.200	21.500	MISSING	303
3	1019	16.200	40.230	.940	72.600	109.100	77	35.900	17.200	8.800	331
4	1020	27	62.450	.930	65.200	101	96.400	49.600	15	12	358
5	1021	18.900	51.820	1.040	70.800	116.800	90	42.600	23	9	289
6	1022	20.930	60.550	1.020	72.300	106.400	90.500	51.200	23.200	6.800	296
7	1023	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
8	1024	15.530	68.180	.920	70.800	112.100	93.600	60.900	31.300	MISSING	MISSING
9	1041	MISSING	MISSING	.770	MISSING	MISSING	93.500	55.600	24.500	5.800	258
10	1042	13.500	65.450	.940	68.100	113.600	95.800	56	26	6.200	300
11	1043	14.850	77.450	.940	59.300	97.700	95.600	66.500	28	6.400	273
12	1044	14.850	62.050	.840	74.800	111.200	91.700	52	12.300	6	343
13	1045	15.300	71.730	1.030	69.300	123.100	105	65.500	27.700	8	348
14	1046	31.050	67.640	.930	60.400	100.600	98	58	33	8.100	343
15	1047	20.930	61.360	1.010	70.700	114.800	94.300	53	26.800	MISSING	MISSING
16	1048	14.850	75	.900	73.900	115.700	99.200	66.100	24.900	7.900	255
17	1065	20.930	75	.970	68.900	129.900	115.600	61.300	23.700	7	291
18	1066	26.330	37.090	1.040	83	122.400	69.500	26.100	5.400	9.100	289
19	1067	22.950	69.550	.880	60.700	93.800	89.500	62.300	31.900	6.800	348
20	1068	27.680	74.730	.970	43.700	95.400	95.400	68.800	32.700	6.700	298
21	1069	25.650	73.090	.970	64.400	101.900	95.700	63.200	23.300	6.700	340
22	1070	24.300	55.360	.880	70.400	102.100	78.800	51.800	21.800	10.100	MISSING
23	1071	14.850	48.540	.950	62.400	124.400	93.100	45	24.700	8	MISSING
24	1072	27	61.640	.900	MISSING	111.300	92.200	55.700	25.800	10.200	366

CONTROL GROUP

CASE NO.	LABEL	34 PR	35 QRS	37 VOLN15	38 VOLN1Ø	39 VOLN5	4Ø VOLØ	41 VOL5	42 VOL1Ø	43 VOL15	44 VOL2Ø
1	1Ø17	.Ø45Ø	.Ø138	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
2	1Ø18	.Ø413	.Ø125	Ø	.1ØØ	.95Ø	1	8.55Ø	9.9ØØ	1Ø.6ØØ	11
3	1Ø19	.Ø463	.ØØ88Ø	Ø	.19Ø	.39Ø	.69Ø	7.99Ø	1Ø.19Ø	1Ø.89Ø	11.29Ø
4	1Ø2Ø	.Ø475	.Ø138	Ø	Ø	Ø	.24Ø	8.29Ø	1Ø.Ø4Ø	1Ø.74Ø	11.Ø4Ø
5	1Ø21	.Ø425	.Ø113	Ø	.27Ø	.37Ø	1.Ø7Ø	8.87Ø	1Ø.87Ø	11.57Ø	11.87Ø
6	1Ø22	.Ø475	.Ø113	Ø	.15Ø	.15Ø	1.15Ø	8.6ØØ	1Ø.Ø5Ø	1Ø.6ØØ	1Ø.95Ø
7	1Ø23	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
8	1Ø24	MISSING	MISSING	Ø	.27Ø	.32Ø	1.47Ø	9.Ø2Ø	1Ø.17Ø	1Ø.77Ø	11.Ø7Ø
9	1Ø41	.Ø48Ø	.Ø16Ø	Ø	.Ø7ØØ	.32Ø	1.37Ø	7.57Ø	9.Ø7Ø	9.77Ø	1Ø.17Ø
1Ø	1Ø42	.Ø425	.Ø125	Ø	Ø	.2ØØ	1.4ØØ	8.15Ø	9.9ØØ	1Ø.55Ø	11
11	1Ø43	.Ø45Ø	.Ø125	Ø	.Ø5ØØ	.2ØØ	1.35Ø	8.Ø5Ø	9.85Ø	1Ø.35Ø	1Ø.95Ø
12	1Ø44	.Ø438	.ØØ88Ø	Ø	.18Ø	.18Ø	1.58Ø	8.Ø3Ø	9.68Ø	1Ø.28Ø	1Ø.78Ø
13	1Ø45	.Ø54Ø	.Ø15Ø	Ø	.1ØØ	.2ØØ	.9ØØ	8.65Ø	1Ø.6ØØ	11.25Ø	11.7ØØ
14	1Ø46	.Ø425	.ØØ9ØØ	Ø	.79Ø	.79Ø	.89Ø	8.59Ø	1Ø.19Ø	1Ø.79Ø	11.Ø9Ø
15	1Ø47	MISSING	MISSING	Ø	Ø	Ø	1.Ø6Ø	1Ø.36Ø	11.16Ø	11.41Ø	11.46Ø
16	1Ø48	.Ø525	.Ø125	Ø	Ø	Ø	.99Ø	9.19Ø	9.99Ø	1Ø.29Ø	1Ø.59Ø
17	1Ø65	.Ø538	.Ø175	Ø	Ø	1.21Ø	1.46Ø	1Ø.71Ø	11.56Ø	11.96Ø	12.Ø6Ø
18	1Ø66	.Ø475	.Ø115	Ø	.16Ø	.16Ø	1.26Ø	8.86Ø	11.16Ø	11.91Ø	12.26Ø
19	1Ø67	MISSING	MISSING	Ø	.2ØØ	.45Ø	.9ØØ	8.15Ø	9.1ØØ	9.6ØØ	9.9ØØ
2Ø	1Ø68	.Ø4ØØ	.Ø113	Ø	Ø	Ø	1.Ø1Ø	1Ø.96Ø	11.21Ø	11.36Ø	11.41Ø
21	1Ø69	.Ø475	.Ø138	Ø	.14Ø	.24Ø	1.24Ø	8.Ø9Ø	9.54Ø	1Ø.Ø9Ø	1Ø.64Ø
22	1Ø7Ø	MISSING	MISSING	Ø	.23Ø	.28Ø	.73Ø	7.48Ø	9.13Ø	9.88Ø	1Ø.13Ø
23	1Ø71	MISSING	MISSING	Ø	.Ø5ØØ	.15Ø	1.35Ø	8.5ØØ	1Ø.55Ø	11.25Ø	11.75Ø
24	1Ø72	.Ø488	.Ø125	Ø	.Ø9ØØ	.19Ø	1.Ø9Ø	7.64Ø	9.69Ø	1Ø.39Ø	1Ø.89Ø

CONTROL GROUP

CASE NO.	LABEL	45 VOL25	49 DHEFR50	50 DHEFR25	52 ISOFLOW	53 PCO2	54 PO2	55 PH	56 CO2RESP
1	1017	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	93.900
2	1018	11.250	10.500	13.700	13.100	42.800	79.300	7.417	96.300
3	1019	11.440	21.500	7.300	10.200	MISSING	MISSING	MISSING	92.500
4	1020	11.240	14.600	8.100	15.900	41.800	74.500	7.428	55.700
5	1021	12.320	12.800	12	1.500	MISSING	MISSING	MISSING	144.500
6	1022	11.150	18.600	14.800	11.600	MISSING	MISSING	MISSING	170.200
7	1023	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	70.600
8	1024	11.190	24.700	25.200	3.700	44.800	70.500	7.403	123
9	1041	10.200	23.300	18.900	MISSING	41.100	98.600	7.390	MISSING
10	1042	11.150	27.400	10.700	7.900	43.800	81.200	7.400	39.700
11	1043	11.100	22.700	8.400	9	48.200	68.700	7.374	94.100
12	1044	10.830	18	12.800	2.100	45.700	84.300	7.351	81
13	1045	11.900	9.800	12.600	MISSING	MISSING	MISSING	MISSING	71.400
14	1046	11.300	14.300	6.500	1.500	44.900	73.100	7.390	143.200
15	1047	11.560	4.700	9.600	11.900	MISSING	MISSING	MISSING	37.100
16	1048	10.610	25.900	14.800	1.600	45.400	91.100	7.420	103.500
17	1065	12.210	-4.400	8.800	1.500	43.300	85.900	7.429	125
18	1066	12.510	21.900	1.600	7.600	MISSING	MISSING	MISSING	123.200
19	1067	9.900	11.300	-2.500	25.600	MISSING	MISSING	MISSING	107
20	1068	11.510	21.100	1.200	8.200	MISSING	MISSING	MISSING	MISSING
21	1069	10.740	30.500	15.400	5.800	45.400	75.100	7.422	105.400
22	1070	10.250	15.900	8.100	2.800	MISSING	MISSING	MISSING	187
23	1071	11.850	19.200	12.900	MISSING	MISSING	MISSING	MISSING	85.600
24	1072	11.090	29.800	15	0	MISSING	MISSING	MISSING	68.700

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	1217	1.550	5.500	60	.0800	.280	9.960	10.630	3.800	11.750	.200
26	1218	1.870	5	84	.870	.270	9.260	10.710	3.500	11.820	.212
27	1219	1.740	3.040	64	.590	.380	9.930	11.760	3.870	11.980	.157
28	1220	1.910	5.500	79	1.010	.270	10.600	11.910	3.150	12.860	.221
29	1221	1.820	6.500	48	.880	.210	10.020	10.810	3.420	11.670	.139
30	1222	2.070	4	70	1.470	.220	10.350	10.680	3.200	11.490	.188
31	1223	1.550	5.170	57	.180	.350	10.210	11.250	3.720	12.170	.155
32	1224	2.110	4.830	43	1.230	.350	9.580	11.230	3.300	11.940	.111
33	1241	1.710	5.250	70	.270	.290	10.300	11.460	3.760	12.520	.200
34	1242	1.600	4	58	.140	.470	10.470	11.700	2.710	13.320	.135
35	1243	1.500	5	56	.300	.270	MISSING	MISSING	MISSING	MISSING	MISSING
36	1244	1.550	5	66	.300	.390	10	10.800	3.340	11.820	.175
37	1245	1.580	5.670	62	.360	.350	9.350	10.600	3.300	12.150	.178
38	1246	1.750	6.040	89	MISSING	MISSING	8.770	10.380	3.840	10.980	.189
39	1247	1.670	4.670	60	.350	.400	10.930	12.210	4.370	14.240	.175
40	1248	1.860	7	70	.170	.410	10.060	10.950	4.270	11.930	.167
41	1265	1.860	5.750	70	.430	.370	10.230	11.300	2.930	12.430	.189
42	1266	1.760	5.330	56	.380	.480	10.940	12.010	3.450	13.800	.167
43	1267	1.550	4.500	62	.290	.370	9.940	11	3.350	11.660	.168
44	1268	1.690	5	59	.540	.370	9.070	10.180	3.730	11.130	.159
45	1269	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
46	1270	1.570	5.330	76	.250	.340	10.360	11.590	3.440	12.770	.185
47	1271	1.670	5.500	62	.190	.360	10.690	11.380	2.780	12.540	.182
48	1272	1.570	7	71	.0700	.260	9.950	10.910	3.060	12.330	.147

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	22 PST	23 V30	24 QSCCS	32 VMAX	26 PEF	27 EFR50	28 EFR25	29 EFR10	30 M1M0	33 HR
25	1217	32.400	87	.830	MISSING	119.800	111.300	65.600	31.100	10.100	442
26	1218	MISSING	MISSING	.810	MISSING	MISSING	MISSING	MISSING	MISSING	5.700	353
27	1219	17.550	51.820	.930	70.800	101.400	80.200	45.900	25.600	7	338
28	1220	27	72.950	.990	64.400	124.200	111.700	59.800	27.700	5.200	333
29	1221	20.250	42.950	.950	80.100	102.700	67	38	16.100	10.300	397
30	1222	13.500	73.640	.970	71.100	110.600	94.700	62.700	29.200	10.100	321
31	1223	17.550	53.450	.940	68.400	102.500	80.800	50.600	23.500	MISSING	MISSING
32	1224	16.200	49.090	.950	83.300	89.400	61.600	43.200	28.100	6.800	338
33	1241	21.260	67.500	.990	76.300	105.300	89.500	54.900	21	8.100	437
34	1242	14.850	69.820	.950	73.200	126	97.800	63.100	31.400	5.600	MISSING
35	1243	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	297
36	1244	15.530	67.640	.920	68.900	101.200	89.500	62.500	25.900	9.300	340
37	1245	13.500	70.360	.920	68.900	114.900	98.300	62.700	34.600	5.900	307
38	1246	21.600	74.460	.850	72.100	116.700	97.900	64.100	30.200	8.400	400
39	1247	18.900	64.770	.930	76.700	122.100	86.300	58.500	27.400	5.100	MISSING
40	1248	10.800	69	.920	77.500	122.300	94.900	60.900	26.900	9.100	288
41	1265	16.200	49.090	.990	66.700	109	89.500	42	22.300	7.700	271
42	1266	12.150	71.730	1.050	69.600	113.900	97.400	65.400	27.300	5.800	344
43	1267	22.950	70.360	.950	71.900	124.900	100.700	54.100	24.900	8.700	312
44	1268	31.050	64.360	.920	77	108.500	84.300	57.200	32.400	7.900	343
45	1269	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	303
46	1270	16.200	84	.950	66.700	129.300	110.500	69.600	28.600	7.300	345
47	1271	20.250	64.090	.890	67.900	122.900	99.200	55	31.400	9.700	344
48	1272	18.900	52.360	.910	79.300	112	78.100	47.900	22.300	7.100	284

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	34 PR	35 QRS	37 VOLN15	38 VOLN10	39 VOLN5	40 VOL0	41 VOL5	42 VOL10	43 VOL15	44 VOL20
25	1217	.0438	.00880	0	.380	.480	.780	7.030	9.380	10.080	10.580
26	1218	.0450	.0150	0	.660	1.400	1.460	7.660	9.560	10.160	10.460
27	1219	MISSING	MISSING	0	1.260	1.430	1.830	9.980	10.830	11.130	11.430
28	1220	.0495	.0100	0	.620	.720	1.320	8.970	10.720	11.370	11.720
29	1221	.0475	.0175	0	.0200	.150	.790	7.030	9.460	10.220	10.580
30	1222	.0450	.00750	0	.0200	.120	.320	7.970	9.620	10.220	10.520
31	1223	MISSING	MISSING	0	0	0	1.040	10.140	10.640	10.940	11.040
32	1224	.0438	.0100	0	.0500	.350	1.650	8.200	10.150	10.650	11.050
33	1241	MISSING	MISSING	0	.0600	1.010	1.160	8.660	10.260	10.910	11.160
34	1242	MISSING	MISSING	0	.0300	.180	1.230	8.680	10.630	11.280	11.630
35	1243	.0500	.0113	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
36	1244	.0438	.0113	0	0	.100	.800	8.300	9.800	10.250	10.600
37	1245	.0450	.0100	0	.0500	.150	1.250	8.300	9.650	10.100	10.450
38	1246	.0425	.0100	0	.310	.510	1.610	7.410	9.110	9.710	10.010
39	1247	MISSING	MISSING	0	0	0	1.280	9.300	11.180	11.630	12.080
40	1248	.0475	.0100	0	0	0	.890	8.480	10.190	10.640	10.890
41	1265	.0425	.0163	0	.170	.370	1.070	8.770	10.270	10.820	11.070
42	1266	.0475	.0100	0	0	0	1.070	10.770	11.470	11.720	11.870
43	1267	.0425	.0100	0	0	.860	1.060	10.010	10.560	10.810	10.860
44	1268	.0475	.0175	0	.310	.610	1.110	8.410	9.710	9.960	10.110
45	1269	.0475	.0138	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
46	1270	.0400	.0100	0	.0300	.0800	1.230	8.330	10.330	10.980	11.430
47	1271	.0488	.0125	0	.0800	.430	.680	7.630	9.980	10.730	11.080
48	1272	.0460	.0138	0	.0600	.360	.960	7.910	9.760	10.260	10.760

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	45 VOL25	49 DHEFR50	50 DHEFR25	52 ISOFLOW	53 PCO2	54 PO2	55 PH	56 CO2RESP
25	1217	10.780	120.800	72.800	MISSING	43.200	122.700	7.353	79.600
26	1218	10.710	MISSING	MISSING	MISSING	38.200	92.900	7.420	88.800
27	1219	11.830	8.600	10.600	.500	MISSING	MISSING	MISSING	135.200
28	1220	11.820	-4.400	15.600	59.500	MISSING	MISSING	MISSING	39.700
29	1221	10.780	21	11	7	MISSING	MISSING	MISSING	48.400
30	1222	10.570	12.100	6.400	11.100	MISSING	MISSING	MISSING	108.300
31	1223	11.290	30.300	12.700	3.600	42.400	83.800	7.388	113.500
32	1224	11.150	5.900	-7.600	5.400	45.100	82.400	7.411	74
33	1241	11.410	33.900	19.900	0	40.200	96.300	7.375	119.300
34	1242	11.730	21.800	14.800	5.600	36.700	109.100	7.444	95.200
35	1243	MISSING	MISSING	MISSING	MISSING	45.400	61	7.338	103.500
36	1244	10.800	17.300	12.200	0	MISSING	MISSING	MISSING	114.800
37	1245	10.500	9.400	6.600	14.600	MISSING	MISSING	MISSING	74.700
38	1246	10.360	20.200	3.400	12.400	43.100	99.200	7.385	133.800
39	1247	12.210	33	19.400	12.500	42.600	102.700	7.409	67.800
40	1248	10.950	33.300	22.700	4.700	MISSING	MISSING	MISSING	179.400
41	1265	11.320	6.600	8.800	1	MISSING	MISSING	MISSING	86.100
42	1266	12.010	27.200	13.200	0	MISSING	MISSING	MISSING	114.500
43	1267	11.060	27	16.700	5.800	MISSING	MISSING	MISSING	MISSING
44	1268	10.180	14.600	6.100	19.900	37.800	73	7.447	98.700
45	1269	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	121.100
46	1270	11.480	20.700	7.500	1.500	53.100	72.800	7.376	185.400
47	1271	11.430	26	.200	6.700	MISSING	MISSING	MISSING	33.800
48	1272	10.960	37.700	21.100	5.300	43.100	105.300	7.426	105.100

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	1417	1.900	6	72	.480	.310	10.460	10.900	3.970	11.920	.182
50	1418	2.050	7.250	80	1.790	.220	10.030	11.560	2.910	12.890	.183
51	1419	1.740	7.330	86	.840	.280	10.470	10.930	3.430	12.120	.195
52	1420	1.740	5.500	73	.160	.330	9.660	10.450	2.540	11.280	.190
53	1421	1.910	4	59	.160	.490	9.630	10.750	3.880	11.480	.158
54	1422	1.680	5	62	.290	.490	10.090	11.210	3.170	12.350	.193
55	1423	1.830	6.580	89	.370	.240	10.030	10.480	3.340	11.630	.166
56	1424	1.450	5.250	67	.330	.290	9.570	10.260	2.880	11.170	.161
57	1441	1.690	5.750	60	.370	.290	9.390	10.440	3.090	11.140	.145
58	1442	1.500	7	62	.470	.330	9.980	10.740	3.990	12.300	.197
59	1443	1.670	6	64	.450	.200	9.470	10.350	2.850	11.460	.157
60	1444	1.460	5.250	63	.540	.390	10.680	11.660	3.500	13.040	.155
61	1445	1.790	4.250	52	.270	.410	10.780	11.740	3.770	13.080	.143
62	1446	1.620	5.500	61	.220	.390	10.670	11.850	3.150	14.380	.192
63	1447	1.700	5.920	76	.740	.290	10.200	11.140	4.750	12.050	.200
64	1448	1.820	5.500	66	.150	.390	11	11.750	2.880	MISSING	MISSING
65	1465	1.700	6.170	65	.250	.300	10.800	11.290	3.660	12.080	.179
66	1466	1.830	5	63	.130	.700	11.530	12.640	3.600	13.960	.210
67	1467	1.760	5.500	66	.170	.400	10.840	11.810	3.220	13.100	.197
68	1468	1.880	5	64	.210	.540	11.540	12.680	3.450	14.600	.221
69	1469	1.820	6	85	1.460	.500	10.460	11.290	3.350	12.490	.193
70	1470	1.620	5.500	60	.170	.380	10.600	11.500	3.190	12.630	.200
71	1471	1.700	7.330	64	.220	.310	10.560	11.950	3.680	13.550	.208
72	1472	1.730	5.920	71	.210	.300	11.100	11.750	2.670	13.270	.195

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	22 PST	23 V30	24 QSCCS	32 VMAX	26 PEF	27 EFR50	28 EFR25	29 EFR10	30 M1M0	33 HR
49	1417	13.500	67.640	.990	64.700	111.100	91.200	57.100	32.700	13.900	333
50	1418	28.350	68.180	.920	73.300	107.900	96.300	57.900	23	6	352
51	1419	21.600	49.640	1.010	72.400	88.700	69.900	39.800	20	11.300	328
52	1420	13.500	72.270	.950	60.300	101.900	93.300	60.500	29.300	10.700	364
53	1421	16.200	75.820	.850	65.200	118.200	106.700	69.700	24	8.400	258
54	1422	20.250	62.730	1.010	63.300	105	96.300	56.100	27.200	7.100	335
55	1423	16.200	58.910	.940	77.800	104.900	87	51.800	25	MISSING	MISSING
56	1424	24.300	60.550	.900	78.100	106.800	81.900	53	26.600	9.400	245
57	1441	18.900	54	.930	71	110.500	82.500	50.900	25.600	9.700	331
58	1442	21.600	59.460	.920	60.400	93.900	87.800	50.300	22.300	5.900	257
59	1443	27	60.270	.800	72.100	108.900	91.300	49.700	18.800	8.300	308
60	1444	39.150	51.550	.950	77	100.100	75.500	48	21.700	7	345
61	1445	20.250	75.680	1.010	72.600	134.400	104.200	68.300	28	8	234
62	1446	MISSING	60	.950	67.300	128.200	97.100	53.200	23.300	4.500	293
63	1447	MISSING	69.820	.940	78.500	105	95	56.900	16.700	10.700	378
64	1448	MISSING	MISSING	1	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	378
65	1465	16.200	69	1.040	65.900	110.900	94.200	61.900	31.300	13.900	245
66	1466	28.350	74.730	1.030	71.300	132.500	106	66.200	27.200	7.100	309
67	1467	21.600	69.550	1	67.600	128.700	109.200	57	25.900	8.900	MISSING
68	1468	31.730	70.360	1.100	74.100	127.600	99	49.400	11.800	5.300	364
69	1469	30.110	71.730	1.010	65	113.600	102.800	55.700	22.900	9.700	320
70	1470	21.600	74.180	1.030	68.900	121.100	104.200	64.700	31.500	9.300	282
71	1471	24.300	70.230	.990	79.900	119.100	92	64.700	32.200	6.500	MISSING
72	1472	21.600	64.360	1	75.300	132.100	89.200	59.300	24	8.400	311

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	34 PR	35 QRS	37 VOLN15	38 VOLN10	39 VOLN5	40 VOL0	41 VOL5	42 VOL10	43 VOL15	44 VOL20
49	1417	.0375	.0100	0	.0400	.140	.440	8.490	9.640	10.340	10.840
50	1418	.0438	.0125	0	1.030	1.130	1.530	7.730	9.930	10.680	11.130
51	1419	.0513	.0156	0	.100	.110	.460	8.210	9.760	10.360	10.660
52	1420	.0425	.0138	0	.0900	.140	.790	7.540	9.190	9.790	10.190
53	1421	.0475	.0150	0	.120	.120	1.120	7.070	9.420	10.120	10.520
54	1422	.0463	.0125	0	.0300	.430	1.130	8.080	9.930	10.730	10.930
55	1423	MISSING	MISSING	0	.140	.190	.440	6.990	9.040	9.740	10.240
56	1424	.0475	.0138	0	0	0	.690	8.740	9.690	9.990	10.090
57	1441	.0425	.0113	0	.0500	.350	1.050	7.950	9.350	9.900	10.250
58	1442	.0463	.0113	0	0	0	.760	9.710	10.160	10.360	10.560
59	1443	.0488	.0138	0	.180	.230	.880	6.530	8.980	9.580	10.080
60	1444	.0438	.0100	0	.580	.930	.980	7.730	10.090	10.880	11.380
61	1445	.0463	.0100	0	0	0	.950	10.250	11.050	11.450	11.550
62	1446	.0488	.0150	0	.0800	.480	1.180	8.530	10.680	11.230	11.580
63	1447	.0438	.0150	0	.0400	MISSING	.940	8.640	9.940	10.540	11.140
64	1448	.0425	.0100	0	.550	.600	.750	8.400	10.750	11.550	11.950
65	1465	.0488	.0150	0	.0100	.190	.490	8.440	10.290	10.840	11.090
66	1466	.0500	.0125	0	.110	.260	1.110	9.410	11.410	12.060	12.510
67	1467	MISSING	MISSING	0	.0700	.120	.970	8.420	10.570	11.170	11.570
68	1468	.0475	.0100	0	0	0	1.140	11.640	12.140	12.390	12.540
69	1469	.0513	.0188	0	0	.380	.830	8.830	10.230	10.880	11.230
70	1470	.0465	.0113	0	0	.150	.900	8.150	10.200	10.950	11.300
71	1471	MISSING	MISSING	0	.0900	.190	1.390	7.990	10.390	11.140	11.590
72	1472	.0450	.0125	0	.0500	.150	.650	8.150	10.350	11	11.450

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	45 VOL25	49 DHEFR50	50 DHEFR25	52 ISOFLOW	53 PCO2	54 PO2	55 PH	56 CO2RESP
49	1417	10.940	18.700	17.900	3.200	48.700	66.700	7.422	244.700
50	1418	11.530	11.100	16.400	0	MISSING	MISSING	MISSING	102.100
51	1419	10.960	22	17.200	12.800	MISSING	MISSING	MISSING	177.100
52	1420	10.290	12.900	1.600	22.900	41.800	69.100	7.405	86.700
53	1421	10.620	4.800	-4.100	MISSING	MISSING	MISSING	MISSING	69.700
54	1422	11.130	13.500	14.200	15.600	43.500	71.500	7.401	94.600
55	1423	10.440	10.900	-.100	25.600	46.300	63.100	7.483	135.800
56	1424	10.190	14.400	6.200	2.300	MISSING	MISSING	MISSING	69.700
57	1441	10.300	23.400	17.200	4.600	45.400	79.300	7.385	173.800
58	1442	10.760	19.700	14	1.100	MISSING	MISSING	MISSING	95.500
59	1443	10.380	26.900	10.600	0	46.500	78.500	7.371	MISSING
60	1444	11.480	20.800	11.600	0	MISSING	MISSING	MISSING	127
61	1445	11.700	19.400	3.400	11.700	48	75.100	7.361	149.800
62	1446	11.930	18.100	12.600	2.500	MISSING	MISSING	MISSING	35.700
63	1447	11.140	21.900	8.600	0	46	92.800	7.341	84.100
64	1448	12.250	MISSING	MISSING	MISSING	44.200	64.900	7.400	53.800
65	1465	11.290	23.800	13.200	13	MISSING	MISSING	MISSING	62.200
66	1466	12.640	16.400	6.200	0	45.900	72.400	7.405	76.300
67	1467	11.720	22.800	17.100	4.900	MISSING	MISSING	MISSING	MISSING
68	1468	12.680	7.200	-14.700	38.200	43.900	103.300	7.391	95.200
69	1469	11.290	16.800	15.100	5	MISSING	MISSING	MISSING	59.200
70	1470	11.500	19.200	9.500	0	MISSING	MISSING	MISSING	100.900
71	1471	11.890	17.100	8.400	14.700	MISSING	MISSING	MISSING	114.500
72	1472	11.650	15.400	.300	0	MISSING	MISSING	MISSING	136.900

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	1617	1.300	6.250	83	.750	.170	9.040	9.540	2.870	10.330	.0990
74	1618	1.660	5.500	83	.540	.250	9.660	10.390	2.390	11.230	.124
75	1619	1.390	8	82	.320	.160	8.460	9.340	2.360	10.080	.124
76	1620	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
77	1621	1.440	7.330	84	1.470	.130	9.060	9.810	2.510	10.510	.124
78	1622	1.550	6.690	93	.290	.290	8.170	9.160	3.040	10.130	.130
79	1623	1.780	7	79	.750	.220	9.110	10.160	3.270	10.680	.120
80	1624	1.170	7.510	108	MISSING	MISSING	9.020	9.440	2.860	10.460	.110
81	1641	1.470	6.820	150	.410	.270	8.920	9.960	3.090	10.330	.134
82	1642	1.490	7.310	120	MISSING	MISSING	8.300	9.130	2.300	9.530	.105
83	1643	1.480	7	121	.180	.250	8.880	9.880	2.140	10.600	.113
84	1644	1.340	7.510	82	.370	.240	8.230	9.490	2.700	10.180	.147
85	1645	1.510	7.010	83	.560	.230	9.870	10.450	4.020	11.100	.148
86	1645	1.290	5.830	112	MISSING	MISSING	9.180	10.230	3.170	10.960	.140
87	1647	1.590	5	75	.570	.140	9.420	10.090	2.800	11.150	.136
88	1648	1.750	7.190	103	.180	.290	9.550	10.910	3.540	11.820	.123
89	1665	1.490	4.350	104	.180	.380	9.760	10.460	2.910	11.580	.124
90	1666	1.300	5.840	99	.360	.260	9.180	9.830	2.790	10.460	.136
91	1667	1.480	4.800	117	1.520	.180	10.150	10.860	2.780	11.750	.145
92	1668	1.520	6.250	113	.230	.150	8.560	9.310	2.670	9.940	.112
93	1669	1.460	6	58	.260	.430	10.540	11.380	3.710	12.440	.151
94	1670	1.650	5.930	110	.160	.320	9.560	10.600	2.850	11.880	.143
95	1671	1.740	7.500	117	.130	.290	9.260	10.080	3.100	10.660	.138
96	1672	1.430	7.500	130	.130	.320	8.410	9.560	2.250	10.340	.139

20 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	22 PST	23 V30	24 QSCCS	32 VMAX	26 PEF	27 EFR50	28 EFR25	29 EFR10	30 M1M0	33 HR
73	1617	27	63.270	.800	68.600	105.800	91.600	58.900	23.300	15	279
74	1618	18.900	63.540	.920	54.800	100.400	91.300	47.500	19.400	9.300	261
75	1619	14.850	62.050	.790	65.800	110.900	93.800	53.500	23.700	MISSING	239
76	1620	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	327
77	1621	21.600	46.910	.830	62.200	93.800	75.900	34.300	8	9.200	MISSING
78	1622	17.550	49.090	.770	74.800	101.200	84.400	40	10.400	8.100	MISSING
79	1623	16.200	70.360	.920	66.800	118	97.200	54.300	24.500	MISSING	MISSING
80	1624	29.700	70.230	.820	63.700	112.100	101.900	61.500	24	MISSING	MISSING
81	1641	28.350	55.090	.810	66.700	105.600	87.100	46.200	17.800	12	MISSING
82	1642	16.200	70.910	.770	64.100	109	93.700	58.200	24.900	13.500	282
83	1643	18.630	58.090	.850	63.700	88.900	83.900	52.600	22.500	7.700	304
84	1644	22.280	56.180	.750	61.500	101.800	90	45.900	20.900	8.200	356
85	1645	28.350	66	.920	71.100	118.200	94.300	54.900	25.900	MISSING	MISSING
86	1646	24.300	64.910	.920	63	104.400	95.500	55.900	23.200	MISSING	MISSING
87	1647	22.950	65.460	.840	60	90.400	80.500	60.800	21.400	10.300	MISSING
88	1648	33.080	75.820	.970	71.900	114.600	96.200	67.900	24.500	7.900	275
89	1665	13.500	60	.940	69	109.100	92.600	48.800	18.500	8.100	306
90	1666	29.700	68.730	.920	69.500	153.100	117.800	71.800	32.400	13.900	300
91	1667	33.750	73.090	.970	62.100	128.500	110.100	57.500	28.200	11.100	337
92	1668	14.180	65.180	.870	63.700	120.200	99.800	52.200	22.700	13.300	333
93	1669	16.200	80.050	1.010	71.400	126.400	103.900	60.200	19.600	10	247
94	1670	14.850	68.180	.920	79.300	121.400	101.600	59.400	20.200	7.700	276
95	1671	27	56.180	.810	76	104.500	81.900	46.400	23	13.600	316
96	1672	27	62.460	.820	74.100	108.900	95.100	53.700	18.300	8.800	414

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	1617	.Ø463	.Ø125	Ø	.1ØØ	.2ØØ	.5ØØ	6.85Ø	8.4ØØ	8.9ØØ	9.3ØØ
74	1618	.Ø5ØØ	.Ø1ØØ	Ø	.33Ø	.48Ø	.73Ø	7.43Ø	9.23Ø	9.88Ø	1Ø.13Ø
75	1619	.Ø475	.Ø15Ø	Ø	.Ø8ØØ	.Ø8ØØ	.68Ø	6.83Ø	8.28Ø	8.83Ø	9.Ø8Ø
76	162Ø	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING
77	1621	MISSING	MISSING	Ø	Ø	.65Ø	.75Ø	7.3ØØ	9.35Ø	9.9ØØ	1Ø.15Ø
78	1622	MISSING	MISSING	Ø	.Ø1ØØ	.14Ø	.99Ø	6.79Ø	8.19Ø	8.64Ø	8.99Ø
79	1623	MISSING	MISSING	Ø	.Ø6ØØ	.21Ø	1.Ø6Ø	8.Ø1Ø	9.16Ø	9.61Ø	9.86Ø
8Ø	1624	MISSING	MISSING	Ø	.12Ø	.22Ø	.42Ø	6.37Ø	8.32Ø	8.82Ø	9.22Ø
81	1641	MISSING	MISSING	Ø	Ø	Ø	1.Ø5Ø	8.35Ø	9.35Ø	9.6ØØ	9.85Ø
82	1642	.Ø513	.Ø138	Ø	.22Ø	.32Ø	.82Ø	6.97Ø	8.22Ø	8.62Ø	9.Ø2Ø
83	1643	.Ø525	.Ø175	Ø	Ø	Ø	1	8.5ØØ	9.4ØØ	9.55Ø	9.8ØØ
84	1644	.Ø5ØØ	.Ø175	Ø	.Ø6ØØ	.16Ø	1.26Ø	6.76Ø	8.46Ø	8.91Ø	9.26Ø
85	1645	MISSING	MISSING	Ø	.Ø8ØØ	.18Ø	.58Ø	6.38Ø	9.Ø8Ø	9.88Ø	1Ø.18Ø
86	1646	MISSING	MISSING	Ø	.24Ø	.44Ø	1.Ø4Ø	8.Ø4Ø	9.34Ø	9.89Ø	1Ø.Ø4Ø
87	1647	MISSING	MISSING	Ø	.Ø7ØØ	.32Ø	.67Ø	7.37Ø	9.Ø7Ø	9.52Ø	9.87Ø
88	1648	.Ø49Ø	.Ø125	Ø	Ø	Ø	1.36Ø	9.46Ø	1Ø.16Ø	1Ø.51Ø	1Ø.76Ø
89	1665	.Ø463	.Ø125	Ø	.Ø1ØØ	.56Ø	.71Ø	8.Ø6Ø	9.41Ø	1Ø.Ø1Ø	1Ø.31Ø
9Ø	1666	.Ø475	.Ø138	Ø	.24Ø	.44Ø	.64Ø	7.49Ø	8.84Ø	9.49Ø	9.64Ø
91	1667	.Ø45Ø	.Ø1ØØ	Ø	.31Ø	.46Ø	.71Ø	8.21Ø	9.71Ø	1Ø.31Ø	1Ø.71Ø
92	1668	.Ø53Ø	.Ø13Ø	Ø	.Ø5ØØ	.25Ø	.75Ø	7.45Ø	8.55Ø	9	9.15Ø
93	1669	.Ø45Ø	.Ø125	Ø	.Ø4ØØ	.24Ø	.84Ø	8.44Ø	1Ø.14Ø	1Ø.89Ø	11.24Ø
94	167Ø	.Ø45Ø	.Ø15Ø	Ø	.Ø4ØØ	.19Ø	1.Ø4Ø	7.99Ø	9.44Ø	1Ø.Ø4Ø	1Ø.44Ø
95	1671	.Ø45Ø	.ØØ88Ø	Ø	.11Ø	.31Ø	.81Ø	7.21Ø	8.81Ø	9.51Ø	9.81Ø
96	1672	.Ø475	.Ø113	Ø	.25Ø	.35Ø	1.15Ø	6.9ØØ	8.45Ø	8.95Ø	9.35Ø

20 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	45 VOL25	49 DHEFR5Ø	5Ø DHEFR25	52 ISOFLOW	53 PCO2	54 PO2	55 PH	56 CO2RESP
73	1617	9.5ØØ	-4.5ØØ	-5.2ØØ	14.9ØØ	41	73.3ØØ	7.424	93.6ØØ
74	1618	1Ø.48Ø	12.6ØØ	8.5ØØ	1Ø.7ØØ	44	67.8ØØ	7.554	29.6ØØ
75	1619	9.38Ø	14.5ØØ	9.9ØØ	Ø	43.5ØØ	73.2ØØ	7.395	1Ø3.6ØØ
76	162Ø	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	MISSING	1Ø5.2ØØ
77	1621	1Ø.5ØØ	6.3ØØ	-4.8ØØ	38.3ØØ	MISSING	MISSING	MISSING	113.2ØØ
78	1622	9.24Ø	11.9ØØ	16.8ØØ	Ø	44.8ØØ	64.8ØØ	7.449	12Ø.6ØØ
79	1623	1Ø.Ø6Ø	19	6.8ØØ	12.2ØØ	MISSING	MISSING	MISSING	99.3ØØ
8Ø	1624	9.42Ø	17.1ØØ	3.6ØØ	15.5ØØ	MISSING	MISSING	MISSING	1Ø1.8ØØ
81	1641	1Ø.Ø5Ø	19.2ØØ	11.5ØØ	Ø	MISSING	MISSING	MISSING	54.3ØØ
82	1642	9.Ø7Ø	12.7ØØ	6.9ØØ	1.4ØØ	42.3ØØ	63.9ØØ	7.4Ø6	31.7ØØ
83	1643	9.88Ø	11.7ØØ	9.8ØØ	13.9ØØ	MISSING	MISSING	MISSING	1Ø5.7ØØ
84	1644	9.51Ø	22.6ØØ	11.9ØØ	6.8ØØ	4Ø.5ØØ	116.6ØØ	7.361	85.1ØØ
85	1645	1Ø.45Ø	22.2ØØ	18	1Ø	44.8ØØ	7Ø.8ØØ	7.41Ø	1Ø3.4ØØ
86	1646	1Ø.23Ø	14.3ØØ	12	7.8ØØ	4Ø.3ØØ	1Ø2.2ØØ	7.382	66.8ØØ
87	1647	1Ø.17Ø	16.4ØØ	2.7ØØ	1.7ØØ	42	64.7ØØ	7.395	68.3ØØ
88	1648	1Ø.91Ø	22	9	Ø	43.1ØØ	7Ø.1ØØ	7.4Ø9	51.9ØØ
89	1665	1Ø.46Ø	11	14.5ØØ	3.4ØØ	MISSING	MISSING	MISSING	64.5ØØ
9Ø	1666	9.83Ø	2Ø.7ØØ	1Ø.3ØØ	2.2ØØ	MISSING	MISSING	MISSING	87.4ØØ
91	1667	1Ø.95Ø	2Ø.3ØØ	1Ø	6.4ØØ	MISSING	MISSING	MISSING	MISSING
92	1668	9.31Ø	17.2ØØ	11.9ØØ	3.1ØØ	MISSING	MISSING	MISSING	MISSING
93	1669	11.34Ø	21.8ØØ	16.4ØØ	14.3ØØ	MISSING	MISSING	MISSING	119.4ØØ
94	167Ø	1Ø.54Ø	18.8ØØ	4.3ØØ	6.4ØØ	43.7ØØ	62	7.4Ø3	37.9ØØ
95	1671	1Ø.Ø6Ø	2Ø.8ØØ	6.2ØØ	8.6ØØ	MISSING	MISSING	MISSING	112.2ØØ
96	1672	9.4ØØ	7.3ØØ	.2ØØ	3.6ØØ	MISSING	MISSING	MISSING	84.6ØØ

APPENDIX E

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	5 DRYWT	6 OHPR	7 PROTEIN	8 DNA	9 ELASTIN
1	1017	312.100	3.130	198.300	6.540	8.210
2	1018	280.700	2.940	176.100	5.820	7.360
3	1019	256.300	2.350	160	5.350	6.540
4	1020	361	3.290	220	7.480	8.910
5	1021	318	2.970	202.700	6.750	8.100
6	1022	275.200	2.830	176.300	5.550	7.040
7	1023	303.500	2.730	195.400	6.320	7.620
8	1024	298.900	2.750	183.600	6.150	7.930
9	1041	263.400	2.430	166.700	5.350	6.730
10	1042	380.500	3.690	239.300	7.790	9.430
11	1043	277.100	2.540	172.600	5.870	7.170
12	1044	281	2.550	177.100	5.720	7.260
13	1045	385.200	3.540	237.600	7.980	9.160
14	1046	272	2.540	167.300	5.690	7.180
15	1047	304.900	3	194.200	6.410	7.670
16	1048	234.100	2.310	140.600	5.040	6.140
17	1065	307.400	2.940	196.600	6.540	7.940
18	1066	316.900	3.060	201.200	6.690	8.240
19	1067	291.700	2.840	183.300	5.970	7.630
20	1068	314.100	3	196.900	6.500	8.190
21	1069	303.200	2.970	190.900	6.120	7.760
22	1070	302	2.900	192.200	6.130	7.860
23	1071	286.600	2.750	183.100	5.770	7.550
24	1072	312.400	3.070	197	6.450	7.900

2 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	5 DRYWT	6 OHPR	7 PROTEIN	8 DNA	9 ELASTIN
49	1417	268.500	2.840	176	5.940	6.870
50	1418	331.300	3.240	213.200	7.080	8.140
51	1419	319.700	3.200	192.600	7	8.360
52	1420	392.800	4.640	245.700	7.960	9.620
53	1421	262.900	3.340	160.200	5.960	6.990
54	1422	282.100	2.880	181.100	6.070	7.380
55	1423	301.800	3.020	186	6.430	7.120
56	1424	376.600	3.920	235.900	7.440	9.670
57	1441	355	4.250	222.100	7.110	9.190
58	1442	346.500	3.710	225.700	7.130	8.660
59	1443	318	3.280	193.900	6.620	8.060
60	1444	312.600	3.350	194.200	6.400	8.020
61	1445	284.100	3.010	176.400	6.400	8.110
62	1446	325.900	3.410	193.100	7.330	8.450
63	1447	351.800	3.720	212.300	7.560	9.220
64	1448	MISSING	MISSING	MISSING	MISSING	MISSING
65	1465	312.400	3.310	189.700	6.720	8.360
66	1466	346.500	3.620	218.600	7.660	8.890
67	1467	298.800	3.230	184.600	6.470	7.700
68	1468	357	3.820	222.700	7.470	9.250
69	1469	326	3.410	203.900	6.990	8.500
70	1470	319.700	3.300	199.500	6.570	8.120
71	1471	367.200	3.780	236.500	7.370	9.340
72	1472	316.300	3.490	195	6.950	8.080

10 mg SiO_2/m^3 GROUP

CASE NO.	LABEL	5 DRYWT	6 OHPR	7 PROTEIN	8 DNA	9 ELASTIN
25	1217	294.500	2.890	184.200	6.400	7.440
26	1218	360.700	3.300	220.100	7.060	9.460
27	1219	330.400	3.090	202.100	6.990	8.960
28	1220	323.900	3.440	208.900	6.850	8.390
29	1221	294.900	3.030	190.200	6.230	8.380
30	1222	323.800	3.160	208.200	6.780	7.780
31	1223	288.100	2.820	177.200	5.760	7.650
32	1224	296.800	2.790	182.500	6.370	7.170
33	1241	317.400	3.120	193.500	6.540	7.930
34	1242	334.300	3.490	210.300	6.880	8.330
35	1243	MISSING	MISSING	MISSING	MISSING	MISSING
36	1244	320.100	3.370	199.600	6.440	8.420
37	1245	321.800	3	198.500	6.900	8.740
38	1246	294.800	2.820	182.500	6.290	7.850
39	1247	292.100	3.050	178.100	6.230	8.270
40	1248	315.500	3.120	198.800	6.680	8.290
41	1265	344.300	3.260	216	7.110	8.940
42	1266	355.200	3.800	223.800	7.360	9.010
43	1267	327.200	3.390	211.100	6.620	8.330
44	1268	325.800	3.240	210.100	6.730	8.370
45	1269	350.800	3.660	219.100	7.430	9.020
46	1270	342.900	3.540	215.600	6.950	8.640
47	1271	304.100	3.060	195.500	6.540	7.970
48	1272	292	2.930	185.400	6.180	7.430

20 mg SiO_2 GROUP

CASE NO.	LABEL	5 DRYWT	6 OHPR	7 PROTEIN	8 DNA	9 ELASTIN
73	1617	566.900	5.530	247.400	8.840	9.710
74	1618	657.700	5.770	310	10.460	10.560
75	1619	542.800	4.040	233.400	8.570	9.030
76	1620	642.700	4.830	347.800	10.300	9.920
77	1621	453.700	4.360	217	7.720	9.680
78	1622	852.700	6.660	402.700	13.570	13.490
79	1623	599.300	4.980	288.900	8.920	10.300
80	1624	602.100	5.170	296.100	9.060	10.690
81	1641	659.900	5.520	347.400	10.160	10.510
82	1642	672.100	6.040	302.600	9.600	11.570
83	1643	650.500	5.560	297	10.670	11.830
84	1644	528.300	4.960	270.500	8.600	11.040
85	1645	517.300	4.470	251.800	9.030	10.750
86	1646	550.300	4.650	291.700	9.480	10.190
87	1647	497	4.100	223.900	7.850	10
88	1648	647.900	5.410	314.200	10.570	11.650
89	1665	514.900	4.490	257.300	9.350	10.450
90	1666	621.300	5.030	309.200	10.540	11.400
91	1667	664.400	4.870	352.900	10.270	11.990
92	1668	MISSING	MISSING	MISSING	MISSING	MISSING
93	1669	444.400	4.120	235	7.690	9.890
94	1670	593.800	5.230	280.800	8.910	10.740
95	1671	666.400	5.390	356.800	10.390	12.080
96	1672	636.500	5.240	335.400	9.760	11.390